Biological Illustration:
Kekkon6 localization and biological pacemakers

A Major Qualifying Project Report

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By

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Abstract

Illustrations have helped mankind understand the world we inhabit since antiquity. Today biological illustration helps us to understand the biological world. This project demonstrates my abilities as an artist and knowledge as a scientist. As biological illustrator, I have done this by illustrating for two projects. One pertains to localization of Kekkon6, a transmembrane protein, in *Drosophila melanogaster*. The other relates to developing biological pacemakers with the use of stem cell implants.

Acknowledgements

I first would like to thank Jill Rulfs, my academic advisor and head of the committee of professors supporting my interdisciplinary individually created Biological Illustration major. Thank you for guiding me through my time here at WPI. And for encouraging me to embrace my passion for biology and the arts with the realization of this Biological Illustration major. And thank you for your endless help and support pertaining to this MQP.

I would also like to thank Joe Duffy and Glenn Gaudette for extending interest in my MQP proposal and allowing me to work with you. Your support and reassurance in my abilities and direction as an artist and a scientist were fundamental in this project.

To Joe Farbrook, thank you for teaching me everything you have as my professor and allowing me to grow as an artist. And for your support as a committee advisor for the creation of my major and believing that what I was pursuing as an artist has a future.

Also, thank you to Dan Gibson and Mike Buckholt for showing support by being committee advisors for my Biological Illustration major. Thank you both for the confidence and reassurance in this academic endeavor.

Development of Illustration in the Sciences

In every form of life, no matter the complexity of the organism or its role on earth, there is a necessity for communication. Be it within the organism, or between organisms, communication leads life. Single cells can signal to the nucleus when a particular the cell is damaged, so the nucleus can initiate apoptosis, or programmed cell death. Receptors on a sunflower plant tell it which way to face to receive the most sunlight. Our bodies have a nervous system that carries electrochemical impulses to communicate pain and other sensory information. Many organisms possess the ability to vocalize in order to communicate with others.

In the evolution of humanity, our modes of communication have grown, from simple vocalization to now expressing ideas and thoughts electronically over the internet. Beyond the vocalization of primitive man, primitive visual aid developed, and the idea that something could be better understood if it was not only heard, but also seen.

In what was ancient Rome, stone models of organs have been found. The small sculptures of ears, eyes, or intestines were used as charms to defend against the illness of these parts (Ford, 1993). These charms must have been meant to communicate with someone or something somewhere to protect these areas. If the believed gods of this time were godly in the peoples' minds, would they not also be literate? Why did the ancient Romans not write "protect my eyes from illness" on a slate? There is something safe, uncontroversial and unmistakable about using visual depictions to communicate, even with omniscient beings.

Cro-magnon illustrations on cave walls give us further insight into how important mankind thought communication was. These drawings with blood, clay, juices from berries and charred wood depict attack patterns for hunting and killing large prey. They also illustrate specific target areas for young hunters to aim at, to better maim the beast (Ford, 1993). When life required hunting and with hunting came the risk of dying, being careful and accurate was as important as being successful. Hunting, in that time, was something that required training, skill, knowledge and discipline; it was as much a science then as science is now. These illustrations represent the earliest attempts to use visual representation to convey an important concept of a science. This would serve as the possible birth of scientific illustration, if not by today's definition of the term, then by the literal meaning of the words that compose today's definition.

Mankind has a natural need to understand, and desire to teach others what we've learned. As history progressed, man wanted to understand more. Australian aboriginal "x-ray" drawings crudely depict the spinal cord, pelvis and other anatomical structures of the kangaroo. In 11th-century England, diagrams were drawn to depict medical procedures (Ford, 1993). An explosion of medical and scientific illustrations emerged as man progressed and came to understand the importance of these images.

Many artists have influenced various fields of study and realms of life. Let's take a look at a few artists who helped shape the modern illustration of scientific, medical, and natural subjects.

Leonardo da Vinci was a man of science, whose interests and work spanned the realms of zoology, botany, geology, anatomy, optics, aerodynamics, hydrodynamics and many others (Boston Museum of Science). He was a great inventor. His ability to take his ideas and his work and represent it on paper led to his greatness. In this way, as he created inventions in the fields of architecture, canal building, military engineering and weapons development (Boston Museum of Science).

However it is his role in medical and scientific illustration that is of interest here. He was apprenticed to Verrocchio of Florence, an artist who was recognized for his mastery of perspective. With the skills learned from his master, da Vinci started to create a new path for medical illustration. Prior to da Vinci's work, most informational art was visually two dimensional, having firm outlines and lacking in form, weight and structure. His mastery of chiaroscuro shading gave his figures and depictions these necessary qualities to depict with exact accuracy and put the viewer in awe. His works were created with realism and life, when earlier eras relied on figurative representation to convey the idea of an image (Boston Museum of Science).

As a medical scientist, he studied ancient texts and works. However, he noticed and understood their limitations. He dissected over 30 human cadavers in his efforts to understand the body. Through his sharp observation and truthful depictions, his understanding surpassed the accepted ideas of the time and he in turn understood more. Because of this he corrected countless fallacies in the accepted understanding of anatomy and corrected techniques used in medieval

procedures (Boston Museum of Science). This was his method; precise dissection, direct observation, accurate depiction and truthful conclusion. It was a systematic, genuine, and literal approach to observational information. This mentality in the fields of natural sciences would persist as the accepted method into the 19th century (Boston Museum of Science). It is with the combination of his talents in the arts, discipline in the sciences, and passion for both that his accomplishments have resonated throughout the development of modern scientific illustration.

John James Audubon, born in 1785, was a French-American ornithologist. He was also a naturalist and a painter. This began with his hobby of studying and drawing birds. Audubon's great work is his book *Birds of America*. This was not the first undertaking of the sort. Alexander Wilson created a similar compilation of birds; however, Audubon's work surpassed Wilson's. In the 18 years of his life that it took to complete his book, Audubon illustrated and painted 435 life size images of North American birds. Avian artists of the 20th and 21st centuries are still compared to the immensely talented and famed Audubon (National Audubon Society).

Today, if someone mentions medical illustration, or visual aspect of anatomy, the name Netter, Frank H. Netter, is undoubtedly brought up. Netter was born in 1906. His career in medical illustration started in the 1930's, when he was commissioned by a pharmaceutical company. They hired him to do drawings of human pathology and organs. These first drawings were so successful that they were published in a book. His life's work became completing a series of anatomic atlases, The Netter Collection of Medical Illustrations (Netter Medical Images). His illustrations were immensely detailed and lifelike. He was the modern master in medical illustration and even after his death in 1991. His works are still the bar when measuring other artists' medical illustrations. This one man, whose notebooks in school were filled with drawings rather than traditional notes, changed the modern standard and understanding of anatomic art (Netter Medical Images).

The following sections of this project will feature the two projects that this MQP focused on and the illustrations I have done for them. All of the images seen here were either taken or created by myself.

Biological Illustration: Visual Insight into Junction Biology

Drosophila melanogaster (commonly known as the fruit fly, figure 1) females have two ovaries each containing approximately 18-20 ovarioles. Each ovariole consists of a chain of egg chambers at different developmental stages (stages 1-14), with an egg chamber being comprised of an oocyte and its associated nurse cells all enclosed in a monolayer of epithelial cells termed the follicular epithelium. Essential to oogenesis, tissue formation and subsequently embryonic development is the necessity of cells to communicate and react to their local environment. In this case this means other cells within that environment, meaning proper communication between the somatic (epithelial) and germline (oocyte and nurse) cells. One way that this happens is through cell-cell junctions. A more recently discovered type of cell-cell junction is the Tricellular Junction (TCJ). TCJs represent the region where three cells are in contact (figure 2). Initial work from the Duffy lab characterized Kekkon6 (Kek6) exclusion from TCJs and localization in "portals" in bi-cellular regions in the epithelium of the egg chamber. My work pertaining to this area focused on studying, imaging and representing Kek6. The goal of this section of the project is to accurately and clearly represent the localization of Kek6 at different levels of *Drosophila*, tissue, cell, and subcellular.

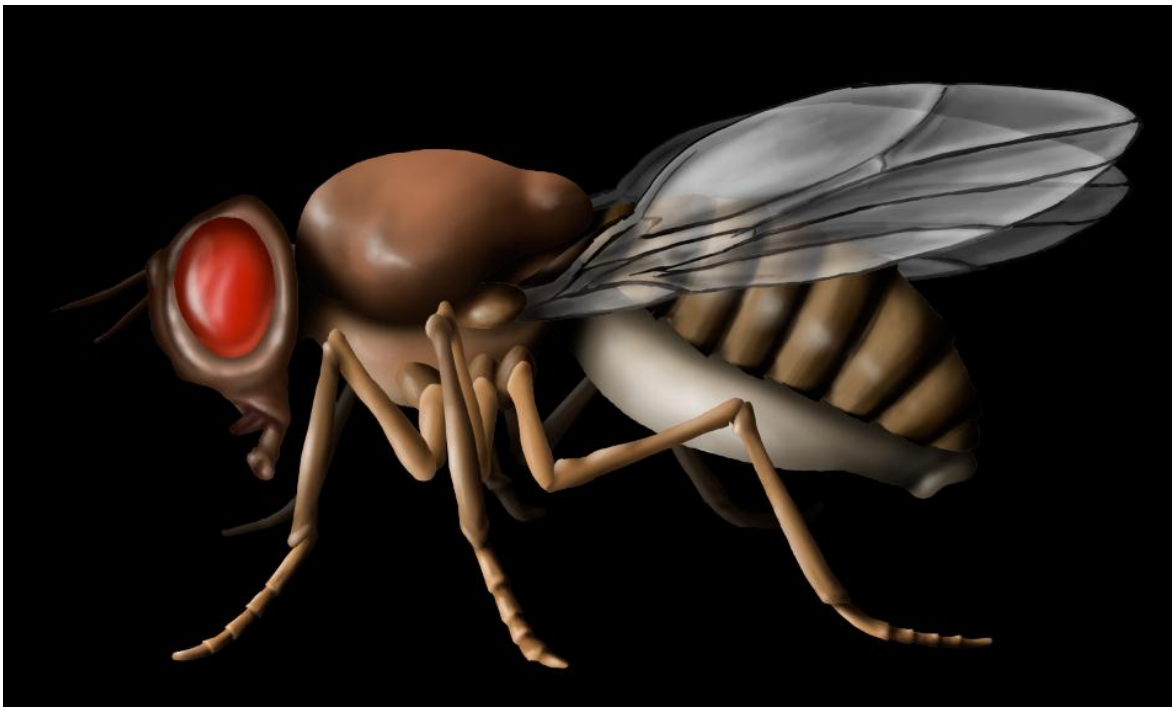


Figure 1. Lateral view of *Drosophila melanogaster,* carrying the Kek6-GFP transgene.

Kek6 is a member of the Kekkon family of six related transmembrane proteins in *Drosophila* (Arata, 2011). Transmembrane means these proteins extend from the inside of the cell and traverse through the plasma membrane to the outside of the cell. The Kek family is included in the larger LIG family of proteins that all contain both Leucine-Rich Repeats (LRRs) and an Immunoglobulin domain (Ig) in their extracellular region. While the number of each repeat or domain varies among LIG family members, the Kek family proteins each have 7 LRRs and one Ig domain. Interestingly, while studying members of the Kek family the Duffy lab discovered that Kek6 appears to be the only known protein specifically excluded from TCJ's. This suggests that Kek6 has a unique relationship with the structure and function of TCJs and gives impetus to determine what precise function it has (Arata, 2011).

While working on her thesis studying Kek6, Michelle Arata discovered the existence of Kek6's unique bicellular localization and tricellular exclusion. Bicellular junctions are the regions in which two cells are in contact (figure 2). Kek6 localizes in unique, geometric appearing patterns where its expression is seen around the edge of ring like structures that have been informally dubbed "portals" (Figures 9 and 10). In addition, Kek6 is only expressed in bicellular regions when both neighboring cells express Kek6. When one cell expresses Kek6 and its neighboring cell does not express Kek6, its expression is lost from the bicellular junction between these two cells. This information gives us additional insight that the extracellular portion of Kek6 could play a large role in its localization and perhaps its function. This is also consistent with the lack of involvement of the intercellular cytoplasmic domain in Kek6's localization as discussed below (Arata, 2011).

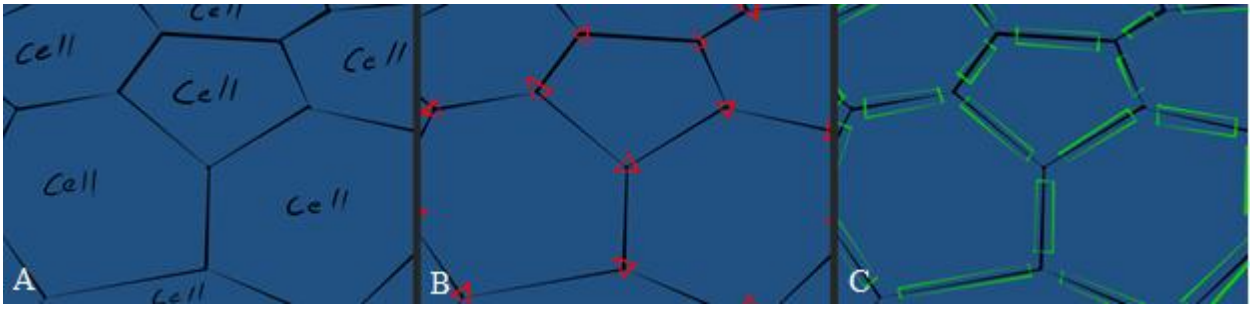


Figure 2. Graphic of epithelial cells. (A) Basic two dimensional representation of epithelial cell monolayer. (B) Tricellular regions are outlined in red. This is where we see exclusion of Kek6. (C) Bicellular regions are outlined in green. These regions are where the "portal" structures are found.

In contrast to Kek6, Kek5, Kek6's closest relative in the Kek family, localizes throughout the membrane in epithelial cells. It was initially hypothesized that this difference in localization might depend on differences in the cytoplasmic sequences between Kek6 and Kek5. However, swapping cytoplasmic sequences between both proteins had no effect on their localization, suggesting that the intracellular sequences are not responsible for the localization of these proteins. This, combined with the bicellular interaction between Kek6 and non-Kek6 expressing cells shown above, argues strongly that the extracellular portion of Kek6 influences its localization in the membrane of epithelial cells (Arata, 2011).

The following section features images created to represent Kekkon6 loaclization at various levels of detail. These images range from the dissection of the ovaries out of the female fly (figure 3), to the ovaries themselves (figure 4), to an ovariole removed from an ovary (figure 5), to an oocyte (figure 6), down to confocal images and representations of Kek6 localization (figures 7-12). These confocal micrographs are possible because the protein Kekkon6 has been tagged with Green Fluorescent Protein (GFP). When GFP is excited by the proper wavelength of laser stimulation, it fluoresces in the visible spectrum, specifically green. To remain consistent, many of the representation have been created using the color green as well. The captions below describe the concept or message depicted in the images.

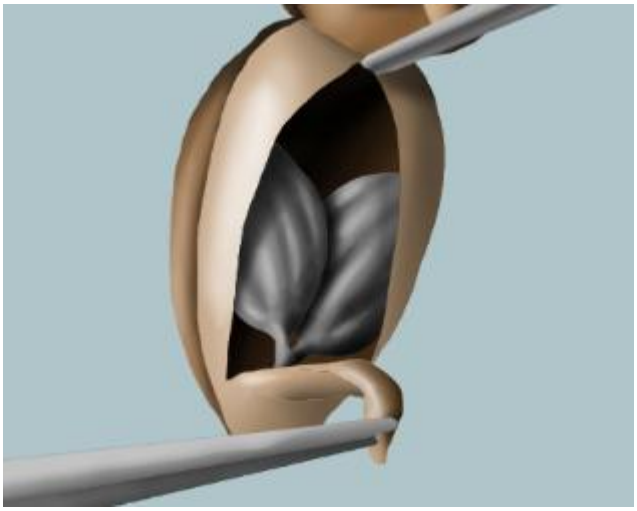


Figure 3. Ventral view of *Drosophila* dissection, in which the ovaries are extracted out to obtain the epithelial tissue encasing the oocyte within an ovariole. After the tissue is obtained we can prepare it for confocal microscopy to view Kek6-GFP expression.

Figure 4. Representation of *Drosophila* ovaries dissected out of an adult female. Here we can see the ovarioles that make up the ovary.

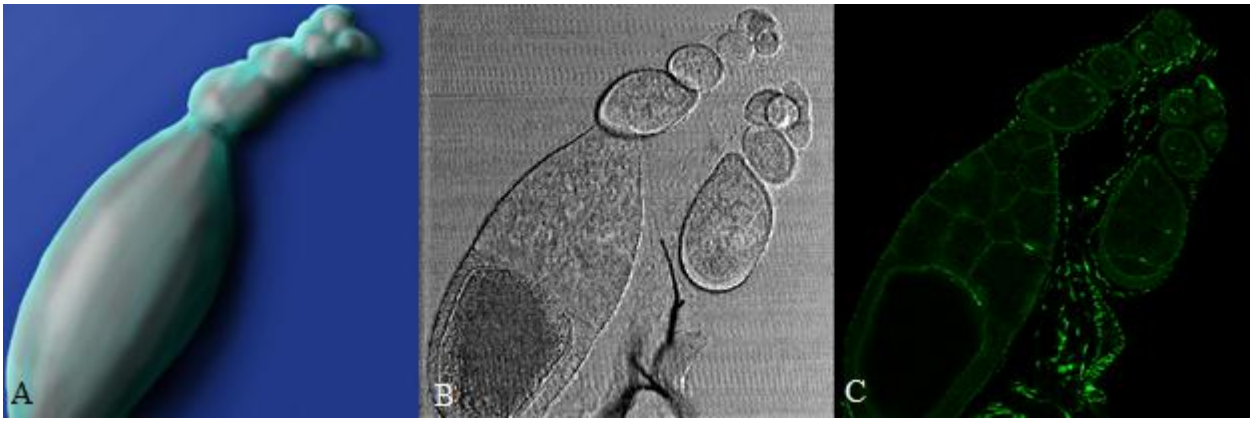


Figure 5. Ovariole dissected out from the ovary of an adult carcass. (A) Representation of an ovariole with oocytes at various developmental stages at approximantely THIS magnification. (B) Confocal image of a dissected ovariole taken with a 1.25x aperture and a 40x objective immersed in oil. (C) Confocal image of the same ovariole with GFP excited so we can see Kek6's localization ovariole taken with a 1.25x aperture and a 40x objective immersed in oil.

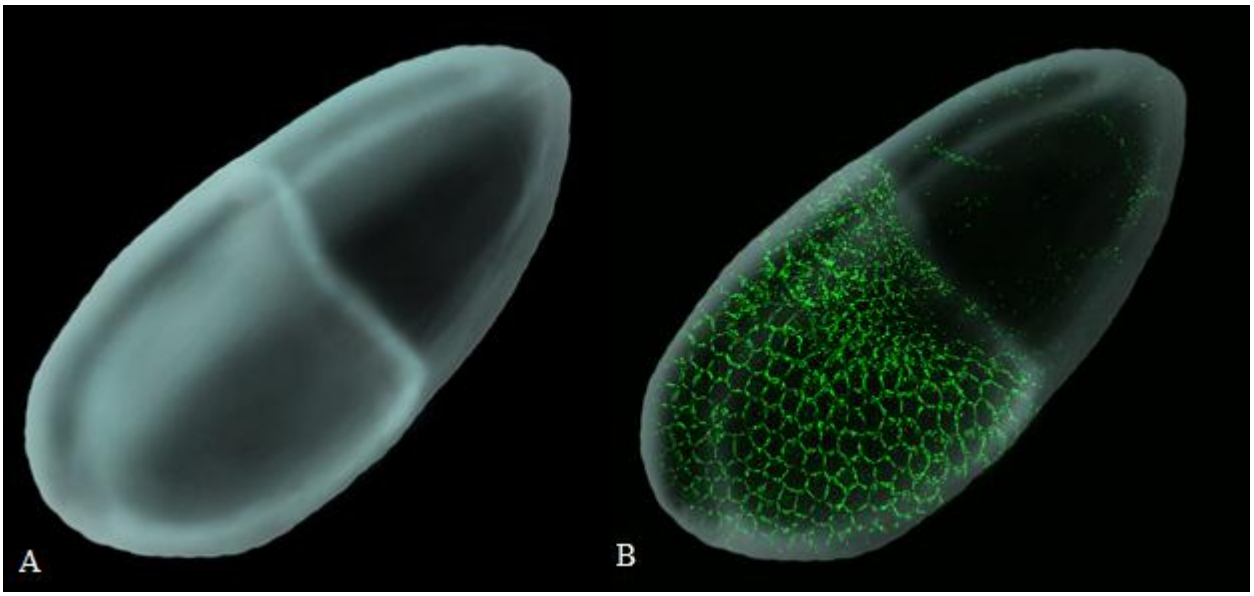


Figure 6. (A) Visual representation of an oocyte extracted from its ovariole. (B) The same oocyte inlayed with an image of excited GFP tagged Kekkon6 ovariole taken with a 1.25x aperture and a 40x objective immersed in oil.

Figure 7. A screen shot of view of Kek6 GFP expression in epithelial cells taken with a 1.25x aperture and a 40x objective immersed in oil.. The Microscope took many sliced sections of images and compiled them into a three dimensional rotational object. This shot was a still taken from the movie file created from this data.

Figure 8. (A) A three dimensional “x-ray” structural representation of the epithelial monolayer surrounding an oocyte. (B) Another representation of the same monolayer of cells with “solid” walls to better show the division of cells. (C) The same “solid” walled image as image B with a black base to add a variety of images so the viewer may better see and understand the cellular environment.

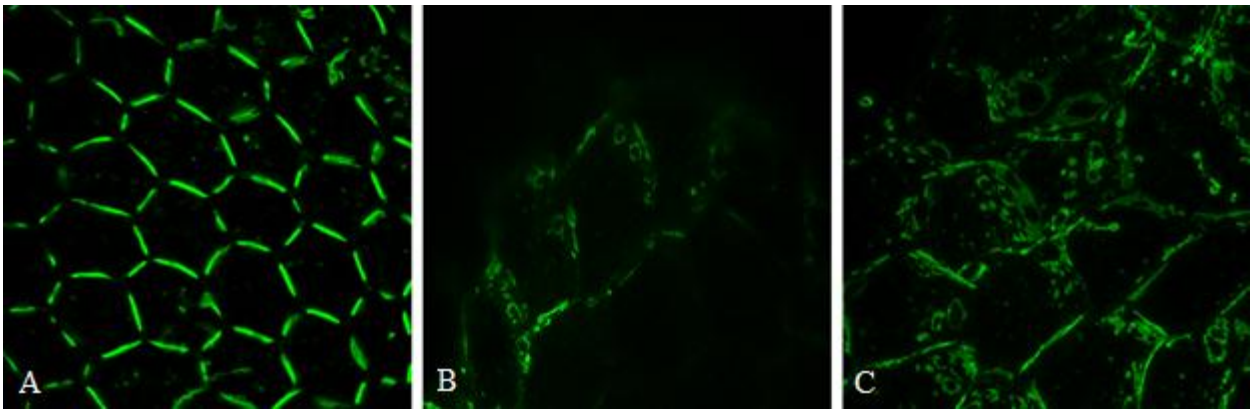


Figure 9. (A) Tricellular exclusion of GFP tagged Kek6 taken with a 1.25x aperture and a 40x objective immersed in oil. (B) Image of GFP tagged Kek6 of the portal structures seen in bicellular regions taken with a 1.4x aperture and a 63x objective immersed in oil. (C)Another confocal image of Kek6 and the portal structures seen in bicellular regions taken with a 1.4x aperture and a 63x objective immersed in oil.

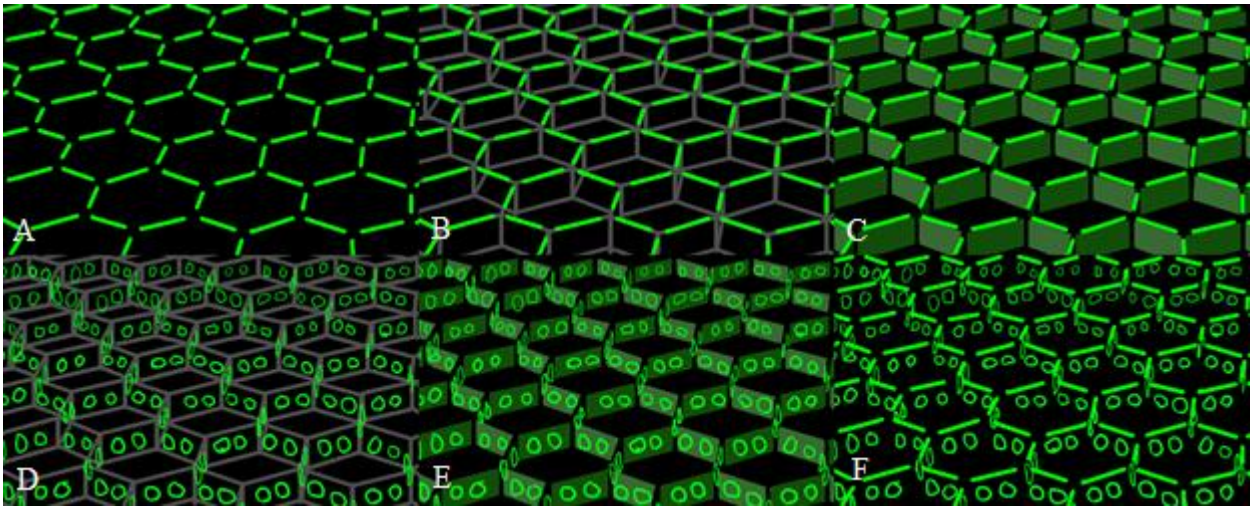


Figure 10. Graphic representations of Kekkon6 localization. (A) Geometric representation of Kek6 expression and exclusion from TCJs in follicle cell epithelium. (B) Same representational exclusion of GFP tagged Kek6 from Tricellular junctions overlaid onto a structural representation of the epithelial cells they localize in. (C) Tricellular exclusion overlaid onto a solid represention of the structure of epithelial cells. (D) Structural representation of epithelial cells overlaid with a representational image of the bicellular portals seen in Kek6 expression. (E) Solid walled structural representation of epithelial cells overlaid with the same image of the bicellular portals. (F) Combined representation of the tricellular exclusion and bicellular portal concepts.

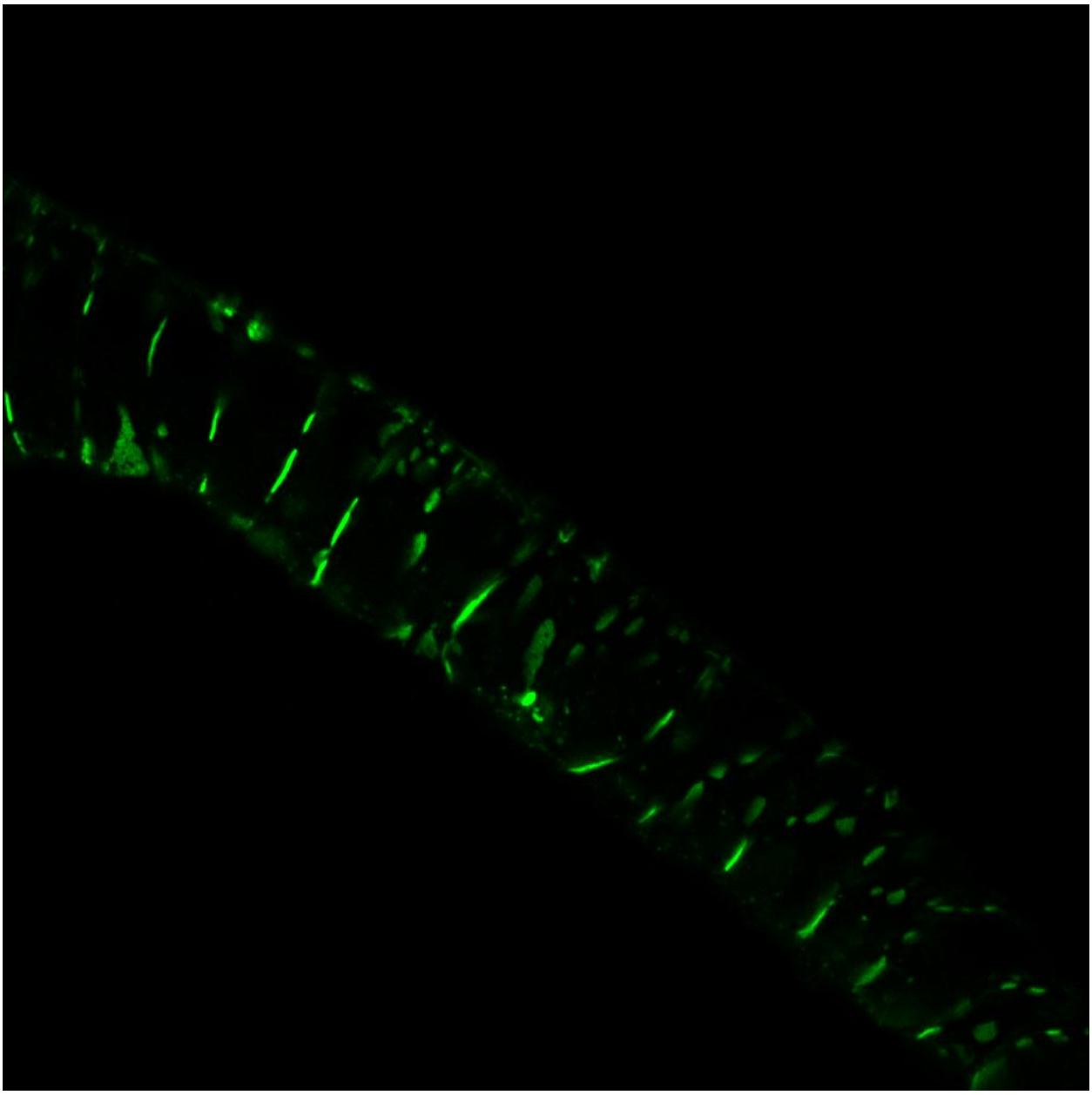


Figure 11. Cross sectional image taken with a 1.25x aperture and a 40x objective immersed in oil of GFP tagged Kek6. This shows where Kek6 is expressed most, which can be seen is apically (closer to the inside of the egg, towards the bottom left) rather than basally (closer to the outside of the egg, towards the upper right).

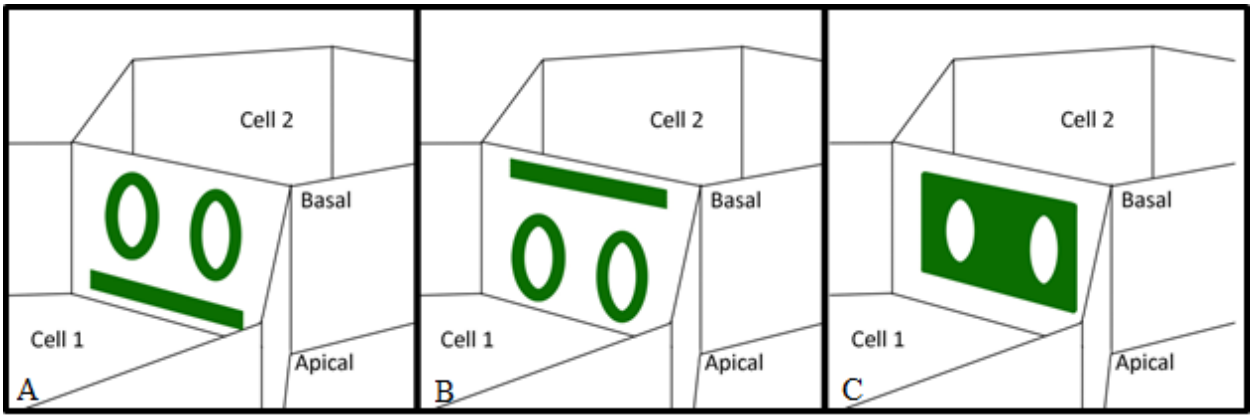


Figure 12. Although we are learning more about Kek6 and how it localizes in the monolayer of epithelial cells, it is unclear as to how its localization looks two dimensionally from the side, more specifically whether morse of the expression is seen apically (closer to the inside of the egg) or basally (closer to the outside of the cell). These three three-dimensional representations depict a few propositions. (A) Tricellular exclusion is seen more apically than the bicellular portals. (B) Tricellular exclusion is seen more basally than bicellular portals. (C) Tricellular exclusion and bicellular portals are within the same expressional structure and it is unclear whether the combined structure localizes basally or apically.

Biological Illustration: Visualization of the Biological Pacemaker Concept

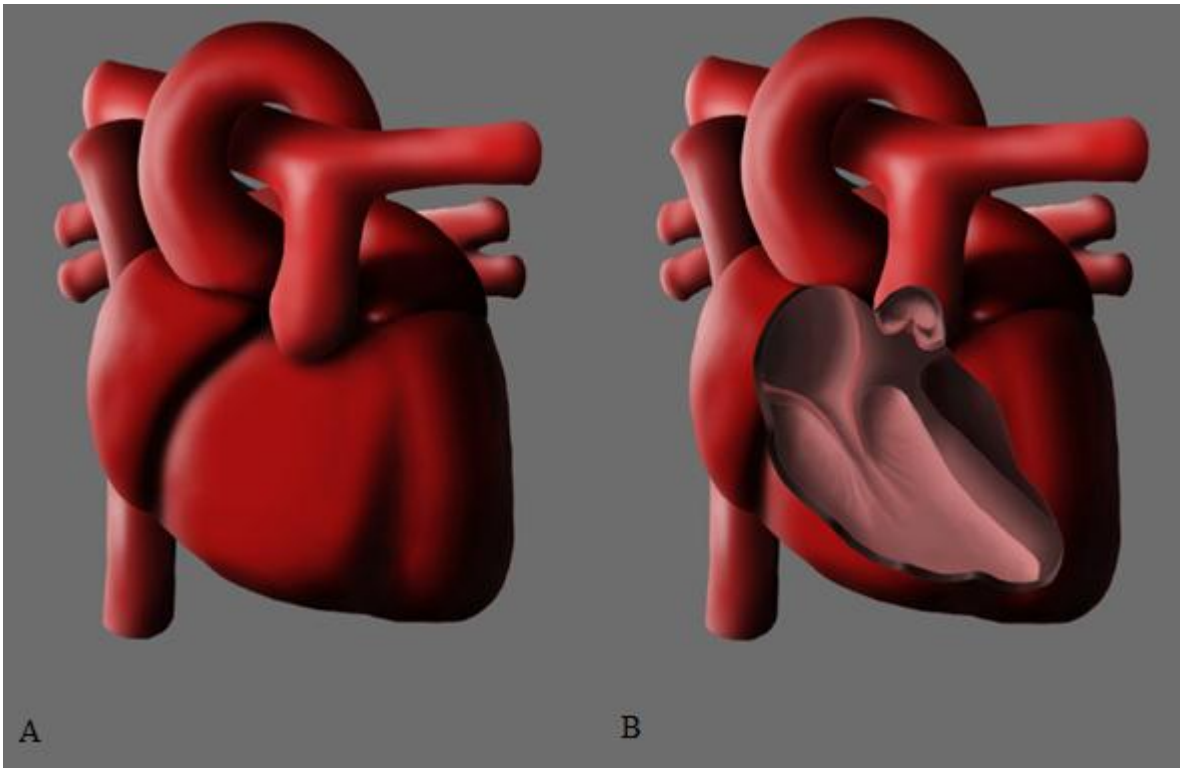


Figure 13. (A) Ventral view of the human heart. (B) A windowed look at the internal structures of the heart.

Glenn Gaudette's work involves the heart (figure 13). Basic anatomical analysis shows the heart to have two sides, right and left. Each side is made up of two chambers, an atrium and a ventricle. Each side also has one inlet and one outlet. The inlets each connect to a vein that brings blood back into the heart. Both outlets connect to an artery that carries blood away from the heart. In the right side of the heart there are two nodes, the sinoatrial nodes and the atrioventricular node, responsible for the electrical signal that causes the muscle cells in the heart to contract and therefor pump blood. The proper path of signal propagation starts in the sinoatrial node, carries through the right atria, reaches the atrioventricular node, continues into the septum to the bundle of His, then the path diverges and follows the septum until it reaches the base and fully diverges and propagates throughout both ventricles (figure 14).

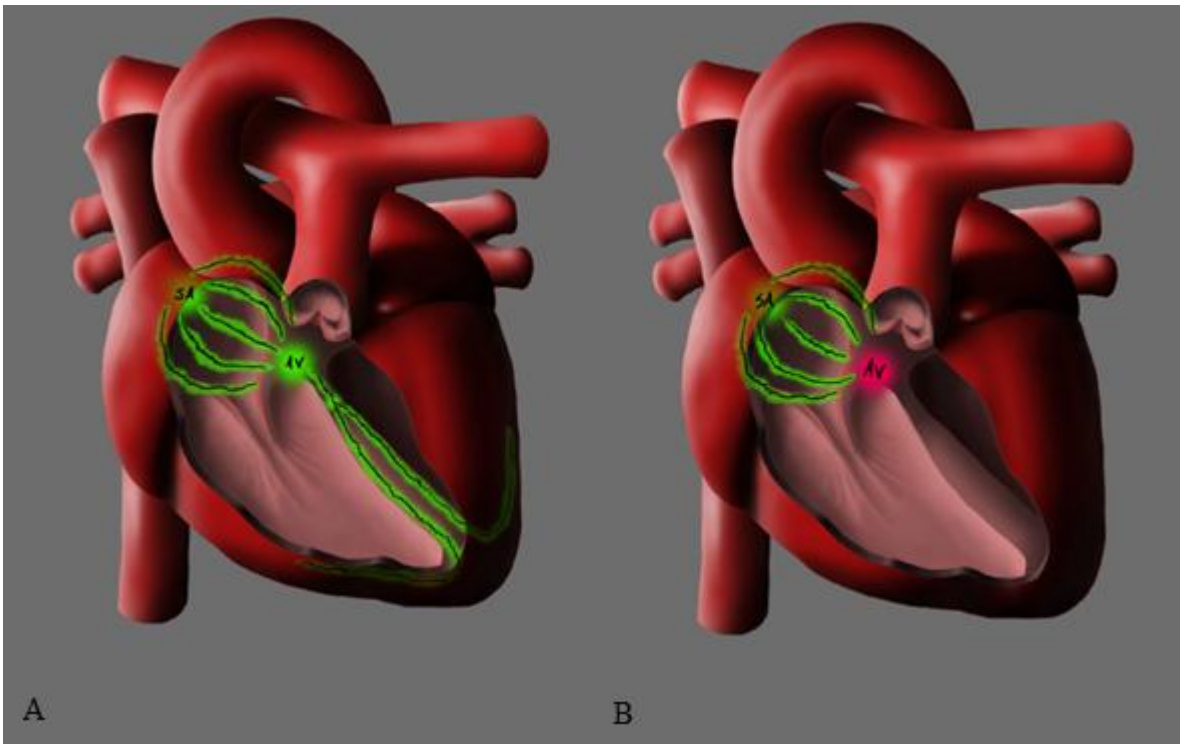


Figure 14. (A) This image shows the location of the Sinoatrial and Atrioventricular Nodes as well as the natural path of propagation shown in green. (B) When the current released by the SA node reaches dysfuntional AV tissue, the current can no longer carry forward.

Unfortunately, sometimes an individual's electric pathway in the heart is rendered dysfunctional either during development, by aging or by traumatic event. There is however technology today to help compensate for the loss of function enough that the individual can live. I am refereeing to the use of electronic pacemakers. The Gaudette lab is working to develop a biological pacemaker using human mesenchymal stem cells, or HMSCs, to bypass the dysfunctional tissue, obviating the need for these electronic pacemakers.

Currently available electrical mechanical pacemakers have certain limitations and negative attributes. Because of the nature of the device, its function can be hindered by electromagnetic interference. This of course is dangerous for the individual with the pacemaker. These pacemakers also have batteries, which require changing. Each surgery done to do so is also in itself a risk. They also have leads that conduct the charge which can fail and render the pacemaker dysfunctional. These devices are mechanical, so they are not responsive to biological stimuli such as physical or neurological stresses. This means they cannot adjust to meet the needs

cardiac needs of the individual. They are also, by comparison, large physical implants, that cannot grow with the patient. This creates issue as the patient grows and so does their heart (Gaudette, 2012).

The biological pacemaker developed by Gaudette and his lab could solve or reduce the impact of these issues. They would be part of the hearts biological structure and therefor would be responsive to the physiological changes imposed by the body. The pacing would be much more reliable. It would also greatly lessen the need for repetitive surgeries to change the battery, electrodes and other parts in the mechanical pacemaker. Also, because of the greatly reduced size of the biological pacemaker and its catheter style delivery, the implantation is much less invasive and therefor reduces resulting infection and inflammation when compared to the surgery needed to implant electrical pacemakers (Gaudette, 2012).

There are four design concepts (1a, 1b, 2a, and 2b) for creating a functioning biological pacemaker. Aims 1a and 1b create a new charge after the dysfunctional AV tissue, and 2a and 2b carry the existing current from the SA node to another region of functioning tissue. For the explanation of these aims, a non-functioning AV node will be used as an example. In this case, the charge released by the SA node ceases to flow as the non-functioning AV node does not allow it to further propagate (figure 14).

Aim 1a

Aim 1a uses hMSCs to create a new current after the dysfunctional AV tissue contained in a polyurethane mesh bag. These cells partially extend past the polyurethane mesh and form gap junctions with the neighboring myocytes (figure 15). The concept is that these cells are induced to accumulate ions which will eventually result in the cell reaching its depolarization threshold causing an action potential to be generated and the charge expelled through the gap junctions with the myocytes (Cohen, 2012). Because of its placement, the current reaches the neurological syncytium within the heart's structure and propagates forward throughout the rest of the heart. However these hMSCs have the tendency to migrate, so they are implanted just below the damaged AV tissue in a polyurethane mesh "bag" to keep them localized. This mesh bag bag has small enough holes to keep the cells where they are, but big enough to allow the stem cells to

project through the mesh in order to form gap junction with the neighboring myocytes. These muscle cells receive the expelled charge from the stem cells and carry it forward to the bundle of His.

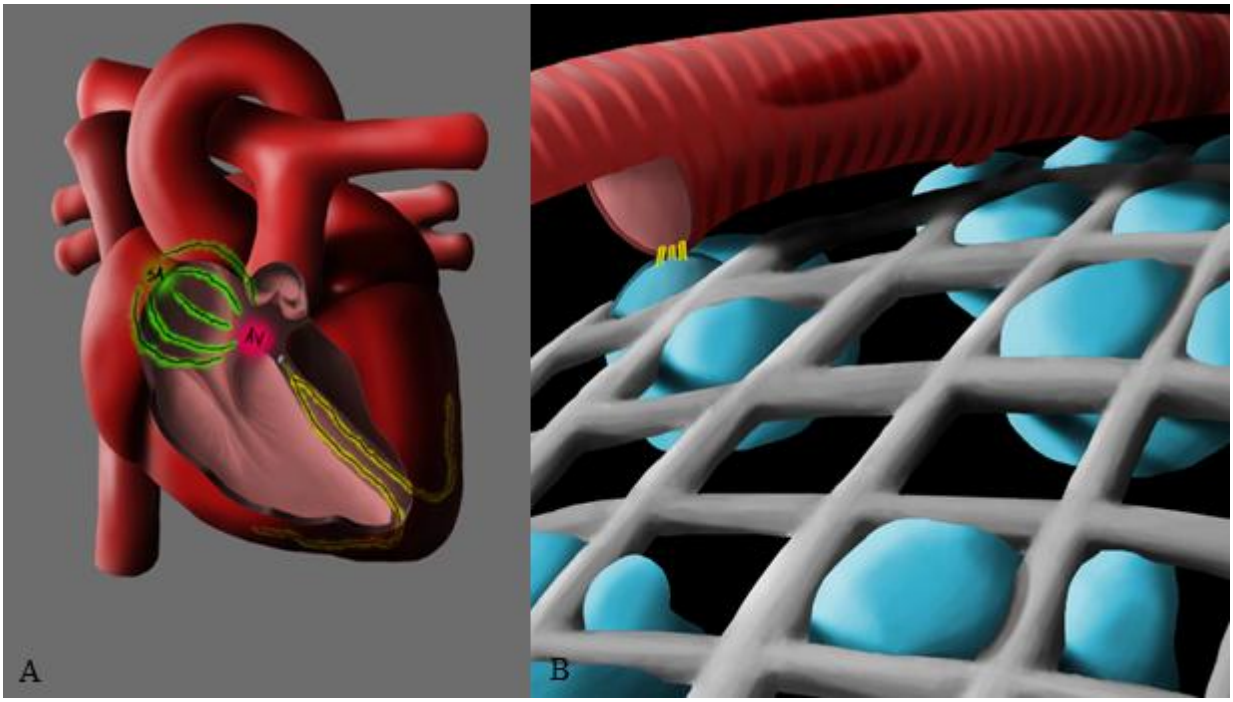


Figure 15. (A) The implanted mesh bag of hMSCs generates and releases a new current, represented in yellow. (B) A close up of a stem cell projecting through the polyurethane mesh bag and forming gap junctions (yellow tubes) with a myocyte.

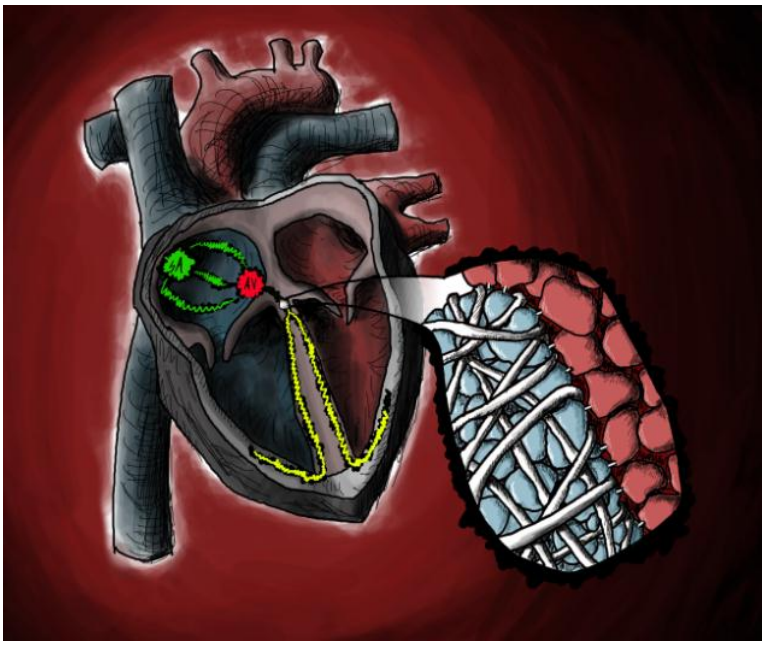


Figure 16. Conceptual image of the generated current released from the bag of hMSCs show in yellow. The inset shows a close up image of the stem cells making gap junctions with neighboring myocytes.

Aim 1b

Aim 1b uses induced pluripotent stem cells (iPS cells) to create a new current after the dysfunctional AV tissue contained on a microthread. These stem cells have been developed from fully differentiated cells through genetic reprogramming and are not as apt to migrate, so they do not need to be contained in the PU bag. Because of this, they are seeded onto an electrospun microthread. The thread is implanted just below the damaged AV node. Again the concept is that the cells will accumulate ions until the threshold is reached and expel a charge through gap junctions with the myocytes, stimulating the muscle and causing it to contract (figure 17).

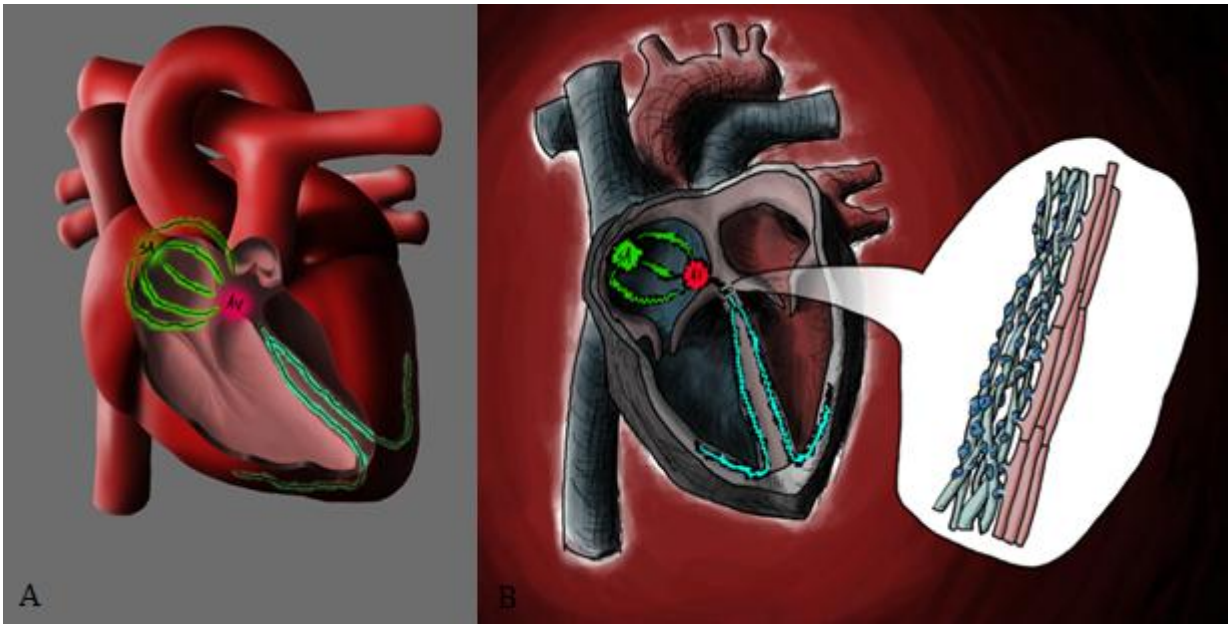


Figure 17. (A) The implanted micro thread seeded with iPS cells generates and releases a new current, represented in teal. (B) Conceptual image of the generated current released from the microthread of iPS cells shown in teal. The inset shows a close up image of the stem cells making gap junction with neightboring myocytes.

Aim 2a

For 2a, hMSCs contained in a polyurethane mesh tube will be used to conduct an existing current from the functional SA node, around a dysfunctional AV node to functioning tissue further down the path of propagation. The tube is designed in such a way that the ends of the tube only have one layer of polyurethane mesh whereas the length of the tube has multiple layers, making it much more difficult for the stem cells inside the tube to make gap junctions in this thicker region. However gap junctions are easily made at the ends of the tube by the mechanism described earlier. This tube runs from between the SA and AV nodes and past the damaged AV node. The cells receive the charge from the functioning SA node and carry it around the damaged AV node. The thicker wall here inhibits the charge from escaping into the damaged tissue. Once the charge reaches the other end of the tube, the thinner walls again allow gap junctions to be made so the current can be passed from the stem cells to the healthy myoctes for further propagation (figure 18).

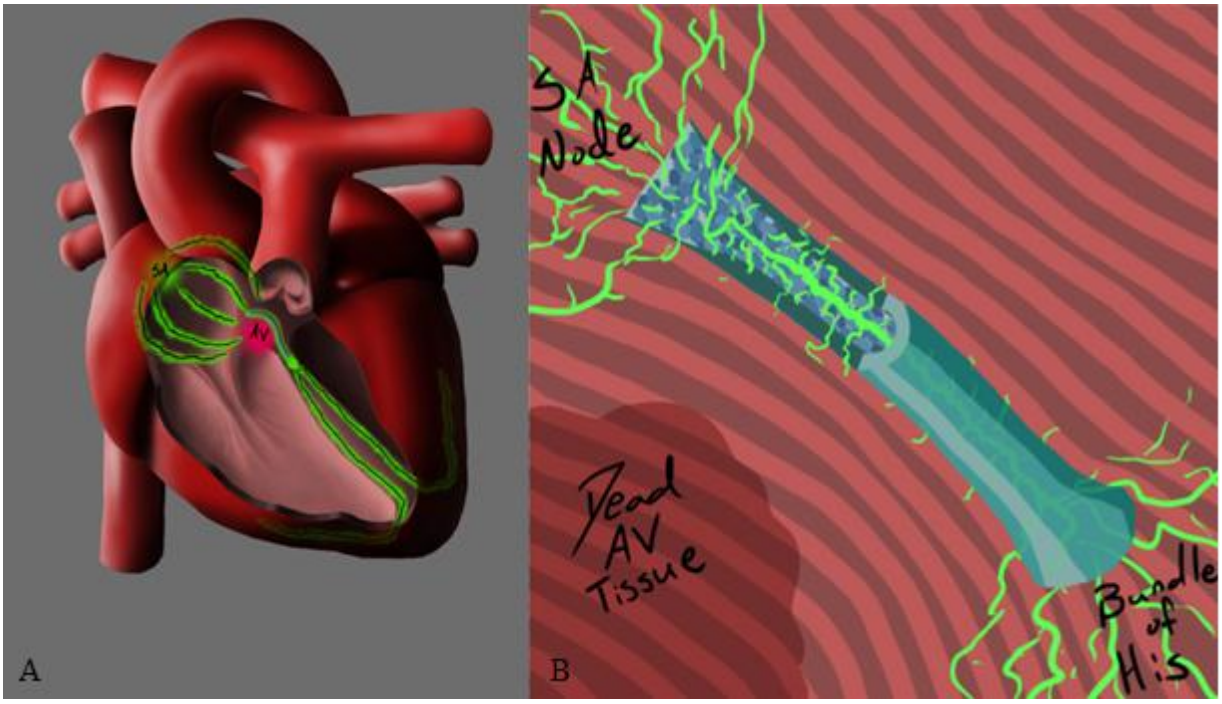


Figure 18. (A) The current released by SA node is received by the hMSCs in the polyurethane mesh tube and carried around the dysfunctional AV node to an area of functioning myocytes where the current then continues to propogate. (B) A close up image of the stem cells within the mesh tube carrying the charge from the SA node around the AV tissue. The charge can enter the tube because of the thinner mesh allowing gap junctions. The length of the tube has thicker mesh that makes gap junctions difficult to form, however some do get made. Again the thinner mesh at the end of the tube allows the charge to leave the tube because gap junctions can be made with the myocytes in this region.

Aim 2b

Aim 2b uses iPS cells seeded on longer microthread in order to conduct an existing current from the functional SA node, around a dysfunctional AV node to functioning tissue further down the path of propagation. This seeded micrthread would be implanted between the SA and AV nodes and continue around the dead AV node. As above in 2a, these cells receive the charge from the SA node and carry it along the microthread around the AV node until it reaches healthy tissue, where the charge is carried on by healthy myocytes (figure 19).

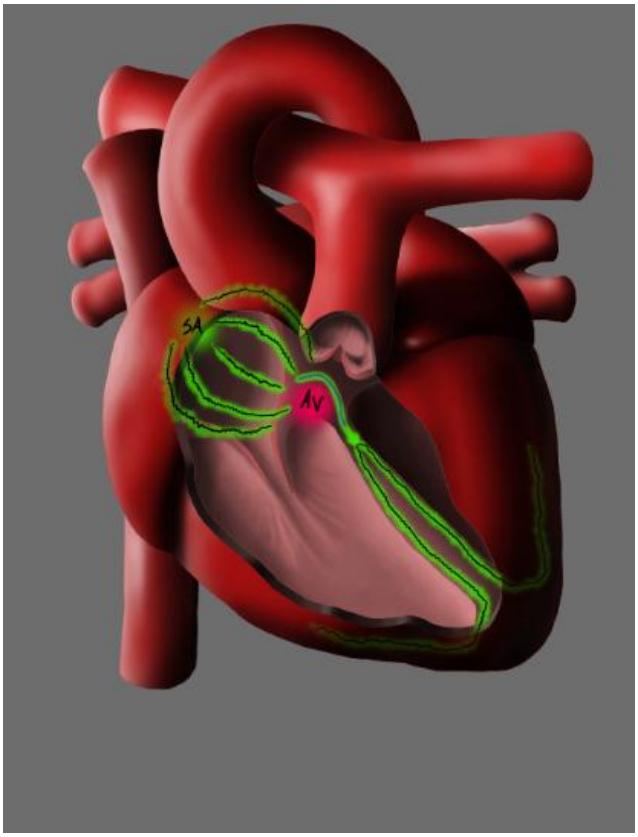


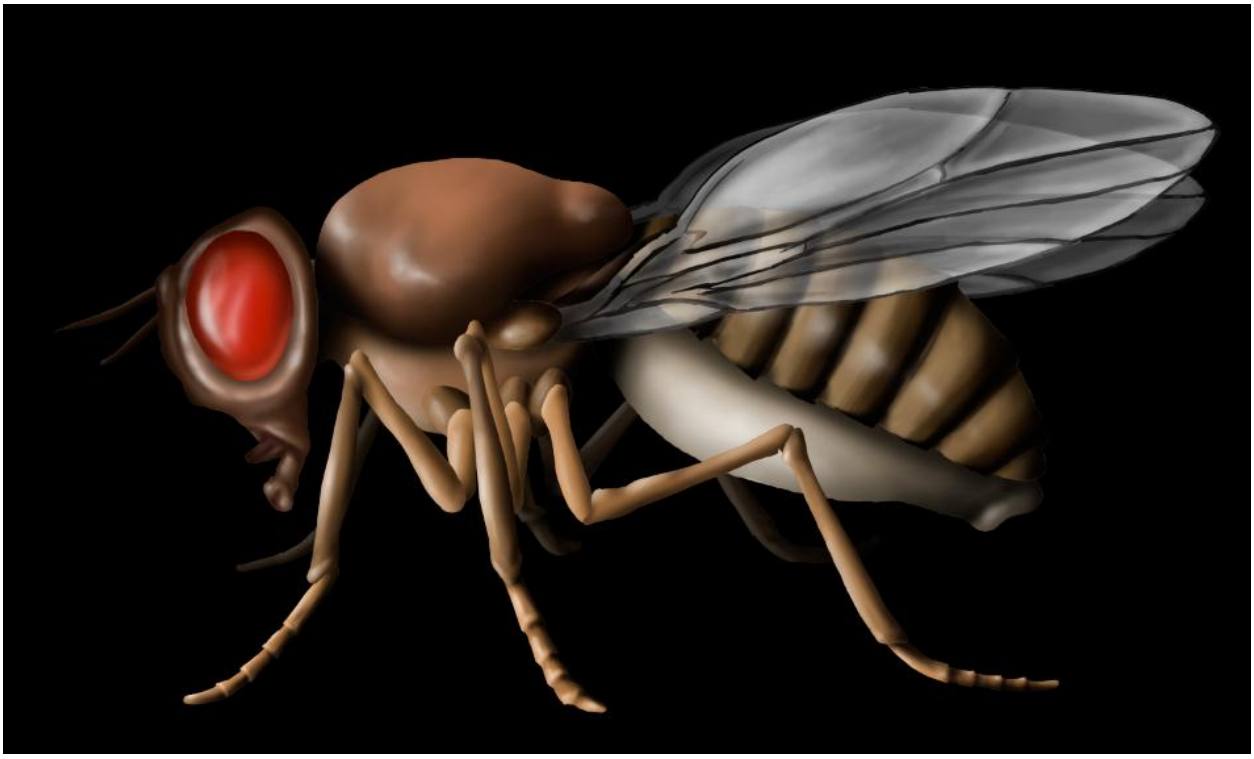
Figure 19. the current released by the SA node is received by the iPS cells on the microthread and carried around the dysfunctional AV node to an area of funtioning myocytes where the current continues to propagate.

Biological Illustration: Representations of Biological Subjects

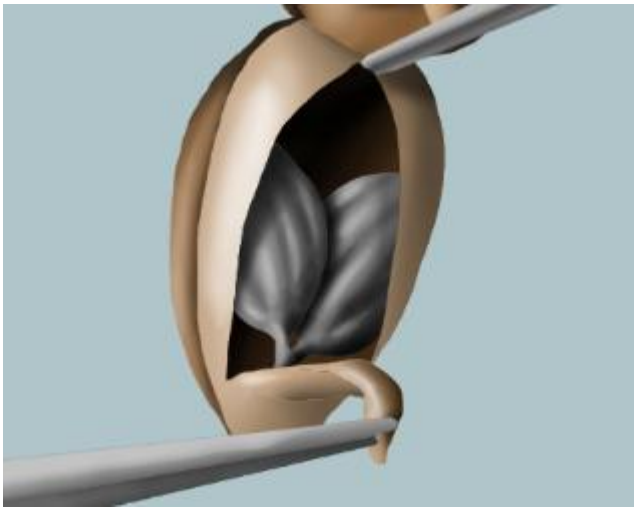
The work I've done for Professor Duffy and Professor Gaudette are each very different in content and in nature. For Professor Duffy's Kekkon6 project I was representing complicated images and science that was just being discovered. In Professor Gaudette's Bio-pacemaker project I transferred a concept into representational reality. In both I had to establish a macro understanding of the subject matter for the viewer before delving into what was being studied. However, in the bio-pacemaker project the focus was representing a physical object being placed into an understood environment with a clear hypothesized result. There was very clear instruction and set of deliverables, and as a contractual artist it is easy to deliver when you know exactly what your goals are. It is challenging and enjoyable to have a list and to meet the requirements. In the Kekkon6 project the focus was on interpreting visual data that is not fully understood and into a comprehensible representation. I had a lot of creative license with this project because science does not, at the moment, understand the "why" of what is being seen, so we are not sure of the best way to represent what we do see. This was interesting because as a scientist I could speculate, and as an artist I could imagine.

Professor Duffy's Kekkon6 Project

The work I did for Professor Duffy was very hands on to start. Before any real drawing could be done, other than rough sketches, I had to obtain the tissue needed to take images of Kekkon6. I first had to separate female flies from the males, dissect the ovaries out of the female fly, then dissect out the ovarioles apart from each other, and then separate the oocytes of the each ovariole. After that was done, the tissue had to be fixed and mounted onto slides to be viewed under the microscope. It was then my job to represent what was seen through the microscope in a way that was clear and accurate.

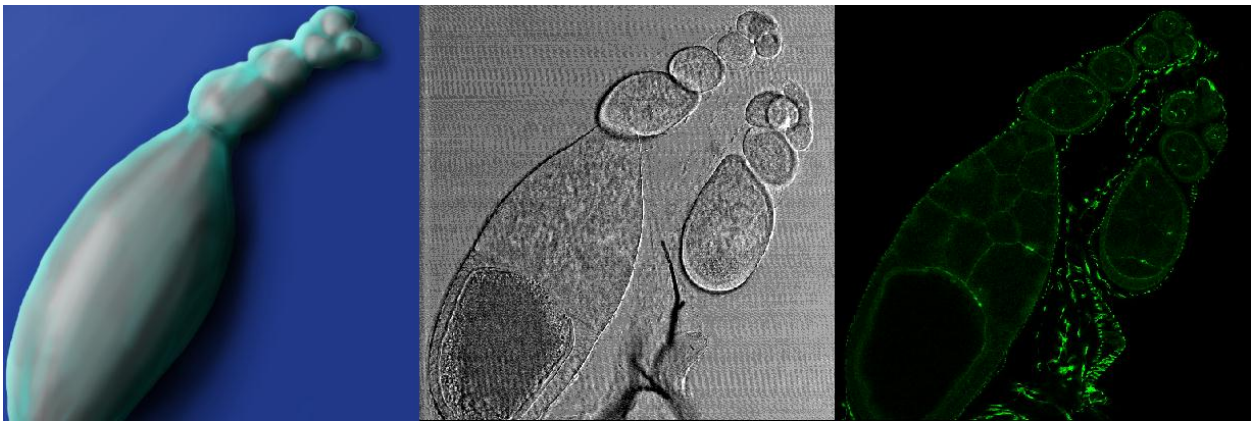


This is a female member of the species *Drosophila melanogaster*. To many biology majors the fact that *Drosophila melanogaster* is the scientific name for the common fruit fly is common knowledge. To an art major, this information or even what a fruit fly looks like up close may not be so common. I thought it was important to understand every level of studying Kekkon6, starting with a visual depiction of the organism.

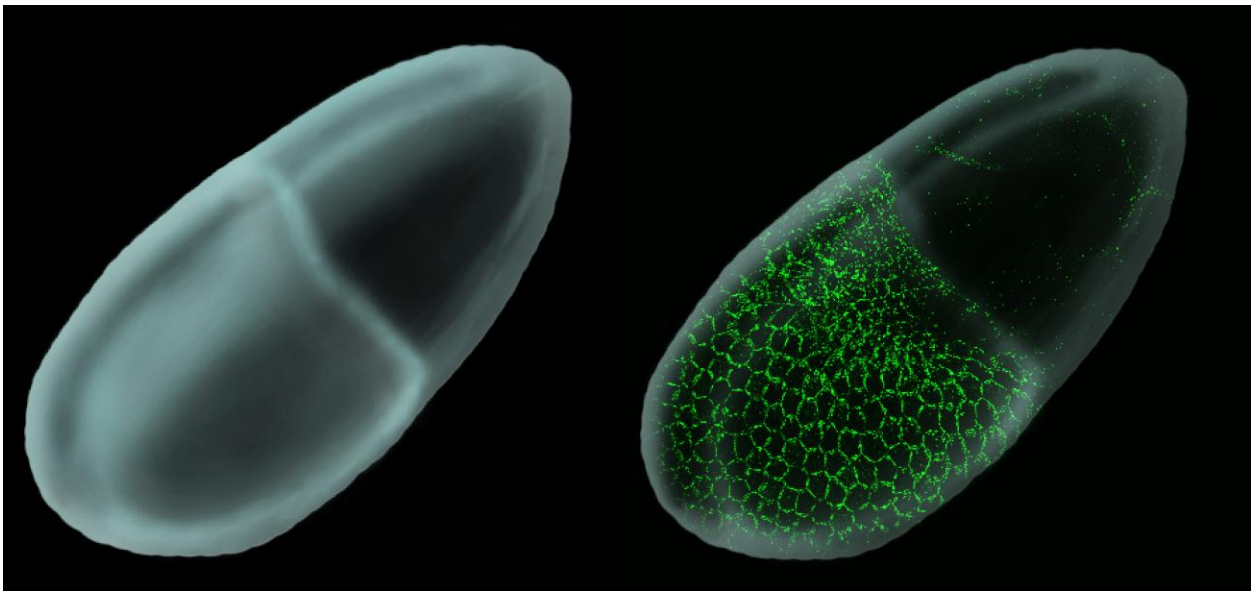


This image depicts the dissection of the fly to remove the ovaries. I thought it was important also to understand how this tissue is obtained. An image like this was also a good transition from the large fly to the next image of just the ovaries. It shows anatomically where they are within abdomen of the organism.

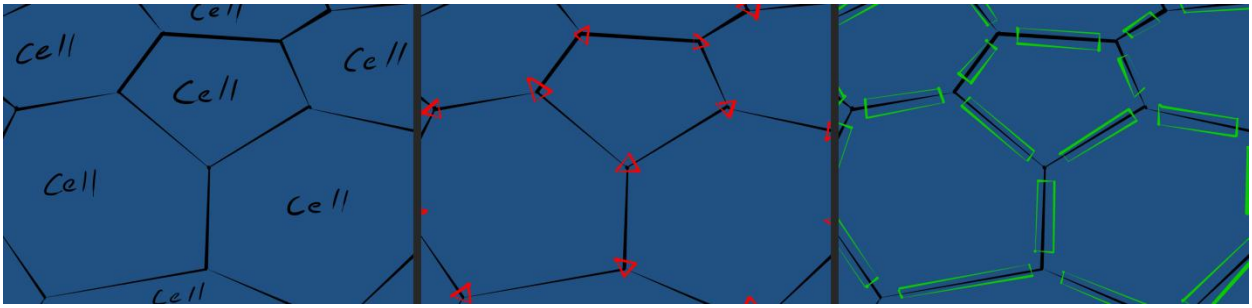
This image shows a representation of the ovaries extracted from a fly. These image, like many of the others, was created in layers. At first I shaded each individual ovariole from light to dark. Although I do not talk about it above in the biological portion the ovarioles within each ovary are encased in a sheath of tissue that make them one individual ovary. I wanted to depict that for biological accuracy and decided to add a layer of shading that wrapped around all of the ovarioles to help the eye understand that something thin is encasing them into one unified object.



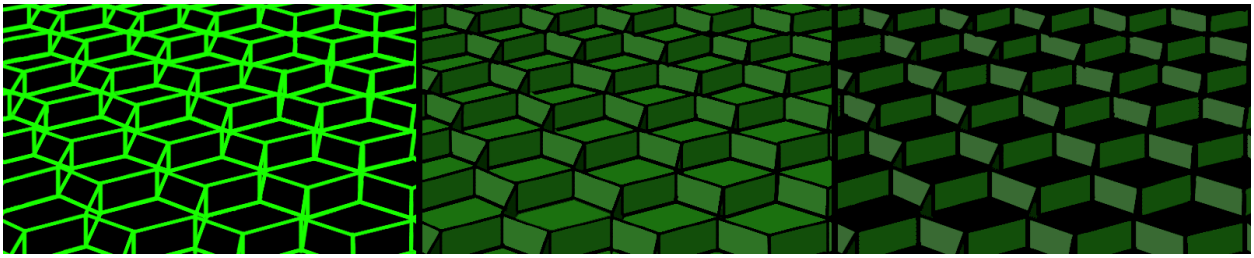
These images show an individual ovariole, containing oocytes at various stages. Using the two microscope images to the right as a base I recreated what the actual ovariole being imaged might have looked like. I thought that seeing the oocyte on different visual levels would help the viewer understand the structure of the oocyte better. Pertaining to this project there was no reason to capture the middle image, but combined with the other two images more can be understood about the oocyte internally. Again, after completing the individual oocytes I added a top layer, this time of color, to help show that the oocytes are wrapped together by tissue to make one ovariole.



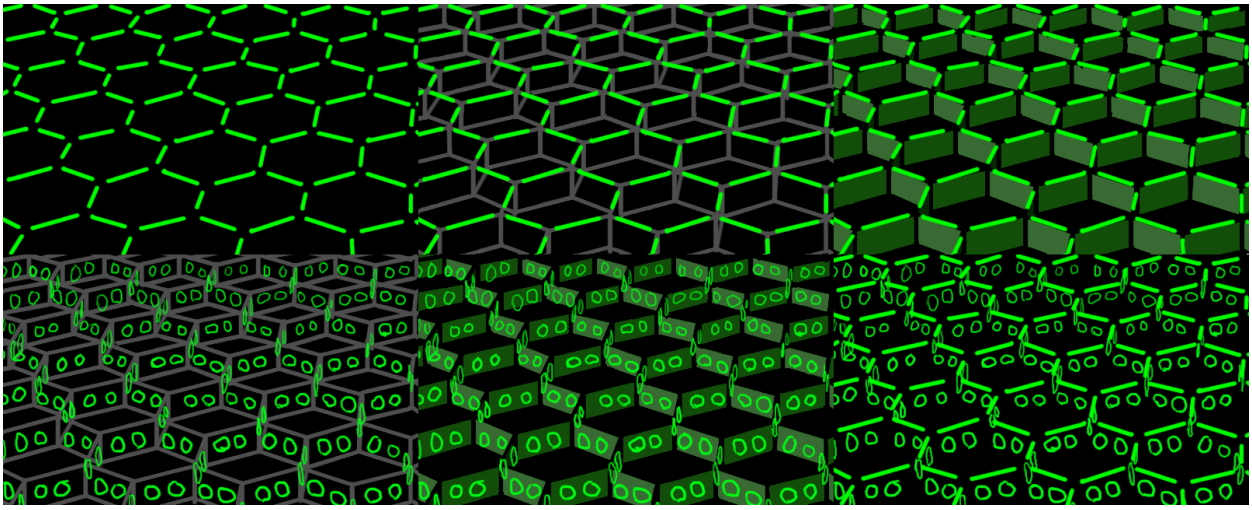
This image depicts a single oocyte. Using an actual image taken with a microscope I drew a representation of the oocyte being imaged. This time however I made the part of the drawing transparent and laid the image inside of the drawing. I thought this was an interesting way of understanding the differences and similarities between what can be seen with the naked eye through a microscope and what can be seen with microscopic enhancements.



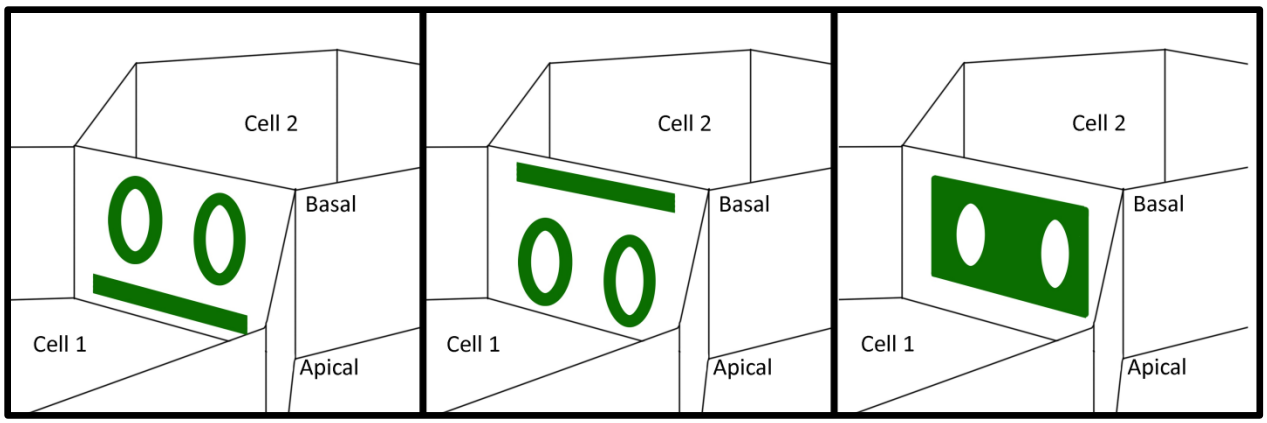
This image of neighboring cells was placed early in the biological portion in order to give the viewer a basis for understanding where tricellular regions (red) and bicellular regions (green) were. In many of the other images the focus is realistic representation. I went for simplicity here. Often a message can be crowded by or lost in unnecessary and extraneous information. The colors were chosen simply for contrast. In any field it is important to know when less is more.



Again, these images showing three-dimensional cell structure have been kept simple. Even so, it appears there is a lot going because of the repetition of shape. The first image shows what can be likened to a skeletal view, showing only edges of planes, in which other structures can be seen through the structure. Contrasting that image, the other two have solid “walls” that show individual rooms that represent individual cells. I included two versions because when it comes to shapes and light, people often perceive images of this nature differently. I was hoping to accommodate for this by including both images.



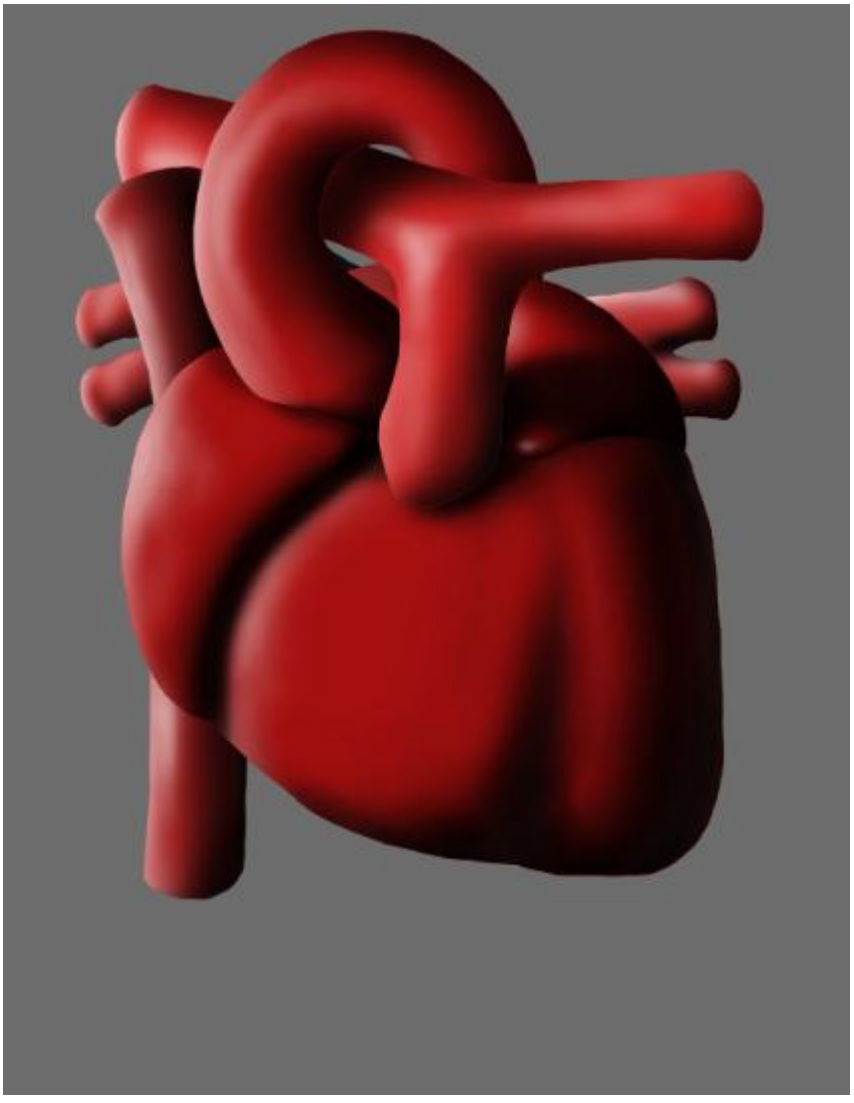
After giving the viewer an understanding of how the tissue is arranged, I simplified the ideas of Kek6 exclusion. The first top panel shows a graphic representation of what the microscope saw, Kek6 localized along bicellular region and no expression in tricellular regions. The next panel depicts the same thing but with a greyed version of the skeletal structural image to clarify the location of cells. The same was done with one of the walled version. Again including both was to help ensure the idea gets across as everyone sees things differently. The first and second bottom panels show simplified portals along bicellular surfaces. The last image shows both observed localizations together. In doing so a pseudo-structure was created with no actual structural image behind it. It was by doing this that the question crossed my mind: Where are these patterns pertaining to the cell membrane?



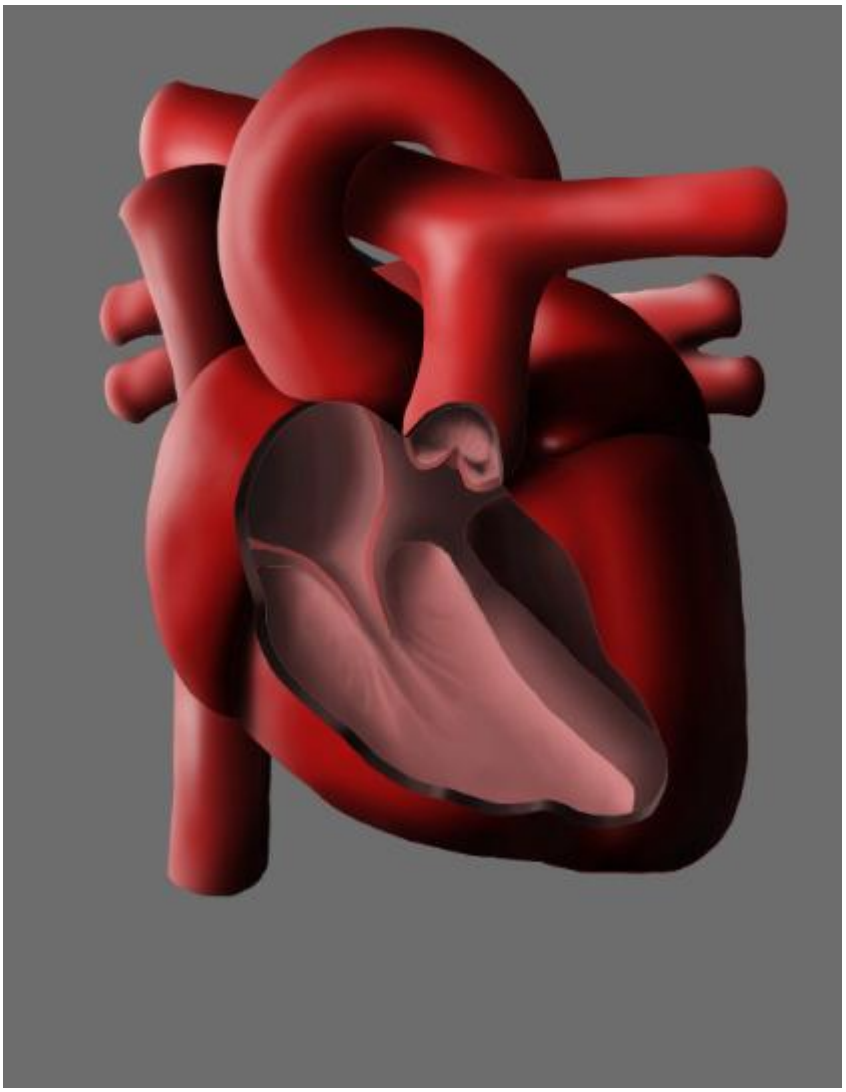
The answer to the question above had no answer. We observed higher concentrations of Kek6 expression apically. However this observation is not enough. After discussion with Professor Duffy, three proposed concepts were created. More images would need to be taken to fully understand this. One way that this could be better understood is to compare Kek6 expression with other proteins known to localize in certain regions of the membrane. However we did not have to time to obtain the resources needed to do this and it could in fact be an entire MQP in and of itself.

Professor Gaudette's Biological Pacemaker Project

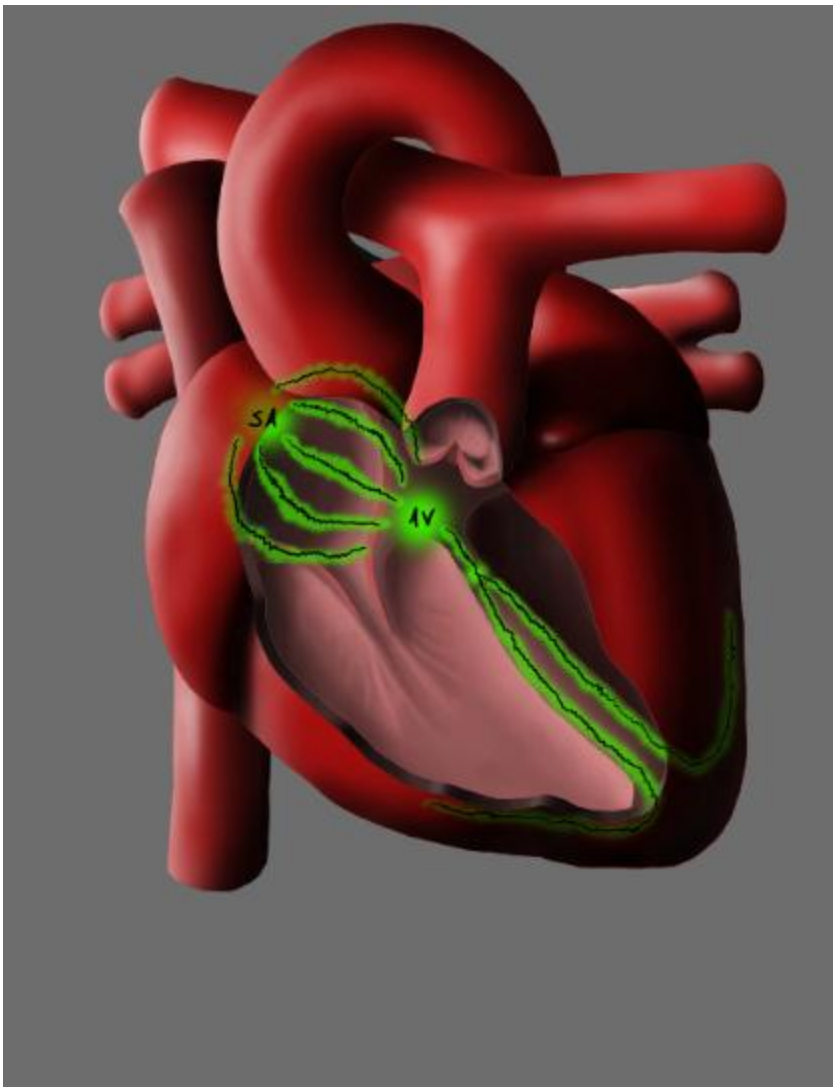
Professor Gaudette's project moved forward very predictably from concept, to drafting, to approval, creating a final image, having it critiqued, and then executing the alterations. As some whose ideal career is contractual biological illustration, having clear and concise requirements to satisfy is ideal. That was what I enjoyed most about this project, other than the interesting biology of the concept.



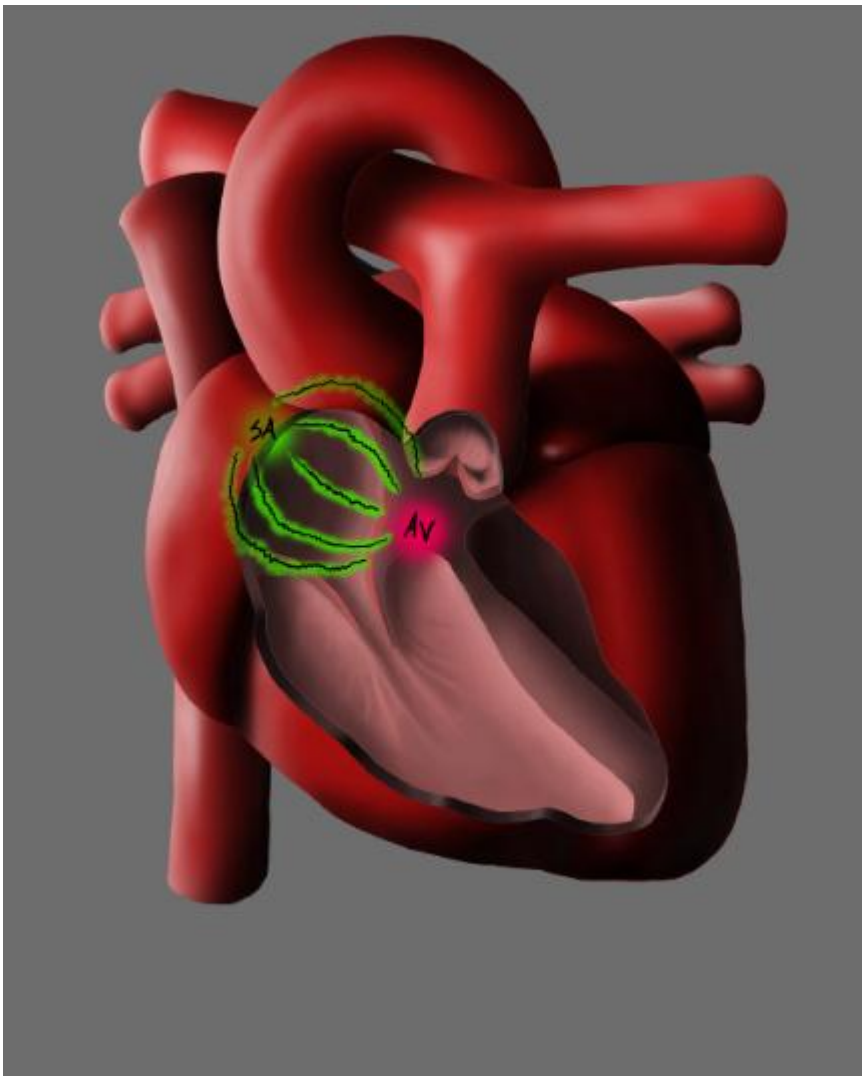
Like the *Drosophila* project I felt it was necessary to get an all-encompassing view of the organ being discussed. The focus here was the shape and features of the heart rather than showing it inside of a human body with other organs and structures in the background. I felt that everyone know the hearts location within their own body.



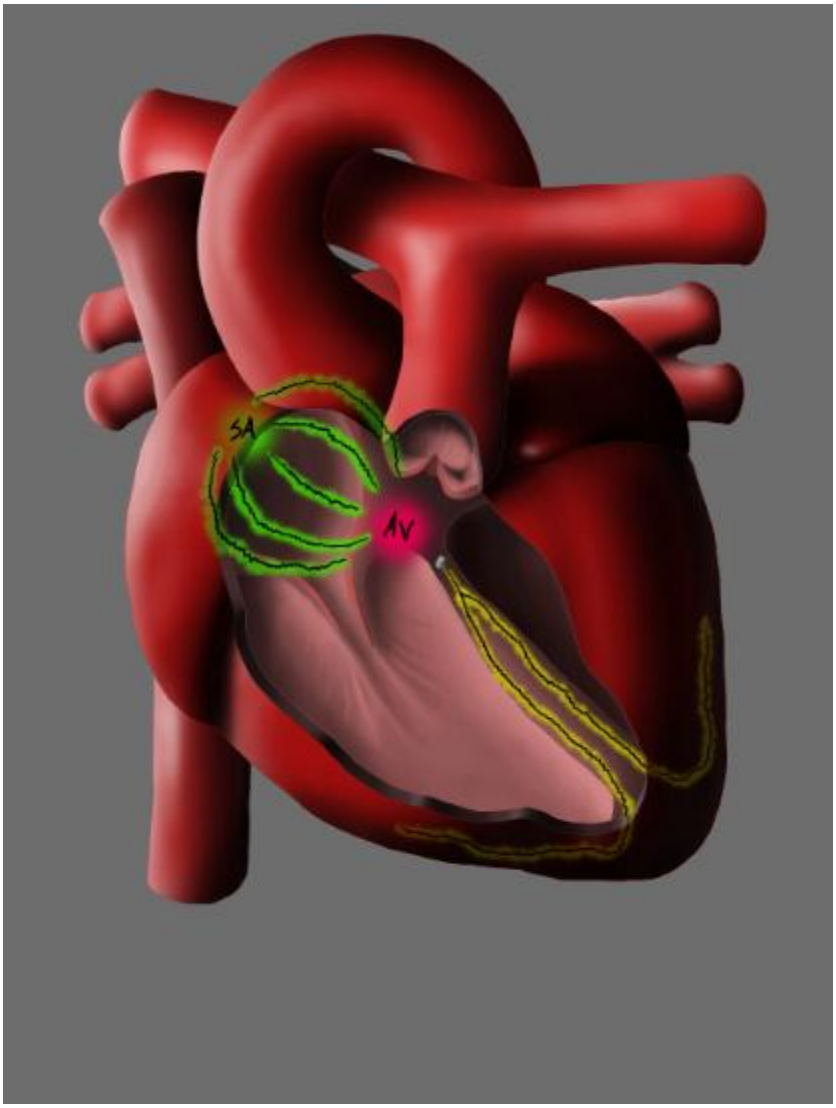
This is a windowed view of the internal makings of the area of the heart we are interested in. This look “into” the heart is not what it looks like however. After having the previous image of the heart drawn out, it didn’t make sense to cut away from that image and draw the internal structures. What is seen here is a layer on top of the heart with the internal structures drawn, essentially, on the surface of the heart to make it appear as if the heart had been cut into. When this layer is removed, the heart appears whole again. Here I enjoyed imagining three dimensionally where certain walls and structures would begin and end.



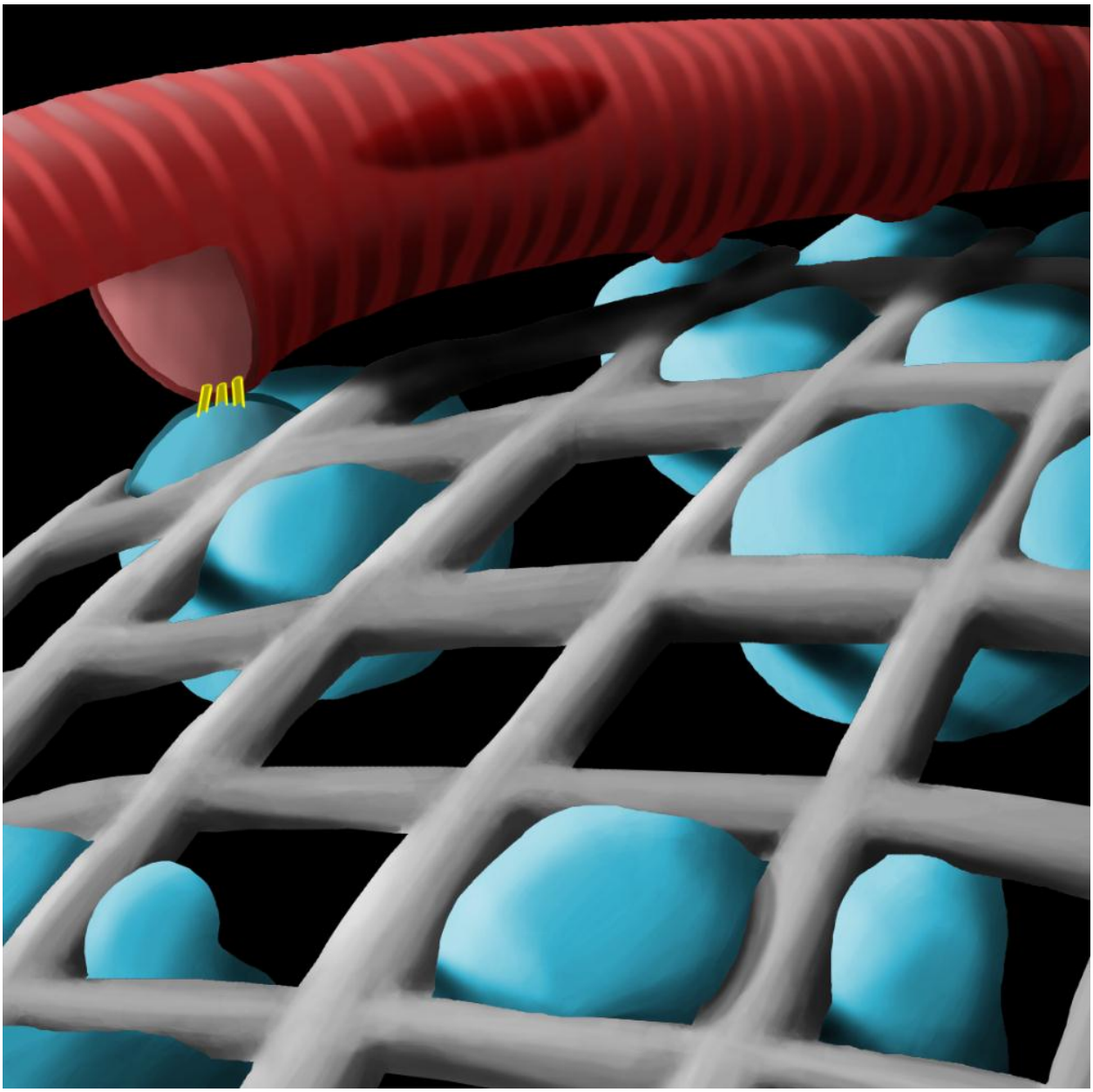
Depicting the path of current propagation was fairly easy. Some of the path carries the current through areas that are not seen “into”. I made those sections of the path less vibrant to show that the path is beneath the exterior of the heart, but still visible so the viewer knows it persists through these areas.



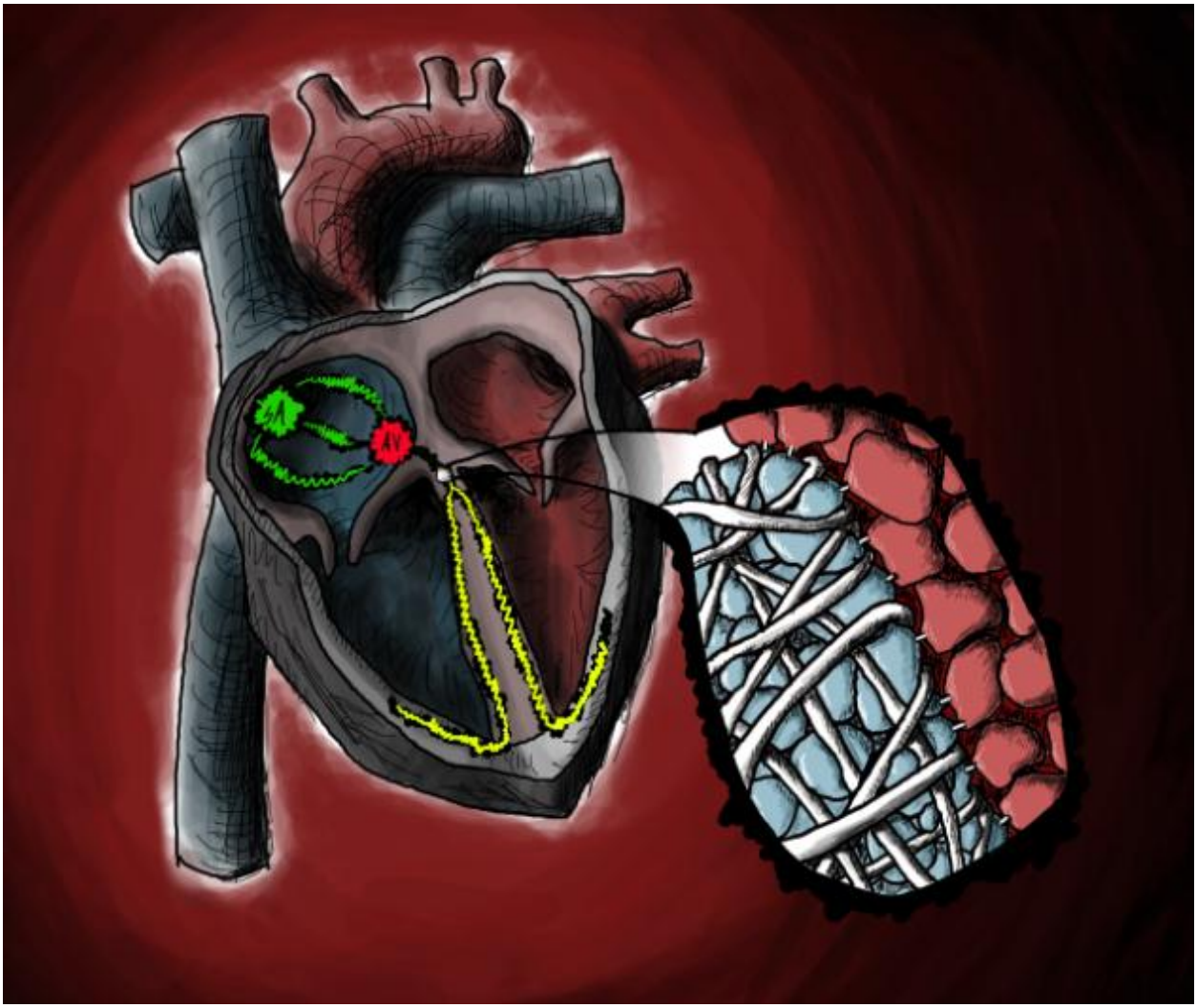
Here you can see that the dysfunctional AV node inhibits the current released by the SA node from carrying through to the rest of the heart.



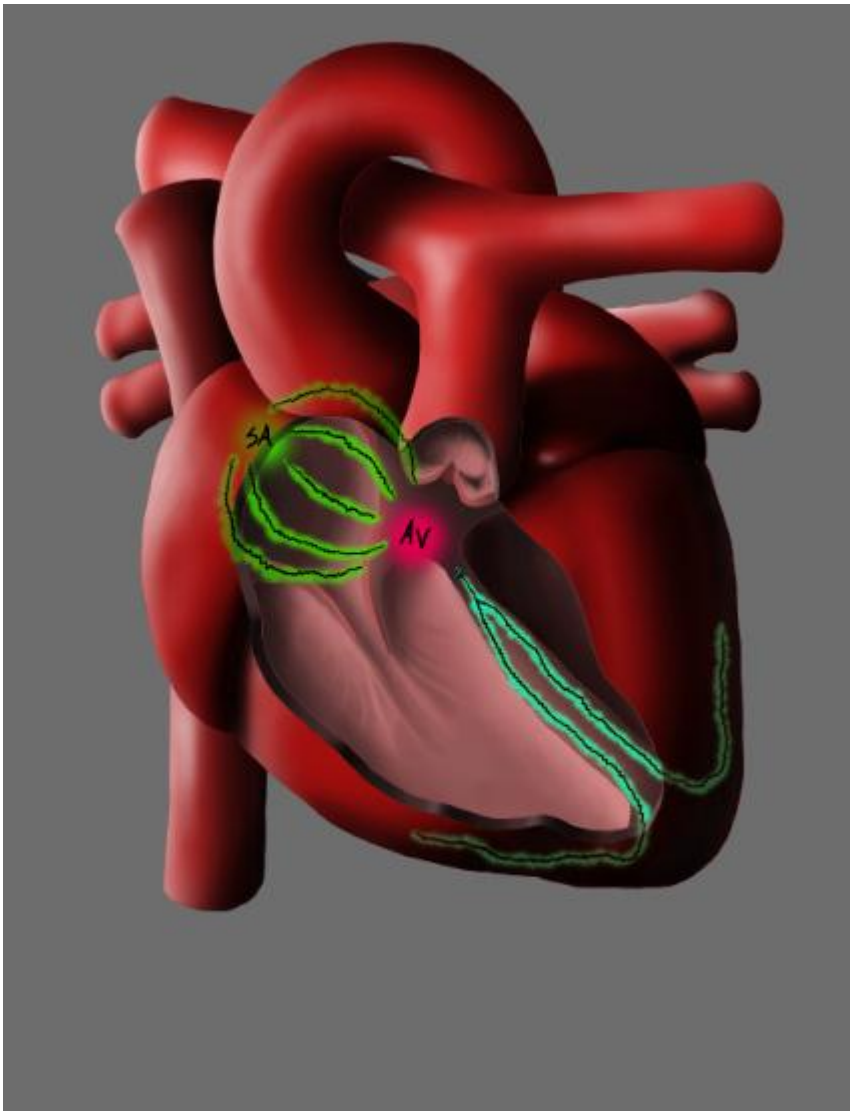
This image shows aim 1a, hMSCs in the polyurethane mesh bag. This concept creates a new charge, separate from the one released by the SA node. This new charge was depicted in the same fashion, but with a different color. This was done in order to relay that it's the same type of event, but it is new and separate from the original current.



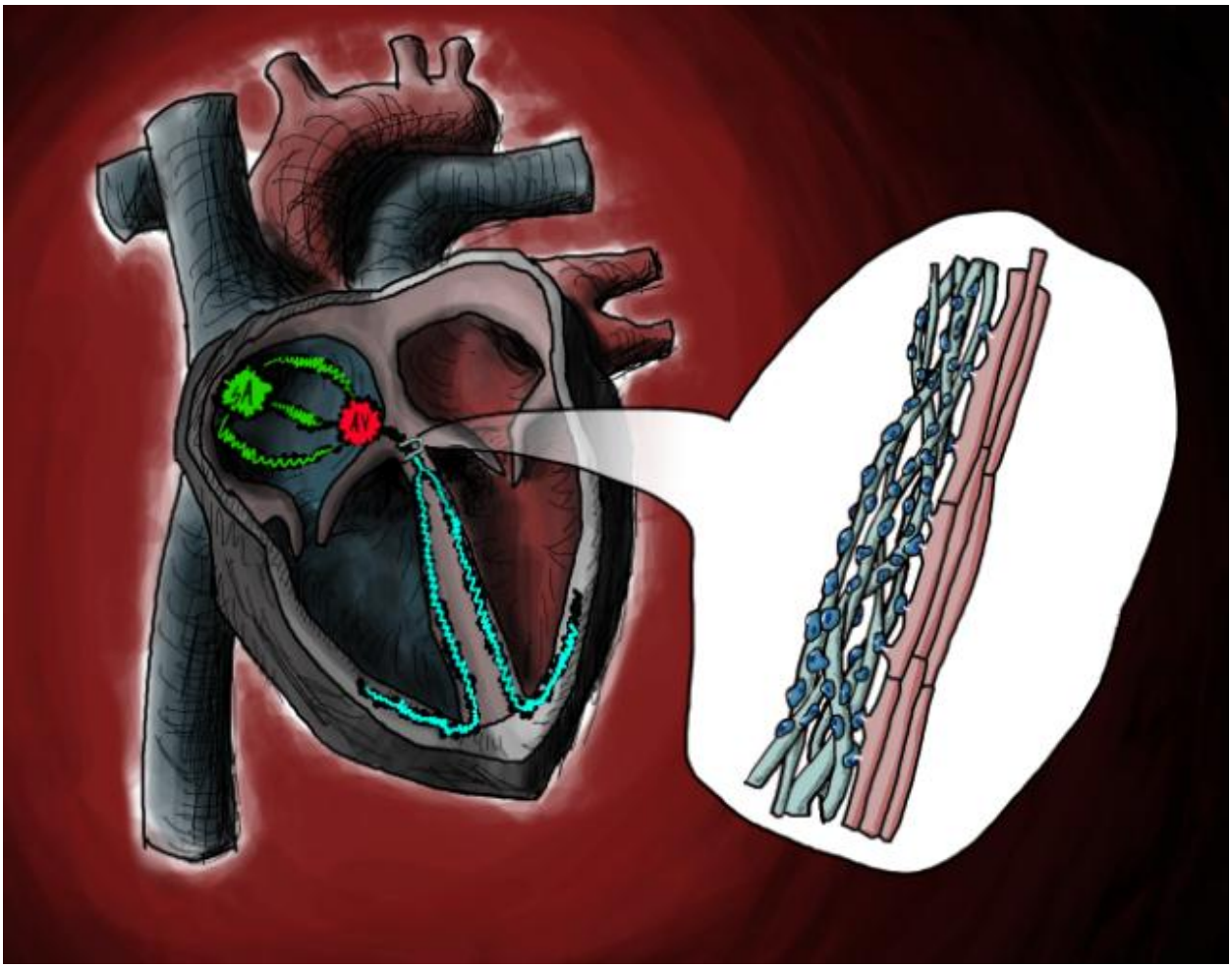
This image is a close up of aim 1a. The stem cells (colored blue) are seen reaching through the grey polyurethane mesh to form gap junctions (yellow) with the neighboring myocyte (red). It is through these junctions that the new charge travels through to begin propagating.



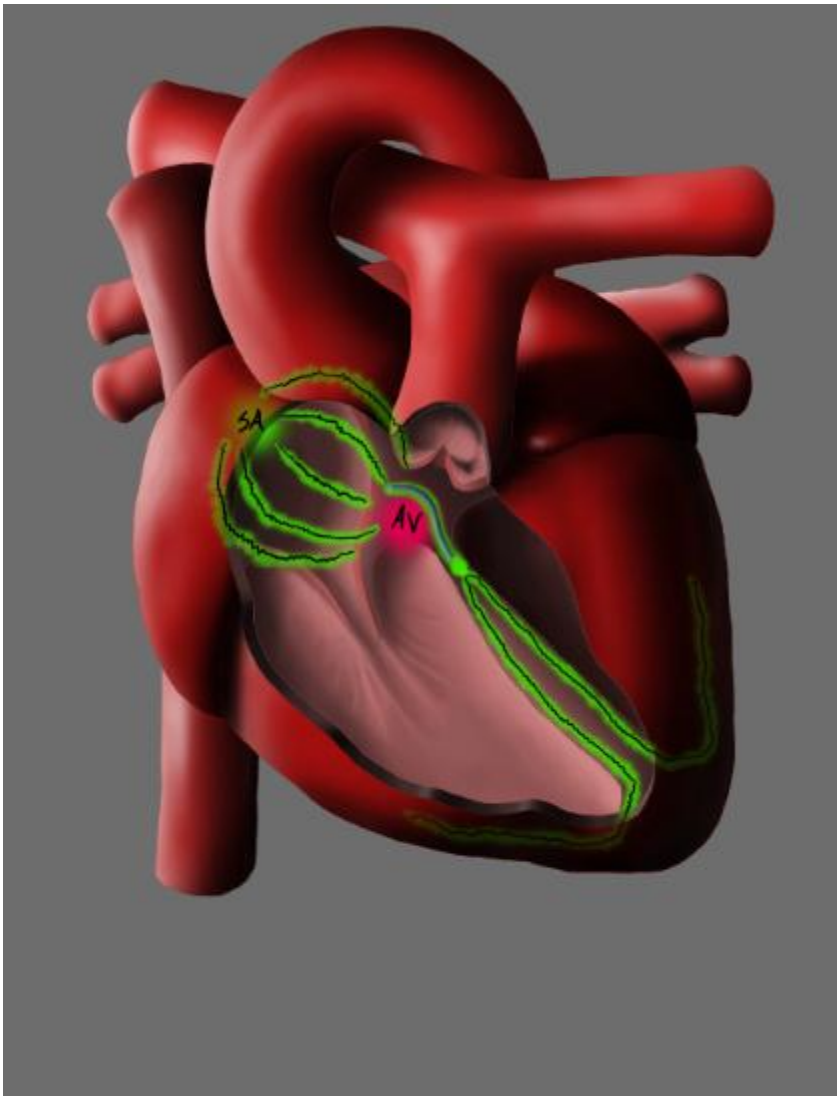
This is another, earlier version of the heart and close-up of aim 1a done with a very different style of illustration. Much of my drawing history stemmed from reading comic books. I taught myself how to draw many things by recreating my favorite images out of comic books. I wanted to relate what I was doing now in a similarly loose, free and graphic style of illustration. This image was done mainly to relay the concept of gap junctions between the stem cells in the bag and the myocytes surrounding it. In contrast, the above close-up is very biologically accurate, in terms of size and visual representation of the myocyte.



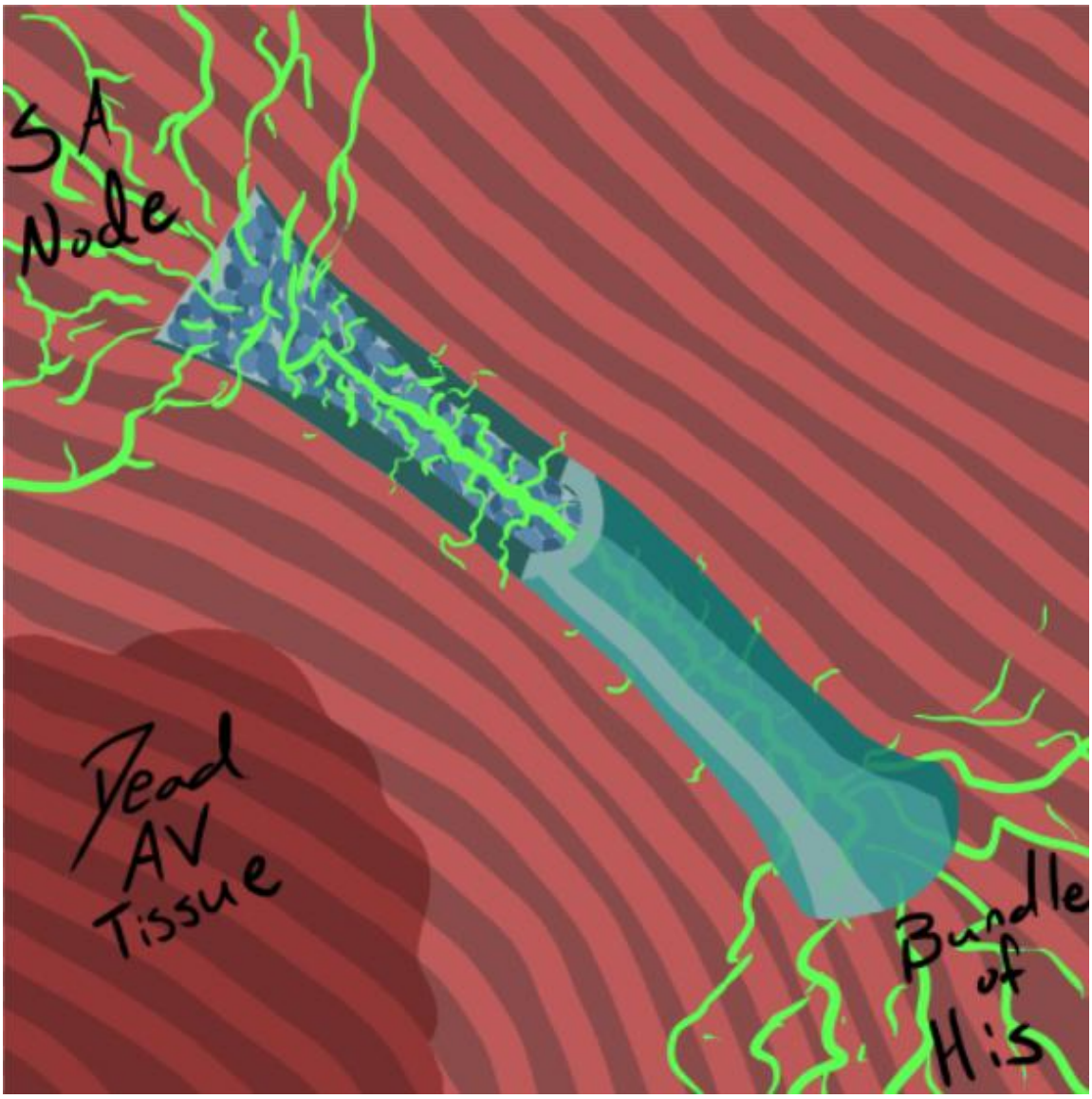
Here we see aim 1b, iPS cells seeded on a thread that would again create a new current that would then propagate forward. The color of the iPS current is again different from the SA node current and even the previous hMSC current.



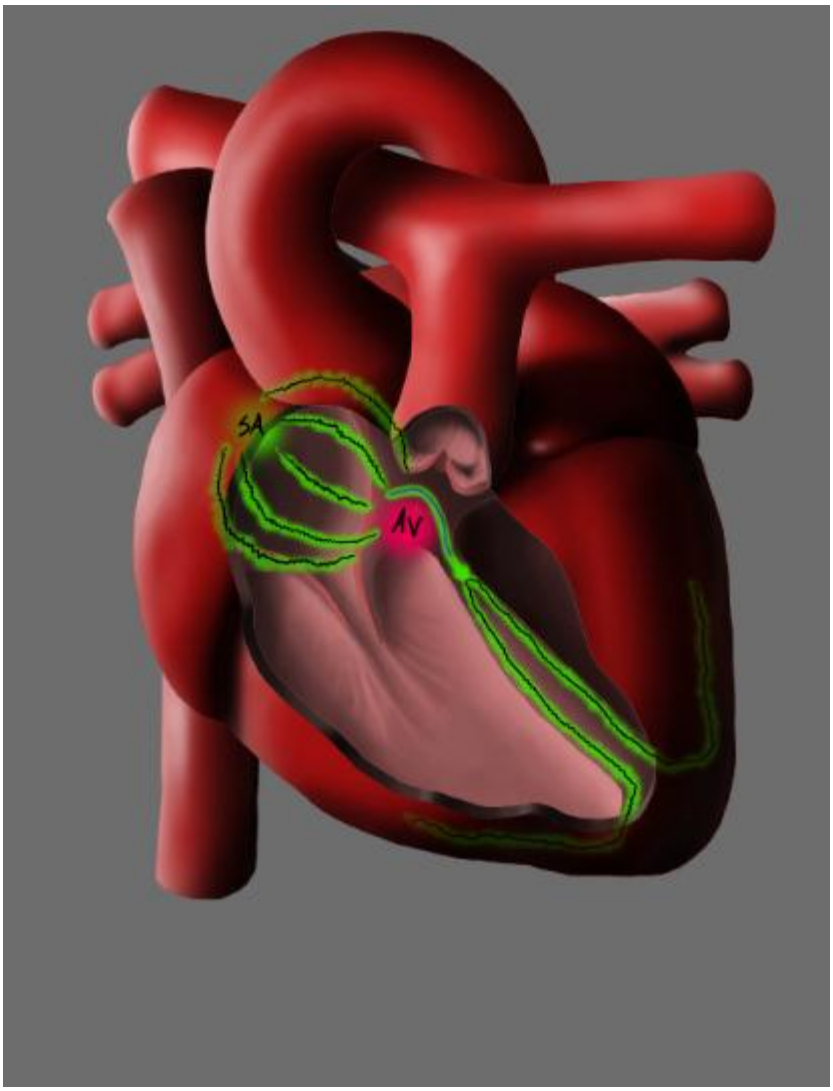
Here I've shown the close up of the comic-like version of aim 1b. The iPS cells on the electrospun microthread can be seen forming gap junctions with the mycoytes to the right.



This image shows aim 2a, hMSCs inside the polyurethane mesh tube that receives the original charge from the SA node and carries it around the dysfunctional AV tissue to a healthy region of myocytes. As you can see the color of the current after the implant is the same as the SA current because it is the same charge that has been relayed past the AV node.



This close up of aim 2a was done in a minimalistic and almost abstract style. There is no light and dark value seen here, except on the tube to show that it has be “cut away” to reveal the hMSCs and current inside. The striped tissue around the tube represents the myoctes in the heart. The charge from the SA node gets into the tube because the single layer of mesh allows many gap junctions to be made. The multiple layers of mesh makes it very difficult for gap junctions to be made. However it is not impossible, that is why some of the charge is seen escaping through the sides of the tube. And again the single layer of mesh allows the current to be released to the healthy tissue past the AV node. Again because the charge inside the tube is partially hidden, I dulled its color to allow the viewer to see the current but still know that the tube is wrapping around it.



This image shows aim 2b. iPS cells seeded onto the microthread that would carry the original charge from the SA node around the AV node to the functioning myocytes. Again the charge is the same color because it is the same charge that was released by the SA node.

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