

**AWARD NUMBER:** W81XWH-16-1-0101

**TITLE:** Engineering of Pulsatile Conduits from Human Pluripotent Stem Cell-Derived Cardiomyocytes

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**REPORT DATE:** June 2018

**TYPE OF REPORT:** Annual

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

**DISTRIBUTION STATEMENT:** Approved for Public Release;  
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE June 2018		2. REPORT TYPE Annual		3. DATES COVERED 1 June 2017 - 31 May 2018	
4. TITLE AND SUBTITLE Engineering of Pulsatile Conduits from Human Pluripotent Stem Cell-derived Cardiomyocytes				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-16-1-0101	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Yibing Qyang, PI  E-Mail: yibing.qyang@yale.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Yale University 300 College Street New Haven, CT 06511				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel 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 The primary goal of this project is to develop tissue-engineered pulsatile conduits (TEPCs) to treat single ventricle congenital heart defect using cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSCs). The PI's group has successfully generated unlimited numbers of functional hiPSC-CMs based on robust cardiac differentiation and lactate-based metabolic selection. Engineered heart tissues (EHTs) made by seeding hiPSC-CMs and primary human cardiac fibroblasts into decellularized porcine myocardium matrix has produced robust contractility. The PI has also successfully scaled up EHTs (15x14.5mm) by seeding seven million hiPSC-CMs and three million human cardiac fibroblasts. Additionally, the PI's group has developed novel fibroblast-derived biological glue to wrap EHTs onto the decellularized umbilical artery scaffold effectively to generate TEPCs. Moreover, the PI has established an efficacious bioreactor approach with which TEPCs can be cultured under conditions of biomechanical stretch that enhance the development of contractility and pressure generation of TEPCs. With robust hiPSC-CM derivation, efficient EHT generation and the novel bioreactors that provide biomechanical training to mimic native heart tissue formation, the PI is poised to develop efficacious TEPCs and then evaluate the therapeutic efficacy by implanting TEPCs as the venous interposition grafts in rats in the coming research period.					
15. SUBJECT TERMS Human induced pluripotent stem cells, cardiomyocytes, contractile force					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  Unclassified	18. NUMBER OF PAGES  23	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT  Unclassified	b. ABSTRACT  Unclassified	c. THIS PAGE  Unclassified			19b. TELEPHONE NUMBER (include area code)

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**10. INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The primary objective of this proposal is to establish tissue-engineered pulsatile conduits (TEPCs) using cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSCs) for surgical correction for patients with single ventricle cardiac anomalies that afflicts approximately 1 in 1000 live births. TEPCs will be transplanted into the inferior vena cava of nude rats in order to investigate whether TEPCs can develop into functional pulsatile conduits. Establishment of TEPCs using hiPSC-derived CMs will set the stage for the development of autologous tissue engineered pulsatile conduits for clinical intervention in single ventricle patients.

**11. KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Tissue-engineered pulsatile conduits, human induced pluripotent stem cells, cardiomyocytes

**12. ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

The major goals of the project are to derive unlimited numbers of functional cardiomyocytes from human induced pluripotent stem cells (hiPSCs), establish approaches to highly enrich these cardiomyocytes (hiPSC-CMs) for effective cardiac tissue engineering, develop optimal scaffolds or matrix to support hiPSC-CMs for cardiac tissue formation, and establish robust bioreactor system that can provide biomechanical training for tissue engineered pulsatile conduits (TEPCs) for efficacious contractile force development. The pulsatility and pressure generation of TEPCs will be examined in a rat venous interposition graft model in vivo.

The PI's group has made significant progress on deriving large quantities of highly enriched hiPSC-CMs based on robust cardiac differentiation of hiPSCs and lactate-based metabolic selection. Engineered heart tissues (EHTs) made by seeding hiPSC-CMs and commercially available human cardiac fibroblasts (HCFs) into the decellularized porcine myocardium matrix has showed robust contractility. Large (15x14.5mm) EHTs seeded with  $7 \times 10^6$  hiPSC-derived cardiomyocytes and  $3 \times 10^6$  human cardiac fibroblasts has been used to wrap onto the decellularized human umbilical artery scaffold for TEPC engineering. The PI's group has also developed novel fibroblast-derived biological glue to adhere EHTs onto the decellularized umbilical artery scaffold effectively. Moreover, the PI has established an efficacious bioreactor with which TEPCs can be developed under biomechanical stretch in order to enhance the development contractility and force production of TEPCs. Additionally, the study of marker expression of hiPSC-CMs revealed the absence of pluripotency markers and the presence of cardiomyocyte marker in hiPSC-CMs, indicating that hiPSC-CMs are differentiated cells that do not show pluripotent potential. The karyotyping analysis of hiPSCs revealed the normal karyotype in the PI's previously reported manuscript. The karyotyping and teratoma analyses of hiPSC-CMs are ongoing in the PI's group and will be updated in the near future.

With the availability of robust hiPSC-CMs, efficient EHTs and novel bioreactors that readily provide biomechanical training to mimic native heart tissue formation, the PI's group has made significant progress on the major tasks of this important TEPC project, and is poised to move forward to the major task three by generating efficacious TEPCs and then implanting them as the venous interposition grafts in nude rats in vivo in the coming research period.

### **What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

### **1. Major Activities**

I was an invited speaker at the Samuel and Jean Frankel Cardiovascular Center and Department of Cardiac Surgery Seminar Series, University of Michigan (7/14/2017); and at The University of Alabama at Birmingham Biomedical Engineering Seminar Series (2/28/2018). I also attended and gave talks at International Society for Stem Cell Research (ISSCR) 2017 annual meeting in Boston, American Heart Association Basic Cardiovascular Sciences 2017 Scientific Sessions in Portland, 2018 New England Cardiovascular Tissue Engineering Symposium in New Haven, Connecticut, 2018 NHLBI Cardiovascular Bioengineering Symposium, Birmingham, Alabama, and 2018 Keystone Symposia Conferences iPSCs: A Decade of Progress and Beyond in Olympic Valley, California. I reviewed manuscripts for Am J Physiol, Acta Biomaterialia, Circulation Research, Stem Cells, and Stem Cell Report. I also reviewed Yale scholar grants. My group had biweekly joint meetings on engineered heart tissue with Drs. Stuart Campbell and Laura Niklason. I have attended Yale cardiology faculty meeting, Yale Cardiology Grand Round and Yale VBT Program Seminar Series, Yale Stem Cell Research Forum, and the annual retreat of Yale VBT Program and Yale Stem Cell Center. I have been directing Yale Stem Cell Research Forum and co-directing Yale Myocardial Biology Seminar Series. I also taught a class provided by Yale Stem Cell Center (GENE 655). Furthermore, I mentored graduate students to write NIH F31 (2), AHA (1), NSF (1) predoctoral fellowships and a postdoctoral researcher to write AHA fellowship.

### **2. Specific Objectives**

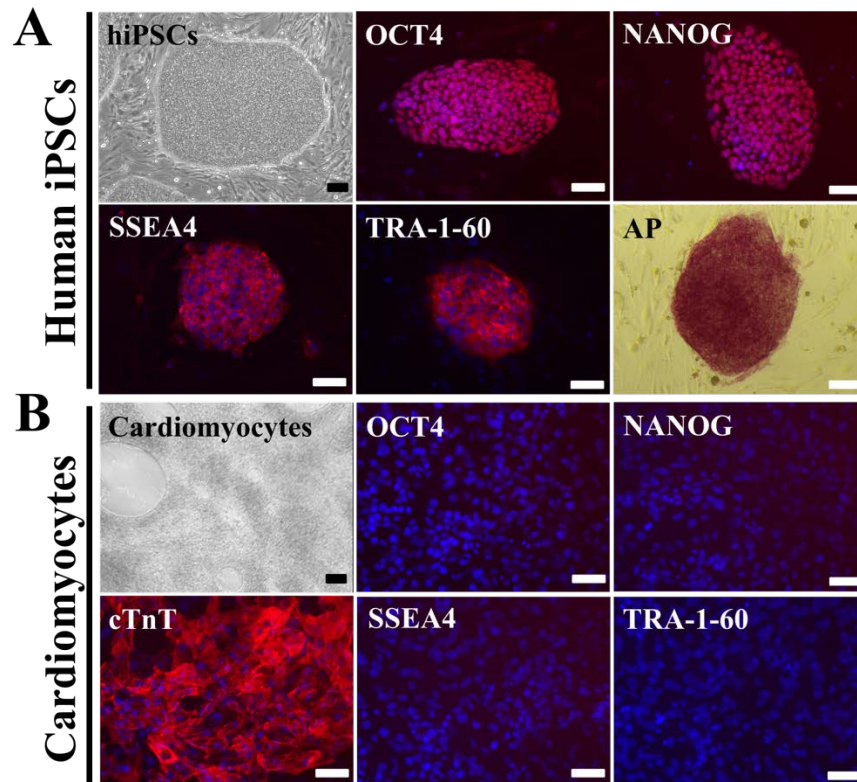
In this research period, the PI would like to examine the expression of pluripotent and cardiomyocyte markers in human iPSC-derived cardiomyocytes. The optimal size of up-scaled large scaffold will be determined in order to generate functional tissue-engineered pulsatile conduits. Additionally, the PI will figure out an efficacious biological adhesive for stable adhesion of the engineered heart tissue to the umbilical artery. Furthermore, the PI will develop a bioreactor approach to effectively train engineered pulsatile conduits under conditions of biomechanical stretch that mimic native heart tissue formation.

### 3. Significant Results or Key Outcomes

#### 3.1. Examination of pluripotency and cardiac marker expression in human iPSC-derived cardiomyocytes (hiPSC-CMs)

We have employed an iPSC line derived by the Yale Stem Cell Center Core from a healthy donor. We examined the expression of pluripotency markers including Oct4, Nanog, SSEA4 and TRA 1-60 and alkaline phosphatase (AP) (Figure 1A). Immunofluorescence staining for pluripotency biomarkers (OCT4, NANOG, SSEA-4 and TRA-1-60) were performed on both human iPSCs and differentiated cardiomyocytes (hiPSC-CMs) (Figure 1). This was done to show that there are no pluripotent cells remaining after differentiation, as evidenced by the lack of expression of pluripotency markers (Figure 1B). To induce a cardiac differentiation, hiPSCs had their Wnt signaling enhanced by a glycogen synthase kinase 3 inhibitor CHIR99021 and subsequently inhibited by IWP4 at the early stage. These cells start to beat around 8<sup>th</sup> to 10<sup>th</sup> day of after initial introduction of CHIR99021. Once the cells start to beat, the PI's group uses metabolic selection by incubating the beating cardiomyocytes and contaminating cells in glucose free, lactate enriched media. This protocol leverages the ability of cardiomyocytes to metabolize lactate which is not possible for other cell types in the population. Our results showed that while human iPSCs were pluripotent evidenced by the expression of pluripotent markers OCT4, NANOG, SSEA4, TRA-1-60 and alkaline phosphatase positivity (Fig. 1A), human iPSC-derived cardiomyocytes were fully differentiated cells that expressed the cardiomyocyte marker (cardiac troponin T: cTnT) but not the pluripotency markers (Fig. 1B).

To further confirm the lack of pluripotency and the safety of using hiPSC-CMs *in vivo*, the PI's group recently injected either two million or five million hiPSC-CMs in left hind limb of Rag2-/-;Il2rg-/- mice along with the same number of hiPSCs in the right hind limb of the same animal as a positive control. The research results will be available 8 weeks after the injection, and reported as the next progress update.



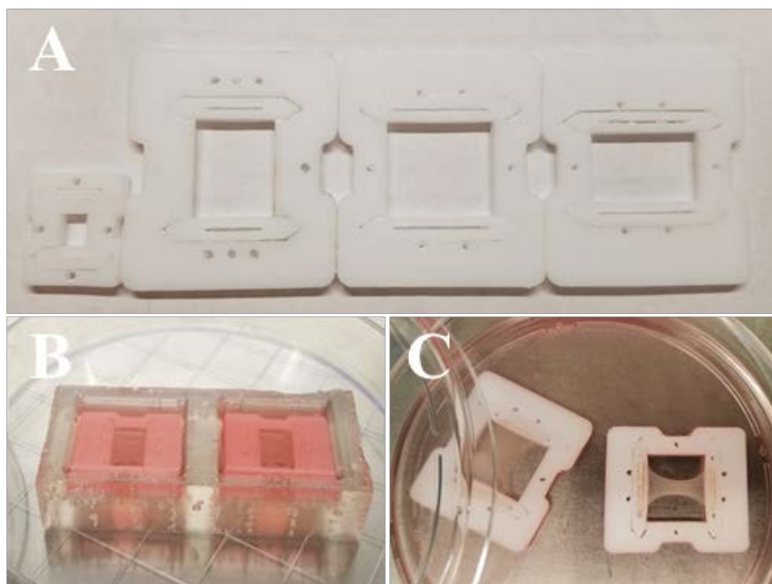
**Figure 1.** Characterization of hiPSCs and hiPSC-CMs. These cells were incubated with primary antibodies diluted in blocking solution (5% normal goat serum in PBS) overnight at 4°C and were washed three times with PBS, then incubated with appropriate secondary antibodies diluted 1:500 in blocking solution for 1 hour at room temperature, and then washed again three times. (A) Typical hiPSC colony and immunostaining with pluripotency markers (OCT4, NANOG, SSEA-4 and TRA-1-60). hiPSCs were positive for all pluripotency markers and alkaline phosphatase (AP). (B) hiPSC-CMs were seeded in 48-well plate and displayed positive staining for cardiac troponin T (cTnT) and were negative for pluripotency markers. Scale bars=100 um

### 3.2. Up-scaled large scaffold to dimensions to generate TEPC

In the last funding period, we compared and contrasted multiple biomimetic scaffold materials, such as polyglycolic acid (PGA), rat tail collagen type I and laser-cut scaffold made from thin sections of decellularized porcine myocardium, and investigate which would be an efficacious material for establishing tissue-engineered pulsatile constructs with hiPSC-CMs. Engineered heart tissues (EHTs) were made by seeding hiPSC-CMs and commercially available human cardiac fibroblasts (HCFs) into decellularized porcine myocardium matrix, and showed effective contractility. We also discovered that decellularized human umbilical artery (HUA) may be suitable as the scaffold to generate pulsatile tubular conduits by wrapping EHT around it.

In the current funding period, different size EHTs were tested for wrapping umbilical arteries to have the most coverage of the vessel width while also having enough length to wrap >1 full revolution about the vessel circumference. At first, tissues were elongated to match the circumference of the HUA at 40mmHg (bioreactor training pressure). However, extra dead length was needed to account for the clips that were handled during manual wrapping in order to avoid CM death from touching the tissue with metal forceps. In the final iteration, extra width

was added to the tissue to account for the general trend towards thinning of the EHT into an hourglass shape during static culture. These large (15x14.5mm) EHTs are derived from 150um thick section of porcine left ventricular myocardium that are laser cut into the specified dimensions and mounted into Teflon frames (Figure 2A). These tissues are subsequently decellularized using 0.5% SDS solution and primed with media overnight. The following day, prepared scaffolds are seeded with  $7 \times 10^6$  hiPSC derived cardiomyocytes and  $3 \times 10^6$  human cardiac fibroblasts acquired from a commercial vendor. Cells are seeded onto the scaffold inside a polydimethylsiloxane (PDMS) seeding bath designed by the PI's group (Figure 2B). Seeded EHTs statically culture in the Teflon frame for 4 days before wrapping a vessel (Figure 2C).



**Figure 2.** Up-scaled large scaffold to dimensions to generate TEPC suitable for rat IVC implantation. (A) Original frame and up-scaled from the original dimensions to produce EHTs of three different sizes (3x4mm, 10x14mm, 15x14 and 15x10mm). (B and C) 15x14mm of frame currently employed for producing large scaffolds, since it was suitable for wrapping HUA. (B) Seeded 15x14mm EHTs in PDMS seeding well. (C) 15x14mm EHTs 4 days after seeding. Total 10 million cells (7 million of CMs and 3 million of HCFs) were seeded onto a large scaffold in the seeding bath.

### 3.3. Trials for wrapping EHT on HUA and the discovery of novel human cardiac fibroblast-based biological glue for beating conduit production

A biological adhesive was necessary for stable adhesion of the engineered heart tissue to the umbilical artery. This has been a big challenge for beating conduit engineering. Very excitingly, after investigating multiple biocompatible glues for adhesive efficacy and ease of handling (Table 1 below), the PI's group has made a huge progress and discovered a novel, efficacious bio-glue derived from human cardiac fibroblasts, as described below.

First, gelatin glue was tested in a 100mg/mL concentration both with and without a crosslinking agent (EDC). While gelatin appeared to work initially, the gel degraded and the tissue detached at 37°C. To address this, a crosslinking agent (EDC) was added to the gelatin glue to add stability at higher temperatures. Several titrations were assessed between 100mg/mL gelatin and 50mg/mL EDC for a mixture that showed good adhesiveness without becoming too stiff (Figure 3A). However, this glue was still weakly adhesive and the tissue would become detached during static culture.



Next, a fibrin based glue was tested. A mixture of fibrin and thrombin were mixed to a final concentration of 72mg/ml and 10U/ml respectively. The mixture contained 10mM of calcium to aid in coagulation. Immediately after mixing, fibrin glue mixture was coated around the HUA and EHT. However, all tested combinations of fibrin/thrombin made a thick encasing around the vessel which impeded beating (Figure 3B).

A covalent crosslinker, polyethylene glycol (PEG), was tested as the next adhesive candidate because it covalently crosslinks collagen fibers to one another. Seeing as collagen is abundant in the decellularized umbilical artery and the engineered heart tissue, this seemed like a natural choice. PEG was dissolved in DMSO and diluted in PBS to a final concentration of 5-10mg/ml. Decellularized umbilical arteries were coated in these mixtures for 30 minutes at room temperature on a rod before wrapping. This crosslinker generally showed weak adhesion of the EHT to the vessel that would unravel over time (Figure 3C).

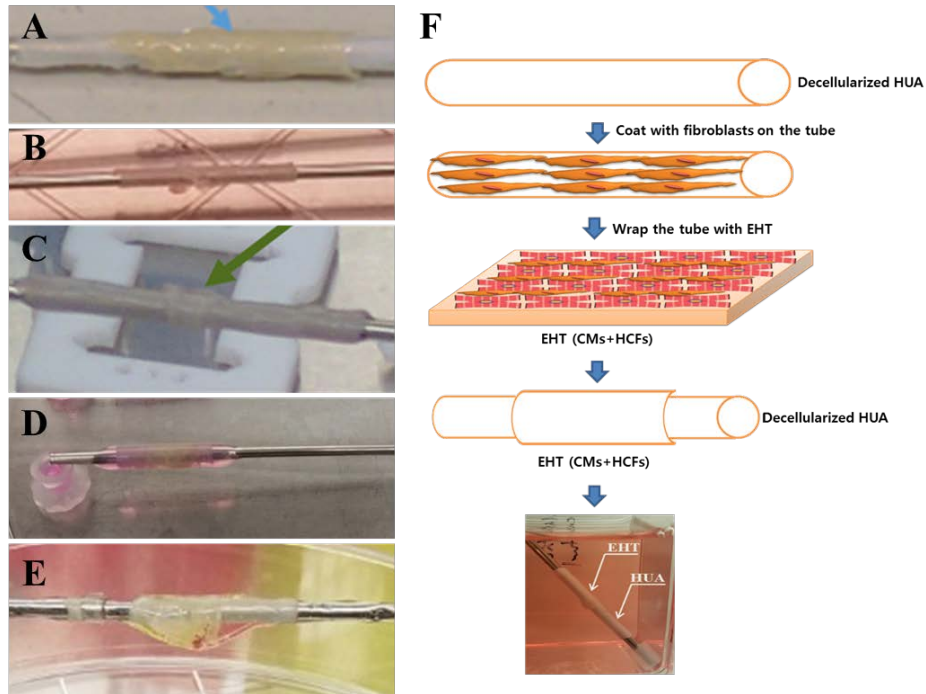
Next PI's group tested a collagen gel encasing around the wrapped vessel to hold the tissue in place (Figure 3D). 500ul of a 2 mg/ml or 3mg/ml solution of collagen gel, (10xDMEM and 1M NaOH mixed at pH10) was coated around the vessel and EHT inside a 1ml syringe and polymerized for 1hour in the cell incubator. These collagen tubes were too weak to be sutured into the bioreactor for training.

A recent publication noted that chitosan could be used as a biological adhesive during surgery, and this chitosan formula was tested in our hands. Chitosan was dissolved at 2.5% in AES buffer before wrapping. However, after wrapping, there was visible damage to the cells in addition to an unraveling of the tissue (Figure 3E). This may be due to chitosan's solubility being capped for solutions at pH <6.5.

Finally, the PI's group tested a coating of fibroblasts on the exterior of the HUA to help adhere the tissue through focal adhesions with the EHT cardiomyocytes (Figure 3F). 1.5 million fibroblasts in 400ul of media were coated on the HUA after pre-coating with 0.1% gelatin for 30 min in a petridish for 1 hour. An EHT was manually wrapped on the HUA to produce a TEPC. TEPC was cultured in T75 flask for five days. This novel strategy shows an excellent adhesion efficacy and is very consistent. The PI's group is continuing experiments using this exciting and efficacious adhesion method.

**Table 1.** Summary of glues used for wrapping

Glue type	effects of glue
Gelatin glue	weak adhesion
Fibrin gel glue	made EHT solidified
Polyethylene Glycols (PEG)	not consistent and weak adhesion
Commercial super glue	made EHT solidified
collagen gel (2mg/ml and 3mg/ml)	both of dose were too weak to mount to bioreactor
Chitosan glue	weak adhesion and damage to cells
fibroblasts	highly consistent and working very well



**Figure 3.** Several types of glues were tested for wrapping. (A) Gelatin glue. (B) Fibrin gel glue. (C) Polyethylene Glycols. (D) Collagen gel. (E) Chitosan. (F) Schematic of using human cardiac fibroblasts as a bio-natural glue, which has showed an excellent efficacy in assisting the adhesion of engineered heart tissue (EHT) around decellularized human umbilical artery for the development of beating conduit.

### 3.4. Bioreactor training and mechanical assessment

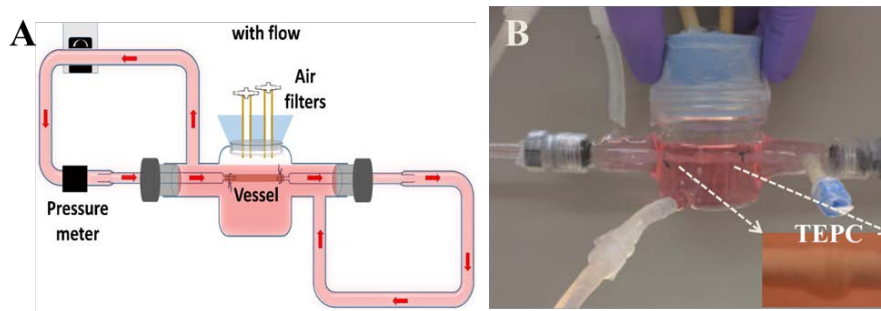
In order to produce a robust TEPC, the PI's group is developing an in vitro bioreactor training strategy to improve cardiomyocyte contractility by providing mechanical stimulus to the TEPC via luminal stretch. The current training strategy was intended to mimic the rat IVC environment as this is the animal model the PI's group intends to use for in vivo studies with trained TEPCs.

Using a peristaltic pump connected to a flow bioreactor (Figure 4A) media can be perfused to impart varying degrees of luminal stretch based on the pulse pressure extruded through the TEPC lumen. TEPCs are trained for 14 days in this bioreactor under cyclic stretching with a maximum luminal pressure value of 40mmHg. At the end of the 14 day training period, a pressure sensor is hooked up to the bioreactor and the plastic tubing preceding the vessel is clamped to reduce noise in the measurement. There seemed to be a small conduit pressure when the pump was off (Table 2 below). Additionally, the PI's group has observed under the microscope that the vessel showed appreciable beating after the 14-day bioreactor training period. To confirm whether such pressure is generated by the TEPCs, the PI's group will prepare decellularized HUA without the wrapping of EHT, and then place it in the bioreactor under the same biomechanical training conditions.

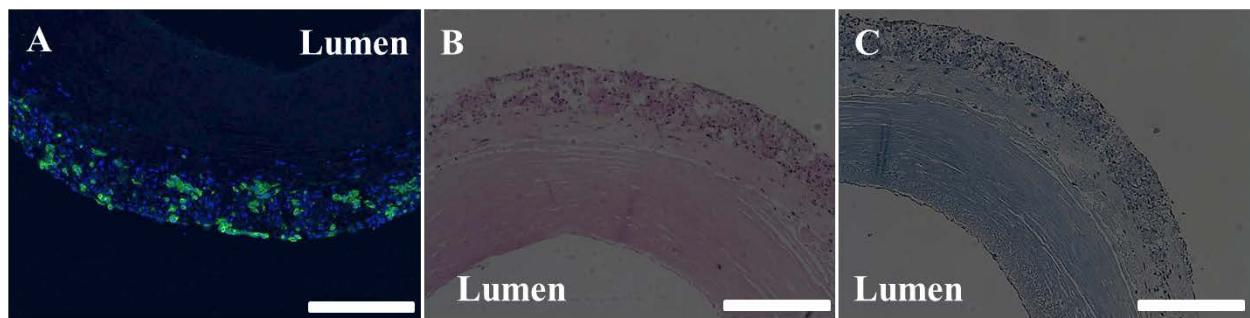
Moreover, there are several ways that the PI's group is currently investigating how to enhance TEPC contractility and pressure generation. One possibility is to increase the compliance of the umbilical artery since it may be too stiff for the cardiomyocytes to contract. Another potential issue could be that the cardiomyocytes in the tissue are not contracting in synchrony due to the

immature phenotype of iPSC derived CMs. To this end, the PI's group is currently developing a strategy to electrically pace the TEPC during force measurements and during the training phase to improve electrical handling of the tissue.

The PI's group is also currently performing histological analyses on trained tissues (Figure 5). Cardiac Troponin T (cTnT) is used as a marker for cardiomyocytes to determine their distribution within the tissue (Figure 5A). Hematoxylin and Eosin (H&E) stains are assessed in conjunction with Masson's Trichrome to observe tissue level organization and potential remodeling of the extracellular matrix by the cardiac fibroblasts (Figure 5B and C). CMs seemed to be throughout the engineered heart tissue wrapped around the umbilical artery, and EHT maintained as an intact tissue outside of umbilical artery. There was minimal or no cell infiltration from the engineered heart tissue to the umbilical artery in this 14-day period. Longer studies are required to follow up the remodeling of TEPCs.



**Figure 4.** Training of TEPC. (A) Schematic of flow bioreactor system used to train TEPC. Media is aspirated from main reservoir via peristaltic pump and passed through lumen to impart mechanical force to the vessel via luminal stretch. Media is filtered back into main reservoir to provide adequate mixing of media in this closed loop system. (B) Image of TEPC in flow bioreactor system.



**Figure 5.** Histological assessment of TEPC after training in bioreactor. TEPC was stained by cTnT to determine arrangement of cardiomyocytes in the tissue (A), H&E (B) and Trichrome (C) staining was performed to look at the general topology of the tissue. Scale bars=100  $\mu$ m.

**Table 2.** Summary of the measurement of force from TEPCs

Exp#	Experiment Duration	Final Cycle Frequency	Final Pressure that pump applies	Conduit pressure when pump is off
1	7 days	0.5 Hz	38 mmHg	0.56 mmHg
2	7 days	0.5 Hz	38 mmHg	0.71 mmHg
3	7 days	0.5 Hz	38 mmHg	0.54 mmHg
4	8 days	0.5 Hz	39 mmHg	0.63 mmHg
5	7 days	0.5 Hz	19mm Hg	1.19 mmHg
	10 days	1.2 Hz	24 mmHg	1.36 mmHg
	14 days	2 Hz	39 mmHg	1.49 mmHg

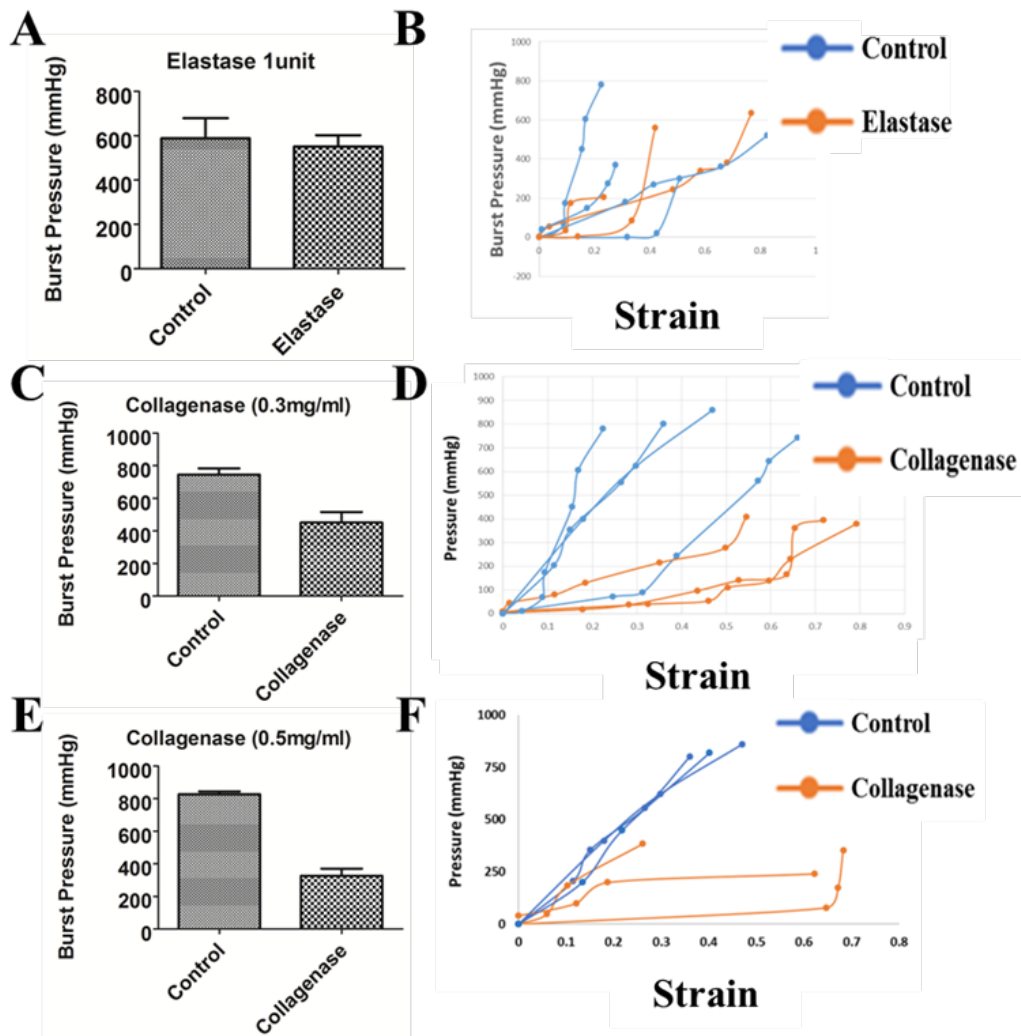
### 3.5. Optimizing mechanical parameters of decellularized human umbilical artery for production of contractile TEPC

In order to obtain more effective pressure generation from TEPCs, the PI has attempted to increase the compliance of the umbilical artery scaffold, since the umbilical artery may be too stiff for the EHT to contract. To look into this hypothesis, the PI's group has tested the mechanical properties of decellularized umbilical arteries before and after different protease treatments to look for changes in compliance of the vascular scaffold (Figure 6).

Several sample vessels were incubated with either elastase or collagenase of a given concentration in 37°C for 20 minutes. These treated vessels would then be sutured into a specialized flow chamber that would impart luminal pressures of known values into the vessel. A high-resolution camera was used to track distension of the vessels at different luminal pressure values and the strain was subsequently calculated from this data. Plots of pressure vs. strain are shown in figure 6 as a proxy for the compliance of the treated umbilical arteries. After collecting this deformation data, burst pressure was assessed by gradually increasing luminal pressure until the failure point of the vessel was reached.

Elastase treatment was tested at 1unit but did not have a discernable effect on the burst pressure or compliance of the vessel (Figure 6A, B). Elastase may not have been able to make a discernable change due to a low abundance of elastin in distal arteries. However, the PI's group is currently testing elastase treatments at higher concentrations to be thorough.

Collagenase treatments were tested within the range of 0.1-0.5mg/ml. At 0.1mg/ml, there was no discernable change in the mechanical properties (compliance and burst pressure) when compared to the untreated controls (Data not shown). However, at 0.5mg/ml, the vessel became too weak to implant into the bioreactor (Figure 6E, F). Collagenase treatment at 0.3mg/ml showed an increase in compliance when compared to untreated controls (Figure 6C, D).



**Figure 6** Protease treatment for the optimization of compliance of TEPC scaffolds. We measured the burst pressure and made burst pressure to strain curve by the treatment of 1 unit of elastase (A and B), 0.3 mg/ml of collagenase (C and D) and 0.5 mg/ml of collagenase (E and F) with control.

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

*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Dr. Jinkyu Park has further broadened his research expertise from stem cell biology into cardiovascular tissue engineering. Specially, he has developed a novel fibroblast-derived biological glue approach for wrapping engineered heart tissue onto the decellularized human umbilical artery scaffold. Additionally, he has collaborated with Dr. Laura Niklason group to develop a bioreactor system to enhance the contractility and pressure generation of the engineered pulsatile conduits. Finally, Christopher Anderson, a graduate student in the PI's group, has mastered cardiomyocyte derivation technology using human iPSCs. Moreover, he has upscaled small EHTs into large EHTs for pulsatile tissue construct development.

**How were the results disseminated to communities of interest?**

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

*Nothing to Report*

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

*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

The PI's group plans to pursue several avenues to improve upon the current design strategy for producing TEPCs in the coming research period. In terms of the vascular scaffold, protease treatments to increase the compliance of the tube are ongoing. Our group is hopeful that these treatments will produce a vessel that can be easily contracted by our engineered heart tissue to produce a robust luminal pressure. As a potential alternative, the PI's group is also investigating alternative scaffolds. The PI's group is currently looking into a promising new scaffold material derived from 3D printed vessels available from the Geibel lab here at Yale. These vessels are produced by 3D printing cell laden hydrogel inks in successive layers. The layer closest to the lumen contains vascular endothelial cells, followed by several layers of vascular smooth muscle cell containing ink. These vessels are cultured statically for four days to allow for initial remodeling of the tissue. Video provided from the Geibel lab showed a very sturdy, yet elastic product. More in depth mechanical assessments of these vessels are currently in progress by the PIs group. Using this synthetically produced scaffold is expected to increase both the efficacy of our TEPCs in producing luminal pressure and increase the stability of our product. One challenge noted in using biologically derived scaffolds is that the exact mechanical properties are

not identical between samples derived from different donors. As seen in figure 6, this variability can be magnified in mechanical assessments of these tissues. Using a synthetically derived scaffold will ensure the mechanical properties of the vessel are tightly conserved between batches and within the control of the PI's group to optimize for our needs.

Additionally, the PI's group is also developing modifications to the current bioreactor system to impart electrical field stimulation to train and test the TEPC. One potential reason the previous TEPC batches have not been producing robust pressure changes is that the cardiomyocytes may not be contracting in synchrony. Acute electrical pacing during mechanical assessment would ensure that all cardiomyocytes are synchronized during the test. Additionally, the induction of electrical stimulus during the training phase is expected to improve electrical handling of the tissue overall. Cells within the TEPC are expected to spontaneously beat at the same frequency, as determined by the electrical training protocol. Cardiomyocytes that undergo electrical training have also been noted to increase gap junction formation to better allow conduction of electrical signals through the tissue itself. The PI's group is also modifying their mechanical training protocol to provide both systole and diastole conditions to the TEPC as a biomimetic training approach. The PI expects to develop TEPCs with significant contractility and pressure generation that can be implanted into a rat venous interposition graft model after the above optimizations.

The PI will continue the joint group meetings with Drs. Stuart Campbell, Lawrence Young and Laura Niklason, to expand his research in cardiovascular tissue engineering and physiology. To further support his research in cardiovascular biology, he will also join Yale Cardiology Grand Round and Yale VBT Seminar Series, the Yale Pathology Grand Round, the Yale Cardiology Faculty meeting, the Yale Cardiology/VBT annual retreat, the Yale Stem Cell Center annual retreat, and the Yale Stem Cell Center monthly Research Forum. Additionally, he will plan to attend external scientific meetings such as American Heart Association's Scientific Sessions. He will continue to direct the monthly Yale Stem Cell Research Forum and Yale Myocardial Biology Seminar Series to help to stimulate the collaborations amongst Yale stem cell and cardiac researchers. He will commit himself to assist and contribute to the cardiovascular and stem cell research communities through reviewing grant proposals and scientific manuscripts. The PI plans to submit one research manuscript and two review manuscripts (Invited reviews) based on the support from this funding. Additionally, the PI would like to submit one external proposal to obtain extra funding to further support this important TEPC project.

- 4. IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

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

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

The engineered heart tissue (EHT) technology allows for controllable, and directed fiber orientation by seeding hiSPC-derived cardiomyocytes onto decellularized porcine heart matrix. Additionally, these EHTs are optimizable in terms of cell composition. These innovations will improve the efficiency of force generation of the tissue. The novel application of biomechanical stretch and electrical stimuli to train TEPCs *in vitro* enables robust contractile force generation. This will allow for a second level of optimization and quality control that is not possible with *in situ* development strategies, leading to novel therapeutic interventions in treating single ventricle congenital heart disease.

### **What was the impact on other disciplines?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

The approach of using biomechanical stretch and electrical stimuli to train engineered pulsatile conduits *in vitro* for highly efficient contractile force generation will likely inspire the development of other engineered tissue constructs under electrical and biomechanical stimulation for effective extracellular matrix production as well as tissue remodeling and maturation in the near future.

### **What was the impact on technology transfer?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

*Nothing to Report.*

### **What was the impact on society beyond science and technology?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*



*Nothing to Report.*

- 5. CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

**Changes in approach and reasons for change**

*Describe any changes in approach during the reporting period and reasons for these changes.*

*Remember that significant changes in objectives and scope require prior approval of the agency.*

*Nothing to Report.*

**Actual or anticipated problems or delays and actions or plans to resolve them**

*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

*Nothing to Report.*

**Changes that had a significant impact on expenditures**

*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

*Nothing to Report.*

*reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

*Nothing to Report.*

**Significant changes in use or care of vertebrate animals.**

*Nothing to Report.*

**Significant changes in use of biohazards and/or select agents**

*Nothing to Report.*

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

**Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

*Nothing to Report.*

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Cardiac Tissue Engineering (Invited book chapter ready for submission), Encyclopedia of Tissue Engineering and Regenerative Medicine, Luke Batty, Matthew Ellis, Christopher Anderson and Yibing Qyang.
2. Cardiovascular Regenerative Medicine: Tissue Engineering and Clinical Applications (Invited book chapter in preparation). Springer Nature, Matthew Ellis, Christopher Anderson, Luke Batty and Yibing Qyang

**Other publications, conference papers, and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (\*) if presentation produced a manuscript.*

*Nothing to Report.*

- **Website(s) or other Internet site(s)**

*List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

*Nothing to Report.*

- **Technologies or techniques**

*Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.*

*Nothing to Report.*

- **Inventions, patent applications, and/or licenses**

*Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

*Nothing to Report.*

- **Other Products**

*Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the*

*understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

*Nothing to Report.*

## **7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

### **What individuals have worked on the project?**

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”*

Name:	Yibing Qyang, Ph.D.
Project Role:	PI
Nearest person month worked:	1.0
Contribution to Project:	Dr. Qyang leads the project as the principal investigator and is responsible for the general management and development of this proposed study

Name:	Jinkyu Park, Ph.D.
Project Role:	Other personnel (Postdoctoral scientist)
Nearest person month worked:	5.0
Contribution to Project:	Dr. Park has derived unlimited numbers of functional cardiomyocytes from human induced

pluripotent stem cells (hiPSCs), developed engineered heart tissues, and collaborated with Dr. Niklason's group to generate tissue-engineered pulsatile conduits (TEPCs) by wrapping the EHT around the decellularized umbilical artery scaffold.

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

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

*Nothing to Report.*

**What other organizations were involved as partners?**

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*

*Organization Name:*

*Location of Organization: (if foreign location list country)*

*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

*Nothing to Report.*

## **8. SPECIAL REPORTING REQUIREMENTS**

*Not applicable.*

**COLLABORATIVE AWARDS:** N/A

**QUAD CHARTS:** None

## **9. APPENDICES:** None