Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University – Of The Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Research Project 17: Stem Cell Medicated Repair of the Ischemic Heart

7. **Start and End Date of Research Project:** 01/01/2011 – 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Steven R. Houser, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 157,353.99

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
N/A			

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Houser, Steven R.	Director, CVRC	10%
Berretta, Remus	Laboratory Manager	5%
Thomas Sharp	Research Assistant – Graduate Student	5%
Hajime Kubo	Laboratory Director	5%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
AD Instruments	Ability to visualize pressure-volume relationship	46,828

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes___X_____ No_____

If yes, please indicate the source and amount of other funds:

Departmental funding was used to support the purchase of supplies for this project.

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X___ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
HL33921 that just received a 9% at NHLBI	X NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	11/5/2013	$2,649,458	$2,044,762

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes__X____ No_____

If yes, please describe your plans:
We have used the information obtained from these projects to apply for a new NIH R01 grant. The new NIH grant was just reviewed and obtained a score that should be within the current pay line. Our goal is to extend the science into a test of a novel stem cell that we believe might enhance cardiac repair after injury.

12. Future of Research Project. What are the future plans for this research project?

We are planning to determine if altering the function of the class of Calcium channels (t-type Calcium channels) studied with State support can induce proliferation and commitment of cardiac stem cells into new cardiac tissue.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X___ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female			0	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other			1	
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No___x_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___x_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. <u>Provide detailed results of the project.</u> Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations

at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Background. Ischemic heart disease commonly results in myocardial infarction (MI). MI causes the death of affected cardiac myocytes and supporting tissues, leading to pathological cardiac remodeling and depressed cardiac pump function, subsequently leading to heart failure (HF). One major goal of the field is to study novel therapies which may improve cardiac function after MI. We investigated the hypothesis that intramyocardial injection of bone marrow-derived stem cells would aid in the regenerative process by replacing the myocytes and vasculature that have been lost by the ischemic event decreasing pathological remodeling and dysfunction.

The Swine Ischemia-reperfusion model: We have the capacity to perform surgical procedure to induced an ischemic event and permit reperfusion (post ischemia) to mimic the events observed with patients who present to the Emergency Department with an MI. This facility also allows for the follow-up evaluation of these large animals for a prolonged period of time to observed changes in structure and function, with or without stem cell intervention. Ischemia is produced by placing an angioplasty balloon within the left anterior descending coronary artery (LAD). The balloon is then inflated for certain time (usually 90 minutes). This time period inhibits blood flow to distal areas creating an infarction to a region of the myocardium. In previous publications we have demonstrated that each procedure is consistent and that the infarct size and region are predictable with no significant difference. When animals were randomized to treatment group, they will receive the therapy immediately post MI via injection into the myocardium through a catheter-based delivery system. The animals have been followed out over a period of three months to be able to observed any change in cardiac function with or without treatment. This large animal model is vital for preclinical testing of novel therapies and delivery systems to demonstrate efficacy and safety. This model is the gateway between newly developed therapeutics and their targets to become eligible for commercialization and FDA approval for clinical use.

Cell and Gene Therapy Development:

We have developed methodology to isolate a sub-population of stem cells from a bone marrow aspirate. The cells are selected by specific markers found on the cell surface. The cells are then expanded in culture and labeled with green-fluorescent protein (GFP) for proof-of-concept. This allowed us to identify newly formed myocytes and vascular tissue from the exogenous cells introduced post MI. Gene Therapy: We have utilized the virus core which has developed novel constructs to target molecular pathways and modify protein expression to also limit the level of pathological remodeling and dysfunction observed post MI. Constructs that have been tested include an AAV-mediated gene transfer of bARK-CT (to normalize adrenergic signaling defects) and a novel caveolin targeted Ca channel blocker, termed Cav-Rem1-265.

Aim 1. We will determine if, and by what mechanism cKit+ bone marrow derived stem cells improves cardiac structure and function in adult pigs after MI

Progress made on aim 1:
Aim 1, is under completion. We've characterized the pig model and are now performing stem cell injections to observe changes in cardiac pump function and morphometric analysis. Ischemia reperfusion-induced MI was performed in all twelve minipigs to characterize the dysfunction over a three month period. Animals were then evaluated for a 12 week period post MI. To evaluate pathological manifestation and treatment, we performed serial transthoracic echocardiography at 5 time points throughout the duration of the study (Baseline, 4, 8, 12 weeks post MI). NOGA electromechanical mapping was also utilized for assessment of function.

METHODS

Ischemia-reperfusion induced Myocardial Infarction

The right femoral artery was cannulated with a 6-French percutaneous sheath and femoral venous access was also acquired with a 5-French percutaneous sheath for administration of any necessary pharmacological intervention. To prevent thrombosis throughout the duration of the procedure, heparin was administered with a loading dose of 100U/kg and a maintenance dose of 40U/kg every hour. A 5-French pigtail catheter was placed in the left ventricle and a ventriculography was performed. A 6-French guide catheter was used to cannulate the left coronary artery to perform arteriography (figure 1). Next, a 0.014-in angioplasty guide wire was place in the left anterior descending coronary artery (LAD), then a 3.0mm x 12mm Quantum Maverick, monorail, balloon (Boston Scientific, M.A. USA) was placed in the LAD just distal to the first diagonal branch, all placements were performed under fluoroscopic guidance and recorded. The balloon was then inflated to 8.0 atmospheres for a period of 90 minutes and cessation of flow distal to the balloon was confirmed with another angiography (figure 1). ST-segment elevation was also used to assess onset on infarction (figure 1). After deflation of the balloon, the guide wire and balloon were removed and reperfusion of the LAD was confirmed by angiography (figure 1). Next, the guide catheter was removed and the animal was given 30 minutes of reperfusion prior to any subsequent treatment being performed.

Transthoracic Echocardiography

Transthoracic echocardiography was performed at baseline, 4, 8 and 12 weeks post MI with the Zonare z. one ultra Ultrasound system on all animals. Images were acquired in the 2D-mode in long- and shot- axis; while m-mode images were taken in the short axis. All measurements including end-diastolic dimensions, end-systolic dimensions, end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF) and fractional shortening (FS) were calculated by the American Society of Echocardiography guidelines.

NOGA Electromechanical Mapping and Intramyocardial Injections

Electromechanical assessment was performed pre- and post-MI to confirm the ischemia-reperfusion MI induction. This catheter based technique acquires 80-100 discrete points by contact with the endocardial surface. We're able to assess the changes in conductance and motion of the left ventricular myocardium. This technique also allows us to localize our injection sites, for accurate delivery of therapy via transendocardial injections (figure 2).

Tissue Processing & Gross Morphometric Analysis

Cardiectomy was performed under general anesthesia 12 weeks post MI. The heart was weighed; then the bottom third was sliced in cross-section. This portion was cut into two short-axis slices; the basal slice was then sectioned and frozen immediately in liquid nitrogen, then stored at -80°C for future protein analysis; the apex was submersion fixed in 10% formalin for histological analysis. The intact two-thirds of the heart were fixed via gravity perfusion with 2-liters of 10% formalin. Once fixed, this portion was sectioned into 3 short-axis slices. Each slice (including slice for protein analysis) was measured for mean thickness prior to further processing. The basal surface of each slice was photographed using a Nikon DS-F11 camera. The infarct area was identified by the scar, while the viable area was healthy tissue and both were measured using NIH Image J software. The infarct volume was calculated by multiplying the infarct area of each slice by the average thickness, this calculation was also performed for the viable area. The percent infarct volume of each heart was determined by adding the volume of all slices and dividing by the total infarct area.

RESULTS

Gross and Histological Analysis Post MI

Upon cardiectomy, the infracted region is distinguished from other cardiac tissue through coloration and anatomical location (pale white/anterior-apical) (Figure 2). This gross visual observation confirms the location of the infarct to be distal of the occlusion via balloon angioplasty performed within the LAD (observed on fluoroscopy [Figure 1]). To assess hypertrophy we weighed the hearts of control (n=6) and post MI animals (n=12) and looked at the heart-to-body weight ratio (HW/BW ratio). There was a significant increase in the HW/BW ratio in the post MI group versus control, demonstrating the manifestation of cardiac hypertrophy at 12 weeks post MI (Figure 2). After fixation, we then sliced the heart into cross-section to observe the scar on the anterior, septal, and apical regions (Figure 2). To quantify the infarct, we assessed the infarct volume as a percent of the total ventricle volume at 12 weeks post MI to be 17.29 ± 3.56% (Figure 2). There was no significant difference in the infarct size between control MI and RBX treated animals (data not shown), suggesting any functional benefit observed was due to the administration or absence of acute RBX treatment.

After tissue processing for histological analysis we performed H&E staining to investigate changes in cellular morphometry. In Figure 3, we see representative H&E images using bright-field microscopy (Column 1 [left]) of three distinct zones which are created due to the ischemia-reperfusion injury (Infarct Zone [I.Z.], Border Zone [B.Z.] and the Remote Zone [R.Z.]). The I.Z. bright-field image, Figure 3A (Row1) demonstrates that there is a frank loss of myocytes within this region and is replaced by fibrous tissue. With immunohistochemistry we observed a lack of α- sarcomeric actin positive cells but saw preservation of the vasculature

structure with smooth muscle actin positive vessels at 12 weeks post MI. The B.Z. bright-field and immunohistochemistry, Figure 3B (Row 2), allows us to distinguish between viable and non-viable tissue with the absence or presence of α-sarcomeric actin. We are still able to observe intact vasculature structure within the B.Z. The R.Z., Figure 3C (Row 3), demonstrate clear intact structure of the myocardium with no significant amount of fibrous tissue at 12 weeks post MI. The confocal images of the R.Z. recapitulate the findings of H&E staining. To confirm the observation of hypertrophy in the HW/BW ratio at 12 weeks post MI, we measured myocyte-cross sectional area from H&E stained tissue sections of the control and MI groups. In figure 4, we illustrate the distinct differences in myocyte cross section between group's at all three zones (IZ, BZ and RZ). The mean LV myocyte cross-sectional area was significantly increased at 12 weeks post MI vs. control (572.66 ± 30.98 μm^2 vs. 362.26 ± 30.47 μm^2, respectively). There was a significant increase in myocyte cross-sectional area when analyzing the two distinct anatomical regions of viable tissue (B.Z. and R.Z.). There was a significant increase in the BZ post-MI (653.25 ± 43.59 μm^2) versus control (360.92 ± 27.46 μm^2) (Figure 4). The large increase in myocyte cross-sectional area in the BZ suggests that the cells within this zone are under great mechanical stress; therefore to compensate for this we see an increase in cell size. The RZ post-MI (504.58 ± 45.73 μm^2) as compared to control (363.89 ± 34.57 μm^2) was also significantly increased (Figure 4).

Cardiac Function Analysis

A decrease in cardiac pump function was observed over the three month period post MI (Figure 5). This confirms that we are indeed creating an animal model where there is significant loss of myocardium, leading to a deficit in cardiac pump function. The LVEF was significantly reduced from 69.27 ± 1.92% at baseline to 46.30 ± 3.53% at just 4 weeks post MI. Dysfunction continued to decline at 12 weeks post MI where the LVEF was 32.68 ± 2.88%. The LVEF over the 12 week period declined at 4 weeks post MI but did not significantly change at 8 weeks post MI; this observation demonstrates that the heart is in a state of compensation, despite there being some dysfunction. The continued decrease in LVEF from 8 weeks post MI to 12 week post MI suggests that the heart has entered a state of decompensated HF at this time point. A significant decline in the FS was also observed from baseline to 4 weeks post MI (37.89 ± 1.50% to 22.91 ± 2.12%, respectively). At 12 weeks post MI the FS was reduced to 15.54 ± 1.67%. These data implicate there is decrease in function 4 weeks post MI which is sustained through 8 weeks, and at 12 weeks post MI the contractile function of the heart decompensates.

Echocardiography also demonstrated a significant increase in chamber dilation through 12 weeks (Figure 5 & 6). This confirmed that the heart was remodeling over a 12 week period due to the induced cardiac injury. In Figure 5C., the EDV significantly increased at 8 weeks post MI, from 39.01 ± 3.47 ml at baseline to 63.6 ±5.55 ml, respectively. Further increase in the EDV was observed at 12 weeks post MI. Similar results were observed in the ESV; there was a significant increase from baseline (13.71 ± 2.47 ml) through 8weeks, to 12 weeks post MI (52.017 ± 5.53 ml). These data indicate that the left ventricle is dilating post infarct confirming that cardiac remodeling is taking place (Figure 6). Mean diastolic and systolic LV internal diameters (Figure 6C. & D.) reached 42.94 ± 2.02 ml and 35.57 ± 2.18 ml, respectively, at 12 weeks post MI.

CONCLUSION

These functional parameters confirm that MI induces significant decreases in cardiac pump function, and through a 12 week period the heart goes through a compensated state which then becomes decompensated at 12 weeks post MI. The volumetric and morphometric measurements illustrate the remodeling and dysfunction which occurs after acute MI over a 12 week period confirming the manifestation of HF.

After characterizing the acute myocardial infarction/ heart failure model, we began to look at our novel therapeutics to attenuate the dysfunction observed. In aim 1, we injected stem cells into the border zone of the left ventricle which was accessed via NOGA mapping. A NOGA myostar injection catheter was used to make ten trans-endocardial injections of our isolated stem cells. We then followed the animals out one month to observe functional changes and to see whether we could observe the cells within the host myocardium. Functional changes are observed at one month post MI + SC (Figure 7). We observed preservation of LVEF and fractional shortening, while attenuating the LV chamber dilation, as observed in the end-diastolic & end-systolic volumes. Here, we are able to conclude that stem cell injections into the myocardium post MI protect against the adverse remodeling which is classically seen after myocardial infarction.

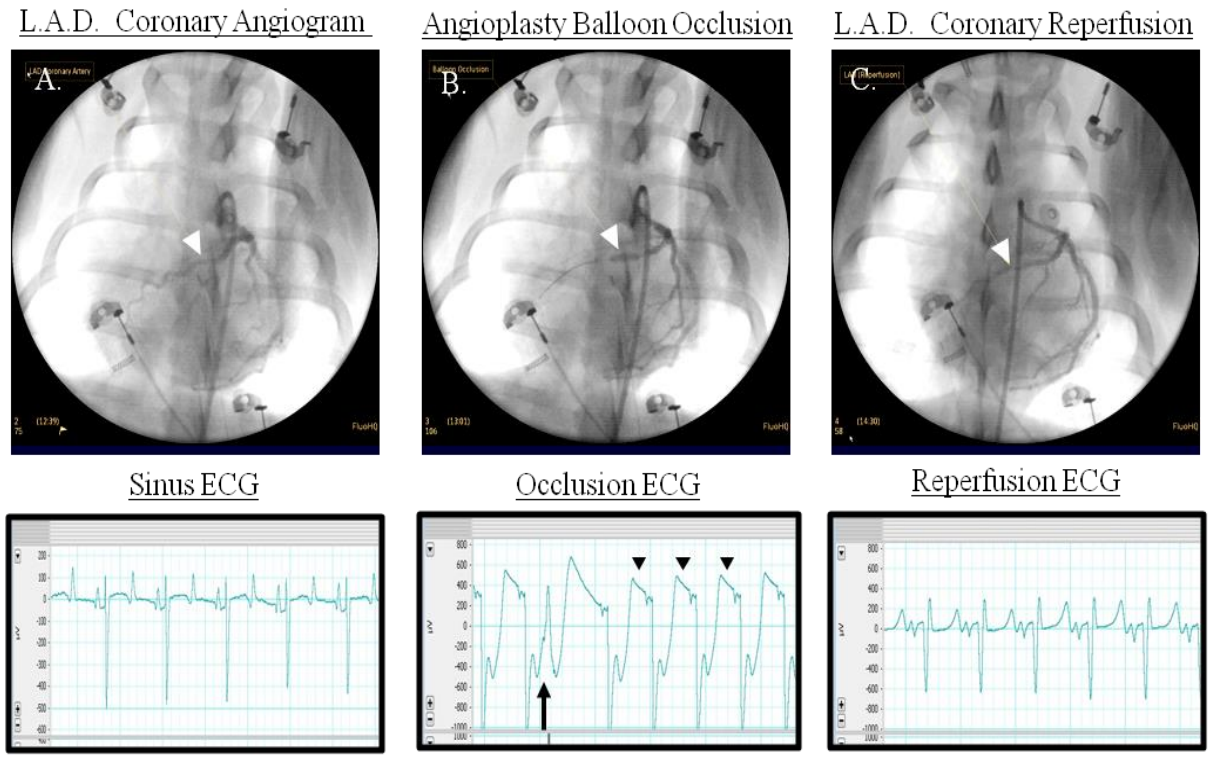


Figure 1. Representative fluoroscopic images and electrocardiogram from ischemia-reperfusion induced MI in Göttingen minipig. **A.** Left coronary angiogram illustrating the baseline perfusion of the left anterior descending (LAD) coronary artery (white arrow head). The baseline electrocardiogram illustrates the sinus rhythm (lower panel). **B.** Left coronary angiogram demonstrating the occlusion of the LAD. The angioplasty balloon (white arrow head) creates cessation of flow distal to the inflation (left of the white arrow head). The electrocardiogram (below) shows premature ventricular contraction ([PVC], black arrow) and S-T segment elevation throughout the duration of the occlusion (black arrow heads). **C.** LAD coronary angiogram demonstrating reperfusion distal of the angioplasty balloon inflation (white arrow head). The electrocardiogram post-MI retains a sinus waveform but with some abnormalities due to the procedure (lower panel).

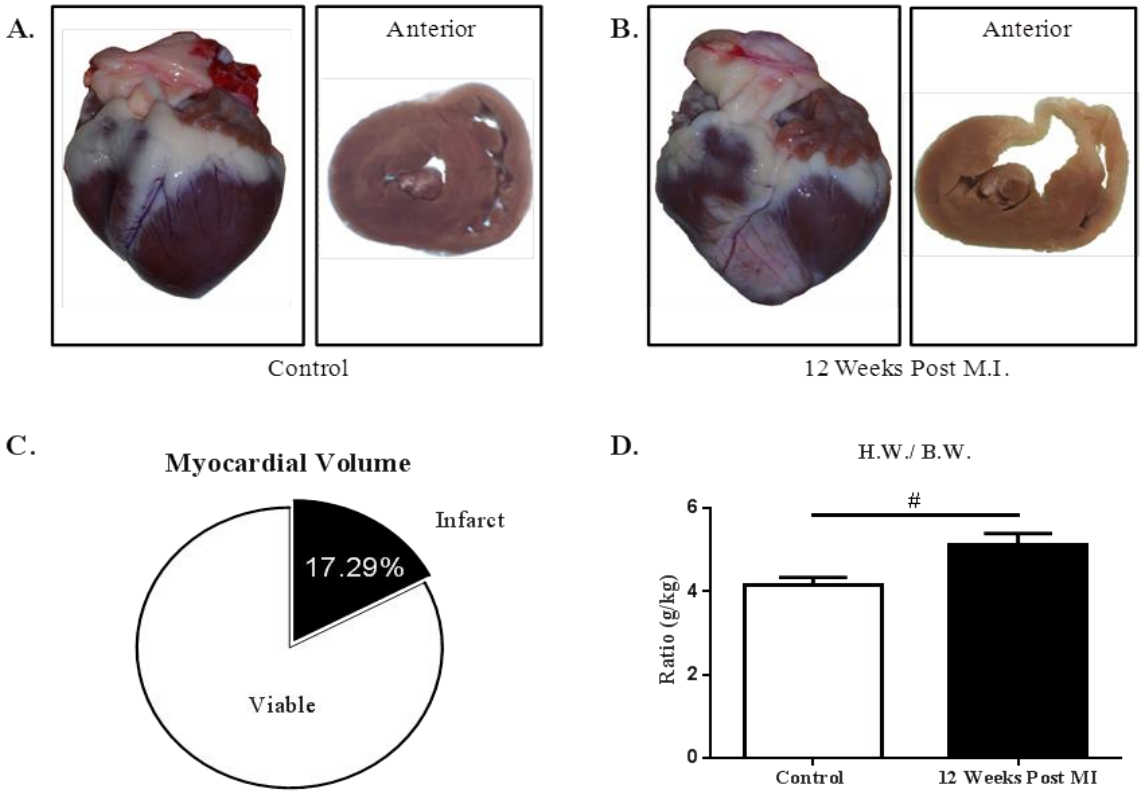


Figure 2. Representative gross anatomy and infarct size analysis. A. Gross images of control heart, whole organ (left panel) and cross-section of mid-myocardium (right panel) **B.** Gross images of 12 week post-MI heart, whole organ (left panel) and cross-section of mid-myocardium (right panel). **C.** Infarct size analysis (viable in white and infarct percentage in black) **D.** Heart weight-to- body weight ratio. (#), Control vs. 12 post MI (student's *t*-test).

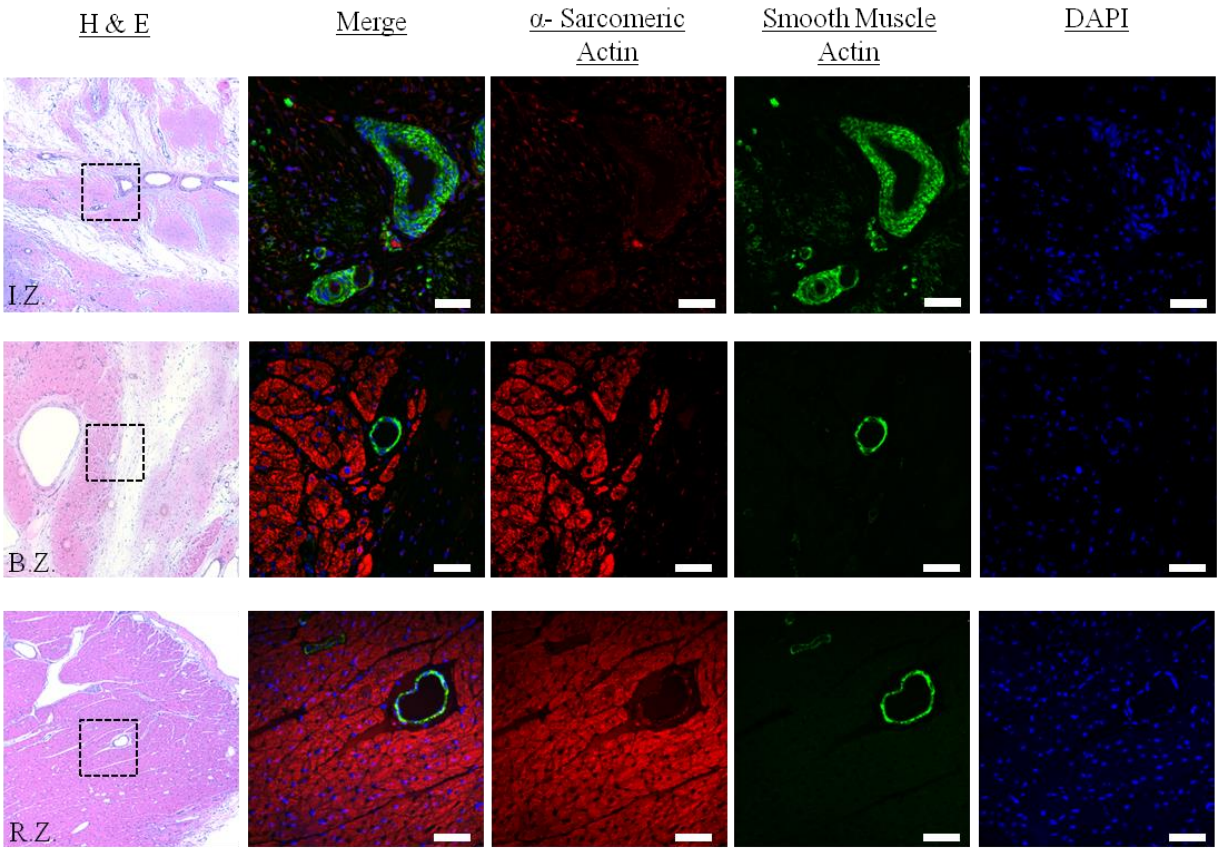
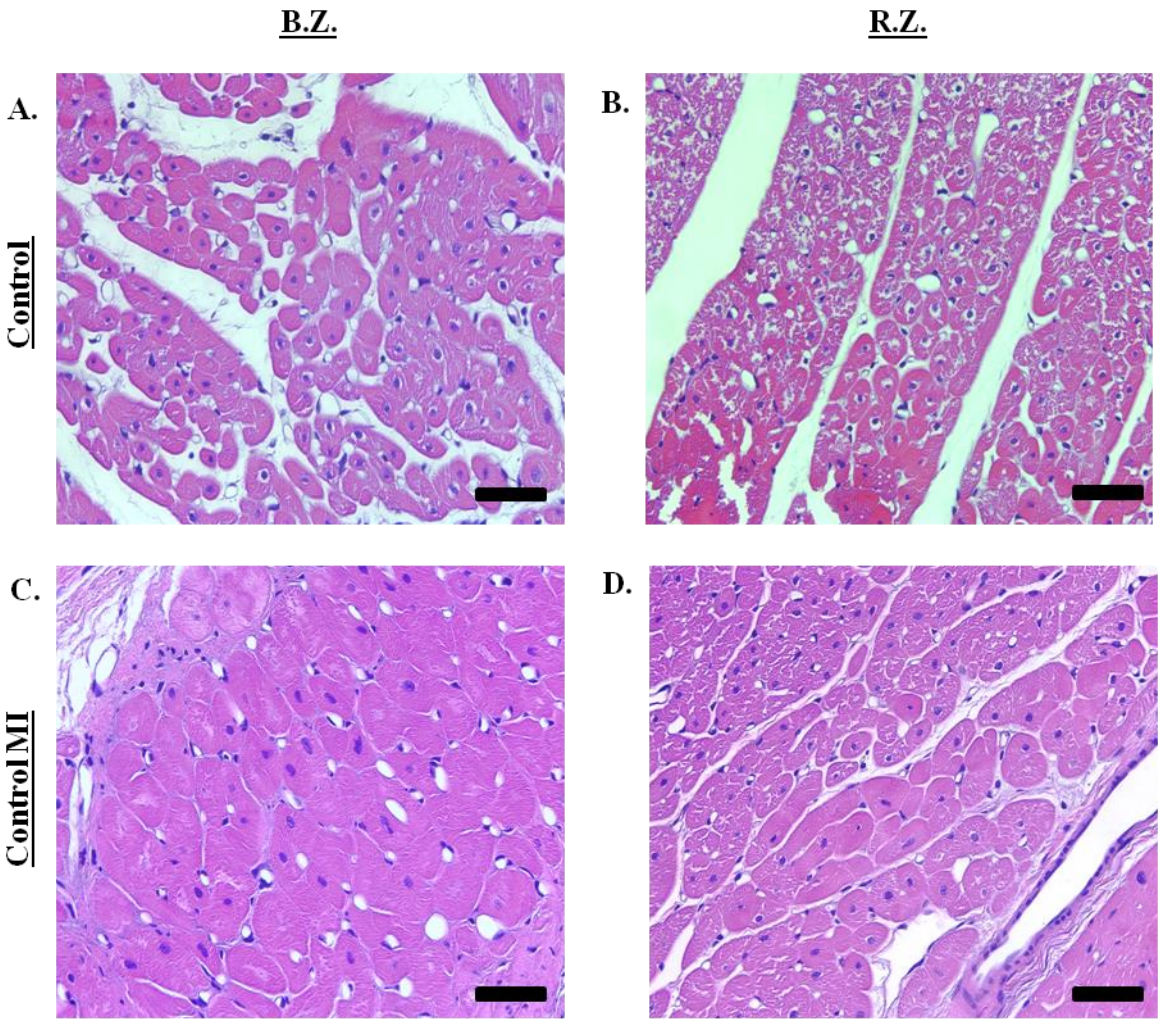


Figure 3. Representative bright-field and confocal images from the three distinct zones created in the ischemia-reperfusion induced MI in Göttingen minipig. Images (left-to-right) represent bright-field 10X H&E, 40X confocal images: merged, confocal image of cardiac protein α-sarcomeric actin (red), vascular smooth muscle actin (green), and nuclear DAPI staining (blue). **A.** Represents the infarct zone (IZ) (Row 1) **B.** Represents the border zone (BZ) (Row 2) **C.** Represents the remote zone (RZ) (Row 3). Black square = 40X area for confocal imaging. Scale bar = 50 μm



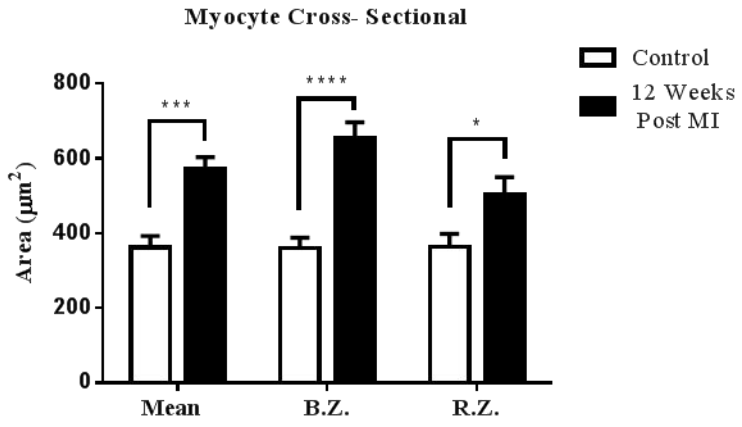


Figure 4. Representative H &E images of myocyte cross-section. A. & **B.** H&E stained slides under light microscope at 40X magnification of control minipig heart tissue from the B.Z. or R.Z. (left and right top panels, respectively). **C.** & **D.** H&E stained slides under light microscope at 40X magnification of 12 week post-MI heart tissue from the B.Z. or R.Z. (left and right top panels, respectively). Scale bar = 50 μm. **E.** Myocyte cross-sectional area calculated from the two representative zone (shown above, B.Z. and R.Z.) in control animals versus 12 week post-MI animals.

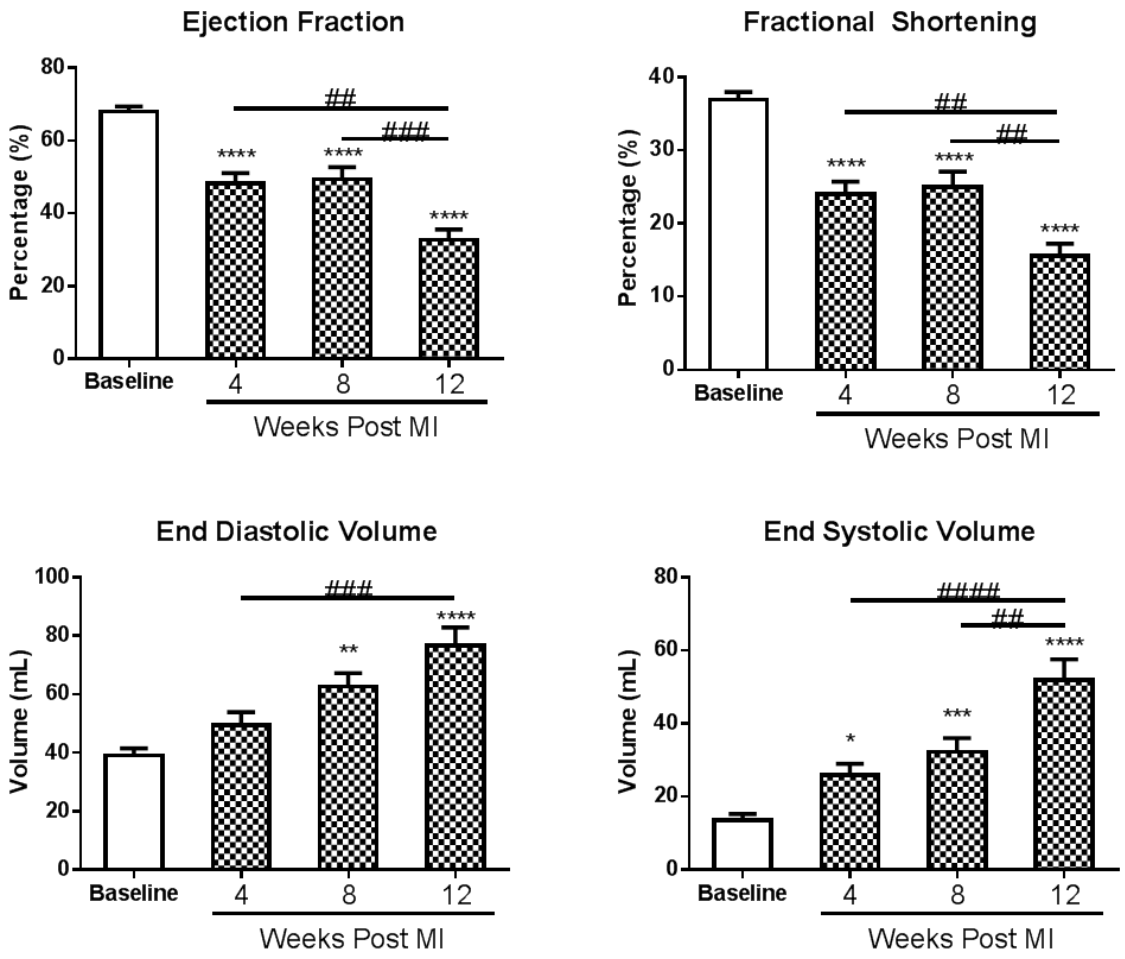


Figure 5. Cardiac functional measured by echocardiography. Animals underwent MI procedure and received follow-up serial echocardiography at 4, 8, 12 weeks post MI. **A.** & **B.** Functional parameters derived from short axis m-mode images at each time point. **C.** & **D.** Volume measurements derived from short axis m-mode images at each time point. 4,8, 12 weeks post MI vs. Baseline (two-way ANOVA); (+), 4 and 8 weeks post MI vs. 12 weeks post MI; (##/###/####).

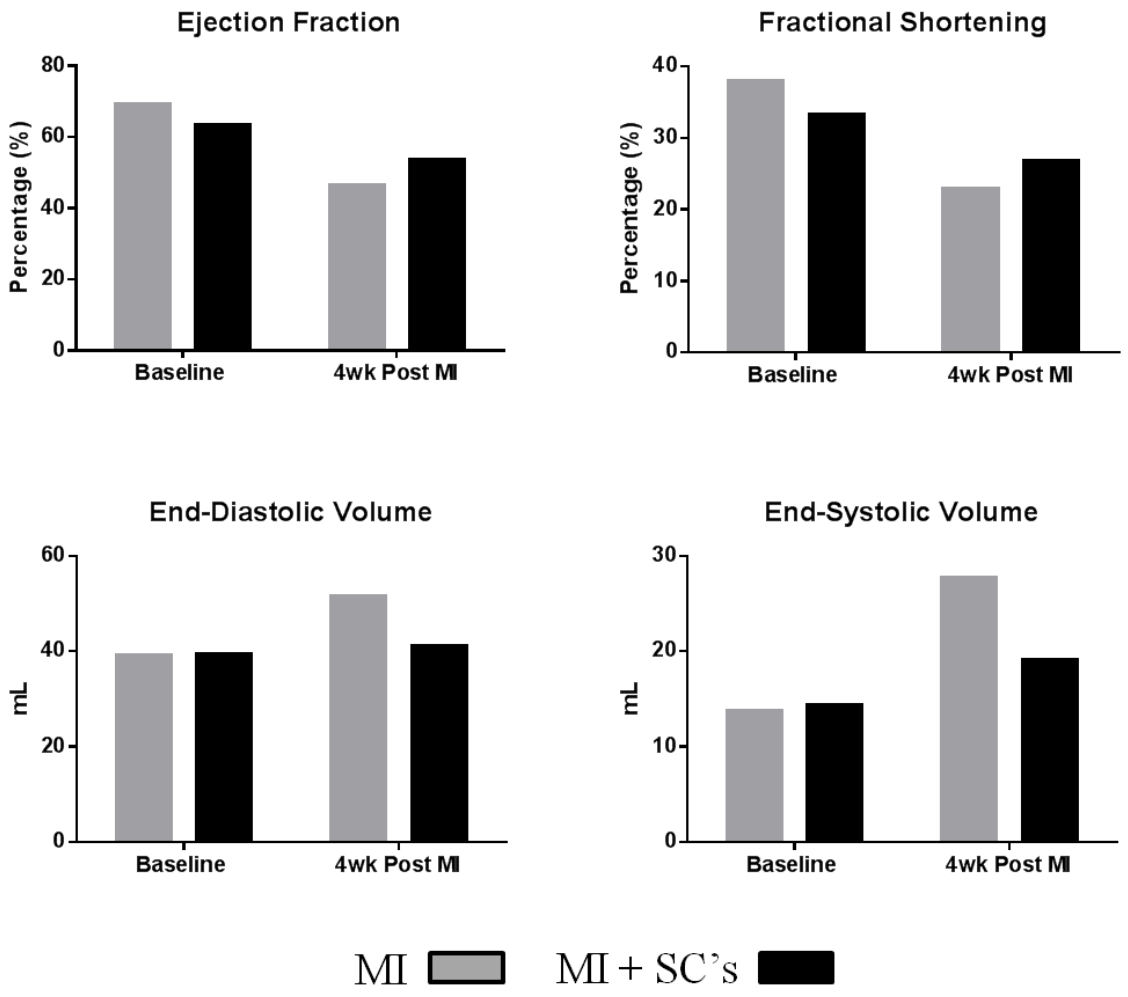


Figure 7. Preservation of cardiac function by echocardiography by stem cell injection.. Animals underwent MI procedure and Then received stem cells immediately post MI. Follow-up echocardiography was performed 1 month later. **A & B** Functional parameters derived from short axis m-mode. **C & D** Volume measurements derived from short axis m-mode.

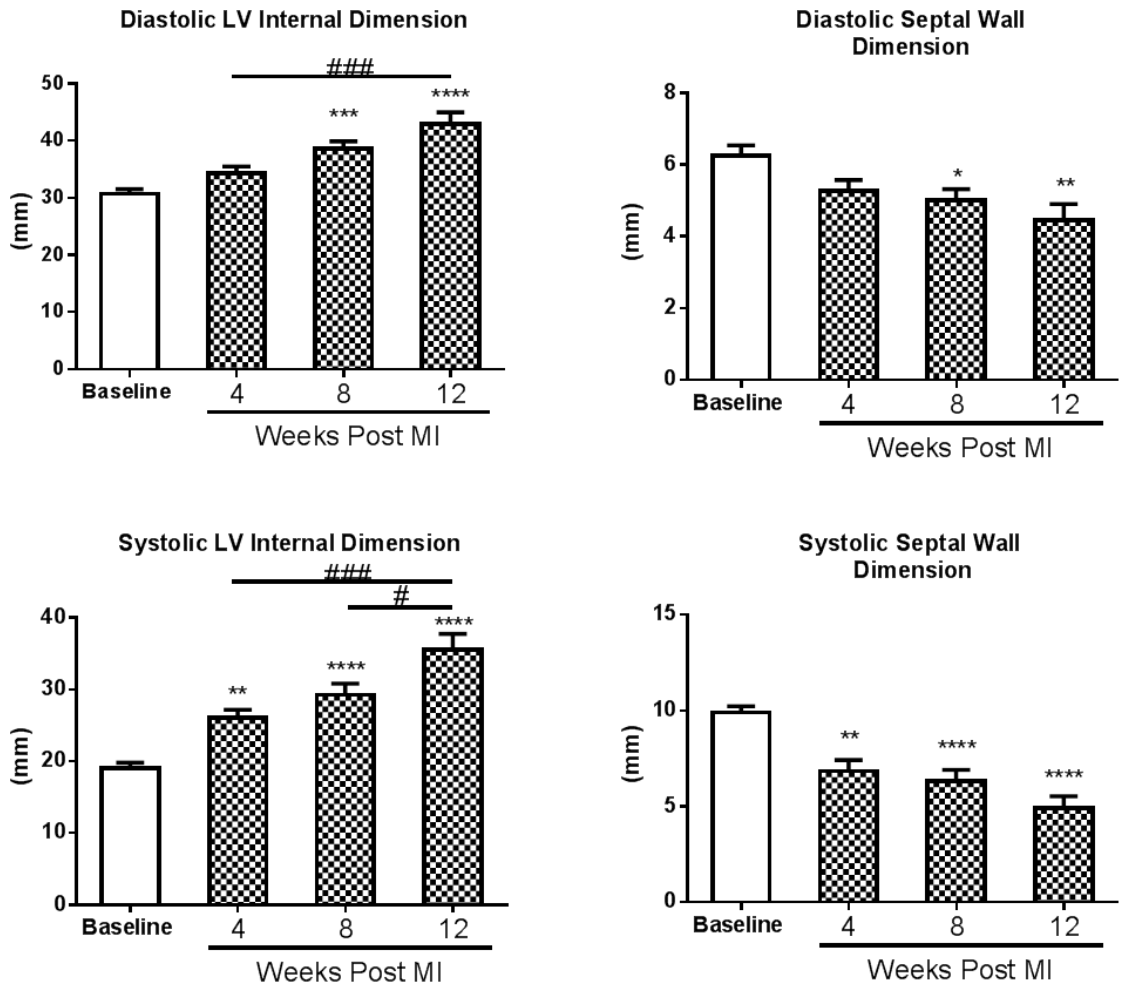


Figure 6. Structural parameters measured by echocardiography. Animals underwent MI procedure and received follow-up serial echocardiography at 4, 8, 12 post MI. **A & B** are diastolic dimensions; **C & D** are systolic dimensions. (**/***/****), 4,8, 12 weeks post MI vs. Baseline (two-way ANOVA); (#/###).

Aim 2. We will determine if, and by what mechanism βARK-CT (via AAV6 gene therapy) improves cardiac structure and function in adult pigs (with adrenergic signaling similar to that found in humans) after MI

Progress made on aim 2:

Aim2, is in progress and has not yet been achieved. After characterizing the pig model in aim 1, we began to embark on utilizing gene therapy through an AAV6 construct to attenuate the functional deficits observed post MI. Ischemia reperfusion-induced MI was performed in four minipigs (AAV-GFP [n=2] & AAV BARK-CT [n=2]) to characterize the dysfunction over a one month period, then follow them out two months to observe whether or not the gene therapy has any effect. To evaluate pathological manifestation and treatment, we performed serial transthoracic echocardiography at 3 time points throughout the duration of the study (Baseline, 2, 4 and 8).

METHODS

Ischemia-reperfusion induced Myocardial Infarction

The right femoral artery was cannulated with a 6-French percutaneous sheath and femoral venous access was also acquired with a 5-French percutaneous sheath for administration of any necessary pharmacological intervention. To prevent thrombosis throughout the duration of the procedure, heparin was administered with a loading dose of 100U/kg and a maintenance dose of 40U/kg every hour. A 5-French pigtail catheter was placed in the left ventricle and a ventriculography was performed. A 6-French guide catheter was used to cannulate the left coronary artery to perform arteriography. Next, a 0.014-in angioplasty guide wire was place in the left anterior descending coronary artery (LAD), then a 3.0mm x 12mm Quantum Maverick, monorail, balloon (Boston Scientific, M.A. USA) was placed in the LAD just distal to the first diagonal branch, all placements were performed under fluoroscopic guidance and recorded. The balloon was then inflated to 8.0 atmospheres for a period of 90 minutes and cessation of flow distal to the balloon was confirmed with another angiography. ST-segment elevation was also used to assess onset on infarction. After deflation of the balloon, the guide wire and balloon were removed and reperfusion of the LAD was confirmed by angiography. Next, the guide catheter was removed and the animal was given 30 minutes of reperfusion prior to any subsequent treatment being performed.

Transthoracic Echocardiography

Transthoracic echocardiography was performed at baseline, 2, 4 and 8 weeks post MI with the Zonare z. one ultra Ultrasound system on all animals. Images were acquired in the 2D-mode in long- and shot- axis; while m-mode images were taken in the short axis. All measurements including end-diastolic dimensions, end-systolic dimensions, end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF) and fractional shortening (FS) were calculated by the American Society of Echocardiography guidelines.

Hemodynamics

Invasive hemodynamic were performed at sacrifice looking at the β-adrenergic response of the heart between control animals and those who received BARK-CT. A 5-french pigtail conductance catheter (Millar Instruments) was placed within the LV and escalating doses of dobutamine were administered through venous access (Figure 1). The dP/dT maximum and minimum were derived and used to assess contractility and relaxation properties of the myocardium.

Molecular Analysis

Upon tissue harvest, sections of the myocardium were collected for molecular analysis along with other major organs to observe localized and systemic expression of the gene targets. Western blotting was performed to investigate the expression of either GFP (control) or BARK-CT.

RESULTS

Cardiac functional Analysis

Transthoracic echocardiography was performed at baseline, 2, 4 and 8 weeks post MI. There was no significant difference in LVEF or fractional shortening throughout the study, nor was

there any significant difference in end-diastolic and end-systolic volumes (Figure 1). At euthanasia, we performed invasive hemodynamics to observe any change in contractility or relaxation due to a dobutamine challenge. There were no significant differences in dose response between groups with dP/dT max. or min. (Figure 1). In seeing no significant difference in function between control (AAV-GFP) and BARK-CT animals, we sought to understand if the construct was expressing and if it had localized properly to heart tissue; for this we utilized western blotting to look at expression of GFP and BARK-CT.

Western Blotting

In the investigation of protein expression we observed robust AAV-GFP expression in viable tissue within the left and right ventricles (Figure 2.). Lower expression was observed in the border zone regions of the myocardium (Figure 2.). There was no expression in the liver, suggesting that this AAV- construct is highly cardiotropic. In looking for BARK-CT expression we observed none (data not shown).

CONCLUSION

At the end of our investigation, we took a look back at what errors may have occurred in the process to not allow us to see the over expression of BARK-CT. We later discovered that the viral vector (AAV-BARK-CT) was made improperly and that a new construct needed to be made. The GFP expression upon administration of the AAV was robust, which gives promise to this aim 2. We will correct the problem and plan to further investigate BARK-CT gene therapy as an avenue to attenuate the dysfunction observed post MI.

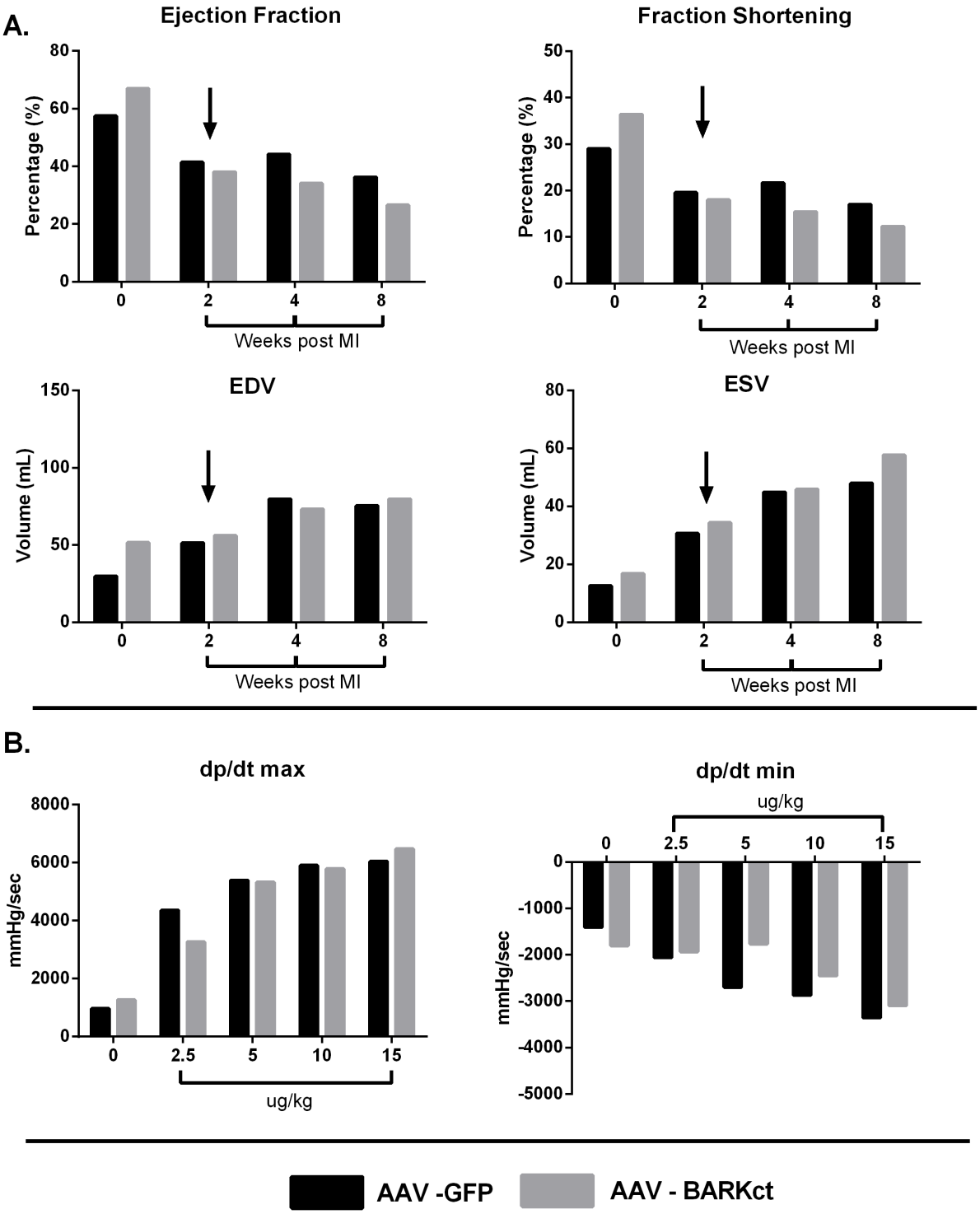


Figure 1. Cardiac functional data and hemodynamics. A. All measurements were taken from short axis m-mode transthoracic echocardiography images at baseline, 2, 4, 8 weeks post MI. The black arrow represents when the gene therapy was administered systemically. **B.** Hemodynamic measurements we acquired via invasive hemodynamics with a millar catheter system at 8 weeks post MI (6 weeks post gene therapy). A dobutamine challenege was givien 0, 2.5, 5, 10 and 15 ug/kg. GFP (n=2), BARKct (n=2). EDV, end-diastolic volume; ESV, end-systolic volume.

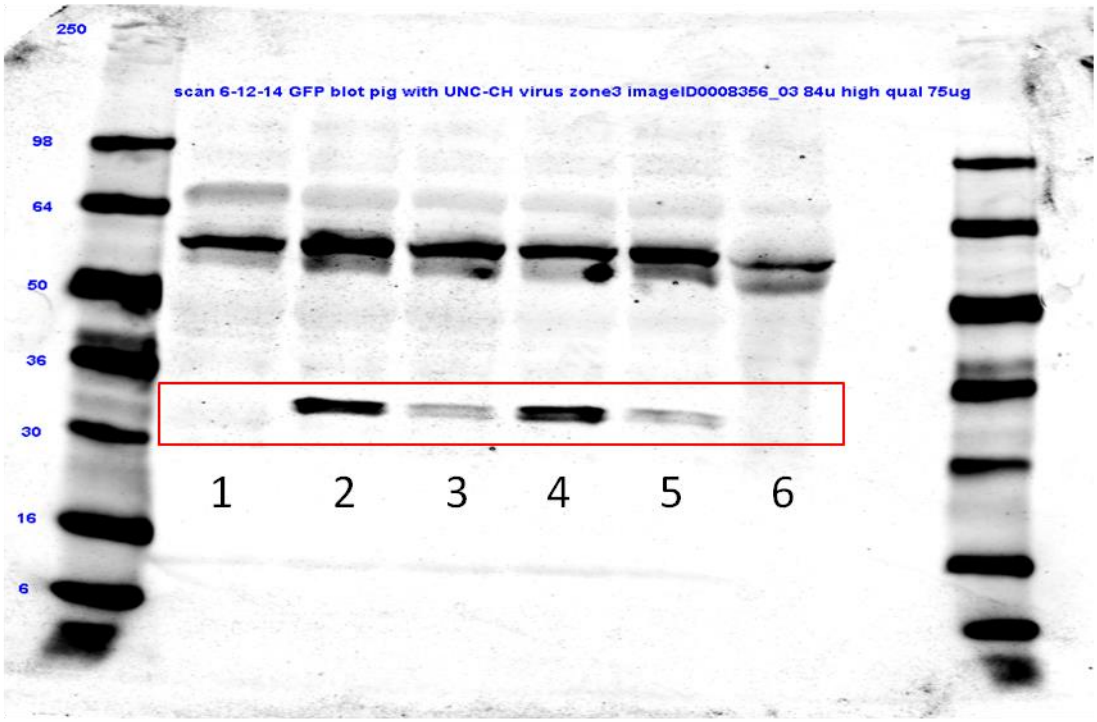


Figure 2. Western blot analysis of GFP expression . GFP expression amongst isolated heart tissue 8 weeks post MI. 1=non infected control pig; 2=LV zone3; 3=septum zone3; 4=RV zone 3; 5= RV zone2; 6= liver; Zone2 is boarder zone, zone 3 viable

Aim 3. We will determine if, and by what mechanism Rem^{1-265}-Cav (via AAV6 gene therapy) improves cardiac structure and function in adult pigs (with Ca^{2+} signaling similar to that found in humans) after MI

Progress made on aim 3:
Aim 3, is not completed and has just been started. The viral construct is being tested *in vitro* and will soon be moved into a small animal model to test viability and reproducibility. Once confirmed we will begin to move this construct into our established large animal model. The reason for such slow progress in aim 3, was the difficulty in producing the AAV6 construct. Now that the construct has been made, we plan to forge ahead with this project and yield further data on the attenuation of cardiac remodeling post MI.

Abstract of Research Progress:
Introduction: Myocardial Infarction (MI) is a major health problem that causes cardiac dysfunction and often leads to heart failure. Previously, we have demonstrated that in a rodent model that administration of cell therapy post MI can be beneficial to the heart. This enhancement of cardiac function has been linked to reduce infarct size, increased paracrine factor secretion, new blood vessel formation, and regeneration of lost tissue. In the present study we tested the effects of a sub-population of stem cells from the bone marrow, on cardiac remodeling and function in a swine MI model.

Hypothesis: Cell therapy will help to regenerate the loss of functional cardiac tissue due to MI therefore altering scar formation, pathological remodeling, chamber dilation and decrease function observed post MI

Methods and Results: MI was induced in swine (6-7 months of age) by occlusion of the left anterior descending coronary artery (LAD) using an inflated percutaneous transluminal coronary angioplasty (PTCA) balloon for 90 minutes, followed by reperfusion. Cardiac structure and function was evaluated using echocardiography before and 4, 8, and 12 weeks post MI. In the control MI group, during the 12 weeks after MI there was a progressive depression of pump function (Ejection Fraction [EF] 66.52% ± 1.16 [baseline] to 32.66 % ± 1.02 [12 weeks after MI] $p < 0.0001$) and dilation of the LV volumes (End-diastolic volume [EDV] was 40.38 ml ± 3.01[baseline] to 75.73 ml ± 3.18 [12 weeks post MI] $p = 0.0123$ and End-systolic volume [ESV] was 15.14 ml ± 2.32 to 50.75 ml ± 1.86 $p = 0.0004$). Inhibiting the dilation of the chamber indicates that the cell therapy is slowing the progression of the pathological state towards dilated cardiomyopathy and CHF. Cell treatment significantly increased EF (from 32.66% ± 1.02 to 41.43% ± 2.87 $p = 0.0282$). Cell therapy also significantly reduced EDV and ESV (from 75.73 ml ± 3.18 to 63.58 ml ± 4.06 $p = 0.0494$, and from 50.75 ml ± 1.86 to 37.45 ml ± 3.52 $p = 0.0156$ respectively).

Conclusion: These data show that acute cell therapy treatment reduces infarct size preserves cardiac function post MI.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics

_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include

the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We are extending our work and with the new data to be obtained we plan to submit peer-reviewed reports over the next few years.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis,

or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

NONE

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

NONE

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:

Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Houser, Steven, R.	Professor and Chair of Physiology
eRA COMMONS USER NAME (credential, e.g., agency login)	
SRHOUSER	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Eastern College	BA	1973	Biology, Chemistry
Temple University School of Medicine	Ph.D.	1977	Physiology
Temple University School of Medicine	Post Doc	1979	Cardiovascular
University of CA at San Francisco	Visiting Sci	1992	Sabbatical (Simpson)

A. Personal Statement I have been an NIH funded faculty member at Temple for more than 30 years. During this time my group has helped define many fundamental features of the normal cardiac myocyte as well as identifying defective molecular and cellular processes that produce abnormal cardiac myocyte function in cardiovascular disease. When I started my laboratory it was not clear how Ca^{2+} entered cardiac myocytes and how it initiated contraction. My group was among the first to show that Ca^{2+} entry through the L-type Ca^{2+} channel is the major trigger for induction of Ca^{2+} release from the sarcoplasmic reticulum. We were at the forefront of the fundamental biology of cardiac myocyte Ca^{2+} handling and excitation-contraction coupling. My group documented that Ca^{2+} entry through the L-type Ca^{2+} channel is the major if not the exclusive trigger for SR Ca^{2+} release. My laboratory was one of the first (maybe the first) to study the idea that Ca^{2+} influx through the L-type Ca^{2+} channel is abnormal in cardiac disease and is centrally involved in the disrupted Ca^{2+} regulation that causes reduced contractility reserve in heart failure, and provokes cell death signaling that ultimately leads to heart failure progression. We dissecting out the fundamental molecular and cellular defects in Ca^{2+} regulation in failing human myocytes. We also were one of the first groups to show that Ca^{2+} also enters diseased myocytes through another class of channels (T-type Ca^{2+} channels). The functional significance of this Ca^{2+}influx is still not clear is being studied currently by a number of groups including ours. Currently my group is exploring how Ca^{2+} entry through distinct channels and transporters can direct physiological growth and pathological hypertrophy and how Ca^{2+} influx pathways into cardiac progenitors influence the generation of new myocytes. A major focus of the lab is now mechanisms of cardiac injury and repair, with Ca^{2+} regulation as the hub. New data from our group and others suggests that the Ca^{2+} influx that regulates “signaling” (hypertrophy is one example) occurs in “microdomains” that are physically distinct from the Ca^{2+} influx that determines “contractile” Ca^{2+}. We have also recently found that diseased (myocardial infarction; MI) heart structure and function can be improved by injection of cardiac or bone derived stem cells into the MI border zone. In the present research we are exploring novel approaches to define the mechanisms of improved cardiac structure and function by cell therapy and will determine if altering the Ca^{2+} influx into injected stem cells via enhanced Cn43 mediated contact of stem cells to cardiac myocytes or by altering T-type Ca^{2+} channels will enhance the reparative function of injected cells.

B. Positions and Honors

1979 – 85 Assistant Professor of Physiology, Temple University School of Medicine, Department of Physiology, Philadelphia, PA

1985 – 91 Associate Professor of Physiology, Temple University School of Medicine, Department of Physiology, Philadelphia, PA

1991- Professor of Physiology, Temple University School of Medicine, Department of Physiology, Philadelphia, PA

1973 Magna Cum Laude, Eastern College

1977 Summa Cum Laude, Temple University School of Medicine (Ph.D.)

1983 A.N. Richards Research Prize Award Winner

1984 RFA Reviewer (NIH) "Molecular Mechanisms of Cardiac Hypertrophy"

1988-92 Member, Cardiovascular (A) Study Section

1998-2003 Co-Director, Cardiovascular Research Group, Temple University School of Medicine 2003-present Director, Cardiovascular Research Center

2003-2006 Chair, Cardiac Contractility, Hypertrophy and Failure Study section

2006-present Chair, Department of Physiology, Temple University School of Medicine

2009-2013 Chair, NHLBI K08 study section

2012-2013 NIH Study Section Members conflict SEPs

C. Selected Peer Reviewed Publications

1: Duran JM, Makarewich CA, Sharp TE, Starosta T, Zhu F, Hoffman NE, Chiba Y,Madesh M, Berretta RM, Kubo H, Houser SR. Bone-derived stem cells repair the heart after myocardial infarction through transdifferentiation and paracrine signaling mechanisms. Circ Res. 2013 Aug 16;113(5):539-52.

2: Wang F, Gao H, Kubo H, Fan X, Zhang H, Berretta R, Chen X, Sharp T, Starosta T, Makarewich C, Li Y, Molkentin JD, Houser SR. T-type Ca^{2+} channels regulate the exit of cardiac myocytes from the cell cycle after birth. J Mol Cell Cardiol. 2013 Sep;62:122-30.

3: Wang WE, Yang D, Li L, Wang W, Peng Y, Chen C, Chen P, Xia X, Wang H, Jiang J, Liao Q, Li Y, Xie G, Huang H, Guo Y, Ye L, Duan DD, Chen X, Houser SR, Zeng C. Prolyl hydroxylase domain protein 2 silencing enhances the survival and paracrine function of transplanted adipose-derived stem cells in infarcted myocardium. Circ Res. 2013 Jul 19;113(3):288-300.

4: Gandhirajan RK, Meng S, Chandramoorthy HC, Mallilankaraman K, Mancarella S, Gao H, Razmpour R, Yang XF, Houser SR, Chen J, Koch WJ, Wang H, Soboloff J, Gill DL, Madesh M. Blockade of NOX2 and STIM1 signaling limits lipopolysaccharide-induced vascular inflammation. J Clin Invest. 2013 Feb 1;123(2):887-902.

5: Mancarella S, Potireddy S, Wang Y, Gao H, Gandhirajan RK, Autieri M, Scalia R, Cheng Z, Wang H, Madesh M, Houser SR, Gill DL. Targeted STIM deletion impairs calcium homeostasis, NFAT activation, and growth of smooth muscle. FASEB J. 2013 Mar;27(3):893-906.

6: Duran JM, Taghavi S, Berretta RM, Makarewich CA, Sharp Iii T, Starosta T, Udeshi F, George JC, Kubo H, Houser SR. A characterization and targeting of the infarct border zone in a swine model of myocardial infarction. Clin Transl Sci. 2012 Oct;5(5):416-21.

7: Gao H, Wang F, Wang W, Makarewich CA, Zhang H, Kubo H, Berretta RM, Barr LA, Molkentin JD, Houser SR. Ca(2+) influx through L-type Ca(2+) channels and transient receptor potential channels activates pathological hypertrophy signaling. J Mol Cell Cardiol. 2012 Nov;53(5):657-67..

8: Houser SR, Margulies KB, Murphy AM, Spinale FG, Francis GS, Prabhu SD, Rockman HA, Kass DA, Molkentin JD, Sussman MA, Koch WJ; on behalf of the American Heart Association Council on Basic Cardiovascular Sciences, Council on Clinical Cardiology, and Council on Functional Genomics and Translational Biology. Animal Models of Heart Failure: A Scientific Statement From the American Heart Association. Circ Res. 2012 Jun 22;111(1):131-150. Epub 2012 May 17. PubMed PMID: 22595296.

9: Raake PW, Zhang X, Vinge LE, Brinks H, Gao E, Jaleel N, Li Y, Tang M, Most P, Dorn GW 2nd, Houser SR, Katus HA, Chen X, Koch WJ. Cardiac G-protein-coupled receptor kinase 2 ablation induces a novel Ca2+ handling phenotype resistant to adverse alterations and remodeling after myocardial infarction. Circulation. 2012 May 1;125(17):2108-18. Epub 2012 Apr 10. PubMed PMID: 22496128.

10: Makarewich CA, Correll RN, Gao H, Zhang H, Yang B, Berretta RM, Rizzo V, Molkentin JD, Houser SR. A caveolae-targeted L-type Ca^{2}+ channel antagonist inhibits hypertrophic signaling without reducing cardiac contractility. Circ Res. 2012 Mar 2;110(5):669-74. Epub 2012 Feb 2. PubMed PMID: 22302787; PubMed Central PMCID: PMC3324037.

11: Zhang H, Makarewich CA, Kubo H, Wang W, Duran JM, Li Y, Berretta RM, Koch WJ, Chen X, Gao E, Valdivia HH, Houser SR. Hyperphosphorylation of the cardiac ryanodine receptor at serine 2808 is not involved in cardiac dysfunction after myocardial infarction. Circ Res. 2012 Mar 16;110(6):831-40. Epub 2012 Feb 2.PubMed PMID: 22302785; PubMed Central PMCID: PMC3322671.

12: Goonasekera SA, Hammer K, Auger-Messier M, Bodi I, Chen X, Zhang H, Reiken S, Elrod JW, Correll RN, York AJ, Sargent MA, Hofmann F, Moosmang S, Marks AR, Houser SR, Bers DM, Molkentin JD. Decreased cardiac L-type Ca^{2+} channel activity induces hypertrophy and heart failure in mice. J Clin Invest. 2012 Jan 3;122(1):280-90. Epub 2011 Dec 1. PubMed PMID: 22133878; PubMed Central PMCID: PMC3248289.

13: Ladage D, Tilemann L, Ishikawa K, Correll RN, Kawase Y, Houser SR, Molkentin JD, Hajjar RJ. Inhibition of PKCα/β with ruboxistaurin antagonizes heart failure in pigs after myocardial infarction injury. Circ Res. 2011 Dec 9;109(12):1396-400. Epub 2011 Oct 13. PubMed PMID: 21998327; PubMed Central PMCID: PMC3237728.

14: Angert D, Berretta RM, Kubo H, Zhang H, Chen X, Wang W, Ogorek B, Barbe M, Houser SR. Repair of the injured adult heart involves new myocytes potentially derived from resident

cardiac stem cells. Circ Res. 2011 May 13;108(10):1226-37. Epub 2011 Mar 31. PubMed PMID: 21454756; PubMed Central PMCID: PMC3322670.

15: Zhang H, Chen X, Gao E, MacDonnell SM, Wang W, Kolpakov M, Nakayama H, Zhang X, Jaleel N, Harris DM, Li Y, Tang M, Berretta R, Leri A, Kajstura J, Sabri A, Koch WJ, Molkentin JD, Houser SR. Increasing cardiac contractility after myocardial infarction exacerbates cardiac injury and pump dysfunction. Circ Res. 2010 Sep 17;107(6):800-9. Epub 2010 Jul 29. PubMed PMID: 20671241; PubMed Central PMCID: PMC3021375.

D. Research Support

Ongoing Research Support

Title: "Ca^{2+} Regulation in Newly Formed Ventricular Myocytes"
Agency: National Heart Lung and Blood Institute, HL033921 **Dates**: 06/01/85 – 07/31/14
Principal Investigator: Steven R. Houser, Ph.D. 2.4 calendar
Objectives: The long-standing goals of this research program are to determine the cellular and molecular bases of the myocytes electromechanical abnormalities induced by pathological processes that lead to sudden death and heart failure. This work was chosen for an NHLBI MERIT award after the last review (4 years ago), and was strongly recommended for continuation by NHLBI staff. However, NHLBI council did not support the NHLBI staff request, without explanation, but presumably because of the current fiscal climate.

Title: Inflammatory Proteases, Ubiquitin Proteasome System, and Myocyte Death
Agency: National Heart Lung and Blood Institute, HL076799 **Dates**: 09/15/04-08/30/14
Co-Investigator : Steven R. Houser, Ph.D. .8 calendar
Objectives: The research proposed in this application will delineate the role of ubiquitin ligases in focal adhesion signaling alterations caused by inflammatory proteases and will determine if serine proteases and the ubiquitin ligase Cbl could be novel targets for therapies that diminish myocyte death and dysfunctional remodeling after myocardial IR injury.

Title: "Ca2+ Influx Mediated Damage and Regeneration of Adult Myocardium"
Agency: National Heart Lung and Blood Institute, HL091799 **Dates**: 05/01/08-03/31/2014
Co-Investigator : Steven R. Houser, Ph.D. .6 calendar
Objectives: The major goal of this study is to identify many novel targets for CHF therapy and would change the thinking from current approaches that seek to increase myocyte force generation to those that seek to reduce myocyte death and promote myocyte regeneration. This PPG will end soon and the current application is a new project that was derived from the work supported by this grant.

Title: "Improving Cardiac Function after Myocardial Infarction"
Agency: National Heart Lung and Blood Institute, HL108806 **Dates**: 05/07/2012

– 03/21/17
Principal Investigator : Steven R. Houser, Ph.D. 3.60 calendar
Objectives: This PPG will test 3 approaches to reduce the consequences of MI. All projects will include therapeutic testing in a large animal model of cardiac injury so that beneficial approaches can be rapidly translated to humans.