Stem Cell Orchestra: interactive didactic animation for cardiac tissue engineering

by
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ABSTRACT

A potential treatment of heart failure from myocardial infarction is to replace damaged tissue with a cardiac patch, a bioengineered construct of cardiomyocytes and stem cell-supported capillaries grown on a bioscaffold. Tissue engineers must have a thorough understanding of the cues that guide multiple cell types to form functional tissue. As the field of cardiac tissue regeneration develops novel protocols for stem cell-based therapies, visualizations that didactically convey *in vitro* spatiotemporal cell-cell interactions become increasingly important to provide to students and the general scientific community.

An interactive web-based animation and educational module, *Stem Cell Orchestra*, was designed and developed to introduce students to case-based examples of biomedical research that are directly related to fundamental tissue engineering principles. A conceptual flowchart was created to establish the navigational structure of the module and connections between educational topics. The module focused on visualizing the development of a cardiac patch as a model bioengineered tissue through the creation of a 3D animation depicting cell-cell interactions within a cardiac patch. Animation content was derived from confocal microscopy and transmitted light microscopy datasets in combination with the results of a literature review of current cardiac regenerative medicine techniques. The educational module presents the 3D animation within the context of supplemental educational material on mechanotransduction cues related to cardiac patch development.

This interactive animation platform introduces students to examples of primary research and accurately showcases tri-culture of a cardiac patch from cardiomyocyte elongation and synchronization to the formation of endothelial capillaries supported by the novel human adipose derived stem cells. Supplemental material contextualizes mechanotransduction presented within the animation. This project resulted in the development of a novel workflow and educational module that can be expanded to include additional tissue engineering concepts.

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ABBREVIATIONS

Myocardial Infarction – MI

Neonatal Rat Ventricular Cardiomyocytes – NRVCM

Human Umbilical Vein Endothelial Cells – HUVEC

Human Adipose Derived Stromal Stem Cells – hASC

Alpha-Smooth Muscle Actin – αSMA

Vascular Endothelial Growth Factor – VEGF

Induced Pluripotent Stem Cell – iPS

INTRODUCTION

Engineering Tissue for the Heart

The heart myocardium is the thick layer of muscle in the walls of both atria and ventricles. It is composed primarily of contractile cardiomyocytes. The myocardium has limited regenerative potential after an injury such as myocardial infarction (MI), which induces formation of scar tissue. This area of scar tissue has no contractile ability and often contributes to heart failure that typically occurs within five years of an MI event (Mather 2016, Radisic 2016, Stoppel 2016). Current heart failure treatments include heart transplantation, drug therapy, and left ventricular assist devices. The limitations of these therapies spurred the development of alternative treatments that include replacement of the damaged vasculature and cellular microenvironment (Radisic, 2016, Parsa, 2016).

Replacement of damaged native myocardium could be achieved with a tissue-engineered construct called a cardiac patch (Iyer 2008, Lesman 2010, Mather 2016, Menasche 2015). Successful integration into native heart tissue requires that the cardiac patch be viable, non-immunogenic capable of electrical impulse propagation, and synced rhythmic contraction (Liau, 2011) with a robust vascular network (Lesman 2010, Sun 2016) (**Figure 1**).

Smaller tissue constructs designed for the skin, cartilage, or cornea, rely on oxygen diffusion from native vascularization after implantation, with a maximum distance of 100-200 μm from cell to capillary (Novosel, 2011). However, cardiomyocytes are metabolically demanding within tissue constructs. Prevascularization strategies for thick tissue constructs are being developed to address high *in vivo* metabolic demand and prevent ischemia and cell death (Lesman, 2010). These strategies require simultaneous cultivation of lineage-specific cell types, prompting deeper research into the role of physical stimuli in cell differentiation. To create a functional vascularized cardiac patch, cell lineage specificity is directed through carefully orchestrated spatiotemporal cues and cell-cell interactions (Choi 2010, Iyer 2008, Morrissette-McAlmon 2017)

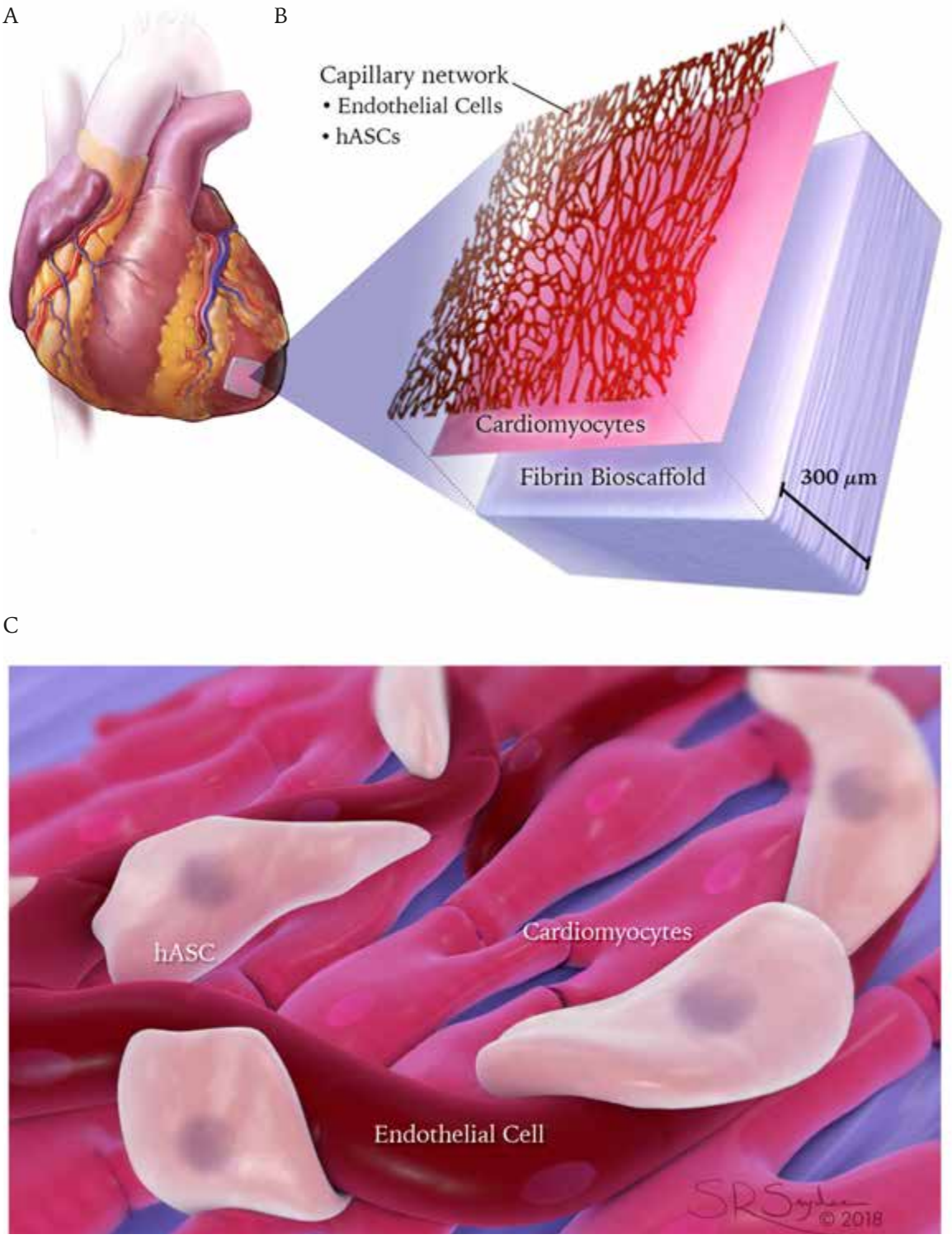


Figure 1. Components of a cardiac patch. (A) Heart with cardiac patch applied over area of myocardial infarction. (B) Cardiac patch components expanded for examination. (C) Cardiac patch cellular microenvironment on the fibrin bioscaffold.

Cell Types in the Native Myocardium

It is necessary to first understand the anatomical and physiological conditions of native myocardium in order to prepare *in vitro* cardiomyocytes for *in vivo* stresses (Mather, 2016). While cardiomyocytes represent the bulk of the myocardium, constituting ~75% of total heart volume, their number accounts for <40% of all heart cells, the majority being highly adaptive supporting cell types, such as fibroblasts, circulating blood cells, vascular smooth muscle cells, and endothelial cells (**Figure 2**). These additional cells are critical for supporting the tissue architecture that delivers oxygen to the energetically demanding cardiomyocytes.

Cardiomyocytes

Native heart tissue contracts 60-100 times per minute, enduring contractile stresses of 15-30 kiloPascals (kPa). Only 1% of cardiomyocytes control the electrical conduction rate that signals contraction; the rest are triggered to contract via gap junctions and voltage-gated ion channels that control internal and external ion levels (Stoppel, 2016).

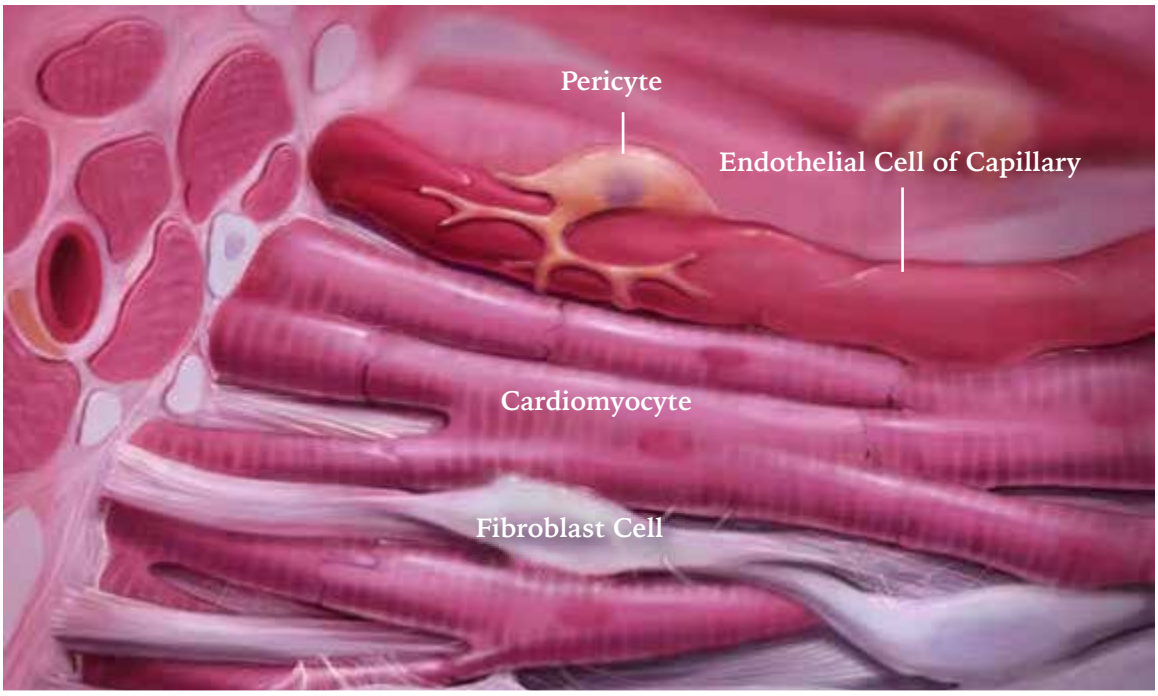


Figure 2. Conceptual illustration of the native heart microenvironment. Pericytes stabilize endothelial cells, which constitute the walls of capillaries. Fibroblasts secrete collagen, which aligns cardiomyocytes into a parallel configuration and provides structural support.

Cardiomyocytes do not regenerate, but the heart does undergo periods of growth due to age-related mechanical stimuli. This suggests that cardiomyocytes are capable of undergoing local changes in size (e.g. hypertrophy) in response to external forces. Phenotypic cardiomyocyte change is guided by the native heart extracellular matrix (ECM), which is primarily constructed of perimysial collagen fibers (~0.5-10 μm in diameter). The cardiomyocytes interact with collagen fibers through various mechanotransduction pathways (Parsa, 2016, Mather, 2016) that, early in development, pack the cells into a parallel-oriented alignment. Cardiomyocytes sense differences in substrate stiffness through integrin binding, receptor tyrosine kinase activation, and GTPase activation at the cell membrane, which affects cell-signaling pathways. This is an adaptive, temporal process that allows cells to induce transient or permanent tissue modifications, and ultimately determines cell fate decisions (van Helvert, 2018). Understanding the development and architecture of native heart tissue is critical for constructing a bioscaffold on which to grow cardiomyocytes and capitalize on the physical constraints that regulate heart tissue at the microscale.

Endothelial Cells

Endothelial cells form the inner layer of blood vessels. They are generally thin and slightly elongated, and in the heart, contribute greatly to cardiovascular homeostasis. In healthy heart tissue, the endothelium is the principle regulator of vascular permeability, blood vessel caliber, and cell transport to and from the bloodstream. Through the expression, activation and release of vasoactive and bioactive molecules (Brutsaert, 2003), endothelial cells communicate with cardiomyocytes to regulate contraction. When cultured with endothelial cells, cardiomyocytes align along the outside of the developing capillaries, form gap junctions, and help coordinate contraction (Hsieh, 2006).

Mural Cells

Mural cells such as pericytes and smooth muscle cells stabilize thin-walled capillaries. Endothelial cells actively recruit pericytes to the outside of growing multicellular cords during angiogenesis and vasculogenesis. The pericytes adhere and

differentiate to form the outside of the new capillary. In cellular culture, however, mural cell use is limited due to an inability to obtain adequate cell numbers for induced pluripotent stem cell therapies (Merfeld-Clauss, 2010).

Fibroblast Cells

Fibroblasts are found throughout cardiac tissue, surrounding the cells and bridging the gaps between myocardial tissue layers. They synthesize many of the extracellular matrix proteins found in the heart, including collagen, which maintains tissue architecture during ventricular contraction and relaxation (Dostal, 2001).

Human Adipose-Derived Stem Cells in Tissue Engineering

Widely used in tissue engineering applications, human adipose-derived stem cells (hASCs) are a clinically available source of stem cells and endothelial progenitor cells (Hutton, 2015). Stem cells are undifferentiated cells that have multilineage potential, long-term viability and great potential for self-renewal. In adipose tissue, hASCs are predominantly localized to the periendothelial layer of capillaries and are both phenotypically and functionally equivalent to capillary pericytes (Merfeld-Clauss, 2010). When attached to a substrate *in vitro*, hASCs display a spindle-shaped morphology and lack the intracellular lipid droplets found in adipocytes. In comparison with bone marrow-derived mesenchymal stem cells, hASCs show higher collagen production and are more stable in long-term cultures, with lower cell death and higher proliferation capacities (Frese 2016).

When cultured with endothelial cells, hASCs guide the self-assembly of endothelial-derived vascular networks (Hutton, 2015), express functional properties of pericytes, and establish multilayered functional vessels (Merfeld-Clauss, 2010), which may accelerate tissue construct vascularization *in vivo* rather than having to rely solely on angiogenic ingrowth from native myocardium (Hutton, 2015). Factors secreted by hASCs, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and angiogenin, promote endothelial cell survival and multicellular cord formation. In co-culture with endothelial cells, hASCs secrete extracellular matrix proteins (Merfeld-Clauss, 2010) and enable endothelial cells

to form 3D vascular networks, even in hypoxic environments, such as those likely to be encountered after implantation before the integration of host vasculature. Additionally, when cultured together with endothelial cells and cardiomyocytes, hASCs may integrate with cardiomyocytes and possibly contribute to contraction (data unpublished, Morrissette-McAlmon, 2018). The minimally invasive method of harvest, simple isolation procedure, and great potential for self-renewal means hASCs could hold great promise for future cell-based therapies.

Building a Cardiac Patch

At the Grayson Lab for Craniofacial and Orthopaedic Tissue Engineering, a vascularized cardiac patch was created on fibrin microfiber sheets utilizing a cellular tri-culture approach. Three cell types were selected: neonatal rat ventricular cardiomyocytes (NRVCMs), hASCs, and human vein umbilical endothelial cells (HUVECs). Tri-culturing of NRVCM:hASC:HUVEC, with the optimal ratio of 1500:37.5:150, resulted in robust vascular network formation with cardiomyocytes retaining conduction velocities and action potential durations similar to NRVCM-only experiments (data unpublished, Morrissette-McAlmon 2018).

Fibrin microfiber sheets are produced through electrospinning **(Figure 3)**, which uses electric force to draw charged threads out of polymer solution to create tunable fiber diameters. Electrospun fibrin microfiber sheets were used to provide alignment cues to guide cardiomyocyte elongation.

Fibrin microfiber sheets of two different stiffness values were used. Fibrinogen concentration of the solution, concentrations of calcium and thrombin of the collection bath, and the rotational speed of the bath all impact fibrin microfiber sheet stiffness.

During electrospinning of a fibrin microfiber sheet, a thread of fibrinogen hyrdogel is ejected from an syringe and onto a rotating collection wheel to form a crosslinked disc of parallel fibrin threads. To begin, the collection wheel is set to a speed of ~45 RPM and 50 mL of collection solution (20 U/ml thrombin to 50 mM $CaCl_2$) is added to the outside rim of the wheel. This creates a band of liquid on

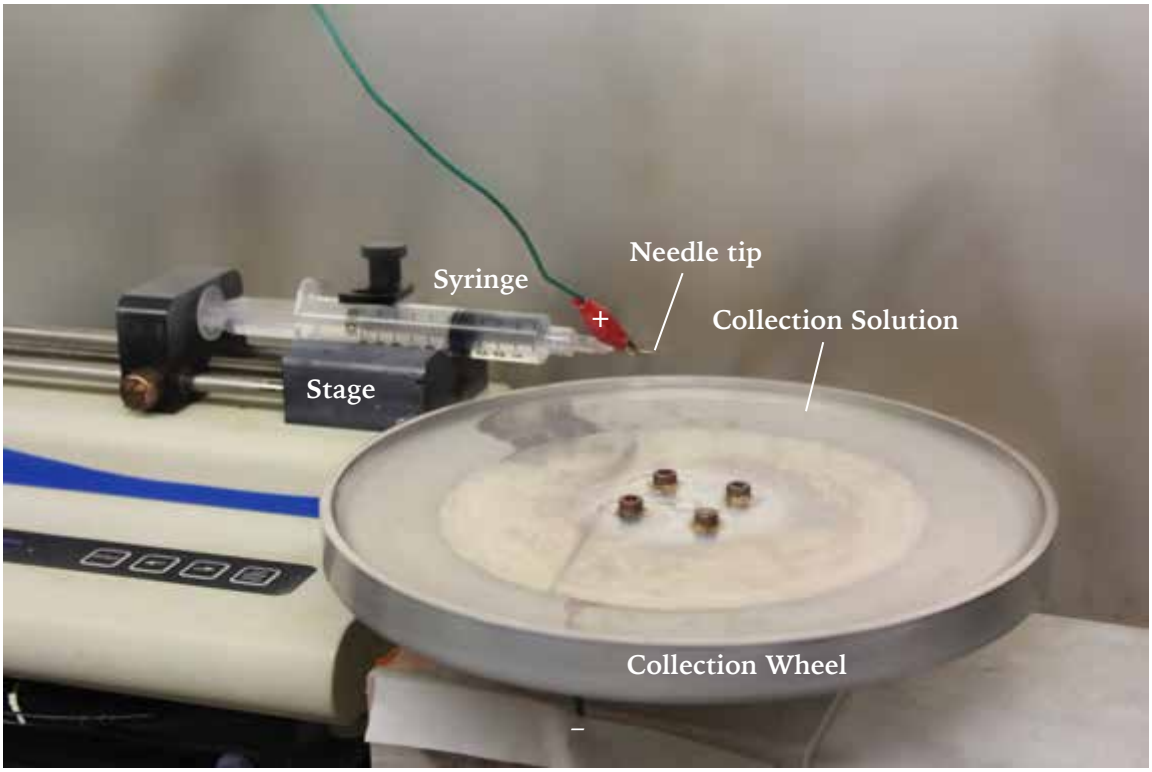


Figure 3. Electrospinning of a fibrin microfiber sheet. The syringe dispenses a thread of fibrinogen hydrogel which crosslinks into fibrin when it lands in the collection solution. The collection wheel rotates as the syringe moves back and forth, forming a thin sheet of parallel fibrin on the surface of the collection solution.

the outer margins of the rotating plate with no liquid in the center of the collection wheel. With the ground (negative) electrode propped against the underside of the rotating collection wheel, the syringe containing 5 mL of fibrinogen hydrogel is loaded into the syringe pump and set to dispense (5.0 ml/hr). The syringe pump is set on a linear stage (~3 cm/min over 1.5 cm) and the syringe needle tip (positive electrode) is placed over the collection wheel. The fibrinogen thread is pumped out and onto the collection wheel after the activation of the power supply (4.5 kV).

After 17-20 minutes, the power is shut off and the fibrin sheet sits for an additional 2 minutes in the collection bath to ensure full crosslinkage of the microfibers. After being wrapped around a Mylar frame (1 cm x 1 cm), the resulting fibrin bioscaffold is 300-500 microns thick.

The cardiomyocytes (suspended in NRVC media), are seeded onto the fibrin

bioscaffold at 1.5 x 10^6 cells/cm^2. They immediately begin to attach to the substrate and Aprotinin (10%) supplemented into the media prevents the cardiomyocytes from eroding the fibrin bioscaffold. Washed with NRVCM media containing Aprotinin every other day, the cells align into a parallel configuration due to the topographical properties of the fibrin bioscaffold. Spontaneous contraction begins four to seven days after seeding. After thirteen days, mature intercalated discs form at the endplates of touching cardiomyocytes, and gap junctions between the neighboring cardiomyocytes in the cell sheet promote synchronized contraction.

Fourteen days after cardiomyocyte seeding, hASCs and HUVECS (3.7 x 10^4 hASC/cm^2 and 1.5 x 10^5 HUVEC/cm^2 in 20 µL of media) with an optimized composition are added to the fibrin bioscaffold. All the cells are nourished with tri-culture media (3mL). The media is refreshed every other day as the endothelial cells elongate and begin to form multicellular cords. During this time, the hASCs migrate and cluster around the multicellular cords as the cords fuse into a capillary network. The cords become stabilized from the hASCs and the vascularized myocardial tissue can remain in culture for up to two months (**Figure 4**).

The survival, morphology and electrophysiological and contractile properties of cardiomyocytes on these fibers were described and it was found that (1) cardiomyocytes formed gap junctions and developed mature electrophysiological parameters, (2) culture of hASCs and endothelial cells resulted in dense capillary networks, and (3) fibrin microfiber sheets may serve as a suitable bioscaffold on which to grow a functional vascularized cardiac patch (data unpublished, Morrissette-McAlmon et al., 2018).

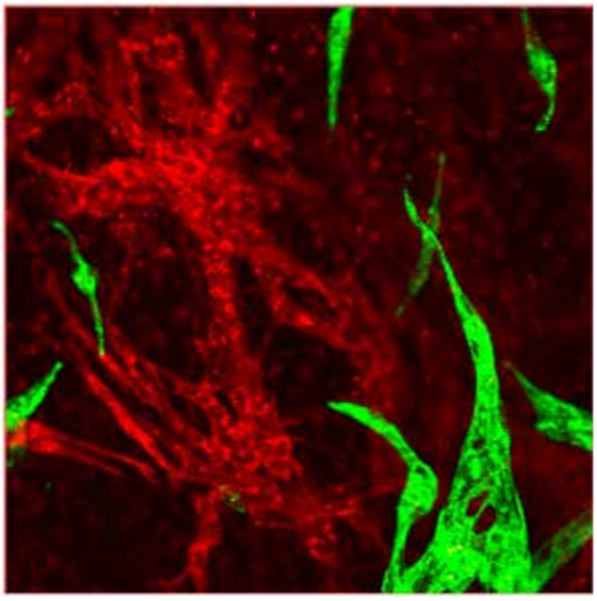


Figure 4. Confocal microscopy data of tri-cultured cardiac patch. Red channel shows mature, aligned cardiomyocytes, green channel shows endothelial cells. Data unpublished, Morrissette-McAlmon et al. 2018

Current teaching materials for tissue engineering

At Johns Hopkins University School of Medicine, the course Principles of Tissue Engineering is offered to senior undergraduate and first-year Masters, PhD and MD/PhD students by Dr. Warren Grayson. The course covers topics relevant to the field of tissue engineering, including cellular structure development and function and is intended to introduce students to applications of fundamental engineering concepts for the design of biological tissue substitutes. The information is presented in several ways, including text, photography, tables, graphs, SEM images, confocal microscopy images and 2D illustrations.

Informal discussion with student consultants at the initial stage of this project informed the content design and objectives of the educational material. In addition, detailed review and critique of the course materials identified areas that could benefit from the creation of visual didactic materials. While some course content included lectures with dynamic visual displays of data and case examples of current research, the following limitations to the current educational materials were identified.

First, the majority of cellular events presented in lecture are captured and displayed through confocal microscopy. Confocal microscopy is an optical imaging technique for capturing microscopic assemblies by illuminating specific structures tagged with immunofluorescent antibodies. By capturing multiple 2-dimensional images at different depths in an organic sample, a three-dimensional (3D) volume of a specimen can be reconstructed. Limitations to these visualizations included the fact that (i) not all cells or structures are antibody-tagged, which leaves cut-out dark shapes where untagged cells reside, (ii) cellular bioscaffolds are not targeted, so 3D bioscaffolds appear as a negative space and 2D bioscaffolds do not appear at all.

Additionally, cellular bioscaffolds are only visualized through scanning electron microscopy (**SEM**). SEM visualization is restricted to the outermost material and in multi-layer tissue constructs, the deeper layers of tissue remain inscrutable. They indicated that the individual visualizations of each component of bioengineered tissues make it difficult to conceptualize the cellular microenvironment as a whole.

Cellular motion cannot be captured through immunofluorescent imaging as cells are fixed (dead) during the visualization process. Rather, cellular motion is generalized by capturing cells in different stages of development over an interval of time and presented in a multipart sequence. The intricacies of cellular motion throughout each stage of tissue culture are not captured through other means.

Course lectures contained few 3D structures. Volumetric reconstructions were used to visualize cells in multi-layer constructs and alignment in relation to a 3D bioscaffold, which can appear as a negative space in immunofluorescent imaging. However, in 2D bioscaffold cell culture, there is no negative space as the cells are grown into a confluence on top of the bioscaffold. When shown in lecture, it can be difficult to convey the intricacies of 2D physical stimuli with volumetric reconstructions alone.

As a result of the course content review and informal discussions, it was determined that the course would benefit from the inclusion of more bioengineering data presented in a visual way to show the complexity of a system or tissue and that more specific examples of bioengineering research would provide engaging and memorable ways to learn new content and help students decide in which lab they would like to work.

Understanding cellular microenvironment dynamics is critical for every step of successfully culturing multiple cell types together. Although there are many reliable techniques to visualize *in vitro* cellular culture, these techniques fail to convey the intricacies when three different cell types are cultured together. Because cardiac tissue engineering is an evolving discipline, audiences such as students and the general scientific community may have difficulty interpreting novel research results and integrating raw data into an accurate representation for educational purposes. Multiple audiences could benefit greatly from the development of a didactic, interactive 3D animation-based module that utilizes novel data to visualize and teach tissue-engineered principles.

Interactive Media in Biomedical Engineering

Bioengineering students are introduced to the complex fundamental processes that guide stem cell fate in lectures before they experience lab work. However, because cardiac tissue engineering is a rapidly emerging field (Radisic, 2016), visuals of the intricacies of organized *in vitro* cardiac cell interactions and tissue growth are extremely limited. In addition, raw datasets do not show all of the cell types and structures within a cardiac patch simultaneously, making intricate cellular activity difficult to visualize for novice students.

As students working towards their degree may require flexibility for scheduling learning activities, educational formats are being developed to accommodate a variety of teaching and learning styles (Caron 2015). Supplementary online educational formats offer students a range of learning opportunities outside the classroom. Learners can have increased control over the content and pace of information presentation and view educational materials independently (Kalyuga 2015). For bioengineering students with a focus on tissue engineering, access to novel interactive animations integrated into an online learning module could be used to connect real-world laboratory research with examples of fundamental tissue-engineering principles. This resource could serve to guide students through visual case-based research examples that integrate different research datasets into comprehensive teaching visualizations.

Learning Theories

Cognitive load theory suggests that learning happens best under conditions aligned with the learner's cognitive architecture. New information is stored in short term memory, which is limited in the number of elements it can hold simultaneously. Learning materials that reduce working memory in learners should be designed by (1) physically placing sources of information together (e.g. labels near structures they define) (2) reducing unnecessarily repetitive information and (3) cueing visual and auditory information simultaneously (Sweller 1999). In scientific animation, these principles can be utilized to reduce extraneous cognitive load imposed by the attentional requirements of processing relevant elements (Amadieu F 2011).

Objectives

This project focused on visualization of the development of a cardiac patch as a model bioengineered tissue through the following objectives:

1. Design a web-based platform that contextualizes fundamental tissue engineering principles and techniques with examples of laboratory research.
2. Create didactic 2D and 3D animations of the cardiac patch building process with supplemental interactive 3D models and learning materials accessible from the animation timeline that allow users to study cellular interactions and environmental cues.
3. Using 3D models built from segmented data, the interactive animations will:

    a. Visualize external and internal forces that drive cellular interactions and impulse conduction between cardiomyocytes.

    b. Communicate biophysical and mechanotransduction principles of substrate-cell interactions and cell-cell interactions of cardiomyocytes, endothelial cells, and hASCs.

Audience

The primary audience for this interactive animation and website is biomedical engineering undergraduates and graduate students specializing in tissue engineering. The secondary audience is professional biomedical engineers and biologists working in related research areas.

MATERIALS AND METHODS

Software Overview

A range of software was utilized to create art assets and final animations for this project. Fiji (Fiji is Just ImageJ)® and Imaris® from Bitplane were used to segment tri-culture cardiac patch and cardiac cell t-tubule confocal microscopy datasets to create surface meshes and analyze 3D volumetric renderings of the supplemental cardiomyocytes. Meshlab® was used to remove artifacts and decimate surface meshes. ZBrush® from Pixologic was used to optimize the segmented meshes, sculpt, and create new models for the 3D animation. Cinema 4D® and Adobe After Effects® were used to create and render 3D scenes and assemble the 3D animation. Storyboards and additional pieces of content were created with Adobe Photoshop®, Adobe Illustrator®, and Procreate® for Apple iPad. TouchCast® Studio with Webinar and Livestream was used to compose the final interactive animation. Wordpress® was used to create the Stem Cell Orchestra website.

Content Aggregation

All materials related to cell adhesion, mechanotransduction, and vascularization that are taught in the graduate course *Principles of Tissue Engineering* were studied in preparation for design of the website and animation. Information from peer-reviewed research articles was gathered to assess current methods of growing a cardiac patch, and information on the cellular microenvironment was gathered from cellular biology and histological textbooks.

The hierarchy of information to be presented on the website was developed from conversations with the project preceptor, Dr. Warren Grayson. The website content was further organized through the aforementioned informal discussions with students. A series of questions (**Appendix A**) was utilized to guide discussions and specific educational goals were defined and prioritized.

An outline of fundamental tissue engineering subjects and educational topics to be included in the website was created. The following topics in the course were identified as critical learning goals: (1) Stem Cell Differentiation (2) Tissue Structure

and Function – Native and Engineered (3) Cellular Adhesion and Mechanobiology (4) Bioscaffold – construction and application (5) Vasculogenesis and (6) Transplantation of Large Tissue Constructs.

Of the current biological areas of interest in regenerative medicine, *Heart Muscle* was chosen as the primary subject to develop as a prototypical pathway for the entire website. The information hierarchy for the *Heart Muscle* section was developed and revised with feedback from the student consultants. Development of a cardiac patch was chosen to serve as a real-world example of laboratory research and a model for bioengineered tissue to be visualized. Learning topics were identified to link the web-based content in the *Heart Muscle* section with the laboratory research visualization entitled, *Building a Cardiac Patch*. A conceptual flowchart was created to establish the navigational structure of the website and interlinkage between educational topics and the cardiac patch visualization. The flowchart was modified based on consultant feedback.

Script and Storyboard Development

For the cardiac patch visualization, a script (**Appendix B**) was created based off the defined educational topics and unpublished data on cardiac patch tri-culture provided by the Warren Grayson Lab. Time points for user interactivity were identified within the script. A detailed animation timeline with points of interactivity was then created based on the previously outlined goals to match the interactivity of the website flowchart. Aforementioned principles derived from cognitive load theory (Sweller 1999) were applied to the animation design in order to reduce extraneous cognitive load.

Interactive media from the *Heart Muscle* webpage relevant to the module, *Building a Cardiac Patch*, was chosen for development as a complete educational module with interactive animation. These areas were indicated by numerals within the flowchart.

Storyboards for the selected educational module and interactive animation were created to reflect the topics outlined in the website flowchart and the user interface was designed. Iterative student feedback was used to revise website storyboards.

Data Visualization

Datasets Utilized

Confocal microscopy datasets of tri-culture (NRVCM:hASC:HUEVC) cells were provided by the Grayson Lab for Craniofacial and Orthopaedic Tissue Engineering. These datasets contained stacks of two-dimensional images captured on a Zeiss LSM 510 Meta confocal microscope using a 5x or 20x objective lens. The .lsm file format was used for storage of each set of confocal data.

Bill Louch and Michael Frisk of the Louch Lab at Oslo University Hospital provided an additional dataset (.lsm and .tif) of a single cardiomyocyte cultured on a hard substrate, captured with a 63x objective lens.

Molly Maleckar and the Assay Development team at the Allen Institute for Cell Science provided three additional datasets of live cardiomyocytes (transmitted light microscopy) with a 40x objective lens and fixed, immature induced pluripotent stem cell (**iPS**) cardiomyocytes (.tif) with a 63x objective lens.

Of the available datasets, seven (**Table 1**) were selected to use as visualization aids that show optimal cardiomyocyte and capillary characteristics.

#-#	Source	Objective	Structures	Width x Height x Depth
1-1	Grayson Lab	5x	Vascular Channels	1800 x 1800μm x 8 images
1-2	Grayson Lab	20x	Vascular Channels Tri-culture	450 x 450μm x 8 images
1-3	Grayson Lab	5x	Cardiomyocyte Tri-culture	450 x 450μm x 15 images
1-4	Grayson Lab	20x	Cardiomyocyte Tri-culture	450 x 450μm x 36 images
2-1	Louch Lab	63x	Isolated Cardiomyocyte and T-tubule data	111.87 x 55.93μm x 188-197 images
3-1	Allen Institute for Cell Science	63x	Isolated Immature iPS Cardiomyocytes	153.17 x 144.05μm x 225 images
3-2	Allen Institute for Cell Science	40x	Contracting Immature iPS Cardiomyocytes	250x176μm x 120 images

Table 1. Selected datasets used as visual references throughout the entirety of the project.

Component	Before Seeding	14-21 days after culture
NRVCM	10μm	8-13μm diameter, 55-64μm length
hASC	20μm	100μm length
Endothelial Cell	10μm	126-145μm length
Endothelial Cord	--	12-14μm diameter, 45-126 mm length

Table 2. Measurements of cellular elongation gathered from the aforementioned datasets.

Review of Literature

A table of relevant measurements (**Table 2**) was compiled from a review of the literature, which included vessel diameter and length of neovascularization in a tri-cultured cardiac patch, and cellular tri-culture dimensions before and after seeding onto a bioscaffold. These measurements were used to inform the creation of the reference illustrations and 3D models including cardiomyocytes, hASCs and endothelial cells upon seeding, during temporal cellular change, and after growth.

Mesh Creation & Optimization

FIJI was used to view the datasets and create surface meshes that informed 3D model sculpting. To create a 3D model of a sheet of cardiomyocytes that were aligned from external bioscaffold cues, #1-4 (Table 1) cardiomyocyte tri-culture data was used. In this dataset, two antibodies were used to label cardiomyocytes and endothelial cells. Cardiomyocytes were visualized in the red channel (cardiac troponin 1), and endothelial cells visualized in the green channel (CD31) (**Figure 5**). To isolate the cardiomyocyte channel, the file channels were split using **Image>Color>Split Channels**. Binarization of the red channel was performed using **Process>Binary>Make Binary** (**Figure 6**) in order to divide the fluorescent data from the background. A 3D volume rendering was made using **Plugins>3D viewer** with the pre-selected settings (**Figure 7**). In the FIJI 3D Viewer, the volume rendering was then converted into a surface mesh by selecting **Edit>Display as>Surface**, and exporting the surface mesh, **File>Export surfaces>STL(ASCII)**. This process was also used for additional capillary networks (datasets #1-1, #1-2) and cardiomyocyte dataset (#1-3) (Table 1).

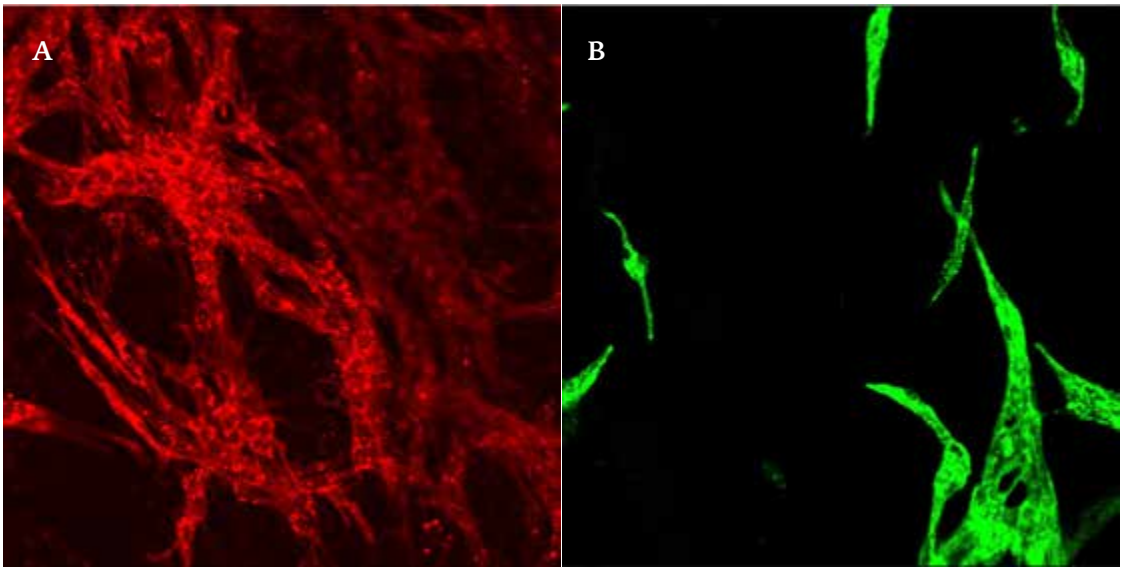


Figure 5. Confocal microscopy of tri-culture datasets. (A) Cardiomyocyte channel, **cardiac troponin 1.** (B) Endothelial cords, **CD31.**

The confocal microscopy datasets were imported into Imaris for Bitplane for semi-manual and automatic segmentation. Automatic surface creation began by selecting the **Surfaces** button in the navigational menu and following the creation wizard. After the surface mesh was created, it was exported to VRML (Virtual Reality Modeling Language) format, **Surpass>Export** selected object to vrml, for further optimization.

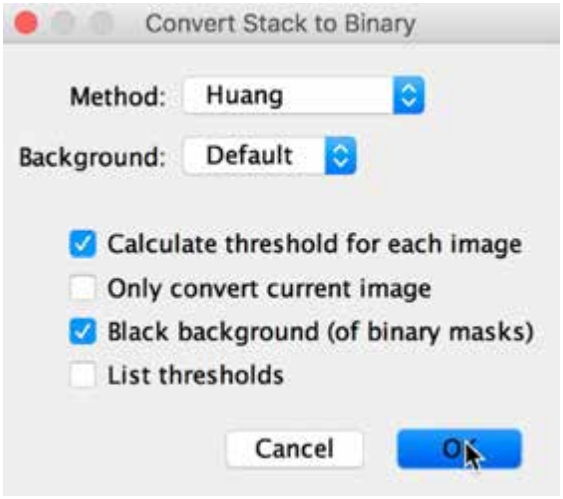


Figure 6. Binarization settings for removal of background.

The exported surface mesh of the vascular channels (dataset #1-1) was then decimated by applying **Filters>Remeshing, Simplification and Reconstruction>Quadric Edge Collapse Decimation** (**Figure 8**) in the software Meshlab. Additional processing, **Filter>Cleaning and Repairing>Remove isolated pieces**, was performed to remove unwanted unattached mesh data.

Reference Drawings for Model Creation

Cell measurements were acquired from confocal microscopy datasets (dataset #2-1) using FIJI. These measurements were used to guide the creation of the initial 3D models of cardiomyocytes and capillaries.

Figure 7. FIJI 3D Viewer settings.

Using dataset #1-3 (Table 1), an illustration was created that guided the creation of a continuous cardiomyocyte sheet. The illustration was created in Procreate on the Apple iPad, and then imported into ZBrush where it was projected onto the background plane and used as a reference image for sculpting and refining the 3D models into a connected cardiomyocyte sheet.

Data-Derived 3D Assets

Surface meshes exported from MeshLab were imported into a new ZBrush file as a subtool. The subtool was duplicated, renamed, and hidden to preserve a high resolution, unaltered reference surface mesh. All models in ZBrush were sent to Cinema 4D using the **Tools>GoZ plugin**. Cinema 4D models were often refined in ZBrush using the **Script>Manager>Go FromCinema4DToZBrush** plugin.

Cardiomyocyte Models

A Cylinder subtool was appended into a new ZBrush workspace with an illustration of the cardiomyocyte sheet loaded into the background. The **Scale** and **Move** tools were used to manipulate the Cylinder to roughly the same size as a cardiomyocyte cell body in the illustration. The cylinder was duplicated. With the new cylinder as a base, the **CurveTube** brush was selected from the **Brush** palette and used to sculpt the branches extending from the cylinder. These were matched to the underlying drawing. With the **CurveTube** spline activated and editable, the

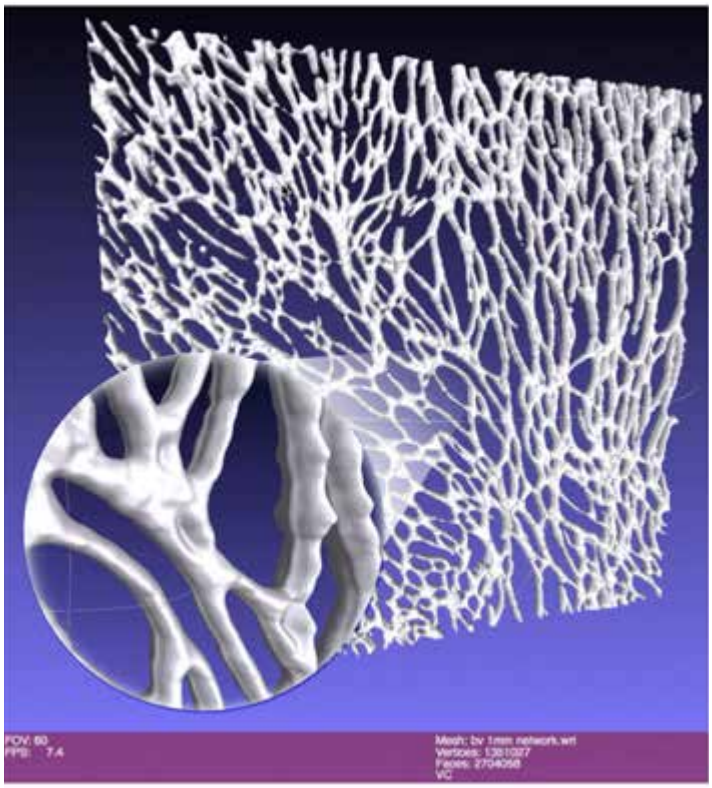


Figure 8. Decimated capillary network (dataset #1-1) in Meshlab. Text not intended to be read.

Draw Size was used to control the diameter of the tube. Adjustment of the **Draw Size** and manipulation of the **CurveTube** spline when in **Edit Mode** was used to form the base of a cardiomyocyte. By pressing Ctrl and clicking to exit the spline edit mode, a second press of Ctrl and click merged the CurveTube mesh with the cylinder mesh to allow sculpting with other brushes. These methods were repeated to approximate the other cell bodies of the cardiomyocyte and then to create a sheet of cardiomyocytes. The **Smooth, Move Topological, and Inflate** brushes were used to manipulate the cardiomyocyte models.

The main cardiomyocyte was partially sculpted from 3D t-tubule data from the Louch Lab data (#2-1). In FIJI, a 3D surface mesh of t-tubule data was created as described above and imported into a new ZBrush file (**Tool>Import**). A new subtool was appended into the file and sculpted to overlap the t-tubule surface mesh (**Figure 9**). The **CurveTube** brush was used to further sculpt branches onto this mesh and to edit the model, which was fit into the cardiomyocyte sheet.

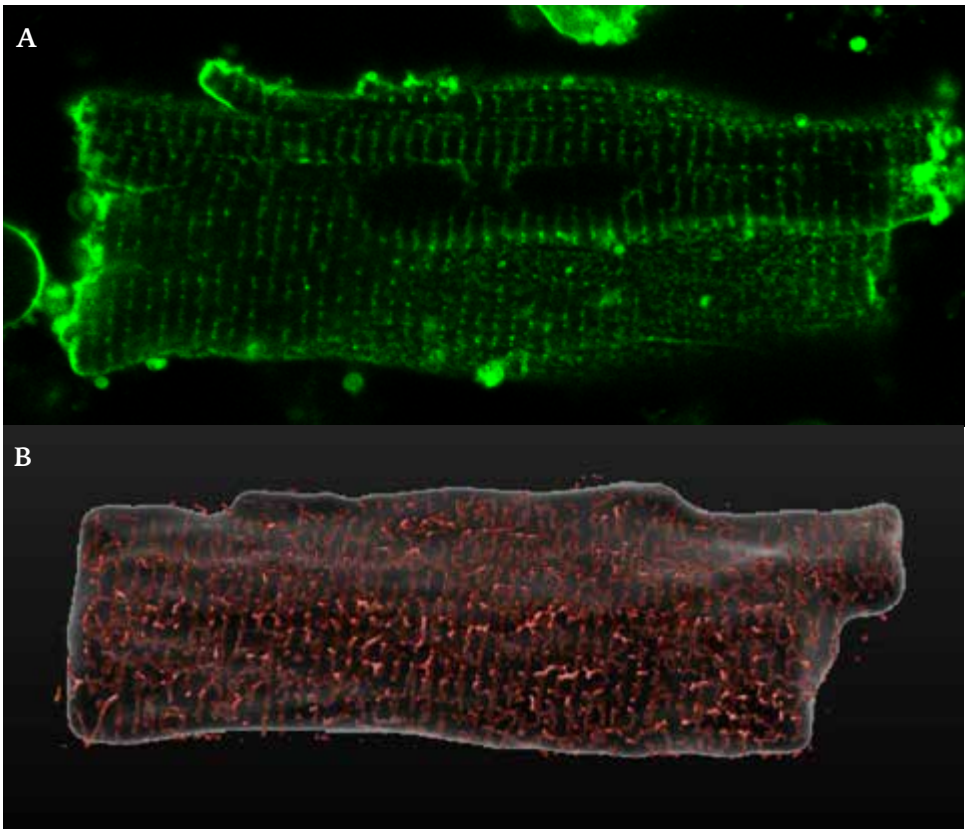


Figure 9. Cardiomyocyte visual reference. (A) Confocal microscopy of single cardiomyocyte (Frisk et al., AJP Heart and Circ, 2014). (B) Cardiomyocyte reconstruction in ZBrush.

Capillary Bed Model

Further optimization of the capillary surface mesh was done in ZBrush. Undifferentiated (round) cells that remained attached to the endothelial vessel networks were removed. Artefacts and missing areas of data were extrapolated by using the **CurveTube** brush to approximate areas of the missing mesh components.

Sculpted 3D Assets

Human Adipose Derived Stem Cell Models

As there was no confocal microscopy data available on the appearance of the hASCs in tri-culture of the cardiac patch, they were created *de novo* based on measurements (**Table 2**), images found in the literature (Hutton, 2015), and discussions with the project preceptor. In Cinema 4D, a Sphere object was created and in the **Object panel,** the radius was reduced to 90, the **Segments** increased to

55, and **Type** set to **Icosahedron**. For those that were to be **Pose Morph** animated, the object was changed from **Parametric** to **Editable**.

Capillary Growth Model

The capillaries used in the cellular scene (**Figure 10**) were created *de novo* yet highly informed by the data segmentation. By selecting **Enable Snap>Vertex Snap** and **Create>Spline>Sketch** in Cinema 4D, a spline was created that draped over the cardiomyocytes and onto the fibrin bioscaffold. Other splines were drawn to create an accurately vascularized field of capillaries no more than 200 μm from any cardiomyocyte.

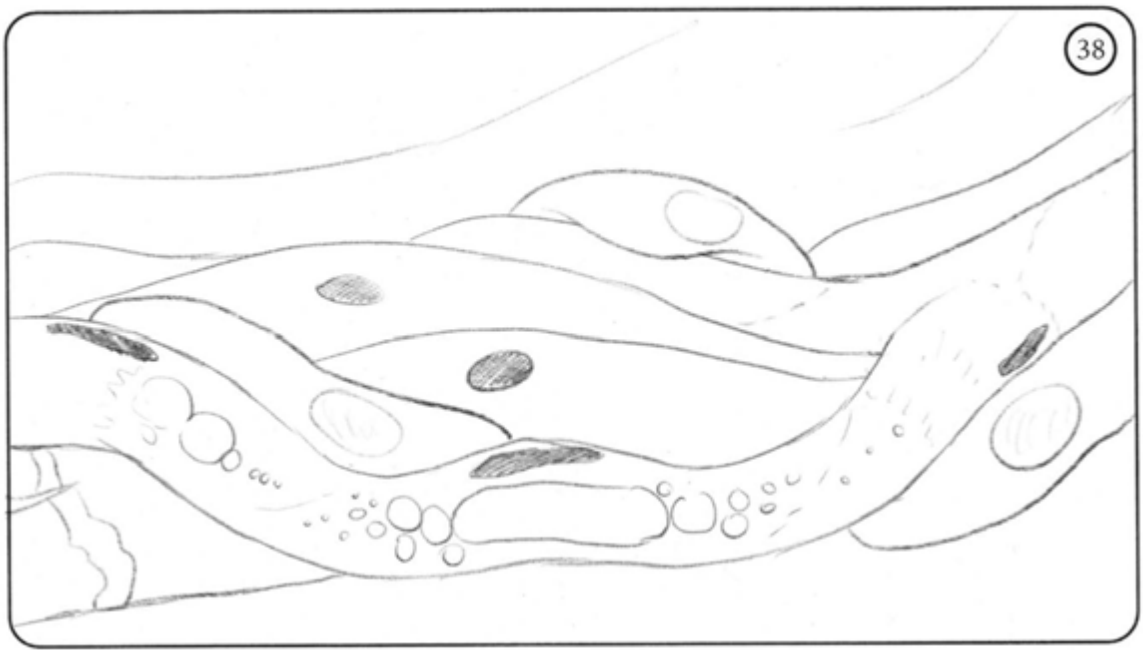


Figure 10. Storyboard # 38, capillary within cellular scene.

The two splines were paired with a Circle (**Create>Spline>Circle**), duplicated, and placed under a **Sweep Generator** (**Create>Generators>Sweep)**. The sweep was then transformed into a polygon object by using the **Object>Current State to Object** function. The generated child objects were then connected to create a single object (**Object>Connect Objects+Delete**). Further surface details were sculpted by sending the model to ZBrush using the **GoFromCinema4DToZBrush** plugin.

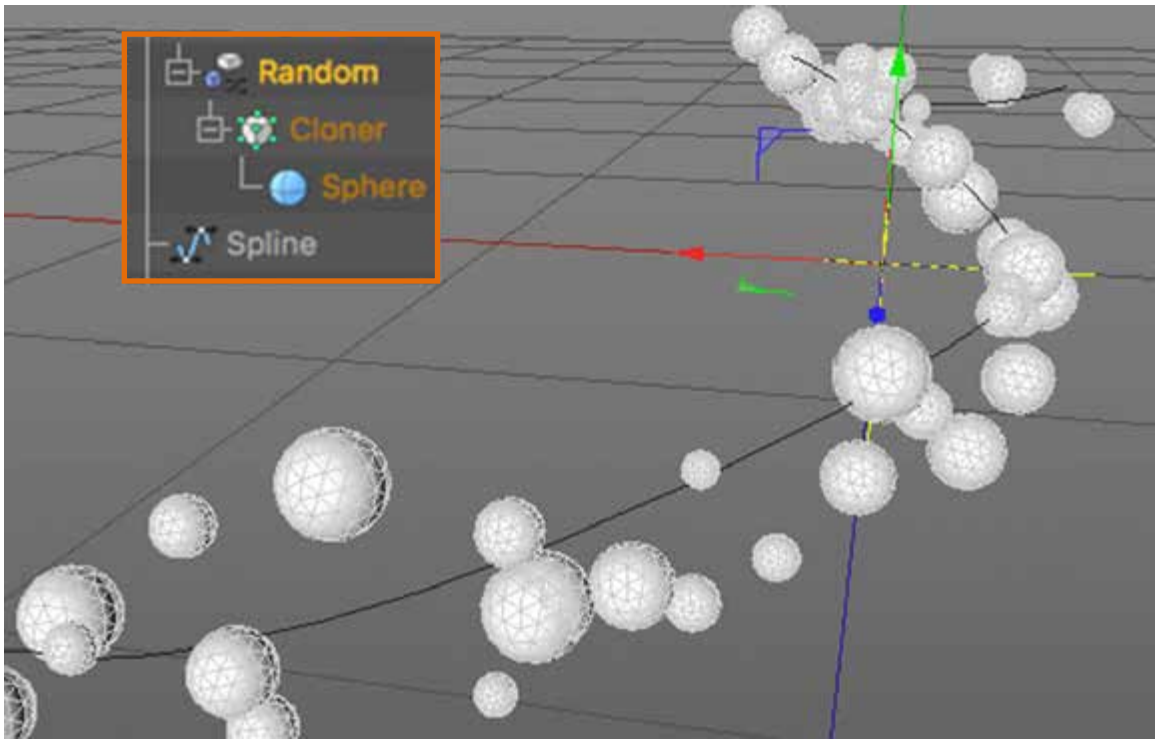


Figure 11. Lumenization effect I. Random effector applied to Cloner object following spline.

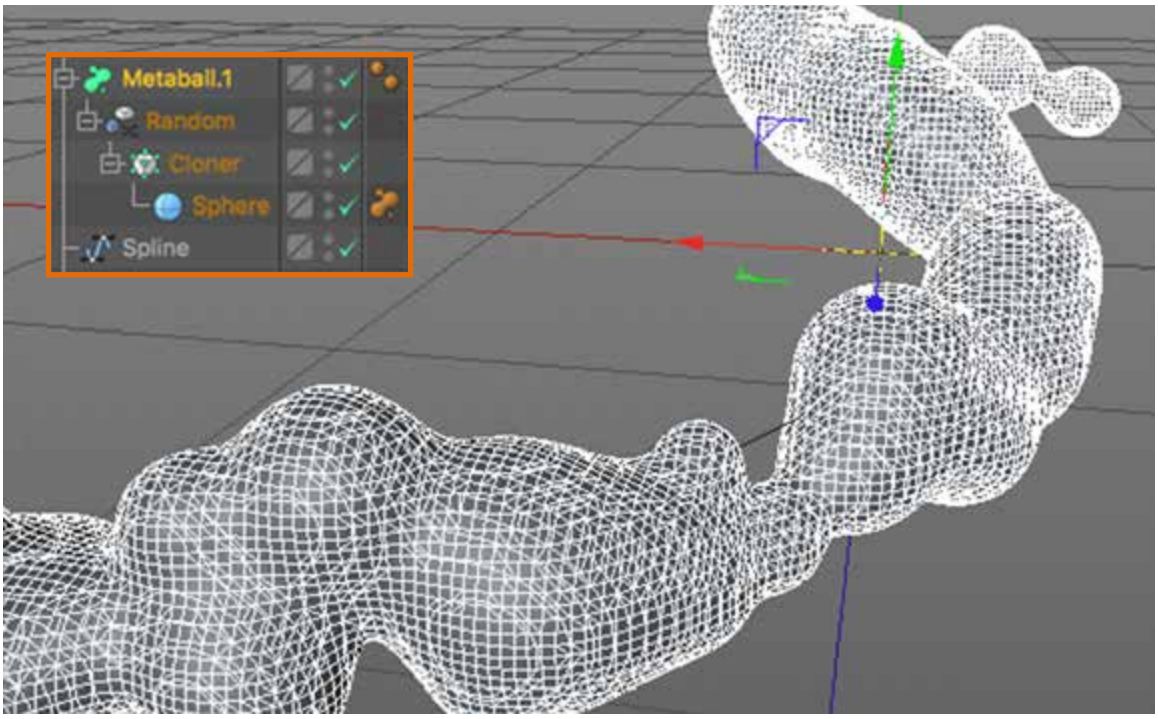


Figure 12. Lumenization effect II. Metaball Object applied over randomized cloner object.

In Cinema 4D, the duplicated splines were used to create the lumenization effect. A Sphere object (**Create>Object>Sphere**) was created and placed under a **Cloner Object (MoGraph>Cloner**) which was set to **Object Mode**. The spline was then dragged into the **Cloner Object field**. The **Distribution** was set to **Step,** step set to 11, and **Offset Variation to 5%**. A **Random Effector** (**MoGraph>Effector>Random**) was created and made a parent of the **Cloner** (**Figure 11**). Lastly, a **Metaball Object** (**Create>Modeling>Metaball**) was made the parent of the **Random Effector** (**Figure 12**). The **Editor Subdivision** was lowered to 2 cm. A **Metaball tag** was applied to the sphere object (**Tags>Cinema 4D Tags>Metaball**) and the radius set to **16 cm**.

Endothelial Cells

As three individual endothelial cells were needed (**Figure 13**) in order to depict capillary growth, a new Sphere object was created, the radius was reduced to **50**, the **Segments** increased to **44**, and **Type** set to **Icosahedron**. Models of the endothelial cell elongation were created in ZBrush. The capillary growth model mesh, created as described above, was used as a reference to align the position of the endothelial cells.

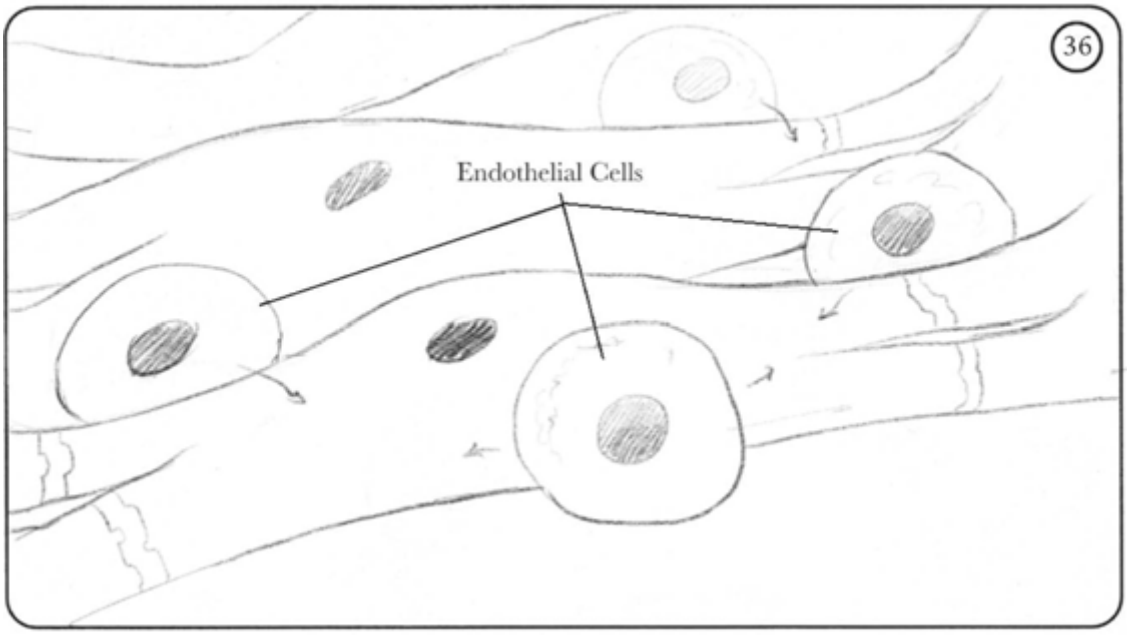


Figure 13. Storyboard #36. Main endothelial cells to teach elongation.

Fibrin Bioscaffold Creation

The fibrin bioscaffold, on which the cellular interactions take place, was created in Cinema 4D. A SEM image of a 2D fibrin bioscaffold microfiber sheet was used for reference (**Figure 14**). A plane object (**Object>Width segments = 50, Height segments = 124**) was created and then a **Displacer** deformer was applied. In the **Displacer**, **Object Height** channel was set to **10 cm**, and a **Noise** layer was created.

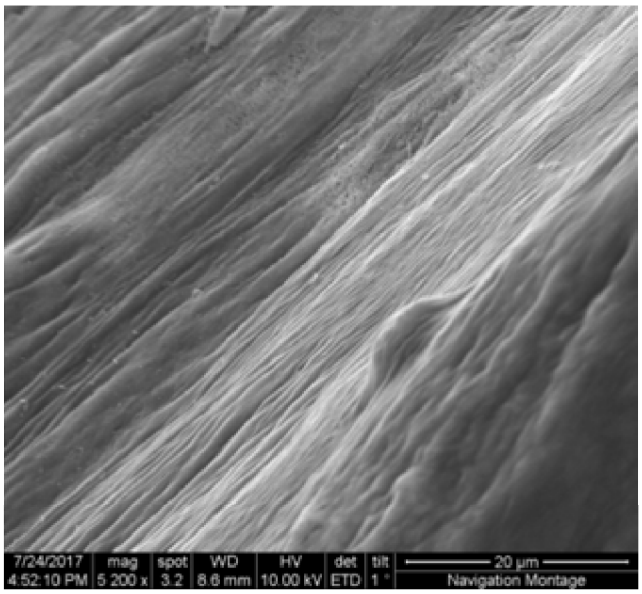


Figure 14. SEM reference of fibrin microfiber sheet. Text not intended to be read.

A **Smoothing** deformer was created, **Stiffness** was set to **75%**, and then the **Displacer–Plane** tree was made a child of the **Smoothing** deformer. This relaxed points that overlapped on the plane as a result of the noise in the **Displacer Shader** channel.

The object tree was converted into an editable object and further sculpted in Cinema 4D and ZBrush. Once the cardiomyocyte models were in place, the **Grab** brush, set between 100-200 width, was used to raise and lower individual pieces of the fibrin microfiber sheet mesh, to make the cardiomyocytes appear to be lying on the surface.

Macro Cardiac Patch Components

The capillary mesh from the tri-culture confocal microscopy dataset was imported as an .obj into a new Cinema 4D file. As the microscopy resolution was 1800 x 1800 μm, the capillary sheet was scaled to 1800 x 1800 cm for easy future scaling by powers of 10. Two cubes were created, aligned to the capillary object and scaled to represent the cardiomyocyte layer and fibrin bioscaffold layer.

Creation of 3D Cellular Scenes

Animating Cell Growth and Contraction

Cinema 4D was used to animate the cellular interaction scenes. To sculpt the different stages of growth of the cardiomyocytes, endothelial cells, and hASCs, models of each stage were first created based off of the storyboards.

The **Pose Morph** tag was used to create smooth animated transitions between each stage of cell growth. The **Pose Morph** tag was applied to a given stage of a cell model. From that model, additional poses and meshes were created, edited with Cinema 4D sculpting tools, and animated by adjusting strength of deformation of desired parameters (Point, Position, etc.) The **Pose Morph** tag only functions when meshes in the **Pose Morph** tree have the exact same number of points. For the cellular models with complex geometry (e.g. cardiomyocytes), a model with a high point count was first created and then smoothed for transitions to simple models. For the cellular models with simplified geometry (ex. hASCs), an editable sphere was used and posed as the sphere moved along an animated path.

To pose morph cellular adherence, the **Pose Morph** tag was applied, "Points" selected in the **Basics** tab, and the mesh was manipulated with Cinema 4D sculpting brushes (**Sculpt>Brushes>Grab**) to give the appearance of attachment to the fibrin bioscaffold. For the animation of cellular adherence, the "Animate" tab was selected, the sliders adjusted and then the models were keyframed.

Next, the high-polygon count models were given **Pose Morph** tags and, working in reverse of the animation timeline, were sculpted into simpler forms for each stage of the animation. As each mesh had to be carefully manipulated from one step in elongation to the next, instead of creating a new pose, which would create a duplicate of the base pose, a new mesh was created from the last pose (**Right click>New Mesh**) and then sculpted outside of the **Pose Morph** tree. This same **Pose Morph** workflow was used to modify the base models of endothelial cells.

To animate the models, the **Pose Morph** mode was changed from **Edit** to **Animate**. The sliders were keyframed by pressing "Record Sliders" at desired

intervals of time and at the desired strength. For complex morphing, many slider strengths were keyframed to overlap one pose into the next (**Figure 15**).

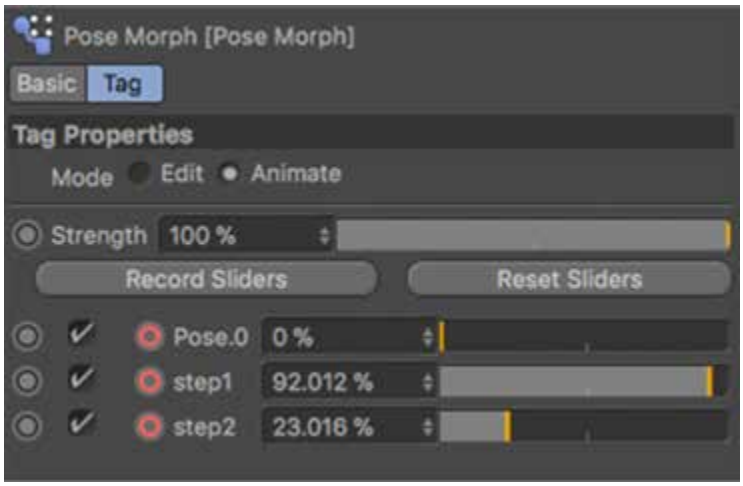


Figure 15. Pose Morph animation sliders.

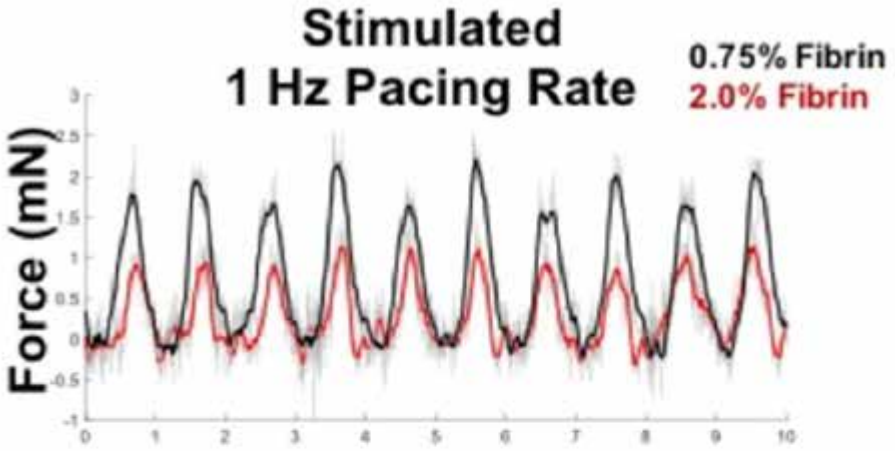
As the cardiomyocytes first contract as isolated units and then as a whole, two methods of simulating contraction were used. As isolates, cardiomyocytes were animated to contract rhythmically along the Z axis, by scaling them from 1 to ~.9, while a Bulge deformer was increased in strength, from 0% to ~5%. For finer timing control, the keyframe spline Bezier curves were matched to cardiac patch force of contraction rates during stimulated pacing (**Figure 16**).

To animate the sheet of cardiomyocytes contracting synchronously, the Null containing all the cardiomyocytes was keyframed to scale with a **Bulge** deformer in a similar manner as described above. These keyframes were repeated for the scene.

Creating Materials in Cinema 4D

Sarcomeres can be observed within the cardiomyocytes #3-1, #3-2 (Table 1) datasets (**Figure 17**). A drawing of sarcomeres based on electron micrograph data was created. The drawing was manipulated in Adobe Photoshop to create a seamless 2048 x 2048 ppi pattern. A new material was then created in Cinema 4D. The drawing was loaded into the **Material Alpha Channel** (**Alpha>Texture>Load Image**) and in the **Texture Tag**, the **Projection** was changed from **Spherical** to **Flat**. By changing the viewport from **Object mode** to **Texture mode,** the axis of the texture tag was adjusted to align the sarcomere pattern with the longitudinal axis of the cardiomyocyte cell (**Figure 18**) This material was animated to contract by

A



B

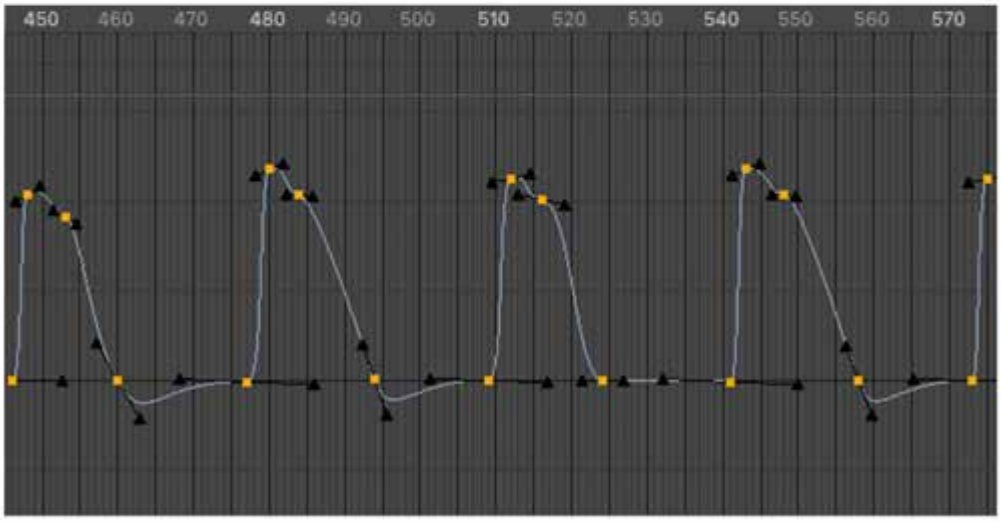


Figure 16. Animating cardiomyocytes to contract. (A) Stimulated pacing rate from tri-culture studies (data unpublished, Morrissette-McAlmon et al., 2018). (B) animated contraction (30 frames per second) F-stop curves were adjusted in Cinema 4D to match stimulated pacing rate of acutal cardiomyocytes.

keyframing the **Length U** and **Length V** percentages in Cinema 4D.

A second material was created with a dark red in the color channel that provided a colored base under the Alpha material.

Animating in Cinema 4D

Interactions between hASCs, capillaries, and cardiomyocytes were created with the **Collision** and **Smoothing** deformers.

These two deformers were made children of the main hASC mesh. A section of the capillary object was identified for the hASC to collide around. Then, a Cylinder (**Create>Object>Cylinder**) was created and set into the same orientation as the section of the capillary object. The Cylinder was made invisible in the Viewport and dragged into the Collision Deformer **Collider Object Field. Outside (Volume)** was selected in the **Solver tab**. Additional hASC cell-cell interactions were animated through use of the Pose Morph tag as described previously.

Interactive Illustration Content

An additional piece of illustrative content was created to demonstrate web-based interactivity within the animation. After review of the provided *Principles of Tissue Engineering* course materials, *Cell Biology* (Pollard, Earnshaw, Lippincott-Schwartz, Johnson, 3rd edition), and additional literature (Stoppel 2016), three illustrations were created on the subject of mechanobiology.

After review with preliminary sketches, the illustrations were scanned into Adobe Photoshop and adjusted into the final layout. This illustration was exported as a .png image and uploaded into a webpage.

Interactive Animation Creation and Website Development

The software **TouchCast Studio** was used to add interactivity to the animation. A new interactive video was first created on an Apple iPad Pro by entering the TouchCast Studio application and selecting **Annotate a Video**. The video, which

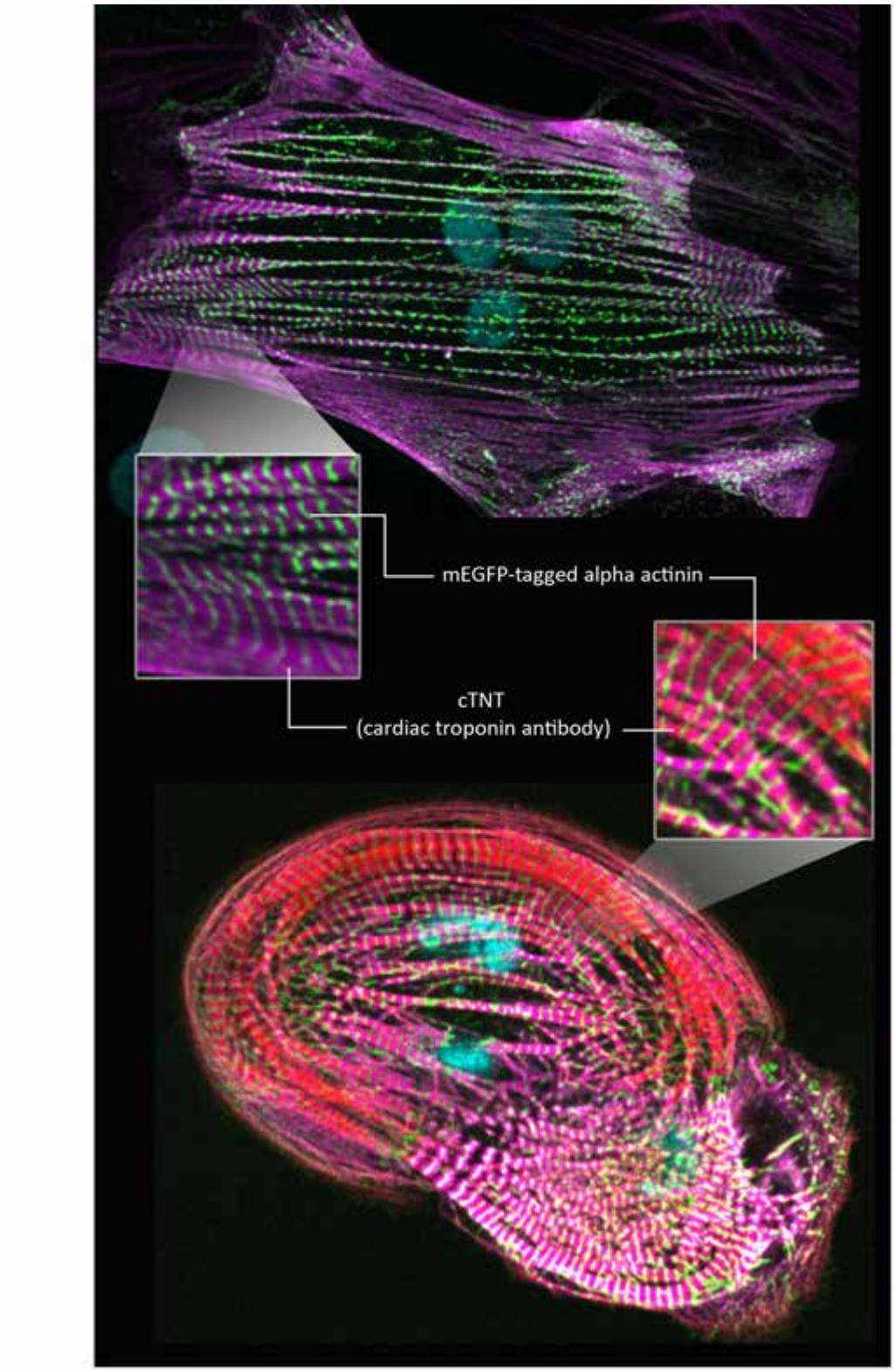


Figure 17. Sarcomere appearance of iPS cardiomyocytes (#3-1). Image credit: unpublished data from Allen Institute for Cell Science, a division of the Allen Institute.

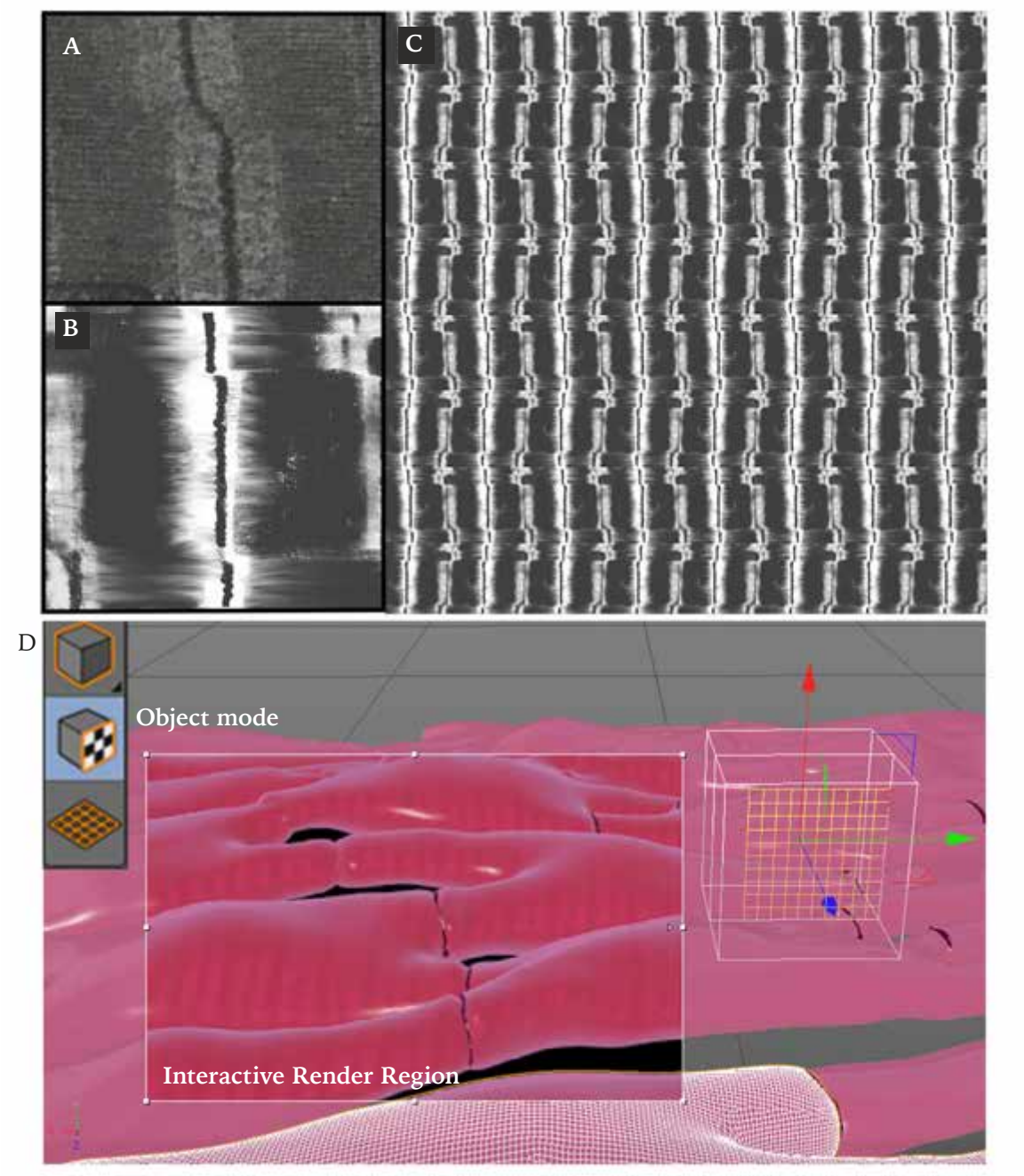


Figure 18. Sarcomere creation. (A) Electron micrograph of sarcomere (*Bailey's Textbook of Microscopic Anatomy*). (B) Drawing of sarcomere. (C) Seamless sarcomere pattern created in Photoshop. (D) Pattern projection onto cardiomyocytes in Cinema 4D.

was pre-rendered and uploaded to a cloud sharing service (Dropbox), was imported through the **TouchCast Cloud importer**. A new **vApp** was created (**vApps>Add a vApp>Webpage**). **Webpage** was chosen and a URL was loaded. This creates a **vApp** that hyperlinks to the webpage of choice, in this case, Stem Cell Orchestra: Mechanobiology webpage. This **vApp** was saved into the **vApp tray**.

To add the vApp to the animation, a new recording session was started (**Click>Record**). At the time indicated in the narration for interactivity, the recording was paused and the **Mechanobiology webpage vApp** was loaded into the video (**Figure 19**). After resuming play for about 15 seconds, the video was paused again and the vApp removed. The recording session resumed. At the end of the session, the new TouchCast interactive animation was uploaded to the user's channel. The interactive video was then embedded into a webpage.

The website was developed through **Wordpress** with the **Optimizer** theme and the modular website building plugin, **Page Builder by SiteOrigin.**

Figure 19. Interactive animation creation. TouchCast interactive video creation interface on Apple iPad Pro, with vApp placed in animation.

RESULTS

Flowchart and Storyboards

A flowchart (**Figure 20**) was created to organize the content of the website into main topics, subtopics, and additional categories containing education material. User interaction with educational material is indicated.

A set of storyboards (**Figure 21.1-4**) was created from selected pathways within the flowchart to define the user interface and website navigation. Numbers on the flowchart correspond to numbers on the storyboards. Selected sections include the following pages: (1) Stem Cell Orchestra homepage, (2) Heart Muscle topic navigation, (3) Stem Cell & Tissue Types interactive topic webpage, (4) interactive media webpage.

A detailed outline of the animation and interactivity (**Figure 22**) was created to indicate interactive content as reflected in the flowchart.

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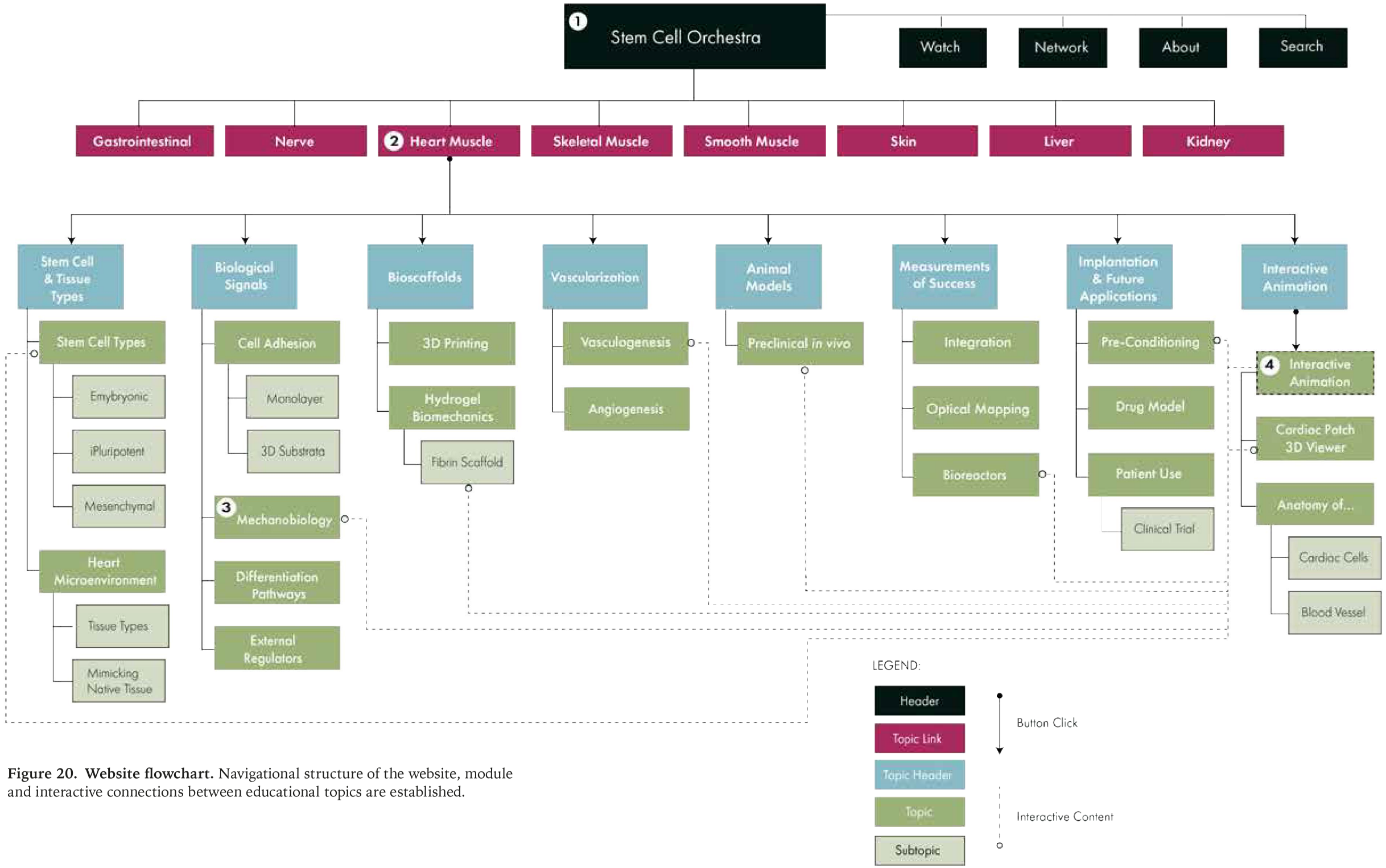
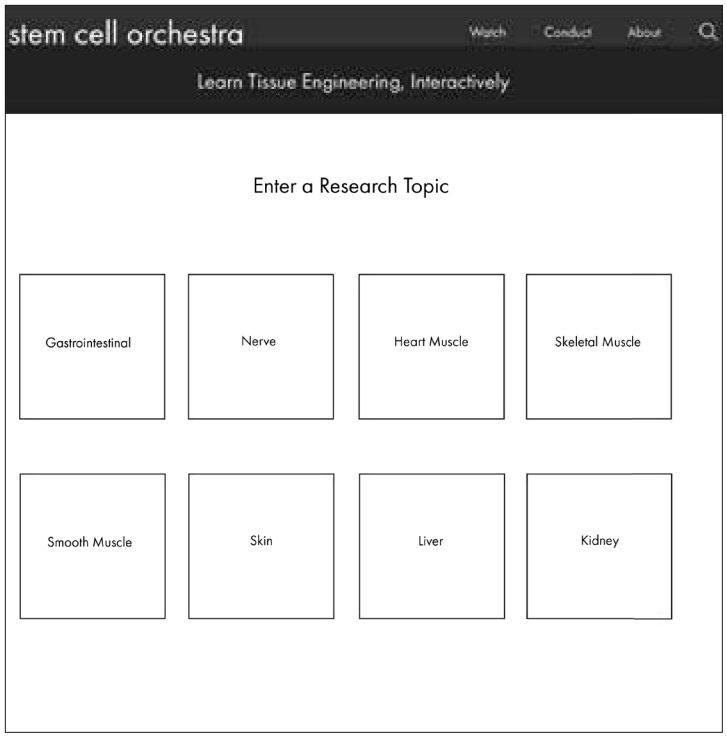


Figure 20. Website flowchart. Navigational structure of the website, module and interactive connections between educational topics are established.



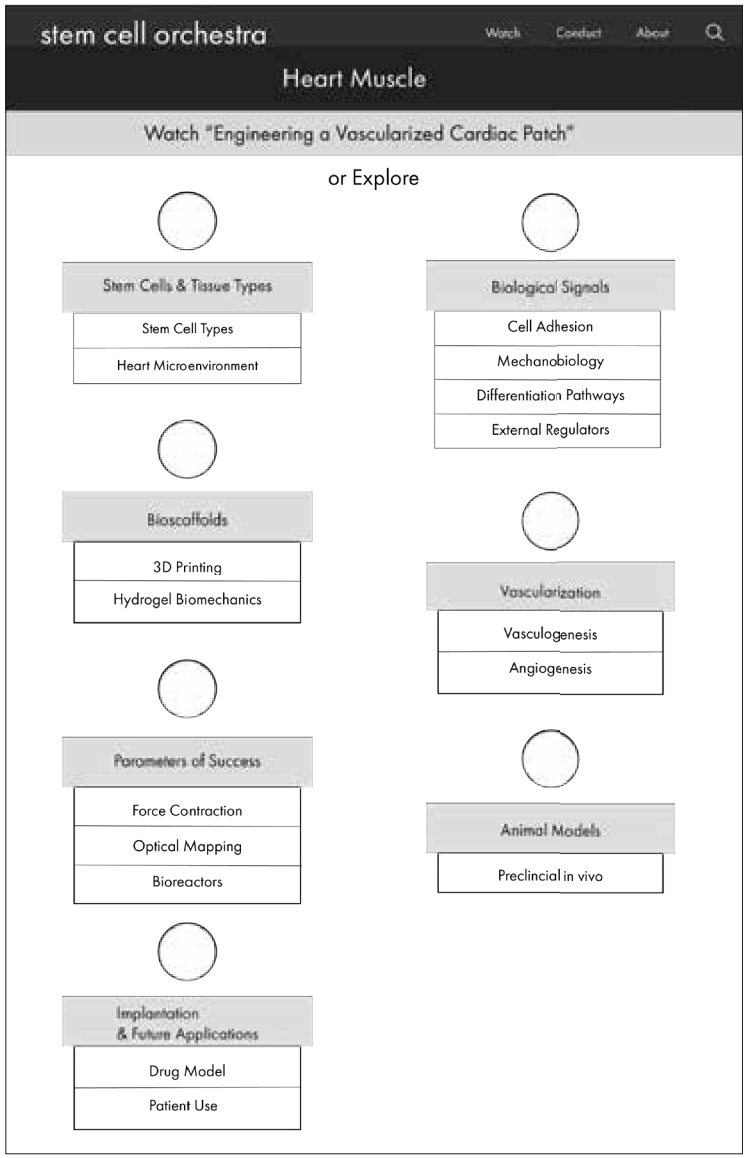


Figure 21.1-2. Website storyboards. 1) Stem Cell Orchestra homepage. 2) Clicking of *Heart Muscle* topic navigates to *Heart Muscle* page (text not intended to be read).

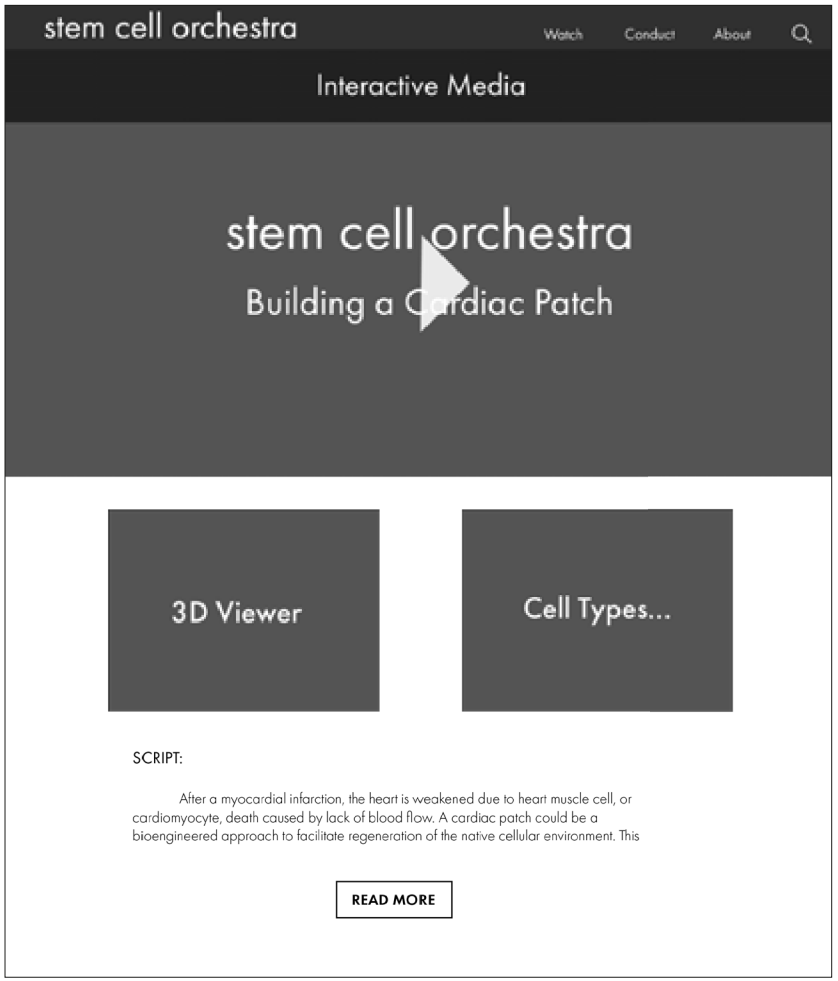


Figure 21.3-4. Website storyboards. 1) Educational Mechanobiology resource. 2) Interactive Media webpage with interactive animation (text not intended to be read).

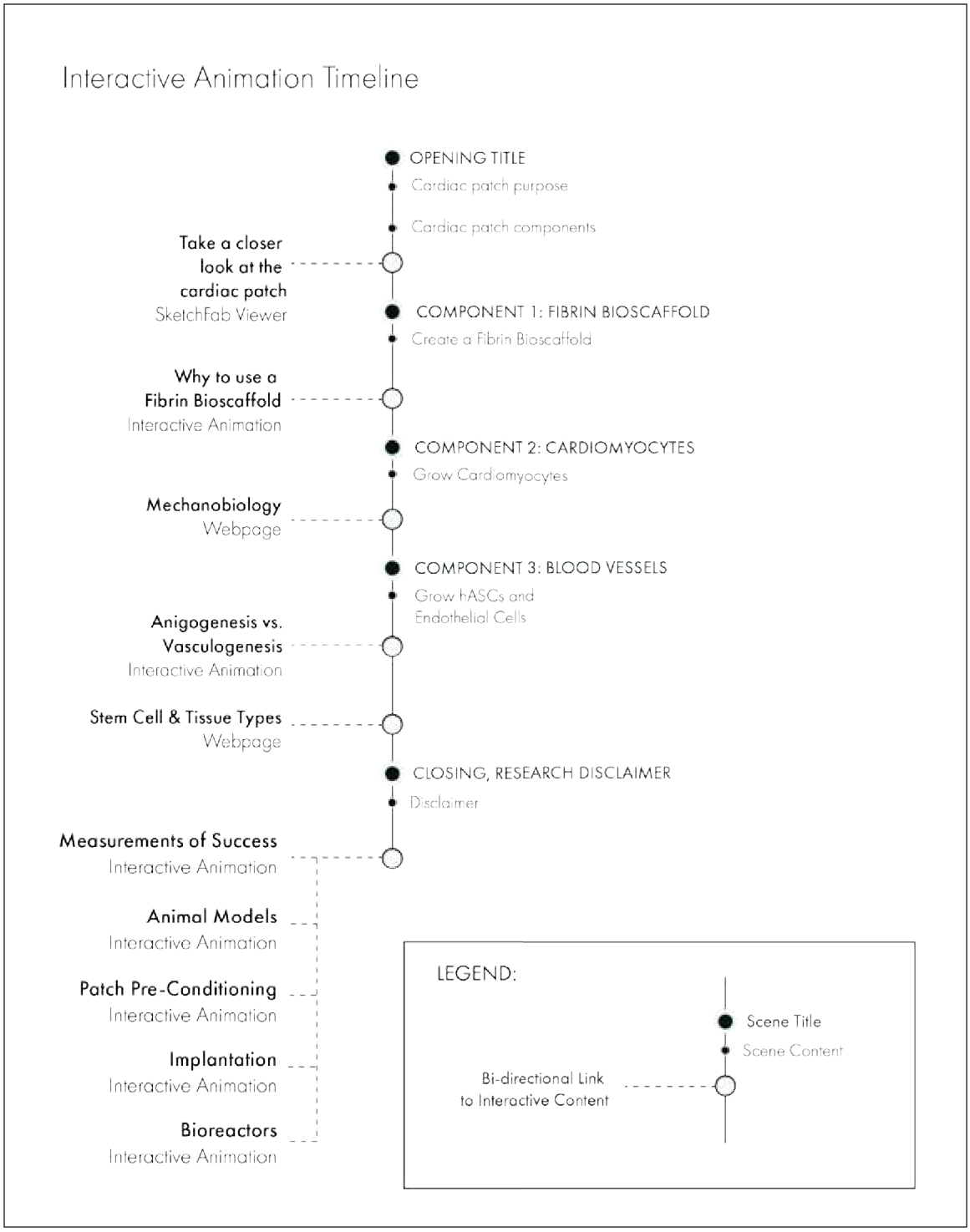


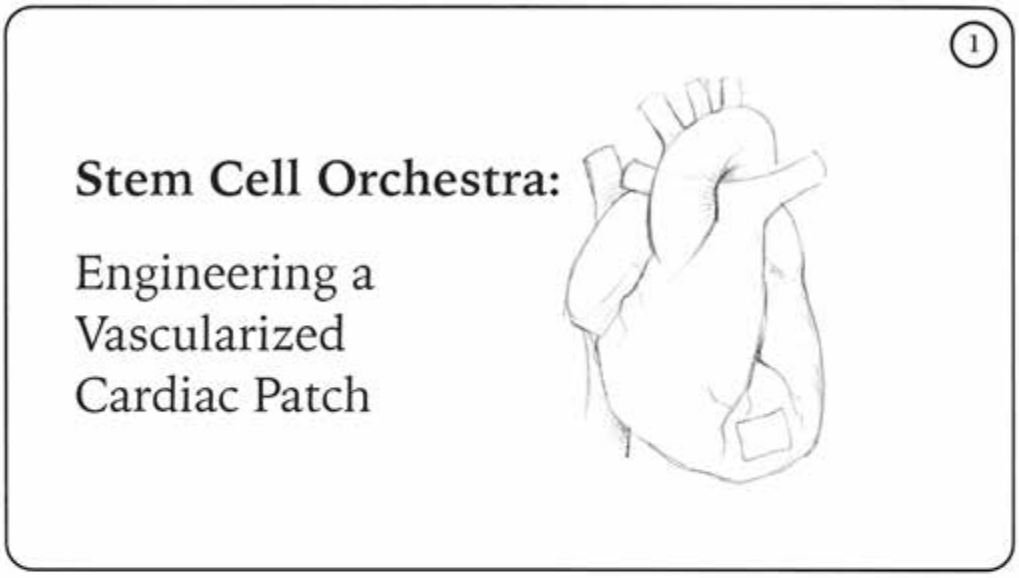
Figure 22. Detailed outline of animation and interactivity for the complete video of ***Building a Cardiac Patch*****.**

Cellular Animation

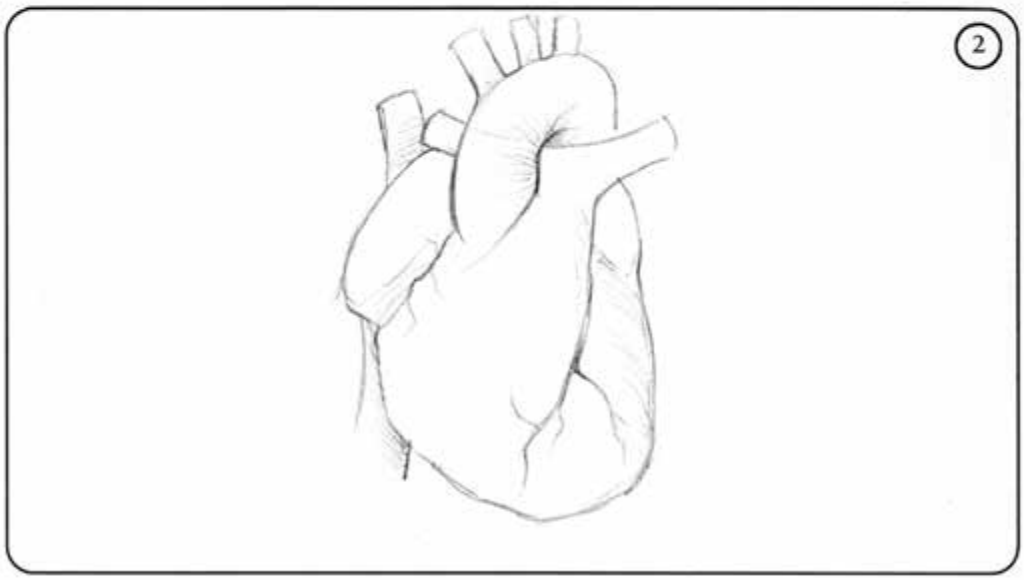
A script (**Appendix B**) and storyboards (**Figures 23.1-27**) were created following the detailed outline of the animation and interactivity. “Visuals” briefly describe the purpose behind the sketch and make note of motion not conveyed in the sketch. “Audio” was directly taken from the script and reformatted to align with multiple drawings. “Interactive Points” was derived from the detailed outline to present the interactive module title and briefly describe the proposed content, as well as show where the viewer would have to click on the screen to access interactive content.

Stem Cell Orchestra: Engineering a Vascularized Cardiac Patch

Scene 1. Cardiac Patch Purpose and Components



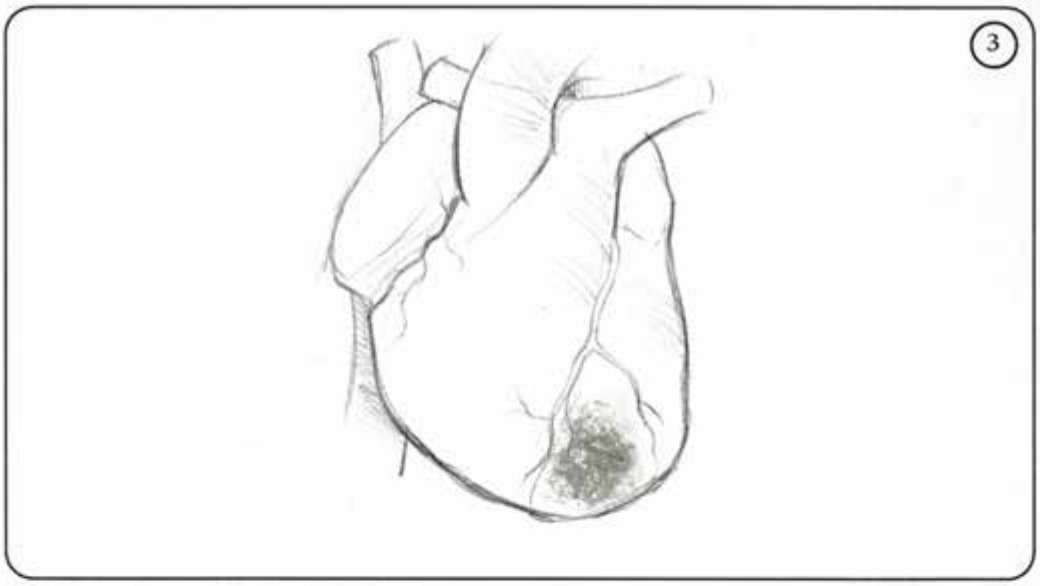
1. Visuals: Title text.



2. Visuals: Transition into anatomical heart (2D drawing), no patch.

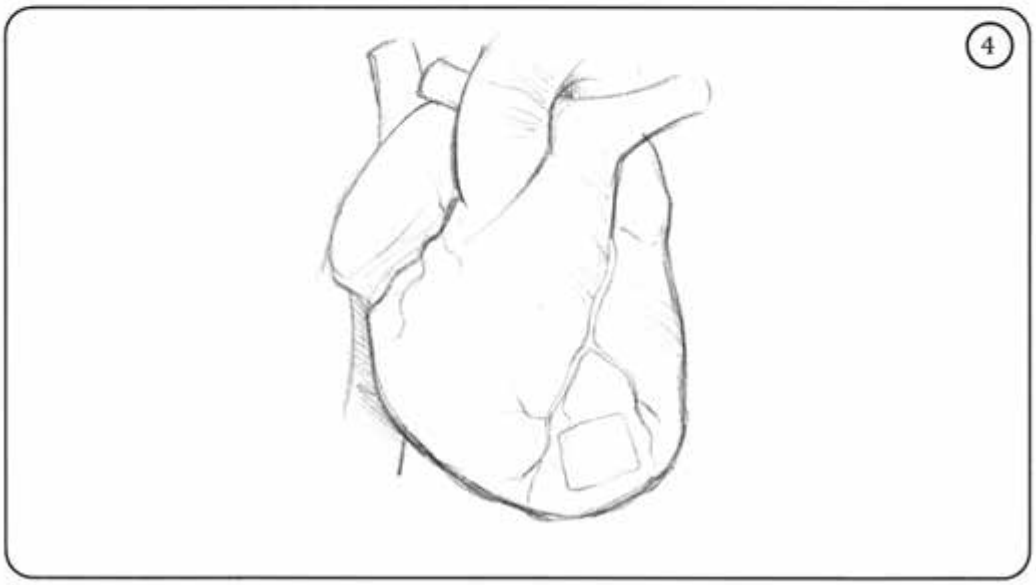
Audio: "After a myocardial infarction, the heart is weakened..."

Figure 23.1. Animation storyboards 1-2.



3. Visuals: Dark stain appears on left ventricle.

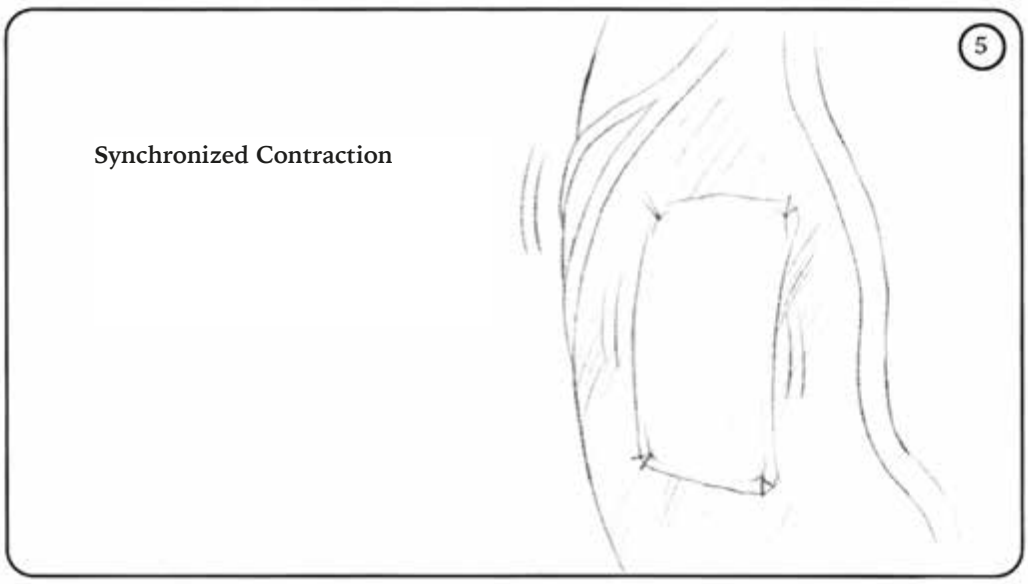
Audio: "...due to death of cardiomyocytes, or heart muscle cells, caused by lack of blood flow. Cardiomyocytes do not regenerate, and so the heart can fail over time."



4. Visuals: Cardiac patch is applied to heart.

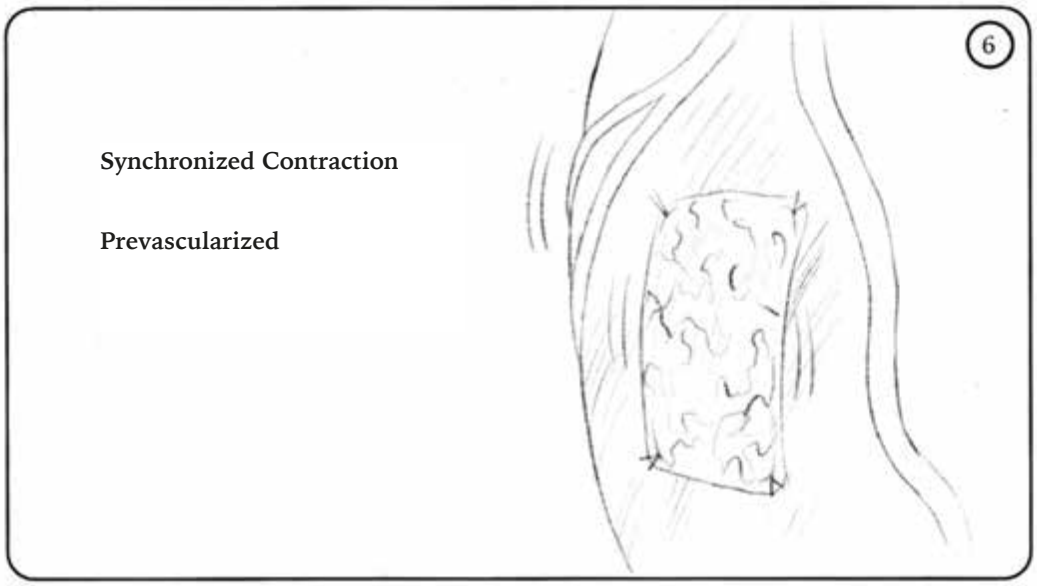
Audio: "This section focuses on one method of creating a bioengineered cardiac patch to support heart function."

Figure 23.2. Animation storyboards 3-4.



5. Visuals: Cut to patch on heart, show heart pumping and patch contracting in time.

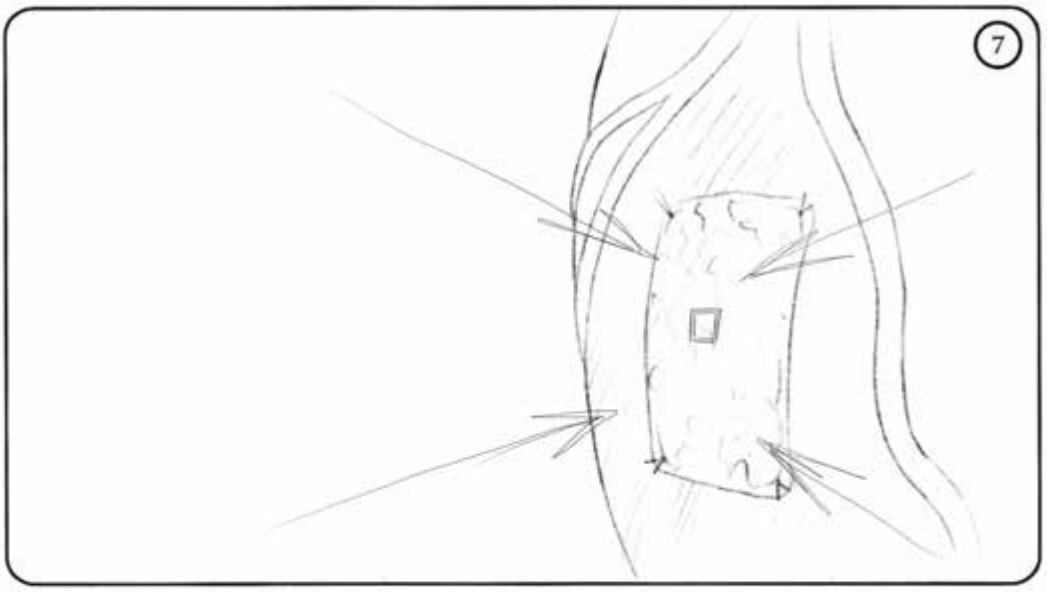
Audio: "Cardiomyocytes grown on the patch beat in time with the cells of the living heart."



6. Visuals: Blood perfuses the patch.

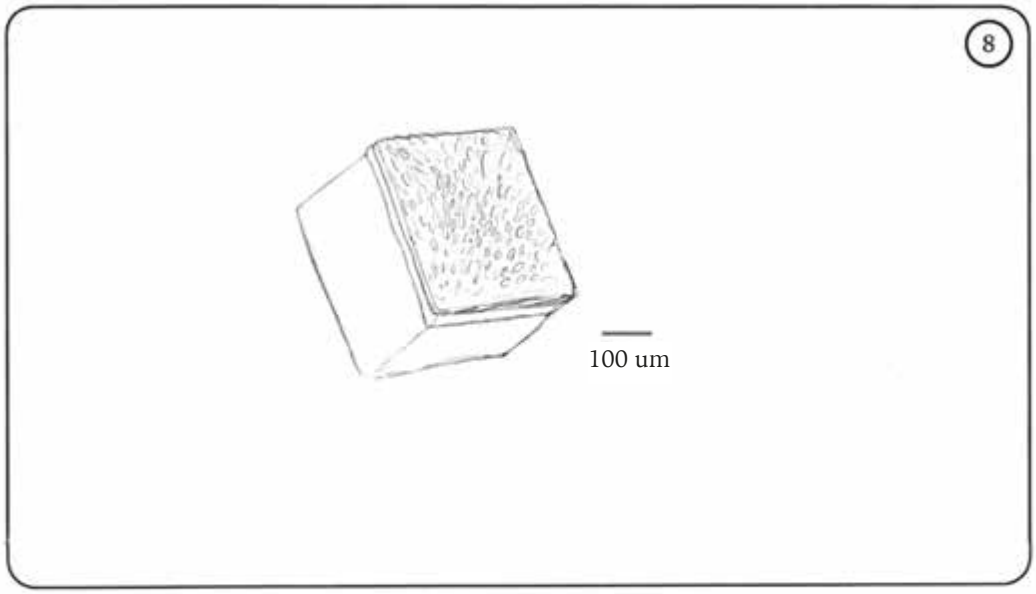
Audio: "The patch is also prevascularized so that it contains blood vessels, which integrate with the heart's vasculature."

Figure 23.3. Animation storyboards 5-6.



7. Visuals: Box fades onto patch, zoom into box.

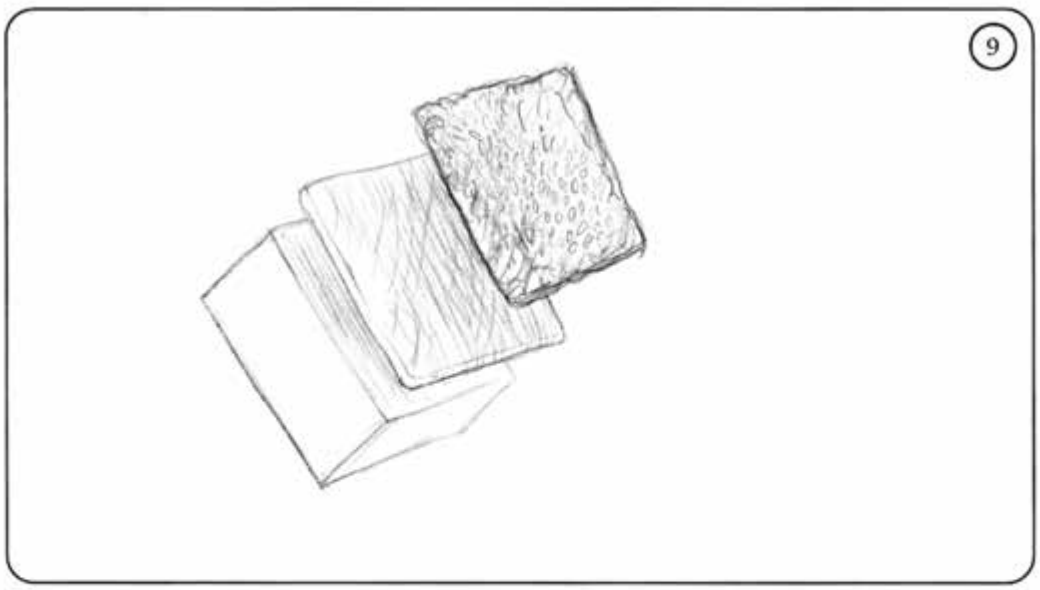
Audio: "A closer examination of one small piece of the patch…"



8. Visuals: Cut to that patch, larger, with scale bar, no heart.

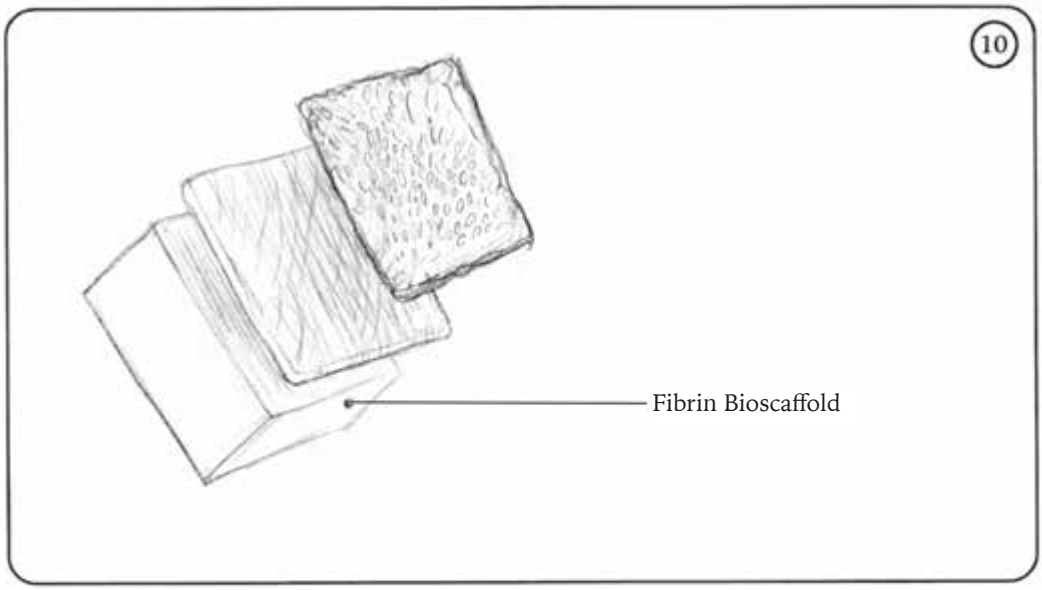
Audio: "Reveals these elements in greater detail."

Figure 23.4. Animation storyboards 7-8.



9. Visuals: Components expand.

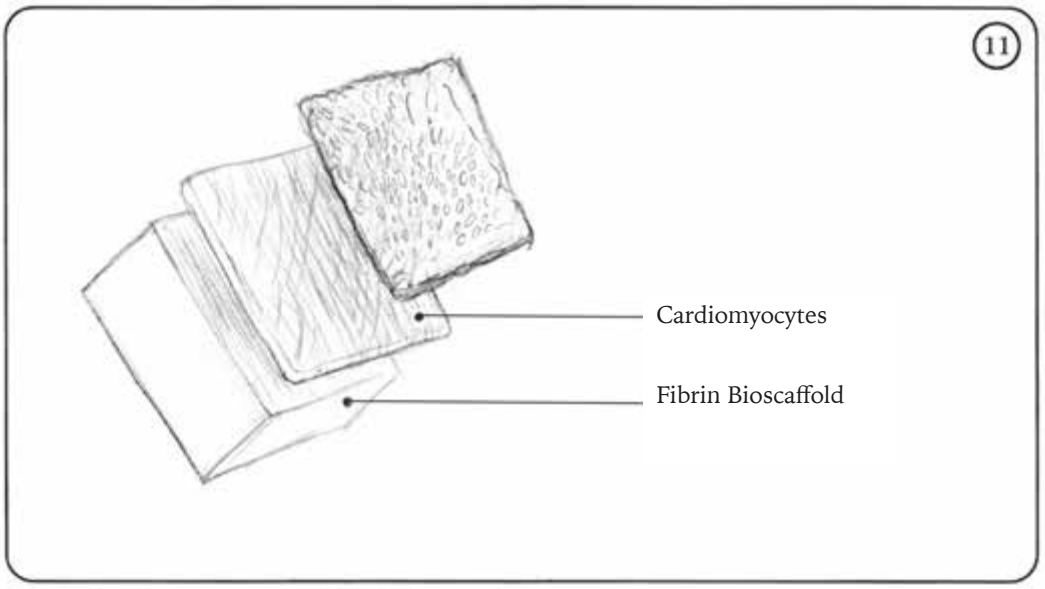
Audio: "This cardiac patch can be broken down into three main components."



10. Visuals: Components labeled as read.

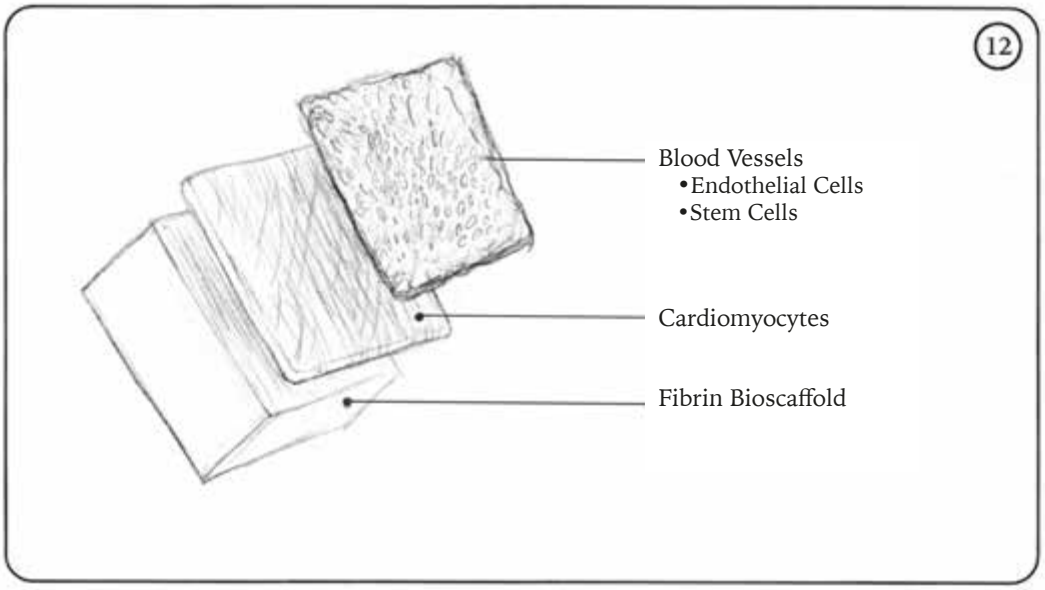
Audio: "The base is a biodegradable fibrin bioscaffold."

Figure 23.5. Animation storyboards 9-10.



11. Visuals: Components labeled as read.

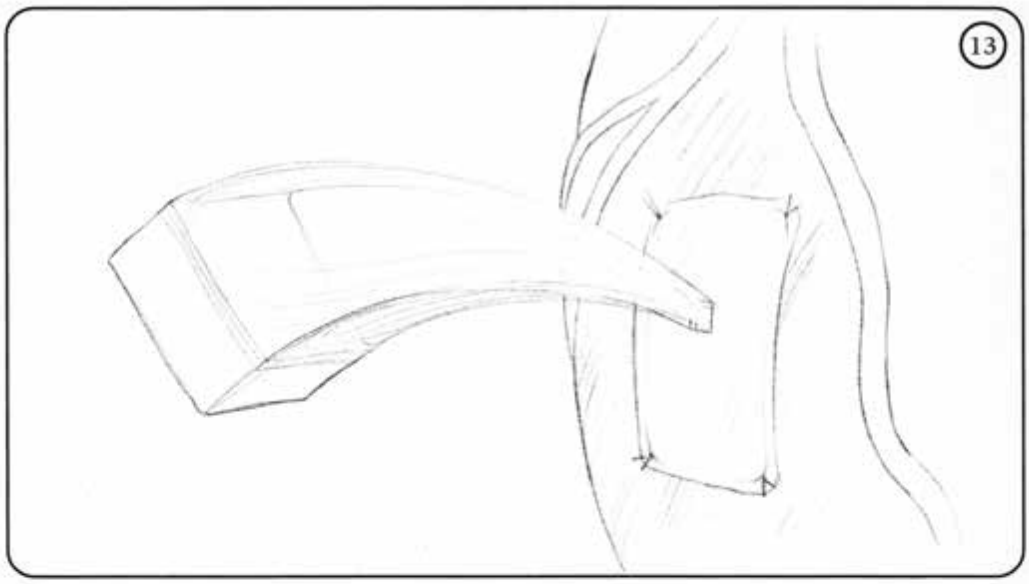
Audio: "Cardiomyocytes are grown on the bioscaffold."



12. Visuals: Components labeled as read.

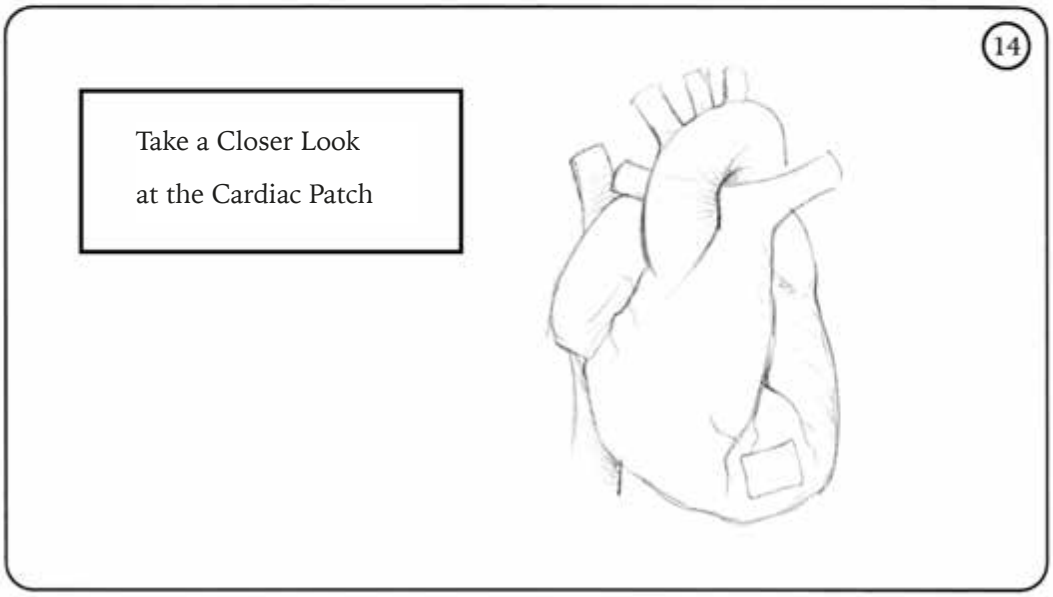
Audio: "Blood vessels, formed from endothelial cells and fibroblast-mimicking stem cells, are grown on top of the cardiomyocytes."

Figure 23.6. Animation storyboards 11-12.



13. Visuals: Text disappears, layers fit back into larger cardiac patch sutured to heart.

Audio: "A carefully orchestrated set of events transforms progenitor cells into…"

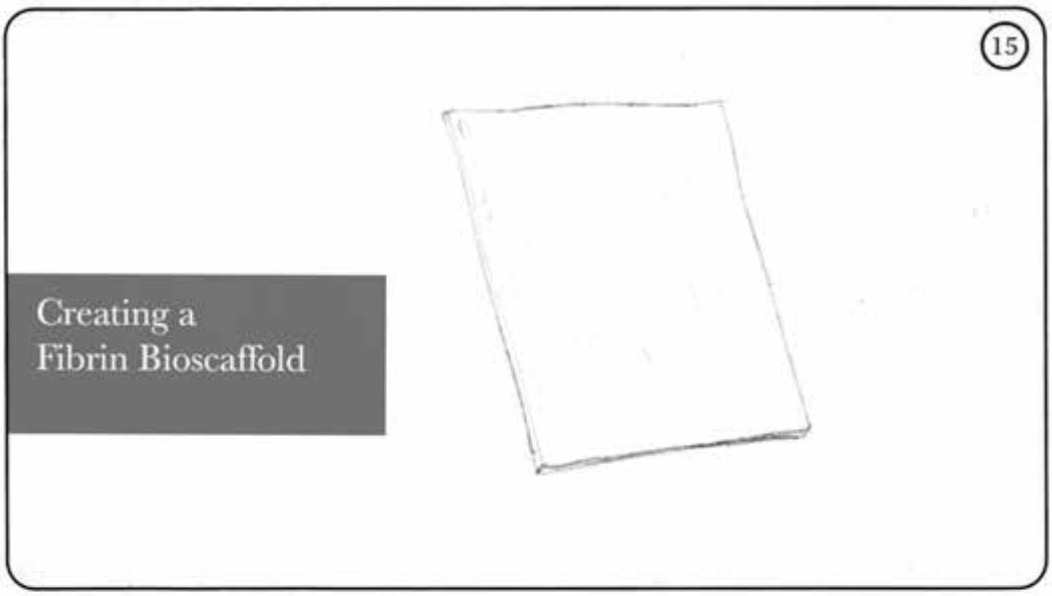


14. Visuals: Cross-fade back to drawing with patch sutured over infarction.

Audio: "…this functional cardiac patch for implantation onto damaged heart tissue. Click to take a closer look at the cardiac patch."
Interactive (15 secs): Take a closer look at the cardiac patch. (Sketchfab viewer)

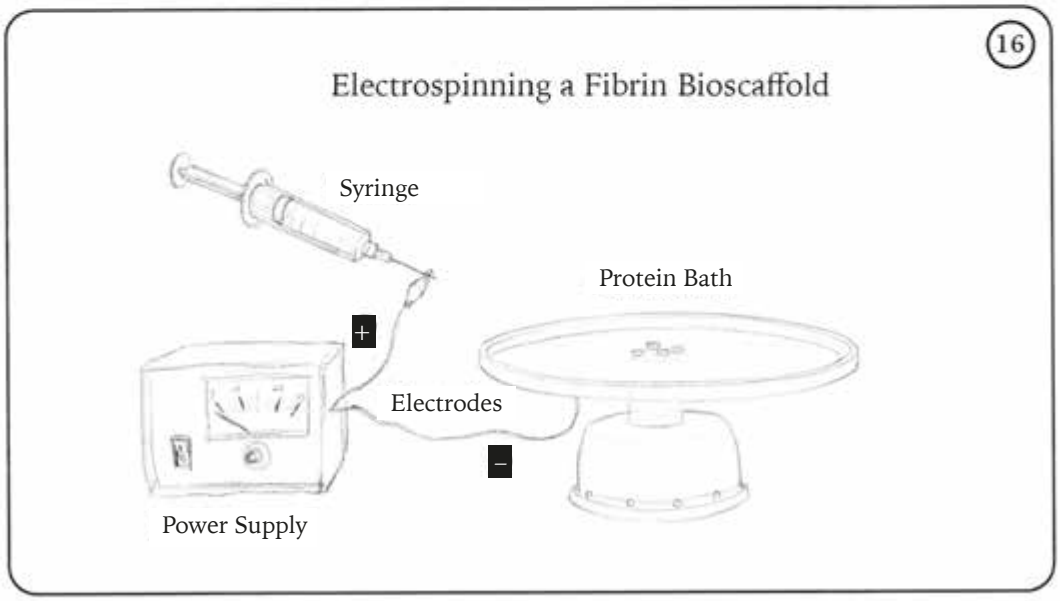
Figure 23.7. Animation storyboards 13-14.

Scene 2. Component 1: Fibrin Bioscaffold. Possibly show as video.



15. Visuals: Fibrin layer appears, title "Create a Fibrin Bioscaffold".

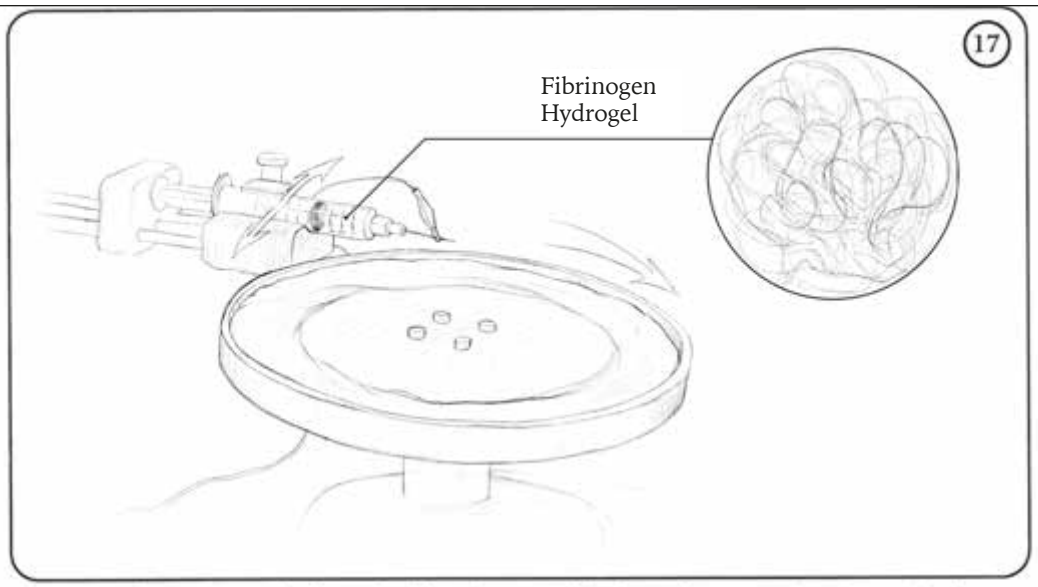
Audio: "A fibrin bioscaffold can be created to replicate key elements of native heart structure."



16. Visuals: Cut to: Electrospinning set up (labeled), syringe with power supply, rotating bath, blue hydrogel in syringe.

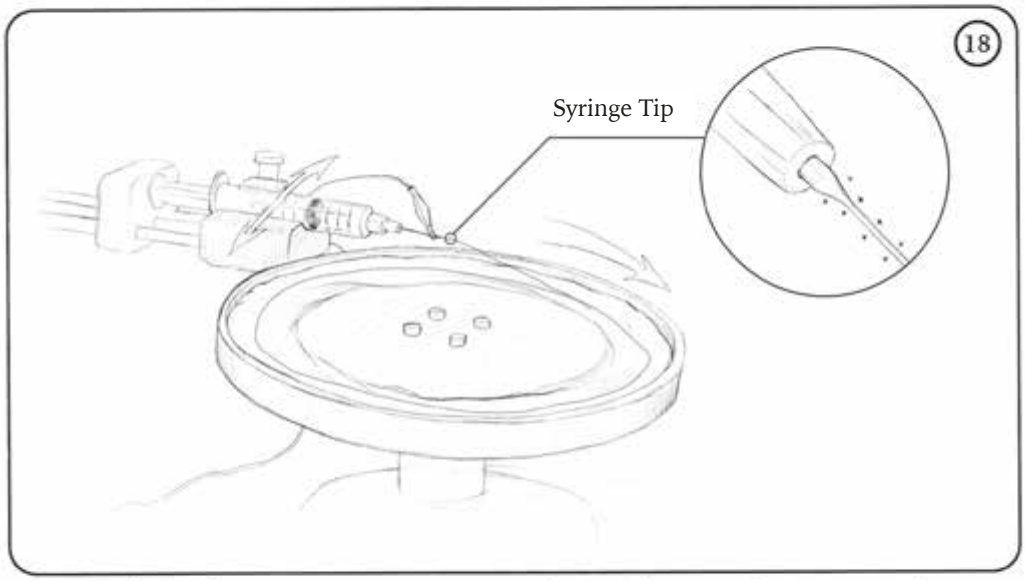
Audio: "A sheet of fibrin bioscaffold is produced by electrospinning a fibrinogen hydrogel into a swirling protein bath."

Figure 23.8. Animation storyboards 15-16.



17. Visuals: Camera shift to slightly above and looking down into bath. Leader line from syringe contents to circle: showing microscopic tangle of fibrinogen (label).

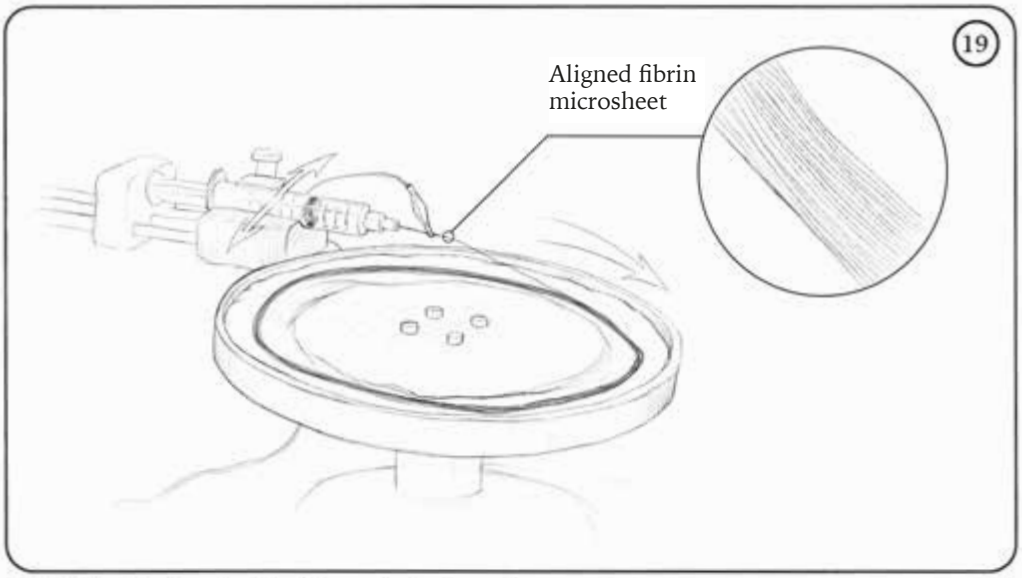
Audio: "The hydrogel consists of fibrinogen, a natural blood clotting polymer of unlinked microfibers, suspended in distilled water."



18. Visuals: Back out at previous view, strand ejects from nozzle tip into swirling protein bath. Leader line from syringe tip to circle: show tangle of fibrinogen in syringe, ejecting through nozzle in parallel alignment. Syringe oscillates slowly, one rotation of bath, strand from syringe lands next to first strand in bath.

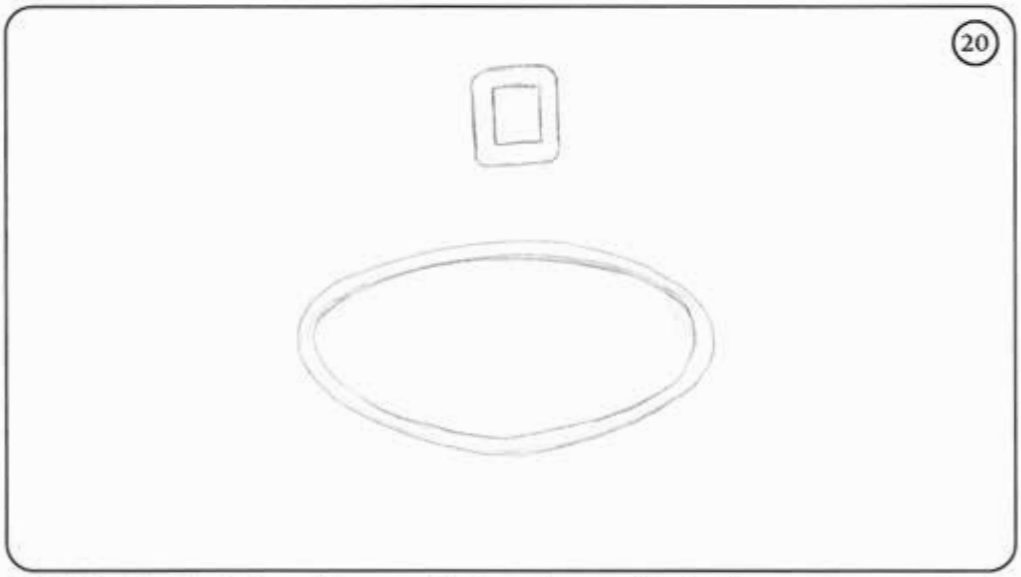
Audio: "A slight charge holds the strand together as a uniform stream. The slow oscillation of the syringe causes the subsequent strand of fibrinogen to land next to the previously deposited fibrinogen strand."

Figure 23.9. Animation storyboards 17-18.



19. Visuals: Leader line from the bath surface to circle shows crosslinking of microfibers.

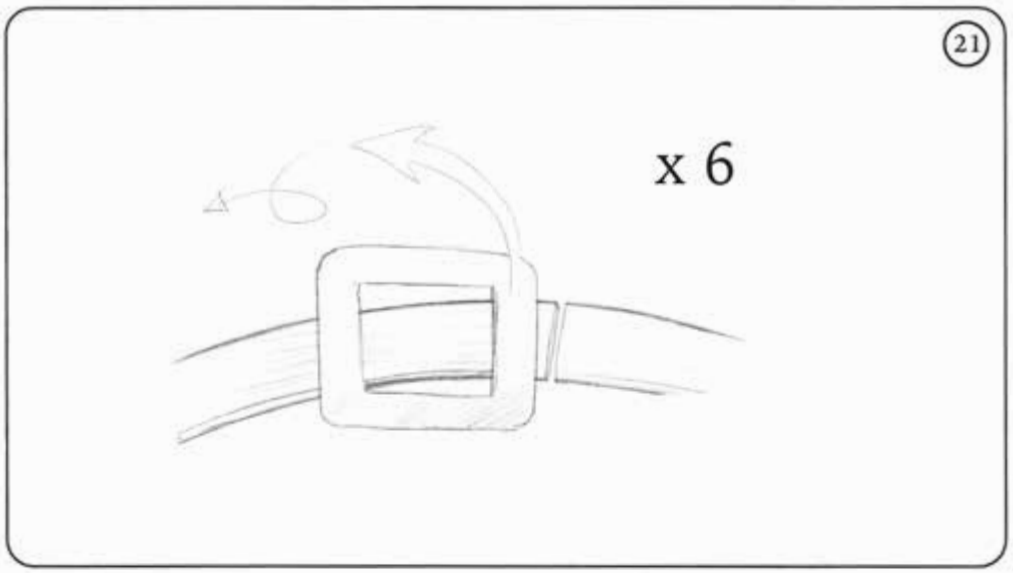
Audio: "Thrombin in the bath causes the microfibers to crosslink. This results in a sheet of parallel cross-linked fibrin."



20. Visuals: Fibrin sheet lifts out of bath; syringe and bath fall out of frame and disappear. Mylar frame fades into view. Camera pans as it drops onto fibrin sheet.

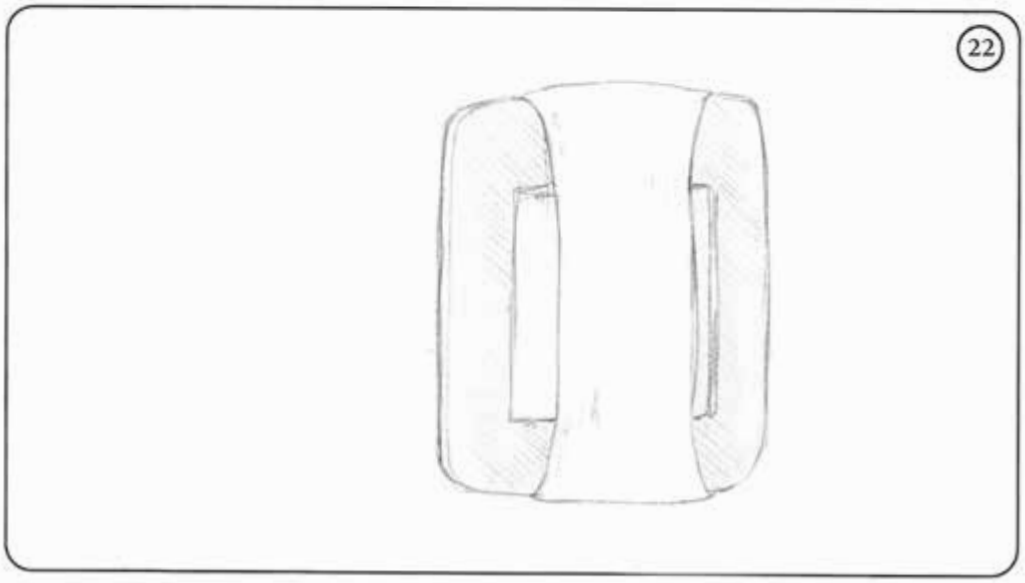
Audio: "The fibrin sheet..."

Figure 23.10. Animation storyboards 19-20.



21. Visuals: Zoom into one part; show empty Mylar frame, sheet begins to wrap about it.

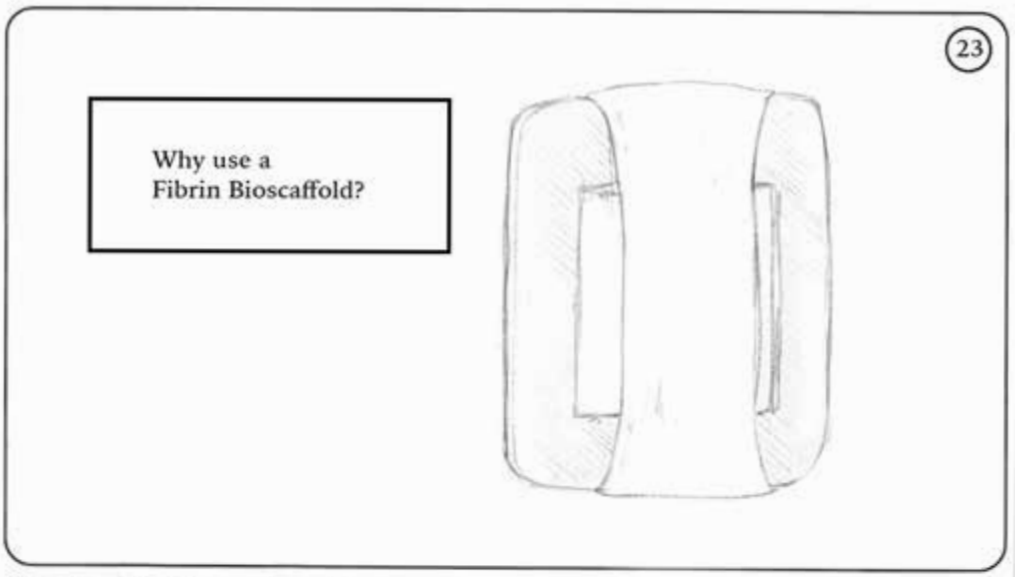
Audio: "...is spun around a Mylar frame up to six times to create a single thin sheet about 300 μm thick."



22. Visuals: Cut to it fully wrapped sheet, same as the beginning of this scene.

Audio: "It is ready to be seeded with cells."

Figure 23.11. Animation storyboards 21-22.

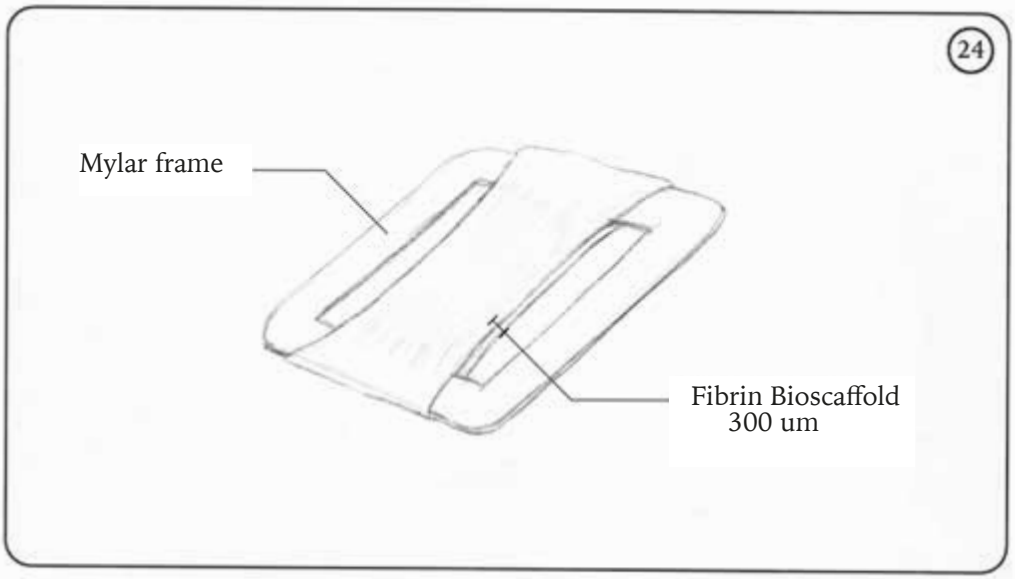


23. Visuals: Arrow alters viewer to icon at "here".

Audio: "Click 'here' to learn more about why fibrin is used as a bioscaffold material."
Interactivity: Why use a Fibrin Bioscaffold?

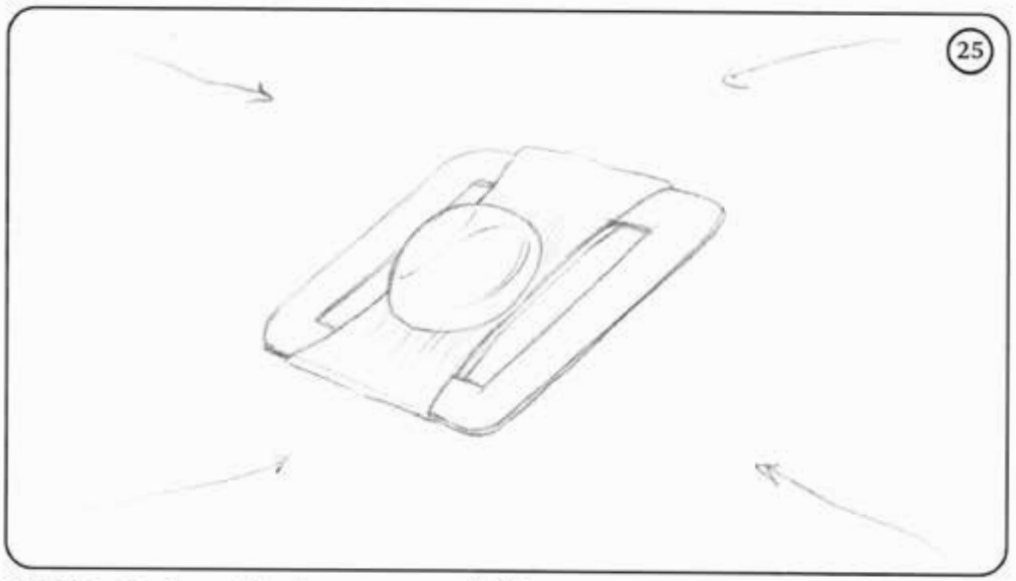
Figure 23.12. Animation storyboard 23.

Scene 3. Growing Cardiomyocytes



24. Visuals: Label mylar frame and fibrin bioscaffold. Fibrin bioscaffold turn to side when size is mention, brackets indicate which dimension is being referred to.

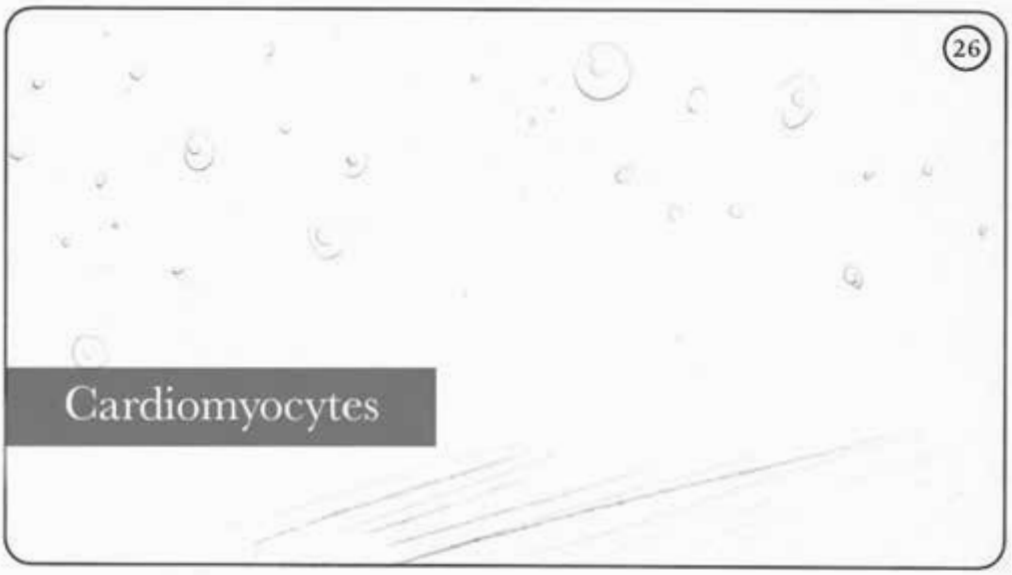
Audio: "To create a cardiac patch, various cell types are seeded on to a fibrin bioscaffold which is wrapped around a mylar frame and approximately 300 μm thick."



25. Visuals: Round droplet appears on fibrin.

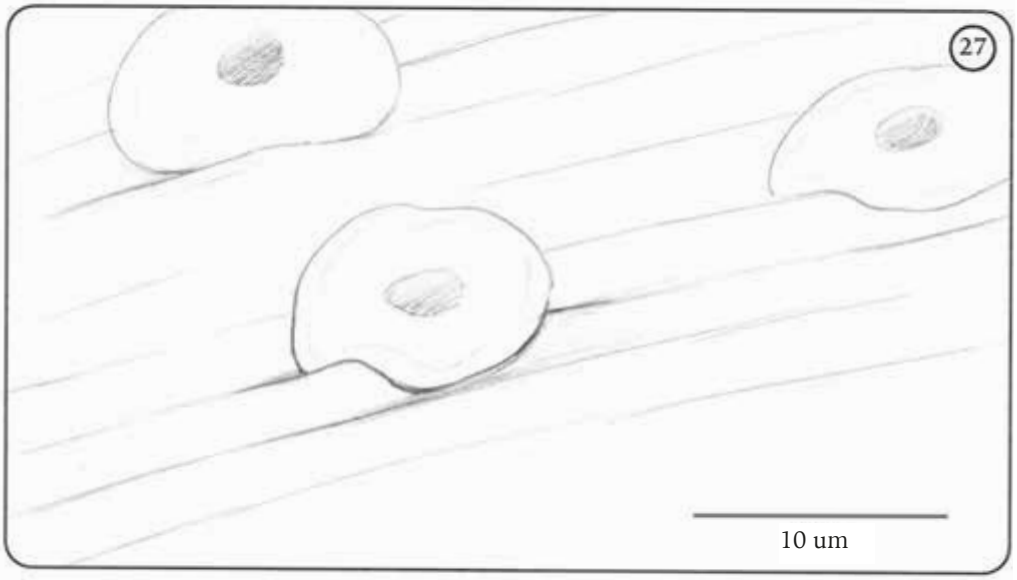
Audio: "A droplet of solution is placed on the fibrin."

Figure 23.13. Animation storyboards 24-25.



26. Visuals: Power zoom into droplet contents. See many cardiomyocytes slowly drifting downwards. Fibrin sheet below. Scale bar in corner.

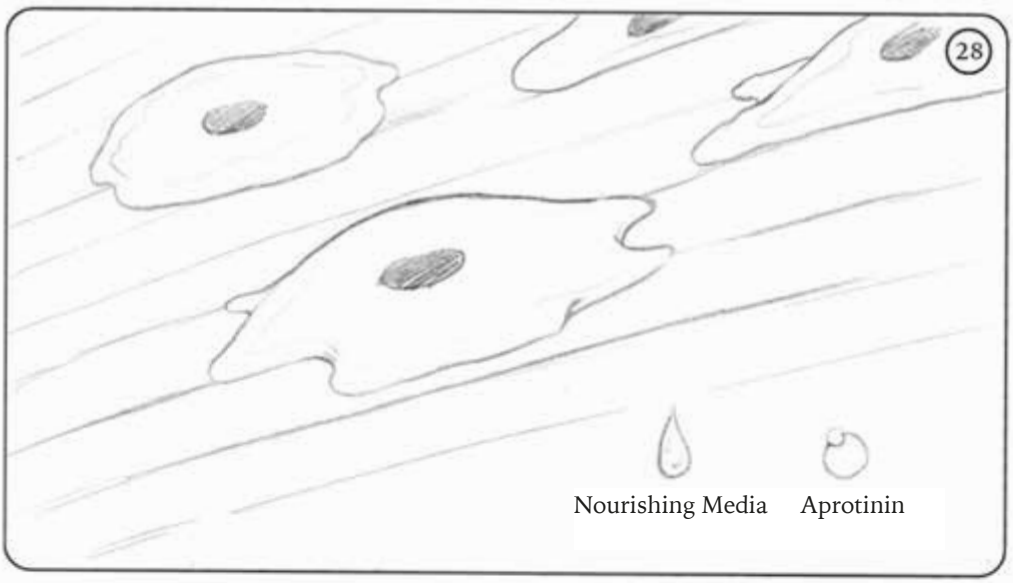
Audio: "The solution contains cardiomyocytes, which..."



27. Visuals: Cut to fibrin bioscaffold, one cardiomyocyte gently lands on scaffold. Scale bar adjusts.

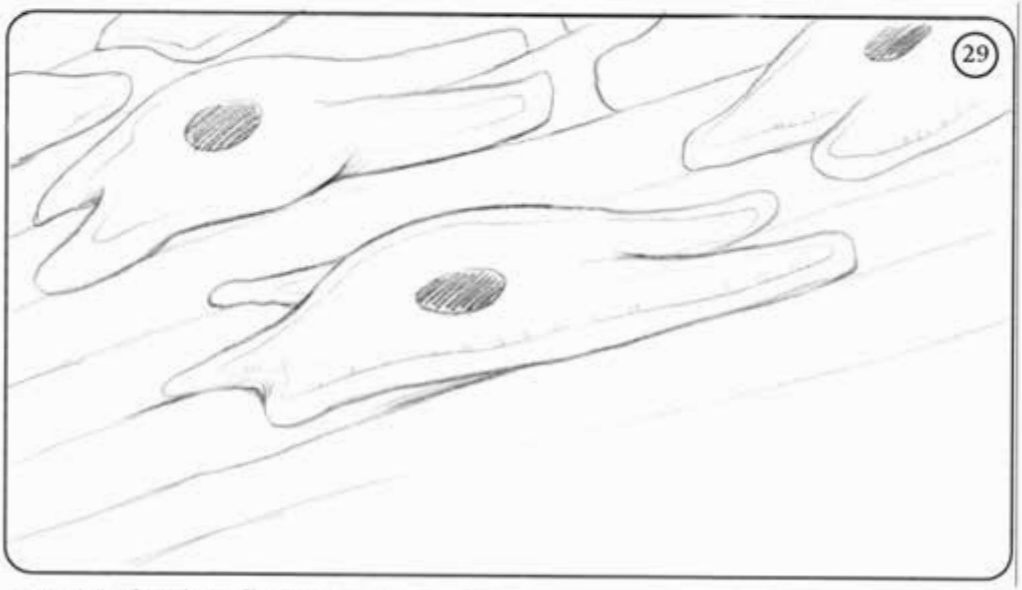
Audio: "...attach to the fibrin bioscaffold."

Figure 23.14. Animation storyboards 26-27.



28. Visuals: More cells adhere to fibrin bioscaffold. "Nourishing media" and "Aprotinin" appear as spoken. Wave for diffuse non-specific particles or fluid wash over.

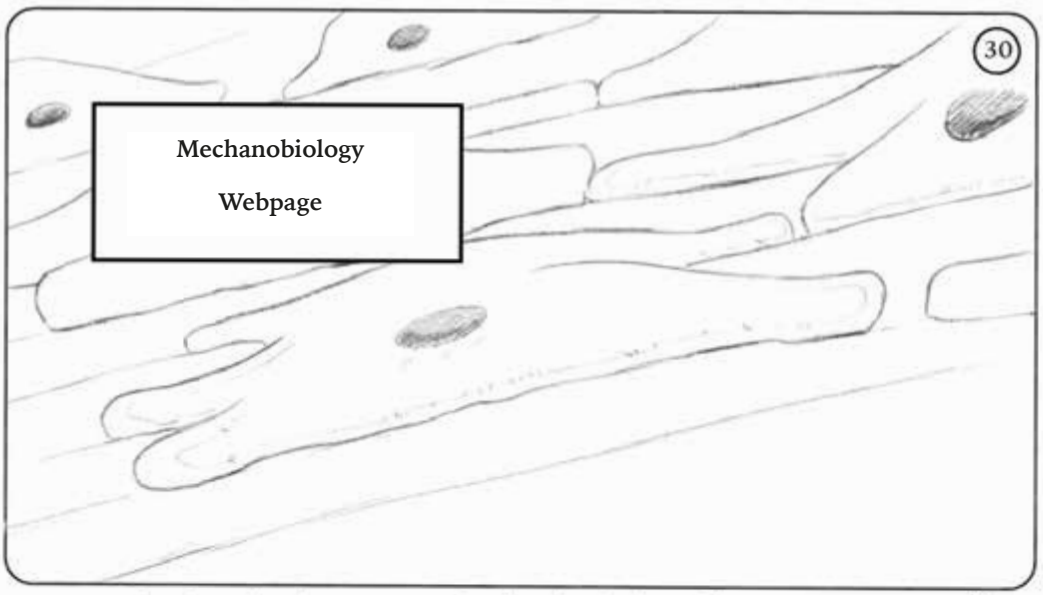
Audio: "The cells are immersed in solution that contains nourishing media and Aprotinin, which inhibits break down of the fibrin bioscaffold."



29. Visuals: The cells flatten into the fibrin bioscaffold.

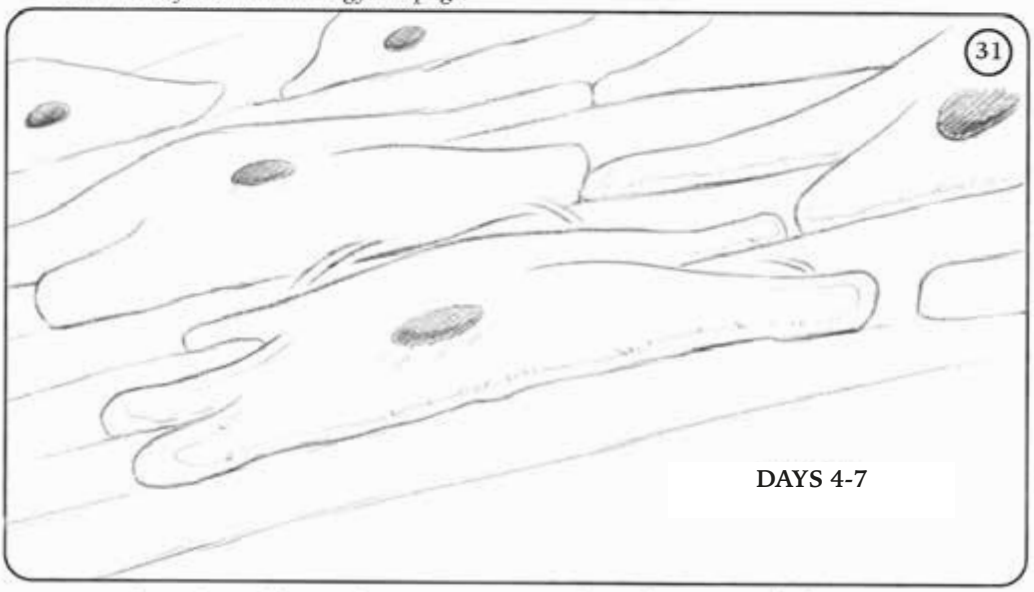
Audio: "The solution is refreshed every other day as the cardiomyocytes elongate..."

Figure 23.15. Animation storyboards 28-29.



30. Visuals: The cells elongate. Branched ends of other cells come into view, to fill onscreen gaps.

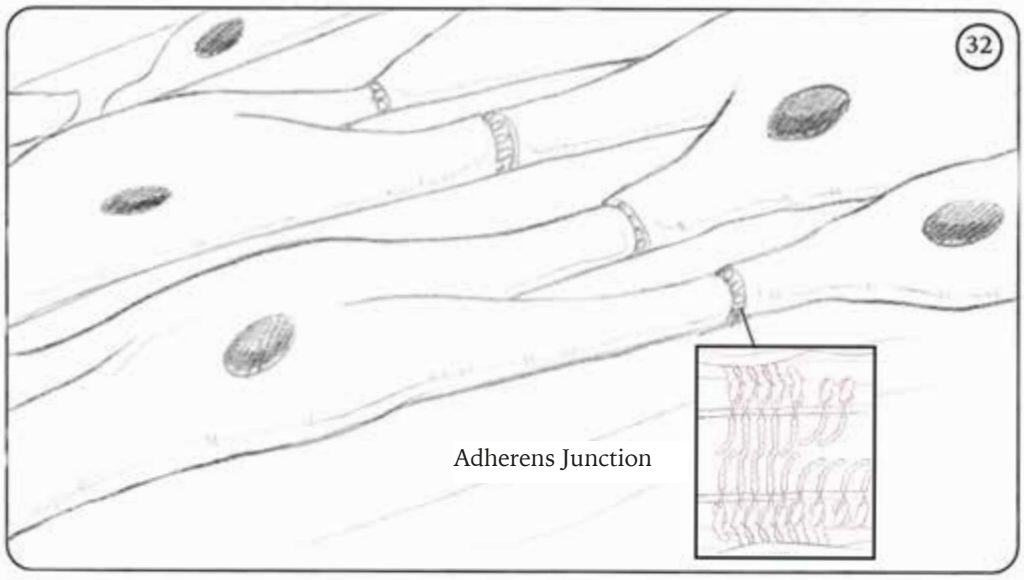
Audio: "...guided by the parallel alignment of the fibrin bioscaffold. Click to learn more about how bioscaffolds influence cell growth."
Interactivity: Mechanobiology webpage



31. Visuals: A few cells are shown contracting for a short period of time.

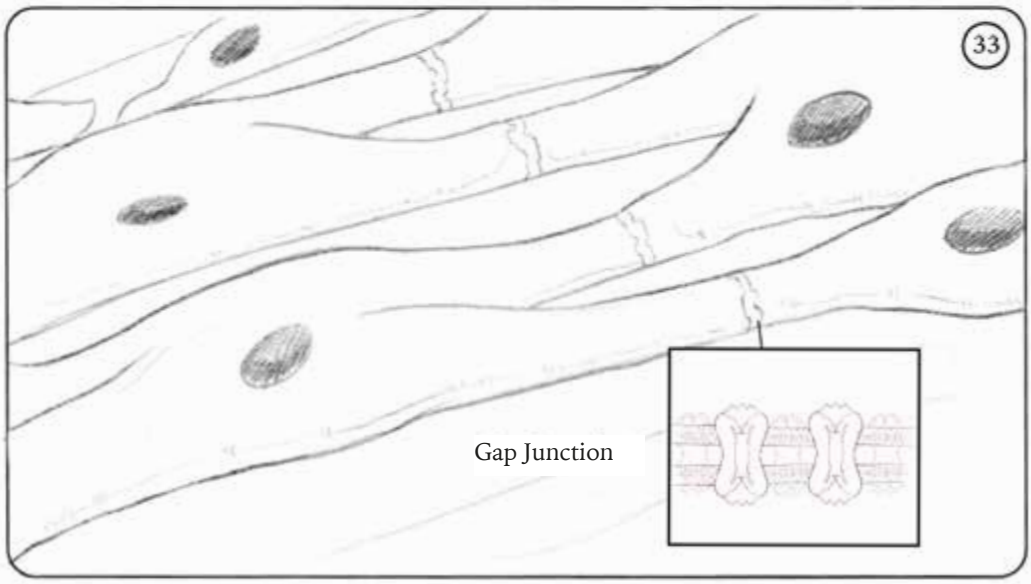
Audio: "Between days 4 to 7, the cardiomyocytes begin to spontaneously contract."

Figure 23.16. Animation storyboards 30-31.



32. Visuals: Cell ends begin to join, glow as ends join Slight camera shift to bring ends more into center of view.

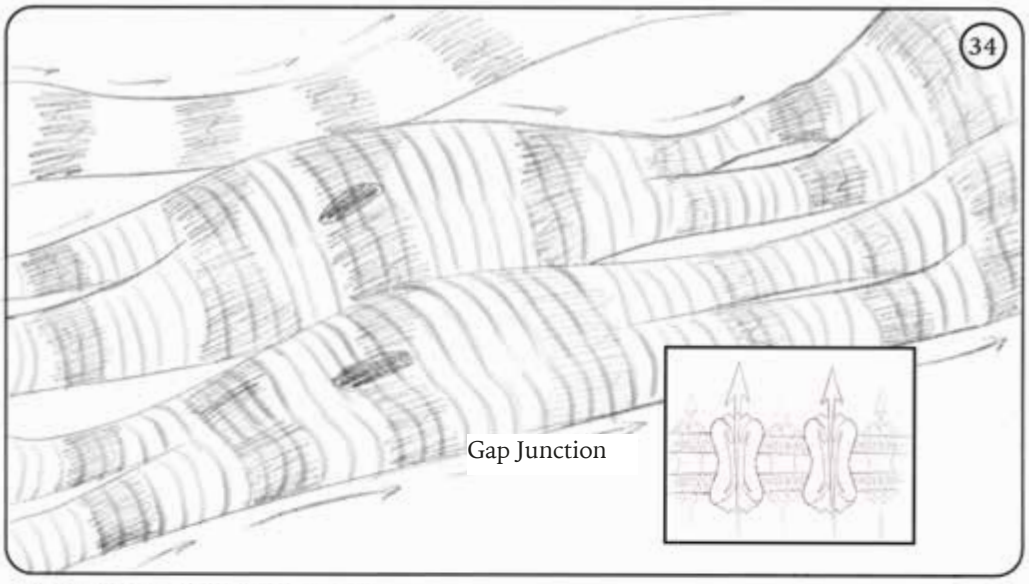
Audio: "Adherens junctions bind the ends of growing cardiomyocytes together, which become intercalated discs."



33. Visuals: Cells contract together; end plates thicken (intercalated disc labeled), branches become more round and robust.

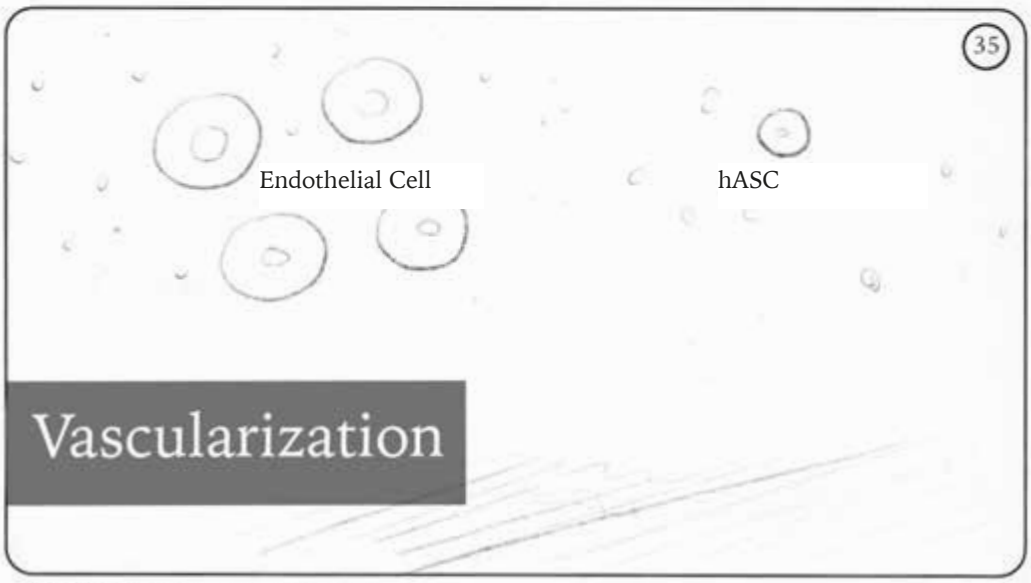
Audio: "Gap junctions at the intercalated discs..."

Figure 23.17. Animation storyboards 32-33.



34. Visuals: Camera shifts back to see cells, screen darkens, band of light travels through cells, illuminates all from the inside in waves, indicates cells contracting together.

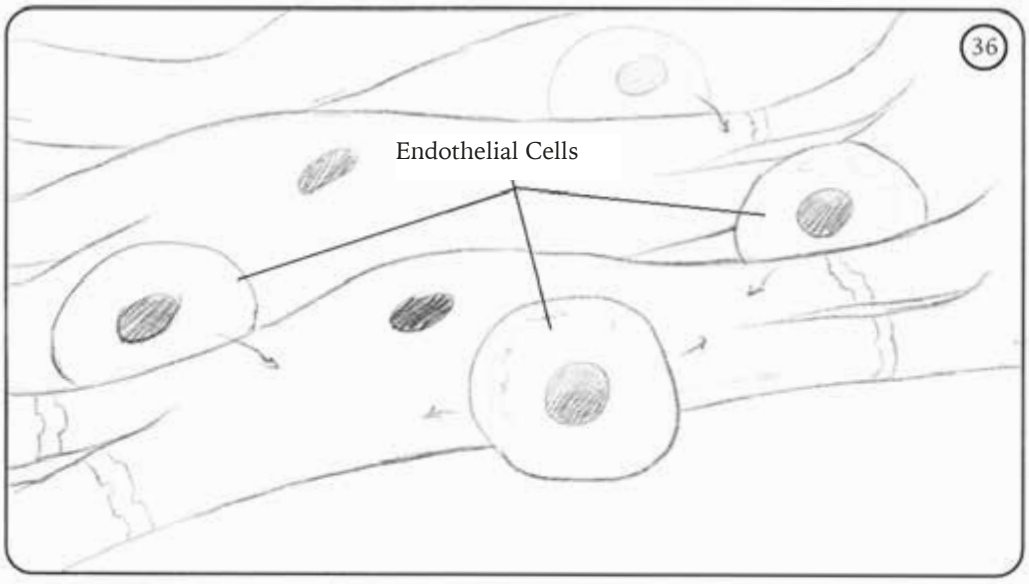
Audio: "... allowing the cardiomyocytes to become electrically synchronized."



35. Visuals: Cut to see new cells (in 4:1 ratio) suspended in media (like the cardiomyocytes). Cells are labeled.

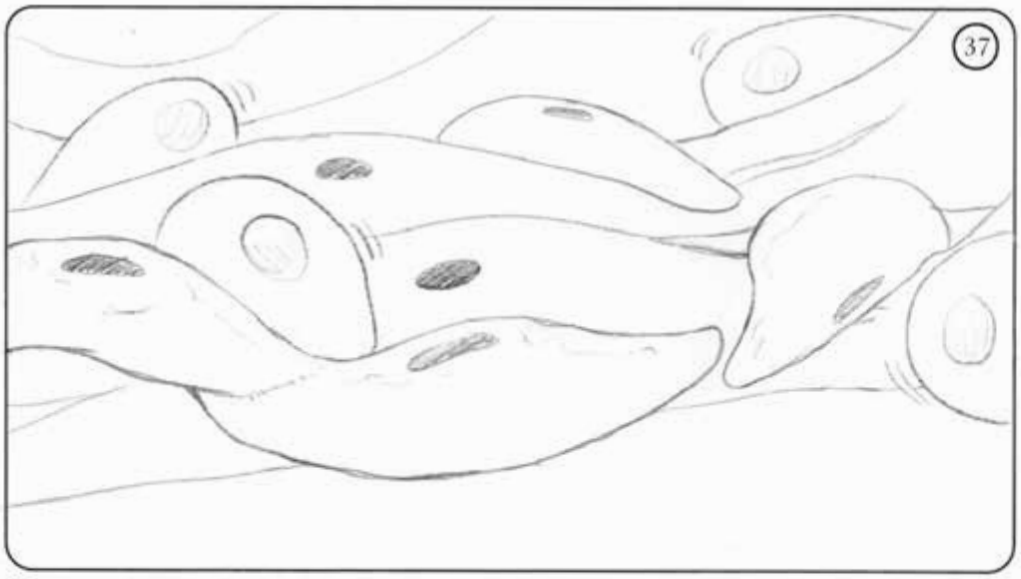
Audio: "After 14 days, a four to one ratio of endothelial cells and human adipose derived stem cells, or hASCs, are seeded onto the cardiomyocytes to start the growth of the blood vessels."

Figure 23.18. Animation storyboards 34-35.



36. Visuals: Cut to endothelial cells land on cardiomyocyte layer. Label endothelial cells.

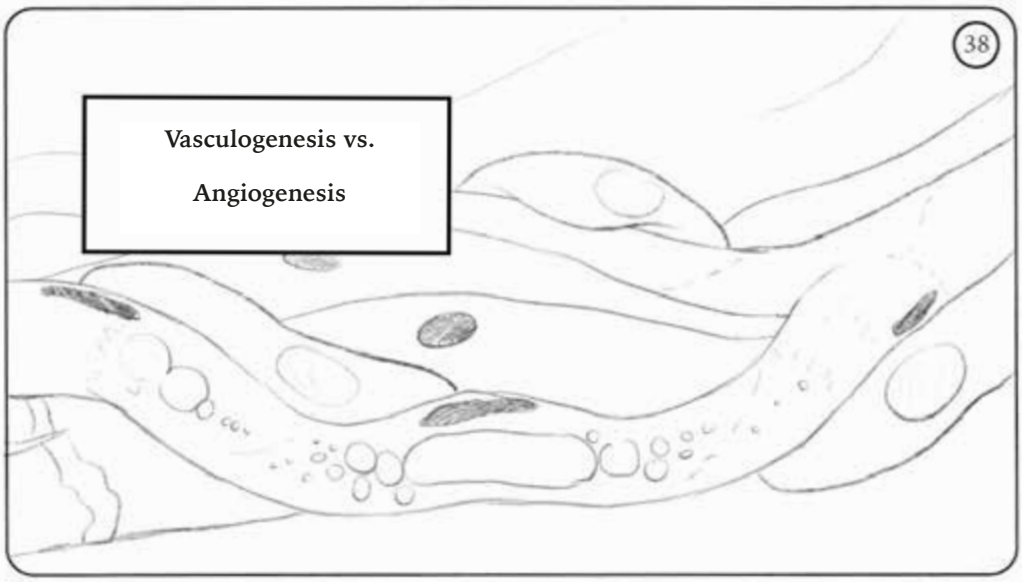
Audio: "Refreshed every other day with a composite of stem cell and endothelial growth media, the endothelial cells elongate..."



37. Visuals: Three endothelial cells elongate and join end to end, forming vessel cords.

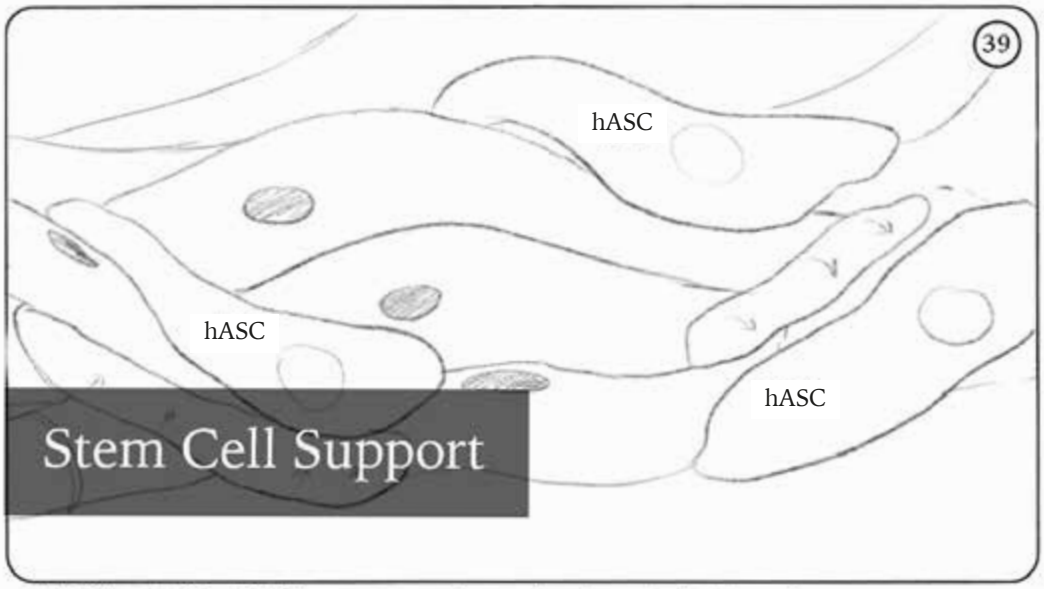
Audio: "...and undergo vasculogenesis to form new blood vessels."

Figure 23.19. Animation storyboards 36-37.



38. Visuals: Front most cord becomes transparent, vacuoles form inside and merge into a lumen.

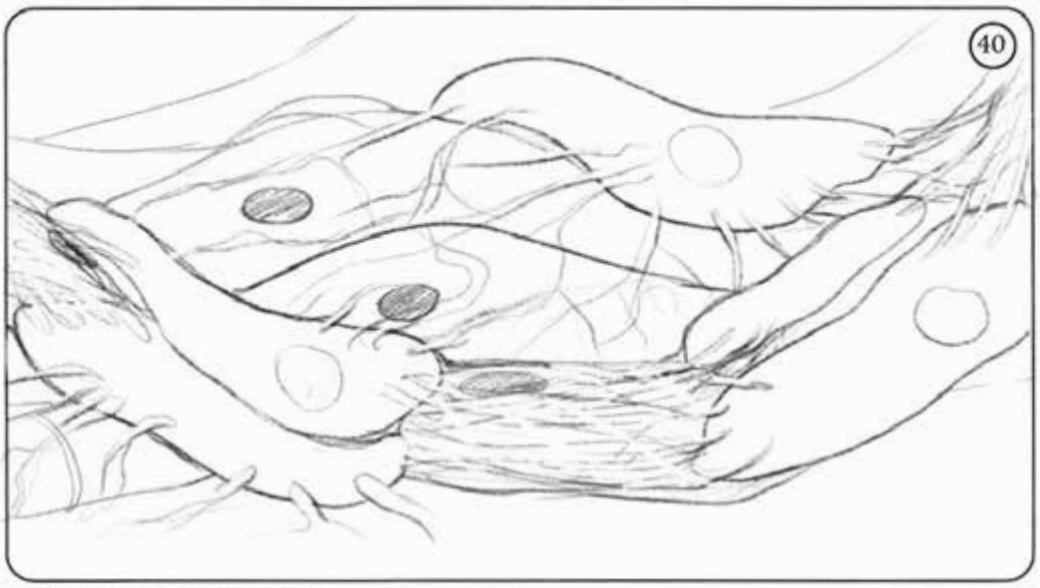
Audio: "The cells elongate into multicellular cords. Intercellular vacuoles unify to create a hollow lumen. Click here to find out more about Vasculogenesis."
Interactivity: The difference between Angiogenesis vs. Vasculogenesis



39. Visuals: The hASCs wrap around vessels, elongate. Label again.

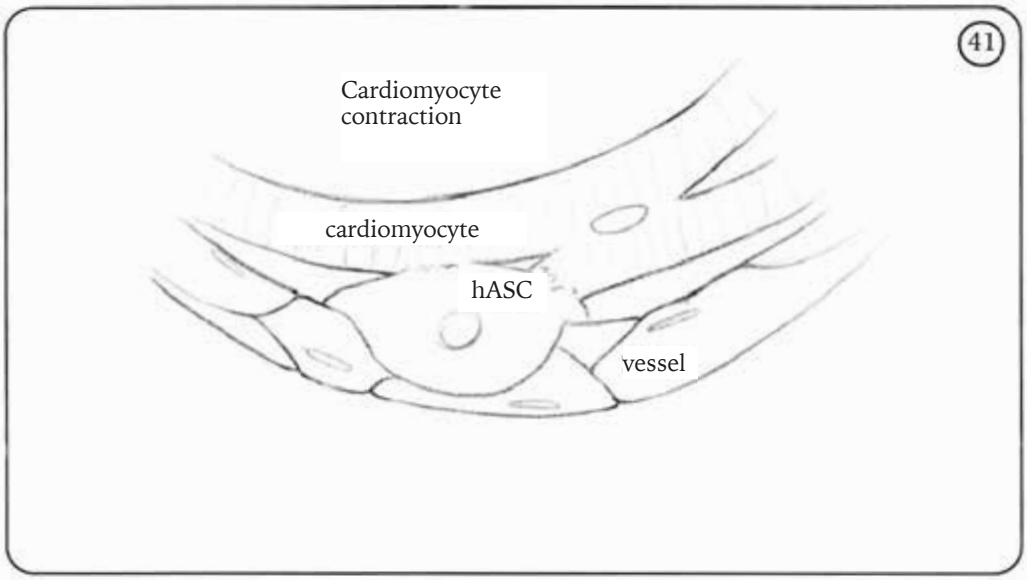
Audio: "The hASCs are mesenchymal stem cells. They can mimic perivascular cells by stabilizing the endothelial vessels."

Figure 23.20. Animation storyboards 38-39.



40. Visuals: The hASCs elongate, secrete matrix over vessels and cardiomyocytes.

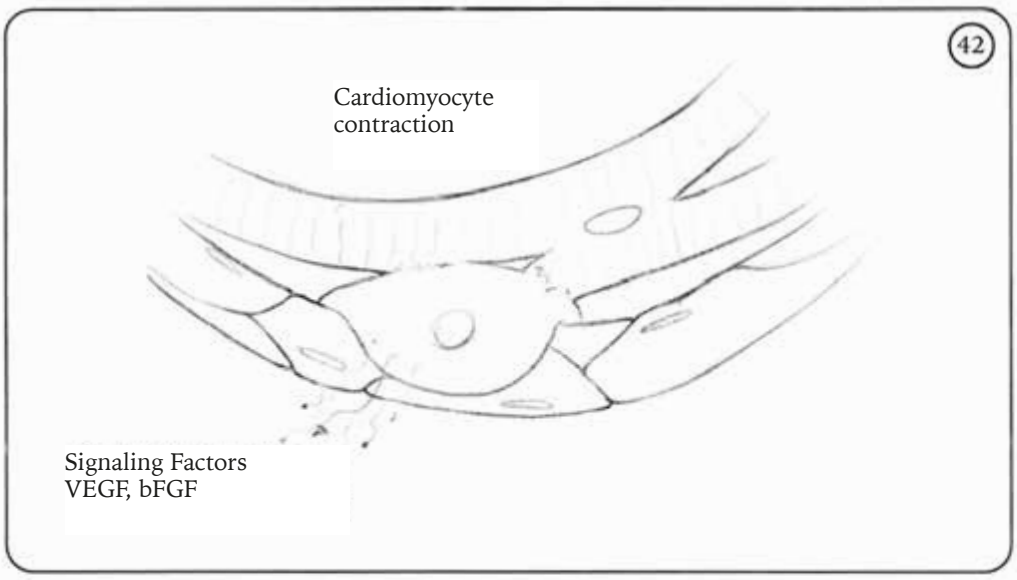
Audio: "The hASCs also mimic fibroblast cells by secreting an extracellular matrix that envelopes the endothelial vessels and cardiomyocytes."



41. Visuals: Overlay, graphic representation of cells, no details. Cardiomyocyte layer, yellow hASC integrated and contracting with cardiomyocytes.

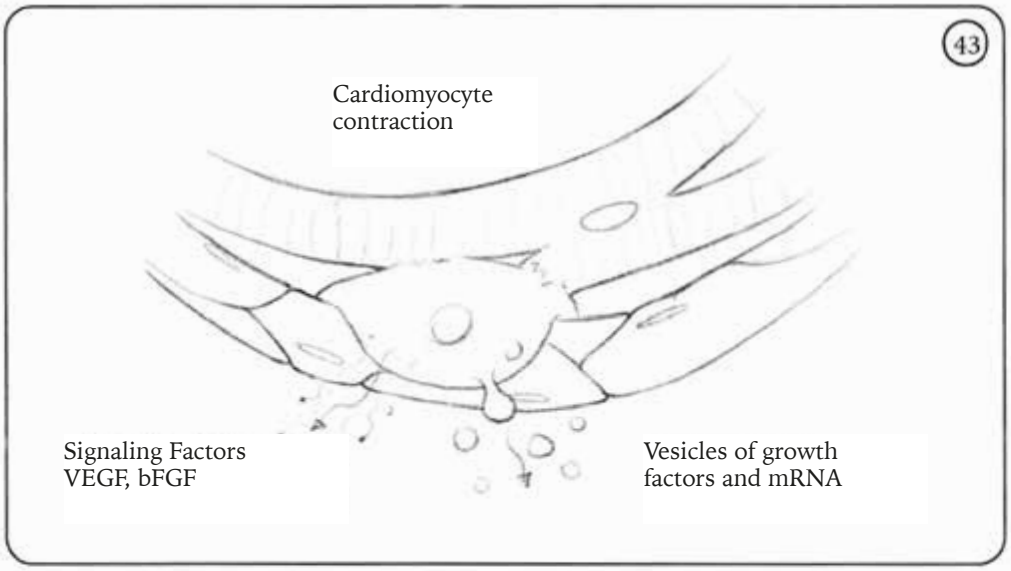
Audio: "New research results suggest that hASCs may form gap junctions with cardiomyocytes and contribute to contraction."

Figure 23.21. Animation storyboards 40-41.



42. Visuals: New graphic, yellow hASC secretes particles, labeled as read.

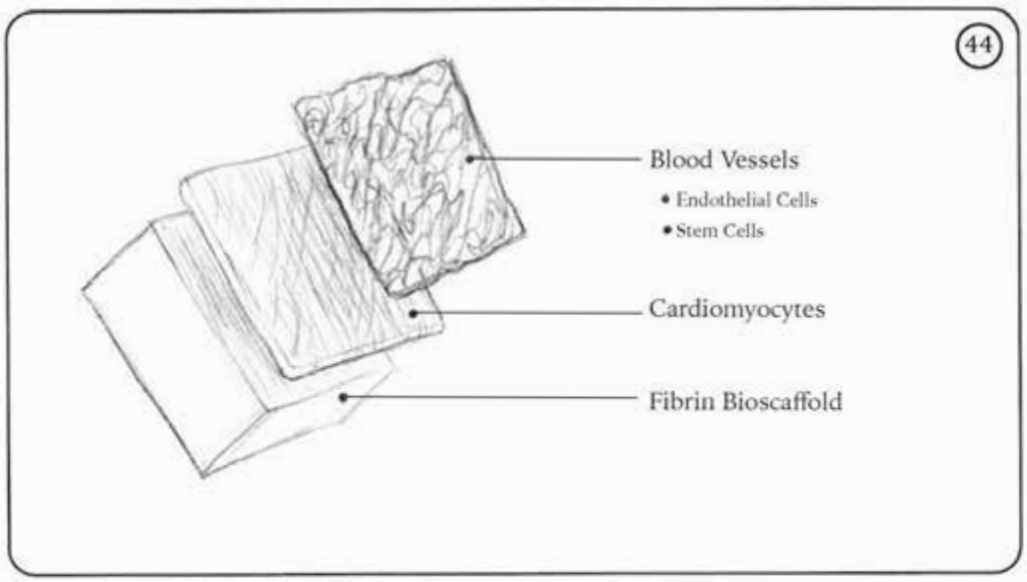
Audio: "hASCs may also promote angiogenesis by secreting paracrine signaling factors such as VEGF, FGF, and bFGF."



43. Visuals: New graphic, yellow hASC membrane buds off into small round vesicles, labeled as read.

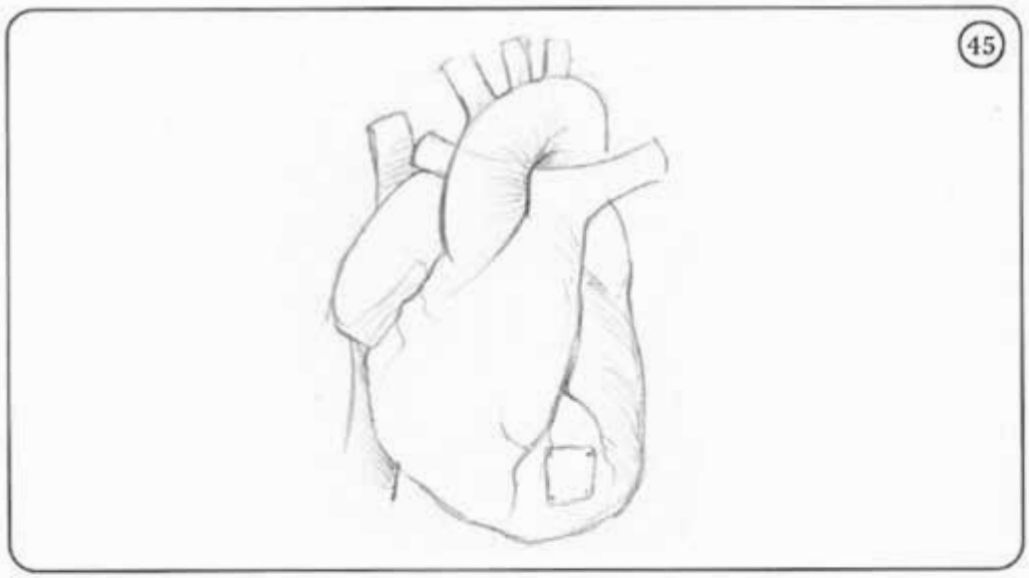
Audio: "They also secrete exosomes, or vesicles filled with growth factors and microRNA, the effects of which are still unknown."

Figure 23.22. Animation storyboards 42-43.



44. Visuals: Show three main components, labeled.

Audio: "Each of these cell types and processes impact the assembly and function of the cardiac patch as well as its ability to integrate with the native heart."

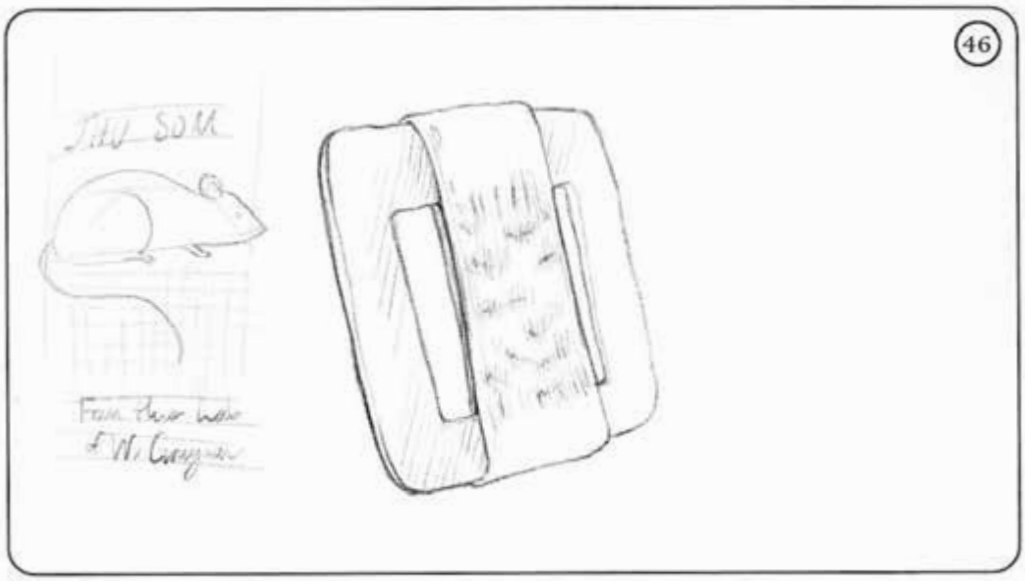


45. Visuals: Cut to heart with patch applied.

Audio: "A functional vascularized cardiac patch can support heart function after myocardial infarction by repopulating the damaged area with new cells."

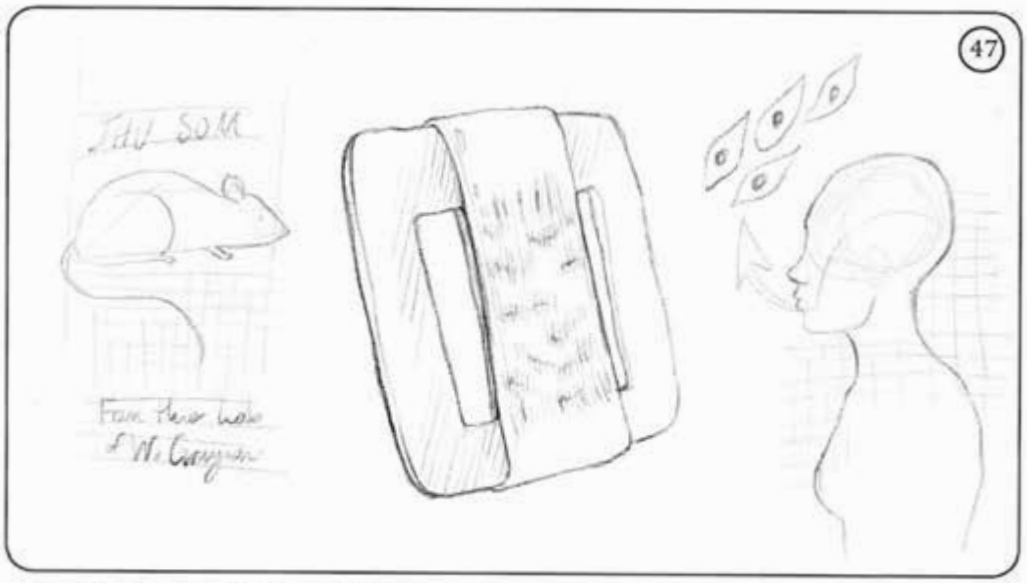
Figure 23.23. Animation storyboards 44-45.

Scene 5. Closing Research Disclaimer



46. Visuals: Background switches to white grid on blue background (architect paper), rat is 'drawn' in sketchy fashion.

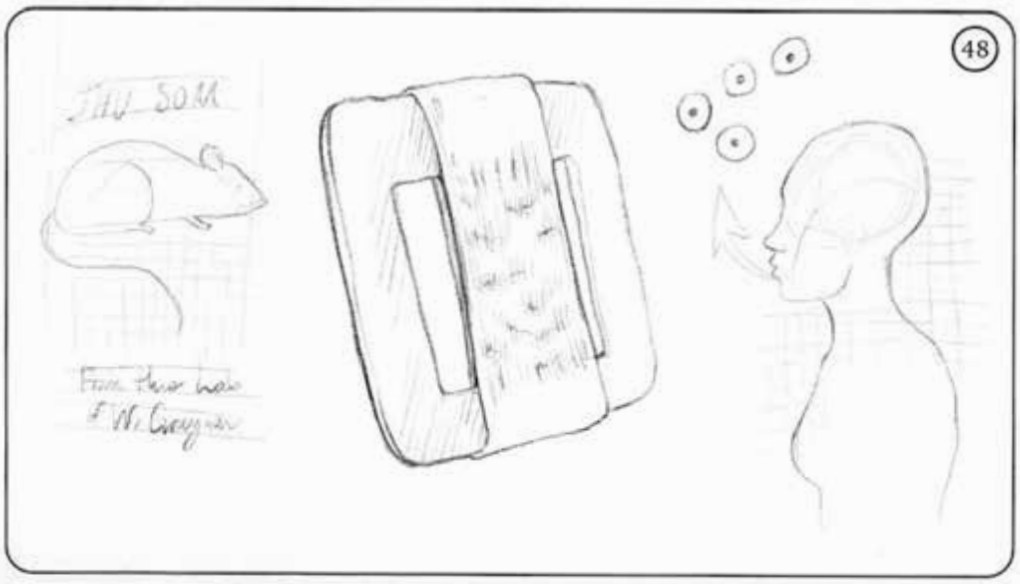
Audio: "The research presented in this animation is based on data collected from animal models."



47. Visuals: Human outline is 'drawn' in sketchy fashion, and then differentiated cells 'drawn' nearby.

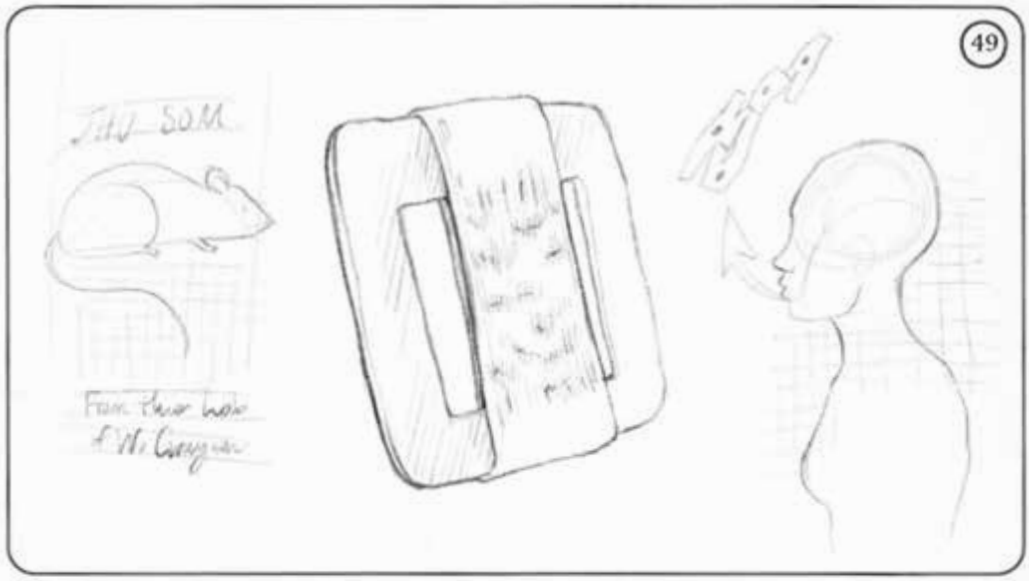
Audio: "In the future, the idea is to harvest some of the patient's own cells..."

Figure 23.24. Animation storyboards 46-47.



48. Visuals: Differentiated cells erased, replaced with blank stem cell type.

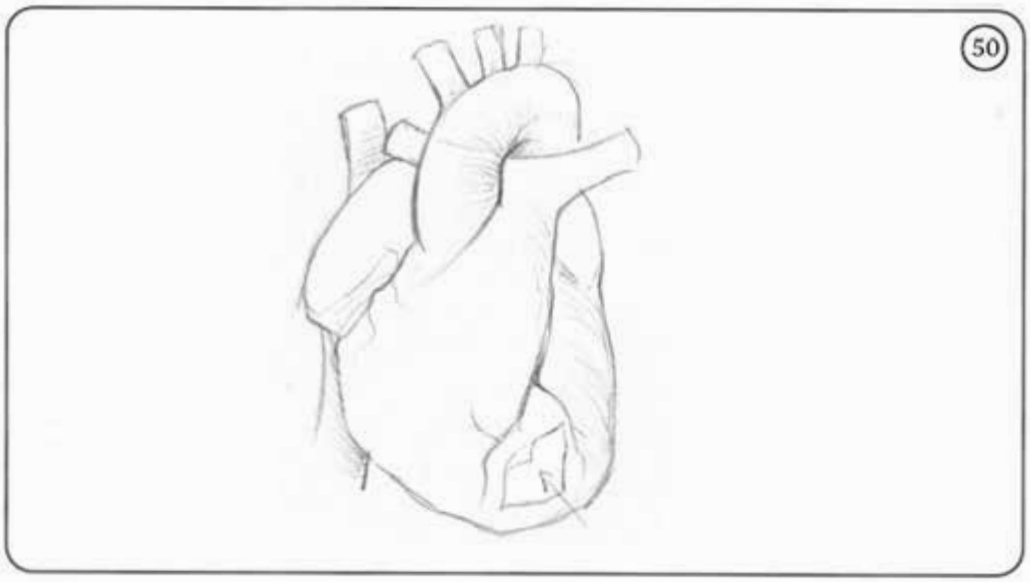
Audio: "...turn them into induced pluripotent stem cells..."



49. Visuals: Blank stem cell type erased, 'drawn' into twitching cardiomyocytes.

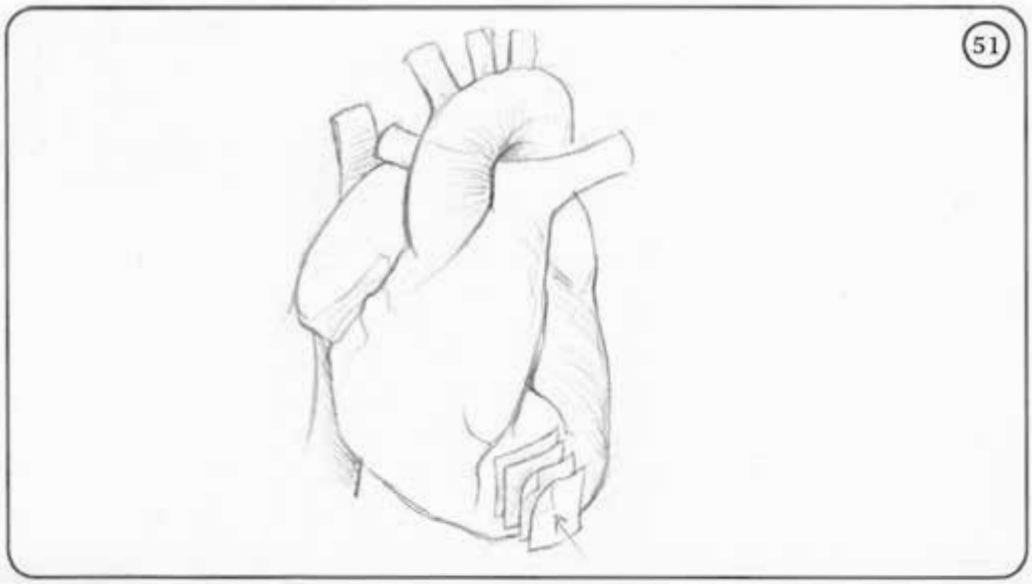
Audio: "...and grow those into functional vascularized cardiac patch."

Figure 23.25. Animation storyboards 48-49.



50. Visuals: Heart drawing replaces cardiac patch in the middle, one small patch flies onto area of infarct...

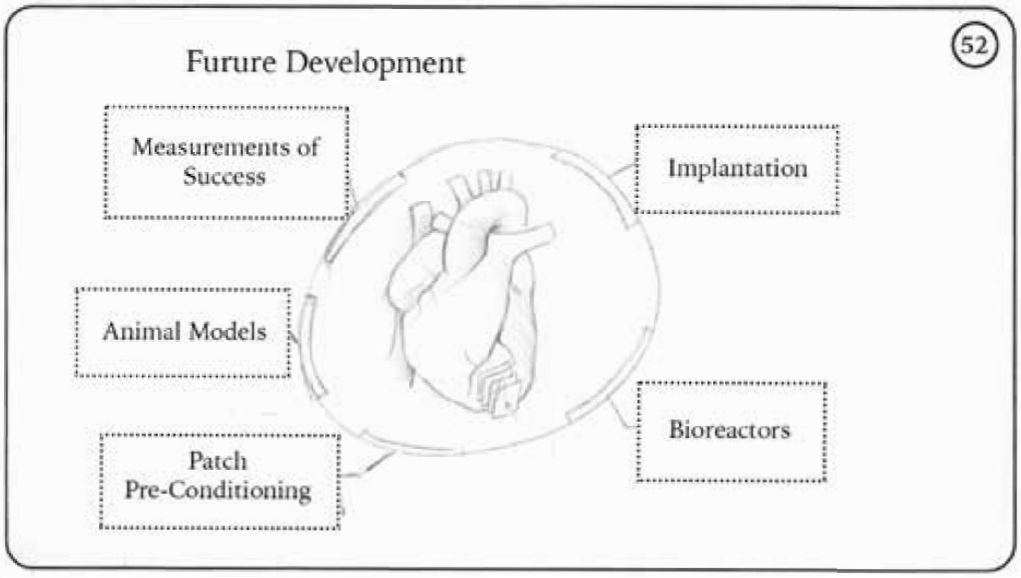
Audio: "Apply the patch..."



51. Visuals: Then many layers fly onto area of infarct.

Audio: "Or perhaps many layers of the patch, to regenerate the tissue that was damaged by myocardial infarction."

Figure 23.26. Animation storyboards 50-51.



52. Visuals: Title appears, circle graphic encircles heart with patch, links to additional ent content pop up, connected to circle.

Audio: "To learn more about tissue engineering of cardiac patches, click any of these buttons to explore the website."

Figure 23.27. Animation storyboard 52.

Interactive Animation

A section of the storyboards was adapted and developed into an interactive animation (**Figure 24.1-25**). The animation shows: (1) theoretical surgical application of the patch on a heart, (2) macroscopic view of the fibrin bioscaffold, (3) seeding and stages of growth of cardiomyocytes, (4) cardiomyocyte intercalated disc formation and electrical conduction, (5) seeding and stages of growth of endothelial cells, (6) seeding and properties of hASCs, (7) additional properties of hASCs, and (8) macroscopic view of the completed patch on the fibrin bioscaffold. The learning module was integrated into a webpage on the final website.

Figure 24.1. Animation still. Stem Cell Orchestra opening title.

Figure 24.2. Animation still. Corresponds to storyboard #24.

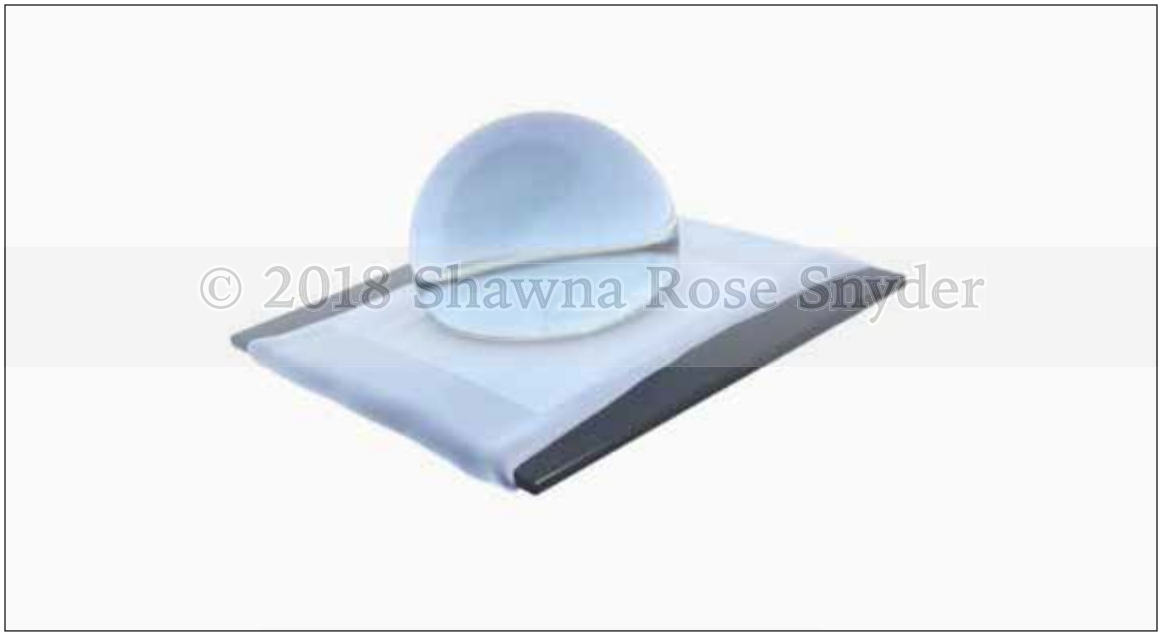


Figure 24.3. Animation still. Corresponds to storyboard #25.

Figure 24.4. Animation still. Corresponds to storyboard #26.

Figure 24.5. Animation still. Corresponds to storyboard #27.

Figure 24.6. Animation still. Corresponds to storyboard #28.

Figure 24.7. Animation still. Use of arrows to emphasize narration.

Figure 24.8. Animation still. Corresponds to storyboard #29.

Figure 24.9 Animation still. Demonstrates interactive link to educational topic, corresponds to storyboard #30. Text not intended to be read.

Figure 24.10. Animation still. Corresponds to storyboard #31.

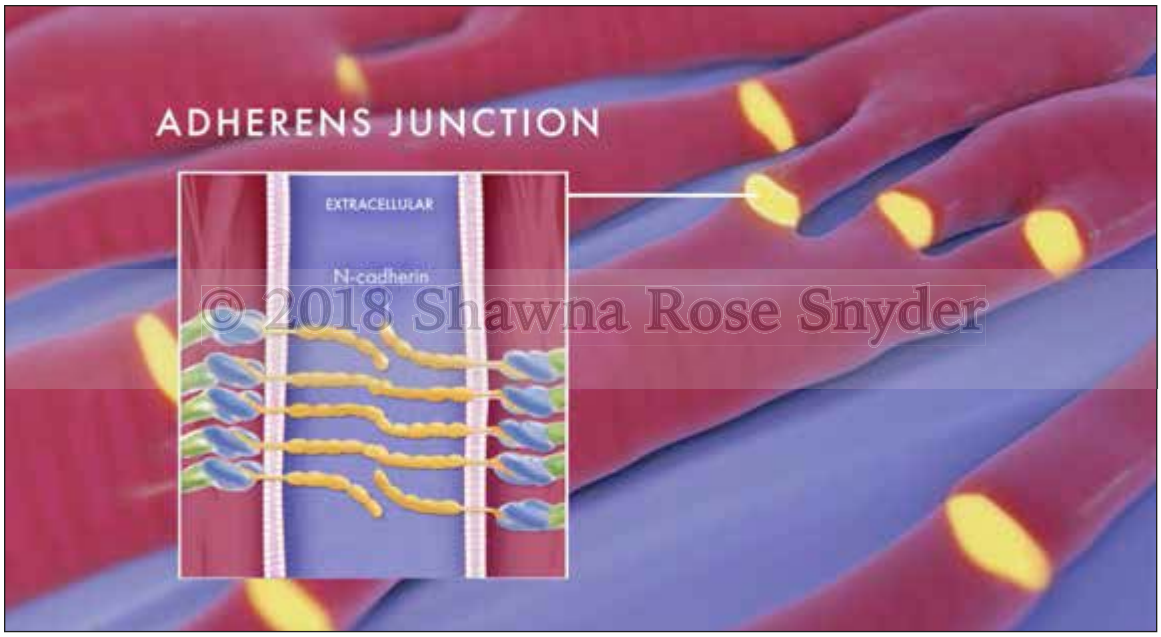


Figure 24.11. Animation still. Corresponds to storyboard #32. Text not intended to be read.

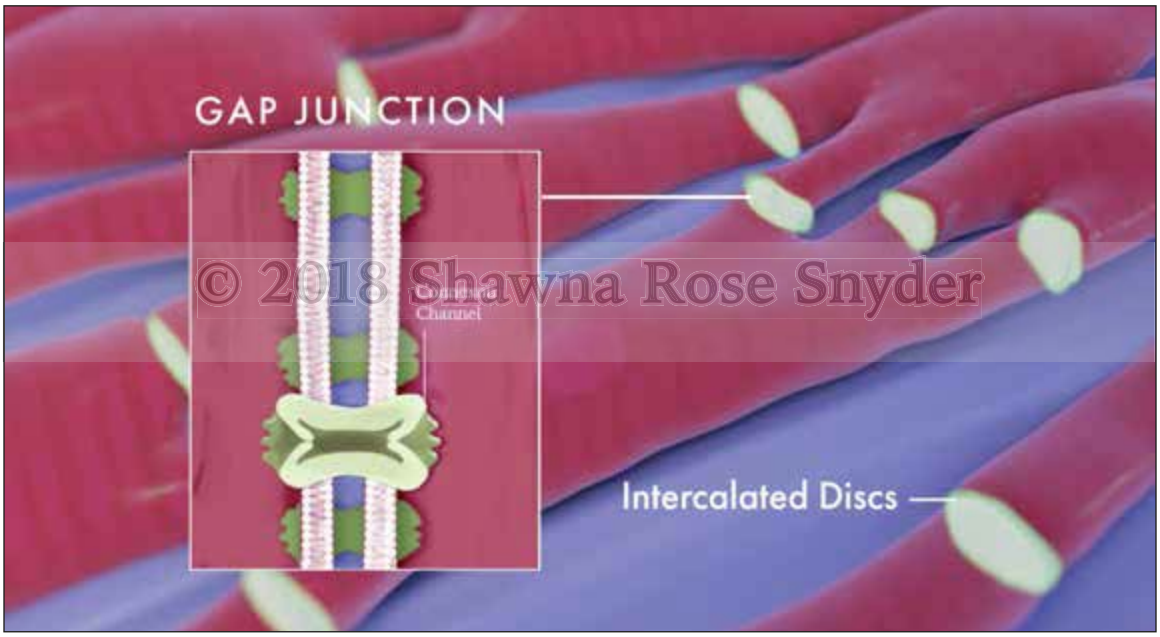


Figure 24.12. Animation still. Corresponds to storyboard #33. Text not intended to be read.

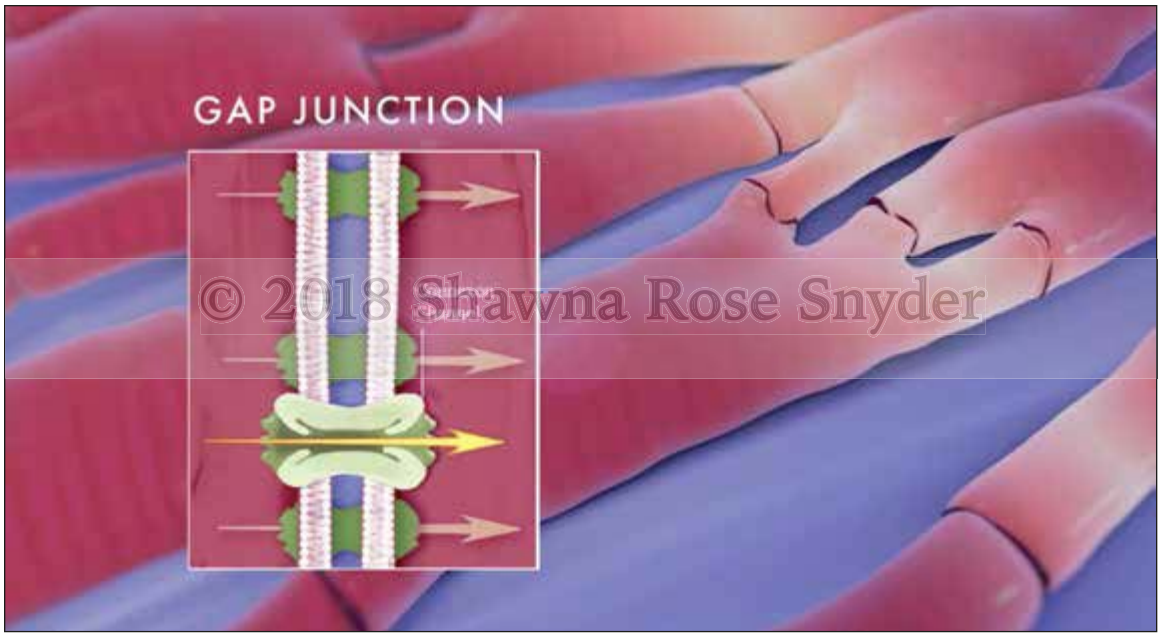


Figure 24.13. Animation still. Corresponds to storyboard #34. Text not intended to be read.

Figure 24.14. Animation still. Corresponds to storyboard #35.

Figure 24.15. Animation still. Corresponds to storyboard #36.

Figure 24.16. Animation still. Corresponds to storyboard #37.

Figure 24.17. Animation still. Corresponds to storyboard #38.

Figure 24.18. Animation still. Corresponds to storyboard #39.

Figure 24.19. Animation still. Corresponds to storyboard #40.

Figure 24.20. Animation still. Corresponds to storyboard #41. Text not intended to be read.

Figure 24.21. Animation still. Demonstrates interactive link, corresponds to storyboard #42.

Figure 24.22. Animation still. Corresponds to storyboard #43.

Figure 24.23. Animation still. Demonstrates interactive link, corresponds to storyboard #44.

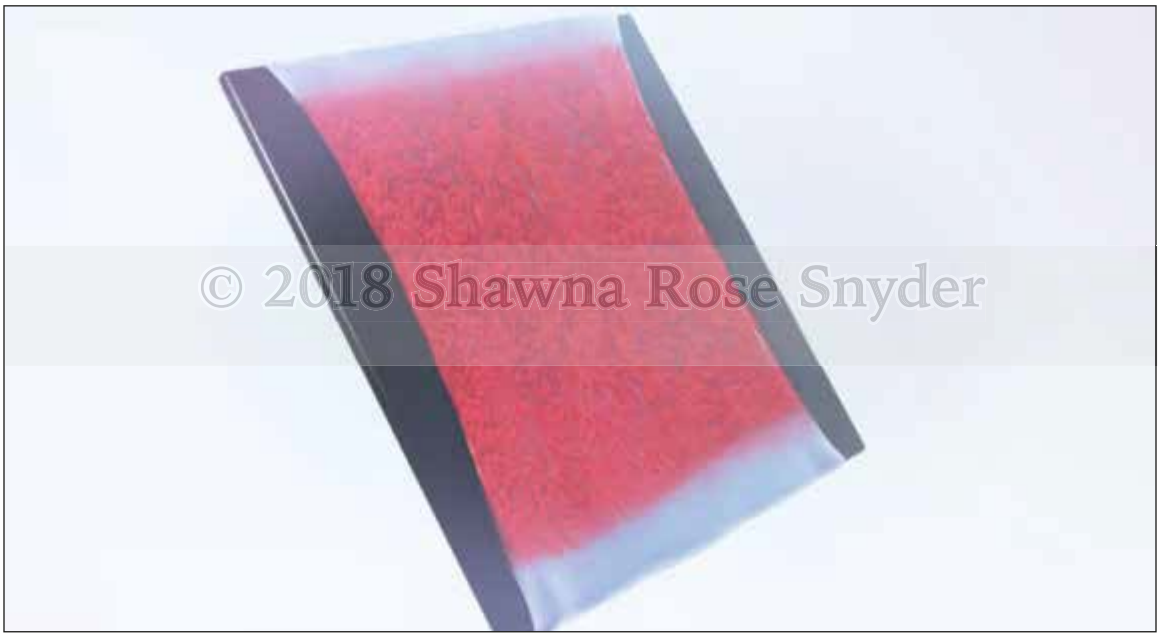


Figure 24.24. Animation still. Macro view of the cardiac patch on Mylar frame.

Figure 24.25. Animation still. Corresponds to storyboard #45.

Mechanotransduction Illustration

A didactic illustration (**Figure 25.1-3**) was created to demonstrate web-based interactivity. The illustration teaches: (1) adult stem cell differentiation (2) a focal adhesion pathway, (3) how substrate stiffness influences tissue engineered cell constructs, and (4) how these concepts are important to cardiac regenerative medicine.

Deployed Interactive Website

The website "Stem Cell Orchestra" was developed and launched as an interactive educational website. A set of icons (**Figure 26)** were developed to represent each topic within the *Heart Muscle* section. Within the *Heart Muscle* section, the module *How to Build a Cardiac Patch* was developed to demonstrate the navigation, user interface, educational benefits, and functionality of the web-based interactive animation. Screenshots of the website and interactive animation are shown (**Figures 27-30**).

Figure 25.1. Mechanotransduction Illustration I. Dashed line indicates end of webpage.

Mechanotransduction Illustration

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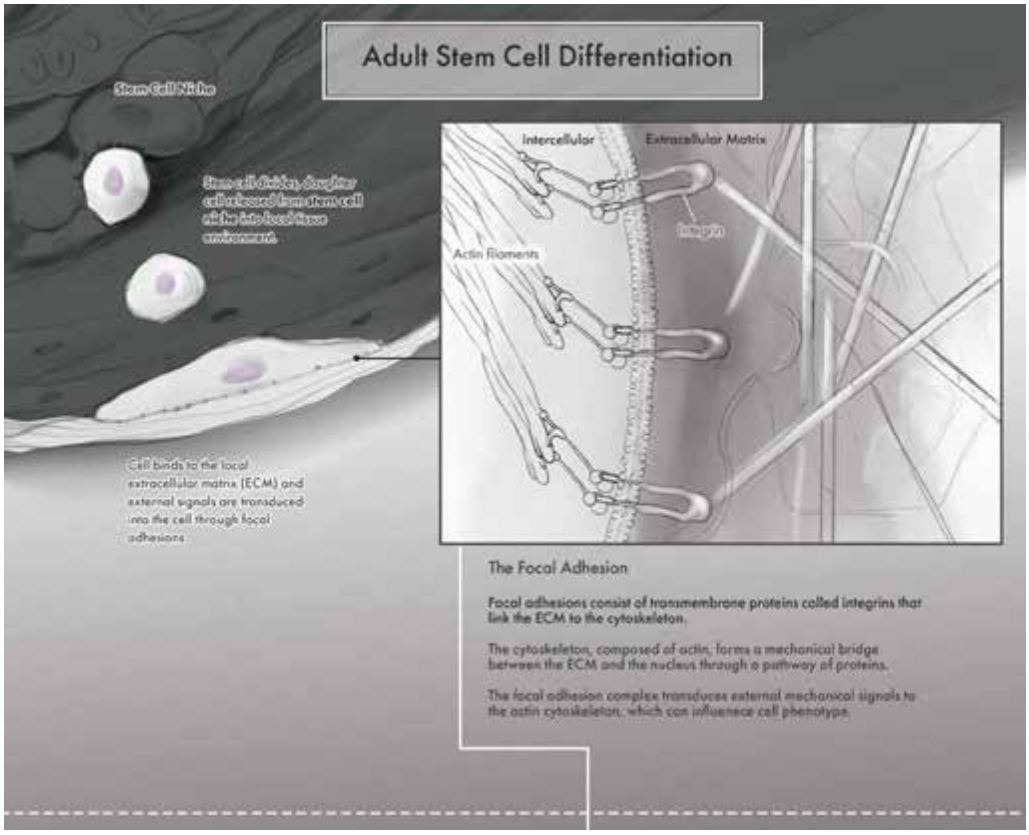


Figure 25.1. Mechanotransduction Illustration I. Dashed line indicates end of webpage.

Figure 25. Mechanotransduction web illustration II-II. Continuation of previous illustration. Text not intended to be read.

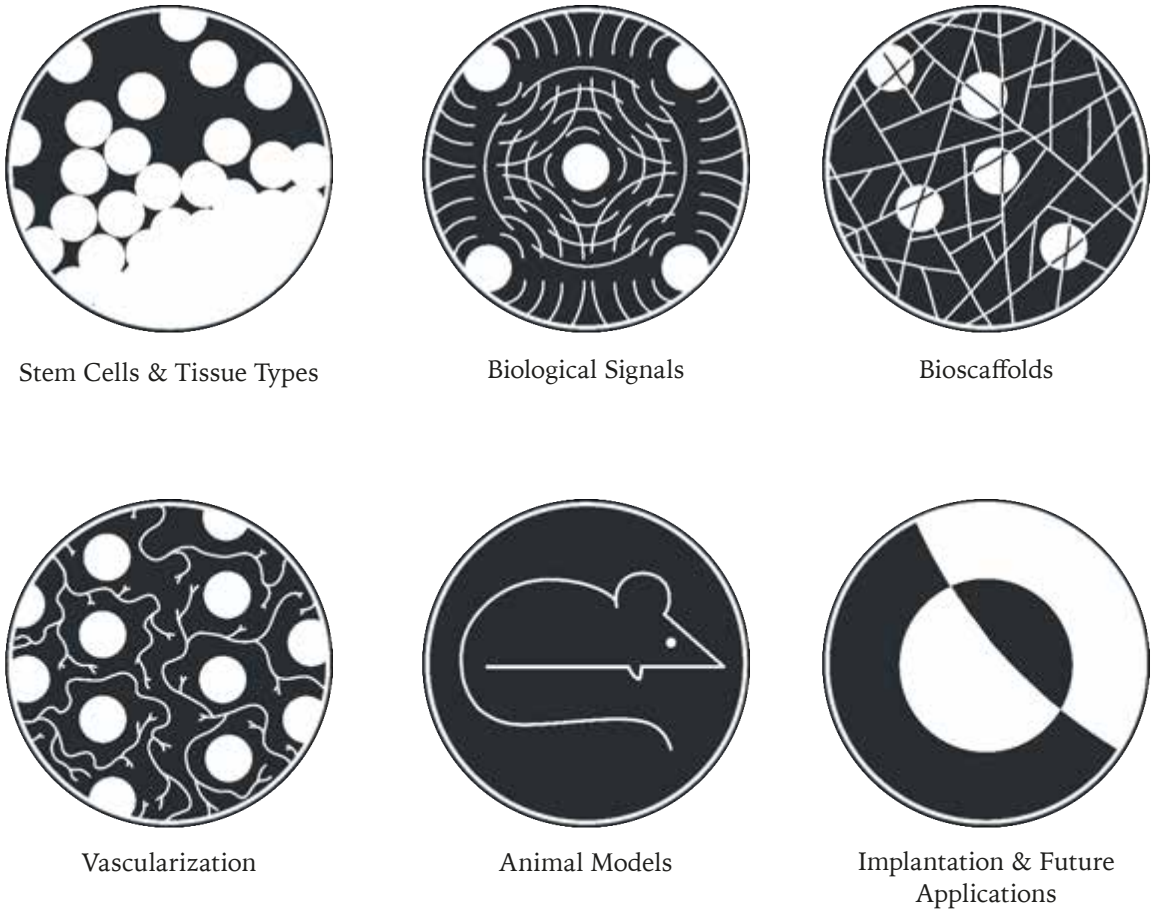


Figure 26. Icons developed for *Heart Muscle* **navigation.**

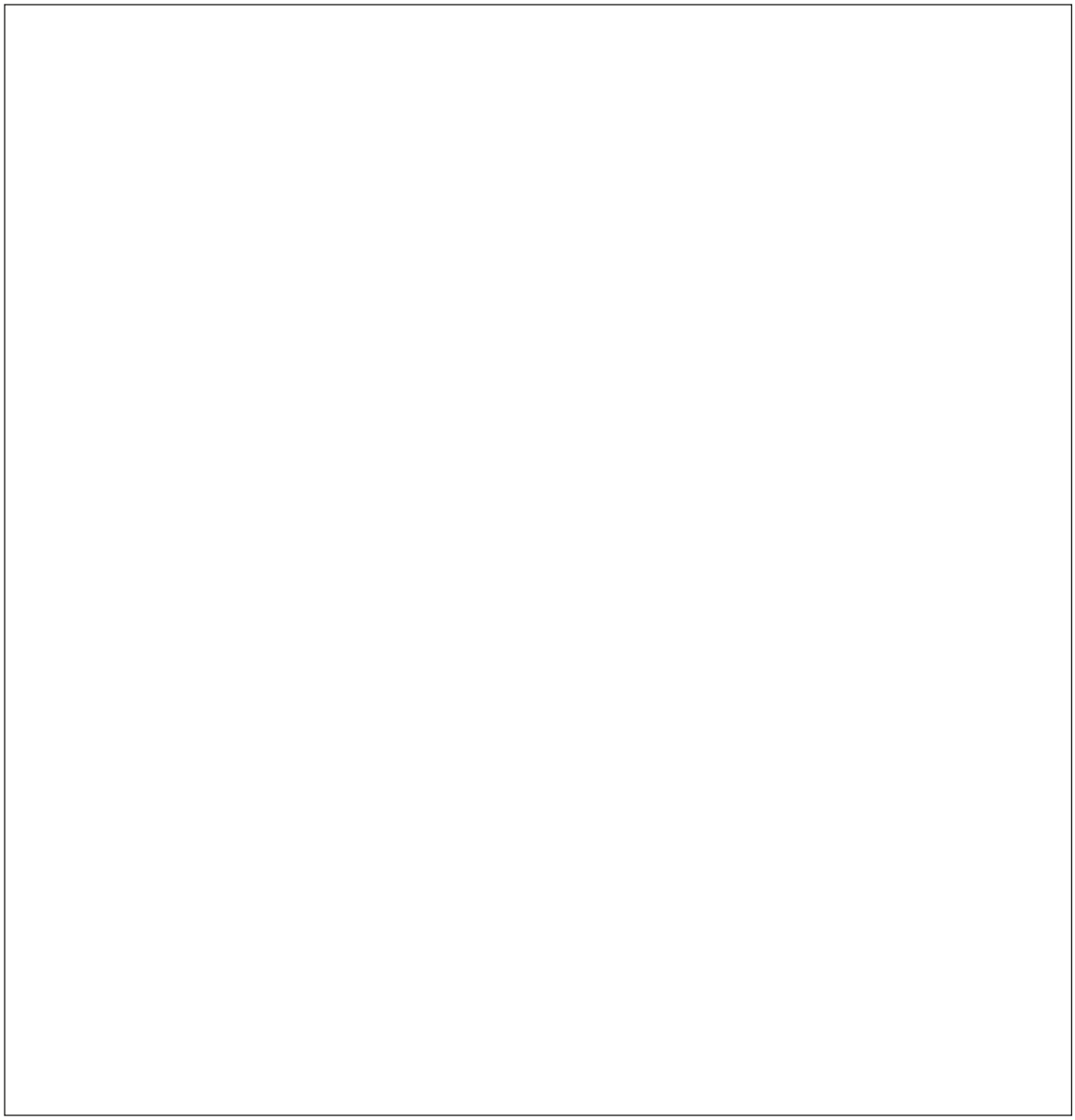


Figure 27. Stem Cell Orchestra homepage. Text not intended to be read.

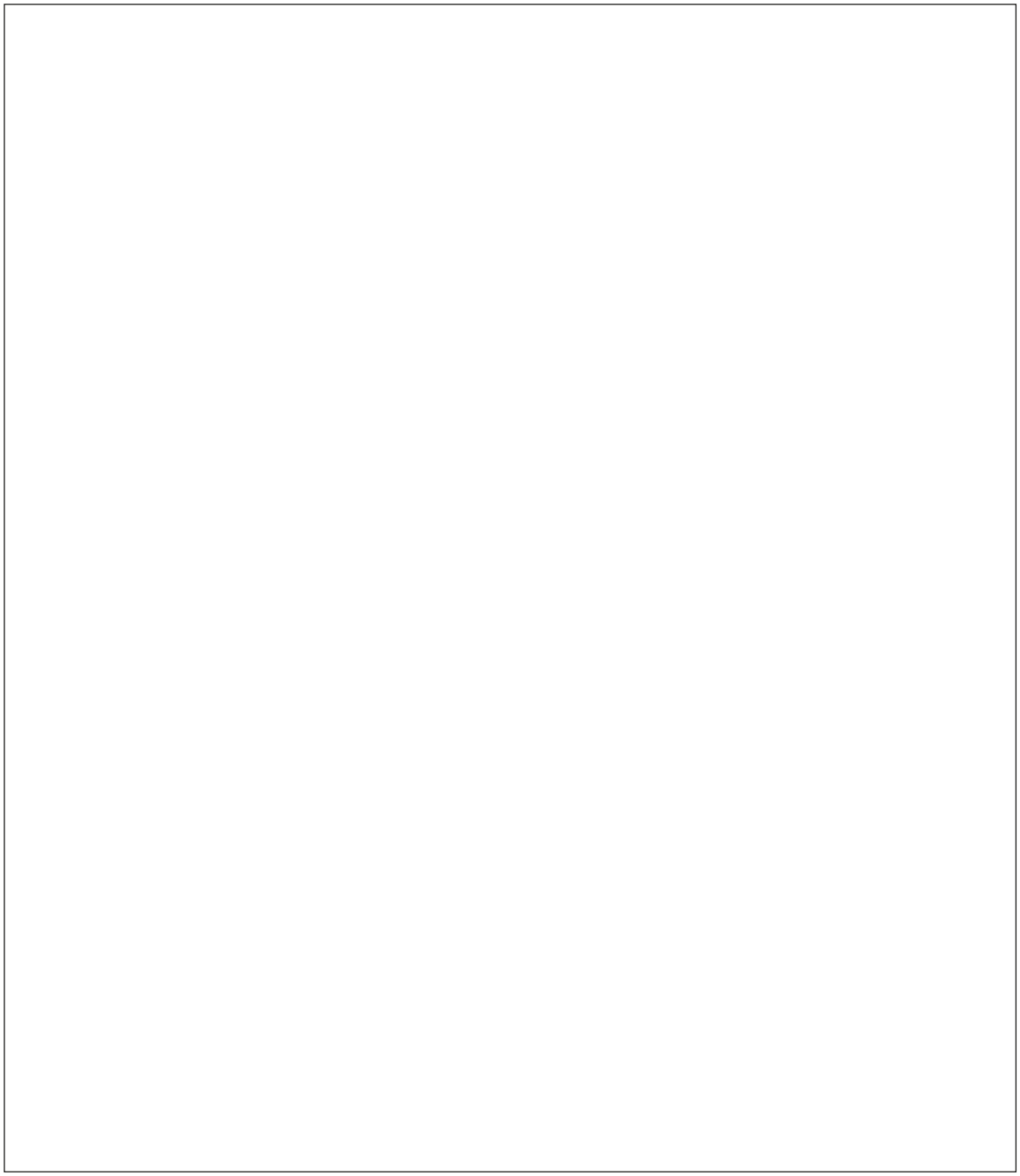


Figure 28. Stem Cell Orchestra website, *Heart Muscle* topic navigation and link to interactive animation. Text not intended to be read.

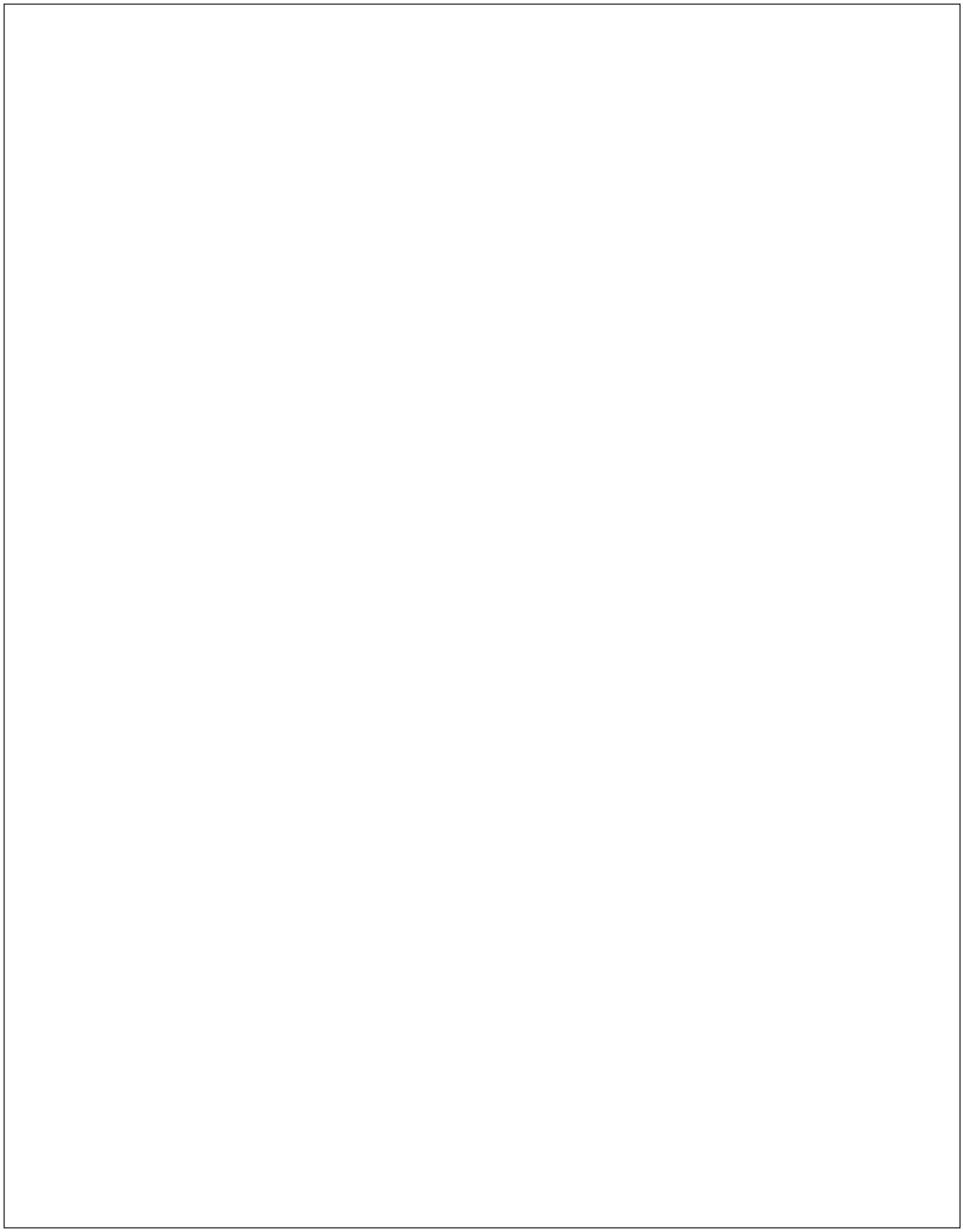
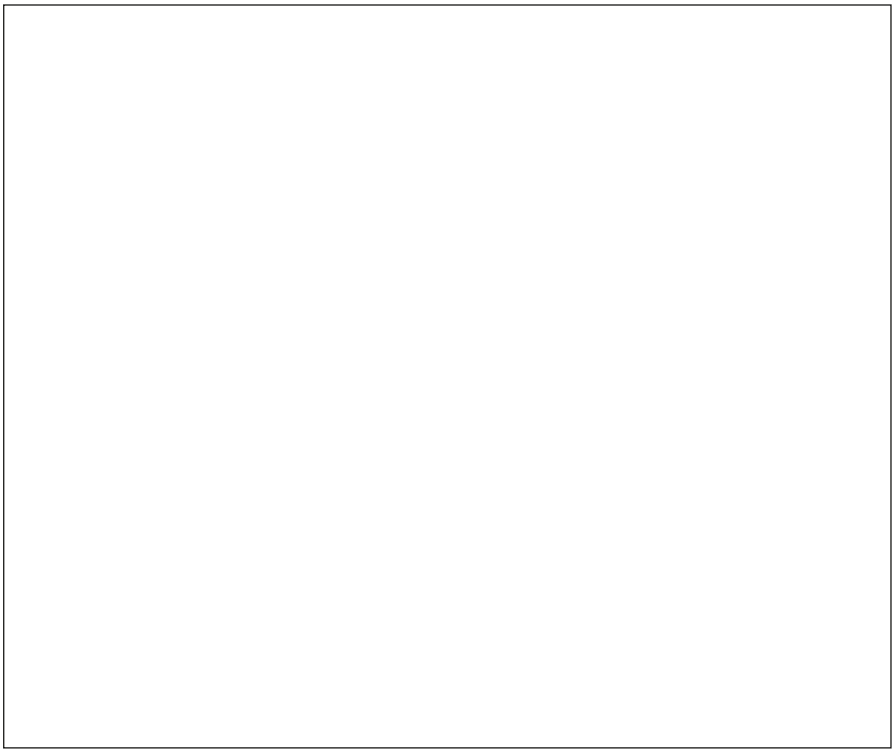


Figure 29. Stem Cell Orchestra website, interactive media webpage. Text not intended to be read.

A



B

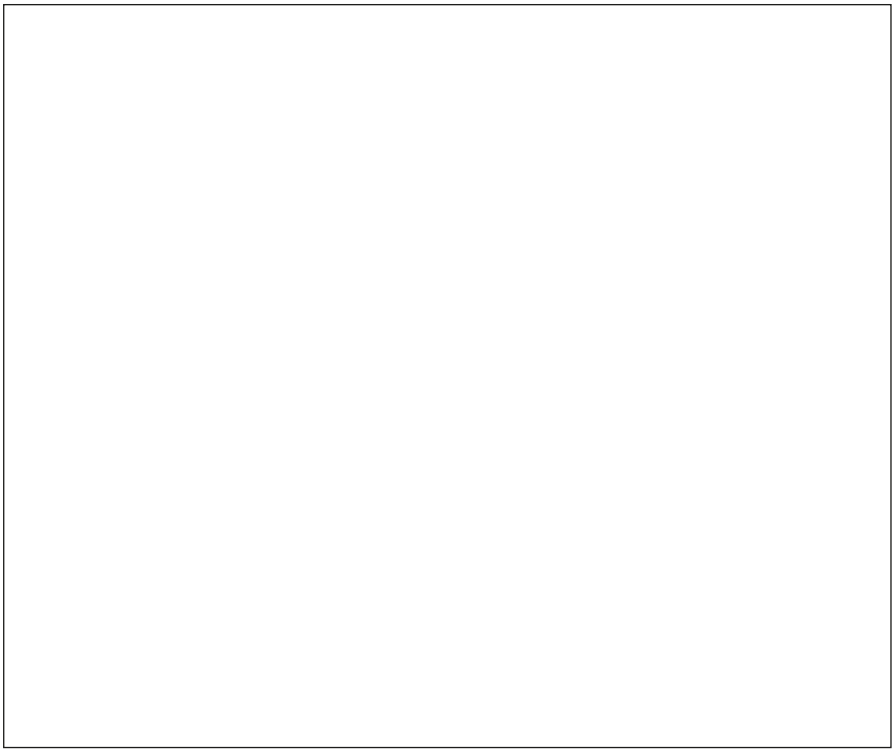


Figure 30. Demonstration of timeline web interactivity. (A) Interactive link (orange outline added for clarity) in cellular animation timeline on Interactive Media homepage. (B) Clicking the interactive link takes the viewer to the Mechanobiology webpage, still within the cellular animation video player (orange outline added for clarity). Text not intended to be read.

Access to Assets

The website created from this thesis is hosted online and can be accessed at www.stemcellorchestra.com. Please contact the author at srsnyderillustration@gmail.com for more information or through the contact form on her website www.srsnyderillustration.com. The author can also be contacted through the Department of Art as Applied to Medicine at Johns Hopkins University School of Medicine at http://medicalart.johnshopkins.edu/.

DISCUSSION

A Visual Solution to Teach Tissue Engineering

The primary goal of this project was to create an interactive didactic animation hosted on a web-based platform that introduces senior-year undergraduate students, and first-year Masters and PhD students to the topic of engineering a cardiac patch. The primary visualization, *Building a Cardiac Patch*, achieves this by showing the external and internal forces that drive cellular interactions and impulse conduction between cardiomyocytes with 3D models built from segmented volumetric datasets. Informal discussions with those who have taken the course, *Fundamentals of Tissue Engineering*, taught at Johns Hopkins University School of Medicine informed the content of this project. Survey questions can be found in **Appendix A**. The preliminary discussion and review of course materials detailed in the Introduction indicated that dynamic 3D visualizations presenting case examples of primary research would be beneficial material for students to access before, during and after the class. No such previously developed didactic tissue engineering research resource is known to the author. Suggestions from the graduate students were iteratively collected and incorporated into the design of the animation and website.

Designing *Stem Cell Orchestra* began with a thorough literature review and survey of existing educational materials. Available print and online resources often depict cardiomyocyte interactions in flat, still images. Some primary research resources, such as "Maturing human pluripotent stem cell-derived cardiomyocytes in human engineered cardiac tissues," (Radisic, Feric, 2016) contain confocal microscopy stills of cardiac tissue ultra-structure; others, such as *Bailey's Textbook of Microscopic Anatomy,* (Kelly, Wood, Enders, 18th edition) depict the non-syncytial ventricular muscle through electron micrograph cross-sections.

In order for tissue engineers to construct viable cardiac patches, they need a thorough understanding of the active, temporal forces at play within cellular culture. Cell fate decisions are ultimately induced by transient or permanent bioscaffold parameters that are controlled by the tissue engineer (Hartman 2016, Stoppel 2016, Liau 2016, Morrissette-McAlmon 2017). It is difficult to visualize this dynamic

setting due to the novelty of the tri-culture system and shortcomings of current visualization methods. Real-time imaging techniques such as confocal laser scanning microscopy can capture cellular events such as mitosis, but not cardiomyocyte contraction. When 3D image sets of fixed cells are visualized as a volume rendering, the cells are displayed only by their fluorescing antibody and the bioscaffold on which they are cultured is absent. These resources fail to depict the spatiotemporal cell-cell interactions that occur in a tri-culture system of a cardiac patch.

An interactive animation platform was proposed to teach student audiences and provide a self-paced, in-depth look into examples of primary research. A website-based interactive animation was chosen as the most appropriate educational media for depicting how to create this particular bioengineered model. As the interactive animation allows the user to be in control of their learning, review of supplemental educational materials can be customized to the learner's experience level before they progress onto the next stage of the animation. Additionally, this web-based interactive animation is available for students to review at any time from any computer or mobile device.

Interactive Animation Development

By pre-rendering the *Stem Cell Orchestra: Building a Cardiac Patch* 3D animation and incorporating the interactivity within a separate application, the animation will stream on any device, dependent only on Internet access.

Several aspects should be considered when designing an animation for timeline based interactivity. Adobe Animate (formerly Adobe Edge) uses a timeline and JavaScript to create web-based interactive vector graphics animations. Image sequences can be added to the timeline to create an animation on playback in a web browser. During initial technology background research for this project, a low resolution (72 dpi) image sequence of a 3D animation was added to an Adobe Animate project, JavaScript was added for interactivity, and the file was published to a web browser. Upon publishing, the image sequence is converted into a Spritesheet and added to the HTML file for playback. A significant reduction of speed was found

at 50 images, or a Spritesheet of 5.4MB. Adobe Animate also has the capability to progressively download a video from a web server, which allows videos to easily be embedded into a website. A new ActionScript file was created and a test 3D animation (FLV or F4V) was loaded. A preset user video playback interface was generated and the file was published to the desktop. However, the video playback controls are set and cannot be programmed for interactivity so the viewer cannot explore the website through the video timeline. Additionally, small, short duration videos can be embedded directly into the Animate document and can be published online as a part of the SWF file. This significantly increases the size of the published file and is only suitable for small videos less than 10 seconds in length. Given these drawbacks, it was determined that Adobe Animate was not a good fit for this project.

TouchCast is a smart video broadcast platform that allows viewers to interact with shared content without leaving the video stream. TouchCast is freely available to download and use on Apple iPad Pro and PC desktop computers (a Mackintosh desktop program is in development). Primarily used in live video broadcast, creators can quickly record, annotate live video and publish to a live stream. Or, users can upload pre-rendered video and annotate the video timeline with video apps (vApps) within the program to be published later for audience interactivity. Content includes web pages, photos, maps, twitter streams, headlines, photo galleries, video, and can be added anywhere in the video timeline and on the video screen. The creator has control over how long vApps are shown in the video, what size the vApp button is, and if a still thumbnail is shown or if there is immediate playback. Once annotated, the video is published to the creator's TouchCast channel as an interactive video, with a link that can be embedded anywhere on the Internet.

Once public, viewers can engage with annotated content within the video timeline, either by directly selecting the annotation within the video timeline or selecting the created hotspot within the video without leaving the video stream. Once selected, the content is displayed in full. The original video, which resides in the corner of the screen for the user to return to at any time, resumes playback from exactly where the user left off. This means a three-minute animation can

be extended into a longer educational experience through the addition of didactic materials into key moments of the animation timeline.

Currently, TouchCast is being used for classroom education, major company communications (BBC, Pfizer), and personal video creation. To the author's knowledge, this is the first instance of TouchCast interactive video being used to create biomedical education materials.

Data Segmentation and 3D modeling

The confocal microscopy datasets available for this project were taken with a low objective (5x to 20x), and while fluorescent labels show some structures (nuclei, gap junctions, etc.) other structures remained invisible (ASCs, fibrin bioscaffold). First FIJI, then Imaris were used to manually segment areas of the cellular data from available datasets. Additional resources from the Allen Institute of Cell Science and Louch Lab at the University of Oslo offered higher objective images (63x). For example, the appearance of sarcomeres was needed within the cardiomyocytes. Sarcomeres are arranged end to end into long, rod-shaped myofibrils that run the length of the cell. They have a striated appearance due to the overlapping nature of thin and thick filaments within an individual the myofibril, which make up the contractile apparatus of striated muscle cells.

For this project, confocal microscopy datasets acquired at the 40-63x magnification were utilized for visualizing 5-30μm cellular structural data. Segmented t-tubule data was used to reconstruct an additional single cardiomyocyte, which provided reference data for depicting the final elongated cardiomyocytes.

Future Development of the Website

A novel workflow for the production of an interactive, didactic, data-driven animation depicting a bioengineered tissue construct was developed. Newly developed materials can be incorporated into the website. Future development could focus on flipped-classroom instructor videos, and space for researchers to actively contribute research and publications. The described workflow, 3D modeling techniques, and interactivity software could also be applicable to the production of

didactic animations of other biomedical subject matter and may be adapted for other audiences to offer alternative didactic resources.

Future Applications of This Technology

Scientific animations are a potential source of insight and research dissemination; however, it can be difficult to determine the degree to which visualizations are informed by scientific evidence (Jantzen 2015). In peer-reviewed scientific publications, there is an established system of citation, which is difficult to replicate in scientific animation, as there is no way to link references to individual features of the display. Even when animations include a bibliography in the end credits, it is up to the viewer to examine references and make assumptions as to where and how they were used to inform the work, which leads to a lack of clarity regarding the credibility of scientific animation (Jantzen 2015).

With this technology, 3D models within scientific animations can be annotated with citations. As a 3D model is shown, a link can be established to an image, webpage, even a peer-reviewed journal article, that shows the reference material from which the 3D model was derived from. This would be informative in molecular animations derived from the Protein Databank, or in cellular animation that uses particular datasets. Future investigations are needed to demonstrate how this technology can best clarify scientific animations.

CONCLUSION

Teaching temporal cellular dynamics and cell-cell interactions is challenging due to the difficulty of obtaining real-time datasets over a long culture time. By combining the newest tri-culture method, cutting edge interactive video, and a review of cellular datasets, a web-based "Stem Cell Orchestra" was created. Future work can be done to incorporate primary research into the website and produce interactive, data-derived visualizations of cellular interactions.

Lastly, a novel workflow for creation of data-driven cellular 3D animation supplemented with educational cellular study materials in an interactive video timeline was developed to visualize the development of a cardiac patch as a model bioengineered tissue. This workflow can be expanded to encompass any type of bioengineered tissue, as it describes teaching needs assessment, data segmentation, 3D modeling and animation with web-based interactivity.

The novel integration of web-based resources with didactic animation in teaching bioengineering concepts fills the gap in educational resources available for biomedical engineering students. This project lays the foundation for the complete development of the *Stem Cell Orchestra* website and a series of animations for tissue engineering research. This innovative approach provides a powerful new workflow that can be used in multi-disciplinary scientific and biological visual communication projects.

APPENDIX A

1. Were you familiar with any tissue engineering concepts before this class? If so, what undergrad courses or lab experience introduced you to these concepts?
2. Have you ever cultured cells before? Y/N
3. What specific topic of tissue engineering were you most interested in learning about? What was the moment you decided you wanted to pursue this field?
4. Were there any unclear topics or lectures throughout the course? Or, were any topics particularly difficult to understand?
5. Was any content about engineering tissue constructs particularly memorable? What was not clear?
6. Would an animation of cell growth clarify the importance of vasculogenesis in large tissue constructs?
7. What concepts did you research outside of class? Did you find any supplemental websites for teaching tissue engineering concepts?
8. We are considering developing a visual teaching tool to help students learn this subject. What type of visualization would you like to help better understand the material? Please rank the following:

    a. Schematics and drawings

    b. 2D animation with narration

    c. 3D animation with narration interactive 3D models (click structures on and off)

    d. ability to rotate and manipulate a model, show models change over time, ability to zoom in and out.

9. Which would you most like to see animated?

    a. Cell-cell interactions

b. Cell growth on specific substrates

c. Vascularization of tissue

10. Do you think a resource that teaches components of tissue engineering through a visual case example would be useful for students of this class? How much time would you spend outside of class using it?

11. Is there anything that we haven't mentioned that you would like to add?

APPENDIX B

Stem Cell Orchestra Script

Cardiomyocytes

To create a cardiac patch, various cell types are seeded on to a fibrin bioscaffold which is wrapped around a Mylar frame and approximately 300 microns thick.

A droplet of solution is placed on the fibrin.

The solution contains cardiomyocytes, which attach to the fibrin bioscaffold.

The cells are immersed in solution that contains nourishing media and Aprotinin, which inhibits break down of the fibrin bioscaffold.

The solution is refreshed every other day as the cardiomyocytes elongate guided by the parallel alignment of the fibrin bioscaffold. Click to learn more about how bioscaffolds influence cell growth.

Between days 4 to 7, the cardiomyocytes begin to spontaneously contract.

Binding sites called adherens junctions form structural connections between adjacent cardiomyocytes. These sites develop into intercalated discs.

Gap junctions that also form at the intercalated disc allow electrical signal propagation between cardiomyocytes, facilitating synchronized contraction.

Vascularization.

After 14 days, a four to one ratio of endothelial cells and human adipose derived stem cells, or hASCs, are seeded onto the cardiomyocytes to start the growth of the blood vessels.

Refreshed every other day with a composite of stem cell and endothelial growth media, the endothelial cells elongate and undergo vasculogenesis to form new blood vessels.

The cells elongate into multicellular cords. Intercellular vacuoles unify to create a

hollow lumen.

Stem Cell Support

The hASCs are mesenchymal stem cells. They can mimic perivascular cells by stabilizing the endothelial vessels.

The hASCs also mimic fibroblast cells by secreting an extracellular matrix that envelopes the endothelial vessels and cardiomyocytes.

New research results suggest that hASCs may form gap junctions with cardiomyocytes and contribute to contraction.

hASCs may also promote angiogenesis by secreting paracrine signaling factors such as VEGF, FGF, and bFGF.

They also secrete exosomes, or vesicles filled with growth factors and microRNA, the effects of which are still unknown.

These carefully orchestrated interactions between endothelial cells, hASCs, and cardiomyocytes form new functional tissue. The complete vascularized cardiac patch is able to integrate with native heart tissue and support heart function after myocardial infarction.

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CURRICULUM VITAE

Shawna Snyder was born in Ames, Iowa. Growing up on a pig farm near Huxley, Iowa, her love of science and drawing began at an early age.

Shawna's wonderful family and teachers inspired her to pursue higher education at Iowa State University. There, while working in the studio one night, she met Dean Biechler, a veteran biological illustrator and the head of the Biological/Pre-Medical Illustration (BPMI) program. He introduced her to the dual world of illustration and science, and she joined the BPMI program later that month. It was a perfect fit. While working and studying at ISU, she found success in the BPMI program, earned numerous awards for her artwork and academic achievements, and enjoyed the structure and company she had there.

In August 2016, she continued her education in the Medical and Biological Illustration program in the Department of Art as Applied to Medicine at the Johns Hopkins University School of Medicine. While studying for her graduate degree, Shawna received the Orville Parks Best in Show from the Association of Medical Illustrators and recieved a Vesalius Research Grant for her thesis research. She anticipates recieving her Master of Arts degree in May of 2018.

After graduation, Shawna will be pursuing a career in biomedical visualization and hopes to continue to take on new, challenging projects that stretch her abilities and require her to learn new things in order to teach medicine and science.