Numerical and Biological Approaches to Investigate the Role of Fibrosis as a Modifier of the Vulnerable Substrate for Atrial Fibrillation

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Ann Arbor, Michigan

Atrial Fibrillation

- The most common cardiac arrhythmia seen in clinical practice.
- The most important cause of stroke

AF: A Silent Killer!

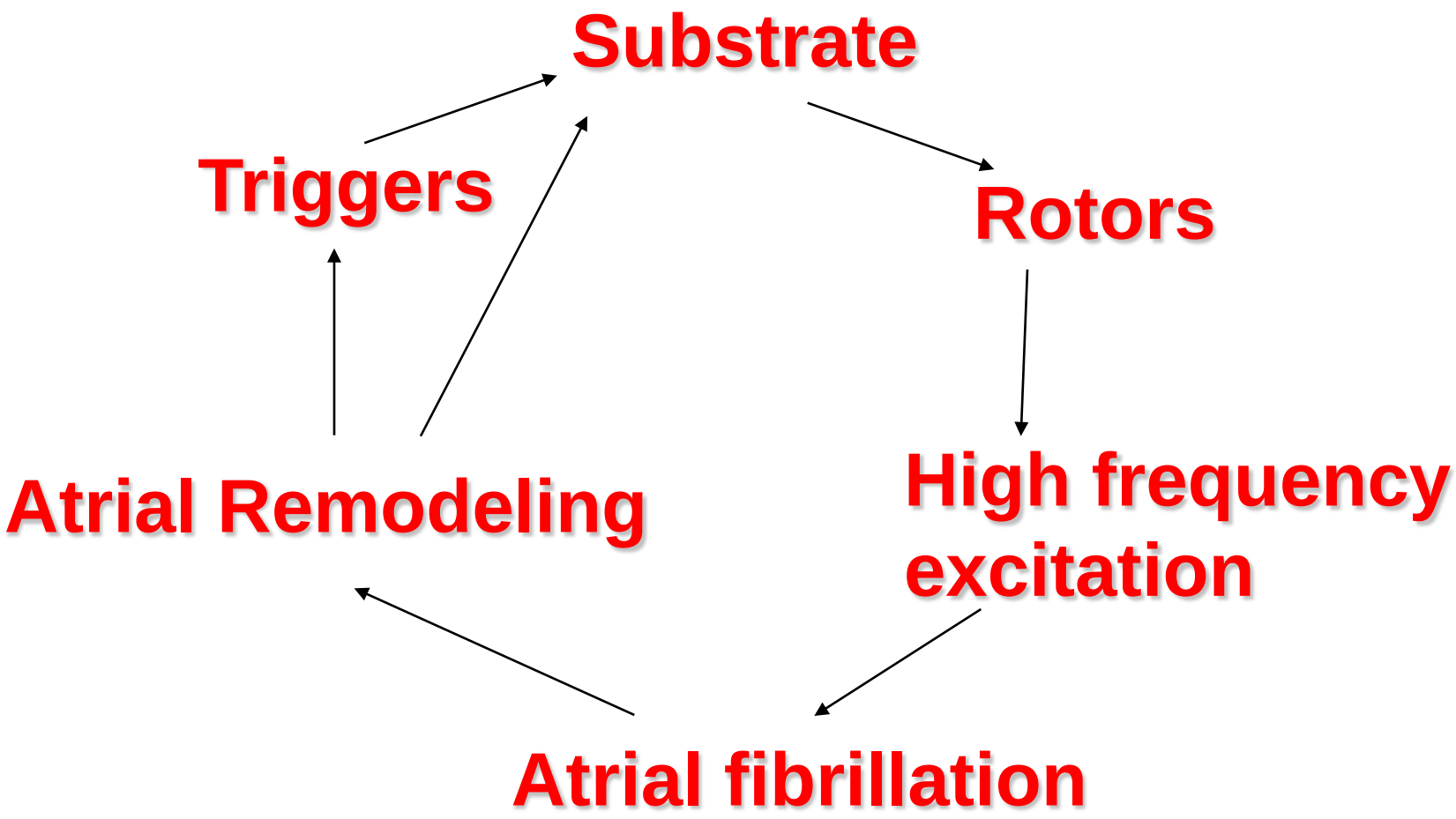
AF may lead to:

- 400% to 500% increased risk for stroke
- 200% increased risk for dementia,
- 300% increased risk for heart failure,
- 40% to 90% increased risk for overall mortality.

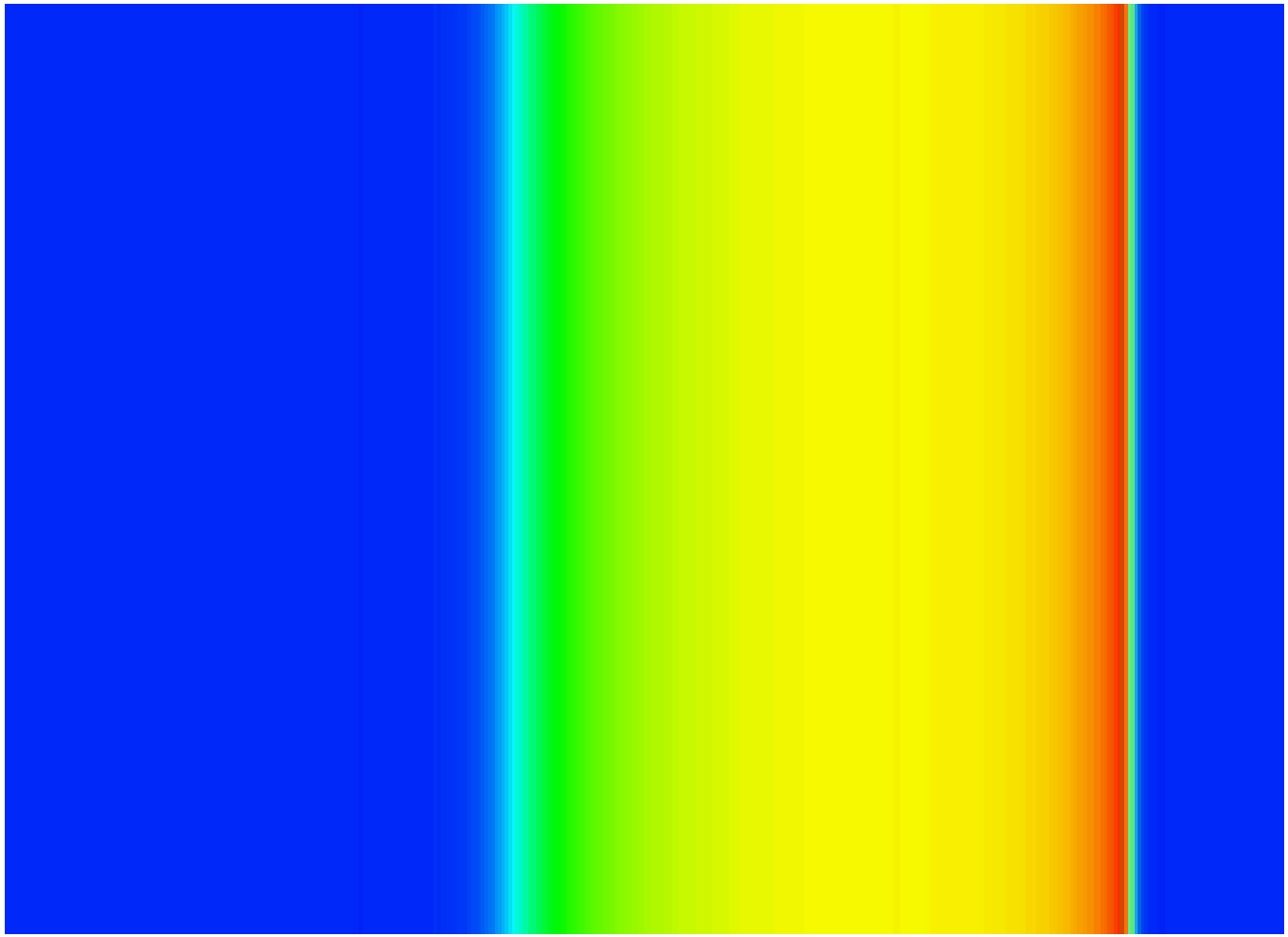
Benjamin E, et al, 2009

The mechanisms that maintain AF in the human heart are poorly understood.

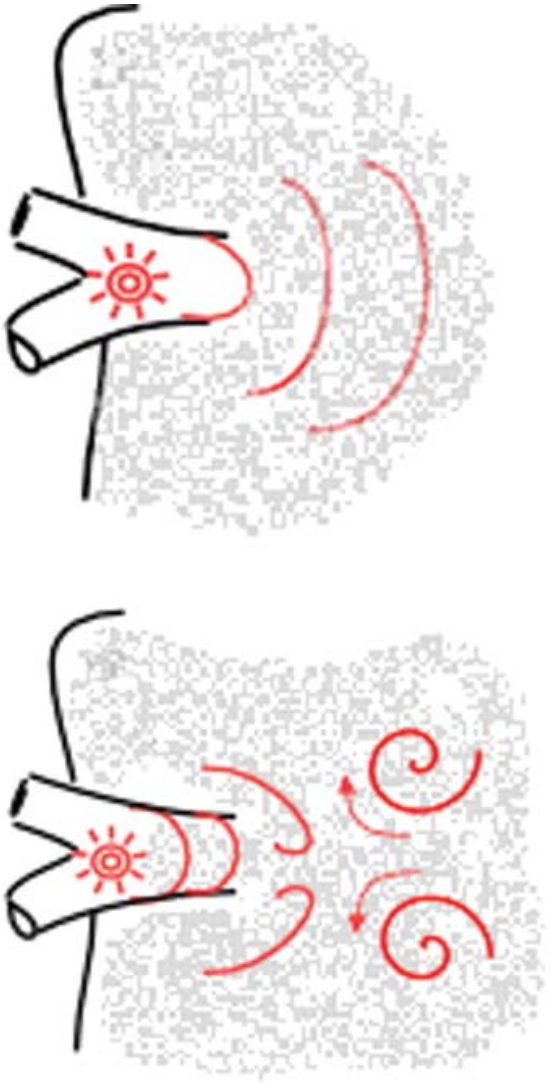
In particular, we simply do not know how chronic AF is maintained


Substrate
Rotors
High frequency excitation
Atrial fibrillation
Atrial Remodeling
Triggers

Rotor formation in a 2D model of electrically coupled cardiac cells

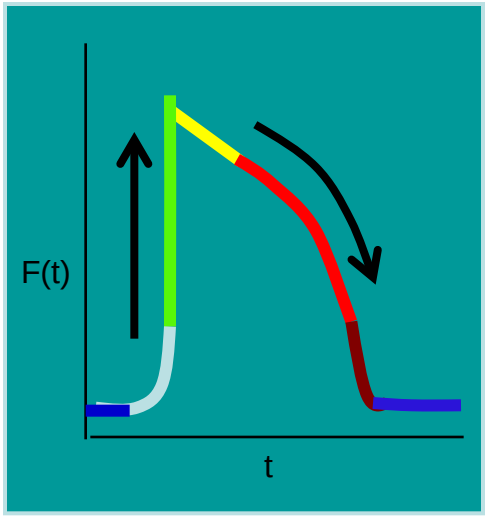


Rotors may be initiated by triggered discharges in a pulmonary vein



Cholinergic AF in isolated sheep heart

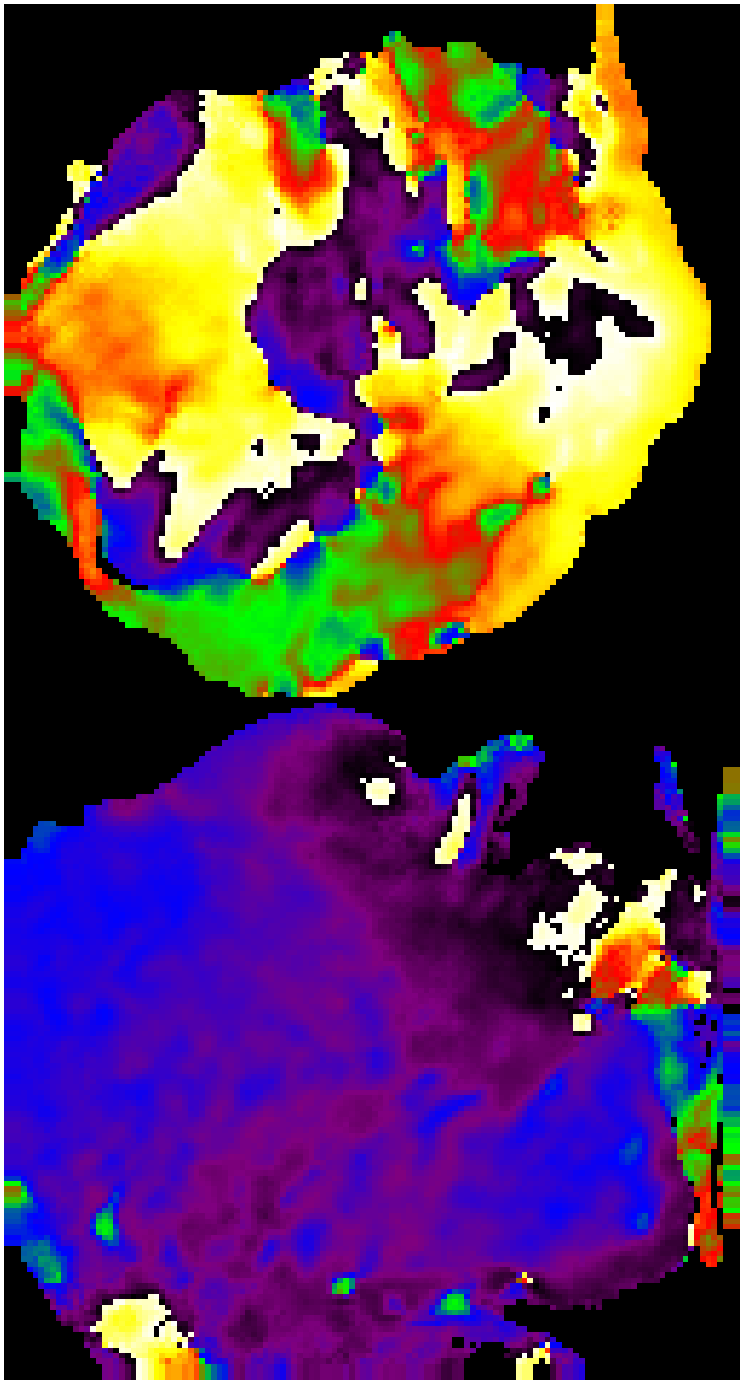
Phase Movie



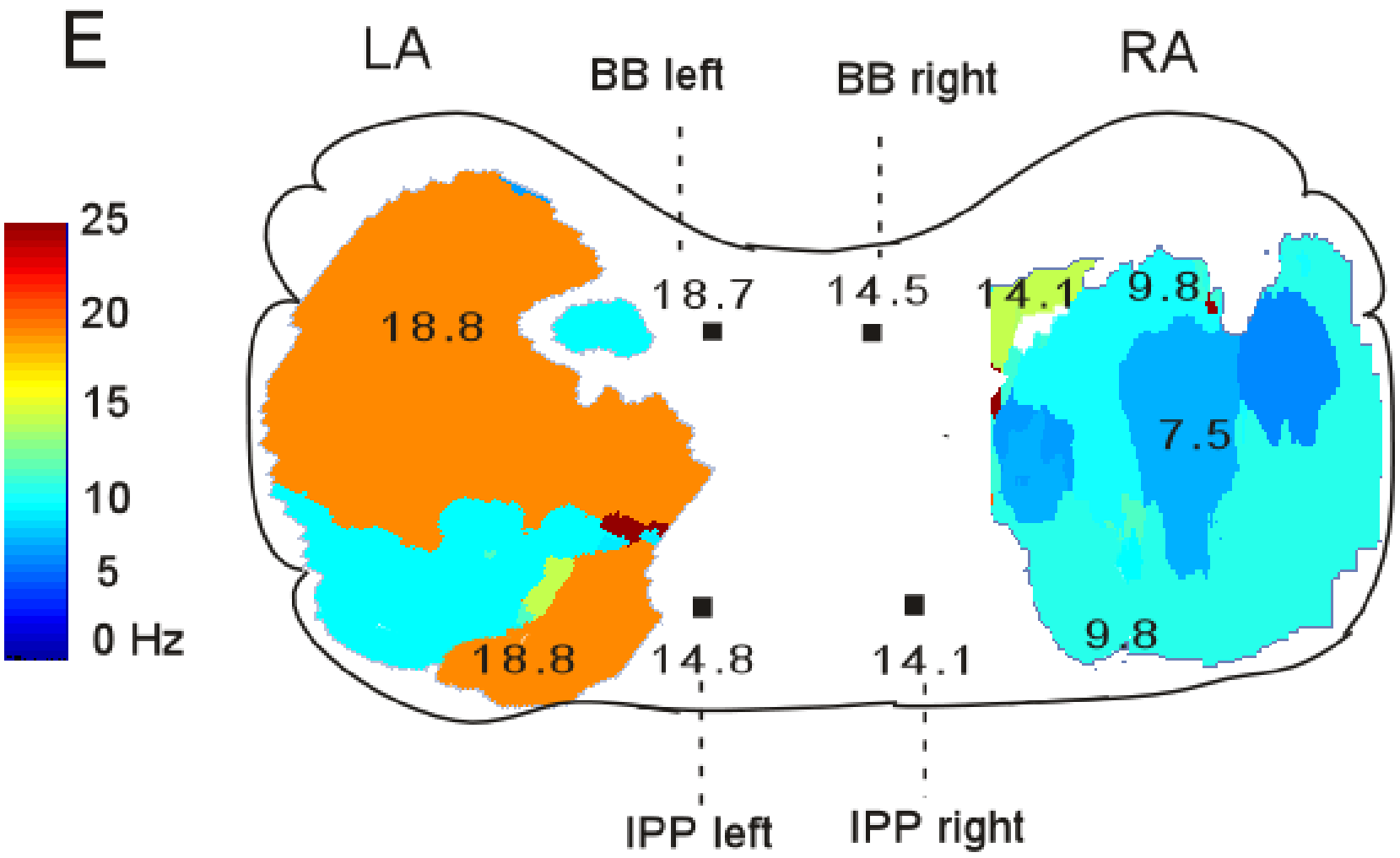
Potentiometric dye (Di-4-ANEPPS)
2 CCD cameras, 16,000 pixels each
200 frames/sec

RA

LA

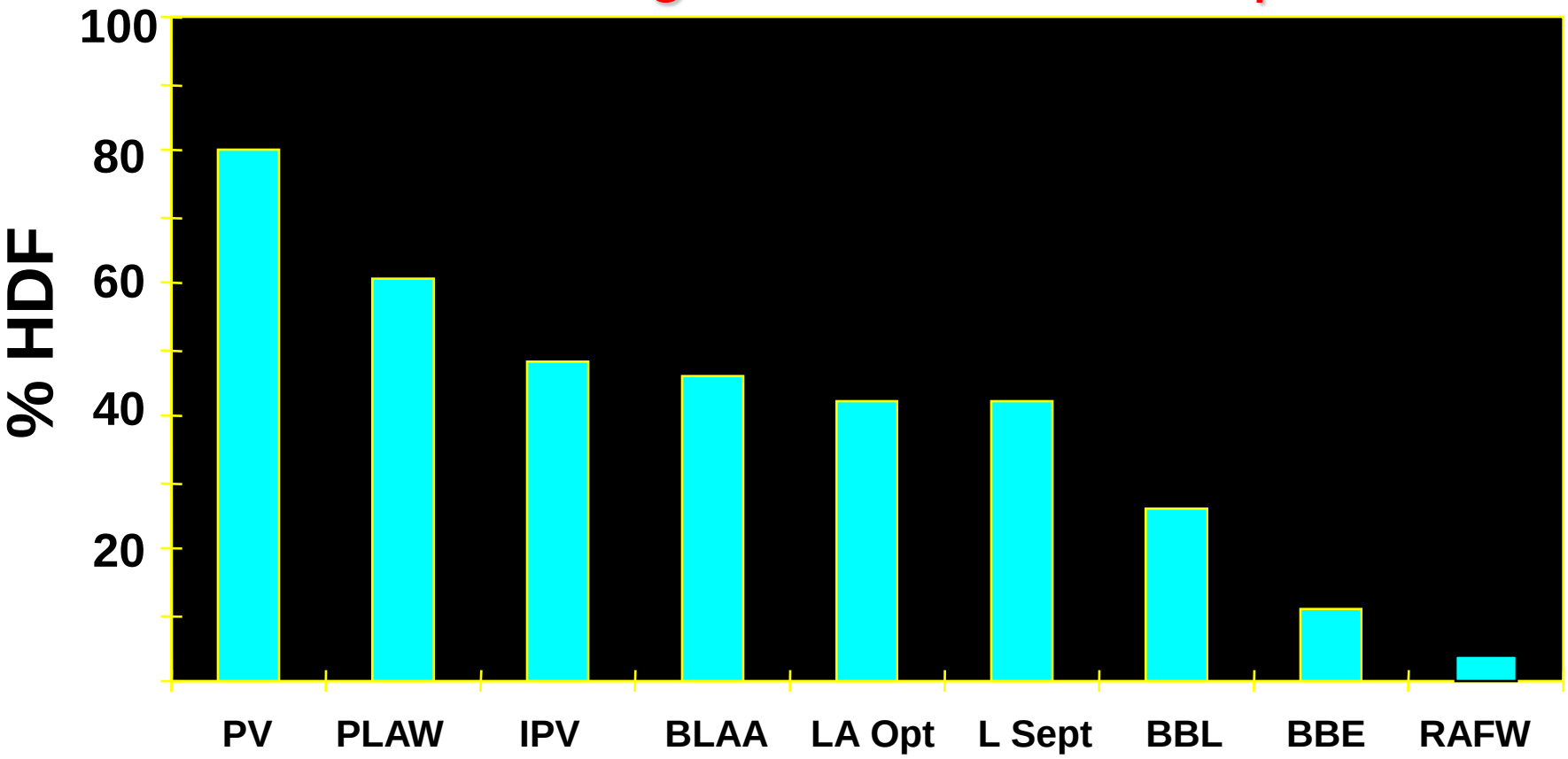


Dominant Frequency Map



Mansour et al, Circulation, 2001

Distribution of Highest Dominant Frequencies

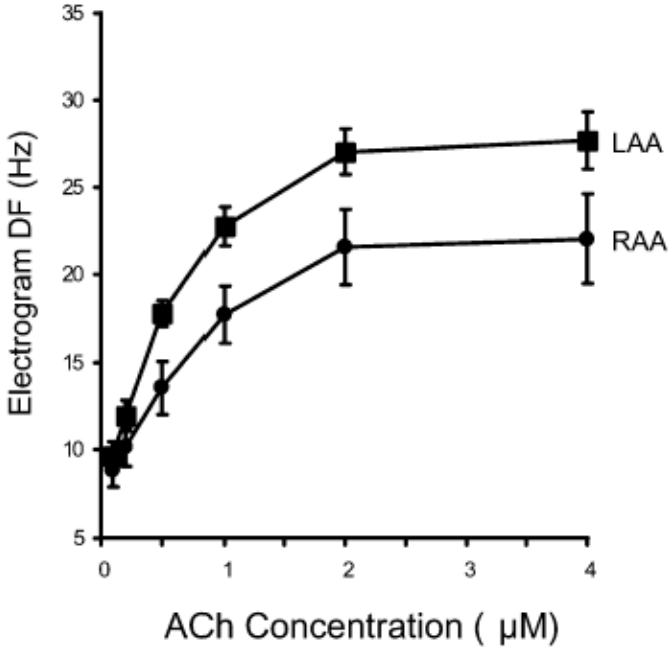


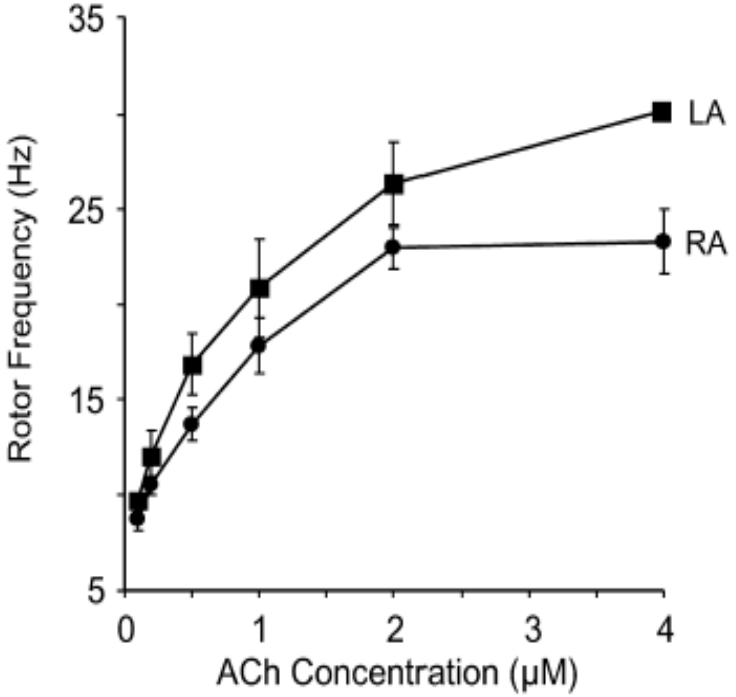
Mandapati R, et al *Circulation*. 2000;101:194-199

How do we explain the DF and DF gradients in this animal model?

Cholinergic atrial fibrillation: $I_{K,ACh}$ gradients determine unequal left/right atrial frequencies and rotor dynamics

Farzad Sarmast, Arun Kolli, Alexey Zaitsev, Keely Parisian, Amit S. Dhamoon, Prabal K. Guha, Mark Warren, Justus M.B. Anumonwo, Steven M. Taffet, Omer Berenfeld*, José Jalife





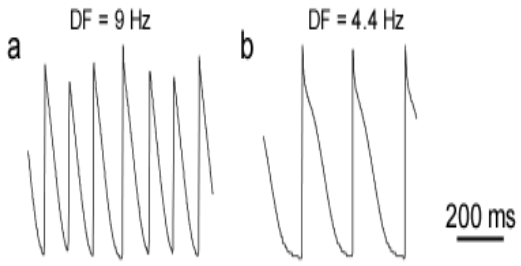
Cardiovascular Research 59 (2003) 863–873

How do we explain the DF and DF gradients in this animal model?

In silico 2D model of paroxysmal AF

0.5 μM ACh

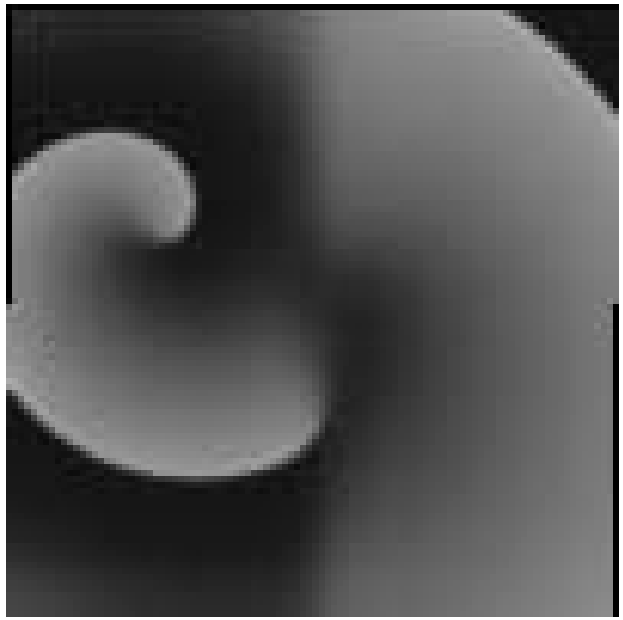
LA RA

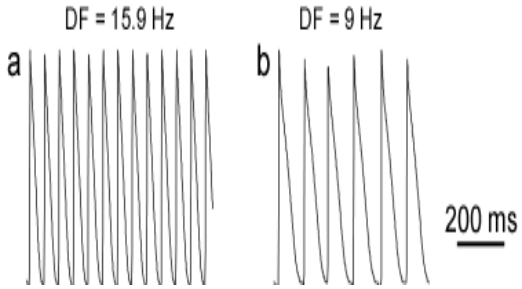


LA-to-RA gradient: 4.6 Hz

2.0 μM ACh

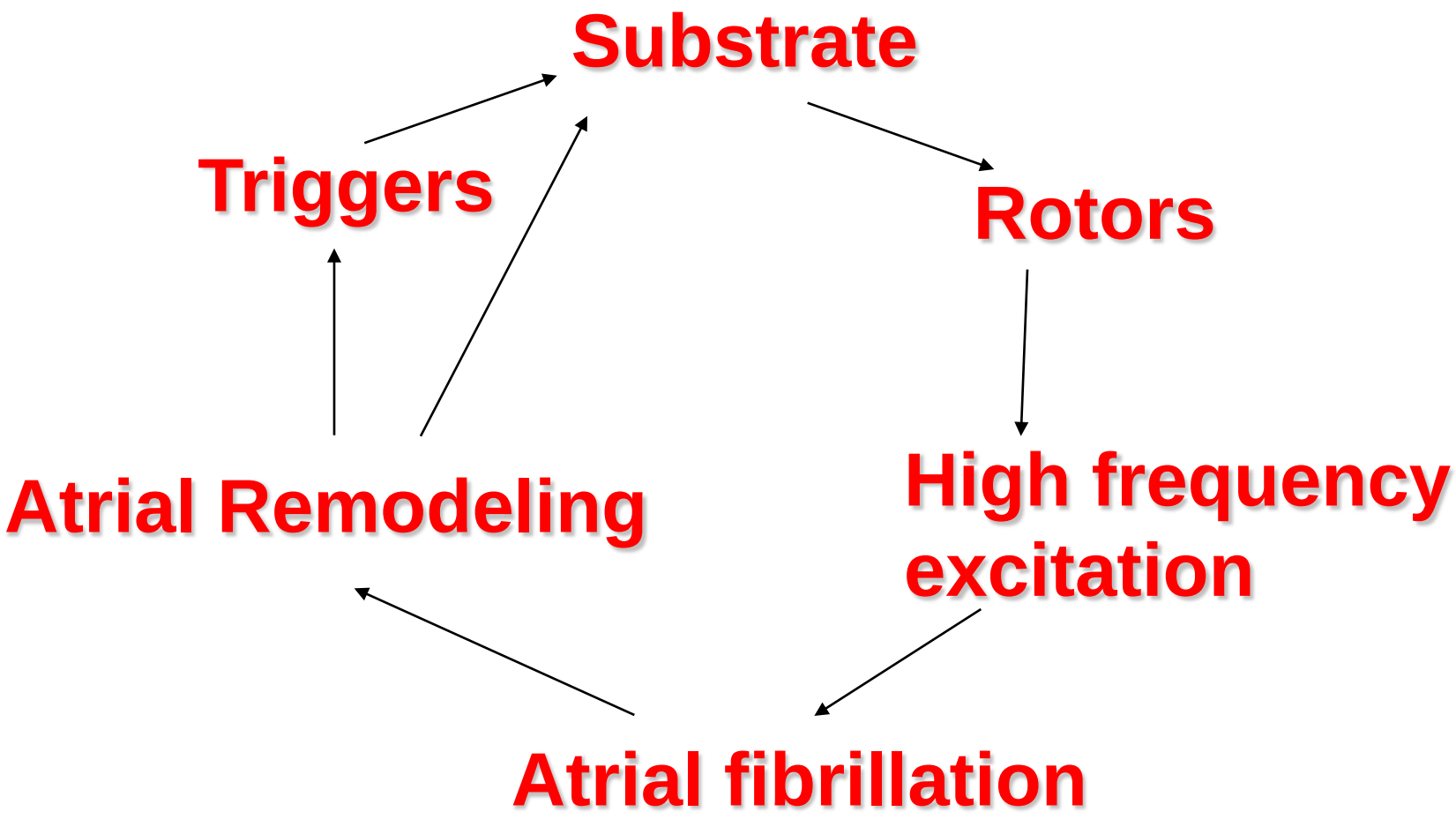
LA RA





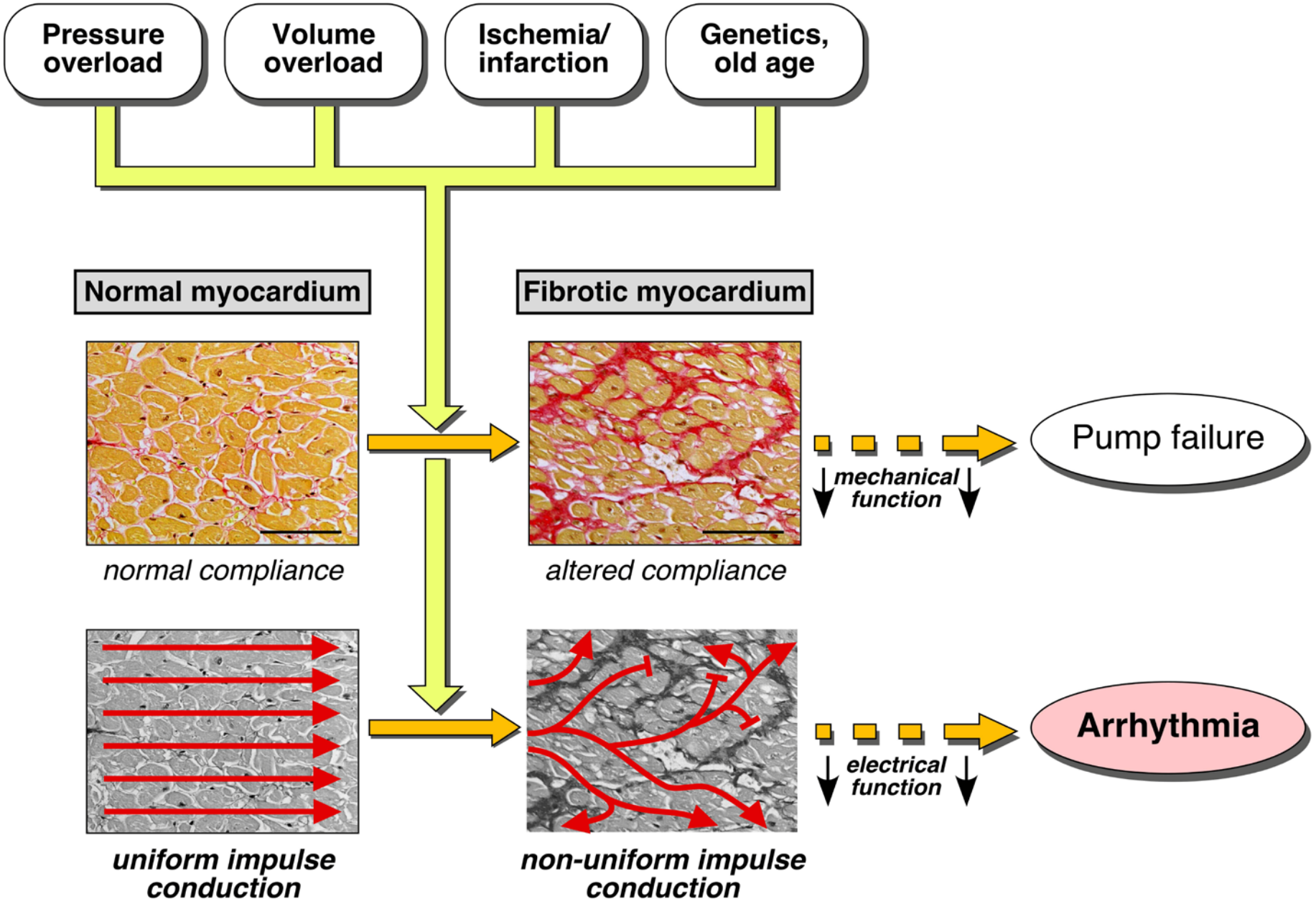
LA-to-RA gradient: 6.9 Hz

Which factors may contribute to atrial remodeling and to the multiplication of arrhythmic sources in chronic AF?


Substrate
Rotors
High frequency excitation
Atrial fibrillation
Atrial Remodeling
Triggers

Fibrosis is ubiquitous in the human heart and increases substantially in Chronic AF.

Cardiac fibrosis is a crucial determinant of myocardial heterogeneity and the propensity for reentrant arrhythmias



S. Rohr, Heart Rhythm in press

Questions:

Is fibrosis nothing more than a passive obstacle to wave propagation?

or

Do the fibroblasts that replace the myocytes during remodeling couple electrically with the myocytes and actively modulate propagation?

If so, how does fibrosis affect wave propagation complexity during AF?

and

How does fibrosis affect AF frequency?

Lecture Outline

Part I. Myocyte-Fibroblast interactions as an arrhythmogenic substrate in 2D

Part II. Fibrosis and AF in a heart failure model in the rabbit heart

Part III. Left atrial fibrosis in patients developing AF after cardiac surgery

Part I

A simplified biological model of 2-dimensional reentry in a controllable fibrotic substrate

Hypothesis

Cardiac fibrosis (i.e., replacement of myocytes with fibroblast):

- Increases the complexity of reentry dynamics
- Reduces reentry frequency
- Increases susceptibility to spontaneous reentry initiation.

Methods

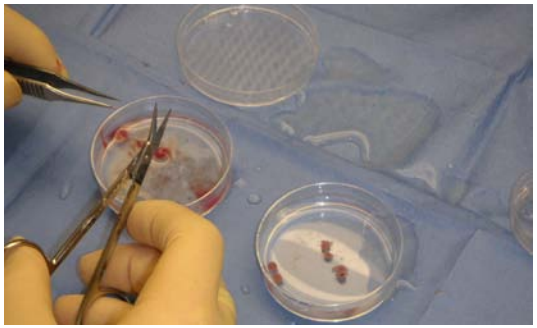
- Optical mapping of myocyte-myofibroblast co-cultures
- Numerical simulations

Cardiocyte cultures

1-2 days old neonatal rats

Heart removal

Ventricles minced



Enzymatic cell isolation

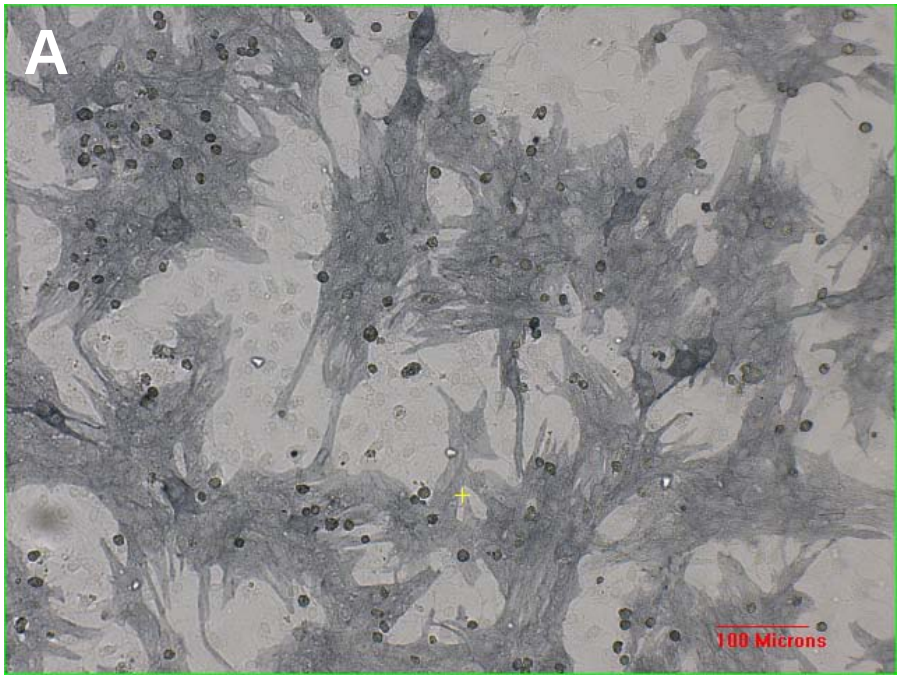
Differential Cell Pre-plating

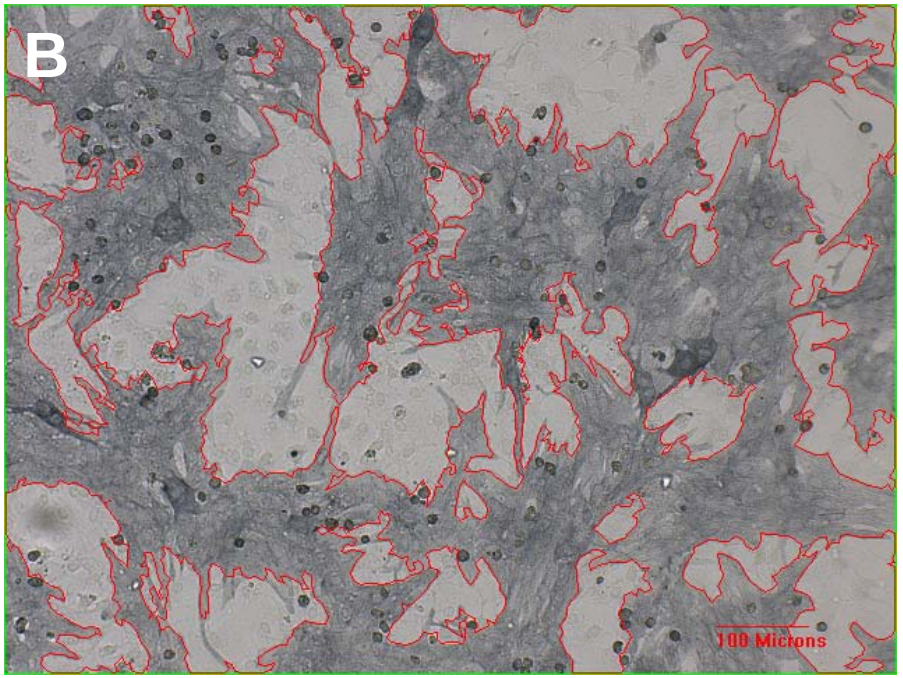
Myocytes

Fibroblasts

Quantification of areas of fibrosis.

neonatal rat co-culture after peroxidase stain.





A. Dark, cells positive for sarcomeric α-actinin (myocytes).

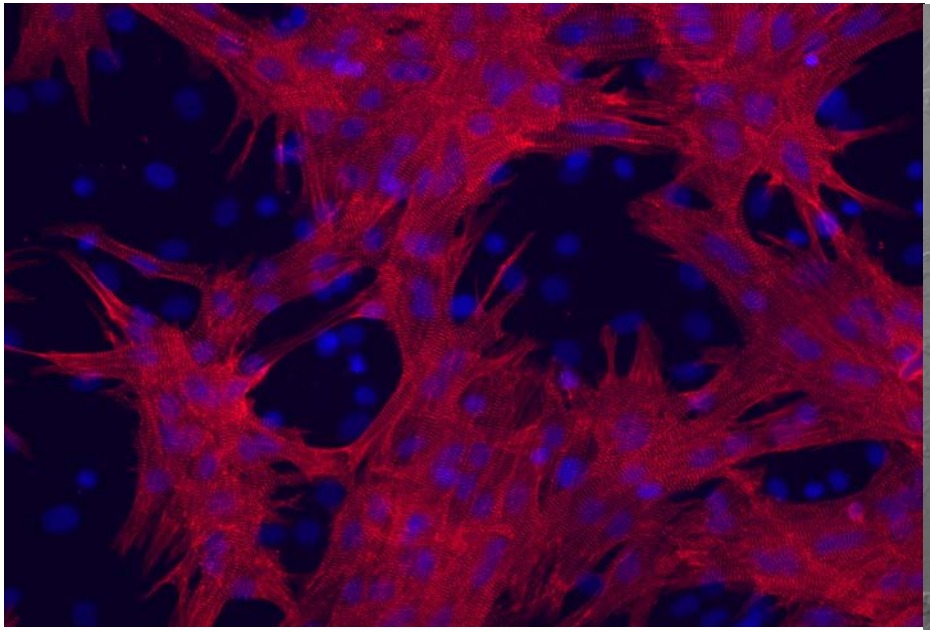
B. Areas of fibrosis were quantified using BioQuant software based on color contrast (26% of the area in this particular example).

Criteria used to assess the phenotype of cardiocytes after 6 days in culture using immunofluorescence microscopy and Western immunoblots

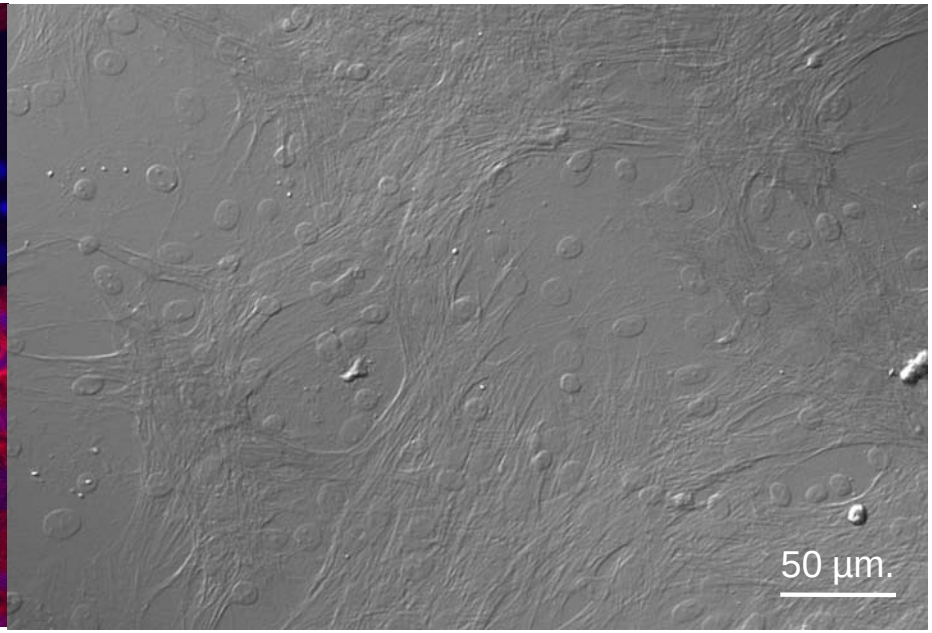
Anti-body	Myocytes	Endothelial cells	Smooth muscle cells	Fibroblasts	Myofibroblasts
SA	+	-	-	-	-
α-SMA	-	-	+	-	+
DDR2	-	-	-	+	+
VWF	-	+	-	-	-

Antibodies against: sarcomeric α-actinin (SA), α-smooth muscle actin (α-SMA), discoidin domain receptor 2 (DDR2) and Von Willebrand Factor (VWF)

Confluent Co-Cultured Monolayer Confirming Sarcomeric α Actinin Antibody Specificity to Myocytes

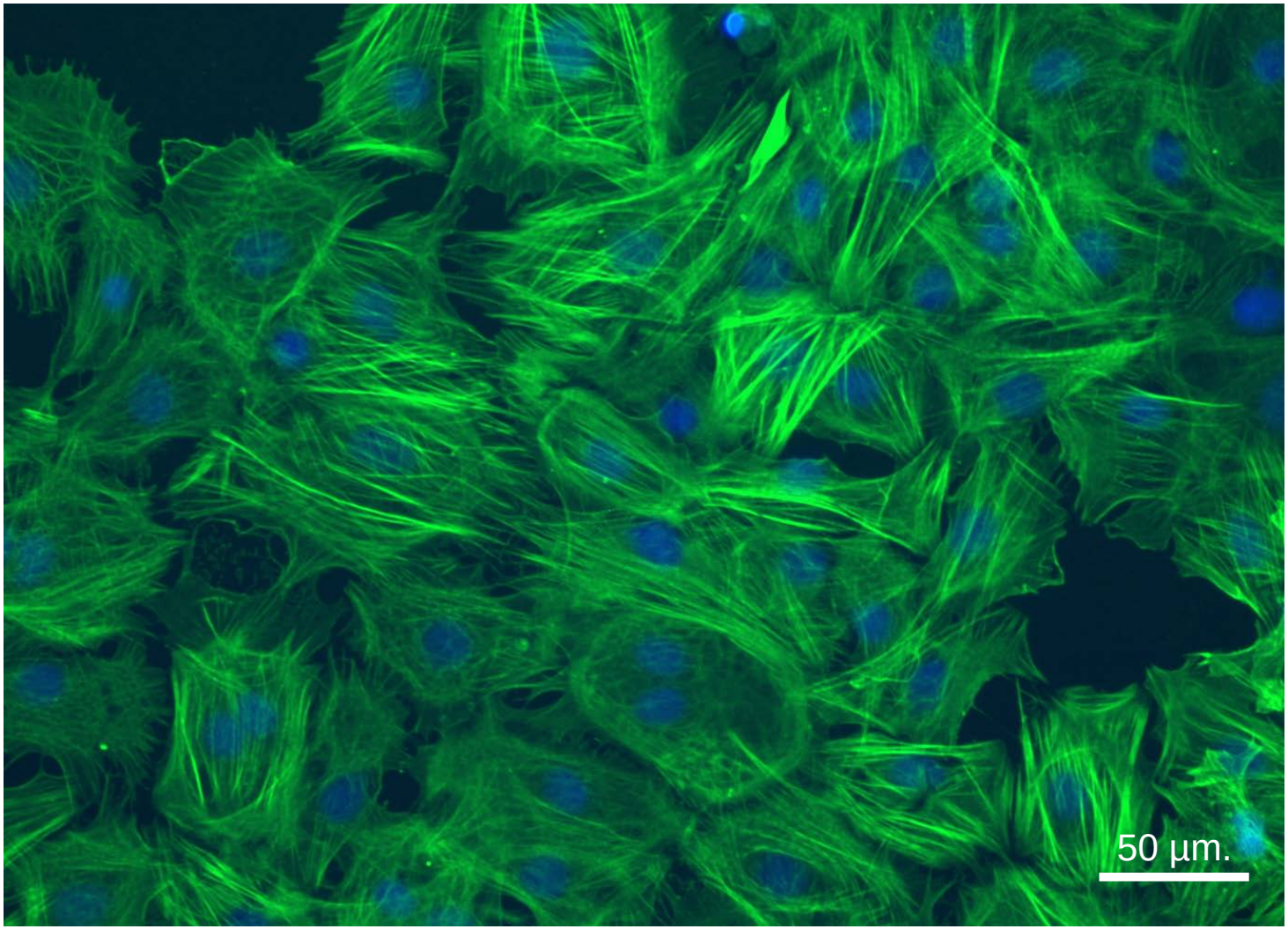


sarcomeric α actinin



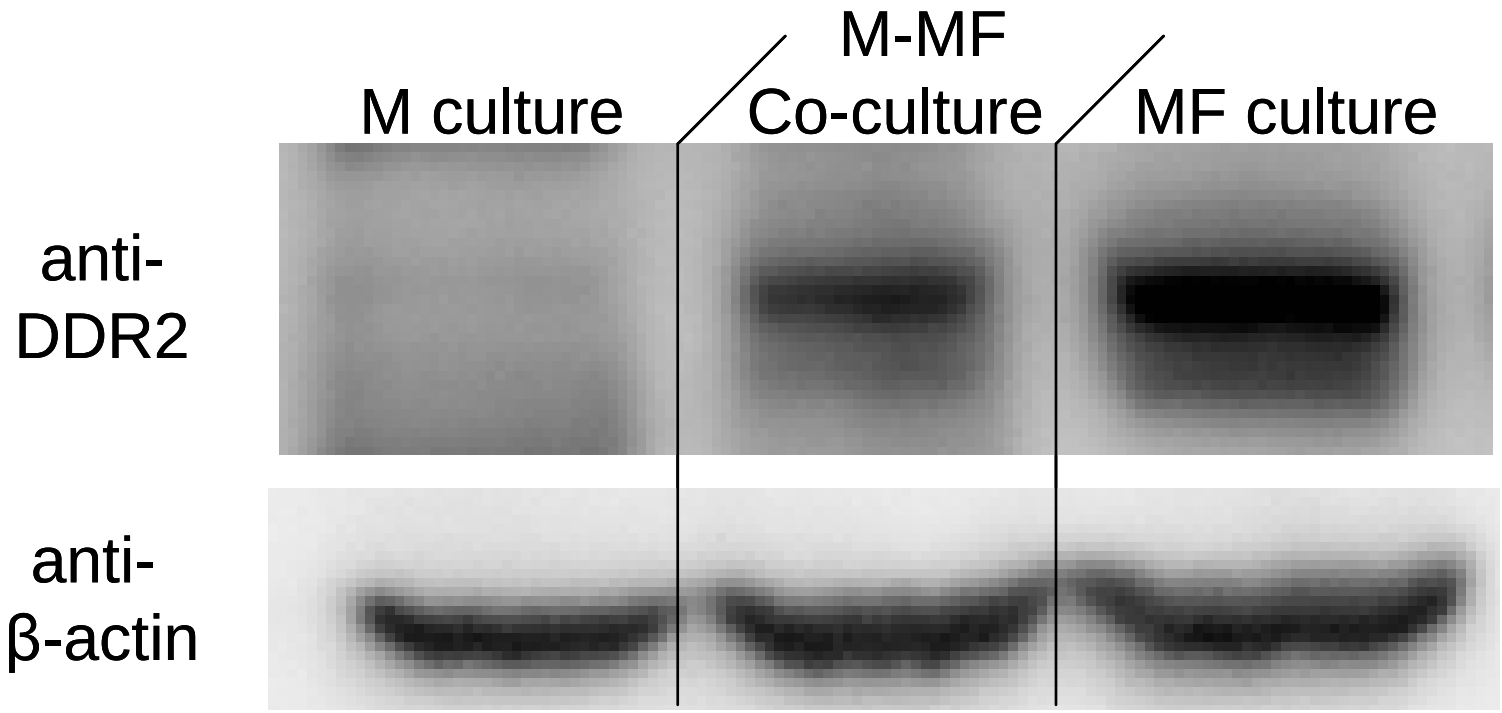
phase contrast

Non-myocytes expressing smooth muscle actin (α-SMA) in culture.



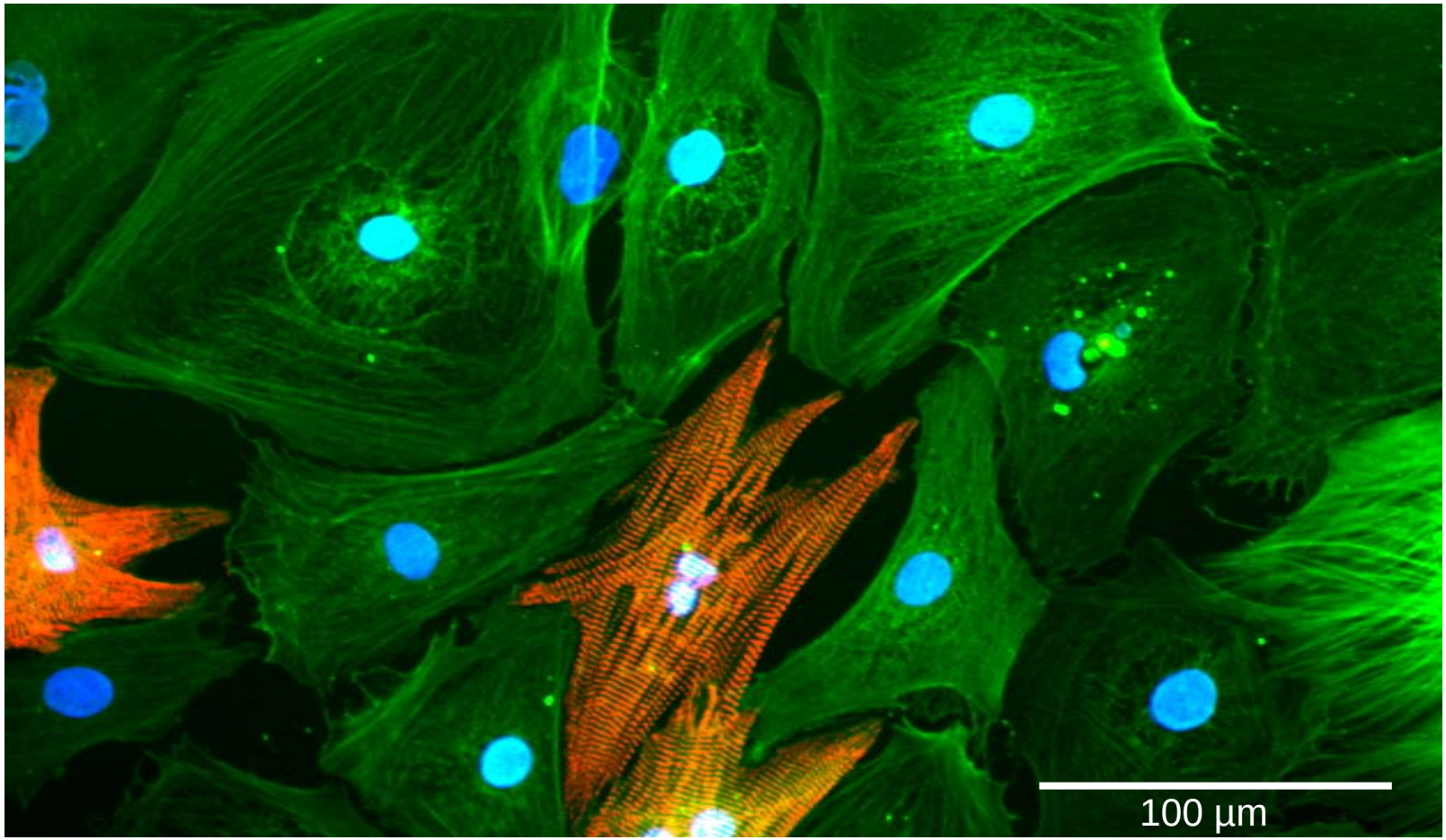
Data obtained from 10 myofibroblast-rich monolayers immunostained for αSMA showed that non-myocytes expressed α-SMA in a stress fiber related pattern

Western immunoblot showing DDR2 expression only in cultures containing myofibroblasts.



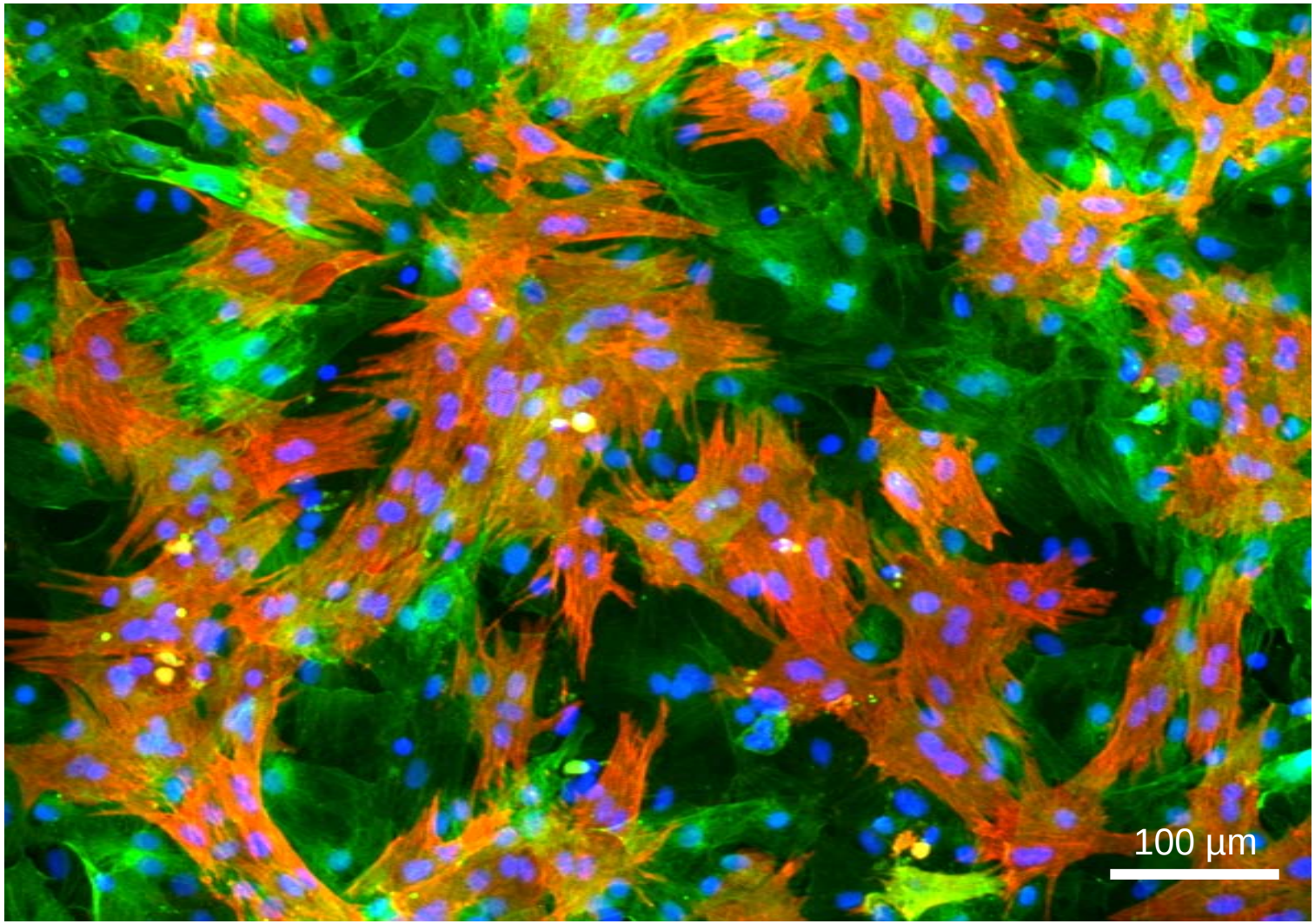
M = myocytes, MF = myofibroblasts.

confluent monolayer triple-stained for sarcomeric α-actinin (red), α-SMA (green), and DAPI nuclear probe (blue)



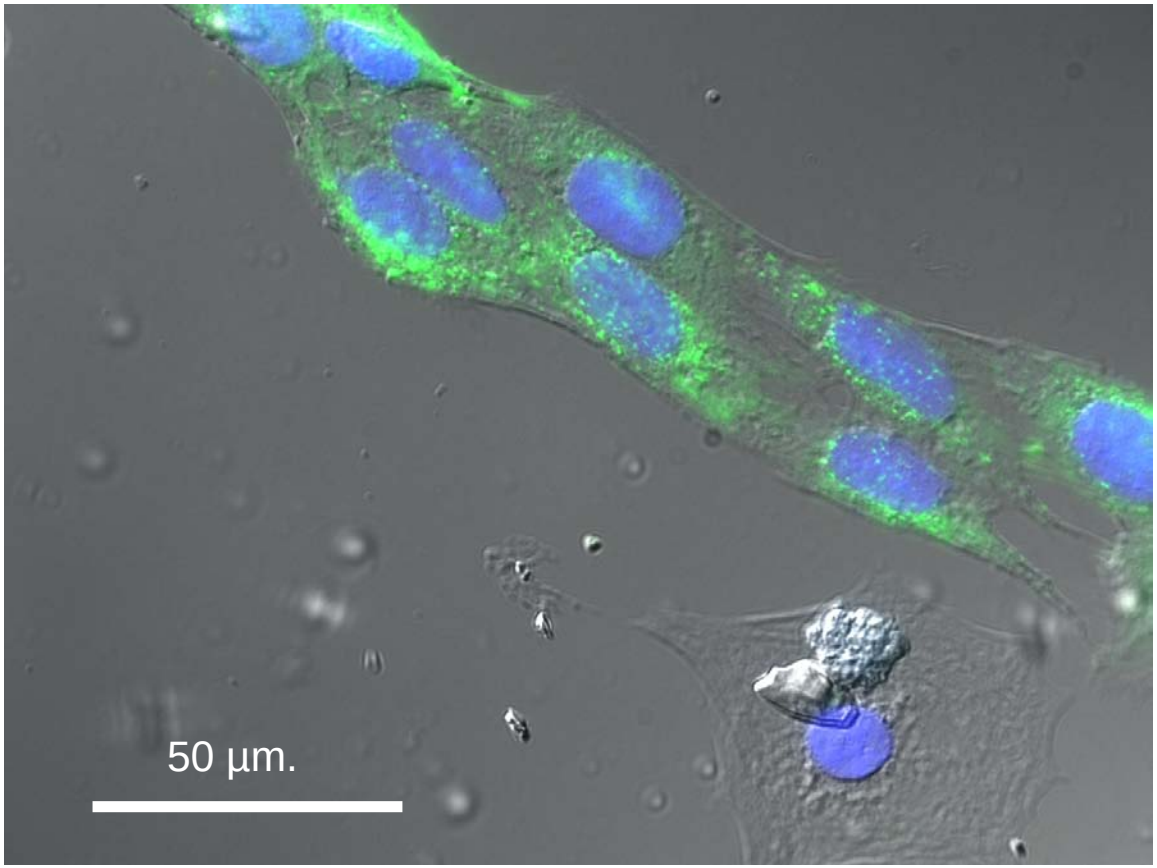
Cells are either cardiomyocytes (red) or myofibroblasts (green)

confluent monolayer triple-stained for sarcomeric α-actinin (red), α-SMA (green), and DAPI nuclear probe (blue)



Cells are either cardiomyocytes (red) or myofibroblasts (green)

Expression of Von Willebrand Factor (green).



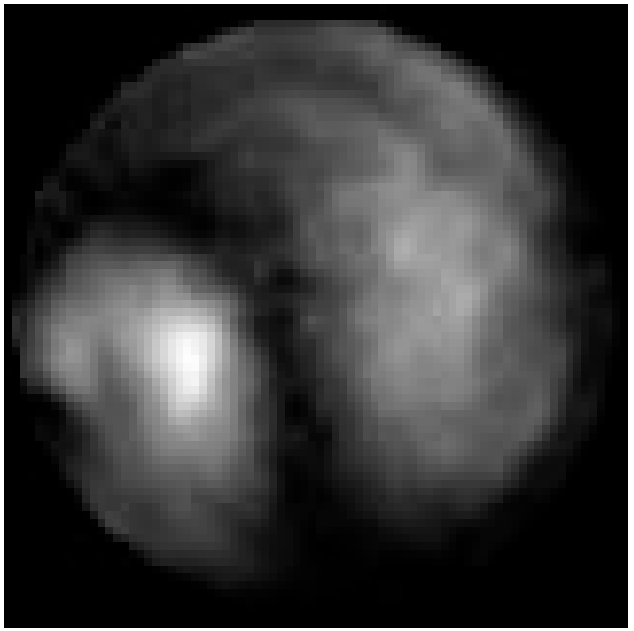
(Blue, nuclear specific DAPI stain)

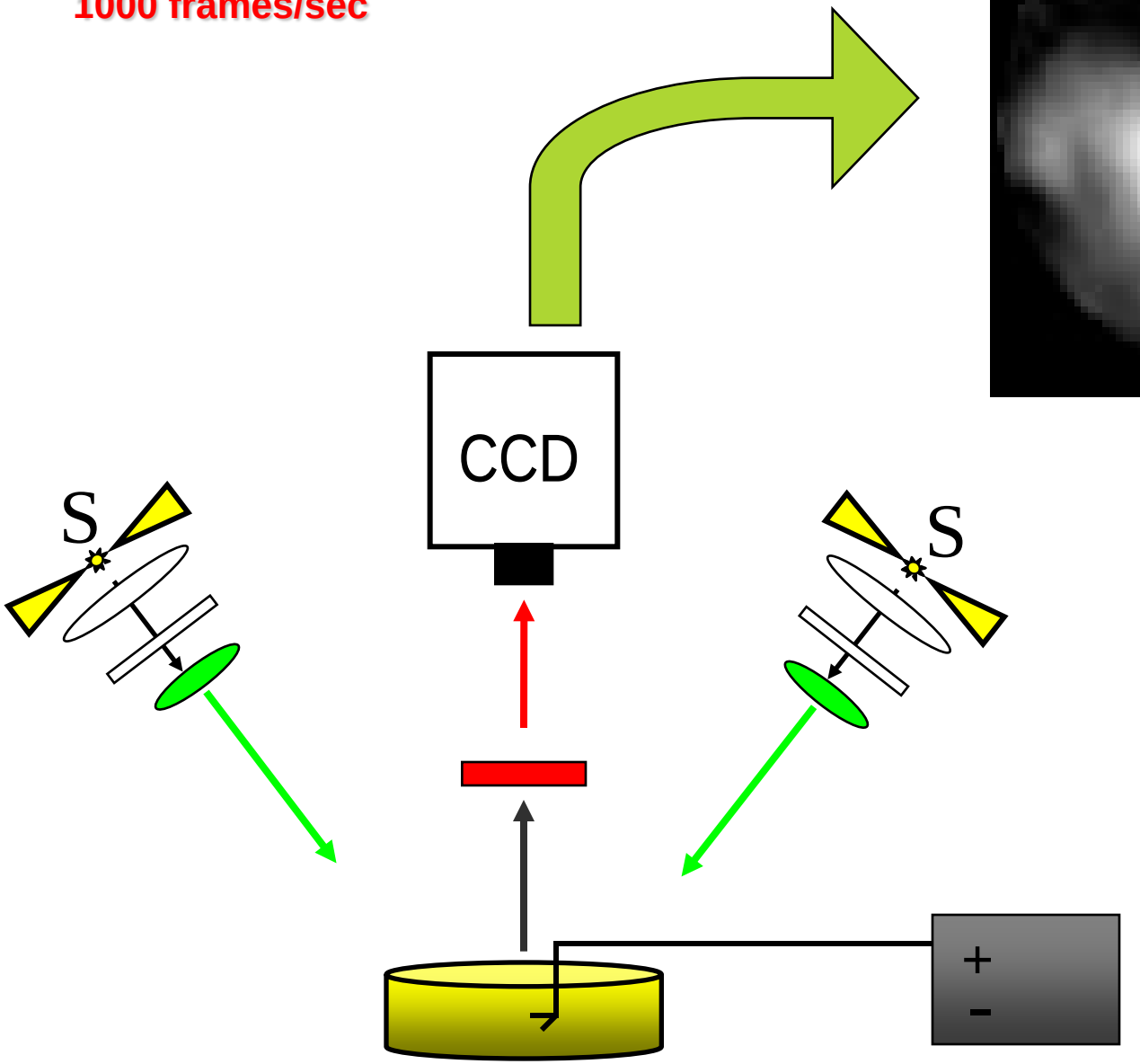
Out of thousands of cells examined, only the 9 cells were identified as endothelial cells.

Question:

How does the presence of randomly distributed myofibroblasts affect myocyte-to-myocyte electrical propagation and reentry dynamics in the monolayer?

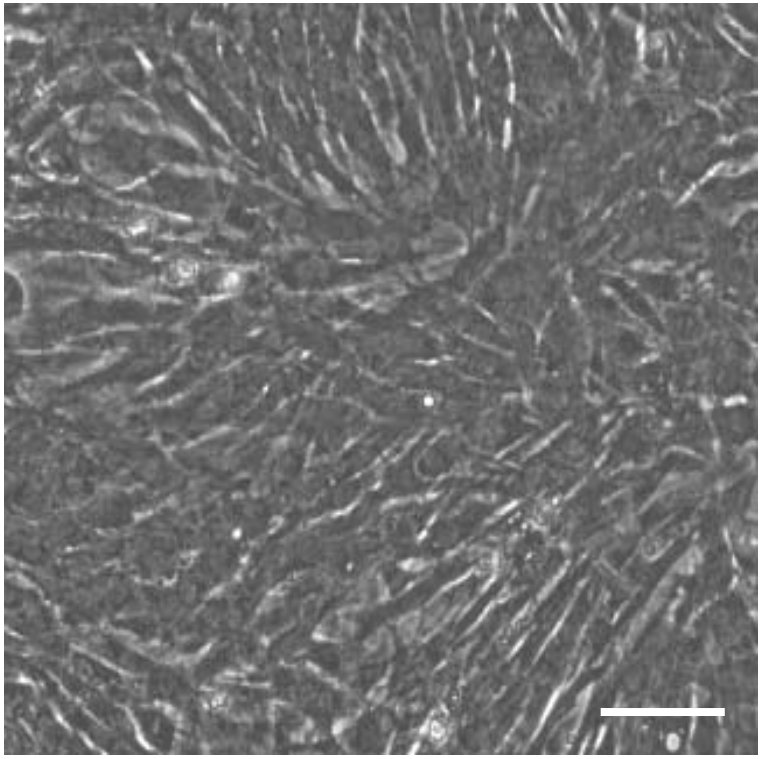
Voltage-sensitive Dye Di-8-ANEPPS
CCD camera (80x80 pixels)
1000 frames/sec



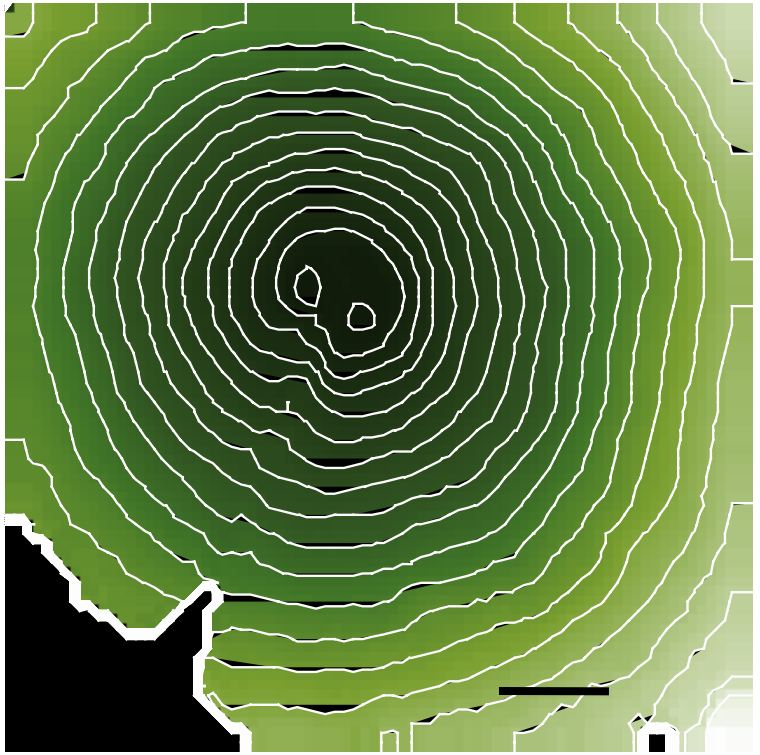


Propagation of Electrical Waves in Confluent Monolayer

A

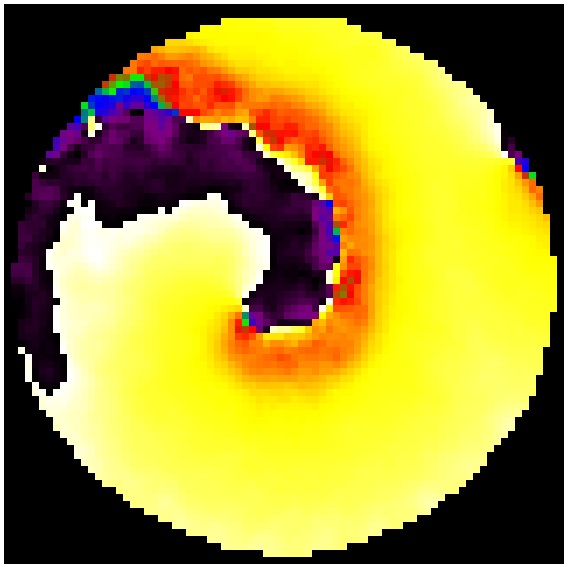


B

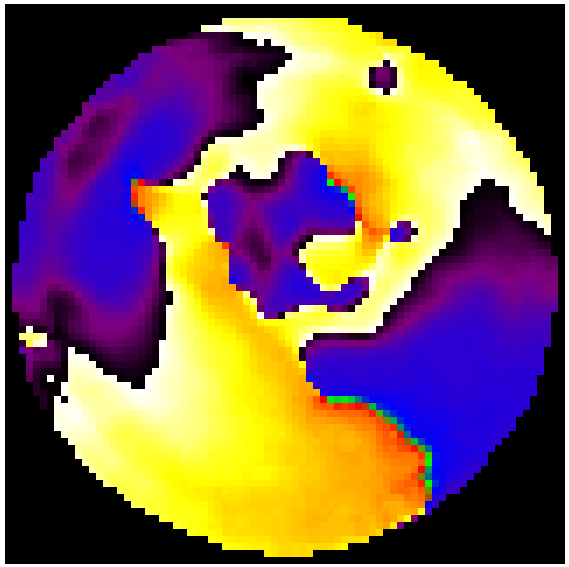


Di-8-ANEPPS
CCD camera (80x80 pixels)
1000 frames/sec

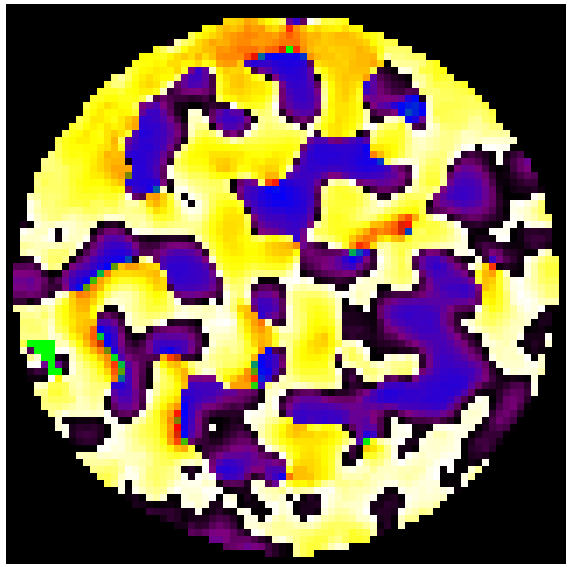
Effects of randomly distributed fibrosis on reentry dynamics



5% fibroblasts

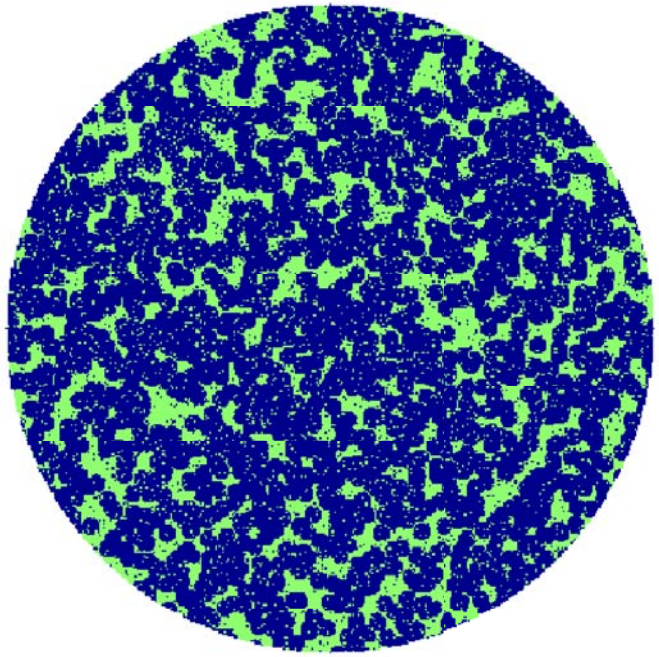


30% fibroblasts



60% fibroblasts

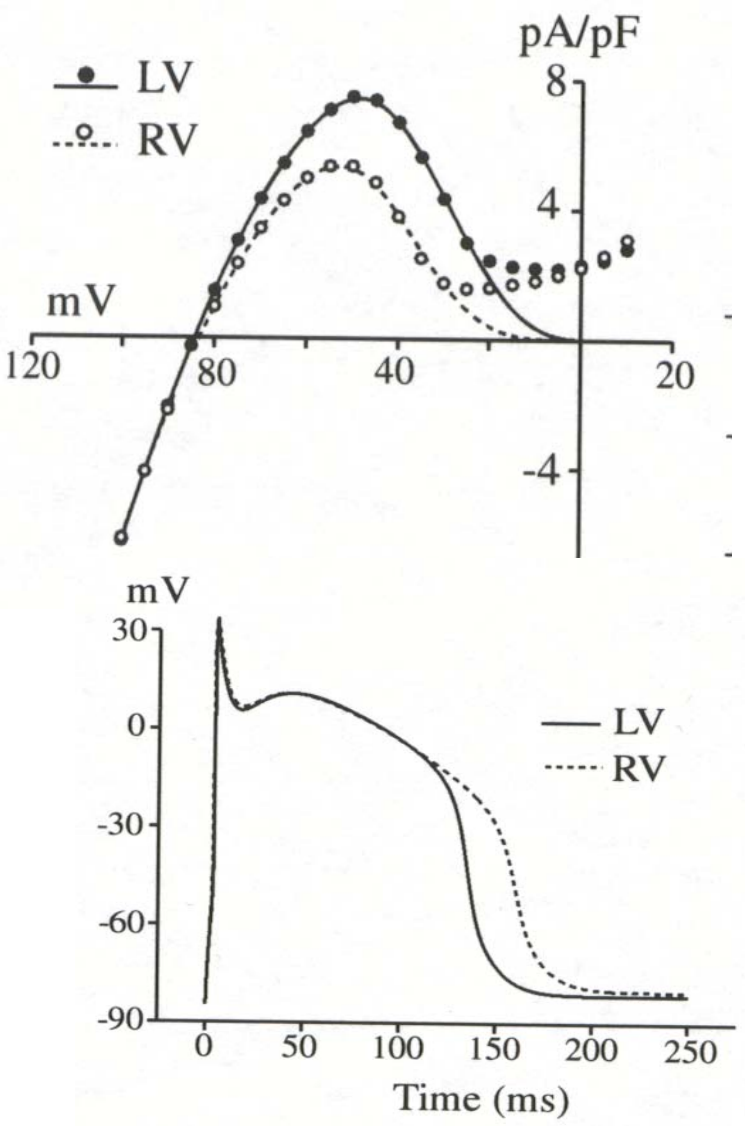
Computer simulations using Luo & Rudy model. 2-D sheets (35 x 35 mm) with randomly distributed fibrosis and various degrees of myocyte-fibroblast coupling



Areas of fibrosis were quantified in neonatal rat myocyte monolayer using BioQuant software based on color contrast

Electrophysiological Properties of Myocytes

- Excitable (active) cells.
- Low membrane resistance.
- High V_{rest} (~-85mV)

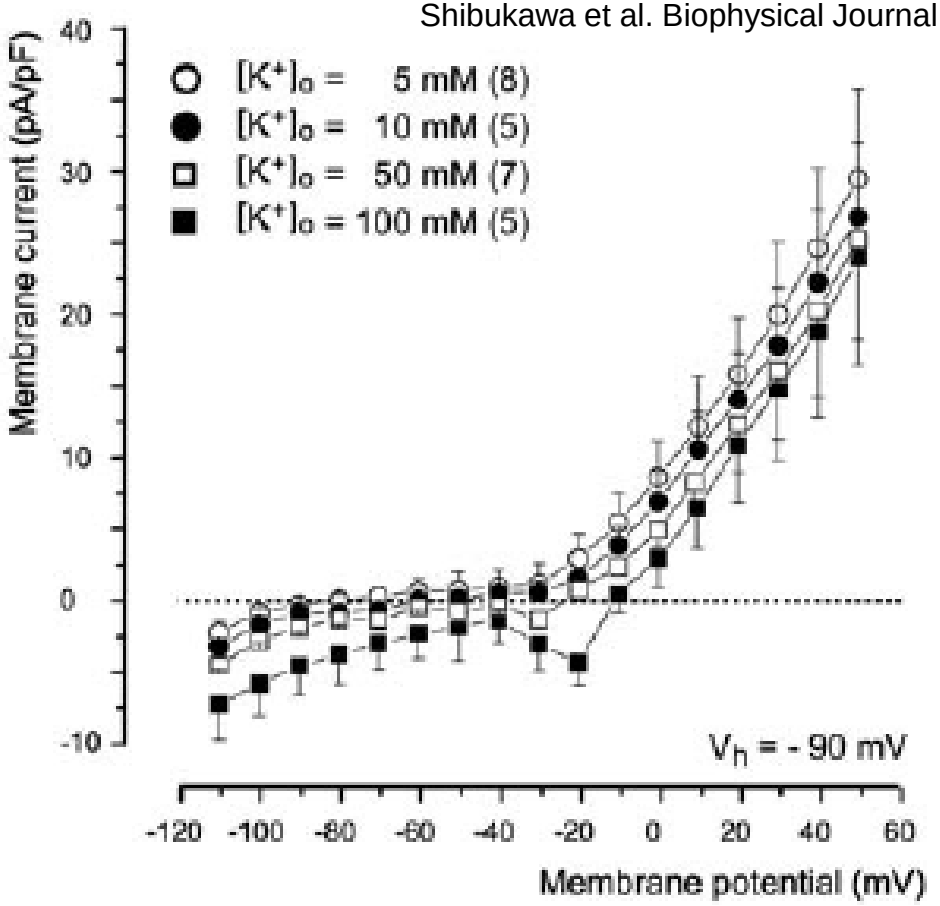


Samie et al. Circ Res. 2001

Electrophysiological Properties of Fibroblasts

- Non-excitable (passive) cells.
- High membrane resistance.
- Low V_{rest} (~-38 mV)
- Heterocellular coupling:

Shibukawa et al. Biophysical Journal 2005



$$D_f = \kappa_f D$$

$$D_{fibroblast/myocyte} = (2D \cdot D_f / D + D_f) = (2\kappa_f / 1 + \kappa_f) D$$

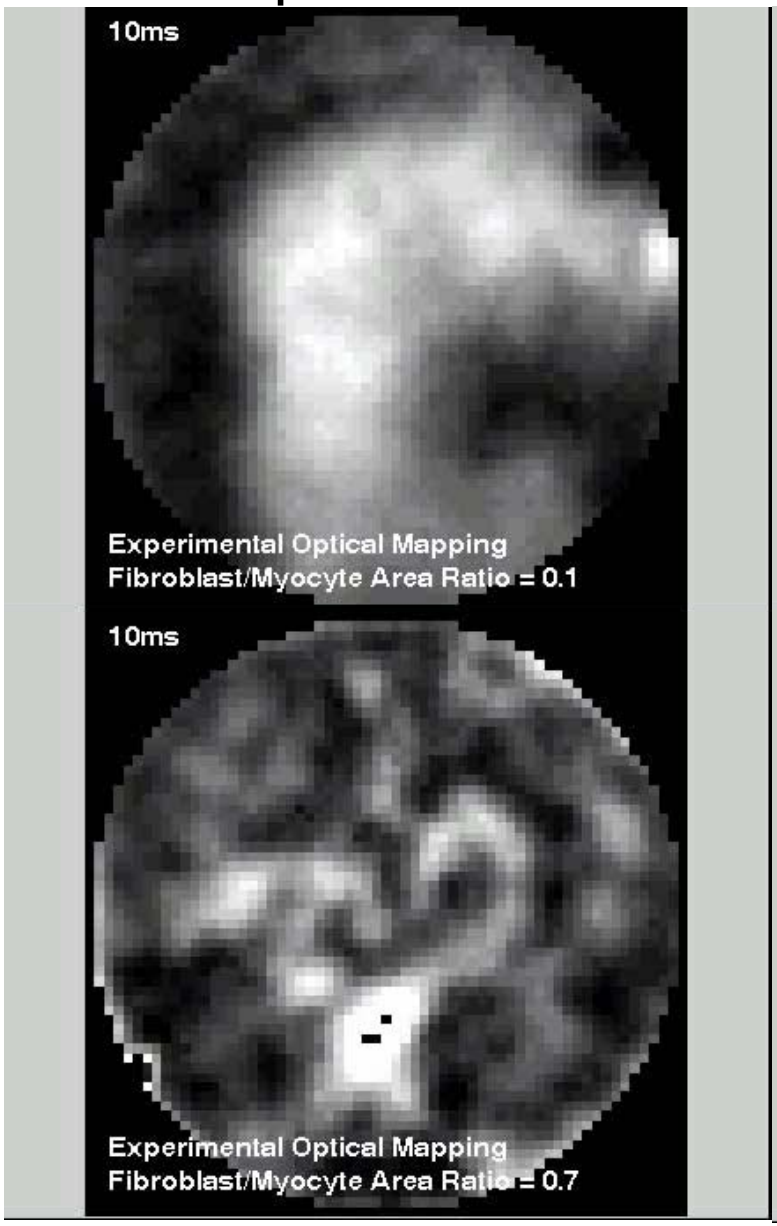
Effects of Changing Fibroblast/Myocyte Ratio

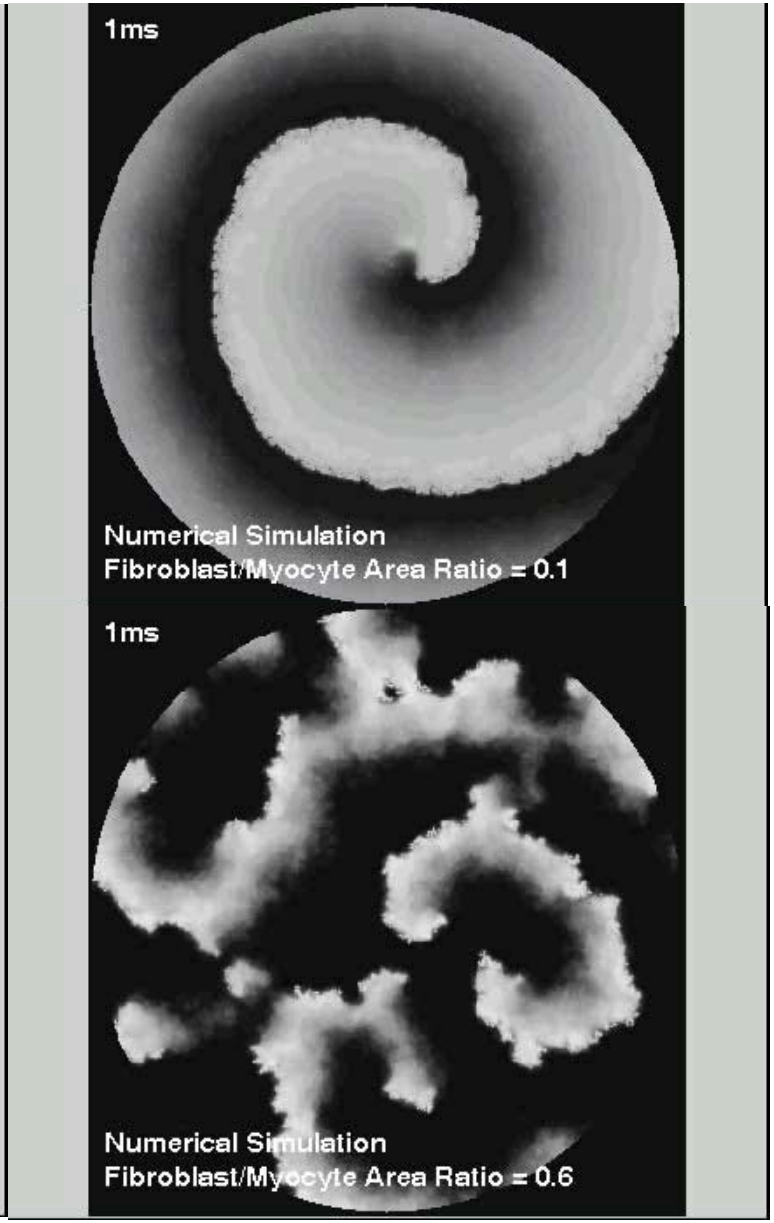
Experimental

Numerical

10% Fibroblasts

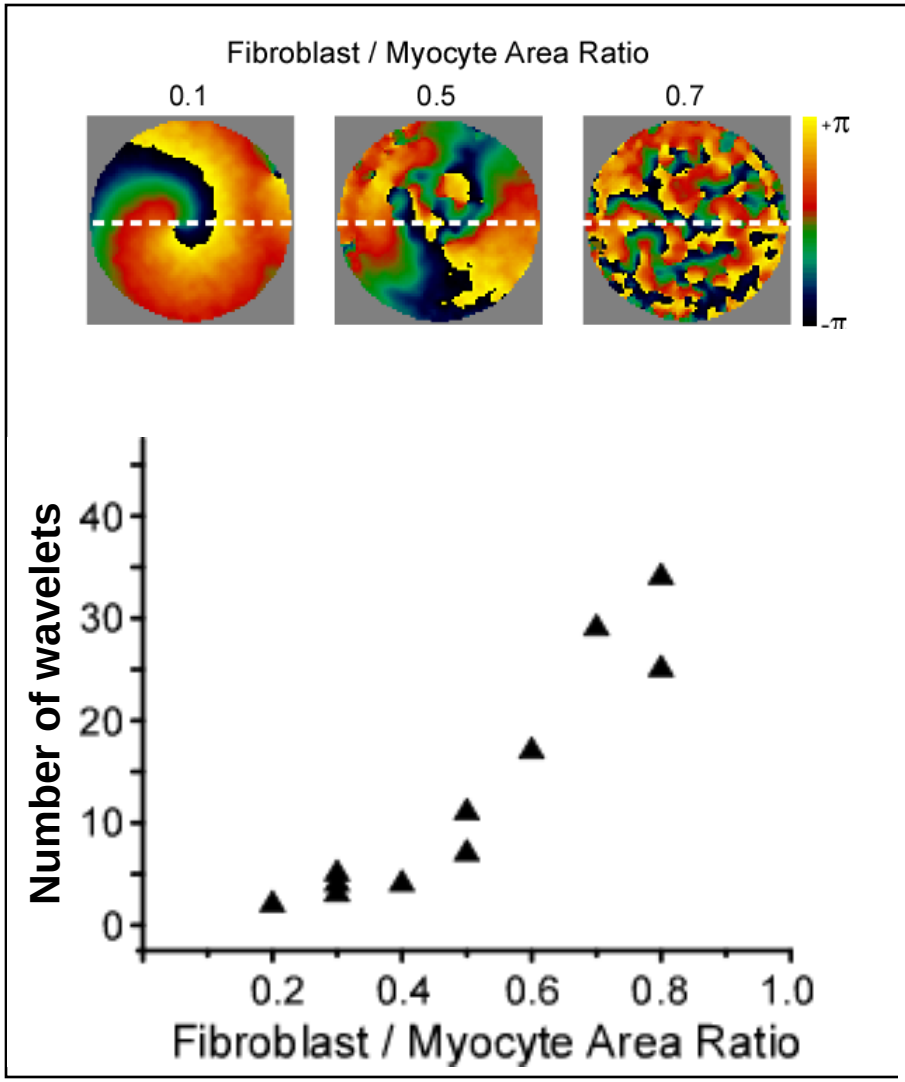
70/60% Fibroblast



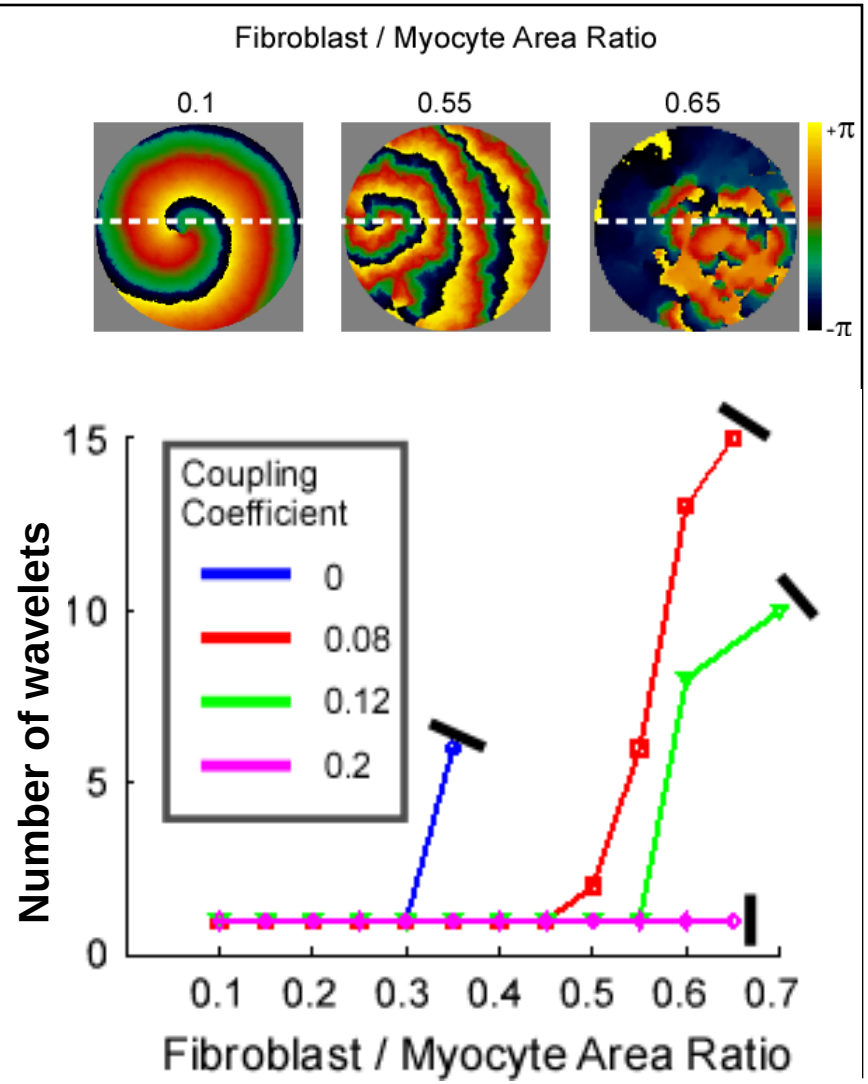


Number of Wavelets

Experimental



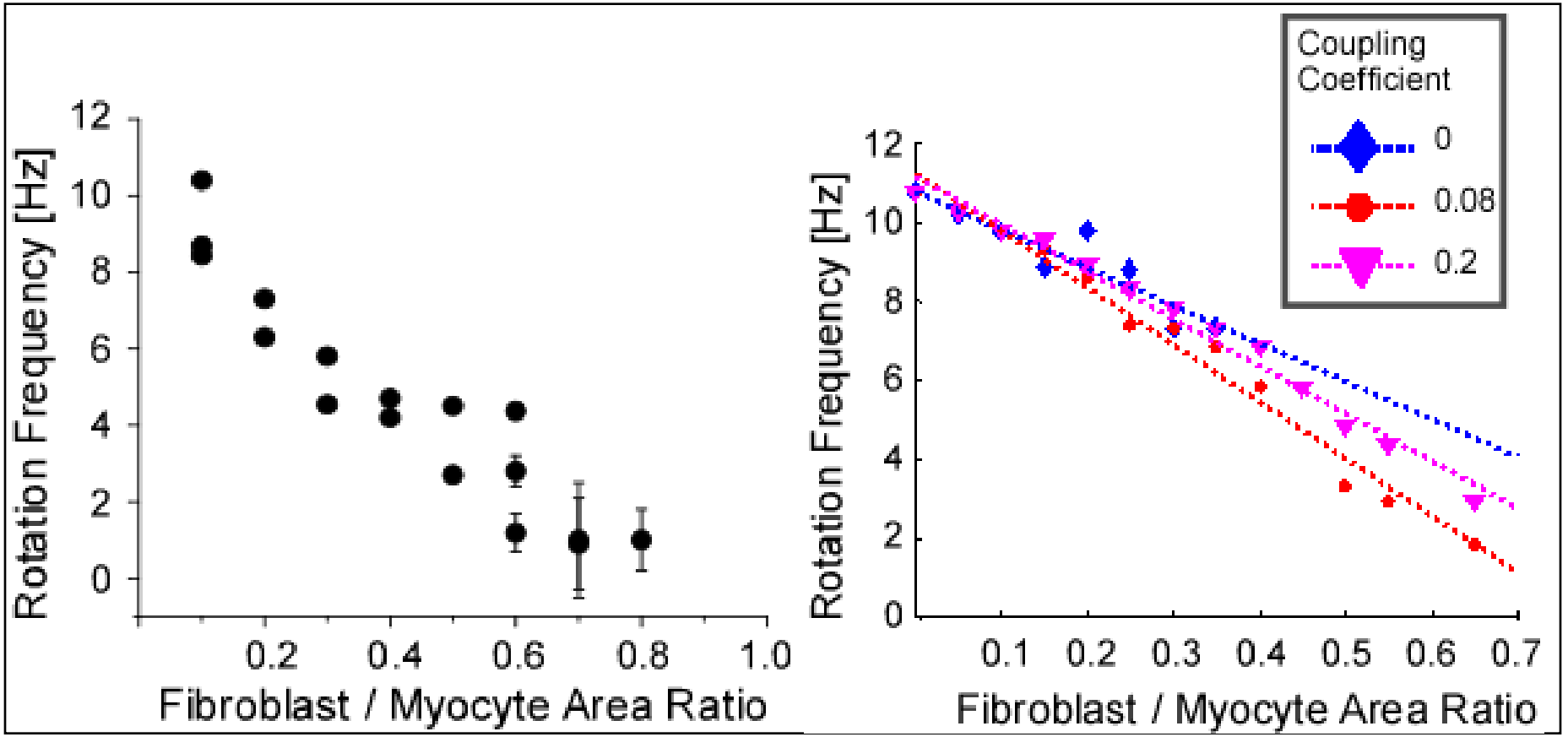
Numerical



Frequency of Rotation

Experimental

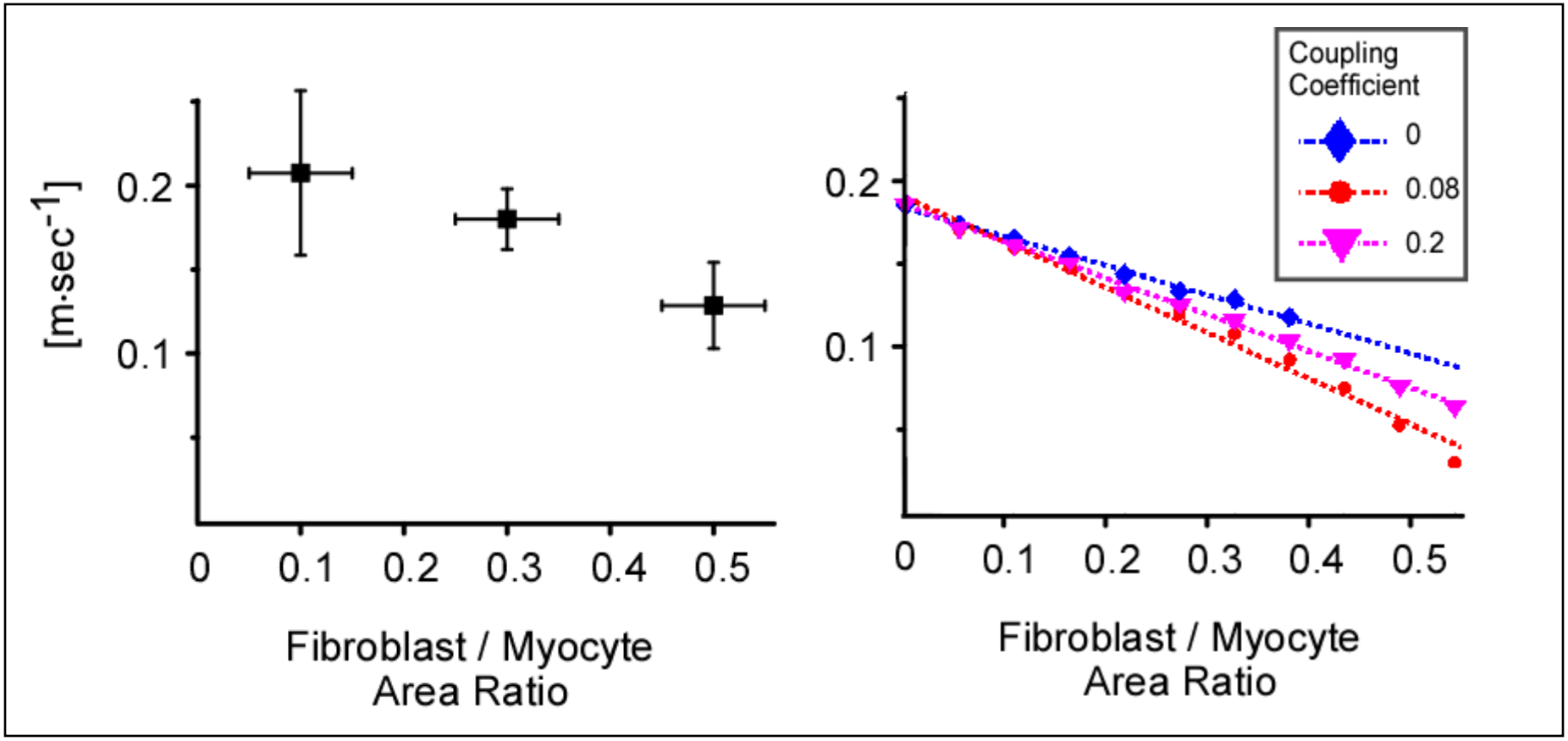
Numerical



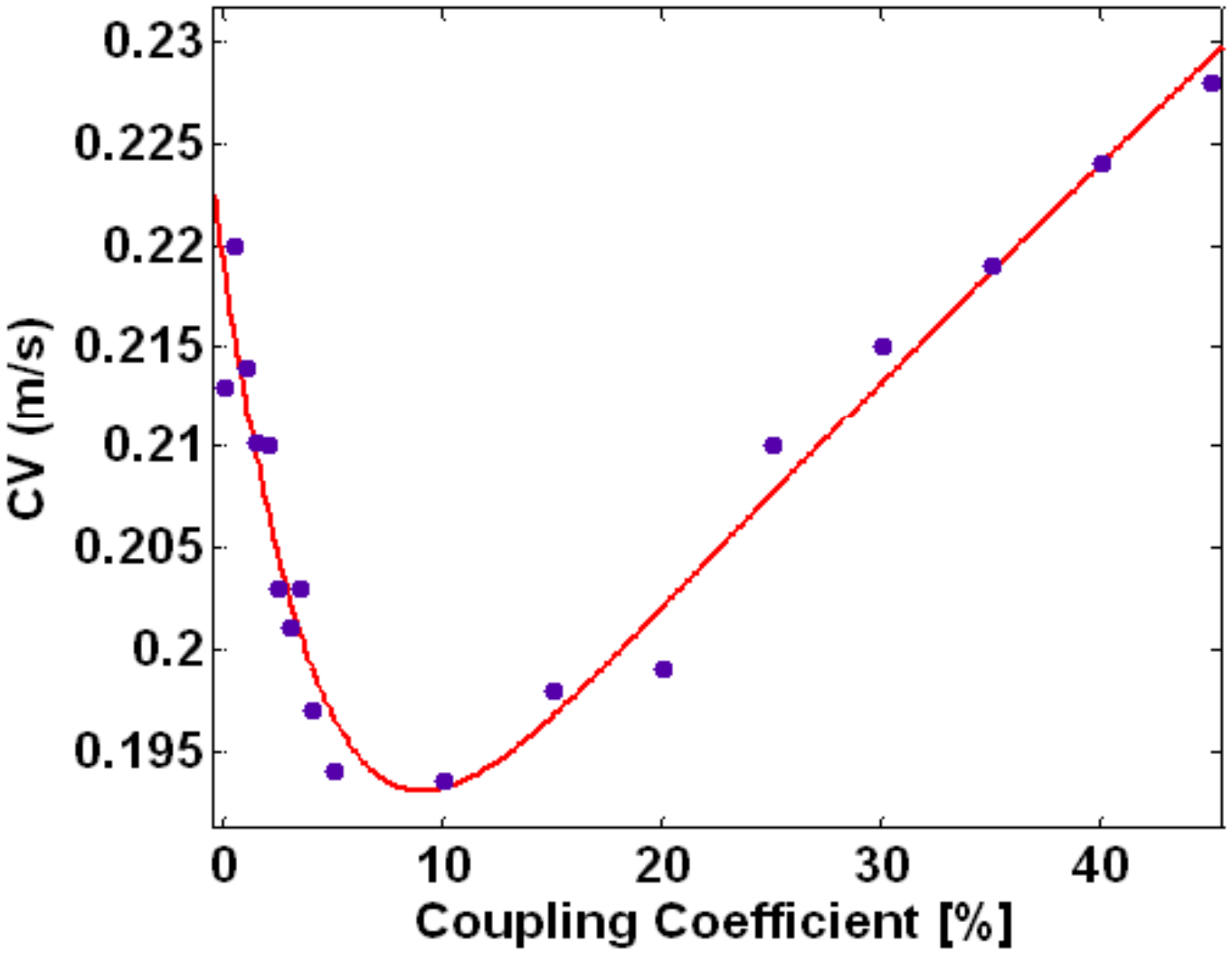
Conduction Velocity (pacing at 2Hz)

Experimental

Numerical



Effect of Electrical Coupling on CV is Biphasic



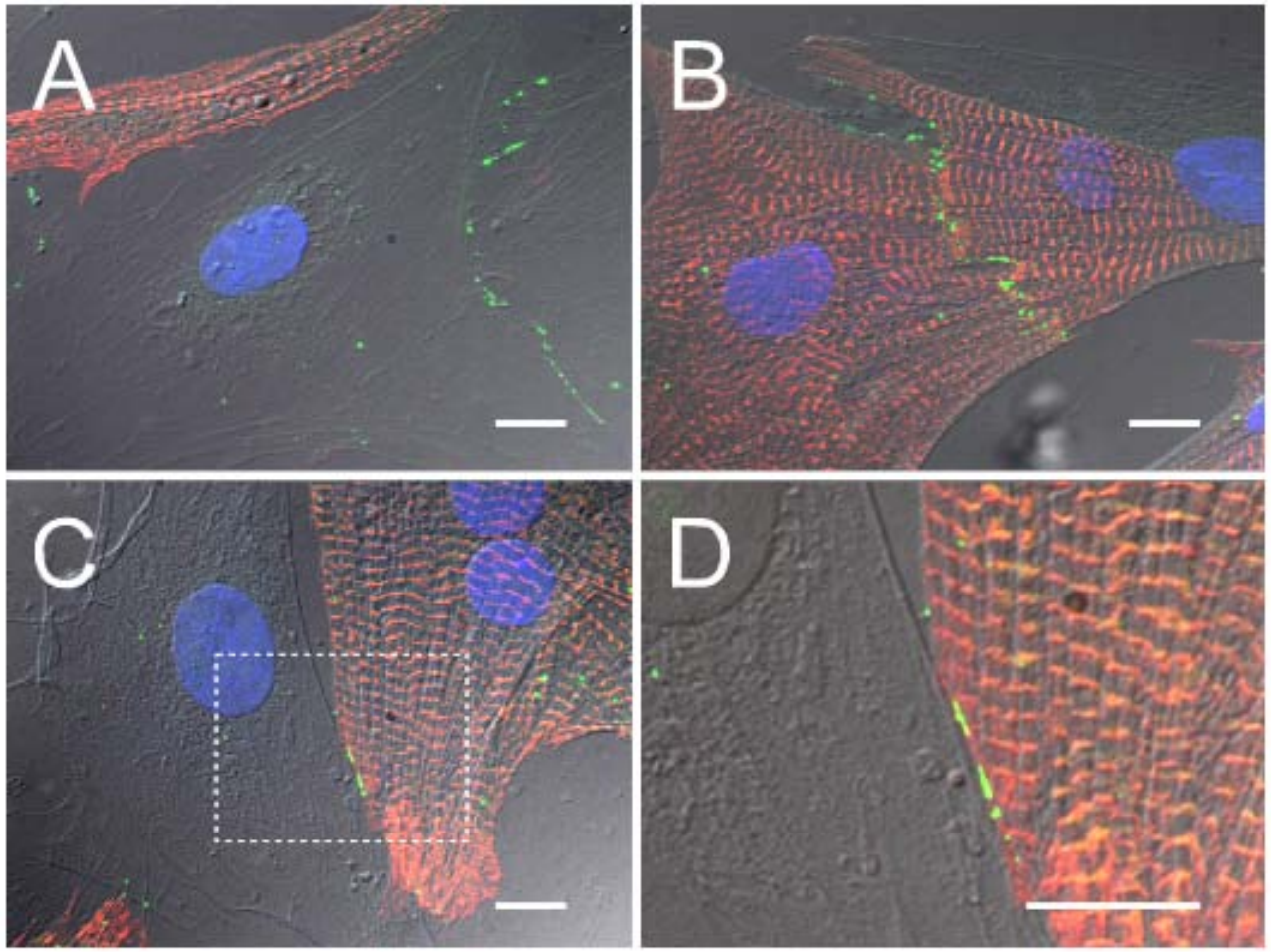
Questions:

1. Do fibroblasts couple electrically with myocytes through Cx43 gap junctions?

If so,

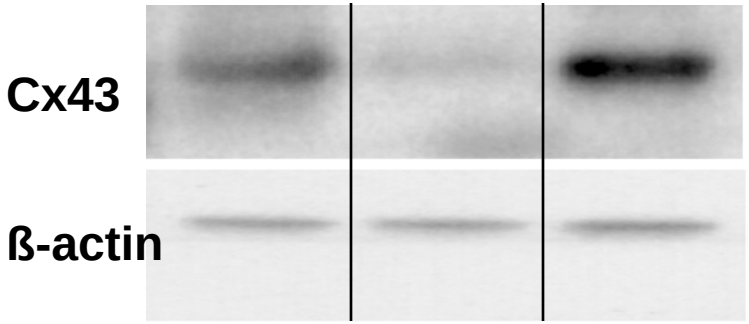
2. Can one verify in the experiment the model prediction of a biphasic dependence of CV on electrical coupling by somehow changing the degree of expression of Cx43?

Connexin43 Expression

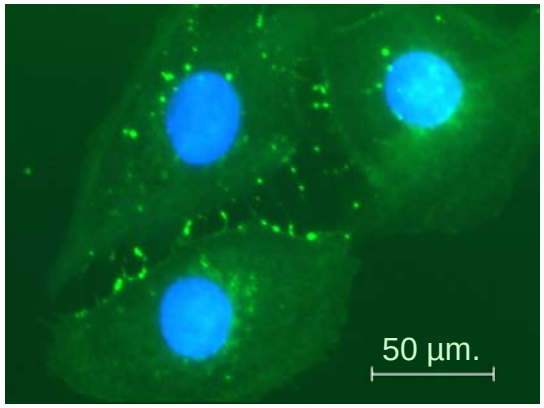


Anti-Cx43 **Anti-sarcomeric a-actinin** **Nuclear DAPI**

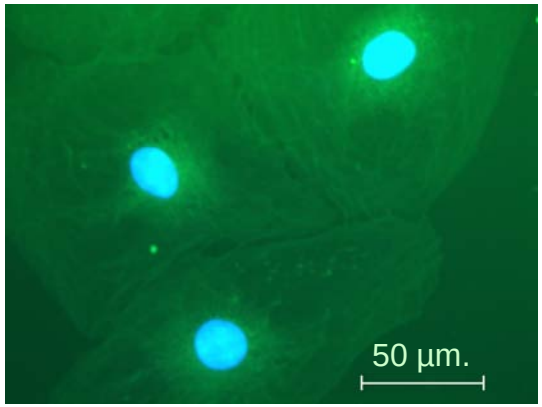
Modifying Fibroblast Connexin43 Expression



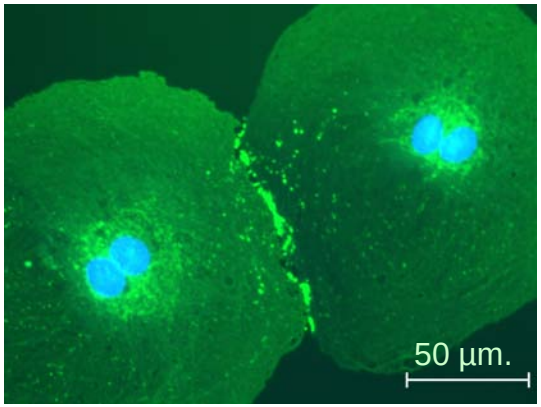
Non-treated



Silenced
Cx43 RNAi

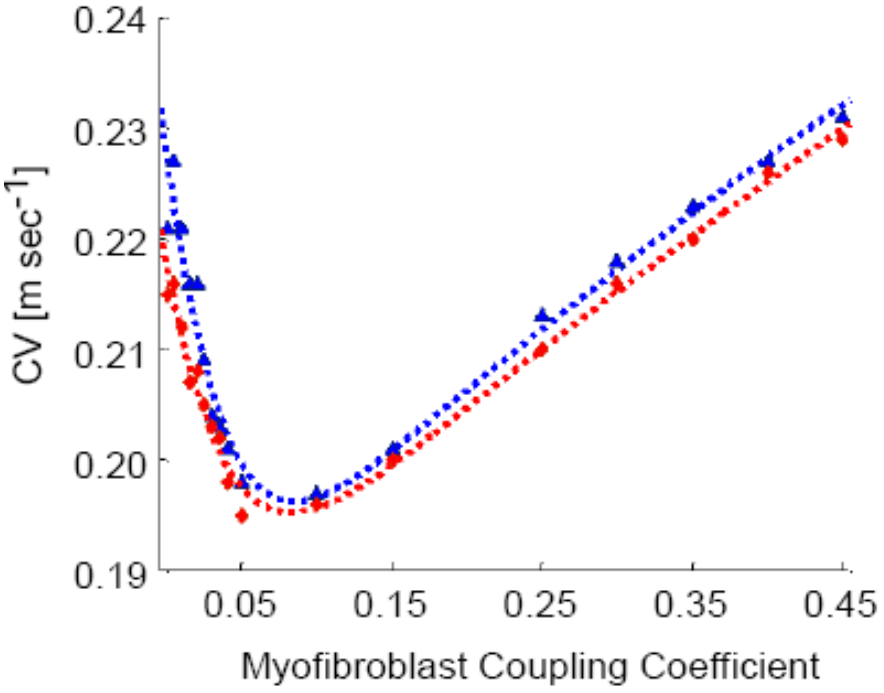


Over-expressed

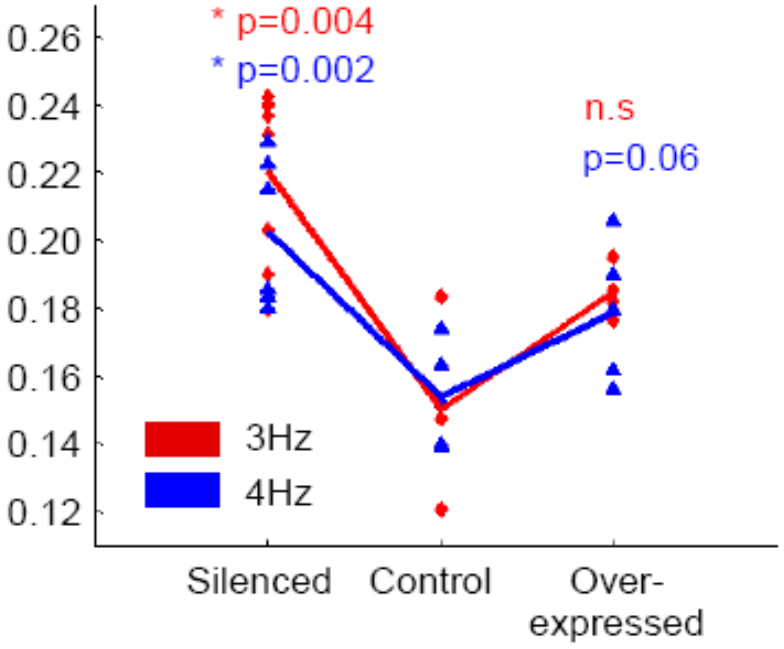


Effect of Electrical Coupling on CV is Biphasic

Numerical Prediction



Experimental Verification



p-values, comparison to control

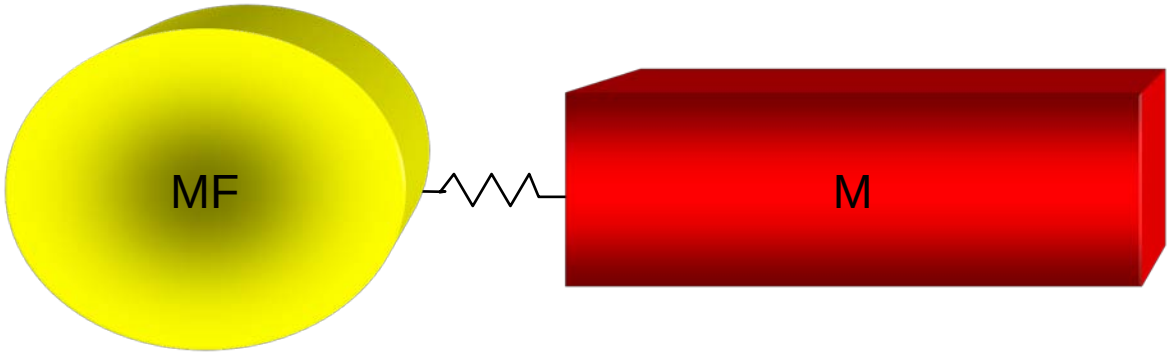
* statistically significant

Myofibroblast:

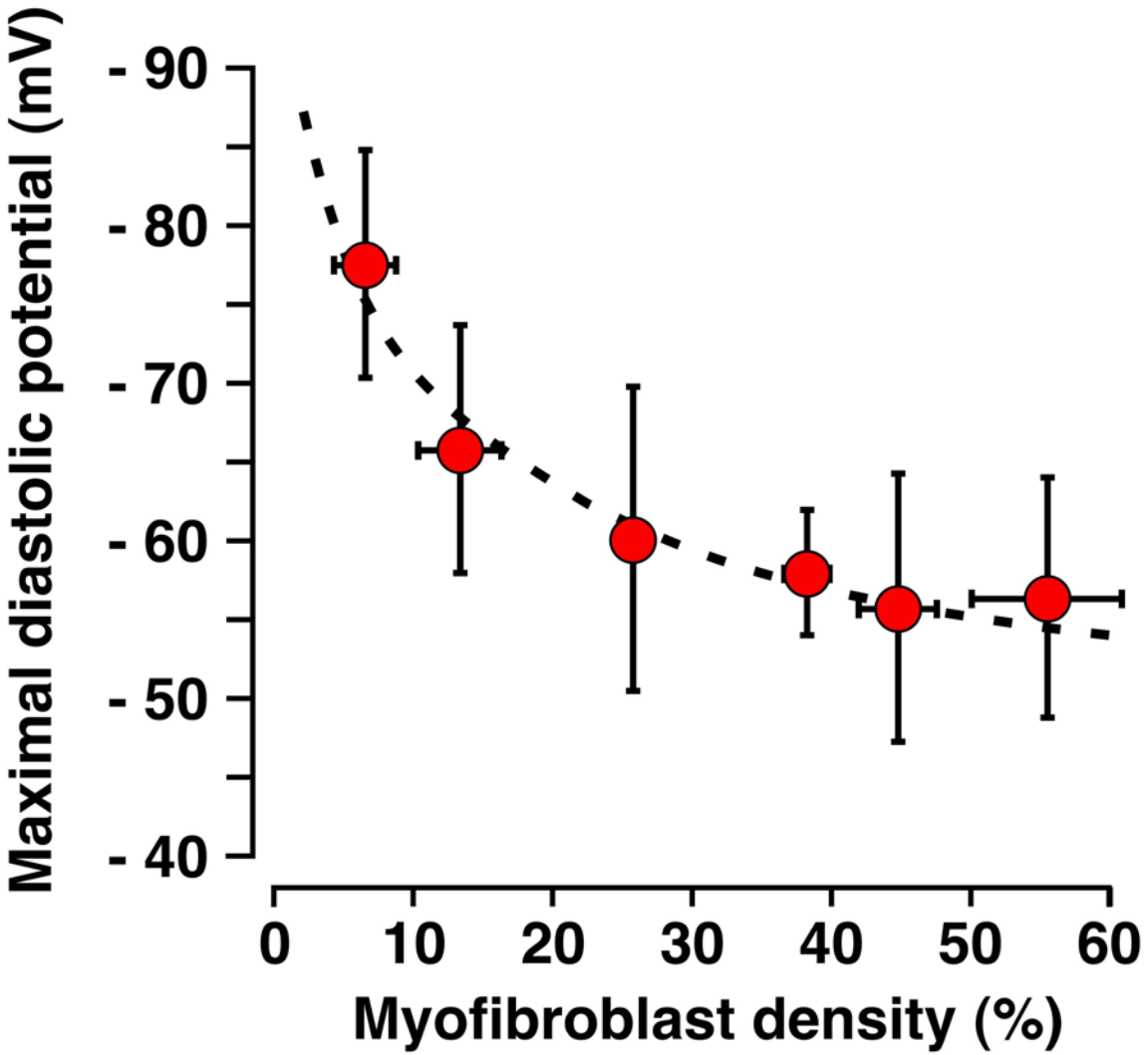
- Non-excitable (passive)
- High membrane resistance.
- Low V_{rest} (~-15mV)

Myocyte:

- Excitable (active).
- Low membrane resistance.
- High V_{rest} (~-85mV)

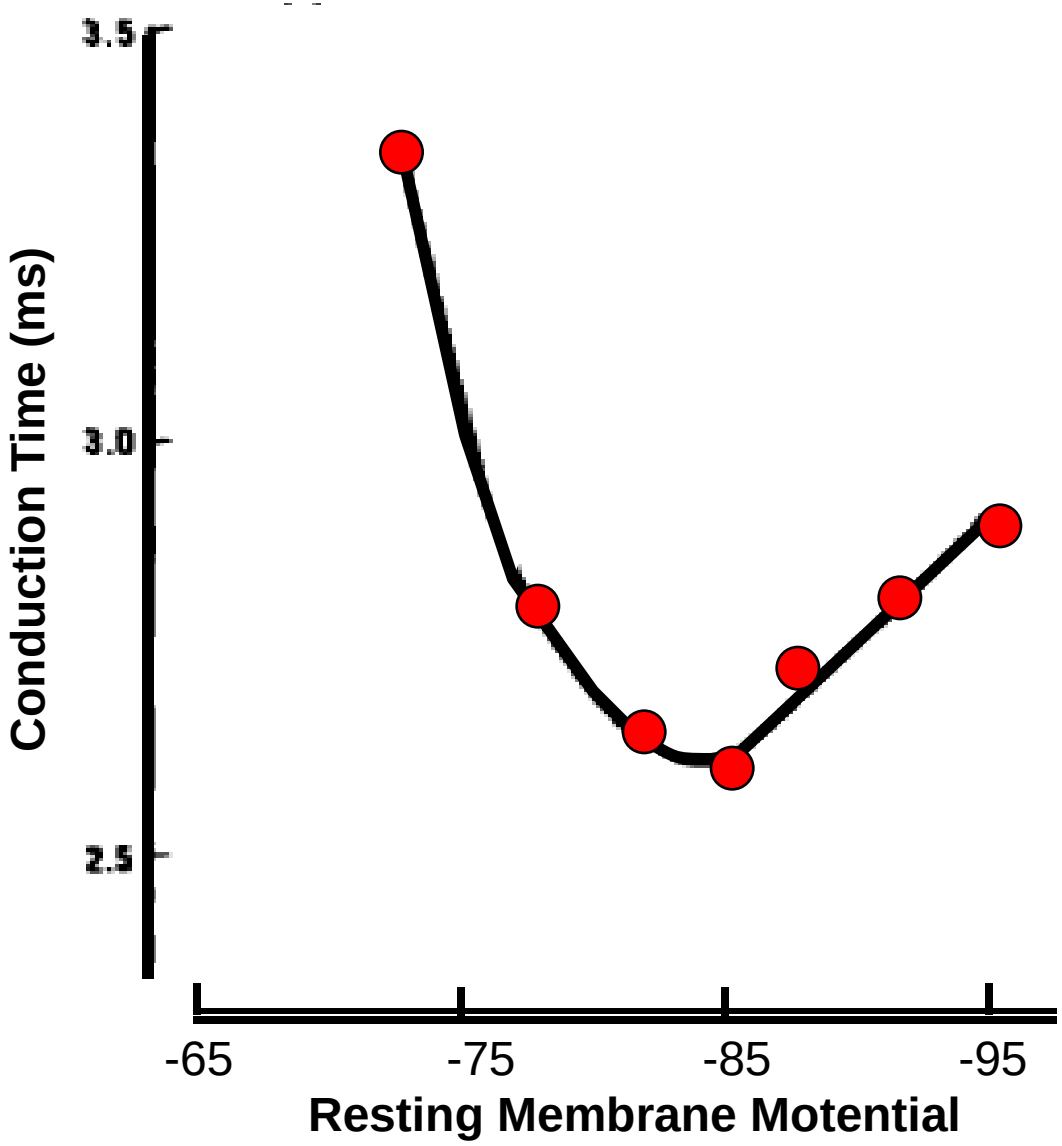


Effects of heterocellular coupling on myocyte membrane potential



Miragoli M. et al. Circ. Res. 2006;98:801-810

Relationship between resting potential and conduction time



Modified from Peon, J, Ferrier GR, Moe GK, Circ Res. 1978;43;125-135

Proposed Mechanisms for Biphasic Effect of F-M Coupling on CV

Up to a certain threshold, which depends on fibrosis ratio, fibroblasts act as current SINKS, not exciting downstream cells, and therefore velocity decreases with coupling level.

Above threshold, fibroblasts can transmit enough current to depolarize downstream cells – i.e. current SOURCE, and velocity increases with coupling level.

Summary and Conclusions

►Fibrosis promotes the formation of multiple stable rotors.

►The presence of fibrosis reduces the frequency of reentry in experiments and in computer simulations.

► Modifications of electrical coupling between myocytes and fibroblasts might be a potentially useful antiarrhythmic approach for the prevention of spontaneous initiation and/or maintenance of reentry.

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The End