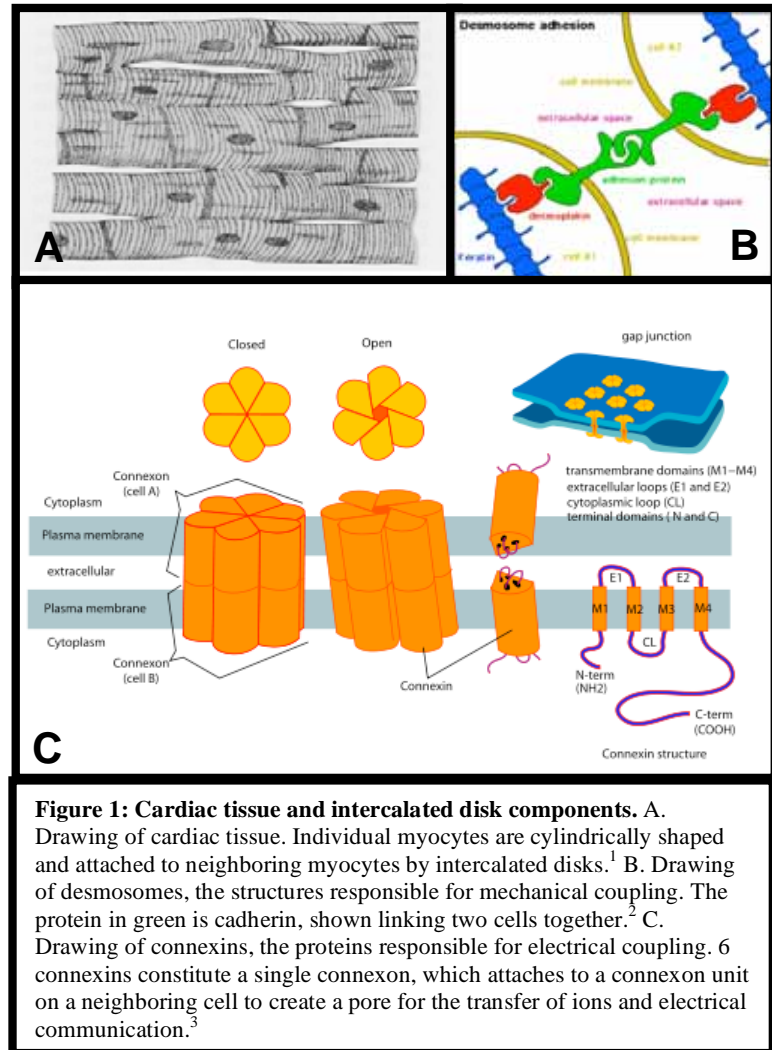Megan McCain
ES240 – FEM Final Project
December 19, 2006

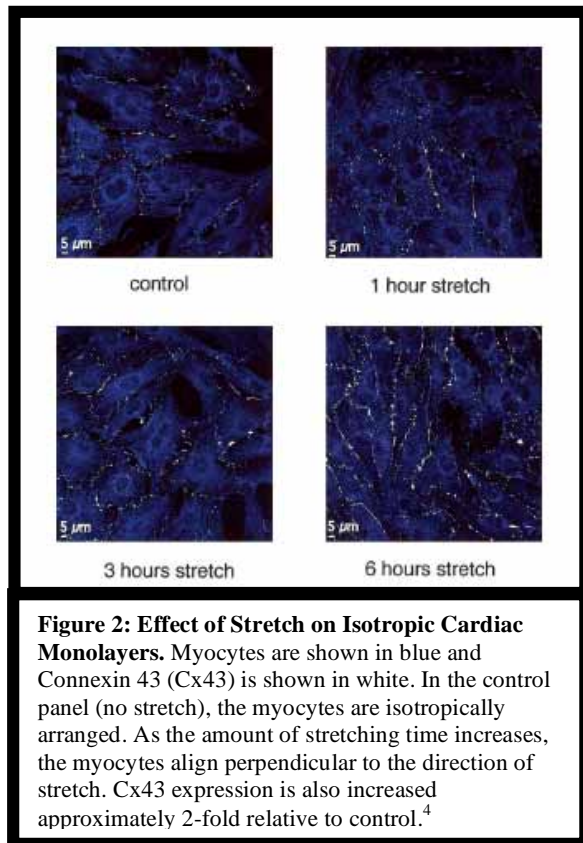# Stretching Cardiac Myocytes: A Finite Element Model of Cardiac Tissue

Cardiac myocytes are the cells that constitute the working muscle of the heart. These cells are cylindrically shaped, and because of the arrangement of their contractile apparatus, they shorten longitudinally during contraction. Myocytes are coupled to each other through intercalated disk structures, which are located on the transverse faces of neighboring cells (see Figure 1A). Intercalated disks are responsible for both mechanical and electrical coupling through a collection of different proteins. Desmosomes are the structures responsible for mechanical coupling and primarily consist of cadherin proteins[2] (see Figure 1B). Gap junctions are the structures responsible for electrical coupling and consist of connexin proteins[3] (see Figue 1C).

As shown in Figure 1C, six connexins arrange cylindrically into a connexon, which forms a pore through the cell membrane. Connexons from one cell will attach to connexons on another cell, therefore creating a tiny channel between two cells. Ions and other small molecules can travel through these connexon channels, which allows for efficient electrical communication between cells. Because electrical excitation leads to contraction,

Figure 1: Cardiac tissue and intercalated disk components. A. Drawing of cardiac tissue. Individual myocytes are cylindrically shaped and attached to neighboring myocytes by intercalated disks.[1] B. Drawing of desmosomes, the structures responsible for mechanical coupling. The protein in green is cadherin, shown linking two cells together.[2] C. Drawing of connexins, the proteins responsible for electrical coupling. 6 connexins constitute a single connexon, which attaches to a connexon unit on a neighboring cell to create a pore for the transfer of ions and electrical communication.[3]

electrical information needs to be quickly spread throughout the tissue to ensure synchronous contraction of the entire muscle of the ventricle. All the myocytes of the ventricle need to contract synchronously to create enough force to pump blood throughout the body.[3]

If the electrical propagation in the heart is disturbed, the heart could experience an arrhythmia, which is any condition in which the heart contracts irregularly, leading to a decrease in its blood pumping ability. Because connexins are crucial for normal electrical propagation,

and irregular electrical propagation can lead to an arrhythmia and decreased function of the heart, connexins play a critical role in maintaining the normal, healthy function of the heart. Therefore, it is important to understand the behavior and responses of connexins to different conditions. Because connexins are very dynamic proteins (they have a turnover rate of about one hour[4]), they can remodel themselves and change the overall electrical behavior of a tissue on a relatively short timescale, which can be dangerous if the electrical behavior becomes irregular.

Figure 2: Effect of Stretch on Isotropic Cardiac Monolayers. Myocytes are shown in blue and Connexin 43 (Cx43) is shown in white. In the control panel (no stretch), the myocytes are isotropically arranged. As the amount of stretching time increases, the myocytes align perpendicular to the direction of stretch. Cx43 expression is also increased approximately 2-fold relative to control.[4]

It has already been shown that cardiac myocytes and connexins remodel in response to pulsatile stretch. This was shown by growing cardiac myocytes isotropically on elastic substrates and stretching them for different amounts of time. This process involved digesting the heart from a neonatal rat to dissolve the tissue structure and create a mixture of single cells. The myocyte mixture was then adhered to the substrate by covering it in fibronectin, an extracellular matrix protein that will also bind to the cell surface. This process creates isotropic monolayers: the cells are randomly oriented on the substrate and lack any discernable pattern (Figure 2, top left). After being stretched, the myocytes aligned themselves perpendicular to the direction of stretch (Figure 2, bottom right), because this orientation experiences the least amount of substrate deformation. The connexins change their location as well as their protein expression levels: the amount of connexin 43 (the most common connexin in the ventricle) doubles after being exposed to pulsatile stretch (see Figure 2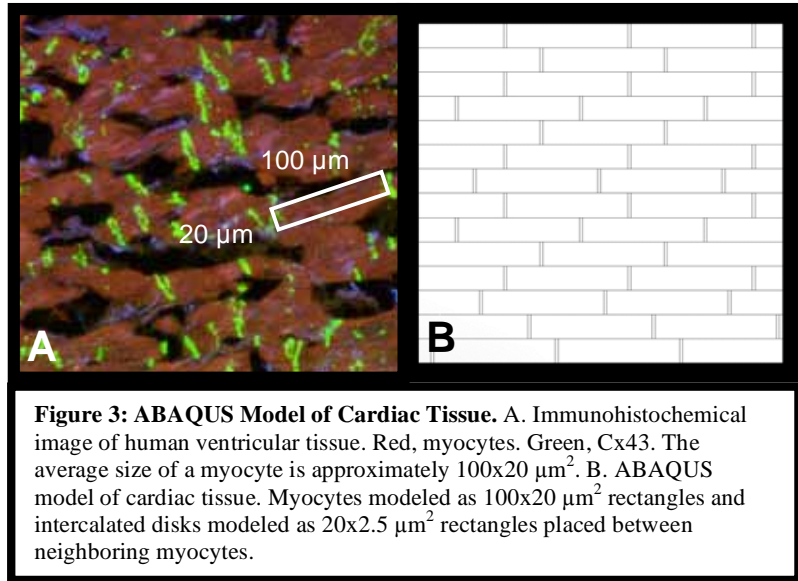). This is an important result, because any disturbance of the normal connexin distribution will change the normal pattern of electrical propagation through the heart, which can be highly arrhythmogenic. The main disadvantage to this model is that it lacks some physiological relevance: cardiac myocytes are not arranged isotropically in the heart, as they are in this model. As described above, myocytes in the heart are actually arranged in the cylindrical end-to-end pattern shown in Figure 1A.

Advances in tissue engineering have made it possible to create *in vitro* 2-dimensional anisotropic monolayers of myocytes arranged in a striated pattern closely resembling that of actual cardiac tissue. This is possible through a micropatterning process, during which a striated pattern of fibronectin (an extracellular matrix protein that binds to the cell surface) is placed on a substrate before seeding with neonatal rat ventricular myocytes. The fibronectin guides the adhesion of the myocytes, forcing the cells to adopt the same striated pattern. These 2D monolayers of cardiac tissue are an excellent model for studying many problems in cardiac physiology. Part of my PhD thesis will involve stretching these anisotropic monolayers and observing the changes in connexin expression. A finite element model of the stress and strain

distribution will be useful for correlating stress to changes in connexin localization, expression, and function. This project investigated the stress distribution in anisotropic monolayers subjected to pulsatile stretch in two different orientations: transverse and longitudinal. The goal was to determine the stress and strain distributions throughout the tissue, especially the differences applied to myocytes versus intercalated disks.

The first part of this project involved creating a model of cardiac tissue. Based on an image of human ventricular tissue I collected as an undergraduate (see Figure 3A), I determined the average size of a cardiac myocyte to be 20 μm x 100 μm. I decided to model the intercalated disks as 20 μm x 2.5 μm rectangles located between neighboring myocytes. The Young's modulus of a cardiac myocyte is approximately 35 kPa and the Poisson's ratio is 0.5, as measured by atomic force microscopy.[5] I could not find any values for the Young's modulus of intercalated disks, probably because this structure is a combination of many proteins so it has a highly variable value. For my model, I assumed a Young's modulus of 60 kPa, because the intercalated disks should be stiffer than the myocytes, but the stiffness should be on the same order of magnitude as myocytes. I maintained a Young's modulus of 0.5, which is average for biological tissue. In ABAQUS, this step involved creating a single part representing a square of tissue, and partitioning the different shapes and properties of myocytes and intercalated disks (see Figure 3B).

Figure 3: ABAQUS Model of Cardiac Tissue. A. Immunohistochemical image of human ventricular tissue. Red, myocytes. Green, Cx43. The average size of a myocyte is approximately $100 \times 20\ \mu m^2$. B. ABAQUS model of cardiac tissue. Myocytes modeled as $100 \times 20\ \mu m^2$ rectangles and intercalated disks modeled as $20 \times 2.5\ \mu m^2$ rectangles placed between neighboring myocytes.
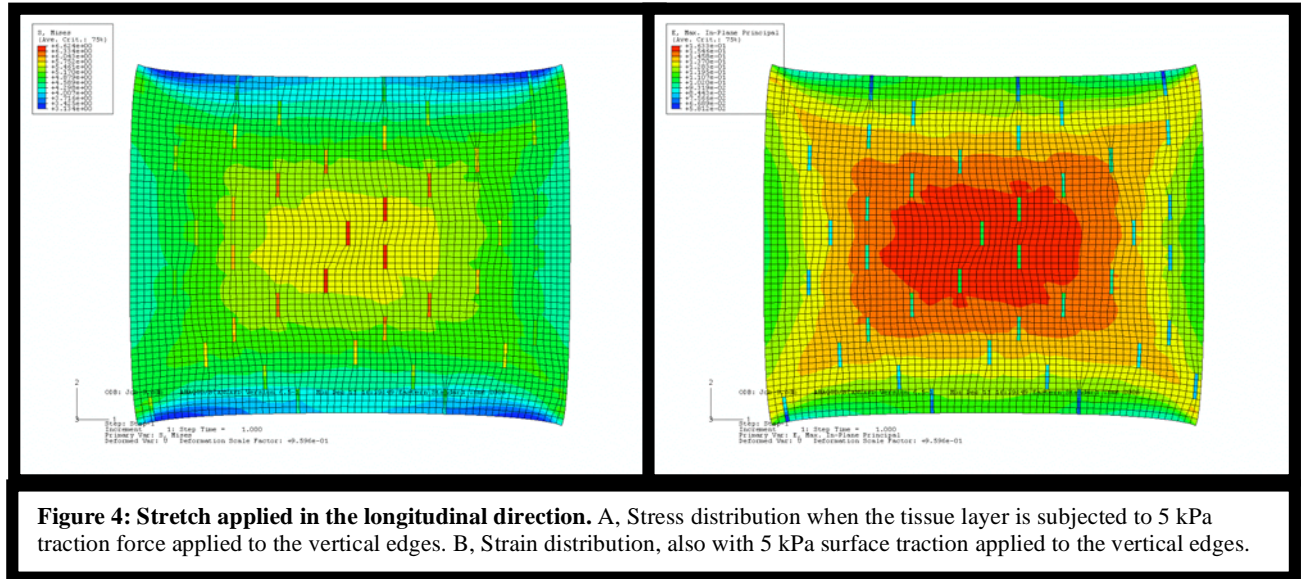
The next step was to stretch the layer of tissue in either the longitudinal or transverse direction. Because I wanted a strain of 0.1-0.2, I applied a surface traction of 5 kPa, based on the simple stress-strain equation:

$$\sigma = E\varepsilon \qquad E = 35\ \text{kPa},\ \varepsilon \approx 0.15,\ \text{Solve for } \sigma = 5\ \text{kPa}$$
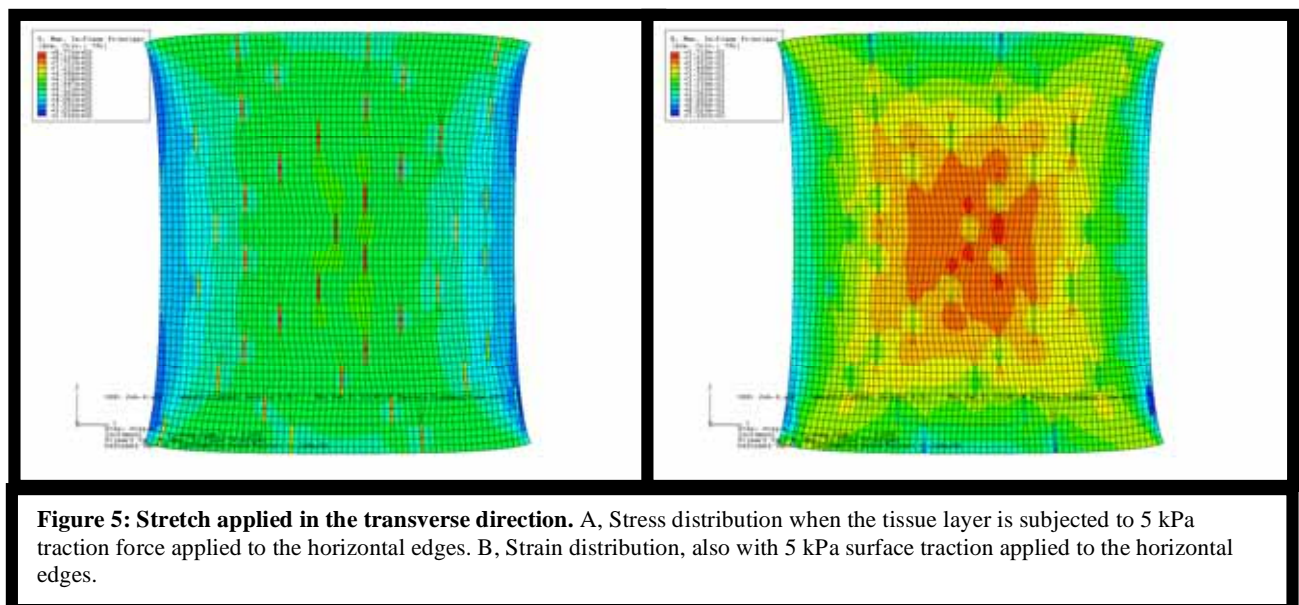
The surface traction of 5 kPa was applied to two opposite edges in the normal direction. I also applied two boundary conditions: symmetry along the edge that was experiencing the surface traction to maintain the width of the tissue along those two edges, and zero rotation along the two edges not experiencing the surface traction.

The results for longitudinal stretch are shown in Figure 4A. As expected, the stress is higher in the middle of the tissue (5.8 kPa) compared to the edges (4.5 kPa). The stress is also higher at the intercalated disks (max: 6.6 kPa) compared to the myocytes (max: 5.8 kPa). The strain is shown in Figure 4A. The strain is also higher in the center (0.16) compared to the edges

(0.11). The myocytes (max: 0.16) experience more strain than the intercalated disks (max: 0.10). The differences in stress and strain can easily be explained by Young's modulus and the basic stress-strain equation: σ = Eε. Because intercalated disks have a higher Young's modulus than myocytes, the intercalated disks will stretch less (lower strains) and also have higher stresses.

Figure 4: Stretch applied in the longitudinal direction. A, Stress distribution when the tissue layer is subjected to 5 kPa traction force applied to the vertical edges. B, Strain distribution, also with 5 kPa surface traction applied to the vertical edges.
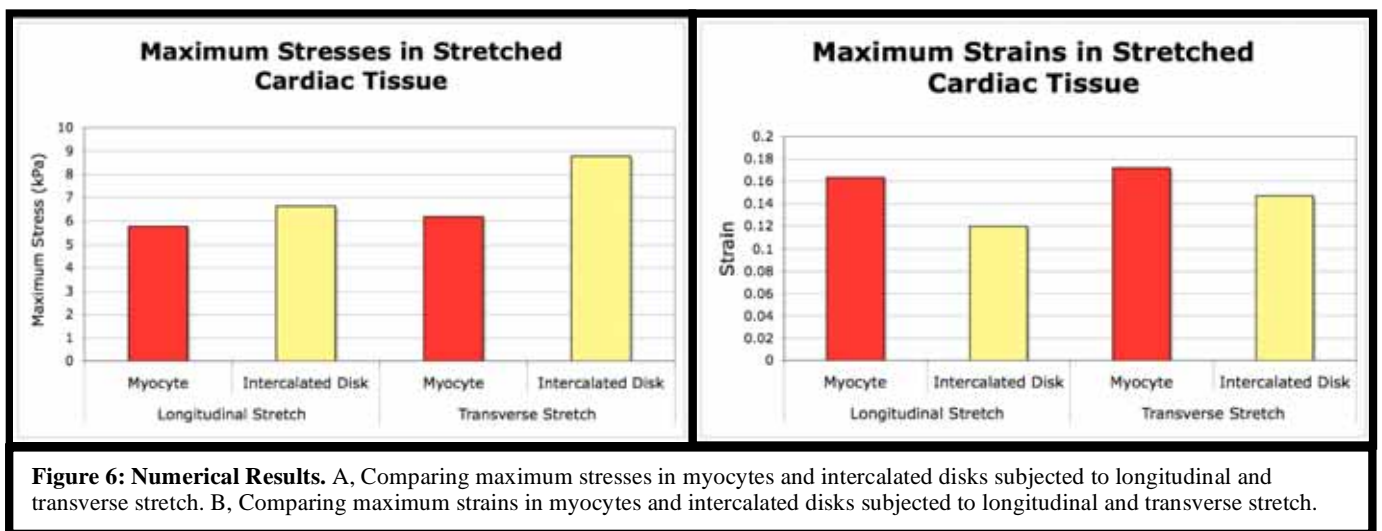
The results for transverse stretch are shown in Figure 5. The stress distribution is slightly different than in the longitudinal stretch situation. Instead of concentrating in the center and decreasing concentrically (as in longitudinal stretch), the stresses are highest along the entire vertical axis (5.6 kPa) and decrease towards the vertical edges (3.6 kPa). Again, the intercalated disks experience more stress (max: 8.8 kPa) than the myocytes (max: 5.6 kPa). Interestingly, the strain still concentrates in the center (0.18) and decreases concentrically to the edges (0.11), similar to longitudinal stretch. Again, myocytes experience more strain (max: 0.17) than intercalated disks (max: 0.14) for the same reasons as longitudinal stretch. Another interesting

Figure 5: Stretch applied in the transverse direction. A, Stress distribution when the tissue layer is subjected to 5 kPa traction force applied to the horizontal edges. B, Strain distribution, also with 5 kPa surface traction applied to the horizontal edges.
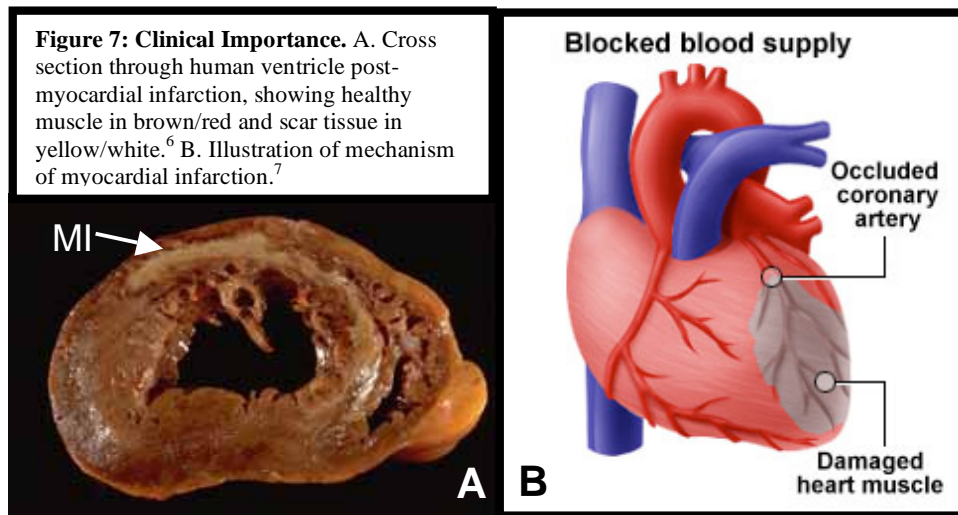
observation is the ability of the specific position of the intercalated disks to influence the strain in the myocytes. For example, near the center of Figure 5B, the area of myocytes shown in red are strained the most because they are located vertically between two intercalated disks. This is an excellent example of the ability of specific tissue architecture to dramatically effect the stress and strain distribution.

Figure 6 compares the stresses and strains for myocytes and intercalated disks during longitudinal and transverse stretch. As explained above, myocytes experience less strain and more stress under all conditions. Comparing longitudinal to transverse stretch, the stress and strain for the myocytes do not significantly change between the two directions. However, the intercalated disks experience a larger difference in both stress and strain when comparing longitudinal to transverse stretch. This is most likely due to the different sizes of the two different parts. Because the intercalated disks are smaller and stiffer, they are more sensitive to the direction of stretch. Both the myocytes and the intercalated disks are made of rectangles with large aspect ratios, so basic geometry alone cannot fully explain the differences. The smaller size and higher Young's modulus must be the most important factors contributing to the differences between longitudinal and transverse stretch for intercalated disks.

Figure 6: Numerical Results. A, Comparing maximum stresses in myocytes and intercalated disks subjected to longitudinal and transverse stretch. B, Comparing maximum strains in myocytes and intercalated disks subjected to longitudinal and transverse stretch.

There is also important clinical significance to this model. If a patient suffers a myocardial infarction (heart attack), one of the coronary arteries becomes blocked for any variety of reasons (blood clot, cholesterol build up, etc.). As shown in Figure 7, this causes a portion of the muscle in the ventricle to lose blood flow and die, thus becoming a scar that has a much higher Young's modulus than the surrounding living myocytes. The scar tissue will not contract, but the surrounding myocytes will still continue to contract with every heartbeat. Therefore, the scar tissue imposes a stretch on the surrounding myocardium. For example, if a myocyte is located on the boundary of the scar, the myocytes surrounding it on the other sides will contract, but the scar will not. The scar is therefore causing the myocyte to be stretched unevenly. Depending on the orientation of the myocyte to the scar, the stretch can be in any direction – longitudinal, transverse, and anything in between. Therefore, it is important to understand the stresses myocytes and their intercalated disks experience when being stressed at different orientations, and to be able to relate these stresses to any remodeling that occurs (differences in gene

Figure 7: Clinical Importance. A. Cross section through human ventricle post-myocardial infarction, showing healthy muscle in brown/red and scar tissue in yellow/white.[6] B. Illustration of mechanism of myocardial infarction.[7]

expression, etc.). Myocytes are known to remodel around a myocardial infarction, and these areas are also known to be very arrhythmogenic. It is obvious that stretch has a powerful effect on cardiac tissue, which usually proves to be dangerous and even fatal.

There are several improvements I could make as future directions for my model. First, I neglected the substrate that the tissue would be growing on (silicon). I could add this layer underneath the tissue layer to make it more realistic to the experimental conditions. This would probably change the magnitude of stresses and strains, but not their overall distribution. I could also stretch the tissue at many different angles, because stresses in the heart will not be limited to the longitudinal or transverse directions. My model also needs a more precise value of Young's modulus for the intercalated disk structures. I also simplified the model by placing intercalated disks only at the transverse borders, but myocytes are also coupled along the longitudinal borders (although not nearly as densely). It would also be interesting to change the aspect ratios of the shape of the myocytes, because normal myocytes are rectangular with an aspect ratio of 1:7 and myocytes in hypertrophied hearts have an aspect ratio of 1:5. This would change the stress-strain distributions and provide interesting insight into the disease of hypertrophy.

References

[1] http://www.math.utah.edu/~keener/lectures/defib/theory1/cell_structure2.jpg

[2] http://en.wikipedia.org/wiki/Desmosomes

[3] http://en.wikipedia.org/wiki/Connexin

[4] Zhuang J, KA Yamada, JE Saffitz, AG Kleber. Pulsatile Stretch Remodels Cell-to-Cell Communication in Cultures Myocytes. Circulation Research. 2000;87:316.

[5] Lieber SC, et al. Aging Increases Stiffness of Cardiac Myocytes Measured by Atomic Force Microscopy Nanoindentation. Am J Physiol Heart Circ Physiol 287: H645-H651,2004.

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[7] http://forte.fh-hagenberg.at/cardiac/images/medical/infarct.jpg