Investigating the Utility of in vivo Bio-Impedance Spectroscopy for the Assessment of Post-Ischemic Myocardial Tissue

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Abstract — **Increased myocardial structural heterogeneity in response to ischemic injury following myocardial infarction (MI) is purported as the mechanism of ventricular arrhythmogenesis. Current modalities for in vivo assessment of structural heterogeneity for identification of arrhythmogenic substrate are limited due to the complex nature of the structural microenvironment post-MI.**

We investigated the utility of in vivo bio-impedance spectroscopy (BIS) in a large post-infarct animal model for differentiation between normal and infarcted tissue. We also investigated the quantitative effects of adipose and collagen on BIS assessment of myocardium. The results indicate that the degree of myocardial injury following chronic post-infarction remodeling could be reliably quantified (performed in triplicates) using BIS. Furthermore, the presence of intramyocardial adipose tissue that develops in conjunction with collagen within the infarct zone had a greater and significant influence on BIS then collagen tissue alone. These preliminary results indicate a potential role of BIS for quantitative assessment and characterization of complex arrhythmogenic substrates in ischemic cardiomyopathy.

I. INTRODUCTION

The electrical impedance of a tissue sample is dependent on its structure, hence, bio-impedance spectroscopy (BIS) has been used to assess the condition of animal tissues; although it still requires further refinement, research and development to deliver a system that has sufficient reliability [1]. Previous studies have demonstrated that BIS is able to differentiate between various disease states of myocardium post-myocardial infarction [1, 2]. Whilst these studies were able to distinguish between absolute normal or diseased states, there is a paucity of knowledge regarding whether the complex nature of the post-ischemic myocardial architecture could be interpreted using BIS. Recent evidence in humans and large animal models of chronic myocardial infarction, indicate that the remodeling cascade of myocardium attributing to risk of arrhythmogenesis includes the deposition of collagen, proliferation of intramyocardial adipose tissue, and degeneration of viable myocytes [3]. Due to such variation in sub-cellular structure within infarct territory, we hypothesize that each of these properties would contribute to variation in tissue impedance. As such, the aims of the study were to 1) Assess the role of BIS for quantitative assessment of the degree of myocardial remodeling in the chronic phase of myocardial infarction, and 2) assess the differential role of adipose tissue and collagen on the variance of BIS assessment of myocardium.

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II. METHODS

A. Percutaneous Myocardial Infarct Induction

Three sheep, weighing 45 kilograms or greater and approximately 1 year of age, were used in this experiment.

Following premedication with Xylazine and Atropine intramuscularly, anesthesia was induced using intravenous Propofol. Myocardial Infarction was induced using a revised method proposed by Reek et al [4]. This was achieved by inflating a 3.0 × 20 mm angioplasty balloon in the left anterior descending artery equivalent, for 3 hours followed by reperfusion. This time is representative of an approximate time, from where a patient would experience symptoms of ischemia (symptom onset), to the time the patient arrived at a hospital and received revascularization therapy (TIMI3 reperfusion time) [5]. Animals were then sent back to their grazing habitat for recovery.

B. Bioimpedance Spectroscopy Measurement Process

A tetrapolar impedance method was utilized to generate an impedance frequency sweep of the normal and infarcted myocardial tissue using a Hioki Impedance analyzer (IM3570, Hioki EE Corporation, Japan). The impedance between voltage sensing connections V+ and V- was measured while a current was supplied via force connections I+ and I-, over a range of frequencies.

Myocardial infarction results in transmural thinning of the myocardium (normal ~1.4-1.9mm, scar border ~0.8-1.5mm and dense scar ~0.5-0.8mm thick). As such, multi-electrode plunge needles, designed to be inserted and rotated, were utilized and consisted of 2, 3 or 4 electrodes (N1-4). Due to such variable transmural thickness, only two poles were used for the interfacing electrodes (V+, I+). Further, V+ was moved along the electrodes to get measurements throughout the differing layers of the myocardial tissue whilst ensuring reliable electrode-myocardium interfacing. The layout of the integrated measurement circuit is shown in "Fig. 1."

A quadripolar electrophysiology catheter (St Jude Medical, MN, USA) was deployed alongside the plunge needles to provide a common reference point and the second set of connections (C1-4) for the four pole measurement. The negative terminals (I-, V-) were positioned within the blood pool as it had a constant and relatively lower

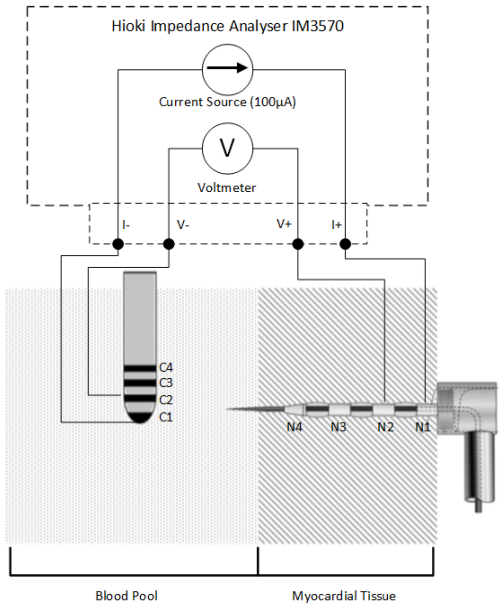

Figure 1. Schematic diagram depicting the four terminal method used for performing bioimpedance measurements in myocardial tissue. The circuit consists of (1) a multielectrode plunge needle at the interface of myocardium, (2) a reference catheter interfacing the cardiac blood pool, and (3) the Hioki impedance analyzer.

impedance, which ensured preferential conduction between the negative and positive terminals. This negated the compounding impedance effects due to the interfacing of the tissue structure at the negative terminus.

The impedance measurement parameters included: Frequency sweep from 500Hz – 500kHz, later refined to 31.5 kHz based on our findings, current input of 0.15 mA and 16 measurements across the frequency range.

C. *Validation of the Bioimpedance Measurement*

The bench testing procedure utilized a biological 0.9% saline solution, to model the physiology within the myocardium. The four components tested included: The temperature of the saline solution at 20^0C and at 37^0C to compare in vitro and in vivo conditions. The salinity of the solution was defined at 0.9%, 0.675%, and 0.45% to validate the proportional results of needle conductivity. Needle lengths and corresponding electrode numbers varied from 2 - 4 electrodes depending on myocardial thickness at the site of deployment. This was used to validate the needles by comparing the readings in varying conditions and electrode configurations. The distance between the needle and catheter was examined to assess the influence by variation in inter-catheter-electrode distance.

D. *In Vivo Quantitative Bioimpedance Spectral Assessment of Post-Ischemic Myocardial Architecture*

Once the animal was fully recovered from the infarct induction procedure (5 weeks recovery), a left thoracotomy was performed to expose the heart. A grid of 20 multi-electrode plunge needles were positioned in a grid spanning the apex to base and anterior to lateral aspect of the left ventricle, within infarcted and normal myocardium. The number of electrodes on the needle was dependent upon the thickness of the myocardium, as determined by palpitation and anticipated thickness.

The quadripolar electrophysiology catheter was positioned within the left ventricle percutaneously, ascending the femoral vein.

The bioimpedance values were collected from every single pole combination on the twenty needles using the process outlined in section B. This allowed for all layers of the tissue to be examined over different areas. They were taken as triplicates to show replication and remove experimental bias. The readings were taken in beating hearts first and once the animal was sacrificed, asystole readings were also collected. The data was then analyzed with the statistical software package SPSS (PASW Statistics for Macintosh, Version 18.0, SPSS Inc., Chicago).

E. *Histological Processing and Analysis*

One centimeter squared tissue blocks were excised around the mapped myocardial regions and histologically prepared as detailed by Pouliopoulos et al [6]. In essence, it involved processing the tissue before staining the sections with Gomori Tri-Chrome, for distinguishing collagen from muscle tissue. An example slide is provided in "Fig. 2B," where the myocardial tissue is stained red, the collagen blue and the adipose as white space.

Automated histological analysis was undertaken on in-house software to differentiate between the viable and non-viable myocardium. This process eliminated operator error and reduced the effects of artefacts introduced during the histological procedure as previously validated [6].

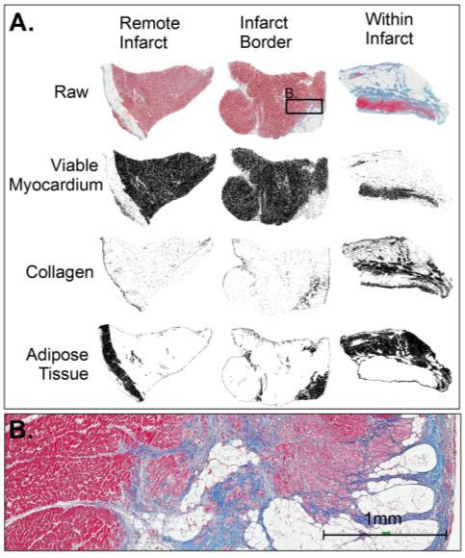

Figure 2. **A.** Automated histological analysis of myocardium excised from Normal (no-scar), Peri-infarct (peripheral scarring), and infarct (dense scarring) demonstrating differentiation between viable myocardium, collagen, and adipose tissue. **B.** Architectural organisation of myocardium from an area of ischemic injury and subsequent chronic remodelling. The section of tissue (x μm thickness) was stained using Gomori Trichrome to differentiate between collagen (blue), and viable myocardium (red). Adipocytes have morphological characteristics indicative of an absence of cytoplasmic material, relatively circular cell membranes, with close interconnected arrangement.

III. RESULTS

A. Validation of the Bioimpedance Measurement

For each of the variables analyzed in this in vitro study, the results were in accordance to the anticipated results. For each °C increase in temperature, there was a decrease of 0.86Ω in magnitude. For every 0.1% decrease in saline concentration, there was a 6.88Ω decrease in magnitude. The readings of the different needle type all produced the same results. And for every cm increase in the distance between the needle and catheter, there was an 8.33Ω increase in the magnitude.

B. Effect of Myocardial Viability

Areas with significant myocardial injury (<50% viability) following healed myocardial infarction exhibited greatest attenuation of magnitude compared to areas with >50% viability (Fig. 3). Impedance magnitude was observed to be systematically increased across all viability categories during asystole compared to sinus rhythm, with the relationship between magnitude and viability remaining similar.

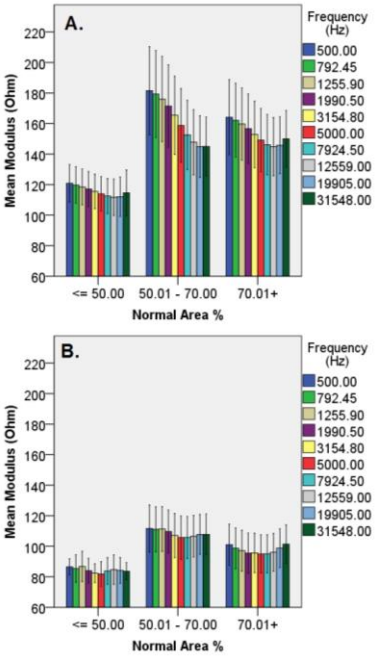

Figure 3. The effect of local myocardial viability (Normal Area %) on magnitude measured across the 500 – 31548Hz spectrum. **A.** Measurment performed during cardiac asystole and normoxic conditions. **B.** Measurements performed during sinus rhythm and normoxic conditions.

C. Effect of Adipose and Collagen

Pairwise comparisons between low (<5%), moderate (5-15%) and high (>15%) collagen and adipose densities were performed. It was shown in "Fig. 4" that, cumulative magnitude was directly and significantly influenced by both the presence of collagen and adipose tissue during asystole. However, this relationship was not significant during sinus rhythm. In addition, attenuation in cumulative magnitude appeared to be greatest in areas with >5% adipose density.

D. Effects over Frequency Spread

After adjusting for the effects of intramyocardial collagen and adipose density, the bioimpedance spectrum in "Fig. 5A" showed a distinct separation between the levels of myocardial viability in asystole conditions. During sinus rhythm there was a greater spread in the trends but not of an equal spread as shown in "Fig. 5B."

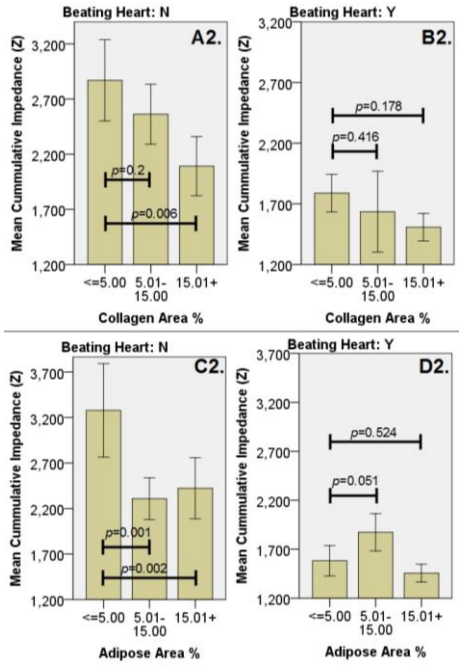

Figure 4. Effect of collagen (**A2, B2**) and adipose (**C2, D2**) density on cummulative impedance magnitude, independent of viability. **A2, C2** = Measurements performed during cardiac asystole and normoxic conditions. **B2, D2** = Measurements performed during sinus rhythm and normoxic conditions.

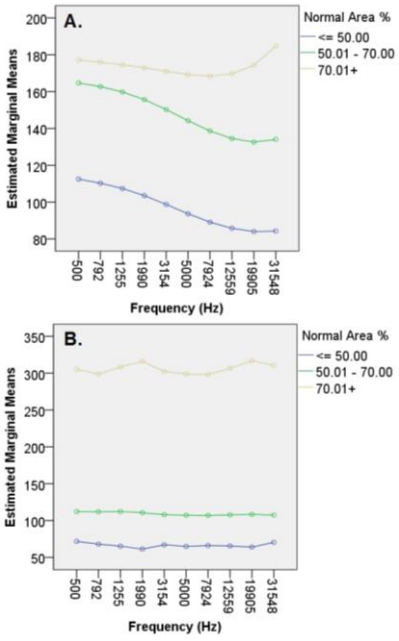

Figure 5. Analysis of Covariance Estimated Adjusted "Marginal" Means Plots of magnitude for each level of myocardial viability (Normal Area %), measured across the 500 - 31548Hz spectrum. **A.** Asystole normoxic conditions. **B.** Beating and normoxic conditions.

IV. DISCUSSION

This study was designed to describe and validate a more refined in vivo technique and equipment setup, than what is current for BIS. Validation of this modality was performed using automated histological assessment of the myocardium, co-registered with impedance spectrograms and confirmed electrode-tissue contact. Further, bench testing validated the equipment and parameters chosen. All of which provided a more refined collective methodology.

The results of the bench testing showed that the temperature, electrode distance, salinity and needle design, did not contribute to significant changes in impedance magnitude or phase, above the variance reported in vivo for each histological category.

The effect of myocardial viability across the frequency spectrum showed significant effect on magnitude as shown in "Fig 3." More pronounced in the cardiac asystole environment, the magnitude values showed a ~65% increase over the sinus rhythm values. These increases were believed to be related to the relative flow of current in the two states. Based on ANCOVA analysis, myocardial viability was the greatest contributor to the variation in impedance magnitude followed by intramyocardial adipose. These findings were complimentary to that by Salazar et al because they showed that impedance phase was the determining factor of the tissue's composition instead of the magnitude [2]. The differences in the results was most likely attributed to the method of assessment and the design of the impedance needles, validated by our in vitro testing.

Pairwise comparisons carried out on the three density bands in "Fig. 4" provided evidence that both collagen and adipose significantly affected the impedance magnitude measurements. In contrast to previous studies [2], the phase angles were once again not significantly affected by the collagen or adipose tissue content. Adipose and collagen magnitude attenuation was greatest in the asystole environment and at lower composition values as compared to the sinus rhythm values; consistent with observations relating to myocardial viability. As such, impedance magnitude was inversely correlated to local collagen or adipose quantity. It is hypothesized that the collagen and adipose infiltration within the myocardial tissue increased the number of conduction pathways within the myocardium, causing the magnitude decrease as density increased [3].

The most important finding of this study was the relationship between the frequency range and the differentiation between the viable myocardial bands as shown in "Fig. 5." The magnitude of the impedance measured across the frequency spectrum was adequate enough to differentiate between the three viable area % bands with regards to adipose and collagen content. The measured impedance as described previously was dependent not only on the specific impedance at the measured frequency but also on the myocardium and its composite cellular components.

The difference between the values obtained in the asystole and rhythmic heart conditions, even though the sheep were well oxygenated prior to inducing asystole for 10 minutes, was not completely understood. It is hypothesized that in cardiac asystole, the tissue began to undergo ischemia [7]. Through the early stages of this breakdown process, ion channels and gap junctions within the myocardium progressively closed and inhibited the flow of ions. This caused an increase in the paths along which the current needed to travel in the measurement process, in turn increasing the impedance magnitude by the simple law of fluid physics [8]. It is therefore unknown how interlinked this affect is within our study with further analysis required.

The limitations of this study include: being performed on ovine hearts which provided models for human hearts but were not identical in terms of physiology and anatomy. Although they were different, the similarities allowed for a close enough comparison between the two. The impedance measurement process was not robust or powerful enough to automate readings to a high degree of fidelity. For this reason we only took 16 points between the 500 Hz and 50 kHz frequency range. And although histological data was derived from two dimensional sections, the data represented a three dimensional model. The orientations of tissue excision prior to sectioning were therefore randomized to account for this. All these issues however will be addressed in the next round of experimentation, adapting a catheter based approach or including the measurement process in devices such as ICD leads to allow for differentiation.

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