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The development of a three-dimensional scaffold for *ex vivo* biomimicry of human acute myeloid leukaemia

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ABSTRACT

Acute myeloid leukaemia (AML) is a cancer of haematopoietic cells that develops in three-dimensional (3-D) bone marrow niches *in vivo*. The study of AML has been hampered by lack of appropriate *ex vivo* models that mimic this microenvironment. We hypothesised that fabrication and optimisation of suitable biomimetic scaffolds for culturing leukaemic cells *ex vivo* might facilitate the study of AML in its native 3-D niche. We evaluated the growth of three leukaemia subtype-specific cell lines, K-562, HL60 and Kasumi-6, on highly porous scaffolds fabricated from biodegradable and non-biodegradable polymeric materials, such as poly (L-lactic-co-glycolic acid) (PLGA), polyurethane (PU), poly (methylmethacrylate), poly (D, L-lactade), poly (caprolactone), and polystyrene. Our results show that PLGA and PU supported the best seeding efficiency and leukaemic growth. Furthermore, the PLGA and PU scaffolds were coated with extracellular matrix (ECM) proteins, collagen type I (62.5 or 125 μg/ml) and fibronectin (25 or 50 μg/ml) to provide biorecognition signals. The 3 leukaemia subtype-specific lines grew best on PU scaffolds coated with 62.5 μg/ml collagen type I over 6 weeks in the absence of exogenous growth factors. In conclusion, PU-collagen scaffolds may provide a practical model to study the biology and treatment of primary AML in an *ex vivo* mimicry.

1. Introduction

Haematopoietic stem cells (HSCs) require a unique microenvironment in order to sustain blood cell formation [1]. This microenvironment incorporates the 3-D bone marrow niches [2–4] formed by stroma cells, such as macrophages, adipocytes, and fibroblasts. The stroma cells elaborate extracellular matrix proteins including fibronectin, collagen, vitronectin and tenascin, which create specialised compartments with localised chemokines and cytokines resulting in the regulation of proliferation, differentiation and self-renewal of HSCs [5]. The organisation of the bone marrow niches is critical for the function or dysfunction of normal or malignant bone marrow [6]. Acute myeloid leukaemia is one such malignant bone marrow condition [7].

Although traditional two-dimensional (2-D) cultures and *in vivo* animal models of AML have helped to elucidate the molecular determinants of leukaemogenesis, the cellular and microenvironmental elements that enhance leukaemia growth and protect the leukaemic stem cells from chemotherapy are difficult to investigate [8,9]. This difficulty arises from three major limitations of the current techniques used in the study of AML: i) 2-D cultures cannot support long-term primary leukaemic cell growth without exogenous growth factors or stromal cell support, ii) 2-D culture conditions cannot structurally provide the native haematopoietic microenvironment (HM) for human AML cells, especially the 3-D niches which protect the leukaemic stem cell, and iii) animal models, although superior to 2-D cultures, cannot completely replicate the human microenvironment and require extensive animal facilities and expertise for use [10,11]. Development of an *ex vivo* 3-D mimicry of the human haematopoietic microenvironment for the study of AML could overcome these challenges [12].

Traditional bone marrow culture is fundamentally based on the 2-D culture developed by Dexter in tissue culture flasks/plates [13]. However HSCs cannot be cultivated *ex vivo* without the addition of, usually, high concentrations of cytokines [14]. The culture of BM

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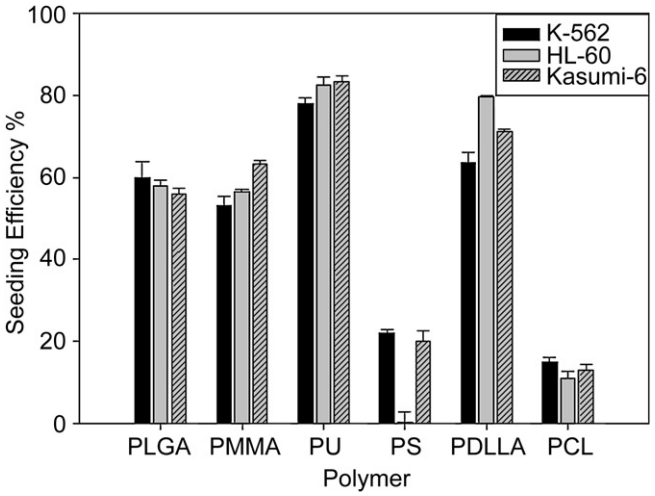

Fig. 1. Cell seeding efficiency of uncoated scaffolds. Seeding efficiency was determined 24 h following inoculation into the scaffolds for each of the 3 AML cell lines, K-562, HL-60 and Kasumi-6. PLGA, PMMA, PU and PDLLA displayed a greater than 50% seeding efficiency with PLGA and PDLLA showing a statistically significant higher ($p < 0.05$) seeding efficiency than the rest. Data were obtained in 3 separate instances, each in triplicate.

haematopoietic cells in 3-D has been investigated as an alternative since it provides a more physiologically accurate microenvironment. Several methods including the use of collagen microspheres [15] and the use of a novel 3-D tantalum-coated biomaterial (TCPB) [16] have been reported. These cultures provide a different microenvironment from that of the 2-D flask cultures and can be used as a model for *ex vivo* haematopoiesis. In order to recreate the 3-D BM microenvironment for the investigation of AML *ex vivo*, polymeric scaffolds need to be fabricated with desirable properties, such as adequate pore size and a high surface area to volume ratio through the presence of a network of channels and interconnected pores, which will facilitate enhanced penetration by cells and the formation of cellular associations [17,18]. Specifically, the fabricated synthetic polymeric scaffolds should have a high porosity and a pore size distribution and architecture similar to that of normal human bone marrow [19]. Furthermore, polymeric scaffolds need to be biocompatible and should offer biorecognition signals that support cellular growth and tissue formation through, possibly, hybridisation with ECM proteins.

Herein, 3 different cell lines, namely K-562 (erythroleukaemia), HL-60 (acute promyelocytic leukaemia) and Kasumi-6 (acute myeloid leukaemia with differentiation), were used as a substitute for human primary AML, a heterogeneous disorder, in order to account for differences in the growth requirements of the different AML subtypes as well as assist in the development of the *ex vivo* 3-D leukaemia model. The leukaemic cell lines were tested on 6 different polymers, all FDA-approved for biologic use and used extensively in biomedical engineering [20,21]: poly (D, L-lactade) (PDLLA), poly (L-lactic-co-glycolic acid) PLGA, polystyrene (PS), poly (methyl-methacrylate) (PMMA), poly (caprolactone) (PCL) and polyurethane (PU). Biorecognition signals were provided to selected scaffolds by coating with either high or low concentration of fibronectin and/or collagen in order to provide an adhesive interface (fibronectin) and strong mechanical contact (collagen) between the scaffold material and the AML cells [22].

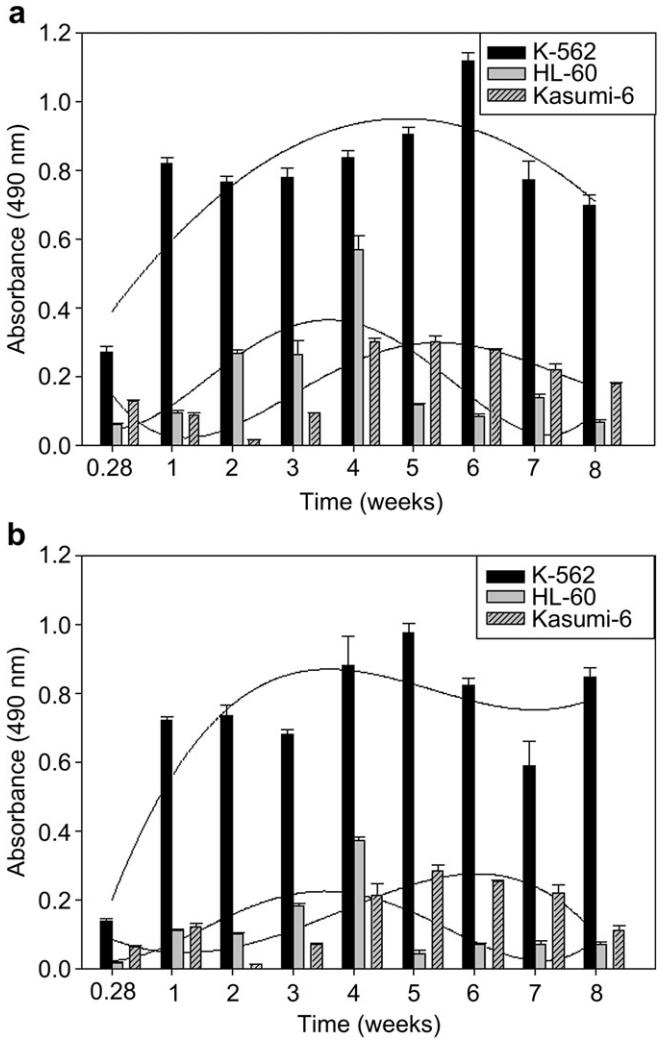

Fig. 2. Cellular growth of AML cell lines on PU and PLGA. The MTS assay was used as a measure of proliferation of each leukaemic cell line on PU (a) and PLGA (b). K562 displayed a significantly higher ($p < 0.01$) growth kinetics in comparison to HL-60 and Kasumi-6 for both scaffolds. The lines represent the growth trends (obtained by regression analysis) for the three AML cell lines. An initial growth phase was observed prior to stabilisation of the cultures. The cultures were performed in parallel on 2 separate occasions, each in triplicates.

2. Materials and methods

2.1. Scaffold fabrication

Dimethylcarbonate (DMC, 99% pure; Sigma–Aldrich, Dorset, UK) was used for the fabrication of PLGA and PDLLA foams (Purasorb® PDLG (53/47), Purasorb® PL; PURAC Biochem, Gorinchem, The Netherlands). Dioxan (99.8% pure; Sigma–Aldrich, UK) was used for the fabrication of PU (Noveon, Belgium) and PMMA (Röhm GmbH & Co, Germany) foams. The polymers, dimethylcarbonate and dioxan were used without further purification. The foams (pore size 100–250 μm, porosity 90–95% [20]) were fabricated by thermally-induced phase separation (TIPS) of polymer solutions (5% w/v in the appropriate solvent) and subsequent solvent sublimation, as described by Safinia et al. [23]. The polymer solution was frozen in liquid nitrogen (−196 °C) for PLGA, PDLLA, PMMA, PCL and PS and at −86 °C for PU and maintained at that temperature for 2 h. The solvent was removed by freeze-drying in an ethylene glycol bath maintained at −15 °C for 3 days. Prior to cell seeding, the scaffolds were cut into cubes of $5 \times 5 \times 5$ mm^3. Sterilisation was done using a combination of 8 min under UV light (230v, 50 Hz, 0.14A, Kendro Laboratory Products UK) and immersion for 2 h in Ethanol (70% v/v); the scaffolds were then washed twice for 5 min in PBS before adding the media and placing in a humidified incubator for 3 days at 37 °C and 5% CO_2 prior to use. The porous morphology of the scaffolds was examined with scanning electron microscopy (SEM), as described below.

2.2. Scaffold coating

Scaffolds were coated with collagen type I from calf skin and/or fibronectin from bovine plasma (Sigma–Aldrich). The foams were pre-wetted by immersion in ethanol (70% v/v) for 1 min and then transferred into phosphate buffered saline (PBS; Gibco, UK) for 20 min. Scaffolds were centrifuged for 10 min at 3630 g and then

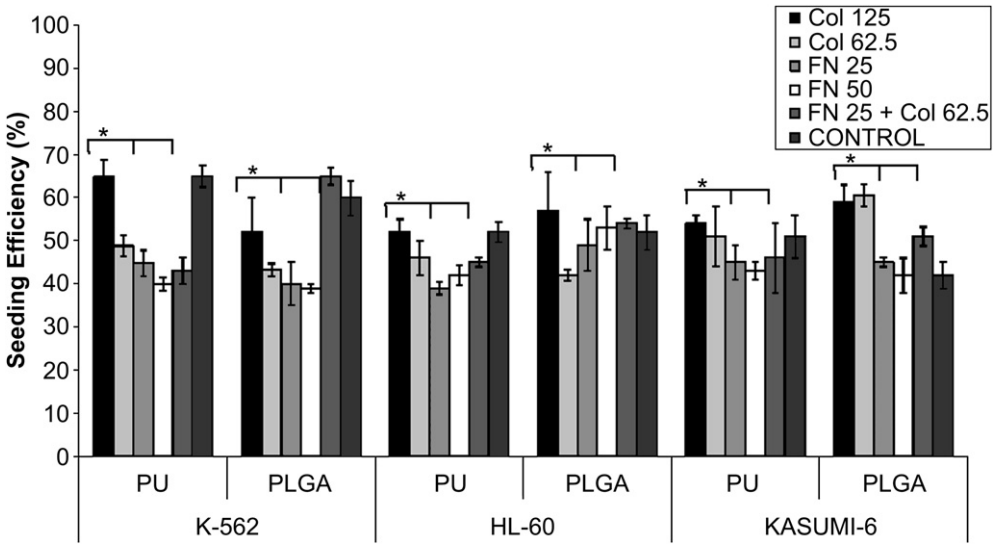

Fig. 3. Cell seeding efficiency of ECM-coated scaffolds. The PLGA and PU scaffolds were coated with collagen type I (125 μg/ml or 62.5 μg/ml), fibronectin (50 μg/ml or 25 μg/ml), and collagen and fibronectin (62.5 μg/ml and 25 μg/ml, respectively). The AML cell lines used were K-562, HL-60 and Kasumi-6. For all cell lines and scaffolds tested, coating with collagen type I at 125 μg/ml consistently displayed a seeding efficiency of over 50% ($p < 0.05$). The cultures were performed in parallel on 2 separate occasions, each in triplicates.

transferred into the different protein concentrations: 125 μg/ml or 62.5 μg/ml of collagen type I soluble in 0.1 M of acetic acid (Fisher Scientific, UK) and dissolved in deionised water from a NANOpure (Barnstead, Duque, IA, conductivity 1.8 MΩ/cm). Alternatively, 25 μg/ml or 50 μg/ml of fibronectin were diluted in deionised water. The foams were centrifuged at 1420 g for 20 min. In order to unblock the surface pores, and allow the cells seeded to penetrate deeper into the scaffold, the scaffolds were centrifuged one more time at 910 g for 10 min in PBS. The protein-coated scaffolds were placed in the orbital incubator at 3 g (Stuart Scientific UK, SI 50) for 3 d at ambient temperature.

2.3. Cell culture

Three human leukaemic cell lines were studied: K-562 (human erythromyeloblastoid leukaemia cell line ATCC®, UK; CCL-243), originally derived from a patient with blast crisis of chronic myeloid leukaemia, HL-60 (ATCC®, UKCCL-240) from a patient with acute promyelocytic leukaemia, and Kasumi-6 (ATCC®, UK; CRL-2775) from a patient with acute myeloid leukaemia with differentiation. These particular cell lines are routinely used for the study of leukaemia in 2-D cultures and were chosen because of the different properties and stages of leukaemic differentiation inherent in each which would enable the optimisation of scaffolds for future use with most leukaemic subtypes. The cells were cultivated on standard polystyrene non-pyrogenic tissue culture flasks (Fisher Scientific) and in a humidified incubator at 37 °C in an atmosphere of 5% CO_2 using the protocol recommended by the cell line supplier, with a seeding density of 3.5×10^4 cells/cm^3. For scaffold cultures, 100 μl of cell suspension (5×10^5 cells/scaffold) were seeded onto the sterile scaffolds (both protein- and non-protein-coated), placed in 24-well tissue culture plates and incubated for 15 min at 37 °C and 5% CO_2 in order to allow the cells to settle into the scaffolds prior to adding 1.5 ml of culture medium. Scaffold cultures seeded with cells underwent a half-medium exchange every other day and were otherwise treated in the same way as cell cultures in flasks.

K-562 and HL-60 cells were cultivated in IMDM Medium (Invitrogen Ltd, UK) supplemented with 10% and 20% v/v, respectively, of foetal bovine serum (FBS heat inactivated, Invitrogen Ltd). Kasumi-6 cells were grown in RPMI-1640 Medium (ATCC®) supplemented with 20% v/v FBS and GM-CSF 2 ng/ml (R&D Systems Europe Ltd). For K-562 cells, medium was changed every second day and for HL-60 and Kasumi-6 cells every three days.

2.4. Cell proliferation assay

Seeding efficiency was determined by counting the number of cells remaining in the media, that are not seeded into the scaffold, after 24 h. Cell proliferation was quantitatively assessed by changes in the number of metabolically active cells using the tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfophenyl)-2H-tetrazolium; MTS, Promega, CellTiter96® AQ_{ueous} Solution Cell Proliferation Assay) [24]. MTS was added and incubated with the scaffolds seeded with cells over 3 h at 37 °C and 5% CO_2; the absorbance was measured at 490 nm.

2.5. Scanning electron microscopy (SEM)

Following cultivation of the leukaemic cell lines on the scaffolds for 48 h, 14 d and 28 d, the scaffolds were examined by SEM (JEOL JSM-840A, JEOL Ltd., Welwyn Garden City, U.K.). Briefly, the scaffolds were fixed with 2.5% v/v PBS-buffered glutaraldehyde solution (Fluka BioChemika, Switzerland) for 40 min at 4 °C and washed twice with PBS. Serial dehydration in ethanol (50, 70, 90, 95, and 98%) followed, each for 2–10 min, after which the samples were dried in an aseptic environment for 4 h. The specimens were cut with a razor blade to enable examination of longitudinal and transverse sections. The specimens were sputter-coated with gold in argon atmosphere for 2 min prior to sectioning and examination by SEM at an acceleration voltage of 20 kV.

2.6. Histochemistry

For each condition, two samples of each scaffold were fixed in 2.5% v/v glutaraldehyde solution, as described above, and then embedded with a xylene replacement (Paraclear™; Polyscience Inc, Warrington, PA) and sectioned at 8 μm thickness using a Shandon Finesse® ME Microtome (Shandon, Pittsburgh, Pennsylvania, USA). Xylene was not used due to its incompatibility with the polymeric scaffolds which lead to the dissolution of the scaffolds. Instead Paraclear (Polysciences Inc., Warrington, USA), a xylene replacement was used. After dewaxing, the thin-sectioned slides were immersed for 30 s in Haematoxylin (Sigma–Aldrich), then rinsed with tap water, and counterstained with 1% v/v Eosin (Sigma–Aldrich) for 2 min.

2.7. Statistical analysis

The experiments evaluating the 3 cell line cultures for each of the 6 different polymers were performed on 3 separate occasions, in triplicate for each occasion. The long-term culture experiments (over 8 weeks) on PLGA and PU were done in triplicate on 2 separate occasions ($N = 2$) and representative data are provided. Statistical significance of the results was evaluated by using one-way analysis of variance [25] with a level of significance $p < 0.05$ or $p < 0.01$. Data were analyzed by linear regression using Sigma Plot (Jandel Scientific, San Rafael, CA).

3. Results

3.1. Unmodified scaffold evaluation

The six polymer scaffolds, PLGA, PMMA, PU, PS, PDLLA and PCL, were evaluated for their ability to support the growth of each of three AML cell lines, HL-60, K-562 and Kasumi-6. As shown in Fig. 1, PLGA, PMMA, PU and PDLLA displayed a seeding efficiency higher than 50% 24 h following inoculation. However, only PU and PLGA were able to support long-term (over a period of 2 months) cellular growth, as shown in Fig. 2a and b, respectively. In contrast, PCL and

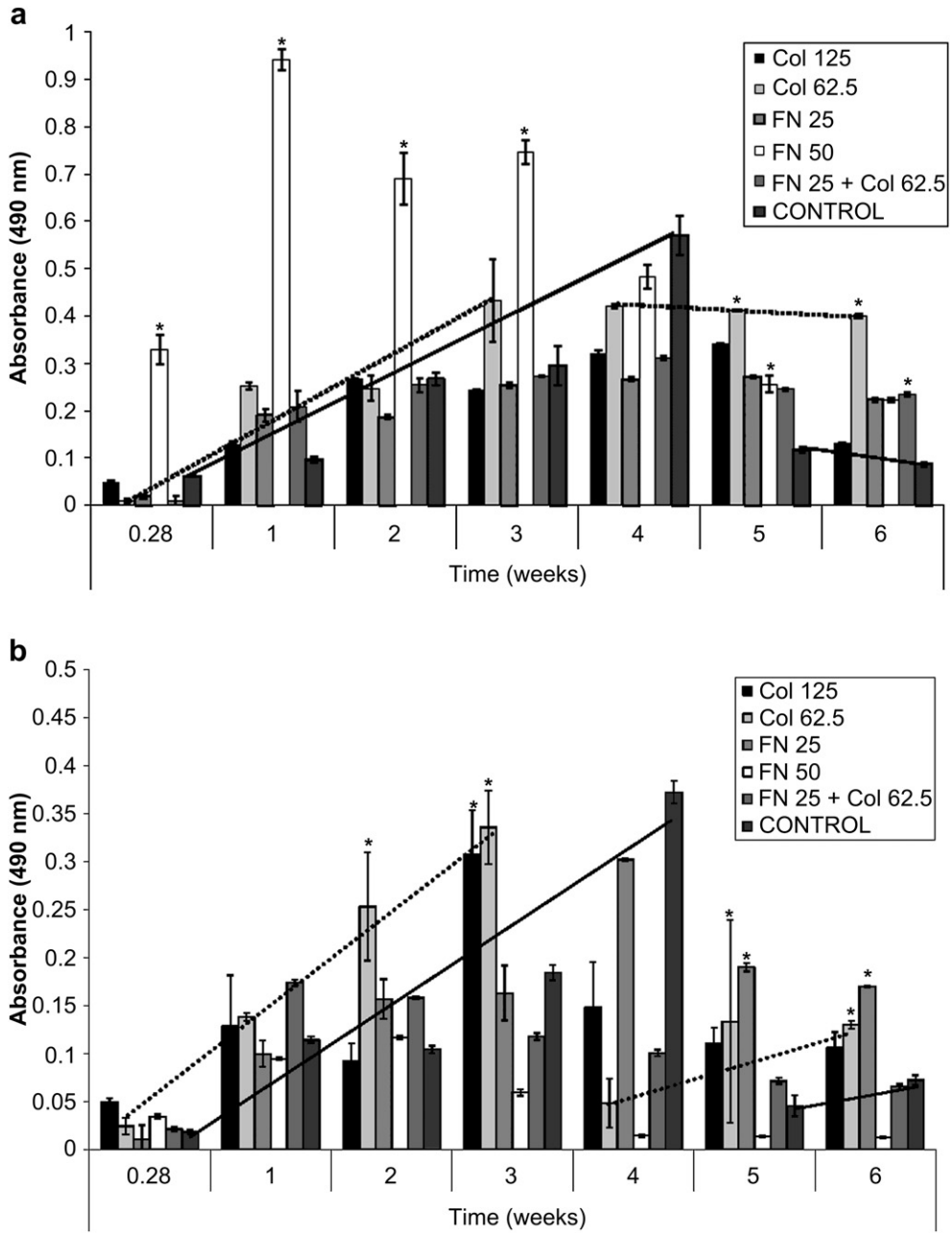

Fig. 4. Cellular proliferation of HL-60 cells on ECM protein-coated PU and PLGA scaffolds. Cell proliferation of the HL-60 cells seeded in PU (a) and PLGA (b) coated with collagen type I and/or fibronectin was assessed. A comparison of the growth trends on the 62.5 μg/ml collagen-coated scaffolds (dotted line) and the uncoated controls (straight line) revealed a significant difference ($p < 0.05$). The cultures were performed in parallel on 2 separate occasions, each in quadruplicates.

PS degraded in less than 2 weeks in culture, and PMMA and PDLLA could not maintain cell growth after one week resulting in cell death (data not shown). Since K-562 has a doubling time of 12 h, three times higher than that of either Kasumi-6 or HL-60 [26], faster growth kinetics, especially over the first 48 h, was observed for both the PU (Fig. 2a) and PLGA (Fig. 2b) scaffolds. Specifically, K562 cellular growth was significantly higher ($p < 0.01$) in both scaffolds throughout the 8-week culture period. K562 culture was established within 1 week after seeding and remained relatively constant for the duration of the 2-month culture time in both scaffolds, albeit with a peak at week 7. In contrast, the Kasumi-6 and HL-60 cell lines exhibited a slower cellular growth. Specifically, AML-cell-line-specific cell growth kinetics was observed that was consistent in both the PU (Fig. 2a) and PLGA (Fig. 2b) scaffolds. For HL-60, two-stage growth kinetics was evident with the first stage peaking at week 4, whereas the slower-growing Kasumi-6 displayed a peak at week 6–7.

3.2. Scaffold coating with ECM proteins

In order to mimic the bone marrow microenvironment and enhance the cell culture, PU and PLGA scaffolds were coated with two of the main bone marrow ECM proteins, collagen type I and fibronectin, evaluated at either high (125 μg/ml for collagen type I and 50 μg/ml for fibronectin) or low concentrations (62.5 μg/ml for collagen type I and 25 μg/ml for fibronectin) [27]. The effect of protein coating of PU and PLGA on the seeding efficiency and cell growth of the AML lines was compared with that on uncoated scaffolds. Coating with ECM proteins did not improve the seeding efficiency of the AML cell lines with the exception of collagen coating of the PLGA scaffold

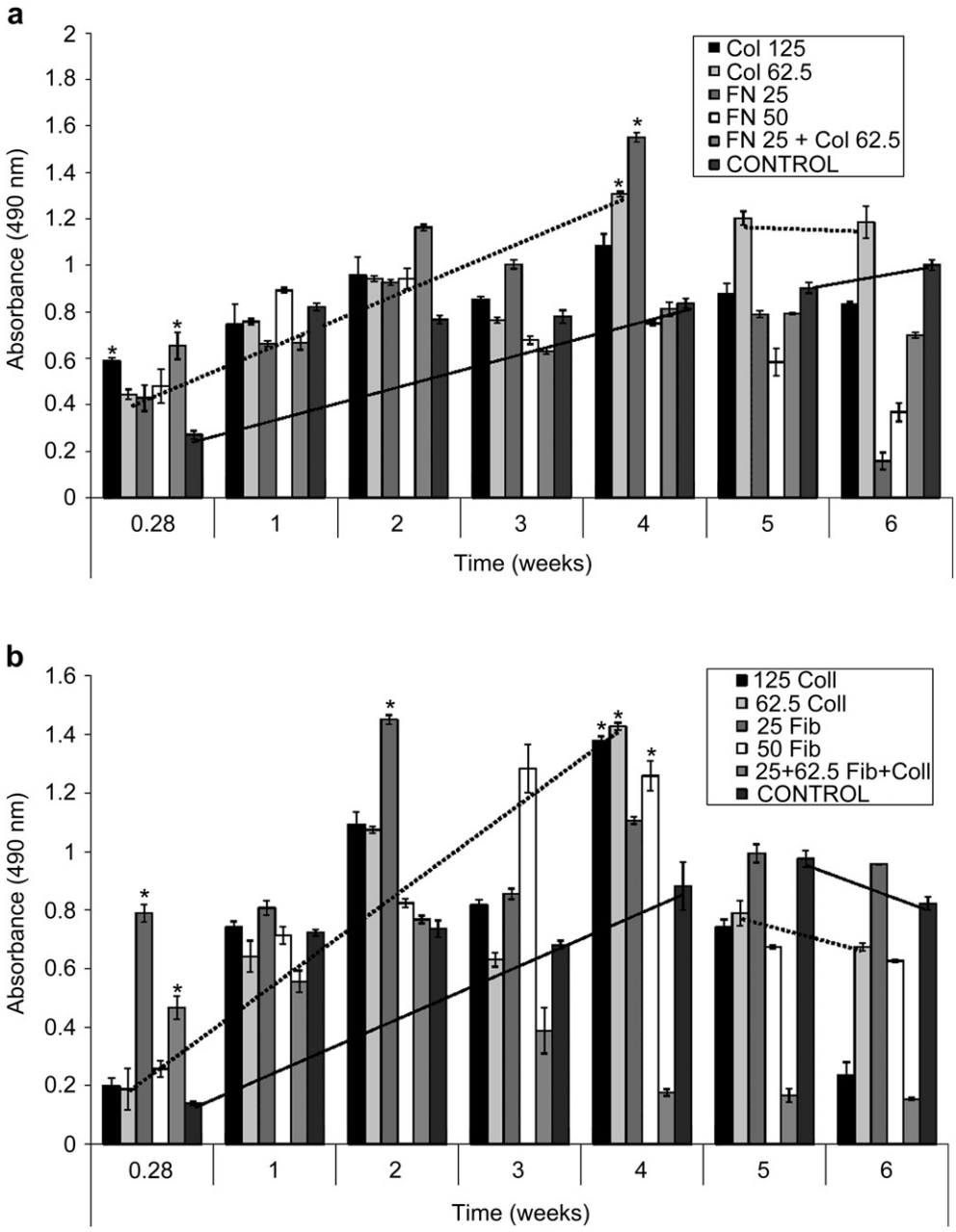

Fig. 5. Cellular proliferation of K562 cells on ECM protein-coated PU and PLGA scaffolds. Cell proliferation of the K562 cells seeded in PU (a) and PLGA (b) coated with collagen type I and/or fibronectin was assessed. A comparison of the growth trends on the 62.5 μg/ml collagen-coated scaffolds (dotted line) and the uncoated controls (straight line) revealed a significant difference ($p < 0.05$). The cultures were performed in parallel on 2 separate occasions, each in triplicates.

for the slow-growing Kasumi-6 line (Fig. 3). However, for all three AML lines, the seeding efficiency was consistently over 50% in the scaffolds coated with collagen type I at the high concentration (125 μg/ml). In contrast, coating the scaffolds with fibronectin, irrespective of concentration (50 or 25 μg/ml), resulted in significantly lower seeding efficiency ($p < 0.05$) when compared with the collagen type I-coated scaffolds at 125 μg/ml (Fig. 3).

Cellular proliferation of the 3 AML cell lines cultivated on ECM-coated PU and PLGA was measured using the MTS assay over a period of 6 weeks. Although different growth kinetics were observed for each AML cell line cultured on the different scaffolds, it was evident that coating of the scaffolds with ECM proteins promoted cell growth when compared with the uncoated scaffolds ($p < 0.05$) (Figs. 4–6). Specifically, HL-60 growth was enhanced by coating PU with fibronectin (50 μg/ml; Fig. 4a) whereas for PLGA coating with 62.5 μg/ml collagen type I represented the best outcome (Fig. 4b). The rapidly growing K-562 cells displayed enhanced cellular growth when cultured on either PU or PLGA scaffolds ($p < 0.05$) coated with 25 μg/ml fibronectin (Fig. 5a and b) compared with that on the uncoated scaffold cultures. Finally, the Kasumi-6 AML cell line appeared to grow better ($p < 0.05$) when cultured on either PU or PLGA scaffolds coated with 62.5 μg/ml of collagen type I (Fig. 6a and b).

3.3. Examination of the 3-D in vitro microenvironment

The 3-D microenvironment provided by the PU and PLGA scaffolds was characterised using SEM at various time points during the culture. Progressively the AML cells, which have cell line-dependent growth kinetics, migrated into the pores of the

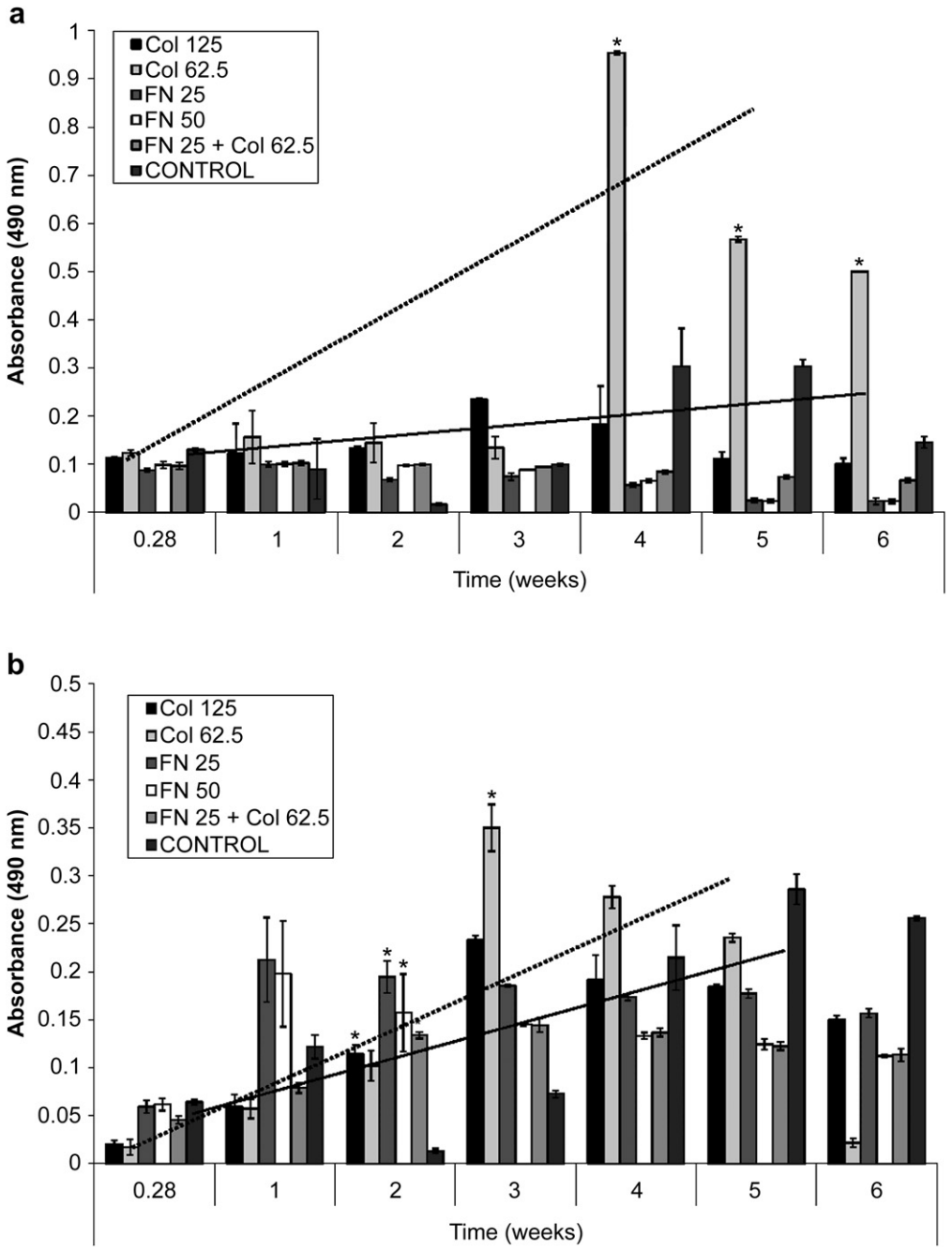

Fig. 6. Cellular proliferation of Kasumi-6 cells on ECM protein-coated PU and PLGA scaffolds. Cell proliferation of the Kasumi-6 cells seeded in PU (a) and PLGA (b) coated with collagen type I and/or fibronectin was assessed. A comparison of the growth trends on the 62.5 μg/ml collagen-coated scaffolds (dotted line) and the uncoated controls (straight line) revealed a significant difference ($p < 0.05$). The cultures were performed in parallel on 2 separate occasions, each in triplicates.

scaffold establishing defined areas of growth, or "niches", as shown in Fig. 7. Cellular density in the pores increased with culture time and by week 4 the internal pores of the scaffolds (mid-section) were populated by AML cells that had adhered to the walls of the pores and had formed cell aggregates, regardless of the AML cell line tested. Consistent with the previous data, scaffolds coated with ECM proteins, especially the PU ones, supported a higher cell growth. Histological examination of representative (mid-section) thin-sections stained with Haematoxylin & Eosin (H&E) showed that coating with ECM proteins appeared to be uniform and penetrate the inner section of the PU and PLGA scaffolds (Fig. 8). As expected, cell aggregates were evident in accordance with the SEM results.

4. Discussion

We have developed a novel *ex vivo* 3-D AML culture system with the use of highly porous scaffolds fabricated from PU and PLGA and coated with collagen and/or fibronectin. Our results indicate that the AML cells grew in 3-D niches simulating the microenvironment that is critical in haematopoiesis [28].

The culture of leukaemic cells *in vitro* is extremely difficult to accomplish with most attempts having failed [29,30]. In 1996 Sutherland et al. developed an optimised cocktail of growth factors that maintained the leukaemic cell culture [31], although it did not promote leukaemic stem cell expansion. To date, the challenge remains and no appropriate culture models of AML exist.

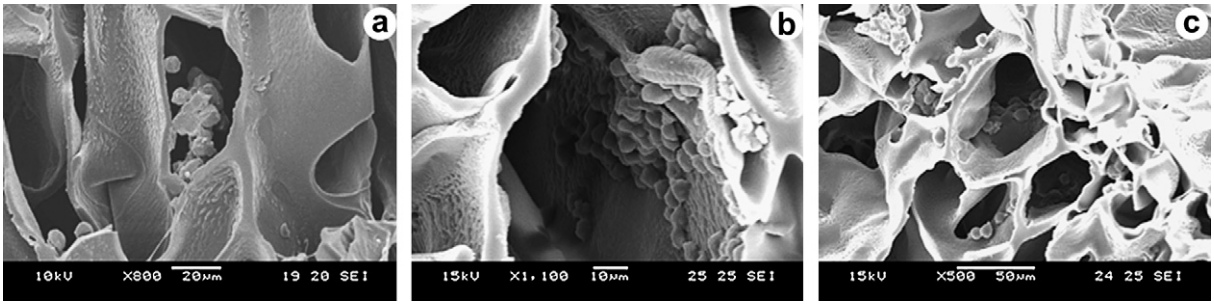

Fig. 7. Scanning electron micrographs of PU scaffolds coated with ECM proteins. Panel (a) shows the PU scaffold coated with 25 μg/ml fibronectin cultured with the Kasumi-6 cells at day 2 of culture. The cells have penetrated the pores of the scaffold and cell aggregates can be seen. Panel (b) shows the PU scaffold coated with 125 μg/ml collagen cultured with the HL-60 cells at week 4 of cultures. Colonies of cells can be seen deep within the pores of the inner section of the scaffold. Panel (c) shows the PU scaffold coated with 50 μg/ml fibronectin cultured with the K-562 cells at week 4 of culture. Cell growth appears extensive with the pores of the scaffold being occupied.

Traditionally, haematopoietic cell cultures have relied on the 2-D growth configuration provided by flasks or well-plates, which do not recapitulate the haematopoietic inductive microenvironment required for supporting the development of multiple cell types at different maturation stages as well as the maintenance of the haematopoietic stem cells. In contrast, ample evidence exists that 3-D culture systems provide a growth environment that promotes cell–cell and cell–matrix contact, recreating the haematopoietic niches, as well as being able to support high cell density; attributes that render 3-D culture systems advantageous [16,32]. Hence, direct comparison between 2-D and 3-D cultures is not appropriate in terms of their growth kinetics and requirements.

Cellular support matrices can be designed to mimic the *in vivo* environment by providing the required architecture, structural properties, as well as the necessary biosignals. Scaffold properties, such as surface area, porosity and pore size, are critical in influencing cell adhesion, spreading, proliferation and differentiation [33]. Several scaffolds have been employed for the culture of stem cells, including the ones evaluated in this work: PLGA has been utilised to expand human chrondrocytes [34], PDLLA for the growth of osteoblasts, chrondrocytes, and lung carcinoma cells [35], PCL for the expansion of dermal fibroblasts [36], PS in tissue culture plastics [37], PMMA for bone remodelling [38], and PU for growth and proliferation of rabbit bone marrow stromal cells [39].

Although the AML cell lines have already been adapted to 2-D cytokine-independent static cultures, their 3-D culture has not been demonstrated. Selecting a scaffold that supports the growth of all three AML lines, would provide a suitable growth environment for the culture of primary AML cells. It became evident that the 6 scaffolds tested provided widely different growth environments to the AML cells due to their differences in physical and chemical properties. Surface hydrophobicity could have caused the low seeding efficiency (less than 20%) observed in PCL and PS, resulting in limited cell proliferation and the early demise of the culture. In contrast, the relatively more hydrophilic PLGA, PDLLA, PMMA and PU, supported a 60% (comparable among them) seeding efficiency. However, only PLGA and PU supported the long-term growth (beyond 4 weeks) of the AML cells; the small pore size of PMMA and the two phase structure of PDLLA were not suitable for long-term culture. The AML cells displayed cell line-specific growth kinetics. K-562, which has a doubling time of 12 h that is three times higher than that of either Kasumi-6 or HL60 [26], established rapidly in the 3-D culture systems generated by PU and PLGA. Even though, PU and PLGA have different properties in terms of porosity (PLGA = 90.1% and PU = 85.6%) and specific pore volume (PLGA = 11.76 cm^3/g and PU = 12.31 cm^3/g) no difference was observed between the two scaffolds for the fast-growing K562 cells suggesting that the scaffolds did not present any mass transport limitations for 3-D leukaemia cultures. In general, the higher the porosity the higher the cellular growth. Other architectural differences between PU and PLGA include the fact that PU has homogenously sized pores whereas PLGA displays a combination of micro- ($\leq$50 μm) and macro-pores ($\leq$300 μm). It was observed, primarily, that in the PLGA scaffolds only the macro-pores were accommodating the AML cells and fostering the formation of cell aggregates and niches. In this respect, the PU scaffolds were considered advantageous for use in developing an *ex vivo* model of leukaemia.

A clear difference between the PU and PLGA scaffolds was observed with the slow-growing HL-60 cells where PU was superior. This difference can be attributed to the surface properties of the PU and PLGA scaffolds and in particular, hydrophobicity, which does affect cell growth. PLGA is more hydrophobic than PU [40] and therefore possesses high interfacial free energy in aqueous solutions that tends to unfavourably influence cells and tissues during the initial stages of contact. The cells adhere and grow better on hydrophilic scaffolds, such as PU [41]. To overcome the challenge of hydrophobic surfaces and to provide biorecognition signals on the surface of support matrices, coating scaffolds with ECM proteins has been employed, which has been demonstrated to improve cell

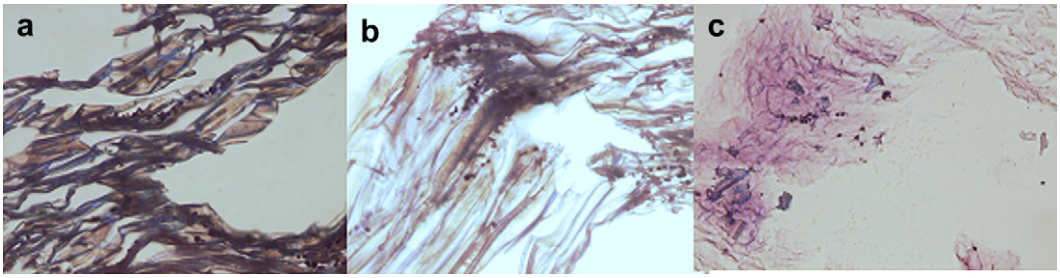

Fig. 8. Histochemical (H&E) staining of PU and PLGA scaffold coated with ECM proteins. Thin-sections were obtained from the mid-section of the scaffolds. Panels (a–b) show the PU scaffold coated with 62.5 μg/ml collagen and cultured with HL-60 cells at week 2 week of culture. Panel (c) shows the PLGA scaffold coated with 125 μg/ml of collagen type I and cultured with the K-562 cells at day 2 of culture. The circles identify areas of cell growth.

expansion. This was in agreement with our results, which showed that coating with collagen, primarily, or fibronectin was beneficial in an AML cell type-specific manner. Collagen can accelerate cell adhesion when compared with other ECM proteins [42] and may explain the superiority of the collagen-coated scaffolds with the slower-growing cell lines in particular. Interestingly, 2-D culture of haematopoietic progenitor cells coated with fibronectin enhanced preservation of the normal progenitors but not abnormal chronic myelogenous leukaemia cells (CML) in the same culture [43]. Therefore, creating an *ex vivo* model of heterogeneous systems, such as AML, requires a new paradigm that revolves around the generation of multiple niches that can accommodate the different cell types at different maturational stages. This model would include multiple biosignals involved in recognition, cell attachment, cell growth and differentiation that would be spatially and, potentially temporally, distributed.

5. Conclusion

We have shown that a highly porous scaffold (PU and PLGA), similar to that defined by the bone marrow architecture, combined with bone marrow-specific ECM proteins (collagen type I and fibronectin) can provide a 3-D structure for sustainable *in vitro* cultivation of AML cells lines for at least 8 weeks and provides a platform for the study of primary AML. On balance, PU coated with collagen type I at a concentration of 62.5 μg/ml sustained the growth of all AML cell lines tested *in vitro* well, although each cell line preferentially grew with different combinations and concentrations of collagen, fibronectin and scaffold type in 3-D at different phases of the cultures. These microenvironmental differences may provide specific insight, in that both cell–matrix and cell–cell interactions can be interrogated in this novel experimental system *ex vivo*, in order to investigate the pathogenesis of disease and to explore novel treatments for leukaemia.

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Author disclosure statement

No competing financial interests exist.

Appendix

Figures with essential colour discrimination. Fig. 8 of this article is difficult to interpret in black and white. The full colour images can be found in the online version, at doi:10.1016/j.biomaterials.2009.11.094.

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