Modeling Fibroblast-Mediated Conduction in the Ventricle

C Vasquez[1], AP Moreno[2], EJ Berbari[2]

[1]Purdue University, West Lafayette, IN, USA
[2]Indiana University Purdue University Indianapolis, Indianapolis, IN, USA

Abstract

This study used a model of a cardiac muscle fiber composed of myocytes (M) and a centrally located insert of fibroblasts (F). Myocytes were represented with the Luo-Rudy model with physical dimensions of cultured neonatal mouse cardiac myocytes. Fibroblasts were represented as passive cells with negligible transmembrane currents. Gap junctional conductance values were set to 15, 4.7 and 4 nS for MM, MF, and FF junctions respectively. Conduction delay, velocity, and changes in dV/dt max were studied for inserts composed of 1-4 cells, with varying fibroblast length. Results showed that propagation is sustained for inserts of up to 3 cells with significant conduction delays and depressed conduction velocities. Action potentials from myocytes distal to the insert had depressed dV/dt max and long foot potentials. Fibroblasts intercalated in a cardiac muscle fiber may allow propagation of the electrical activity through electrotonic interactions, introducing long propagation delays and changes in conduction velocity. These electrical interactions may explain phenomena such as fractionated electrograms and microreentry.

1. Introduction

Following myocardial infarction, scar tissue composed of an array of fibroblast-like cells embedded into a collagen fibrous matrix is formed. In the infarct border zone there is an extensive change in the organization of surviving myocytes as they become trapped in the scar tissue. This remodeling results in disruption of intercellular connections with a subsequent slowing in conduction, and may serve as a substrate for lethal arrhythmias.

Cardiac electrograms recorded from infarct border zones of the ventricles have a characteristic long duration and fractionated appearance. Figure 1 shows and example of a clinical electrophysiology lab recording from an infarct region in a patient with recurrent ventricular tachycardia. Note the lateness and multiphasic character of the LV electrogram. Although these characteristics are assumed to be due to meandering and slow conduction, there is no widely accepted theory to explain this observation.

Our current hypothesis is that fibroblasts, which are present in large numbers in the scar tissue, may be interconnected via gap junctions and provide an electrical link between surviving myocytes in the infarct border zone. Such fibroblast-mediated propagation of electrical activation would result in significant conduction delays and fractionated electrograms. In support of this idea, recent animal studies have shown the presence of fibroblasts expressing gap junctional proteins within infarct regions of the ventricles (1).

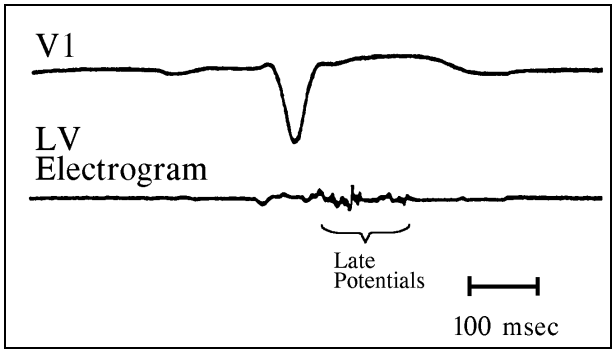

Figure 1. Examples of ECG (V1) and electrogram (LV) recordings from the infarct border zone of a patient with recurrent ventricular tachycardia.

Several *in-vitro* studies have shown that myocytes and fibroblasts establish gap junctional communication at the single cell level (2,3). In addition, high resolution optical mapping has shown that regions of fibroblasts constitute a slow conduction region when co-cultured with cardiomyocytes (4). This notion has been extended more recently with the observation that fibroblasts are capable of synchronizing electrical activation of multicellular cardiac tissue over extended distances through electrotonic interactions (5). Such fibroblast-mediated propagation of the electrical impulse is characterized by long conduction delays in the order of tens of milliseconds. Our previous modeling studies have also

found that relatively low levels of coupling are enough to allow propagation of electrical activation through a fibroblast, with delays dependent on the degree of coupling and the physical dimensions of the fibroblast (6).

This study used a model of a cardiac muscle fiber composed of myocytes and a centrally located fibroblast insert to study the characteristics of fibroblast-mediated conduction of the electrical activation in the ventricles. Conduction delay, conduction velocity for fibroblast-mediated propagation, and changes in maximum dV/dt introduced by fibroblasts were studied for inserts composed of fibroblasts with varying cell length.

2. Methods

This study used a one-dimensional cardiac fiber model composed of myocytes and a centrally located insert composed of 1-4 fibroblasts, for a total of 70 cells connected through resistive gap junctions.

Myocytes were represented with the Luo-Rudy (LRd) model (7). Fibroblasts were represented as passive cells with negligible transmembrane currents, consistent with previous measurements (3,8). Action potential propagation along the multicellular fiber was initiated by applying a suprathreshold stimulus to the first cell in the fiber, and was reconstructed thereafter as described previously (9).

In order to compare results from the theoretical simulations to ongoing *in-vitro* experiments being performed at our laboratory, cell length and radius were adjusted from the LRd model formulation to reported physical dimensions of cultured neonatal mouse cardiocytes, obtained by confocal microscopy methods (10). Myocyte and fibroblast cell radius was set to 4 μm. Cell length was fixed for myocytes at 32 μm. Fibroblast cell length varied from 28 – 38 μm. These parameters give volume to surface area ratios in the same order of the original LRd model. Using a typical specific capacitance of 1μm/cm^2 gives cell capacitance values similar to those calculated from voltage clamp experiments using cultured neonatal mouse cardiocytes, as obtained in our laboratory (Cm = 13.1±0.9,25 pF (mean±SEM,n)) and reported by Nuss et al (11).

Gap junctional conductance (Gj) varied along the fiber depending on the cells forming the junction. Electrophysiological recordings of gap junctional currents between cultured neonatal mouse myocyte-myocyte (MM), myocyte-fibroblast (MF), and fibroblast-fibroblast (FF) pairs were carried out in our laboratory using the dual cell voltage clamp technique. From these measurements, Gj for each pair type was calculated and introduced in the model. Gap junctional conductance values for MM, MF, and FF junctions were set to 15, 4.7, and 4 nS respectively. Axial cytoplasmic resistivity and specific capacitance were set to typical values of 150 Ωcm and 1 μm/cm^2 respectively.

Conduction delay, defined as the difference between time of maximum dV/dt of myocytes distal and proximal to the fibroblast insert, conduction velocity for fibroblast-mediated propagation, and changes in maximum dV/dt introduced by the fibroblast insert were studied for the different cell length and number of cells in insert scenarios.

3. Results and discussion

Results showed that propagation is sustained for fibroblast inserts of up to 3 cells for all cell lengths considered, with significant conduction delays. Conduction block ensued for 4-cell long fibroblast inserts.

Figure 2 shows a plot of transmembrane potential for the fibroblasts and the myocytes proximal and distal to the insert, for a 3 cell long insert. Fibroblast transmembrane potentials (dotted traces) show passive charging of the cellular membrane, and have a compound shape as a result of electrotonic current flow initially from the myocytes proximal and then distal to the insert. The distal myocyte has a long foot-potential, which is consistent with slow charging of the cellular membrane. This is a consequence of the electrotonic (non-regenerative) propagation through the fibroblast insert, and the decrease in axial current due to the reduced coupling between MF and FF (as compared to MM coupling.)

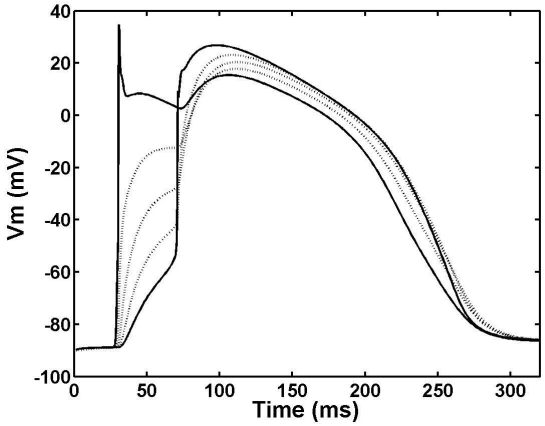

Figure 2. Transmembrane potential (Vm) for fibroblasts composing the insert (dotted traces) and the myocytes proximal and distal to the insert (solid lines.) Number of cells in insert = 3 cells, fibroblast length = 32 μm.

Table 1 shows the propagation delay times and conduction velocities across the fibroblast insert for

different insert and fibroblast lengths. Propagation delay across fibroblast inserts increased with an increasing number of cells in the insert, ranging from 5.7-6.9, 15.8-20.0, and 35.3-50.4 ms for one, 2 and 3 fibroblast long inserts respectively. For a fixed number of fibroblasts, increasing cell length resulted in longer delay times.

Table 1. Delay times and conduction velocity across fibroblast insert for one, 2 and 3-cell long inserts and varying fibroblast cell length.

Insert length	Fibroblast length (μm)	Delay (ms)	Velocity (mm/s)
1 cell	28	5.73	4.89
	33	6.31	5.23
	38	6.89	5.51
2 cells	28	15.79	3.55
	33	17.88	3.69
	38	20.00	3.80
3 cells	28	35.28	2.38
	33	41.92	2.36
	38	50.38	2.26

Propagation velocity across the fibroblast insert decreased with an increasing number of cells in the insert, ranging from 4.9-5.5, 3.6-3.8, and 2.3-2.4 mm/s for one, 2, and 3 fibroblast long inserts, respectively. Normal conduction velocity for the fiber was 35.5 mm/s. Moreover, the effect of increasing fibroblast length on conduction velocity was dependent on the number of cells in the insert. Increasing fibroblast length increased conduction velocities in one and 2-cell inserts, but had the opposite effect in 3-cell inserts.

This paradoxical effect may be explained in the following fashion. Given the non-excitable nature of fibroblasts, a fibroblast intercalated in a model of a cardiac fiber acts as an RC electrical connection between healthy myocytes (12). As such, the time delay introduced by a fibroblast in the fiber has a capacitive component, dependent on the surface area of the cell, and a resistive component, which is given by the large gap junctional resistance and the smaller intracellular myoplasmic resistance.

Therefore, for inserts with a fixed number of cells, increasing individual fibroblast length has two effects that affect the overall conduction velocity of electrical activation across the insert. The first effect of increasing cell length is increasing cell capacitance and myoplasmic resistance, both of which cause an increase in the conduction delay introduced by each fibroblast. Longer delays mean slower conduction. The second effect is an increase in the total length of the insert, which, if not outweighed by the increase in conduction delay, would cause an increase in the overall conduction velocity across the fibroblast insert.

In one and 2-cell inserts, the increase in time delay is offset by the increase in the total length of the insert, resulting in an overall increase of the conduction velocity across the insert. On the other hand, in 3-cell inserts the cumulative increase in delay over the length of the insert prevails over the increase in total insert length, resulting in a decrease of the conduction velocity of the electrical activation across the fibroblast insert. Thus, increasing the number of cells in the insert and the individual fibroblast length results in a smaller attainable velocity before conduction failure ensues.

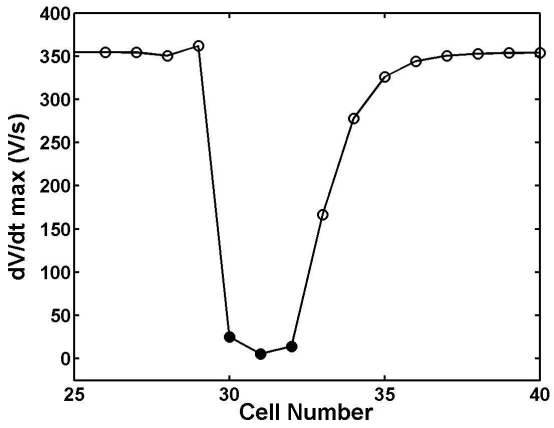

Figure 3. Maximum dV/dt for fibroblasts composing the insert (solid circles, cell numbers 30-32) and myocytes proximal and distal to the insert (open circles.) Number of cells in insert = 3 cells, fibroblast length = 32 μm.

Maximum dV/dt decreases in the insert region, consistent with the passive charging of the fibroblast cellular membranes. Figure 3 shows a plot of maximum dV/dt for fibroblasts in the insert (solid circles) and several myocytes proximal and distal to the insert (open circles) for a 3-cell insert. In distal myocytes, maximum dV/dt stays depressed in several cells after the insert, consistent with partial inactivation of sodium channels due to slow subthreshold charging, also evidenced by the long foot potentials as shown in Figure 2. This effect is less pronounced for shorter insert lengths.

4. Conclusions

The degree of gap junctional communication between ventricular fibroblasts and myocytes has not been evaluated *in-vivo*. However, fibroblast expressing gap junctional proteins have recently been identified within

infarct regions in animal models of myocardial infarction (1). Moreover, in other regions of the heart (SA node) fibroblasts appear to be functionally linked to myocytes (13). Evidence of propagation of electrical activation across cardiac suture scars (14) also suggests a functional electrical connection between fibroblastic cells in the scar tissue and myocytes.

This study shows that fibroblasts intercalated in a theoretical cardiac muscle fiber may allow propagation of the electrical activity through electrotonic interactions. Such fibroblast-mediated conduction is characterized by complex source-load interactions, long propagation delays and significantly reduced conduction velocities, which are dependent on the number and physical dimensions of the fibroblasts. The electrophysiological basis for slow fibroblast-mediated conduction resides in the lack of excitability of fibroblastic cells, and the reduction in intercellular communication due to the expression of different gap junction proteins (15). These electrical interactions may explain phenomena such as fractionated electrograms, microreentry, and cardiac late potentials recorded from infarct border zones of the ventricles.

Acknowledgements

This research was partially funded by the NSF Integrative Graduate Education and Research Training (IGERT) Program in Therapeutic and Diagnostic Devices Grant DGE-99-72770, and by the NIH R01HL63969 and Cardiovascular Endowment Fund, Methodist Research Institute.

References

[1] Camelliti P, Devlin GP, Matthews KG, Kohl P, Green CR. Spatially and temporally distinct expression of fibroblast connexins after sheep ventricular infarction. Cardiovascular Research 2004; 62(2):415-425.

[2] Rook MB, Jongsma HJ, de Jonge B. Single channel currents of homo- and heterologous gap junctions between cardiac fibroblasts and myocytes. Pflugers Arch 1989; 414(1):95-98.

[3] Rook MB, van Ginneken AC, de Jonge B, el Aoumari A, Gros D, Jongsma HJ. Differences in gap junction channels between cardiac myocytes, fibroblasts, and heterologous pairs. Am J Physiol 1992; 263(5 Pt 1):C959-C977.

[4] Fast VG, Darrow BJ, Saffitz JE, Kleber AG. Anisotropic activation spread in heart cell monolayers assessed by high-resolution optical mapping. Role of tissue discontinuities. Circ Res 1996; 79(1):115-127.

[5] Gaudesius G, Miragoli M, Thomas SP, Rohr S. Coupling of Cardiac Electrical Activity Over Extended Distances by Fibroblasts of Cardiac Origin. Circ Res 2003; 93(5):421-428.

[6] Vasquez C., Moreno AP, Berbari EJ. A Model of the Biophysical Basis of Fractionated Electrograms and Microreentry Based on Gap Junction Coupling Between Myocytes and Fibroblasts. NASPE 24th Annual Scientific Sessions. 2003.

[7] Luo CH, Rudy Y. A dynamic model of the cardiac ventricular action potential. I. Simulations of ionic currents and concentration changes. Circ Res 1994; 74(6):1071-1096.

[8] Feld Y, Melamed-Frank M, Kehat I, Tal D, Marom S, Gepstein L. Electrophysiological modulation of cardiomyocytic tissue by transfected fibroblasts expressing potassium channels: a novel strategy to manipulate excitability. Circulation 2002; 105(4):522-529.

[9] Shaw RM, Rudy Y. Ionic mechanisms of propagation in cardiac tissue. Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. Circ Res 1997; 81(5):727-741.

[10] Thomas SP, Bircher-Lehmann L, Thomas SA, Zhuang J, Saffitz JE, Kleber AG. Synthetic Strands of Neonatal Mouse Cardiac Myocytes : Structural and Electrophysiological Properties. Circ Res 2000; 87(6):467-473.

[11] Nuss HB, Marban E. Electrophysiological properties of neonatal mouse cardiac myocytes in primary culture. J Physiol 1994; 479 (Pt 2):265-279.

[12] Vasquez C., Siddiqui RA, Moreno AP, Berbari EJ. A fibroblast-myocyte model which accounts for slow conduction and fractionated electrograms in infarct border zones. Computers in Cardiology 2002;29:245-248.

[13] Camelliti P, Green CR, LeGrice I, Kohl P. Fibroblast Network in Rabbit Sinoatrial Node. Structural and Functional Identification of Homogeneous and Heterogeneous Cell Coupling. Circ Res 2004;01.

[14] Rothman SA, Miller JM, Hsia HH, Buxton AE. Radiofrequency ablation of a supraventricular tachycardia due to interatrial conduction from the recipient to donor atria in an orthotopic heart transplant recipient. J Cardiovasc Electrophysiol 1995; 6(7):544-550.

[15] Elenes S, Martinez AD, Delmar M, Beyer EC, Moreno AP. Heterotypic docking of Cx43 and Cx45 connexons blocks fast voltage gating of Cx43. Biophys J 2001; 81(3):1406-1418.

Address for correspondence

Edward J. Berbari
Department of Biomedical Engineering, SL-174
Indiana University Purdue University Indianapolis
723 West Michigan Street
Indianapolis, IN 46202
eberbari@iupui.edu