A Fibroblast-Myocyte Model which Accounts for Slow Conduction and Fractionated Electrograms in Infarct Border Zones

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Abstract

The biophysical mechanisms behind the fractionated character of cardiac electrograms from infarct border zones have not been clearly identified. The working hypothesis of this study is that fibroblasts in the scar tissue are electrically coupled to myocytes via gap junctions, acting as RC connections between healthy cardiac cells. This study examined the physical characteristics of cultured fibroblasts and calculated an average surface area of 74.4 E-5 cm^2*. Using reported gap junction conductance for myocyte-fibroblast-myocyte triplets, typical myoplasmic resistivity, and specific capacitance yields a total RC time constant in the range of 35-40 ms. This would cause major delays in propagation between two healthy myocytes conjoined by a fibroblast, and could explain phenomena such as micro-reentry and the fractionated electrogram.*

1. Introduction

Sudden cardiac death (SCD) is a major health problem with great economic and social impacts, since it claims approximately 220,000 lives per year in the US alone.[1] Most SCDs are due to ventricular arrhythmias where the heart fails to efficiently contract because of a lack of electrical synchrony. Scar tissue from previous myocardial infarctions (MI) is found in about two thirds of SCD victims, and often constitutes the substrate for these lethal arrhythmias. Understanding of the cellular mechanisms associated with arrhythmia generation in these substrates is important to aid physicians in the recognition of risk factors in post-MI patients, and to guide them in their therapeutic approaches for the prevention of SCD.

Following MI, scar tissue composed of an array of fibroblast-like cells (myofibroblasts) embedded into a collagen-fibrous matrix is formed. This array has a dynamic nature, and as the MI heals, muscle fibers are deformed and surviving cells become trapped in the scar as necrotic myocardial cells are replaced by fibrous tissue.[2] The separation and disorganization of the muscle bundles in the infarct border zones, caused by the invasion of fibrous tissue from the adjacent infarct, disrupts intercellular connections and slows conduction. These changes are mostly due to structural alterations, since animal studies have demonstrated that transmembrane potentials are not severely depressed in healing infarcts and are nearly normal in healed infarcts.[3]

Cardiac electrograms recorded at MI border zone regions of the ventricles have a characteristic long duration and fractionated appearance, which is consistent with slow inhomogeneous activation that forms the substrate for lethal arrhythmias. Different convoluted explanations, mostly based on clinical data, have been proposed to explain the fractionated nature of these recordings, but the biophysical mechanisms behind them have not been clearly identified.

Our working hypothesis is that fibroblasts, which are largely present within these healed MI scar zones, may be interconnected via gap junctions and provide a direct electrical connection between healthy myocytes, but instead of being primarily a resistive connection it is an RC connection due to the membrane capacitance of the fibroblast. *In-vitro* electrophysiological coupling between fibroblasts and myocytes has been previously demonstrated. Rook et al. observed electrotonic interactions in pairs of fibroblasts and myocytes, and demonstrated that non-excitable cardiac cells were capable of passive impulse conduction in a myocyte-fibroblast-myocyte triplet.[4] Similarly, Fast et al. noted that fibroblasts can be electrically coupled to myocytes and serve as sinks for electrotonic currents.[5]

This work presents the calculation of the RC time constant of a fibroblast in a myocyte-fibroblast-myocyte triplet. The calculations are based on a first order electrical model of a fibroblast with realistic surface area, as determined by confocal microscopy images of cultured neonatal rat myofibroblasts.

2. Methods

2.1. Fibroblast isolation and dye staining

The neonatal myofibroblasts were isolated using an isolation system from Worthington Biochemical Corporation, Lakewood, New Jersey. Hearts were harvested from 1- to 3-day-old Wister rats under sterile conditions and placed in ice-cold Ca^{2+} and Mg^{2+} free

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Hanks Balanced Salt Solution. The isolated hearts were cleared of connective tissue and atria, minced in approximately 1 mm blocks, and then incubated with

trypsin for overnight digestion at 4° C. The next day, trypsin activity was neutralized and the tissues were further digested with collagenase. A single cell suspension was made by filtering the cells through a 70 μm cell strainer. Cells were plated at 37° C for 30 minutes in a humidified incubator (95% O_2/5% CO_2). After this time had elapsed, the flasks were gently tapped to dislodge the cardiomyocytes, and the medium containing unattached cardiomyocytes was removed. F-10 media was added to the attached fibroblasts.

To image the cells with the confocal microscope, fibroblasts were seeded onto glass coverslips and loaded with 50 μM Cell Tracker Green (Molecular Probes Inc. C-2925), for 60 minutes at 37° C.

2.2. Estimation of cell surface area

Confocal microscopy uses optical sectioning techniques to produce thin optical sections of cells and tissues with high resolution and sensitivity. This allows the assessment of live cell morphology and structure. Typical resolution values for confocal microscopy are 0.1 to 0.2 μm in the lateral X and Y directions, and 0.5 to 1 μm in the Z direction.[6]

In order to determine an average fibroblast surface area, Z-series consisting of a stack of optical sections where collected from four stained rat fibroblasts with a BioRad Radiance 2000 confocal system (Figure 1). The number of optical slices per cell varied between 25-42, with steps sizes between 0.5-1.0 μm.

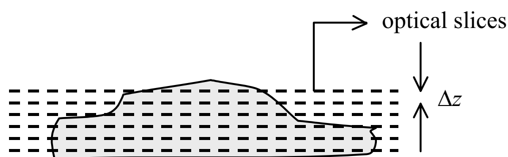

Figure 1. Schematic diagram of Z-series.

The digital images obtained from the confocal system were analyzed using the image processing and analysis software Metamorph®. The cell perimeter for each optical slice of each cell was obtained by using the automated object-measuring feature after manually setting an intensity threshold. The surface area (A) of each cell was next estimated as

$$A = \sum_{i=1}^{N} perimeter_i \cdot \Delta z \qquad (1)$$

where N is the total number of optical slices, $perimeter_i$ is the perimeter of the ith slice, and Δz is the step size in the Z direction. Similarly, the cell volume can be calculated by replacing the perimeter of the optical slice by the area of each slice in equation 1.

2.3. Electrical model of fibroblast

An electrical model of a single muscle fiber can be developed based on concepts from electric circuits. The fiber is represented as a cable subdivided into separate regions by the inclusion of gap junctions.[7,8] Each region is roughly analogous to a physical cell and the gap junctions are modeled as purely resistive based on experimental evidence that indicates that the interactions between cells are passive for gap junction conductances greater than 1-2 nS.[4] Figure 2 shows a schematic diagram of a portion of a muscle fiber, which includes two myocytes electrically interconnected by a fibroblast.

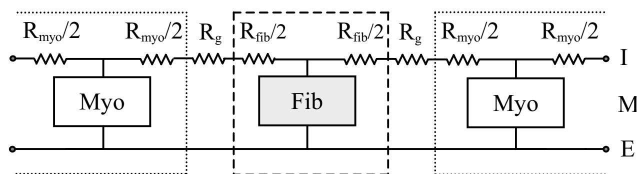

Figure 2. Schematic diagram of cardiac fiber including two myocytes interconnected by a fibroblast. R_g = resistive gap junctions, R_{fib} and R_{myo} = fibroblast and myocyte intracellular axial resistance. I = intracellular space, M = cellular membrane, E = extracellular space.

A first order electrical representation of a single fibroblast can be obtained based on the parallel conductance model. If fibroblasts are strictly considered to be electrically passive cells, an equivalent representation of the cellular membrane of a single cell consists of a fixed conductance in parallel with a capacitor, where the conductance represents the conductance of the membrane to transmembrane current flow, and the capacitance is the biophysical consequence of the lipid bilayer. Previous voltage clamp analysis on neonatal rat ventricular fibroblasts has shown that transmembrane conductance (G_m) is very small on the order of 0.04 – 0.3 pS.[4,9] If transmembrane conductance is neglected based on these observations, the electrical representation of a fibroblast in a fiber, as depicted in figure 2, is reduced to an RC circuit, where R is the sum of the cellular axial resistance and the gap junction resistances, and C_m is the total membrane capacitance, calculated as the surface area times the specific membrane capacitance (1 $\mu F/cm^2$). This is illustrated in Figure 3.

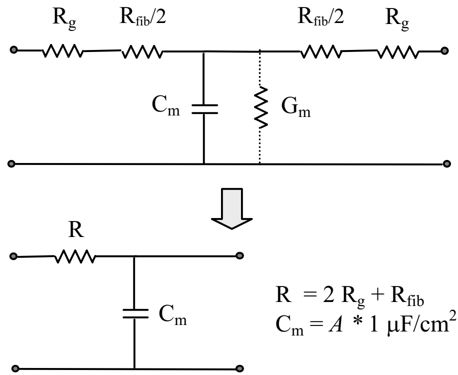

Figure 3. Electrical representation of a fibroblast as an RC circuit with G_m assumed to be zero. A = cell surface area.

3. Results

Table 1 shows the calculated surface area for each fibroblast.

Table 1. Fibroblast surface area

Cell number	*Surface Area (cm^2)*
Fibroblast 1	7.46620 E-5
Fibroblast 2	7.45052 E-5
Fibroblast 3	7.47195 E-5
Fibroblast 4	7.37120 E-5

The average fibroblast surface area from these measurements was 7.44 E-5 cm^2. The total volume of the cells averaged 4.4 E-6 μL. Using a specific membrane capacitance of 1 μF/cm^2, which is accurate for most non-muscle cells, yields a total fibroblast membrane capacitance (C_m) of 74.4 pF.

Based on experimental observations, a fibroblast can be morphologically modeled as a rectangular parallelepiped with a cross-sectional dimension of 2 x 10 μm and a 100 μm length. These particular dimensions give a volume to surface area ratio in the same order for a real cell, as calculated from the aforementioned average measurements. Using a typical axial myoplasmic resistivity of 150 Ωcm yields a total axial resistance of 7.5 MΩ.

Rook et al. reported series conductivities of gap junctions between a myocyte-fibroblast (MF) pair as ranging between 310 pS and 8 nS. Junctional conductivities for a myocyte-fibroblast-myocyte (MFM) triplet (equivalent to two myocyte-fibroblast junctions in series) ranged between 150 pS and 3 nS.[4] Using a MFM total junctional conductance of 2 nS, the total resistance (R) adds up to 507.5 MΩ. As a result, the RC time constant is 37.76 ms.

4. Discussion and conclusions

Previous studies have shown that myocytes and myofibroblasts in culture can be electrically coupled by gap junctions, and that this coupling contributes to the achievement of regular beating in monolayer cultures of heart cells.[10] Even though junctional conductances are reportedly significantly less in fibroblast-fibroblast (FF) and MF pairs than between myocyte-myocyte pairs (MM), this relatively small conductance has been enough to allow propagation between myocytes in MFM triplets in culture, with delays dependent on the degree of coupling between the cells.[4] Although it is not currently known whether such connections occur *in-vivo*, it is possible that these electrical interactions play a role in the disturbances in conduction observed in infarct border zones.

Besides gap junction conductance, electrical coupling and conduction are also dependent on the surface area of the cells and the membrane conductance. In this work, an average surface area of neonatal rat myofibroblasts was calculated and used with reported values for specific capacitance, myoplasmic axial resistivity, and gap junction conductance to estimate a realistic RC time constant of the fibroblast in a MFM triplet. Using an intermediate gap junction conductance of 2 nS, the calculated time constant was 37.76 ms, which would cause a substantial conduction delay considering that the activation between well-coupled MM pairs is almost synchronous. This delay could be longer for greater degrees of uncoupling or if myocytes become separated by more than one fibroblast, as in scar tissue within an MI border region. This would cause slow discontinuous propagation and produce fractionated electrograms at MI border zones, while creating the substrate for micro-reentry and subsequent lethal ventricular arrhythmias.

These biophysical observations need further evaluation, but could provide primary data for models of arrhythmogenesis and abnormal cardiac conduction as measured by late potentials.

Acknowledgements

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