Design of a Tetrapolar Probe for Electrical Characterization of the Left Atrial Appendage from 0.1 Hz to 100 kHz

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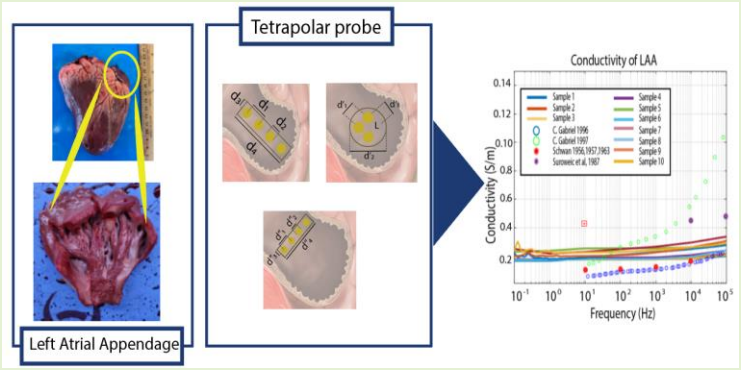
***Abstract*—Recently, non-thermal pulsed-field-ablation using electroporation has generated a lot of interest as a potential treatment for Atrial Fibrillation. The electrical properties, and specially the conductivity of cardiac tissues, are used in the in treatment planning of non-thermal pulsed field ablation. However, there is no standard approach to measure the conductivity, particularly for the left atrial appendage (LAA). Electrical conductivity characterization studies, focusing on cardiac tissues, have used different probe typologies with different electrodes sizes. Furthermore, no study has investigated the effect of the probe design on the acquired conductivity. In this study, common leading probe typologies with different electrode size are compared in terms of accuracy and measurement repeatability. Using liquid phantoms, differences in term of measurement accuracy and repeatability are observed between the probe designs which suggests that the measurement probe design influences the data acquisition quality.**



Based on these results, a custom probe design is proposed suitable for the characterization of the conductivity of the LAA. The probe is tested using ten *ex vivo* bovine tissue samples between 0.1 Hz and 100 kHz. The mean conductivity of the LAA acquired from the ten samples is 2.5 mS/cm with a standard deviation of 0.24 mS/cm which is in line with conductivity of cardiac tissues in the literature. The conductivity values of the left atrial appendage may be useful in electroporation treatment planning, furthermore this study suggests that adapting the probe design to the tissue under study can be important.

***Index Terms*— Tetrapolar probe, conductivity, atrial fibrillation, biological tissue impedance, electroporation, irreversible electroporation, Left atrial appendage, non-thermal pulsed-field ablation.**

I. Introduction

ATRIAL fibrillation (AF) is a cardiac arrhythmia where the normal electrical activity directed by the sinus node is not synchronized with the different impulses firing from other locations in the atria as intermittent or persistent patterns. The left atrial appendage (LAA) has been identified as a frequent source of these aberrant impulses [1], [2]. The result is a disordered heartbeat, primarily in the atria and disruption in blood flow from the atria to the ventricles. The atrioventricular node controls aberrant impulse propagation to an extent and so the activity of the ventricles is typically less affected. AF is the most frequent cardiac arrhythmia that leads to blood clots, stroke, heart failure and other heart-related complications [3]. AF is growing in prevalence globally and is an ever-increasing health challenge. In 2017, over 403 person per million worldwide were diagnosed with AF and predictions estimate that between 6–12 million people will suffer from AF in the US by 2050 and 17.9 million people in Europe by 2060 [4], [5].

These epidemiological predictions on the expected substantial rise in AF cases have led to a lot of interest and investigations into new treatments for the condition. The current methods used to treat AF are pharmacological, cardioversion, catheter-based management, and surgical [6]. Pharmacological options are frequently the first line: targeting restoration of normal heart rhythm and control of the heart

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rate. Concurrently, patients may be put on agents, such as anticoagulants, to reduce the risk of secondary conditions, such as stroke. In some cases, other options may be needed to control AF with cardioversion (controlled electric shock to restore rhythm), catheter treatments (including ablation), and surgery (ranging from pacemaker implantation to Cox-Maze procedure).

Recently non-thermal pulsed-field ablation using electroporation has generated a lot of interest as a potential treatment for AF. Multiple feasibility studies using monophasic or biphasic waveforms have been presented, typically using energy sources that disrupt the cell membrane [7]. This disruption leads to an ablation of the LAA cells [8], [9].

The field of electroporation has been an area of research since about 1950 [10], and has recently received increased attention as a modality for the manipulation of tissue cells [2], [11]–[13]. Electroporation induces changes in the nature of cell membrane, forming pores in the membrane due to the applied, external electric field (electropermeabilization). These changes in membrane permeability can be reversible or irreversible. Reversible electroporation consists of applying a controlled electric field in which the pores are transient and membrane integrity is restored after a period of time depending on the cell type. Irreversible electroporation (IRE) consists of applying a controlled electric field which permanently disrupts the cell membrane and causes cell death [10]. The use of IRE in atrial tissue can result in a curative treatment for AF as the causative electrically aberrant atrial tissue is ablated [14].

The electrical properties, specifically conductivity of the LAA are used for treatment planning of IRE [14]. The electrical conductivity of tissues is a common input for biomedical device design and treatment planning, it can be seen in diverse applications including electrical impedance tomography [10], [15], human blood count monitoring [16], neuron-stimulation systems [2], [12], [13], devices for obstetric applications which compare the electrical impedance of the gravid and non-gravid uterus and cervix [14], [15], and in applications related to oncology comparing normal to cancerous tissues [3], [4], [9], [16]–[19].

Typically, the electrical characterization studies of biological tissues use the 4-electrode method to acquire the conductivity of tissues. The 4-electrode method is used as the measured impedance is less influenced by polarization or charging currents at electrode interface if compared to the 2- or 3-electrode methods. As a result, 4-electrode probes have a larger measurement range compared to other configurations [20]. A variety of probe typologies in terms of electrode configuration and the electrode size have been used in electrical characterization studies of cardiac tissues [21]–[24]. However, no study has investigated if the diverse range of probes used in the literature affects the acquired conductivity. Furthermore, no study has proposed a standard probe design for the electrical characterization of cardiac tissues, especially LAA.

Hence, the aim of this paper is to assess the influence of the probe typology and the electrode size on the accuracy and the repeatability of electrical conductivity measurements. The measurement accuracy and repeatability of commonly used probe designs from the literature is assessed in liquid phantoms in the frequency range between 0.1 Hz and 100 kHz. This frequency range is examined in this work, as electrical currents below 100 kHz typically flow in the extracellular spaces: the charges do not penetrate the cell membrane but flow around the cells. By contrast, higher frequency current, above 100 kHz, [18], [19], [25]. Based on the results of the accuracy and repeatability study, the most accurate probe from the proposed probes with the best repeatability is proposed to be used to characterize the LAA conductivity.

The remainder of this paper is structured as follows: Section II describes the background of the paper regarding the probe design and the literature review on the characterization of the electrical conductivity of cardiac tissues. Section III is dedicated to the methodology including the electrical conductivity acquisition process and rationale for the probe design. Section III also includes the description of the sample acquisition, the dissection of LAA and the measurement process.

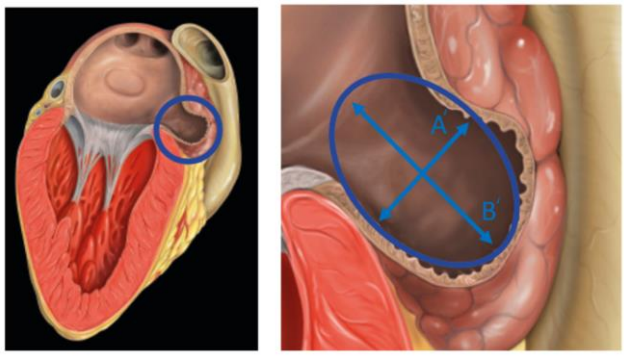
The results are presented in Section IV. The influence of different typologies and the size of the electrodes on the accuracy and repeatability of the data acquired is examined. Furthermore, the conductivity values of the LAA from ten *ex vivo* bovine samples acquired by the proposed probe are presented. Finally, Section V concludes the work.

II. Background

A. Background on cardiac tissue measurement

Cinca *et al.* [20] in 1997 characterized cardiac tissues by monitoring the myocardial electrical impedance induced by coronary artery occlusion on 13 *in vivo* porcine samples. The study used a probe consisting of four platinum electrodes (5 mm long, 0.4 mm in diameter), mounted as a linear array on an insulating substrate separated by an interelectrode distance of 2.5 mm. The study used a tetrapolar probe, as the electrode polarization affects tissue measurements to a lesser extent than when a bipolar probe is used.

A similar study was reported by Ellenby *et al.* [21] in 1987 to detect reversible myocardial ischemic injury through the measurement of myocardial electrical impedance in the *ex vivo* left ventricle acquired from 12 dogs. The study uses four electrode gold-plated brass pins, 3.125 mm in length and 0.625 mm in diameter. The four-electrode method was used to keep the current induction (outer electrodes) and the voltage monitoring (inner electrodes) separate. Local ambient electrical activity of the myocardium was monitored by turning off the injected current while using the amplifier and filter components to record bipolar electrograms between the inner two voltage-sensing electrodes. This would not be possible with another impedance measuring technique (two or three electrode method).



(a) (b)

Fig. 1. Transesophageal echocardiography view illustration of (a) Heart and (b) LAA [26]. The elliptical topology of the LAA is shown and can be defined by a minor axis (A') and major axis (B').

Gabriel *et al*. [27] in 2009 investigated the electrical conductivity of multiple biological tissues, including the heart (atrial) from *in vivo* porcine, updating the previous data reported by Gabriel et al. in 1996 [28] and focusing on conductivities at frequencies below 1 MHz. The study uses linear four terminal probe design with specification for the tissue, and a rectilinear array of four platinum-blacked platinum pin electrodes embedded in synthetic fluoropolymer of tetrafluoroethylene for blood.

Hahn *et al*. [24] in 1980 researched the electrical conductivity of porcine cardiac tissues to assess heat transfer problems associated with heating by ultrasound, microwaves, or radio frequency. The assessment of the electrical conductivity was done using a nine-needle electrode probe (eight electrode in a circle centered around the ninth probe). The outer eight needles form a grounded coaxial guard with respect to the inner active electrode, so the measurement was using the 2-electrode method to acquire the complex frequency dependent impedance. The outer eight needles were 1.5 cm long, while the inner needle was 1.0 cm long. The needles were chosen to be able to penetrate the multilayer tissues and monitor the diffusion of the heat inside the multilayer tissue.

The background literature displays a range of cardiac tissue conductivity which varies from 0.5 mS/cm to 9 mS/cm. The conductivity range varies from study to study and that can be explained by the different origin of the samples (*ex vivo* or *in vivo*, different animal source or human) and the difference in tissue samples (measurement in different part of the heart). However, focusing only on the *ex vivo* animal studies, the conductivity averages at 3 ± 0.5 mS/cm in the frequency range from 10 Hz to 100 kHz.

TABLE I
SUMMARY OF THE TETRAPOLAR PROBE USED TO CHARACTERIZE BIOLOGICAL TISSUE WITH THEIR RESPECTIVE SIZE. THE TWO MOST COMMON PROBES ARE THE COLLINEAR PROBE AND THE POLAR PROBE.

Ref	Typology	Electrode size	Tissue type
Cinca et al. [21]	Colinear	5 x 0.4 mm^2	coronary artery (porcine)
Ellenby et al. [22]	colinear	3.1 x 0.6 mm^2	left ventricle (canine)
Gabriel et al. [27]	Colinear	-	Atrial (porcine)
Hahn et al.[24]	9 needles	0.35 mm radius	Cardiac muscle (porcine)
Jokhi R. et al. [29]	Polar	0.6 mm radius	Cervix (human)
B. Filho [30]	Polar	1 mm radius	Forearm Skin (human)

B. Background of probe design for electrical tissue characterization

Several probe typologies have been proposed to investigate the electrical conductivity of tissue as seen in Table. I. In 1800s, the bipolar probe was implemented [30], this probe design consists of a pair of electrodes that inject the current across them, with the resulting voltage measured by the same electrode pair [31].

The three-electrode probe uses two electrodes to induce current and two electrode to measure voltage with one electrode shared between injecting and measuring electrodes. The third common electrode lowers electrode polarization. [28], [32].

The tetrapolar probe (introduced by Bouty in 1884 as stated in Campbell 1984 [33]) consists of four electrodes: one pair injects the stimulus current, and the other pair measures the resulting voltage [34]. The tetrapolar probe has been used in many studies and in multiple typologies, for example, a polar probe (electrodes in a square arrangement) was used to characterize biological tissue in [29], while a four concentric electrodes probe was used to identify skin cancer in [36], [37] and a collinear probe was used to distinguish normal and cancerous human hepatic tissue in [31].

The tetrapolar probe is less susceptible to electrode polarization or charging currents at electrode interface when compared to two or three electrode method. As a result, the probes proposed for this work are all tetrapolar probes [19]. Furthermore, from comparing the probes in literature used on tissue the two most common probes typologies operated to be the collinear probe (four electrodes in line) and the polar probe (four electrodes in a square configuration).

This paper compares the most common tetrapolar probe typologies (the collinear and polar typology) regarding the accuracy and repeatability. Furthermore, the effect of electrode size on the repeatability and accuracy is assessed, comparison between the typologies and the different electrode sizes lines up with aim of the paper which is to investigate the effect of the different probe design of the conductivity of tissues. Based on the results of the accuracy and the repeatability a custom probe is proposed to characterize the LAA.

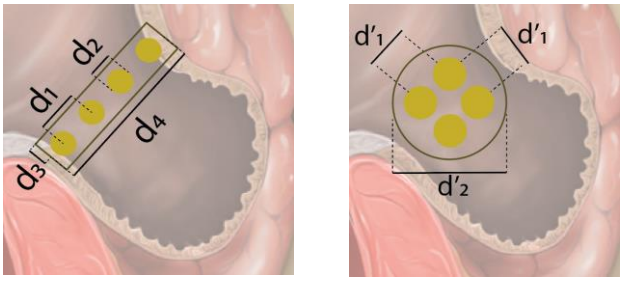
III. Methodology

A. Probe description

To assess the effect of the typology on the acquired conductivity, the collinear and polar probe were proposed. The proposed typologies have the same electrode size and the same electrode spacing of 10 mm for a fair comparison.

The probe footprint is mainly driven by the electrode size and the electrode spacing. In addition to limitations of the measuring area in the LAA, the collinear probe and the polar probe are designed to fit in opening of the LAA where the conductivity is desired. The probes are shown overlaid with the LAA respectively in Fig. 3(a) and Fig. 3(b) to provide a visual representation of the size of probes with the LAA. With $d_1 = 10$ mm, $d_2 = 6$ mm $d_3 = 4$ mm, $d_4 = 40$ mm; $d'_1 = 10$ mm, $d'_2 = 25$ mm; Fig. 4 shows the manufacture prototypes of the collinear probe and polar probe used during the experiments.

The electrodes are fabricated from pure copper and have a thickness of 0.5 mm. Copper electrodes were used as they are low-cost, robust and have suitable conductivity. During the manufacture, a 3D printed resin holder was designed to ensure that the electrodes were mounted at the same level, and to keep constant spacing between the electrodes. In addition, the holder was designed to attach to a retort stand to maintain stability of probe in the set up.



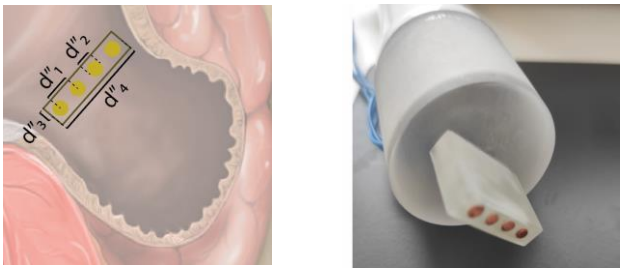
(a) (b)

Fig. 2. Diagram showing the dimensions of the tow proposed typologies of tetrapolar probe used in this paper (a) collinear probe (b) polar probe. The illustration shows the footprint of the probes on the surface of the LAA.

(a) (b)

Fig. 3. Photos of the manufactured prototypes of the proposed tetrapolar probes (a) the collinear probe (b) polar probe.

To assess the effect of the electrode size on the measurement accuracy and repeatability, the electrode size of the collinear probe was miniaturized. Due to the limitations of the measuring area in the LAA, a small probe footprint was desired for the miniaturization. Knowing that the probe footprint is mainly driven by the electrode size and the electrode spacing. Electrodes smaller than 1 mm diameter can introduce undesired effects such as non-linearities, increased electrode impedance and increased electrode polarization. The electrode polarization introduced by the electrodes smaller than 1 mm can introduce parasitic effect that are as large as the impedance of the sample under study [29], [32], [33]. Therefore, the proposed miniaturized collinear probe uses electrodes of 1.5 mm radius spaced 5 mm apart (the collinear probe has been miniaturized by 50%). The total footprint of the proposed probe is 20 mm by 4 mm. The miniaturized collinear probe is shown in Fig. 5(b), and a schematic representation overlaid on the LAA in Fig. 5(a), where the probe characteristics are d''_1 = 5 mm, d''_2 = 3 mm, d''_3 = 2mm, d''_4 =20 mm.



(a) (b)

Fig. 5. (a) Diagram showing the dimensions of the proposed collinear probe with small electrodes. The illustration shows the footprint of the probe on the surface of the LAA, (b) Photo of the manufactured prototype of the proposed collinear probe with small electrodes.

B. Data acquisition

The acquisition of the conductivity passed from acquiring the frequency-dependent complex impedance and it was performed using the methods described in [18], [34], [35] using a PGSTAT204 (Autolab, Kanaalweg Den haag, The Netherlands) in galvanostat mode at room temperature with Nova 2.1 software. The galvanostatic mode is used with a 100 µA current flowing between the inducing electrodes. The measurement setup consists of the PGSTAT204 connected to the proposed probe (i.e., working electrode, counter electrode, reference electrode and sensing electrode) and the PGSTAT204 connected to a computer that uses the Nova 2.1 to control the PGSTAT204. The full set up used is shown in Fig. 6.

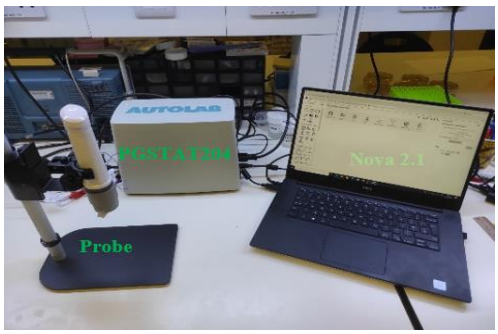


Fig. 6. Measurement setup with the probe, the laptop running NOVA 2.1 software in the foreground. PGSTAT204 potentiostat/galvanostat and the probe holder used to lift and keep the probe fixed.

The measurement accuracy and repeatability are quantified using the average error, the coefficient of variation (CV) and standard deviations (SD). The measurement accuracy is defined by the average error, which is the difference between the measured NaCl solutions conductivity and the reported conductivity in the literature [36]. To compute the conductivity the two-step method from [16] is used. The CV represents the ratio of the standard deviation to the mean, the lower the ratio of the standard deviation to mean return the better the acquired data is considered.

The first step of computing the conductivity is determining the cell constant (k), k is the factor that relates measured conductance and the corresponding reference conductivity. The k for the proposed collinear probe is 0.066 m, 0.039 m for the polar probe and 0.032 m for the collinear probe with small

electrodes for the frequency range from 0.1 Hz to 100 kHz (with the Pearson correlation coefficient R between the measured conductance and the reference conductivity of 0.95 for the collinear probe, 0.85 for the polar probe and 0.99 for the collinear probe with small electrodes). The second step is calculating the electrical conductivity from the measured impedance data and the cell constant using (1) [14].

$$k = \frac{G}{\sigma} \tag{1}$$

where σ is electrical conductivity of the standard liquids and G is the measured conductance of the probe in the targeted frequency range (conductance derived from impedance).

The targeted samples of interest are biological tissue samples from the heart, specifically the LAA. These samples are semisolid with a high water content. Biological samples themselves are variable and the properties are subject to change due to environmental factors, contact force and other confounders. Hence, to compare the probes, a repeatable, reliable test platform was desired with known and understood electrical properties and minimal confounding factors. To the authors' knowledge, there are no standard semisolid phantoms with known electrical properties in the expected range.

Therefore, the characterization of the proposed probes in this work used standard liquid phantoms. The chosen phantoms are commonly used as standards for electrical characterization, the properties of the liquid can be tuned to the correct, expected range for the samples of interest, standard protocols for achieving consistent and reliable results exist, probe contact can be visually verified, the samples are homogeneous and easy to make accurately.

In particular, ionic aqueous solutions (NaCl solutions) have no dielectric dispersions at frequencies below 1 MHz [37]. The acquired conductivity is frequency independent and only varies with the concentrations of the ionic aqueous solutions (NaCl solutions). Furthermore, the electrical properties of ionic aqueous solutions (NaCl solutions) are known and standardized [36].

Specifically, for this study, 0.01 M, 0.05 M and 0.1 M of NaCl solutions at room temperature (21 ± 0.5 °C) are used to characterize the probes (k) and analyze the accuracy and repeatability of the probes. The accuracy of the probe was investigated with measurements taken over the 0.1 Hz to 100 kHz band with 10 frequency points per decade so 60 points per measurement. The total number of measurements taken for the accuracy test is 15 measurements, 5 measurements for each NaCl concentration solution. The measurement on the NaCl phantoms were conducted in a cylindrical container of a diameter of 9 cm and the container was filled with a 1.5 cm depth liquid to match the depth of the LAA.

The repeatability is investigated for the probes in regard to the total 15 measurements. The SD and the CV is calculated using (2) and (3) [38]:

$$SD = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}|X_i - \mu|^2} \tag{2}$$

$$\mathrm{CV} = 100\frac{\mathrm{SD}}{\mu} \tag{3}$$

With μ is the mean, N is the total number of measurements, and X_i are the measurement values. These accuracy and repeatability measures are used to identify the custom probe used to characterize the LAA.

C. Methodology of LAA measurement

This section describes the acquisition process in terms of the tissue handling and measurement set-up. The animal tissue was obtained from the local slaughterhouse immediately after excision and transported to the Translational Medical Device Lab, Galway, Ireland in vacuum sealed containers. The samples arrived at the laboratories within two hours from excision. The total number of samples was ten heart bovine samples. The ten hearts were embedded in their fat capsule to limit the dehydration of the tissue. The LAA was dissected from the heart and the internal membrane was removed to have measurement directly on the LAA pectinate muscle. The position of the measurement was chosen to be on the endocardium of the LAA where the electroporation treatment usually occurs.

The temperature was monitored during all experiments, the initial and final temperature for all samples was stable at room temperature 20 °C ± 0.5 °C.

The dissected LAA from sample one and the position of the measurement can be seen in Fig. 7. Each sample was measured three times in the position shown in Fig. 7. The pressure applied by the probe was 2 N ± 0.5 N to ensure a firm contact while avoiding the tissue moving (sliding) due to excess pressure [29].

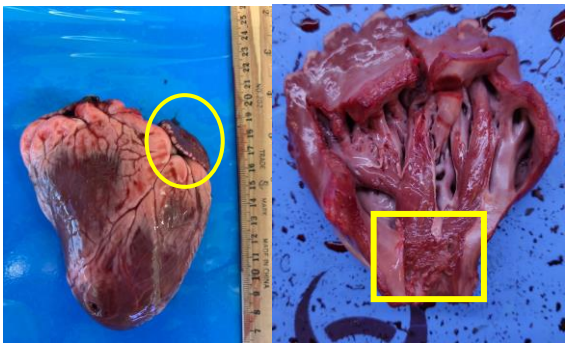


Fig. 7. Left picture shows the first sample bovine heart with the LAA highlighted. The right picture shows the dissected LAA from sample one. The yellow square shows the position of the LAA pectinate muscle where the measurements were taken.

IV. Results

A. Effect of the typology of the probe and electrode size on the data acquired

The measured conductivity values from the three NaCl solution 0.01 M, 0.05 M and 0.1 M phantoms were used to assess the accuracy of the probes. Table II displays the average conductivity plus the SD of the measurements with each probe between 0.1 Hz and 100 kHz with the reference conductivity values from [34]. Variance in the acquired conductivity is primarily due the probe characteristics, as the absolute magnitude of drift error of the setup is less than +2%

from the pre- and post-validation measurement for all experiments.

To assess the effect of probe typology on the acquired conductivity we compare the accuracy and repeatability of the colinear probe and the polar probe. The conductivity values acquired with the polar probe shows a maximum difference of 32% from the reported values in literature [36]. The collinear probes correlate with literature [36] as it has a maximum difference of 12%. From the data acquired from the polar probe and the collinear probe, we observe that the accuracy is significant different, and the collinear probes provides a better accuracy in the NaCl phantoms from 0.1 Hz to 100 kHz.

TABLE II
AVERAGE CONDUCTIVITY PLUS SD OF NACL PHANTOM ACQUIRED WITH THE PROPOSED PROBES WITH THE REFERENCE CONDUCTIVITY VALUES

NaCl (M)	Col. σ ± SD (S/m)	Polar σ ± SD (S/m)	Small Col. σ ± SD (S/m)	Ref. [36] σ (S/m)
0.01	0.096 ±0.003	0.079 ±0.009	0.121 ± 0.01	0.11
0.05	0.45 ±0.02	0.33 ± 0.05	0.483 ± 0.02	0.49
0.1	0.97 ± 0.01	0.94 ± 0.07	1.02 ± 0.03	0.96

CV is used to estimate the repeatability of the data acquired during the measurement and the results from all proposed probes are shown in Fig. 8. The CV is displayed as a percentage and are considered to be low (CV ≈ 0% represent better results). All proposed probes report a CV below 5% for across 0.1 Hz to 100 kHz, implying that there was low variability between measurements. Thus, the measurements are repeatable.

To assess the effect of the typology on the conductivity acquired. The CV of the polar probe and collinear probe both are compared, both probes show a good CV (<5% and <1% respectively); however, it is difficult to assess if the difference means that collinear probe provides more repeatable data than the polar probe as the difference is insignificant to conclude.

Using similar analysis to assess the effect of electrode size on the accuracy. We compare the difference of performances of the collinear probe and the collinear probe with small electrodes. We can see that the collinear probe with small electrodes provides an accuracy of 10% and the collinear probe has 12% accuracy from 0.1 Hz to 100 kHz. The slight variation +2% of the accuracy can be explained by the electrode size and the electric filed between the measuring electrodes, as the collinear probe consists of considerably larger electrodes, introducing more electrode impedance and small spacing ratio between electrodes that induces higher electrode polarization [39].

In similar way, to assess the effect of electrode size on the conductivity acquired, we compare the CV of the collinear probe and the collinear probe with small electrodes. The collinear probe and the collinear probe with small electrodes show a good CV less than 1%, and similarly to the comparison of the collinear probe to the polar probe, it is difficult to draw conclusion on the effect of the electrode size on repeatability in this case as the difference is insignificant.

The effect of electrode typology can is observed from simulation in Fig. 8. The simulation focused on investigating the electric field distribution at point *A*: in the center of the inducing electrodes (x=0, y=0) and point *B*: 10 mm from the center on the y axes (x=0, y= 10)).

From the simulation the accuracy of the probes corelate negatively with electric field distribution between the measuring electrodes. The small colinear probe displays the best accuracy from all the proposed probes (6.02V/m), followed by the collinear probe (1.29V/m) then the polar probe (1.19V/m).

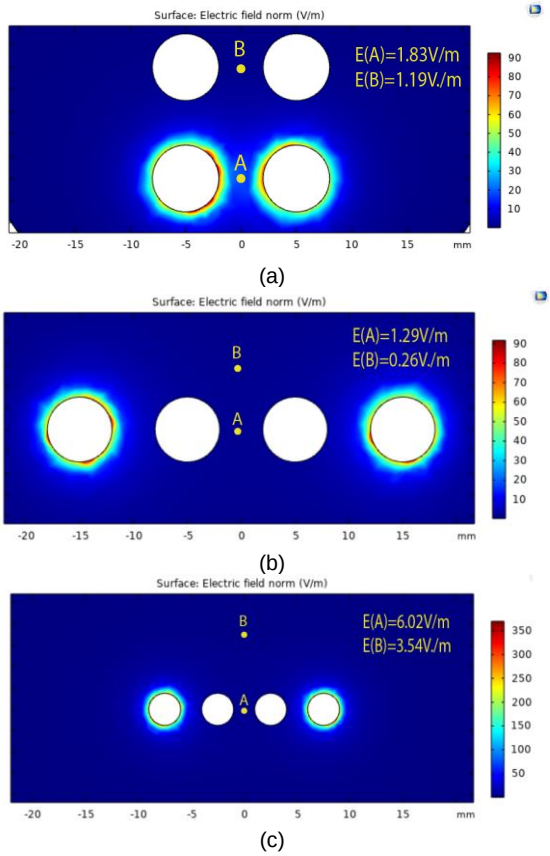


Fig . 8. The electric field distribution by the proposed probes focusing on point A and B. (a) filed distribution of the polar probe (V/m). (b) electric field distribution of the collinear probe (V/m). (c) electric field distribution of the small collinear probe (V/m).

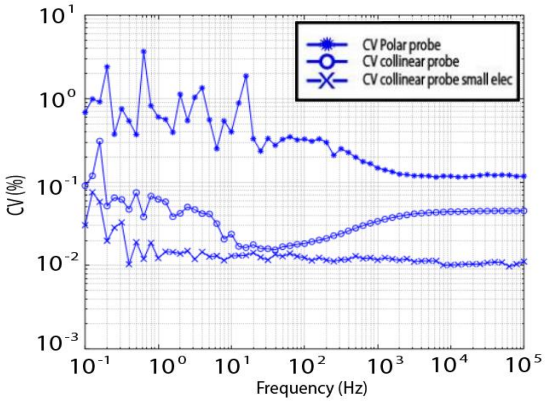


Fig. 9. Coefficient of variation (CV) of the polar probe, collinear probe and the collinear probe with small electrodes. CV shown in the totality targeted frequency range from .1 Hz to 100 kHz.

From Fig. 9, CV is below 5% for all the different typologies with the different electrode sizes, which suggest that the typology and the electrode size proposed has limited influence

on the repeatability of the data acquisition in this case. However, the absolute values of the conductivity acquired from the NaCl phantoms from each probe is different (10% to 32%) suggesting that the electrode size and typologies of the probe affect the data acquisition.

The superiority of the small electrode probe over the large electrode on the basis of 2% improvement in accuracy (from 12% to 10%), a value which is likely smaller than the measurement error can be highlighted. However, if taking in consideration *R* which represents the relationship between the measured data and the reference data from the literature. *R* measures from the collinear probe with small electrodes (0.99) is seen stronger than R from the collinear probe (0.95), the closer the value of *R* is to 1 the stronger the correlation is. Therefore, if taking in consideration the accuracy of 10%, the highest *R* of 0.99, CV of 1% the small collinear probe has the best performances. Thus, it was further tested to acquire the conductivity of the LAA. The probe was tested using ten *ex vivo* bovine LAA tissue samples from 0.1 Hz to 100 kHz.

B. Assessing the conductivity of the LAA using the collinear probe with mall electrodes.

Measurement of electrical conductivity of the bovine LAA were performed with a 4-electrode probe method. The conductivity was computed from the electrical complex impedance acquired using a PGSTAT204 in galvanostatic mode) in combination with the proposed collinear probe with small electrodes probe describe in Section III.

The measured complex electrical impedance was used to calculate the electrical conductivity of the ten LAA samples. To further validate the measurement set up, three pre and post validation measurements with 0.15M NaCl solution and were acquired to show any drift in the measurement set up.

The results from the LAA measurement were compared to the conductivity of cardiac tissue from the literature. The literature displays a wide range of conductivity of cardiac tissue as discussed in Section II. The conductivity of the cardiac tissue form literature ranges from 0.5 mS/cm to 9 mS/cm. The variation of the conductivity of the cardiac tissues can be explained by the origin of the tissue (*ex vivo* or *in vivo*, different animal source or human) and the difference in tissue samples (measurement in different parts of the heart) [23], [24], [27], [40]–[45]. The average of the three measurements of each sample is shown in Fig. 10, with the reference values from literature from conductivity of cardiac tissue. The computed conductivity from the ten bovine samples is 2.5 mS/cm with a SD of 0.24 mS/cm.

Furthermore, the drift from the pre and post validation measurement show a characteristic zero drift and sensitivity drift that averages at 2.5%. The computed conductivity from the LAA is observed to be in line with the literature conductivity range.

The results from the NaCl phantoms and the LAA suggest that not adapting the probe design to the tissue under study can impair the data acquisition from the electrical (different accuracies for different probes).

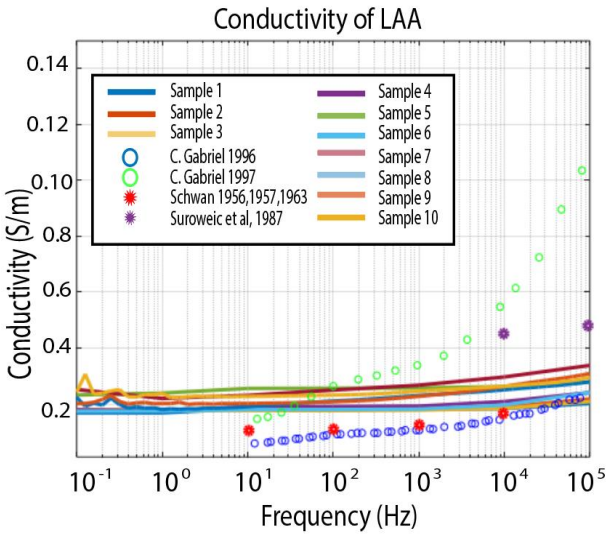


Fig. 10. Conductivity acquired from ten bovine LAA samples at room temperature 20 ºC and the reference cardiac conductivity.

V. Conclusion

Non-thermal pulsed field ablation is a new treatment proposed for treating AF. The treatment planning relies on the electrical conductivity of the LAA to provide the treatment usefully and safely. However, there is no standard approach in terms of probe design for measuring the impedance of cardiac tissues and specially the LAA. The lack of standard can be observed from the diverse typologies and different probe/electrodes sizes in previous studies to characterize the electrical conductivity of biological tissues. This paper investigated the influence of the probe typology on the conductivity measurement accuracy and repeatability.

Two common probe types, the polar probe and the collinear probe, were investigated. The measurements using each probe were repeatable as the showed low variance in the acquired conductivity, so the effect of the typology is difficult to define is this case. However, the accuracy results varied (by 12% to 32%) across the conductivity acquired by the different probe from the same phantoms. These results suggest that the probe typology can influence the accuracy of the measured electrical conductivity.

Regarding the influence of the electrode size on the measurement, we used the collinear probe with different electrode sizes. Similar trends are seen for the repeatability as the probes are displaying low CV and SD therefore the repeatability is not influenced by the electrodes size in the case of the proposed probes. However, the difference in accuracy between the collinear probe and collinear probe with small electrodes (12% and 10%) is highlighting the potential effect of not adapting the probe on the data acquired during the measurement.

Based on the results of the accuracy and the repeatability, the collinear probe with small electrodes was concluded to be the best performing and are further tested using ten *ex vivo* bovine LAA tissue samples at frequency range from 0.1 Hz to 100 kHz. The mean conductivity of the LAA acquired from the ten samples is 2.5 mS/cm with standard deviation of 0.24 mS/cm. The observation from results suggests that the

acquired LAA conductivity is in line with the muscle cardiac tissue from the literature. The conductivity values of the LAA may be useful in electroporation treatment planning.

The results from investigating the effect of the typology of the probe and the electrode size on the conductivity acquired during the measurement, suggest that not adapting the probe design to the tissue under study can impair the conductivity acquisition from the electrical characterization of biological tissues.

The probes analyzed in this work are promising candidate designs based on the literature, however, the results from this study does not conclusively demonstrate that the proposed probes are optimal and further simulations and experimental analysis would be required to investigate this topic further. Specifically, extended simulations and experimental results should include additional typologies with non-equidistant electrodes, a larger range of electrode sizes as well as additional typologies such as curvilinear electrodes. Furthermore, future studies should also look at the development of standardized semi-solid reference samples to more accurately model the target tissues.

REFERENCES

[1] S. Saygi, "Atrial fibrillation and the role of LAA in pathophysiology and clinical outcomes?," *J. Atr. Fibrillation*, vol. 5, no. 3, pp. 153–160, 2012.

[2] M. L. Yarmush, A. Golberg, G. Serša, T. Kotnik, and D. Miklavčič, "Electroporation-based technologies for medicine: Principles, applications, and challenges," *Annu. Rev. Biomed. Eng.*, vol. 16, pp. 295–320, 2014.

[3] Y. Guo, G. Y. H. Lip, and S. Apostolakis, "Inflammation in atrial fibrillation," *J. Am. Coll. Cardiol.*, vol. 60, no. 22, pp. 2263–2270, 2012.

[4] B. P. Krijthe *et al.*, "Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060," *Eur. Heart J.*, vol. 34, no. 35, pp. 2746–2751, 2013.

[5] N. J. Patel *et al.*, "Contemporary trends of hospitalization for atrial fibrillation in the united states, 2000 through 2010 implications for healthcare planning," *Circulation*, vol. 129, no. 23, pp. 2371–2379, 2014.

[6] J. Xu, J. G. Y. Luc, and K. Phan, "Atrial fibrillation: Review of current treatment strategies," *J. Thorac. Dis.*, vol. 8, no. 9, pp. E886–E900, 2016.

[7] P. G. K. Wagstaff *et al.*, "Irreversible electroporation: State of the art," *Onco. Targets. Ther.*, vol. 9, pp. 2437–2446, 2016.

[8] R. N. Doshi, "The state of atrial fibrillation in 2020," *J. Innov. Card. Rhythm Manag.*, 2021.

[9] J. C. Weaver and Y. A. Chizmadzhev, "Theory of electroporation: A review," *Bioelectrochemistry Bioenerg.*, 1996.

[10] K. N. Aycock and R. V. Davalos, "Irreversible Electroporation: Background, Theory, and Review of Recent Developments in Clinical Oncology," *Bioelectricity*, vol. 1, no. 4, pp. 214–234, 2019.

[11] A. R. Liboff, "Toward an Electromagnetic Paradigm for Biology and Medicine," *J. Altern. Complement. Med.*, vol. 10, no. 1, pp. 41–47, 2004.

[12] D. C. Chang, J. A. Saunders, B. M. Chassy, and A. E. Sowers, "1 - Overview of Electroporation and Electrofusion," D. C. Chang, B. M. Chassy, J. A. Saunders, and A. E. B. T.-G. to E. and E. Sowers, Eds. San Diego: Academic Press, 1992, pp. 1–6.

[13] T. Y. Tsong, "Electroporation of cell membranes," *Biophys J. 1991 Aug;60(2)297-306.*, pp. 297–306, 1991.

[14] J. C Weaver, K. C Smith, E. Axel T, S. S, Reuben, and G. T R, "A brief overview of electroporation pulse strength-duration space: A region where additional intracellular effects are expected," *Bioelectrochemistry.*, 2012.

[15] B. McDermott *et al.*, "Stable tissue-mimicking materials and an anatomically realistic, adjustable head phantom for electrical impedance tomography," *Biomed. Phys. Eng. Express*, vol. 4, no. 1, 2018.

[16] N. Istuk, A. La Gioia, H. Benchakroun, A. Lowery, B. McDermott, and M. O'Halloran, "Relationship Between the Conductivity of Human Blood and Blood Counts," *IEEE J. Electromagn. RF Microwaves Med. Biol.*, vol. PP, no. 00, pp. 1–7, 2021.

[17] T. K. Neal RE 2nd, Millar JL, Kavnoudias H, Royce P, Rosenfeldt F, Pham A, Smith R, Davalos RV, "In vivo characterization and numerical simulation of prostate properties for non-thermal irreversible electroporation ablation," *Prostate.*, vol. 74(5):458-.

[18] H. Benchakroun, D. O. Loughlin, N. Ištuk, M. O. Halloran, and A. La Gioia, "Evaluation of the Feasibility of Three Custom-made Tetrapolar Probes for Electrical Characterization of Cardiac Tissue," *Eur. Conf. Antennas Propag. 2021*, 2021.

[19] Q. Castellví, B. Mercadal, and A. Ivorra, "Handbook of Electroporation," pp. 1–20, 2016.

[20] G. J. A. M. Brom-Verheijden, M. H. Goedbloed, and M. A. G. Zevenbergen, "A Microfabricated 4-Electrode Conductivity Sensor with Enhanced Range," *Proceedings*, vol. 2, no. 13, p. 797, 2018.

[21] J. Cinca *et al.*, "Changes in myocardial electrical impedance induced by coronary artery occlusion in pigs with and without preconditioning: Correlation with local ST-segment potential and ventricular arrhythmias," *Circulation*, vol. 96, no. 9, pp. 3079–3086, 1997.

[22] M. I. Ellenby, K. W. Small, R. M. Wells, D. J. Hoyt, and J. E. Lowe, "On-line Detection of Reversible Myocardial Ischemic Injury by Measurement of Myocardial Electrical Impedance," *Ann. Thorac. Surg.*, vol. 44, no. 6, pp. 587–597, 1987.

[23] C. Gabriel, S. Gabriel, and E. Corthout, "The dielectric properties of biological tissues," *Phys. Med. Biol.*, vol. 41, no. 11, pp. 2231–2249, 1996.

[24] G. Hahn, G.M., Kernahan, P., Martinez, A., Pounds, D., Prionas, S., Anderson, T. and Justice, "SOME HEAT TRANSFER PROBLEMS ASSOCIATED WITH HEATING BY ULTRASOUND, MICROWAVES, OR RADIO FREQUENCY," *Ann. New York Acad. Sci. 335 327-346*, 1980.

[25] L. Yang *et al.*, "Ex-vivo characterization of bioimpedance spectroscopy of normal,ischemic and hemorrhagic rabbit brain tissue at frequencies from 10 Hz to 1 MHz," *Sensors (Switzerland)*, vol. 16, no. 11, 2016.

[26] Patrick J. Lynch, "My medical illustrations." 2021.

[27] C. Gabriel, A. Peyman, and E. H. Grant, "Electrical conductivity of tissue at frequencies below 1 MHz.," *Phys. Med. Biol.*, vol. 54, no. 16, pp. 4863–4878, 2009.

[28] S. Gabriel, R. W. Lau, and C. Gabriel, "The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz," *Phys. Med. Biol.*, vol. 41, no. 11, pp. 2251–2269, 1996.

[29] R. P. Jokhi, V. V. Ghule, B. H. Brown, and D. O. C. Anumba, "Reproducibility and repeatability of measuring the electrical impedance of the pregnant human cervix-the effect of probe size and applied pressure," *Biomed. Eng. Online*, vol. 8, no. May 2014.

[30] P. Bertemes Filho, "Assessing Applied Pressure in Impedance Probe by Single-zone Force Sensing Resistors," *Athens J. Technology Eng.*, vol. 4, no. 1, pp. 7–16, 2017.

[31] S. Laufer, A. Ivorra, V. E. Reuter, B. Rubinsky, and S. B. Solomon, "Electrical impedance characterization of normal and cancerous human hepatic tissue," *Physiol. Meas.*, vol. 31, no. 7, pp. 995–1009, 2010.

[32] D. R. H. and B. H. B. D.M. Jones, R.H. Smallwood, "Constraints on tetrapolar tissue impedance measurements," *Electron. Lett.*, 2005.

[33] H. KalvØy, C. Tronstad, B. Nordbotten, S. Grimnes, and

Ø. G. Martinsen, "Electrical impedance of stainless steel needle electrodes," *Ann. Biomed. Eng.*, vol. 38, no. 7, pp. 2371–2382, 2010.

[34] A. Peyman, C. Gabriel, and E. H. Grant, "Complex permittivity of sodium chloride solutions at microwave frequencies," *Bioelectromagnetics*, vol. 28, no. 4, pp. 264–274, 2007.

[35] A. Peyman, C. Gabriel, and E. H. Grant, "Complex permittivity of sodium chloride solutions at microwave frequencies," *Bioelectromagnetics*, vol. 28, no. 4, pp. 264–274, 2007.

[36] J. A. Canchola, "Correct Use of Percent Coefficient of Variation (%CV) Formula for Log-Transformed Data," *MOJ Proteomics Bioinforma.*, vol. 6, no. 3, 2017.

[37] P. Ben Ishai, M. S. Talary, A. Caduff, E. Levy, and Y. Feldman, "Electrode polarization in dielectric measurements: A review," *Meas. Sci. Technol.*, vol. 24, no. 10, 2013.

[38] O. Casas *et al.*, "In vivo and in situ ischemic tissue characterization using electrical impedance spectroscopy." Ann N Y Acad Sci, 1999.

[39] M. Lounsbury and E. T. Crumley, "New practice creation: An institutional perspective on innovation: Michael Lounsbury and Ellen T. Crumley," *Organ. Stud.*, vol. 28, no. 7, pp. 993–1012, 2007.

[40] M. A. Fallert *et al.*, "Myocardial electrical impedance mapping of ischemic sheep hearts and healing aneurysms," *Circulation*, vol. 87, no. 1, pp. 199–207, 1993.

[41] L. A. Geddes and L. E. Baker, "The specific resistance of biological material-A compendium of data for the biomedical engineer and physiologist," *Med. Biol. Eng.*, vol. 5, no. 3, pp. 271–293, 1967.

[42] J. Malvern Benjamin, H. Schwan, C. F. Kay, and J. H. Hafkenschiel, "The Electrical Conductivity of Living Tissues as it Pertains to Electrocardiography I. Review of the Problem of Homogeneity vs. Nonhomo-geneity, an Outline of the Technical Aspects of Tissue Resistivity Measurements, and a Critical and Experi-mental Analysis of Certain Pertinent Experiments," 1950.

[43] J. Z. Tsai *et al.*, "In-vivo measurement of swine myocardial resistivity," *IEEE Trans. Biomed. Eng.*, vol. 49, no. 5, pp. 472–483, 2002

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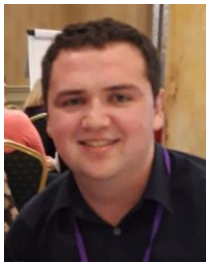

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