AWARD NUMBER: W81XWH-20-1-0193

TITLE: Engineered Development: Human Cardiac Developmental Tissue Model for Multifactorial Screening for the Onset of Congenital Heart Defects

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REPORT DATE: January 2022

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JANUARY 2022	Final	04/01/2020 – 09/30/2021

4. TITLE AND SUBTITLE

Engineered Development: Human Cardiac Developmental Tissue Model for Multifactorial Screening for the Onset of Congenital Heart Defects

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-20-1-0193

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General Hospital
55 Fruit Street
Boston, MA 02114-2696

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this project, we propose to create a tissue model of congenital heart disease (CHD) by combining the principles of tissue engineering and developmental biology. The proposed tissue model will be treated with different toxic agents known to create CHDs in the body including alcohol, cigarette smoke, and the psychiatric drug lithium. We will look to understand whether these toxins affect heart tissue formation and their role on the genetic makeup of different heart cell types. This proposed research would be the first three-dimensional tissue model that can act as a disease-in-a-dish model of CHD. The proposed model will be useful going forward as a way to potentially predict the formation of CHD, act as a toxin screening model for the formation of CHD, and act as a drug screening model to help treat CHD in the future.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	25	19b. TELEPHONE NUMBER *(include area code)*

1. Introduction

The heart is the first organ to develop during embryogenesis. It starts out as a simple tube that then undergoes a looping phenomenon which transforms the tubular structure into the beginnings of the adult four-chambered organ. Looping aligns the heart tube in a way that leads to further development of more specialized forms of the cardiac tissue; including the electro-conduction system, valves, and trabeculation. Incomplete looping causes misalignment of the developing heart resulting in congenital heart disease (CHD). CHD's permanent malformations of the heart require major surgical interventions for the duration of the child's life. In the most extreme cases, CHD can result in perinatal mortality of the child. CHD is the most prevalent cause for congenital malformations, accounting for 28 percent of all congenital malformations, which corresponds to 1.35 million live births every year.

There are many different factors that could lead to the generation of CHD, broadly described in three different ways; either genetic (single gene defects), chromosomal (abnormality in the number of chromosomes), or environmental due to teratogenic agents (including drugs, toxins, infectious agents, and mechanical forces). There is a limited understanding of which individual factors correspond to the different specific CHD. Current understanding of the formation of CHD relies on either *in vitro* cell culture or on mouse genetic defect models. Both of these strategies have discovered important genetic pathways for the formation of CHD. However, these models have not yielded much success in improving our understanding of multifactorial etiologies, especially those stemming from teratogenic factors, and are inherently bad at isolating specific effects on heart development. Therefore, there is a critical need for a better model system to investigate the onset of CHD and the roles that the different factors have in the various malformations of the heart.

To address this critical need, we proposed to create such a tissue model through the highly innovative approach of "engineered development". We planned on combining tissue engineering principles with development biology expertise to create a three-dimensional human tissue model of cardiac development that would then be used as a CHD disease-in-a-dish model. The long-term goal of this proposal is to create a three-dimensional tissue model system that recapitulates cardiac development to act as a tool to examine the pathophysiology of CHD. To build such a model, tissue engineering principles will be combined with knowledge from developmental biology. Through this approach of *"engineered development"*, we hypothesize that a tissue model of early cardiac development can be established and used to examine and predict the teratogenic induction of congenital heart disease.

2. Keywords

Tissue Engineering; Bioreactor; Congenital Heart Disease; Cardiac Looping; Perfusion;

3. Accomplishments

a. What were the major goals of the project?

Specific Aim 1: ***Heart Tube Morphogenesis Tissue Model Development and Teratogenic Testing***	**Timeline**
Major Task 1: Generation of Embryonic Heart Tube Tissue Model	**Months**
Subtask 1: Finalize and Verify Heart Tube Formation Design	1-2
Subtask 2: Finalize Bioreactor Design and Build Working Bioreactor	1-2
Subtask 3: Investigate Looping of Heart Tube in Bioreactor	3-6
Milestone Achieved: Looped Embryonic Heart Tube Model	6
Major Task 2: Validate Design as a Tissue Model for Congenital Heart Disease	
Subtask 1: Treat Tissue Model with Teratogenic Agents and Measure Dose Response	6-9
Milestone(s) Achieved: Verification of CHD Tissue Model	9
Specific Aim 2: ***Teratogenic Effects on the Genetic and Chromosomal Footprint***	
Major Task 3: Investigation of Teratogenic Agents' Effects on 2D Cell Culture	
Subtask 1: Treat Cell Cultures with Different Teratogenic Agents	1-3
Subtask 2: Analyze Cardiomyocyte Cell Cultures After Treatment	2-7
Milestone(s) Achieved: Investigation of teratogenic agents effects on the genetics/chromosomal footprint of developing heart cell models	6
Major Task 4: Investigation of Teratogenic Agent Affected Cells Ability to Form Heart Tissues	
Subtask 1: Treat Cell Cultures with Different Teratogenic Agents	6-7
Subtask 2: Attempt to Form Looped Heart Tissues with Treated Cells	8-12
Milestone(s) Achieved: Investigation of teratogenic agents affected cardiomyocytes ability to form tissues	12

b. What was accomplished under these goals?

Specific Aim #1: Heart Tube Morphogenesis Model Development and Teratogenic Testing

Major Task 1: Generation of Heart Tube Tissue Model:

Subtask 1: Engineered Heart Tube Fabrication

The majority of the funding and time spent from this award was focused on the development of a replicable methodology to fabricate the embryonic heart tube tissue model such that the engineered product had features that best mimic the complex anatomical features of the developing heart (Figure 1). The developing heart tube has a large acellular cardiac jelly matrix that makes up the majority of the tissue which is surrounded by a thin ring of differentiating cardiomyocytes and cardiac progenitor cells, within the inner portions of the cardiac jelly there is a thin ring of endocardial cells. The main focus was to create a heart tube that is small in diameter and length that is perfusable and cannulated such that fluid flow could be provided within the inner lumens of the engineered tube. Along with that focus was to create a tube that had a large inner acellular portion that is largely collagenous to mimic the cardiac jelly and then surrounding the cardiac jelly portion. Once a tube was able to be fabricated with this unique geometry, we would then focus on lining the inner lumen with an endothelial cell layer, eventually to be replaced by differentiated endocardial cells. To create the engineered heart tube, we utilized an injection molding technique (Figure 2). Two different molds were created (Figure 2a), the first mold creates the large inner portion of the final tube which would be cast out of a cardiac jelly mimic and the second mold creates the thin outer cellularized layer. The molds are created with 5 wells for tube creation per mold. The mold is split in half so that the needle assembly, described in more detail later in this section, can be placed within the mold and the final engineered tube can be removed from the mold. The engineered tube needs to be cannulated and mechanically robust in order to eventually hook the engineered tubes into a mechanical stimulation bioreactor that is being designed in conjunction with the development of the fabrication strategy to engineer the heart tube. To allow for the cannulation, on either end of the molds, an 18-gauge dispensing needle (Figure 2b) is held in place and the tubes are casted surrounding the needles. In order to facilitate the connection between the needle and the engineered heart tube, small diameter polytetrafluoroethylene (PTFE) tubing is wrapped around the outside of the needle. PTFE tubing is microporous such that the casted tube would be able

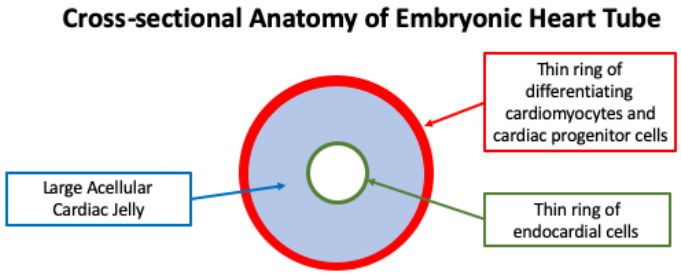

Figure 1: Diagram of the Anatomical Cross-section of the Embryonic Heart Tube

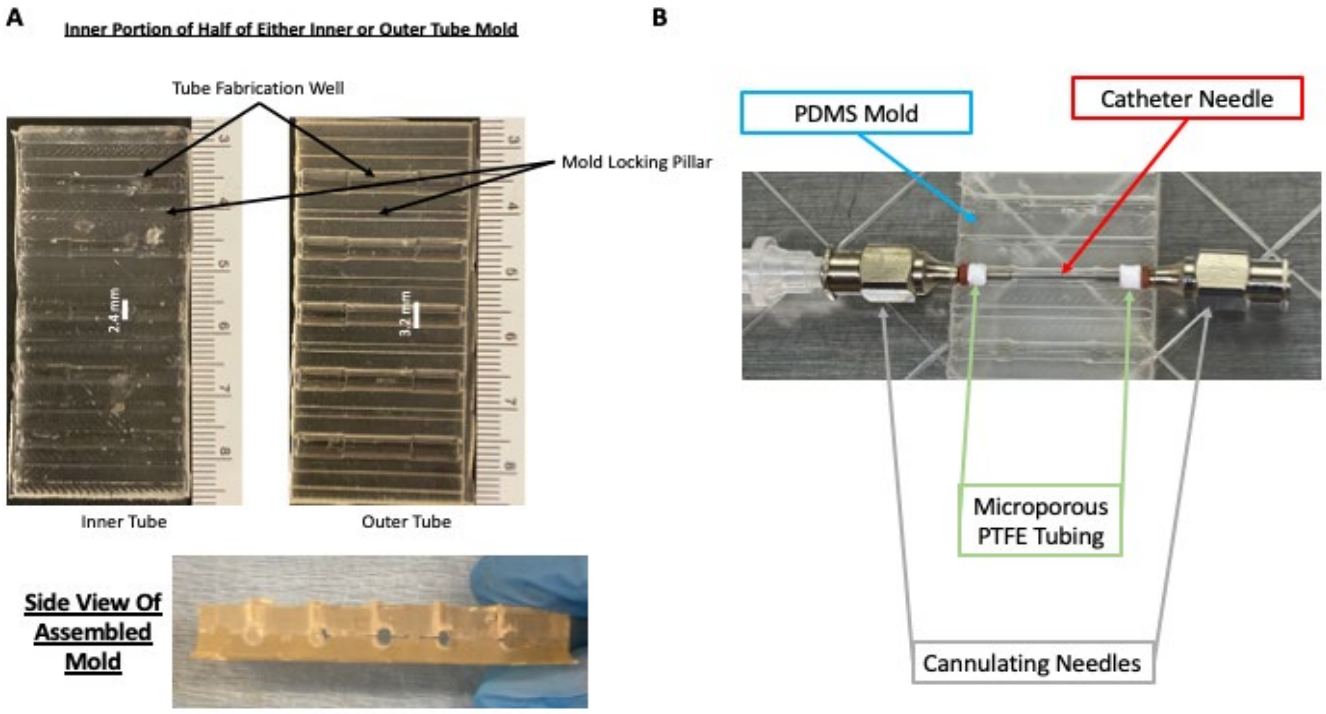

Figure 2: PDMS Molds to Engineered Embryonic Heart Tube Model: (A) PDMS molds for the inner and outer portions of the heart tube were designed. (B) To create the embryonic heart tube, dispensing needles are held in place within the mold that have PTFE microporous tubing surrounding the needle end. To create the inner lumen of the tube, a catheter needle connects the two needles.

to seep into the PTFE tubing which is snuggly fitting around the end of the needle. The two needles with the PTFE tubing are held in opposite sides of the molds with the needlepoints facing each other. The two needles are connected through their inner lumens by a 20-gauge catheter needle. The catheter is important for a variety of reasons, the first being that it holds the two needles in place within the molds, and the second reason is that the catheter creates the inner lumen of the engineered heart tube to allow for perfusion of endothelial cells and eventually media when placed inside the created bioreactors.

Using the described molds and cannula design to fabricate the tubes, we then tested a variety of materials to cast the tubing out of. One of the major concerns with this was the overall mechanical robustness of the finished tube as eventually we want to mechanically stimulate the tube with forces to cause it to undergo the looping phenomenon. To look into this,

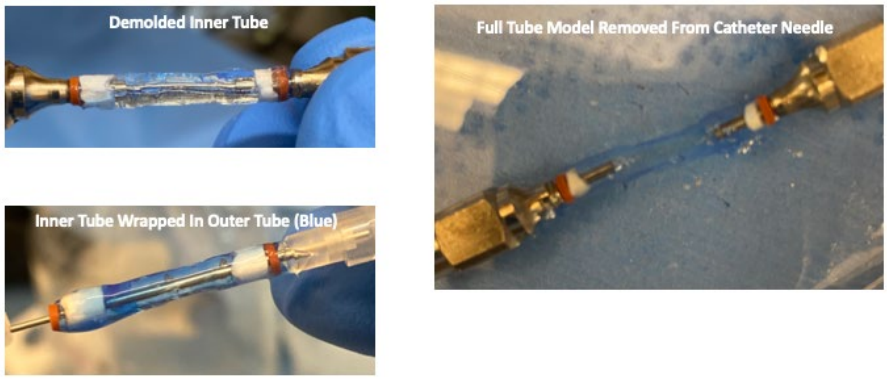

Figure 3: Heart Tubes Can Be Engineered Out of Gelatin. The outer tube was dyed blue and was found to fully encapsulate the inner portion.

we tested a variety of concentrations of collagen, matrigel, and gelatin for both the inner and outer portions of the engineered tube. For both the inner and outer tubes, it was found that using 10% gelatin which was treated with a 3% transglutaminase slurry was the best in terms of creating a robust tube and one that a thin outer tube could be cast. Collagen and matrigel were too weak and when the tubes were demolded, the catheter needle would rip right through the created tube. Similarly, when using either collagen or matrigel as just the outer tube, the outer tube would delaminate from the inner gelatin tube. The treatment of the gelatin with the transglutaminase allowed for the gelatin to be thermally stable at normal incubation temperatures (37°C), mechanically strong to withstand the demolding, and also acts to crosslink the two tube layers to themselves such that the final engineered tube is complete. From this testing, the final engineered heart tube would be created with this transglutaminase treated gelatin for both the inner and outer portions of the tube (Figure 3).

Subtask 2: Mechanical Stimulation Bioreactors

The developing heart tube undergoes a looping phenomenon that transforms the simple tube shape into a looped structure which further develops into the adult four chambered organ. This looping phenomenon is incited from external forces that are exhibited upon the tube from other structures in the developing embryo. For the engineered heart tube model, a mechanical stimulating bioreactor was needed to be designed in order to mimic these external forces that cause the looping phenomenon. To better design this bioreactor, the external forces needed to be simplified and visualized (Figure 4). The tube first undergoes bending in the ventral direction due to inward forces from either end of the tube in the cranial and caudal directions resulting in a c-shape (Figure 4a). Then the tube undergoes growth and forces cause a deflection in the tubes caudal end towards the dorsal direction (Figure 4b). Finally, the tube undergoes a rightward rotation on its cranial side due to uneven growth of the tube, couples this with further tube growth and shortening resulting in the final looped tube (Figure 4c). After visualizing

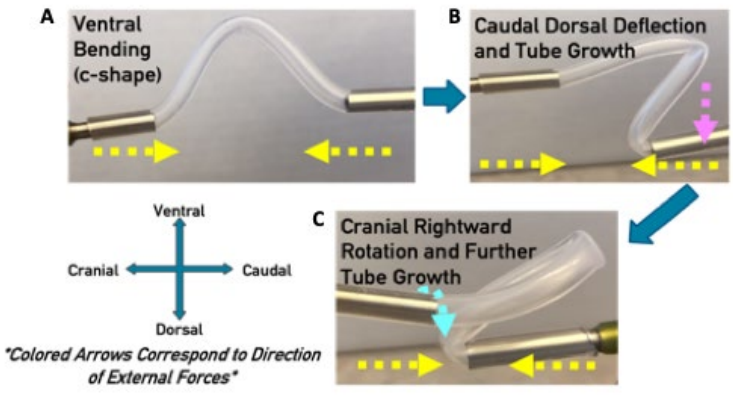

Figure 4: Visualization of External Forces Seen During Heart Looping Phenomenon. (A) Heart tube begins it looping through ventral bending resulting in a c-shape. (B) Next there is a dorsal deflection in the caudal end of the tube due to continue tube growth. (C) Finally, there is a rightward rotation on the cranial end due to uneven growth of the tube which results in the fully looped organ.

these forces, we decided to make two separate sets of bioreactors that would exhibit forces in the same directions seen during the looping phenomenon.

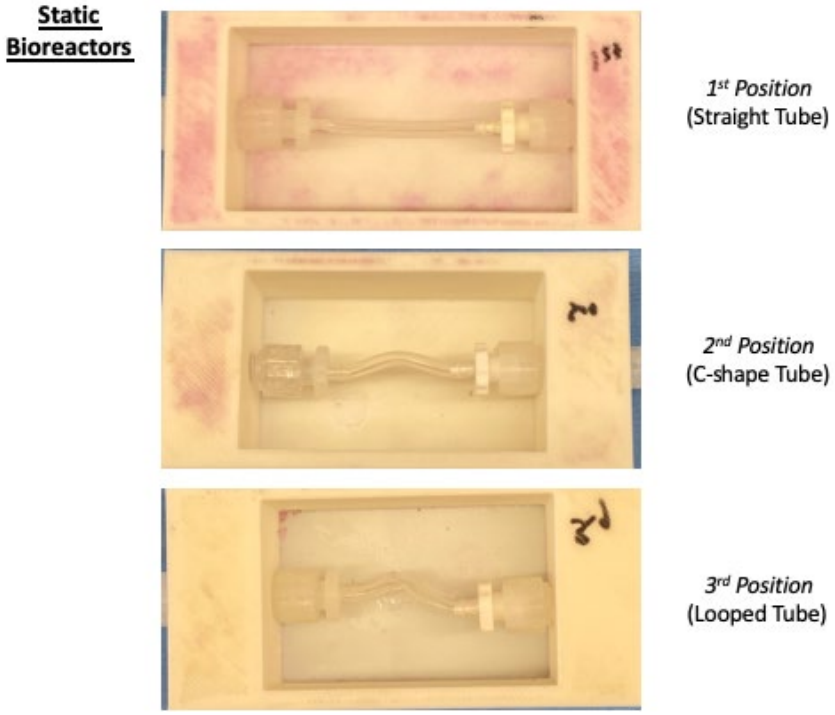

Figure 5: Static Bioreactors were designed to hold the engineered heart tubes in three different positions from different timepoints from the looping process.

The first set of bioreactors engineered are static and hold the engineered tube in three positions that are snapshots in time from the looping phenomenon (Figure 5). The first static bioreactor holds the engineered tube in place as a straight but stretched out tube, called first position. The second static bioreactor shortens the length of the tube and also has a small deflection down on one of the sides (acting as the caudal end with the dorsal deflection) which results in the tube being in the c-shape, called second position. The third and final static bioreactor further shortens the tube and also has a slight rotation on the cranial side and a further deflection in the caudal side resulting in a looped tube, called third position. These static bioreactors are designed to be used in a few different ways. One way is that the bioreactors could be used to compare the effects of the different forces on the developing tubes when held in place. The second way is that a singular tube could be moved from one position to the next to the next in order to simulate the entire looping phenomenon. To potentially account for difficulties in using these static bioreactors, whether to move tubes from one position to the next or for loading the tubes within the tight confines of the bioreactors, a more complex dynamic bioreactor system was also designed.

The second set of bioreactors designed are dynamic system (Figure 6) that mimics the different forces exhibited on the tube in a more controllable way than the static bioreactors. The dynamic bioreactor was designed to have one side act as the cranial side of the embryo and the second side act as the caudal. Both sides are attached to sliding carriages to allow for tube elongation and shortening. The caudal side can slide up and down to mimic the dorsal deflection of the tube. The cranial side is designed to be rotated to mimic the

rotation of the tube that elicits the final aspects of the looping phenomenon. The bioreactor is designed to have luerlock connectors to allow for perfusion through the cannulated engineered heart tube. The bioreactor was designed using a 3d computer aided design program (Figure 6a) and was 3d printed out of polylactic acid (PLA) (Figure 6b). The bioreactor was tested with a silicone tube to verify whether it could result in the proper looping of a tube (Figure 6c). The tested silicone tubing was able to go through the whole looping process and was highly controllable.

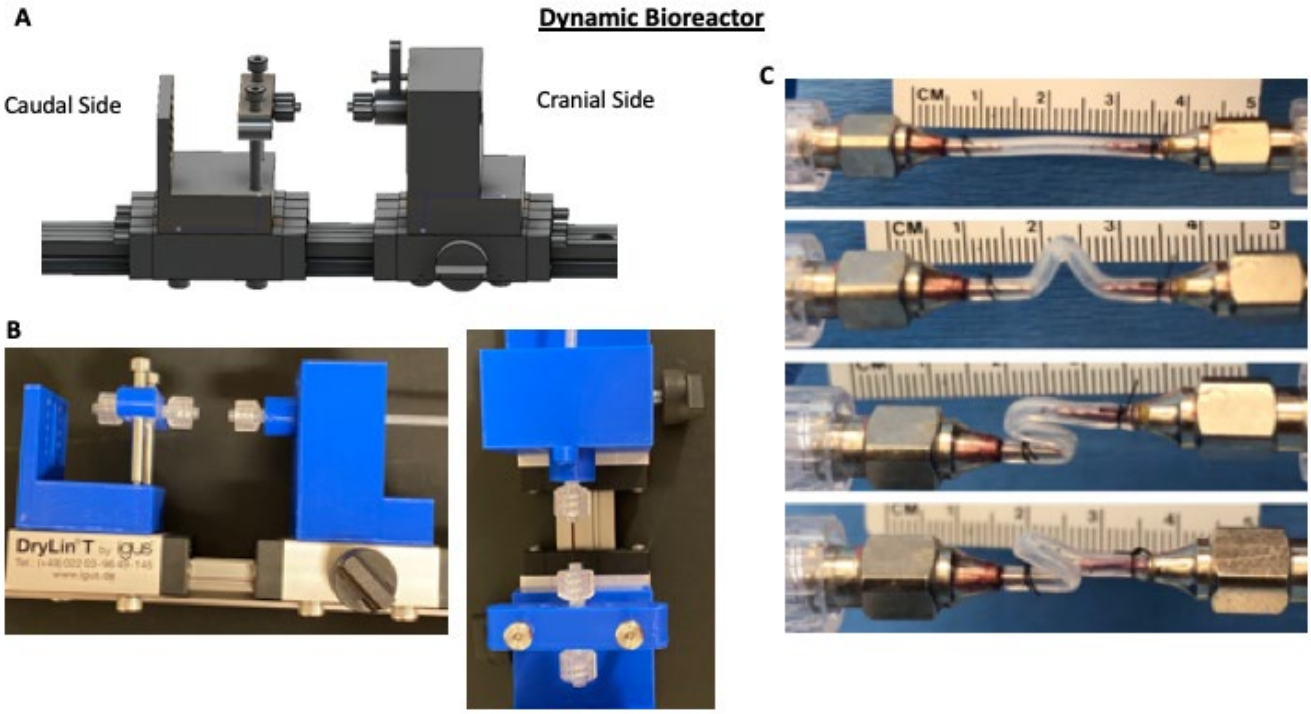

Figure 6: Dynamic Bioreactor Systems Were Designed to Fully Recreate the External Forces Leading to Cardiac Looping. (A) Computer designs of the dynamical bioreactor system with each side representing the caudal or cranial end of the embryo lbeled. (B) These designs were fabricated using a 3D printer out of PLA. (C) The dynamic bioreactor system can induce the full heart tube looping process.

Subtask 3: Heart Tube Validation and Looping from Bioreactor Stimulation

We sought to validate the fabrication of the engineered heart tube and the designed mechanical bioreactors through tissue creation and culture. First, we looked to test the functionality of acellular engineered tubes for their perfusability, mechanical manipulation, and ability to undergo looping within the designed bioreactor. Heart tubes were created as described above with both an inner and outer layer comprised of the transglutaminase treated gelatin. After the tubes were demolded, suture loops were ligated onto the PTFE tubing to further secure the engineered tube down onto the cannulas for manipulation (Figure 7a). Next, these tubes were perfused with a red dye (Figure 7b), to test its perfusability. The red dye was easily perfused throughout the entire lumen of the tube and red dye was able to be collected from the opposite cannula indicating that the entirety of the tube remained perfusable and was water-tight without any noticeable leakage, further demonstrating that the designed heart tube would be usable in a perfusion-based bioreactor system.

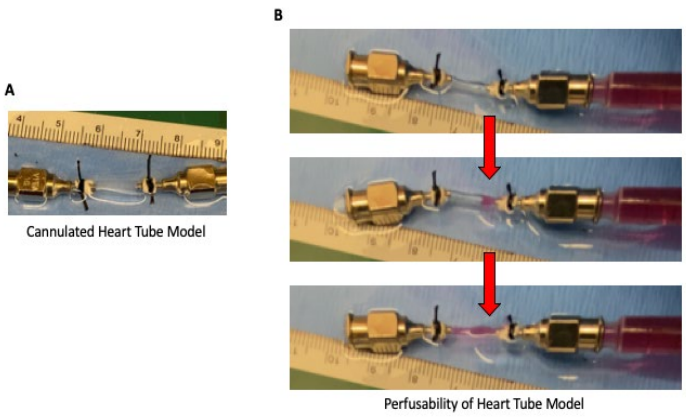

Figure 7: Perfusability of Engineered Heart Tube. (A) Heart tubes were tied tight down onto the cannulae after being demolded. (B) Red dye was perfused through the lumen of the heart tube and was found to flow completely through the tube with limited to no leakage.

After successfully testing the perfusability of the tube, we next looked to investigate whether the engineered tube could undergo looping when placed within the dynamic bioreactor system. The acellular engineered tube was easily attached to the luerlock connectors when originally loaded into the bioreactor system (Figure 8a).

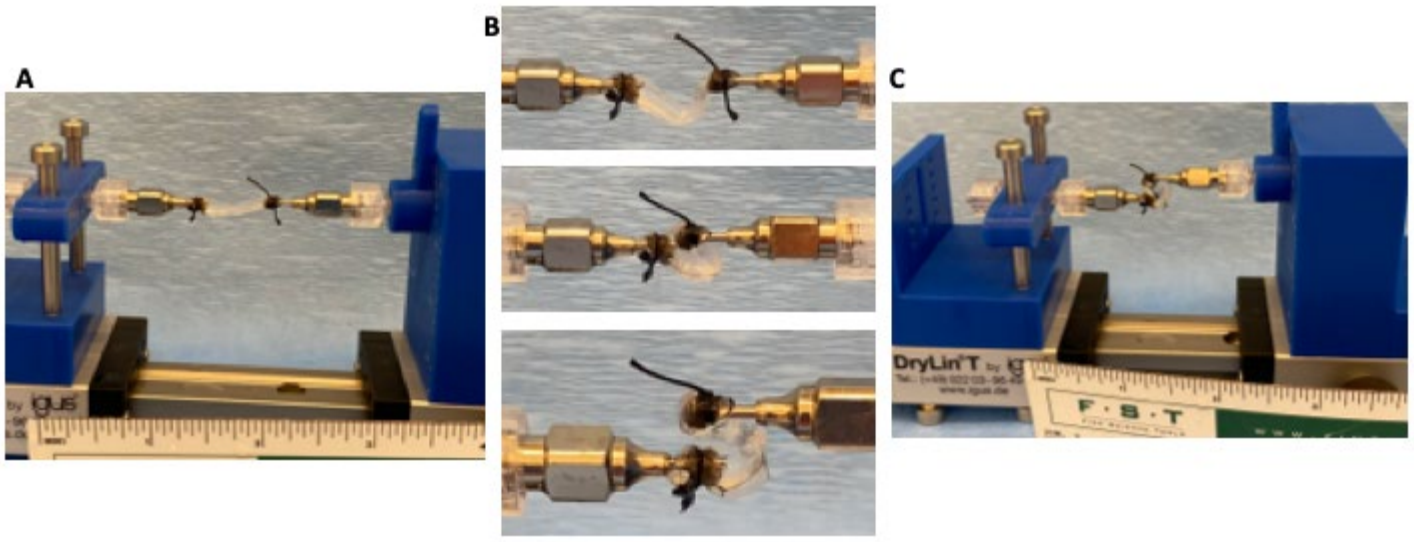

Figure 8: Engineered Heart Tube Undergoes Looping in Dynamic Bioreactor System.(A) Heart tubes were easily loaded into both sides of the bioreactor. (B) The bioreactor is able to induce the looping in the engineered heart tube. (C) The heart tube was able to be held in place in its looped form.

Using the carriages of the bioreactor, the tube was able to be stretched and compressed prior to the start of the looping process. Upon shortening of the tube, the tube began to undergo the looping phenomenon (Figure 8b). It began to have a c-shape upon the shortening. Then upon the deflection of the pillar representing the caudal end of the embryo, the tube started to transform into the s shape. Finally, upon the rotation on the cranial end of the tube and further shortening of the tube, the tube was able to fully loop and was held in place in the looped position (Figure 8c). This test highlights that the engineered tube can undergo the looping process within the designed dynamical bioreactor.

Heart tubes were next engineered with a cellularized cardiomyocyte outer layer to fully demonstrate the tissue engineered design. The first set of heart tubes were engineered with a mature cardiomyocyte population that were isolated from neonatal ventricles. Cardiomyocytes were mixed within the gelatin prior to being injected into the molds for the outer layer portion of the engineered heart tube. After gelation, the heart tubes were demolded and cultured in cardiomyocyte media for 24 hours prior to fixation. Tubes were sectioned and stained with a mason's trichrome stain to visualize the tube architecture (Figure 9a). There is a visible large collagen section surrounding the inner lumen of the tube which mimics the cardiac jelly, surrounding the collagen section is a cellularized ring of cardiomyocytes. This was further verified with immunofluorescent staining (Figure 9b-e).

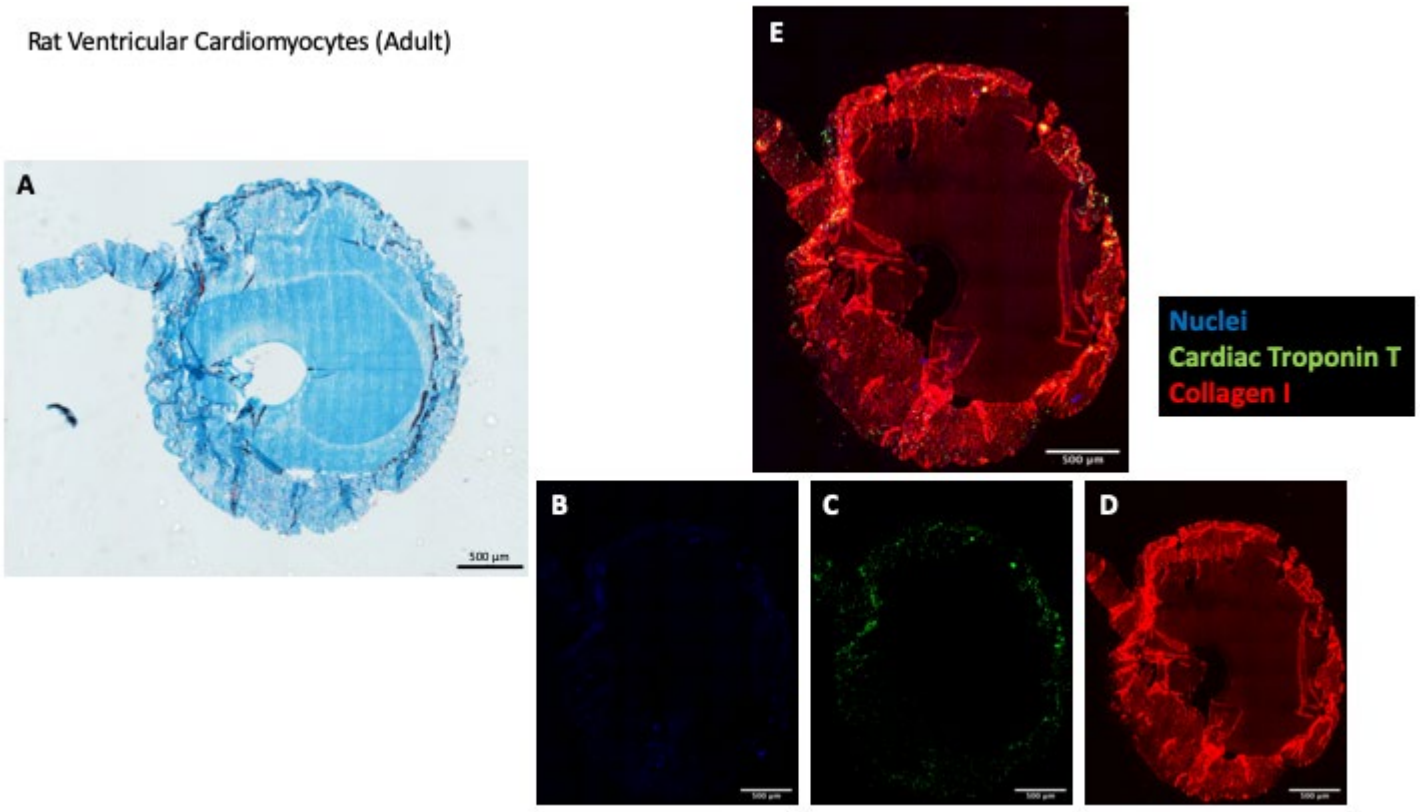

Figure 9: Heart Tubes Can Be Engineered Out of Rat Ventricular Cardiomyocytes. (A) Trichrome staining of the engineered heart tube showing the large inner collagen layer that is surrounded by a cellularized ring. (B-E) Immunofluorescent staining further shows the cross-section of the cardiac tissue

Tubes were stained with DAPI for nuclei (Figure 9b), Cardiac Troponin T (Figure 9c), and Collagen I (Figure 9d). The visualized section (Figure 9e) aligned with what the results from the trichrome stain and demonstrated that the heart tube could be engineered with cardiomyocytes.

We next looked to apply this tissue engineering approach with human induced pluripotent stem cell derived cardiomyocytes. This was performed in order to better demonstrate the tissue model's ability to act as a humanized tissue model and one that could theoretically be used as a patient specific model as induced pluripotent stem cells have the potential to be derived from a patient specific cell population in order to screen for specific genetic defects. To best mimic the developing heart *in vivo* we chose to use the induced pluripotent stem cells that were in the middle of the differentiation process towards mature cardiomyocytes (Figure 10a). Cells were used are from the fifth day of the differentiation process as these cells are the most closely reminiscent to what is seen *in vivo* in the developing heart tube and are considered a cardiac progenitor cell. Cells were incorporated into the outer layer of the heart tube as was previously described with the rat ventricular cardiomyocytes. Tubes were cultured for four days in the cardiomyocyte media after being demolded and were subsequently fixed. Human iPS derived cardiomyocytes were found to be fully incorporated into the outer layer of the engineered heart tube, as demonstrated by hematoxylin and eosin staining (Figure 10b). This was further

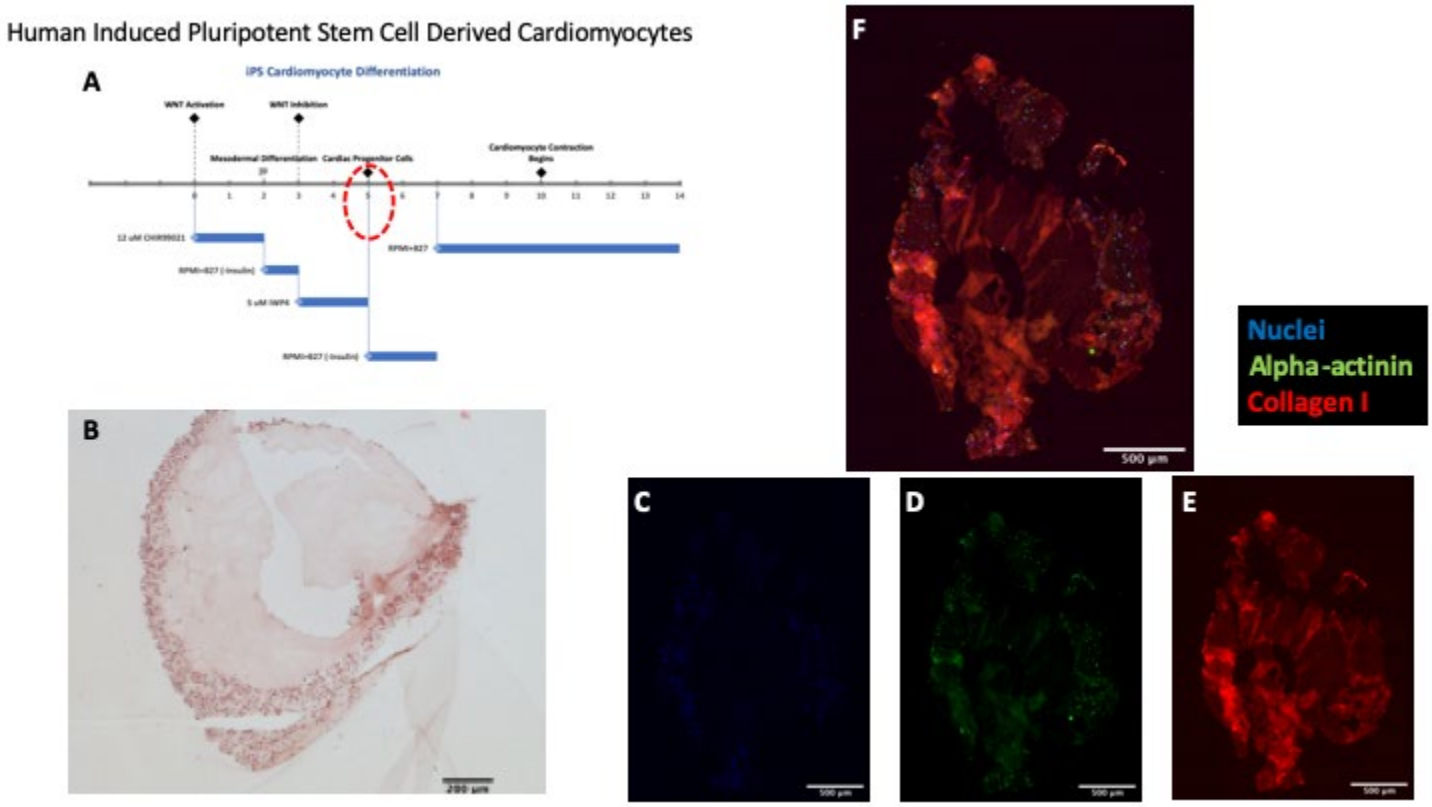

Figure 10: Heart Tubes Can Be Engineered Out of Human Induced Pluripotent Stem Cell Derived Cardiomyocytes. (A) Induced pluripotent stem cells were differentiated into cardiomyocytes. Cells on the day 5 of this process were used to engineer heart tubes as these are considered cardiac progenitor cells and are reminiscent of the developing embryonic heart tube. (B) Hematoxylin and Eosin staining of the engineered heart tube showing the large inner collagen layer that is surrounded by a cellularized ring. (C-F) Immunofluorescent staining further shows the cross-section of the cardiac tissue.

verified through immunofluorescent staining (Figure 10c-f). Of note, there was positive staining of alpha-actinin (Figure 10d) signifying that the iPS cells continued with their differentiation while seeded within the engineered heart tubes and did not de-differentiate out of the cardiac lineage.

Finally, to test the whole system, cellularized heart tubes were cultured in the static bioreactors to investigate whether the system could keep the cells alive within the tubes. Heart tubes were engineered with iPS derived cardiomyocytes in the outer cellularized section and were placed into the three static bioreactors. Tubes were able to be placed into the static bioreactor that holds the tubes straight in the first position without any difficulty. However, there were problems loading the tubes into the bioreactors that hold the tube in the second and third position as the tubes would rupture along where the needles are cannulated within the tube. Going forward in the future, these static bioreactors will need to be re-designed to account for this difficulty. The re-designed bioreactors would have more room and less of a shortening of the tube. The tubes that were placed successfully in the first positioned static bioreactors were cultured through perfusion of media through its inner channel for four days. The bioreactors' luerlock connectors were connected to a perfusion circuit through which the media was pumped from a reservoir media in a container, through the inner channel of the tube and then back to the media reservoir (Figure 11a). The tube was found to be held in place and not disrupted upon start of media perfusion (Figure 11b). Similarly, after the four days of culture, the tube was inspected and found to be intact and still held in place within the bioreactor (Figure 11c). After the four days of culture, tubes were removed from the bioreactor, fixed in 4% paraformaldehyde, embedded in OCT and cryo-sectioned cross-sectionally. The resulting cross-sections were then stained for apoptosis using a DeadEnd Fluorometric TUNEL System fluorescence stain (Promega Corporation). There was some apoptosis found upon imaging of the stained sections (Figure 10d-f), where the nuclei are stained blue with a DAPI stain (Figure 11d) and apoptotic cells are stained green from the TUNEL kit (Figure 11e). These results (Figure 11f) indicate that tubes can be cultured within the perfusion-based bioreactor system. Due to the prescience of some apoptosis, we would need to work to improve culture and viability as we advance with this work. Going forward, we want to progress the use of the dynamic bioreactor system as it seems like loading and controlling the looping within this system is more easily performed when compared to the static system. The major holdup with this dynamic system is designing how best to create the perfusion circuit and container to hold the complex rail, carriage, and bioreactor parts. Upon submission of this report, this dynamic system has yet to be fully engineered but designs to hold three bioreactor

systems within the same container have been developed and we look to further developing this design for more complete dynamic looping cultured of the engineered tissues.

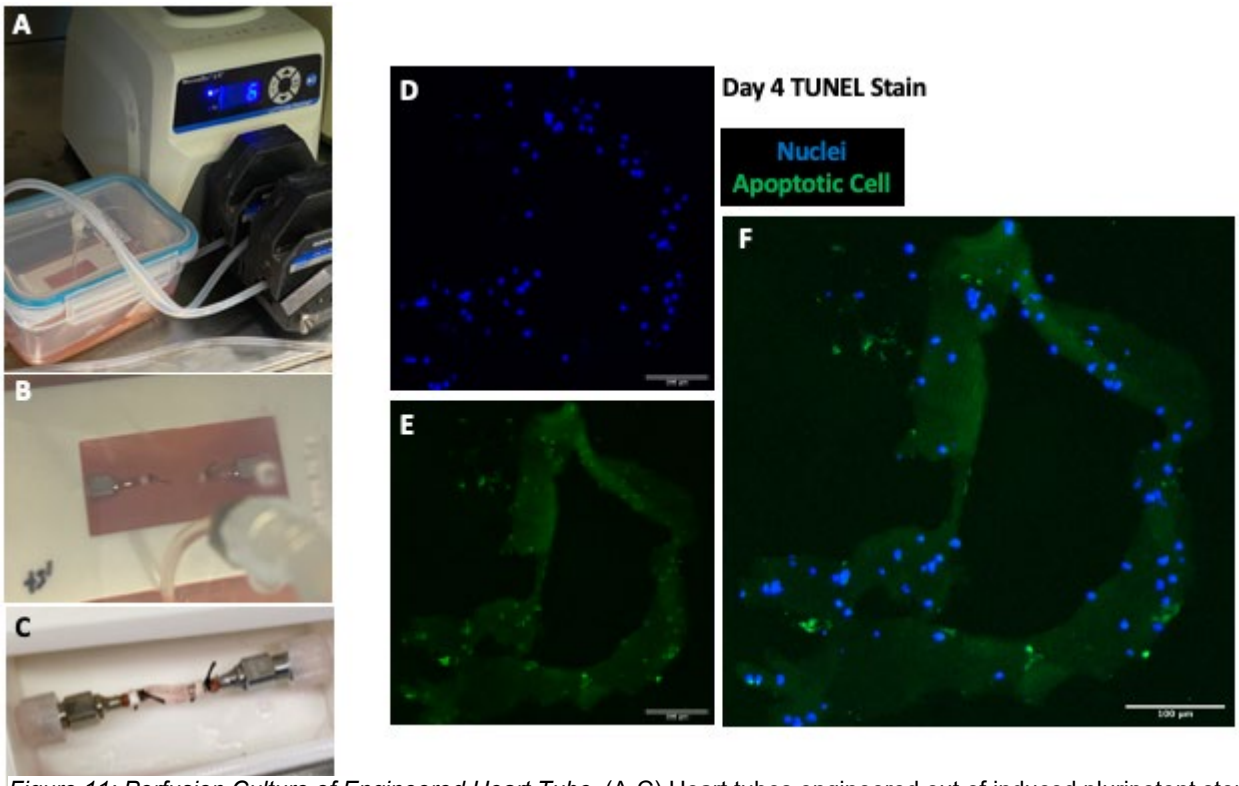

Figure 11: Perfusion Culture of Engineered Heart Tube. (A-C) Heart tubes engineered out of induced pluripotent stem cell derived cardiomyocytes were cultured for four days in a perfusion bioreactor. (D-F) TUNEL staining shows some apoptotic cells but largely the tissue was found to have viable cells indicating these tissues can be cultured this way.

Bioreactor modifications were undertaken in order to limit tube rupture and to have a smaller footprint in order to improve upon reproducibility and sterility. New bioreactors were designed to replace both the previous static and also the dynamic bioreactors. Taking from the study of external forces that lead to heart tube looping (Figure 6c, 12a), bioreactors were simplified. New bioreactors (Figure 12b-d) were designed based off of the concept of a peg board, where the two sides of the heart tube (cranial and caudal) were designed with simple machines that can then be moved towards each other on pegs. To reflect the cranial end's rotation due to uneven growth in the embryo, it was designed to be a wheel that has 180° rotation. The peg board on the caudal end has a stepwise displacement in the z-direction to reflect the displacement seen on that end of the tube in development. The z-direction displacement grows greater as the tube ends get closer up to a 300% displacement.

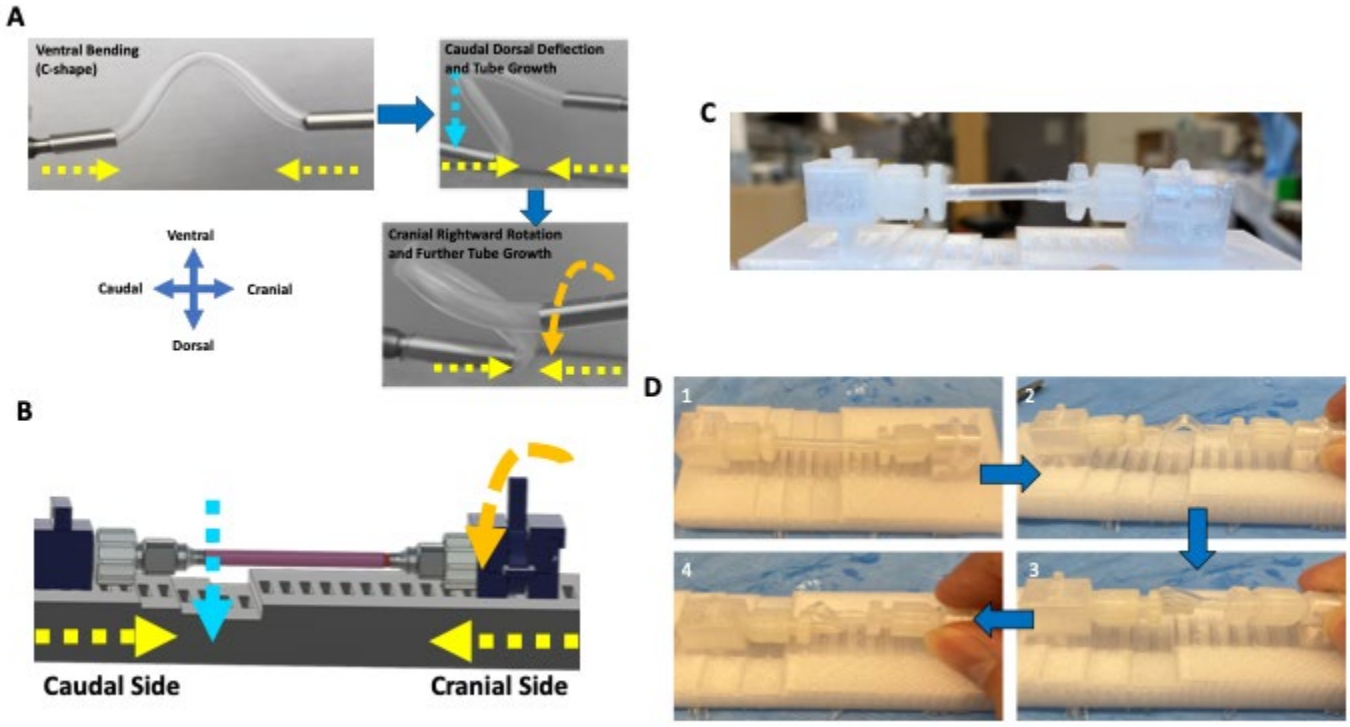

Figure 112: Heart Tube Tissue Looping Bioreactor Design and Verification: (A) External forces leading to the induction of heart tube looping were modeled with a silicon tube. (B) Computer design of the bioreactor corresponding to the proper external forces seen *in vivo*. Yellow arrows show tissue shortening seen on both the cranial and caudal end of the heart tube. The cranial side of the bioreactor is also designed to allow for tube rotation as seen by the orange arrow. The caudal side of the bioreactor is also designed to undergo a dorsal deflection as identified with the blue arrow. (C) A 3D printed fabrication of the bioreactor was (D) used to cause looping in a silicon tube to match what is seen by the *in vivo* heart tube.

With the simplified bioreactor, we sought to perform looping with engineered heart tubes. Engineered heart tubes were cultured via perfusion in the looping bioreactor (Figure 4a). Perfusion was performed with inlet and outlet flow rates of 1 ml/min and tubes were also sitting in a pool of media outside of the tube to fully mimic the

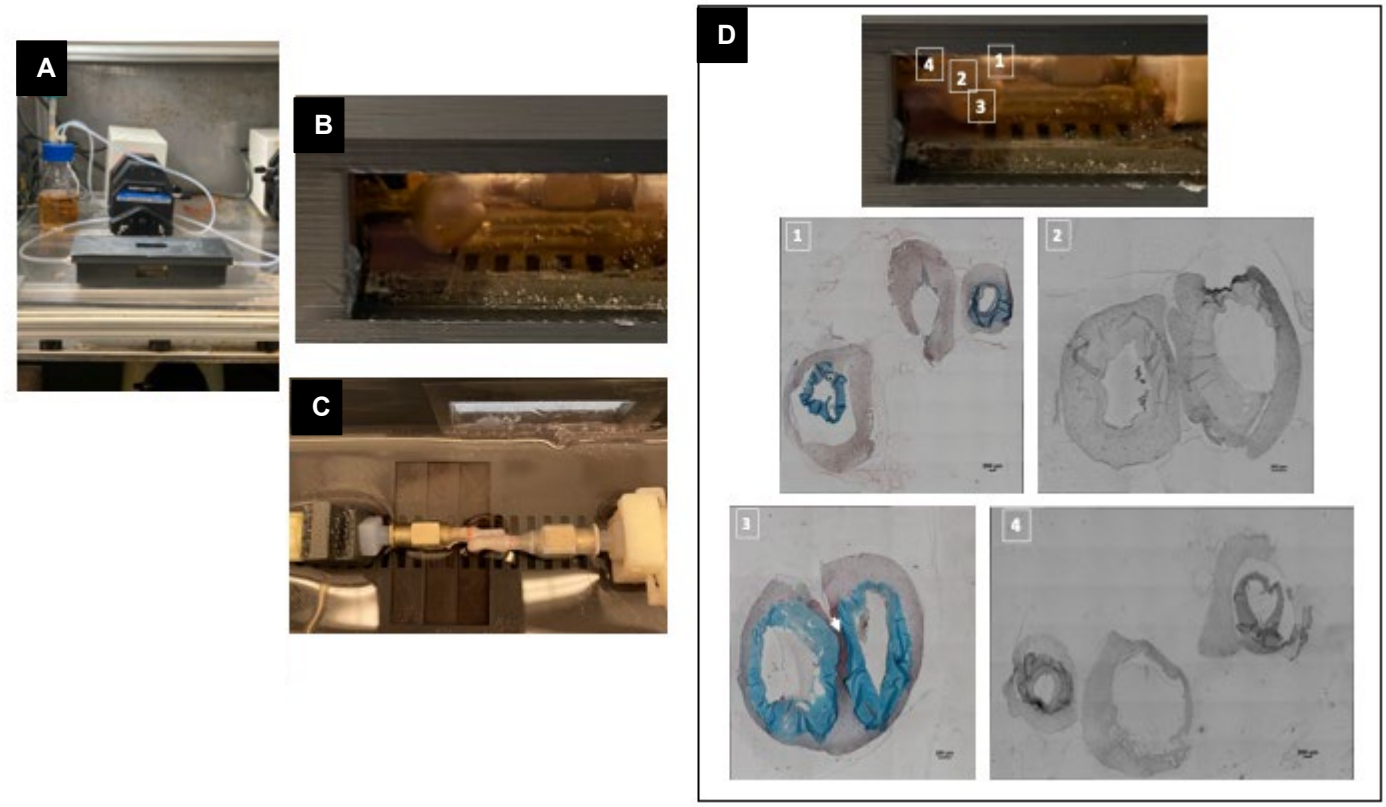

Figure 13: Preliminary Engineered Heart Tube Perfusion Culture and Looping: (A) Heart tubes were perfusion cultured at 1 mL/min. (B, C) Tubes were able to be looped and held in a looped shape for perfusion culture for 7 days. (D) After 7 days, looped tubes were fixed and stained with a Mason's Trichrome Stain. Sections taken from different parts of the looped tube highlight the change in tube orientiation going from the (1) Cranial end to (4) Caudal. Tissues begin to come together (2) and fuse together (4), potentially showing the formation of a ventricular septum as seen by the white arrow.

fluid seen in the embryo and to best keep the tubes alive. Tubes were able to be looped using the bioreactor's external forces (Figure 4b, c). Tubes were cultured for 1 week held statically in the looped position to study the feasibility of this approach. After 1 week of culture, tubes were fixed and stained using a Mason's Trichrome Stain. Tissue cross-sections were taken at different portions of the engineered heart tube (Figure 4d). As the tube goes from the cranial end (1) towards the caudal end (4), the tube can be seen to come together (2) and eventually the tissue fuses together (3, indicated by the white arrow). This fusion looks to be possible ventricular septum formation, this needs further investigation however to be substantiated.

Specific Aim #1: Major Task #2, and Specific Aim #2: Due to Specific Aim #1, Major Task #1 taking longer than anticipated and with restrictions due to staffing from COVID-19 safety precautions, these major tasks do not have any reportable results. However, from the results from Major Task #1, we have shown that the heart tube tissue model is in development and can provide with a viable tissue model that has anatomical features mimicking the developing heart tube, along with bioreactors that can induce the looping phenomenon. Thereby, going forward, further work will be accomplished towards investigating the Major Task #2 and Specific Aim #2 with this developed system and look to incorporate teratogenic agents to our model system.

c. What opportunities for training and professional development has the project provided?

NOTHING TO REPORT

d. How were the results disseminated to communities of interest?

NOTHING TO REPORT

e. What do you plan to do during the next reporting period to accomplish the goals?

NOTHING TO REPORT as this is the final reporting period

4. Impact

a. What was the impact on the development of the principal discipline(s) of the project?

The work performed within this project has resulted in the foundation for a tissue engineering system that models the developing embryonic heart. During normal human development, the heart begins as a simple tube that undoes a phenomenon that causes the heart to loop and fold over itself. The looped embryonic heart then begins to further develop into the adult four-chambered organ. When there is a disruption of the looping phenomenon, birth defects, known as congenital heart defects (CHDs) are formed which result in improperly developed hearts. There are many factors that can contribute to improper looping of the embryonic heart tube. Currently, there are no good tissue-based systems that can test these different factors in order to better understand the development of CHDs. The systems in place to study this are either based upon cells grown in a dish or in mouse models, both of which do not account for the complexities of the human embryo nor do they allow for the control of inciting the CHD through multiple factors. Thereby, this project was focused on developing a tissue engineering system that could mimic the embryonic heart looping phenomenon in order for it to eventually be used as a disease-in-a-dish type of system for further study and potential treatment of CHDs. During this project, we were able to design an engineered tissue that has features reminiscent of the normal heart tube found in the embryo. This heart tube has a small opening which allows for fluid flow throughout it to perform nutrient exchange for proper growth. Furthermore, this tissue was designed to be engineered with human stem cells, which could be used to study specific genetic defects within a patient-specific population. To accompany the engineered tissue, a tissue culture system was engineered. This tissue culture system is able to exert forces upon the engineered tube that mimic the forces seen in the embryo which cause the looping phenomenon to take place. The dynamic culture system has the ability to be highly controllable in order to mimic when these external forces do or do not exhibit themselves properly, further building towards the onset of CHD. Within the reported results from this project, we have demonstrated the foundational work for the development of this tissue model. Going forward, this foundational tissue model could be expanded to further study CHD and be used to study how multiple different factors can shape the onset of CHDs.

b. What was the impact on other disciplines?

NOTHING TO REPORT

c. What was the impact on technology transfer?

This project has the potential to have an impact on technology transfer and technology development. The described engineered tissue model and bioreactor system are both novel in their design and purpose. We have not seen any work prior to this that looked to create such a system. Based upon our results and the potential for our system to be used as an effective disease-in-a-dish system to study whole organ development, we will look to partner with our institution's technology transfer department to try and begin the patent process. As we continue to develop this technology, and if we are able to acquire a patent, protecting our rights to the technology, we would then look to partner our technology with industry. This technology has the potential to be used in drug screening capabilities, and can be expanded upon specific desires from interested industrial partners.

Outside of a strictly technology development standpoint, the results from this project also begin the describe a new thought process we are looking to further develop. We are looking to promote the idea of "engineered development" where we are looking to apply developmental biology knowledge with engineering principles and techniques. Our idea for "engineered development" is for it to become a new practice within the field for which new tissue or organ models could be developed for further study of embryogenesis and the onset of a wide variety of birth defects. Upon the eventual publishing of our current and future results in a scientific article, we aim to describe this new thought process and hope to establish collaborations with other who would share this view point but with specific expertise in different tissue and organ systems.

d. What was the impact on society beyond science and technology?

The described project has the potential to be used as a disease screening platform to study the onset of CHDs. This has a wide-ranging impact on society as the platform could provide new medicines and insights into whether different chemical or environmental agents could affect CHD onset. With this in mind, new understanding of environmental toxins leading to this deadly disease could be identified and highlighted. This identification could lead to the need to develop new technologies and environmental agents. Furthermore this identification could also provide for the need to vocalize the potential dangers found.

5. Changes/Problems

a. Changes in approach and reasons for change

NOTHING TO REPORT

b. Actual or anticipated problems or delays and actions or plans to resolve them

There were some delays in problems in completing aspects of the originally proposed work. This was mainly due to two main reasons. The first being that the first specific aim's major task of creating the tissue model system took longer to design and devise. This was due to some complexities in the creation of a small, perfusable, mechanically robust hydrogel tube that could be loaded into a mechanical stimulating bioreactor. The amount of time needed to test different molds, hydrogels, and crosslinking agents that allowed for a replicable tube to be created was longer than originally anticipated. The second main reason for delays in completing the work is due to constrictions stemming from COVID-19 safety concerns. Due to lessen amount of time from these constrictions, we decided to focus more so on the creation of the tissue model in the first aim due to the complexity of that work. We do believe that with the work completed, we have established a strong foundation to move forward with this work after the terminus of the project award and will look to continue with investigations towards using our established tissue model to study the onset of CHD.

c. Changes that had a significant impact on expenditures

NOTHING TO REPORT

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

NOTHING TO REPORT

6. Products

a. Publications, conference papers, and presentations

NOTHING TO REPORT TO DATE but there is scientific article in preparation with the results described above

b. Website(s) or other Internet site(s)

NOTHING TO REPORT

c. Technologies or techniques

The technology created from this award includes the tissue model system described above. This system, along with the idea of “engineered development” shared in a scientific article that is currently being prepared for submission.

d. Inventions, patent applications, and/or licenses

Currently, there are no inventions or patent applications for the described work. We are beginning to work with our institution’s technology transfer department to try and get patent protection for the tissue model system that was described above.

e. Other Products

NOTHING TO REPORT

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	*Joshua Gershlak*
Project Role:	*Postdoctoral Fellow Research Scholar*
Researcher Identifier (e.g. ORCID ID):	*eRA Commons User Name: jgershlak*
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Gershlak has designed the tissue model system and performed all the analysis to test the tissue model*
Funding Support:	*(this award)*

Name:	*Harald Ott*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*eRA Commons User Name: ottxx057*
Nearest person month worked:	1.2
Contribution to Project:	*Dr. Ott oversaw the creation of the tissue model system and aided in dissemination of the results*
Funding Support:	*(this award)*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

NOTHING TO REPORT

What other organizations were involved as partners?

NOTHING TO REPORT

8. Special Reporting Requirements

NOT APPLICABLE FOR THIS AWARD

9. Appendices

NOTHING TO REPORT