**NARRATIVE**: Longitudinal Left Ventricular Remodeling Following Myocardial Infarction

### *Rationale, Hypothesis, and Objectives:*

The development and progression of congestive heart failure has reached epidemic proportions in the United States as well as worldwide. The incidence of emergent hospital admissions due to congestive heart failure have increased exponentially and currently over 10 million patients suffer from this condition. One of the common causes for congestive heart failure is the long term effects of a heart attack- myocardial infarction (MI). An MI is due to a period of blood flow interruption to the heart muscle that subsequently causes injury and heart cell death. Despite significant advances in our abilities to reopen and restore blood flow to the heart muscle, methods to prevent the long term effects of the damaged myocardium have not been forthcoming. A structural milestone in the development and progression of congestive heart failure secondary to MI is myocardial remodeling. This is defined as changes in left ventricular (LV) geometry and structure which in turn can reduce pumping efficiency. It is now recognized that the region of myocardium surrounding the MI changes shape and size and that this in turn is translated into overall changes in LV geometry. This phenomenon is termed "infarct expansion" and has been identified as an important therapeutic target to minimize post-MI remodeling, subsequent LV remodeling, and in turn reduce the progression to heart failure. Exacerbated infarct expansion in the early post-MI period has been hypothesized to be an independent predictor for accelerated LV dilation and, potentially, progression to the development of heart failure post-MI. Accordingly, the goal of this study will be to longitudinally measure regional and global LV geometry in the same post-MI pigs to develop a relationship between early regional changes in infarct geometry (regional infarct expansion) to later increase in LV dimensions (global LV remodeling).

MI invariably results in heterogeneous changes in LV geometry, with the LV wall of the MI region progressively thinning and the wall of the non-MI region thickening over the course of LV remodeling post-MI. Therefore, imaging modalities that can provide primarily 2-dimensional information of the LV (echocardiography, ventriculography, for example) are not optimal for serial tracking of LV remodeling post-MI. Accordingly, this study will use magnetic resonance imaging (MRI) in a well established porcine MI model to develop a temporal relationship between regional and global changes in LV geometry in the same pigs post-MI.

### *Proposed use of animals*

Species: Yorkshire Pigs (20 kg), Castrated Males

Animals will be purchased from an approved vendor (commercial pork producer in near University ) and individually housed in 4X6 ft. runs with raised vinyl flooring in rooms with a 12:12 light cycle and an ambient temperature of 70-80º F.

<u>Phase 1: Control (non-MI) pigs</u>

Pigs will be allowed to acclimatize within University facilities for a minimum of 7 days. For the first five days that the pigs are in-house, all pigs will be administered erythromycin

(250 mg PO, TID). On the day prior to instrumentation, on the day of instrumentation, and the day following instrumentation, the pigs will be administered Naxcel (ceftiofur sodium, 3.0 to 5.0 mg/kg, intramuscularly). The animals will be fasted for 24 hours, and on the morning of surgery, a 100 μg fentanyl patch (5 μg/kg/day, release rate of 50 μg/hr) will be applied in addition to an intramuscular injection of buprenorphine (0.05-0.1 mg/kg im). Just prior to surgery, anesthesia will be induced with ketamine (22 mg/kg), acepromazine (0.04 mg/kg), and atropine (0.04 mg/kg) by staff and the pigs will be placed in a custom designed pig sling. An ear vein will be accessed and the venous cannula left in place to administer i.v. fluids (e.g. Lactated Ringer's) and other pharmacologic agents (e.g. antiarrhythmics, such as lidocaine) if needed. An ECG and pulse oximetry will be established. Anesthetic induction will be established using a face mask delivering isoflurane (3% 1.5 L/min) and nitrous oxide (0.5 L/minute). The animal will then be intubated with a cuffed endotracheal tube and ventilated at a flow rate of 22 mL/kg/minute. Regulation of the delivery of isoflurane will be used to maintain a stable heart rate and blood oxygenation, and will be increased if either of these parameters rises by over 10% from ambient levels. Oxygen saturation and heart rate will be monitored continuously to provide a sensitive means to ensure a complete and stable surgical plane of anesthesia.

A lidocaine infusion will be initiated with a 3 mg/kg bolus followed by a constant infusion of 120 mg/hr. A sterile left thoracotomy will be performed. A purse string will be made in the thoracic aorta and a catheter connected to an access port (7F) will be advanced to the aorta at the level of the diaphragm. The access port connected to the catheter will be placed in a subcutaneous pocket and secured in place using silk ties. The incision will be closed in layers, with irrigation utilized as necessary. The animals will be weaned off anesthesia and moved to a continually-monitored recovery area when breathing on their own. On recovery of locomotion, the animals will be moved into housing cages, where they will be monitored at least twice daily. Fentanyl patches will be removed 3 days following surgery. Additional analgesics or antibiotics will be administered per recommendations of the staff veterinarian. Pigs will be imaged at 7, 14, 28, 42, and 56 days post-surgery and terminally studied after imaging at 56 days post-surgery.

Phase 2: MI pigs

Pigs will be allowed to acclimatize in facilities for a minimum of 7 days. For the first five days that the pigs are in-house, all pigs will be administered erythromycin (250 mg PO, TID). On the day prior to instrumentation, on the day of instrumentation, and the day following instrumentation, the pigs will be administered Naxcel (ceftiofur sodium, 3.0 to 5.0 mg/kg, intramuscularly). The animals will be fasted for 24 hours, and on the morning of surgery, a 100 μg fentanyl patch (5 μg/kg/day, release rate of 50 μg/hr) will be applied in addition to an intramuscular injection of buprenorphine (0.05-0.1 mg/kg im). Just prior to surgery, anesthesia will be induced with ketamine (22 mg/kg), acepromazine (0.04 mg/kg), and atropine (0.04 mg/kg) by trained staff and the pigs will be placed in a custom designed pig sling. An ear vein will be accessed and the venous cannula left in place to administer i.v. fluids (e.g. Lactated Ringer's) and other pharmacologic agents (e.g. antiarrhythmics, such as lidocaine) if needed. An ECG and pulse oximetry will be established. Anesthetic induction will be established using a face mask delivering isoflurane (3% 1.5 L/min) and nitrous oxide (0.5 L/minute). The animal will then be intubated with a cuffed endotracheal tube and

ventilated at a flow rate of 22 mL/kg/minute. Regulation of the delivery of isoflurane will be used to maintain a stable heart rate and blood oxygenation, and will be increased if either of these parameters rises by over 10% from ambient levels. Oxygen saturation and heart rate will be monitored continuously to provide a sensitive means to ensure a complete and stable surgical plane of anesthesia.

A lidocaine infusion will be initiated with a 3 mg/kg bolus followed by a constant infusion of 120 mg/hr. A sterile left thoracotomy will be performed and a pericardiectomy performed exposing the LV free wall, the left atrium, the circumflex artery and the obtuse marginals. A purse string will be made in the thoracic aorta and a catheter connected to an access port (7F) will be advanced to the aorta at the level of the diaphragm. The access port connected to the catheter will be placed in a subcutaneous pocket and secured in place using silk ties. Obtuse marginal arteries (OM) from the circumflex coronary artery will be identified. Ligatures (Proline 4.0) will be placed around the origin of OM1 and OM2. MI will be induced by permanent ligation of OM1 and OM2. The incision will be closed in layers, with irrigation utilized as necessary. The animals will be weaned off anesthesia and moved to a continually-monitored recovery area. On recovery of locomotion, the animals will be moved into housing cages, where they will be monitored at least twice daily. Fentanyl patches will be removed 3 days following surgery. Additional analgesics or antibiotics will be administered per recommendations of the staff veterinarian.

**Blood Sampling at 24 hours Post-MI Induction:** In order to provide an estimate of MI size, an aortic blood sample will be drawn from the subcutaneous access port. The pig will be sedated with up to 200 mg of benzodiazepam mixed in a meatball formulation administered 2 hours prior to study (ESI-Elkins-Sinn Inc, NJ), and placed in a custom designed sling that allows the animal to rest comfortably in a non-restrained fashion. The area around the access port (5cm) will be washed and prepped and a sterile field created. The access port will be entered with a custom needle (Huber) and 3cc of aortic blood drawn. The access port will then be flushed with heparinized saline (1000 U/mL) supplemented with cefazolin. This entire procedure will last approximately 15 minutes.

**Serial Imaging Measurements:**

<u>Sedation and anesthetic induction</u>

Animals will be sedated with 200 mg of benzodiazepam mixed in a meatball formulation administered 2 hours prior to study (ESI-Elkins-Sinn Inc, NJ), and placed in a custom designed sling that allows the animal to rest comfortably. The animals will be intubated with a cuffed intubation tube and allowed to self-ventilate with 0.5-3.0% isoflurane delivered through a portable anesthesia machine (capable of running on battery backup). Heart rate and rhythm will be continually monitored using surface ECG recordings (ECG machine will be a defibrillator with a minimum of 2 hours on battery backup).

The skin over the vascular access port on the back will be shaved and prepared in a sterile fashion with alternating wipes of betadine and alcohol. The access port will be entered with a custom needle (Huber) and drawback of arterial blood confirmed. The access

port with the associated intravenous line will then be capped using sterile supplies and housed in a custom-designed pouch.

Transport

For imaging studies, the anesthetized animals will be transported to the MRI facility that houses a 3 Tesla instrument in the animal facility approved vehicle (enclosed van). Studies will be performed in the late afternoon to evening hours so that there is no scheduling conflict with other studies being performed at this research imaging facility. Animals will be loaded and unloaded from the vehicle in such a manner that visibility to the public will be minimized through careful selection of transport route and utilization of enclosed loading bays. This vehicle will be specifically outfitted to transport the sling and anesthesia machine (with associated monitoring equipment) in a locked position so that the animals being transported remain stationary relative to the vehicle. In addition, a high capacity power inverter will be installed to provide power to the anesthesia machine and monitoring equipment while in the transport van.

Imaging

Longitudinal measurements of LV geometry and function will be performed using MRI (3T, Siemens). MRI images will be cardiac and respiratory gated to ensure consistent spatial positioning of the heart during each acquisition. A complete set of high temporal resolution images will be acquired in the short-axis plane. Noninvasive tagging of cardiac tissue in magnetic resonance images will be achieved by perturbing the local magnetization using spatial modulation of magnetization (SPAMM) to create MRI-visible tags within the myocardial wall. As these tags move with the underlying myocardial wall, the motion of the tags during the cardiac cycle reveals the internal motion of the otherwise featureless myocardial wall allowing for the measurement of regional strain. The MRI will be performed using a fast-gradient echo pulse sequence with a SPAMM preparatory pulse and the following variables: field of view: 22 cm, acquisition matrix: 256 × 128, flip angle: 15 degrees, repetition time to echo time: 8.8/2.2 ms, slice thickness: 6 mm, interslice gap: 0 (zero), tag spacing: 5 mm, and 2 k-space lines acquired per cardiac frame. These images will be stored for off-line analysis.

Image analysis will be performed using custom-written cardiac MRI analysis routines. Endocardial and epicardial LV contours will be drawn for each slice in the short-axis stack. The contours for the remaining phases are then automatically determined using an optical flow method, which tracks each point on the contour during the entire cardiac cycle, resulting in a complete set of contours being generated. The set of epicardial contours at end-diastole forms the basis for the three-dimensional model of the LV. The vertices from all the two-dimensional contours are input to a Delaunay tessellation algorithm to generate a three-dimensional surface model of the LV, represented as a mesh of interlocking triangles. A spline function fit will be used to improve the overall smoothness of the model. LV volumes at end-systole and end-diastole will be calculated from the endocardial contours. Regional motion (i.e. akinesia and/or dyskinesia) of the generated polygons will be used to determine the MI region.

As an additional measure of perfusion, gadolinium (Gd) enhanced contrast MRI images will be recorded. Gd-DTPA will be manually injected at a dose of 0.1 mmoles/kg through the vascular access port. Images will be acquired during the uptake and washout phase of the contrast agent for every R-R interval. These contrast-enhanced MRI images will be analyzed to determine the extent of the perfusion defect, which will be then quantitated as the area of the MI region.

Containment and cleaning of MRI facilities

While the animal is at the MRI facilities, the animal will be placed atop a containment field, which will prevent potential seepage of fluid(s) to the instrument and/or the flooring of the facility. The containment field will be constructed with disposable absorbent material as well as separation of the animal and the collection area such that the animals will not be in prolonged contact with any fluid/excretion.

Following the completion of the imaging studies, surfaces of the MRI equipment within proximity of the animal will be wiped down with wipes soaked in a freshly diluted 10% bleach (sodium hypochlorite) solution. In addition, disinfectant aerosol will be sprayed in all ante rooms (using MRI safe bottles) to minimize the potential of odors.

Recovery

Following the completion of imaging studies, the animals will be transported back to housing facilities at the Strom Thurmond Building. The access port will then be flushed with heparinized saline (1000 U/mL) supplemented with cefazolin. The Huber needle will be removed. The animals on which future imaging studies will be performed will be weaned off anesthesia, extubated, and then returned to cages once recovered from anesthesia. The animals in which the final set of imaging studies are completed, will be processed for terminal studies.

**Terminal Studies**

Following the final set of MRI studies at 56 days post-MI, the pigs will be anesthetized for assessment of global and regional LV function, microdialysis measurements and hemodynamics. Anesthetic induction will commence using isoflurane (3%) in a mixture of oxygen and nitrous oxide (67%:33%, 1.5 L/minute) delivered by face mask. Once the pig is adequately anesthetized, peripheral venous access (ear) will be obtained and a 2 μg/kg dose of sufentanyl (ESI-Elkins-Sinn Inc, NJ) will be injected through the ear vein cannula. A 0.1mg/kg dose of etomidate (Amidate, ESI, NJ) and a 10mg dose of vecuronium will then be administered intravenously after ensuring that the animal remains adequately anesthetized. This results in a rapid deepening of the already established surgical plane of anesthesia. An endotracheal tube will be surgically placed via a midline submandibular incision and mechanical ventilation established. Anesthesia will be maintained throughout the procedure by delivery of 0.5% isoflurane and intravenously administered morphine (ESI, 3 mg/kg/hr). The delivery of isoflurane, nitrous oxide and morphine will be titrated to maintain stable

physiologic hemodynamic and respiratory profiles. Intensive and continuous monitoring of various vital signs will provide the necessary means to ensure a complete and stable surgical plane of anesthesia. Following stabilization of this surgical anesthetic plane, an intravenous infusion of vecuronium (15mg/hr) will be initiated. This infusion will be titrated as needed to provide continuous muscle relaxation which facilitates appropriate mechanical ventilatory control and a stable surgical field. Additional 5 mg boluses of vecuronium will be administered as needed to support these goals. In the eventuality that vecuronium is unavailable, intravenous pancuronium will be used to effect paralysis. Pancuronium will be delivered through the implanted central line and additional boluses administered as necessary. The heart rate and blood pressure will be carefully monitored to ensure that the animals remain at a stable surgical plane of anesthesia throughout the procedure.

A multi-lumened thermodilution catheter (7.5F, Baxter Healthcare Corp., Irvine, CA) will be positioned in the pulmonary artery via the right external jugular vein. An 8F introducer with side-arm will be placed in the right carotid for blood pressure measurements and arterial access. The aortic access port will also be connected to monitor systemic pressures. A Foley bladder catheter will be surgically placed and secured via a midline suprapubic retroperitoneal incision. A sternotomy will be performed and a vascular ligature placed around the inferior vena cava in order to perform transient caval occlusion. A previously calibrated microtipped transducer (7.5 F, Millar Instruments Inc, Houston, TX) will be placed in the LV through a small apical stab wound. Piezoelectric crystals (2 mm, Sonometrics, Ontario) will be positioned in the LV endocardium in order to provide an orthogonal myocardial dimension across the short axis in 2 regions: the MI region and the remote region. The remote regions will be defined as the area served by the LAD. From this crystal array, LV dimension and wall thickness will be recorded at a sampling frequency of 100 Hz and digitized (Pentium-Sonolab, Sonometrics). A pair of microdialysis probes containing a 4 mm membrane (CMA/Microdialysis, North Chelmsford, MA) will be inserted in the mid-myocardial region between the crystal pairs. The microdialysis probes will be connected to a precision infusion pump and controller system (CMA, MA). A flow rate of 2.5 μL/min will be established and the iso-osmotic dialysis solution containing MMP fluorescent substrates will be infused into each microdialysis probe. Following a 30 min equilibration period, dialysate samples will be collected into chilled tubes at 30 minute intervals. The microdialysis samples will first undergo fluorescent readings and then immediately frozen for subsequent cytokine assay. Thus, MMP fluorescent activity and interstitial concentrations can be measured from the same microdialysate sample. Following the placement of the instrumentation, baseline LV function, regional myocardial function, and microdialysis samples will be collected. Thermodilution derived cardiac output and stroke volume will be obtained from the pulmonary artery catheter in triplicate. All measurements will be simultaneously recorded with the ventilator temporarily suspended in order to prevent respiratory artifact in the LV pressure recordings. Following steady-state measurements, LV preload will be altered by sequential occlusion and release of the inferior vena cava and measurements recorded during occlusion and release. LV peak positive dP/dt will be determined from the digitized LV pressure signal. Pulmonary and systemic vascular resistances will be computed from the thermodilution cardiac output and pressure measurements using standard formulae. From the digitized piezoelectric crystal data, LV myocardial wall thickness throughout the cardiac cycle will be determined and LV end-diastolic myocardial wall thickness considered to be the minimum wall thickness measurement obtained during the cardiac cycle and will be temporally aligned with the R-wave of the simultaneously digitized ECG. LV end-systolic wall

thickness will be defined as maximum myocardial wall thickness. LV myocardial velocity of circumferential fiber shortening, corrected for heart rate (Vcfc) will be computed from the digitized LV crystal and pressure data as described previously. The isochronal LV Vcfc-end systolic wall stress relation will be determined following release of the caval occlusion. Furthermore, the LV systolic segmental shortening-pressure relation will also be determined from the points obtained during and following the caval occlusion. Thus, several load independent indices of LV ejection performance will be obtained. Moreover, this will allow for the construction of LV pressure-dimension loops and the computation of the area circumscribed by this relation.

**issue Collection and Euthanasia**

Following completion of the protocol described above, isoflurane delivery will be increased to 5%, and maintaining full anesthesia, cardioplegic arrest will be induced through delivery of a 24 mEq potassium solution in lactated Ringers through the aortic root. The heart will be harvested and the LV isolated and placed in chilled Krebs solution. The LV will then be cut in circumferential sections (5mm) perpendicular to the long axis and each section weighed. A mid circumferential section will then be placed in a solution containing a tetrazolium salt which will identify the region of the MI. Total infarct size will be defined using tetrazolium staining methods in which digital images of the stained sections will be used to define infarct size by planimetry. A second mid-circumferential section will be processed for fluorescent microsphere measurements. Sections from the MI, border and remote regions will be processed for histology. Finally, regions comprising the MI, border and remote regions will be dissected and flash frozen for subsequent biological assays.

***Rationale for the use of animals and determination that alternatives to their use do not exist***

There are no *in vivo* models that simulate regional and global remodeling in the LV following MI. Therefore, there are no alternatives to performing these procedures in animals.

***Why the chosen species is the most appropriate. List the experimental groups (including controls)***

Pigs have been shown to be an excellent model for performing studies for determining changes in the myocardial ECM in a number of simulated cardiac disease states. Importantly, it has been demonstrated that pigs most accurately reflect the coronary anatomy of humans and respond in a similar fashion to myocardial ischemia/infarction (Gross, DR. Animal models in cardiovascular research. Kluwer Academic Pub, Dordrecht, 153:pp428-436, 1994). Secondly, pigs can be obtained in consistent sizes and weights and therefore, reducing variability between experimental observations. Our laboratory has developed a computerized database of normal baseline hemodynamics, cardiac morphology, ultrastructure, and metabolism for this species.

All data will be collected and coded throughout the protocol and the code broken at the completion of the study. The dependent variables will be compared using analysis of variance for repeated measures across time, and by t-test for single measurements between the 2 groups. For the different treatment-time interventions, a multi-way analysis of variance using a factorial design will be utilized. If the analysis of variance reveals significant differences, pairwise tests of individual group means will be compared using Bonferroni

probabilities. The relative change in indices of LV geometry and function will also be compared to baseline values using a paired t-test. Values of $p<0.05$ will be considered to be statistically significant. The sample size estimates for the MI studies were predicated upon the known variability in changes in regional myocardial dimensions following MI as well as the attrition rate from MI induction. The sample size estimates for the *in vivo* measurements is predicated on several factors. First, that the baseline values in each preparation will be comparable to those randomized the treatment protocols. Second, that the attrition rate in each MI group will not exceed 12-15% (ventricular fibrillation, instrumentation failures), resulting in a minimum sample size of 10 in each treatment group. And third, that the risk of a type I error will be maintained at less than 5% and the risk of a type II error will not exceed 25%. Finally, time-matched non-MI control animals will provide for a comparison and improve the sensitivity of the measurements. Therefore, a total of 20 pigs will be needed for this protocol (n=10 in the non-MI group for Phase 1 and n=10 in the MI group for Phase 2).

***Veterinary care of animals involved***

Upon receipt, and prior to terminal studies, the pigs will be under the veterinary care provided by the University animal care and use program. An assurance statement is on file with OPRR/DHSS detailing the program for animal care and the institution has full accreditation from AAALAC within the last year. The animals will be under the direct care of the veterinary staff and we will consult with them regarding the status of the animals being used in this study.

***Potential adverse effects of the experiment***

Preoperative analgesia will be delivered through a 100 μg fentanyl patch (5 μg/kg/day, release rate of 50 μg/hr) in addition to an intramuscular injection of buprenorphine (0.05-0.1 mg/kg im). A surgical plane of anesthesia will be provided through the use of inhalation isoflurane. The animal will be recovered from the surgery in an intensive care unit under the direction of the staff veterinarians. Buprenorphine 0.005-0.02 mg/kg will be utilized for immediate post-surgical pain. For prolonged post-thoracotomy pain and more prolonged analgesia, ketoralac tromethamine will be administered. Animals with severe post-operative complications or potential hemodynamic compromise will be euthanized with 5% isoflurane and an overdose of Nembutal.

***Euthanasia***

Pigs will be euthanized by exsanguination under a surgical plane of isoflurane anesthesia. Animals deemed to be in distress will be euthanized by a barbiturate overdose. These methods are consistent with the recommendations of the Panel of Euthanasia of the American Veterinary Medical Association.

***Alternatives to the procedures***

Alternatives to the use of animals for this research proposal and alternatives to any painful procedure have been carefully considered. However, there are no non-animal models that simulate the changes in myocardial geometry, biochemistry, structure, and function that occur following MI.

This work was carefully researched with respect to alternative methods to achieve the objectives outlined in this project. Sources used for this research included the National Library of Medicine, Ovid Web, and PubMed. Keywords used for these searches include: myocardial infarction, myocardial remodeling, left ventricle, left ventricular remodeling, animal welfare, and animal research alternatives. Online computerized searches for relevant published articles were initiated and the latest search was performed on March 16, 2011. Literature searches using the same parameters will be repeated monthly.

***Duplication of work***

There are no publications with respect to the relationship between early infarct expansion and late LV dilation post-MI. Therefore, the proposed studies do not duplicate previous research.