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Development of Long-Term Practical Solutions to the Universal Problem of Biofilm Contamination of Dental Unit Waterlines

A thesis submitted to the University of Dublin, Trinity College, in fulfilment of the requirements for the degree Doctor of Philosophy by

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'All that is valuable in human society depends upon the opportunity for development accorded the individual'

Albert Einstein

Summary

Microbial contamination of dental chair unit (DCU) output water by biofilm growth in dental unit waterlines (DUWLs) is a universal problem and a potentially significant source of cross-infection. At the outset of this project the emphasis on DUWL biofilm in the literature focused on describing the problem and attempts at its control using a range of DUWL treatment agents. However, no long-term studies on the efficacy of DUWL disinfection using a large number of DCUs have been reported. Furthermore, until recently, DCU manufacturers have been slow in developing DCU design changes to facilitate DUWL biofilm control.

In the early 2000s a collaboration was established between this laboratory and the DCU manufacturer Planmeca (Helsinki, Finland) to develop DUWL disinfection systems integrated into DCUs. The purpose of the first two parts of the present study was to investigate whether two such prototype systems could effectively control DUWL biofilm in the long-term and to identify and rectify design, microbiological and human factors that contribute to DUWL disinfection failure. The first part of the study investigated the long-term (21-months) efficacy of the Planmeca Waterline Cleaning System (WCS™), a semi-automated DUWL cleaning system retrofitted to 10 Planmeca Prostyle Compact DCUs, to maintain the quality of DUWL output water below the American Dental Association (ADA) standard of ≤200 cfu/ml of aerobic heterotrophic bacteria using once weekly disinfection with the hydrogen peroxide and silver ion-containing disinfectant Planosil. Microbial quality of water was monitored by culture on R2A agar. In the majority of disinfection cycles investigated (272/300), once weekly disinfection maintained DUWL water quality below the ADA standard by effective DUWL biofilm removal as determined by electron microscopy. However, during the first 9-months a high prevalence (28/300 disinfection cycles; 9.3%) of intermittent DUWL disinfection failure occurred in 8/10 DCUs due to operator omission to disinfect all DUWLs (10/28 failed cycles), incorrect compressed air pressure failing to distribute the disinfectant properly (4/28 failed cycles) and physical blockage of disinfectant intake valves due to disinfectant corrosion effects (14/28 failed cycles). On rectification of these faults and omissions through component redesign and procedural changes, no further cases of intermittent DUWL disinfection failure were observed. Independently of these factors, a rapid and consistent decline in efficacy of DUWL disinfection occurred in 4/10 DCUs following the initial nine months due to strongly catalase-positive *Novosphingobium* and *Sphingomonas* bacterial species, indicating selective pressure for their maintenance by prolonged disinfectant usage. Planosil was reformulated to a more concentrated form (Planosil Forte) and when used once weekly in 6 DCUs consistently maintained the bacterial density in DUWL output water below the ADA standard for up to a week post-disinfection during a 17 week study period.

In the second part of the study the microbial quality of DUWL output water from a new Planmeca Compact i DCU equipped with the novel Water Management System (WMS), an integrated and automated DUWL cleaning system, was investigated over a 12-month period with the DUWL disinfectants Planosil and Planosil Forte. Disinfection of DUWLs once weekly with Planosil for 10 weeks resulted in a dramatic reduction in bacterial density immediately following disinfection (mean average 26 cfu/ml). However, bacterial density rose steadily between disinfections and by seven days post-disinfection, DUWL water quality failed (mean average 384 cfu/ml) the ADA standard due to the selection of strongly catalase-positive *Sphingomonas* spp. The DCU was then disinfected once weekly for 40 weeks with Planosil Forte. The average bacterial density immediately post-disinfection was 20 cfu/ml and seven days post-disinfection was 113 cfu/ml, below the ADA standard. Improved output water quality following disinfection with both disinfectants was associated with marked elimination of DUWL biofilm.

The objective of the third part of the study was to develop a centralised, automated water quality and biofilm management system serving the distribution network providing water to Dublin Dental School and Hospital's 103 DCUs, capable of maintaining DUWL supply and output water at better than potable quality standards in the long-term and requiring a minimum of human intervention. This project was undertaken in collaboration with the electrochemically-activated (ECA) disinfectant manufacturer Trustwater (Clonmel, Ireland). To achieve this objective mains water of varying quality was treated by specifically selected automated filtration units to provide DCUs with water of consistent chemical composition. This water was then automatically disinfected using the ECA solution Ecasol™ (2.5 ppm) prior to distribution to DCUs. Microbiological quality of processed DUWL supply and output water was monitored weekly by culture on R2A agar for 10 sentinel DCUs for a 100-week period. The chemical composition of processed mains water consistently bettered potable water standards. The microbiological quality of processed DUWL supply water (average <1 cfu/ml) and output water (average 18.1 cfu/ml) also bettered potable water standards from the 10 DCUs. This correlated with the absence of biofilm in DUWLs. No adverse effects due to Ecasol™ treatment of supply water were observed for DUWLs or DCU instruments.

The research described in this thesis has shown by a process of long-term field-testing that optimisation of integrated DUWL biofilm control systems provide an effective long-term solution to DUWL biofilm in individual DCUs. The development of the fully automated, centralised system provides an effective long-term solution to DUWL biofilm simultaneously in large numbers of DCUs, such as found in dental hospitals. Finally, the applied and translational research described in this thesis demonstrate the benefits of collaboration between DCU manufacturers, water technology professionals and applied microbiologists in developing effective practical solutions to microbial contamination of DUWLs.

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Abbreviations

ADA	American Dental Association
ANOVA	Analysis of variance
ANSI	American National Standards Institute
B.V	Besloten Vennootschap, Private limited liability company (Dutch)
bar	Unit of pressure.
bp	Base pair
BMS	Building's Management System
CDC	Centers for Disease Control and Prevention
cfu	Colony-forming unit
cm	Centimetre
CMA	Centre for Microscopy and Analysis
CMV	Cytomegalovirus
DCU	Dental chair unit
DCU's	Dental chair units
DDSH	Dublin Dental School and Hospital
DNA	Deoxyribonucleic acid
DPD	*N,N*-diethyl-*p*-phenylenediamine
DUWL	Dental unit waterline
DUWLs	Dental unit waterlines
E. coli	*Escherichia coli*
e.g.	For example
EC	European Community
ECA	Electrochemical activated
EDX	Energy Dispersive X-ray
EMBL/GenBank	Public data base of nucleotide and amino acid sequences
et al.	And others
EU	European Union
FAC	Free available chlorine
FEM	Flow-through Electrolytic Module
Fig	Figure

g	Acceleration due to gravity at the Earth's surface
h	Hour(s)
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
i.e.	That is
ICP	Infection control and prevention
IgG	Immunoglobulin G
IgM	Immunoglobulin M
in situ	In place
in vitro	Performing a given procedure in a controlled environment outside of a living organism – artificial environment outside the living organism – usually undertaken in a laboratory
Inc.	Incorporated
KDF	Kinetic Degradation Fluxion
kb	Kilo base
l	Litre
L. pneumophila	*Legionella pneumophila*
LPS	Lipopolysaccharide
MI	Michigan
min	Minute(s)
ml	Millitre
mV	MegaVolt
n	Number
ng	Nanogram
No	Number
NSF	National Sanitation Foundation
N. subarctica	*Novosphingobium subarctica*
Oy	Postfix indicating a Finnish Limited company
P. aeruginosa	*Pseudomonas aeruginosa*
PCR	Polymerase chain reaction

PE	Polyethylene
pH	A measure of the acidity or basicity of a solution.
ppm	Parts per million
Prof.	Professor
PTFE	Polytetrafluorethylene
PVCc	Chlorinated polyvinyl chloride
PVCu	Unplasticised polyvinyl chloride
PVDF	Polyvinylidene fluoride
RNA	Ribonucleic acid
s	Second(s)
S. paucimobilis	*Sphingomonas paucimobilis*
S.p.a.,	Public limited company (Italian)
SARS	Severe Acute Respiratory Syndrome
spp.	Species
™	Trademark
TSE	Transmissible spongiform encephalopathies
UK	United Kingdom
USA	United States of America
UV	Ultraviolet light
v/v	Volume-volume percentage solution
vCJD	Variant Creutzfeldt-Jakob disease
w/v	Weight-volume percentage solution
WCS™	Waterline Cleaning System
WMS™	Waterline Management System
µm	Micrometre
°C	Degree Celsius or centigrade
<	Less than
>	Greater than
~	Approximately
≤	Less than or equal to

Publications Resulting from Work Described in this Thesis

Off prints of published manuscripts are included at the end of this thesis.

O'Donnell MJ, Boyle M, Swan J, Russell RJ, Coleman DC. (2009). A centralised, automated dental hospital water quality and biofilm management system using neutral Ecasol maintains dental unit waterline output at better than potable quality: A 2-year longitudinal study. *Journal of Dentistry* **37**:748-762. PMID:19573971. Digital Object Identifier: 10.1016/j.jdent.2009.06.001

Coleman DC, O'Donnell MJ, Shore AC, Russell RJ. (2009). Biofilm problems in dental unit water systems and its practical control. *Journal of Applied Microbiology* **106**:1424-1437. PMID:19187140. Digital Object Identifier: 10.1111/j.1365-2672.2008.04100.x

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Coleman DC, O'Donnell, MJ. (2007a). Guest editorial. *Journal of Dentistry* **35**:699-700. Digital Object Identifier: 10.1016/j.jdent.2007.07.001

Coleman DC, O'Donnell MJ, Shore AC, Swan J, Russell RJ. (2007b). The role of manufacturers in reducing biofilms in dental chair waterlines. *Journal of Dentistry* **35**:701-711. PMID: 17576035. Digital Object Identifier: 10.1016/j.jdent.2007.05.003

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Chapter 1

General Introduction

1.1. Introduction

For more than a century the dental chair unit (DCU) has been the single most essential item of equipment necessary for the practice of dentistry. Its original function was simply to provide support for patients while enabling easy access to the oral cavity by dental clinicians undertaking dental procedures. Over the decades DCUs have evolved considerably, combining all operating essentials into a single, compact assembly. Modern DCUs consist of a variety of complex, integrated equipment systems that provide the services (e.g. air supply, water, electrical power) and instruments necessary for a diverse range of dental procedures (O'Donnell *et al.*, 2005; O'Donnell *et al.*, 2006a; Coleman *et al.*, 2007; Coleman *et al.*, 2009). They now are adjustable, have a pivotally connected back, seat and footrest section. DCUs use water to cool a range of DCU-associated instruments (e.g. conventional dental handpieces, high-speed turbine dental handpieces, three-in-one air/water syringes and ultrasonic scalers) and also to irrigate tooth surfaces during dental procedures, as the heat generated during instrument operation can be injurious to teeth (Stanley, 1971; Langeland, 1972; Siegel and von Fraunhofer, 2002). DCU-supplied water is also used for oral rinsing by patients (water supplied *via* the cup filler outlet) and to wash out the DCU spittoon bowl, or cuspidor, after oral rinsing (water supplied *via* the bowl-rinse outlet). An intricate network of interconnected narrow-bore tubes called dental unit waterlines (DUWLs) supplies water to all of the DCU-supplied instruments, cup-filler and bowl-rinse outlets (O'Donnell *et al.*, 2006a; Coleman *et al.*, 2007; Coleman *et al.*, 2009).

Modern DCUs are categorised as medical devices under the European Union (EU) Medical Devices Directive (Anonymous, 1993). Microbial contamination of a diverse range of medical devices has been shown to be an important cause of cross-contamination and cross-infection, especially in healthcare environments (Weinstein, 1998; Bagshaw and Laupland 2006; O'Donnell *et al.*, 2006a; Fretz *et al.*, 2009; Hota *et al.*, 2009). A single DCU can be in used in the treatment of many patients each day and microbial contamination of specific component parts can be a significant potential source of cross-infection (O'Donnell *et al.*, 2005; O'Donnell *et al.*, 2006a). This becomes quite significant where immunocompromised, oral surgery or endodontic patients are treated (O'Donnell *et al.*, 2006a). DCU components that come into direct contact with the patient's oral cavity are of particular concern, including dental handpieces, ultrasonic scalers, three-in-one air/water syringes and suction hoses.

Aerosols, splashes and contact contamination contribute to a microbially contaminated environment in the vicinity of a DCU (Dutil *et al.*, 2009). Output water provided by a DCU may also be of concern as a potential source of infection as it comes directly from the DCUs extensive network of DUWLs, which can harbour biofilms, and enters the oral cavity of the patient during treatment. Furthermore, aerosols and droplets produced by dental instruments connected to DUWLs may be inhaled by patients and dental healthcare personnel (Leggat and Kedjarune, 2001; Siegel and von Fraunhofer, 2002; Harrel and Molinari, 2004; O'Donnell *et al.*, 2005). This chapter provides an overview of microbial biofilm formation in DUWLs, consequent contamination of DUWL output water and strategies that have been developed to control it and their limitations.

1.1.1. Microbial Contamination of Dental Unit Waterline Output Water

In the early 1960s, the first reports began to appear in the literature describing the presence of high levels of microorganisms, primarily bacterial species, in DUWL output water (Blake, 1963; Belting *et al.*, 1964). This was followed by a few additional reports in the 1970s and 1980s (Abel *et al.*, 1971; McEntegart and Clark 1973; Clark, 1974; Gross *et al.*, 1976; Kelstrup *et al.*, 1977; Dayoub *et al.*, 1978; Kellett and Holbrook 1980; Scheid *et al.*, 1982; Fitzgibbon *et al.*, 1984; Furuhashi and Miyamae 1985; Martin, 1987) and by a plethora of reports in the 1990s and 2000s (Mayo *et al.*, 1990; Pankhurst and Philpott-Howard 1993; Williams *et al.*, 1993; Barbeau *et al.*, 1996; Pankhurst *et al.*, 1998; Mills, 2000; Walker *et al.*, 2000; Putnins *et al.*, 2001; Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; Walker *et al.*, 2004; Coleman *et al.*, 2007; Coleman *et al.*, 2009). Bacterial densities up to 10^8 colony-forming units (cfu) per ml of output water have been reported (Souza-Gugelmin, 2003). Microbial contamination of DUWLs originates primarily from the DCU water supply, which usually contains low levels of microorganisms (Pankhurst *et al.*, 1998; Tuttlebee *et al.*, 2002). In a typical modern DCU, the waterline network consists of many metres of plastic DUWL tubing having an internal diameter of 1-2 mm (Fig. 1.1). Water flow within these narrow bore tubes is laminar and thus the flow at the lumen surfaces is almost negligible compared to that at the centre of the lumen (Costerton, 1995). A conditioning pellicle of chemical compounds, mainly from the supply water, builds on this inner surface over time providing an easier attachment substrate for microorganisms (Shearer, 1996; Wirthlin *et al.*, 2003). Thus microorganisms in DCU supply water attach to the internal surfaces of the DUWL tubing and form microcolonies that eventually give rise to biofilms. Dental

chair units connected to municipal water supplies usually contain low numbers of a wide variety of environmental bacterial species that eventually give rise to multispecies biofilm in DUWLs (Pankhurst *et al.*, 1998; Mills, 2000; Tuttlebee *et al.*, 2002). Water stagnation within DUWLs when the equipment is not being used encourages the proliferation of biofilm (Williams *et al.*, 1993; Barbeau *et al.*, 1996). Thus DUWL biofilm acts as a reservoir for ongoing contamination of DUWL output water and can act as a potential source of cross-infection.

1.1.2. Microbial biofilms in Dental Unit Waterlines

In natural environments, microorganisms (i.e. bacteria, fungi, protozoa and amoebae) usually coexist in multicellular communities, governed by competition for common nutritional resources with other community members (Donlan and Costerton, 2002). These communities exist in structures known as biofilms and this arrangement is the preferred mode of existence for both single and multiple species of microorganisms. Biofilms form most readily in aquatic, moist or damp environments. Biofilms are complex communities and begin with motile bacterial species approaching and forming an association with a surface and/or other microbes previously attached to the surface. Once a stable association has been made with the surface, motile bacteria move along the surface in two dimensions until other bacteria are encountered and a microcolony is either formed or enlarged. These microorganisms secrete complex exopolysaccharides into the surrounding environment, a phenomenon that is characteristic and essential for biofilm formation (Vuong *et al.*, 2004). A wide variety of bacteria are able to secrete exopolysaccharides during biofilm formation, which contributes to cell protection against adverse environmental conditions, aids attachment to surfaces and nutrient acquisition. These exopolysaccharides are insoluble and help to shield the microorganisms from displacement and from attack by predator organisms. Microorganisms within biofilms are also protected from the action of antibiotics, other antimicrobial agents and disinfectants such as chlorine, which penetrate poorly into biofilms.

Microbial biofilms in DUWLs are comprised mainly of a matrix of highly hydrated complex exopolysaccharides secreted by bacteria, housing microcolonies and single cells, heterogeneously interspersed by channels or pores (Davey and O'Toole, 2000; Watnick and Kolter, 2000). DUWL biofilm matrix also contains both inorganic and organic material derived from supply water and dead microorganisms. Low

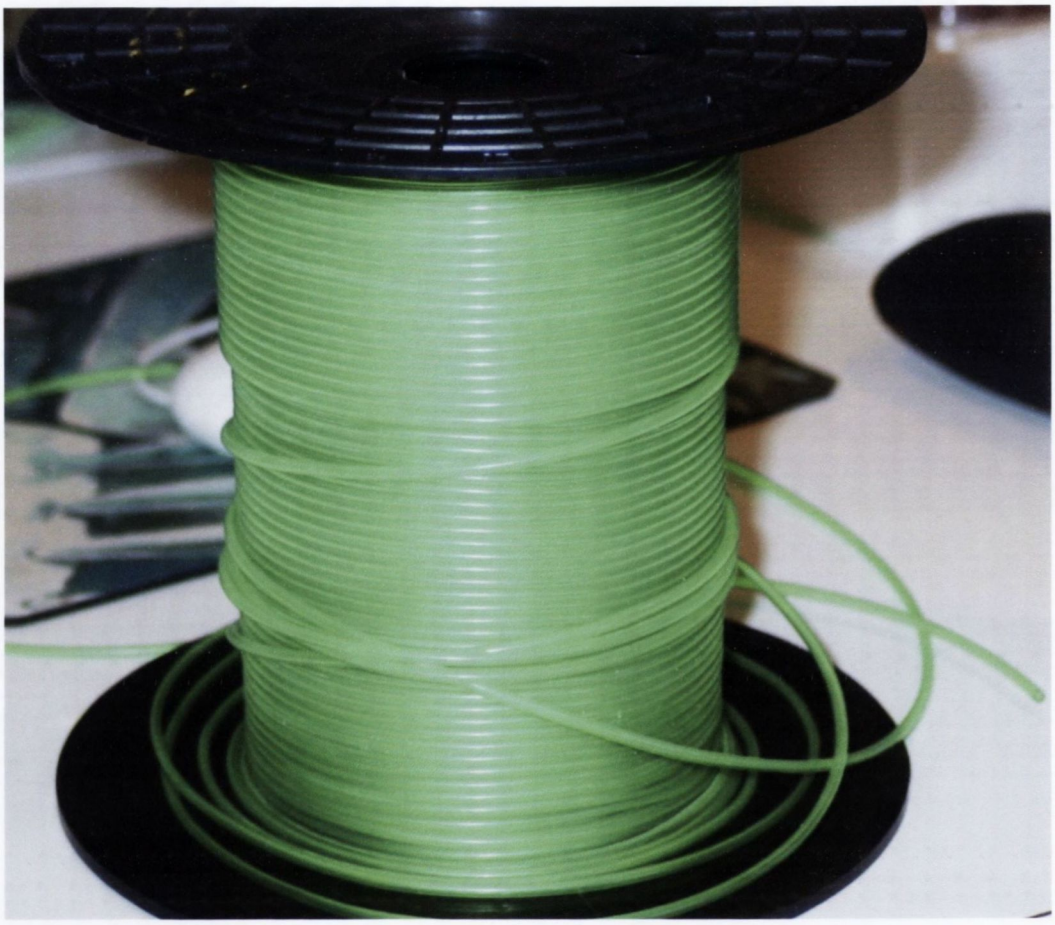


Fig. 1.1. Spool of dental unit waterline tubing with an internal diameter of approximately 2 mm.

boundary flow allows biofilm to form readily in DUWLs (Williams *et al.*, 1993; Walker *et al.*, 2003; Wirthlin *et al.*, 2003). The external surface layer of microorganisms in biofilms is faster growing and thus in DUWLs pieces of biofilm may detach, and along with planktonic forms of microorganisms, are shed to seed biofilm formation elsewhere in the waterline network or are transferred directly into the mouths of patients during dental procedures. Dental handpieces and ultrasonic scalers also aerosolise such biofilm components. These aerosols and fine droplets can enter the lungs of patients and dental healthcare staff (Fotos *et al.*, 1985; Reinthaler *et al.*, 1988; Atlas *et al.*, 1995; Putnins *et al.*, 2001; Pankhurst *et al.*, 2005; Dutil *et al.*, 2007). Thus, DUWL biofilm acts as a reservoir for ongoing contamination of DUWL output water and can act as a potential source of cross-infection. Sterilisation of the handpieces, syringes and associated instruments attached to DUWLs has no impact at all on biofilm within DUWLs (O'Donnell *et al.*, 2006a). Microbial contamination of DUWL output water is a universal problem and all untreated DUWLs in standard DCUs are subject to contamination and will harbour resident biofilms. Studies have shown that once a new DCU is connected to the mains water supply, a biofilm will form within its DUWLs within eight hours, even if no dental treatment has been carried out (Williams *et al.*, 1995; Barbeau *et al.*, 1996). Figure 1.2 shows examples of biofilms on the internal surfaces of DUWLs.

1.1.3. Microorganisms Found in Dental Unit Waterline Output Water

The majority of microbial species found in DUWL output water are Gram-negative aerobic heterotrophic environmental bacterial species of very low pathogenicity, although they may be of concern in the treatment of immunocompromised and medically compromised patients (Pankhurst *et al.*, 1998; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002; Singh *et al.*, 2003; O'Donnell *et al.*, 2006a, O'Donnell *et al.*, 2006b; O'Donnell *et al.*, 2007; Pankhurst and Coulter, 2007). Yeasts, fungi, protozoa and amoebae may also be present in DUWL output water (Kelstrup *et al.*, 1977; Porteus *et al.*, 2003; Singh and Coogan, 2005; Szymanska, 2005; Göksay *et al.*, 2008). Human pathogens detected in DUWLs include *Pseudomonas* spp., *Legionella* spp., and non-*tuberculosis Mycobacterium* spp., (Fotos *et al.*, 1985; Mayo *et al.*, 1990; Atlas *et al.*, 1995; Schulze-Robbecke *et al.*, 1995; Porteus *et al.*, 2004; Dutil *et al.*, 2007). *Pseudomonas* spp., principally *Pseudomonas aeruginosa*, can survive and grow in low nutrient environments and often exhibit resistance to antimicrobial agents, disinfectants

and biocides. However, not only waterborne bacterial pathogens have been identified in DUWLs; in a recent DNA based study using polymerase chain reaction (PCR) technology by Montebugnoli *et al.* (2004) it was demonstrated that DUWLs have the potential to transmit periodontal bacterial pathogens. As mentioned above, fungi (including yeasts) and amoebae have also been identified in DUWLs, although contamination with these microorganisms is less widespread and the organisms are present at lower densities compared to bacterial species As *Legionella* bacteria often live within a variety of species of amoebae and protozoa (Fields *et al.*, 1984; Wadosky *et al.*, 1988; Fields *et al.*, 1989; Barbeau and Buhler, 2001), the presence of the latter in DUWLs may predispose to *Legionella* contamination of DUWL output water. Table 1.1 shows a list of microorganisms previously isolated from DUWL output water.

1.1.3.1. Dental Unit Waterline Contamination by Oral and Skin Microbes

Dental instruments that are connected to DUWLs and that are used in the patient's mouth (e.g. turbine and conventional handpieces, three-in-one air/water syringes and ultrasonic scalers) should contain integrated antiretraction valves or devices that prevent backflow or back siphonage of fluids from the oral cavity into the DUWLs (Walker *et al.*, 2004; Coleman *et al.*, 2007; Coleman *et al.*, 2009). The need for antiretraction devices has been highlighted by previous studies that demonstrated that oral fluids can be retracted into DUWLs during DCU instrument use. The detection of oral bacterial species and other human-derived microorganisms in DUWL output water has provided convincing evidence for likely failure of antiretraction devices (Tuttlebee *et al.*, 2002; Berlutti *et al.*, 2003; Montebugnoli *et al.*, 2004; Petti and Tarsitani, 2006). Recently, an Italian study of 54 DCUs, comprising 18 different models by 6 different DCU manufacturers reported an antiretraction device failure rate of 74% (40/54 DCUs tested) (Berlutti *et al.*, 2003). Thus retraction of oral fluids (saliva and blood) during use of dental instruments attached to DUWLs can add to the range of microorganisms present in DUWL biofilms, and therefore in DUWL output water, and also increase the potential for transmission of pathogenic microbes. In order to minimise the potential impact of antiretraction device failure, the current Centers for Disease Control and Prevention (CDC) guidelines for infection control in dental health-care settings recommend that DCU handpieces should be operated to discharge water and air for a minimum of 20–30 seconds after each patient session (Kohn *et al.*, 2003). All dental

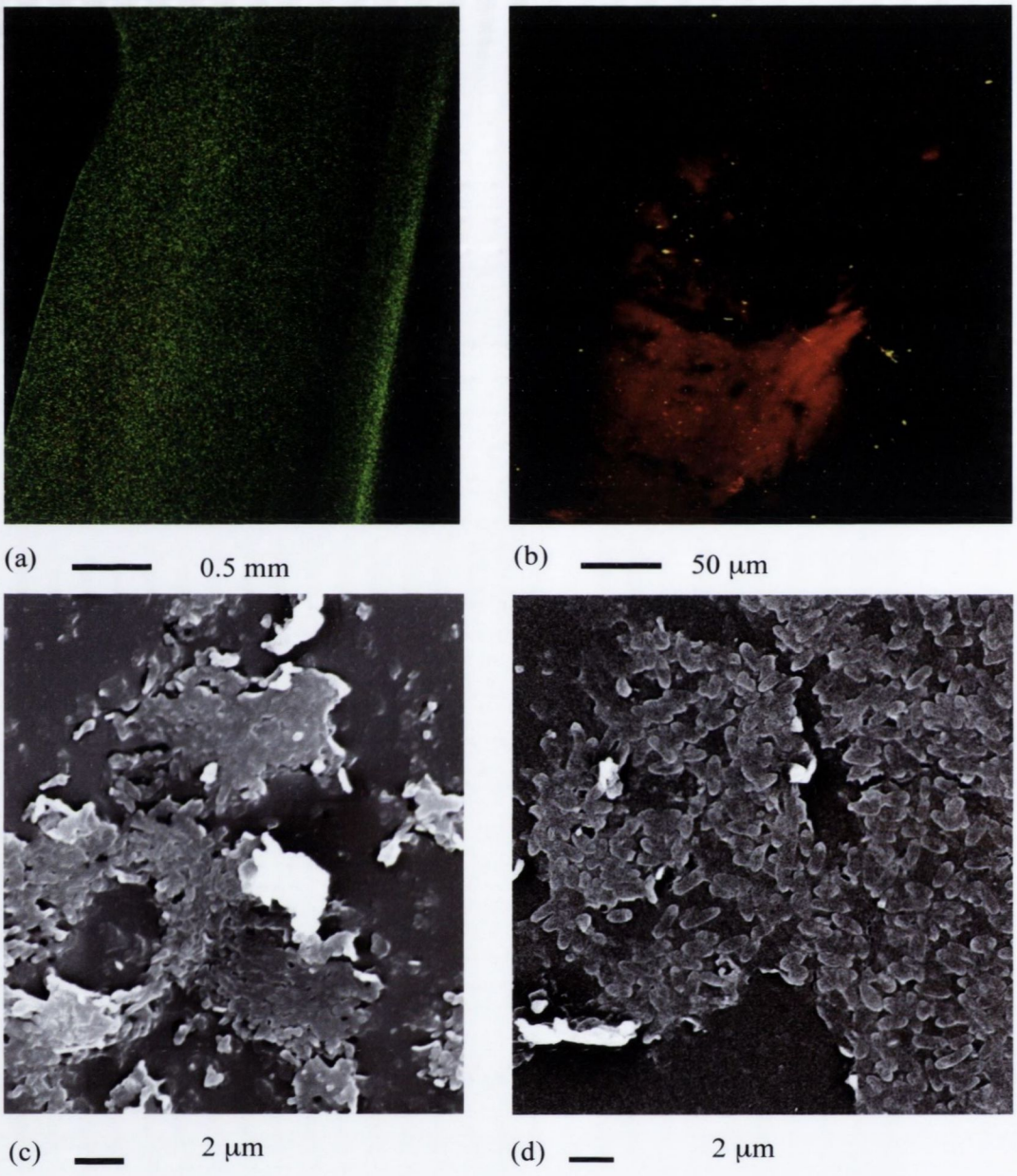


Fig. 1.2. Biofilms in dental unit waterlines. Panels (a) and (b) show confocal micrographs of longitudinal sections of the internal surfaces of DUWL tubing showing extensive biofilm. Living microorganisms populate areas stained green, whereas areas stained red contain predominantly dead microorganisms. The internal surface of the DUWL tubing shown in panel (a) is completely covered with biofilm containing mostly living microorganisms, hence the overall intense green staining. In contrast, the DUWL tubing shown in panel (b) is populated mainly by dead microorganisms, hence the predominant red staining. Panels (c) and (d) show high magnification images of biofilm in DUWLs. Extensive mats of rod shaped bacteria are evident in panels (c & d).

handpieces connected to DUWLs should be cleaned, lubricated and sterilised by heat sterilisation after each patient use.

Clearly, DCU manufacturers should provide clear and unambiguous data on the efficacy of antiretraction devices in DCUs that they market. Furthermore, manufacturers should provide clear and concise guidance in relation to periodic testing and maintenance of antiretraction devices. This should form part of routine DCU maintenance schedules. Manufacturers also have a responsibility to undertake ongoing research and development in this area to improve the quality, robustness and efficacy of antiretraction devices.

Reservoir bottles in DCUs can easily become contaminated with skin organisms such as *Staphylococcus epidermidis* and *Staphylococcus aureus*, the latter a common human pathogen, thus introducing additional human microorganisms into DUWLs (Lancellotti *et al.*, 2007; Table 1.1). To avoid this, reservoir bottles should be handled carefully and should be cleaned and disinfected regularly. Preferably, reservoir bottles that can be sterilised by heat sterilisation after cleaning should be used (Coleman *et al.*, 2009).

1.1.3.2. Detection of Blood in Dental Unit Waterlines

A number of researchers have found traces of blood in DUWL output water suggesting contamination by retraction of oral fluids. In a study by Walker *et al.* in 2004, non-haemolysed traces of blood were detected in 24% and 17% of Greek DUWL output water samples from the three-in-one air/water syringe and dental handpiece DUWL's, respectively. Similarly non-haemolysed traces of blood were detected in 20% of Irish DUWL output water samples from both three-in-one air/water syringe and dental handpiece DUWLs (Walker *et al.*, 2004).

1.1.3.3. Presence of Viruses in Dental Unit Waterlines

It is very likely that viruses present in the oral cavity (e.g. herpes viruses), or in saliva or blood (e.g. Epstein-Barr virus, Hepatitis C virus (HCV) and Hepatitis B Virus (HBV)) can be retracted into DUWLs from dental handpieces used in the oral cavity, either due to the absence of antiretraction valves or due to their malfunction. It is important to emphasise that currently there is no direct evidence in the literature linking transmission of a viral infection to dental patients from virally contaminated DUWLs. However, there is a conspicuous dearth of scientific studies in the literature that have investigated

this possibility. In 1984 Bagga *et al.* reported that patient material (e.g., oral microorganisms, blood, and saliva) could be retracted into DUWLs during patient treatment with turbine handpieces lacking antiretraction valves (Bagga *et al.*, 1984). These authors reported that each time the turbine handpiece is stopped while the bur is still in the patient's mouth, that almost one ml of oral fluid may be aspirated back into the DUWL supplying the handpiece with water. This contaminated water could subsequently enter the mouth of the next patient, potentially initiating an oral, upper respiratory tract infection or blood-borne infection. One recent study reported the detection of HCV RNA in DUWLs from DCUs where the anti-retraction valves had been deactivated and from DCUs without anti-retraction valves following treatment of known HCV-infected patients (Artini *et al.*, 2008). Viral RNA was detected in DUWLs using PCR technology with HCV-specific primers. The fact that HBV and HCV can be present in the saliva of patients with chronic infection highlights the potential risk of transmission of HCV from contaminated DUWLs, particularly if antiretraction valves fail (Davison *et al.*, 1987; Artini *et al.*, 2008; Chen, 2009).

While many viruses are present in the upper respiratory tract and oral cavity, viruses present in saliva or blood are unlikely to survive for prolonged periods outside the body and are unlikely to withstand drying. However, some enveloped viruses such as HBV, can survive for significant periods outside the body (Bond *et al.*, 1981). In 2006, a review of the literature found that viruses from the respiratory tract such as coronaviruses, coxsackie viruses, influenza viruses, Severe Acute Respiratory Syndrome (SARS) virus and rhinoviruses can survive on surfaces for a few days, whereas viruses from the gastrointestinal tract such as astroviruses, hepatits A virus (HAV), polio virus or rotaviruses can persist for approximately two months. Blood borne viruses such as HBV or Human Immunodeficiency Virus (HIV) can persist for more than one week. Herpes viruses such as cytomegalovirus (CMV) or Herpes Simplex Virus (HSV) types one and two, have been shown to persist from only a few hours for up to seven days. (Kramer *et al.*, 2006). Whether oral, salivary or blood-borne viruses can survive in water is largely unknown, but it is possible that some can for short periods of time. Thus the interval between patient treatment and the next patient may be sufficient for the transmission of viable viruses from contaminated DUWLs to subsequent patients. A recent American study found that a surrogate coronavirus remained infectious in water for up to 22 days at ambient temperatures of 25°C and highlighted the fact that contaminated water is a potential vector of transmission of

Table 1.1. Microorganisms isolated from dental unit waterlines

Bacterial species*	References
Achromobacter denitrificans (Alcaligenes denitrificans)	Williams *et al.*, 1993; Uzel *et al.*, 2008
Achromobacter xylosoxidans	Williams *et al.*, 1993
Achromobacter spp.	Meiller *et al.*, 1999
Acidovorax temperans	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Acinetobacter calcoaceticus	Barbeau *et al.*, 1996; Uzel *et al.*, 2008
Acinetobacter spp.	Williams *et al.*, 1993; Kelstrup *et al.*, 1977; Shepherd *et al.*, 2001;Singh *et al.*, 2003
Actinomyces spp.	Sciaky and Sulitzeanu 1962; Scheid *et al.*, 1982; Barbeau *et al.*, 1996
Aeromonas hydrophila	Uzel *et al.*, 2008
Aeromonas salmonicida	Tuttlebee *et al.*, 2002
Aeromonas sobria	Uzel *et al.*, 2008
Aeromonas spp.	Kelstrup *et al.*, 1977
Afipia spp.	Singh *et al.*, 2003
Agrobacterium radiobacter	Tuttlebee *et al.*, 2002
Alcaligenes faecalis	Meiller *et al.*, 1999
Alcaligenes spp.	Kelstrup *et al.*, 1977; Shepherd *et al.*, 2001
Alphaproteobacteria (Alpha proteobacterium)	Singh *et al.*, 2003
Arthrobacter agilis	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Bacillus cereus	Merne *et al.*, 2000
Bacillus licheniformis	Uzel *et al.*, 2008
Bacillus subtilis	Uzel *et al.*, 2008
Bacillus spp.	Kellett and Holbrook 1980; Williams *et al.*, 1993; Barbeau *et al.*, 1996; Meiller *et al.*, 1999; Shepherd *et al.*, 2001; Singh *et al.*, 2003
Brevundimonas diminuta (Pseudomonas diminuta)	Szymanska, 2006a
Brevundimonas vesicularis (Pseudomonas vesicularis)	Williams *et al.*, 1993; Barbeau *et al.*, 1996; Meiller *et al.*, 1999. Merne *et al.*, 2000; Szymanska, 2006a and 2007; Uzel *et al.*, 2007
Burkholderia cepacia	Williams *et al.*, 1993; Meiller *et al.*, 1999. Tuttlebee *et al.*, 2002; Uzel *et al.*, 2008
Burkholderia gladioli	Uzel *et al.*, 2008
Burkholderia pickettii	Williams *et al.*, 1993; Meiller *et al.*, 1999
Caulobacter leidyia	Singh *et al.*, 2003
Chryseobacterium indologenes (Flavobacterium indologenes)	Williams *et al.*, 1993; Tuttlebee *et al.*, 2002; Sacchetti *et al.*, 2006
Chryseomonas acidovorans	Tuttlebee *et al.*, 2002
Chryseomonas luteola	Uzel *et al.*, 2008
Citromyces spp.	Szymanska, 2005
Comamonas acidovorans	Williams *et al.*, 1993; Barbeau *et al.*, 1996; Merne *et al.*, 2000; Tuttlebee *et al.*, 2002; Sacchetti *et al.*, 2006; Uzel *et al.*, 2008
Comamonas testosteroni (Pseudomonas testosteroni)	Williams *et al.*, 1993
Corynebacterium spp.	Singh *et al.*, 2003

Continued overleaf

Table 1.1. Continued

Bacterial species*	References
Cupriavidus pauculus (CDC gr. IVc-2)	Williams *et al.*, 1993; Tuttlebee *et al.*, 2002
Diphtheroids	Sciaky and Sulitzeanu, 1962
Enterococcus spp.	Scheid *et al.*, 1982; Fitzgibbon *et al.*, 1984
Escherichia coli	Singh *et al.*, 2003
Exophiala mesophila	Porteous *et al.*, 2003
Flavobacterium	Shepherd *et al.*, 2001
Fluoribacter bozemanae (Legionella bozemanii)	Pankhurst *et al.*, 1990; Zanetti *et al.*, 2000
Fusobacterium spp.	Kelstrup *et al.*, 1977; Walker *et al.*, 2000
Galactomyces geotrichum (Geotrichum candidum)	Szymanska, 2005
Haemophilus spp.	Scheid *et al.*, 1982
Klebsiella pneumoniae	Clark, 1974; Williams *et al.*, 1993
Kocuria palustris	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Legionella dumoffii	Zanetti *et al.*, 2000
Legionella longbeachae	Atlas *et al.*, 1995
Legionella pneumophila	Oppenheim *et al.*, 1987; Pankhurst *et al.*, 1990; Challacombe and Fernandes, 1995; Atlas *et al.*, 1995; Williams *et al.*, 1996; Walker *et al.*, 2004; Singh and Coogan, 2005; Rice *et al.*, 2006; Veronesi *et al.*, 2007; Ma'ayeh *et al.*, 2008
Legionella spp.	Williams *et al.*, 1993; Pankhurst *et al.*, 2003; Walker *et al.*, 2004; Rice *et al.*, 2006; Singh and Coogan 2005; Veronesi *et al.*, 2007; Castiglia *et al.*, 2008
Mannheimia haemolytica (Pasteurella haemolytica)	Williams *et al.*, 1993
Methylobacterium mesophilicum	Whitehouse *et al.*, 1991; Barbeau *et al.*, 1996; Kohno *et al.*, 2004; Uzel *et al.*, 2008
Micrococcus luteus	Williams *et al.*, 1993; Singh *et al.*, 2003; Szymanska, 2006a and 2007; O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Micrococcus lylae	Szymanska, 2006a and 2007
Moraxella lacunata	Szymanska, 2007
Moraxella osloensis	Meiller *et al.*, 1999
Moraxella spp.	Kelstrup *et al.*, 1977; Szymanska, 2007
Mycobacterium mucogenicum	Porteous *et al.*, 2004
Mycobacterium simiae	Porteous et al., 2004
Mycobacterium spp.	Walker *et al.*, 2000; Walker *et al.*, 2004
Myroides odoratus (Myroides odoratum)	Meiller *et al.*, 1999
Neisseria elongata	Whitehouse *et al.*, 1991
Neisseria sicca	Merne *et al.*, 2000
Nocardia spp.	Williams *et al.*, 1993
Non-faecal coliforms	Walker *et al.*, 2004
Novosphingobium resinovorum (Novosphingobium subartica)	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007

Continued overleaf

Table 1.1. Continued

Bacterial species*	References
Ochrobactrum anthropi	Williams *et al.*, 1993; Tuttlebee *et al.*, 2002; Uzel *et al.*, 2008
Oral anaerobes	Walker *et al.*, 2004
Oral streptococci	Walker *et al.*, 2004; Petti and Tarsitani, 2006
Paracoccus solventivorans	Singh *et al.*, 2003
Paracoccus thiocyanatus	Singh *et al.*, 2003
Pasteurella spp.	Williams *et al.*, 1993
Porphyrobacter tepidarius	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Pseudomonas aeruginosa	Martin, 1987; Williams *et al.*, 1993; Barbeau *et al.*, 1996; Epstein *et al.*, 2002; Zanetti *et al.*, 2003; Walker *et al.*, 2004; Galli *et al.*, 2004; Schel *et al.*, 2006; Sacchetti *et al.*, 2006; Veronesi *et al.*, 2007; Al-Hiyasat 2007; Uzel *et al.*, 2008
Pseudomonas alcaligenes	Tuttlebee *et al.*, 2002
Pseudomonas chlororaphis	Tuttlebee *et al.*, 2002
Pseudomonas fluorescens	Williams *et al.*, 1993; Barbeau *et al.*, 1996; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002; Uzel *et al.*, 2008
Pseudomonas putida	Barbeau *et al.*, 1996; Uzel *et al.*, 2008
Pseudomonas stutzeri	Williams *et al.*, 1993; Meiller *et al.*, 1999; Merne *et al.*, 2000; Singh *et al.*, 2003; Kohno *et al.*, 2004; Uzel *et al.*, 2008
Pseudomonas spp.	Clark 1974; Kellett and Holbrook, 1980; Furuhashi and Miyamae, 1985; Kelstrup, *et al.*, 1977; Zanetti *et al.*, 1999; Walker *et al.*, 2000; Shepherd *et al.*, 2001; Dutil *et al.*, 2006; Castiglia *et al.*, 2008
Psychrobacter phenylpyruvicus (Psychrobacter phenylpyruvica)	Meiller *et al.*, 1999
Ralstonia pickettii	Tuttlebee *et al.*, 2002; Szymanska, 2006a and 2007; Uzel *et al.*, 2008
Rhodococcus fascians	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Sclerotinia sclerotiorum (Sclerotium sclerotiorum)	Szymanska, 2005
Serratia marcescens	Williams *et al.*, 1993
Sphingobium yanoikuyae (Sphingomonas yanoikuya)	Singh *et al.*, 2003
Sphingomonas aerolata	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Sphingomonas paucimobilis (Pseudomonas paucimobilis)	Whitehouse *et al.*, 1991; Williams *et al.*, 1993; Barbeau, *et al.*, 1996. Meiller *et al.*, 1999; Kohno *et al.*, 2004; Merne, *et al.*, 2000; Szymanska, 2006a and 2007; O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007; Uzel *et al.*, 2008
Sphingomonas trueperi	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Sphingomonas spp.	Shepherd *et al.*, 2001; Singh *et al.*, 2003; O'Donnell *et al.*, 2006
Sphingopyxis alaskensis (Sphingomonas laska)	Singh *et al.*, 2003
Staphylococcus aureus	Sciaky and Sulitzeanu, 1962; Kellett and Holbrook, 1980; Scheid *et al.*, 1982; Meiller *et al.*, 1999; Zanetti *et al.*, 2003
Staphylococcus capitis (Staphylococcus capitus)	Williams *et al.*, 1993

Continued overleaf

Table 1.1. Continued

Bacterial species*	References
Staphylococcus cohnii	Szymanska, 2006 and 2007; Uzel *et al.*, 2008
Staphylococcus epidermidis	Singh *et al.*, 2003; Szymanska, 2006b
Staphylococcus haemolyticus	Sacchetti *et al.*, 2006
Staphylococcus hominis	Sacchetti *et al.*, 2006; Szymanska, 2007
Staphylococcus lentus	Szymanska, 2006a
Staphylococcus pasteuri	Singh *et al.*, 2003
Staphylococcus saprophyticus	Williams *et al.*, 1993
Staphylococcus warneri	Williams *et al.*, 1993; Singh *et al.*, 2003; Sacchetti *et al.*, 2006
Staphylococcus spp.	Williams *et al.*, 1993; Szymanska, 2006a and 2007
Staphylococcus, coagulase negative	Meiller *et al.*, 1999
Stenotrophomonas maltophilia	Williams *et al.*, 1993; Barbeau *et al.*, 1996; Szymanska, 2007
Streptococcus hemolyticus	Sciaky and Sulitzeanu, 1962
Streptococcus intermedius	Shepherd *et al.*, 2001
Streptococcus mitans	Shepherd *et al.*, 2001
Streptococcus pyogenes	Scheid *et al.*, 1982
Streptococcus salivarius	Shepherd *et al.*, 2001
Streptococcus sanguis	Shepherd *et al.*, 2001
Streptococcus sobrinus (Streptococcus mutans subspecies *sobrinus)*	Scheid *et al.*, 1982; Shepherd *et al.*, 2001
Streptococcus viridans	Sciaky and Sulitzeanu, 1962
Streptococcus spp.	Williams *et al.*, 1993; Shepherd *et al.*, 2001; Szymanska, 2006a and 2007
Streptomyces albus (Staphylococcus albus)	Kellett and Holbrook, 1980; Szymanska, 2006a and 2007
Tatlockia micdadei (Legionella micdadei)	Zanetti *et al.*, 2000
Variovorax paradoxus	O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007
Xanthomonas maltophilia	Meiller *et al.*, 1999; Szymanska, 2006a
Xanthomonas spp.	Shepherd *et al.*, 2001
Yeast species	
Yeasts	Barbeau *et al.*, 1996
Candida spp.	Epstein 2002; Walker *et al.*, 2000; Walker *et al.*, 2004
Candida albicans	Szymanska, 2005 and 2006b
Candida curvata	Szymanska, 2005 and 2006b
Fungi	
Aspergillus fumigatus	Szymanska, 2005 and 2006b
Aspergillus glaucus	Szymanska, 2006b
Aspergillus repens	Szymanska, 2005 and 2006b
Cephalosporium	Kelstrup *et al.*, 1977
Citromyces spp.	Szymanska, 2005 and 2006b
Cladosporium	Kelstrup *et al.*, 1977
Eurotium amstelodami (Aspergillus amstelodami)	Szymanska, 2005 and 2006b

Continued overleaf

Table 1.1. Continued

Fungi	References
Galactomyces geotrichum *(Geotrichum candidum)*	Szymanska, 2005 and 2006b
Penicillium aspergilliforme	Szymanska, 2005
Penicillium glabrum *(Penicillium frequentans)*	Szymanska, 2006b
Penicillium pusillum	Szymanska, 2005 and 2006b
Penicillium turolense	Szymanska, 2005 and 2006b
Sclerotinia sclerotiorum *(Sclerotium sclerotiorum)*	Szymanska, 2005 and 2006b
Protozoa and amoebae	
Acanthamoeba spp.	Barbeau and Buhler, 2001
Amoebae	Barbeau and Buhler, 2001
Bitrichous bodo spp.	Barbeau and Buhler, 2001
Filamoeba nolandi	Barbeau and Buhler, 2001
Hartmanella vermiformis	Barbeau and Buhler, 2001
Naegleria spp.	Barbeau and Buhler, 2001
Vahlkampfia spp.	Barbeau and Buhler, 2001
Vanella platypodia *(Vannella platypodia)*	Barbeau and Buhler, 2001
Vannella miroides *(Vanella miroides)*	Barbeau and Buhler, 2001

*Species names shown in smaller font in parenthesis relate to older species names that have been changed. In each case, the current species name is shown above the older names. In some of the references cited, the spelling of some species names was incorrect. These have been corrected for this table.

coronaviruses if an aerosol is generated (Casanova *et al.,* 2009). In a review of the literature in relation to airborne and droplet disease transmission within the dental surgery from 2004, the authors identified SARS virus as a candidate virus that could be potentially spread in this manner. Notwithstanding the fact that patients who are acutely ill with SARS would not attend for routine dental treatment, there is the theoretical possibility that in the event of antiretraction valve malfunction that SARS and other viruses could be aspirated back into DUWLs (Samaranayake and Peiris, 2004).

1.1.3.4. Presence of Prions in Dental Unit Waterlines

Currently there are no proven cases of transmission of transmissible spongiform encephalopathies (TSEs) associated with dental treatment (Walker *et al.*, 2008). However, animal studies have detected prion proteins in a range of oral tissues, suggesting that transmission is possible (Mathiason *et al.,* 2006). In humans, prion proteins have been detected in tonsilar tissue, but the majority of oral tissues examined were negative, with the exception of the trigeminal ganglia. Furthermore, there have been four probable cases of variant Creutzfeldt-Jakob disease (vCJD) derived from transfusion of whole blood (Head *et al.*, 2003; Llewelyn *et al.*, 2004). Overall, these findings suggest that transmission of prion proteins following retraction into DUWLs during dental treatment is very unlikely.

1.1.4. Dental Chair Unit Water Heaters

Some DCUs are equipped with water heaters that heat DUWL output water so that it is more comfortable for the patient at temperatures that can range from approximately 20-30°C (Coleman *et al.,* 2007). DCU water heaters generally consist of a low volume metallic reservoir with an electrically heated element fed by DCU supply water and, which in turn supplies heated water to DUWLs. An example of a DCU water heater is shown in Fig. 1.3. Heating DUWL water to >20°C may promote the proliferation of *Legionella* bacteria present in DCU supply water within DUWLs or within the DCU water heater itself. Naturally occurring *L. pneumophila* readily multiply at temperatures between 25 and 37°C (Wadowsky *et al.,* 1985). It is also feasible that heating DCU water may promote the proliferation of human-derived bacterial species that prefer higher temperatures, compared to environmental bacteria, which generally grow at lower temperatures. Particular environmental bacterial species such as *Comamonas acidovorans*, which has been associated with infections in immunocompromised

patients, may be more prevalent in DCU water with a higher temperature (Stampi *et al.*, 1999). DCUs should not be equipped with water heaters unless adequate control measures are present (Coleman *et al.*, 2007).

1.1.5. Infections and Diseases Associated with Contaminated Dental Unit Waterlines

In 1987, Martin reported that abscesses caused by strains of *P. aeruginosa* in two immunocompromised patients were associated with exposure to contaminated DUWL output water. The same study reported the isolation of *P. aeruginosa* from the oral cavities of 78 healthy patients for three to five weeks following exposure to DUWL output water contaminated with *P. aeruginosa* (Martin, 1987). Pairs of *P. aeruginosa* isolates, one isolate recovered from the patient's oral cavity and one recovered from DUWL output water from the DCU used to treat the patient, had the same pyocin type in the case of both patients, respectively. Different pyocin types were recovered from each of the two patients with abscesses. These findings have been cited many times as providing convincing evidence for disease transmission from contaminated DUWL output water. However, there are only a limited number of *P. aeruginosa* pyocin types and the discriminatory power of pyocin typing is limited. Thus, the study of Martin (1987) does not provide definitive proof that the patient isolates belonged to the same strain as the isolates recovered from DUWLs.

Legionella spp., (*L. pneumophila* and approximately 41 other species are often found in water distribution networks and can cause Legionnaire's disease (pneumonia resulting from inhalation) or Pontiac fever (non-pneumonic legionellosis) (Helbig *et al.*, 2006). *Legionella* bacteria live within a variety of amoebae and protozoa commonly found in soil and water and are often found in association with biofilms, including DUWL biofilms. Barbeau and Buhler found that the density of free living amoeba was up to 300 times higher in DUWL output water samples compared to tap water within the same clinical environment (Barbeau and Buhler, 2001). There are numerous reports of transmission of legionnellosis in healthcare settings and in the community, either by direct contact with aerosols generated from contaminated water, or indirectly following exposure to contaminated aerosols generated by respiratory therapy equipment (Woo *et al.*, 1992; Crnich *et al.*, 2005; Leoni *et al.*, 2006). However, there is no definitive published evidence, so far, that any patient has ever contracted legionellosis following exposure to contaminated DUWL output water. Many studies certainly have reported

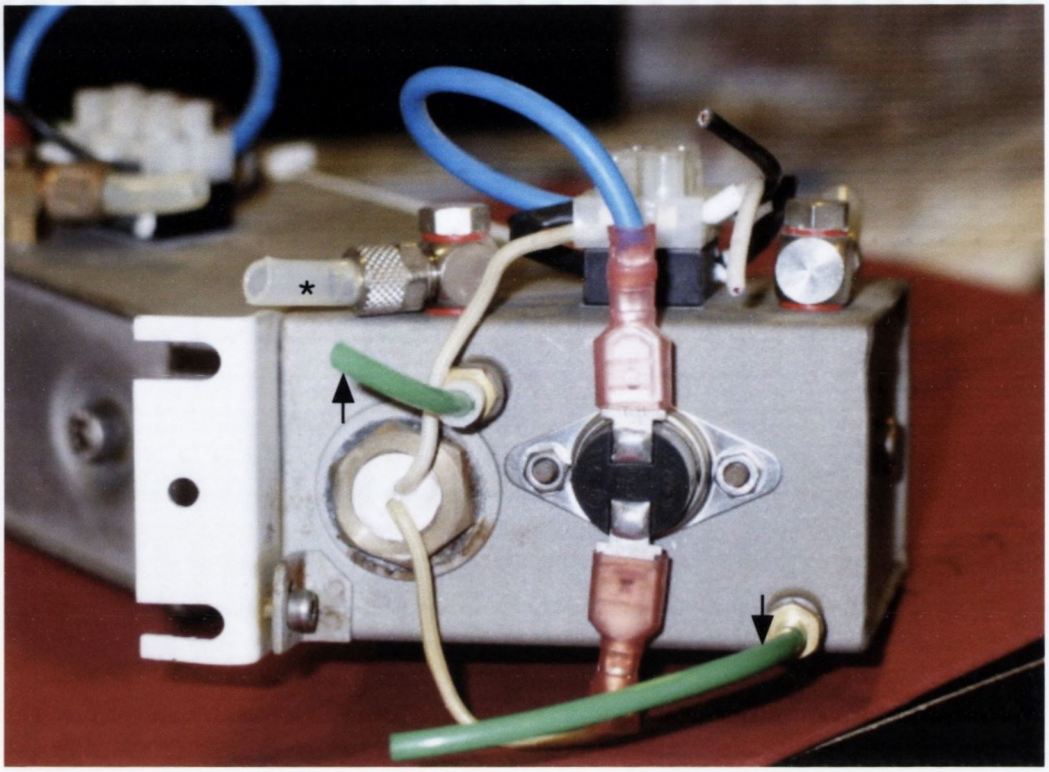


Fig. 1.3. View of a dental chair unit water heater after removal from the chair unit. The green coloured tubes indicated by arrowheads are DUWLs. The heating unit essentially consists of a rectangular vessel containing an electric heating element. Water is supplied to the heater by a wide-bore tube (marked *) and following heating exits the vessel via DUWLs, which then distributes the heated water to the various DCU water outlets supplied by DUWLs, including instrument hoses, patient cup filler and bowl-rinse outlets.

the presence of legionellae in DUWLs (Atlas *et al.*, 1995; Singh and Coogan 2005; Tanzi *et al.*, 2006; Dutil *et al.*, 2007; Pankhurst and Coulter 2007; Veronesi *et al.*, 2007). In 1995, Atlas *et al.* reported the death of a dentist in California resulting from Legionnaire's disease, which was possibly due to exposure to DUWL output water. High levels of *Legionella* species (>10,000 organisms per ml) were detected by monofluorescent antibody and PCR in the DUWL output water in the dentist's dental surgery. Low levels (<100 organisms per ml) were detected from the dentist's domestic water supply. In this case it was not possible to definitively correlate the cause of death to DUWL output water, but the high levels of *Legionella* bacteria detected in DUWL output water would seem to have been the source of this fatal infection (Atlas *et al.*, 1995). Occupational exposure to aerosols of waterborne bacteria generated by dental instruments attached to DUWLs may lead to colonisation of dental staff and also cause a higher prevalence of antibodies to *Legionella* (Fotos *et al.*, 1985; Atlas *et al.*, 1995; Borella *et al.*, 2008). One study of a group of dental staff with more than two years clinical experience revealed that 23% were IgG antibody-positive and 19% were IgM antibody-positive for *L. pneumophila*, compared to IgG antibody-positive levels of 8% for individuals who had no clinical experience (Fotos *et al.* 1985). Few instances of cross-infection related to contaminated DUWL output water have been reported in the literature (Martin, 1987; Atlas *et al.*, 1995), however, it is possible that such infections have gone undetected and unreported because of the failure to associate exposure to DUWL output water and aerosols generated from this water with the development of specific infections (Pankhurst and Coulter, 2007). Sporadic infections not requiring hospital admission are also less likely to be investigated.

A recent case report highlighted the risks that may be associated with amoebae in DUWL output water (Barbeau, 2007). A patient with contact lenses received a stream of water from a dental handpiece into her right eye during dental treatment. The patient subsequently experienced pain in the eye, and consulted several ophthalmologists, who discovered abrasive lesions of the cornea and inflammation. Despite antibacterial and anti-inflammatory therapy, the patient's eye condition worsened. A microbiological examination nearly two months later revealed amoebae in corneal samples and a lawsuit against the dental practitioner was initiated, which was unsuccessful on the grounds of failure to definitively establish a causal relation with the dental treatment and the presence of amoebae in the patient's eye. The patient used to routinely rinse her contact lenses using tap water and this may have been the source of the amoebae. However, this

case highlights that high densities of amoebae in DUWL output water can present a risk if a patient with pre-existing corneal lesions is splashed. This case reinforces the importance of having patients wear safety glasses during dental treatments, and more importantly of ensuring good quality DUWL output water.

Dental unit waterline output water is also a potent source of bacterial endotoxin, composed of lipopolysaccharide (LPS) released from the cell walls of dead Gram-negative bacteria, and levels ranging from 500 to 2560 endotoxin units per ml have been reported (Putnins *et al.*, 2001; Pankhurst and Coulter, 2007). In contrast, the maximum level of endotoxin permissible in sterile water for irrigation in the USA is 0.25 endotoxin units per ml. Endotoxin can cause localised inflammation, fever and shock in susceptible individuals. Interestingly, in medical devices that are prone to biofilm contamination and endotoxin build-up such as humidifiers, a hypersensitivity pneumonitis triggered by contaminating endotoxin is well documented (Pankhurst and Coulter, 2007). Inhaled endotoxin can precipitate reactive airway symptoms (Schwartz, 2001) and asthma severity is directly correlated with the concentration of endotoxin (Michel *et al.*, 1996). Furthermore, results from a single, large, practice-based cross-sectional study reported a temporal association between occupational exposure to contaminated DUWL output water with aerobic bacterial counts of >200 colony-forming units per millitre (cfu/ml) at 37°C and development of asthma in the sub-group of dentists in whom asthma arose following the commencement of dental training (Pankhurst *et al.*, 2005). Finally, Putnins *et al.*, (2001) suggested that endotoxin present in DUWL output water might stimulate the release of pro-inflammatory cytokines in gingival tissue during surgery and adversely affect healing.

1.1.6. Dental Unit Waterline Output Water Quality

The presence of large numbers of microorganisms in DUWL output water conflicts absolutely with best practice in modern infection control and prevention (ICP). Furthermore, from an ethical perspective, it is totally inappropriate to knowingly expose patients, dental healthcare personnel and support staff to heavily contaminated water even if the assessable cross-infection risk is thought to be low. However, there are currently no official standards or legislation that regulate the microbial quality of DUWL output water (Coleman *et al.*, 2007). Furthermore, until recently, there has been hardly any specific guidance from DCU manufacturers on DCU supply water quality (Coleman *et al.*, 2007). In reality, responsibility for ensuring that DCUs provide good

quality output water has by and large been considered to rest on the shoulders of dental practitioners and/or dental clinic management. The principal contributing factor to this situation is that DUWL output water is not intended for human consumption, but it does enter the oral cavity and can be swallowed during dental treatment. It would therefore be sensible, reasonable and responsible to expect that the quality of DUWL output water should at least approximate potable water standards.

The present potable water standards for aerobic heterotrophic bacteria in the EU and the United States of America (USA) do not specify an upper limit, although water sold in bottles or containers in the EU should not exceed 100 cfu/ml (Anonymous, 1998; Anonymous, 2002). The American Dental Association (ADA) Council on Scientific Affairs affirmed that contact with water of poor microbial quality is inconsistent with patient expectations of safety and standards of modern dentistry and set a goal for the year 2000 that water used for dental treatment should contain ≤200 cfu/ml of aerobic heterotrophic bacteria (Anonymous. 1996). This policy has been endorsed by many experts in the field but in reality has not been widely achieved (Coleman and O'Donnell, 2007; Coleman *et al.*, 2007; Pankhurst and Coulter, 2007; Coleman *et al.*, 2009). The current CDC guidelines for infection control in dental health-care settings recommend that DUWL output water should contain ≤500 cfu/ml of aerobic heterotrophic bacteria (Kohn *et al.*, 2003). A symposium entitled *Microbiology of dental unit water lines; setting standards for the future* at the Pan European Federation/International Association for Dental Research meeting held at Trinity College, Dublin, during September 2006 debated setting a standard for DUWL output water quality (Coleman and O'Donnell, 2007). The symposium was the first occasion that scientists and clinicians from academia and dental practice came together in Europe to discuss the universal problem of DUWL biofilm and practical solutions. The consensus from the symposium was that in the absence of any EU standard for DUWL output water quality, every effort should be deployed to ensure that DUWL output water quality in Europe complies with the ADA standard of ≤200 cfu/ml of aerobic heterotrophic bacteria (Coleman and O'Donnell, 2007). It is important to note that such counts should not include human pathogens.

1.1.7. Dental Chair Unit Supply Water Quality

The quality of DUWL output water is directly influenced by the quality of the supply water. Water supplied to DCUs is generally provided either in independent bottle

reservoirs (see Fig. 1.4) or directly from a municipal mains water supply (Coleman *et al.*, 2007; Coleman *et al.*, 2009). In dental hospitals and in dental clinics equipped with multiple DCUs, water supplied to DCUs frequently comes from large holding tank reservoirs supplied by mains water, whereas in smaller clinics, DCUs may have individual mains supplies (Coleman *et al.*, 2007; Coleman *et al.*, 2009). Within the EU, the majority of DCUs are supplied with municipal mains water (Walker and Marsh, 2004). Retraction or backflow of water from DCUs into the mains supply pipe work could occur if the pressure in the mains supply drops below that in DUWLs. Some DCU models incorporate an air gap in their DUWLs to prevent this possibility (O'Donnell *et al.*, 2006b). Some European countries legally require an air gap between the mains supply and dental surgeries (Anonymous, 2001). It seems prudent that DCU manufacturers should include, as standard, an integrated air gap system within the waterline network in all DCU models.

The quality of DUWL output water is directly influenced by the physical content (i.e. presence of suspended material and particulate matter), chemical content (i.e. organic material and dissolved and suspended inorganic compounds) and microbiological quality (i.e. density of microorganisms present) of the inward supply water (Volk and LeChevallier, 1999; Coleman *et al.*, 2007) and indirectly by environmental conditions such as temperature. The condition of the mains water distribution network pipework, maintenance works thereon and storage tanks (where present) also play a major role in water quality (Volk and LeChevallier 1999; Coleman *et al.*, 2007; Coleman *et al.*, 2009). The poorer the quality of water supplied to DUWLs, the more readily biofilm is likely to form on its internal surfaces. Environmental aerobic heterotrophic bacteria present in supply water can convert organic material dissolved or suspended in supply water into biomass, locally (Flemming, 2002). Previous studies on water distribution systems revealed that a range of factors influence biofilm growth, including the level of dissolved inorganic nutrients present, water temperature, type of pipe material used, corrosion control and levels of organic matter present (Volk and LeChevallier, 1999).

The chemical and microbial content of municipal water supplied to DCUs will vary depending on the geographic locality and the degree and efficacy of water treatment by public authorities. The degree of water hardness depends on the levels of dissolved magnesium and calcium minerals. If hard water (~200 parts per million (ppm) hardness minerals) is supplied to DCUs, minerals are deposited within DUWLs and

(a) (b)

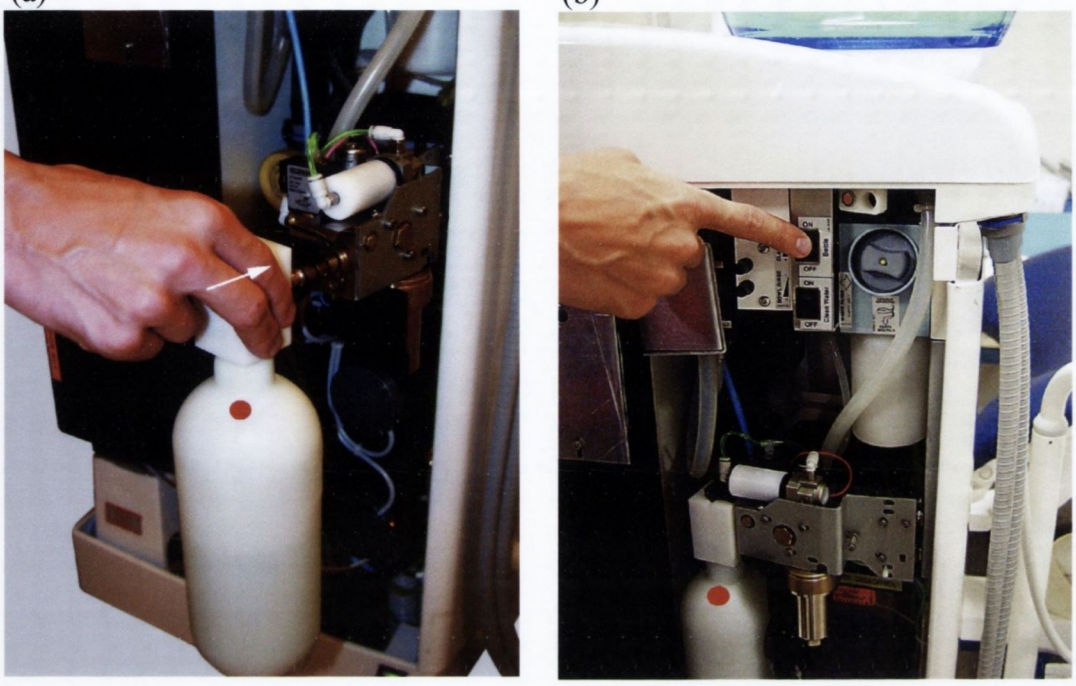


Fig. 1.4. Views of a dental chair unit independent reservoir water bottle. Panel (a) a shows a water reservoir bottle (●) being replaced in the body of a DCU having being filled with water. Panel (b) shows the filled reservoir water bottle (●) *in situ* in the body of the DCU.

associated valves and within DCU water heaters, where present. Such deposits increase the surface area within DUWLs, thus allowing more biofilm to form. Water softening applied to DCU supply water is vital in hard water areas, and is most easily achieved using an ion exchange unit that removes calcium and magnesium ions. Water softening units require periodic maintenance, regeneration and disinfection (Coleman *et al.*, 2007). Proper maintenance is essential, although often overlooked, as biofilm can develop within the unit's resin bed after a period of use, thus adding considerable microbial loads to water entering DCUs downstream of the softening units.

1.1.8. Control and Eradication of Biofilm in Dental Unit Waterlines

As stated earlier, until relatively recently, the onus for tackling the problem of biofilm in DUWLs and poor quality output water has rested with the dental practitioner. Only in recent years have DCU manufacturers responded to the evident need for DCU engineering and design changes to control DUWL biofilms (Coleman *et al.*, 2007). Over the last two decades, numerous approaches, both chemical-based and non-chemical based, for reducing the microbial density in DUWL output water have been proposed but none that is both efficient at eliminating biofilm, compatible in the long-term with the material components of DUWL networks and dental instruments attached to DUWLs, as well as being safe for patients has been universally adopted (Coleman *et al.*, 2007). Table 1.2 lists the various approaches that have been undertaken to control and/or eradicate DUWL biofilm. One widely used procedure for reducing the microbial burden in DUWL output water involves flushing DUWLs with fresh water (Scheid *et al.*, 1982; Rice *et al.*, 2006). However, whereas this procedure does reduce the density of microbes in output water somewhat, it does not remove biofilm and is ineffective as a means of controlling the quality of DUWL output water (Pankhurst and Philpott-Howard, 1993; Furuhashi and Miyamae, 1985; Mills, 2000). Another procedure used to improve quality of DUWL output water involves point-of-use microbial filters at the ends of DUWLs near the instrument attachment sites. These can be very effective but filters have to be changed regularly as they become clogged readily and thus add to ongoing maintenance expense (Dayoub *et al.*, 1978; Pankhurst *et al.*, 1990; Murdoch-Kinch *et al.*, 1997). Microbial filters attached to the DCU supply water line suffer from similar drawbacks. Filters have no effect whatsoever on existing biofilms in DUWLs. The use of sterile water, deionized water or distilled water in independent bottle reservoirs also has no effect on resident biofilms in DUWLs. Another approach that has

been used in an effort to control contamination of DUWL output water involved draining DUWLs of water after use and drying them by passing air through the waterlines (Fiehn and Larsen, 2002). However, this approach did not reduce the number of viable bacteria in DUWL output water following reconnection of the DUWLs to the water supply. It is likely that the highly hydrated nature of DUWL biofilm protected the microorganisms contained within the biofilm from being killed by dessication.

The most efficient means of achieving good quality DUWL output water is regular treatment/disinfection of DUWLs with a chemical, biocide or cleaning agent that removes biofilm from DUWLs effectively, resulting in good quality output water (Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; Walker and Marsh, 2004; Coleman *et al.*, 2007; O'Donnell *et al.*, 2007; Walker and Marsh, 2007). Biofilm re-growth in DUWLs seeded from supply water and/or dental instruments connected to DUWLs used in the oral cavity due to antiretraction device failure usually occurs shortly following disinfection/cleaning and so DUWLs need be treated regularly (Pankhurst and Philpott-Howard, 1993; Williams *et al.*, 1993; Meiller *et al.*, 2000; Mills, 2000; Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; O'Donnell *et al.*, 2006b; O'Donnell *et al.*, 2007). Many studies have demonstrated the efficacy of a broad range of commercially available treatment products for DUWLs that efficiently remove biofilm and reduce bacterial density in DUWL output water to potable water quality or better (Furuhashi and Miyamae 1985; Karpay *et al.*, 1999; Smith *et al.*, 2001; Pederson *et al.*, 2002; Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; Wirthlin *et al.*, 2003; Porteous and Cooley 2004; Martin and Gallagher, 2005; Yabune *et al.*, 2005; Schel *et al.*, 2006; O'Donnell *et al.*, 2006b; Szymanska 2006a and 2006b; O'Donnell *et al.*, 2007; Walker and Marsh 2007; Zhang *et al.*, 2007). However, many of these studies have been conducted *in vitro* and relatively few have actually investigated the efficacy of DUWL treatment products to achieve these desired effects in DCUs (Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; McDowell *et al.*, 2004; Spratt *et al.*, 2004; O'Donnell *et al.*, 2006b; Schel *et al.*, 2006; O'Donnell *et al.*, 2007). Furthermore, only a small minority of studies has yet investigated the long-term efficacy of DUWL treatment agents in DCUs in the clinical setting (O'Donnell *et al.*, 2006b; Coleman *et al.*, 2007; O'Donnell *et al.*, 2007; Coleman *et al.*, 2009), whilst comprehensive controlled comparative efficacy studies have yet to be performed.

Table 1. 2. Approaches used to control microbial contamination of dental unit waterline output water

Approach	Comments	Reference
Anti-retraction valves	Used to prevent back-siphonage of oral fluids into DUWLs during use of dental handpieces in the oral cavity. Studies have shown that they frequently fail resulting in contamination of DUWLs with oral microorganisms	Bagga *et al.*, 1984; American Dental Association, 1996; Kohn *et al.*, 2003
Use of point-of-use microbial filters at the ends of DUWLs near the instrument attachment sites. Use of microbial filters on DCU supply water line	Can be very effective but have to be changed regularly as they become clogged readily and thus add to ongoing maintenance expense. Filters have no effect whatsoever on existing biofilms in DUWLs	Dayoub *et al.*, 1978; Pankhurst *et al.*, 1990; Murdock-Kinch *et al.*, 1997; Copenhagen, 2006
Drying of DUWLs with pressurised air	No significant reduction in bacterial density in DUWL output water	Fiehn and Larsen, 2002
Use of distilled water/sterile DUWL supply water provided from reservoir bottles	No significant reduction in bacterial density in DUWL output water. No affect on resident DUWL biofilm	Mills *et al.*, 1986; Marais and Brozel, 1999; Depaola *et al.*, 2002; Kettering *et al.*, 2002
Pasteurisation of tap water	No significant reduction in bacterial density in DUWL output water. No effect on resident DUWL biofilm	Kim *et al.*, 2000
Flushing of DUWLs with fresh water	Temporarily reduces the density of microbes in output water somewhat, but does not remove biofilm and is ineffective as a means of controlling the quality of DUWL output water	Thé and Van't Hof, 1975; Gross *et al.*, 1976; Scheid *et al.*, 1982; Furuhashi and Miyamae, 1985; Mayo *et al.*, 1990; Whitehouse *et al.*, 1991; Pankhurst and Philpott-Howard, 1993; Williams *et al.*, 1994; ADA, 1996; Mills, 2000; Teixera, 2002; Cobb *et al.*, 2002; Spratt *et al.*, 2004; Rice *et al.*, 2006; Al-Hiyasat, 2007; Watanabe *et al.*, 2008
Use of polyvinylidene fluoride (PVDF) coated tubing	Effective in preventing biofilm formation using an *in vitro* DUWL model	Yabune *et al.*, 2008
Application of water softening and water heating to DUWL supply water	Not effective at significantly improving the microbial quality of DUWL output water. Significant growth of *P. aeruginosa* was found in the softened and heater water	Stampi *et al.*, 1996
Use of chemical agents to treat biofilm in DUWLs	The most effective means of controlling the microbial quality of DUWL output water. Treatment agents that efficiently remove biofilm have been found to be the most useful	See Table 1.3. for a comprehensive list of DUWL treatment agents and relevant references

Table 1.3. Chemical agents used to treat DUWLs to improve the microbiological quality of output water

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Chlorhexidine			
Bio 2000 (BioBlue); chlorohexidine gluconate 0.12%, ethyl alcohol 12%.	http://www.micrylium.com	Tested in 10 new DCUs for six weeks. Left in DUWLs each night. Reduced bacterial density ≤ 200 cfu/ml. Only partial elimination of DUWL biofilm **(I)**	Kettering *et al.*, 2002
Bio 2000 (BioBlue); Chlorohexidine gluconate 0.12%, ethyl alcohol 12%.	http://www.micrylium.com	Tested in model system only. No viable bacteria in output water after 16 h contact and 53.2% removal of biofilm coverage	Walker *et al.*, 2003
Chlorhexidine 0.2%	http://www.sigmaaldrich.com	Tested in model system only. No viable bacteria in output water after 16 h contact and 31.77% removal of biofilm coverage	Walker *et al.*, 2003
Chlorhexidine gluconate 0.12%	Generic product	Tested in three DCUs for 12 weeks. Initially used overnight for six consecutive nights and thereafter once a week overnight. Bacterial density ≤ 200 cfu/ml for eight consecutive weeks **(I)**	Porteous and Cooley 2004
Chlorhexidine 0.2%	Generic product	Tested for 48 h on DUWL tubing from a routinely used DCU. 92% - 95% reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Chlorine dioxide			
Activated chlorine dioxide, 50 ppm	http://www.billydump.com/3r/purogeneinfo.htm	Tested in 1 DCU. After one overnight treatment bacterial density in DUWL output water samples ≤ 200 cfu/ml	Smith *et al.*, 2001
Chlorine dioxide 0.1% and sodium phosphate mouthrinse 0.5%	http://www.rowpar.com/	Tested in 15 ultra sonic reservoir bottle units. Reduced bacterial density in output water ≤ 200 cfu/ml	Wirthlin and Marshall, 2001
Stabilized chlorine dioxide; dilution 1:10 100 ppm	Not stated	Tested in three DCUs for 15 weeks Bacterial density in DUWL output water > 200 cfu/ml. *Exophiala mesophila* proliferated **(R)**	Porteous *et al.*, 2003
Chlorine dioxide; Part A; sodium chlorite. Part B; phosphoric acid, lactic acid and non toxic catalyst (concentrations not given)	http://www.frontierpharm.com/studies.php	Tested in 4 DCUs which were initially shock treated, drained and dried overnight. Daily maintenance solution. Reduced bacterial density in output water ≤ 200 cfu/ml **(I & R)**	Wirthlin *et al.*, 2003
Chlorine dioxide stabilised; chlorine dioxide, trisodium phosphate, purified water	http://www.rowpar.com/	Tested in five DCUs for ten day period. Overnight treatment after initial shock treatment with concentrated product. Daily maintenance solution. Reduced bacterial density in output water ≤ 200 cfu/ml **(I & R)**	Wirthlin *et al.*, 2003

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Dioxiclear; undisclosed concentration of chlorine dioxide	http://www.frontierpharm.com/studies.php	Tested in model system only. No viable bacteria in output water after 16 h contact and 94.7% removal of biofilm coverage	Walker *et al.*, 2003
Glutaraldehyde			
Glutaraldehyde 2%	http://www.castellini.com	Tested in one DCU over 40 weeks. DUWLs seeded with bacterial spores and DUWL isolated microorganisms. DUWLs treated for 6 h daily. Did not consistently maintain bacterial density ≤ 200 cfu/ml **(I)**	Douglas and Rothwell, 1991
Glutaraldehyde 3%	Generic product	Tested for 15 h on DUWL tubing from a routinely used DCU. No viable bacteria in output water. Biofilm not eliminated after 5 applications	Meiller *et al.*, 1999
Gigasept Rapid; glutaraldehyde, formaldehyde, didecyldimethyl-ammoniumchloride 4%	http://www.uk.schulke-mayr.com/dentalhomepage.html	Tested in model system only. No viable bacteria in output water after 16 h contact and 34% removal of biofilm coverage	Walker *et al.*, 2003
Sanicide T4; glutaraldehyde formulated with quarternary ammonium salts	http://www.consealint.com/cms/	Tested in two DCUs for 15 days with overnight treatment; required safety procedures to prevent accidental ingestion. Reduced bacterial density in DUWL output water ≤ 200 cfu/ml **(I)**	Gigola, 2006
Sodium hypochlorite			
Sodium hypochlorite 15%; chlorination 0.5 – 1 ppm	Generic product	Tested in 250 DCUs over 30 weeks. DUWLs treated for 10 mins daily. Reduced bacterial density in DUWL output water to a few hundred cfu/ml **(I)**	Fiehn and Henriksen, 1988
Clorox; sodium hypochlorite, 5,000 ppm free available chlorine (FAC)	http://www.theCloroxcompany.com/	Tested in 10 DCUs. Ten minute weekly treatment followed by residual treatment with 3 ppm FAC. Study duration not stated. Bacterial density in DUWL output water ≤ 200 cfu/ml **(I/R)**	Karpay *et al.*, 1999
Sodium hypochlorite 5.25%	Generic product	Tested for 15 h on DUWL tubing from a routinely used DCU. No viable bacteria in output water. Biofilm not eliminated after 5 applications	Meiller *et al.*, 1999
Sodium hypochlorite 5,000 ppm and 1,500 ppm diluted with pasteurised water	Generic product	One DCU was treated with 5,000 ppm and one with 1,500 ppm for 10-minutes. Bacterial density < 200 cfu/ml in DUWL output water after one application **(I)**	Kim *et al.*, 2000

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Sodium hypochlorite 0.5%	http://www.prlabs.co.uk/	Tested in model system only. No viable bacteria in output water and 94.4% elimination of biofilm coverage	Walker *et al.*, 2003
Sodium hypochlorite 5.25% and phenol combined	Generic product	Tested for 48 h on DUWL tubing from a routinely used DCU. 96-98% reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Sodium hypochlorite 35%	Generic product	Study carried out on tubing taken from a routinely used DCU. 89% reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Hydrogen peroxide/alkaline peroxide			
Dentosept; hydrogen peroxide 1.4%	http://www.sirona.com/	Tested in 10 new DCUs for six weeks. Left in DUWLs each night. Bacterial density in DUWL output water ≤ 200 cfu/ml. Only partial elimination of DUWL biofilm **(I)**	Kettering *et al.*, 2002
Dentosept P; hydrogen peroxide, silver ions 0.014%	http://www.i-apteka.pl/product-pol-784-DENTOSEPT-plyn-100ml.html	Tested in 11 DCUs for up to eight weeks, (average 6.9 weeks). Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 91% of DUWL output water samples **(R)**	Schel *et al.*, 2006
Dialox; hydrogen peroxide, peracetic acid, acetic acid.	http://www.uk.schulke-mayr.com/hospital/Product%20Range/H%20special/dialox.html	Tested in model system only. No viable bacteria in output water after 16 h contact and 99.7% removal of biofilm coverage	Walker *et al.*, 2003
Hydrogen peroxide 35%	Generic product	Tested for 48 h on DUWL tubing from a routinely used DCU. 35-41% reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Oxygenal; hydrogen peroxide 0.4%	http://www.kavo.com/Default.aspx?navid=6700&oid=002&lid=En	Tested in model system only. No viable bacteria in output water after 16 h contact and 99.2% removal of biofilm coverage	Walker *et al.*, 2003
Oxygenal 6; hydrogen peroxide, silver ions 0.02%	http://www.kavo.com/Default.aspx?navid=6700&oid=002&lid=En	Tested in 15 DCUs up to eight weeks (average 6.9 weeks). Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 91% of DUWL output water samples **(R)**	Schel *et al.*, 2006
Oxygenal 6; hydrogen peroxide 6%	http://www.kavo.com/Default.aspx?navid=6700&oid=002&lid=En	Tested in 25 DCUs. Hydrogen peroxide 0.25% used initially for 30 mins, then hydrogen peroxide 0.2% used continuously for two weeks. Bacterial density in majority of DUWL output water samples ≤ 200 cfu/ml **(I/R)**	Szymanska, 2006

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Hydrogen peroxide/alkaline peroxide			
Oxygenal 6; hydrogen peroxide, silver 0.02%	http://www.kavo.com/Default.aspx?navid=6700&oid=002&lid=En	Tested in biofilm model system only using a variety of bacterial and yeast species. When used with 16-18 h contact time, no biofilm dispersal observed and virtually no loss in bacterial viability in biofilms	Gawande *et al.*, 2008
Sanosil; hydrogen peroxide 1% and silver 0.001%	http://www.sanosil.com/	Tested in four DCUs for eight weeks using once weekly overnight treatment. Reduced bacterial density on DUWL output water ≤ 200 cfu/ml for up to a week post-treatment. Biofilm almost completely eliminated **(I)**	Tuttlebee *et al.*, 2002
Sanosil; hydrogen peroxide 1% and silver 0.001%	http://www.sanosil.com/	Tested in model system only. No viable bacteria in output water and complete elimination of biofilm coverage	Walker *et al.*, 2003
Sanosil Super 25; hydrogen peroxide and silver 5% solution	http://www.sanosil.com/	Tested in 30 DCUs for up to eight weeks (average 6.9 weeks) using overnight treatment. Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 83% of DUWL output water samples **(I)**	Schel *et al.*, 2006
Sterilex Ultra; alkaline peroxide 0.5%	http://www.sterilex.com/sterilex/	Tested in 23 pre-clinical simulators (not used for treating patients) for 5 weeks using overnight treatment . Bacterial density in DUWL output water ≤ 200 cfu/ml by week four **(I)**	Linger *et al.*, 2001
Sterilex Ultra; hydrogen peroxide 0.5%	http://www.sterilex.com/sterilex/	Tested in 6 DCUs 7-20 weeks using once weekly overnight treatment. Reduced bacterial density in DUWL output water ≤ 200 cfu/ml for up to a week post-treatment. Biofilm almost completly eliminated. Clogging of DUWLs evident after 4th week in some DUWLs. Residual product present in DUWL output water following treatment **(I)**	Tuttlebee *et al.*, 2002; Tuttlebee, 2004
Sterilex Ultra; alkaline peroxide 5%	http://www.sterilex.com/sterilex/	Tested in six DCUs. Initially used overnight for three consecutive nights and thereafter once or more weekly for six weeks. Initially reduced bacterial counts to < 100 cfu/ml. Bacterial counts rose to > 104 cfu/ml thereafter **(I)**	Larsen and Fiehn, 2003
Sterilex Ultra; alkaline peroxide 5%	http://www.sterilex.com/sterilex/	Tested in model system only. No viable bacteria in output water and 97.3% elimination of biofilm coverage	Walker et al., 2003

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Hydrogen peroxide/alkaline peroxide			
Sterilex Ultra; hydroperoxide ion-phase transfer catalyst 5%	http://www.sterilex.com/sterilex/	Tested in 24 DCUs over a six week period. Initial shock treatment overnight for three nights followed by once weekly overnight treatment. Good elimination of DUWL biofilm and reducted bacterial density in output water ≤ 200 cfu/ml **(I)**	Shepherd *et al.*, 2001
Sterilex Ultra; alkaline peroxide 5%	http://www.sterilex.com/sterilex/	Tested in 10 DCUs for up to eight weeks (average 6.9 weeks) using overnight disinfection. Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 78% of DUWL output water samples **(I)**	Schel *et al.*, 2006
Sterilex Ultra; alkaline peroxide 5%	http://www.sterilex.com/sterilex/	Tested in biofilm model system only using a variety of bacterial and yeast species. When used with 16-18 h contact time, no biofilm dispersal observed, but complete loss of bacterial viability in biofilms	Gawande *et al.*, 2008
Ultra Kleen; alkaline peroxide product. Sodium carbonate peroxyhydrate, *N*-alkyldimethylbenzyla-mmonium chloride dihydrate, EDTA	http://www.sterilex.com/sterilex/	Tested in 5 DCUs overnight for 3 days in week one and two days in week two. Reduced bacterial density in output water ≤ 200 cfu/ml after both sets of shock doses but could not maintain following treatment **(I)**	Wirthlin *et al.*, 2003
Electrically activated water			
Sterilox; superoxidized water 2.5%	http://www.puricore.com/	Tested in model system only. No viable bacteria in output water and 99.3% elimination of biofilm coverage	Walker *et al.*, 2003
Electro-chemically activated water, pH 7.4	http://www.radicalwaters.com/	Tested in seven DCUs for five weeks. Bacterial density in DUWL output water <1cfu/ml by end of week one and the biofilm was removed after five weeks **(R)**	Marais and Brözel, 1999
Acid electrolyzed water; pH 2.7, ORP of 1100 mV, dissolved chlorine 32 ppm	http://www.aqua.med.kyushu-u.ac.jp	Tested in seven DCUs. Bacterial density in DUWL output water < 1 cfu/ml after five weeks continuous treatment **(R)**	Kohno *et al.*, 2004
Optident / Sterilox; super-oxidised water 5%	http://www.puricore.com/	Tested in 10 DCU's that had not been used for 3 weeks before study. Study of 14 weeks duration. Bacterial density in DUWL output water ranged from <1-36 cfu/ml. Bacteria cultured at 37°C **(R)**	Martin and Gallagher, 2005

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Electrically activated water			
Sterilox	http://www.puricore.com/	Tested in 10 DCUs for up to 8 weeks. Initial shock treatment for 8 hours using full-strength product followed by residual treatment using 2% solution. Reduced bacterial density in DUWL output water to almost 0 cfu/ml. DUWL biofilms degraded over the period of the study **(I/R)**	Zhang *et al.*, 2007
Mouthrinses			
Scope mouthwash; 1:10 dilution in distilled water	http://www.getclose.com/	Tested in two DCU over 42 months. No viable bacteria detected in DUWL output water for more than three years **(R)**	Eleazer *et al.*, 1997
Rembrandt mouthrinse	http://www.jnj.com/connect/healthcare-products/consumer/	Tested in 10 new DCUs for six weeks. Left in DUWLs each night. Reduced bacterial density in DUWL output water ≤ 200 cfu/ml. Only partial elimination of DUWL biofilm **(I)**	Kettering *et al.*, 2002
Listerine; ethanol 0.22 ml, benzoic acid 1.5 mg, thymol 0.64 mg, cineole 0.92 mg	http://www.jnj.com/connect/healthcare-products/consumer/	Tested in 10 new DCUs for six weeks. Left in DUWLs each night. Reduced bacterial density ≤ 200 cfu/ml. Only partial elimination of DUWL biofilm **(I)**	Kettering *et al.*, 2002
Miscellaneous treatment agents			
Cavicide; isopropanol 15.3%	http://www.sybrondental.com/	Tested for 15 h on DUWL tubing from a routinely used DCU. No viable bacteria in output water. Biofilm not eliminated after 5 applications	Meiller *et al.*, 1999
Combizyme; proteinases and carbohydrases 1.25%	http://www.biocatalysts.com/	Tested in model system only. 70% reduction in viable bacteria in output water after 16 h contact and 45% removal of biofilm coverage	Walker *et al.*, 2003
Ethylenediaminetetra - acetic acid (EDTA) 1%	Generic product	Tested for 48 h on DUWL tubing from a routinely used DCU. 56-62% reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Grotanat Bohrerbad; calcium hydroxide, propanol, ethylhexanol	http://www.uk.schulke-mayr.com/dentalhomepage.html	Tested in model system only. No viable bacteria in output water after 16 h contact and almost total removal of biofilm coverage	Walker *et al.*, 2003
Grotanol Flussig; chloro-methylphenol chlor-benzylphenol, biphenol 2%	http://www.uk.schulke-mayr.com/dentalhomepage.html	Tested in model system only. No viable bacteria in output water after 16 h contact and almost total removal of biofilm coverage	Walker *et al.*, 2003
Grotanol; triazine-triethanol, sodium hydroxide 3%	http://www.uk.schulke-mayr.com/dentalhomepage.html	Tested in model system only. No viable bacteria in output water after 16 h contact and 64.9% removal of biofilm coverage	Walker *et al.*, 2003

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Miscellaneous treatment agents			
ICX A-dec; sodium percarbonate, silver nitrate and cationic surfactants	http://www.a-dec.com/	Tested in 6 DUWL model systems for 16 weeks. Bacterial density in DUWL output water ≤ 200 cfu/ml at both high and low water hardness levels and prevented biofilm buildup in the DUWLs **(I)**	McDowell *et al.*, 2004
Ozone; 200 mg/h (liquid and gas phase = 10 min)	http://www.onnic.co.uk/products.html	Tested in model system only. 65% reduction in viable bacteria in output water after 16 h contact and 57.8% removal of biofilm coverage	Walker *et al.*, 2003
Parmetol; butylhydroperoxide 1%	http://www.schuelke-mayr.com/	Tested in model system only. 94.8% reduction in viable bacteria in output water after 16 h contact and 31.3% removal of biofilm coverage	Walker *et al.*, 2003
Peracetic acid, 0.26%	http://www.castellini.com/	Tested in six DCUs for one week. Bacterial density in12/144 DUWL output water samples > 200 cfu/ml. Between patient disinfection method. Very time consuming **(I)**	Montebugnoli *et al.*, 2004
PeraSafe; sodium perborate, monohydrate 1.62% w/v	Castellini S.p.a., Bologna, Italy	Tested in biofilm model system only using a variety of bacterial and yeast species. When used with 16-18 h contact time, no biofilm dispersal observed, but complete loss of bacterial viability in biofilms	Gawande *et al.*, 2008
Povidone-iodine 10% used at approximately 1 ppm free iodine	Generic product	Tested in 5 DCUs. Left in DUWLs for 12 h each night for a period of 22 days. DUWL output water remained free of bacterial contamination for 72 h and thereafter increased. By day 22 all 5 DCUs yielded heavily contaminated DUWL output water (approx. 1x 10^5 cfu/ml) **(I)**	Mills *et al.*, 1986
Betadine; povidone iodine 10%	http://www.ssl-international.com	Tested in model system only. No viable bacteria in output water after 16 h contact and 97.3% removal of biofilm coverage	Walker *et al.*, 2003
Povidine iodine 1%	Generic product	Tested for 48 h on DUWL tubing from a routinely used DCU. 12-51% reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Sodium dodecyl sulphate (SDS) 1%	Generic product	Tested for 48 h on DUWL tubing from a routinely used DCU. 16-42% reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Sodium fluoride 0.5%	Generic product	Tested in 10 new DCUs for six weeks. Left in DUWLs each night. Reduced bacterial density ≤ 200 cfu/ml. Only partial elimination of DUWL biofilm **(I)**	Kettering *et al.*, 2002

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Miscellaneous treatment agents			
Sodium metaperiodate 0.1%, sodium dodecyl sulfate 0.1%, sodium bicarbonate 0.1%	http://www.sigmaaldrich.com	Tested in biofilm model system only using a variety of bacterial and yeast species. When all three products used together with 16-18 h contact time, > 90% biofilm dispersal observed. 4-5 $\log_{10}$ reduction in biofilm-embedded bacteria	Gawande *et al.*, 2008
Ster4spray; tetraacetylethylenediamine (TAED), sodium perperborate, peracetic acid 0.26%	http://www.castellini.com/	Tested in 6 DCU's with 5 min disinfection cycle for 10 day period. 90% of DUWL output water samples yielded bacterial densities ≤ 200 cfu/ml. Product very unstable **(I)**	Montebugnoli and Dolci, 2002
Ster4Spray; sodium perborate, EDTA 2%	http://www.castellini.com	Tested in 5 DCUs up to eight weeks (average 6.9 weeks) using overnight treatment. Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 60% of DUWL output water samples **(I)**	Schel *et al.*, 2006
Spor-Klenz; hydrogen peroxide 22%, peracetic acid 4.5%, acetic acid <10%	http://www.steris.com/	Tested in model system only. No viable bacteria in output water and 92.6% elimination of biofilm coverage	Walker *et al.*, 2003
Tetra Acetyl Ethylene Diamine, sodium perborate, peracetic acid 0.26%	http://www.castellini.com	*In vitro* DUWL model system. Flushed for 2, 5 and 10 mins for 3-6 days. Bacterial counts and biofilm reduced to below detectable levels	Spratt *et al.*, 2004
Tegodor; benzalkoniumchloride, formaldehyde, glutaraldehyde 1%	TH Goldschmidt Ltd, Ruislip, Middlesex, UK	Tested in model system only. No viable bacteria in output water and 33% elimination of biofilm coverage	Walker *et al.*, 2003
Tween 20; 4%	Generic product	Tested for 48 h on DUWL tubing from a routinely used DCU. 62% - reduction in biofilm density on DUWL tubing	Liaqat and Sabri, 2008
Alpron products			
Alpron BRS solution; sodium hypochlorite 1-2%, and citric acid 70%	http://www.alpro-medical.co.uk/alpron—brs-system-2-c.asp	Tested in model system only. No viable bacteria in output water after 16 h contact and complete removal of biofilm from tubing	Walker *et al.*, 2003
Alpron BRS; sodium hypochlorite 1-2% and citric acid 70% (Initial Treatment)	http://www.alpro-medical.co.uk/alpron—brs-system-2-c.asp	Tested in 37 DCUs for up to eight weeks (average 6.9 weeks). Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 87% of DUWL output water samples **(I)**	Schel *et al.*, 2006

Continued overleaf

Table 1.3. Continued

Product name[a] / chemical agent(s)	Manufacturer	Comments[b]	Reference
Alpron products			
Alpron; citric acid and sodium-p-toluolsulpon echloramide <0.2% and EDTA and sodium tosylchloramide	http://www.alpro-medical.co.uk/alpron--brs-system-2-c.asp	Two-phase treatment product. Tested in 6 DCU's. One application of the biofilm removal product and continuous use of Alpron 1% for 13 weeks. Bacterial density in DUWL output water samples < 10 cfu/ml for up to 10 weeks. Thereafter, rose to > 200 cfu/ml at week 10 of the study **(I/R)**	Smith *et al.*, 2002
Alpron; p-hydroxybenzoeicacid ester 3%, polyaminoprophylbiguanid < 5%, 1,2-prophyenglycol 15–30%	http://www.alpro-medical.co.uk/alpron—brs-system-2-c.asp	Tested in four new DCUs for two weeks. Bacterial density in DUWL output water <10 cfu/ml **(R)**	Özcan *et al.*, 2003
Alpron; sodium hypochlorite 1-2% and citric acid 70%	http://www.alpro-medical.co.uk/alpron--brs-system-2-c.asp	Once off application. Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 87% of DUWL output water samples **(R)**	Schel *et al.*, 2006
Alpron Mint; sodium-p-toluolsulfonechloramide <0.2%, EDTA1-5%	http://www.alpro-medical.co.uk/alpron—brs-system-2-c.asp	Tested in model system only. No viable bacteria in output water after 16 h contact and complete removal of biofilm from tubing	Walker *et al.*, 2003
Alpron Mint; sodium-p-toluol-sulfonechloramide, 0.2%, EDTA	http://www.alpro-medical.co.uk/alpron—brs-system-2-c.asp	Tested in 37 DCUs for up to eight weeks (average 6.9 weeks). Reduced bacterial density in DUWL output water to ≤ 200 cfu/ml in 87% of DUWL output water samples **(R)**	Schel *et al.*, 2006

[a] The purpose of the table is to provide an in depth overview of the range of DUWL treatment agents that have been described in the literature. For the purpose of conciseness, not every published study with individual treatment agents is included. Treatment agents have been placed into groups based on their active ingredients. Some treatment agents contained more than one active ingredient, but were placed into specific groups based on individual ingredients for convenience in order to simplify the table.

[b] Many of the studies referred to in the table lacked specific details or were ambiguous in relation to frequency of treatment with specific treatment agents. Please refer to the individual studies listed in the reference list provided for more details.

Abbreviations: ORB, oxidation-reduction potential (redox); (I), intermittent treatment; (R), residual or continuous treatment; (I/R), intermittent treatment with treatment agent followed by continuous treatment with the same or another treatment agent. Throughout the table, (I) or (R) designations have only be given in the case studies undertaken in DCUs and have not been provided for studies undertaken using models systems or with DUWL tubing alone.

1.1.8.1. Dental Unit Waterline Treatment Agents

DUWL treatment agents are generally divided into two categories, including agents for intermittent DUWL treatment (e.g. once-weekly) and agents for continuous or residual DUWL treatment. Table 1.3 lists the range of DUWL treatment agents that have been used to control biofilm in DUWLs. A variety of DUWL disinfectants are available for use but both laboratory and clinical studies have shown that their efficacy varies widely. Biofilms are notoriously resistant to the use of chemical biocides and disinfectants and evade antimicrobial challenges by multiple mechanisms because some of the cells in the biofilm are nutritionally deficient and exist in a slow growing state (Costerton *et al.*, 1999). According to Shepherd *et al.* and Coleman *et al.* treatment agents that remove DUWL biofilm comprise the best approach for controlling the release of planktonic bacteria (Shepherd *et al.*, 2001; Coleman *et al.*, 2007). A recent review by Walker and Marsh appraised the range of chemical DUWL treatment agents that are currently available (Walker and Marsh, 2007). They reported that only some DUWL treatment agents have been shown to successfully remove biofilm and consistently reduce the microbial load of DUWL output water to <200 cfu/ml, whereas others did not. The more common DUWL treatment agents are based on a range of chemical compounds including hydrogen peroxide, hydrogen peroxide combined with silver ions, sodium hypochlorite (1:10 dilution), chlorine dioxide, chlorhexidine, peracetic acid and citric acid. Other products such as electrochemically-activated water have also shown good potential for microbial control of DUWL output water. In a large EU study in which a range of DUWL treatment agents were tested for efficacy in DCUs (n = 134) in general practice in several European countries, some of the most effective treatment agents evaluated contained hydrogen peroxide or a combination of hydrogen peroxide and silver ions, which efficiently remove DUWL biofilm. Overall, the continuously applied products performed better than those applied intermittently. It is important to note that many DUWL treatment agents have not been developed or endorsed by DCU manufacturers, but rather have been developed by other manufacturers in response to an evident market need. Thus, there is significant potential for incompatibility of DUWL treatment agents with components of the DUWL network as well as with instruments connected to this network (Coleman *et al.*, 2007; Coleman *et al.*, 2009).

1.1.8.2. Adverse Affects of Dental Unit Waterline Treatment Agents

A few studies have reported adverse effects associated with the application of DUWL treatment agents, but, as mentioned previously, only a very few long-term studies of the effectiveness of DUWL treatment agents have been reported thus far. Furthermore, in the case of residual DUWL treatment agents, there is a conspicuous lack of independent studies in the literature on potential interactions of such agents and their by-products on oral tissues and teeth. A number of recent studies reported that some DUWL treatment agents, (e.g. 3-ppm sodium hypochlorite; a 1:10 dilution of Listerine mouthrinse; bio 2000, (a 0.12% w/v chlorhexidine gluconate- and 12% v/v ethyl alcohol-containing product); 0.224% BioClear, (a citric acid containing product) may adversely affect bonding of composite material to both enamel and dentine (Roberts *et al.*, 2000; Taylor-Hardy *et al.*, 2001). With the extended and more widespread use of DUWL treatment agents, it is likely that such adverse effects may become clinically relevant in the case of residual DUWL treatments.

In 2002, a study from this laboratory reported clogging of DUWLs by the accumulation of disinfectant deposits in 3/6 DCUs treated with the alkaline hydrogen peroxide DUWL treatment agent Sterilex Ultra (Tuttlebee *et al.*, 2002). Clogging became evident after the fourth consecutive week of once-weekly treatment in the three DCUs, and in one of these, after 14 weeks it became impossible to aspirate water or treatment agent through the three-in-one air/water syringe DUWL, which had to be replaced. Additionally, the pH of DUWL output water in these DCUs remained persistently alkaline (e.g. pH 8.4) for several days post DUWL treatment. This was in contrast to the DCU supply water (pH 7.0) and DUWL output water from other DCUs (pH 7.0) in the same clinic, which were treated with a different hydrogen peroxide treatment agent not associated with DUWL clogging (Tuttlebee *et al.*, 2002; Tuttlebee 2004; Coleman *et al.*, 2007). These findings suggested that the residual DUWL treatment agent was present in DUWL output water in the DCUs that exhibited clogging for a considerable time after treatment.

A recent study by Schel *et al.* (2006) reported a range of adverse affects associated with several DUWL treatment agents, including dissolution of waterline tubing and the plastic delivery bottle in one DCU following use of Sterilex Ultra and blocking of DUWLs in three DCUs and two DCUs following use of Sterilex Ultra and

Steri4Spray, respectively. This study also reported staining of dental equipment surfaces in four DCUs following use of Alpron.

Modern DCUs are equipped with a suction system that has a variety of purposes. Primarily, the suction system is used to remove oral fluids and debris from the oral cavity during dental procedures and also to minimise aerosol release into the dental clinic environment during the use of dental instruments attached to DUWLs, especially high-speed turbine handpieces (O'Donnell *et al.*, 2005). Oral fluids and spent DUWL output water removed by DCU suction hoses and from the DCU cuspidor is eventually released as waste-water following particle removal, dental amalgam removal, and disinfection. Special amalgam separators are used to remove amalgam particles generated during the placement and removal of amalgam dental restorations in patients as amalgam contains mercury (Fan *et al.*, 2002; Coleman *et al.*, 2007). A recent study has shown that the performance of amalgam separators can vary and that the total mercury concentration and total dissolved (ionic) mercury concentration in DCU waste-water varied widely for each of 12 amalgam separators tested. (Fan *et al.*, 2002). A recent study from the USA reported that iodine-releasing resin cartridges, an effective residual treatment used to control bacterial growth in DUWLs by constant release of low levels of iodine into DUWL output water, may have a detrimental affect on the environment by mobilising mercury from dental amalgam in DCUs with the release of highly toxic dissolved mercury into the environment from DCU waste-water. Dissolved mercury levels in waste-water increased almost 21-fold (Stone *et al.*, 2006). However, the findings of this study were recently challenged by other authors who claimed that chloramine, used to disinfect municipal water, was more likely to have caused the increase in mercury levels rather than iodine (Hammarback *et al.*, 2007). This suggestion was discounted by a more recent study by Stone *et al.* (2009) who undertook a comprehensive investigation of the mobilisation of mercury from dental amalgam by both chloride and chloramine. The results of the study demonstrated that chlorine mobilises 24.8 times more mercury from dental amalgam than chloramine (Stone *et al.*, 2009). Other studies reported that a range of disinfectants and cleaning agents used to treat DCU waste-water lines also cause the release of mercury from dental amalgam when tested in the laboratory. Strong chlorine-containing agents were reported to cause the release of more mercury than other products (Roberts *et al.*, 2005; Batchu *et al.*, 2006). Based on these findings, it is conceivable that DUWL treatment agents that contain chlorine could also mobilise mercury from dental amalgam collected in

amalgam filters, traps and separators and in DCU waste-water lines and pipes and release it into the environment. The safe disposal of waste amalgam from dental practice is regulated by legislation in the EU and in many other countries. It is very likely that in the future that levels of mercury discharged in waste-water will be further regulated by additional legislation in order to further reduce the levels of toxic metals being released into the environment. Further research is required to develop procedures for testing the efficiency of amalgam separators in removing small amalgam particles and dissolved mercury and minimising the dwell time within the DCU. Dental Chair Unit manufacturers have an important responsibility for being proactive in regard to these matters.

It is evident that it is very important that DCU manufacturers provide very clear guidance to their customers on the types of DUWL treatment agents that they recommend for use in their DCUs. In doing so DCU manufacturers have a responsibility to ensure patient and dental healthcare personnel safety, to ensure that DUWL treatment agents do not exhibit adverse effects on DCU components in the short- and long-term and to ensure that DUWL treatment agents do not have adverse effects on the environment. All of this can only be achieved by significant investment in ongoing research and development.

1.1.9. Dental Unit Waterline Composition and Biofilm Formation

DCU manufacturers should also focus on developing or using novel DUWL materials that are less conducive to biofilm formation. To date, very little applied work has been undertaken in this area with regard to dental equipment. In other clinical fields, a variety of approaches have been undertaken to prevent/reduce biofilm formation on, for example, urinary catheters, including incorporation of antimicrobial agents such as silver and the biocide triclosan. Previous studies with a range of materials commonly used in the construction of water systems demonstrated that some materials were very good at limiting colonisation and biofilm formation by a wide range of bacterial species whereas other materials were very poor. Copper was the best at limiting colonisation and biofilm formation, followed by polybutylene and stainless steel, whereas biofilm formed more readily on polyethylene, chlorinated polyvinyl chloride (PVCc), unplasticised polyvinyl chloride (PVCu), steel and ethylene-propylene (Rogers *et al.*, 1994; Yabune *et al.*, 2005). A study from Japan showed that DUWLs composed of polyvinylidene fluoride (PVDF) were effective at inhibiting biofilm formation and

reducing bacterial density in DUWL output water (Yabune *et al.*, 2005). A more recent study from Italy on the influence of DUWL composition on output water quality reported that the heterotrophic bacterial plate count at 22°C from polytetrafluorethylene (PTFE) was lower than output water from DUWLs made from polyethylene (PE). Furthermore, whereas *P. aeruginosa* was regularly isolated from output water from DUWLs made from PE, it was never recovered from water from DUWLs made from PTFE (Sacchetti *et al.*, 2007). The research and development of novel DUWL materials with antimicrobial and/or anti-biofilm properties is a logical and potentially very fruitful area for research and development by DCU manufacturers with applications in many other areas. Distributing DCU supply water using copper pipes may also be helpful in improving the microbial quality of DCU supply water, as copper has been shown to possess significant antimicrobial advantages over drinking water pipework of other composition. An example of extensive biofilm in a section of plastic Qual-PEX water supply pipework is shown in Fig. 1.5.

1.1.10. Integrated Dental Unit Waterline Cleaning Systems for Dental Chair Units

DUWL treatment agents can be introduced into DUWLs from independent reservoir containers, or from treatment agent delivery devices connected to the DCU water supply inlet. In the case of DCUs connected to a municipal water mains supply, it is essential that the connection is turned off prior to DUWL treatment to avoid contamination of mains water with treatment agent chemicals. After treatment, DUWLs should be flushed thoroughly with clean water before DCUs are used for patient treatments to avoid exposing patients to residual disinfectant. At least one DCU manufacturer has developed a new generation of DCUs with integrated DUWL cleaning systems that facilitate and simplify control of biofilms in DUWLs by cleaning and disinfection with consequent consistent good quality DUWL output water. In 2002 a study from this laboratory reported on the effectiveness of the Planmeca Waterline Cleaning System (WCS™), a semi-automated DUWL cleaning system developed by the Finnish DCU manufacturer Planmeca, to control DUWL biofilm in two separate Planmeca Prostyle Compact DCUs over a 20-week period using the hydrogen peroxide and silver ion-containing disinfectant Sanosil (Tuttlebee *et al.*, 2002). The WCS™ was found to be very effective at eliminating DUWL biofilm in these DCUs when used with Sanosil and consistently provided output water with bacterial densities below the ADA

recommended level of ≤200 cfu/ml of aerobic heterotrophic bacteria for up to seven days post-disinfection.

1.1.11 New Approaches to Dental Unit Waterline Biofilm Control

The problem of poor microbial quality DUWL output water, its causes and the approaches that have been used for its control have been reviewed comprehensively in this chapter. It is now over four decades since the problem was first identified, and even though considerable progress has been made to control this problem, DUWL contamination is still a significant current issue of concern. The absence of official standards and legislation to control the quality of DUWL output water and the relatively small amount of applied research in this field have contributed significantly to slow progress in the development of effective practical solutions. This has been compounded by little interaction between DCU manufacturers and end users and especially by the lack of interaction of DCU designers and engineers with applied microbiologists. At the outset, the main objective of this project was to remedy this situation by collaborating with a DCU manufacturer and other manufacturers in developing practical solutions to the problem of DUWL contamination and by perfecting these solutions through long-term studies in multiple DCUs. This work was undertaken in the Dublin Dental School and Hospital.

1.1.12 Aims of the Study

- To investigate the long-term efficacy of the semi-automated Planmeca DUWL Wateline Cleaning Sytem (WCS™) at controlling DUWL biofilm in long-term studies and to identify and rectify human, microbial, DUWL treatment agent and DCU design factors that contribute to DUWL disinfection failures.

- To investigate the long-term efficacy of the automated Planmeca DUWL Waterline Management System (WMS™) at controlling DUWL biofilm in long-term studies and to identify and rectify human, microbial, DUWL treatment agent and DCU design factors that contribute to DUWL disinfection failures.

- To develop a centralised dental hospital water quality and biofilm management system that automatically maintains DUWL supply water and output water at

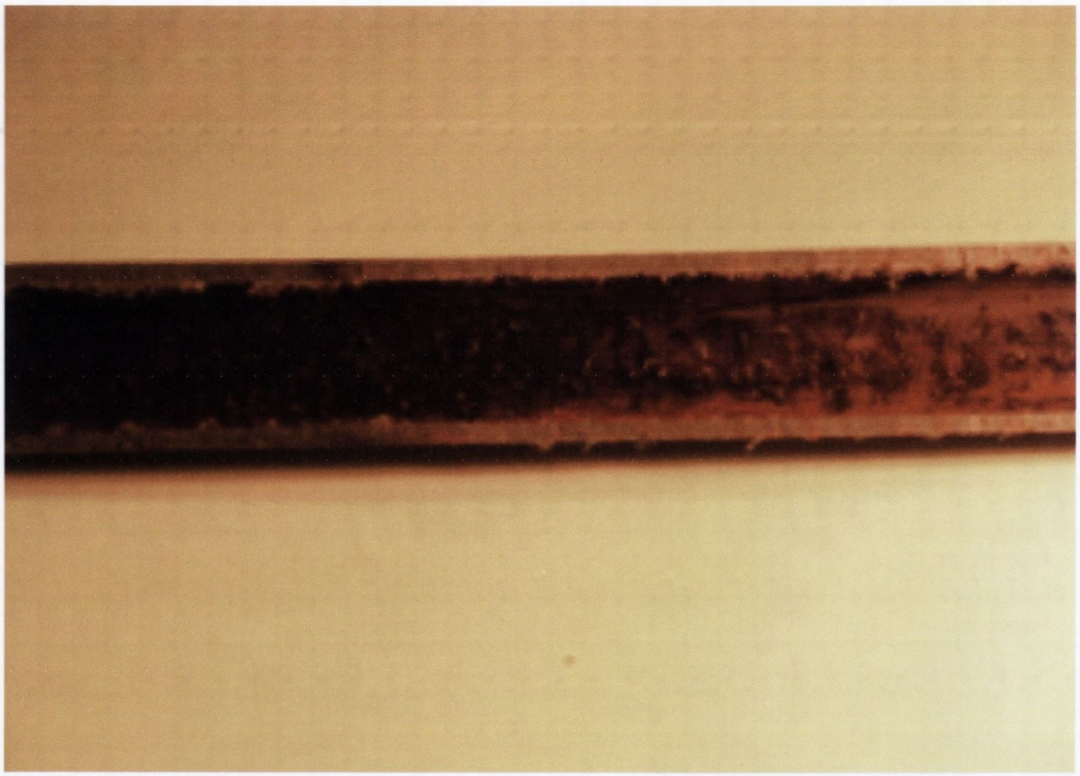


Fig. 1.5. Cross section of a section of Qual-Pex plastic water supply pipe. The presence of heavy biofilm and deposits are evident on the internal surface.

better than potable quality simultaneously in a large number of DCUs (>100) in the long-term.

Chapter 2

General Materials and Methods

2.1. General Methods, Reagents and Consumable Materials

2.1.1. Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade or molecular biology grade and, unless otherwise specified, were purchased from Sigma-Aldrich Ireland Ltd. (Vale Road, Arklow, Co. Wicklow, Ireland).

2.1.2. Disposable Laboratory Plasticware

Sterile, individually wrapped laboratory pipettes (1 ml, 5 ml, 10 ml and 20 ml) were purchased from Greiner (Greiner Bio-One GmbH, Kremsmünste, Austria). Microfuge tubes (1.5 ml and 0.5 ml) were purchased from Eppendorf (Eppendorf AG, Hamburg, Germany). Screw cap tubes (2 ml) with conical bases and a skirt were purchased from Sarstedt (Sarstedt AG and Co., Nümbrecht, Germany). Pipette tips (1-10 µl and 1-200 µl), with and without filters, were purchased from Starlab GmbH, Ahrensburg, Germany). Sterile 50 ml conical plastic tubes with caps, sterile plastic inoculating loops, spreaders, universal containers were purchased from Greiner.

2.1.3. Laboratory Culture Media

R2A agar was the principal culture medium used to isolate bacterial species from water samples. Two types of R2A agar were used; the first type used in the studies described in Chapters Three and Four was purchased in powder form from Difco (Becton Dickinson, Le Pont de Claix, France) and was prepared according to the manufacturer's instructions (per litre: 0.5 g yeast extract, 0.5 g proteose peptone, 0.5 g casamino acids, 0.5 g dextrose, soluble starch 0.5 g, sodium pyruvate 0.3 g, dipotassium hydrogen phosphate 0.05 g and agar 15 g). For the study described in Chapter Five, R2A agar purchased from Lab M Ltd. (Bury, Lancashire, United Kingdom) was used and prepared according to the manufacturer's instructions (per litre: 0.5 g yeast extract, 0.5 g meat peptone, 0.5 g casamino acids, 0.5 g glucose, soluble starch 0.5 g, sodium pyruvate 0.3 g, dipotassium hydrogen phosphate 0.05 g and agar No. 2 15 g). Luria-Bertani agar (LA; pH 7.4) (Lennox, 1955) was used to purify bacterial isolates recovered on R2A agar and also to prepare some bacterial inocula for total genomic DNA isolation.

2.2. Microbial Culture and Analysis of Water

2.2.1. Mains DCU Supply Water Pre-treatment

All 103 DCUs with which the Dublin Dental Hospital is equipped are supplied with municipal mains water. During the time period the studies described in Chapter Three and Chapter Four were undertaken the chlorinated municipal mains supply to the Dublin Dental School and Hospital destined for DCUs was automatically pre-treated, firstly by particle filtration, (removal of particles greater that 10 μm), then ion exchange (to remove calcium and magnesium ions) and finally by UV treatment (to reduce the numbers of viable microorganisms) (see Fig. 2.1). Following treatment, water was stored in an 8,000-l holding supplying a ring main with connections to each of the 103 DCUs. Once water entered the DUWL network of a DCU, a non-return valve prevents backflow of water into the ring main. The ring main pipework was disinfected annually by maintenance staff using hydrogen peroxide (Tuttlebee *et al.*, 2002).

2.2.2. Chemical Analysis of Water Samples

Chemical analysis of water samples was undertaken at the Trinity College Dublin (TCD) Centre for Microscopy and Analysis using standard procedures including inductively coupled plasma mass spectrometry (ICP-MS), gas chromatography-mass spectrometry (GC-MS) and titration as described previously (Tuttlebee *et al.*, 2002). The chemical quality of the water supplied to the DCUs was tested regularly and was found to consistently comply with the limits advocated by the European Community (EC) Directive on the Quality of Water for Human Consumption and by the United States Environmental Protection Agency National Primary Drinking Water Regulations (Anonymous, 1998; Anonymous, 2002; Tuttlebee *et al.*, 2002). No significant difference in the chemical composition of the processed mains water supplied to DCUs and DUWL output water was found following disinfection with either Planosil or Planosil Forte.

2.2.3. Collection of Dental Unit Waterline Supply and Output Water Samples

For the studies described in Chapter Three and Chapter Four, samples of water (50 ml) were collected from the operator's three-in-one air/water syringe waterline in sterile glass bottles from DCUs immediately prior to DUWL disinfection and immediately following DUWL disinfection with either the DUWL disinfectants Planosil or Planosil

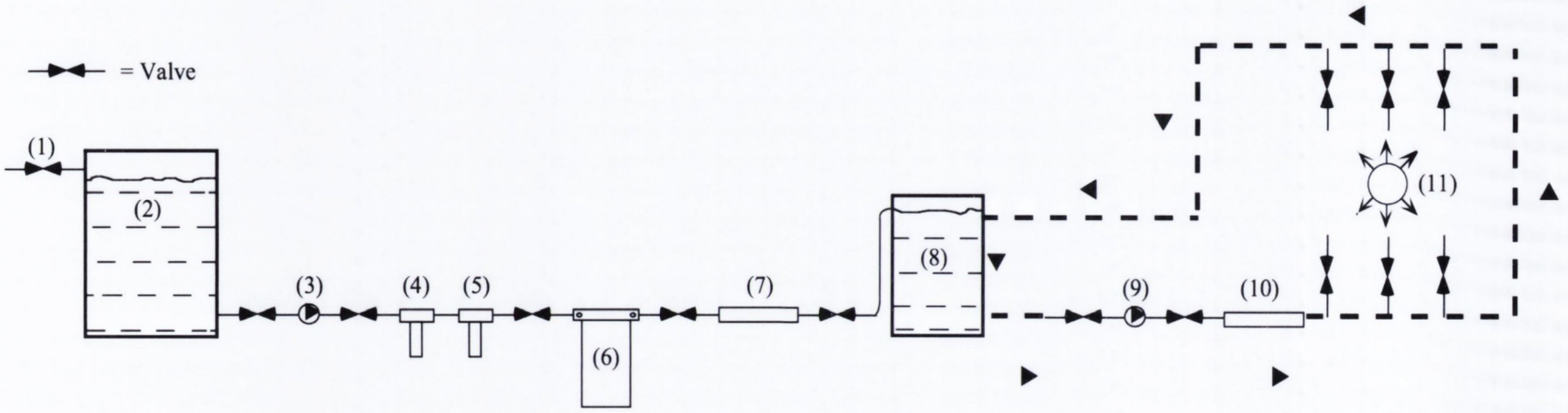


Fig. 2.1. Schematic diagram of treatment system for chlorinated municipal mains water destined for dental chair units in the Dublin Dental Hospital. This water treatment system was in place in the Dublin Dental Hospital during the time period the studies described in chapters 3 and 4 were undertaken. The system was redesigned for the study described in chapter 5. Numbers refer to specific components of the system as follows:(1) mains water intake, (2) 12,000-l water storage tank, (3) transfer pump, (4) 30 mm particle filter, (5) 10 mm particle filter, (6) water softener, (7) 254 $_{nm}$ UV source, (8) 8,000-l ring-main DCU supply water storage tank, (9) ring main pumps, (10) 254 $_{nm}$ UV source, (11) dental chair unit outlets. The ring main providing water to the dental unit outlets is highlighted as a series of thick dashed lines on the right-handside of the figure. The direction of flow of water around the ring main is indicated by a series of black arrowheads.

Forte. Prior to sampling, the operator's three-in-one air/water syringe was flushed for one minute. Sampling was carried out between 7.30 a.m. and 8.30 a.m. before the start of clinical sessions. Samples were transported to the microbiology laboratory within 5 min at ambient temperature and processed immediately. Additional water samples were taken on intervening days between disinfection cycles. Water samples were also taken from the mains water supplied to the DCUs at intervals (see Chapters Three to Five).

2.2.4. Culture of Bacteria from Dental Unit Waterline Supply and Output Water Samples

After collection, water samples were taken immediately to the microbiology laboratory and cultured in duplicate on R2A agar to determine total aerobic heterotrophic bacterial density as described previously (Reasoner and Geldreich, 1985; Tuttlebee *et al.*, 2002). R2A agar was used to determine total aerobic heterotrophic bacterial density because it is particularly efficient at isolating aerobic heterotrophic bacteria from water (Reasoner and Geldreich, 1985; Calabrese and Bissonnette, 1990). For the studies described in Chapter Three and Chapter Four, R2A agar purchased from Difco was used and prepared according to the manufacturer's instructions. For the study described in Chapter Five, R2A agar purchased from Lab M Ltd. was used and prepared according to the manufacturer's instructions. R2A agar plates were prepared in sterile 90 mm triple vented Petri dishes (Greiner) and contained 25 ml agar medium. Agar plates were poured freshly for all experiments and were air dried in a Class II laminar air flow safety cabinet (Microflow model M51424-2; Bioquell UK Ltd., Hampshire, UK), ensuring that no water droplets remained either on the surface of the agar, the lids or the inner sides of the Petri dishes. Samples from test DCUs were filtered both neat and after dilution by a factor of 10 with autoclaved ultra-pure water (18.2 MΩ cm resistivity at 25°C) from a Milli-Q Biocel® (Millipore, MA, USA) water purification system prior to plating in duplicate on to R2A agar. Negative controls consisted of samples of autoclaved ultra-pure water. The R2A plates were incubated in a static incubator (Gallenkamp model IPR150.XX1.5, Leicester, UK.) between 20-22°C for 10 days, after which time they were examined and colonies counted using a Stuart Scientific colony counter (Bibby Sterilin Ltd., Staffordshire, UK) (Chapter Three and Chapter Four) or using a Flash and Go™ automatic colony counter (IUL Instruments Ltd., Barcelona, Spain) (Chapter Five). The total number of colonies and the relative abundance of different colony types were recorded as the average of the duplicate plates, and selected

examples of all the colony types present on each plate were purified by subculture and stored on nutrient agar slopes (Cruinn Diagnostics Ltd., Parkwest Business Park, Dublin 12, Ireland) at room temperature in the dark for subsequent identification.

2.2.5. Identification of Bacterial Isolates

Bacterial isolates recovered from water samples were Gram stained using a Gram stain kit (Sigma-Aldrich) and tested for catalase (see Section 2.1.5.1) and oxidase production (see Section 2.1.5.2). As the majority of organisms recovered were Gram-negative, formal identification was undertaken, where possible, using the API 20NE system (bioMérieux, Marcy l'Etoile, France) (see Section 2.1.5.3), which facilitates the identification of non-fastidious Gram-negative rods not belonging to the *Enterobacteriaceae* within 48 h. Definitive identification of isolates was undertaken by determining the DNA sequence of a segment of the small ribosomal subunit rRNA gene and by comparing the sequences with consensus sequences for individual bacterial species in the EMBL/GenBank nucleotide sequence databases (O'Donnell *et al.*, 2006b) (see Section 2.2.5.4. below).

2.2.5.1. Catalase Test

Bacterial isolates recovered from DUWL supply and output water were routinely tested for their ability to break down hydrogen peroxide by catalase production. First, a 30% (v/v) hydrogen peroxide (H_2O_2) stock solution (Sigma-Aldrich) was diluted to 5% (v/v) with autoclaved ultra-pure water. Colonies of each bacterial isolate grown on R2A for 7 days were tested for catalase production by the addition of 50 µl of 5% (v/v) hydrogen peroxide. For each isolate, five separate colonies were tested for catalase production on each occasion. An arbitrary scale for catalase activity was used based on the visual observance of the evolution of oxygen bubbles following the addition of H_2O_2. If the colony then became rapidly effervescent, converting the hydrogen peroxide to water and oxygen, the isolate was considered strongly catalase-positive. Colonies that produced only minor effervescence following the addition of H_2O_2 were considered weakly positive, while those that yielded no effervescence after one min were considered to be catalase negative (Table 2.1).

Table 2.1. Arbitrary Scale for Catalase Activity Used in this Study

Catalase activity	Definition of catalase activity
+++++	Very strongly catalase-positive with instantaneous and substantial evolution of gas bubbles
++++	Strongly catalase-positive with almost instantaneous and considerable evolution of gas bubbles
+++	Catalase-positive with immediate but moderate evolution of gas bubbles
++	Catalase- weakly positive – minor evolution of gas bubbles
-	Catalase-negative – no evolution of gas bubbles

An arbitrary scale for catalase activity was used based on the visual observance of the evolution of oxygen bubbles from a bacterial colony following the addition of 50 µL of 5% (v/v) H_2O_2. For each isolate tested, at least five separate colonies grown on R2A agar medium were tested for catalase production.

2.2.5.2. Oxidase Test

Prior to testing, bacterial isolates were streak-innoculated onto fresh R2A agar plates and incubated in a static incubator between 20°C and 22°C for 7 to 10 days. Following incubation, isolates were tested for oxidase production using the BBL™ DrySlide ™ (Becton Dickinson and Co., Maryland, USA) system, according to the manufacturer's instructions. Briefly a single colony was removed from a fresh R2A plate using a sterile disposable plastic 1 µl loop (Cruinn Diagnostics Ltd,.) and rubbed onto the filter paper surface of a Dry Slide™ surface. This test detects the presence of bacterial cytochrome oxidase which oxidises cytochrome *c*, which in turn oxidizes reagents like *N*,*N*,*N'*,*N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (present in the DrySlide™) to form a purple coloured compound. Bacteria containing cytochrome *c* as part of their respiratory chain are oxidase positive and turn the reagent purple; bacteria lacking cytochrome *c* as part of their respiratory chain do not oxidize the reagent, leaving it colourless within the time limits of the test, and are oxidase negative.

2.2.5.3. Bacterial Identification Using the API 20NE System

As the majority of isolates recovered from DUWL supply and output water were Gram-negative rods, formal identification was initially attempted using the API 20NE system

(bioMérieux, Marcy l'Etoile, France). The API 20NE system can distinguish between and identify 61 bacterial species not belonging to the *Enterobacteriaceae* within 48 h. Each API 20NE plastic test strip consists of microtubes containing dehydrated media and substrates. The microtubules containing conventional tests are inoculated with a standardised inoculum of each isolate for identification and the strip incubated at 30°C for 24-48 h, following which, the metabolic end products are detected by indicator systems or the addition of reagents. The substrate microtubules contain assimilation tests and are inoculated with a minimal medium. If the bacteria are capable of utilising the corresponding substrate, they will grow. The isolate can then be identified on the basis of the pattern of growth or reactions in the individual tubules.

Each 20NE strip was supplied with an incubation tray and lid. Five ml aliquots of sterile ultra-pure water were placed into each tray to provide humidity during incubation and preventing the 20NE strips from drying out. The external flap on the incubation tray was labelled with indelible pen to identify the isolate being tested. For each isolate to be tested, one 20NE test strip was removed from its packaging and immediately placed into the prepared and labelled incubation tray. Using a sterile inoculating loop, a well-isolated colony (2-3 mm diameter) was picked from a Luria-Bertani agar (LA; pH 7.4) (Sambrook and Russell, 2001) plate grown for seven days at 25°C and placed into an ampoule containing 2 ml of sterile NaCl 0.85% (w/v) solution and thoroughly resuspened to achieve a turbidity equal to a 0.5 McFarland standard. For each 20NE strip, the microtubes containing potassium nitrate, tryptophane, glucose, arginine, urea, esculin, gelatine (with Indian ink) and *p*-nitrophenyl-ß-D galactopyranoside (see Table 2.2) were inoculated with 200 µl of the bacterial suspension. Care was taken to prevent the cupules from being over filled or under filled, as this can result in inaccurate results. Mineral oil was added to the tubules containing glucose, arginine and urea. The inoculum for the remaining tubules was prepared by adding 200 µl of the bacterial suspension to a 7 ml ampoule of AUX medium supplied with the 20NE strips (per litre: ammonium sulphate 2 g, agar 1.5 g, mineral base 82.8 mg, amino acids 250 mg, vitamin and nutritional supplements 35.9 mg in 0.04 M phosphate buffer at a final pH of 9.0-7.2). The contents were mixed gently by aspiration with a pipette, avoiding the formation of bubbles. Aliquots containing 350 µl of this suspension were then used to fill the entire microtube and cupule of those containing, glucose, aribanose, mannose, mannitol, N-acetyl-glucosamine maltose, gluconate, caprate, adipate, malate, citrate, phenyl-acetate (see Table 2.2). Following inoculation,

Table 2.2. Tests included in the API 20NE strip

Tests	Substrates	Reactions/ Enzymes	Results	
			Negative	Positive
N03[a]	Potassium Nitrate	Nitrate reduction to nitrites	**NIT 1 + NIT 2 for 5 min** Colourless	Pink/Red
		Nitrates reduction to nitrogen	**Zinc powder for 5 min** Pink	Colourless
TRP[a]	Tryptophane	Indole production	**James reagent / immediate** Colourless/ Pale green / Yellow	Pink
GLU[ab]	Glucose	Acidification	Blue To Green	Yellow
ADH[ab]	Arginine	Arginine dihydrolase	Yellow	Orange/pink/red
URE[ab]	Urea	Urease	Yellow	Orange/pink/red
ESC[a]	Esculin	Hydrolysis (β-glucosidase)	Yellow	Grey/brown/black
GEL[a]	Gelatine (With India Ink)	Hydrolysis (protease)	No Pigment Diffusion	Diffusion of black pigment
PNPG[a]	P-Nitrophenyl-B-D-galactopyranoside	β-galactosidase	Colourless	Yellow
GLU[c]	Glucose	Assimilation	Transparent[c]	Opaque[c]
ARA[c]	Arabinose	Assimilation	Transparent	Opaque
MNE[c]	Mannose	Assimilation	Transparent	Opaque
MAN[c]	Mannitol	Assimilation	Transparent	Opaque
NAG[c]	*N*-Acetyl-Glucosamine	Assimilation	Transparent	Opaque
MAL[c]	Maltose	Assimilation	Transparent	Opaque
GNT[c]	Gluconate	Assimilation	Transparent	Opaque
CAP[c]	Caprate	Assimilation	Transparent	Opaque
ADI[c]	Adipate	Assimilation	Transparent	Opaque
MLT[c]	Malate	Assimilation	Transparent	Opaque
CIT[c]	Citrate	Assimilation	Transparent	Opaque
PAC[c]	Phenyl-Acetate	Assimilation	Transparent	Opaque

[a]Tubules were inoculated with 200 µl bacterial suspension in 0.85% (w/v) NaCl.

[b]A mineral oil was added to tubules containing glucose, arginine and urea before incubation.

[c]Tubules were inoculated with 350 µl bacterial suspension prepared by addition of 200 µl bacterial suspension in 0.85% (w/v) NaCl into 7 ml AUX Medium (bioMérieux)

An opaque appearance in the microtubule indicates growth of test isolate, while a transparent appearance indicates absence of growth

the lid was replaced on each incubation tray and the trays incubated statically at 30°C for 24-48 h.

Following 24 h incubation, each incubation tray was removed from the incubator. The nitrate test was performed; one drop of Nitrate 1 (bioMérieux; sulfanilic acid 0.4 g, acetic acid 30 g and H_2O 70 ml) and one drop of Nitrate 2 (bioMérieux; N,N-dimethyl-1-napthylamine 0.6 g, acetic acid 30 g, H_2O 70 ml) reagents were added to the NO_3 tubule. After five min, a red colour indicates a positive reaction, which was recorded. If no colour change was observed, 2-3 mg of zinc dust was added to the tubule in case the bacteria had further reduced the nitrogen to nitrogen gas. If, after a further five min, no colour change was observed, a positive reaction was recorded. However, if a red colour appeared after the addition of the zinc dust, the reaction was recorded as negative because the zinc reduced the nitrates to nitrogen within the tubule. In the case of the indole production test, one drop of James reagent (bioMérieux; compound J 2183 [composition confidential – not disclosed by bioMérieux] 0.5 g and HCL 1 N per 100 ml) was added to the TRP tubule. If after five min, the cupule remains colourless, a positive reaction is recorded. A pink-red cupule indicates a negative reaction. The reactions in the remaining 17 tubules were then recorded and the strip reincubated for a further 24 hours (having first removed the reagents from the NO_3 and TRP cupules and then covered these tests with mineral oil).

The tests are separated into groups of three. The following numerical value is assigned to each positive reaction recorded:

1 - Positive reaction in the first test of the group
2 - Positive reaction in the second test of the group
4 - Positive reaction in the third test of the group

Having recorded all of the test results as either positive or negative, a substrate assimilation profile of each isolate was converted into a seven-digit numerical profile. By adding the values corresponding to positive reactions in each group, a seven digit numerical profile is obtained. The numerical profiles were then cross-referenced in the 20NE (Version 6) database of the APILAB software package (bioMérieux) to obtain the identification of each isolate. In the database, each numerical profile is listed with a percentage of identification, which is an estimate of how closely the profile corresponds to that of a particular taxon, relative to all other tests in the database and the T index, which is an estimate of how closely the profile corresponds to the most typical set of

reactions for a particular taxon. Based on these parameters, a profile that closely resembles those of a particular taxon will be classed as 'excellent', or 'good' identification, yielding identification to the genus or species level, whereas atypical results are classed as having low discriminatory powers and are usually unable to yield good identification. Identification is possible to taxon, species or genus level. If the profile is considered unacceptable, it is not listed. Database profiles are listed along with the following information; percentage identification (%id), T index value and comments on the quality of identification selected on the %id as follows:

Excellent identification	% id 99.9 and T > 0.75
Very good identification	% id 99.9 and T > 0.5
Good identification	% id 99.9 and T > 0.25
Acceptable identification	% id 80.0 and T > 0

2.2.5.4. Bacterial Identification by Sequence Analysis of the 16S rDNA Gene

Total genomic DNA from bacterial isolates was prepared using the Qiagen DNeasy kit system (Qiagen, Crawley, West Sussex, UK.) according to the manufacturer's instructions. Bacterial isolates were cultured on either R2A agar or on LA agar for 5–7 days at 25°C in a static incubator. Following incubation, approximately 10 colonies of each bacterial isolate were resuspended in 250 µl of lysis buffer (1 M Tris–EDTA and 10 mg lysozyme) in 1.5 ml Eppendorf microfuge tubes. The bacterial suspensions were then incubated at 37°C for two–three h with occasional vortexing to allow lysis of bacterial cells to occur. Genomic DNA was then extracted using the Qiagen DNeasy kit. Briefly, proteinase K and buffer AL (both reagents supplied with the DNeasy kit), were added to the lysed bacterial cells and the samples incubated at 70°C for 30 min. Following incubation, a 200 µl aliquot of 100% (v/v) ethanol was then added and the mixture transferred to a DNeasy mini spin column (Qiagen) and centrifuged at 6000 × *g* for one min. Following centrifugation, each sample was washed with the buffers supplied with the DNeasy kit and the purified DNA was eluted using 100 µl of the elution buffer supplied with the kit.

A variable segment of the 16S rDNA gene of each bacterial isolate was amplified by PCR as described previously by Singh *et al.* 2003 using approximately 30

ng of purified genomic DNA as template with the universal primers 533F (5′-AGAGTTTGATC/TA/CTGGCTCAG-3′) and 142R (5′-CGGC/TTACCTTGTTACGA-3′). These primers amplify a region of approximately 950 bp to 1.5 kb of the 16S rDNA gene of all bacterial species (Singh *et al.*, 2003). Amplified PCR products were purified using the QIAEX II gel extraction kit (Qiagen) according to the manufacturer's instructions prior to cloning into the pGEM T-Easy Vector System I (Promega Corporation, Madison, Wisconsin, USA). Transformation of competent *Escherichia coli* DH5α cells (Hanahan, 1983) [F- ϕ80d, *lacZ*Δm15, *end*A1, *rec*A1, *hsd*R17, (rK-mK+), *sup*E44, *thi*-1, d-, *gyr*A96, Δ (*lac*ZYA-*arg*F), U169] prepared using $CaCl_2$ and identification of *E. coli* derivatives harbouring recombinant plasmids were performed using standard methods (Sambrook and Russell, 2001). DNA sequencing of cloned amplimers was performed commercially by Lark Technologies (Essex, UK.) using an automated Applied Biosystems 373A DNA sequencer (Foster City, California, USA), dye-labelled terminators and the primers T7 (5′-TAATACGACTCACTATAGGG-3′) and SP6 (5′-ATTTAGGTGACACTATAGAAT-3′) that are specific for the pGEM T-Easy Vector (Promega). Analysis of chromatograms and sequences was carried out using the 373A data analysis software programme version 1.2.0 (Applied Biosystems) and DNA Strider 1.3f11 software (CEA/Saclay, Gif-sur-Yvette, France), respectively. The identity of individual bacterial isolates was determined by comparing their 16S rDNA sequences to those in the EMBL and GenBank nucleotide sequence databases using the BLAST family of computer programmes (http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul *et al.*, 1990). Molecular identification of bacterial isolates was undertaken by Dr. Anna Shore from the Dublin Dental School and Hospital Microbiology Research Laboratory.

2.3. Electron Microscopy and Energy Dispersive X-ray Analysis

2.3.1. Electron Microscopy of Dental Unit Waterline Biofilm

Two-centimeter long samples of DUWL tubing supplying water to dental handpieces were removed from DCUs and cut longitudinally through the lumen using a sterile scalpel. Then the internal walls of both halves were examined for the presence of biofilm without prior fixation, by electron microscopy using an Hitachi model S-3500N (Hitachi Ltd., Tokyo, Japan) variable pressure scanning electron microscope (Tuttlebee *et al.*, 2002).

2.3.2. Energy Dispersive X-ray Analysis of Corrosion Deposits

Energy Dispersive X-ray (EDX) is an analytical technique used for the elemental analysis of a sample and relies on the investigation of a sample through interactions between electromagnetic radiation and matter. Characteristic X-rays are emitted by a sample when a beam of X-rays is focused on the sample. As the energy of the emitted X-rays is characteristic of the elements from which they were emitted, the elemental composition of the specimen can be measured. The elemental composition of corrosion deposits of Planmeca WCS™ disinfectant delivery modules was examined by EDX analysis using an Oxford Instruments (Oxford, UK) INCA EDX Drycool SiLi detector during electron microscopy at the TCD Centre for Microscopy and Analysis (O'Donnell *et al.*, 2007).

2.4. Dental Unit Waterline Disinfectants Used in the Study

The DUWL disinfectants used in the studies described in Chapter Three and Chapter Four were Planosil (1.5% (v/v) hydrogen peroxide, 0.003% (w/v) silver, 0.0015% (v/v) ortho-phosphoric acid) and Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver, 0.0025% (v/v) ortho-phosphoric acid), both of which are marketed by Planmeca Oy (Helsinki, Finland) (Tuttlebee *et al.*, 2002; O'Donnell *et al.*, 2006b). Hydrogen peroxide and silver ions are the active antimicrobial ingredients in both disinfectants, while ortho-phosphoric acid is used as a stabilising agent. Both of these disinfectant products contain a dark blue tracking dye to aid visual observation of complete filling of DUWLs during the disinfectant feeding cycle.

2.4.1. Dental Chair Units Used in the Study

2.4.1.1. The Planmeca Prostyle Compact DCU used in the Study

Modern DCUs consist of a number of integrated components and equipment systems designed to support and enhance the delivery of dental treatment to patients. In the Planmeca Prostyle Compact DCU model configuration used in this study, the DCU consists of the chair that provides physical support and comfort for the entire body of the dental patient and allows for smooth and efficient dental team interaction during treatment of the patient. The instrument console is located on a moveable console arm and is positioned so that the equipment required by the operator is delivered across the chest of the supine patient (see Fig 2.2). The air, water and electricity supplies are

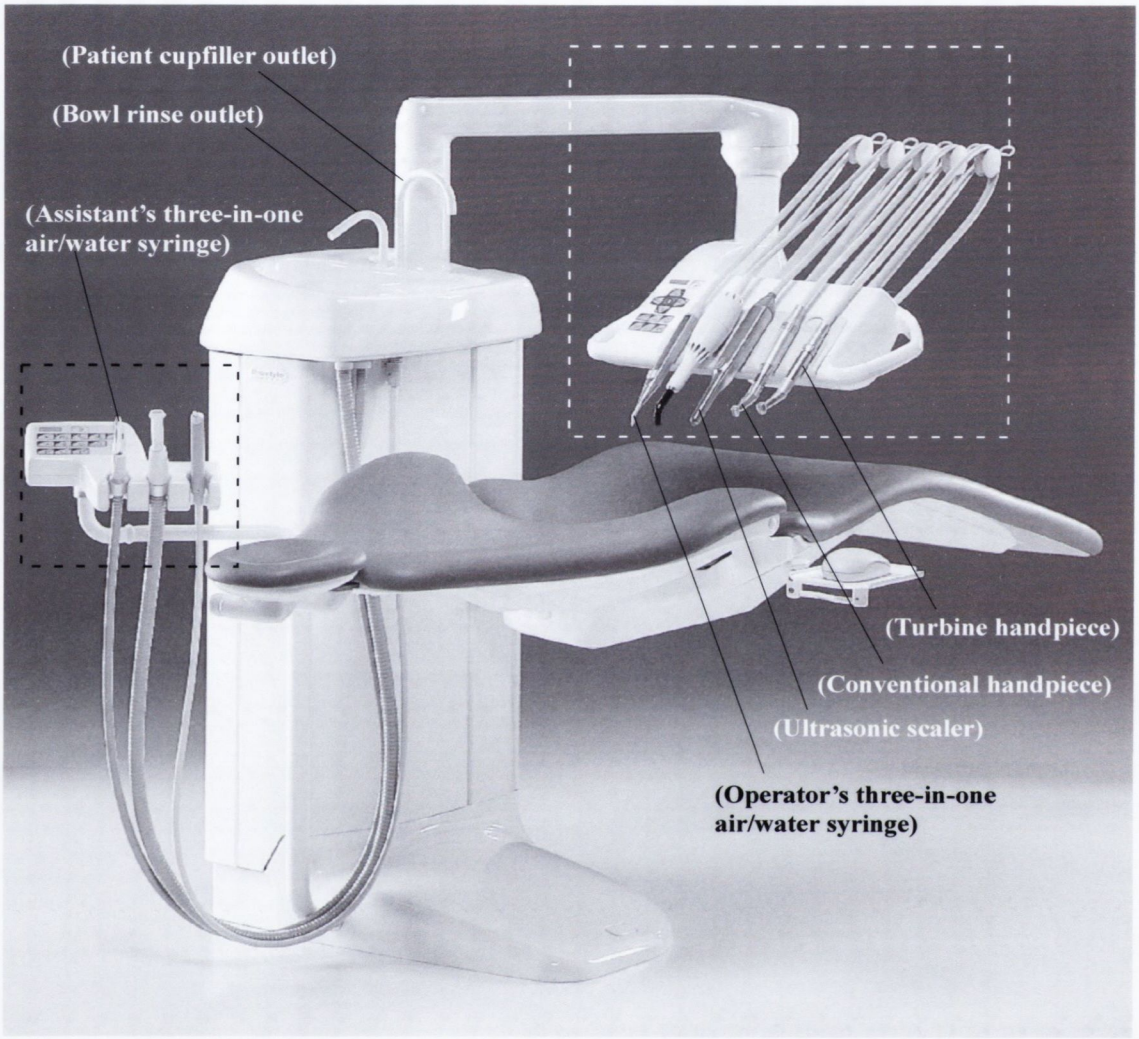


Fig. 2.2. View of the Planmeca Prostyle Compact dental chair unit configuration used in the present study showing all water outlets. The operator's instrument console containing the instrument hoses with instruments attached is highlighted with white dashed lines in the upper right portion of the figure. The assistant's instrument console with the assistant's three-in-one air/water syringe and the high- and low-volume suction system hoses is highlighted with black dashed lines in the mid-left portion of the figure.

routed up through the console arm and are delivered directly to the instrument hoses attached to the instrument console. The instrument console has five instrument hoses, which are attached to the rear of the instrument console. Each hose has its own built-in identification-coding that selects the appropriate settings when the instrument is connected. The handpieces that are generally attached to the instrument console are the three-in-one air/water syringe, turbine and conventional handpieces, ultra-sonic scaler and a light-curing unit. The Planmeca Prostyle Compact DCU has a touch sensitive control panel on the instrument console and is used to control and programme the instruments and the DCU (see Fig 2.2). The suction arm, with the dental nurses control panel, is attached to the left side of the unit (see Fig 2.2). Water, oral fluids and debris are aspirated through the suction hoses and are drawn down into the suction drain tubes and into the central suction system *via* a ring main, first passing through a coarse filter which retains all particles larger than one mm. Beneath the coarse filter is an amalgam collector designed to collect > 95% of the waste amalgam particles and dust removed from dental restorations. The patient cup filler water supply and the spittoon or cuspidor are positioned to the left of the patient and are operated by either the operator's or the dental nurse's control panel (see Fig. 2.2). The operating light is used to illuminate the oral cavity during dental procedures and is attached to the DCU.

Chapter 3

Optimisation of the Long-Term Efficacy of Dental Chair Waterline Disinfection by the Identification and Rectification of Factors Associated with Waterline Disinfection Failure

3.1. Introduction

Dental chair units (DCUs) supply water to irrigate the oral cavity and to cool a range of DCU-linked instruments and tooth surfaces during dental procedures (Siegel and von Fraunhofer, 2002; O'Donnell *et al.*, 2006a). Water supplied to the DCU may come in either of two ways, either in replaceable independent bottle reservoirs or directly from a potable water supply. DCU water is distributed through an intricate network of narrow-bore tubing called dental unit waterlines (DUWLs). Many studies have demonstrated that DUWL output water is frequently contaminated with high densities of microorganisms originating from microbial biofilm on internal DUWL surfaces (Scheid *et al.*, 1982; Martin, 1987; Pankhurst and Philpott-Howard, 1993; Williams *et al.*, 1993; Barbeau *et al.*, 1996; Meiller *et al.*, 2000; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; O'Donnell *et al.*, 2006a). This biofilm is populated from the DCU water supply, which itself usually contains only relatively low densities of microorganisms. The microorganisms in DCU supply water are predominantly aerobic heterotrophic Gram-negative environmental bacterial species. They attach to the internal surfaces of DUWLs forming microcolonies that in due course give rise to multispecies biofilm (Pankhurst *et al.*, 1998; Mills, 2000). These biofilms consist mainly of highly hydrated bacterial exopolysaccharide, in which single cells and microcolonies are heterogeneously interspersed with pores or channels (Davey and O'Toole, 2000; Watnick and Kolter, 2000). As the biofilm develops, planktonic cells and by-products are released into the water entering the mouths of patients during dental procedures. Accordingly, DUWL biofilm functions as a reservoir for continuous contamination of DCU output water.

The presence of high densities of microorganisms in DCU output water (up to 10^8 cfu/ml has been reported) is a potential infection risk to patients and dental healthcare personnel and is contrary to good cross-infection control practices (Mayo *et al.*, 1990; Tuttlebee *et al.*, 2002; Souza-Gugelmin *et al.*, 2003; O'Donnell *et al.*, 2006a). Previous studies have demonstrated that bacteria and fragments of biofilm in DCU output water are aerosolised during DCU instrument use and that patients and dental staff are exposed to these microorganisms (Fotos *et al.*, 1985; Martin, 1987; Reinthaler *et al.*, 1988; Atlas *et al.*, 1995; Pankhurst *et al.*, 2005; Dutil *et al.*, 2009). The presence in DCU output water of overt bacterial pathogens, such as *L. pneumophila*, and opportunistic bacterial pathogens, such as *P. aeruginosa* and non-*tuberculosis*

Mycobacterium spp., is of particular concern for the treatment of medically compromised and immunocompromised patients (Martin, 1985; Atlas *et al.*, 1995; Schulze-Röbbecke *et al.*, 1995; Singh and Coogan, 2005). At present there is no mandatory EU quality standard for DCU output water. However, as many DCUs are supplied with municipal drinking water it is not unreasonable to expect that DCU output water quality should be consistent with potable water standards. The present potable water standard for aerobic heterotrophic bacteria in the EU and the USA do not specify an upper limit, although water sold in bottles or containers in the EU should not exceed 100 cfu/ml (Anonymous, 1998; Anonymous, 2002). The current Centers for Disease Control and Prevention (CDC) guidelines for infection control in dental health-care settings recommend a maximum level of aerobic heterotrophic bacteria in DCU output water of ≤500 cfu/ml, whereas the ADA in 1995 established a target of ≤200 cfu/ml for the year 2000 (Kohn *et al.*, 2003; Anonymous, 1996). A symposium entitled Microbiology of dental unit water lines; setting standards for the future at the Pan European Federation/International Association for Dental Research meeting held at Trinity College, Dublin, during September 2006 debated setting a standard for DUWL output water quality (Coleman and O'Donnell, 2007). The consensus from the symposium was that in the absence of any EU standard for DUWL output water quality, every effort should be deployed to ensure that DUWL output water quality in Europe complies with the ADA standard of <200 cfu/ml of aerobic heterotrophic bacteria (Coleman and O'Donnell, 2007).

One of the most effective and practical approaches to maintaining good microbial quality DCU output water is regular treatment of DUWLs using a disinfectant or biocide that removes biofilm or inhibits biofilm growth (Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; O'Donnell *et al.*, 2006a). A wide range of commercially available DUWL cleaning products and systems has been marketed in recent years. Some of the latter are designed to be retrofitted to existing DCUs (Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; Walker *et al.*, 2004; O'Donnell *et al.*, 2006a). However, few of these microbial control methods have been tested extensively in a large number of DCUs over extended time periods (O'Donnell *et al.*, 2006a).

In 2002 a study from this laboratory investigated the effectiveness of the Waterline Cleaning System™ (WCS™), a DUWL cleaning system developed by the Finnish DCU manufacturer Planmeca Oy (Helsinki, Finland) (Tuttlebee *et al.*, 2002). This system was designed to maintain good quality DCU output water by controlling

DUWL biofilm in Planmeca Prostyle Compact DCUs using hydrogen peroxide-containing disinfectants. The WCS™ was designed to be retrofitted to existing Planmeca DCU models and delivers disinfectant periodically to DUWLs from a specially designed external reservoir. This was found to be very effective at eliminating DUWL biofilm and delivered DUWL output water at bacterial densities below the ADA recommended threshold of ≤200 cfu/ml for up to one week post-disinfection. Following on from these studies, in 2003 all 103 DCUs in the Dublin Dental School and Hospital (DDSH) were retrofitted with the WCS™ and a once-weekly DUWL disinfection regimen was introduced using the hydrogen peroxide and silver ion-containing disinfectant, Planosil. However, during the first nine-month period following the introduction of the WCS™ hospital-wide, routine monitoring of DCU output water identified a significant number of instances of intermittent waterline disinfection failure. The quality of DUWL output water was seen to fail the ADA standard of ≤200 cfu/ml, immediately following or, within 48 h of disinfection. Furthermore, during the next six-months the quality of DUWL output water from several of the hospital's DCUs consistently fell below the ADA standard ≤200 cfu/ml by three to four days post-disinfection despite the disinfection regimen.

3.1.1. Aims

Very few studies have prospectively investigated the long-term effectiveness of DUWL disinfection in multiple DCUs. The aim of this part of the present study was to undertake a long-term study of DUWL disinfection simultaneously in multiple DCUs of the same model and to investigate human, microbiological, DUWL treatment agents and DCU design and engineering factors associated with waterline disinfection failure.

There were three main objectives for this part of the study:

- To investigate the long-term (21-months) efficacy of the Planmeca Waterline Cleaning System (WCS™) to maintain the quality of DUWL output water below the ADA recommended standard of ≤200 cfu/ml of aerobic heterotrophic bacteria using once-weekly disinfection with the hydrogen peroxide and silver ion-containing disinfectant Planosil.

- To identify and rectify factors associated with intermittent waterline disinfection failure in DCUs equipped with the WCS™ system.

- To investigate and resolve the problem of rapid deterioration of DUWL output water quality following disinfection observed in several problematic DCUs.

3.2. Materials and Methods

3.2.1. Dental Chair Units and Disinfection of Waterlines Using the Planmeca Waterline Cleaning System™

Ten Planmeca Prostyle Compact DCUs (Fig. 3.1, panel a) retrofitted with the Planmeca WCS™ were used in the present study (see Chapter Two Section 2.4.1 for a detailed description of the Prostyle Compact DCU configuration used in the present study). The DUWLs on these DCUs were connected to a treated potable water supply (see Chapter Two, Section 2.2.1). The DCUs were located in the Accident and Emergency/Oral Surgery Clinic (four DCUs), Clinic one (three DCUs) and Clinic two (three DCUs) in the Dublin Dental School and Hospital (DDSH). Between July 2003 and March 2005 (21 months) the efficacy of the WCS™ units in maintaining good microbiological quality of DUWL output water was investigated while the DCUs were in daily use for dental patient treatments. The DCUs were disinfected once per week following the afternoon clinical session. The four DCUs in Accident and Emergency/Oral Surgery Clinic were disinfected solely by the author or by Professor D. Coleman, whereas dental nursing staff assigned to the other clinics disinfected the remaining six DCUs.

The microprocessor-controlled WCS™ was developed to be retrofitted to existing Planmeca DCUs having a municipal mains water supply. All DUWLs, apart from the cuspidor rinse DUWL are included in the microprocessor-controlled process whereby DUWLs are automatically filled with disinfectant and after a specified disinfectant contact time, subsequently automatically flushed with fresh clean water during the disinfection cycle. If the cycle is not completed properly or an electrical power failure occurs during the cycle, an error message is displayed on the DCU's instrument console digital display and all DCU functions are locked preventing the DCU from being used (Tuttlebee *et al.*, 2002). The WCS™ is semi-automated and requires the operator to fill the disinfectant container with disinfectant, to turn off a valve on the mains water supply and to place DCU instrument hoses into special receivers. DUWL cleaning is activated from a keypad on the DCUs control console and disinfectant is automatically fed into each DUWL. Following this the DCU electrical supply is switched off to allow the disinfectant *in situ* to take effect. When the electrical power is turned back on, a code message on the DCU control console display prompts the operator to flush the DUWLs. This is initiated by disconnecting the disinfectant container module, and

turning on the mains water supply switch. Each DUWL is then rinsed and flushed automatically with fresh mains water, after which time the DCU is ready for use.

3.2.1.1. Description of the Planmeca WCS™ System

In late spring of 2003 all 103 Planmeca Prostyle Compact DCUs with which the DDSH is equipped were retrofitted with the Planmeca WCS™ and a once-weekly DUWL disinfection regimen was introduced using the hydrogen peroxide and silver ion-containing disinfectant, Planosil. The Planmeca WCS™ system disinfection module consists of two parts; the disinfection module receiver (Fig. 3.2, panel a), which is fitted permanently to the dental unit, provides a mechanism whereby the internal DUWLs can be isolated from the ring-main water supply by closing a valve (Figs. 3.2, panels c and d). Once the DUWLs are isolated, the disinfection module (Figs. 3.2, panels b and d) is attached. This consists of an aluminium block that clips on to the module receiver attached to the dental unit and is connected to a 200 ml plastic water bottle *via* an aluminium collar (Fig. 3.2, panel b). A plastic tube runs from inside the water bottle into the block, which in turn is connected directly to the DUWLs. This enables the aspiration of the contents of the bottle into the DUWLs. During disinfection, the DUWLs in each test unit are isolated from the ring-main water supply, as described above. When using the WCS™, the DCU instrument hoses containing the DUWLs are first placed into instrument hose receivers located in a specially designed receptacle (Fig. 3.1, panel b). In the configuration of the Prostyle Compact DCUs used in this study, the instrument hoses containing DUWLs supplying the dental operators instruments (i.e. turbine handpiece, conventional handpiece and ultrasonic scaler) and three-in-one air/water syringe are all located together on an instrument console attached to a moveable arm (Fig. 3.1, panel b), whereas the assistant's three-in-one air/water syringe is located separately on the assistant's console along with the high volume and low volume suction hoses (Fig. 3.1, panel a). All DUWLs, apart from the cuspidor rinse DUWL, are included in a microprocessor-controlled process whereby DUWLs are automatically filled with disinfectant and subsequently automatically flushed with clean water during the disinfection cycle. If the cycle is not completed properly or a power failure occurs during the cycle, an error message is displayed on the DCU's instrument console. While the error message is displayed all DCU functions are locked preventing the DCU from being used. Pressing an activation key on an integrated keyboard on the DCU instrument console (Fig. 3.1, panels a and b) starts the cleaning cycle. A mains

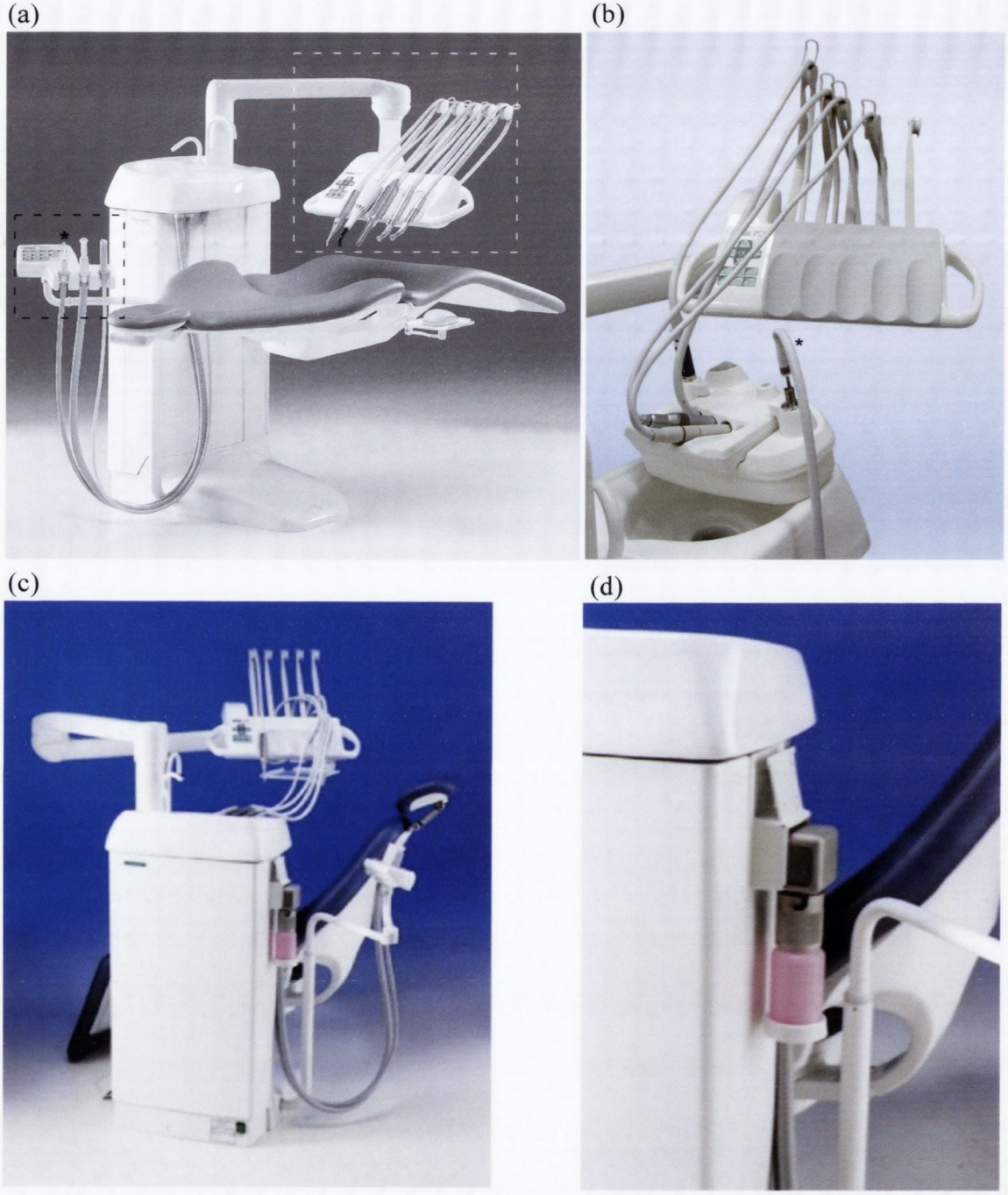


Fig. 3.1. The Planmeca Prostyle Compact dental chair unit model used in the present study. (a) Overview of the Prostyle Compact DCU. The operator's instrument console containing the instrument hoses with instruments attached is highlighted with white dashed lines in the upper right portion of the panel. The assistant's console with the assistant's three-in-one air/water syringe (*) and the suction system hoses attached is highlighted with black dashed lines in the lower left portion of the panel. (b) View of the DCU instrument hoses *in situ* in the flushing receivers during a disinfection cycle. The position of the assistant's three-in-one air/water syringe is indicated (*). (c) View of Prostyle Compact DCU with disinfection module attached. (d) Close-up view of removable disinfection module attached to a fixed receiver on a Prostyle Compact DCU.

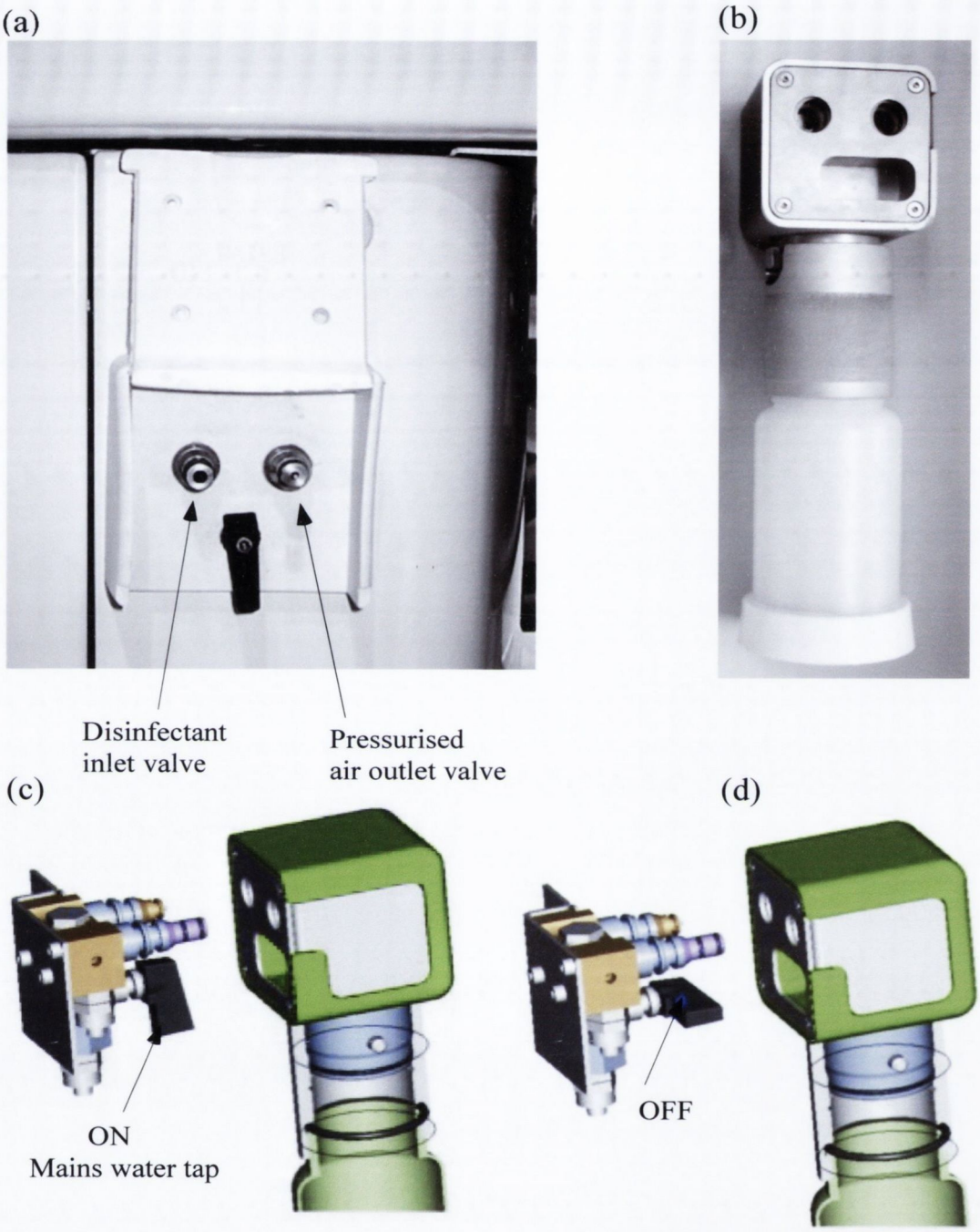


Fig. 3.2. Components of the Planmeca WCS™ used to disinfect DUWLs. Panel (a) shows a receiver module integrated into a DCU. The disinfectant intake valve and the air valve are indicated. The switch shown in the lower part of the figure is used to shut off the mains water supply to the DCU during DUWL disinfection. Panel (b) shows a disinfectant delivery module. The head of the module is designed to interface with the WCS™ receiver shown in panel (a). Panels (c) and (d) show schematics that demonstrate that the disinfectant delivery module can only interface with the receiver module when the mains water switch present on the latter is in the off position.

water supply switch to the DCU is then turned off (Fig. 3.2, panel a) and the WCS™ disinfectant container module filled with disinfectant is then attached to an integrated receiver module on the DCU. This receiver module contains a disinfectant intake valve and an air valve, the latter supplying pressurised air from the DCU's medical quality air supply (Fig. 3.2, panel a). Air from the air valve pressurises disinfectant in the disinfectant container bottle until a pressure of 1.5 bar is reached, after which the disinfectant intake valve opens and disinfectant is fed from the container, first to the instrument hose DUWLs, and then to the cup-fill DUWL. The disinfectant, which contains a dark blue marker dye, flows through the DUWLs until observed emitting from the outlet of each DUWL. The electrical supply to the DCU is then switched off leaving the DUWLs full of disinfectant overnight (15 h). When the electrical supply to the DCU is turned on again, a code on the DCU instrument console is displayed prompting the operator to disconnect the disinfectant container module, fill it with warm tap water and reattach it to the unit to start an initial flushing cycle of the instrument waterlines (30 s) and cup-fill line (20 s). After this cycle, a code prompts the operator to remove the container module and to turn on the mains water supply switch. This triggers an automatic flushing cycle whereby clean mains water is used to sequentially flush the instrument DUWL and cup-fill DUWL for a total duration of four min. After this automatic flushing and rinsing cycle, the DCU is ready for normal use.

3.2.2. Collection of Water Samples

Samples of water (50 ml) were collected from the three-in-one air/water syringe DUWL of 10 Planmeca Prostyle Compact DCUs immediately prior to disinfection and immediately following the disinfection and flushing procedure using either the waterline disinfectant Planosil or Planosil Forte (see Chapter Two, Section 2.2.3.). Additional water samples were also taken on intervening days each week between disinfection cycles. Water samples were also taken from the mains water supplied to the DCUs at monthly intervals throughout the study period.

3.2.3. Dental Chair Unit Supply Water

During the time period this study was undertaken the DDSH chlorinated mains water supplied to DCUs was automatically pre-treated prior to delivery to DCUs (see Chapter Two, Section 2.2.3.).

3.2.4. Laboratory Processing of Water Samples

Water samples were cultured for aerobic heterotrophic bacteria on R2A agar as described in Chapter Two, Section 2.2.4. Bacterial isolates were identified, where possible, using the API 20NE system as described in Chapter Two, Section 2.2.5. Definitive identification of bacterial isolates was undertaken by determining the DNA sequence of a segment of the small ribosomal subunit rRNA gene as described in Chapter Two, Section 2.2.5.4. Chemical analysis of water samples was undertaken as described in Chapter Two, Section 2.2.2.

3.2.5. Electron Microscopy of Waterline Biofilm

Two-centimetre lengths of DUWL waterline tubing (supplying water to the three-in-one air/water syringe close to the syringe) were removed at intervals before and immediately following disinfection with both Planosil and Planosil Forte and the internal surfaces examined for the presence of biofilm as described in Chapter Two, Section 2.3.1.

3.2.6. Energy Dispersive X-ray Analysis of Corrosion Deposits

The elemental composition of corrosion deposits of WCS^{TM} disinfectant delivery modules was examined by Energy Dispersive X-ray (EDX) analysis as described in Chapter Two, Section 2.3.2.

3.2.7. Statistical Analysis

The relative abundance of colonies of *Novosphingobium* and *Sphingomonas* spp., cultured from DUWL output water in the 10 DCUs used in this study was statistically analysed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test.

3.3. Results

3.3.1. Effectiveness of Once-Weekly Dental Unit Waterline Disinfection with Planosil During the Initial Nine Month Period

The main purpose of the present study was to prospectively investigate the effectiveness of the Planmeca WCS™ using once-weekly DUWL disinfection with Planosil over a prolonged period (21 months, between July 2003-March 2005), as prior to this study no studies on the long-term efficacy of DUWL disinfection have been reported in the literature. Ten representative DCUs were selected for study from the hospital's three main treatment clinics and the microbiological quality of their DUWL output water was investigated. During the nine-month period July 2003-March 2004, the DUWL output quality from the 10 DCUs was each tested during a total of 30 individual, once-weekly disinfection cycles (i.e. water quality was tested following a total of 300 separate disinfection cycles of the 10 DCUs investigated). During this period the average density of aerobic heterotrophic bacteria in the processed DCU supply water was 66 cfu/ml (range 60-112 cfu/ml). The microbiological quality of DCU output water remained below the ADA standard of ≤200 cfu/ml for up to a week following DUWL disinfection for 272/300 (90.7%) of the disinfection cycles investigated Fig. 3.3, panel (a) shows representative data from five consecutive disinfection cycles from one DCU. For the remaining 28/300 (9.3%) disinfection cycles DCU output water quality failed the ADA standard, in every case immediately or within 48 h following disinfection. These 28 failed disinfection cycles occurred in 8/10 DCUs investigated and were intermittent throughout the nine-month period July 2003-March 2004, with the first two failed cycles being detected within one month of starting the study. Apart from two exceptions, no two consecutive rounds of DUWL disinfection in individual DCUs resulted in output water quality that failed the ADA standard. During the course of this study several samples of DUWL tubing from the assistant's three-in-one air/water syringe DUWL were detached from the DCUs and the internal surfaces examined for the presence of biofilm by electron microscopy. Samples from DCUs that yielded output water within the ADA standard up to seven days post-disinfection had no detectable biofilm present immediately following disinfection with Planosil, although isolated patches of biofilm were evident by day seven post-disinfection. Patches of biofilm were evident immediately following disinfection, however, in DUWLs from DCUs that exhibited intermittent failure of DUWL disinfection (Fig. 3.4).

3.3.2. Investigation of Factors Associated with Intermittent Waterline Disinfection Failure and their Rectification

In 4/28 (14.3%) of the *failed* disinfection cycles, the DCU valve controlling the pressure of medical quality air supplied to dental handpieces had been inadvertently turned down in two separate DCUs. This pressurised air supply is also used by the WCS™ to force disinfectant from the WCS™ disinfectant reservoir into DUWLs during waterline disinfection (see Section 3.2.1.1). Thus in these four disinfection cycles, disinfectant was not aspirated into DUWLs resulting in waterline disinfection failure. The air pressure in these two DCUs concerned was correctly adjusted to 1.5 bar and the water quality of DUWL output water from subsequent disinfection rounds remained below the ADA standard for up to a week post-disinfection (Fig. 3.3, panel b).

In 10/28 (35.7%) of the failed disinfection cycles, which were approximately evenly distributed over the nine-month period July 2003 to March 2004, it was found that the assistant's three-in-one air/water syringe waterline had not been disinfected in six of the DCUs due to operator error. Using the WCS™, following overnight disinfection with Planosil, all DUWLs are flushed with clean water to remove disinfectant. Planosil contains a dark blue tracking dye so that DUWL filling and flushing of disinfectant from DUWLs can be observed visually. The assistant's three-in-one air/water syringe, which is not attached to the DCU's main instrument console (Fig. 3.2, panel a) can be inadvertently excluded by the operator from the disinfection cycle just by neglecting to place it into its flushing receiver during set-up. This does not interfere with disinfection of the other DUWLs. By ensuring that the assistant's three-in-one air/water syringe DUWL was included when disinfecting with Planosil in subsequent disinfectant cycles, output water quality was maintained below the ADA standard for up to a week post-disinfection (Fig. 3.3, panel c).

In 14/28 (50%) of the failed disinfection cycles, all of which occurred during the period January to March 2004 after six-months of continuous once-weekly disinfection with Planosil, DUWL disinfection failure was directly associated with blockage of and/or leakage from WCS™ disinfectant intake valves, and corrosion of components of disinfectant delivery module bottles (Fig. 3.3, panel d). On initiating the disinfection cycle disinfectant either failed to leave the disinfectant bottle or only a portion of the disinfectant left the disinfectant bottle and thus DUWLs were not filled or were incompletely filled with disinfectant. In the WCS™, the disinfectant container module

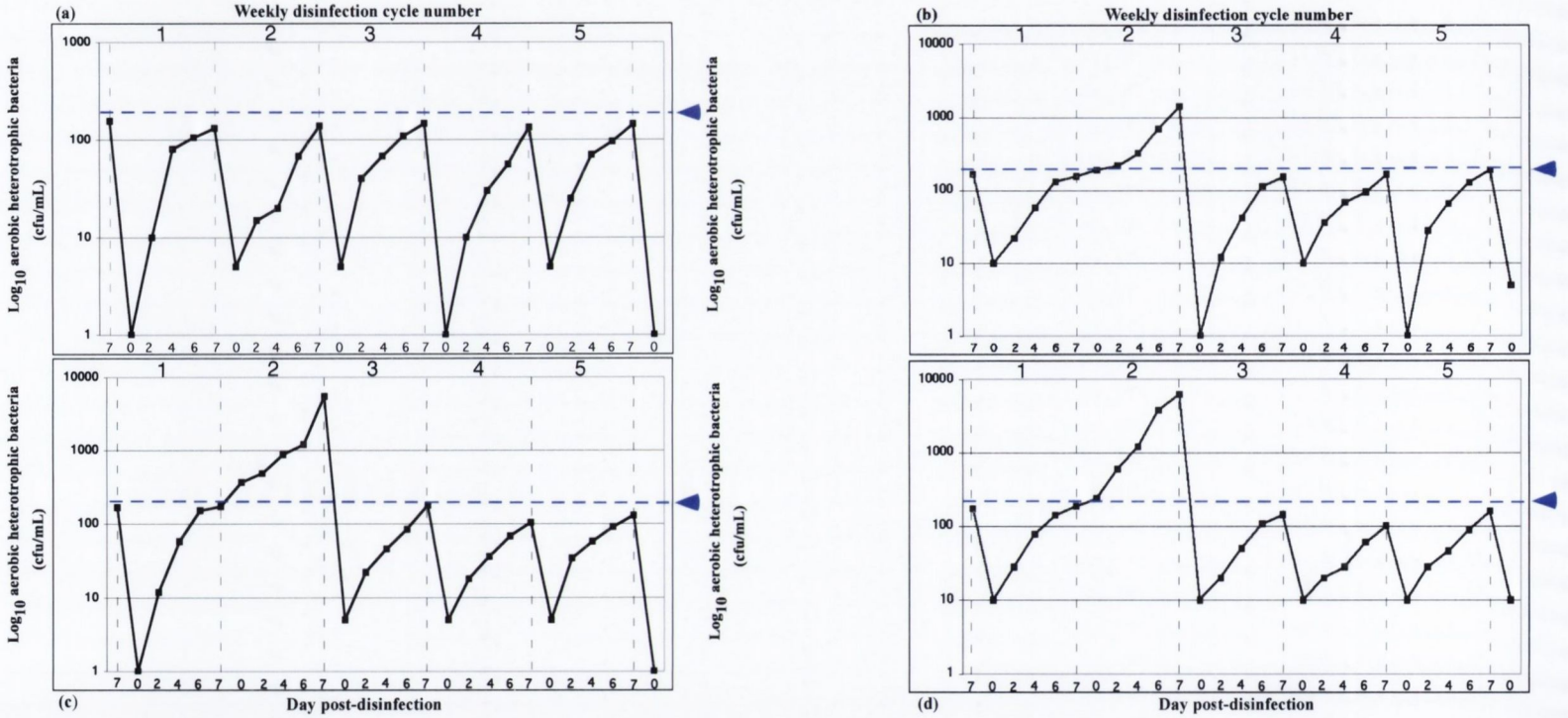


Fig. 3.3. Density of aerobic heterotrophic bacteria in three-in-one air/water syringe waterline output water from DCUs fitted with the Planmeca WCS™ during consecutive cycles of once weekly disinfection with Planosil. In 272/300 (90.7%) individual disinfection cycles with 10 DCUs between July 2003-March 2004 the microbiological quality of DUWL output water remained below the ADA standard of ≤ 200 cfu/ml of aerobic heterotrophic bacteria (blue dashed lines) following once a week (15 h) disinfection with the DUWL disinfectant Planosil. Panel (a) shows an example of typical data obtained in five consecutive successful disinfection cycles from a DCU where water quality remained within the ADA limit for up to seven days post-disinfection. Panels (b)-(d) show data obtained in five consecutive disinfection cycles from DCUs that exhibited intermittent failure of DUWL disinfection due to (b) low air pressure (occurred in 4/300 disinfection cycles with the 10 DCUs tested), (c) operator failure to include the assistant's three-in-one air/water syringe DUWL in the disinfection cycle (occurred in 10/300 disinfection cycles with the 10 DCUs tested) and (d) blockage and/or leakage of the disinfectant intake valve (occurred in 14/300 disinfection cycles with the 10 DCUs tested). The failed disinfection cycle is shown as cycle number 2 in each case.

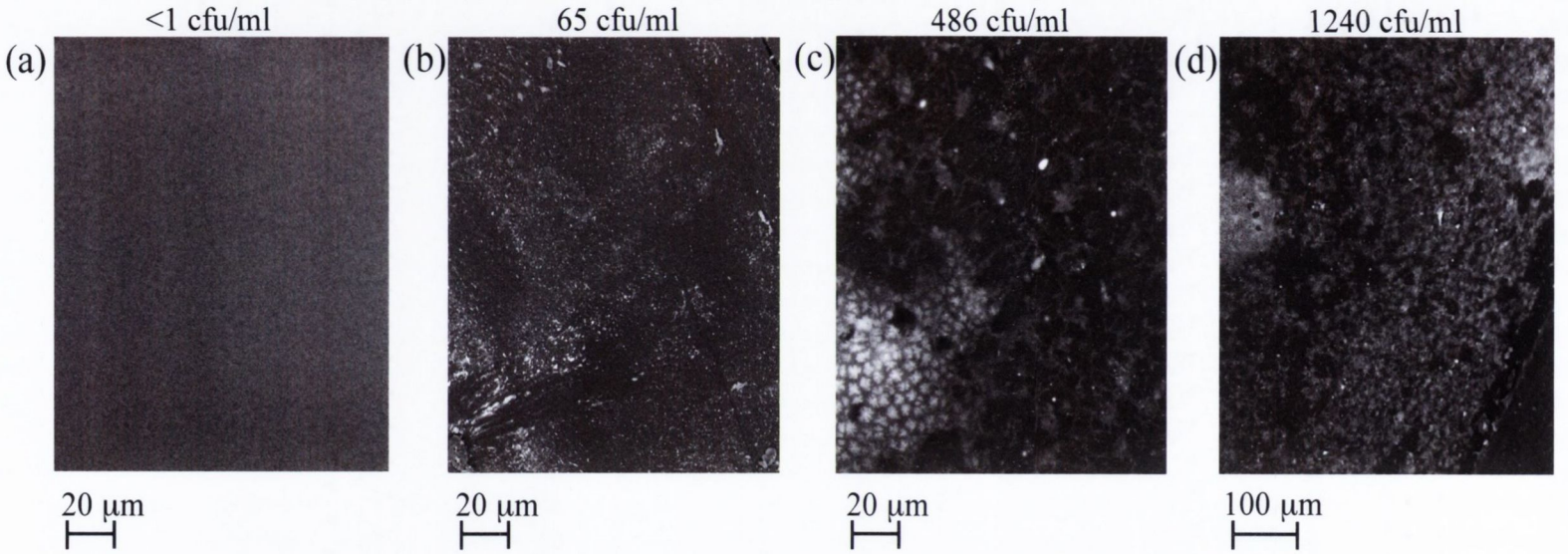


Fig. 3.4. Electron micrograph images of sections of the internal surface of DUWL tubing from the operator's air/water syringe taken from one of the four DCUs that exhibited rapid deterioration of output water quality following disinfection with Planosil during the second phase of the study. (a) section taken immediately post-disinfection showing the complete absence of biofilm. (b) section taken two days post-disinfection showing sparse biofilm. (c) and (d) sections taken four and seven days post-disinfection, respectively, showing the presence of extensive patchy biofilm. The corresponding bacterial density in output water samples taken immediately prior to removal of DUWL sections for electron microscopy are shown above each panel.

attaches to a receiver module on the DCU unit (Figs. 3.1, 3.2 and 3.5). The receiver module consists of a disinfectant intake valve, an air valve and a mains water cut-off switch (see Figs. 3.1, 3.2 and 3.5). During the disinfection process, the disinfectant container (Fig. 3.2, panel b) interfaces with the receiver module and the disinfection cycle is initiated electronically from the DCU control console. Air from the DCU's medical quality air supply is forced through the air valve pressurising the disinfectant in the disinfectant container. When the pressure reaches 1.5 bar, the disinfectant inlet valve opens allowing disinfectant to fill the DUWLs. Dismantling of the WCS™ receiver modules in all eight affected DCUs and examination of the disinfectant intake valves revealed that all the valves were blocked or partially blocked and/or leaky. Furthermore, examination of the disinfectant delivery module units revealed the presence of patches of powdery corrosion of aluminium components comprising the head of the module and the aluminium collar that connects the module head with the polyethylene disinfectant bottle container (Fig. 3.6). The intake valves in the remaining two non-affected DCUs were found to be functioning normally and were not blocked or leaky. These findings suggested that a constituent(s) of the disinfectant used (i.e. Planosil) may have been responsible for the corrosion following prolonged use and that the powdery deposits associated with the corrosion may have been responsible for blocking the disinfectant intake valves. Examination of the deposits by EDX analysis during electron microscopy yielded elemental spectra consistent with aluminium oxides, which were the predominant constituent (Fig. 3.7) This strongly suggested that oxidation of aluminium by hydrogen peroxide contained in Planosil disinfectant was responsible for the corrosion deposits. As an interim measure, blocked valves were replaced by maintenance personnel in the eight DCUs concerned and several of them together with a number of corroded disinfectant container modules were forwarded to the DCU Manufacturer Planmeca Oy (Helsinki, Finland) for detailed investigation.

Examination of the blocked valves and corroded disinfectant delivery modules by Planmeca engineering personnel concluded that two factors had contributed to blockage of the disinfectant intake valves, the first of which was the construction of the valves themselves and the second was corrosion of aluminium components of the disinfectant delivery module by hydrogen peroxide contained in the disinfectant. The valves consist of a head and a base section that were held together with glue (Figs. 3.5, panels b and c). Inside the valve, a valve needle mounted on a spring keeps the valve shut until pressurised disinfectant from the disinfectant bottle forces the needle back

against its spring allowing disinfectant to enter the DUWLs. Examination of the blocked valves revealed that glue and deposits had blocked the valve lumens in each case. Furthermore, in some cases loss of glue at the junction of the two valve sections resulted in valve leakage, whereas in other cases, the presence of glue and deposits in the valve lumen prevented the valve from closing after the disinfectant container had been emptied during DUWL filling. This allowed disinfectant to leak from the DUWLs. Corrosion of aluminium components appeared to be the major contributory factor as valve blockage in experimental DCUs became exacerbated when exposed to higher concentrations of hydrogen peroxide (Planmeca, personal communication). To circumvent these problems, new valves and disinfectant delivery modules were designed by Planmeca and manufactured. The head and base sections of the new valves are threaded and are screwed together with an O ring in between to give a tight seal without the requirement for glue (Fig. 3.5, panel d). In the newly designed disinfectant delivery modules, the aluminium components of the head and collar of the original modules have been replaced with acid-resistant steel and hard plastic (Figs. 3.6, panels a and b).

For the 10 DCUs used in this study replacement of the disinfectant intake valves and disinfectant delivery modules by the newly designed valves and disinfectant delivery modules eliminated further instances of DUWL disinfectant failure due to blocked disinfectant intake valves. During the summer of 2004, all DCUs in the DDSH had their WCS™ valve units replaced with the new-design valve units. No further instances of WCS™ valve leakage or blockage have been experienced since.

3.3.3. Decline of Efficacy of Once-Weekly Waterline Disinfection with Planosil

The second phase of the study was undertaken with the same 10 DCUs during the 12 month period April 2004–March 2005. During this period, between April-September 2004, the microbiological quality of DUWL output water from 4/10 (40%) DCUs consistently failed (i.e. 22 consecutive once-weekly disinfection cycles) the ADA standard of ≤200 cfu/ml of aerobic heterotrophic bacteria by day seven post-disinfection (Figs. 3.8, panels a and b). In all of these cases, no detectable biofilm was evident in DUWLs by electron microscopy immediately following disinfection and the bacterial density in DUWL output water was well below the ADA standard immediately following disinfection but rapidly deteriorated, and in most cases exceeded the ADA standard by day four post-disinfection (Figs. 3.8, panels a and b). This type of

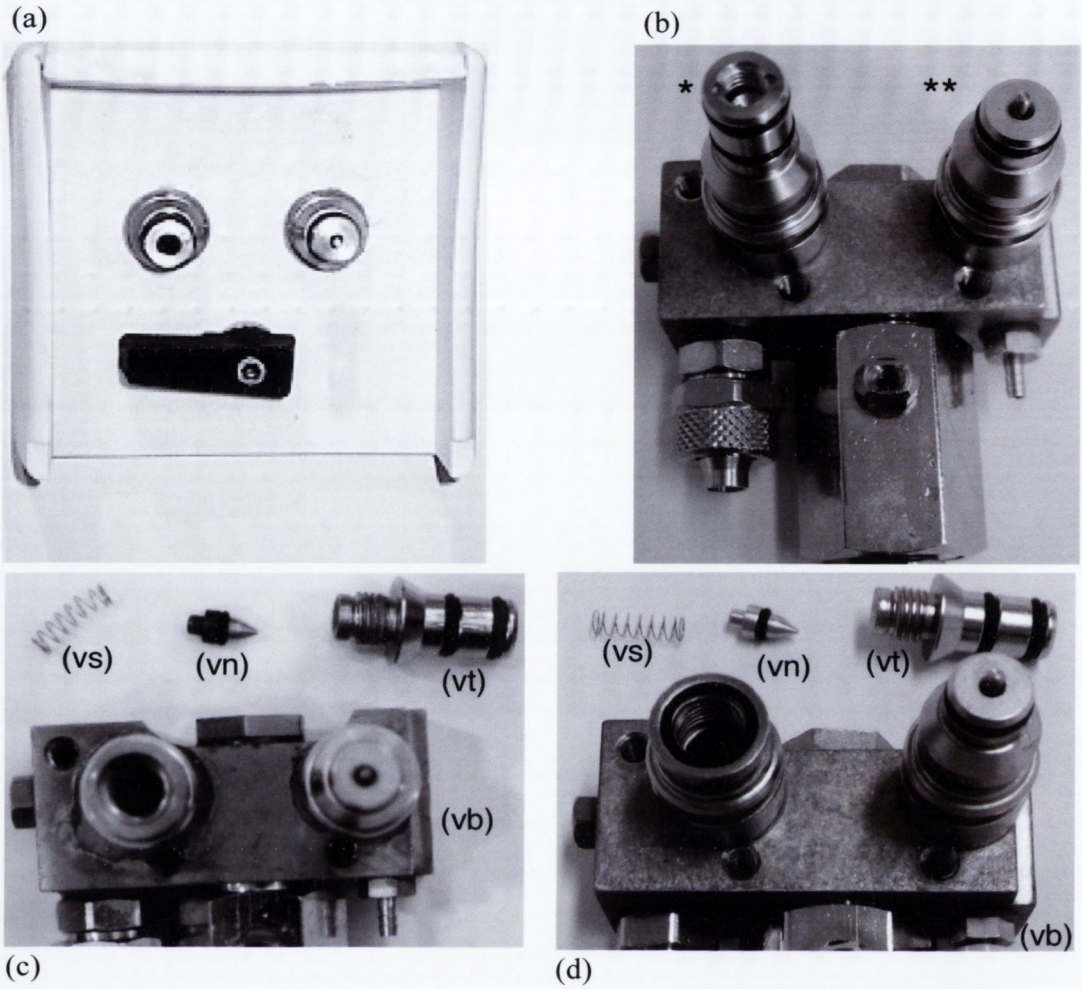


Fig. 3.5. Photographs of the disinfectant intake valve and the air valve of a WCS™ disinfectant module receiver on a Planmeca Prostyle Compact DCU used in this study. Panel (a) shows a receiver module. The disinfectant intake valve is located in the upper left and the air valve in the upper right. The switch shown in the lower portion of the photograph is used to shut off the mains water supply to the DCU during waterline disinfection. Panel (b) shows an example of an isolated WCS™ disinfectant intake valve (*) and air valve (**) unit. Panel (c) shows an example of a WCS™ valve unit with the disinfectant intake valve disassembled. In the WCS™ units fitted to the DCUs used in present study the top section of the valve (vt) was glued to the valve base (vb) section with the valve needle (vn) and spring (vs) housed within the lumen of the assembled valve. Following six months of once weekly disinfection with Planosil, the disinfectant intake valves of 8/10 of the DCUs tested during this study became blocked and/or leaked preventing uptake of disinfectant into DUWLs during the disinfection process. Panel (d) shows a new design valve unit with the disinfectant intake valve disassembled. In the new valve units the top section of the disinfectant intake valve (vt) screws into the valve base (vb) section, both of which are threaded for this purpose, with an O ring in between both components to ensure a watertight seal. As with the old valves, the valve needle (vn) and spring (vs) are housed within the lumen of the assembled valve. Replacement of the valve units in the DCUs with the new design valve units and the use of new design disinfectant delivery modules (Fig. 3.6) completely eliminated waterline disinfection failure due to blocked and/or leaky disinfectant intake valves.

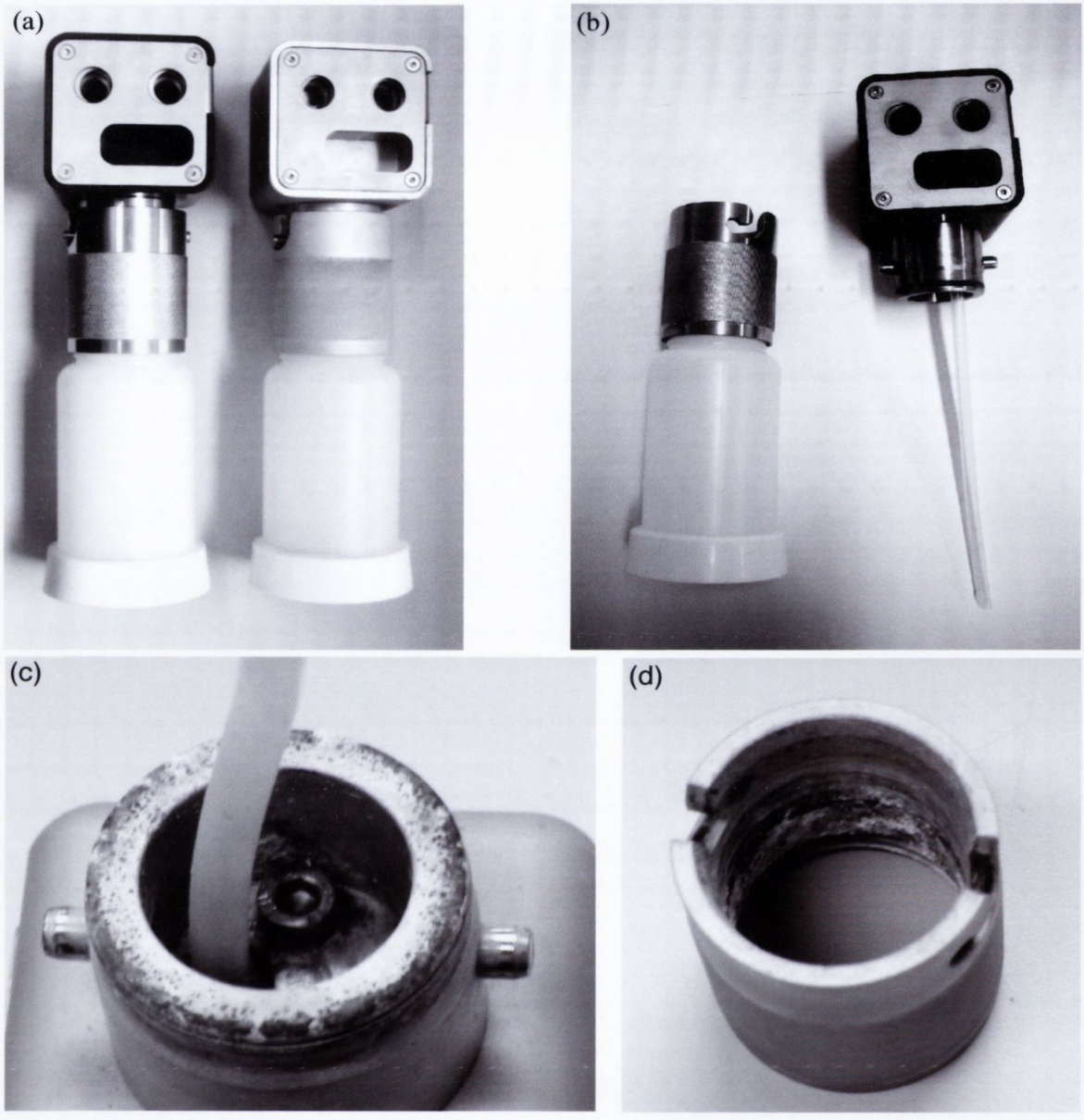
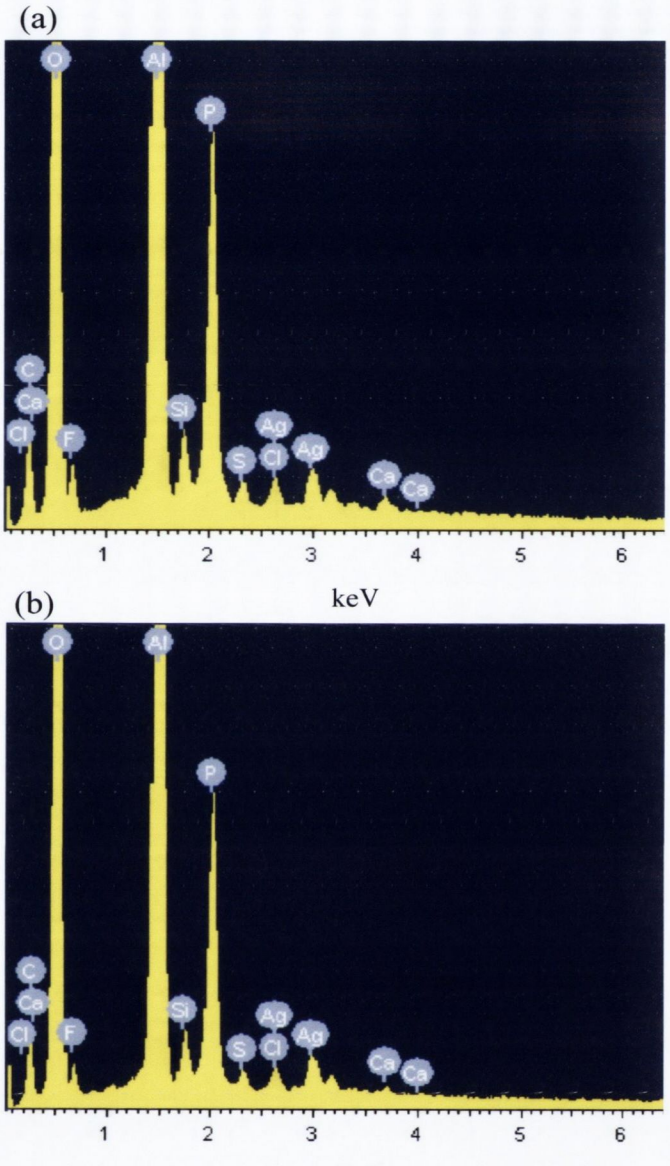


Fig. 3.6. Disinfectant delivery bottle modules. Panel (a) shows an original design (right of figure) and new design (left of figure) disinfectant delivery module used in the present study. The head of the modules are designed to interface with the WCS™ receiver (Fig. 3.5 panel (a)) units fitted on DCUs. Both modules function in the same way, have the same overall dimensions and each consist of three components including an identical polyethylene bottle container (lower portion of each module), a central collar unit into which the bottles are screwed and an upper head interfacing unit. Both the collar and head units of the old type modules were manufactured from aluminium and were prone to corrosion by hydrogen peroxide contained in the Planosil disinfectant used in this study. Panel (b) shows an example of the new type module with the head unit disassembled from the remainder of the module. In these new modules the collar unit and neck of the head unit are manufactured from high-quality, acid-resistant steel that is resistant to corrosion by hydrogen peroxide. The head unit is manufactured from hard plastic. Panels (c) and (d) show Planosil-induced corrosion on the aluminium neck of the head unit and the internal surface of an aluminium collar unit, respectively, from an old-design disinfectant delivery module.



Elemental spectrum	C	O	F	Al	Si	P	S	Cl	Ca	Mn	Fe	Ag	Total (w/w)
(a)	11.93	53.31	5.53	20.84	0.79	5.08	0.34	0.40	0.19	-0.02	0.15	1.45	100.00
(b)	11.20	54.49	3.88	21.63	0.82	5.29	0.36	0.42	0.12	-0.03	0.08	1.75	100.00
Standard deviation	0.61	0.92	0.87	0.51	0.07	0.19	0.04	0.06	0.05	0.07	0.05	0.16	

Fig. 3.7. Energy Dispersive X-ray (EDX) analysis of corrosion deposits. Panel (a) taken from deposits of the neck region and panel (b) collar region (see Fig. 3.6 panels (c) and (d)) of a disinfectant bottle module using to deliver Planosil to DCUs in the present study. The principal components of the deposits are aluminium oxides.

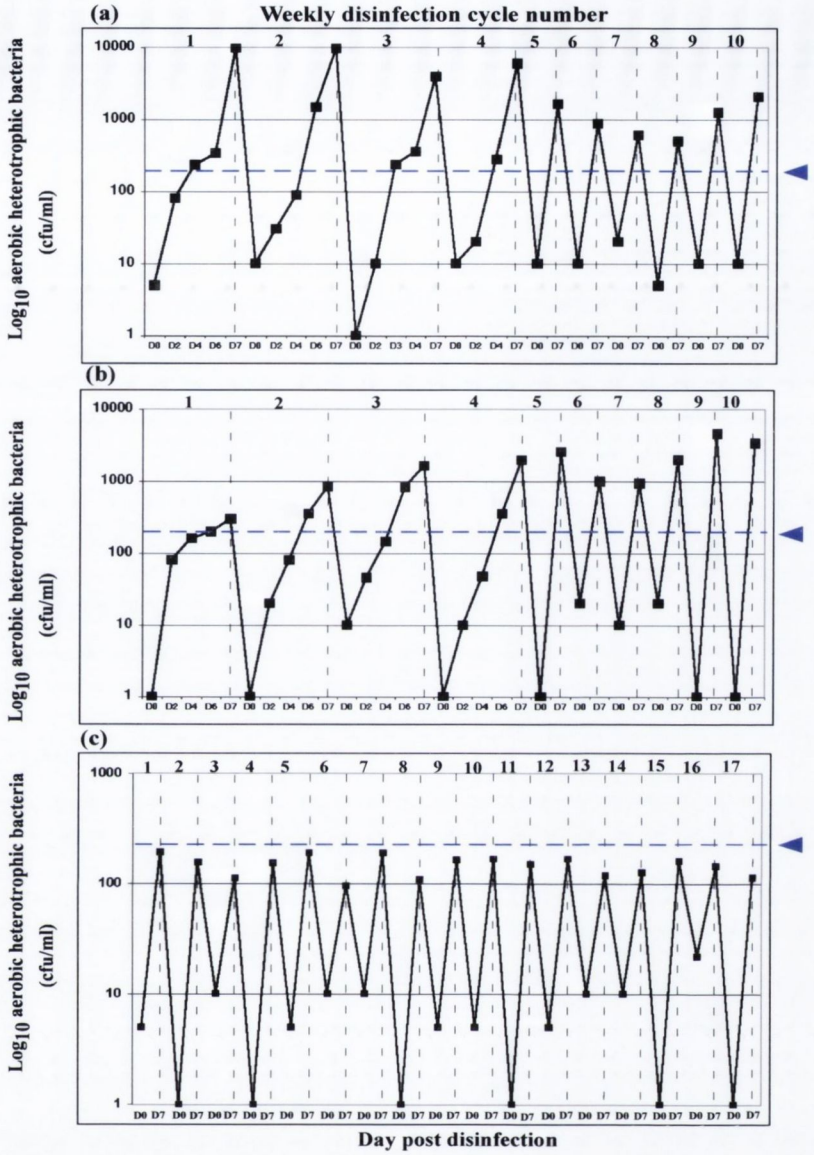


Fig. 3.8. Density of aerobic heterotrophic bacteria in three-in-one air/water syringe DUWL output water from DCUs fitted with the WCS™ during consecutive cycles of once weekly disinfection with Planosil during the second study period (April 2004-March 2005). The blue horizontal dashed lines indicate the maximum ADA limit of 200 cfu/ml of aerobic heterotrophic bacteria. Panels (a) and (b) show bacterial density levels in DUWL output water from two separate DCUs (May-July 2004) that failed to maintain the ADA standard for up to a week post disinfection. In both cases, bacterial density levels exceeded the ADA maximum limit between days 4-6 post disinfection. The predominant bacterial species present in DUWL output water from these two DCUs were strongly catalase-positive *N. subarctica* strains and strains of several *Sphingomonas* species. Panel (c) shows the bacterial density levels in DUWL output water from a DCU (the same DCU used to obtain data shown in panel (a) above) disinfected once weekly for 17 consecutive disinfection cycles (September 2004-January 2005) with Planosil Forte. For each cycle, bacterial levels remained below the ADA maximum limit up to seven days post disinfection.

contamination event was associated with the presence of patchy biofilm on the internal surfaces of DUWLs as detected by electron microscopy. During this period the average density of aerobic heterotrophic bacteria in the processed DCU supply water was 70 cfu/ml (range 46-118 cfu/ml). Careful examination of the DCUs showed that their WCS™ units were all functioning correctly and the rapid deterioration in DUWL output water quality observed in these DCUs following disinfection could not be attributed to any of the factors that were responsible for intermittent failure of waterline disinfection in the first part of the study described above.

The diversity and distribution of aerobic heterotrophic bacterial species in DUWL output water from these DCUs was investigated in order to determine whether the relative abundance of individual species types could be associated with the rapid decline in DUWL output water quality following disinfection. Bacterial isolates recovered from DCU supply water and from DUWL output water samples from the first phase (July 2003-March 2004) and second phase (April 2004-March 2005) of this study were included for comparison. Approximately 80% of bacterial isolates recovered on R2A agar plates were Gram-negative. Representative isolates of each colony type recovered on R2A agar were identified to the species level based on the DNA sequence of a variable region of the 16S rDNA gene. The most common bacterial isolates recovered from the processed mains water supply to the DCUs during the entire study period were Gram-positive, catalase-positive organisms that were identified as *Micrococcus luteus* (Table 3.1). These organisms were also identified in output water from the 10 DCUs used in the study following disinfection with Planosil. There was no obvious difference in the distribution of bacterial species in DUWL output water during the first and second study periods. However, one group of organisms was significantly more prevalent in DUWL output water from the four DCUs that exhibited rapid decline in output water quality following disinfection during the second study period between April-September 2004. These were Gram-negative, catalase-positive organisms identified as *Novosphingobium subarctica*, *Sphingomonas paucimobilis* or other *Sphingomonas* spp., (mean average prevalence of 37.1% of all colonies recovered on R2A agar, range between 20-50%). In contrast, these organisms were a minority (<1% of all colonies recovered on R2A agar) of the aerobic heterotrophic bacterial populations recovered in DCU supply water throughout the study, in all 10 DCUs during the first study period and from the six DCUs during the second study period that did not exhibit rapid decline in DUWL output water quality following disinfection. The

prevalence of *Novosphingobium* and *Sphingomonas* spp., in the four DCUs that exhibited rapid decline in DUWL output water quality following disinfection during the second phase of the study was significantly different from the corresponding prevalence in the remaining six DCUs and from the prevalence of these organisms in all ten DCUs during the first phase of the study as determined by one-ANOVA testing (d.f. = 9, F = 216.6, $P < 0.0001$). Further analysis with Tukey's *post hoc* test showed a significant difference between each of four DCUs that exhibited rapid decline in water quality and each of the remaining six DCUs ($P < 0.001$). All of the *N. subarctica* and other *Sphingomonas* isolates were very mucoid and found to be very strongly catalase-positive (Table 3.1) and this latter feature may have been a significant contributory factor in the rapid decline of DUWL output water quality following disinfection with Planosil due to its reliance on hydrogen peroxide as one of the main active ingredients of this disinfectant.

3.3.4. Replacement of Planosil with Planosil Forte

In order to develop an effective practical solution to the problem of rapid decline in DUWL output water quality following once-weekly disinfection with Planosil (1.5% (v/v) hydrogen peroxide, 0.003% (w/v) silver, 0.0015% (v/v) ortho-phosphoric acid), the four DCUs that exhibited this phenomenon during the study period April-September 2004 and two that did not were subject to 17 separate once-weekly DUWL disinfection cycles with Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver, 0.0025% (v/v) phosphoric acid) during October 2004-March 2005. DUWL output water quality was again monitored by determining the density of aerobic heterotrophic bacteria per ml of water following culture on R2A agar medium. For all six DCUs, the bacterial density in DUWL output water consistently remained below the ADA standard of $\leq$200 cfu/ml for up to a week post-disinfection. Representative data from one DCU is shown in Fig. 3.8, panel c; this DCU was one of the four DCUs that exhibited rapid decline in DUWL output water quality with Planosil during the period April-September 2004. Electron microscopy of the internal surfaces of samples of DUWLs demonstrated the absence of detectable biofilm immediately post-disinfection with Planosil Forte, although patches of biofilm were detectable by electron microscopy at seven days post-disinfection. Colonies of *N. subarctica* and other *Sphingomonas* spp., continued to be isolated from DUWL output water from the DCUs disinfected with Planosil Forte and from the DCU supply water. In the majority of cases these species formed a minority

Table 3.1. Bacterial species isolated from the air/water syringe waterline of the dental chair units used in this study

Bacterial species*	Gram-stain	Catalase activity**
Novosphingobium subarctica	Negative	+++++
Sphingomonas paucimobilis	Negative	+++++
Sphingomonas aerolata	Negative	+++++
Sphingomonas trueperi	Negative	+++++
Acidovorax temperans	Negative	-
Porphyrobacter tepidarius	Negative	-
Pseudomonas fluorescens	Negative	+++
Arthrobacter agilis	Positive	++++
Variovorax paradoxus	Positive	++++
Rhodococcus fascians	Positive	+++
Kocuria palustris	Positive	+++
Micrococcus luteus	Positive	+++

*Identified by DNA sequence analysis of the variable region of 16S rDNA gene.

**An arbitrary scale for catalase activity was used based on the visual observance of the evolution of oxygen bubbles from a bacterial colony following the addition of 50 µl of 5% (v/v) H_2O_2. +++++ = very strongly catalase-positive with instantaneous and substantial evolution of gas bubbles; +++ = catalase-positive with immediate but moderate evolution of gas bubbles; - = catalase-negative. For each isolate tested, at least 5 separate colonies grown on R2A agar medium were tested for catalase production.

population (<1% of all colonies recovered on R2A agar). These findings demonstrated that replacement of Planosil with Planosil Forte was an effective practical solution to the problem of rapid decline of DUWL output water quality following once-weekly disinfection with Planosil.

From October 2005, Planosil Forte was introduced as the standard DUWL disinfectant throughout the DDSH. Between October 2005 and June 2006, monthly sampling of DUWL output water from six DCUs, two selected from each of the hospital's three main treatment clinics, showed the continued efficacy of Planosil Forte at maintaining the quality of DUWL output water below the ADA standard, up to seven days post-disinfection.

3.4. Discussion

During the last decade many published studies from around the world have highlighted that high densities of microbes, principally bacterial species, including several known human pathogens and opportunistic pathogens such as *L. pneumophilia* and *P. aeruginosa*, respectively, frequently contaminate output water supplied by DUWLs. (Abel *et al.*, 1971; Scheid *et al.*, 1982; Martin, 1985; Williams *et al.*, 1993; Atlas *et al.*, 1995; Barbeau *et al.*, 1996; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002; Pankhurst *et al.*, 2005). This phenomenon is a universal problem in DCUs and is caused by the development and proliferation of biofilm in narrow-bore DUWLs. Many health-care authorities and dental professional organisations have affirmed that it is unacceptable to expose patients (especially immunocompromised or medically compromised patients), dental and support staff and maintenance personnel to contaminated DUWL output water or aerosols generated during use of dental instruments connected to DUWLs (Kohn *et al.*, 2003; O'Donnell *et al.*, 2006b). In recent years, the focus on contaminated DUWL output water has centered on the development of effective practical solutions for implementation in dental clinics. At present a diverse range of commercial DUWL treatment products and systems are marketed to control or eradicate DUWL biofilm (Table 1.3). However, very few have been tested extensively in DCUs in long-term studies (O'Donnell, *et al.*, 2006b). Because modern DCUs are classified as medical devices, manufacturers have an essential role to play in developing new types of DCUs with integrated, robust, easy to use and effective DUWL cleaning systems. In the author's view, all DCUs should be equipped with DUWL cleaning systems as standard and not as an optional extra. In 2002 a study from this laboratory described the effectiveness of the Planmeca WCS™, a DUWL cleaning system developed by the Finnish DCU manufacturer Planmeca Oy, to eradicate and control DUWL biofilm in two separate Planmeca Prostyle Compact DCUs over a 20 week period using the hydrogen peroxide and silver ion-containing disinfectant Sanosil (Tuttlebee *et al.*, 2002). The WCS™ was found to be very effective at eradicating DUWL biofilm in these DCUs and consistently provided output water with bacterial densities below the ADA recommended threshold of ≤200 cfu/ml for up to seven days post-disinfection.

In the present study, the ability of the WCS™ to maintain the microbiological quality of DUWL output water below the ADA standard was investigated over a much longer period (21-months) with a much larger number of DCUs (10 DCUs) using the

hydrogen peroxide and silver ion-containing DUWL disinfectants Planosil (identical formulation to Sanosil) and Planosil Forte. Dental nursing staff disinfected the DUWLs in six of the DCUs, whereas DUWLs in the remaining four DCUs were disinfected by the author or Professor D. Coleman. This approach was adopted specifically to investigate the efficacy of the WCS^{TM} in busy working dental clinics. Apart from one other study from this laboratory with a DCU equipped with a different DUWL disinfection system to that used here, (see Chapter Four), the present study is the only prospective study on the long-term efficacy of DUWL disinfection yet to be reported. During this period the WCS™ performed well in the majority of cases providing DUWL output water in compliance with the ADA standard for up to seven days post-disinfection. However, two distinct kinds of anomalous DUWL post-disinfection outcomes were observed during the course of the study, including intermittent failure of DUWL disinfection that occurred in the first period of the study (July 2003-March 2004) and secondly, rapid decline in the microbiological quality of DUWL output water following disinfection that occurred during the second period of the study (between April-September 2004). In the first nine-month period of the study (July 2003-March 2004) the microbiological quality of DUWL output water remained below the ADA standard for up to a week following DUWL disinfection with Planosil in 272/300 (90.7%) of the disinfection cycles investigated (Fig. 3.3, panel a). During this period the average density of aerobic heterotrophic bacteria in the processed DCU supply water was 66 cfu/ml (range 60-112 cfu/ml). However, 28/300 (9.3%) of disinfection cycles yielded DUWL output water that failed the ADA standard by day seven post-disinfection (Fig. 3.3). These results contrasted with our previous, shorter-term study where WCS™ units retrofitted to two identical DCUs as used in the present study, maintained the quality of DUWL output water below the ADA standard for up to seven days post-disinfection for all 20 of the once-weekly disinfection cycles investigated using a DUWL disinfectant with an identical formulation to Planosil. In the present study, a DUWL disinfection failure rate of slightly less than 10% for the 300 disinfection cycles investigated was deemed unacceptable and investigations were undertaken to identify and rectify the causes.

In 4/28 (14.3%) of the failed disinfection cycles the air supply to the dental handpieces and the WCS™ disinfectant container receiver module (Fig. 3.1, panel b) had been inadvertently reduced resulting in insufficient pressure (<1.5 bar) being generated in the disinfectant container module to open the disinfectant intake valve

during the disinfection process. The nursing staff subsequently reported that the WCS™ units on the DCUs were faulty and maintenance staff corrected the air pressure the following morning. Not all 103 DCUs in the DDSH have their DUWLs disinfected on the same day for logistical reasons. Clusters of DCUs have their DUWLs disinfected once-weekly on specific days (Tuesday to Friday) to facilitate the clinical commitments of nursing staff. Because of the low air pressure problem, the DUWLs in the two DCUs were not disinfected until the next disinfection cycle seven days later and when water samples were taken immediately prior to DUWL disinfection, the bacterial density in DUWL output water reflected a 14 day period without disinfection and thus failed the ADA standard. Clinical staff and students were instructed not to adjust the air pressure setting on any of the DCUs and no further instances of DUWL disinfection failure due to low air pressure were encountered during the study period.

In 10/28 (35.7%) of the failed disinfection cycles involving six DCUs, failure was due to operator error whereby nursing staff undertaking DUWL disinfection inadvertently neglected to include the assistant's three-in-one air/water syringe in the disinfection process. In the Planmeca Prostyle Compact DCUs retrofitted with WCS™ units used in the present study, disinfection of the DUWLs is microprocessor controlled. During the disinfection process, disinfectant is automatically fed to the DUWLs supplying the instrument hoses and the patient's cup-fill DUWL from the pressurised disinfectant container module. The instrument hoses are first placed in their flushing receivers, the disinfection cycle is activated from the DCU instrument console and each DUWL is filled with disinfectant in a specified order dictated by the microprocessor. However, if either three-in-one air/water syringe line is not in its flushing receiver, but all of the other lines are, the disinfection process can still be activated from the instrument console. If a three-in-one air/water syringe DUWL is not disinfected it will continue to contaminate output water from this DUWL. Furthermore, because all DUWLs in DCUs are interconnected, this DUWL can rapidly contaminate the other DUWLs, as many of the bacterial species present in DUWL biofilm are motile. It was fortunate in the present study that flushing of disinfectant from DUWLs following overnight (15 h) disinfection in the 10 DCUs included in the study, and the collection of DUWL output water samples for microbiological analysis were undertaken by either the author or Professor D. Coleman. Because of this it was possible to observe and record that the assistant's three-in-one air/water syringe DUWL had not been included in the disinfection cycle (i.e. the assistant's three-in-one air/water syringe hose was still

attached to the assistant's console (Fig. 3.1, panel a) during 10 disinfection cycles involving six DCUs. In all 10 of these failed disinfection cycles, DUWL output water from the assistant's three-in-one air/water syringe failed the ADA standard, either immediately following the DCU disinfection process or within 24-48 h (representative data from one DCU is shown in Fig. 3.3, panel c). In contrast, DUWL output water from the operator's three-in-one air/water syringe DUWL from the six DCUs during the 10 failed disinfection cycles was well below the ADA standard immediately post-disinfection, but quality rapidly deteriorated and failed the ADA standard within three days post-disinfection (data not shown). Contamination from the assistant's three-in-one air/water syringe DUWL was probably the cause of this rapid deterioration of output water from the operator's three-in-one air/water syringe DUWL as suggested above. To prevent further occurrences of this type of intermittent DUWL disinfection failure, clinic managers were requested to ensure that nursing staff undertaking DUWL disinfection were vigilant not to neglect to disinfect the assistant's three-in-one air/water syringe DUWL. This type of problem could be eliminated completely by incorporating error detection systems into DCUs, which detect failure to position hoses for decontamination and display an alarm code on the DCU instrument console.

In 14/28 (50%) of the failed disinfection cycles involving eight DCUs, all of which occurred following six months of continuous once-weekly disinfection with Planosil, failure was directly associated with blockage of and/or leakage from WCS™ disinfectant intake valves, and corrosion of components of disinfectant delivery module bottles. Detailed investigations revealed that the disinfectant intake valves of the WCS™ disinfectant container receiver units on the DCUs had a previously unidentified defect in that the upper and lower valve sections were joined with glue (Figs. 3.5, panels b and c). Following six months of regular once-weekly disinfection with Planosil the valves began to block and/or leak. It is likely that the strongly oxidising capacity of hydrogen peroxide in the disinfectant was responsible for damaging the integrity of the glue sealing both sections of the valves. This would give rise to leakage of disinfectant from the junction of the two valve sections and probably also caused sections of glue to become dislodged into the valve lumen. Indeed, examination of blocked valves revealed the presence of glue in the valve lumen. These problems with the valves were exacerbated by corrosion of aluminium components of the disinfectant delivery container modules, again most likely caused by oxidisation of aluminium by hydrogen peroxide present in the Planosil disinfectant. Areas of corrosion were often

characterised by the presence of loose, flaky deposits on the metal surfaces involved. These deposits very likely contributed to blockage of disinfectant intake valves. Valve blockage prevented disinfectant being fed into DUWLs during the disinfection process resulting in DUWL disinfection failure. Likewise, leakage of disinfectant from the intake valve would prevent sufficient disinfectant being fed to the DUWLs to ensure adequate disinfection. This would particularly affect the assistant's three-in-one air/water syringe DUWL, as it is the last DUWL to be filled with disinfectant during the disinfection process. The design of new valves, both sections of which are machined to screw together with an O ring in-between (Fig. 3.5, panel d), and the design of new disinfectant container modules, with acid-resistant steel instead of aluminium (Fig. 3.6, panels a and b), completely solved the problem of valve blockage and leakage and eliminated this particular cause of intermittent DUWL disinfection failure. This type of problem very likely occurs with many other types of medical devices apart from DCUs, as decontamination chemicals and methods change and points to the necessity for long-term compatibility testing and communication between manufacturers and end users.

During the second phase of the April 2004–March 2005 study, between April-September 2004, the microbiological quality of DUWL output water from 4/10 (40%) DCUs consistently failed the ADA standard by day seven post-disinfection (Figs. 3.8, panels a and b). In contrast to the cases of intermittent DUWL disinfection failure recorded between July 2003-March 2004 described above, in these cases the bacterial density in DUWL output water was well below the ADA standard immediately following disinfection but rapidly and consistently deteriorated, and in most cases exceeded the ADA standard by day four post-disinfection (see Figs. 3.8, panels a and b). This correlated with the presence of patchy biofilm on the internal surfaces of DUWLs as detected by electron microscopy. During this period the average density of aerobic heterotrophic bacteria in the DCU supply water was 70 cfu/ml (range 46-118 cfu/ml). Examination of the four DCUs concerned revealed that the rapid deterioration in DUWL output water quality observed in these DCUs post-disinfection could not be attributed to any of the factors responsible for intermittent failure of waterline disinfection detected in the first phase of this study (see above). However, detailed analysis of the DUWL output water from these four DCUs showed that one group of organisms (*N. subarctica*, *S. paucimobilis* or other *Sphingomonas* spp.; Table 3.1) was significantly more prevalent ($p < 0.0001$) compared with output water from the other six DCUs or the DCU supply water. *Novosphingobium subarctica*, *Sphingomonas*

paucimobilis or other *Sphingomonas* spp., accounted for between 20-50% of all bacterial isolates recovered from DUWL output water from these four DCUs, but were a minority population (<1% of all bacterial colonies recovered on R2A agar) in all other DUWL output water samples throughout the study. Isolates of these species were found to be very strongly catalase-positive and thus had the ability to break down hydrogen peroxide. It is very likely that this latter feature was a significant factor in the rapid deterioration of DUWL output water quality in the four DCUs concerned. Furthermore, a more recent study from this laboratory investigating a more advanced integrated DUWL cleaning system fitted to a single Planmeca Compact i DCU, also demonstrated rapid deterioration of DUWL output water quality by day seven post-disinfection with Planosil (see Chapter four). In 9/10 consecutive once-weekly disinfection cycles with this DCU, output water quality failed the ADA standard by day seven post-disinfection. The most prevalent bacterial species recovered from output water were also *N. subarctica*, *S. paucimobilis* or other *Sphingomonas* spp., *Novosphingobium* and *Sphingomonas* spp., have previously been isolated from DUWLs and they produce copious amounts of viscous exopolysaccharides that enable the organisms to adhere to surfaces and give rise to dense protective biofilms (Singh *et al.*, 2003; O'Donnell *et al.*, 2006b; Szymanska, 2006a). Some of these organisms have been responsible for colonisation of mechanical ventilators and other medical equipment in hospitals and have been responsible for episodes of nosocomial infection (Lemaitre *et al.*, 1996; Hsueh *et al*, 1998; Perola *et al.*, 2002; Gomila *et al.*, 2005; Ensminger *et al.*, 2006).

The use of hydrogen peroxide-containing disinfectants to control DUWL biofilm over extended periods has the potential for the development of biocide resistance or tolerance within DUWL biofilm by adaptation and/or selection following prolonged use. Bacteria within biofilms are notoriously more difficult to eradicate with antimicrobials or biocides than planktonic organisms (Elkins *et al.*, 1999; Costerton *et al.*, 1987; Campanac *et al.*, 2002; Grobe *et al.*, 2002; O'Donnell *et al.*, 2005). In DUWL biofilms it is likely that catalase-positive bacteria can be selected by and/or adapt to hydrogen peroxide contained in disinfectants used to eradicate biofilm following prolonged use. Tolerance to hydrogen peroxide exhibited by biofilm bacteria has been reported previously to be accompanied by enhanced catalase activity. A previous study with *P. aeruginosa* biofilm demonstrated that organisms in biofilms were significantly less susceptible to killing by hydrogen peroxide compared to planktonic forms and that significant catalase induction occurred in biofilms following exposure to hydrogen

peroxide (Elkins *et al.*, 1999). A different study reported that for sufficiently thick biofilms, bacterial cells in biofilms implement adaptive responses more effectively than planktonic cells (Szomolay *et al.*, 2005). Another study with *P. aeruginosa* biofilms proposed that new genes are expressed when the bacteria attach to a surface and begin to form biofilm and that some of the proteins expressed by these genes could reduce the susceptibility of the bacteria to oxidative biocides such as hydrogen peroxide (Cochran *et al.*, 2000). Interestingly, a recent study from Poland on DUWL disinfection with Oxygenal 6, a different hydrogen peroxide and silver ion-containing disinfectant to that used in the present study, reported that isolates of *S. paucimobilis* were significantly more prevalent (~prevalence of 80%) in DUWL output water post-disinfection compared to output water pre-disinfection (~prevalence 10%) (Szymanska, 2006a). This finding together with the results of the present study suggest that disinfection of DUWLs with hydrogen peroxide and silver ion-containing disinfectants can select for the maintenance of bacterial species such as *Novosphingobium* or *Sphingomonas* spp. It is interesting to speculate why only 4/10 DCUs used in the present study exhibited rapid deterioration of DUWL output water quality in the second phase of the present study. This may have been associated with a requirement for a critical density of strongly catalase-positive *Novosphingobium* or *Sphingomonas* organisms in DUWLs of individual DCUs as these species were only predominant in DCUs that showed rapid decline in output water quality. It is very likely that all 10 DCUs used in the present study, indeed all 103 DCUs with which the DDSH is equipped, would all eventually have succumbed to the problem of rapid deterioration of DUWL output water quality following prolonged, once-weekly disinfection with Planosil. In order to circumvent this, it was decided to investigate the efficacy of Planosil Forte, an alternative DUWL disinfectant with a stronger concentration of hydrogen peroxide and silver ions.

In the present study, the use of Planosil Forte with its higher concentration of hydrogen peroxide and silver ions compared to Planosil effectively dealt with the problem of rapid deterioration of DUWL output water quality in the four DCUs where this problem was encountered. Similarly, the use of Planosil Forte effectively dealt with the problem of rapid decline of DCU output water quality in another study with the Planmeca Compact i unit referred to above (see Chapter Four). In the present study, it is likely that rapid deterioration of DCU output water was also occurring in some of the remaining 93 DCUs in the DDSH. Following this study, routine DUWL disinfection in the DDSH was undertaken with Planosil Forte. However, it is likely that prolonged use

of Planosil Forte will eventually result in reduced efficacy of this product to control DUWL biofilm by the development of biofilm tolerance. To prevent such difficulties developing in DUWLs disinfected with hydrogen peroxide following protracted use, the periodic use of a different disinfectant with a different mode(s) of action would be prudent. We are currently evaluating the efficacy of a range of alternative DUWL disinfectants for periodic use. Furthermore, we are also developing systems that remove nutrients from DCU supply water, thus making it more difficult for biofilm-forming bacterial species to grow in DUWLs (see Chapter Five).

3.5. Conclusions

The present study is the only prospective study to date to document the long-term efficacy of DUWL disinfection with multiple DCUs. The study identified and appropriately rectified causes of DUWL disinfection failure using the Planmeca WCS™ due to operator error, design flaws and the selection of disinfectant-tolerant bacterial species. All of these factors are likely to adversely affect the long-term efficacy of other DUWL cleaning and disinfection systems. It is important to emphasise that DCU manufacturers have an important role to play in further developing and improving DUWL cleaning systems. Only by undertaking ongoing long-term field studies with DCUs used in clinical practice can problems with DUWL cleaning and disinfection be identified and rectified, thus ensuring good quality DUWL output water for patients and dental healthcare staff.

Chapter 4

A Novel Automated Waterline Cleaning System that Facilitates Effective and Consistent Control of Microbial Biofilm Contamination of Dental Chair Unit Waterlines: A One-Year Study

4.1. Introduction

The modern dental chair unit (DCU) is a complex medical device designed to provide the equipment and services necessary for a wide variety of dental procedures (O'Donnell *et al.*, 2005). Water is used to cool and irrigate a variety of DCU-supplied instruments and tooth surfaces during dental procedures, as the heat generated during operation can be detrimental to teeth (Stanley, 1971; Langeland, 1971; Siegel and von Fraunhofer, 2002). Water is also required for oral rinsing during and following dental treatment and to flush the DCU cuspidor, after the patient has finished rinsing. In order to supply water as a coolant and irrigant to turbine handpieces, ultrasonic scalers, three-in-one air/water syringes, as well as providing water for the patient rinse cup filler and cuspidor, water has to be delivered by an intricate network of interconnected narrow-bore tubes referred to as dental unit waterlines (DUWLs).

As described in detail in Chapter One, many studies have shown that water from DUWLs is often contaminated with high concentrations of bacteria. This is a widespread problem and virtually all DUWLs in standard DCUs are likely to be contaminated (Abel *et al.*, 1971; Scheid *et al.*, 1982; Williams *et al.*, 1993; Atlas *et al.*, 1995; Challacombe and Fernandes, 1995; Barbeau *et al.*, 1996; Meiller *et al.*, 2000, Walker *et al.*, 2000; Tuttlebee *et al.*, 2002). Bacterial contamination of DUWLs is considered to originate in the DCU water supply, which usually contains low levels of microorganisms (Pankhurst and Philpott-Howard, 1993; Tuttlebee *et al.*, 2002). In a typical modern DCU, the network of DUWLs can comprise several meters of plastic tubing with an internal diameter of a few millimeters in which water can stagnate when the equipment is not being used (Pankhurst and Philpott-Howard, 1993; Barbeau *et al.*, 1996). Microorganisms in water supplied to DCUs, mainly aerobic heterotrophic Gram-negative environmental bacteria, attach to the internal surfaces of the DUWLs and form microcolonies that eventually give rise to multispecies biofilm. (Pankhurst *et al.*, 1998; Mills, 2000). DUWL biofilms are composed mainly of highly hydrated bacterial exopolysaccharide that contains both microcolonies and single cells interspersed heterogeneously with channels or pores (Davey and O'Toole, 2000; Watnick and Kolter, 2000). Biofilm forms because the water at the internal surface of the narrow-bore DUWLs flows more slowly than water at the centre and thus there is little disturbance to the microorganisms present (Williams *et al.*, 1993). This allows the microorganisms to proliferate before eventually dispersing through the water supply as

planktonic forms where they may be deposited at other sites within the waterline network or are delivered directly into the mouths of patients during dental procedures. Thus, DUWL biofilm acts as a reservoir for ongoing contamination of DCU output water.

The presence of high concentrations of microorganisms in DCU water is a potential risk of infection for dental patients and staff and is incompatible with good cross-infection control practices (Mayo *et al.*, 1990; Tuttlebee *et al.*, 2002). Previous studies have shown that waterborne bacteria are aerosolised during dental procedures and that dental personnel and patients are exposed to these microorganisms and fragments of biofilm (Fotos *et al.*, 1985; Martin, 1987; Reinthaler *et al.*, 1988; Atlas *et al.*, 1995; Denton *et al.*, 1998). DUWL contamination is of particular concern in the treatment of immunocompromised and medically compromised individuals. It is important to emphasise that only a few cases of infectious disease transmission related to DUWLs and related biofilm have been reported in the literature (Martin, 1987; Atlas *et al.*, 1995). However, the possibility still remains that DUWL-associated infections have gone unrecognised or unreported because of the failure to associate exposure to DUWL output water and aerosols generated from this water with the development of specific infections. Sporadic infections not requiring hospital admission are also less likely to be investigated or notified. However, there is considerable potential for infection with some bacterial pathogens found in dental unit water such as *P. aeruginosa*, *L. pneumophila* and non-*tuberculosis Mycobacterium* spp., (Martin, 1987; Atlas *et al.*, 1995; Schulze-Röbbecke *et al.*, 1995).

Currently there are no microbial quality standards imposed for DCU output water within the EU. However, it is not unreasonable to expect that the quality of DCU output water should range within potable water standards. In 1995, the ADA established a goal for the year 2000 of ≤200 cfu/ml of aerobic heterotrophic bacteria for DCU output water (Anonymous, 1996). However, this goal has not received widespread achievement. The current CDC guidelines for infection control in dental health-care settings recommend that DCU output water should be ≤500 cfu/ml of aerobic heterotrophic bacteria (Kohn *et al.*, 2003).

As reviewed in detail in Chapter One, the most efficient means of maintaining good quality DCU output water is regular disinfection of DUWLs with a disinfectant or biocide that removes biofilm from the waterlines resulting in output water of potable quality (Tuttlebee *et al.*, 2002). A wide variety of commercial waterline cleaning

products and systems are available, some of which can be retrofitted to existing DCUs (Meiller *et al.*, 2000; Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; Martin and Gallagher, 2005). However, very few studies published in the peer-reviewed literature have actually investigated the efficacy of disinfectants to achieve these desired effects in DCUs and detailed comparative studies have yet to be published (Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; Spratt *et al.*, 2004). Furthermore, there is an onus on DCU manufacturers to consider the problem of DUWL biofilm contamination when designing DCUs (Tuttlebee *et al.*, 2002; Montebugnoli *et al.*, 2004; Coleman *et al.*, 2007). In 2002 we reported on the effectiveness of the Planmeca WCS™ DUWL cleaning system retrofitted to several Planmeca Prostyle Compact DCUs using once-weekly DUWL disinfection with the hydrogen peroxide and silver ion-containing disinfectant Sanosil (Tuttlebee *et al.*, 2002) and showed that the system consistently maintained DUWL output water quality below the ADA standard of ≤200 cfu/ml for up to a week post-disinfection. However, as described in Chapter Three, further long-term studies identified shortcomings with the system that resulted in episodes of DUWL disinfection failure or rapid deterioration of DUWL output quality water following disinfection. These failings were due to a combination of operator error, equipment component design flaws and to the selection of disinfectant tolerant bacterial species following prolonged DUWL disinfectant use. All of the factors that contributed to DUWL disinfection failure were resolved in collaboration with the DCU manufacturer Planmeca. Following on from the success of this collaboration, Planmeca developed a more advanced and fully automated DUWL disinfection system called the Water Management System (WMS™) that was integrated into novel DCU models at the time of manufacture. The purpose of this part of the present study was to investigate the effectiveness of the WMS™ at maintaining DUWL output water quality using once-weekly disinfection in a long-term study.

4.1.1. Aims

- To investigate the effectiveness of the Planmeca WMS™ integrated at manufacture in a new Planmeca Compact i DCU at maintaining DUWL output quality water below the ADA standard of ≤200 cfu/ml for up to one week post-disinfection with the DUWL disinfectants Planosil and Planosil Forte in a long-term (21-month) study.

- To identify human and DCU design factors that contribute to DUWL disinfection failure during the study period, if any, and to rectify them.

- To determine whether *Novosphingobioum*, *Sphingomonas* or other bacterial species are selected in DUWLs following prolonged use of Planosil and Planosil Forte with the Planmeca WMS™ in long-term (21-month) study and whether this can result in rapid deterioration of DUWL output quality post-disinfection.

4.2. Materials and methods

4.2.1. Dental Chair Unit

A new Planmeca Compact i DCU (Planmeca Oy, Helsinki, Finland) equipped with the novel integrated Planmeca WMS^{TM} was used in the present study (Fig. 4.1, panel a). The DCU was installed in the Oral Surgery Clinic in the DDSH in December 2003. From February 2004 to January 2005 the efficacy of the WMS^{TM} to maintain good microbial quality of DUWL output water was investigated while the DCU was in daily use for dental patient treatments. To facilitate use during clinical sessions, the DCU was disinfected once a week, on the same day each week, following the afternoon clinical session. The Planmeca WMS^{TM} can be operated in three separate modes: (A) mains water supply mode, (B) bottle water supply mode and (C) waterline cleaning mode. The WMS^{TM} contains a physical air gap designed to comply with the requirements of European Standard EN1717 for dental unit installations (Anonymous, 2001). This integrated back-flow prevention device consists of a vertical air gap of 20 mm and prevents contamination of the mains water supply with water from the DCU waterlines (Fig. 4.2).

In the mains water supply mode, the DCU is connected to the mains water supply line. Mains water is conveyed across the vertical air gap under gravity to an atmospheric reservoir from where it is pumped to a pressurised bottle container (Figs. 4.1, panel b and Fig. 4.2). Water is then fed from the pressurised bottle *via* DUWLs to the dental instruments (ultrasonic scaler, three-in-one air/water syringe, low-speed handpiece and high-speed turbine handpiece) (Fig. 4.1, panel c and Fig. 4.2) and to the patient cup-fill line (Figs 4.1, panel d and Fig. 4.2) by using constant pressure of 2.8 bar. The bottle container has two sensors (upper and lower) that monitor the water level. When the water level goes below the upper sensor, a pump engages and fills the container automatically (Fig. 4.2).

In clean water mode, the DCU uses clean water from the bottle container that is filled manually. The user initially removes the bottle container from the DCU by activating a container control switch to depressurise the container and then fills it with potable or sterile water. The filled container is then replaced back in the DCU and pressurised by activating the container control switch. The pressurised container supplies clean water to the instruments and to the cup-fill line. The lower sensor of the bottle container recognises when the water level in the container reaches a minimum

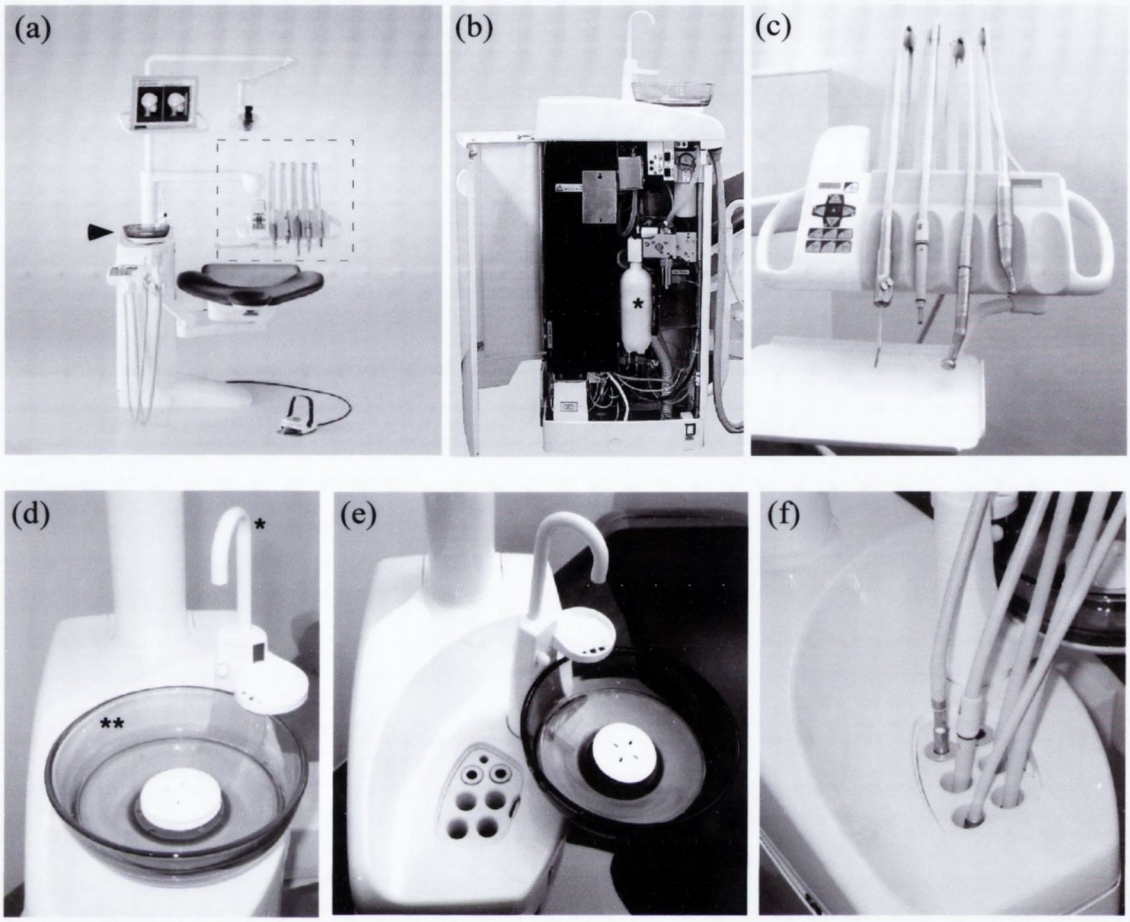


Fig. 4.1. Components of the Planmeca Compact i DCU equipped with the integrated Planmeca WMS™ that was used in the present study. (a) Overview of the Compact i DCU. The instrument console is highlighted with dashed lines and an arrowhead indicates the location of the cuspidor. (b) Side view of the Compact i DCU with the pedestal door open. The pressurised bottle container is marked with an asterisk. (c) Close up view of the DCU instrument console. Four instrument hoses containing DUWLs with instruments attached (from left to right: air/water syringe, ultrasonic scaler, high-speed handpiece, low-speed handpiece) are shown in the upper right section of the panel. A keypad and visual display unit to activate various DCU functions are shown to the left of the instrument hoses. (d) View of the DCU cuspidor (**) and cup-fill water outlet (*). (e) View of the instrument hose receivers used during waterline disinfection. The receivers are located beneath the cuspidor, the latter of which is swiveled sideways from its normal position when the receivers are in use. (f) View of the DCU instrument hoses *in situ* in the flushing receivers during a disinfection cycle.

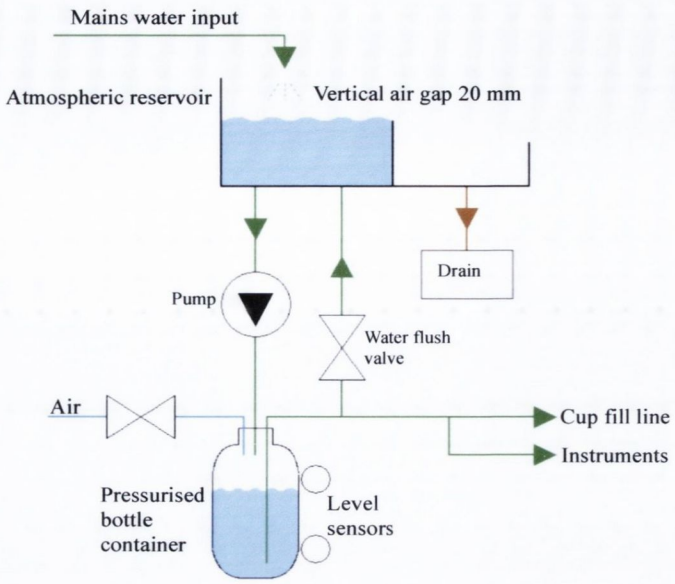


Fig. 4.2. A schematic representation of the Planmeca WMS™. This system can be operated in three separate modes. In the mains water supply mode, mains water is delivered to an atmospheric reservoir under gravity across a 20 mm vertical gap. The air gap functions as a back-flow prevention device that prevents contamination of the mains water supply with water from the DCU. Water is pumped from the reservoir to the pressurised bottle container that supplies water to the DUWLs. The bottle container has two sensors (upper and lower) that monitor the water level. When the water level goes below the upper sensor, the pump fills the bottle container automatically from the water stored in the atmospheric reservoir. In clean water mode, the DCU uses clean water from the bottle container. The lower sensor of the bottle recognises when the water level reaches a minimum level and the unit sends an electronic signal to a visual display on the DCU instrument console that the container needs to be refilled. In the waterline-cleaning mode, the instrument hose lines are first placed into the integrated instrument-flushing receivers (Fig. 4.1, panels (d)-1(f)). The cleaning cycle is started by pressing an activation key on an integrated keyboard on the DCU instrument console (Fig. 4.1 panel (c)), the bottle is filled with disinfectant solution and replaced in the DCU. Disinfectant is then automatically fed from the bottle container to the instrument DUWLs and cup-fill DUWL. This is followed by automatic opening of a water flush valve, starting of the pump and pumping of the disinfectant to the atmospheric reservoir. When the lower sensor reads that the bottle container is empty of disinfectant, the pump stops, the water flush valve closes and the DCU visual display shows a code indicating that the feeding cycle is completed. The electrical supply to the DCU is then switched off for a set time period (15 h in the present study) to allow the disinfectant to take effect. When the DCU is turned on again an automatic flushing cycle begins. Initially, disinfectant in the atmospheric reservoir is voided to a drain; mains water fills the reservoir and is pumped to the bottle container and back to a drain outlet in the atmospheric reservoir container. This cycle is repeated three times automatically to purge disinfectant from the bottle container, pump and atmospheric reservoir. Finally mains water is pumped from the atmospheric reservoir to the bottle container, and from there is used to flush the instrument waterlines and cup fill line for a duration of 4 minutes. After this automatic flushing and rinsing cycle the DCU is ready for normal use.

level and the unit sends an electronic signal to the user by means of a visual display on the DCU instrument console that the container has to be refilled (see Figs. 4.1 and 4.2).

In the waterline-cleaning mode, the instrument hoses containing the DUWLs are first placed into the integrated instrument hose flushing receivers situated under the cuspidor (Figs. 4.1, panels d–f). For this purpose, the cuspidor can be swivelled to one side revealing the instrument hose receivers (Fig. 4.1, panel e). The cleaning cycle is started by pressing an activation key on an integrated keyboard on the DCU instrument console (Fig. 4.1, panel c). Then the bottle container is depressurised, removed and filled with disinfectant solution and the bottle replaced in the DCU. Disinfectant is then automatically fed from the container to the instrument hoses and then to the cup-fill waterline. This is followed by automatic opening of a water flush valve, starting of the pump and pumping of the disinfectant to the atmospheric reservoir (Fig. 4.2). When the lower sensor reads that the bottle container is empty of disinfectant, the pump stops automatically, the water flush valve closes automatically and the DCU visual display shows a code indicating that the feeding cycle (total time <two min) is completed. The electrical supply to the DCU is then switched off to allow the disinfectant to take effect (15 h in the present study). When the electrical supply to the DCU is turned on again an automatic flushing cycle begins. Initially, disinfectant in the atmospheric reservoir is voided to a drain and then mains water fills the reservoir, is pumped to the bottle container and back to a drain outlet in the atmospheric reservoir container (Fig. 4.2). This cycle is repeated three times automatically (total time = approx. nine min) to purge disinfectant from the bottle container, pump and atmospheric reservoir. Subsequently fresh mains water is pumped from the atmospheric reservoir to the bottle container, and from there is used to flush the instrument waterlines and cup fill line for a duration of four min. After this automatic flushing and rinsing cycle the DCU is ready for normal use.

If the DCU is left switched on following the disinfectant feeding cycle, disinfectant purging and waterline flushing is initiated automatically after a time lapse of 8 h. When the DCU's waterline cleaning mode is activated, an inbuilt safety feature inactivates all other DCU functions, thus preventing use of the DCU for patient treatment.

4.2.2. Disinfection of Waterlines Using the Planmeca Water Management System™

The microbiological quality of waterline output water from the Planmeca model Compact i DCU equipped with the Planmeca WMS™ was assessed over a 50-week period using the waterline disinfectants Planosil (1.5% (v/v) hydrogen peroxide, 0.003% (w/v) silver, 0.0015% (v/v) phosphoric acid) and Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver, 0.0025% (v/v) phosphoric acid) using once-weekly, overnight (15 h) disinfection. Hydrogen peroxide and silver-ions are the active antimicrobial ingredients in both disinfectants, while ortho-phosphoric acid is used as a stabilising agent. Both of these disinfectant products contain a dark blue tracking dye so that complete filling of waterlines can be observed visually by the elution of the coloured disinfectant from each waterline outlet during the disinfectant feeding cycle. The Compact i DCU waterlines were initially disinfected with Planosil for a period of 10 weeks and then with Planosil Forte for a further period of forty weeks. Prior to testing the Compact i DCU with Planosil, the unit's waterlines were not disinfected for a four-week period to allow biofilm to form naturally in its waterlines. During this period the DCU was connected to the mains water supply and was not used for treating patients. Following the last Planosil disinfection cycle, the DCU waterlines were not disinfected for a two-week period. The total study period was 55 weeks.

4.2.3. Collection and Laboratory Processing of Water Samples

Samples of water (approximately 50 ml) were collected from the Compact i DCU three-in-one air/water syringe DUWL, immediately prior to disinfection and immediately following disinfection with both disinfectants as described in Chapter Two, Section 2.2.3. Two to three additional water samples were also taken on separate days each week between disinfection cycles. Samples were collected in sterile glass bottles directly from the operator three-in-one air/water syringe DUWL, transported to the microbiology laboratory at ambient temperature within five min and processed immediately as described in Chapter Two, Section 2.2,4. Water samples were also taken from the mains water supplied to the Compact i DCU on six occasions at intervals throughout the 55-week study period.

4.2.4. Identification of Bacterial Isolates

Bacterial isolates were identified, where possible, by means of the API 20NE system for identifying non-enterobacterial Gram-negative bacteria as described in Chapter Two, Section 2.2.5.3. Definitive identification of isolates was undertaken by determining the DNA sequence of a segment of the small ribosomal subunit rRNA gene as described in Chapter Two, Section 2.2.5.4.

4.2.5. Electron Microscopy of Waterline Biofilm

Two-centimetre long samples of DUWL waterline tubing supplying water to the three-in-one air/water syringe close to the syringe attachment site were removed at intervals before and immediately following disinfection with both Planosil and Planosil Forte. The samples were prepared and examined by electron microscopy as described in Chapter Two, Section 2.3.1.

4.2.6. Mains Supply Water

During this part of the study, municipal mains water supplied to DCUs was automatically pre-treated prior to delivery to DCUs as described in Chapter Two, Section 2.2.1.

4.2.7. Chemical Analysis of Water

Water samples from the mains supply to the Compact i DCU and samples from the three-in-one air/water syringe waterline were subject to chemical analysis as described in Chapter Two, Section 2.2.2.

4.2.8. Statistical Analysis

Bacterial density levels in DUWL output water obtained following disinfection with either Planosil or Planosil Forte were compared using a two-sample unpaired *t* test, assuming equal variances. Comparison of bacterial density levels in DUWL output water seven days post-disinfection relative to the ADA DUWL output water quality standard of $\leq$200 cfu/ml was carried out using one-sample *t* tests.

4.3. Results

4.3.1. Compact i Dental Chair Unit with Water Management System™

Operation of the DCU in waterline cleaning mode was straightforward and was facilitated by its automated nature. All that is required by the operator is to place the instrument hoses into receivers beneath the DCU cuspidor, activate the DUWL cleaning function on the DCU console keypad, fill the DCUs bottle container with disinfectant and wait approximately two min until all of the DCUs DUWLs are automatically filled with disinfectant and then switch the unit off. The following morning, the operator switches the DCU on again and the DUWLs are automatically purged of disinfectant and thoroughly flushed. The entire process takes about 15 min after which time the DCU is ready for use. An inbuilt safety function prevents the DCU from being operated until the DUWL purging and rinsing cycles have been completed automatically.

4.3.2. Disinfection of Dental Unit Waterlines

The microbial quality of DUWL output water from the Compact i DCU equipped with the novel integrated Planmeca WMS™ was tested over a 55-week period with once-weekly DUWL disinfection for 50 weeks with the hydrogen peroxide and silver ion-containing disinfectants Planosil and Planosil Forte. Planosil was selected for use because a previous report from this laboratory in 2002 showed that a product with a similar formulation (Sanosil) was very effective at controlling biofilm in DCU waterlines (Tuttlebee *et al.*, 2002). However, since then we have observed a decline in the efficacy of this product following prolonged use, possibly due to the prevalence of catalase-positive bacteria in DUWL biofilm (see Chapter Three, Section 3.3.3, and Table 3.1). In order to circumvent similar problems in the present study, Planosil Forte, with its higher concentrations of hydrogen peroxide and silver ions was also used.

At the beginning of the study the DCU was connected to the processed mains water supply provided to all DCUs in the DDSH and was not subject to DUWL disinfection for a period of four weeks to allow biofilm to form naturally in its waterlines. Following this initial period the units DUWLs were subject to a 15 h (i.e. overnight) disinfection once-weekly for a period of 10 weeks with Planosil. After these 10 weekly disinfection cycles the DCU waterlines were not disinfected for one week and then subjected to a further 40 weekly disinfection cycles with the disinfectant Planosil Forte. During the entire 55-week period water samples from the DCU operator

three-in-one air/water syringe were taken four to five times a week and tested for the presence of aerobic heterotrophic bacteria. These organisms were chosen as marker organisms for DUWL contamination as many previous studies have shown that these are the predominant microorganisms present in DUWLs (Pankhurst and Philpott-Howard, 1993; Barbeau *et al.*, 1996; Pankhurst *et al.*, 1998; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002).

The microbial quality of processed mains water supplied to the Compact i DCU was tested the day the DCU was initially connected to the mains supply and on five separate occasions throughout the 55-week study period and was found to contain low levels of bacteria with a mean average of 77 cfu/ml (range 49–122 cfu/ml). During the four-week period following connection of the DCU to the mains water supply the bacterial density in DUWL output water rose steadily and reached 15,400 cfu/ml on day 28 (Fig. 4.3, panel a). In contrast, immediately following disinfection and seven days post-disinfection with Planosil, DUWL output water had a markedly reduced bacterial density during each of the 10 weeks the DCU was disinfected with Planosil (Fig. 4.3, panel a). The average bacterial density immediately following disinfection with Planosil was 26 cfu/ml (range 10–45 cfu/ml). Bacterial levels rose steadily each week between disinfections and the mean average bacterial density in output water seven days post-disinfection was 384 cfu/ml (range 180–620 cfu/ml). The majority (9/10; 90%) of water samples taken seven days post-disinfection with Planosil exceeded the microbial quality threshold of ≤200 cfu/ml set by the ADA for DUWL output water (Fig. 4.3, panel a). Fourteen days following the final disinfection with Planosil the bacterial density in DUWL output water increased to 8,600 cfu/ml (Fig. 4.3, panel a). Following the period the DCU was disinfected with Planosil, the DCU was initially subjected to once-weekly disinfection with Planosil Forte for 10-weeks. Similar to the results obtained with Planosil, DUWL output water had a markedly reduced bacterial density compared to the densities recorded 28 days post-connection of the DCU to the mains water supply during each of the 10 weeks the DCU was disinfected with Planosil Forte (Fig. 4.3, panel b). The average bacterial density immediately following disinfection with Planosil Forte was 18 cfu/ml (range 11–27 cfu/ml). Here again, bacterial levels increased during each week between disinfections but not to the same extent as during Planosil disinfection, and the mean average bacterial density in output water seven days post-disinfection was 112 cfu/ml (range 15–178 cfu/ml). All 10 (100%) water samples taken seven days post-disinfection with Planosil Forte were below the microbial quality

threshold of ≤200 cfu/ml set by the ADA for DUWL output water (Fig. 4.3, panel b). There was a highly significant difference between the bacterial density in DUWL output water seven days post-disinfection with Planosil and Planosil Forte. The bacterial density in DUWL output water seven days post-disinfection with Planosil during the 10-week period the DCU was disinfected with this product (average 384 cfu/ml) was significantly higher than the corresponding density recorded during the 10-week period the DCU was disinfected with Planosil Forte (average 112 cfu/ml) ($p = 0.00001$). In order to investigate whether consistent results would be obtained with Planosil Forte over a longer period of time, the Compact i DCU was subjected to a further 30 cycles of once-weekly disinfection with this disinfectant and similar results were obtained (Fig. 4.4). The average bacterial density immediately following disinfection and seven days post-disinfection with Planosil Forte over the entire 40-week period the DCU was disinfected with this product was 20 cfu/ml (range 10–32 cfu/ml) and 113 cfu/ml (range 15–190 cfu/ml), respectively. These results demonstrated that both disinfectant products were effective at controlling levels of aerobic heterotrophic bacteria in DUWL output water following once-weekly disinfection but that Planosil Forte was significantly more effective. Output water from DUWLs disinfected with Planosil Forte was consistently below the ADA waterline output water quality threshold of ≤200 cfu/ml for each of the seven days in each disinfection cycle, whereas by day seven the majority of samples from DUWLs disinfected with Planosil had exceeded 200 cfu/ml (Fig. 4.3, panel a).

4.3.3. Electron Microscopy of Dental Unit Waterline Biofilm

Two centimetre samples of waterline tubing close to the outlet of the three-in-one air/water syringe of the Compact i DCU used in this study were removed from the DCU at the outset of the study after the unit had been connected to the mains water supply for a period of 28 days. Additional samples were removed at intervals immediately prior to disinfection with both Planosil and Planosil Forte and seven days post-disinfection. All samples were examined for the presence of biofilm by electron microscopy. Extensive biofilm was evident on the internal surface of the waterline tubing sample 28 days post-connection of the DCU to the mains water supply (Fig. 4.5, panel a). Biofilm was also apparent on the internal surface of the waterline tubing seven days post-disinfection with either Planosil or Planosil Forte, but was less extensive (Fig. 4.5, panels b and c). However, biofilm had been virtually eliminated from the internal surface of the waterline tubing immediately post-disinfection with either product (Fig. 4.5, panel d).

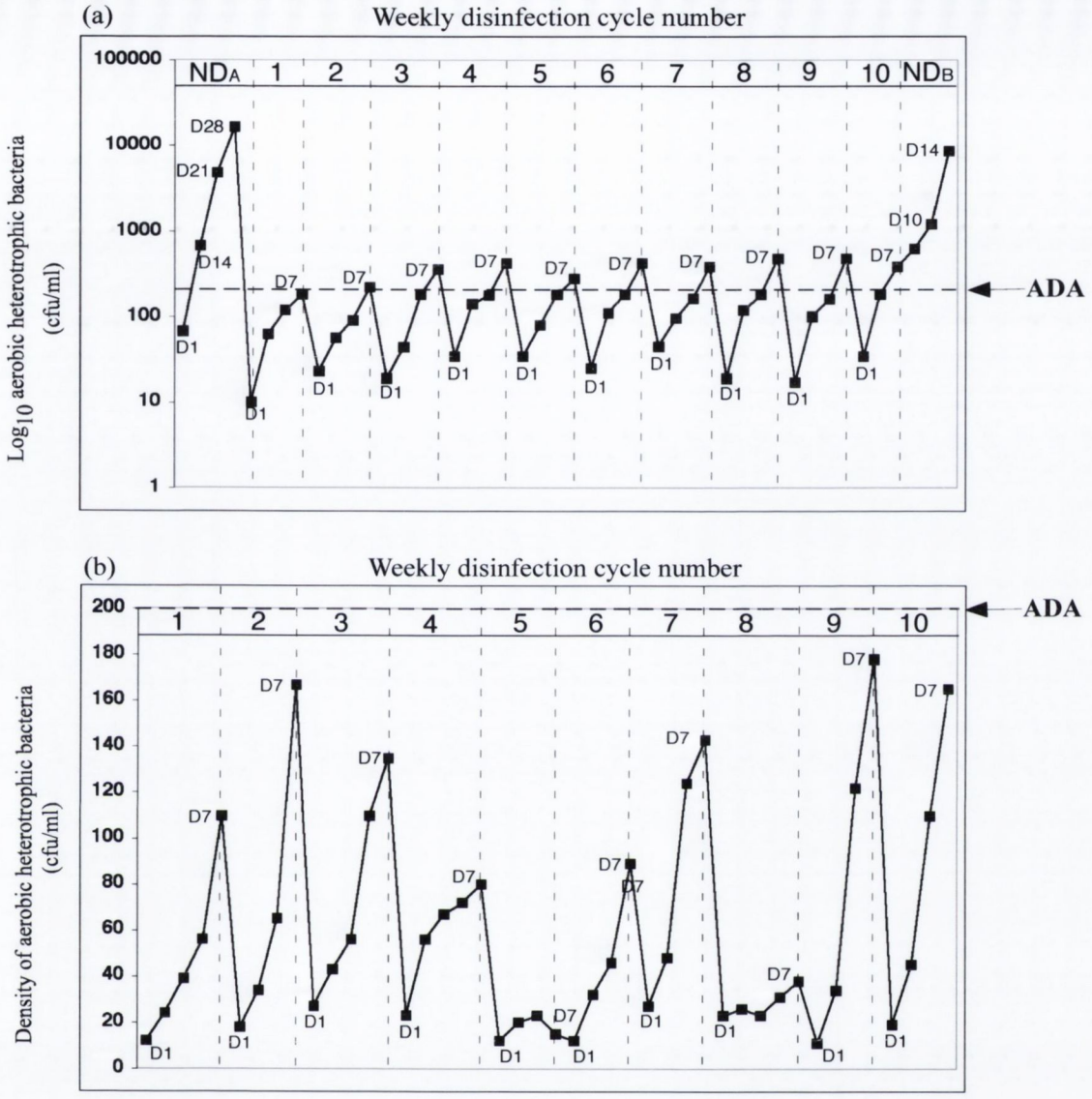


Fig. 4.3. Density of aerobic heterotrophic bacteria in air/water syringe output water from the Compact i DCU during ten cycles of once weekly disinfection with (a) Planosil or (b) Planosil Forte. The DCU was not disinfected for a four-week period prior to disinfection with Planosil. D1, day 1 immediately following disinfection; D7, day 7 post-disinfection and immediately prior to the next disinfection episode. At the beginning of the study the DCU was connected to the mains water supply for a four-week period during which the DCUs waterlines were not disinfected (ND_A). Water samples were taken during this period on days 1, 14, 21 and 28 post-connection to the mains supply. Following the tenth disinfection episode the waterlines were not disinfected for two weeks (ND_B). The horizontal dashed lines shown represent the maximum bacterial density (≤200 cfu/ml) recommended by the ADA guideline for DCU output water quality.

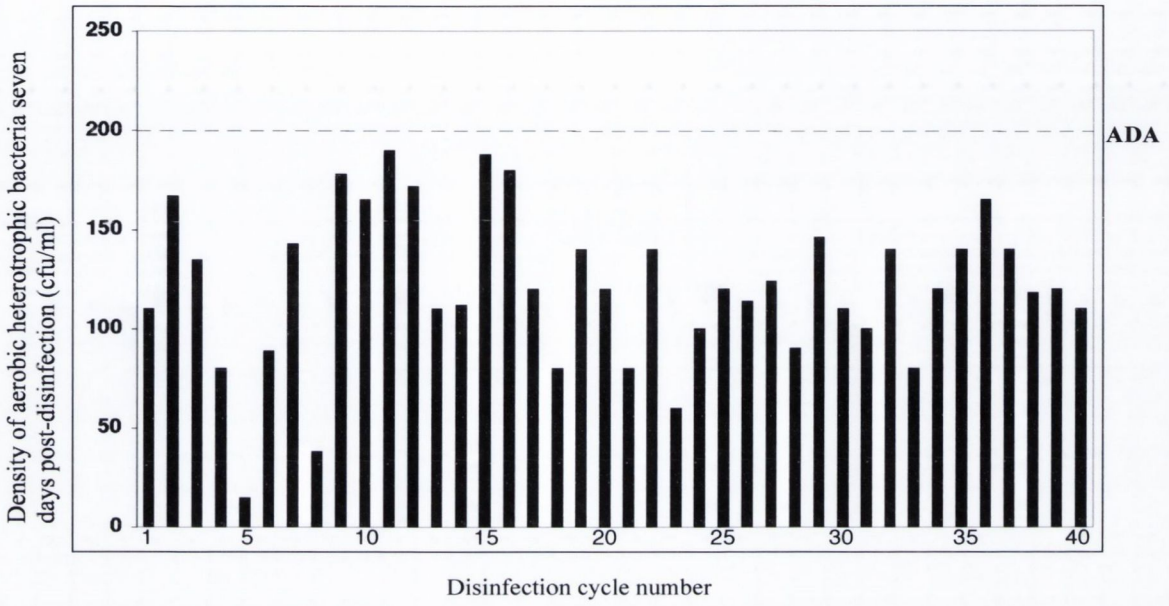


Fig. 4.4. Density of aerobic heterotrophic bacteria in the three-in-one air/water syringe output water from the Compact i DCU seven days post-disinfection during 40 cycles of once weekly disinfection with Planosil Forte. The horizontal dashed line shown represents the maximum bacterial density (≤200 cfu/ml) recommended by the ADA guideline for DCU output water quality.

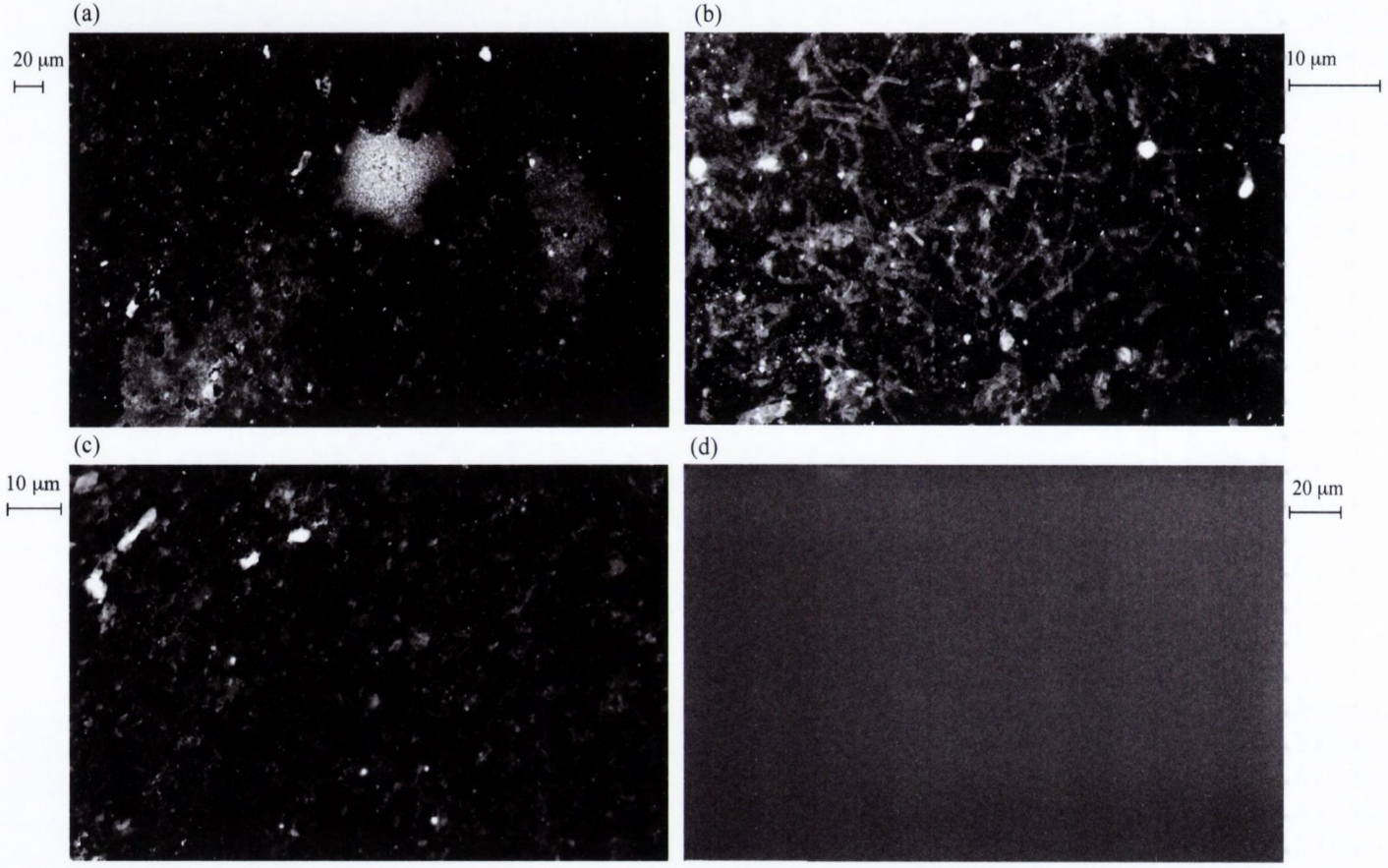


Fig. 4.5. Electron micrographs of longitudinal sections of DUWL tubing from the air/water syringe waterline. The Electron micrographs compare the amounts of biofilm on the internal surfaces from the (a) non-disinfected DCU 28 days post-connection to the mains supply water, (b), Planosil-disinfected DCU seven days post-disinfection, (c) Planosil Forte-disinfected DCU seven days post-disinfection and (d) Planosil Forte-disinfected DCU immediately following disinfection. Similar results to those shown in (d) were obtained following disinfection with Planosil.

4.3.4. Chemical Analysis of Water

In order to investigate whether disinfection of DUWLs with either Planosil or Planosil Forte adversely affected the chemical quality of DUWL output water, mains water supplied to the DCU and several DUWL output water samples taken at different times during the study period from the three-in-one air/water syringe immediately post-disinfection were analysed. The chemical quality of all the water samples tested was consistently found to be well within the limits advocated by the EC Directive on the Quality of Bottled Water for Human Consumption and by the United States Environmental Protection Agency National Primary Drinking Water Regulations (Anonymous, 1998; Anonymous, 2002). Furthermore, no significant difference was found between the chemical composition of mains supply water and DUWL output water following disinfection (Table 4.1).

4.3.5. Bacterial Species Detected in Water

In the present study R2A agar was used to determine the total aerobic heterotrophic bacterial density in processed mains water and DUWL output water. These organisms were chosen as markers for microbial contamination as many previous studies have demonstrated that these are the predominant microbes present in DUWL output water (Pankhurst and Philpott-Howard, 1993; Barbeau *et al.*, 1996; Pankhurst *et al.*, 1998; Walker *et al.*, 2000, Tuttlebee *et al.*, 2002). Selected isolates of each colony type recovered on R2A agar were purified, Gram-stained and stored for subsequent identification. The majority of isolates recovered on R2A agar plates were Gram-negative (approximately 80%). Initially, attempts were made to identify Gram-negative isolates using the API 20NE system but many of the isolates could either not be satisfactorily identified with this system or could be identified only to the genus level (data not shown). In order to circumvent this problem, it was decided to identify all selected isolates to the species level based on the DNA sequence of a variable region of the 16S rDNA gene. The universal primer pair 533F/142R, which can be used to amplify a region of approximately 950 bp to 1.5 kb of the 16S rDNA gene of all bacterial species was used in separate PCR amplification experiments with template DNA from each of the isolates selected for identification. A single PCR amplimer was obtained with template DNA from each isolate, which was subsequently cloned and its nucleotide sequence determined. Each amplimer sequence obtained shared 99–100%

homology with the 16S rDNA consensus sequence of only one bacterial species in each case, in the EMBL/GenBank nucleotide sequence databases and thus all of the isolates were identified unequivocally to the species level (Table 4.2). The most common bacterial isolates recovered from the mains water supply during the 55-week study period were Gram-positive and catalase-positive organisms that were identified as *Micrococcus luteus*. These organisms were also identified in output water from the Compact i DCU three-in-one air/water syringe waterline following disinfection with either Planosil or Planosil Forte. However, the most prevalent bacterial isolates recovered from DUWL output water were Gram-negative, catalase-positive organisms identified as *Novosphingobium subarctica*, *Sphingomonas paucimobilis* or other *Sphingomonas* spp., All of these *Sphingomonas* isolates were found to be very strongly catalase-positive (see Table 4.2).

Table 4.1. Chemical analysis of mains supply water and water from the air/water syringe waterline from the Compact i DCU immediately following disinfection with Planosil and Planosil Forte

Test	DUWL[a] water (Planosil) (mg/l)	Processed[b] mains water (mg/l)	DUWL[a] water (Planosil Forte) (mg/l)	Processed[b] mains water (mg/l)	EC[c] (mg/l)	US[d] (mg/l)	Method[e] of analysis
Calcium	15.4	15.2	16.4	16.2	N/A	N/A	ICP-MS[f]
Magnesium	1.9	1.8	2.5	2.5	N/A	N/A	ICP-MS[f]
Sodium	7.5	7.6	7.0	7.0	200.0	N/A	ICP-MS[f]
Potassium	0.048	0.125	0.8	0.9	N/A	N/A	ICP-MS[f]
Iron	0.017	0.022	0.002	0.002	0.20	0.30	ICP-MS[f]
Manganese	<0.001	<0.001	<0.002	<0.002	0.05	0.05	ICP-MS[f]
Copper	0.035	0.007	0.044	0.042	2.00	1.3	ICP-MS[f]
Lead	<0.001	<0.001	<0.001	<0.001	0.01	0.0	ICP-MS[f]
Zinc	0.32	0.004	0.023	0.004	N/A	5.0	ICP-MS[f]
Silver	0.003	0.003	0.004	<0.002	N/A	0.1	ICP-MS[f]
Carbonate	61.05	61.0	30.0	40.0	N/A	N/A	Titration
Chloride	13.8	13.9	12.0	12.0	250.0	250.0	Colourimetric assay
Sulphate	40.0	39.0	41.0	34.0	250.0	250.0	Turbidometric assay
Nitrate as NO_3	3.82	4.64	3.3	3.1	50.0	10.0	Colourimetric assay
Nitrite as NO_2	<0.05	<0.05	<0.05	<0.05	0.5	1.0	Colourimetric assay
Ammonia as NH_4	0.03	0.02	0.02	0.02	0.5	N/A	Colourimetric assay
Fluoride	0.79	0.81	0.9	0.6	1.50	4.0	Colourimetric assay
ortho-Phosphate as PO_4	<0.15	<0.15	<0.03	<0.03	N/A	N/A	Colourimetric assay
pH	7.57	7.68	7.56	7.61	6.5–9.5	6.5–8.5	Meter
Conductivity at 25 °C (μS/cm)	148.4	143.9	156.0	156.0	N/A	N/A	Meter

[a]Water samples tested immediately following disinfection of DUWLs with Planosil or Planosil Forte, respectively.
[b]Mains water supplied to the DCU was initially processed by particle filtration, ion exchange and UV treatment (see Section 4.2).
[c]European Commission maximum permissible concentration in water for human consumption (Anon., 1998).
[d]Maximum permissible concentration in drinking water advocated by the United States Environmental Protection Agency drinking water regulations (Anon., 2002).
[e]Used in the present study and based on standard methods as described previously (Tuttlebee *et al.*, 2002).
[f]ICP-MS, inductively coupled plasma-mass spectrometry.
N/A: no maximum permissible concentration in water for human consumption.

Table 4. 2. Bacterial species isolated from the air/water syringe waterline of the Compact i dental chair unit used in the present study

Bacterial species[a]	Gram-stain	Catalase activity[b]
Novosphingobium subarctica	Negative	+++++
Sphingomonas paucimobilis	Negative	+++++
Sphingomonas aerolata	Negative	+++++
Sphingomonas trueperi	Negative	+++++
Acidovorax temperans	Negative	−
Porphyrobacter tepidarius	Negative	−
Arthrobacter agilis	Positive	++++
Micrococcus luteus	Positive	+++++
Variovorax paradoxus	Positive	++++
Rhodococcus fascians	Positive	+++
Kocuria palustris	Positive	+++

[a]Identified by DNA sequence analysis of the variable region of 16S rDNA gene.

[b]An arbitrary scale for catalase activity was used based on the visual observance of the evolution of oxygen bubbles from a bacterial colony following the addition of 50 µL of 5% (v/v) H2O2. +++++, very strongly catalase-positive with instantaneous and substantial evolution of gas bubbles; +++, catalase-positive with immediate but moderate evolution of gas bubbles; −, catalase-negative. For each isolate tested, at least five separate colonies grown on R2A agar medium were tested for catalase production.

4.4. Discussion

Over the last decade numerous studies from many countries have highlighted that output water from DUWLs can contain very high microbial loads of mainly bacterial species including several known human pathogens such as *P. aeruginosa* and *L. pneumophilia* (Abel *et al.*, 1971; Martin, 1987; Williams *et al.*, 1993; Atlas *et al.*, 1995; Challacombe and Fernandes, 1995; Barbeau *et al.*, 1996; Meiller *et al.*, 2000; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002). This phenomenon results from biofilm formation in narrow-bore DUWLs and is a universal problem in DCUs. It is now generally accepted by health care authorities and professional bodies that it is not acceptable from an infection control and prevention (ICP) perspective to expose patients (especially medically compromised or immunocompromised patients), dental staff and support staff to heavily contaminated dental unit water or aerosols generated during use of dental instruments connected to DUWLs. In recent years the focus on DUWL biofilm contamination has shifted from investigating the problem to developing effective solutions that can be implemented in dental practice. Currently there are a wide variety of commercial DUWL treatment products available that are claimed by manufacturers to control waterline contamination and/or eliminate DUWL-associated biofilm (Meiller *et al.*, 2000; Pederson *et al.*, 2002; Tuttlebee *et al.*, 2002, Walker *et al.*, 2003; Kohno *et al.*, 2004; McDowell *et al.*, 2004; Martin and Gallagher, 2005). However, only a few of these products have been extensively tested *in situ* in DCUs for extended periods. Furthermore, extensive comparative studies of the effectiveness of these products in DCUs await to be undertaken.

Because the phenomenon of DUWL biofilm contamination is essentially a consequence of the design of DCUs, i.e. the requirement for narrow-bore DUWL tubing to carry water to cool dental instruments and tooth surfaces during dental procedures, there have been calls for DCU manufacturers to consider the problem of DUWL biofilm contamination when designing and developing new DCU models (Tuttlebee *et al.*, 2002; Montebugnoli *et al.*, 2004; Coleman *et al.*, 2007). This is particularly appropriate as modern DCUs are classified as medical devices. In 2002, we reported on the effectiveness of the Waterline Cleaning System (WCS^{TM}), a DUWL cleaning system developed by the Finnish DCU manufacturer Planmeca Oy (Helsinki, Finland), to control DUWL biofilm in Planmeca Prostyle DCUs using hydrogen peroxide-containing disinfectants (Tuttlebee *et al.*, 2002). At that time the WCS^{TM} was designed

to be retrofitted to existing Planmeca DCU models and delivered disinfectant to DUWLs from an external container and was found to be very effective at eliminating DUWL biofilm (Tuttlebee *et al.*, 2002). DUWL output water from DCUs disinfected with the WCS^{TM} contained bacterial densities below the ADA recommended threshold of ≤200 cfu/ml for up to a week post-disinfection. However, more detailed studies undertaken over a 21-month period showed that the retrofitted system had a number of drawbacks, including the requirement to manually flush some of the waterlines following disinfection, intermittent problems associated with disinfectant leakage from retrofitted valves, component corrosion by Planosil, and the systems effectiveness depended on strict compliance with the operating procedure (see Chapter Three).

The Planmeca Compact i DCU investigated during the present study equipped with the novel WMS^{TM} represents the next stage in the development of DCUs as it has been specifically designed to contain a fully integrated and automated waterline cleaning system that is installed during DCU manufacture. The WMS^{TM} cleaning procedure is easy to perform and is facilitated by its automation. All that is required of the operator is to (i) place the instrument hoses into dedicated receivers, (ii) activate the waterline cleaning function on the DCU console, (iii) add disinfectant to the DCU bottle container, (iv) allow the waterlines to fill with disinfectant automatically (v) and then switch the unit off. This process takes less than five minutes. Following disinfection, DUWLs are automatically purged of disinfectant and then automatically flushed extensively with clean water. The WMS^{TM} is also equipped with a significant additional safety feature, consisting of a vertical air gap that prevents backflow contamination of the mains water supply with water from the DCU waterline network. The WMS^{TM} is designed so that all parts of the water distribution network within the DCU, including DUWLs, atmospheric reservoir, bottle container and pump are all subject to disinfection when the waterline-cleaning mode is activated (Figs. 4.1 and 4.2).

During the 55-week long period the Compact i DCU was investigated, the microbiological quality of DUWL output water was monitored following 50 separate, once-weekly disinfection cycles with the hydrogen peroxide and silver ion-containing disinfectants Planosil and Planosil Forte. These products were selected for DUWL disinfection because our previous studies demonstrated that hydrogen peroxide-containing disinfectants are very efficient at removing DUWL biofilm, a key requirement for an effective DCU waterline disinfectant. Planosil (1% (v/v) hydrogen peroxide, 0.003% (w/v) silver) essentially has the same chemical formulation as

Sanosil, one of the disinfectant products that we previously demonstrated was effective at controlling biofilm formation in DUWLs in the retrofitted DCUs referred to above, whereas Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver) contains higher concentrations of peroxide and silver (Tuttlebee *et al.*, 2002; Chapter Three). Both disinfectant products were found here to effectively and consistently reduce the density of aerobic heterotrophic bacteria, that were used as marker organisms for microbial contamination, in DUWL output water immediately following disinfection to levels well below the ADA recommended threshold of ≤200 cfu/ml of output water (mean = 26 cfu/ml for Planosil and 20 cfu/ml for Planosil Forte, respectively, (Figs. 4.3, panel b and 4.4). Between weekly disinfections with both disinfectants, the bacterial density in DUWL output water rose steadily, and in the case of DUWLs disinfected with Planosil, exceeded the ADA threshold of ≤200 cfu/ml before the seventh day post-disinfection in 9/10 (90%) disinfection cycles with a mean average bacterial density of 384 cfu/ml (Fig. 4.3, panel a). In contrast, the bacterial density in output water from DUWLs disinfected with Planosil Forte consistently remained below ADA threshold of ≤200 cfu/ml throughout the 40 weekly cycles the DCU was disinfected with Planosil Forte, even seven days post disinfection where the mean average bacterial density was 112 cfu/ml (Figs. 4.3, panel b and 4.4). The density of aerobic heterotrophic bacteria in DUWL output water seven days post-disinfection with Planosil was significantly higher than the ADA threshold of ≤200 cfu/ml ($p = 0.00085$, 95% confidence interval for the mean: 289.6–478.4), while the corresponding density seven days post-disinfection with Planosil Forte was significantly lower than the ADA threshold of ≤200 cfu/ml ($p = 0004$, 95% confidence interval for the mean: 72.01–151.99). In the case of DUWL disinfection with both Planosil and Planosil Forte, the marked reduction in bacterial density recorded in DUWL output water was directly associated with elimination of biofilm from the internal surfaces of DUWLs as determined by electron microscopy (Fig. 4.5). Biofilm was observed to have re-grown in DUWLs seven days post-disinfection with both disinfectants, but determining whether there were any quantitative differences in the amount or composition of the biofilm present was beyond the scope of this study. Re-growth of biofilm following DUWL disinfection and consequent deterioration of the microbiological quality of output water has been described in several studies, hence the requirement for regular DUWL disinfection (Furuhashi and Miyamae, 1985; Pankhurst and Philpott-Howard, 1993; Williams *et al.*, 1993; Mills, 2000; Meiller *et al.*, 2000; Tuttlebee *et al.*, 2002). The results of the present

study demonstrate that the Planmeca WMS^{TM} used in combination with Planosil Forte consistently maintains the quality of DUWL output water below the ADA standard of ≤200 cfu/ml for up to seven days post-disinfection using a once-weekly overnight disinfection. Planosil was not as effective as Planosil Forte in maintaining output water quality, but in 9/10 disinfection cycles the output water from DUWLs disinfected with Planosil was above the ADA standard of ≤200 cfu/ml five days post-disinfection (Fig. 4.3, panel a). Clearly the higher hydrogen peroxide and silver ion content of Planosil Forte is more effective at eliminating biofilm, although as mentioned above, electron microscopy of DUWLs revealed that both disinfectants were very effective at removing waterline biofilm. It is conceivable that residual biofilm was present in some parts of the waterline network following Planosil disinfection and that this occurred to a lesser extent with Planosil Forte because of its higher concentrations of hydrogen peroxide and silver ions.

Chemical and pH analysis of paired samples of mains water supplied to the Compact i DCU and output water from the three-in-one air/water syringe waterline immediately following disinfection with either disinfectant showed no significant differences (Table 4.1) indicating the absence of chemical residues in output water following disinfection. This is an important finding, as patient safety has to be paramount when selecting products for DUWL cleaning. Good quality supply water is essential for DCUs as heterotrophic bacteria found in supply water can convert organic material dissolved in supply water into biomass locally (Flemming, 2002). The poorer the quality of the water supplied to DUWLs, the more readily biofilm will form on its surfaces (Coleman *et al.*, 2009) In this regard, it is advisable that DCUs connected to a mains water supply should be processed to remove particulate matter, chemical residues, and soluble organic matter (Coleman *et al.*, 2007; Coleman *et al.*, 2009).

A number of DUWL treatment products involve permanent treatment of water in DUWLs and have been claimed to provide continuous protection of the waterlines against biofilm formation. These products, a number of which have been shown independently to be effective at controlling DUWL biofilm, are added to water supplied to DCUs, usually from an internal bottle reservoir or are released by devices connected to the DCU water supply, and essentially have a residual effect within the DUWLs (Pederson *et al.*, 2002; Kohno *et al.*, 2004; Martin and Gallagher, 2005). By their very nature these waterline-cleaning products enter the mouths of patients during dental procedures and are claimed not to be detrimental to human health. However, in the

authors' view it is prudent to question whether DUWL cleaning products should be introduced into the oral cavities of patients, very often without their knowledge, if effective alternative approaches that do not expose patients to cleaning agents are available. Interestingly, a recent study by Stone *et al.* 2006 reported that iodine-releasing cartridges, an effective residual treatment used to control bacterial growth in DUWLs, may have a detrimental affect on the environment by mobilising mercury from dental amalgam in DCUs with the release of highly toxic dissolved mercury into the environment from DCU wastewater.

Many of the Gram-negative bacterial isolates representative of the colony types recovered on R2A agar from DCU water samples could not be identified using the commercially available API 20NE system. Other studies have also reported that some bacterial isolates recovered from DUWLs are not amenable to identification by conventional means (Singh *et al.*, 2003). To circumvent this problem, all isolates were identified unequivocally to the species level by comparative sequence analysis of a PCR amplified variable region of the 16S rDNA gene. Interestingly, many isolates with different types of colony morphology were found to belong to the same species (data not shown). This was particularly evident with *Novosphingobium subarctica* and *Sphingomonas* spp., and isolates of *Micrococcus luteus*. *Sphingomonas* spp., have been detected previously in DUWLs (Singh *et al.*, 2003). These organisms secrete viscous exopolysaccharides that aid in their ability to adhere to surfaces. *Sphingomonas paucimobilis* (isolated from DUWL output water in the present study) has been previously associated with colonisation of mechanical ventilators and other medical devices in the hospital environment, as well as being responsible for episodes of nosocomial infection (Lemaitre *et al.*, 1996; Hsueh *et al.*, 1998; Perola *et al.*, 2002). *Novosphingobium subarctica* and *Sphingomonas* species were the most prevalent aerobic heterotrophic bacterial species recovered from DUWL output water, but not in the DCU supply water. These results parallel the findings of the study on the Planmeca WCS™ described in Chapter Three, where these species were associated with rapid deterioration of DUWL output water quality following prolonged DUWL disinfection with Planosil in individual DCU units. Similar results were also noted in the present study with the Compact i DCU unit in the first part of the study using Planosil. It is highly likely that the prolonged use of Planosil to disinfect DUWLs in the first phase of the study with the Compact i DCU selected for the maintenance of these strongly catalase-positive species because of their ability to destroy hydrogen peroxide. As

occurred with the study on the Planmeca WCS™ described in Chapter Three, replacing Planosil with Planosil Forte, the latter with its higher concentration of hydrogen peroxide and silver ions, prevented subsequent episodes of rapid deterioration of DUWL output water quality due to proliferation of *Novosphingobium subarctica* and *Sphingomonas* spp. All of the bacterial species identified in the present study were environmental bacterial species. The narrow range of species identified probably reflects the good quality of the mains supply water provided to DCUs in the DDSH as demonstrated by chemical analysis and the relatively low density of aerobic heterotrophic bacteria present in DCU supply water (average 77 cfu/ml; range 49–122 cfu/ml). Interestingly, many of the bacterial isolates identified in DUWL output water were strongly catalase-positive and thus capable of breaking down hydrogen peroxide. It is interesting to speculate that some of these organisms survived in DUWLs following disinfection with Planosil (1% (v/v) hydrogen peroxide) and were subsequently responsible for the earlier deterioration of DUWL output water quality compared to DUWLs disinfected with Planosil Forte (2.5% (v/v) hydrogen peroxide).

In the present study the combination of hydrogen peroxide and silver ion-containing disinfectants was shown to be particularly efficient at eradicating waterline biofilm. Silver ions destabilise the biofilm matrix by binding to electron donor groups of biological molecules leading to reductions in the number of binding sites for hydrogen bonds and electrostatic and hydrophobic interactions and, thus, the destabilisation of the biofilm structure (Chaw *et al.*, 2005). In contrast, the antimicrobial activity of hydrogen peroxide stems from its intense oxidizing properties, which allow it to disrupt membrane lipids, DNA and other essential cell components. One potential concern about the prolonged use of hydrogen peroxide-containing disinfectants to clean DUWLs is the potential for the development of biocide tolerance or resistance within DUWL biofilm by adaptation or selection following prolonged treatment. Biofilm bacteria are notoriously more difficult to destroy with antimicrobial agents or biocides than planktonic organisms. (Costerton *et al.*, 1987; Campanac *et al.*, 2002; Grobe *et al.*, 2002; O'Donnell *et al.*, 2005). In DUWL biofilms it is conceivable that catalase-positive bacteria could adapt to exogenous hydrogen peroxide used to eradicate biofilm following long-term use. Tolerance by biofilm bacteria against hydrogen peroxide has been previously reported to be accompanied by an enhanced catalase activity (Furuhashi and Miyamae, 1985). A previous study with *P. aeruginosa* biofilm showed that biofilm organisms are significantly less susceptible to killing by hydrogen peroxide compared

to planktonic forms and that significant catalase induction occurred in biofilms following exposure to hydrogen peroxide (Elkins *et al.*, 1999). Another study reported that for sufficiently thick biofilms, bacterial cells in biofilm implement adaptive responses more effectively than planktonic cells (Szomolay *et al.*, 2005). To prevent such potential problems arising in DUWLs disinfected with hydrogen peroxide following prolonged use, the periodic use of an alternative disinfectant with a different mode(s) of action (i.e. not containing hydrogen peroxide) would be advisable.

4.5. Conclusions

In conclusion, the results of this study demonstrate that the Planmeca Compact i DCU equipped with the novel, automated WMS™ effectively and consistently maintains the quality of DUW output water below the ADA recommended standard of ≤200 cfu/ml of aerobic heterotrophic bacteria for up to seven days using once-weekly disinfection with Planosil Forte. Automation of the WMS™ eliminates problems associated with manually operated waterline cleaning systems where strict compliance with procedure is necessary for effective control of waterline biofilm. For dental clinics with large numbers of DCUs, such as Dental Hospitals and other public clinics, DCUs with automated waterline cleaning systems such as the WMS™ will facilitate the efficient disinfection of large numbers of DCUs. For institutions equipped with many DCUs, it is conceivable that DCUs equipped with the WMS™ could be adapted so that many DCUs could be disinfected simultaneously with disinfectant being supplied from a central reservoir using a dedicated disinfectant delivery line to provide disinfectant to individual DCUs. Control of the process could be mediated electronically from a central desktop computer. The development of new types of DCU, such as the Planmeca Compact i equipped with the WMS™, with automated DUW cleaning systems will undoubtedly make significant contributions to dealing with the problem of DUW biofilm in clinical dental practice.

Chapter 5

Development of a Centralised, Automated Dental Hospital Water Quality and Biofilm Management System using Neutral Ecasol™ Maintains Dental Unit Waterline Output at Better than Potable Quality: A Two-Year Longitudinal Study

5.1. Introduction

Numerous studies over the last four decades have shown that output water from DUWLs is usually heavily contaminated with microorganisms primarily aerobic Gram-negative heterotrophic bacterial species of environmental origin, although oral and skin microorganisms may also be present. (Martin, 1987; Mayo *et al.*, 1990; Pankhurst *et al.*, 1993; Williams *et al.*, 1993; Atlas *et al.*, 1995; Barbeau *et al.*, 1996; Meiller *et al.*, 1999; Stampi *et al.*, 1999; Mills, 2000; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002; Singh *et al.*, 2003; Walker *et al*, 2003; Coleman *et al.*, 2007; Szymańska, 2007; Coleman *et al.*, 2009). The high levels of microorganisms in DUWL output water originate from microbial biofilms formed on the internal surfaces of DUWLs (Coleman *et al.*, 2007; Coleman *et al.*, 2009). These biofilms are composed mainly of highly hydrated bacterial exopolysaccharides, in which single cells and microcolonies are generally heterogeneously interspersed with pores or channels (Davey and O'Toole, 2000; Watnick and Kolter, 2000; Coleman *et al.*, 2009). As the biofilm grows and matures, planktonic cells and by-products such as bacterial endotoxin are released into the water and are aerosolised by DCU-supplied instruments such as turbine dental handpieces and ultrasonic scalers, thus exposing patients and staff to these microorganisms and to fragments of biofilm and bacterial endotoxins (Putnins *et al.*, 2001; Huntington *et al.*, 2007; Pankhurst and Coulter, 2007).

At present there is no obligatory EU quality standard for DCU output water. However, DCUs are classified as medical devices under the European Medical Devices Directive (Anonymous, 1993) and because many DCUs are supplied directly with municipal mains water of potable quality, the quality of DCU output water quality should be consistent with, or at least approximate, potable water quality standards (Anonymous, 1996; Coleman *et al.*, 2007; Coleman *et al.*, 2009). The present potable water standards for aerobic heterotrophic bacteria in the EU and the USA do not specify an upper limit, although water sold in bottles or containers in the EU should not exceed 100 cfu/ml (Anonymous, 1998; Anonymous, 2002). CDC guidelines for infection control in dental health-care settings recommend a maximum level of aerobic heterotrophic bacteria in DUWL output water of $\leq$500 cfu/ml (Kohn *et al.*, 2003). Furthermore, the ADA in 1995 established a target limit of $\leq$200 cfu/ml for the year 2000, but this has been quite difficult to achieve in practice (Anonymous, 1996; Coleman *et al.*, 2007; Coleman *et al.*, 2009).

The most effective approach to maintaining good microbial DCU output water quality has been regular treatment of DUWLs using a disinfectant, biocide or cleaning agent that removes biofilm or inhibits biofilm growth (Karpay *et al.*, 1999; Smith *et al.*, 2001; Tuttlebee *et al.*, 2002; Walker *et al.*, 2003; Porteous *et al.*, 2004; Walker *et al.*, 2004; O'Donnell *et al.*, 2006; Schel *et al.*, 2006; Szymańska, 2006a and 2006b; Coleman *et al.*, 2007; O'Donnell *et al.*, 2007; Walker and Marsh, 2007; Coleman *et al.*, 2009).

A wide range of commercially available DUWL treatment products have been marketed in recent years, including both agents for intermittent use and for continuous use, many of which have been reported to be effective at controlling DUWL biofilm (Coleman *et al.*, 2007; Coleman *et al.*, 2009). They have been reviewed in detail in Chapter One. However, only a few of these products have been tested extensively in a large number of DCUs over extended time periods (O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007). Furthermore, some manufacturers have developed DCU models with integrated semi-automated DUWL cleaning systems that facilitate the regular cleaning of DUWLs with effective disinfectants that eliminate biofilm (Tuttlebee *et al.*, 2002; O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007). Studies on the long-term efficacy of two of these systems in the DDSH, including the Planmeca WCS™ and the Planmeca WMS™ are described in Chapter Three and Chapter Four, respectively. These studies showed that both were very effective at eliminating DUWL biofilm and provided output water at bacterial densities below the ADA recommended threshold of ≤200 cfu/ml for up to one week post-disinfection when using the hydrogen peroxide and silver ion-containing DUWL disinfectants Planosil and Planosil Forte (Tuttlebee *et al.*, 2002; O'Donnell *et al.*, 2006; Coleman *et al.*, 2007; O'Donnell *et al.*, 2007; Coleman *et al.*, 2009). However, these studies also showed that consistent provision of good quality DUWL output water was dependent on meticulous implementation of the disinfection protocol by staff undertaking DUWL disinfection (Chapter Three; O'Donnell *et al.*, 2007).

In the DDSH all 103 DCUs with which the hospital is equipped are supplied with water from a central 8,000-l storage tank supplied with potable quality mains water. For several years this water supply in association with once-weekly disinfection of DUWLs (see Chapter Three and Chapter Four) consistently provided DUWL output water complying with the ADA recommended level of ≤200 aerobic heterotrophic bacteria per ml (Tuttlebee *et al.*, 2002; O'Donnell *et al.*, 2006b; Coleman *et al.*, 2007;

Coleman *et al.*, 2009). In June 2006, during a period of warm weather, ongoing routine monitoring detected a bacterial bloom of *Pseudomonas fluorescens* in the 8,000-l DCU storage tank, which developed over the course of a week between weekly samplings, where the bacterial density rose to >100,000 cfu/ml. Concomitantly, routine weekly testing of DUWL output water from several sentinel DCUs showed bacterial densities >100,000 cfu/ml despite the once-weekly DUWL disinfection regime with Planosil Forte. This incident necessitated disconnecting all of the hospital's DCUs from the tank supply and providing each with fresh potable quality water in clean independent reservoir bottles until the contamination problems with the tank could be resolved. These findings highlighted the necessity for effective control of water quality throughout the DCU supply water network in dental hospitals and multi-DCU clinics and not only within DUWLs.

Electrochemical activation (ECA) technology was pioneered as a specialised discipline of electrochemistry by Professor Vitold Bakhir in the 1970s in the former Soviet Union (Bakhir, 1997; Bakhir *et al.*, 2001; Bakhir, 2005). Electrochemically activated solutions were generated by passing a dilute salt solution through an electric field in a Flow-through Electrolytic Module (FEM), segregating the ions formed and producing two oppositely charged solutions possessing altered physical and chemical properties (Bakhir, 2005). The activation process changes the state of the salt solution from a stable to a metastable state. The positively charged solution (anolyte) typically has a redox value of +600mV, and is composed of a mixture of unstable mixed oxidants (predominantly hypochlorous acid) in a physically excited state which is highly microbicidal and able to penetrate biofilms. The negatively charged antioxidant solution (catholyte) has detergent like properties, typically a pH of 11, a redox value of -600mV and contains predominantly sodium hydroxide in an excited state. These active ion species and free radicals are short lived with a half-life of typically less than 48 hours (Bakhir, 2005). Activation also generates electrically and chemically active microbubbles of electrolytic gas, 0.2 to 0.5 μm in diameter, which enhance the redox potential of anolyte and catholyte (Bakhir, 2005). Different kinds of ECA solutions can be generated by different types of ECA generators, each with specific properties and applications (Bakhir, 2005). Over the years, several generations of FEM were developed, the FEM-3 being one of the more recent (Bakhir, 1997 and 2005). ECA solution production with consistent quality and characteristics was difficult to achieve prior to the development of FEM-3 technology. FEM-3-based ECA technology outside

Russia is now owned by and has been further refined by the Trustwater Group, Clonmel, Ireland. Anolyte (Ecasol™) produced by Trustwater Group generators has a neutral pH, very much in contrast to anolyte produced by earlier generators from other manufacturers, which was often acidic and corrosive (Bakhir, 1997; Bakhir *et al.*, 2001; Bakhir, 2005).

5.1.1. Aim

- To develop a centralised DCU supply water pre-treatment system capable of automatically and consistently maintaining both the DUWL supply and output quality below the EU potable bottle water standard maximum of 100 cfu of aerobic heterotrophic bacteria per ml. To achieve this we decided to use automated enhanced filtration of mains water destined for DCUs followed by automated disinfection using the electrochemically activated (ECA) solution Ecasol™.

5.2. Materials and Methods

5.2.1. Measurement of Residual Free Available Chlorine in Dental Unit Waterline Output Water

Residual Free Available Chlorine (FAC) in Ecasol™-treated processed mains water and in DUWL output water was measured using a Hach Pocket Colorimeter II (Hach Company, Iowa, USA) analysis system which uses *N,N*-diethyl-*p*-phenylenediamine (DPD) to react with free chlorine and form a red solution, whose colour intensity is proportional to the chlorine concentration. The equipment was used according to the manufacturer's instructions.

5.2.2. Mains Water Filtration

Water from the mains potable supply to the DDSH was subjected to sequential filtration through a number of specific water filter types in order to provide DCU supply water of consistent physical and chemical composition (Fig. 5.1). The final configuration of filters used, was arrived at by preliminary small scale testing. Mains water was initially filtered through a self-cleaning Depura Automatic particle filter (GEL S.p.a., Acona, Italy) fitted with a 95 μm micron stainless steel filtration cartridge (Fig. 5.2, panel a). This system requires minimal maintenance because the filtration cartridge is cleaned by timed automated back washing with mains water when there is no demand on the water supply (Coleman *et al.*, 2007; Coleman *et al.*, 2009). This is combined with an effective mechanical brushing system. The water supply was next subjected to water softening by ion exchange using Crystal-Right™ zeolite crystals (Aqua Solutions Inc., Hazelton, PA, USA), a medium manufactured from sodium aluminosilicate. The crystals have an irregular surface and have a greater capacity for ion exchange compared to uniform spherical bead resins used in most conventional water softeners. This medium provides excellent water softening, iron and manganese removal, while at the same time, raising pH levels of acidic water (O'Donnell *et al.*, 2009). The supply water was then filtered through a granulated activated carbon filter (Norit Nederland B.V., Amersfoort, the Netherlands) to remove chlorine, organic compounds and volatile organic compounds. Figure 5.2, panel b shows the water softening unit and activated carbon filter unit used in this study. The carbon medium was supplemented with 15% (v/v) Kinetic Degradation Fluxion (KDF) KDF-85 filter medium (KDF Fluid Treatment Inc., Three Rivers, MI, USA), which is effective in controlling the build-up of bacteria, algae, fungi

and scale in granular activated carbon beds as well as reducing iron and hydrogen sulphide from municipal or other water supplies. KDF filter media consist of granulated, high-purity copper and zinc alloys that are designed to inhibit microbial growth in filters, remove or reduce chlorine content, iron, heavy metals and hydrogen sulphide by a redox (approx. -500 mV) process. The final stage of pre-treatment consisted of passing the water through a KDF-55 fine filter unit (Fig. 5.3). All filters were sized to permit a minimum flow rate of 3,000 litres of water per hour. The KDF-85 filter medium and the granular activated charcoal medium are certified to NSF International Standard 61 for water treatment plant applications (Anonymous, 2008). KDF-55 filter medium is certified to NSF International Standard 042 for drinking water treatment units (Anonymous, NSF/ANSI 042). The KDF filter, the water softener and the activated carbon filter were each equipped with an electronic regeneration controller, which permitted each filter to regenerate by backwashing out of phase at night with fresh mains water when there was no demand on the water supply and the effluent in each case was discharged to drain. Following filtration, the water destined for DCUs was stored in an 8,000-l storage tank that supplied water to each of the DDSH's 103 DCUs *via* a dedicated re-circulating ring main (Fig. 5.1). Each of the regeneration controllers was configured to send an electronic signal to the hospital's Building's Management System (BMS) computer following each regeneration cycle where the information was then recorded.

5.2.3. Use of Ecasol™ to Disinfect Dental Unit Waterlines and Associated Water Supply Network

The electrochemically-generated disinfectant solution Ecasol™ (Trustwater Group) was used as a residual disinfectant to minimise microbial proliferation and biofilm formation in DUWLs and throughout the dedicated ring-main water distribution network supplying water to DCUs. This included the 8,000-l water tank, the ring-main pipework and pumps and the DUWLs in each of the 103 DCUs of the DDSH (Fig. 5.1). Ecasol™ was produced by electrochemical activation (ECA) using a Trustwater Model 120 ECA generator equipped with four FEM-3 (Bakhir and Zadorozhny, 1997) type flow-through electrolytic modules (FEMs) (Figs 5.4 and 5.5). The generator was supplied with water pre-treated as described above, together with a saturated NaCl solution to give a final concentration of 0.2% (w/v). This generated a solution (Ecasol™) at neutral pH having an oxidation-reduction potential of +600 mV ±100mV and consisting of approximately

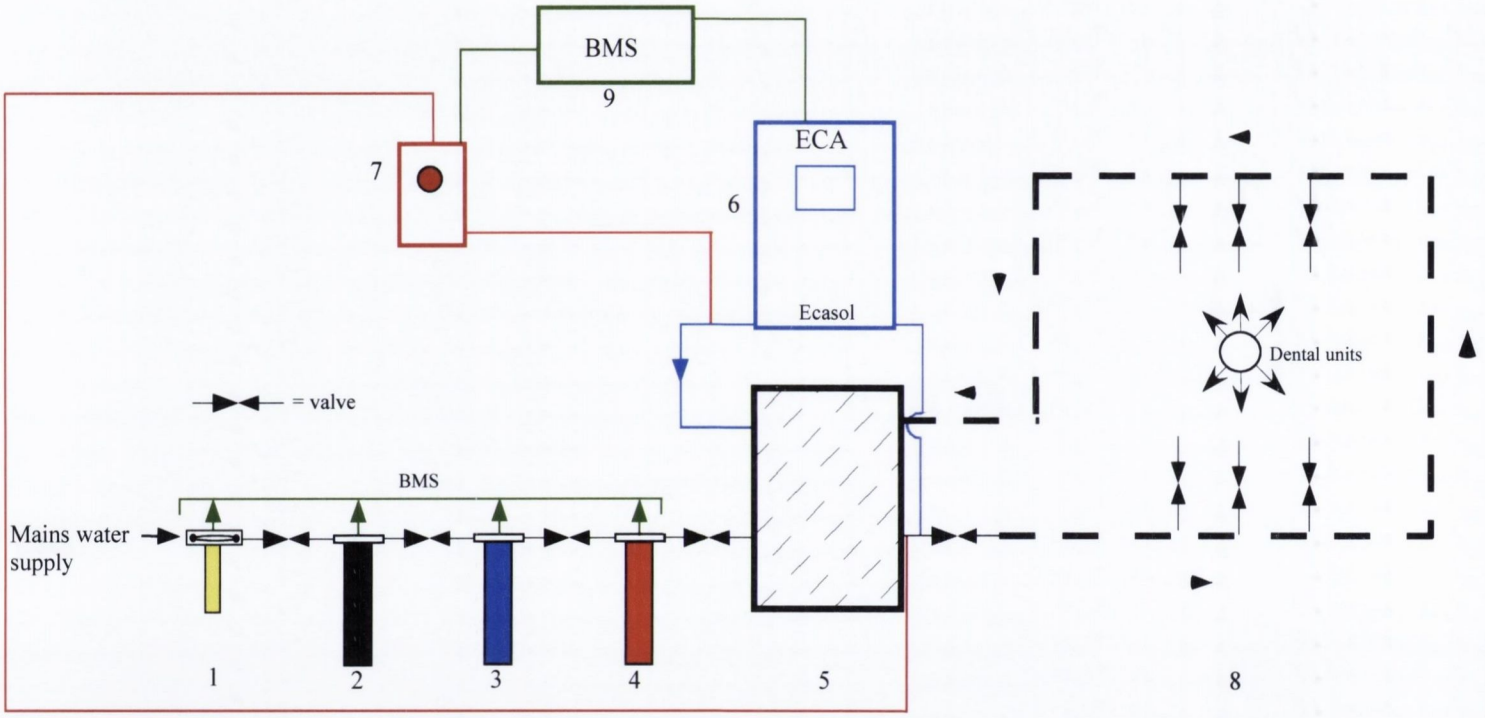


Fig. 5.1. Schematic diagram of the integrated and automated prefiltration and Ecasol™ disinfection system for maintaining the quality of DUWL supply and output water at better than potable quality at the Dublin Dental Hospital. Key: (1) self-cleaning particle filter, (2) water softening unit, (3) granular activated carbon filter with 15% (v/v) KDF-85 media, (4) KDF-55 filter, (5) 8,000-l processed water storage tank, (6) Trustwater Model 120 Ecasol™ generator, (7) Hach chlorine analyser, (8) recirculating ring main supplying water to DCUs with the direction of flow indicated by arrowheads and (9) connections to Buildings Management System (BMS) computer and remote site monitoring station.

(a) (b)

Fig. 5.2. Views of individual components of the centralised water treatment system used in the present study. Panel (a) shows a view of the self-cleaning Depura automatic particle filter fitted with a 95 mm stainless steel filtration cartridge. The filtration cartridge is cleaned by timed automated back-washing with mains water when there is no demand on the water supply. Panel (b) shows a view of the activated carbon filter (left) unit and the water softening unit (right). Each unit is fitted with an electronic regeneration controller (*).

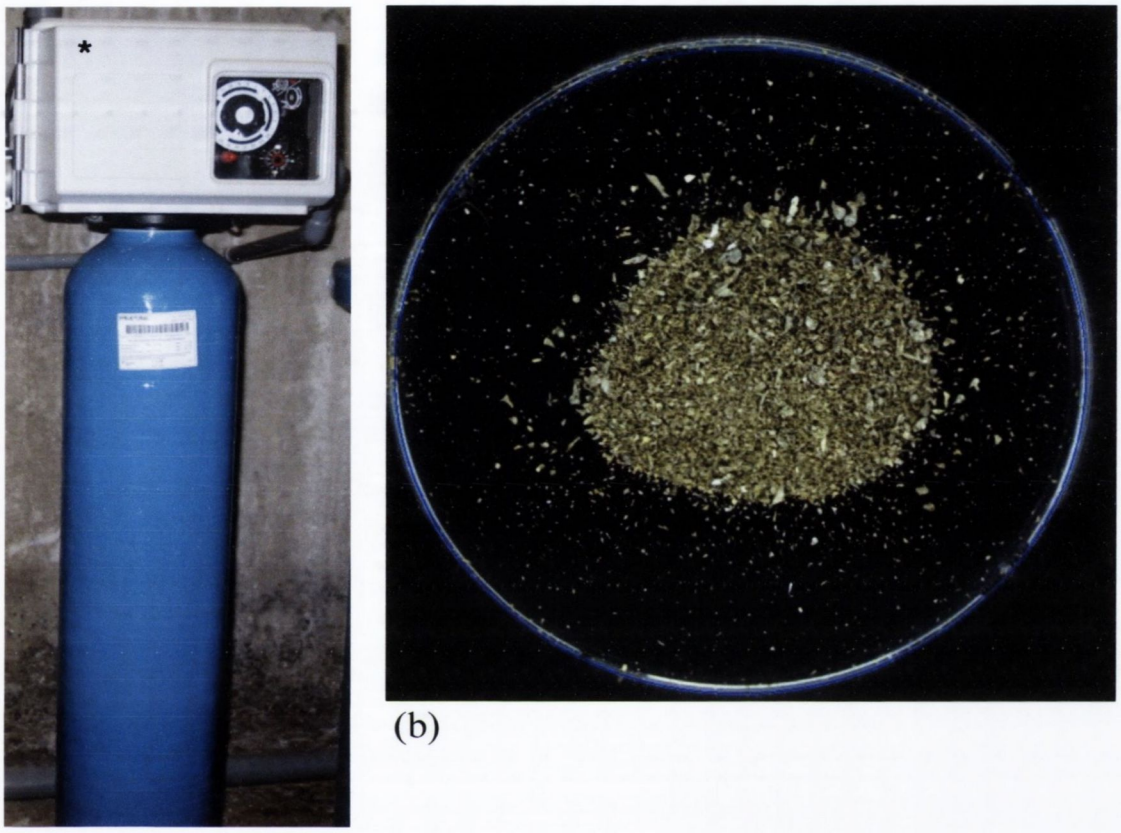


Fig. 5.3. View of a KDF filter containing granulated KDF 55 filter medium. Panel (a) shows the filter unit fitted with an electronic regeneration controller (*). Panel (b) shows a sample of KDF 55 medium in a plastic Petri dish. KDF media consist of granulated high-purity copper and zinc alloys that are designed to inhibit microbial growth in filters, remove or reduce chlorine, iron, heavy metals and hydrogen sulphide by a redox process.

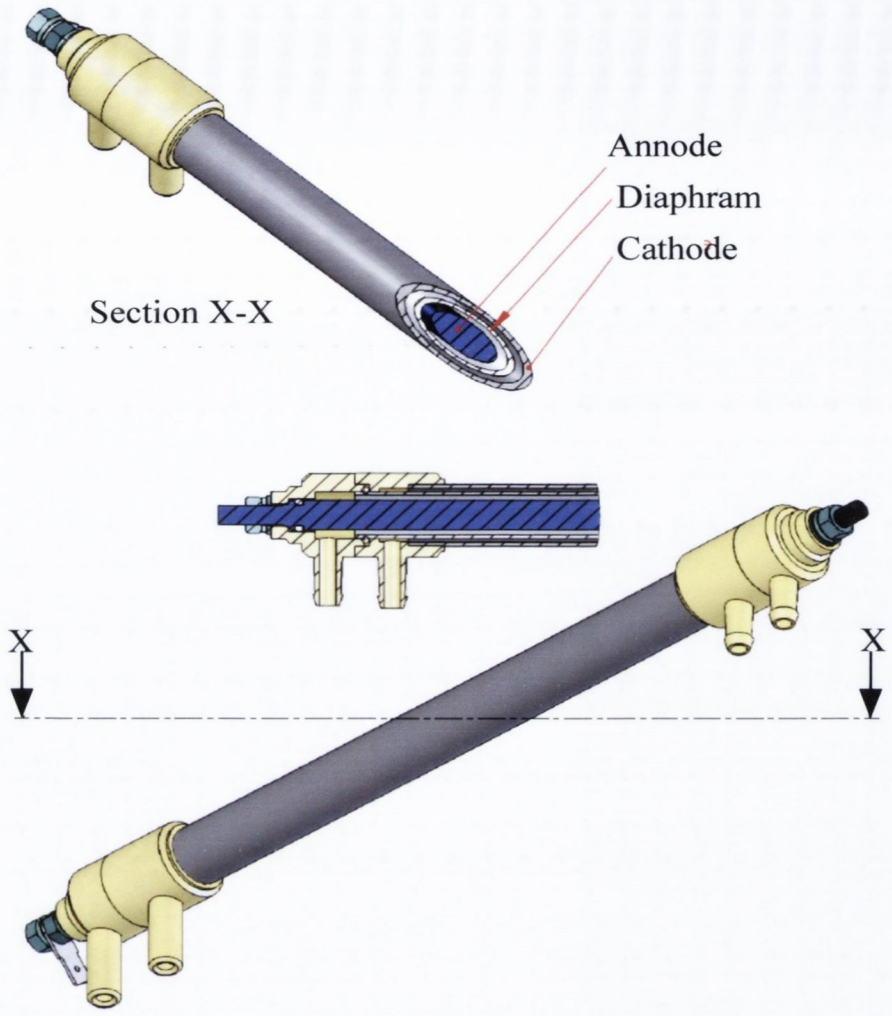


Fig. 5.4. Schematic diagram of a Trustwater FEM 3 module. Electrochemically activated solutions are generated by passing a dilute salt solution through an electric field in the FEM, segregating the ions formed and producing two oppositely charged solutions with altered physical and chemical properties. The activation process changes the state of the salt solution from a stable to a metastable state. The positively charged solution (anolyte) typically has a redox value of +600 mv, and is composed of a mixture of unstable mixed oxidants (predominantly hypochlorous acid) in a physically excited state which is highly microbicidal. The negatively charged antioxidant solution (catholyte) has detergent like properties, typically a pH of 11, a redox value of -600 mv and contains predominantly sodium hydroxide in an excited state. These active ion species and free radicals are short lived with a half-life of typically less that 48 h. Activation also generates electrically and chemically active microbubbles of electrolytic gas, 0.2 to 0.5 μm in diameter, which enhance the redox potential of anolyte and catholyte. Anolyte (Ecasol™) produced by the FEM 3 has a neutral pH.

(a) (b)

Fig. 5.5. View of a Trustwater ecaflo 120 ECA generator. Panel (a) shows an outside view of the generator. Panel (b) shows a view of the inside of the generator. The unit is equipped with four FEM 3 modules, which are visible in the lower left-hand corner of the unit. The outer two FEM 3 modules are labelled with a blue asterisk.

200 ppm metastable oxidants (predominantly hypochlorous acid ~158 ppm, hypochlorite ion ~ 42 ppm, ozone < 1 ppm, chlorine dioxide < 2.5 ppm, chloric acid <1.5 ppm and chlorous acid <3 ppm) (Ecasol SDS, 2009; O'Donnell *et al.*, 2009). The activated oxidants (Ecasol's activated state lasts for a period of up to 48 h), which are formed initially are in dynamic equilibrium and gradually revert to the initial ingredients (i.e. water supplied and 0.2% NaCl) over time. The formed substances are additionally in an electrochemically energised state, which relaxes gradually over a period of 24-48 h. Ecasol™ initially contains energised microbubbles formed at the FEMs electrode interface (Bakhir, 1997; Bakhir and Zadorozhny, 1997; Bakhir *et al.*, 2001; Bakhir, 2005). These also dissipate by cavitation over a period of 12-24 h.

Freshly generated Ecasol™ was automatically dosed by a pump into the 8,000-l water tank supplying water to the hospital's DCUs to give a final concentration of 2.5 ppm FAC (Fig. 5.1). This level was consistently monitored by *in situ* probes and automatically adjusted as required by a Hach CL17 Chlorine Analyzer (Hach Company, Iowa, USA) (Fig. 5.1). As a safeguard, an upper FAC limit (usually 5 ppm) causes immediate cessation of dosing Ecasol™ into the tank and sends an alarm signal to the hospital's Buildings Management System (BMS) computer (see Fig. 5.1) and short message service (SMS) text messages to mobile phones of designated personnel.

5.2.4. Flushing of DUWLs Following Periods of Stagnation

To ensure the aerobic heterotrophic bacterial density of DUWL output water was consistently lower than the EU bottled potable water maximum (<100 cfu/ml) following periods of water stagnation (e.g. during the night, at weekends and during holiday periods) an optimised DUWL flushing protocol was developed. Each DUWL was flushed for a period of two min prior to the start of each clinical day and each bowl rinse DUWL was flushed for one min because its bore is larger than the other waterlines. In the Planmeca Prostyle Compact DCUs used in the DDSH this process was facilitated by the ability to programme the DCUs to automatically sequentially flush each instrument DUWL and the cup filler DUWL (O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007). Following this procedure, the bowl rinse DUWL had to be flushed separately by pressing the appropriate function key on the DCUs instrument console. In the latest Prostyle Compact DCU models, all DUWLs can be programmed to automatically sequentially flush all DUWLs once the instrument hoses containing the DUWLs have been placed in flushing holders.

5.2.6. Maintenance of the Dental Chair Unit Supply Water Filtration and Disinfection System

Efficient operation of the integrated DCU supply water filtration and disinfection system required occasional maintenance. Consumable reagents for specific components required replenishing, including the saturated 0.2% (w/v) NaCl solution for the Ecasol™ generator (once-monthly), the NaCl for the brine tank supplying the water softening unit (once-monthly), citric acid descaler for the FEM-3 cells (once-monthly) and calibration chemicals for the Hach CL17 Chlorine Analyzer (once-monthly). Planned preventive maintenance was also instituted on a six-monthly basis. This was undertaken under contract by the Trustwater Group and consisted of simple equipment performance evaluation and calibration checks and component cleaning. The KDF and granular activated carbon media require replacement after five years and the water-softening medium after three years. Periodically, some Ecasol™ was added to the brine tank supplying the water softener to eliminate any biofilm that had formed in the unit's ion-exchange media and from the downstream carbon filter's activated carbon medium.

5.2.7. Collection of Water Samples

After flushing for one min, samples (50 ml) of filtered, Ecasol™-treated water (hereafter referred to as processed water, (see Sections 5.2.7 and 5.2.8 below)) were collected each week for 100 consecutive weeks directly from the operator's three-in-one air/water syringe waterline on 10 Planmeca Prostyle Compact DCUs located in three separate clinics in the DDSH as described previously (see Chapter Two, Section 2.2.3). Prior to sampling, the operator's three-in-one air/water syringe was flushed for one minute. At the same time, weekly water samples were also taken from the unprocessed mains water supply to the DDSH and from the 8,000-l storage tank supplying processed water to the hospital's 103 DCUs. Additional processed water samples were also taken periodically from the bowl rinse and cup filler outlet from each DCU. At six-monthly intervals, individual processed water samples from operator three-in-one air/water syringe waterlines from 25 other DCUs were also tested. All samples were processed immediately in the adjacent microbiology laboratory.

5.2.8. Laboratory Processing of Water Samples

Water samples were cultured in duplicate on R2A agar plates (Lab M Ltd., Bury, Lancashire, UK.) to determine total aerobic heterotrophic bacterial density as described in Chapter Two, Section 2.2.4). After 10 days incubation at 20-22°C, plates were examined and colonies counted using a Flash and Go™ automatic colony counter (IUL Instruments Ltd., Barcelona, Spain). R2A agar is the medium of choice for monitoring heterotrophic bacterial counts in water as it permits the recovery of significantly more organisms than conventional, more nutritious culture media, at 20°C compared to 35°C. Higher counts of bacteria are recovered on this medium following prolonged incubation (i.e. 10 days) ensuring that the maximum number of bacteria are detected (Reasoner and Geldreich, 1985). The inclusion of sodium pyruvate in R2A medium also leads to enhanced recovery of chlorine-stressed bacteria from water (Reasoner and Geldreich, 1985; Calabrese and Bissonnette, 1990).

The total number of colonies and the relative abundance of different colony types were recorded as the average of the duplicate plates, and representative examples of all colony types present on each plate were purified by subculture and stored on nutrient agar slopes in the dark, for subsequent identification. These were Gram-stained, tested for Catalase and oxidase production and then identified as described in Chapter Two Section 2.2.5.4. For 100 duplicate processed water samples (80 from DCUs and 20 from the 8,000-l tank), one sample in each case was supplemented with 1% (w/v) sodium thiosulphate to neutralise residual chlorine. No significant differences in the density or types of aerobic heterotrophic bacteria recovered were observed following culture on R2A agar as described above and so subsequent processed samples were not treated with thiosulphate. Experiments were also performed to demonstrate that free available chlorine present in concentrations of Ecasol used to treat processed water destined for DCUs (i.e. maximum of 2.5 ppm) was inactivated upon contact with R2A agar. Fresh R2A agar (25 ml) in 90 mm Petri dishes was flooded with 5 ml of Ecasol™ solution at 2.5, 5 and 10 ppm FAC, respectively. After 10 min contact, the liquid was withdrawn with a sterile pipette and the free available chlorine levels measured as described in Section 5.2.3. For each concentration of Ecasol™ tested, no FAC was detected following contact with R2A agar, indicating neutralisation.

5.3. Results

5.3.1. Management of Dental Unit Waterline Output Water Quality

In June 2006, during a period of warm weather, routine weekly microbiological monitoring detected overgrowth of *P. fluorescens* (i.e. >100,000 cfu/ml) in the 8,000-l water storage tank supplying the hospital's DCUs. This bloom was associated with an elevated iron concentration (5 ppm) in the mains supply water, 250 times higher than the average level present in mains water supplied to the DDSH in the 24-month period prior to June 2006 (data not shown). For approximately one year prior to June 2006, the average density of aerobic heterotrophic bacteria in the mains supply and from the tank supplied by it was 81 and 136 cfu per ml, respectively (Fig. 5.6, panel a) and the iron concentration averaged 0.02 ppm.

Maintaining DUWL output water quality below the ADA standard of ≤200 cfu/ml of aerobic heterotrophic bacteria with once-weekly disinfection of DUWLs with Planosil Forte clearly could not be sustained if the microbiological and/or chemical quality of the DCU supply water provided by the 8,000-l tank deteriorated on a regular basis. For this and other reasons (see below) we decided to develop a centralised DCU supply water pre-treatment system capable of automatically and consistently maintaining DUWL supply and output quality below the EU potable bottle water standard maximum of 100 cfu of aerobic heterotrophic bacteria per ml regardless of seasonal fluctuations in ambient temperature or variations in the chemical quality of mains supply water. We also wanted to develop a centralised system for maintaining good quality DUWL output water that would (a) reduce the time spent by nursing staff on DUWL disinfection using our existing disinfection protocol using Planosil with the Planmeca WCS™ system (up to 50 hours per week for the hospital's 103 DCUs), (b) reduce the use and handling of chemicals in DUWL disinfection, (c) introduce more uniformity into DUWL disinfection and (d) reduce incidences of human error in relation to DUWL disinfection.

5.3.2. Enhanced Pre-Filtration of Mains Water

In order to ensure that the chemical quality of water supplied to DCUs was consistent regardless of fluctuations in the municipal mains supply, we first applied enhanced filtration to mains water prior to its delivery to the 8,000-l tank that provides water to the DDSH 103 DCUs (Fig. 5.1). A series of new filters, each capable of processing

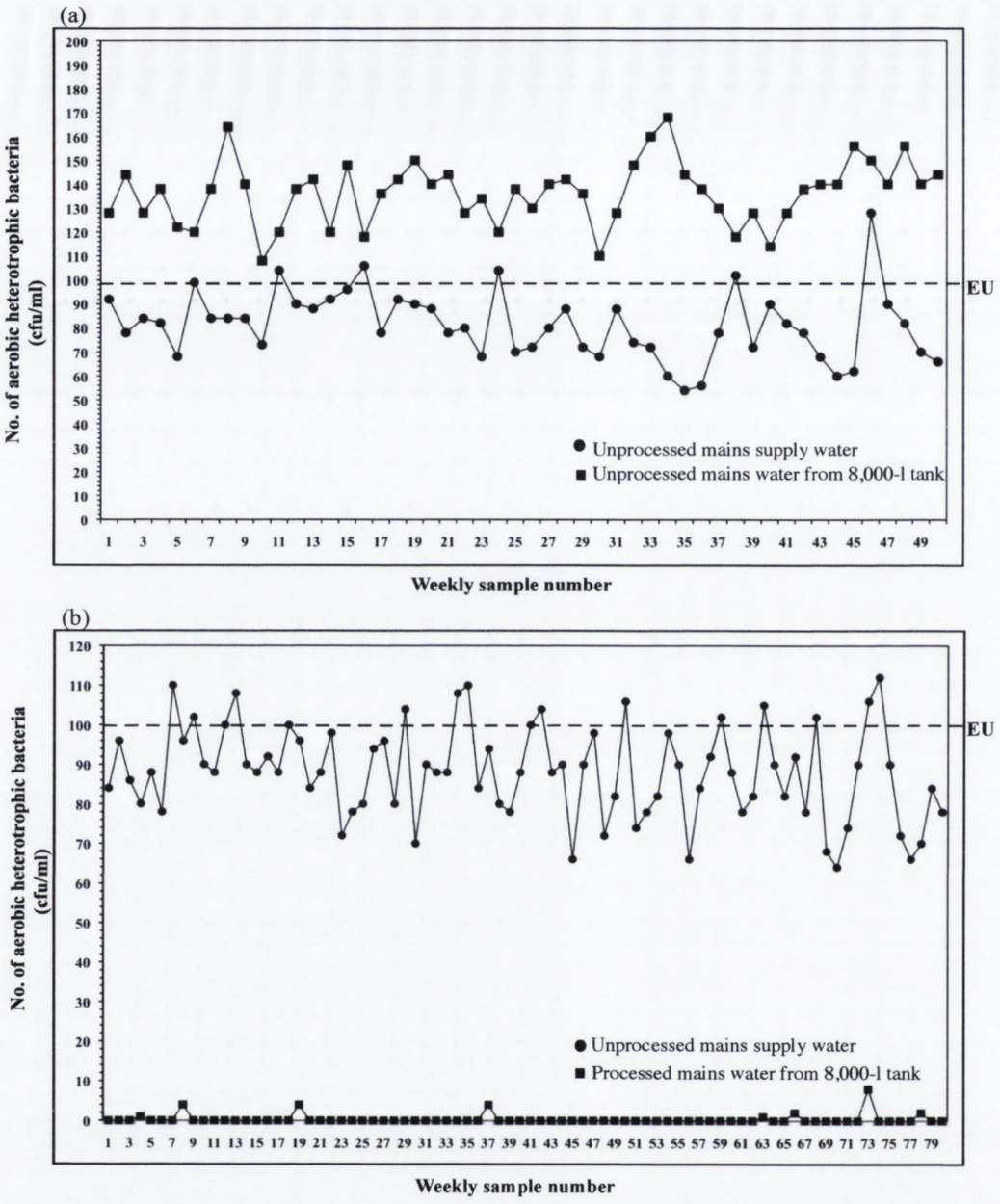


Fig. 5.6. Density of aerobic heterotrophic bacteria in unprocessed and processed mains water destined for DCUs in the Dublin Dental Hospital. Panel (a) shows the bacterial density in unprocessed mains water (average density 81 cfu/ml) and in water from the 8,000-l mains water storage tank (average density 136 cfu/ml) supplying the hospital's DCUs for a 50-week period between June 2005 and May 2006. Panel (b) shows the bacterial density in unprocessed mains water (average density 88 cfu/ml) and in processed (i.e. filtered and Ecasol™-treated) water from the 8,000-l mains water storage tank (average density <1 cfu/ml) supplying the hospital's DCUs for a 100-week period between September 2006 and August 2008. The dashed line marked EU shown in both panels indicates the maximum density of aerobic heterotrophic bacteria in cfu/ml permissible in bottled potable water according the EU Directive on water for human consumption as no upper limit is specified for potable quality mains water (Anonymous, 1998).

3,000-l of municipal mains water per hour, was installed (Fig. 5.1). Mains water was initially filtered through a particle filter, (to remove large particles and sediment >90 μm), followed by water softening using ion exchange, (to remove calcium, magnesium and iron), followed by filtration through a granular activated carbon filter, (to remove organic compounds), and finally by filtration through a KDF filter (to remove chlorine, iron and heavy metals). Between July 2006 and September 2008 the chemical composition of this processed water was analysed every two months, both from the 8,000-l storage tank and from DUWLs and in each case was found to be consistently well below the maximum limits detailed in the EU Directive on the quality of bottled water for human consumption (Table 5.1).

5.3.3. Efficacy of Automated Ecasol™ Disinfection of Dental Chair Unit Supply Water and Dental Unit Waterline Output Water

For a period of 100 consecutive weeks, between September 2006 and August 2008, unprocessed mains water supplied to the DDSH and processed mains water (i.e. filtered and Ecasol™-treated water) from the 8,000-l tank supplying the hospital's 103 DCUs was tested weekly for density of aerobic heterotrophic bacteria. During this period, the average density in unprocessed mains water and processed water was 88 cfu/ml and <1 cfu/ml, respectively (Fig. 5.6, panel b). The average bacterial density in DUWL output water from the operator's three-in-one air/water syringe DUWL from the 10 sentinel DCUs included in the 100-week study period was 18.1 cfu/ml (Table 5.2). Of the 10 DCUs included in the study, 3/10 (300 samples in total) yielded an average bacterial density in DUWL output water of <3 cfu/ml (range 0-20 cfu/ml) over 100 consecutive weekly water samples tested per DCU (Table 5.2). The microbiological quality of every sample from these DCUs was well below the maximum permissible density of 100 cfu/ml permissible in bottled water for human consumption (Fig. 5.7). For the remaining seven DCUs, 92.3% (646/700) DUWL output water samples tested also yielded bacterial densities well below 100 cfu/ml, however, the remaining 7.7% (54/700) water samples from these seven DCUs exceeded 100 cfu/ml (Table 5.2). In 53/54 cases the high bacterial counts were intermittent and did not occur in consecutive samples (Fig. 5.8). Examination of the circumstances surrounding these 54 water samples revealed that in each case either the DCU concerned had not been used for several days prior to water sampling and its DUWLs had been left stagnant, or one or more DCU water outlets (i.e. the cup filler and/or the bowl rinse waterline) had been

turned off by clinical staff or students resulting in water stagnation in these DUWLs. Ensuring that the water supply to each DUWL in each DCU was turned on and flushed each morning (see Chapter Five, Section 5.2.4) and reiterating that DCUs that were not being used must have their DUWLs flushed each morning ensured that the bacterial density in DUWL output water remained well below 100 cfu/ml. In the one DCU that yielded DUWL output water with a bacterial density >100 cfu/ml in two consecutive weekly samples (see Fig. 5.8, panel a; DCU No. 5; Table 5.2), it was discovered that the DCU had been taken out of service for repairs not relating to the DUWL-supplied dynamic instruments and had not been used for two weeks. Examination of a section of DUWL tubing from this DCU's operator's three-in-one air/water syringe revealed the presence of extensive biofilm (see Fig. 5.9, panels b and c). Flushing of its DUWLs every day for a week resulted in bacterial densities well below 100 cfu/ml in all subsequent water samples tested. Water from the cup filler and bowl rinse DUWLs of the 10 sentinel DCUs sampled at 12 week intervals during the study period yielded average bacterial densities of 7 and 9 cfu/ml, respectively. Furthermore, water samples from the operator's three-in-one air/water syringe DUWL from 25 other DCUs sampled at six-monthly intervals during the study period yielded an average bacterial density of 9 cfu/ml.

Samples of Ecasol™-treated water from the 8,000-l tank and from DUWLs were investigated at four-monthly intervals for the presence of trihalomethanes. Trihalomethanes are formed as a by-product when chlorine or bromine are used to disinfect water for drinking. They result from the reaction of chlorine and/or bromine with organic material present in the water being treated. The levels of trihalomethanes detected in Ecasol™-treated water were consistently within the limits detailed in the EU Directive on water for human consumption (Table 5.1).

5.3.4. Bacterial Species Detected in Water

R2A agar was used to determine the total aerobic heterotrophic bacterial density in unprocessed mains water, processed mains water and in DUWL output water. These organisms were chosen as markers for microbial contamination as many previous studies have shown that they are the predominant microorganisms present in DUWL output water (Barbeau *et al.*, 1996; Pankhurst *et al.*, 1998; Walker *et al.*, 2000; Tuttlebee *et al.*, 2002; O'Donnell *et al.*, 2007). The majority of species recovered from unprocessed mains water on R2A agar were Gram-negative (Table 5.3). However, the

Table 5.1. Chemical analysis of processed supply water for dental units in the Dublin Dental School and Hospital

Test	Processed[a] mains water (mg/l)	Potable water EC maximum conc.[b] (mg/l)	Method of analysis[c]
Calcium	2.00	N/A	ICP-MS
Magnesium	0.95	N/A	ICP-MS
Sodium	54.80	200.00	ICP-MS
Potassium	0.41	N/A	ICP-MS
Iron	0.02	0.20	ICP-MS
Manganese	0.00	0.05	ICP-MS
Copper	0.03	2.00	ICP-MS
Lead	<0.001	0.01	ICP-MS
Zinc	0.39	N/A	ICP-MS
Silver	<0.001	N/A	ICP-MS
Chromium	<0.01	0.05	ICP-MS
Cadmium	<0.001	0.01	ICP-MS
Nickel	<0.001	0.02	ICP-MS
Carbonate	25.00	N/A	Titration
Chloride	69.70	250.00	Photometry
Sulphate	<40.00	250.00	Photometry
Nitrate as NO_3	4.63	50.00	Photometry
Nitrite as NO_2	0.09	0.50	Photometry
Ammonia as NH_4	0.00	0.50	Photometry
Fluoride	0.87	1.50	Photometry
Ortho Phosphate as PO_4	0.27	N/A	Photometry
Total organic carbon	<2.0	N/A	Photometry
Chloroform	<0.01	0.1	GC-MS
Bromodichloromethane	<0.01	0.1	GC-MS
Dibromochloromethane	<0.01	0.1	GC-MS
Bromoform	<0.01	0.1	GC-MS
Trichloroethane	<0.01	0.01	GC-MS
Tetrachloroethane	<0.01	0.01	GC-MS
1,2-dichloroethane	<0.001	0.003	GC-MS
pH	7.60	6.5-9.5	Meter
Conductivity at 25°C (µS/cm)	460.00	2500.00	Meter

[a]Chemical analysis of processed DCU supply water was undertaken every two months between September 2006 and August 2008. The data shown is representative of the results obtained during this period.

[b]European Community maximum permissible concentration in water for human consumption (Anonymous, 1998).

[c]Abbreviations: ICP-MS, inductively coupled plasma-mass spectrometry; GC-MS, gas chromatography-mass spectrometry; N/A, no maximum permissible concentration in water for human consumption.

Table 5.2. Density of aerobic heterotrophic bacteria in 100 consecutive weekly water samples from DUWLa output water from ten DCUs supplied with Ecasol™-treated water

DCU No	Average bacterial density cfu/ml[a]	Bacterial density range	No. weekly samples/100 yielding bacterial density > 100 cfu/ml[b]
1	1.6	0-18	0 (0%)
2	2.3	0-20	0 (0%)
3	1.7	0-16	0 (0%)
4	28.3	0-840	8 (8%)
5	38.1	0-1060	7 (7%)
6	29.3	0-960	9 (9%)
7	22.2	0-222	5 (5%)
8	17.0	0-400	8 (8%)
9	21.7	0-306	7 (7%)
10	19.4	0-610	10 (10%)
Overall	**18.1**	**0-1060**	**54 (5.4%)**

[a]Water samples were taken from the operator's three-in-one air/water syringe from each DCU.

[b]The maximum permissible density of aerobic heterotrophic bacteria in bottled water for human consumption is 100 cfu/ml (Anonymous, 1998).

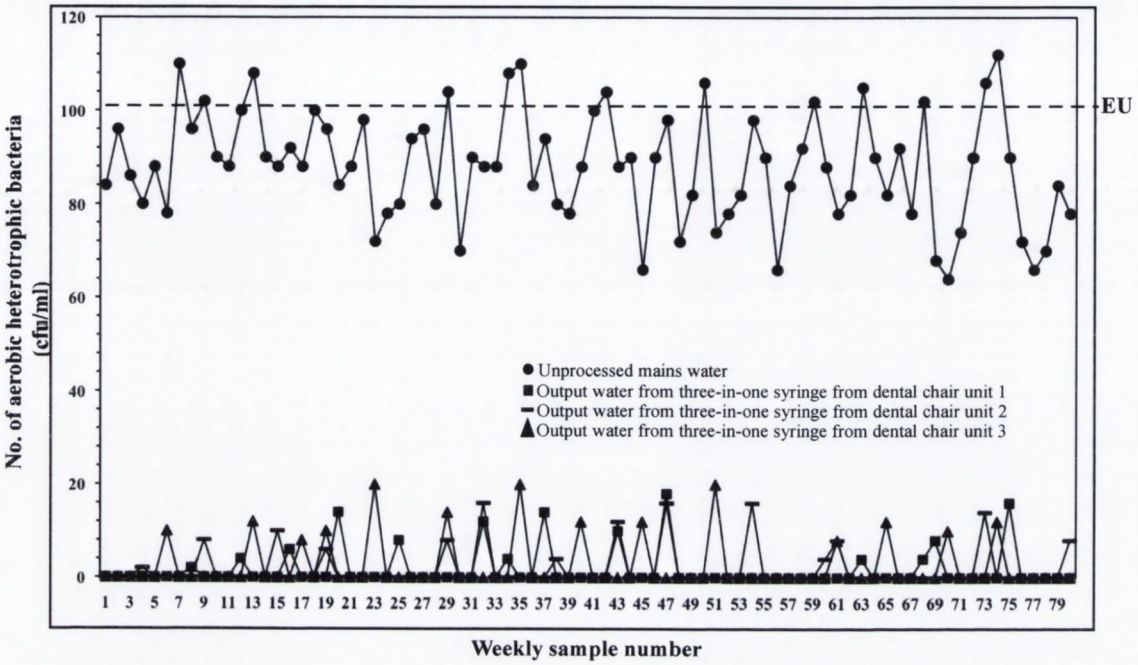


Fig. 5.7. Density of aerobic heterotrophic bacteria in unprocessed mains water supplied to the Dublin Dental hospital and in processed (i.e. filtered and Ecasol™-treated) DUWL output water from the operator's three-in-one air/water syringe DUWL from DCUs Nos. 1-3 (Table 5.2) with average bacterial densities of 1.6, 2.3 and 1.7 cfu/ml, respectively, during the100-week study period between September 2006 and August 2008. The dashed line marked EU indicates the maximum density of aerobic heterotrophic bacteria in cfu/ml permissible in bottled potable water according the EU Directive on water for human consumption as no upper limit is specified for potable quality mains water (Anonymous, 1998).

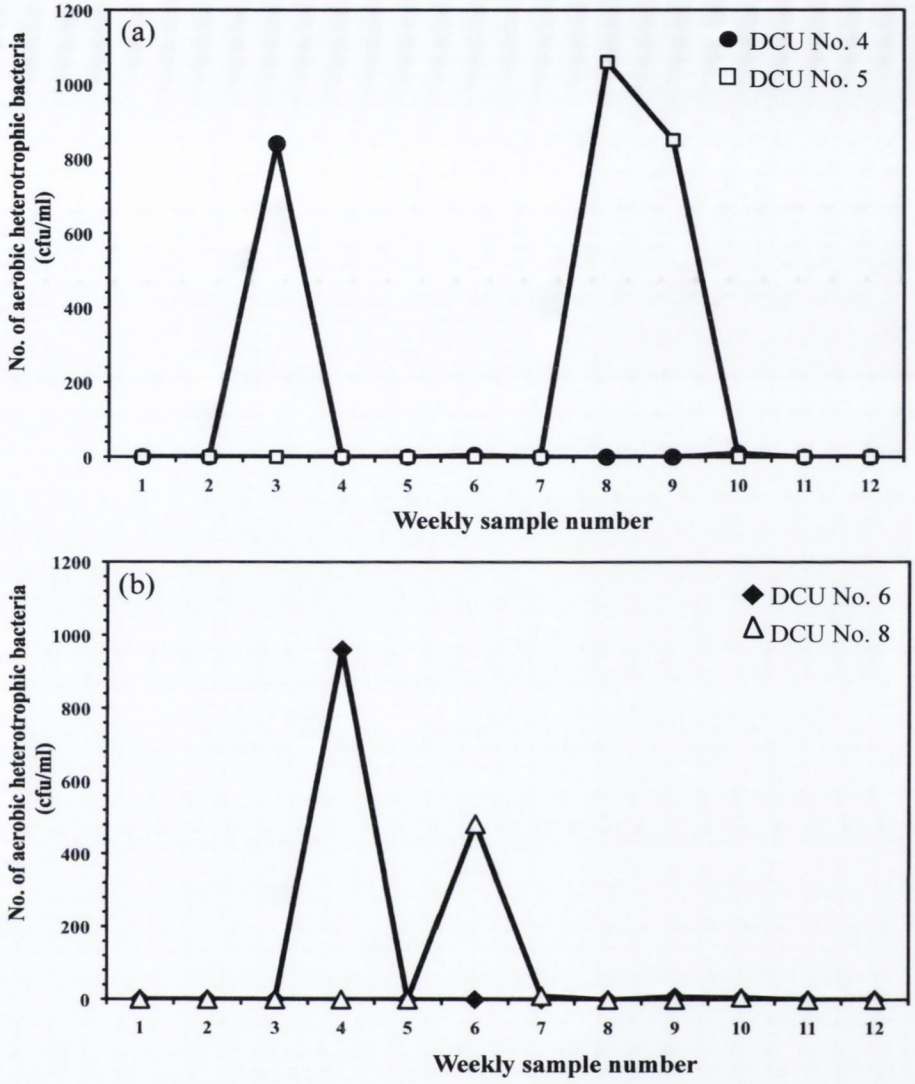


Fig. 5.8. Panels (a & b) show the density of aerobic heterotrophic bacteria in processed (i.e. filtered and Ecasol-treated) DUWL output water from the operator's three-in-one air/water syringe DUWL from four separate DCUs for a period of 12 consecutive weeks during the 100-week study period showing instances of intermittent poor quality output water. In all cases the cause of poor quality output water was found to be due to water stagnation in the DUWLs in the DCUs concerned, either because the DCU was not used for a period of up to two weeks due to breakdown and its DUWLs had not been flushed daily, or because the patient cup filler DUWL and/or the cuspidor DUWL had been turned off in the DCUs concerned. All of the DUWLs in DCUs are interconnected and stagnation in one can result in biofilm formation that can contaminate other waterlines.

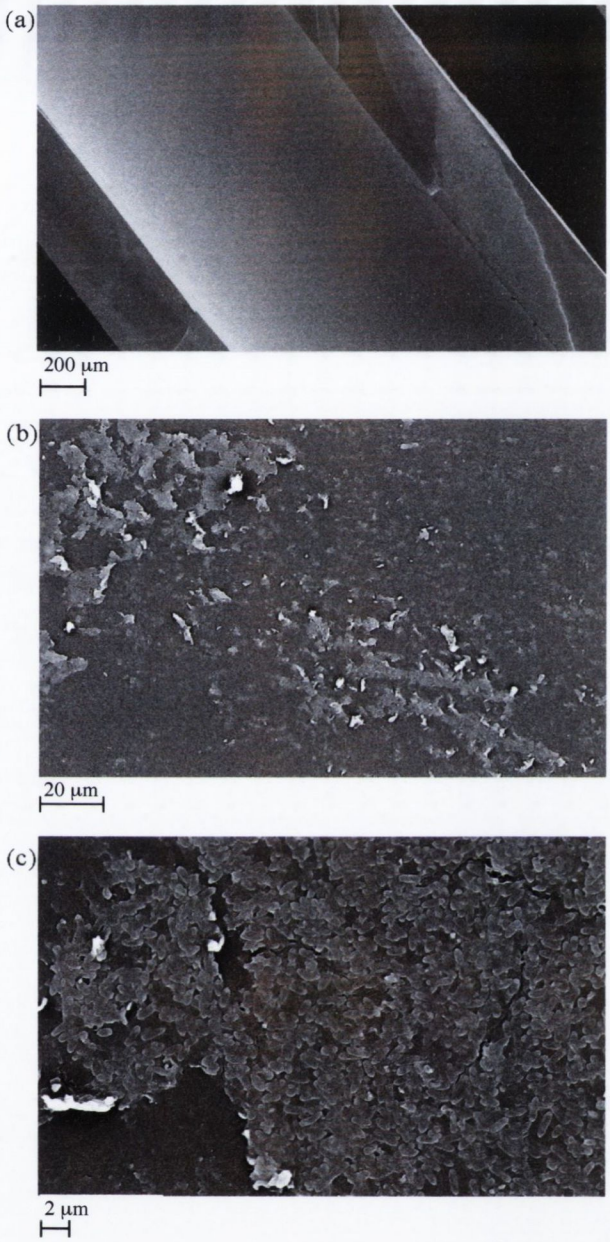


Fig. 5.9. Electron micrographs of longitudinal sections of DUWL tubing from thee-in-one air/water syringe DUWLs supplied with filtered and Ecasol™-treated water. Panel (a) shows a low magnification view of a section of DUWL from DCU No. 2 showing the absence of biofilm. Output water from this DUWL yielded an average density of aerobic heterotrophic bacteria of 2.3 cfu/ml over the 100-week study period (Table 5.2). Panels (b & c) show medium and high magnification views of the same DUWL section from DCU No. 5 taken after the DCU had been out of service for two weeks due to a mechanical problem during which time its DUWLs had been left stagnant (see also Table 5.2 and Fig. 5.4). Extensive biofilm is evident in both views.

Table 5.3. Bacterial species isolated on R2A agar from unprocessed mains water supplied to the Dublin Dental School and Hospital during the 100-week study period

Bacterial species*	Gram-stain
Aeromonas salmonicida	Negative
Acidovorax temperans	Negative
Comamonas acidovorans	Negative
Novosphingobium subarctica	Negative
Sphingomonas paucimobilis	Negative
Sphingomonas aerolata	Negative
Sphingomonas trueperi	Negative
Porphyrobacter tepidarius	Negative
Pseudomonas fluorescens	Negative
Arthrobacter agilis	Positive
Kocuria palustris	Positive
Micrococcus luteus	Positive
Rhodococcus fascians	Positive
Variovorax paradoxus	Positive

*Identified by DNA sequence analysis of the variable region of 16S rDNA gene (O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007).

Gram-positive organism *Micrococcus luteus* was the most common bacterial species identified. Bacterial isolates were hardly ever recovered from processed (i.e. filtered and Ecasol™-treated) mains water (average density <1 cfu/ml), but when they were, they belonged to the same species present in unprocessed mains water and had a similar distribution. Similarly, bacterial isolates recovered from DUWL output water reflected the species and distribution present in unprocessed mains water.

5.3.5. Electron Microscopy of Dental Unit Waterlines

For each of the 10 sentinel DCUs included in the study, samples of DUWL tubing supplying the operator's three-in-one air/water syringe were taken at intervals throughout the study and examined for the presence of biofilm by electron microscopy. Water samples were taken from the respective DUWLs for bacterial culture immediately prior to taking the DUWL samples. In every case, examination of the internal surfaces of the DUWLs showed the absence of microbial biofilm, which correlated with DUWL output water densities <10 cfu/ml (an example is shown in Fig. 5.9, panel a). Figure 5.5, panels b and c show the presence of extensive biofilm in a DUWL section from DCU No. five taken after the DCU had been out of service for two weeks during which time its DUWLs had been left stagnant (Table 5.2 and Fig. 5.8).

5.3.6. Ecasol™ Corrosion Effects

Throughout this study, metal component parts of DUWL networks in the DCUs were examined at intervals for potential corrosive effects of Ecasol at the working concentration used (maximum of 2.5 ppm). No corrosive effects were observed. Two instrument multiplexer units (which distribute water *via* DUWLs to dental instrument hoses) from DCUs included in this study were examined independently by the DCU manufacturer Planmeca who reported no corrosive effects. Finally, dental instruments attached to DUWLs that were supplied with Ecasol™-treated water showed no corrosive effects either. Examination of DUWL tubing from several of the test DCUs used in this study revealed no detectable deterioration.

5.4. Discussion

In the DDSH for the past several years, the microbial quality of DUWL output water from the hospital's 103 Prostyle Compact DCUs was consistently maintained below the ADA recommended standard of ≤200 cfu/ml of aerobic heterotrophic bacteria by once-weekly disinfection using the hydrogen peroxide and silver ion-containing DUWL disinfectants Planosil or Planosil Forte (O'Donnell *et al.*, 2006). This changed in June 2006 when a bloom of *P. fluorescens* occurred in the 8,000-l mains water storage tank supplying the hospital's DCUs, which coincided with an increased level of iron (250 times greater than average) in the mains water supply probably caused by works on the mains water distribution network by municipal authorities. Iron is essential for bacterial growth and it is likely that a combination of increased tank water temperature and available iron were significant factors in the overgrowth of *P. fluorescens* (Mossialos *et al.*, 2007). The quality of DUWL output water is directly influenced by the physical content (i.e. presence of particulate matter and suspended material), chemical content (i.e. dissolved and suspended inorganic compounds and organic material) and microbiological quality (i.e. density of microorganisms present) of the supply water (Volk and LeChevallier, 1999; Coleman *et al.*, 2007; Coleman *et al.*, 2009) and indirectly by environmental conditions such as temperature (Coleman *et al.*, 2007; Coleman *et al.*, 2009). The condition of the mains water pipework, maintenance works undertaken on the mains water distribution system, the type of pipe material used, corrosion controls and the presence of water storage tanks and their condition also play a major role in supply water quality (Mossialos *et al.*, 2007; Coleman *et al.*, 2007; Coleman *et al.*, 2009).

The overall aim of the present study was to develop a centralised and automated water quality and biofilm management system for the DDSH encompassing the entire water distribution network providing water to DCUs which could maintain DUWL output water at better than potable quality simultaneously in all of the hospital's 103 DCUs and require a minimum of human intervention. Because the hospital's DCUs are in constant use during the working week, the necessity for uninterrupted water supply requires a mains water-supplied storage tank rather than having an individual mains supply for each DCU. The presence of the 8,000-l water storage tank did influence the microbiological quality of the water supplied to DCUs. For approximately one year prior to overgrowth of *P. fluorescens* in the 8,000-l DCU supply tank in June 2006, the

average density of aerobic heterotrophic bacteria in the mains supply was 81 cfu/ml, whereas the corresponding average density in tank water was 136 cfu/ml (see Fig. 5.6, panel a). The increased density in tank water was more than likely due to the release of planktonic bacteria from biofilm on the inner surfaces of the tank originally seeded from bacteria present in mains water. Our approach to developing a centralised and automated water quality and biofilm management system for the hospital's DUWL supply network consisted of firstly developing an automated control system to maintain consistency in the chemical composition of DUWL supply water, and secondly to develop an automated control system to minimise the microbial density in both DUWL supply and output water. We achieved our first objective of maintaining consistent chemical composition in DUWL supply water despite temporal fluctuations in the chemical content of the inward supply by using a series of sequential water filters for treating mains water (Fig. 5.1 and Table 5.1). Mains water was initially subjected to particle filtration to remove sediment and suspended particles >90 μm, followed by water softening, granular activated carbon filtration and finally filtration through a KDF-55 filter. Each filter had an integrated facility programmed to automatically back-wash the filters with fresh mains water at night, out of phase, when there was no demand on the water supply. This facility increases the working life of the filters by eliminating contaminants and helps to reduce microbial proliferation in the filters. Proper maintenance of water filters and ion exchange units is essential, although often overlooked, as biofilm can develop within them, particularly ion-exchange media, after a period of use, thus adding significant microbial loads to water entering DCUs downstream (Coleman *et al.*, 2007; Coleman *et al.*, 2009). In the present study, nightly automated backwashing ensured the water-softening unit worked efficiently, whereas periodic disinfection of the softening medium with Ecasol™ minimised microbial contamination. The granular activated carbon filter medium used in this study to remove organic material was supplemented with 15% (v/v) KDF-85 medium to reduce the proliferation of microorganisms in the carbon filter bed as well as to remove other contaminants such as iron, chlorine and sulphides (KDF homepage, 2009). This combination of filters used to process the incoming mains water provided water of consistent chemical composition over the two-year period of the study, well under the maximum limits required by the EU Directive on bottled water for human consumption, without completely demineralising the water (Table 5.1; Anonymous, 1998). Highly demineralised water can have corrosive effects on metal components under specific

conditions and is an important consideration when dealing with sensitive equipment such as medical devices.

We achieved our objective of minimising microbial contamination in DUWL supply and output water by using the ECA solution Ecasol™ as a residual disinfectant in the filtered mains water supply provided to DCUs. Between September 2006 and August 2008, unprocessed mains water supplied to the DDSH had an average aerobic heterotrophic bacterial cell density of 88 cfu/ml, whereas processed (i.e. filtered and Ecasol™-treated water) from the 8,000-l water storage tank had an average bacterial density of <1 cfu/ml (Fig. 5.6, panel b). The average bacterial density in DUWL output water, tested weekly, from 10 sentinel DCUs included in the 100-week study period was 18.1 cfu/ml (see Fig. 5.7). These results demonstrated unequivocally that our centralised water treatment system for DUWL supply water was capable of maintaining the microbiological quality of DUWL supply and output water at significantly better than potable water quality for the 100-week period of the study. Electron microscopy of the internal surfaces of samples of DUWL waterline tubing taken periodically from the 10 sentinel DCUs revealed the absence of biofilm, demonstrating that the use of Ecasol™ as a residual disinfectant is effective at controlling biofilm formation in DUWLs (Fig. 5.9, panel a). Preliminary tests at the outset of the study revealed that the efficacy of Ecasol™ at maintaining DUWL output water at better than potable quality was dependent on daily flushing of each DCU's DUWLs and so a daily flushing policy was developed whereby each DUWL was flushed for two minutes each morning, apart from the bowl rinse DUWL, which was flushed for just one minute (see Section 5.2.9). Implementation of this procedure in the DDSH DCUs was facilitated by the ability to programme each DCU to flush its DUWLs automatically in the morning prior to the start of clinics (O'Donnell *et al.*, 2007). All that is required is for a member of the dental staff to initiate the process by placing the DCU's instrument hoses containing the DUWLs into flushing holders to direct the effluent to the wastewater outlet and activating the flushing procedure by pressing the appropriate key on the DCU's electronic instrument console. Despite this protocol, 54/1000 (5.4%) of the DUWL output water samples taken from the 10 sentinel DCUs over the 100-week period yielded bacterial densities above the EU potable bottled water maximum of 100 cfu/ml (Table 5.2). These 54 'failures' from seven DCUs occurred intermittently in 53/54 cases (i.e. not in consecutive weeks) (see Fig. 5.8). In every case, the reason for poor quality DUWL output water was found to be due to failure to implement the daily DUWL

flushing protocol in the DCUs concerned, where either the DCU concerned had not been used for several days prior to water sampling and its DUWLs had been left stagnant, or one or more DUWL outlets (i.e. the cup filler and/or the bowl rinse waterline) had been turned off by clinical staff or students resulting in water stagnation in these DUWLs. These findings highlight the fact that even when effective DUWL biofilm control protocols are implemented for individual DCUs, no matter how automated; even if minimal human intervention is required, the long-term efficacy of the process is dependent on rigorous adherence to the procedure. In busy dental clinics, the often-heavy demands placed on dental healthcare staff can result in poor compliance with DUWL cleaning protocols with resulting poor quality output water. In a previous study from this laboratory, 14/28 (50%) of failed DUWL disinfection cycles in eight DCUs using the Planmeca WCS™ DUWL cleaning system with Planosil disinfectant over a nine-month period were found to be due to improper implementation of the DUWL disinfection protocol (see Chapter Three; O'Donnell *et al.*, 2007).

A variety of ECA solutions, also referred to as superoxidised water, anolyte and various other names, have been used previously as a residual disinfectant to control biofilm in DUWLs and studies have shown that such solutions can be very effective (Marais and Brözel, 1999; Kohno *et al.*, 2004; Martin and Gallagher, 2005; Zhang *et al.*, 2007). However, not all ECA solutions are the same and some can have the potential for adverse effects on DUWLs and DCU instruments connected to them following prolonged use if the parameters for electrochemical activation are sub-optimal or if the product used is too concentrated or too acidic (Coleman *et al.*, 2007; Coleman *et al.*, 2009) Data from this laboratory have shown that exposure of DUWLs to insufficiently dilute anolyte or non pH neutral anolyte from a number of sources could cause some DUWLs to deteriorate and cause corrosion damage to other DCU components (Coleman *et al.*, 2007). In the present study, very dilute Ecasol™ (i.e. 2.5 ppm FAC initial dosing) was found to be quite sufficient at minimising microbial growth in a relatively large water distribution system supplying 103 DCUs in the DDSH. No adverse effects were observed on DUWLs throughout the study on metal components associated with DUWLs (i.e. solenoid valves) or with dental instruments supplied by DUWLs containing water treated with 2.5 ppm Ecasol™. To a great extent, early work on disinfection using anolyte solutions used generators that produced inconsistent and extremely aggressive acidic forms that could also cause corrosion and harm to some materials (Coleman *et al.*, 2007; Coleman *et al.*, 2009). When using ECA

to control biofilm in DUWLs it is essential that the ECA generator is capable of consistent quality output at neutral pH, such as that achieved by the generator equipped with FEM-3 modules used in the present study. The ECA product concentration in DUWL output water does not need to exceed 1-2 ppm FAC, as it is so effective. This requires accurate dosing into DUWL supply water as anolyte produced by ECA generators, including the Trustwater 120 Model ECA generator used in this study, is usually much more concentrated (e.g. 200 ppm FAC). In the present study, the use of in situ probes to monitor FAC levels in the DCU supply water distribution network and constant automated monitoring using an FAC analyser ensured that the level of FAC within treated water was strictly maintained at low levels (Fig. 5.1).

Other researchers who investigated the use of ECA solutions to control biofilm in DUWLs reported that it can take up to five weeks continuous treatment to eliminate resident biofilm from DUWLs (Marais and Brözel, 1999). Prior to the present study in the DDSH, DUWLs were disinfected once-weekly with the hydrogen peroxide and silver-ion containing disinfectant Planosil Forte, which completely eliminated biofilm from DUWLs and maintained the bacterial density in DUWL output water at <200 cfu/ml for up to seven days post-disinfection. Biofilm began to form again within a few days post-disinfection but this was then eliminated by the next weekly disinfection cycle (Tuttlebee *et al.*, 2002; O'Donnell *et al.*, 2006; O'Donnell *et al.*, 2007). Thus for several years prior to the start of this study the hospital's DUWLs were essentially free of extensive biofilm and when the DCUs began to be supplied with processed mains water, the presence of residual Ecasol™ was able to maintain the DUWLs free from biofilm. In heavily fouled DUWLs with extensive biofilm, it can take several weeks for dilute Ecasol™ to eliminate the biofilm, but once it is eliminated, continued use of dilute Ecasol™ as a residual disinfectant is effective at maintaining DUWLs free of biofilm (O'Donnell *et al.*, 2009).

Installation of our centralised and automated water treatment system to maintain DUWL output water at better than potable quality was very cost-effective. Prior to installation of the new system management of DUWLs, cleaning throughout the hospital using the Planmeca WCS™ system integrated in each DCU, required about 50 hours per week to implement. Taking this time into account, together with the cost of the DUWL disinfectant, the overall annual expenditure for managing the quality of DUWL output water was approximately €80,000. The automated system described in the present study requires minimal human intervention, although it does require

monthly replenishment of consumable reagents to specific components, which takes less than 30 min to implement and six-monthly planned preventive maintenance on equipment and pumps etc. Filter media only have to be replaced every three to five years. Overall, the operating costs are less than €20,000 per annum. Furthermore, the system is environmentally friendly, does not produce harmful waste products, spent filter media are recyclable, and staff do not have to handle potentially hazardous chemicals. It also reports its status in real time to facilities management and is capable of remote diagnostics, which are also time–saving features.

There are few quantitative scientific publications outside of Russia that consider the health effects of ECA solutions, although marketed widely in the US and Japan for both human and animal use. Researchers in the USA, Italy and Mexico have used pH neutral ECA solutions very successfully to aid wound healing and reduce infection in a wide range of patients with severe wounds, including patients with limb amputations, ulcers and burns (Allie, 2006; Altamirano, 2006; Paola, 2006; Wolvos, 2006). The US Food and Drug Administration consider Ecasol suitable for food processing applications. ECA solutions have been used extensively in Russia for over 30 years, for drinking water disinfection, swimming pool disinfection, as the general disinfectant in hospitals, as wound irrigants, as nebulised inhalant sprays and many other infection control applications with no apparent harmful effects (Bakhir, 1997; Bakhir *et al.*, 2001; Bakhir, 2005; Coleman *et al.*, 2007; Coleman *et al.*, 2009).

5.5. Conclusions

We have developed a centralised and automated biofilm management system for mains water distribution networks supplying water to DCUs that consistently maintains DUWL supply and output water at better than potable quality simultaneously in 103 DCUs in a Dental Hospital setting. The first part of the system consists of automated filtration of mains water to yield DCU supply water of consistent chemical composition, despite fluctuations in the chemical composition of the inward mains supply. The second part of the system automatically disinfects filtered mains water using dilute (i.e. 2.5 ppm) Ecasol™, a neutral ECA anolyte solution, and over the 100-week study period maintained the average microbiological quality of DUWL supply water and output water at <1 cfu/ml and 18.1 cfu/ml, respectively, of aerobic heterotrophic bacteria, significantly better than potable water. The system requires minimal human intervention, is cost-effective, environmentally friendly and provides a robust solution

to the universal problem of biofilm formation in DUWLs. The current system is ideally suited to Dental Hospitals and large dental clinics equipped with many DCUs but has immense potential for managing water quality in other healthcare facilities, including acute hospitals.

Chapter 6

General Discussion

6.1. General Discussion

6.1.1. The Problem of Biofilms in Dental Unit Waterlines

Modern DCUs are categorised as medical devices according to the EU Medical Devices Directive, which stipulates that a medical device is "*any instrument, apparatus, appliance, material or other article, whether used alone or in combination, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of diagnosis, prevention, monitoring, treatment or alleviation of disease*" (Anonymous, 1993). Microbial contamination of an extensive range of medical devices, including catheters, equipment used for the administration of anaesthetics, laryngoscopes, endoscopes, gastroscopes, colonoscopes, mechanical ventilators, resuscitation equipment and a wide variety of prostheses has been shown to be an important cause of cross-contamination and cross-infection, especially in healthcare environments (Lemaitre *et al.*, 1996; Weinstein, 1998; Bagshaw and Laupland 2006; O'Donnell *et al.*, 2006a; Fretz *et al.*, 2009; Hota *et al.*, 2009). Wet, damp or humid parts or surfaces of medical devices have been particularly associated with cross-infection and cross-contamination because such areas are favourable to the establishment and growth of microbial biofilm (Lemaitre *et al.*, 1996; Hsueh *et al.*, 1998; Perola *et al.*, 2002).

An individual DCU can be in used in the treatment of many patients each day and microbial contamination of specific component parts can be important potential sources of cross-infection (O'Donnell *et al.*, 2005; O'Donnell *et al.*, 2006a; Coleman *et al.*, 2007; Coleman *et al.*, 2009). This becomes quite significant where immunocompromised or other medically compromised patients are treated. DCU components that come into direct contact with the patient's oral cavity are of particular significance, including dental unit handpieces, ultrasonic scalers, three-in-one air/water syringes and suction hoses. Output water provided by a DCU is also of particular concern as a potential source of infection as it comes directly from the DCUs extensive network of DUWLs, which can harbour microbial biofilms (Coleman *et al.*, 2007; Coleman *et al.*, 2009). Consequently, DUWL output water is often contaminated with high densities of microorganisms, predominantly bacterial species. This is a universal problem in untreated DUWLs and has been recognised as an important challenge for dentistry for over two decades (Coleman *et al.*, 2007; Coleman *et al.*, 2009). However, ensuring that DCUs provide good quality output water has generally been considered to

be the responsibility of dental practitioners or dental practice management, very often in the absence of specific guidance from DCU manufacturers. Ironically, the problem of DUWL biofilms is essentially a consequence of the inherent design of DCUs and the requirement for water to cool dental instruments and tooth surfaces during dental treatment. According to the EU Medical Devices Directive *"the devices and manufacturing processes must be designed in such a way as to eliminate or reduce as far as possible the risk of infection to the patient, user and third parties"* (Anonymous, 1993). Thus DCU manufacturers have an important responsibility to design for reduced risk of biofilms and also to provide detailed and specific instructions in relation to control of DUWL biofilm in the DCUs they manufacture and market to dental clinics and dental practitioners (Coleman *et al.*, 2007). Unfortunately, until recently, DCU manufacturers have provided very little specific guidance in this regard and have been slow to redesign DCUs to facilitate DUWL biofilm control.

One of the most important contributing factors to this conspicuous absence of direction on DUWL biofilm control by DCU manufactures is the absence of specific standards or regulations governing the microbiological quality of DUWL output water. This is because DUWL output water is not intended for human ingestion, although it frequently is swallowed, aspirated and/or inhaled. Because of the nature of dental treatment and the requirement for DUWL output water to cool dental instruments and tooth surfaces and to function as an oral irrigant, it is impossible to avoid some level of ingestion or aspiration of DUWL output water by patients receiving dental treatment, or indeed to avoid exposure of dental staff to aerosolised DUWL output water. For these reasons, the current consensus from experts in the field affirms that it is reasonable to expect that the quality of DUWL output water should approximate potable water standards (Coleman and O'Donnell, 2007; Coleman *et al.*, 2007; Pankhurst and Coulter, 2007; Walker and Marsh, 2007).

6.1.2. Control of Biofilms in Dental Unit Waterlines

As mentioned above, until recently, responsibility for dealing with the problem of biofilm in DUWLs has largely been considered the responsibility of dental practitioners. DCU manufacturers have been slow to respond to the obvious need for DCU engineering and design changes to control DUWL biofilms. As reviewed in detail in Chapter One, over the last twenty years numerous approaches, both chemical based and non-chemical based, for reducing the bacterial density in DUWL output water have

been suggested, many of which do not remove biofilm and are unsatisfactory as a means of controlling the quality of DUWL output water (Chapter One Tables 1.1 and 1.2). The most effective means of maintaining good quality DUWL output water is regular treatment/disinfection of DUWLs with a chemical, biocide or cleaning agent that removes biofilm, resulting in good quality output water. However, biofilm re-growth in DUWLs usually occurs within one week following disinfection/cleaning and so DUWLs need be treated regularly or continuously with a residual treatment agent. A large number of studies have demonstrated the efficacy of a broad range of commercially available treatment products for DUWLs that efficiently remove biofilm and reduce bacterial density to potable water quality or better (see Chapter One, Table 1.2). However, many of these studies have been conducted *in vitro* and relatively few studies have actually investigated the efficacy of DUWL treatment agents to achieve these desired effects in DCUs (Coleman *et al.*, 2007; Coleman *et al.*, 2009). Furthermore, only a small minority of studies has yet investigated the long-term efficacy of DUWL treatment agents in DCUs in the clinical setting (O'Donnell *et al.*, 2006; Coleman *et al.*, 2007; O'Donnell *et al.*, 2007), whilst comprehensive controlled comparative efficacy studies have yet to be undertaken.

DUWL treatment agents can broadly be divided into two basic categories, including agents for periodic DUWL treatment (e.g. once-weekly) and agents for continuous or residual DUWL treatment (Chapter One, Table 1.2). The majority of all currently available DUWL treatment agents have not been developed or endorsed by DCU manufacturers, but rather have been developed by other third-party manufacturers in response to an obvious market requirement (Coleman *et al.*, 2007). Until recently, this situation has been a significant shortcoming among DCU manufacturers. Because of this, there is significant potential for incompatibility of DUWL treatment agents with components of the DCU waterline network as well as with instruments connected to this network. A small number of studies have reported adverse affects associated with the application of DUWL treatment agents (Coleman *et al.*, 2007), but, as mentioned above, only a very few long-term studies of the efficacy of DUWL treatment agents have been reported to date. Furthermore, in the case of residual DUWL treatment agents, there is a noticeable paucity of independent studies in the literature on potential interactions of such agents and their by-products on oral and dental tissues (Coleman *et al.*, 2007). It is evident that it is very important that DCU manufacturers provide very clear guidance to their customers on the types of DUWL treatment agents that they recommend for use in

their DCUs. In doing so DCU manufacturers have a responsibility to ensure patient and dental healthcare personnel safety, to ensure that DUWL treatment agents do not exhibit adverse effects on DCU components in the short- and long-term and to ensure that DUWL treatment agents do not have adverse effects on the environment. All of this can only be achieved by significant investment in ongoing research and development and close collaboration between DCU designers, end-users and applied microbiologists knowledgeable in the control, behavior and interaction of microorganisms in natural and man-made environments.

6.1.3. Development of Effective Systems for Long-Term Control of Dental Unit Waterline Biofilm

In an effort to develop effective practical solutions to the problem of DUWL biofilms, in the late 1990's Professor David Coleman from the DDSH Microbiology Laboratory initiated a collaboration with the Finnish DCU manufacturer Planmeca (Helsinki, Finland) to develop integrated systems for DUWL biofilm control. This collaboration resulted in the design of the Planmeca Waterline Cleaning System (WCS™) (Tuttlebee *et al.*, 2002). The microprocessor controlled WCS™ was originally developed to be retrofitted to existing Planmeca DCUs having a mains water supply. All DUWLs, apart from the cuspidor rinse DUWL, are included in a microprocessor-controlled process whereby DUWLs are automatically filled with disinfectant and subsequently automatically flushed with clean water during the disinfection cycle. If the cycle is not completed properly or a power failure occurs during the cycle, an error message is displayed on the DCU's instrument console. While this error message is displayed all DCU functions are locked preventing the DCU from being used. The WCS™ is semi-automated and requires the operator to fill the disinfectant container with disinfectant, to turn off a valve on the mains water supply and to place DCU instrument hoses into special receivers. DUWL cleaning is activated from a keypad on the DCUs control console and disinfectant is automatically fed into each DUWL. Following this the DCU electrical supply is switched off to allow the disinfectant *in situ* to take effect. When the electrical power is turned back on, a code message on the DCU control console display prompts the operator to flush the DUWLs. Disconnecting the disinfectant container module and turning on the mains water supply switch initiate this process. Each DUWL is then rinsed and flushed automatically with fresh mains water, after which time the DCU is ready for use. In 2002 we reported on the effectiveness of the WCS™ to control

DUWL biofilm in two separate Planmeca Prostyle Compact DCUs over a 20-week period using the hydrogen peroxide and silver-ion containing disinfectant Sanosil (Tuttlebee *et al.*, 2002). The WCS™ was found to be very effective at eradicating DUWL biofilm in these DCUs when used with Sanosil and consistently provided output water with bacterial densities below the ADA recommended threshold of ≤200 cfu/ml for up to seven days post-disinfection.

In the present study, the ability of the WCS™ to maintain the microbiological quality of DUWL output water below the ADA standard was investigated over a much longer period (18-months) with a much larger number of DCUs (10 DCUs) using the hydrogen peroxide and silver-ion containing DUWL disinfectants Planosil and Planosil Forte. In the majority of cases, once-weekly DUWL treatment using the WCS™ provided output water below the ADA standard for up to a week post-disinfection. During the first nine-months a high incidence (28/300 disinfection cycles; 9.3%) of intermittent DUWL disinfection failure occurred in 8/10 DCUs due to operator omission to disinfect all DUWLs (10/28 failed cycles), operator tampering with air pressure regulators resulting in inadequate compressed air pressure failing to distribute the disinfectant properly (4/28 failed cycles) and physical blockage of disinfectant intake valves due to corrosion effects of Planosil (see Chapter Three, Figs. 3.3, 3.5 and 3.6). Following six-months of continuous once-weekly (15 h overnight) disinfection with Planosil, some instances of DUWL disinfection failure were directly associated with blockage of and/or leakage from disinfectant intake valves, and corrosion of aluminum components of disinfectant delivery module containers. Valve leakage was associated with damage to an internal glue seal, whereas valve blockage was caused by a combination of dislodged glue and oxidized aluminum deposits from the corroded disinfectant delivery containers. Hydrogen peroxide with its strong oxidizing properties was identified as the disinfectant component most likely responsible for the adverse effects on the DCUs concerned (see Chapter Three, Fig. 3.7). The problems were completely resolved in collaboration with the DCU manufacturer Planmeca who developed replacement valves that were resistant to leakage following prolonged exposure to Planosil disinfectant and by replacing the aluminium components of the disinfectant delivery containers with hard plastic and acid-resistant stainless steel (Chapter Three, Figs. 3.5 and 3.6). On rectification of these faults and operator errors through engineering redesign and procedural changes, no further cases of intermittent DUWL disinfection failure were observed. This study highlights the importance of

investigating the long-term effects that DUWL treatment agents can have on DCU components and also highlights the important role that DCU manufacturers have in constantly developing and improving their DCUs. The findings of the study also emphasise the potential for operator error with a semi-automated DUWL cleaning system in busy dental clinics where there are heavy demands placed on staff. Independently of these factors, a rapid and consistent decline in efficacy of DUWL disinfection occurred in 4/10 DCUs following the initial nine months of once-weekly disinfection. There was a highly significant difference ($P < 0.0001$) in the prevalence of strongly catalase-positive (an enzyme that breaks down hydrogen peroxide) *Novosphingobium* and *Sphingomonas* bacterial species (mean average prevalence of 37.1%) in DUWL output water from these four DCUs compared to the other six DCUs and DCU supply water (prevalence <1%), which correlated with biofilm presence in the DUWLs and indicated selective pressure for maintenance of these species by prolonged disinfectant usage (Chapter Three, Fig. 3.4). On recommendation from this research group, Planosil was reformulated by the DCU manufacturer Planmeca as a more concentrated form of hydrogen peroxide and silver-ions (Planosil Forte) and when used once-weekly was found to maintain bacterial density in output water below the ADA standard for all 10 DCUs during the 17 consecutive weeks studied (Chapter Three, Fig. 3.8).

These type of long-term problems identified in the present study with the WCS™ and Planosil very likely occur with other DCU brands, models and DUWL disinfectants and point to the necessity for long-term compatibility testing of DUWL treatment products with DCUs, communication between manufacturers and end users and the development of DUWL cleaning systems that are automated and require minimal human input. The longer-term goal should be for fully automated disinfection systems that can be validated. The selection of disinfectant-tolerant bacterial species in DUWLs following prolonged use of a particular disinfectant, such as Planosil in the present study and Oxygenal 6 in the recent study by Szymańska (2006a), is a worrying but perhaps not surprising development. To counteract this phenomenon it would be prudent to use alternative DUWL disinfectants with a different mode(s) of action periodically, or to use a disinfectant or biocide with multiple modes of action such as the electrochemically activated mixed oxidant solution Ecasol (described in detail in Chapter Five). In recent years the WCS™ is no longer provided as a retrofitted system but is provided as an integrated DUWL cleaning system as standard in new Planmeca

Prostyle Compact DCUs without increasing the cost of the DCUs to the customer.

In the second part of this study the efficacy of the Planmeca Water Management System (WMS™) to provide good quality DUWL output water was assessed in a long-term study. The WMS™ is a more advanced microprocessor-controlled DUWL cleaning system with significant enhancements over the WCS™ system investigated in the first part of the study. The WMS™ is a fully integrated and automated DUWL cleaning system installed in DCUs during manufacture that requires minimal effort on the part of the operator. The WMS™ also contains many additional features, including an air gap, and was designed based on our experience obtained with the WCS™ to minimise materials incompatibility with DUWL disinfectants. Results from the present study with a Planmeca Compact i DCU equipped with the WMS™ demonstrated that the system consistently provided DUWL output water that passed the ADA quality standard of ≤200 cfu/ml for up to seven days after once-weekly disinfection with Planosil Forte during a test period of 40 consecutive weeks (Chapter Four, Fig. 4.4). No instances of intermittent DUWL disinfection failure were encountered with the WMS™ system during the year-long study period, although preliminary studies with Planosil did reveal that this disinfectant was not able to maintain DUWL output water below the ADA of ≤200 cfu/ml for up to a week post-disinfection, due to the prevalence of strongly catalase-positive *Novosphingobium* and *Sphingomonas* bacterial species (Chapter Four, Fig. 4.3).

6.1.4 Development of a Centralised, Automated Dental Hospital Water Quality and Biofilm Management System

In DCUs provided with a mains water supply, the most effective approaches at controlling DUWL biofilm have been focused at the level of the DCU by implementing periodic or residual treatment of the DUWLs with chemical disinfectants or biocides. However, the microbiological and chemical quality of DUWL output water is directly influenced by the quality of the incoming supply water, which in turn is influenced by the condition of the water distribution network pipes and storage tanks, where present (Coleman *et al.*, 2007; Coleman *et al.*, 2009). The poorer the quality of water supplied to DUWLs, the more readily biofilm is likely to form on its internal surfaces. Heterotrophic bacteria present in supply water convert organic material in supply water into biomass (Flemming, 2002). Thus it is very difficult to maintain the quality of DUWL output water below the ADA standard of ≤200 cfu/ml if the DCU supply water

contains significant levels of microorganisms. In the third part of the present study we set out to develop a centralised, automated water quality and biofilm management system for the DDSH, which was capable of providing DCU supply water of consistent chemical quality, and with an integrated disinfectant treatment system capable of maintaining the microbial load in DUWL supply and output water at a minimum. This development was prompted in 2006 by high summer temperatures, which resulted in overgrowth of *P. fluorescens* in the DCU supply water holding tanks supplied with mains water (Chapter Two, Fig. 2.1) and it was impossible to maintain DUWL output water below the ADA standard of ≤200 cfu/ml despite regular DUWL disinfection. In order to achieve our objective, we initiated a collaboration with the Irish ECA technology company Trustwater (Clonmel, Ireland) in conjunction with the Finnish DCU manufacturer Planmeca. This collaboration sought at the outset to combine the applied microbiology expertise available in our laboratory with the DCU manufacturing and engineering expertise available at Planmeca and the ECA technology expertise available at Trustwater in order to develop a fully automated, centralised DUWL biofilm management system. Our approach to developing a centralised system consisted of two stages, the first of which consisted of using particle filtration, water softening, activated carbon filtration and KDF filtration to process incoming mains water to ensure chemical consistency despite fluctuations in the composition of the incoming potable quality supply water. This approach worked very effectively and provided supply water with a chemical composition that complied consistently with and better than the limits advocated by the EC Directive on the Quality of Bottled Water for Human Consumption (Anonymous, 1998) and by the United States Environmental Protection Agency National Primary Drinking Water Regulations (Anonymous, 2002) (Chapter Five, Table 5.1). The second stage involved using the ECA solution Ecasol as a residual disinfectant in processed supply water, which was then provided *via* a recirculation loop to the hospital's 103 DCUs. Sensors in the loop constantly monitor the level of Ecasol in the water and automatically trigger adjustments when required (Chapter Five, Fig. 5.1). Results from the two-year longitudinal study described in Chapter Five demonstrated that the automated system maintained the average density of aerobic heterotrophic bacteria in DUWL supply water and output water simultaneously in a large number of DCUs at <1 cfu/ml and 18.1. cfu/ml, respectively, (Chapter Five, Figs. 5.6 and 5.7), significantly better than the EU potable water and bottled water standards (Anonymous, 1998), with the complete absence of biofilm in DUWLs (Chapter 5, Fig.

5.9, panel a).

The development and long-term testing of our centralised biofilm management system has actually achieved the long-term term goal referred to earlier in this chapter for fully automated disinfection systems for DUWLs that can be validated. The system has been shown to be far superior to the WCS™ and WMS™ DUWL cleaning systems investigated in long-term studies in Chapters Three and Four, respectively. Recently our centralised biofilm management system has been installed by Planmeca and Trustwater at the Copenhagen Dental School and tenders for additional installations at other Dental Hospitals have been approved for installation in the near future.

As reviewed in Chapter One, Section 1.1.8.2, chlorine has been reported to mobilise mercury from dental amalgam particles trapped in DCU suction systems with the release of highly toxic dissolved mercury into the environment from DCU waste-water (Stone *et al.*, 2006; Stone *et al.*, 2009). Because Ecasol consists predominantly of metastable hypochlorous acid providing FAC, it is conceivable that Ecasol used to disinfect DUWLs could cause the release of ionic mercury from dental amalgam particles trapped in DCU suction systems. Spent DUWL output water ultimately is voided to the waste-water stream using pipe work shared with the DCU suction system. However, this possibility is very unlikely for two reasons; firstly the level of FAC in spent DUWL output water from the centralised system is very low (< 2 ppm). Secondly because of the metastable nature of Ecasol, the small concentration present in spent DUWL output water should ensure that it is inactivated upon contact with organic matter in the DCU waste-water pipe work before being released as waste-water. Nonetheless, we intend to investigate the potential for mobilisation of ionic mercury from dental amalgam by Ecasol in the near future.

6.1.5. Future Work

Results from the studies described in this thesis demonstrate that the WCS™ and WMS™ DUWL cleaning systems are excellent for consistently maintaining DUWL output quality water below the ADA standard ≤200 cfu/ml and these systems will undoubtedly be used in dental clinics for many years to come to provide good quality DUWL output water. However, the superior biofilm control provided by the centralised system and its total automation makes it an obvious system for ongoing development. The next challenge is to adapt the basic concepts of our centralised biofilm management system for use with individual DCUs and for dental clinics equipped with several

DCUs. We have already embarked on this task and a prototype system has been developed by Trustwater, which will be subjected to field-testing at the DDSH in the near future. This automated miniature system utilises reverse osmosis to pretreat water to yield DUWL supply water with a consistent chemical composition and automated Ecasol treatment to prevent biofilm formation in DUWLs. The prototype system has been designed to provide water for use in independent reservoir bottles in DCUs. Further research and development is in progress to produce an automated centralised system for DUWL biofilm control suitable for medium-sized dental clinics equipped with 10-20 DCUs.

As described in Chapter Five, the negatively charged antioxidant solution (catholyte) generated by ECA generators has detergent like properties, typically a pH of 11, a redox value of -600 mv and contains predominantly sodium hydroxide in an excited state. We are currently investigating the possibility of utilising catholyte as a disinfectant and cleaning agent for DCU suction hoses, together with a novel DCU suction hose disinfection system that automatically fills suction hoses with disinfectant. The achievement of this objective means that our automated, centralised biofilm control system could also be used to disinfect DCU suction hoses. Such a development will have further significant beneficial economic and environmental impacts, obviating the requirement to use expensive suction system disinfectants, together with a reduction in the release of chemicals to the environment from DCU waste-water. This research is currently in progress.

6.1.6. Conclusions

The research described in this thesis has shown that novel DCUs with integral, semi-automated DUWL cleaning systems such as the WCS™ and WMS™ can effectively control DUWL biofilm in the long-term. These studies helped to considerably improve the design of the systems and identified for the manufacturer of these systems a variety of factors that can contribute to failure of DUWL disinfection in the long-term, including human error, corrosion of equipment following prolonged or incompatible disinfectant usage and the natural selection of bacterial species tolerant to particular disinfection regimes. Solutions to all of these problems were achieved by ongoing research and development in close cooperation with the DCU manufacturer and highlighted that automation of DUWL disinfection using systems that could be validated was the best long-term solution to the problem of DUWL biofilm control.

Further research in collaboration with the DCU manufacturer Planmeca and the ECA technology company Trustwater resulted in the design and development of a fully automated water treatment and biofilm management system that consistently maintains both DUWL supply and output water at better than potable quality simultaneously in a large number of DCUs over the long term.

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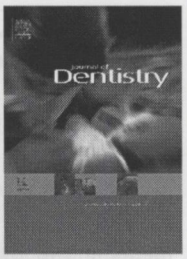

A novel automated waterline cleaning system that facilitates effective and consistent control of microbial biofilm contamination of dental chair unit waterlines: A one-year study

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ABSTRACT

Microbial contamination of dental chair unit (DCU) output water caused by biofilm growth in dental unit waterlines (DUWs) is a universal problem and a potentially significant source of cross-infection. The microbial quality of output water from a Planmeca Compact i DCU equipped with the novel Water Management System (WMS), an integrated and automated DUW cleaning system, was investigated over a 12-month period with the hydrogen peroxide- and silver ion-containing disinfectants Planosil and Planosil Forte. Four weeks after connection to the potable-water quality mains supply the density of aerobic heterotrophic bacteria, rose from the low levels consistently found in the supply water throughout this study (mean average 77 cfu/mL) to 15,400 cfu/mL. Disinfection of DUWs once weekly with Planosil for 10 weeks resulted in a dramatic reduction in bacterial density immediately following disinfection (mean average 26 cfu/mL). Bacterial density rose steadily between disinfections and by 7 days post-disinfection, water quality failed (mean average 384 cfu/mL) the American Dental Association DCU water quality standard of ≤200 cfu/mL. The DCU was then disinfected once weekly for 40 weeks with Planosil Forte. The average bacterial density immediately post-disinfection was 20 cfu/mL and 7 days post-disinfection was 113 cfu/mL. Electron microscopy showed that improved output water quality following disinfection with both disinfectants was associated with marked elimination of DUW biofilm, but deterioration of water quality following disinfection was associated with its regrowth. The most common bacterial species cultured from the mains water and the DCU output water were *Micrococcus luteus* and *Sphingomonas* spp., respectively, the latter of which are known opportunistic pathogens. The findings of this study show that the Planmeca Compact i DCU equipped with the easy to use and automated WMS, that requires minimal effort on the part of the operator, consistently provides output water that passes the ADA quality standard of ≤200 cfu/mL for up to 7 days following once-weekly disinfection with Planosil Forte.

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1. Introduction

The modern dental chair unit (DCU) is a complex medical device designed to provide the equipment and services necessary for a wide variety of dental procedures.[1] Water is used to cool and irrigate a variety of DCU-supplied instruments and tooth surfaces during dental procedures, as the heat generated during operation can be detrimental to teeth.[2–4] Water is also required for oral rinsing during and following dental treatment and to flush the DCU spittoon, or cuspidor, after the patient has finished rinsing. In order to supply water as a coolant and irrigant to turbine handpieces, ultrasonic scalers, three-way air/water syringes, as well as providing water for the patient rinse cup filler and cuspidor, water has to be delivered by an intricate network of interconnected narrow-bore tubes referred to as dental unit waterlines (DUWs).

Many studies have shown that water from DUWs is often contaminated with high concentrations of bacteria. This is a widespread problem and virtually all DUWs in standard DCUs are likely to be contaminated.[5–14] Bacterial contamination of DUWs is considered to originate in the DCU water supply, which usually contains low levels of microorganisms.[14,15] In a typical modern DCU, the network of waterlines can comprise several meters of plastic tubing with an internal diameter of a few millimeters in which water can stagnate when the equipment is not being used.[15,16] Microorganisms in water supplied to DCUs, mainly aerobic heterotrophic Gram-negative environmental bacteria, attach to the internal surfaces of the waterlines and form microcolonies that eventually give rise to multispecies biofilm.[17,18] DUW biofilms are composed mainly of highly hydrated bacterial exopolysaccharide that contains both microcolonies and single cells interspersed heterogeneously with channels or pores.[19,20] Biofilm forms because the water at the internal surface of the narrow-bore DUWs flows more slowly than water at the centre and thus there is little disturbance to the microorganisms present.[8] This allows the microorganisms to proliferate before eventually dispersing through the water supply as planktonic forms where they may be deposited at other sites within the waterline network or are delivered directly into the mouths of patients during dental procedures. Thus, DUW biofilm acts as a reservoir for ongoing contamination of DCU output water.

The presence of high concentrations of microorganisms in DCU water (up to 10^6 cfu/mL has been recorded) is a potential risk of infection for dental patients and staff and is incompatible with good cross-infection control practices.[14,21] Previous studies have shown that waterborne bacteria are aerosolised during dental procedures and that dental personnel and patients are exposed to these microorganisms and fragments of biofilm.[7,9,22–24] DUW contamination is of particular concern in the treatment of immunocompromised and medically compromised individuals. It is important to emphasise that only a few cases of infectious disease transmission related to DUWs and related biofilm have been reported in the literature.[7,9] However, the possibility still remains that DUW-associated infections have gone unrecognised or unreported because of the failure to associate exposure to DUW output water and aerosols generated from this water with the development of specific infections. Sporadic infections not requiring hospital admission are also less likely to be investigated or notified. However, there is considerable potential for infection with some bacterial pathogens found in dental unit water such as *Pseudomonas aeruginosa*, *Legionella pneumophila* and non-tuberculosis *Mycobacterium* species.[7,9,25]

Currently there are no microbial quality standards imposed for DCU output water within the EU. However, it is not unreasonable to expect that the quality of DCU output water should range within potable water standards. The potable water standards set for the EU, the USA and Japan are 100, 500 and 100 cfu/mL, respectively, of aerobic heterotrophic bacteria.[26,27] In 1995, the American Dental Association (ADA) established a goal for the year 2000 of $\leq$200 colony forming units (cfu) per milliliter of aerobic heterotrophic bacteria for DCU output water.[28] However, this goal has not received widespread achievement. The current Centers for Disease Control and Prevention (CDC) guidelines for infection control in dental health-care settings recommend that DCU output water should be $\leq$500 cfu/mL of aerobic heterotrophic bacteria.[29]

The most efficient means of maintaining good quality DCU output water is regular disinfection of DUWs with a disinfectant or biocide that removes biofilm from the waterlines resulting in output water of potable quality.[14] A wide variety of commercial waterline cleaning products and systems are available, some of which can be retrofitted to existing DCUs.[12,14,30,31] However, very few studies published in the peer-reviewed literature have actually investigated the efficacy of disinfectants to achieve these desired effects in DCUs and detailed comparative studies have yet to be published.[14,30,32] Furthermore, there is an onus on DCU manufacturers to consider the problem of DUW biofilm contamination when designing DCUs.[14,33] The purpose of the present study was to evaluate the effectiveness of an advanced DUW cleaning system integrated into a newly designed DCU to control DUW biofilm over a 12-month period using a once weekly disinfection regime with two formulations of a hydrogen peroxide- and silver ion-containing disinfectant.

2. Materials and methods

2.1. *Dental chair unit*

A new Planmeca Compact i DCU (Planmeca Oy, Helsinki, Finland) equipped with the novel integrated Planmeca Water Management System (WMS) was used in the present study (Fig. 1). The DCU was installed in the Oral Surgery Clinic in the Dublin Dental Hospital in December 2003. From February 2004 to January 2005 the efficacy of the WMS to maintain good microbial quality of DUW output water was investigated while the DCU was in daily use for dental patient treatments. To facilitate use during clinical sessions, the DCU was disinfected once a week, on the same day each week, following the afternoon clinical session. The Planmeca WMS can be operated in three separate modes: (A) mains water supply mode, (B) bottle water supply mode and (C) waterline cleaning mode. The WMS contains a physical air gap designed to comply with the requirements of European Standard EN1717

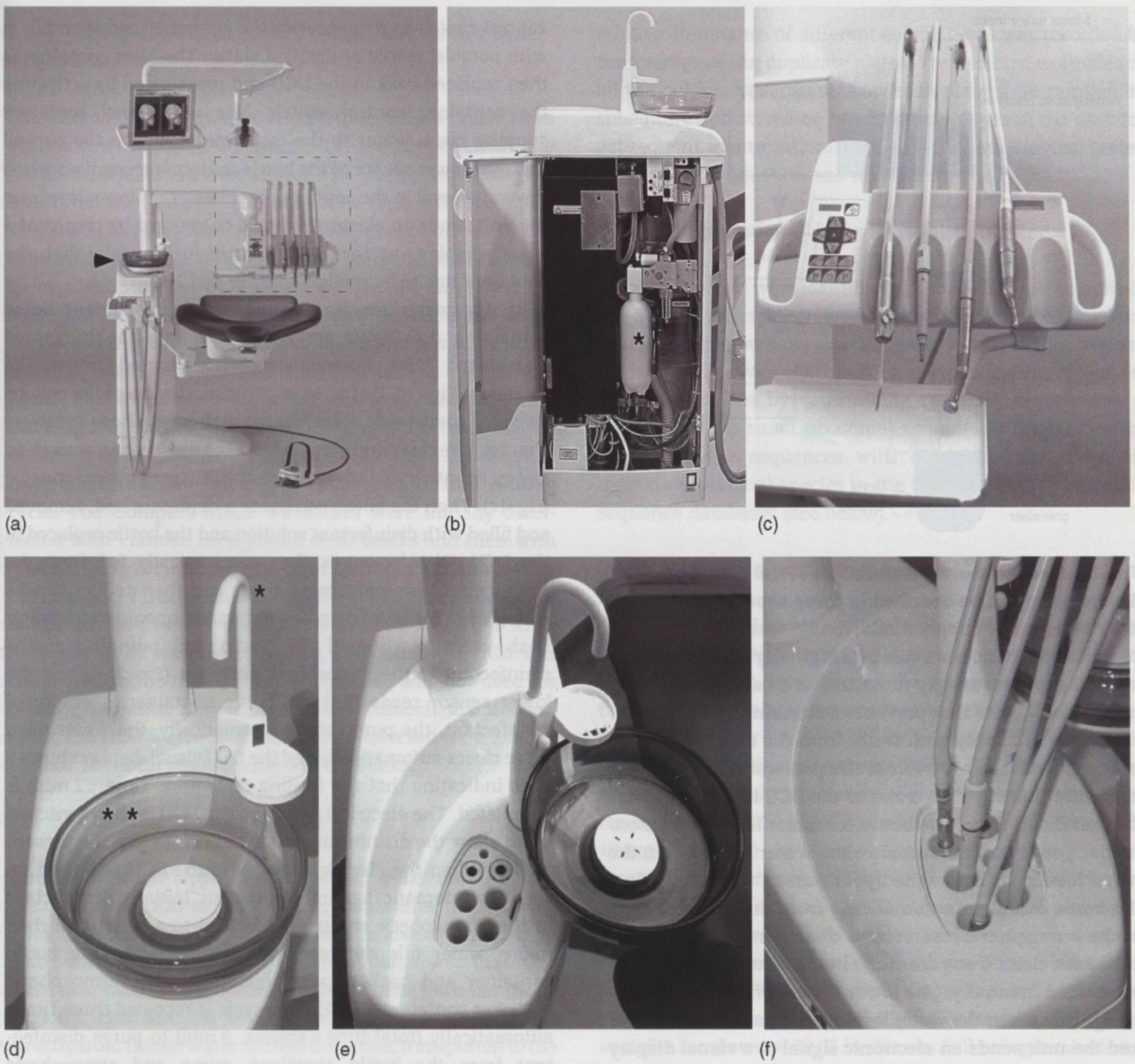

Fig. 1 – Components of the Planmeca Compact i DCU equipped with the integrated Planmeca WMS that was used in the present study. (a) Overview of the Compact i DCU. The instrument console is highlighted with dashed lines and an arrowhead indicates the location of the cuspidor. (b) Side view of the Compact i DCU with the pedestal door open. The pressurised bottle container is marked with an asterisk. (c) Close up view of the DCU instrument console. Four instrument hoses containing DUWs with instruments attached (from left to right: air/water syringe, ultrasonic scaler, high-speed handpiece, low-speed handpiece) are shown in the upper right section of the panel. A keypad and visual display unit to activate various DCU functions are shown to the left of the instrument hoses. (d) View of the DCU cuspidor (**) and cup-fill water outlet (*). (e) View of the instrument hose receivers used during waterline disinfection. The receivers are located beneath the cuspidor, the latter of which is swiveled sideways from its normal position when the receivers are in use. (f) View of the DCU instrument hoses in situ in the flushing receivers during a disinfection cycle.

for dental unit installations.[34] This integrated back-flow prevention device consists of a vertical air gap of 20 mm and prevents contamination of the mains water supply with water from the DCU waterlines (Fig. 2).

In the mains water supply mode, the DCU is connected to the mains water supply line. Mains water is conveyed across the vertical air gap under gravity to an atmospheric reservoir from where it is pumped to a pressurised bottle container (Figs. 1b and 2). Water is then fed from the pressurised bottle via DUWs to the dental instruments (ultrasonic scaler, air/water syringe, low-speed handpiece and high-speed turbine handpiece) (Figs. 1c and 2) and to the patient cup-fill line (Figs. 1d and 2) by using constant pressure of 2.8 bar. The bottle container has two sensors (upper and lower) that monitor the water level. When the water level goes below the upper sensor, a pump engages and fills the container automatically (Fig. 2).

In clean water mode, the DCU uses clean water from the bottle container that is filled manually. The user initially removes the container from the DCU by activating a container

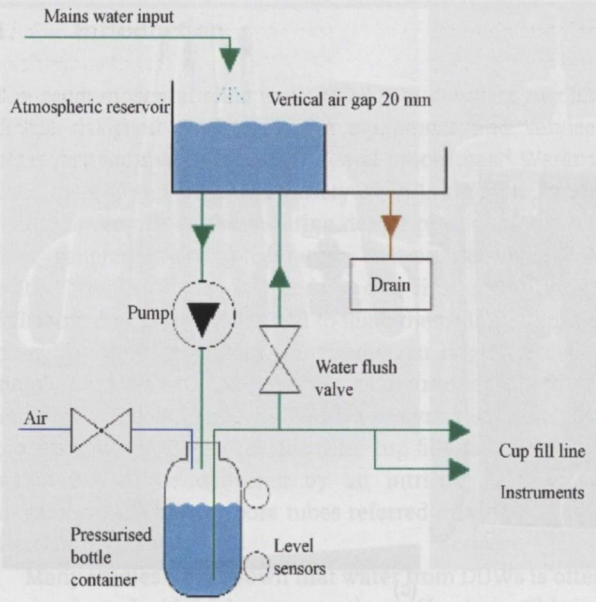

Fig. 2 – A schematic representation of the Planmeca WMS. This system can be operated in three separate modes. In the mains water supply mode, mains water is delivered to an atmospheric reservoir under gravity across a 20 mm vertical gap. The air gap functions as a back-flow prevention device that prevents contamination of the mains water supply with water from the DCU. Water is pumped from the reservoir to the pressurised bottle container that supplies water to the DCU instrument hose lines and cup-filler. The bottle container has two sensors (upper and lower) that monitor the water level. When the water level goes below the upper sensor, the pump fills the bottle container automatically from the water strored in the atmospheric reservoir. In clean water mode, the DCU uses clean water from the bottle container that has been filled manually. The lower sensor of the bottle recognises when the water level reaches a minimum level and the unit sends an electronic signal to a visual display on the DCU instrument console that the container needs to be refilled. In the waterline-cleaning mode, the instrument hose lines are first placed into the integrated instrument-flushing receivers (Fig. 1d–f). The cleaning cycle is started by pressing an activation key on an integrated keyboard on the DCU instrument console (Fig. 1c), the bottle is filled with disinfectant solution and replaced in the DCU. Disinfectant is then automatically fed from the bottle container to the instrument waterlines and cup-fill waterline. This is followed by automatic opening of a water flush valve, starting of the pump and pumping of the disinfectant to the atmospheric reservoir. When the lower sensor reads that the bottle container is empty of disinfectant, the pump stops, the water flush valve closes and the DCU visual display shows a code indicating that the feeding cycle is completed. The electrical supply to the DCU is then switched off for a set time period (15 h in the present study) to allow the disinfectant to take effect. When the DCU is turned on again an automatic flushing cycle begins. Initially, disinfectant in the atmospheric reservoir is voided to a drain; mains water fills the reservoir and is pumped to the bottle container and back to a drain outlet in the atmospheric reservoir container. This cycle is repeated three times automatically to purge disinfectant from the bottle container, pump and atmospheric reservoir. Finally mains water is pumped from the atmospheric reservoir to the bottle container, and from there is used to flush the instrument waterlines and cup fill line for a duration of 4 min. After this automatic flushing and rinsing cycle the DCU is ready for normal use.

control switch to depressurise the container and then fills it with potable, sterile or distilled water. The filled container is then replaced back in the DCU and pressurised by activating the container control switch. The pressurised container supplies clean water to the instruments and to the cup-fill line. The lower sensor of the bottle container recognises when the water level in the container reaches a minimum level and the unit sends an electronic signal to the user by means of a visual display on the DCU instrument console that the container has to be refilled (Figs. 1c and 2).

In the waterline-cleaning mode, the instrument hoses containing the DUWs are first placed into the integrated instrument hose flushing receivers situated under the cuspidor (Fig. 1d–f). For this purpose, the cuspidor can be swiveled to one side revealing the instrument hose receivers (Fig. 1e). The cleaning cycle is started by pressing an activation key on an integrated keyboard on the DCU instrument console (Fig. 1c). Then the bottle container is depressurised, removed and filled with disinfectant solution and the bottle replaced in the DCU. Disinfectant is then automatically fed from the container to the instrument hoses and then to the cup-fill waterline. This is followed by automatic opening of a water flush valve, starting of the pump and pumping of the disinfectant to the atmospheric reservoir (Fig. 2). When the lower sensor reads that the bottle container is empty of disinfectant, the pump stops automatically, the water flush valve closes automatically and the DCU visual display shows a code indicating that the feeding cycle (total time <2 min) is completed. The electrical supply to the DCU is then switched off to allow the disinfectant to take effect (15 h in the present study). When the electrical supply to the DCU is turned on again an automatic flushing cycle begins. Initially, disinfectant in the atmospheric reservoir is voided to a drain and then mains water fills the reservoir, is pumped to the bottle container and back to a drain outlet in the atmospheric reservoir container (Fig. 2). This cycle is repeated three times automatically (total time = approx. 9 min) to purge disinfectant from the bottle container, pump and atmospheric reservoir. Subsequently fresh mains water is pumped from the atmospheric reservoir to the bottle container, and from there is used to flush the instrument waterlines and cup fill line for a duration of 4 min. After this automatic flushing and rinsing cycle the DCU is ready for normal use.

If the DCU is left switched on following the disinfectant feeding cycle, disinfectant purging and waterline flushing is initiated automatically after a time lapse of 8 h. When the DCU's waterline cleaning mode is activated, an inbuilt safety feature inactivates all other DCU functions, thus preventing use of the DCU for patient treatment.

2.2. *Disinfection of waterlines using the Planmeca water management system*

The microbiological quality of waterline output water from the Planmeca model Compact i DCU equipped with the Planmeca WMS was assessed over a 50-week period using the waterline disinfectants Planosil (1.5% (v/v) hydrogen peroxide, 0.003% (w/v) silver, 0.0015% (v/v) phosphoric acid) and Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver, 0.0025% (v/v) phosphoric acid) using once weekly, overnight (15 h) disinfection. Hydrogen peroxide and silver ions are the active antimicrobial ingredients in both disinfectants, while *ortho*-phosphoric acid is used as a stabilising agent. Both of these disinfectant products contain a blue tracking dye so that complete filling of waterlines can be observed visually by the elution of the coloured disinfectant from each waterline outlet during the disinfectant feeding cycle. The Compact i DCU waterlines were initially disinfected with Planosil for a period of 10 weeks and then with Planosil Forte for a further period of 40 weeks. Prior to testing the Compact i DCU with Planosil, the unit's waterlines were not disinfected for a 4-week period to allow biofilm to form naturally in its waterlines. During this period the DCU was connected to the mains water supply and was not used for treating patients. Following the last Planosil disinfection cycle, the DCU waterlines were not disinfected for a 2-week period. The total study period was 55 weeks.

2.3. *Collection of water samples*

Samples of water (approximately 50 mL) were collected from the Compact i DCU air/water syringe waterline, immediately prior to disinfection and immediately following disinfection with both disinfectants. Two to three additional water samples were also taken on separate days each week between disinfection cycles. Samples were collected in sterile glass bottles directly from the air/water syringe waterline, transported to the microbiology laboratory at ambient temperature within 5 min and processed immediately. Water samples were also taken from the mains water supplied to the Compact i DCU on six occasions at intervals throughout the 55-week study period.

2.4. *Laboratory processing of water samples*

After collection, water samples were taken immediately to the microbiology laboratory for analysis and cultured in duplicate on R2A agar plates (Difco, Becton Dickinson, Le Pont de Claix, France) as described previously.[14] Each agar plate consisted of 25 mL of medium contained in 90 mm diameter single-vented Petri dishes (Greiner Bio-One GmbH, Kremsmünste, Austria). R2A agar was used to determine total aerobic heterotrophic bacterial density because it is particularly efficient at isolating aerobic heterotrophic bacteria from water.[14,35] Negative controls consisted of samples of autoclaved ultra-pure water (18.2 MΩ cm resistivity at 25 °C) from a Milli-Q Biocel® (Millipore, MA, USA) water purification system. The R2A plates were incubated at room temperature (approx. 20 °C) for 10 days. After incubation, plates were examined and colonies counted using a Stuart Scientific colony counter (Bibby Sterilin Ltd., Staffordshire, UK). The total number of colonies and the relative abundance of different colony types was recorded as the average of the duplicate plates, and selected examples of all the colony types present on each plate were purified by subculture and stored on nutrient agar slopes in the dark for subsequent identification. These were Gram-stained, tested for catalase production and identified.

2.5. *Identification of bacterial isolates*

Bacterial isolates were identified, where possible, by means of the API 20 NE system for identifying non-enterobacterial Gram-negative bacteria (bio-Mérieux, Marcy l'Etoile, France) as described previously.[14] Definitive identification of isolates were undertaken by determining the DNA sequence of a segment of the small ribosomal subunit rRNA gene and by comparing the sequences with consensus sequences for individual bacterial species in the EMBL/GenBank nucleotide sequence databases (see below).

2.6. *Isolation of bacterial genomic DNA*

Total genomic DNA from bacterial isolates was prepared using the Qiagen DNeasy kit system (Qiagen, Crawley, West Sussex, United Kingdom) according to the manufacturer's instructions. Bacterial isolates were cultured on either R2A agar or on Luria-Bertani agar (LA; pH 7.4)[36] for 5–7 days at 25 °C in a static incubator (Gallenkamp, Leicester, England). Following incubation, approximately 10 colonies of each bacterial isolate were resuspended in 250 μL of lysis buffer (1 M Tris–EDTA and 10 mg lysozyme) in 1.5 mL Eppendorf microfuge tubes (Eppendorf AG, Hamburg, Germany). The bacterial suspensions were then incubated at 37 °C for 2–3 h with occasional vortexing to allow lysis of bacterial cells to occur. Genomic DNA was then extracted using the Qiagen DNeasy kit. Briefly, proteinase K and buffer AL (both reagents supplied with the DNeasy kit), were added to the lysed bacterial cells and the samples incubated at 70 °C for 30 min. Following incubation, a 200 μL aliquot of 100% (v/v) ethanol was then added and the mixture transferred to a DNeasy mini spin column (Qiagen) and centrifuged at 6000 × *g* for 1 min. Following centrifugation, each sample was washed with the buffers supplied with the DNeasy kit and the purified DNA was eluted using 100 μL of the elution buffer supplied with the kit.

2.7. *PCR amplification, cloning and sequencing of the 16S rDNA gene*

A variable segment of the 16S rDNA gene of each bacterial isolate was amplified by PCR as described previously by Singh et al.,[37] using approximately 30 ng of purified genomic DNA as template with the universal primers 533F (5′-AGAGTTTGATC/TA/CTGGCTCAG-3′) and 142R (5′-CGGC/TTACCTTGTTACGA-3′). These primers amplify a region of approximately 950 bp to 1.5 kb of the 16S rDNA gene of all bacterial species.[37]

Amplified PCR products were purified using the QIAEX II gel extraction kit (Qiagen) according to the manufacturer's instructions prior to cloning into the pGEM T-Easy Vector System I (Promega Corporation, Madison, Wisconsin, USA). Transformation of competent *Escherichia coli* DH5α cells prepared using $CaCl_2$ and identification of *E. coli* derivatives

harbouring recombinant plasmids were performed using standard methods.[36] DNA sequencing of cloned amplimers was performed commercially by Lark Technologies (Essex, United Kingdom) using an automated Applied Biosystems 373A DNA sequencer (Foster City, CA, USA), dye-labelled terminators and the primers T7 (5′-TAATACGACTCACTA-TAGGG-3′) and SP6 (5′-ATTTAGGTGACACTATAGAAT-3′) that are specific for the pGEM T-Easy Vector (Promega). Analysis of chromatograms and sequences was carried out using the 373A data analysis software programme version 1.2.0 (Applied Biosystems) and DNA Strider 1.3f11 software (CEA/Saclay, Gif-sur-Yvette, France), respectively. The identity of individual bacterial isolates was determined by comparing their 16S rDNA sequences to those in the EMBL and GenBank nucleotide sequence databases using the BLAST family of computer programmes (http://www.ncbi.nlm.nih.gov/BLAST/).[38]

2.8. *Electron microscopy of waterline biofilm*

Two-centimetre samples of DUW waterline tubing supplying water to the air/water syringe close to the syringe attachment site were removed at intervals before and immediately following disinfection with both Planosil and Planosil Forte. The samples were cut longitudinally through the lumen using a sterile scalpel and then the internal walls examined for the presence of biofilm without prior fixation by electron microscopy as described previously.[14]

2.9. *Mains supply water*

In the Dublin Dental Hospital mains water supplied to DCUs is automatically processed by particle filtration (removal of particles greater that 10 μm) ion exchange (to remove calcium and magnesium ions) and UV treatment (to reduce the numbers of viable microorganisms) prior to delivery to DCUs.[14]

2.10. *Chemical analysis of water*

Water samples from the mains supply to the Compact i DCU and samples from the air/water syringe waterline were subject to chemical analysis using standard procedures as described previously.[14]

2.11. *Statistical analysis*

Bacterial density levels in DUW output water obtained following disinfection with either Planosil or Planosil Forte were compared using a two-sample unpaired t test, assuming equal variances. Comparison of bacterial density levels in DUW output water seven days post-disinfection relative to the ADA DUW output water quality standard of ≤200 cfu/mL was carried out using one-sample t tests.

3. Results

3.1. *Compact i DCU with WMS*

Operation of the DCU in waterline cleaning mode was straightforward and was facilitated by its automated nature. All that is required by the operator is to place the instrument hoses into receivers beneath the DCU cuspidor, activate the waterline cleaning function on the DCU console keypad, fill the DCUs bottle container with disinfectant and wait approximately 2 min until all of the DCUs waterlines are automatically filled with disinfectant and then switch the unit off. The following morning, the operator switches the DCU on again and the waterlines are automatically purged of disinfectant and thoroughly flushed. The entire process takes about 15 min after which time the DCU is ready for use. An inbuilt safety function prevents the DCU from being operated until the waterline purging and rinsing cycles have been completed automatically.

3.2. *Disinfection of DUWs*

The microbial quality of DUW output water from the Compact i DCU equipped with the novel integrated Plammeca WMS was tested over a 55-week period with once weekly DUW disinfection for 50 weeks with the hydrogen peroxide- and silver ion-containing disinfectants Planosil and Planosil Forte. Planosil was selected for use because a previous report from this laboratory in 2002 showed that a product with a similar formulation (Sanosil) was very effective at controlling biofilm in DCU waterlines.[14] However, since then we have observed a decline in the efficacy of this product following prolonged use, possibly due to the prevalence of catalase-positive bacteria in DUW biofilm. In order to circumvent similar problems in the present study, Planosil Forte, with its higher concentrations of hydrogen peroxide and silver ions was also used.

At the beginning of the study the DCU was connected to the processed mains water supply provided to all DCUs in the Dublin Dental Hospital and was not subject to DUW disinfection for a period of 4 weeks to allow biofilm to form naturally in its waterlines. Following this initial period the units DUWs were subject to a 15 h (i.e. overnight) disinfection once weekly for a period of 10 weeks with Planosil. After these 10-weekly disinfection cycles the DCU waterlines were not disinfected for a week and then subjected to a further 40 weekly disinfection cycles with the disinfectant Planosil Forte. During the entire 55-week period water samples from the DCU air/water syringe were taken four to five times a week and tested for the presence of aerobic heterotrophic bacteria. These organisms were chosen as marker organisms for DUW contamination as many previous studies have shown that these are the predominant microorganisms present in DUWs.[13–17]

The microbial quality of processed mains water supplied to the Compact i DCU was tested the day the DCU was initially connected to the mains supply and on five separate occasions throughout the 55-week study period and was found to contain low levels of bacteria with a mean average of 77 cfu/mL (range 49–122 cfu/mL). During the 4-week period following connection of the DCU to the mains water supply the bacterial density in DUW output water rose steadily and reached 15,400 cfu/mL on day 28 (Fig. 3a). In contrast, immediately following disinfection and 7 days post-disinfection with Planosil, DUW output water had a markedly reduced bacterial density during each of the 10 weeks the DCU was disinfected with Planosil (Fig. 3a). The average bacterial density immediately following disinfection with Planosil was 26 cfu/mL

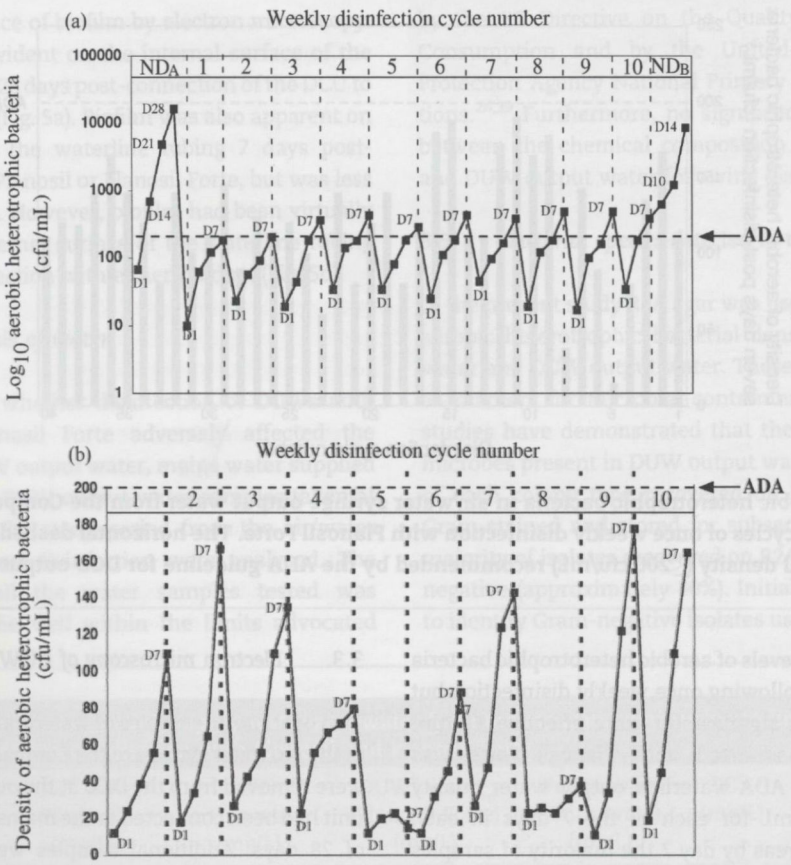

Fig. 3 – Density of aerobic heterotrophic bacteria in air/water syringe output water from the Compact i DCU during 10 cycles of once weekly disinfection with (a) Planosil or (b) Planosil Forte. The DCU was not disinfected for a 4-week period prior to disinfection with Planosil. D1, day 1 immediately following disinfection; D7, day 7 post-disinfection and immediately prior to the next disinfection episode. At the beginning of the study the DCU was connected to the mains water supply for a 4-week period during which the DCUs waterlines were not disinfected (ND_A). Water samples were taken during this period on days 1, 14, 21 and 28 post-connection to the mains supply. Following the tenth disinfection episode the waterlines were not disinfected for 2 weeks (ND_B). The horizontal dashed lines shown represent the maximum bacterial density (≤200 cfu/mL) recommended by the ADA guideline for DCU output water quality.

(range 10–45 cfu/mL). Bacterial levels rose steadily each week between disinfections and the mean average bacterial density in output water 7 days post-disinfection was 384 cfu/mL (range 180–620 cfu/mL). The majority (9/10; 90%) of water samples taken 7 days post-disinfection with Planosil exceeded the microbial quality threshold of ≤200 cfu/mL set by the ADA for DUW output water (Fig. 3a). Fourteen days following the final disinfection with Planosil the bacterial density in DUW output water increased to 8600 cfu/mL (Fig. 3a). Following the period the DCU was disinfected with Planosil, the DCU was initially subjected to once weekly disinfection with Planosil Forte for 10-weeks. Similarly to the results obtained with Planosil, DUW output water had a markedly reduced bacterial density compared to the densities recorded 28 days post-connection of the DCU to the mains water supply during each of the 10 weeks the DCU was disinfected with Planosil Forte (Fig. 3b). The average bacterial density immediately following disinfection with Planosil Forte was 18 cfu/mL (range 11–27 cfu/mL). Here again, bacterial levels increased during each week between disinfections but not to the same extent as during Planosil disinfection, and the mean average bacterial density in output water 7 days post-disinfection was 112 cfu/mL (range 15–178 cfu/mL). All 10 (100%) water samples taken 7 days post-disinfection with Planosil Forte were below the microbial quality threshold of ≤200 cfu/mL set by the ADA for DUW output water (Fig. 3b). There was a highly significant difference between the bacterial density in DUW output water seven days post-disinfection with Planosil and Planosil Forte. The bacterial density in DUW output water seven days post-disinfection with Planosil during the 10-week period the DCU was disinfected with this product (average 384 cfu/mL) was significantly higher than the corresponding density recorded during the 10-week period the DCU was disinfected with Planosil Forte (average 112 cfu/mL) ($p = 0.00001$). In order to investigate whether consistent results would be obtained with Planosil Forte over a longer period of time, the Compact i DCU was subjected to a further 30 cycles of once weekly disinfection with this disinfectant and similar results were obtained (Fig. 4). The average bacterial density immediately following disinfection and 7 days post-disinfection with Planosil Forte over the entire 40-week period the DCU was disinfected with this product was 20 cfu/mL (range 10–32 cfu/mL) and 113 cfu/mL (range 15–190 cfu/mL), respectively. These results demonstrated that both disinfectant products were

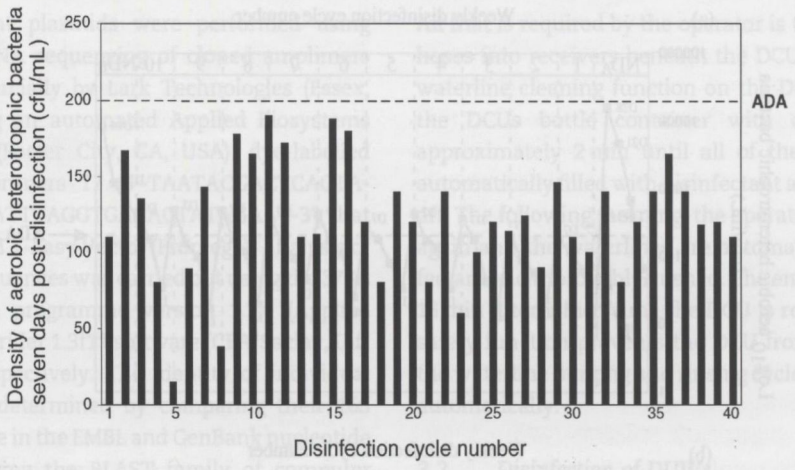

Fig. 4 – Density of aerobic heterotrophic bacteria in air/water syringe output water from the Compact i DCU 7 days post-disinfection during 40 cycles of once weekly disinfection with Planosil Forte. The horizontal dashed line shown represents the maximum bacterial density (≤200 cfu/mL) recommended by the ADA guideline for DCU output water quality.

effective at controlling levels of aerobic heterotrophic bacteria in DUW output water following once weekly disinfection but that Planosil Forte was significantly more effective. Output water from DUWs disinfected with Planosil Forte was consistently below the ADA waterline output water quality threshold of ≤200 cfu/mL for each of the 7 days in each disinfection cycle, whereas by day 7 the majority of samples from DUWs disinfected with Planosil had exceeded the ADA threshold (Fig. 3).

3.3. *Electron microscopy of DUW biofilm*

Two centimetre samples of waterline tubing close to the outlet of the air/water syringe of the Compact i DCU used in this study were removed from the DCU at the outset of the study after the unit had been connected to the mains water supply for a period of 28 days. Additional samples were removed at intervals immediately prior to disinfection with both Planosil and Planosil Forte and 7 days post-disinfection. All samples were

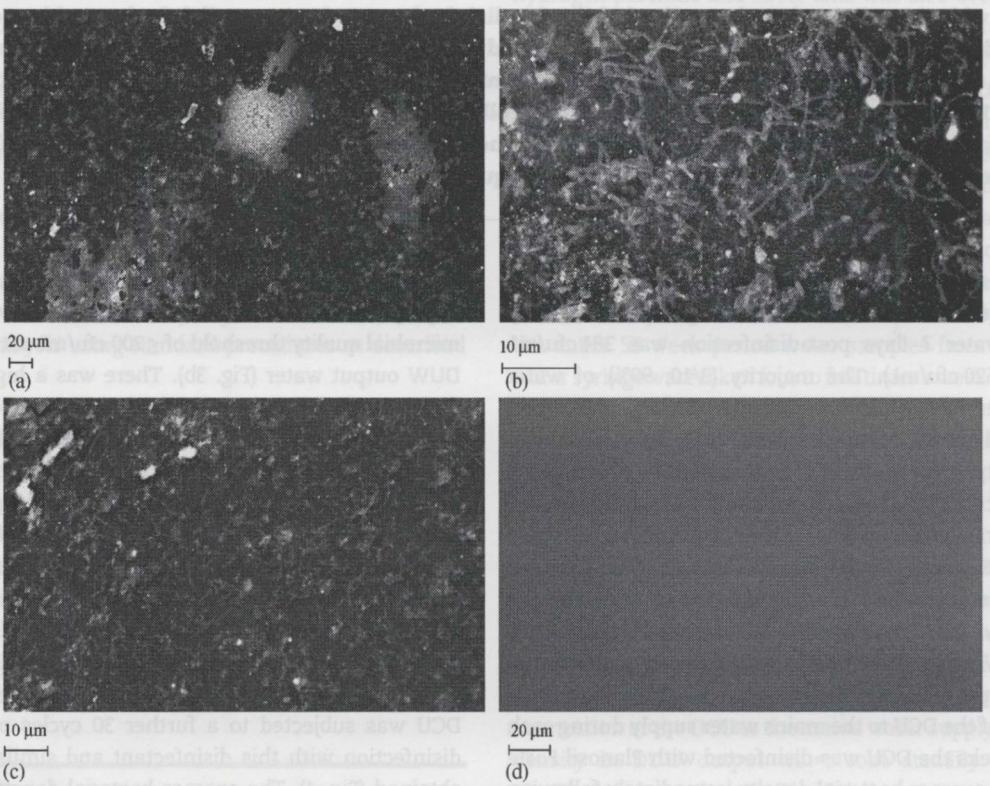

Fig. 5 – Electron micrographs of longitudinal sections of DUW tubing from the air/water syringe waterline comparing the amounts of biofilm on the internal surfaces from the (a) non-disinfected DCU 28 days post-connection to the mains supply water, (b) Planosil-disinfected DCU 7 days post-disinfection, (c) Planosil Forte-disinfected DCU 7 days post-disinfection and (d) Planosil Forte-disinfected DCU immediately following disinfection. Similar results to those shown in (d) were obtained following disinfection with Planosil.

examined for the presence of biofilm by electron microscopy. Extensive biofilm was evident on the internal surface of the waterline tubing sample 28 days post-connection of the DCU to the mains water supply (Fig. 5a). Biofilm was also apparent on the internal surface of the waterline tubing 7 days post-disinfection with either Planosil or Planosil Forte, but was less extensive (Fig. 5b and c). However, biofilm had been virtually eliminated from the internal surface of the waterline tubing immediately post-disinfection with either product (Fig. 5d).

3.4. *Chemical analysis of water*

In order to investigate whether disinfection of DUWs with either Planosil or Planosil Forte adversely affected the chemical quality of DUW output water, mains water supplied to the DCU and several DUW output water samples taken at different times during the study period from the air/water syringe immediately post-disinfection were analysed. The chemical quality of all the water samples tested was consistently found to be well within the limits advocated by the EC Directive on the Quality of Water for Human Consumption and by the United States Environmental Protection Agency National Primary Drinking Water Regulations.[26,39] Furthermore, no significant difference was found between the chemical composition of mains supply water and DUW output water following disinfection (Table 1).

3.5. *Bacterial species detected in water*

In the present study R2A agar was used to determine the total aerobic heterotrophic bacterial density in processed mains water and DUW output water. These organisms were chosen as markers for microbial contamination as many previous studies have demonstrated that these are the predominant microbes present in DUW output water.[13–17] Selected isolates of each colony type recovered on R2A agar were purified, Gram-stained and stored for subsequent identification. The majority of isolates recovered on R2A agar plates were Gram-negative (approximately 80%). Initially, attempts were made to identify Gram-negative isolates using the API 20 NE system

Table 1 – Chemical analysis of mains supply water and water from the air/water syringe waterline from the Compact i DCU immediately following disinfection with Planosil and Planosil Forte

Test	DUW[a] water (Planosil) (mg/L)	Processed[b] mains water (mg/L)	DUW[a] water (Planosil Forte) (mg/L)	Processed[b] mains water (mg/L)	EC[c] (mg/L)	US[d] (mg/L)	Method[e] of analysis
Calcium	15.4	15.2	16.4	16.2	N/A	N/A	ICP-MS[f]
Magnesium	1.9	1.8	2.5	2.5	N/A	N/A	ICP-MS[f]
Sodium	7.5	7.6	7.0	7.0	200.0	N/A	ICP-MS[f]
Potassium	0.048	0.125	0.8	0.9	N/A	N/A	ICP-MS[f]
Iron	0.017	0.022	0.002	0.002	0.20	0.30	ICP-MS[f]
Manganese	<0.001	<0.001	<0.002	<0.002	0.05	0.05	ICP-MS[f]
Copper	0.035	0.007	0.044	0.042	2.00	1.3	ICP-MS[f]
Lead	<0.001	<0.001	<0.001	<0.001	0.01	0.0	ICP-MS[f]
Zinc	0.32	0.004	0.023	0.004	N/A	5.0	ICP-MS[f]
Silver	0.003	0.003	0.004	<0.002	N/A	0.1	ICP-MS[f]
Carbonate	61.05	61.0	30.0	40.0	N/A	N/A	Titration
Chloride	13.8	13.9	12.0	12.0	250.0	250.0	Colourimetric assay
Sulphate	40.0	39.0	41.0	34.0	250.0	250.0	Turbidometric assay
Nitrate as NO_3	3.82	4.64	3.3	3.1	50.0	10.0	Colourimetric assay
Nitrite as NO_2	<0.05	<0.05	<0.05	<0.05	0.5	1.0	Colourimetric assay
Ammonia as NH_4	0.03	0.02	0.02	0.02	0.5	N/A	Colourimetric assay
Fluoride	0.79	0.81	0.9	0.6	1.50	4.0	Colourimetric assay
ortho-Phosphate as PO_4	<0.15	<0.15	<0.03	<0.03	N/A	N/A	Colourimetric assay
pH	7.57	7.68	7.56	7.61	6.5–9.5	6.5–8.5	Meter
Conductivity at 25 °C (μS/cm)	148.4	143.9	156.0	156.0	N/A	N/A	Meter

N/A: no maximum permissible concentration in water for human consumption.

[a] Water samples tested immediately following disinfection of DUWs with Planosil or Planosil Forte, respectively.

[b] Mains water supplied to the DCU was initially processed by particle filtration, ion exchange and UV treatment (see Section 2).

[c] European Commission maximum permissible concentration in water for human consumption.[26]

[d] Maximum permissible concentration in drinking water advocated by the United States Environmental Protection Agency drinking water regulations.[39]

[e] Used in the present study and based on standard methods as described previously.[14]

[f] ICP-MS, inductively coupled plasma-mass spectrometry.

Table 2 – Bacterial species isolated from the air/water syringe waterline of the Compact i dental chair unit used in the present study

Bacterial species[a]	Gram-stain	Catalase activity[b]
Novosphingobium subarctica	Negative	+++++
Sphingomonas paucimobilis	Negative	+++++
Sphingomonas aerolata	Negative	+++++
Sphingomonas trueperi	Negative	+++++
Acidovorax temperans	Negative	–
Porphyrobacter tepidarius	Negative	–
Arthrobacter agilis	Positive	++++
Micrococcus luteus	Positive	+++++
Variovorax paradoxus	Positive	++++
Rhodococcus fascians	Positive	+++
Kocuria palustris	Positive	+++

[a] Identified by DNA sequence analysis of the variable region of 16S rDNA gene.

[b] An arbitrary scale for catalase activity was used based on the visual observance of the evolution of oxygen bubbles from a bacterial colony following the addition of 50 μL of 5% (v/v) H_2O_2. +++++, very strongly catalase-positive with instantaneous and substantial evolution of gas bubbles; +++, catalase-positive with immediate but moderate evolution of gas bubbles; –, catalase-negative. For each isolate tested, at least five separate colonies grown on R2A agar medium were tested for catalase production.

but many of the isolates could either not be satisfactorily identified with this system or could be identified only to the genus level (data not shown). In order to circumvent this problem, it was decided to identify all selected isolates to the species level based on the DNA sequence of a variable region of the 16S rDNA gene. The universal primer pair 533F/142R, which can be used to amplify a region of approximately 950 bp to 1.5 kb of the 16S rDNA gene of all bacterial species, was used in separate PCR amplification experiments with template DNA from each of the isolates selected for identification. A single PCR amplimer was obtained with template DNA from each isolate, which was subsequently cloned and its nucleotide sequence determined. Each amplimer sequence obtained shared 99–100% homology with the 16S rDNA consensus sequence of only one bacterial species, in each case, in the EMBL/GenBank nucleotide sequence databases and thus all of the isolates were identified unequivocally to the species level (Table 2). The most common bacterial isolates recovered from the mains water supply during the 55-week study period were Gram-positive and catalase-positive organisms that were identified as *Micrococcus luteus*. These organisms were also identified in output water from the Compact i DCU air/water syringe waterline following disinfection with either Planosil or Planosil Forte. However, the most prevalent bacterial isolates recovered from DUW output water were Gram-negative, catalase-positive organisms identified as *Novosphingobium subarctica*, *Sphingomonas paucimobilis* or other *Sphingomonas* species. All of these *Sphingomonas* isolates were found to be very strongly catalase-positive (Table 2).

4. Discussion

Over the last decade numerous studies from many countries have highlighted that output water from DUWs can contain very high microbial loads of mainly bacterial species, including several known human pathogens such as *P. aeruginosa* and *Legionella pneumophilia*.[5–14] This phenomenon results from biofilm formation in narrow-bore DUWs and is a universal problem in DCUs. It is now generally accepted by health care authorities and professional bodies that it is not acceptable from an infection control perspective to expose patients (especially medically compromised or immunocompromised patients), dental staff and support staff to heavily contaminated dental unit water or aerosols generated during use of dental instruments connected to DUWs. In recent years the focus on DUW biofilm contamination has shifted from investigating the problem to developing effective solutions that can be implemented in dental practice. Currently there are a wide variety of commercial DUW treatment products available that are claimed by manufacturers to control waterline contamination and/or eliminate DUW-associated biofilm.[14,30,31,40–43] However, only a few of these products have been extensively tested in situ in DCUs for extended periods. Furthermore, extensive comparative studies of the effectiveness of these products in DCUs await to be undertaken.

Because the phenomenon of DUW biofilm contamination is essentially a consequence of the design of DCUs, i.e. the requirement for narrow-bore DUW tubing to carry water to cool dental instruments and tooth surfaces during dental procedures, there have been calls for DCU manufacturers to consider the problem of DUW biofilm contamination when designing and developing new DCU models.[14,33] This is particularly appropriate as modern DCUs are classified as medical devices. In 2002, we reported on the effectiveness of the Waterline Cleaning System (WCS), a DUW cleaning system developed by the Finnish DCU manufacturer Planmeca Oy (Helsinki, Finland), to control DUW biofilm in Planmeca Prostyle DCUs using hydrogen peroxide-containing disinfectants.[14] At that time the WCS was designed to be retrofitted to existing Planmeca DCU models and delivered disinfectant to DUWs from an external container and was found to be very effective at eliminating DUW biofilm.[14] DUW output water from DCUs disinfected with the WCS contained bacterial densities below the ADA recommended threshold of ≤200 cfu/mL for up to a week post-disinfection. However, the retrofitted system had a number of drawbacks, including the requirement to manually flush some of the waterlines following disinfection, intermittent problems associated with disinfectant leakage from retrofitted valves and the systems effectiveness depended on strict compliance with the operating procedure. The Planmeca Compact i DCU investigated during the present study equipped with the novel WMS represents the next stage in the development of DCUs as it has been specifically designed to contain a fully integrated and automated waterline cleaning system. The WMS cleaning procedure is easy to perform and is facilitated by its automation. All that is required of the operator is to (i) place the instrument hoses into dedicated receivers, (ii) activate the waterline cleaning function on the DCU console, (iii) add disinfectant to the DCU bottle container, (iv) allow the waterlines to fill with disinfectant automatically (v) and then switch the unit off. This process takes less than five minutes. Following disinfection, DUWs are automatically purged of disinfectant and then automatically flushed extensively with

clean water. The WMS is also equipped with a significant additional safety feature, consisting of a vertical air gap that prevents backflow contamination of the mains water supply with water from the DCU waterline network. The WMS is designed so that all parts of the water distribution network within the DCU, including DUWs, atmospheric reservoir, bottle container and pump are all subject to disinfection when the waterline-cleaning mode is activated (Fig. 2).

During the 55-week long period the Compact i DCU was investigated, the microbiological quality of DUW output water was monitored following 50 separate, once-weekly disinfection cycles with the hydrogen peroxide- and silver ion-containing disinfectants Planosil and Planosil Forte. These products were selected for waterline disinfection because our previous studies demonstrated that hydrogen peroxide-containing disinfectants are very efficient at removing DUW biofilm, a key requirement for an effective DCU waterline disinfectant. Planosil (1% (v/v) hydrogen peroxide, 0.003% (w/v) silver) essentially has the same chemical formulation as Sanosil, one of the disinfectant products that we previously demonstrated was effective at controlling biofilm formation in DUWs in the retrofitted DCUs referred to above, whereas Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver) contains higher concentrations of peroxide and silver.[14] Both disinfectant products were found here to effectively and consistently reduce the density of aerobic heterotrophic bacteria, that were used as marker organisms for microbial contamination, in DUW output water immediately following disinfection to levels well below the ADA recommended threshold of ≤200 cfu/mL of output water (mean = 26 cfu/mL for Planosil and 20 cfu/mL for Planosil Forte, respectively, Figs. 3 and 4). Between weekly disinfections with both disinfectants, the bacterial density in DUW output water rose steadily, and in the case of DUWs disinfected with Planosil, exceeded the ADA threshold of ≤200 cfu/mL before the 7th day post-disinfection in 9/10 (90%) disinfection cycles with a mean average bacterial density of 384 cfu/mL (Fig. 3a). In contrast, the bacterial density in output water from DUWs disinfected with Planosil Forte consistently remained below ADA threshold of ≤200 cfu/mL throughout the 40 weekly cycles the DCU was disinfected with Planosil Forte, even 7 days post disinfection where the mean average bacterial density was 113 cfu/mL (Figs. 3b and 4). The density of aerobic heterotrophic bacteria in DUW output water 7 days post-disinfection with Planosil was significantly higher than the ADA threshold of ≤200 cfu/mL ($p = 0.00085$, 95% confidence interval for the mean: 289.6–478.4), while the corresponding density 7 days post-disinfection with Planosil Forte was significantly lower than the ADA threshold of ≤200 cfu/mL ($p = 0004$, 95% confidence interval for the mean: 72.01–151.99). In the case of DUW disinfection with both Planosil and Planosil Forte, the marked reduction in bacterial density recorded in DUW output water was directly associated with elimination of biofilm from the internal surfaces of DUWs as determined by electron microscopy (Fig. 5). Biofilm was observed to have regrown in DUWs 7 days post-disinfection with both disinfectants, but determining whether there was any quantitative differences in the amount or composition of the biofilm present was beyond the scope of this study. Regrowth of biofilm following DUW disinfection and consequent deterioration of the microbiological quality of output water has been described in several studies, hence the requirement for regular DUW disinfection.[8,14,15,18,40,44] The results of the present study demonstrate that the Planmeca WMS used in combination with Planosil Forte consistently maintains the quality of DUW output water below the ADA standard of ≤200 cfu/mL for up to 7 days post-disinfection using a once weekly overnight disinfection. Planosil was not as effective as Planosil Forte in maintaining output water quality, but in 9/10 disinfection cycles the output water from DUWs disinfected with Planosil was below the ADA standard of ≤200 cfu/mL 5 days post-disinfection (Fig. 3a). Clearly the higher hydrogen peroxide and silver ion content of Planosil Forte is more effective at eliminating biofilm, although as mentioned above, electron microscopy of DUWs revealed that both disinfectants were very effective at removing waterline biofilm. It is conceivable that residual biofilm was present in some parts of the waterline network following Planosil disinfection and that this occurred to a lesser extent with Planosil Forte because of its higher concentrations of hydrogen peroxide and silver ions.

Chemical and pH analysis of paired samples of mains water supplied to the Compact i DCU and output water from the air/water syringe waterline immediately following disinfection with either disinfectant showed no significant differences (Table 1) indicating the absence of chemical residues in output water following disinfection. This is an important finding, as patient safety has to be paramount when selecting products for DUW cleaning. Good quality supply water is essential for DCUs as heterotrophic bacteria found in supply water can convert organic material dissolved in supply water into biomass locally.[45] The poorer the quality of the water supplied to DUWs, the more readily biofilm will form on its surfaces. In this regard, in the authors view it is advisable that DCUs connected to a mains water supply should be processed to remove particulate matter, chemical residues, and soluble organic matter.

A number of DUW treatment products involve permanent treatment of water in DUWs and have been claimed to provide continuous protection of the waterlines against biofilm formation. These products, a number of which have been shown independently to be effective at controlling DUW biofilm, are added to water supplied to DCUs, usually from an internal bottle reservoir or are released by devices connected to the DCU water supply, and essentially have a residual effect within the DUWs.[31,41,42] By their very nature these waterline-cleaning products enter the mouths of patients during dental procedures and are claimed not to be detrimental to human health. However, in the authors' view it is prudent to question whether waterline cleaning products should be introduced into the oral cavities of patients, very often without their knowledge, if effective alternative approaches that do not expose patients to cleaning agents are available. Interestingly, a recent study by Stone et al.[46] reported that iodine-releasing cartridges, a residual treatment used to control bacterial growth in DUWs, may have a detrimental affect on the environment by mobilising mercury from dental amalgam in DCUs with the release of highly toxic dissolved mercury into the environment from DCU wastewater.

Many of the Gram-negative bacterial isolates representative of the colony types recovered on R2A agar from DCU water

samples could not be identified using the commercially available API 20 NE system. Other studies have also reported that some bacterial isolates recovered from DUWs are not amenable to identification by conventional means.[37] To circumvent this problem, all isolates were identified unequivocally to the species level by comparative sequence analysis of a PCR amplified variable region of the 16S rDNA gene. Interestingly, many isolates with different types of colony morphology were found to belong to the same species (data not shown). This was particularly evident with *Novosphingobium subarctica* and *Sphingomonas* spp. isolates and isolates of *Micrococcus luteus*. *Sphingomonas* spp. have been detected previously in DUWs.[37] These organisms secrete viscous exopolysaccharides that aid in their ability to adhere to surfaces. *Sphingomonas paucimobilis* (isolated from DUW output water in the present study) has been previously associated with colonisation of mechanical ventilators and other medical devices in the hospital environment, as well as being responsible for episodes of nosocomial infection.[47–49] All of the bacterial species identified in the present study were environmental bacterial species. The narrow range of species identified probably reflects the good quality of the mains supply water provided to DCUs in the Dublin Dental Hospital as demonstrated by chemical analysis and the relatively low density of aerobic heterotrophic bacteria present in DCU supply water (average 77 cfu/mL; range 49–122 cfu/mL). Interestingly, many of the bacterial isolates identified in DUW output water were strongly catalase-positive and thus capable of breaking down hydrogen peroxide. It is interesting to speculate that some of these organisms survived in DUWs following disinfection with Planosil (1% (v/v) hydrogen peroxide) and were subsequently responsible for the earlier deterioration of DUW output water quality compared to DUWs disinfected with Planosil Forte (2.5% (v/v) hydrogen peroxide).

In the present study the combination of hydrogen peroxide- and silver ion-containing disinfectants was shown to be particularly efficient at eradicating waterline biofilm. Silver ions destabilise the biofilm matrix by binding to electron donor groups of biological molecules leading to reductions in the number of binding sites for hydrogen bonds and electrostatic and hydrophobic interactions and, thus, the destabilisation of the biofilm structure.[50] In contrast, the antimicrobial activity of hydrogen peroxide stems from its intense oxidizing properties, which allow it to disrupt membrane lipids, DNA and other essential cell components. One potential concern about the prolonged use of hydrogen peroxide-containing disinfectants to clean DUWs is the potential for the development of biocide tolerance or resistance within waterline biofilm by adaptation or selection following prolonged treatment. Biofilm bacteria are notoriously more difficult to destroy with antimicrobial agents or biocides than planktonic organisms.[1,51–53] In DUW biofilms it is conceivable that catalase-positive bacteria could adapt to exogenous hydrogen peroxide used to eradicate biofilm following long-term use. Tolerance by biofilm bacteria against hydrogen peroxide has been previously reported to be accompanied by an enhanced catalase activity.[44] A previous study with *P. aeruginosa* biofilm showed that biofilm organisms are significantly less susceptible to killing by hydrogen peroxide compared to planktonic forms and that significant catalase induction occurred in biofilms following exposure to hydrogen peroxide.[54] Another study reported that for sufficiently thick biofilms, bacterial cells in biofilm implement adaptive responses more effectively than planktonic cells.[55] To prevent such potential problems arising in DUWs disinfected with hydrogen peroxide following prolonged use, the periodic use of an alternative disinfectant with a different mode(s) of action (i.e. not containing hydrogen peroxide) would be advisable. We are currently evaluating a range of alternative waterline disinfectants for periodic use with the WMS described in this study.

5. Conclusions

In conclusion, the results of this study demonstrate that the Planmeca Compact i DCU equipped with the novel, automated WMS effectively and consistently maintains the quality of DUW output water below the ADA recommended standard of ≤200 cfu/mL of aerobic heterotrophic bacteria for up to 7 days using once weekly disinfection with Planosil Forte. Automation of the WMS eliminates problems associated with manually operated waterline cleaning systems where strict compliance with procedure is necessary for effective control of waterline biofilm. For dental clinics with large numbers of DCUs, such as Dental Hospitals and other public clinics, DCUs with automated waterline cleaning systems such as the WMS will facilitate the efficient disinfection of large numbers of DCUs. For institutions equipped with many DCUs, it is conceivable that DCUs equipped with the WMS could be adapted so that many DCUs could be disinfected simultaneously with disinfectant being supplied from a central reservoir using a dedicated disinfectant delivery line to provide disinfectant to individual DCUs. Control of the process could be mediated electronically from a central desk-top computer. The development of new types of DCU, such as the Planmeca Compact i equipped with the WMS, with automated DUW cleaning systems will undoubtedly make significant contributions to dealing with the problem of DUW biofilm in clinical dental practice.

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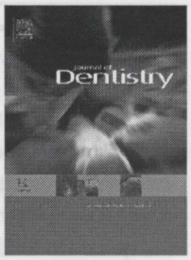

Optimisation of the long-term efficacy of dental chair waterline disinfection by the identification and rectification of factors associated with waterline disinfection failure

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ABSTRACT

Although many studies have highlighted the problem of biofilm growth in dental chair unit waterlines (DUWs), no long-term studies on the efficacy of DUW disinfection using a large number of dental chair units (DCUs) have been reported.

Objectives: To investigate the long-term (21 months) efficacy of the Planmeca Waterline Cleaning System (WCS™) to maintain the quality of DUW output water below the American Dental Association (ADA) recommended standard of ≤200 cfu/mL of aerobic heterotrophic bacteria using once weekly disinfection with the hydrogen peroxide-and silver ion-containing disinfectant Planosil.

Methods: Microbiological quality of DUW output water was monitored by culture on R2A agar for 10 DCUs fitted with the WCS™. The presence of biofilm in DUWs was examined by electron microscopy.

Results: During the first 9 months a high prevalence (28/300 disinfection cycles; 9.3%) of intermittent DUW disinfection failure occurred in 8/10 DCUs due to operator omission to disinfect all DUWs (10/28 failed cycles), incorrect compressed air pressure failing to distribute the disinfectant properly (4/28 failed cycles) and physical blockage of disinfectant intake valves due to corrosion effects of Planosil (14/28 failed cycles). On rectification of these faults through engineering redesign and procedural changes, no further cases of intermittent DUW disinfection failure were observed. Independently of these factors, a rapid and consistent decline in efficacy of DUW disinfection occurred in 4/10 DCUs following the initial 9 months of once weekly disinfection. There was a highly significant difference ($P < 0.0001$) in the prevalence of strongly catalase-positive *Novosphingobium* and *Sphingomonas* bacterial species (mean average prevalence of 37.1%) in DUW output water from these 4 DCUs compared to the other 6 DCUs and DCU supply water (prevalence <1%), which correlated with biofilm presence in the DUWs and indicated selective pressure for maintenance of these species by prolonged disinfectant usage. Planosil was reformulated to a more concentrated form (Planosil Forte) and when used once weekly was found to maintain bacterial density in output water below the ADA standard for all 10 DCUs.

Conclusions: A variety of factors can contribute to failure of DUW disinfection in the long-term, including human error, disinfectant corrosion of equipment and natural selection of naturally disinfectant-tolerant bacterial species.

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1. Introduction

Dental chair units (DCUs) supply water to irrigate the oral cavity and to cool a range of DCU-linked instruments and tooth surfaces during dental procedures.[1,2] Water supplied to the DCU may come in either of two ways, either in replaceable independent bottle reservoirs or directly from a potable water supply. DCU water is distributed through an intricate network of narrow-bore tubing called dental unit waterlines (DUWs). Many studies have demonstrated that DUW output water is frequently contaminated with high densities of microorganisms originating from microbial biofilm on internal DUW surfaces.[3–12] This biofilm is populated from the DCU water supply, which itself usually contains only relatively low densities of microorganisms. The microorganisms in DCU supply water are predominantly aerobic heterotrophic Gram-negative environmental bacterial species. They attach to the internal surfaces of DUWs forming microcolonies that in due course give rise to multispecies biofilm.[13,14] These biofilms consist mainly of highly hydrated bacterial exopolysaccharide, in which single cells and microcolonies are heterogeneously interspersed with pores or channels.[15,16] As the biofilm develops, planktonic cells and by-products are released into the water entering the mouths of patients during dental procedures. Accordingly, DUW biofilm functions as a reservoir for continuous contamination of DCU output water.

The presence of high densities of microorganisms in DCU output water (up to 10^8 cfu/mL has been reported) is a potential infection risk to patients and dental healthcare personnel and is contrary to good cross-infection control practices.[10,12,17] Previous studies have demonstrated that bacteria and fragments of biofilm in DCU output water are aerosolised during DCU instrument use and that patients and dental staff are exposed to these microorganisms.[4,18–22] The presence in DCU output water of overt bacterial pathogens, such as *Legionella pneumophila*, and opportunistic bacterial pathogens, such as *Pseudomonas aeruginosa* and non-tuberculosis *Mycobacterium* species, is of particular concern for the treatment of medically compromised and immunocompromised patients.[4,20,23,24] At present there is no mandatory European Union (EU) quality standard for DCU output water. However, as many DCUs are supplied with municipal drinking water it is not unreasonable to expect that DCU output water quality should be consistent with potable water standards. The current potable water standards for aerobic heterotrophic bacteria in the EU is 100 cfu/mL and in the USA is 500 cfu/mL.[25,26] The current Centers for Disease Control and Prevention (CDC) guidelines for infection control in dental healthcare settings recommend a maximum level of aerobic heterotrophic bacteria in DCU output water of ≤500 cfu/mL, whereas the American Dental Association (ADA) in 1995 established a target of ≤200 cfu/mL for the year 2000.[27,28]

One of the most effective and practical approaches to maintaining good microbial DCU output water quality is regular treatment of DUWs using a disinfectant or biocide that removes biofilm or inhibits biofilm growth.[10–12] A wide range of commercially available DUW cleaning products and systems has been marketed in recent years. Some of the latter are designed to be retrofitted to existing DCUs.[10–12,29] However, few of these microbial control methods have been tested extensively in a large number of DCUs over extended time periods.[12]

In 2002 the authors reported on the effectiveness of the Waterline Cleaning System™ (WCS™), a DUW cleaning system developed by the Finnish DCU manufacturer Planmeca Oy (Helsinki, Finland).[10] This system was designed to maintain good quality DCU output water by controlling DUW biofilm in Planmeca Prostyle Compact DCUs using hydrogen peroxide-containing disinfectants. The WCS™ was designed to be retrofitted to existing Planmeca DCU models and delivers disinfectant periodically to DUWs from a specially designed external reservoir. This was found to be very effective at eliminating DUW biofilm and delivered DUW output water at bacterial densities below the ADA recommended threshold of ≤200 cfu/mL for up to 1 week post-disinfection. Following on from these studies, in 2003 all 103 DCUs in the Dublin Dental School & Hospital were retrofitted with the WCS™ and a once weekly DUW disinfection regimen was introduced using the hydrogen peroxide- and silver ion-containing disinfectant, Planosil. However, during the first 9-month period following the introduction of the WCS™ hospital-wide, routine monitoring of DCU output water identified a significant number of instances of intermittent waterline disinfection failure. The quality of DUW output water was seen to fail the ADA standard of ≤200 cfu/mL, immediately following or, within 48 h of disinfection. Furthermore, during the next 6 months the quality of DUW output water from several of the hospital's DCUs consistently fell below the ADA standard ≤200 cfu/mL by 3–4 days post-disinfection despite the disinfection regimen.

The purpose of the present study was to identify and rectify factors associated with intermittent waterline disinfection failure and to investigate and resolve the phenomenon of rapid deterioration of DUW output water quality following disinfection observed in several problematic DCUs.

2. Materials and methods

2.1. *Dental chair units and disinfection of waterlines using the Planmeca Waterline Cleaning System™*

Ten Planmeca Prostyle Compact DCUs (Fig. 1a; Planmeca Oy, Helsinki, Finland) retrofitted with the Planmeca WCS™ were used in the present study. The DUWs on these DCUs were connected to a treated potable water supply. The DUW cleaning system has been described in detail in a previous study by the authors.[10] The DCUs were located in the Accident and Emergency/Oral Surgery Clinic (4 DCUs), Clinic 1 (3 DCUs) and Clinic 2 (3 DCUs) in the Dublin Dental School & Hospital. Between July 2003 and February 2005 (18 months) the efficacy of the WCS™ units in maintaining good microbiological quality of DUW output water was investigated while the DCUs were in daily use for dental patient treatments. The DCUs were disinfected once per week following the afternoon clinical session. The four DCUs in Accident and Emergency/Oral Surgery Clinic were disinfected solely by two of the authors, whereas dental nursing staff assigned to the other clinics disinfected the remaining six DCUs.

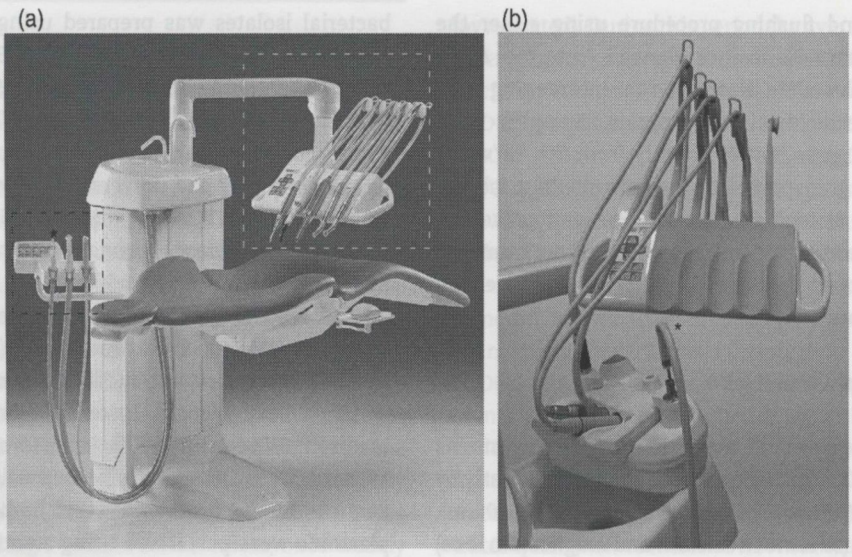

Fig. 1 – The Planmeca Prostyle Compact DCU model used in the present study. (a) Overview of the Prostyle Compact DCU. The operator's instrument console containing the instrument hoses with instruments attached is highlighted with white dashed lines in the upper right portion of the panel. The assistant's console with the assistant's three-in-one air/water syringe (*) and the suction system hoses attached is highlighted with black dashed lines in the lower left portion of the panel. (b) View of the DCU instrument hoses in situ in the flushing receivers during a disinfection cycle. The position of the assistant's three-in-one air/water syringe is indicated (*).

The authors have previously described the operation of the WCS™ in detail.[10] In brief, when using the WCS™, the DCU instrument hoses containing the DUWs are first placed into instrument hose receivers located in a specially designed receptacle (Fig. 1b). In the configuration of the Prostyle Compact DCUs used in this study, the instrument hoses containing DUWs supplying the dental operators instruments (i.e. turbine handpiece, conventional handpiece and ultrasonic scaler) and three-in-one air/water syringe are all located together on an instrument console attached to a moveable arm (Fig. 1a), whereas the assistant's three-in-one air water syringe is located separately on the assistant's console along with the high volume and low volume suction hoses (Fig. 1a). All DUWs, apart from the cuspidor rinse DUW, are included in a microprocessor-controlled process whereby waterlines are automatically filled with disinfectant and subsequently automatically flushed with clean water during the disinfection cycle. If the cycle is not completed properly or a power failure occurs during the cycle, an error message is displayed on the DCU's instrument console. While the error message is displayed all DCU functions are locked preventing the DCU from being used. Pressing an activation key on an integrated keyboard on the DCU instrument console starts the cleaning cycle. A mains water supply switch to the DCU is then turned off and the WCS™ disinfectant container module filled with disinfectant is then attached to an integrated receiver module on the DCU. This receiver module contains a disinfectant intake valve and an air valve, the latter supplying pressurised air from the DCU's medical quality air supply. Air from the air valve pressurises disinfectant in the disinfectant container bottle until a pressure of 1.5 bar is reached, after which the disinfectant intake valve opens and disinfectant is fed from the container, first to the instrument hoses, and then to the cup-fill waterline. The disinfectant, which contains a blue marker dye, flows through the DUWs until observed emitting from the outlet of each DUW. The electrical supply to the DCU is then switched off leaving the DUWs full of disinfectant overnight (15 h). When the electrical supply to the DCU is turned on again, a code on the DCU instrument console is displayed prompting the operator to disconnect the disinfectant container module, fill it with warm tap water and reattach it to the unit to start an initial flushing cycle of the instrument waterlines (30 s) and cup-fill line (20 s). After this cycle, a code prompts the operator to remove the container module and to turn on the mains water supply switch. This triggers an automatic flushing cycle whereby clean mains water is used to sequentially flush the instrument waterlines and cup-fill line for a total duration of 4 min. After this automatic flushing and rinsing cycle, the DCU is ready for normal use.

The DUW disinfectants used in the present study were Planosil (1.5% (v/v) hydrogen peroxide, 0.003% (w/v) silver, 0.0015% (v/v) ortho-phosphoric acid) and Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver, 0.0025% (v/v) phosphoric acid), both of which are marketed by Planmeca.[10,12] Hydrogen peroxide and silver ions are the active antimicrobial ingredients in both disinfectants, while ortho-phosphoric acid is used as a stabilising agent. Both of these disinfectant products contain a blue tracking dye to aid visual observation of complete filling of waterlines during the disinfectant feeding cycle.

2.2. *Collection of water samples*

Samples of water (50 mL) were collected from the air/water syringe waterline of 10 Planmeca Prostyle Compact DCUs immediately prior to disinfection and immediately following

the disinfection and flushing procedure using either the waterline disinfectant Planosil or Planosil Forte.[10,12] Additional water samples were also taken on intervening days each week between disinfection cycles. Samples were collected in sterile glass bottles directly from the air/water syringe waterline, transported to the microbiology laboratory within 5 min at ambient temperature and processed immediately.[12] Water samples were also taken from the mains water supplied to the DCUs at monthly intervals throughout the study period.

2.3. *Mains supply water*

In the Dublin Dental School & Hospital chlorinated mains water supplied to DCUs is automatically processed firstly by particle filtration, (removal of particles greater that 10 μm), then ion exchange (to remove calcium and magnesium ions) and finally by UV treatment (to reduce the numbers of viable microorganisms) prior to delivery to DCUs.[10,12] The chemical quality of the water supplied to the DCUs is tested regularly and has been shown to comply consistently with the limits advocated by the EC Directive on the Quality of Water for Human Consumption and by the United States Environmental Protection Agency National Primary Drinking Water Regulations.[10,12] Previous studies from this laboratory have shown no significant difference between the processed mains water supplied to DCUs and DUW output water following disinfection with either Planosil or Planosil Forte.[12]

2.4. *Laboratory processing of water samples*

After collection, water samples were taken immediately to the microbiology laboratory for analysis and cultured in duplicate on R2A agar plates (Difco, Becton Dickinson, Le Pont de Claix, France) to determine total aerobic heterotrophic bacterial density as described previously.[10,12,30] After 10 days incubation at room temperature (approximately 20 °C) in a static incubator (Gallenkamp, Leicester, England), plates were examined and colonies counted using a Stuart Scientific colony counter (Bibby Sterilin Ltd., Staffordshire, UK). The total number of colonies and the relative abundance of different colony types were recorded as the average of the duplicate plates, and selected examples of all the colony types present on each plate were purified by subculture and stored on nutrient agar slopes in the dark for subsequent identification. These were Gram-stained, tested for catalase production and then identified.

2.5. *Identification of bacterial isolates*

Bacterial isolates were identified, where possible, by means of the API 20 NE system for identifying non-enterobacterial Gram-negative bacteria (bio-Mérieux, Marcy l'Etoile, France) as described previously.[10,12] Definitive identification of isolates was undertaken by determining the DNA sequence of a segment of the small ribosomal subunit rRNA gene and by comparing the sequences with consensus sequences for individual bacterial species in the EMBL/GenBank nucleotide sequence databases.[12] Briefly, total genomic DNA from bacterial isolates was prepared using the Qiagen DNeasy kit system (Qiagen, Crawley, West Sussex, United Kingdom).[12] A variable segment of the 16S rDNA gene of each bacterial isolate was amplified by PCR as described previously, using approximately 30 ng of purified genomic DNA as template with the universal primers 533F (5′-AGAGTTTGATC/TA/CTGGCTCAG-3′) and 142R (5′-CGGC/TTACCTTGTTACGA-3′).[31] These primers amplify a region of approximately 950 bp to 1.5 kb of the 16S rDNA gene of all bacterial species. Amplified PCR products were purified using the QIAEX II gel extraction kit (Qiagen) according to the manufacturer's instructions prior to cloning into the pGEM T-Easy Vector System I (Promega Corporation, Madison, Wisconsin, USA). Transformation of competent *Escherichia coli* DH5alpha prepared using $CaCl_2$ and identification of *E. coli* derivatives harbouring recombinant plasmids were performed using standard methods.[32] DNA sequencing of cloned amplimers was performed commercially by Lark Technologies (Essex, United Kingdom) using an automated Applied Biosystems 373A DNA sequencer (Foster City, California, USA), dye-labelled terminators and the primers T7 (5′-TAATACGACTCACTATAGGG-3′) and SP6 (5′-ATTTAGGTGACACTATAGAAT-3′) that are specific for the pGEM T-Easy Vector (Promega). Analysis of chromatograms and sequences was carried out using the 373A data analysis software programme version 1.2.0 (Applied Biosystems) and DNA Strider 1.3f11 software (CEA/Saclay, Gif-sur-Yvette, France), respectively. The identity of individual bacterial isolates was determined by comparing their 16S rDNA sequences to those in the EMBL and GenBank nucleotide sequence databases using the BLAST family of computer programmes (http://www.ncbi.nlm.nih.gov/BLAST/).[33]

2.6. *Electron microscopy of waterline biofilm*

Two-centimetre lengths of DUW waterline tubing (supplying water to the air/water syringe close to the syringe) were removed at intervals before and immediately following disinfection with both Planosil and Planosil Forte. The samples were cut longitudinally through the lumen using a sterile scalpel and then the internal walls examined for the presence of biofilm, without prior fixation, by electron microscopy using a Zeiss Supra 35 variable pressure field emission scanning electron microscope as described previously.[10] The elemental composition of corrosion deposits of WCS disinfectant delivery modules was examined by Energy Dispersive X-ray (EDX) analysis using an Oxford Instruments (Oxford, UK) INCA EDX Drycool SiLi detector during electron microscopy. All electron microscopy and EDX analysis was undertaken by the Centre for Microscopy and Analysis (CMA), Trinity College, Dublin, Ireland.

2.7. *Statistical analysis*

The relative abundance of colonies of *Novosphingobium* and *Sphingomonas* spp. cultured from DUW output water in the 10 DCUs used in this study was statistically analysed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test.

3. Results

3.1. *Effectiveness of once weekly dental unit waterline disinfection with Planosil during the initial 9-month period*

The main purpose of the present study was to prospectively investigate the effectiveness of the Planmeca Waterline Cleaning System™ (WCS™) using once-weekly DUW disinfection with Planosil over a prolonged period (21 months, between July 2003 and March 2005), as no studies on the long-term efficacy of dental unit waterline disinfection have been reported in the literature. Ten representative DCUs were selected for study from the hospital's three main treatment clinics and the microbiological quality of their waterline output water was investigated. During the 9-month period July 2003–March 2004, the DUW output quality from the 10 DCUs was each tested during a total of 30 individual, once weekly disinfection cycles (i.e. water quality was tested following a total of 300 separate disinfection cycles of the 10 DCUs investigated). During this period the average density of aerobic heterotrophic bacteria in the processed DCU supply water was 66 cfu/mL (range 60–112 cfu/mL). The microbiological quality of DCU output water remained below the ADA standard of ≤200 cfu/mL for up to a week following DUW disinfection for 272/300 (90.7%) of the disinfection cycles investigated (Fig. 2a shows representative data from five consecutive disinfection cycles from one DCU). For the remaining 28/300 (9.3%) disinfection cycles DCU output water quality failed the ADA standard, in every case immediately or within 48 h following disinfection. These 28 *failed* disinfection cycles occurred in 8/10 DCUs investigated and were intermittent throughout the 9-month period July 2003–March 2004, with the first two failed cycles being detected within 1 month of starting the study. Apart from two exceptions, no two consecutive rounds of waterline disinfection in individual DCUs resulted in output water quality that failed the ADA standard.

During the course of this study several samples of DUW tubing from the assistant's three-in-one air/water syringe

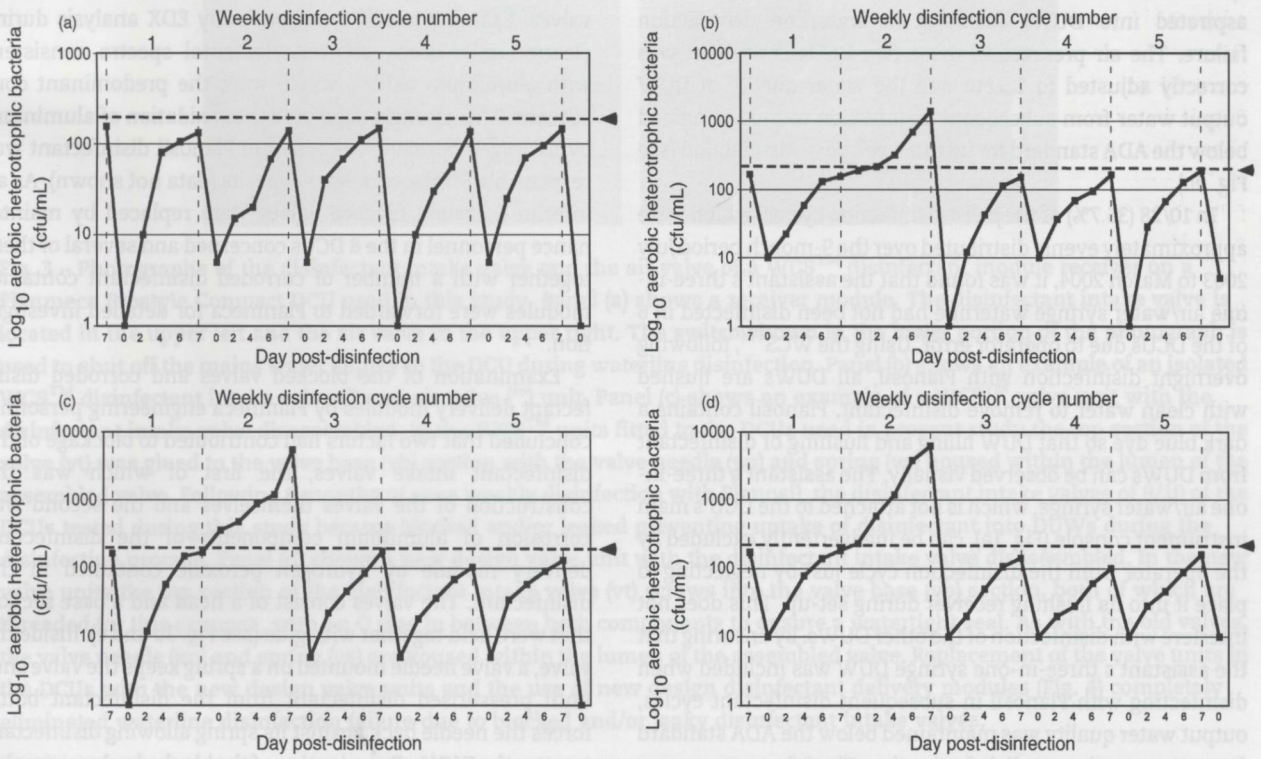

Fig. 2 – Density of aerobic heterotrophic bacteria in three-in-one air/water syringe waterline output water from Planmeca Prostyle Compact DCUs fitted with the Planmeca Waterline Cleaning System™ (WCS™) during consecutive cycles of once weekly disinfection with Planosil. In 272/300 (90.7%) individual disinfection cycles with 10 DCUs between July 2003 and March 2004 the microbiological quality of DUW output water remained below the ADA standard of ≤200 cfu/mL of aerobic heterotrophic bacteria (horizontal dashed lines) following once a week overnight (15 h) disinfection with Planosil. Panel (a) shows an example of typical data obtained in five consecutive successful disinfection cycles from a DCU where water quality remained within the ADA limit for up to 7 days post-disinfection. Panels (b)–(d) show data obtained in five consecutive disinfection cycles from DCUs that exhibited intermittent failure of waterline disinfection due to (b) low air pressure (occurred in 4/300 disinfection cycles with the 10 DCUs tested), (c) operator failure to include the assistant's three-in-one air/water syringe DUW in the disinfection cycle (occurred in 10/300 disinfection cycles with the 10 DCUs tested) and (d) blockage and/or leakage of the disinfectant intake valve (occurred in 14/300 disinfection cycles with the 10 DCUs tested). The failed disinfection cycle is shown as cycle number 2 in each case. By addressing the cause of DUW disinfection failure in these latter three cases (i.e. correctly adjusting the air pressure (b), ensuring to disinfect the assistant's three-in-one air/water syringe DUW (c) and cleaning or replacing the valve (d)) subsequent disinfection cycles resulted in water quality that remained within the ADA limit up to 7 days post-disinfection.

DUW were detached from the DCUs and the internal surfaces examined for the presence of biofilm by electron microscopy. Samples from DCUs that yielded output water within the ADA standard up to 7 days post-disinfection had no detectable biofilm present immediately following disinfection with Planosil, although isolated patches of biofilm were evident by day 7 post-disinfection. Patches of biofilm were evident immediately following disinfection, however, in DUWs from DCUs that exhibited intermittent failure of waterline disinfection (data not shown).

3.2. *Investigation of factors associated with intermittent waterline disinfection failure and their rectification*

In 4/28 (14.3%) of the *failed* disinfection cycles, the DCU valve controlling the pressure of medical quality air supplied to dental handpieces had been inadvertently turned down in two separate DCUs. This pressurised air supply is also used by the WCS™ to force disinfectant from the WCS™ disinfectant reservoir into DUWs during waterline disinfection (see Section 2). Thus in these four disinfection cycles, disinfectant was not aspirated into DUWs resulting in waterline disinfection failure. The air pressure in these two DCUs concerned was correctly adjusted to 1.5 bar and the water quality of DUW output water from subsequent disinfection rounds remained below the ADA standard for up to a week post-disinfection (see Fig. 2b).

In 10/28 (35.7%) of the *failed* disinfection cycles, which were approximately evenly distributed over the 9-month period July 2003 to March 2004, it was found that the assistant's three-in-one air/water syringe waterline had not been disinfected in 6 of the DCUs due to operator error. Using the WCS™, following overnight disinfection with Planosil, all DUWs are flushed with clean water to remove disinfectant. Planosil contains a dark blue dye so that DUW filling and flushing of disinfectant from DUWs can be observed visually. The assistant's three-in-one air/water syringe, which is not attached to the DCU's main instrument console (Fig. 1a), can be inadvertently excluded by the operator from the disinfection cycle just by neglecting to place it into its flushing receiver during set-up. This does not interfere with disinfection of the other DUWs. By ensuring that the assistant's three-in-one syringe DUW was included when disinfecting with Planosil in subsequent disinfectant cycles, output water quality was maintained below the ADA standard for up to a week post-disinfection (see Fig. 2c).

In 14/28 (50%) of the *failed* disinfection cycles, all of which occurred during the period January to March 2004 after 6 months of continuous once weekly disinfection with Planosil, DUW disinfection failure was directly associated with blockage of and/or leakage from WCS™ disinfectant intake valves, and corrosion of components of disinfectant delivery module bottles. On initiating the disinfection cycle disinfectant either failed to leave the disinfectant bottle or only a portion of the disinfectant left the disinfectant bottle and thus DUWs were not filled or were incompletely filled with disinfectant. In the WCS™, the disinfectant container module attaches to a receiver module on the DCU unit (Figs. 3 and 4).[10] The receiver module consists of a disinfectant intake valve, an air valve and a mains water cutoff switch (see Fig. 3a). During the disinfection process, the disinfectant container (see Fig. 4a) interfaces with the receiver module and the disinfection cycle is initiated electronically from the DCU control console. Air from the DCU's medical quality air supply is forced through the air valve pressurising the disinfectant in the disinfectant container. When the pressure reaches 1.5 bar, the disinfectant inlet valve opens allowing disinfectant to fill the DUWs. Dismantling of the WCS™ receiver modules in all 8 affected DCUs and examination of the disinfectant intake valves revealed that all the valves were blocked or partially blocked and/or leaky. Furthermore, examination of the disinfectant delivery module units revealed the presence of patches of powdery corrosion of aluminium components comprising the head of the module and the aluminium collar that connects the module head with the polyethylene disinfectant bottle container (see Fig. 4). The intake valves in the remaining 2 non-affected DCUs were found to be functioning normally and were not blocked or leaky. These findings suggested that a constituent(s) of the disinfectant used (i.e. Planosil) may have been responsible for the corrosion following prolonged use and that the powdery deposits associated with the corrosion may have been responsible for blocking the disinfectant intake valves. Examination of the deposits by EDX analysis during electron microscopy showed elemental spectra consistent with aluminium oxides, which were the predominant constituent. This strongly suggested that oxidation of aluminium by hydrogen peroxide contained in Planosil disinfectant was responsible for the corrosion deposits (data not shown). As an interim measure, blocked valves were replaced by maintenance personnel in the 8 DCUs concerned and several of them together with a number of corroded disinfectant container modules were forwarded to Planmeca for detailed investigation.

Examination of the blocked valves and corroded disinfectant delivery modules by Planmeca engineering personnel concluded that two factors had contributed to blockage of the disinfectant intake valves, the first of which was the construction of the valves themselves and the second was corrosion of aluminium components of the disinfectant delivery module by hydrogen peroxide contained in the disinfectant. The valves consist of a head and a base section that were held together with glue (see Fig. 3b and c). Inside the valve, a valve needle mounted on a spring keeps the valve shut until pressurised disinfectant from the disinfectant bottle forces the needle back against its spring allowing disinfectant to enter the DUWs. Examination of the blocked valves revealed that glue and deposits had blocked the valve lumens in each case. Furthermore, in some cases loss of glue at the junction of the two valve sections resulted in valve leakage, whereas in other cases, the presence of glue and deposits in the valve lumen prevented the valve from closing after the disinfectant container had been emptied during DUW filling. This allowed disinfectant to leak from the DUWs. Corrosion of aluminium components appeared to be the major contributory factor as valve blockage in experimental DCUs became exacerbated when exposed to higher concentrations of hydrogen peroxide (Planmeca, personal communication to D. Coleman). To circumvent these problems, new valves and disinfectant delivery modules were designed by Planmeca and manufactured. The head and base sections of the new valves are threaded and are screwed together with an O ring in between

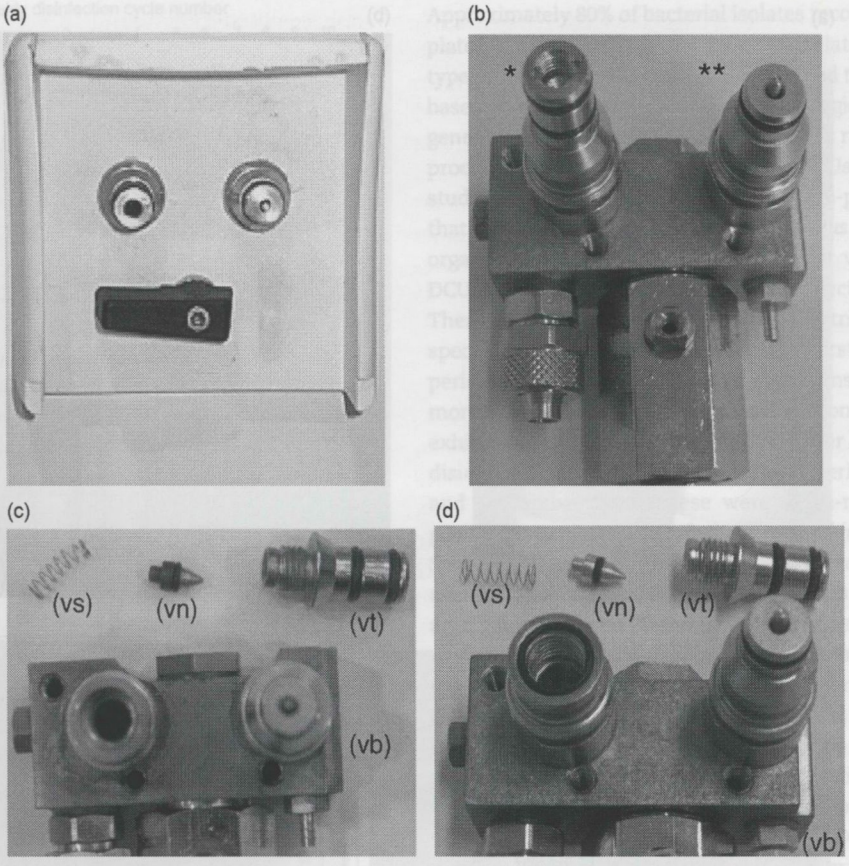

Fig. 3 – Photographs of the disinfectant intake valve and the air valve of a WCS™ disinfectant module receiver on a Planmeca Prostyle Compact DCU used in this study. Panel (a) shows a receiver module. The disinfectant intake valve is located in the upper left and the air valve in the upper right. The switch shown in the lower portion of the photograph is used to shut off the mains water supply to the DCU during waterline disinfection. Panel (b) shows an example of an isolated WCS™ disinfectant intake valve (*) and air valve (**) unit. Panel (c) shows an example of a WCS™ valve unit with the disinfectant intake valve disassembled. In the WCS™ units fitted to the DCUs used in present study the top section of the valve (vt) was glued to the valve base (vb) section with the valve needle (vn) and spring (vs) housed within the lumen of the assembled valve. Following 6 months of once weekly disinfection with Planosil, the disinfectant intake valves of 8/10 of the DCUs tested during this study became blocked and/or leaked preventing uptake of disinfectant into DUWs during the disinfection process. Panel (d) shows a new design valve unit with the disinfectant intake valve disassembled. In the new valve units the top section of the disinfectant intake valve (vt) screws into the valve base (vb) section, both of which are threaded for this purpose, with an O ring in between both components to ensure a watertight seal. As with the old valves, the valve needle (vn) and spring (vs) are housed within the lumen of the assembled valve. Replacement of the valve units in the DCUs with the new design valve units and the use of new design disinfectant delivery modules (Fig. 4) completely eliminated waterline disinfection failure due to blocked and/or leaky disinfectant intake valves.

to give a tight seal without the requirement for glue (see Fig. 3b and d). In the newly designed disinfectant delivery modules, the aluminium components of the head and collar of the original modules have been replaced with acid-resistant steel and plastic (see Fig. 4a and b).

For the 10 DCUs used in this study replacement of the disinfectant intake valves and disinfectant delivery modules by the newly designed valves and disinfectant delivery modules eliminated further instances of DUW disinfectant failure due to blocked disinfectant intake valves. During the summer of 2004, all DCUs in the Dublin Dental Hospital had their WCS™ valve units replaced with the new-design valve units. No further instances of WCS™ valve leakage or blockage have been experienced since.

3.3. *Decline of efficacy of once weekly waterline disinfection with Planosil*

The second phase of the study was undertaken with the same 10 DCUs during the 12-month period April 2004–March 2005. During this period, between April and September 2004, the microbiological quality of DUW output water from 4/10 (40%) DCUs consistently failed (i.e. 22 consecutive once weekly disinfection cycles) the ADA standard of $\leq$200 cfu/mL of aerobic heterotrophic bacteria by day 7 post-disinfection (Fig. 5a and b). In all of these cases, no detectable biofilm was evident in DUWs by electron microscopy immediately following disinfection and the bacterial density in DUW output water was well below the ADA standard immediately following disinfection but rapidly

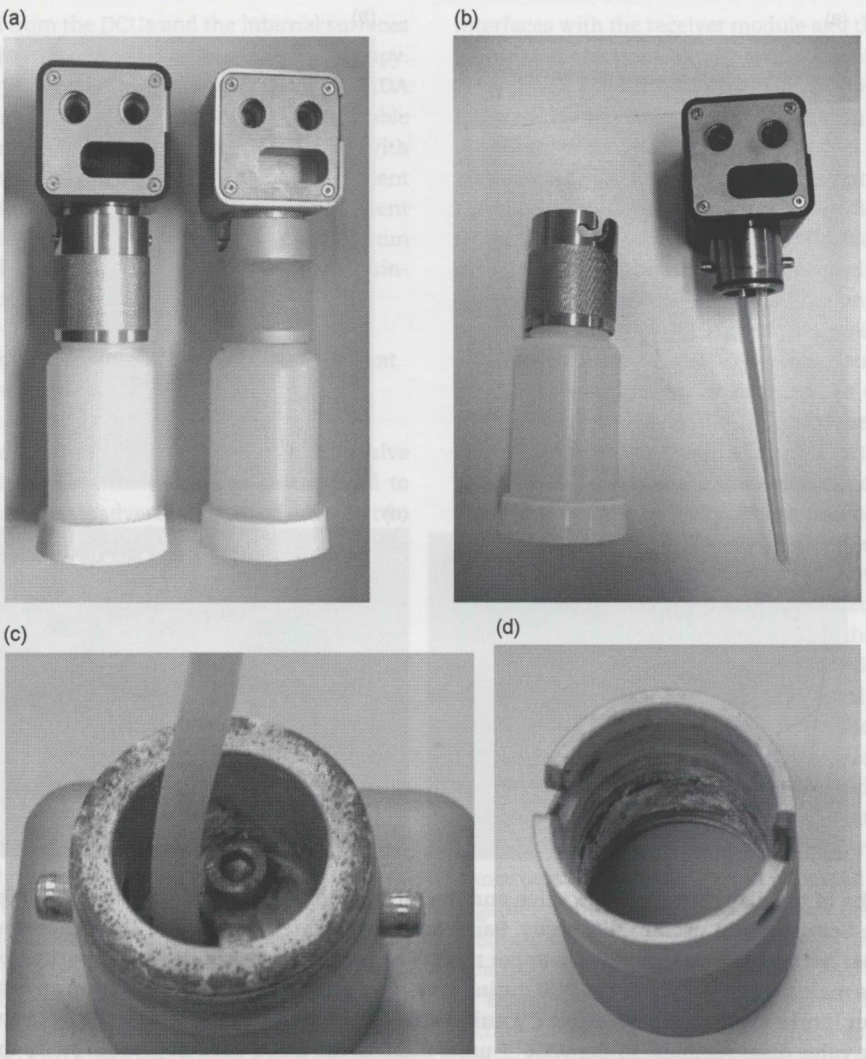

Fig. 4 – Disinfectant delivery bottle modules. Panel (a) shows an original design (right of figure) and new design (left of figure) disinfectant delivery module used in the present study. The head of the modules are designed to interface with the WCS™ receiver (Fig. 2a) units fitted on DCUs. Both modules function in the same way, have the same overall dimensions and each consist of three components including an identical polyethylene bottle container (lower portion of each module), a central collar unit into which the bottles are screwed and an upper head interfacing unit. Both the collar and head units of the old type modules were manufactured from aluminium and were prone to corrosion by hydrogen peroxide contained in the Planosil disinfectant used in this study. Panel (b) shows an example of the new type module with the head unit disassembled from the remainder of the module. In these new modules the collar unit and neck of the head unit are manufactured from high-quality, acid-resistant steel that is resistant to corrosion by hydrogen peroxide. The head unit is manufactured from hard plastic. Panels (c) and (d) show Planosil-induced corrosion on the aluminium neck of the head unit and the internal surface of an aluminium collar unit, respectively, from of an old-design disinfectant delivery module.

deteriorated, and in most cases exceeded the ADA standard by day 4 post-disinfection (Fig. 5a and b). This type of contamination event was associated with the presence of patchy biofilm on the internal surfaces of DUWs as detected by electron microscopy. During this period the average density of aerobic heterotrophic bacteria in the processed DCU supply water was 70 cfu/mL (range 46–118 cfu/mL). Careful examination of the DCUs showed that their WCS™ units were all functioning correctly and the rapid deterioration in DUW output water quality observed in these DCUs following disinfection could not be attributed to any of the factors that were responsible for intermittent failure of waterline disinfection in the first part of the study described above.

The authors decided to investigate the diversity and distribution of aerobic heterotrophic bacterial species in DUW output water from these DCUs in order to determine whether the relative abundance of individual species types could be associated with the rapid decline in DUW output water quality following disinfection. Bacterial isolates recovered from DCU supply water and from DUW output water samples from the first phase (July 2003–March 2004) and second phase (April 2004-March 2005) of this study were included for comparison.

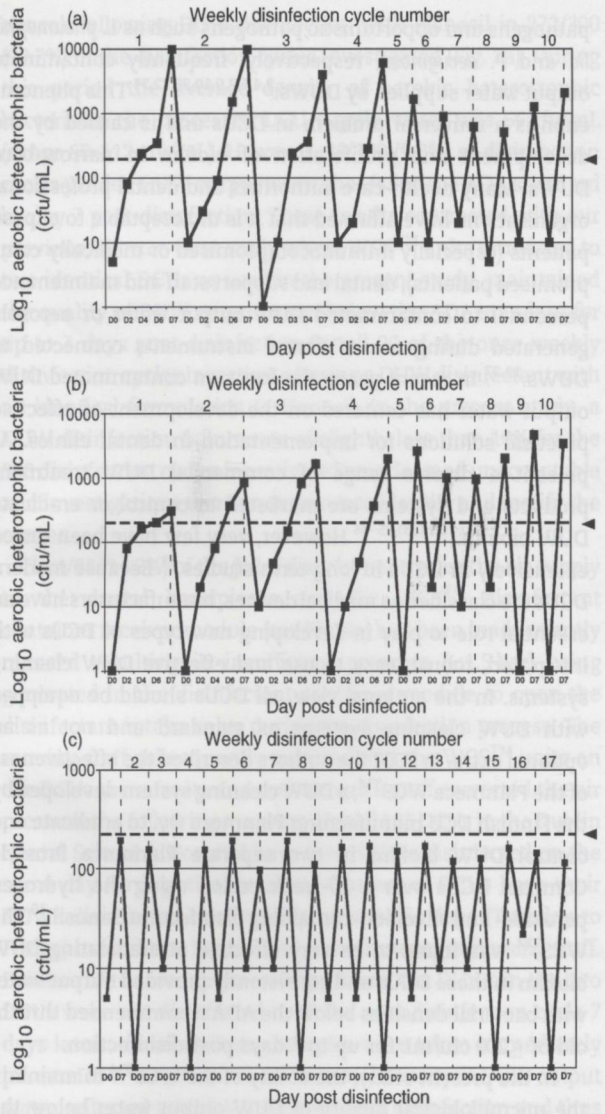

Fig. 5 – Density of aerobic heterotrophic bacteria in three-in-one air/water syringe DUW output water from Planmeca Prostyle Compact DCUs fitted with the Planmeca Waterline Cleaning System™ (WCS™) during consecutive cycles of once weekly disinfection during the second study period (April 2004–March 2005). The horizontal dashed lines indicate the maximum ADA limit of 200 cfu/mL of aerobic heterotrophic bacteria. Panels (a) and (b) show bacterial density levels in waterline output water from two separate DCUs (May–July 2004) that failed to maintain the ADA standard for up to a week post-disinfection with Planosil. In both cases, bacterial density levels exceeded the ADA maximum limit between days 4 and 6 post-disinfection. The predominant bacterial species present in waterline output water from these two DCUs were highly mucoid and strongly catalase-positive *Novosphingobium subarctica* strains and strains of several *Sphingomonas* species. Panel (c) shows the bacterial density levels in DUW output water from a DCU (the same DCU used to obtain data shown in panel (a) above) disinfected once weekly for 17 consecutive disinfection cycles (September 2004–January 2005) with Planosil Forte. For each cycle, bacterial levels remained below the ADA maximum limit up to 7 days post-disinfection.

Approximately 80% of bacterial isolates recovered on R2A agar plates were Gram-negative. Selected isolates of each colony type recovered on R2A agar were identified to the species level based on the DNA sequence of a variable region of the 16S rDNA gene. The most common bacterial isolates recovered from the processed mains water supply to the DCUs during the entire study period were Gram-positive, catalase-positive organisms that were identified as *Micrococcus luteus* (Table 1). These organisms were also identified in output water from the 10 DCUs used in the study following disinfection with Planosil. There was no obvious difference in the distribution of bacterial species in DUW output water during the first and second study periods. However, one group of organisms was significantly more prevalent in DUW output water from the 4 DCUs that exhibited rapid decline in output water quality following disinfection during the second study period between April and September 2004. These were Gram-negative, catalase-positive organisms identified as *Novosphingobium subarctica*, *Sphingomonas paucimobilis* or other *Sphingomonas* species (mean average prevalence of 37.1% of all colonies recovered on R2A agar, range between 20 and 50%). In contrast, these organisms were a minority (<1% of all colonies recovered on R2A agar) of the aerobic heterotrophic bacterial populations recovered in DCU supply water throughout the study, in all 10 DCUs during the first study period and from the 6 DCUs during the second study period that did not exhibit rapid decline in DUW output water quality following disinfection. The prevalence of *Novosphingobium* and *Sphingomonas* sp. in the 4 DCUs that exhibited rapid decline in DUW output water quality following disinfection during the second phase of the study was significantly different from the corresponding prevalence in the remaining 6 DCUs and from the prevalence of these organisms in all 10 DCUs

Table 1 – Bacterial species isolated from the air/water syringe waterline of the dental chair units used in this study

Bacterial species[a]	Gram-stain	Catalase activity[b]
Novosphingobium subarctica	Negative	+++++
Sphingomonas paucimobilis	Negative	+++++
Sphingomonas aerolata	Negative	+++++
Sphingomonas trueperi	Negative	+++++
Acidovorax temperans	Negative	–
Porphyrobacter tepidarius	Negative	–
Pseudomonas fluorescens	Negative	+++
Arthrobacter agilis	Positive	++++
Variovorax paradoxus	Positive	++++
Rhodococcus fascians	Positive	+++
Kocuria palustris	Positive	+++
Micrococcus luteus	Positive	+++

[a] Identified by DNA sequence analysis of the variable region of 16S rDNA gene.

[b] An arbitrary scale for catalase activity was used based on the visual observance of the evolution of oxygen bubbles from a bacterial colony following the addition of 50 μL of 5% (v/v) H_2O_2. +++++ = very strongly catalase-positive with instantaneous and substantial evolution of gas bubbles; +++ = catalase-positive with immediate but moderate evolution of gas bubbles; – = catalase-negative. For each isolate tested, at least 5 separate colonies grown on R2A agar medium were tested for catalase production.

during the first phase of the study as determined by one-ANOVA testing (d.f. = 9, $F = 216.6$, $P < 0.0001$). Further analysis with Tukey's *post hoc* test showed a significant difference between each of 4 DCUs that exhibited rapid decline in water quality and each of the remaining 6 DCUs ($P < 0.001$). All of the *N. subarctica* and other *Sphingomonas* isolates were very mucoid and found to be very strongly catalase-positive (Table 1) and this latter feature may have been a significant contributory factor in the rapid decline of DUW output water quality following disinfection with Planosil due to its reliance on hydrogen peroxide as one of the main active ingredients of this disinfectant.

3.4. *Replacement of Planosil with Planosil Forte*

In order to develop an effective practical solution to the problem of rapid decline in DUW output water quality following once weekly disinfection with Planosil (1.5% (v/v) hydrogen peroxide, 0.003% (w/v) silver, 0.0015% (v/v) ortho-phosphoric acid), the four DCUs that exhibited this phenomenon during the study period April–September 2004 and two that did not were subject to 17 separate once weekly DUW disinfection cycles with Planosil Forte (2.5% (v/v) hydrogen peroxide, 0.012% (w/v) silver, 0.0025% (v/v) phosphoric acid) during October 2004–March 2005. DUW output water quality was again monitored by determining the density of aerobic heterotrophic bacteria per mL of water following culture on R2A agar medium. For all 6 DCUs, the bacterial density in DUW output water consistently remained below the ADA standard of ≤200 cfu/mL for up to a week post-disinfection. Representative data from one DCU is shown in Fig. 5c; this DCU was one of the four DCUs that exhibited rapid decline in DUW output water quality with Planosil during the period April–September 2004. Electron microscopy of the internal surfaces of samples of DUWs demonstrated the absence of detectable biofilm immediately post-disinfection with Planosil Forte, although patches of biofilm were detectable by electron microscopy at 7 days post-disinfection. Colonies of *N. subarctica* and other *Sphingomonas* species continued to be isolated from DUW output water from the DCUs disinfected with Planosil Forte and from the DCU supply water. In the majority of cases these species formed a minority population (<1% of all colonies recovered on R2A agar). These findings demonstrated that replacement of Planosil with Planosil Forte was an effective practical solution to the problem of rapid decline of DUW output water quality following once weekly disinfection with Planosil.

From October 2005, Planosil Forte was introduced as the standard DUW disinfectant throughout the Dublin Dental School & Hospital. Between October 2005 and June 2006, monthly sampling of DUW output water from 6 DCUs, two selected from each of the hospital's three main treatment clinics, showed the continued efficacy of Planosil Forte at maintaining the quality of DUW output water below the ADA standard, up to 7 days post-disinfection.

4. Discussion

During the last decade many published studies from around the world have highlighted that high densities of microbes, principally bacterial species, including several known human pathogens and opportunistic pathogens such as *L. pneumophilia* and *P. aeruginosa*, respectively, frequently contaminate output water supplied by DUWs.[3,4,6,7,9,10,20,22,34] This phenomenon is a universal problem in DCUs and is caused by the development and proliferation of biofilm in narrow-bore DUWs. Many health-care authorities and dental professional organisations have affirmed that it is unacceptable to expose patients (especially immunocompromised or medically compromised patients), dental and support staff and maintenance personnel to contaminated DUW output water or aerosols generated during use of dental instruments connected to DUWs.[12,27] In recent years, the focus on contaminated DUW output water has centered on the development of effective practical solutions for implementation in dental clinics. At present a diverse range of commercial DUW treatment products and systems are marketed to control or eradicate DUW biofilm.[10,11,20,35–44] However, very few have been tested extensively in DCUs in long-term studies.[12] Because modern DCUs are classified as medical devices, manufacturers have an essential role to play in developing new types of DCUs with integrated, robust, easy to use and effective DUW cleaning systems. In the authors' view, all DCUs should be equipped with DUW cleaning systems as standard and not as an optional extra. In 2002 the authors described the effectiveness of the Planmeca WCS™, a DUW cleaning system developed by the Finnish DCU manufacturer Planmeca Oy, to eradicate and control DUW biofilm in two separate Planmeca Prostyle Compact DCUs over a 20-week period using the hydrogen peroxide- and silver ion-containing disinfectant Sanosil.[10] The WCS™ was found to be very effective at eradicating DUW biofilm in these DCUs and consistently provided output water with bacterial densities below the ADA recommended threshold of ≤200 cfu/mL for up to 7 days post-disinfection.

In the present study, the ability of the WCS™ to maintain the microbiological quality of DUW output water below the ADA standard was investigated over a much longer period (18 months) with a much larger number of DCUs (10 DCUs) using the hydrogen peroxide- and silver ion-containing DUW disinfectants Planosil (identical formulation to Sanosil) and Planosil Forte. Dental nursing staff disinfected the DUWs in 6 of the DCUs, whereas DUWs in the remaining 4 DCUs were disinfected by two of the authors. This approach was adopted specifically to investigate the efficacy of the WCS in busy working dental clinics. Apart from one other study from this laboratory with a DCU equipped with a different DUW disinfection system to that used here,[12] the present study is the only prospective study on the long-term efficacy of DUW disinfection yet to be reported. During this period the WCS™ performed well in the majority of cases providing DUW output water in compliance with the ADA standard for up to 7 days post-disinfection. However, two distinct kinds of anomalous DUW post-disinfection outcomes were observed during the course of the study, including intermittent failure of DUW disinfection that occurred in the first period of the study (July 2003–March 2004) and secondly, rapid decline in the microbiological quality of DUW output water following disinfection that occurred during the second period of the study (between April and September 2004). In the first 9-month period of the study (July 2003–March 2004) the microbiological quality of DUW output water remained below the ADA standard for up to

a week following DUW disinfection with Planosil in 272/300 (90.7%) of the disinfection cycles investigated (Fig. 2a). During this period the average density of aerobic heterotrophic bacteria in the processed DCU supply water was 66 cfu/mL (range 60–112 cfu/mL). However, 28/300 (9.3%) of disinfection cycles yielded DUW output water that failed the ADA standard by day 7 post-disinfection. These results contrasted with our previous, shorter-term study where WCS™ units retrofitted to two identical DCUs as used in the present study, maintained the quality of DUW output water below the ADA standard for up to 7 days post-disinfection for all 20 of the once weekly disinfection cycles investigated using a DUW disinfectant with an identical formulation to Planosil. In the present study, a DUW disinfection failure rate of slightly less than 10% for the 300 disinfection cycles investigated was deemed unacceptable and investigations were undertaken to identify and rectify the causes.

In 4/28 (14.3%) of the *failed* disinfection cycles the air supply to the dental handpieces and the WCS™ disinfectant container receiver module (see Fig. 4a) had been inadvertently reduced resulting in insufficient pressure (<1.5 bar) being generated in the disinfectant container module to open the disinfectant intake valve during the disinfection process. The nursing staff subsequently reported that the WCS™ units on the DCUs were faulty and maintenance staff corrected the air pressure the following morning. Not all 103 DCUs in the Dublin Dental School & Hospital have their DUWs disinfected on the same day for logistical reasons. Clusters of DCUs have their DUWs disinfected once weekly on specific days (Tuesday to Friday) to facilitate the clinical commitments of nursing staff. Because of the low air pressure problem, the DUWs in the two DCUs were not disinfected until the next disinfection cycle 7 days later and when water samples were taken immediately prior to DUW disinfection, the bacterial density in DUW output water reflected a 14-day period without disinfection and thus failed the ADA standard. Clinical staff were instructed not to adjust the air pressure setting on any of the DCUs and no further instances of DUW disinfection failure due to low air pressure were encountered during the study period.

In 10/28 (35.7%) of the *failed* disinfection cycles involving 6 DCUs, failure was due to operator error whereby nursing staff undertaking DUW disinfection inadvertently neglected to include the assistant's three-in-one air/water syringe in the disinfection process. In the Planmeca Prostyle Compact DCUs retrofitted with WCS™ units used in the present study, disinfection of the DUWs is microprocessor controlled. During the disinfection process, disinfectant is automatically fed to the DUWs supplying the instrument hoses and the patient's cup-fill DUW from the pressurised disinfectant container module. The instrument hoses are first placed in their flushing receivers, the disinfection cycle is activated from the DCU instrument console and each DUW is filled with disinfectant in a specified order dictated by the microprocessor. However, if either three-in-one syringe line is not in its flushing receiver, but all of the other lines are, the disinfection process can still be activated from the instrument console. If a three-in-one DUW is not disinfected it will continue to contaminate output water from this DUW. Furthermore, because all DUWs in DCUs are interconnected, this DUW can rapidly contaminate the other DUWs, as many of the bacterial species present in DUW biofilm are motile. It was fortunate in the present study that flushing of disinfectant from DUWs following overnight (15 h) disinfection in the 10 DCUs included in the study, and the collection of DUW output water samples for microbiological analysis were undertaken by the authors. Because of this we were able to observe and record that the assistant's three-in-one air/water syringe DUW had not been included in the disinfection cycle (i.e. the assistant's three-in-one air/water syringe hose was still attached to the assistant's console; see Fig. 1a) during 10 disinfection cycles involving 6 DCUs. In all 10 of these *failed* disinfection cycles, DUW output water from the assistant's three-in-one air/water syringe failed the ADA standard, either immediately following the DCU disinfection process or within 24–48 h (representative data from one DCU is shown in Fig. 2c). In contrast, DUW output water from the operator's three-in-one air/water syringe DUW from the 6 DCUs during the 10 *failed* disinfection cycles was well below the ADA standard immediately post-disinfection, but quality rapidly deteriorated and failed the ADA standard within 3 days post-disinfection (data not shown). Contamination from the assistant's three-in-one air/water syringe DUW was probably the cause of this rapid deterioration of output water from the operator's three-in-one syringe DUW as suggested above. To prevent further occurrences of this type of intermittent DUW disinfection failure, clinic managers were requested to ensure that nursing staff undertaking DUW disinfection were vigilant not to neglect to disinfect the assistant's three-in-one air/water syringe DUW. This type of problem could be eliminated completely by incorporating into DCUs error detection systems for failure to position hoses for decontamination.

In 14/28 (50%) of the *failed* disinfection cycles involving 8 DCUs, all of which occurred following 6 months of continuous once weekly disinfection with Planosil, failure was directly associated with blockage of and/or leakage from WCS™ disinfectant intake valves, and corrosion of components of disinfectant delivery module bottles. Detailed investigations revealed that the disinfectant intake valves of the WCS™ disinfectant container receiver units on the DCUs had an unidentified defect in that the upper and lower valve sections were joined with glue (Fig. 3b and c). Following 6 months of regular once weekly disinfection with Planosil the valves began to block and/or leak. It is likely that the strongly oxidising capacity of hydrogen peroxide in the disinfectant was responsible for damaging the integrity of the glue sealing both sections of the valves. This would give rise to leakage of disinfectant from the junction of the two valve sections and probably also caused sections of glue to become dislodged into the valve lumen. Indeed, examination of blocked valves revealed the presence of glue in the valve lumen. These problems with the valves were exacerbated by corrosion of aluminium components of the disinfectant delivery container modules, again most likely caused by oxidisation of aluminium by hydrogen peroxide present in the Planosil disinfectant. Areas of corrosion were often characterised by the presence of loose, flaky deposits on the metal surfaces involved. These deposits very likely contributed to blockage of disinfectant intake valves. Valve blockage prevented disinfectant being fed into DUWs during the disinfection process resulting in DUW disinfection failure. Likewise, leakage of disinfectant from the intake valve would prevent

sufficient disinfectant being fed to the DUWs to ensure adequate disinfection. This would particularly affect the assistant's three-in-one DUW, as it is the last DUW to be filled with disinfectant during the disinfection process. The design of new valves, both sections of which are machined to screw together with an O ring in-between (Fig. 3d), and the design of new disinfectant container modules, with acid-resistant steel instead of aluminium (Fig. 4), completely solved the problem of valve blockage and leakage and eliminated this particular cause of intermittent DUW disinfection failure. This type of problem very likely occurs with many other types of medical devices apart from DCUs, as decontamination chemicals and methods change and points to the necessity for long-term compatibility testing and communication between manufacturers and end users.

During the second phase of the April 2004–March 2005 study, between April and September 2004, the microbiological quality of DUW output water from 4/10 (40%) DCUs consistently failed the ADA standard by day 7 post-disinfection (Fig. 5a and b). In contrast to the cases of intermittent DUW disinfection failure recorded between July 2003 and March 2004 described above, in these cases the bacterial density in DUW output water was well below the ADA standard immediately following disinfection but rapidly and consistently deteriorated, and in most cases exceeded the ADA standard by day 4 post-disinfection (Fig. 5a and b). This correlated with the presence of patchy biofilm on the internal surfaces of DUWs as detected by electron microscopy. During this period the average density of aerobic heterotrophic bacteria in the DCU supply water was 70 cfu/mL (range 46–118 cfu/mL). Examination of the 4 DCUs concerned revealed that the rapid deterioration in DUW output water quality observed in these DCUs post-disinfection could not be attributed to any of the factors responsible for intermittent failure of waterline disinfection detected in the first phase of this study (see above). However, detailed analysis of the DUW output water from these 4 DCUs showed that one group of organisms (*N. subarctica*, *S. paucimobilis* or other *Sphingomonas* species; Table 1) was significantly more prevalent ($P < 0.0001$) compared with output water from the other 6 DCUs or the DCU supply water. *N. subarctica*, *S. paucimobilis* or other *Sphingomonas* species accounted for between 20 and 50% of all bacterial isolates recovered from DUW output water from these 4 DCUs, but were a minority population (<1% of all bacterial colonies recovered on R2A agar) in all other DUW output water samples throughout the study. Isolates of these species were found to be very strongly catalase-positive and thus had the ability to break down hydrogen peroxide. It is very likely that this latter feature was a significant factor in the rapid deterioration of DUW output water quality in the 4 DCUs concerned. Furthermore, a more recent study from this laboratory investigating a more advanced integrated DUW cleaning system fitted to a single Planmeca Compact i DCU, also demonstrated rapid deterioration of DUW output water quality by day 7 post-disinfection with Planosil.[12] In 9/10 consecutive once weekly disinfection cycles with this DCU, output water quality failed the ADA standard by day 7 post-disinfection. The most prevalent bacterial species recovered from output water were also *N. subarctica*, *S. paucimobilis* or other *Sphingomonas* species. *Novosphingobium* and *Sphingomonas* spp. have previously been isolated from DUWs and they produce copious amounts of viscous exopolysaccharides that enable the organisms to adhere to surfaces and give rise to dense protective biofilms.[12,31,45] Some of these organisms have been responsible for colonisation of mechanical ventilators and other medical equipment in hospitals and have been responsible for episodes of nosocomial infection.[46–50]

The use of hydrogen peroxide-containing disinfectants to control DUW biofilm over extended periods has the potential for the development of biocide resistance or tolerance within DUW biofilm by adaptation and/or selection following prolonged use. Bacteria within biofilms are notoriously more difficult to eradicate with antimicrobials or biocides than planktonic organisms.[2,51–53] In DUW biofilms it is likely that catalase-positive bacteria can be selected by and/or adapt to hydrogen peroxide contained in disinfectants used to eradicate biofilm following prolonged use. Tolerance to hydrogen peroxide exhibited by biofilm bacteria has been reported previously to be accompanied by enhanced catalase activity. A previous study with *P. aeruginosa* biofilm demonstrated that organisms in biofilms were significantly less susceptible to killing by hydrogen peroxide compared to planktonic forms and that significant catalase induction occurred in biofilms following exposure to hydrogen peroxide.[54] A different study reported that for sufficiently thick biofilms, bacterial cells in biofilms implement adaptive responses more effectively than planktonic cells.[55] Another study with *P. aeruginosa* biofilms proposed that new genes are expressed when the bacteria attach to a surface and begin to form biofilm and that some of the proteins expressed by these genes could reduce the susceptibility of the bacteria to oxidative biocides such as hydrogen peroxide.[56] Interestingly, a recent study from Poland on DUW disinfection with Oxygenal 6, a different hydrogen peroxide- and silver ion-containing disinfectant to that used in the present study, reported that isolates of *S. paucimobilis* were significantly more prevalent (~prevalence of 80%) in DUW output water post-disinfection compared to output water pre-disinfection (~prevalence 10%).[45] This finding together with the results of the present study suggest that disinfection of DUWs with hydrogen peroxide- and silver ion-containing disinfectants can select for the maintenance of bacterial species such as *Novosphingobium* or *Sphingomonas* species. It is interesting to speculate why only 4/10 DCUs used in the present study exhibited rapid deterioration of DUW output water quality in the second phase of the present study. This may have been associated with a requirement for a critical density of strongly catalase-positive *Novosphingobium* or *Sphingomonas* organisms in DUWs of individual DCUs as these species were only predominant in DCUs that showed rapid decline in output water quality. It is very likely that all 10 DCUs used in the present study, indeed all-103 DCUs with which the Dublin Dental School & Hospital is equipped, would all eventually have succumbed to the problem of rapid deterioration of DUW output water quality following prolonged, once weekly disinfection with Planosil. In order to circumvent this, it was decided to investigate the efficacy of Planosil Forte, an alternative DUW disinfectant with a stronger concentration of hydrogen peroxide and silver ions.

In the present study, the use of Planosil Forte with its higher concentration of hydrogen peroxide and silver ions compared to Planosil effectively dealt with the problem of rapid deterioration of DUW output water quality in the 4 DCUs where this problem was encountered. Similarly, the use of Planosil Forte effectively dealt with the problem of rapid decline of DCU output water quality in our previous study with the Planmeca Compact i unit referred to above.[12] Because in the present study, it is likely that rapid failure of DCU output water was also occurring in some of the remaining 93 DCUs in the Dublin Dental School & Hospital. Routine DUW disinfection is now undertaken with Planosil Forte. However, it is likely that prolonged use of Planosil Forte will eventually result in reduced efficacy of this product to control DUW biofilm by the development of biofilm tolerance. To prevent such difficulties developing in DUWs disinfected with hydrogen peroxide following protracted use, the periodic use of a different disinfectant with a different mode(s) of action would be prudent. We are currently evaluating the efficacy of a range of alternative DUW disinfectants for periodic use. Furthermore, we are also developing systems that remove nutrients from DCU supply water, thus making it more difficult for biofilm-forming bacterial species to grow in DUWs.

5. Conclusions

The present study is the only prospective study to date to document the long-term efficacy of DUW disinfection with multiple DCUs. The study identified and appropriately rectified causes of DUW disinfection failure using the Planmeca WCS™ due to operator error, design flaws and the selection of disinfectant-tolerant bacterial species. All of these factors are likely to adversely affect the long-term efficacy of other DUW cleaning and disinfection systems. It is important to emphasise that DCU manufacturers have an important role to play in further developing and improving DUW cleaning systems. Only by undertaking ongoing long-term field studies with DCUs used in clinical practice can the problems with DUW cleaning and disinfection be identified and rectified, thus ensuring good quality DUW output water for patients and dental healthcare staff.

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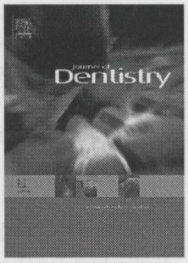

Review

The role of manufacturers in reducing biofilms in dental chair waterlines

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ABSTRACT

Objectives: This paper reviews how dental chair unit (DCU) manufacturers can contribute practically to resolving the problem of biofilm formation in dental unit waterlines (DUWs).

Study selection: The review concentrates on how novel developments and changes in a range of specific areas have, and might contribute to DUW biofilm control. These include (i) DCU engineering and design changes; (ii) improvements to DCU supply water quality; (iii) development of automated DUW treatment procedures that are effective at controlling biofilm in the long-term, safe for patients and dental staff, environmentally friendly and which do not exhibit adverse effects on DCU components after prolonged use.

Sources: The majority of the material contained in this review is based on, or supported by the peer-reviewed literature.

Data: The current consensus from the literature reveals that the emphasis on DUW biofilm and its control has focused on describing the problem and its control using a range of periodic and residual DUW treatment agents. Unfortunately, until recently, DCU manufacturers have provided very little specific guidance in this regard. Indeed, ensuring that DCUs provide good quality output water has generally been regarded to be the responsibility of dental practitioners. Some recent studies have shown that novel DCUs with integral semi-automated or automated DUW cleaning systems can effectively control DUW biofilm in the long-term. However, there are other potential DCU engineering and design changes that DCU manufacturers could undertake to further improve DUW biofilm control.

Conclusions: DCU manufacturers can significantly contribute to controlling the problem of DUW biofilm.

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1. Introduction

1.1. *Biofilms and microbial contamination of dental waterlines*

Modern dental chair units (DCUs) are classified as medical devices under the EU Medical Devices Directive.[1] According to this Directive, a medical device is "any instrument, apparatus, appliance, material or other article, whether used alone or in combination, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of diagnosis, prevention, monitoring, treatment or alleviation of disease". Microbial contamination of a wide range of medical devices, such as anaesthetic equipment, laryngoscopes, endoscopes, gastroscopes, mechanical ventilators, resuscitation tubes, colonoscopes and catheters, has been recognised as an important cause of cross-contamination and cross-infection, especially in the hospital setting.[2] Wet or moist sites or surfaces of medical devices have been particularly associated with cross-infection and cross-contamination because such areas are conducive to the growth of microbial biofilm. Because DCUs are used in the treatment of many patients throughout each day, microbial contamination of specific component parts is an important potential source of cross-infection.[2] Of particular concern are parts of DCUs that come into direct contact with the patient's oral cavity, including dental unit handpieces, three-in-one air/water syringes and suction hoses. DCU output water is also of concern as a source of potential cross-infection as it comes directly from the DCU and enters the oral cavity of the patient during treatment.[2] Furthermore, droplets and aerosols generated by DCU handpieces may be inhaled by patients and dental healthcare personnel.[3–6]

DCUs consist of several complex, integrated equipment systems that are central to the practice of modern dentistry. These systems are designed to provide the instruments and services necessary for a diverse range of dental procedures.[6–8] DCUs use water to cool and irrigate a variety of DCU-supplied instruments and tooth surfaces during dental procedures, as the heat generated during instrument operation can be detrimental to teeth.[4,9,10] DCU-supplied water is also used for oral rinsing by patients during and following dental treatment and to wash out the DCU spittoon, or cuspidor, after oral rinsing. DCUs supply water as a coolant and irrigant to turbine and conventional handpieces, ultrasonic scalers, three-in-one air/water syringes, as well as providing water for the patient rinse cup filler and cuspidor via an intricate network of interconnected narrow-bore tubes called dental unit waterlines (DUWs).[7,8]

Over the last 20 years many studies have shown that water from DUWs is often contaminated with high densities of microorganisms, predominantly bacterial species.[7,8,11–23] This is a universal problem and all untreated DUWs in standard DCUs are subject to contamination and will host resident biofilms. Microbial contamination of DUWs originates predominantly in the DCU water supply, which usually contains low levels of microorganisms.[15,22] In a typical modern DCU the waterline distribution network consists of several meters of plastic DUW tubing with an internal diameter of a few millimeters in which water can stagnate when the equipment is not being used.[16,18] Microorganisms in water supplied to DCUs, predominantly aerobic heterotrophic Gram-negative environmental bacteria, attach to the internal surfaces of the DUW tubing and form microcolonies that eventually give rise to multispecies biofilm.[17,20,22] DUW biofilms are composed mainly of highly hydrated bacterial exopolysaccharide secreted by bacteria that contain microcolonies and single cells interspersed heterogeneously with channels or pores.[23,24] Biofilm primarily forms in DUWs because water at the internal surface of the narrow-bore waterline tubing flows more slowly than water at the centre and thus there is little disturbance to any microorganisms present.[16] This permits the microorganisms to multiply and subsequently disperse through the water supply as planktonic forms where they may be deposited at other sites within the waterline network or are transferred directly into the mouths of patients during dental procedures. For these reasons DUW biofilm acts as a reservoir for continuing contamination of DCU output water. Fig. 1, panels (a) and (b) show examples of biofilm in DUWs. Sterilisation of the handpieces, syringes and associated instruments makes no impact on biofilm within DUWs whatsoever.

The occurrence of high densities of microorganisms in DCU output water (up to 10^6 cfu/mL has been reported) constitutes a potential risk of infection for dental patients and dental healthcare and support staff and is diametrically opposed to good infection control practices.[14,22,25] Furthermore, it is ethically unacceptable to knowingly expose patients, dental healthcare staff and support staff to heavily contaminated water even if the assessable cross-infection risk is considered to be low. Previous studies have shown that bacteria in DCU output water are aerosolised by DCU handpieces during dental procedures and that dental personnel and patients are exposed to these microorganisms, to fragments of DUW-derived biofilm and to waterborne bacterial endotoxin, the latter consisting of lipopolysaccharide released from the cell walls of dead Gram-negative bacteria.[13,26–30] Most of the bacterial species found in DCU output water are Gram-negative aerobic heterotrophic environmental bacterial species that exhibit very low pathogenicity although they may be of concern in the treatment of vulnerable patients, such as immunocompromised and medically compromised individuals.[7,17,20,22] Nonetheless there is considerable potential for infection with some bacterial pathogens found in DCU output water such as *Pseudomonas aeruginosa*, *Legionella pneumophila* and non-tuberculosis *Mycobacterium* species.[13,27,29,31] Only a few instances of cross-infection related to DUWs and associated biofilm have been reported in the literature.[13,29] However, it is still possible that DCU output water-associated infections have gone undetected or unreported because of the failure to associate exposure to DCU output water and aerosols generated from this water with the development of specific infections. Sporadic infections not requiring hospital admission are also less likely to be investigated.

2. Dental chair unit water systems

2.1. *Water quality standards*

According to the EU Medical Devices Directive "the devices and manufacturing processes must be designed in such a way

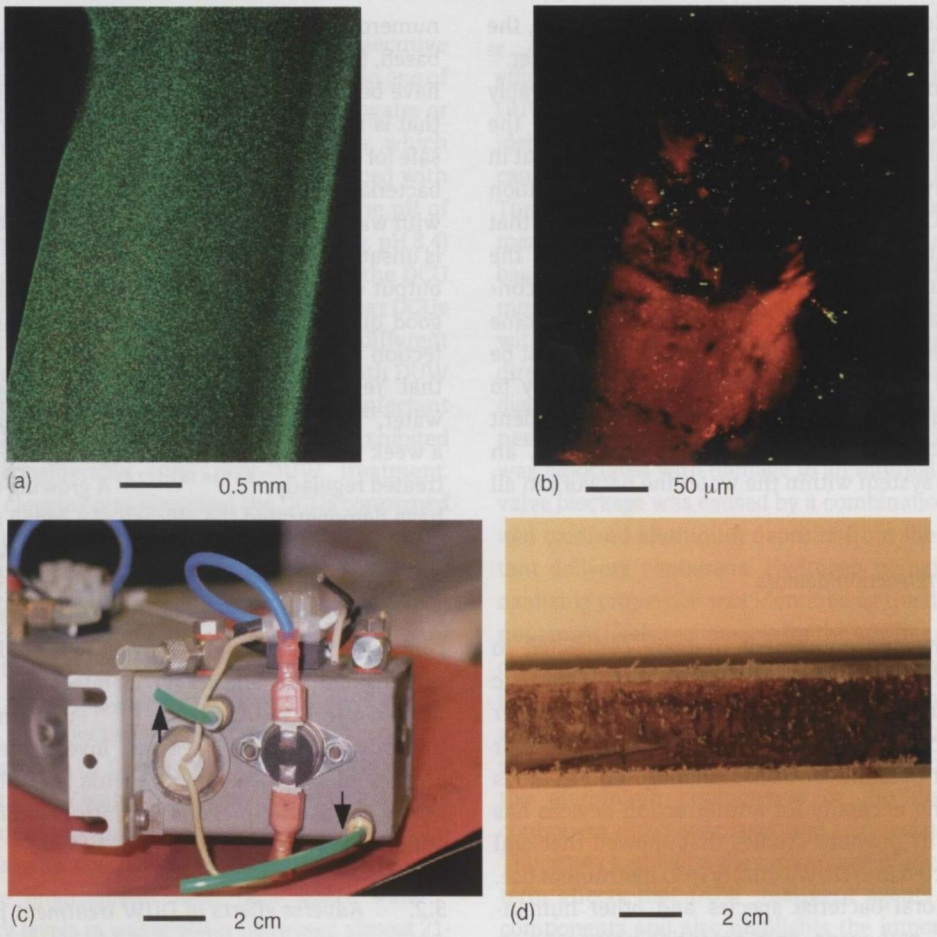

Fig. 1 – Biofilm in DCUs and supply pipework. (a and b) Confocal micrographs of longitudinal sections of the internal surfaces of DUW tubing showing extensive biofilm. Living microorganisms populate areas stained green, whereas areas stained red contain predominantly dead microorganisms. The internal surface of the DUW tubing section shown in panel (a) is completely covered with biofilm containing mostly living microorganisms, hence the overall intense green staining. In contrast, the DUW tubing biofilm shown in panel (b) is populated mainly by dead microorganisms, hence the predominant red staining. (c) View of a DCU water heater removed from a DCU. The green coloured tubes indicated by arrowheads are DUWs. (d) View of a longitudinal section of a plastic water pipe used to supply DCUs with mains water showing the presence of extensive biofilm and deposits on the internal surface.

as to eliminate or reduce as far as possible the risk of infection to the patient, user and third parties".[1] Thus DCU manufacturers have an important responsibility to design for reduced risk of biofilms and also to provide detailed and specific instructions in relation to control of DUW biofilm in the DCUs they manufacture and market to dental clinics and dental practitioners. Unfortunately, until recently, DCU manufacturers have provided very little specific guidance in this regard. Indeed, ensuring that DCUs provide good quality output water has generally been regarded to be the responsibility of dental practitioners or dental clinic management. One of the main contributing factors to this conspicuous absence of direction on DCU biofilm control by DCU manufactures is the absence of specific regulations or standards concerning the microbiological quality of DCU output water. Currently there are no microbiological quality standards imposed for DCU output water within the EU. However, although DCU output water is not intended for human ingestion, it frequently does become so and it is not unreasonable to expect that the quality of DCU output water should therefore approximate potable water standards. The potable water standards set for the EU and the USA are 100 and 500 colony forming units (cfu) per mL, respectively, of aerobic heterotrophic bacteria.[32,33] In 1995, the American Dental Association (ADA) established a goal for the year 2000 of ≤200 colony forming units (cfu) per mL of aerobic heterotrophic bacteria for DCU output water.[34] However, this standard has not been widely achieved, indicating the extent of this problem. The current Centers for Disease Control and Prevention (CDC) guidelines for infection control in dental health-care settings recommend that DCU output water should be ≤500 cfu/mL of aerobic heterotrophic bacteria.[25]

2.2. *DCU water supply*

Water supplied to DCUs is usually provided either in independent bottle reservoirs or directly or indirectly from a municipal mains water supply. In Dental Hospitals and in some large dental clinics equipped with many DCUs, water supplied to DCUs often comes from large holding tanks supplied with mains water, whereas in smaller clinics DCUs

may have individual mains connections. Within the EU, the majority of DCUs are supplied with municipal mains water.[35] Pressure variations in the mains supply could conceivably cause retraction or backflow of water from DCUs into the mains if the pressure in the mains supply drops below that in DUWs. To circumvent this possibility, the water distribution network in some DCU models contains an air gap system that physically separates the water within the DUWs from the supply water, thus preventing backflow of potentially contaminated DCU water into the supply water network.[7] In some European countries the law requires that an air gap must be incorporated into the water supply to a dental surgery to prevent backflow into the mains supply.[36,37] It seems prudent that DCU manufacturers should include, as standard, an integrated air gap system within the waterline network in all DCU models.

2.3. *DCU anti-retraction devices*

Dental instruments and equipment that are connected to DUWs and which enter the patient's mouth (e.g., ultrasonic scalers, turbine and conventional handpieces, or air/water syringes) should contain integrated antiretraction valves or devices to prevent backflow or back siphonage of oral fluids into the DUWs. The necessity for antiretraction devices has been emphasised by previous studies that showed that oral fluids can be retracted into DUWs during DCU instrument use. The detection of oral bacterial species and other human-derived microbial pathogens and potential pathogens in dental unit water has provided convincing evidence for probable failure of antiretraction devices.[22,38,39] Furthermore, a recent Italian study of 54 DCUs, comprising 18 different models by six different DCU manufacturers demonstrated an antiretraction device failure rate of 74% (40/54 DCUs tested).[40] Clearly, DCU manufacturers should provide clear and unambiguous data on the efficacy of antiretraction devices in DCUs that they market. Furthermore, manufacturers should provide clear and concise guidance in relation to periodic testing and maintenance of antiretraction devices. In our view, this should form part of routine DCU maintenance schedules. Manufacturers also have a responsibility to undertake ongoing research and development in this area to improve the quality, robustness and efficacy of antiretraction devices. Currently, best practice recommends that DCU handpieces should be operated to discharge water and air for a minimum of 20–30 s after each patient to flush out patient material and oral fluids that may have been retracted into the handpiece airlines, or waterlines.[25] Furthermore, handpieces should be cleaned, lubricated and sterilised by autoclaving after each patient use.

3. Eradication and control of biofilm in DUWs

3.1. *Biofilm control in DUWs*

As mentioned above, until recently, responsibility for dealing with the problem of biofilm in DUWs has been considered the duty of the dental practitioner. DCU manufacturers have been slow to respond to the obvious need for DCU engineering and design changes to control DUW biofilms. Over the last 20 years numerous proposals, both chemical based and non-chemical based, for reducing the bacterial density in DCU output water have been suggested but none has been universally adopted that is both efficient at eliminating biofilm, as well as being safe for patients.[15,16] One widely used practice for reducing the bacterial density in DCU output water involves flushing DUWs with water.[41] However, flushing does not remove biofilm and is unsatisfactory as a means of controlling the quality of DUW output water.[15,20,42] The most efficient means of maintaining good quality DUW output water is regular treatment/disinfection of DUWs with a chemical, biocide or cleaning agent that removes biofilm, resulting in good quality output water.[22,43,44] Biofilm re-growth in DUWs usually occurs within a week following disinfection/cleaning and so DUWs need be treated regularly.[7,8,15,16,19,20,22,43] A growing number of studies have demonstrated the efficacy of a broad range of commercially available treatment products for DUWs that efficiently remove biofilm and reduce bacterial density to potable water quality or better.[7,8,22,42–53] Many of these studies have been conducted *in vitro* and relatively few studies have actually investigated the efficacy of disinfectants to achieve these desired effects in DCUs.[7,8,22,44,48,54] Furthermore, only a small minority of studies have yet investigated the long-term efficacy of DUW treatment agents in DCUs in the clinical setting, whilst comprehensive controlled comparative efficacy studies have yet to be undertaken.[7,8]

3.2. *Adverse effects of DUW treatment products*

DUW treatment agents can broadly be divided into two basic categories, including agents for periodic DUW treatment (e.g. once weekly) and agents for continuous or residual DUW treatment. The majority of all the currently available DUW treatment agents have not been developed or endorsed by DCU manufacturers, but rather have been developed by other third-party manufacturers in response to an obvious market need. Until recently, this situation has been a significant shortcoming among DCU manufacturers. Because of this, there is significant potential for incompatibility of DUW treatment agents with components of the DCU waterline network as well as with instruments connected to this network. A small number of studies have reported adverse affects associated with the application of DUW treatment agents, but, as mentioned above, only a very few long-term studies of the efficacy of DUW treatment agents have been reported to date. Furthermore, in the case of residual DUW treatment agents, there is a noticeable paucity of independent studies in the literature on potential interactions of such agents and their by-products on oral and dental tissues. Interestingly, a number of recent studies reported that some DUW treatment agents may adversely affect bonding of composite material to both enamel and dentine.[55,56] With the prolonged and more widespread use of DUW treatment agents, it is likely that such adverse effects may become clinically relevant in the case of residual DUW treatments.

In 2002, a study from this laboratory reported that the use of the alkaline hydrogen peroxide DUW treatment agent Sterilex Ultra was associated with clogging or blocking of DUWs in three of six DCUs treated with this product.[22] Clogging was caused by the accumulation of disinfectant

deposits and became apparent after the fourth consecutive week of once weekly treatment in the three units, and in one of these, after 14 weeks it became impossible to aspirate water or disinfectant through the air/water syringe waterline, which had become completely blocked and had to be replaced with new waterline tubing. Furthermore, in these DCUs the pH of DUW output water remained persistently alkaline (e.g. pH 8.4) for up to 5 days post-DUW treatment, in contrast to the DCU supply water (pH 7.0) and DUW output water from other DCUs (pH 7.0) in the same clinic that were treated with a different hydrogen peroxide treatment agent not associated with DUW clogging.[57] These findings suggested that residual disinfectant was present in DUW output water in the DCUs that exhibited clogging for a considerable time post-DUW treatment. Fortunately, once clogging was identified the DCUs concerned were temporarily decommissioned until the problem could be resolved and also to prevent patients from being exposed to DCU output water containing disinfectant residue. Such clogging and decommissioning are expensive in terms of staff and patient time and maintenance costs.

A recent study from the USA reported that iodine-releasing resin cartridges, an effective residual treatment used to control bacterial growth in DUWs by constant release of low levels of iodine into DUW water, may have a detrimental affect on the environment by mobilising mercury from dental amalgam in DCUs with the release of highly toxic dissolved mercury into the environment from DCU waste water.[58] Dissolved mercury levels in waste water increased almost 21-fold. However, the findings of this study were recently challenged by other authors who claimed that chloramine was more likely to have caused the increase in mercury levels rather than iodine.[59] Other researchers have showed that a range of disinfectants and cleaning agents used to treat DCU wasterwater lines also cause the release of mercury from dental amalgam when tested in the laboratory. Chlorine-containing products caused the release of more mercury than other products.[60] These findings have been confirmed by a more recent study.[61] Based on these findings, it is conceivable that DUW treatment agents that contain chlorine could also mobilise mercury from dental amalgam collected in amalgam filters, traps and separators and in DCU waste water lines and pipes and release it into the environment. Modern DCUs are equipped with amalgam separators to remove amalgam particles generated during the placement and removal of amalgam restorations. A recent study has shown that the performance of amalgam separators can vary and that the total mercury concentration and total dissolved (ionic) mercury concentration in DCU waste water varied widely for each of 12 amalgam separators tested.[62] The safe disposal of waste amalgam from dental practice is regulated by legislation in the EU and in many other countries.[63] It is very likely that in the future that levels of mercury discharged in waste water will be further regulated by additional legislation in order to further reduce the levels of toxic metals being released into the environment. Further research is required to develop procedures for testing the efficiency of amalgam separators in removing small amalgam particles and dissolved mercury and minimizing the dwell time within the DCU. DCU manufacturers have an important responsibility for being proactive in regard to these matters.

Another recent study from this laboratory on the long-term efficacy of the hydrogen-peroxide and silver-ion-containing DUW disinfectant Planosil in Planmeca Prostyle Compact DCUs identified several cases of DUW disinfection failure that were caused by adverse disinfectant effects on DCU components.[8] The DCU manufacturer Planmeca (Helsinki, Finland) recommended the use of Planosil for DUW cleaning in their DCUs based on independent comparative studies.[22] Following 6 months of continuous once weekly (15 h overnight) disinfection with Planosil, some instances of DUW disinfection failure were directly associated with blockage of and/or leakage from disinfectant intake valves, and corrosion of aluminum components of disinfectant delivery module containers. Valve leakage was associated with damage to an internal glue seal, whereas valve blockage was caused by a combination of dislodged glue and oxidized aluminum deposits from the corroded disinfectant delivery containers. Hydrogen peroxide with its strong oxidizing properties was identified as the disinfectant component most likely responsible for the adverse affects on the DCUs concerned. The problems were completely resolved in collaboration with the DCU manufacturer Planmeca who developed replacement valves that were resistant to leakage following prolonged exposure to Planosil disinfectant and by replacing the aluminium components of the disinfectant delivery containers with hard plastic and acid-resistant stainless steel.[8] This study highlights the importance of investigating the long-term effects that DUW treatment agents can have on DCU components and also highlights the important role that DCU manufacturers have in constantly developing and improving their DCUs.

Various types of electrochemically activated water (ECA), also known as superoxidised water, have been used for a number of years as a residual treatment for DUWs to control biofilm and studies have shown that it is very effective.[49,51,64] However, electrochemically activated water has the potential for adverse effects on DUWs and DCU instruments connected to them following prolonged exposure if the parameters for electrochemical activation are not optimal or if product used is too concentrated. To generate electrochemically activated water, water of varying mineralization (usually dilute sodium chloride) is passed through an electrocatalytical cell, the design of which permits the generation of two streams of activated water including a negatively charged antioxidant solution (catholyte) and a positively charged oxidant solution (anolyte), the latter of which contains predominantly hypochlorous acid. Data from this laboratory has shown that even short-term exposure of DUWs to undiluted or insufficiently dilute anolyte causes some DUWs to deteriorate rapidly and can cause corrosion damage to other DCU components (D. Coleman and R. Russell, unpublished data). Detailed studies are in progress to investigate these adverse effects in detail. There is a noticeable absence of direct scientific data that confirm or refute the safety of ECA solutions for human exposure. However, ECA technology has been used widely in Russia and the former Soviet Union for many years as a general disinfectant with no apparent harmful effects.[64–67] Nonetheless, a variety of free radicals are produced during the generation of ECA solutions that may potentially have harmful effects. It is important to emphasize that limited exposure of dental patients to the small quantities of free radicals in ECA solutions in DUW water should not pose a health

hazard. Human tissues are adapted to contend with natural exposure to hypochlorous acid generated in inflammatory states by phagocytic cells. However, the safety of ECA should be definitively established *in vitro* and *in vivo* using mammalian tissue culture and animal models.

It is evident that it is very important that DCU manufacturers provide very clear guidance to their customers on the types of DUW treatment agents that they recommend for use in their DCUs. In doing so DCU manufacturers have a responsibility to ensure patient and dental healthcare personnel safety, to ensure that DUW treatment agents do not exhibit adverse effects on DCU components in the short- and long-term and to ensure that DUW treatment agents do not have adverse effects on the environment. All of this can only be achieved by significant investment in ongoing research and development.

3.3. *DUW cleaning and disinfection systems*

DUW treatment agents can be introduced into DUWs from independent reservoir bottles, or from disinfectant delivery devices connected to the DCU water supply inlet. In the case of DCUs connected to a municipal water mains supply, it is imperative that the connection is turned off prior to DUW treatment to prevent contamination of mains water with treatment agent. After treatment, DUWs should be flushed thoroughly using clean water before DCUs are used for patient treatments. There is an onus on DCU manufacturers to consider the problem of DUW biofilm contamination when designing DCUs. In general, DCU manufactures have been slow to respond to this challenge.

To our knowledge at least one DCU manufacturer have developed a new generation of DCUs with integrated DUW cleaning systems that facilitate and simplify DUW disinfection.[7,8] Indeed other DCU manufacturers are likely to follow suit, as the dental community becomes more aware of the problem of DUW contamination. In 2002 we reported on the effectiveness of the Planmeca Waterline Cleaning System (WCS™), a DUW cleaning system developed by the Finnish DCU manufacturer Planmeca, to control DUW biofilm in two separate Planmeca Prostyle Compact DCUs over a 20 week period using the hydrogen peroxide- and silver ion-containing disinfectant Sanosil.[22] The WCS™ was found to be very effective at eradicating DUW biofilm in these DCUs when used with Sanosil and consistently provided output water with bacterial densities below the ADA recommended threshold of ≤200 cfu/mL for up to 7 days post-disinfection. The microprocessor controlled WCS™ was originally developed to be retrofitted to existing Planmeca DCUs having a mains water supply. All DUWs, apart from the cuspidor rinse DUW, are included in a microprocessor-controlled process whereby waterlines are automatically filled with disinfectant and subsequently automatically flushed with clean water during the disinfection cycle. If the cycle is not completed properly or a power failure occurs during the cycle, an error message is displayed on the DCUs instrument console.[8,22] While this error message is displayed all DCU functions are locked preventing the DCU from being used. The WCS™ is semi-automated and requires the operator to fill the disinfectant container with disinfectant, to turn off a valve on the mains water supply and to place DCU instrument hoses into special receivers. DUW cleaning is activated from a keypad on the DCUs control console and disinfectant is automatically fed into each DUW. Following this the DCU electrical supply is switched off to allow the disinfectant *in situ* to take effect. When the electrical power is turned back on, a code message on the DCU control console display prompts the operator to flush the DUWs. This is initiated by disconnecting the disinfectant container module and turning on the mains water supply switch. Each DUW is then rinsed and flushed automatically with fresh mains water, after which time the DCU is ready for use. In recent years the WCS™ is no longer provided as a retrofitted system but is provided as an integrated DUW cleaning system as standard in new Planmeca Prostyle Compact DCUs without increasing the cost of the DCUs to the customer.[8]

In a more recent study from this laboratory, the ability of the WCS™ to maintain the microbiological quality of DUW output water below the ADA standard was investigated over a much longer period (18 months) with a much larger number of DCUs (10 DCUs) using the hydrogen peroxide- and silver ion-containing DUW disinfectants Planosil and Planosil Forte.[8] In the majority of cases, once-weekly DUW treatment using the WCS™ provided output water below the ADA standard for up to a week post-disinfection. During the first 9 months a high prevalence (28/300 disinfection cycles; 9.3%) of intermittent DUW disinfection failure occurred in 8/10 DCUs due to operator omission to disinfect all DUWs (10/28 failed cycles), incorrect compressed air pressure failing to distribute the disinfectant properly (4/28 failed cycles) and physical blockage of disinfectant intake valves due to corrosion effects of Planosil (see Section 3.2 above), (14/28 failed cycles). On rectification of these faults through engineering redesign and procedural changes, no further cases of intermittent DUW disinfection failure were observed. Independently of these factors, a rapid and consistent decline in efficacy of DUW disinfection occurred in 4/10 DCUs following the initial 9 months of once-weekly disinfection. There was a highly significant difference ($P < 0.0001$) in the prevalence of strongly catalase-positive (an enzyme that breaks down hydrogen peroxide) *Novosphingobium* and *Sphingomonas* bacterial species (mean average prevalence of 37.1%) in DUW output water from these four DCUs compared to the other six DCUs and DCU supply water (prevalence < 1%), which correlated with biofilm presence in the DUWs and indicated selective pressure for maintenance of these species by prolonged disinfectant usage. Planosil was reformulated by the DCU manufacturer Planmeca as a more concentrated form of hydrogen peroxide and silver ions (Planosil Forte) and when used once weekly was found to maintain bacterial density in output water below the ADA standard for all 10 DCUs during the 17 consecutive weeks studied. These type of long-term problems very likely occur with other DCU brands and models and point to the necessity for long-term compatibility testing of DUW treatment products with DCUs, communication between manufacturers and end users and the development of DUW cleaning systems that are automated and require minimal human involvement. The longer term goal should be for fully automated disinfection systems that are validatable. Recently Planmeca developed a more advanced microprocessor-controlled DUW cleaning system called the Water Management System (WMS), a fully integrated and automated DUW cleaning system that requires minimal effort on the part of the operator.[7] The WMS is more advanced and automated than the WCS and

also contains many additional features, including an air gap. Studies with a Planmeca Compact i DCU demonstrated that the WMS consistently provided DUW output water that passed the ADA quality standard of ≤200 cfu/mL for up to 7 days after once-weekly disinfection with Planosil Forte during a test period of 40 consecutive weeks.

3.4. *DCU water heaters*

Some DCU manufacturers provide water heaters as an optional extra that heat DUW water so that it is more comfortable for the patient at temperatures that can range from approximately 20 to 30 °C. DCU water heaters generally consist of a low volume metallic reservoir with an electrically heated element fed by DCU supply water and which in turn supplies heated water to DUWs. An example of a DCU water heater is shown in Fig. 1, panel (c). Heating DCU water to >20 °C may encourage the proliferation of *Legionella* bacteria present in the supply water or in the DUWs supplying the heating reservoir. Studies have shown that naturally occurring *L. pneumophila* multiply at temperatures between 25 and 37 °C.[68] It is also conceivable that heating DCU water may encourage the proliferation of human-derived bacterial species that prefer higher temperatures, compared to environmental bacteria, which generally grow at lower temperatures. Furthermore, a previous study has shown that certain environmental bacterial species such as *Comamonas acidovorans* may be more frequent in DCU water with a higher temperature.[69] This species has been associated with infections in immunocompromised patients. In our view, DCU manufacturers should consider these factors and should not equip DCUs with water heaters unless other control measures are present.

3.5. *Water pre-treatment*

The quality of DCU output water is directly influenced by the quality of the incoming supply water. This in turn can then be influenced by the condition of the water distribution network pipes and storage tanks, where applicable. In this regard, quality refers not only to the microbial load but also the concentration of suspended or dissolved organic and inorganic materials present in supply water.[70] The poorer the quality of water supplied to DUWs, the more readily biofilm is likely to form on its surfaces. Heterotrophic bacteria present in supply water can convert organic material dissolved in supply water into biomass locally.[71] Previous studies on water distribution systems have shown that a variety of factors influence biofilm growth. These include the level of dissolved inorganic nutrients, water temperature, selection of pipe material, corrosion control and levels of organic matter (suspended and dissolved).[70] Dental unit manufacturers in general have been slow to consider and provide guidance to dental practitioners on the advantages and significance of DCU water pre-treatment.

The chemical and microbial content of municipal water supplied to DCUs will vary depending on the geographic location and the degree of water treatment by public water authorities. The extent of water hardness depends on the levels of dissolved calcium and magnesium minerals. When hard water (~200 ppm hardness minerals) is used to supply DCUs, minerals are deposited within DUWs and DCU valves and within water heaters. These microscopically rough deposits increase the colonisable surface area within DUWs thus allowing more biofilm to form and may eventually result in DUW clogging. Water softening of DCU supply water is essential in hard water areas. This is easily achieved using a water softening unit that remove calcium and magnesium ions by ion exchange. The water softening unit is plumbed in-line in the DCU water supply and will require periodic regeneration and maintenance. Proper maintenance of water softening units is essential, although often overlooked, as biofilm can develop within the resin after a period of use and this can result in a reservoir effect adding considerable microbial loads to water entering DCUs downstream of the softening units. To circumvent this problem, many modern water softeners have an integrated backwash facility that can be timed to operate when the softening unit is not being used (e.g. at night). This allows the ion exchange resin to be freshly rejuvenated, increasing efficiency and increasing the lifespan of the unit. A wide variety of water softening units is available commercially. Dental practitioners should seek advice from their DCU manufacturer concerning the type of softening unit that is suitable for their dental chair, if required. Proper installation of these units is also important as their location and set-up has often been found to be sub-optimal (R. Russell, unpublished).

A variety of other water pre-treatment systems are available that may be of benefit in treating water destined for DCUs depending on the quality of the supply water. These include sediment pre-filters that remove suspended solids, activated carbon filters that reduce organic contaminants and kinetic degradation fluxion (KDF) filters that remove some dissolved metals. Sediment pre-filters should be fitted in-line with the incoming DCU supply before any other pre-treatment system or device. They prolong the life of the latter by eliminating gross contaminants that would otherwise clog either water softening units or activated carbon filters by reducing the surface area available for absorption. Some sediment pre-filters also are equipped with an integrated backwash function that allows them to restore their full filtration capacity at times when there is no demand on the water supply network (e.g. at night), thus reducing maintenance. Activated carbon filters remove/reduce many volatile organic chemicals, herbicides and pesticides, as well as chlorine, bacterial endotoxins, solvents and many other toxic chemicals found in tap water. Some activated carbon filters are moderately effective at removing some, but not all, heavy metals. Carbon filters are generally not good at removing dissolved inorganic contaminants such as minerals/salts or metals. KDF filters contain a high-purity copper–zinc formulation matrix which uses the basic principle of oxidation/reduction (redox) to remove chlorine, lead, mercury, iron, and hydrogen sulphide from water supplies. The redox process between water and filter creates a potential of approximately +300 mV, which provides a mild but substantial antimicrobial effect. Such redox media remove up to 98% of water-soluble cations (positively charged ions) of lead, mercury, copper, nickel, chromium, and other dissolved metals. When placed in-line before carbon filters, they can greatly extend their life and efficiency.

Currently all of the above mentioned water pre-treatment systems are commercially available from a range of manu-

facturers and are used widely to treat water in a variety of water distribution networks. In the Dublin Dental Hospital, a new water pre-treatment system was installed in 2007 to provide consistent high quality (chemical and microbiological) DCU supply water (D. Coleman, unpublished). During 2005 and 2006 high summer temperatures resulted in overgrowth of *Pseudomonas fluorescens* in DCU supply water holding tanks supplied with mains water, resulting in poor quality DCU supply water despite regular DUW disinfection. Chlorinated municipal mains water supplied to the hospital's 103 DCUs is now automatically processed firstly by a back-washable particle filter, (removal of particles greater that 10 μm), a back-washable water softening unit (ion exchange to remove calcium and magnesium ions), then by KDF filtration to remove dissolved metal ions, especially iron. Finally water is treated with 2 ppm anolyte at neutral pH generated *in situ* using an Ecaflo FA 20 anolyte generator, (Ecaflo, Clonmel, Ireland) to control microbial load and biofilm prior to delivery to DCUs via a dedicated water supply network. The chemical quality of the water supplied to the DCUs is tested regularly and has been shown to comply consistently with, and better than, the limits advocated by the EC Directive on the Quality of Water for Human Consumption and by the United States Environmental Protection Agency National Primary Drinking Water Regulations. Water pre-treatment helps to maintain the condition of the water pipe network supplying DCUs by significantly reducing inorganic deposits, corrosion and biofilm formation. The density of aerobic heterotrophic bacteria in the supply water entering DCUs in the Dublin Dental Hospital is consistently <100 cfu/mL (range 0–60).

During installation, commissioning and initial day to day use, careful and detailed monitoring of chemical and microbiological parameters are essential to provide feedback for the installers to make fine adjustments to the system. This is often omitted or poorly done as typical installation engineers are not equipped to make such detailed measurements or indeed often to integrate different pieces of equipment which may not be entirely compatible.

In our view, DCU manufacturers should consider developing integrated water pre-treatment modules for the DCUs they manufacture. These would dramatically improve the quality of water being supplied to DCUs and would go a long way in helping to reduce the problem of biofilm formation in DUWs. All of the water pre-treatment technology is already available and is relatively simple. However, the choice of such equipment, its compatibility and its integration does require quite a degree of expertise.

3.6. *DUW materials and biofilm formation*

In addition to modifying the design of water contact components within DCUs, manufacturers should also focus on developing or using novel DUW materials that are less conducive to biofilm formation. To date, very little applied work has been undertaken in this area with regard to dental equipment. In other clinical fields, a variety of approaches have been undertaken to prevent/reduce biofilm formation on, for example, urinary catheters, including incorporation of antimicrobial agents such as silver and the biocide triclosan.[72,73] Previous studies with a range of materials commonly used in the construction of water systems demonstrated that some materials were very good at limiting colonisation and biofilm formation by a wide range of bacterial species, whereas other materials were very poor. Copper was the best at limiting colonisation and biofilm formation, followed by polybutylene and stainless steel, whereas biofilm formed more readily on polyethylene, chlorinated polyvinyl chloride (PVCc), unplasticised polyvinyl chloride (PVCu), steel and ethylene-propylene.[74,75] A study from Japan showed that DUWs composed of polyvinylidene fluoride (PVDF) were effective in inhibiting biofilm formation and reducing bacterial density in DUW output water.[76] A more recent study from Italy on the influence of DUW composition on output water quality reported that the heterotrophic bacterial plate count at 22 °C from polytetrafluorethylene (PTFE) was lower than output water from DUWs made from polyethylene (PE).[77] Furthermore, whereas *P. aeruginosa* was regularly isolated from output water from DUWs made from PE, it was never recovered from water from DUWs made from PTFE. The research and development of novel DUW materials with antimicrobial and/or anti-biofilm properties is a logical and potentially very fruitful area for research and development by DCU manufacturers with applications in many other areas. Distributing DCU supply water using copper pipes may also be helpful in improving the microbial quality of DCU supply water, as copper has been shown to possess significant antimicrobial advantages over drinking water pipework of other composition. An example of extensive biofilm in a section of plastic water supply pipework is shown in Fig. 1, panel (d).

4. Conclusions

Despite the increasing complexity and sophistication of DCUs, some of the fundamental issues concerning cross-infection control and biofilm establishment in DUWs have not yet been satisfactorily resolved. These difficulties arise from a number of quarters. First, there is no universally agreed standard for the quality of output water from a DCU and, in fact, there is often no specification regarding the quality of water supplied to the DCU either. These are clearly matters for the attention of DCU manufacturers and within their capability to improve upon by a combination of design, choice of materials and other preventative methods (Table 1). Second, there is often insufficient unambiguous instruction from DCU manufacturers regarding decontamination and disinfection of DUWs in the DCUs they manufacture. This type of information is essential for effective infection control of any medical device, for prevention of blockages and malfunctions and for prevention of damage to the equipment through use of incompatible chemicals or treatments. It is clear that insufficient in-use testing of DCUs is undertaken by manufacturers to properly establish the requirements for cross-infection control or biofilm management and this is, in turn, reflected in the inadequate information provided to the end-users. Third, there is insufficient communication and feedback between operators and DCU manufacturers regarding design problems and operator difficulties with decontamination. Were this information to flow better, the manufacturers' design teams might find it easier to design out potential problems at an early stage. An obvious area of difficulty for engineers is that of the microbiology of biofilms. It

Table 1 – How manufacturers can contribute to reducing biofilms in DUWs

Aspect of DCU/DUW	Manufacturers' contribution to improvement
DUW biofilm control	• Provide detailed evidence-based guidance on DUW treatments that are effective in the long-term, safe for patients and staff, environmentally friendly and do not cause adverse effects with DCU components and ancillary equipment in the long-term
	• Do not provide water heaters in DCUs unless effective controls are also in place to control DUW biofilm
	• Continually develop and improve DUW biofilm control systems based on experience and feedback from operators
	• Develop and incorporate new DUW tubing materials with antimicrobial properties that are not conducive to biofilm formation
DCU engineering and design changes	• Develop integrated and fully automated DUW cleaning/disinfection systems that are easy to use, effective in the long-term and can be validated
	• Improve performance of anti-retraction devices to prevent back-siphonage of oral fluids into DUWs. Provide detailed information on maintenance and periodic performance testing
	• Ensure that the DUWs in all DCUs are separated by an air gap from municipal mains water supplies
Improve DUW supply water quality	• Develop DCU water supply pre-treatment systems to remove suspended organic and inorganic material, dissolved hard water minerals, metals and dissolved organic material. These systems should be available for individual DCUs or on a larger scale for Dental Hospitals or clinics equipped with many DCUs
Biocides	• Help develop effective and safe biocides and application protocols based on lengthy clinical proving trials
User complaints/suggestions	• Establish closer links with users and collect user data
Training	• Provide comprehensive decontamination training
Monitoring test kits	• Develop rapid, accurate and easy-to-use monitoring tests for DUW output water microbial load

is an area of expertise which has only relatively recently been addressed by microbiologists themselves and is quite complex. Close cooperation with experts in this area should be a priority for the manufacturers. Fourth, insufficient data is available from long-term studies monitoring DUW output from DCUs from different manufacturers in different use situations and with different disinfection regimes. This is necessary to address problems with the existing installed base of DCUs and to develop ways of improving their DUW output quality and biofilm control.

Some recent studies have shown that novel DCUs with integral, semi-automated or automated DUW cleaning systems can effectively control DUW biofilm in the long-term.[7,8] This shows that manufacturers of DCUs who do attempt to address the points raised above can achieve success. These studies identified for the manufacturer a variety of factors that can contribute to failure of DUW disinfection in the long-term, including human error, corrosion of equipment following prolonged or incompatible disinfectant usage and the natural selection of bacterial species resistant to particular disinfection regimes. Solutions to these problems were achieved by ongoing research and development by the DCU manufacturer in close cooperation with researchers resulting in successful design changes. However, there are many other potential DCU engineering and design changes that DCU manufacturers could yet address to further improve DUW biofilm control including that of supply water pre-treatment and the development of novel DUW tubing materials that are less conducive to biofilm formation.

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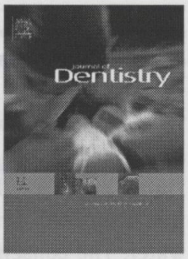

Guest editorial

This issue of the *Journal of Dentistry* contains three review articles that stem from a symposium entitled "Microbiology of dental unit water lines; setting standards for the future" that was held as part of the Pan European Federation/International Association for Dental Research meeting held at Trinity College, Dublin, Ireland, 13–16 September 2006. The three review articles, written by experts in different aspects of the subject field, have been subject to independent peer review.

1. What can manufacturers do to reduce biofilms in dental chairs?

The first review by Coleman and O'Donnell appraises the role of dental chair unit (DCU) manufacturers' in controlling dental unit waterline (DUW) biofilm. Interestingly, a search of the international literature reveals a dearth of articles on this important subject. The main emphasis on DUW biofilm and its control has focused on describing the problem and its control using a range of periodic and residual DUW treatment agents. Indeed, until recently, the problem of DUW biofilm and subsequent poor quality waterline output water has generally been considered to be the responsibility of dental practitioners and until recently manufacturers have provided very little guidance on DUW biofilm control. This review focuses on how novel developments in DCU engineering and design, improvements to DCU supply water quality and the development of integral semi-automated or automated DUW cleaning systems have, and might contribute to DUW biofilm control. The review concludes that DCU manufacturers can significantly contribute to controlling the problem of DUW biofilm.

2. Do contaminated dental unit waterlines pose a risk of infection?

The second review by Pankhurst and Coulter assesses the evidence from the literature that DUWs are a source of occupational and healthcare-acquired infection or other adverse health effects in the dental surgery. This review is significant as very few previous studies have been devoted specifically to this important topic. The majority of micro-organisms isolated from DUWs are Gram-negative environmental bacterial species of low pathogenicity and very few cases of infection associated with DUW output water have been described. However, a small number of studies described infection or colonisation in susceptible individuals with *Legionella* bacteria, *Pseudomonas aeruginosa* and environmental mycobacteria following exposure to DUW output water. This review also explores the potential adverse health effect of exposure to Gram-negative bacterial cell wall endotoxin present in DUW output water. A consequence of indoor endotoxin exposure is the triggering or exacerbation of asthma. The review concludes that although the number of published cases of infection or respiratory symptoms resulting from exposure to DUW output water is limited, there are ethical and medico-legal requirements on dental practitioners to control biofilm in DUWs to ensure good quality output water for patient treatment.

3. Microbial biofilm formation in DUWs and their control using disinfectants

The third review by Walker and Marsh discusses why microbial contamination and biofilms are so prevalent in DUWs, and considers the role of disinfectants and their potential for achieving good microbial quality output water. DUWs are prone to the formation of biofilm and the presence of opportunistic pathogens, irrespective of the source and quality of the supply water. A variety of commercial DUW disinfectants and treatment agents are available, but controlled laboratory and clinical studies have shown that they can vary markedly in their efficacy and suitability for use. Some products have been shown to successfully remove biofilm and consistently reduce the density of aerobic heterotrophic bacteria in DUW output water to <200 cfu/mL, in compliance with the American Dental Association (ADA) DUW output water quality standard. The ADA standard is the only DUW output water quality standard proposed to date. The review concludes that the effective delivery of approved disinfectants can control the level of microorganisms in DUWs at acceptable levels.

4. Overview

The symposium was the first occasion that scientists and clinicians from academia and dental practice came together in Europe to share their thoughts and ideas on the universal problem of DUW biofilm and the quality of waterline output water. The consensus from the symposium was that in the absence of an EU standard for DUW output water quality, every effort should be employed to ensure that DUW output water quality in Europe complies with the ADA standard of <200 cfu/mL.

On behalf of all the speakers at the IADR/PEF symposium, we wish to express our thanks to the symposium sponsors, Planmeca, Helsinki, Finland, for their support without which the symposium would not have been possible.

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REVIEW ARTICLE

Biofilm problems in dental unit water systems and its practical control

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Summary

Dental chair units (DCUs) contain integrated systems that provide the instruments and services for a wide range of dental procedures. DCUs use water to cool and irrigate DCU-supplied instruments and tooth surfaces during dental treatment. Water is supplied to these instruments by a network of interconnected narrow-bore (2–3 mm) plastic tubes called dental unit waterlines (DUWLs). Many studies over the last 40 years demonstrated that DUWL output water is often contaminated with high densities of micro-organisms, predominantly Gram-negative aerobic heterotropic environmental bacteria, including *Legionella* and *Pseudomonas* species. Untreated DUWLs host biofilms that permit micro-organisms to multiply and disperse through the water network and which are aerosolized by DCU instrument use, thus exposing patients and staff to these micro-organisms, to fragments of biofilm and bacterial endotoxins. This review concentrates on how practical developments and innovations in specific areas can contribute to effective DUWL biofilm control. These include the use of effective DUWL treatment agents, improvements to DCU supply water quality, DCU design changes, development of automated DUWL treatment procedures that are effective at controlling biofilm in the long-term and require minimal human intervention, are safe for patients and staff, and which do not cause deterioration of DCU components following prolonged use.

Introduction

For more than a century, the dental chair unit (DCU) has been the single most essential item of equipment necessary for the practice of dentistry. Its original function was simply to provide support for patients while enabling easy access to the oral cavity by dental clinicians undertaking dental procedures. Over the decades DCUs have evolved considerably, combining all operating essentials into a single, compact assembly. Modern DCUs consist of a variety of complex, integrated equipment systems that provide the services (e.g. air supply, water and electrical power) and instruments necessary for a diverse range of dental procedures (O'Donnell *et al.* 2005, 2006a; Coleman *et al.* 2007). DCUs use water to cool a range of DCU-associated instruments (e.g. conventional dental handpieces, high-speed turbine dental handpieces, three-way air/water syringes and ultrasonic scalers) and also to irrigate tooth surfaces during dental procedures, as the heat generated during instrument operation can be injurious to teeth (Stanley 1971; Langeland 1972; Siegel and von Fraunhofer 2002). DCU-supplied water is also used for oral rinsing by patients (water supplied via the cup filler outlet) and to wash out the DCU spittoon, or cuspidor, after oral rinsing (water supplied via the bowl-rinse outlet). An intricate network of interconnected narrow-bore tubes called dental unit waterlines (DUWLs) supplies water to all of the DCU-supplied instruments, cup-filler and bowl-rinse outlets (O'Donnell *et al.* 2006a; Coleman *et al.* 2007).

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Modern DCUs are categorized as medical devices under the European Union Medical Devices Directive (Anon., 1993). Microbial contamination of a diverse range of medical devices has been shown to be an important cause of cross-contamination and cross-infection, especially in healthcare environments (O'Donnell *et al.* 2006a). A single DCU can be in used in the treatment of many patients each day and microbial contamination of specific component parts can be a significant potential source of cross-infection (O'Donnell *et al.* 2005, 2006a). This becomes quite significant where immunocompromised, oral surgery or endodontic patients are treated (O'Donnell *et al.* 2006a). DCU components that come into direct contact with the patient's oral cavity are of particular concern, including dental unit handpieces, ultrasonic scalers, three-in-one air/water syringes and suction hoses. Aerosols, splashes and contact contamination contribute to a microbially contaminated environment in the vicinity of a DCU. Output water provided by a DCU may also be of concern as a potential source of infection as it comes directly from the DCUs extensive network of DUWLs, which can harbour biofilms, and enters the oral cavity of the patient during treatment. Furthermore, aerosols and droplets produced by dental instruments connected to DUWLs may be inhaled by patients and dental healthcare personnel (Leggat and Kedjarune 2001; Siegel and von Fraunhofer 2002; Harrel and Molinari 2004; O'Donnell *et al.* 2005). In this review, we provide an overview of microbial biofilm formation in DUWLs, consequent contamination of the DUWL output water and strategies that have been developed to control it and provide good quality water that is safe for patients and healthcare personnel.

Microbial contamination of DUWL output water

In the early 1960s, the first reports began to appear in the literature describing the presence of high densities of micro-organisms, primarily bacterial species, in DUWL output water (Blake 1963; Belting *et al.* 1964). This was followed by a few additional reports in the 1970s and 1980s (Abel *et al.* 1971; Kelstrup *et al.* 1977; Dayoub *et al.* 1978; Scheid *et al.* 1982; Furuhashi and Miyamae 1985; Martin 1987) and by a plethora of reports in the 1990s and 2000s (Mayo *et al.* 1990; Pankhurst and Philpott-Howard 1993; Williams *et al.* 1993; Barbeau *et al.* 1996; Pankhurst *et al.* 1998; Mills 2000; Walker *et al.* 2000, 2003; Putnins *et al.* 2001; Tuttlebee *et al.* 2002; Walker and Marsh 2004; Coleman *et al.* 2007). Bacterial densities up to 10^6 colony-forming units (CFU) per ml of output water have been reported (Williams *et al.* 1993). Microbial contamination of DUWLs originates principally from the DCU water supply, which usually contains low levels of micro-organisms (Pankhurst *et al.* 1998; Tuttlebee *et al.* 2002). In a typical modern DCU, the waterline network consists of many metres of plastic DUWL tubing having an internal diameter of 1–2 mm. Water flow within these narrow bore tubes is laminar and thus the flow at the lumen surfaces is almost negligible compared with that at the centre of the lumen. A conditioning pellicle of chemicals mainly from the supply water builds on this inner face over time providing an easier attachment substrate for micro-organisms (Shearer 1996; Wirthlin *et al.* 2003). Thus, micro-organisms in DCU supply water attach to the internal surfaces of the DUWL tubing and form microcolonies that eventually give rise to biofilms. DCUs connected to municipal water supplies usually contain low numbers of several bacterial species that eventually give rise to multispecies biofilm in DUWLs (Pankhurst *et al.* 1998; Mills 2000; Tuttlebee *et al.* 2002). Water stagnation within DUWLs when the equipment is not being used encourages the proliferation of biofilm (Williams *et al.* 1993; Barbeau *et al.* 1996).

Microbial biofilms are comprised mainly of a matrix of highly hydrated complex exopolysaccharides secreted by bacteria, housing microcolonies and single cells, heterogeneously interspersed by channels or pores (Davey and O'Toole 2000; Watnick and Kolter 2000). These are highly structured communities with a sophisticated degree of intercommunication, wherein cell phenotypes and function alter considerably (Costerton 2007). DUWL biofilm matrix also contains both inorganic and organic material derived from supply water and dead micro-organisms. Low boundary flow allows biofilm to form readily in DUWLs (Williams *et al.* 1993; Walker *et al.* 2003; Wirthlin *et al.* 2003). From here planktonic forms of micro-organisms and pieces of biofilm are shed to seed biofilm formation elsewhere in the waterline network or are transferred directly into the mouths of patients during dental procedures. Dental handpieces and ultrasonic scalers also aerosolize such biofilm components. These aerosols and fine droplets can enter the lungs of patients and dental healthcare staff (Fotos *et al.* 1985; Reinthaler *et al.* 1988; Atlas *et al.* 1995; Putnins *et al.* 2001; Pankhurst *et al.* 2005; Dutil *et al.* 2007). Thus, DUWL biofilm acts as a reservoir for ongoing contamination of DUWL output water and can act as a potential source of cross-infection. Sterilization of the handpieces, syringes and associated instruments attached to DUWLs has no impact at all on biofilm within DUWLs (O'Donnell *et al.* 2006a). Microbial contamination of DUWL output water is a universal problem and all untreated DUWLs in standard DCUs are subject to contamination and will harbour resident biofilms.

The majority of microbial species found in DUWL output water are Gram-negative aerobic heterotrophic

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environmental bacterial species of very low pathogenicity, although they may be of concern in the treatment of immunocompromised and medically compromised patients (Pankhurst *et al.* 1998; Walker *et al.* 2000; Tuttlebee *et al.* 2002; Singh *et al.* 2003; O'Donnell *et al.* 2006a,b, 2007; Pankhurst and Coulter 2007). Yeasts, fungi and amoebae may also be present in DUWL output water (Kelstrup *et al.* 1977; Porteous *et al.* 2003; Singh and Coogan 2005; Szymanska 2005; Göksay *et al.* 2008). Human pathogens detected in DUWLs include *Pseudomonas* spp., *Legionella* spp. and non-*tuberculosis Mycobacterium* spp. (Fotos *et al.* 1985; Mayo *et al.* 1990; Atlas *et al.* 1995; Schulze-Robbecke *et al.* 1995; Porteous *et al.* 2004; Dutil *et al.* 2007). *Pseudomonas* spp., principally *Pseudomonas aeruginosa*, can survive and grow in low nutrient environments and often exhibit resistance to antimicrobial agents, disinfectants and biocides. In 1987, Martin reported that abscesses caused by strains of *P. aeruginosa* in two immunocompromised patients were associated with exposure to contaminated DUWL output water (Martin 1987). The same study reported the isolation of *P. aeruginosa* from the oral cavities of 78 healthy patients for 3–5 weeks following exposure to DUWL output water contaminated with *P. aeruginosa*. *Legionella* species (*L. pneumophila* and approx. 30 other species) are often found in water distribution networks and can cause Legionnaire's disease (pneumonia resulting from inhalation) or Pontiac fever (non-pneumonic legionellosis). *Legionella* bacteria live within a variety of amoebae and protozoa commonly found in soil and water and are often found in association with biofilms, including DUWL biofilms. There are numerous reports of transmission of legionnellosis in healthcare settings and in the community, either by direct contact with aerosols generated from contaminated water, or indirectly following exposure to contaminated aerosols generated by respiratory therapy equipment (Woo *et al.* 1992; Crnich *et al.* 2005; Leoni *et al.* 2006). However, there is no definitive published evidence, so far, that any patient has ever contracted legionellosis following exposure to contaminated DUWL output water. Many studies certainly have reported the presence of legionellae in DUWLs (Atlas *et al.* 1995; Singh and Coogan 2005; Tanzi *et al.* 2006; Dutil *et al.* 2007; Pankhurst and Coulter 2007; Veronesi *et al.* 2007). In 1995, Atlas *et al.* reported the death of a dentist in California resulting from Legionnaire's disease, which was possibly due to exposure to DUWL output water. Occupational exposure to aerosols of waterborne bacteria generated by dental instruments attached to DUWLs may lead to colonization of dental staff and also cause a higher prevalence of antibodies to *Legionella* (Fotos *et al.* 1985; Atlas *et al.* 1995; Borella *et al.* 2008). One study of a group of dental staff with more than 2 years clinical experience revealed that 23% were IgG antibody-positive and 19% were IgM antibody-positive for *L. pneumophila*, compared with IgG antibody-positive levels of 8% for individuals who had no clinical experience (Fotos *et al.* 1985). Few instances of cross-infection related to contaminated DUWL output water have been reported in the literature (Martin 1987; Atlas *et al.* 1995), however, it is possible that such infections have gone undetected and unreported because of the failure to associate exposure to DUWL output water and aerosols generated from this water with the development of specific infections (Pankhurst and Coulter 2007). Sporadic infections not requiring hospital admission are also less likely to be investigated.

Dental unit waterline output water is also a potent source of bacterial endotoxin, composed of lipopolysaccharide released from the cell walls of dead Gram-negative bacteria, and levels ranging from 500 to 2560 endotoxin units (EU) per ml have been reported (Putnins *et al.* 2001; Pankhurst and Coulter 2007). In contrast, the maximum level of endotoxin permissible in sterile water for irrigation in the USA is 0·25 EU per ml. Endotoxin can cause localized inflammation, fever and shock in susceptible individuals. Interestingly, in medical devices that are prone to biofilm contamination and endotoxin build-up such as humidifiers, a hypersensitivity pneumonitis triggered by contaminating endotoxin is well documented (Pankhurst and Coulter 2007). Inhaled endotoxin can precipitate reactive airway symptoms (Schwartz 2001) and asthma severity is directly correlated with the concentration of endotoxin (Michel *et al.* 1996). Furthermore, results from a single, large, practice-based cross-sectional study reported a temporal association between occupational exposure to contaminated DUWL output water with aerobic counts of >200 CFU per ml at 37°C and development of asthma in the subgroup of dentists in whom asthma arose following the commencement of dental training (Pankhurst *et al.* 2005). Finally, Putnins *et al.* (2001) suggested that endotoxin present in DUWL output water might stimulate the release of pro-inflammatory cytokines in gingival tissue during surgery and adversely affect healing.

DUWL contamination by oral and skin microbes

Dental instruments that are connected to DUWLs and that are used in the patient's mouth (e.g. turbine and conventional handpieces, air/water syringes and ultrasonic scalers) should contain integrated antiretraction valves or devices that prevent backflow or back siphonage of fluids from the oral cavity into the DUWLs (Coleman *et al.* 2007). The need for antiretraction devices has been highlighted by previous studies that demonstrated that

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oral fluids can be retracted into DUWLs during DCU instrument use. The detection of oral bacterial species and other human-derived micro-organisms in DUWL output water has provided convincing evidence for likely failure of antiretraction devices (Tuttlebee *et al.* 2002; Berlutti *et al.* 2003; Montebugnoli *et al.* 2004; Petti and Tarsitani 2006). Recently, an Italian study of 54 DCUs, comprising 18 different models by six different DCU manufacturers reported an antiretraction device failure rate of 74% (40/54 DCUs tested) (Berlutti *et al.* 2003). Thus, retraction of oral fluids (e.g. saliva and blood) during use of dental instruments attached to DUWLs can add to the range of micro-organisms present in DUWL biofilms, and therefore in DUWL output water, and also increase the potential for transmission of pathogenic microbes. To minimize the potential impact of antiretraction device failure, the current Centers for Disease Control and Prevention (CDC) guidelines for infection control in dental healthcare settings recommend that DCU handpieces should be operated to discharge water and air for a minimum of 20–30 s after each patient session (Kohn *et al.* 2003). All dental handpieces connected to DUWLs should be cleaned, lubricated and sterilized by autoclaving after each patient use.

Reservoir bottles in DCUs can easily become contaminated with skin organisms such as *Staphylococcus epidermidis* and *Staphylococcus aureus*, the latter a significant human pathogen, thus introducing additional human micro-organisms into DUWLs (Lancellotti *et al.* 2007). To avoid this, reservoir bottles should be handled carefully and should be cleaned and disinfected regularly. Preferably, reservoir bottles that can be sterilized by autoclaving after cleaning should be used.

DUWL water heaters

Some DCUs are equipped with heaters that warm DUWL water so that it is more comfortable for the patient (Coleman *et al.* 2007). Heating DUWL water to >20°C may promote the proliferation of *Legionella* bacteria present in DCU supply water within DUWLs or within the DCU water heater itself. Naturally occurring *L. pneumophila* readily multiply at temperatures between 25°C and 37°C (Wadowsky *et al.* 1985). It is also feasible that heating DCU water may promote the proliferation of human-derived bacterial species that grow better at higher temperatures, compared with environmental bacteria, which generally grow at lower temperatures. Particular environmental bacterial species such as *Comamonas acidovorans*, which has been associated with infections in immunocompromised patients, may be more prevalent in DCU water with a higher temperature (Stampi *et al.* 1999). DCUs should not be equipped with water heaters unless adequate control measures are present (Coleman *et al.* 2007).

Dental chair unit water

DUWL output water quality

The presence of large numbers of micro-organisms in DUWL output water conflicts absolutely with best practice in modern cross-infection control and prevention. Furthermore, from an ethical perspective, it is totally inappropriate to knowingly expose patients, dental healthcare personnel and support staff to heavily contaminated water even if the assessable cross-infection risk is thought to be low. However, there are currently no official standards or legislation that regulate the microbial quality of DUWL output water (Coleman *et al.* 2007). Furthermore, until recently, there has been hardly any specific guidance from DCU manufacturers on DCU supply water quality (Coleman *et al.* 2007). In reality, responsibility for ensuring that DCUs provide good quality output water has, by and large, been considered to rest on the shoulders of dental practitioners and/or dental clinic management. The principal contributing factor to this situation is that DUWL output water is not intended for human consumption, but it does enter the oral cavity and can be swallowed during dental treatment. It would therefore be sensible, reasonable and responsible to expect that the quality of DUWL output water should at least approximate potable water standards.

The potable water (drinking water quality) standards set for the European Union, the USA and Japan are 100, 500 and 100 CFU ml^{-1}, respectively, of aerobic heterotrophic bacteria (Furuhashi and Miyamae 1985; Anon. 1998, 2002). The American Dental Association (ADA) Council on Scientific Affairs affirmed that contact with water of poor microbial quality is inconsistent with patient expectations of safety and standards of modern dentistry and set a goal for the year 2000 that water used for dental treatment should contain ≤200 CFU ml^{-1} of aerobic heterotrophic bacteria (Anon. 1996). This policy has been endorsed by many experts in the field but in reality has not been widely achieved (Coleman and O'Donnell 2007; Coleman *et al.* 2007; Pankhurst and Coulter 2007). The current CDC guidelines for infection control in dental healthcare settings recommend that DUWL output water should contain ≤500 CFU ml^{-1} of aerobic heterotrophic bacteria (Kohn *et al.* 2003). A symposium entitled *Microbiology of dental unit water lines; setting standards for the future* at the Pan European Federation/International Association for Dental Research meeting held at Trinity College, Dublin, during September 2006 debated setting a standard for DUWL output water quality (Coleman and

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O'Donnell 2007). The symposium was the first occasion that scientists and clinicians from academia and dental practice came together in Europe to discuss the universal problem of DUWL biofilm and practical solutions. The consensus from the symposium was that in the absence of any European Union standard for DUWL output water quality, every effort should be deployed to ensure that DUWL output water quality in Europe complies with the ADA standard of <200 CFU ml^{-1} of aerobic heterotrophic bacteria (Coleman and O'Donnell 2007). It is important to note that such counts should not include human pathogens.

DCU supply water quality

The quality of DUWL output water is directly influenced by the quality of the supply water. Water supplied to DCUs is generally provided either in independent bottle reservoirs or directly from a municipal mains water supply (Coleman *et al.* 2007). In Dental Hospitals and in dental clinics equipped with multiple DCUs, water supplied to DCUs frequently comes from large holding tank reservoirs supplied by mains water, whereas in smaller clinics, DCUs may have individual mains supplies (Coleman *et al.* 2007). Within the European Union, the majority of DCUs are supplied with municipal mains water (Walker and Marsh 2004). Retraction or backflow of water from DCUs into the mains supply pipe work could occur if the pressure in the mains supply drops below that in DUWLs. Some DCU models incorporate an air gap in their DUWLs to prevent this possibility (O'Donnell *et al.* 2006b). Some European countries legally require an air gap between the mains supply and dental surgeries (Anon. 2001).

The quality of DUWL output water is directly influenced by the physical content (i.e. presence of suspended material and particulate matter), chemical content (i.e. organic material and dissolved and suspended inorganic compounds) and microbiological quality (i.e. density of micro-organisms present) of the inward supply water (Volk and LeChevallier 1999; Coleman *et al.* 2007) and indirectly by environmental conditions such as temperature. The condition of the mains water distribution network pipework, maintenance works thereon and storage tanks (where present) also play a major role in water quality (Volk and LeChevallier 1999; Coleman *et al.* 2007). The poorer the quality of water supplied to DUWLs, the more readily biofilm is likely to form on its internal surfaces. Environmental aerobic heterotrophic bacteria present in supply water can convert organic material dissolved or suspended in supply water into biomass, locally (Flemming 2002). Previous studies on water distribution systems revealed that a range of factors influence biofilm growth, including the level of dissolved inorganic nutrients present, water temperature, type of pipe material used, corrosion control and levels of organic matter present (Volk and LeChevallier 1999).

The chemical and microbial content of municipal water supplied to DCUs will vary depending on the geographic locality and the degree and efficacy of water treatment by public authorities. The degree of water hardness depends on the levels of dissolved magnesium and calcium minerals. If hard water (~200 ppm hardness minerals) is supplied to DCUs, minerals are deposited within DUWLs and associated valves and within DCU water heaters, where fitted. Such deposits increase the surface area within DUWLs, thus allowing more biofilm to form. Water softening applied to DCU supply water is vital in hard water areas, and is most easily achieved using an ion exchange unit that removes calcium and magnesium ions. Water softening units require periodic maintenance, regeneration and disinfection (Coleman *et al.* 2007). Proper maintenance is essential, although often overlooked, as biofilm can develop within the unit's resin bed after a period of use, thus adding considerable microbial loads to water entering DCUs downstream of the softening units. To circumvent this problem, many modern water softeners have an integrated backwash facility that can be timed to operate when the softening unit is not being used. This allows the ion exchange resin to be regenerated, increasing efficiency and increasing the lifespan of the resin. Proper installation of water softening units is also important as their location and set-up has often been found to be suboptimal, particularly if used in combination with other types of filtration devices such as carbon filters, used to remove organic matter from water (Coleman *et al.* 2007).

A diverse range of other water pretreatment filters is available that may be of benefit in treating DCU supply water depending on its quality. These include sediment filters that remove suspended solids, activated carbon filters that reduce organic contaminants and Kinetic Degradation Fluxion (KDF) filters that remove some dissolved metals (Coleman *et al.* 2007). The aim should be to install a simple integrated system, suited to the performance needs and which will provide consistent quality despite any fluctuations in the supply water quality. Final treatment or disinfection of a consistent quality supply then becomes a much simpler proposition. Sediment filters should be fitted in-line with the incoming DCU supply before any other pretreatment system or device. They prolong the life of the latter by eliminating gross contaminants that would otherwise clog either the resin beds of water softening units or activated carbon filters by reducing the surface area available for absorption

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(Coleman *et al.* 2007). Activated carbon filters remove/reduce many volatile organic chemicals, pesticides and herbicides, as well as chlorine, endotoxins and solvents (Coleman *et al.* 2007). They should be adequately sized to cope with high contamination episodes. KDF filters contain a high-purity copper–zinc matrix that uses the principle of oxidation/reduction (redox) to remove chlorine, lead, mercury, iron and hydrogen sulfide from water supplies. The redox process between water and filter creates a potential of approx. +300 mV, which provides a mild but substantial antimicrobial effect within the filter matrix and also helps precipitate dissolved minerals. Such redox media can remove up to 98% of water-soluble cations of lead, mercury, copper, nickel, chromium and other dissolved metals (Coleman *et al.* 2007).

Control and eradication of biofilm in DUWLs

Biofilm control in DUWLs

As stated earlier, until relatively recently, the onus for tackling the problem of biofilm in DUWLs and poor quality output water has rested with the dental practitioner. Only in recent years have DCU manufacturers responded to the evident need for DCU engineering and design changes to control DUWL biofilms (Coleman *et al.* 2007). Over the last two decades, numerous approaches, both chemical and nonchemical based, for reducing the microbial density in DUWL output water have been proposed but none that is both efficient at eliminating biofilm, compatible in the long-term with the material components of DUWL networks and dental instruments attached to DUWLs, as well as being safe for patients has been universally adopted (Coleman *et al.* 2007). One widely used procedure for reducing the microbial burden in DUWL output water involves flushing DUWLs with fresh water (Scheid *et al.* 1982; Rice *et al.* 2006). However, whereas this procedure does reduce the density of microbes in output water somewhat, it does not remove biofilm and is ineffective as a means of controlling the quality of DUWL output water (Furuhashi and Miyamae 1985; Pankhurst and Philpott-Howard 1993; Mills 2000). Another procedure used to improve quality of DUWL output water involves point-of-use microbial filters at the ends of DUWLs near the instrument attachment sites. These can be very effective but have to be changed regularly as they become clogged readily and thus add to ongoing maintenance expense (Dayoub *et al.* 1978; Pankhurst *et al.* 1990; Murdoch-Kinch *et al.* 1997). Microbial filters attached to the DCU supply water line suffer from similar drawbacks. Filters have no effect whatsoever on existing biofilms in DUWLs. The use of sterile water, deionized water or distilled water in independent bottle reservoirs also has no effect on resident biofilms in DUWLs.

The most efficient means of achieving good quality DUWL output water is regular treatment/disinfection of DUWLs with a chemical, biocide or cleaning agent that removes biofilm from DUWLs effectively, resulting in good quality output water (Tuttlebee *et al.* 2002; Walker *et al.* 2003; Walker and Marsh 2004, 2007; Coleman *et al.* 2007; O'Donnell *et al.* 2007); (see Table 1 in the recent review by Walker and Marsh (2007) for a list of DUWL treatment agents). Biofilm re-growth in DUWLs seeded from supply water and/or dental instruments connected to DUWLs used in the oral cavity due to antiretraction device failure usually occurs shortly following disinfection/cleaning and so DUWLs need be treated regularly (Pankhurst and Philpott-Howard 1993; Williams *et al.* 1993; Meiller *et al.* 2000; Mills 2000; Tuttlebee *et al.* 2002; Walker *et al.* 2003; O'Donnell *et al.* 2006b, 2007). Many studies have demonstrated the efficacy of a broad range of commercially available treatment products for DUWLs that efficiently remove biofilm and reduce bacterial density in DUWL output water to potable water quality or better (Furuhashi and Miyamae 1985; Karpay *et al.* 1999; Smith *et al.* 2001; Pederson *et al.* 2002; Tuttlebee *et al.* 2002; Walker *et al.* 2003; Wirthlin *et al.* 2003; Porteous and Cooley 2004; Martin and Gallagher 2005; Yabune *et al.* 2005; O'Donnell *et al.* 2006b, 2007; Schel *et al.* 2006; Szymanska 2006; Walker and Marsh 2007; Zhang *et al.* 2007). However, many of these studies have been conducted *in vitro* and relatively few have actually investigated the efficacy of DUWL treatment products to achieve these desired effects in DCUs (Tuttlebee *et al.* 2002; Walker *et al.* 2003; McDowell *et al.* 2004; Spratt *et al.* 2004; O'Donnell *et al.* 2006b, 2007; Schel *et al.* 2006). Furthermore, only a small minority of studies has yet investigated the long-term efficacy of DUWL treatment agents in DCUs in the clinical setting (O'Donnell *et al.* 2006b, 2007), whilst comprehensive controlled comparative efficacy studies have yet to be performed.

DUWL treatment agents

Dental unit waterlines treatment agents are generally divided into two categories, including agents for intermittent DUWL treatment (e.g. once weekly) and agents for continuous or residual DUWL treatment. It is important to note that many DUWL treatment agents have not been developed or endorsed by DCU manufacturers, but rather have been developed by other manufacturers in response to an evident market need. Thus, there is significant potential for incompatibility of DUWL treatment agents with components of the DUWL network as well as with instruments connected to this network (Coleman *et al.* 2007).

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Table 1 Summary of practical measures for controlling biofilms in DUWLs

DUWL biofilm control	Practical approach to controlling biofilms in DUWLs	Comments
DCU	Ensure that DCUs are not equipped with water heaters unless effective controls are also in place to control DUWL biofilm	Seek advice from DCU manufacturer, preferably prior to procurement
	Ensure that independent reservoir bottles supplying water to DUWLs are cleaned regularly, disinfected and sterilized by autoclaving	Ensure several reservoir bottles are available to allow adequate time for reprocessing
	Use aseptic technique when filling reservoir bottles with water to minimize potential contamination with skin micro-organisms	Wear protective clothing and gloves to minimize contamination of reservoir bottles and water
	Use DCUs that are equipped with integrated and automated or semi-automated DUWL cleaning systems	These systems simplify biofilm control in DUWLs
	Ensure DCUs are serviced at least annually by competent personnel, including efficacy testing of antiretraction valves/devices	Seek advice from DCU manufacturer
	Ensure that the DUWLs in DCUs are separated by an air gap from municipal mains water supplies	To prevent contamination of mains water supplies with micro-organisms from DUWL biofilm and/or water
Dental unit supply water	Pretreat dental unit municipal supply water to remove suspended organic and inorganic material, dissolved hard water minerals, dissolved organic material and metals. Water supplied to DUWLs should be at least of potable quality	Seek advice from DCU manufacturer and/or water hygiene professional
	Water supplied to reservoir bottles should be at least of potable quality. The use of sterile water, deionized water or distilled water in independent bottle reservoirs is common, but these can readily become contaminated	Equipment used to produce distilled or deionized water for use in independent reservoirs should be regularly maintained to ensure good quality output water. Ensure that storage of water supplies for reservoirs does not result in deterioration of water quality
DUWL treatment agents	Use DUWL treatment agents that have been independently shown to remove biofilm and that do not exhibit adverse affects on DCUs and attached dental instruments and which do not leave residues in DUWLs	Seek advice from DCU manufacturer on the types of DUWL treatment agent to use in specific DCU models and the duration and frequency of treatment
	Residual DUWL treatment agents come in direct contact with patients and dental staff and should not have any toxic or adverse affects	Seek advice from DCU manufacturer
	The efficacy of DUWL treatment should be tested periodically by measuring the microbiological quality of output water	Seek advice from water hygiene professional
Training	All staff involved in DUWL decontamination should be comprehensively trained	Protocols for DUWL decontamination should be provided by DCU manufacturers

DUWL, dental unit waterline; DCU, dental chair unit.

Adverse affects of DUWL treatment agents

A few studies have reported adverse effects associated with the application of DUWL treatment agents, but, as mentioned previously, only a very few long-term studies of the effectiveness of DUWL treatment agents have been reported thus far. Furthermore, in the case of residual DUWL treatment agents, there is a lack of independent studies in the literature on potential interactions of such agents and their by-products on oral tissues and teeth. A number of recent studies reported that some DUWL treatment agents (e.g. 3-ppm sodium hypochlorite; a 1 : 10 dilution of Listerine mouthrinse; bio 2000, a 0·12% chlorhexidine gluconate- and 12% ethyl alcohol-containing product; and 0·224% BioClear, a citric acid containing product) may adversely affect bonding of composite material to both enamel and dentine (Roberts *et al.* 2000; Taylor-Hardy *et al.* 2001). With the extended and more widespread use of DUWL treatment agents, it is likely that such adverse effects may become clinically relevant in the case of residual DUWL treatments.

In 2002, a study from this laboratory reported clogging of DUWLs by the accumulation of disinfectant deposits in three of six DCUs treated with the alkaline hydrogen peroxide DUWL treatment agent Sterilex Ultra (Tuttlebee

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et al. 2002). Clogging became evident after the fourth consecutive week of once-weekly treatment in the three DCUs, and in one of these, after 14 weeks it became impossible to aspirate water or treatment agent through the air/water syringe waterline, which had to be replaced. Additionally, the pH of DUWL output water in these DCUs remained persistently alkaline (e.g. pH 8·4) for several days post-DUWL treatment. This was in contrast to the DCU supply water (pH 7·0) and DUWL output water from other DCUs (pH 7·0) in the same clinic, which were treated with a different hydrogen peroxide treatment agent not associated with DUWL clogging (Tuttlebee *et al.* 2002; Coleman *et al.* 2007). These findings suggested that residual DUWL treatment agent was present in DUWL output water in the DCUs that exhibited clogging for a considerable time after treatment. Another recent study from this laboratory on the long-term efficacy of Planosil (a hydrogen-peroxide and silver-ion-containing DUWL treatment agent) in Planmeca Prostyle Compact DCUs, identified several episodes of failure to disinfect DUWLs due to adverse effects on a variety of DCU components (O'Donnell *et al.* 2007). After 6 months of continuous once weekly (15 h overnight) DUWL treatment with Planosil, some episodes of DUWL disinfection failure were directly linked with blockage of and/or leakage from disinfectant intake valves, and corrosion of aluminium components of disinfectant delivery containers. Valve leakage was linked with damage to an internal glue seal, whereas valve blockage was caused by a combination of dislodged glue and oxidized aluminium deposits. Protracted exposure to hydrogen peroxide with its strong oxidizing properties was identified as the most likely cause of the adverse affects on the DCUs concerned. The problems were completely resolved in collaboration with the DCU manufacturer, Planmeca, who developed replacement valves and disinfectant delivery containers that were resistant to damage/corrosion following prolonged exposure to Planosil (O'Donnell *et al.* 2007). This study highlights the importance of investigating the long-term effects that DUWL treatment agents can have on DCU components and also highlights the important role that DCU manufacturers have in ongoing research and development to identify problems and to continually improve their DCUs.

Modern DCUs are equipped with a suction system that has a variety of purposes. Primarily, the suction system is used to remove oral fluids and debris from the oral cavity during dental procedures and also to minimize aerosol release into the dental clinic environment during the use of dental instruments attached to DUWLs, especially high-speed turbine handpieces (O'Donnell *et al.* 2005). Oral fluids and spent DUWL output water removed by DCU suction hoses and from the DCU cuspidor is eventually released as waste water following particle removal, dental amalgam removal and disinfection. Special amalgam separators are used to remove amalgam particles generated during the placement and removal of amalgam dental restorations in patients as amalgam contains mercury (Coleman *et al.* 2007). The performance of amalgam separators can vary considerably as can the total mercury concentration and total dissolved (ionic) mercury concentration in DCU waste water (Fan *et al.* 2002). A recent study from the USA reported that iodine-releasing resin cartridges, an effective residual treatment used to control biofilm growth in DUWLs by continuous release of low levels of iodine into DUWL output water, may have a harmful affect on the environment by mobilizing mercury from dental amalgam in DCUs with the release of highly toxic dissolved mercury into the environment from DCU waste water (Stone *et al.* 2006). However, the findings of this study were challenged by other authors who claimed that chloramine used to disinfect municipal water was more likely to have caused the increase in mercury levels rather than iodine (Hammarback *et al.* 2007). Other studies reported that a range of disinfectants and cleaning agents used to treat DCU waste water lines also cause the release of mercury from dental amalgam when tested in the laboratory. Strong chlorine-containing agents were reported to cause the release of more mercury than other products (Roberts *et al.* 2005; Batchu *et al.* 2006). These findings suggest that it is conceivable that DUWL treatment agents that contain chlorine could also mobilize mercury from dental amalgam collected in amalgam filters, traps and separators and in DCU waste water lines and pipes and release it into the environment.

A variety of types of electrochemically activated (ECA), solutions also called superoxidized water, anolyte and various other terms have been used in recent years as a residual treatment to control biofilm in DUWLs and studies have shown that such solutions can very effective (Marais and Brozel 1999; Kohno *et al.* 2004; Martin and Gallagher 2005; Zhang *et al.* 2007). However, some ECA solutions can have the potential for adverse effects on DUWLs and DCU instruments connected to them following extended use if the parameters for electrochemical activation are suboptimal or if the product used is too concentrated (Coleman *et al.* 2007). The literature concerning ECA solutions in all their varieties is confusing due to the use of differing terminologies, technologies and end products. ECA is generated by passing water containing dilute salt solutions (usually dilute sodium chloride) or other mineral solutions through an electrochemical cell designed to generate two streams of activated solution, one a negatively charged antioxidant solution (catholyte) and the other a positively charged oxidant solution (anolyte) (Coleman *et al.* 2007). Much

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early work on disinfection using anolyte solutions, used generators which produced an extremely acidic form which could also cause corrosion and harm to some materials. Data from this laboratory has shown that even short-term exposure of DUWLs to insufficiently dilute anolyte from a number of sources could cause some DUWLs to deteriorate rapidly and cause corrosion damage to other DCU components (Coleman *et al.* 2007). When using ECA to control biofilm in DUWLs it is essential that the ECA generator is capable of consistent quality output at neutral pH. The ECA product concentration in DUWL output water does not need to exceed 1–2 ppm free available chlorine, as it is so effective. This requires accurate dosing into DUWL supply water as anolyte produced by ECA generators is usually much more concentrated (e.g. 200 ppm).

DUWL composition and biofilm formation

Very little applied research has been undertaken to investigate whether the materials used to manufacture DUWLs can influence biofilm formation. One Japanese study reported that DUWLs composed of polyvinylidene fluoride were effective in inhibiting biofilm formation and reducing bacterial density in DUWL output water (Yabune *et al.* 2005). Another study from Italy reported that the aerobic heterotrophic bacterial plate count at 22°C from polytetrafluorethylene was lower than output water from DUWLs made from polyethylene (Sacchetti *et al.* 2007). These findings indicate that the development of novel DUWL materials with antimicrobial and/or anti-biofilm properties is a potentially very productive area for research on DUWL biofilm control. Delivering DCU supply water using copper pipes may also be beneficial in improving the microbial quality of DCU supply water, as copper pipework has been shown to possess significant antimicrobial advantages over drinking water pipework of other composition (Rogers *et al.* 1994a,b).

DUWL cleaning and disinfection systems

Dental unit waterlines treatment agents can be introduced into DUWLs from independent reservoir containers, or from treatment agent delivery devices connected to the DCU water supply inlet. In the case of DCUs connected to a municipal water mains supply, it is essential that the connection is turned off prior to DUWL treatment to avoid contamination of mains water with treatment agent chemicals. After treatment, DUWLs should be flushed thoroughly with clean water before DCUs are used for patient treatments to avoid exposing patients to residual disinfectant.

Integrated DUWL cleaning systems for DCUs

To our knowledge at least one DCU manufacturer has developed a new generation of DCUs with integrated DUWL cleaning systems that facilitate and simplify control of biofilms in DUWLs by cleaning and disinfection with consequent consistent good quality DUWL output water (Tuttlebee *et al.* 2002; O'Donnell *et al.* 2006b, 2007; Coleman *et al.* 2007). In 2002 we reported on the effectiveness of the Planmeca Waterline Cleaning System (WCS™), a semi-automated DUWL cleaning system developed by the Finnish DCU manufacturer Planmeca, to control DUWL biofilm in two separate Planmeca Prostyle Compact DCUs over a 20-week period using the hydrogen peroxide- and silver ion-containing disinfectant Sanosil (Tuttlebee *et al.* 2002). The WCS™ was found to be very effective at eliminating DUWL biofilm in these DCUs when used with Sanosil and consistently provided output water with bacterial densities below the ADA recommended level of ≤200 CFU ml^{-1} of aerobic heterotrophic bacteria for up to 7 days post-disinfection. The microprocessor-controlled WCS™ was originally developed to be retrofitted to existing Planmeca DCUs having a municipal mains water supply. All DUWLs, apart from the cuspidor rinse DUWL are included in the microprocessor-controlled process whereby DUWLs are automatically filled with disinfectant and after a specified disinfectant contact time, subsequently automatically flushed with fresh clean water during the disinfection cycle. If the cycle is not completed properly or an electrical power failure occurs during the cycle, an error message is displayed on the DCUs instrument console digital display and all DCU functions are locked preventing the DCU from being used (Tuttlebee *et al.* 2002). In recent years the WCS™ is provided as an integrated DUWL cleaning system in new Planmeca Prostyle Compact DCUs (O'Donnell *et al.* 2007). In a more recent study from this laboratory, the ability of the WCS™ to maintain the microbiological quality of DUWL output water below the ADA recommended level of ≤200 CFU ml^{-1} of aerobic heterotrophic bacteria was investigated over a longer period (18 months) with a much larger number of DCUs (10 DCUs) using the hydrogen peroxide- and silver ion-containing DUWL disinfectants Planosil and Planosil Forte (O'Donnell *et al.* 2007). In the majority of cases, once-weekly DUWL treatment using the WCS™ yielded output water below the ADA standard for up to a week post-disinfection. During the first 9 months a high prevalence (28/300 disinfection cycles; 9·3%) of intermittent DUWL disinfection failure occurred in 8/10 DCUs due to operator error (14/28 failed cycles), and physical blockage of disinfectant intake valves due to the corrosive effects of Planosil (14/28 failed cycles). These

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problems were resolved by rigorous staff training and procedural changes and by design changes to particular components of the WCS™ system and no further cases of intermittent DUWL disinfection failure were observed. Independently of these factors, a rapid and consistent decline in efficacy of DUWL disinfection occurred in 4/10 DCUs following the initial 9 months of once-weekly disinfection. There was a highly significant difference ($P < 0{\cdot}0001$) in the prevalence of *Novosphingobium* and *Sphingomonas* bacterial species (mean average prevalence of 37·1%) in DUWL output water from these four DCUs compared with the other six DCUs and DCU supply water (prevalence <1%), which correlated with biofilm presence in the DUWLs. These bacterial species are strongly catalase-positive and thus capable of breaking down hydrogen peroxide, one of the active ingredients of Planosil, and indicated selective pressure for maintenance of these species following prolonged disinfectant usage. A recent study from Poland on DUWL disinfection with a different hydrogen peroxide- and silver ion-containing disinfectant (Oxygenal 6) reported that isolates of *Sphingomonas paucimobilis* were significantly more prevalent (80%) in DUWL output water post-disinfection compared with output water pre-disinfection (10%) (Szymanska 2006). Planosil was reformulated by the DCU manufacturer Planmeca as a more concentrated form of hydrogen peroxide and silver ions (Planosil Forte) and when used once weekly was found to maintain bacterial density in output water below the ADA standard for all 10 DCUs during the 17 consecutive weeks studied (O'Donnell *et al.* 2007). These and other types of long-term problems very likely occur with other DCU brands and models and point to the importance of long-term compatibility testing of DUWL treatment products with DCUs, good communication between manufacturers and end users and the development of DUWL cleaning systems that are automated and require minimal human involvement. In our view, the long-term goal should be for fully automated disinfection systems that are validatable. In this regard, it is interesting to note that Planmeca recently developed a more advanced microprocessor-controlled DUWL cleaning system called the Water Management System (WMS), a fully integrated and automated DUWL cleaning system that requires minimal effort on the part of the user (O'Donnell *et al.* 2006b). The WMS is more advanced and automated than the WCS and also contains many additional features, including an air gap. Studies with a Planmeca Compact i DCU demonstrated that the WMS consistently provided DUWL output water that passed the ADA quality standard of ≤200 CFU ml^{-1} for up to 7 days after once-weekly disinfection with Planosil Forte during a test period of 40 consecutive weeks (O'Donnell *et al.* 2006b).

Centralized control of DCU water quality

In dental hospitals and other dental clinics equipped with many DCUs, ensuring consistency in DUWL output water quality from every DCU can be challenging, even if the DCUs are equipped with integrated DUWL cleaning systems. Meeting this challenge requires constant and consistently strict adherence to DUWL cleaning protocols using effective DUWL treatment agents and regular checks on supply water quality, infrastructure and controls. In busy dental hospitals and clinics, this can make considerable demands on already busy dental healthcare and maintenance staff and resources. Between 2005 and 2006 in the Dublin Dental Hospital we decided to centralize control of both DCU supply water quality and DUWL output water quality for the 103 Planmeca Prostyle Compact DCUs with which the hospital is equipped (Coleman *et al.* 2007). This was prompted by overgrowth during warm summer months of *Pseudomonas fluorescens* (approx. 1×10^5 CFU ml^{-1}) in DCU supply water holding tanks fed by mains water (<100 CFU ml^{-1} of aerobic heterotrophic bacteria), resulting in poor quality DCU supply water. Consequently, the quality of DUWL output water from DCUs was unacceptable despite regular disinfection using the WCS™ and emergency measures had to be implemented to reduce the bacterial density in supply water to achieve potable quality. Further deterioration would have necessitated closure of the clinics for patient safety. A centralized DCU supply water pretreatment system was designed and installed in early 2007 to provide consistent high quality (physical, chemical and microbiological) DCU supply water. Chlorinated municipal mains water supplied to the hospital's 103 DCUs is now automatically processed firstly by particle filtration, secondly by activated carbon filtration, thirdly by passage through an ion exchange water softening unit and fourthly by KDF filtration. This processed water is stored in a holding tank and treated with Ecasol (a neutral pH ECA solution) at 2·5 ppm ml^{-1}. This is generated *in situ* from supply water, a small amount of NaCl and electricity, using a Trustwater ECA generator (Trustwater, Clonmel, Ireland) which provides the consistent quality described above. This treatment controls both microbial load and biofilm prior to delivery to DCUs via a dedicated ring-main water supply network. The chemical quality of the water supplied to the DCUs is tested regularly and has been shown to be consistently better than the limits advocated by the EC Directive on the Quality of Water for Human Consumption (Anon. 1998) and by the United States Environmental Protection Agency National Primary Drinking Water Regulations (Anon. 2002). The density of aerobic heterotrophic bacteria in the supply water entering DCUs in the Dublin Dental

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Hospital consistently averages <1 CFU ml^{-1} (Coleman *et al.* 2007). The density of aerobic heterotrophic bacteria in DUWL output water from six sentinel DCUs also consistently averages <1 CFU ml^{-1} (O'Donnell *et al.* 2008) with the complete absence of biofilm in DUWLs. These findings demonstrate that our centralized DCU water treatment system not only maintains water quality at a level better than potable quality standards, but also simultaneously controls DUWL biofilm in a large number of DCUs and provides DUWL output water that more often than not is sterile whilst also providing logistical benefits.

Conclusions

The problem of microbial biofilm formation in DUWLs and poor quality DUWL output water has been recognized for more than four decades. In an age where inadequate infection control and prevention in healthcare facilities is seldom absent from the media, it is vital that dental healthcare professionals endeavour to maintain their DCU output water quality at a level which would at least satisfy the ADA standard of ≤200 CFU ml^{-1} of aerobic heterotrophs, or preferably, potable water standards. Achieving this objective has been difficult to meet consistently, mainly because of the absence of standards or legislation, but also because DCU manufacturers have been slow to address this issue by DCU engineering and design changes and by the provision of specific guidance on DUWL disinfection. Fortunately, this situation has begun to change with some DCU manufacturers developing and continuing to improve effective integrated and automated DUWL disinfection systems for use with specified DUWL treatment agents that are effective in the long-term and compatible with their DCUs. Work described in this review has shown that DUWL biofilm problems when studied in detail and in the longer term can have relatively simple solutions. It is clear that one cannot prescribe a single solution to improve DUWL water quality for all circumstances as the DCUs in dental clinics may be single, multiple, supplied by mains water, by large internal storage and distribution systems supplied with mains water or supplied by separate reservoir bottles. The DCUs may be of different makes and models. The one factor that impacts on biofilm formation in all DCUs and their DUWLs, regardless of make, is consistently high quality supply water. If DCU supply water is of poor quality, DUWL output water will be of poor quality. Practical approachs to providing this have been outlined, together with a centralized automated disinfection system suitable for individual or many DCUs that can provide DUWL output water of consistently better than potable water in the long-term and has many economic advantages in terms of reduced maintenance and equipment down-time.

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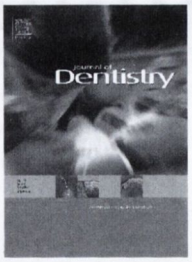

A centralised, automated dental hospital water quality and biofilm management system using neutral Ecasol™ maintains dental unit waterline output at better than potable quality: A 2-year longitudinal study

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ABSTRACT

Most studies addressing biofilm formation in dental chair unit waterlines (DUWLs) have focused on a range of individual dental chair units (DCUs) and no studies on a centralised approach in a large number of DCUs have been reported to date.

Objectives: To develop a centralised, automated water quality and biofilm management system serving the distribution network providing water to Dublin Dental Hospital's 103 DCUs, capable of maintaining DUWL supply and output water at better than potable quality standards in the long-term and requiring a minimum of human intervention. The potable water standard for the European Union does not specify an upper limit of aerobic heterotrophic bacteria, whereas a maximum of 100 cfu/mL is permitted in bottled water.

Methods: Mains water of varying quality was treated by specifically selected automated filtration units to provide DCUs with water of consistent chemical composition. This water was then automatically disinfected using an electrochemically activated solution Ecasol™ (Trustwater Group, Clonmel, Ireland) (2.5 ppm) prior to distribution to DCUs. Microbiological quality of both DUWL supply and output water was monitored weekly by culture on R2A agar for 10 sentinel DCUs for a 100-week period. DUWLs were tested for the presence of biofilm by electron microscopy.

Results: Chemical composition of processed mains water consistently bettered potable water standards. DUWL supply water and output water aerobic heterotrophic bacterial counts averaged <1 and 18.1 cfu/mL, respectively, from the 10 DCUs, compared to 88 cfu/mL for unprocessed mains water. This correlated with the absence of biofilm in DUWLs. No adverse effects due to Ecasol™ treatment of supply water were observed for DUWLs or DCU instruments.

Conclusions: This centralised and automated water treatment and biofilm management system consistently maintains DUWL output water at better than potable quality simultaneously in a large number of DCUs over the long-term.

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1. Introduction

Dental chair units (DCUs) are equipped with several integrated systems that supply the services and instruments needed for a wide range of dental procedures.[1–4] DCUs use water to irrigate the oral cavity and tooth surfaces during dental procedures via three-in-one air/water syringes and provide cooling to the dynamic instruments (e.g., turbine and conventional handpieces and ultrasonic scalers) as heat generated during instrument operation can be detrimental to teeth.[5–7] DCUs also provide water for oral rinsing by patients (water supplied via the cup filler outlet) and to rinse the DCU spittoon, or cuspidor bowl, after oral rinsing (water supplied via the bowl rinse outlet).[1,2] An elaborate network of interconnected narrow-bore plastic tubing called dental unit waterlines (DUWLs) supplies water to all of these DCU water outlets.[3,4,8] In individual dental practices, water supplied to a DCU may be provided either directly from the municipal potable quality mains supply or from bottle-type reservoirs in the DCU that are filled with water as required.[3,4] In dental hospitals and dental clinics equipped with large numbers of DCUs, water is often provided to DCUs via water storage tanks supplied with mains water.[3,4,8]

Numerous studies over the last four decades have shown that output water from DUWLs is usually heavily contaminated with microorganisms, primarily aerobic Gram-negative heterotrophic bacterial species of environmental origin, although oral organisms may also be present.[3,4,9–23] The high levels of microorganisms in DUWL output water originate from microbial biofilms formed on the internal surfaces of DUWLs.[3,4] These biofilms are composed mainly of highly hydrated bacterial exopolysaccharides, in which single cells and microcolonies are generally heterogeneously interspersed with pores or channels.[4,24,25] As the biofilm grows and matures, planktonic cells and by-products such as bacterial endotoxin are released into the water and are aerosolised by DCU-supplied instruments such as turbine dental handpieces and ultrasonic scalers, thus exposing patients and staff to these microorganisms and to fragments of biofilm and bacterial endotoxins.[26–29]

The occurrence of large numbers of microorganisms in DCU output water (up to 10^6 cfu/mL has been reported) is a potential infection, inflammation and allergenic risk to patients, dental staff and maintenance personnel and is incompatible with infection control and prevention best practices.[4,14,27,29] The presence of bacterial pathogens, such as *Legionella pneumophila*, and opportunistic bacterial pathogens, such as *Pseudomonas aeruginosa* and non-tuberculosis *Mycobacterium* species, is of particular concern in the dental treatment of immunocompromised patients and medically compromised patients.[9,13,22,30–32] At present there is no obligatory European Union (EU) quality standard for DCU output water. However, DCUs are classified as medical devices under the European Medical Devices Directive[33] and because many DCUs are supplied directly with municipal mains water of potable quality, the quality of DCU output water quality should be consistent with, or at least approximate, potable water quality standards.[3,4,34,35] The present potable water standard for aerobic heterotrophic bacteria in the EU and the USA do not specify an upper limit, although water sold in bottles or containers in the EU should not exceed 100 cfu/mL.[34,36] The Centers for Disease Control and Prevention (CDC) guidelines for infection control in dental health-care settings recommend a maximum level of aerobic heterotrophic bacteria in DUWL output water of ≤500 cfu/mL.[37] Furthermore, the American Dental Association (ADA) in 1995 established a target limit of ≤200 cfu/mL for the year 2000, but this largely has been quite difficult to achieve in practice.[3,4,38]

The most effective approach to maintaining good microbial DCU output water quality has been regular treatment of DUWLs using a disinfectant, biocide or cleaning agent that removes biofilm or inhibits biofilm growth.[1–4,19,21,22,39–44] A wide range of DUWL treatment products have been marketed in recent years, including both agents for intermittent use and for continuous use, many of which have been reported to be effective at controlling DUWL biofilm.[3,4] However, only a few of these products have been tested extensively in a large number of DCUs over extended time periods.[1,2] Furthermore, some manufacturers have developed DCU models with integrated semi-automated DUWL cleaning systems that facilitate the regular cleaning of DUWLs with effective disinfectants that eliminate biofilm.[1,2,19] We previously reported on the long-term efficacy of two of these systems in the Dublin Dental Hospital, including the Planmeca Waterline Cleaning System (WCS™) and the Planmeca Waterline Management System (WMS™), and showed that both were very effective at eliminating DUWL biofilm and provided output water at bacterial densities below the ADA recommended threshold of ≤200 cfu/mL for up to 1-week post-disinfection when using the hydrogen peroxide and silver ion-containing DUWL disinfectants Planosil.[1–3,19] These studies also showed however, that consistent provision of good quality DUWL output water was dependent on meticulous implementation of the disinfection protocol by staff undertaking DUWL disinfection.[2]

In the Dublin Dental Hospital all 103 DCUs with which the hospital is equipped are supplied with water from a central 8000-L storage tank supplied with potable quality mains water. In June 2006, during a period of warm weather, ongoing routine monitoring detected a bacterial bloom of *Pseudomonas fluorescens* in the 8000-L DCU storage tank, which developed over the course of 1-week between weekly samplings, where the bacterial density rose to >100,000 cfu/mL. Concomitantly, routine weekly testing of DUW output water from several sentinel DCUs showed bacterial densities >100,000 cfu/mL despite the once weekly DUWL disinfection regime with Planosil. This incident necessitated disconnecting all of the hospital's DCUs from the tank supply and providing each with fresh potable quality water in clean independent reservoir bottles until the contamination problems with the tank could be resolved. These findings highlighted the necessity for effective control of water quality throughout the DCU supply water network in dental hospitals and multi-DCU clinics and not only within DUWLs.

Electrochemical activation (ECA) technology was pioneered as a specialised discipline of electrochemistry by Prof. Vitold Bakhir in the 1970s in the former Soviet Union.[45–47] Electrochemically activated solutions were generated by passing a dilute salt solution through an electric field in a flow-through

electrolytic module (FEM), segregating the ions formed and producing two oppositely charged solutions possessing altered physical and chemical properties.[48] The activation process changes the state of the salt solution from a stable to a metastable state. The positively charged solution (anolyte) typically has a redox value of +600 mV, and is composed of a mixture of unstable mixed oxidants (predominantly hypochlorous acid) in a physically excited state which is highly microbicidal and able to penetrate biofilms. The negatively charged antioxidant solution (catholyte) has detergent like properties, typically a pH of 11, a redox value of −600 mV and contains predominantly sodium hydroxide in an excited state. These active ion species and free radicals are short-lived with a half-life of typically less than 48 h.[48] Activation also generates electrically and chemically active microbubbles of electrolytic gas, 0.2–0.5 μm in diameter, which enhance the redox potential of anolyte and catholyte.[48] Different kinds of ECA solutions can be generated by different types of ECA generators, each with specific properties and applications.[48] Over the years, several generations of FEM were developed, the FEM-3 being one of the more recent.[47,49] ECA solution production with consistent quality and characteristics was difficult to achieve prior to the development of FEM-3 technology. FEM-3-based ECA technology outside Russia is now owned by and has been further refined by the Trustwater Group, Clonmel, Ireland. Anolyte (Ecasol™) produced by Trustwater Group generators has a neutral pH, very much in contrast to anolyte produced by earlier generators from other manufacturers, which was often acidic and corrosive.[47–49]

The purpose of the present study was to develop a centralised DCU supply water pre-treatment system capable of automatically and consistently maintaining both the DUWL supply and output quality below the EU potable bottle water standard maximum of 100 cfu of aerobic heterotrophic bacteria per mL. To achieve this we decided to use automated enhanced filtration of mains water destined for DCUs followed by automated disinfection using the electrochemically activated (ECA) solution Ecasol™.

2. Materials and methods

2.1. *Collection of water samples*

After flushing for 1 min, samples (50 ml) of filtered, Ecasol™-treated water (hereafter referred to as processed water, see Sections 2.7 and 2.8 below) were collected each week for 100 consecutive weeks directly from the operator's air/water syringe waterline on 10 Planmeca Prostyle Compact DCUs located in three separate clinics in the Dublin Dental Hospital as described previously.[1,2,19] At the same time, weekly water samples were also taken from the unprocessed mains water supply to the Dental Hospital and from the 8000-L storage tank supplying processed water to the hospital's 103 DCUs. Additional processed water samples were also taken periodically from the bowl rinse and cupfiller outlet from each DCU. At 6-monthly intervals, individual processed water samples from operator air/water syringe waterlines from 25 other DCUs were also tested. All samples were processed immediately in the adjacent microbiology laboratory.

2.2. *Laboratory processing of water samples*

Water samples were cultured in duplicate on R2A agar plates (Lab M Ltd., Bury, Lancashire, United Kingdom) to determine total aerobic heterotrophic bacterial density as described previously.[1–3,19,50] After 10 days incubation at 20–22 °C, plates were examined and colonies counted using a Flash and Go™ automatic colony counter (IUL Instruments Ltd., Barcelona, Spain). R2A agar is the medium of choice for monitoring heterotrophic bacterial counts in water as it permits the recovery of significantly more organisms than conventional, more nutritious culture media, at 20 °C compared to 35 °C. Higher counts of bacteria are recovered on this medium following prolonged incubation (i.e., 10 days) ensuring that the maximum number of bacteria are detected.[50] The inclusion of sodium pyruvate in R2A medium also leads to enhanced recovery of chlorine-stressed bacteria from water.[50,51]

The total number of colonies and the relative abundance of different colony types were recorded as the average of the duplicate plates, and representative examples of all colony types present on each plate were purified by subculture and stored on nutrient agar slopes in the dark, for subsequent identification. These were Gram-stained, tested for catalase production and then identified as described previously (see Section 2.4 below).[1,2,19,52] For 100 duplicate processed water samples (80 from DCUs and 20 from the 8000-L tank), one sample in each case was supplemented with 1% (w/v) sodium thiosulphate to neutralise residual chlorine. No significant differences in the density or types of aerobic heterotrophic bacteria recovered were observed following culture on R2A agar as described above and so subsequent processed samples were not treated with thiosulphate. Experiments were also performed to demonstrate that free available chlorine present in concentrations of Ecasol used to treat processed water destined for DCUs (i.e., maximum of 2.5 ppm) was inactivated upon contact with R2A agar. Fresh R2A agar (25 ml) in 90 mm Petri dishes was flooded with 5 ml of Ecasol™ solution at 2.5, 5 and 10 ppm free available chlorine (FAC), respectively. After 10 min contact, the liquid was withdrawn with a sterile pipette and the free available chlorine levels measured as described in Section 2.3 below. For each concentration of Ecasol™ tested, no FAC was detected following contact with R2A agar, indicating neutralisation.

2.3. *Measurement of residual free available chlorine in DUWL output water*

Residual FAC in Ecasol™-treated processed mains water and in DUWL output water was measured using a Hach Pocket Colorimeter II (Hach Company, Iowa, USA) analysis system which uses *N,N*-diethyl-*p*-phenylenediamine (DPD) to react with free chlorine and form a red solution, whose colour intensity is proportional to the chlorine concentration. The equipment was used according to the manufacturer's instructions.

2.4. *Identification of bacterial isolates*

Bacterial isolates were identified, where possible, by means of the API 20 NE system for identifying non-enterobacterial

Gram-negative bacteria (bio-Mérieux, Marcy l'Etoile, France) as described previously.[1,2,19,52] Definitive identification of isolates was undertaken as described previously by determining the DNA sequence of a segment of the small ribosomal subunit rRNA gene and by comparing the sequences with consensus sequences for individual bacterial species in the EMBL/GenBank nucleotide sequence databases.[1,2]

2.5. *Electron microscopy of waterline biofilm*

Two-centimetre lengths of DUWL waterline tubing (supplying water to the air/water syringe, close to the syringe) were removed aseptically at intervals from each of the 10 sentinel DCUs included in the study. The samples were cut longitudinally through the lumen using a sterile scalpel and the internal walls examined for the presence of biofilm, without prior fixation, by electron microscopy using a Zeiss Supra 35 variable pressure field emission scanning electron microscope as described previously.[1,2,19] All electron microscopy was undertaken commercially by the Centre for Microscopy and Analysis (CMA), Trinity College, Dublin, Ireland.

2.6. *Chemical analysis of water samples*

Chemical analysis of water samples was undertaken commercially by CMA, Trinity College, Dublin, Ireland using standard procedures including inductively coupled plasma mass spectrometry (ICP-MS) and gas chromatography–mass spectrometry (GC–MS), as described previously.[1,2,19]

2.7. *Mains water filtration*

Water from the mains potable supply to the Dublin Dental Hospital was subjected to sequential filtration through a number of specific water filter types in order to provide DCU supply water of consistent physical and chemical composition (Fig. 1). The final configuration of filters used was arrived at by preliminary small scale testing. Mains water was initially filtered through a self-cleaning Depura Automatic particle filter (GEL S.p.a., Acona, Italy) fitted with a 95 micron stainless steel filtration cartridge. This system requires minimal maintenance because the filtration cartridge is cleaned by timed automated backwashing with mains water when there is no demand on the water supply.[3,4] This is combined with an effective mechanical brushing system. The water supply was next subjected to water softening by ion exchange using Crystal-Right™ zeolite crystals (Aqua Solutions Inc., Hazelton, PA, USA), a medium manufactured from sodium aluminosilicate. The crystals have an irregular surface and have a greater capacity for ion exchange compared to uniform spherical bead resins used in most conventional water softeners. This medium provides excellent water softening, iron and manganese removal, while at the same time, raising pH levels of acidic water. The supply water was then filtered through a granulated activated carbon filter (Norit Nederland B.V., Amersfoort, the Netherlands) to remove chlorine, organic compounds and volatile organic compounds. The carbon medium was supplemented with 15% (v/v) Kinetic Degradation Fluxion (KDF) KDF-85 filter medium (KDF Fluid Treatment Inc., Three Rivers, MI, USA), which is effective in controlling the build-up of bacteria, algae, fungi and scale in granular activated carbon beds as well as reducing iron and hydrogen sulphide from municipal or other water supplies. KDF filter media consist of granulated, high-purity copper and zinc alloys that are designed to inhibit microbial growth in filters, remove or reduce chlorine content, iron, heavy metals and hydrogen sulphide by a redox (approx. −500 mV) process. The final stage of pre-treatment consisted of passing the water through a KDF-55 fine filter unit. All filters were sized to permit a minimum flow rate of 3000 L of water per hour. The KDF-85 filter medium and the granular activated charcoal medium are certified to NSF International Standard 61 for water treatment plant applications.[53] KDF-55 filter medium is certified to NSF International Standard 042 for drinking water treatment units.[54] The KDF filter, the water softener and the activated carbon filter were each equipped with an electronic regenera-

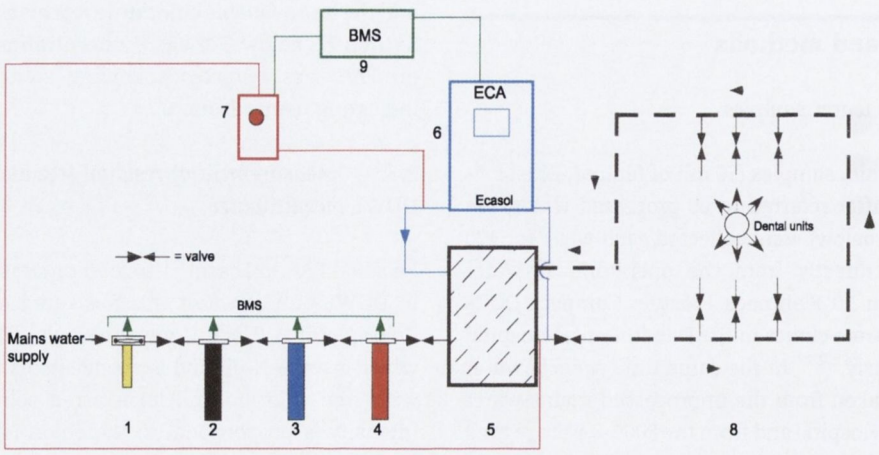

Fig. 1 – Schematic diagram of the integrated and automated prefiltration and Ecasol™ disinfection system for maintaining the quality of DUWL supply and output water at better than potable quality at the Dublin Dental Hospital. Key: (1) self-cleaning particle filter; (2) water softening unit; (3) granular activated carbon filter with 15% (v/v) KDF-85 media; (4) KDF-55 filter; (5) 8000-L processed water storage tank; (6) Trustwater Model 120 Ecasol™ generator; (7) Hach chlorine analyser, (8) recirculating ring main supplying water to DCUs with the direction of flow indicated by arrowheads; (9) connections to buildings management system (BMS) computer and remote site monitoring station.

tion controller, which permitted each filter to regenerate by backwashing out of phase at night with fresh mains water when there was no demand on the water supply and the effluent in each case was discharged to drain. Following filtration, the water destined for DCUs was stored in an 8000-L storage tank that supplied water to each of the Dublin Dental Hospital's 103 DCUs via a dedicated recirculating ring main (Fig. 1). Each of the regeneration controllers was configured to send an electronic signal to the hospital's building's management system (BMS) computer following each regeneration cycle where the information was then recorded.

2.8. *Use of Ecasol™ to disinfect DUWLs and associated water supply network*

The electrochemically-generated disinfectant solution Ecasol™ (Trustwater Group) was used as a residual disinfectant to minimise microbial proliferation and biofilm formation in DUWLs and throughout the dedicated ring main water distribution network supplying water to DCUs. This included the 8000-L water tank, the ring main pipework and pumps and the DUWLs in each of the 103 DCUs of the Dublin Dental Hospital (Fig. 1). Ecasol™ was produced by electrochemical activation (ECA) using a Trustwater Model 120 ECA generator equipped with four FEM-3[49] type flow-through electrolytic modules (FEMs). The generator was supplied with water pretreated as described above, together with a saturated NaCl solution to give a final concentration of 0.2% (w/v). This generated a solution (Ecasol™) at neutral pH having an oxidation–reduction potential of +900 ± 100 mV and consisting of approximately 200 ppm metastable oxidants (predominantly hypochlorous acid ~158 ppm, hypochlorite ion ~42 ppm, ozone <1 ppm, chlorine dioxide <2.5 ppm, chloric acid <1.5 ppm and chlorous acid <3 ppm).[55] The activated oxidants (Ecasol's activated state lasts for a period of up to 48 h), which are formed initially are in dynamic equilibrium and gradually revert to the initial ingredients (i.e., water supplied and 0.2% NaCl) over time. The formed substances are additionally in an electrochemically energised state, which relaxes gradually over a period of 24–48 h. Ecasol™ initially contains energised microbubbles formed at the FEMs electrode interface.[45–49] These also dissipate by cavitation over a period of 12–24 h.

Freshly generated Ecasol™ was automatically dosed by a pump into the 8000-L water tank supplying water to the hospital's DCUs to give a final concentration of 2.5 ppm FAC (Fig. 1). This level was consistently monitored by *in situ* probes and automatically adjusted as required by a Hach CL17 Chlorine Analyzer (Hach Company, Iowa, USA) (Fig. 1). As a safeguard, an upper FAC limit (usually 5 ppm) causes immediate cessation of dosing Ecasol™ into the tank and sends an alarm signal to the hospital's BMS computer (Fig. 1) and short message service (SMS) text messages to mobile phones of designated personnel.

2.9. *Flushing of DUWLs following periods of stagnation*

To ensure the aerobic heterotrophic bacterial density of DUWL output water was consistently lower than the EU bottled potable water maximum (<100 cfu/ml) following periods of water stagnation (e.g., during the night, at weekends and during holiday periods) an optimised DUWL flushing protocol was developed. Each DUWL was flushed for a period of 2 min prior to the start of each clinical day and each bowl rinse DUWL was flushed for 1 min because its bore is larger than the other waterlines. In the Planmeca Prostyle Compact DCUs used in the Dublin Dental Hospital this process was facilitated by the ability to programme the DCUs to automatically sequentially flush each instrument DUWL and the cupfiller DUWL.[1,2] Following this procedure, the bowl rinse DUWL had to be flushed separately by pressing the appropriate function key on the DCUs instrument console. In the latest Prostyle Compact DCU models, all DUWLs can be programmed to automatically sequentially flush all DUWLs once the instrument hoses containing the DUWLs have been placed in flushing holders.

2.10. *Maintenance of the DCU supply water filtration and disinfection system*

Efficient operation of the integrated DCU supply water filtration and disinfection system required occasional maintenance. Consumable reagents for specific components required replenishing, including the saturated 0.2% (w/v) NaCl solution for the Ecasol™ generator (once monthly), the NaCl for the brine tank supplying the water softening unit (once monthly), citric acid descaler for the FEM-3 cells (once monthly) and calibration chemicals for the Hach CL17 Chlorine Analyzer (once monthly). Planned preventive maintenance was also instituted on a 6-monthly basis. This was undertaken under contract by the Trustwater Group and consisted of simple equipment performance evaluation and calibration checks and component cleaning. The KDF and granular activated carbon media require replacement after 5 years and the water softening medium after 3 years. Periodically, some Ecasol™ was added to the brine tank supplying the water softener to eliminate any biofilm that had formed in the unit's ion exchange media and from the downstream carbon filter's activated carbon medium.

3. Results

3.1. *Management of DUWL output water quality*

In June 2006, during a period of warm weather, routine weekly microbiological monitoring detected overgrowth of *P. fluorescens* (i.e., >100,000 cfu/mL) in the 8000-L water storage tank supplying the hospital's DCUs. This bloom was associated with an elevated iron concentration (5 ppm) in the mains supply water, 250 times higher than the average level present in mains water supplied to the Dental Hospital in the 24-month period prior to June 2006 (data not shown). For approximately a year prior to June 2006, the average density of aerobic heterotrophic bacteria in the mains supply and from the tank supplied by it was 81 and 136 cfu per mL, respectively (Fig. 2, panel a) and the iron concentration averaged 0.02 ppm.

Maintaining DUWL output water quality below the ADA standard of ≤200 cfu/mL of aerobic heterotrophic bacteria with once weekly disinfection of DUWLs with Planosil clearly

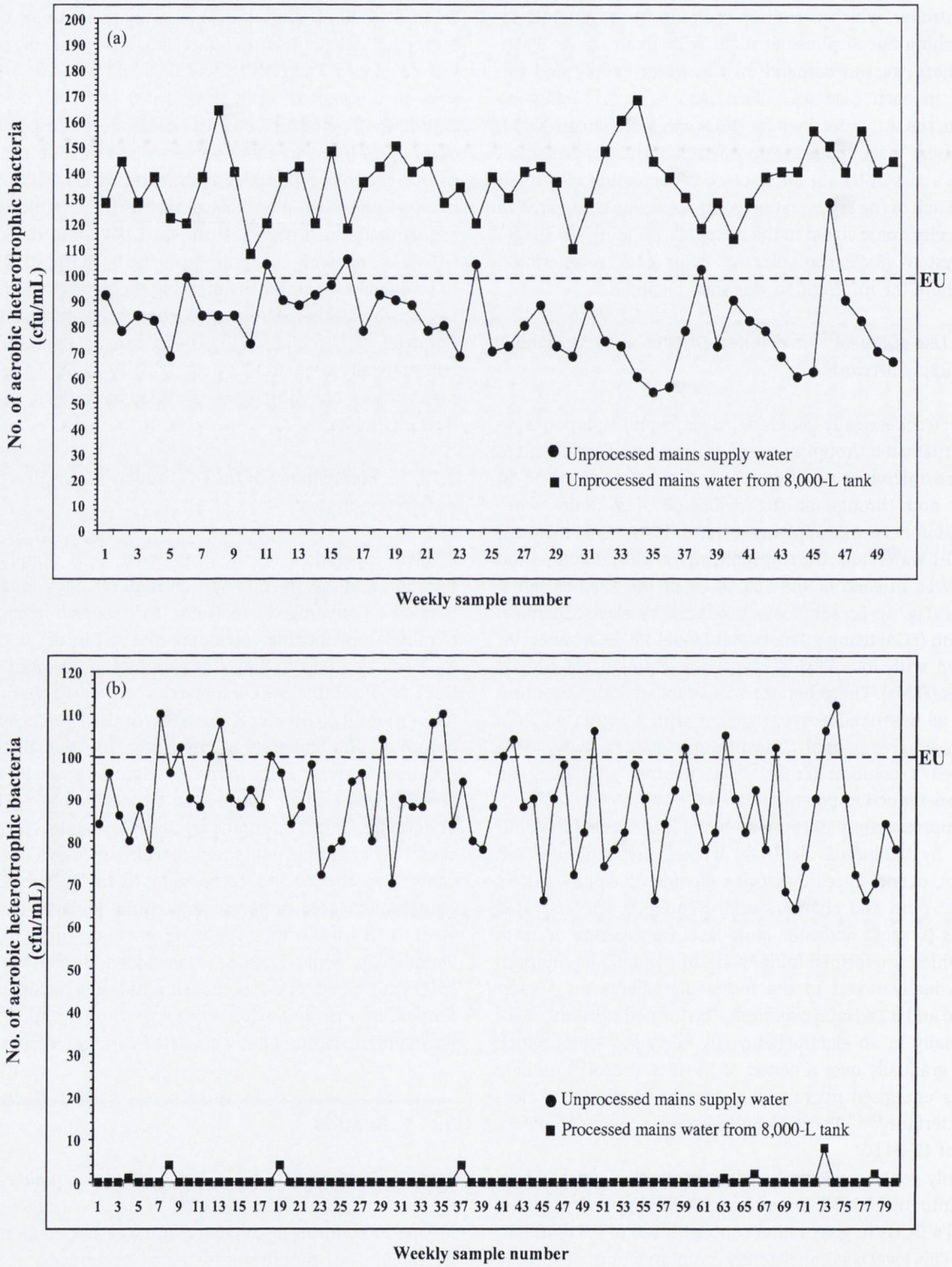

Fig. 2 – Density of aerobic heterotrophic bacteria in unprocessed and processed mains water destined for DCUs in the Dublin Dental Hospital. Panel (a) shows the bacterial density in unprocessed mains water (average density 81 cfu/mL) and in water from the 8000-L mains water storage tank (average density 136 cfu/mL) supplying the hospital's DCUs for a 50-week period between June 2005 and May 2006. Panel (b) shows the bacterial density in unprocessed mains water (average density 88 cfu/mL) and in processed (i.e. filtered and Ecasol™-treated) water from the 8000-L mains water storage tank (average density <1 cfu/mL) supplying the hospital's DCUs for a 100-week period between September 2006 and August 2008. The dashed line marked EU shown in both panels indicates the maximum density of aerobic heterotrophic bacteria in cfu/mL permissible in bottled potable water according the EU Directive on water for human consumption as no upper limit is specified for potable quality mains water.[34]

Table 1 – Chemical analysis[a] of processed supply water for dental units in the Dublin Dental Hospital.

Test	Processed water (mg/L)	Potable water EC maximum conc.[b] (mg/L)	Method of analysis[c]
Calcium	2.00	N/A	ICP-MS
Magnesium	0.95	N/A	ICP-MS
Sodium	54.80	200.00	ICP-MS
Potassium	0.41	N/A	ICP-MS
Iron	0.02	0.20	ICP-MS
Manganese	0.00	0.05	ICP-MS
Copper	0.03	2.00	ICP-MS
Lead	<0.001	0.01	ICP-MS
Zinc	0.39	N/A	ICP-MS
Silver	<0.001	N/A	ICP-MS
Chromium	<0.01	0.05	ICP-MS
Cadmium	<0.001	0.01	ICP-MS
Nickel	<0.001	0.02	ICP-MS
Carbonate	25.00	N/A	Titration
Chloride	69.70	250.00	Photometry
Sulphate	<40.00	250.00	Photometry
Nitrate as NO_3	4.63	50.00	Photometry
Nitrite as NO_2	0.09	0.50	Photometry
Ammonia as NH_4	0.00	0.50	Photometry
Fluoride	0.87	1.50	Photometry
Ortho Phosphate as PO_4	0.27	N/A	Photometry
Total organic carbon	<2.0	N/A	Photometry
Chloroform	<0.01	0.1	GC–MS
Bromodichloromethane	<0.01	0.1	GC–MS
Dibromochloromethane	<0.01	0.1	GC–MS
Bromoform	<0.01	0.1	GC–MS
Trichloroethane	<0.01	0.01	GC–MS
Tetrachloroethane	<0.01	0.01	GC–MS
1,2-dichloroethane	<0.001	0.003	GC–MS
pH	7.60	6.5–9.5	Meter
Conductivity at 25 °C (μS/cm)	460.00	2500.00	Meter

[a] Chemical analysis of processed DCU supply water was undertaken every 2 months between September 2006 and August 2008. The data shown is representative of the results obtained during this period.

[b] European Community maximum permissible concentration in water for human consumption.[34]

[c] Abbreviations: ICP-MS, inductively coupled plasma-mass spectrometry; GC–MS, gas chromatography-mass spectrometry; N/A, no maximum permissible concentration in water for human consumption.

could not be sustained if the microbiological and/or chemical quality of the DCU supply water provided by the 8000-L tank deteriorated on a regular basis. For this and other reasons (see below) we decided to develop a centralised DCU supply water pre-treatment system capable of automatically and consistently maintaining DUWL supply and output quality below the EU potable bottle water standard maximum of 100 cfu of aerobic heterotrophic bacteria per mL regardless of seasonal fluctuations in ambient temperature or variations in the chemical quality of mains supply water. We also wanted to develop a centralised system for maintaining good quality DUWL output water that would (a) reduce the time spent by nursing staff on DUWL disinfection using our existing disinfection protocol using Planosil with the Planmeca WCS™ system (up to 50 h per week for the hospital's 103 DCUs), (b) reduce the use and handling of chemicals in DUWL disinfection, (c) introduce more uniformity into DUWL disinfection and (d) reduce incidences of human error in relation to DUWL disinfection.

3.2. *Enhanced prefiltration of mains water*

In order to ensure that the chemical quality of water supplied to DCUs was consistent regardless of fluctuations in the municipal mains supply, we first applied enhanced filtration to mains water prior to its delivery to the 8000-L tank that provides water to the hospitals 103 DCUs (Fig. 1). A series of new filters, each capable of processing 3000 L of municipal mains water per hour, was installed (Fig. 1). Mains water was initially filtered through a particle filter, followed by water softening using ion exchange, followed by filtration through a granular activated carbon filter, and finally by filtration through a KDF filter. Between July 2006 and September 2008 the chemical composition of this processed water was analyzed every 2 months, both from the 8000-L storage tank and from DUWLs and in each case was found to be consistently well below the maximum limits detailed in the EU Directive on water for human consumption (Table 1).

3.3. *Efficacy of automated Ecasol™ disinfection of DCU supply water and DUWL output water*

For a period of 100 consecutive weeks, between September 2006 and August 2008, unprocessed mains water supplied to the Dublin Dental Hospital and processed mains water (i.e., filtered and Ecasol™-treated water) from the 8000-L tank supplying the hospital's 103 DCUs was tested weekly for density of aerobic heterotrophic bacteria. During this period,

Table 2 – Density of aerobic heterotrophic bacteria in 100 consecutive weekly water samples from DUWL[a] output water from ten DCUs supplied with Ecasol™-treated water.

DCU No.	Average bacterial density (cfu/mL)	Bacterial density range	No. of weekly samples/100 yielding bacterial density > 100 cfu/mL[b]
1	1.6	0–18	0 (0%)
2	2.3	0–20	0 (0%)
3	1.7	0–16	0 (0%)
4	28.3	0–840	8 (8%)
5	38.1	0–1060	7 (7%)
6	29.3	0–960	9 (9%)
7	22.2	0–222	5 (5%)
8	17.0	0–400	8 (8%)
9	21.7	0–306	7 (7%)
10	19.4	0–610	10 (10%)
Overall	18.1	0–1060	54 (5.4%)

[a] Water samples were taken from the operator's three-in-one air/water syringe from each DCU.
[b] The maximum permissible density of aerobic heterotrophic bacteria in bottled water for human consumption is 100 cfu/mL.[34]

the average density in unprocessed mains water and processed water was 88 and <1 cfu/mL, respectively (Fig. 2b). The average bacterial density in DUWL output water from the operator's air/water syringe DUWL from the 10 sentinel DCUs included in the 100-week study period was 18.1 cfu/mL (Table 2). Of the ten DCUs included in the study, 3/10 (300 samples in total) yielded an average bacterial density in DUWL output water of <3 cfu/mL (range 0–20 cfu/mL) over 100 consecutive weekly water samples tested per DCU (Table 2). The microbiological quality of every sample from these DCUs was well below the maximum permissible density of 100 cfu/mL permissible in bottled water for human consumption (Fig. 3). For the remaining seven DCUs, 92.3% (646/700) DUWL output water samples tested also yielded bacterial densities well below 100 cfu/mL, however, the remaining 7.7% (54/700) water samples from these seven DCUs exceeded 100 cfu/mL (Table 2). In 53/54 cases the high bacterial counts were intermittent and did not occur in consecutive samples (Fig. 4). Examination of the circumstances surrounding these 54 water samples revealed that in each case either the DCU concerned had not been used for several days prior to water sampling and its DUWLs had been left stagnant, or one or more DCU water

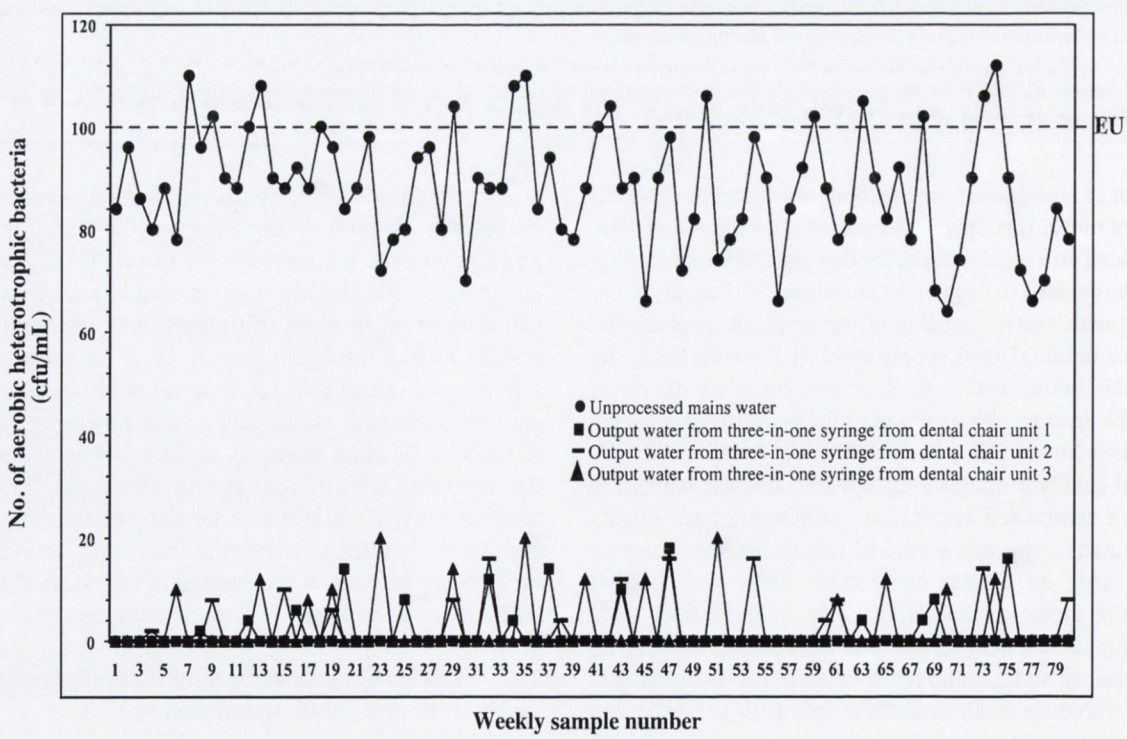

Fig. 3 – Density of aerobic heterotrophic bacteria in unprocessed mains water supplied to the Dublin Dental hospital and in processed (i.e., filtered and Ecasol™-treated) DUWL output water from the operator's air/water waterline from DCUs Nos. 1–3 (Table 2) with average bacterial densities of 1.6, 2.3 and 1.7 cfu/mL, respectively, during the 100-week study period between September 2006 and August 2008. The dashed line marked EU indicates the maximum density of aerobic heterotrophic bacteria in cfu/mL permissible in bottled potable water according the EU Directive on water for human consumption as no upper limit is specified for potable quality mains water.[34]

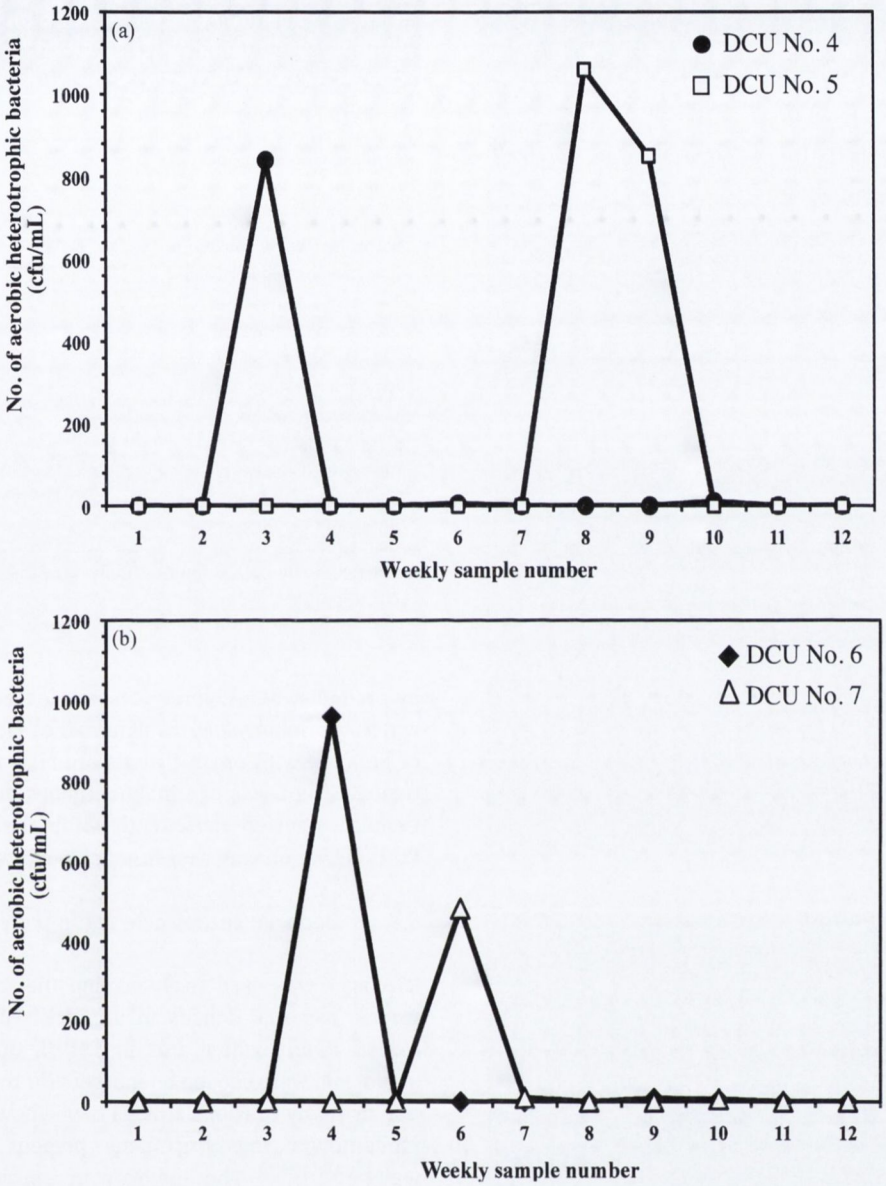

Fig. 4 – Panels (a and b) show the density of aerobic heterotrophic bacteria in processed (i.e., filtered and Ecasol-treated) DUWL output water from the operator's air/water waterline from four separate DCUs for a period of 12 consecutive weeks during the 100-week study period showing instances of intermittent poor quality output water. In all cases the cause of poor quality output water was found to be due to water stagnation in the DUWLs in the DCUs concerned, either because the DCU was not used for a period of up to 2 weeks due to breakdown and its DUWLs had not been flushed daily, or because the patient cup filler DUWL and/or the cuspidor DUWL had been turned off in the DCUs concerned. All of the DUWLs in DCUs are interconnected and stagnation in one can result in biofilm formation that can contaminate other waterlines.

outlets (i.e., the cup filler and/or the bowl rinse waterline) had been turned off by clinical staff or students resulting in water stagnation in these DUWLs. Ensuring that the water supply to each DUWL in each DCU was turned on and flushed each morning (see Section 2.9) and reiterating that DCUs that were not being used must have their DUWLs flushed each morning ensured that the bacterial density in DUWL output water remained well below 100 cfu/mL. In the one DCU that yielded DUWL output water with a bacterial density >100 cfu/mL in two consecutive weekly samples (Fig. 4a; DCU No. 5, Table 2), it was discovered that the DCU had been taken out of service for repairs not relating to the DUWL-supplied dynamic instruments and had not been used for 2 weeks. Examination of a section of DUWL tubing from this DCU's operator's air/water syringe revealed the presence of extensive biofilm (Fig. 5, panels b and c). Flushing of its DUWLs every day for a week resulted (see Section 2.9) in bacterial densities well below 100 cfu/mL in all subsequent water samples tested. Water from the cup filler and bowl rinse DUWLs of the 10 sentinel DCUs sampled at 12-week intervals during the study period yielded average bacterial densities of 7 and 9 cfu/mL, respectively. Furthermore, water samples from the operator's air/water syringe DUWL from 25 other DCUs sampled at 6 monthly intervals during the study period yielded an average bacterial density of 9 cfu/mL.

Samples of Ecasol™-treated water from the 8000-L tank and from DUWLs were investigated at 4-monthly intervals for the presence of trihalomethanes. Trihalomethanes are formed as a

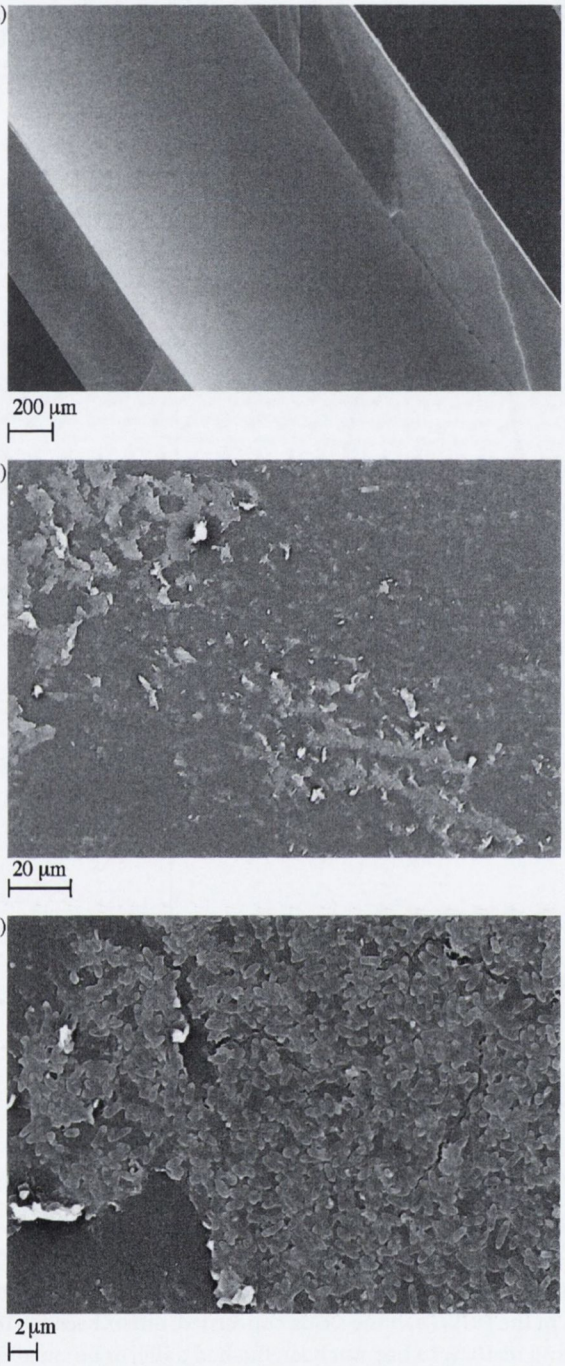

Fig. 5 – Electron micrographs of longitudinal sections of DUWL tubing from air/water syringe waterlines supplied with filtered and Ecasol™-treated water. Panel (a) shows a low magnification view of a section of DUWL from DCU No. 2 showing the absence of biofilm. Output water from this DUWL yielded an average density of aerobic heterotrophic bacteria of 2.3 cfu/mL over the 100-week study period (Table 2). Panels (b and c) show medium and high magnification views of the same DUWL section from DCU No. 5 taken after the DCU had been out of service for 2 weeks due to a mechanical problem during which time its DUWLs had been left stagnant (see also Table 2 and Fig. 4). Extensive biofilm is evident in both views.

Table 3 – Bacterial species isolated on R2A agar from unprocessed mains water supplied to the Dublin Dental Hospital during the 100-week study period.

Bacterial species[a]	Gram-stain
Aeromonas salmonicida	Negative
Acidovorax temperans	Negative
Comamonas acidovorans	Negative
Novosphingobium subarctica	Negative
Sphingomonas paucimobilis	Negative
Sphingomonas aerolata	Negative
Sphingomonas trueperi	Negative
Porphyrobacter tepidarius	Negative
Pseudomonas fluorescens	Negative
Arthrobacter agilis	Positive
Kocuria palustris	Positive
Micrococcus luteus	Positive
Rhodococcus fascians	Positive
Variovorax paradoxus	Positive

[a] Identified by DNA sequence analysis of the variable region of 16S rDNA gene.[1,2]

by-product when chlorine or bromine are used to disinfect water for drinking They result from the reaction of chlorine and/or bromine with organic material present in the water being treated. The levels of trihalomethanes detected in Ecasol™-treated water were consistently within the limits detailed in the EU Directive on water for human consumption (Table 1).

3.4. *Bacterial species detected in water*

R2A agar was used to determine the total aerobic heterotrophic bacterial density in unprocessed mains water, processed mains water and in DUWL output water. These organisms were chosen as markers for microbial contamination as many previous studies have shown that they are the predominant microorganisms present in DUWL output water.[1,2,8,14,18,19] The majority of species recovered from unprocessed mains water on R2A agar were Gram-negative (Table 3). However, the Gram-positive organism *Micrococcus luteus* was the most common bacterial species identified. Bacterial isolates were hardly ever recovered from processed (i.e., filtered and Ecasol™-treated) mains water (average density <1 cfu/mL), but when they were, they belonged to the same species present in unprocessed mains water and had a similar distribution. Similarly, bacterial isolates recovered from DUWL output water reflected the species and distribution present in unprocessed mains water.

3.5. *Electron microscopy of DUWLs*

For each of the 10 sentinel DCUs included in the study, samples of DUWL tubing supplying the operator's air/water syringe were taken at intervals throughout the study and examined for the presence of biofilm by electron microscopy. Water samples were taken from the respective DUWLs for bacterial culture immediately prior to taking the DUWL samples. In every case, examination of the internal surfaces of the DUWLs showed the absence of microbial biofilm, which correlated with DUWL output water densities <10 cfu/mL (an example is shown in Fig. 5, panel a). Fig. 5 panels b and c show

the presence of extensive biofilm in a DUWL section from DCU No. 5 taken after the DCU had been out of service for 2 weeks during which time its DUWLs had been left stagnant (see also Table 2 and Fig. 4).

3.6. *Ecasol™ corrosion effects*

Throughout this study, metal component parts of DUWL networks in the DCUs were examined at intervals for potential corrosive effects of Ecasol at the working concentration used (maximum of 2.5 ppm). No corrosive effects were observed. Two instrument multiplexer units (which distribute water via DUWLs to dental instrument hoses) from DCUs included in this study were examined independently by the DCU manufacturer Planmeca, who reported no corrosive effects. Finally, dental instruments attached to DUWLs that were supplied with Ecasol™-treated water showed no corrosive effects either. Examination of DUWL tubing from several of the test DCUs used in this study revealed no detectable deterioration.

4. Discussion

In the Dublin Dental Hospital for the past several years, the microbial quality of DUWL output water from the hospital's 103 Prostyle Compact DCUs was consistently maintained below the ADA recommended standard of ≤200 cfu/mL of aerobic heterotrophic bacteria by once weekly disinfection using the hydrogen peroxide and silver ion-containing DUWL disinfectant Planosil.[1,2] This changed in June 2006 when a bloom of *P. fluorescens* occurred in the 8000-L mains water storage tank supplying the hospital's DCUs, which coincided with an increased level of iron (250 times greater than average) in the mains water supply probably caused by works on the mains water distribution network by municipal authorities. Iron is essential for bacterial growth and it is likely that a combination of increased tank water temperature and available iron were significant factors in the overgrowth of *P. fluorescens*.[56] The quality of DUWL output water is directly influenced by the physical content (i.e., presence of particulate matter and suspended material), chemical content (i.e., dissolved and suspended inorganic compounds and organic material) and microbiological quality (i.e., density of microorganisms present) of the supply water.[3,4,57] and indirectly by environmental conditions such as temperature.[3,4] The condition of the mains water pipework, maintenance works undertaken on the mains water distribution system, the type of pipe material used, corrosion controls and the presence of water storage tanks and their condition also play a major role in supply water quality.[3,4,56]

The overall aim of the present study was to develop a centralised and automated water quality and biofilm management system for the Dublin Dental Hospital encompassing the entire water distribution network providing water to DCUs which could maintain DUWL output water at better than potable quality simultaneously in all of the hospital's 103 DCUs and require a minimum of human intervention. Because the hospital's DCUs are in constant use during the working week, the necessity for uninterrupted water supply requires a mains water-supplied storage tank rather than having an individual mains supply for each DCU. The presence of the 8000-L water storage tank did influence the microbiological quality of the water supplied to DCUs. For approximately a year prior to overgrowth of *P. fluorescens* in the 8,000-L DCU supply tank in June 2006, the average density of aerobic heterotrophic bacteria in the mains supply was 81 cfu/mL, whereas the corresponding average density in tank water was 136 cfu/mL (Fig. 2, panel a). The increased density in tank water was more than likely due to the release of planktonic bacteria from biofilm on the inner surfaces of the tank originally seeded from bacteria present in mains water. Our approach to developing a centralised and automated system consisted of firstly developing an automated control system to maintain consistency in the chemical composition of DUWL supply water, and secondly to develop an automated control system to minimise the microbial density in both DUWL supply and output water. We achieved our first objective by using a series of sequential water filters for treating mains water (Fig. 1 and Table 1). Mains water was initially subjected to particle filtration to remove sediment and suspended particles >90 μm, followed by water softening, granular activated carbon filtration and finally filtration through a KDF-55 filter. Each filter had an integrated facility programmed to automatically back-wash the filters with fresh mains water at night, out of phase, when there was no demand on the water supply. This facility increases the working life of the filters by eliminating contaminants and helps to reduce microbial proliferation in the filters. Proper maintenance of water filters and ion exchange units is essential, although often overlooked, as biofilm can develop within them, particularly ion exchange media, after a period of use, thus adding significant microbial loads to water entering DCUs downstream.[3,4] In the present study, nightly automated back-washing ensured the water softening unit worked efficiently, whereas periodic disinfection of the softening medium with Ecasol™ minimised microbial contamination. The granular activated carbon filter medium used in this study to remove organic material was supplemented with 15% (v/v) KDF-85 medium to reduce the proliferation of microorganisms in the carbon filter bed as well as to remove other contaminants such as iron, chlorine and sulphides.[58] This combination of filters used to process the incoming mains water provided water of consistent chemical composition over the 2-year period of the study, well under the maximum limits required by the EU Directive on water for human consumption, without completely demineralising the water (Table 1).[34] Highly demineralised water can have corrosive effects on metal components under specific conditions and is an important consideration when dealing with sensitive equipment such as medical devices.

We achieved our objective of minimising microbial contamination in DUWL supply and output water by using the ECA solution Ecasol™ as a residual disinfectant in the filtered mains water supply provided to DCUs. Between September 2006 and August 2008, unprocessed mains water supplied to the Dublin Dental Hospital had an average aerobic heterotrophic bacterial cell density of 88 cfu/mL, whereas processed (i.e., filtered and Ecasol™-treated water) from the 8000-L water storage tank had an average bacterial density of <1 cfu/mL (Fig. 2b). The average bacterial density in DUWL

output water, tested weekly, from ten sentinel DCUs included in the 100-week study period was 18.1 cfu/mL (Fig. 3). These results demonstrated unequivocally that our centralised water treatment system for DUWL supply water was capable of maintaining the microbiological quality of DUWL supply and output water at significantly better than potable water quality for the 100-week period of the study. Electron microscopy of the internal surfaces of samples of DUWL waterline tubing taken periodically from the 10 sentinel DCUs revealed the absence of biofilm, demonstrating that the use of Ecasol™ as a residual disinfectant is effective at controlling biofilm formation in DUWLs (Fig. 5, panel a). Preliminary tests at the outset of the study revealed that the efficacy of Ecasol™ at maintaining DUWL output water at better than potable quality was dependant on daily flushing of each DCU's DUWLs and so a daily flushing policy was developed whereby each DUWL was flushed for 2 min each morning, apart from the bowl rinse DUWL, which was flushed for just 1 min (see Section 2.9). Implementation of this procedure in the hospital's DCUs was facilitated by the ability to programme each DCU to flush its DUWLs automatically in the morning prior to the start of clinics.[2] All that is required is for a member of the dental staff to initiate the process by placing the DCU's instrument hoses containing the DUWLs into flushing holders to direct the effluent to the wastewater outlet and activating the flushing procedure by pressing the appropriate key on the DCU's electronic instrument console. Despite this protocol, 54/1000 (5.4%) of the DUWL output water samples taken from the 10 sentinel DCUs over the 100-week period yielded bacterial densities above the EU potable bottled water maximum of 100 cfu/mL (Table 2). These 54 'failures' from seven DCUs occurred intermittently in 53/54 cases (i.e., not in consecutive weeks) (Fig. 4). In every case, the reason for poor quality DUWL output water was found to be due to failure to implement the daily DUWL flushing protocol in the DCUs concerned, where either the DCU concerned had not been used for several days prior to water sampling and its DUWLs had been left stagnant, or one or more DUWL outlets (i.e., the cup filler and/or the bowl rinse waterline) had been turned off by clinical staff or students resulting in water stagnation in these DUWLs. These findings highlight the fact that even when effective DUWL biofilm control protocols are implemented for individual DCUs, no matter how automated; even if minimal human intervention is required, the long-term efficacy of the process is dependent on rigorous adherence to the procedure. In busy dental clinics, the often-heavy demands placed on dental health-care staff can result in poor compliance with DUWL cleaning protocols with resulting poor quality output water. In a previous study from this laboratory, 14/28 (50%) of failed DUWL disinfection cycles in 8 DCUs using the Planmeca WCS™ DUWL cleaning system with Planosil disinfectant over a 9-month period were found to be due to improper implementation of the DUWL disinfection protocol.[2]

A variety of ECA solutions, also referred to as superoxidised water, anolyte and various other names, have been used previously as a residual disinfectant to control biofilm in DUWLs and studies have shown that such solutions can be very effective.[59–62] However, not all ECA solutions are the same and some can have the potential for adverse effects on DUWLs and DCU instruments connected to them following prolonged use if the parameters for electrochemical activation are sub-optimal or if the product used is too concentrated or too acidic.[3,4] Data from this laboratory have shown that exposure of DUWLs to insufficiently dilute anolyte or non pH neutral anolyte from a number of sources could cause some DUWLs to deteriorate and cause corrosion damage to other DCU components.[3] In the present study, very dilute Ecasol™ (i.e., 2.5 ppm FAC initial dosing) was found to be quite sufficient at minimising microbial growth in a relatively large water distribution system supplying 103 DCUs in the Dublin Dental Hospital. No adverse effects were observed on DUWLs throughout the study on metal components associated with DUWLs (i.e., solenoid valves) or with dental instruments supplied by DUWLs containing water treated with 2.5 ppm Ecasol™. To a great extent, early work on disinfection using anolyte solutions, used generators that produced inconsistent and extremely aggressive acidic forms that could also cause corrosion and harm to some materials.[3,4] When using ECA to control biofilm in DUWLs it is essential that the ECA generator is capable of consistent quality output at neutral pH, such as that achieved by the generator equipped with FEM-3 modules used in the present study. The ECA product concentration in DUWL output water does not need to exceed 1–2 ppm FAC, as it is so effective. This requires accurate dosing into DUWL supply water as anolyte produced by ECA generators, including the Trustwater 120 Model ECA generator used in this study, is usually much more concentrated (e.g., 200 ppm FAC). In the present study, the use of *in situ* probes to monitor FAC levels in the DCU supply water distribution network and constant automated monitoring using an FAC analyzer ensured that the level of FAC within treated water was strictly maintained at low levels (Fig. 1).

Other researchers who investigated the use of ECA solutions to control biofilm in DUWLs reported that it can take up to 5 weeks continuous treatment to eliminate resident biofilm from DUWLs.[59] Prior to the present study in the Dublin Dental Hospital, DUWLs were disinfected once weekly with the hydrogen peroxide and silver ion-containing disinfectant Planosil, which completely eliminated biofilm from DUWLs and maintained the bacterial density in DUWL output water at <200 cfu/mL for up to 7 days post-disinfection. Biofilm began to form again within a few days post-disinfection but this was then eliminated by the next weekly disinfection cycle.[1,2,19] Thus for several years prior to the start of this study the hospital's DUWLs were essentially free of extensive biofilm and when the DCUs began to be supplied with processed mains water, the presence of residual Ecasol™ was able to maintain the DUWLs free from biofilm. In heavily fouled DUWLs with extensive biofilm, it can take several weeks for dilute Ecasol™ to eliminate the biofilm, but once it is eliminated, continued use of dilute Ecasol™ as a residual disinfectant is effective at maintaining DUWLs free of biofilm (D. Coleman and R. Russell, unpublished data).

Installation of our centralised and automated water treatment system to maintain DUWL output water at better than potable quality was very cost-effective. Prior to installation of the new system management of DUWLs, cleaning throughout the hospital using the Planmeca WCS™ system integrated in each DCU, required about 50 h per week to implement. Taking this time into account, together with the cost of the DUWL disinfectant, the overall annual expenditure for managing the

quality of DUWL output water was approximately €80,000. The automated system described in the present study requires minimal human intervention, although it does require monthly replenishment of consumable reagents to specific components, which takes less than 30 min to implement and 6-monthly planned preventive maintenance on equipment and pumps etc. Filter media only have to be replaced every 3–5 years. Overall, the operating costs are less than €20,000 per annum. Furthermore, the system is environmentally friendly, does not produce harmful waste products, spent filter media are recyclable, and staff do not have to handle potentially hazardous chemicals. It also reports its status in real time to facilities management and is capable of remote diagnostics, which are also time-saving features.

There are few quantitative scientific publications outside of Russia that consider the health effects of ECA solutions, although marketed widely in the US and Japan for both human and animal use. The US Food and Drug Administration consider Ecasol suitable for food processing applications. ECA solutions have been used extensively in Russia for over 30 years, for drinking water disinfection, swimming pool disinfection, as the general disinfectant in hospitals, as wound irrigants, as nebulised inhalant sprays and many other infection control applications with no apparent harmful effects.[3,4,45–48]

5. Conclusions

We have developed a centralised and automated biofilm management system for mains water distribution networks supplying water to DCUs that consistently maintains DUWL supply and output water at better than potable quality simultaneously in 103 DCUs in a Dental Hospital setting. The first part of the system consists of automated filtration of mains water to yield DCU supply water of consistent chemical composition, despite fluctuations in the chemical composition of the inward mains supply. The second part of the system automatically disinfects filtered mains water using dilute (i.e., 2.5 ppm) Ecasol™, a neutral ECA anolyte solution, and over the 100-week study period maintained the average microbiological quality of DUWL supply water and output water at <1 and 18.1 cfu/mL, respectively, of aerobic heterotrophic bacteria, significantly better than potable water. The system requires minimal human intervention, is cost-effective, environmentally friendly and provides a robust solution to the universal problem of biofilm formation in DUWLs. The current system is ideally suited to Dental Hospitals and large dental clinics equipped with many DCUs but has immense potential for managing water quality in other health-care facilities, including acute hospitals.

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