Speckle Tracking Echocardiography Detects Strain Changes in Murine Heart during Acute Ischemia Perfusion

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THESIS

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**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>1. Literature Review</td>
<td></td>
</tr>
<tr>
<td>1.1 LV Remodeling and its effects</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Imaging Modalities: Past and current</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Strain and Strain Rate</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Doppler Strain Imaging</td>
<td>6</td>
</tr>
<tr>
<td>1.5 Speckle Tracking Echocardiography</td>
<td>7</td>
</tr>
<tr>
<td>1.6 Mesenchymal Stem cells and role of MSCs in cardiomyocyte repair</td>
<td>8</td>
</tr>
<tr>
<td>II. 2. SPECIFIC AIMS</td>
<td></td>
</tr>
<tr>
<td>2.1 Specific Aim #1</td>
<td>15</td>
</tr>
<tr>
<td>2.2 Specific Aim #2</td>
<td>15</td>
</tr>
<tr>
<td>2.3 Specific Aim #3</td>
<td>16</td>
</tr>
<tr>
<td>III. 3. MATERIALS AND METHODS</td>
<td></td>
</tr>
<tr>
<td>3.1 BM-MSC isolation and in-vitro expansion</td>
<td>17</td>
</tr>
<tr>
<td>3.2 Coronary Ligation &amp; BM-MSC Injection</td>
<td>20</td>
</tr>
<tr>
<td>3.3 Speckle Tracking Measurements</td>
<td>21</td>
</tr>
<tr>
<td>3.4 Statistical Analysis</td>
<td>23</td>
</tr>
<tr>
<td>IV. 4. RESULTS</td>
<td></td>
</tr>
<tr>
<td>4.1 Regional Velocity measurements in the parasternal Apex</td>
<td>24</td>
</tr>
<tr>
<td>4.2 Regional Displacement measurements in the parasternal Apex</td>
<td>26</td>
</tr>
<tr>
<td>4.3 Regional Strain measurements in the parasternal Apex</td>
<td>29</td>
</tr>
<tr>
<td>4.4 Regional Strain rate measurements in the parasternal Apex</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>4.5</td>
<td>Regional Displacement measurements in the short axis Apex</td>
</tr>
<tr>
<td>4.6</td>
<td>Regional Velocity measurements in the short axis Apex</td>
</tr>
<tr>
<td>4.7</td>
<td>Regional Strain measurements in the short axis Apex</td>
</tr>
<tr>
<td>4.8</td>
<td>Regional Strain rate measurements in the short axis Apex</td>
</tr>
<tr>
<td>V.</td>
<td>5. DISCUSSION</td>
</tr>
<tr>
<td>VI.</td>
<td>6. CONCLUSION</td>
</tr>
<tr>
<td>VII.</td>
<td>7. CITED LITERATURE</td>
</tr>
<tr>
<td></td>
<td>VITAE</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

BM-MSC  Bone marrow-derived mesenchymal stromal/stem cells

dP/dt    Derivative pressure product

EDPVR   End-diastolic pressure–volume relation

$E_{es}$ End-systolic elastance

$E_{max}$ Maximum elastance

ESPVR   End-systolic pressure–volume relationship

FBS     Fetal bovine serum

HGF     Hepatocyte growth factor

HSC     Hematopoietic stem cells

IL      Interleukin

LV      Left ventricular

MI      Myocardial infarction

MSC     Mesenchymal stromal/stem cells

PBS     Phosphate buffered saline

TNF     Tumor necrosis factor

VEGF    Vascular endothelial growth factor
Myocardial infarction (MI) is the leading cause of mortality and morbidity worldwide. Rapid loss of cardiomyocytes induced by MI and subsequent reperfusion results in remodeling of left ventricular (LV) cardiac architecture to support the contractile functioning of the heart. But the functioning of such a remodeled heart cannot be compared with a healthy heart due to a variety of reasons.

Even though global strain measurements are considered to be an index to characterize heart function, myocardial dysfunction and the progression to heart failure may not be easily identified using global cardiac function measurements. This is because vital measurement parameters such as the ejection fraction (EF), and LV stroke volume remain within normal limits. So the measurement of LV global function remains insufficient.

As a result, the measurement of regional changes that occur in the LV becomes in predicting the progression of cardiac disease and heart failure. Strain and strain rate are excellent measures that are used as a clinical index to gauge myocardial function as they measure the magnitude and rate of myocardial deformation during both systole and diastole.
2D-Speckle tracking echocardiography (STE) is a novel method for determining both global and regional strain and strain rate. The principle used in STE is the measurement of distance between two stable patterns or “speckles” on a 2D echocardiographic image of a LV segment during a cardiac cycle. Unlike Doppler imaging STE is angle independent and hence is less subjected to variabilities in quantification of global and regional LV function.

Cardiac regenerative therapy (CRT) using stem/progenitor precursor cells have gained considerable research attention with varying degree of beneficial effects for various kinds of cells. Our lab focuses on bone marrow-derived mesenchymal stem cells as a potential tool for CRT due to their ease of isolation, huge expansion potential and effectiveness to enhance repair in many tissues (Boomsma A.R. 2006, Grajales, Garcia et al. 2012). However, others and we have demonstrated a dramatic loss of stem cells within hours of administration in the heart (Boomsma et al. 2006). Since the number of cells available in the region of injury corresponds to their beneficial effects, prevention of loss of stem cells would improve their contribution to positive changes in the functioning of heart. This in turn is measurable by increased strain and strain rate at the site of injury.
Overall, our data demonstrated an increase in strain and strain rate measures with the introduction of stem cells when compared to a sham and a MI group with no stem cells. The presence of stem cells did alter the strain and strain rate values in a positive manner. Though a comprehensive improvement in cardiac function due to the injection of stem cells could not be verified, an encouraging trend was observed.
1. Literature Review:

1.1 LV Remodeling and its effects

The myocardium functions essentially in alternating phases known as systole and diastole. The sarcomere dynamics, geometry of the ventricles, orientation of the fibers, and myocardial wall elasticity determine the extent of shortening and relaxation. Injury to the myocardium by means of a myocardial infarction (MI) causes Left Ventricular (LV) remodeling that is immediately followed by a change in shape, size and function. This in turn is followed by pressure and volume overload, which causes the LV to undergo functional and massive structural changes that can affect the overall cardiac function.

LV remodeling occurs within hours following an infarction and is an early prognostic indicator of long-term survival. Heart failure is a result of progressive myocardial enlargement and dysfunction. The adverse effects of MI and ensuing arrhythmias are LV dilation through adverse remodeling and replacement fibrosis. Replacement fibrosis causes reduction in strain through loss of contractile elements. However, LV remodeling causes increased stress and decreased contractility in the viable myocardium thereby causing a loss of cardiac function.
(Yu Peng 2009). Hence, the assessment of regional myocardial function and LV remodeling after an injury adds therapeutic and prognostic value to treatment including revascularization and stem cell therapy. The evolving infarcts with LV remodeling and increased fibrosis causes reduced tethering within the myocardial fiber bundles leading to cardiac dysfunction (Becker, Bilke et al. 2006).

Previous studies have shown that LV remodeling following MI occurs not only due to myocyte death in the infarct region but also as a myopathic process in the infarct region coupled with apoptosis (Lim, Fallavollita et al. 1999). Also, it has been demonstrated that dysfunctions occurs in adjacent regions after anterior myocardial infarction, which, further depresses the contractile efficiency of the myocardium (Migrino, Zhu et al. 2008). This could be addressed by quantifying the strain changes in the myocardium using various imaging modalities that have been improved over a period of time. These improvements have provided a greater insight about the inherent adaptations post MI for the effective functioning of the myocardium.
1.2 Imaging modalities: Past and Current

The initial evaluation of myocardial function by echocardiography heavily relied on visual detection of the complete endocardial wall motion and its abnormalities and also the assessment of left ventricular ejection fraction. This approach had its disadvantages as it was subjective based on the operator (Hoit 2011). Though myocardial shortening and thickening represent the mechanics of the heart, the effects of longitudinal and circumferential deformation have not been routinely studied. The development of a non-invasive and inclusive echocardiographic gold standard to assess regional myocardial performance is absolutely necessary.

The current clinical standard employed to quantify regional strain measurements is with Tissue Doppler imaging (TDI). In TDI, the velocity of the myocardium is measured from the apical window for longitudinal strain and short-axis scans are employed for radial strain in both systole and diastole and is generally more sensitive to contractile than ejection fraction (Nesbitt GC, Mankad S et al. 2009). This is achieved by quantifying the peak systolic strain from each LV segment. However, the disadvantage with TDI is that the velocities fluctuate
due to translational movement and tethering. This becomes time consuming and also makes it difficult to differentiate the akinetic segments that are pulled along with actively contracting segments (Pislaru CAT, 2002)

1.3 Strain and Strain rate:

Myocardial strain and strain rate measurements have been identified as a clinical index for regional and global myocardial function (Amundsen, Helle-Valle et al. 2006). Strain and strain rate measure the magnitude and rate of myocardial deformation during both systole and diastole. Three kinds of strain measurements are taken into account while analyzing myocardial deformation. These are: circumferential strain and strain rate that account for fiber shortening along the circumferential line. Longitudinal strain measures the myocardial deformation along the long axis of the heart that predicts the adverse effects of LV remodeling post anterior wall acute myocardial infarction. Radial strain relates to extent of wall thickening whereas, radial strain rate relates to velocity of wall thickening (Sun, Niu et al. 2007). As a result, abnormal myocardial deformation can be observed by means of measuring strain and strain rate (Hoit 2011). Apart from global measurements of strain any additional information about regional strain measurements add both diagnostic and prognostic information. This might be
crucial in predicting myocardial recovery post MI and the possibility of any future MI due to regional changes (Hoit 2011).

Strain is defined as the normalized dimensionless measure of deformation of a solid object, when a force or stress acts on the object. There are two types of strain namely the Lagrangian and Natural strain. Lagrangian strain $\varepsilon_l$ is defined as the deformation of an object from its initial length ($L_0$) before any force acted upon it

$$\varepsilon_l = \frac{L(t) - L(t_0)}{L(t_0)}$$

Where $L(t)$ is the length of object at instantaneous time $t$ after deformation and $L(t_0)$ is the length of object when no force acts upon it. However, Natural strain is denoted by $\varepsilon_n$ and is calculated by,

$$\varepsilon_n = \ln \left[ 1 + \varepsilon(t) \right]$$

Natural strain is used to measure strain in case of the Vevo 2100 due to the 3D geometry of the heart and hence instantaneous measures of length of the cardiac muscle fiber are taken into account. $\varepsilon_n$ denotes the natural strain, which is a natural logarithm expressing the deformation of an object relative to the length at a
previous time instance and not the initial length \( (t_0) \). Due to the 3D rendition of the myocardium and the orientation of myocardial muscle fibers there are 3 kinds of strain measurements that are taken into account namely the circumferential, longitudinal and the radial strain which are either shear or normal to the plane of cardiac orientation (Hoit 2011). Strain values measured can be either positive or negative and can denote lengthening or shortening of the muscle fibers. By convention, negative values of strain are associated with circumferential and longitudinal strain and positive values of strain are with radial strain respectively.

1.4 Doppler Strain Imaging:

Strain rate is defined as the strain measured over time or in other words the rate of change of deformation and it has a unit of \( \text{s}^{-1} \). Strain and strain rate are acquired either by Doppler strain imaging (DSI) or 2D Speckle tracking echocardiography (STE). DSI has its disadvantages with respect to measuring strain and strain rate. DSI is angle dependent and since, there are 3 components of strain with perpendicular vectors and differing sign convention, it makes it important to align the ultrasound beam parallel to the region of interest in order to obtain strain data. Also, the angle deviations can make strain rate calculation
difficult. The myocardial deformation takes place in 3D space whereas the Doppler strain calculations are in 1D and strain measurement occurs only in the axis of ultrasound beam propagation. Thus only a few components of strain are calculated instantaneously.

1.5 Speckle Tracking Echocardiography (STE):

Strain measurements by 2D speckle tracking echocardiography adopts a frame-by-frame tracking of speckles or natural acoustic markers to measure myocardial deformation that are generated by the interaction of the incoming ultrasound beam and the myocardium by means of reflection, scattering and interference (Gjesdal, Hopp et al. 2007). STE calculates the natural strain and this is achieved by employing 2 kinds of tracking algorithms. The algorithm may either be a simple absolute sum of differences that essentially fits a weighted data set to calculate regional strain at approximately 3mm interval of LV circumference. The other method by which strain is calculated is by applying Fourier analysis that assumes a shape similar to the tracked geometry using a sequence of intermediate passages in the range of 2 cm to 5-Pixel bands (Leitman, Lysyansky et al. 2004).
The quality of image is critical for accurate tracking and strain measurements by 2D STE. The strain measurements are affected when the heart swings out of the region of interest. Also, when the heart rotates or moves out of the image plane, out-of-plane motion occurs which leads to only a portion of the real motion being acquired (Hoit 2011). Myocardial infarction leads to reduce longitudinal strain and correlates with infarct size and ejection fraction and this leads to LV remodeling (Gjesdal, Hopp et al. 2007). STE is employed to measure deformation and global strains ideally in multiple directions which has the potential to identify infarct size, the extension of a myocardial scar and extent of nonviable myocardium (Jurcut, Pappas et al. 2008)

1.6 MSC s and Role of MSC in Cardiomyocyte Repair

A cell selected form adult tissue, that can be expanded appropriately in population by in-vitro culture, that can retain its ability to differentiate into a specific lineage, is called a proper “stem cell” or MSC (Muraglia A 2000). It is possible to clone and grow millions of MSC from a bone marrow aspirate in-vitro. The need for in-vitro expansion of MSC is due to its inability to come in contact with each other in tissue formation. There are wide varieties of advantages working
with MSC due to their ease of isolation, huge expansion potential, genetic stability, compatibility with tissue engineering principle and potential to enhance repair in many tissues (Jones, Kinsey et al. 2002).

MSCs can be injected allogenically, implanted systemically and be made to differentiate into a phenotype of cardiomyocyte when implanted into a healthy myocardium (Toma 2002, Pittenger 2004). MSCs have the ability to home to the site of injury when injected intravenously after acute infarction. Evidence shows that MSCs injected into infarcted myocardium engrafted and differentiated toward a myogenic lineage with the expression of muscle specific proteins including $\alpha$-actinin, troponin-T, tropomyosin, myosin heavy chain-MHC, phospholamban and other muscle-specific proteins (Shake G.J 2002), (Grajales, Garcia et al. 2012). The mechanism of MSC-mediated improvements in perfusion may be attributed to their ability to secrete a wide variety of angiogenic cytokines (fibroblast growth factor, vascular endothelial growth factor (VEGF), matrix metallo-proteases, platelet derived growth factor, interleukin-1 etc.) that are expressed only from cells injected into the myocardium and not in in vitro cultures of MSCs. However, the myogenic differentiation into a complete structural sarcomere has not been observed after the injection of MSCs in an infarcted myocardium (Pittenger 1999,
MSC cardiomyoplasty has been associated with significant functional improvements in post MI hearts such as prevention of pathologic wall thinning by improvement of end-diastolic wall thickness by 30% and improved post infarction hemodynamics (Shake G.J 2002). Lower end-diastolic pressure in MSC treated animals suggests improved diastolic relaxation and decreased wall stress that are likely to be associated with ventricular remodeling. Lack of improved systolic function may be attributed to the absence of a proper sarcomere organization in the MSCs injected into the damaged region. However, these results show that the injected MSCs do contribute to increased myocardial compliance with better diastolic filling properties (Toma 2002).

“The loss of cardiomyocytes after MI coupled with a lack of endogenous cell repair mechanism is a reason for progressive heart failure” (Pittenger 2004). Myocardial perfusion plays a major role in effective cardiac function. Post myocardial infarction, restoration of cardiac function not only requires the replenishment of lost cardiac myocytes but also revascularization and regeneration of infarcted myocardium. Adult cardiomyocytes essentially have no regenerative capacity and hence, introduction of stem cells may offer a meaningful approach to treat the damaged myocardium (Anversa 2002). Evidence of exogenous cell
lineages that can engraft in the myocardium and offer some functional improvements has paved the way for deeper interest in cell therapeutic applications (Ray C. J 1995).

The physiology of MSCs in wound repair is aided by the ability of the cells to home to the site of injury after being injected into an infarcted myocardium (Taylor 2002, Pittenger 2004). However, the window of MSC homing is limited, wherein 5 million cells injected into the infarcted rat myocardium resulted in a significant cardiac engraftment within 10 minutes of reperfusion. When the cells were injected 2 weeks post reperfusion there was a significant reduction in cardiac engraftment and most of the cells returned to the bone marrow (Pittenger 2004). Factors responsible for such a remarkable specificity of homing has not been clearly identified although the involvement of an inflammatory mediator similar to those responsible involved in macrophage and neutrophil infiltration in injured tissue has been reported (Rombouts and Ploemacher 2003).

A variety of cells have been used to prove that bone marrow derived stem cells differentiate into cardiomyocytes, smooth muscle cells and endothelial cells in vitro (Horwitz, Gordon et al. 2002). Theoretically, an ideal cell chosen for
cellular therapy has to be less committed and that which can undergo cardiomyocyte differentiation to enhance angiogenesis and trigger vasculogenesis. MSC may have the necessary combination of plasticity and viability to do so (Simmons PJ 1991). MSCs are not only capable of \textit{in vitro} transdifferentiation into functional cardiomyocytes in the presence of appropriate culture conditions but also transdifferentiate into functional cells in an acute ischemic model or in a myocardial environment that expresses desmin, troponin-T adrenoreceptor and sarcomereic MHC (Jane S Reese 1999, Lazarus 2001). Transdifferentiation of MSCs into functional cardiomyocytes would lead to reduced scar size, reduced LV remodeling and improved cardiac function. Recent studies that evaluated the functional improvements of MSC transplantation by catheterization demonstrated an attenuation in the +/- dP/dtmax and LV systolic pressure (LVSP) and attenuation in the LV end-diastolic pressure (LVEDP) suggesting that MSCs may contribute to preserved cardiac function (Pittenger 2004).

Furthermore, it has been shown that MSC transplantation inhibits LV remodeling, augments or preserves the elasticity of the myocardium, improves heart function and reduces expression of extracellular matrix genes (Fuchs S 2001). Direct evidence for the improvements in cardiac function by preservation
of residual cardiomyocytes has not been demonstrated by the regional implantation of MSCs (Pittenger 2004)

The underlying mechanisms involved in the angiogenesis post MSC implantation are unclear. The ischemic myocardium might express certain ligands, which facilitate the trafficking, adhesion and homing of MSCs to the site of injury. Also studies have shown the presence of Connexin 43, a gap junction protein that facilitates the adhesion of MSCs through cell-cell attachment leading to potential tissue regeneration. This further highlights the importance of electromechanical coupling properties of MSCs (Fuchs S 2001). Other studies have shown that stem cells fuse directly with the host cardiomyocytes in the ischemic myocardium. Based on these studies it is possible to state that the MSCs implanted into the myocardium undergo cardiogenic differentiation.

From these studies the importance of the LV and the impact it has after an MI has been clearly established. The different imaging techniques that have been employed to calculate regional strain and strain rate have been reviewed. The important role MSC’s play in the repair after an MI and the benefit it provides has also been established. Now it would be ideal to study the retention capabilities of
the MSCs by the myocardium post MI and their positive influence in the functioning of the myocardium by improvement of strain values in the apex of the myocardium where the injury has been induced. A region focused imaging such as the STE that would facilitate the study can achieve this.
2. Specific Aims:

2.1 To establish the ability of regional strain measurements in predicting the effect of LV remodeling post MI:

The following experiments were carried out with a null hypothesis that the ability to measure regional strain changes over the global strain would not help in predicting the amount of LV remodeling that would take place post MI.

2.2 To establish STE as a tool for measuring regional strain changes:

To establish speckle tracking based echocardiography as a tool for measuring the regional strain and strain rate. Previous studies have shown the need and importance of measuring regional strain and strain rate as a parameter for treatment post MI. Vevo-2100 is a specialized echocardiographic tool for small animal imaging. It makes use of the principle of tracking “speckles” or natural acoustic markers that are readily available when sound waves are passed on to the target organ or tissue. The sound waves that are reflected off of the tissue then form an interference pattern with the subsequent incoming sound wave. The distance between two speckles is mapped and traced along the path of the LV. This
is then used to establish the strain values based on the principle of measuring lagrangian strain.

2.3 To study the effect of retention of stem cells and their effective contribution in improving myocardial function post injection:

MSCs are to be introduced in the myocardium intravenously post MI using a PBS solution as a vehicle, and the animals are to be imaged 24 hrs post introduction of stem cells to study the effect of MSCs in improving contractile function in the myocardium. Regional velocity, displacement, strain and strain rate from the radial, longitudinal and circumferential orientation are measured in the parasternal long-axis and short-axis view. It is expected that the animals with injected MSCs are to have a improved regional strain and strain rates at the apex of the heart.
3. Materials and Methods:

3.1 BM-MSC isolation and in-vitro expansion:

BM-MSCs were isolated from C57BL/6 mice (Jackson Laboratory, Maine) as previously described (Boomsma, Swaminathan, & Geenen, 2007). Tibia and femur were stripped of muscle and placed in ice-cold phosphate buffered saline (PBS) + 2% fetal bovine serum (FBS). The epiphyseal ends were removed and the bones were centrifuged at 4,000×g for 1 minute in a microfuge tube containing the cut end of a 1 ml pipette tip inside its other half (see Figure 1). The tips provided support for the bones during centrifugation and allowed the marrow to collect at the bottom of the tube. The bone marrow cells were suspended in ice-cold PBS + 2% FBS, passed through a 70-µm filter and counted with a hemocytometer.

Filtered bone marrow cells were suspended in PBS + 2% FBS + 0.1 g/L phenol red and enriched for lineage negative (Lin⁻) cells using SpinSep system (StemCell Technologies, Canada). The cells were incubated with Murine Progenitor Enrichment Cocktail (anti-CD45, anti-CD45R, anti-CD11b, anti-Gr-1, anti-TER119, and anti-7/4) (StemCell Technologies, Canada) on ice for 30 minutes, washed, and incubated with dense particles on ice for 20 minutes. The
cells were layered on density medium, centrifuged at 1,200×g for 10 minutes, and the layer of cells at the density medium/PBS interface was collected, washed, and counted.

Enriched bone marrow cells were seeded on tissue culture-treated plates at a density of 0.1×10⁶ cells/cm² in murine MesenCult media (StemCell Technologies, Canada) with 100-µg/ml penicillin, 100-µg/ml streptomycin, and 0.25 µg/ml amphotericin B added. The media were changed after 48 hours and adherent cells were maintained in culture with twice weekly media changes. After 4 weeks, the confluent cells were detached with trypsin and split 3:1. Lin⁻ MSCs were characterized for surface antigens using flow cytometry. Antibodies were obtained from BD Pharningen unless otherwise noted. Cultured cells were detached with Trypsin and incubated with 1 µl mouse Fc block (1:50 dilution; clone 2.4G2, rat anti-mouse CD16/CD32; Sigma, Missouri) for 5 minutes on ice. Cells were then incubated with 1-µl fluorescent-conjugated antibodies (1:50 dilution) for 1 hour on ice, washed, and analyzed.
Figure 1: Modified microfuge tube for bone marrow cell isolation. One–ml pipette tip is cut in half, the tip is inserted into the other end and then they are fit on top of a microfuge tube and act as a cradle to hold the bone in place.
3.2 Coronary Ligation & BM-MSC Injection:

BM-MSCs were detached from the cell culture plate with trypsin and washed in injection buffer (Ca\(^{2+}\)/Mg\(^{2+}\) free PBS + 2 mM EDTA + 0.25% bovine serum albumin), passed through a 35-μm-cell strainer and suspended in a final concentration of 10\(^5\)-cells/10 μl. For the other experimental groups for in vivo injections, GFP\(^+\) BM-MSCs were treated overnight with CBX (100 μM), or CBX in injection buffer (100 μM), and vehicle alone were used.

Wild type C57BL/6 mice were initially anesthetized with etomidate (10 mg/kg body weight; i.p.) and intubated with an 18-gauge angiocath sleeve. Surgical anesthesia was maintained using 1.5% isoflurane delivered through a vaporizer with 100% oxygen connected in series to a rodent ventilator with the tidal volume set at 0.2 to 0.3 ml/min (based on body weight) and a respiratory rate of 135 per minute. A left thoracotomy was performed to expose the heart and the pericardium was ruptured. The left coronary artery was ligated 3 mm from the ostium with 8-0 prolene sutures to produce myocardial ischemia. The mouse was maintained on isoflurane for 90 minutes following the coronary ligation after which, the suture was removed and the ischemic area was reperfused. The different groups of animals namely the Sham +BM-MSC and MI+ BM-MSC were injected either with BM-MSCs (1x10\(^5\); suspended in 10 μl) and Sham, MI animals were injected with vehicle alone respectively. They
were injected into the apical myocardium with a 30-gauge microliter syringe (Hamilton Inc, Nevada). Following injection a chest tube was placed in the thoracic cavity and the thoracotomy was closed in three layers (intercostal muscles, pectoral muscles, and skin) followed by evacuation of the chest cavity and removal of the tube. During the surgery and hemodynamic measurements, an adequate plane of anesthesia was judged by respiration, heart rate and toe pinch reflex. Animals received buprenorphine (0.1 ml of 0.03 mg/ml; s.c.) at the time of surgery and were allowed to recover in a heated cage for 24 hours. Sham–operated mice underwent the same procedure as described above except the left coronary artery was not ligated. All subsequent experiments were performed at 24 hours after ischemia/reperfusion.

3.3 Speckle tracking measurements:

Echocardiographic images were obtained using a Vevo-2100 ultrasound imaging system, a high-resolution in-vivo micro imaging system (VisualSonics, ON/Canada). The Vevo-2100 is a specially designed imaging system, which enables in-vivo visualization, assessment, measurement of anatomical structures and hemodynamic measurements for imaging small animals using an array of measurement packages such as cardiac measurement package, abdominal measurement package and vascular measurement package. The in-vivo visualization is obtained by means of simple 2D B-mode, M-
mode, PW (Pulsed Wave) Doppler mode, Color Doppler mode, Power Doppler mode and Contrast mode imaging. We employed simple 2D B-mode & M-mode imaging in the longitudinal parasternal and circumferential short axis orientations for measuring strain and strain rate.

The images were obtained after placing the mouse on a dedicated platform with a continuous supply of anesthesia maintained using 1.5% isoflurane delivered through a vaporizer with 100% oxygen. A MS-400 (256-element) MicroScan array transducer optimized for mouse cardiovascular imaging that delivers a frame rate of over 300 fps was used to obtain real-time visualization of the anatomical target i.e. the LV of the mice. The transducer was fixed on top of the animal stage and attached to the support setup, which offers a high degree of freedom to maneuver over the animal. B-mode images were obtained for locating anatomical structures such as the LV.

The parasternal axis is located after applying the ultrasound gel placed in the warm bath. The transducer is moved and the real-time B-mode images of the LV are recorded. The cine loop frames that record the LV were saved and then the transducer was rotated in the anti-clockwise direction to view the short-axis and record the same. It was ensured that the transducer was not lifted from the surface of the gel while it was rotated in the anti-clockwise direction to maintain continuity and to record images from the same region as
the parasternal axis. The corresponding M-mode images were obtained for each of the animals and stored as cine loops.

The cine loops were later analyzed using the Vevo-2100 software cardiac measurements package. The tracing was done outlining the LV of the heart and the algorithm processed the tracing to display the graphic representation of the Velocity, Displacement, Strain and Strain rate with their corresponding regional values along the stretch of the LV. The same procedure was applied to all the four groups of animals namely the Sham, Sham +MSC, MI and MI+MSC to obtain the corresponding values.

3.4 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed between the sham, sham+MSC, MI and MI+MSC groups to check for overall group differences. The p value was set at 0.05 to determine the level of significance. Significant group differences detected by the ANOVA were further subjected to a Tukey post-hoc multiple comparison test to determine which of the four groups were significantly different from each other. The data were plotted and displayed as a histogram with their means and standard errors of the mean. The histograms were plotted using PRISM form Graph Pad software.
4. Results:

We used four groups of animals for our studies. The four groups were Sham, Sham+MSC, MI and MI+MSC. We imaged all animals 24 hours post their respective surgical procedure. Each animal gave four sets of results namely regional velocity, displacement, strain and strain rate.

4.1 Regional velocity measurements in the parasternal axis:

We successfully recorded cine loops and analyzed the regional radial velocity measurements in the posterior apex and anterior apex of the heart in the parasternal view. The means of the four groups were measured and displayed as a bar graph with their respective standard error of the mean. It was observed that the radial velocity for the posterior apex for all the groups was in the range between 0.64-0.96 cm/s. The anterior apex was observed to be in the range between 0.41-0.47 cm/s. There was a trend that was observed with respect to the velocity measurements where in the MI velocity was the least and the Sham and Sham+MSC group observed to be very closely spaced. This could be attributed to a certain significant drop in the MI velocity post ischemia-reperfusion that happened to rebound with the injection of MSCs in the mouse.
Figure 1A: Mean Radial Velocity values for posterior (Left) and anterior (Right) apex for Sham (0.96±0.08 cm/s), Sham+MSC (0.95±0.11 cm/s), MI (0.64±0.14 cm/s) and MI+MSC (0.84±0.13 cm/s) respectively. 1B Anterior apex for Sham (0.59±0.05 cm/s), Sham+MSC (0.62±0.08 cm/s), MI (0.46±0.06 cm/s) and MI+MSC (0.70±0.09 cm/s) respectively. Figure 1C: Mean Longitudinal Velocity values for posterior (Left) and anterior (Right) apex for Sham (0.63±0.05 cm/s), Sham+MSC (0.68±0.13 cm/s), MI (0.42±0.08 cm/s) and MI+MSC (0.63±0.05 cm/s) respectively. 1D: Anterior apex for Sham (0.41±0.03 cm/s), Sham+MSC (0.46±0.09 cm/s), MI (0.42±0.05 cm/s) and MI+MSC (0.47±0.11 cm/s) respectively.
Longitudinal Velocity:

Similarly cine loops were recorded and analyzed for the regional longitudinal velocity measurements in the posterior apex and anterior apex of the heart in the parasternal view. It was observed that the longitudinal velocity for the post. Apex for all the groups was in the range between 0.42-0.68 cm/s again with MI being the least and the velocity rebounded to comparable levels with the Sham+MSC groups.

4.2 Regional Displacement measurements in the parasternal axis:

We recorded cine loops and analyzed the regional radial displacement measurements in the posterior apex and anterior apex of the heart in the parasternal view. The means of the four groups were measured and displayed as a bar graph with their respective standard error to mean. It was observed that the radial displacement for the post. Apex for all the groups was in the range between 0.17-0.29 mm. The Ant. Apex was observed to be in the ranged between 0.10-0.18 mm. There was a certain trend that was observed with respect to the displacement measurements where in the MI displacement was the least and Sham+MSC group observed to be the greatest. The sham & MI+MSC measured to be the same up to the second decimal. This could be attributed to the magnitude of displacement being comparable in the respective
hearts based on the surgical procedure. Statistically, there wasn’t a significant difference (p>0.05) between the four groups for the displacement to suggest an improvement in displacement upon the injection of MSCs.
Figure 2A: Mean Radial displacement values for posterior (Left) and anterior (Right) apex for Sham (0.29±0.02 mm), Sham+MSC (0.28±0.04 mm), MI (0.17±0.04 mm) and MI+MSC (0.24±0.04) respectively. 2B: Anterior apex for Sham (0.15±0.03 mm), Sham+MSC (0.18±0.03 mm), MI (0.10±0.02 mm) and MI+MSC (0.15±0.03 mm) respectively. Figure 2C: Mean Longitudinal displacement values for posterior (Left) and anterior (Right) apex for Sham (0.10±0.02 mm), Sham+MSC (0.07±0.02 mm), MI (0.35±0.017 mm) and MI+MSC (0.08±0.03 mm) respectively. 2D: Anterior apex for Sham (0.03±0.01 mm), Sham+MSC (0.04±0.009 mm), MI (0.04±0.01 mm) and MI+MSC (0.04±0.009 mm) respectively.
Longitudinal Displacement:

The longitudinal displacement values varied from 0.03 to 0.10 mm in the Post.Apex with least displacement in the MI heart and the maximum displacement in the sham mice. The pattern of similarity in the MI heart measuring the least for all the four parameters being measured was witnessed. This could be due to the severely damaged apex in the myocardium as a result of the injury. Similarly for the Ant Apex the measured displacements ranged from 0.03-0.045 mm. The Sham seemed to have the least value and all the other groups had the same measured displacement value. A linear increase over the different groups in the Ant apex displacement was witnessed.

4.3 Regional strain measurements in the parasternal axis

The Regional strain measurements gave a deep insight into the magnitude of changes that occurred post a particular type of injury or an injury along with injected cells into the myocardium. Strain gives a measure of deformation that occurs in the muscles due to a contraction and relaxation of the heart to pump blood into the system. As a result of the injury the MI animal had a severely damaged apex leading to a severe loss of contractile function. The Radial strain for the post-apex were measured and its the range lied between 5.64-17.89 % starting with MI followed by sham. Both the
Sham+MSC and MI+MSC measured the same. The Ant-apex on the other hand ranged from (6.69±1.36)-(17.31±1.96) % with Sham+MSC being significantly higher than MI+MSC. Statistically, it was found that there was a significant difference (p<0.05) between the different groups as depicted in the graph based out of a post-hoc test.
Figure 3A: Mean Radial strain values for posterior (Left) and anterior (Right) apex for Sham (13.65±3.64 %), Sham+MSC (17.90±1.83 %), MI (5.65±1.36 %) and MI+MSC (17.87±2.93 %) respectively. 3B: Anterior apex for Sham (11.46±3.27 %), Sham+MSC (17.31±1.96 %), MI (6.69±1.36 %) and MI+MSC (15.56±2.92 %) respectively. Figure 3C: Mean Longitudinal strain values for posterior (Left) and anterior (Right) apex for Sham (-21.44±4.03 %), Sham+MSC (-14.60±2.4 %), MI (-8.40±3.44 %) and MI+MSC (-11.65±2.33 %) respectively. 3D: Anterior apex for Sham (-12.45±1.6 %), Sham+MSC (-12.33±2.04 %), MI (-6.79±1.9 %) and MI+MSC (-11.96±3.35 %) respectively.
Longitudinal Strain:

The longitudinal strain values for the post-apex and ant-apex ranged between -21.44 to -8.40 %. Strain is the deformation that is measured as a percentage and hence all values are depicted as per cent. A Higher percentage signified an increased deformation and better contractility of a particular region. There wasn’t a significant difference between the different groups (p<0.05) though the bar graph represents a trend to see a rebound in strain values of the MI+MSC similar to a sham or a Sham+MSC group. The Sham seemed to record the highest strain values in both the post apex and ant apex followed by the Sham+MSC. A sudden drop occurred in the MI region, which again found to increase back to a sham or Sham+MSC level.

4.4 Regional Strain rate in the parasternal axis:

Strain rate measures the rate of change of strain and has a unit of 1/s. The radial strain for the post and ant apex varied between 3.72-5.96 1/s and 3.2-5.94 1/s respectively. As with the other measurements the strain rate for MI was the least with MI+MSC and Sham+MSC being comparable to each other. The trend of increased strain rate as with other measures with MI+MSC in comparison with MI was observed here also. Statistically significant groups
were present in the Ant apex of radial strain rate and the groups that differed from each other was depicted in the adjacent figure.

Figure 4A: Mean Radial strain rate values for posterior (Left) and anterior (Right) apex for Sham (0.59±0.66 1/s), Sham+MSC (5.58±0.38 1/s), MI (3.72±0.68 1/s) and MI+MSC (5.96±0.92 1/s) respectively. 4B: Anterior apex for Sham (3.88±0.36 1/s), Sham+MSC (5.23±0.22 1/s), MI (3.29±0.21 1/s) and MI+MSC (5.94±0.68 1/s) respectively. Figure 4C: Mean Longitudinal strain rate values for posterior (Left) and anterior (Right) apex for Sham (-7.98±1.17 1/s), Sham+MSC (-7.03±1.27 1/s), MI (-5.17±1.36 1/s) and MI+MSC (-5.97±0.88 1/s) respectively. 4D Anterior apex for Sham (0.41 cm/s), Sham+MSC (-2.45±2.09 1/s), MI (-5.33±0.96 1/s) and MI+MSC (-3.95±0.60 1/s) respectively.
Longitudinal strain rate:

Longitudinal strain rate in the post and ant apex varied from -5.17 to -7.9 1/s and -3.95 to -5.63 respectively. In the post apex the strain rate for sham was the highest at -7.98 1/s and the Sham+MSC recorded a lower strain rate compared to sham. On the other hand in the ant apex the MI recorded a strain rate of -3.95 1/s. Sham+MSC and MI+MSC groups recorded a comparable strain rate at -5.3 and -5.6 1/s respectively. There wasn’t a statistical significance that was noted between the four groups.

4.5 Regional velocity measurements in the short axis:

We successfully recorded cine loops and analyzed the regional radial velocity measurements in the posterior wall and anterior septum of the heart in the short axis view. The means of the four groups were measured and displayed as a bar graph with their respective standard error to mean. It was observed that the radial velocity for the post. Wall for all the groups was in the range between 0.77-1.25 cm/s. The Ant. Septum was observed to be in the range between 0.73-0.89 cm/s. There was a certain trend that was observed with respect to the velocity measurements where in the MI velocity was the least and the Sham and Sham+MSC group observed to be very closely spaced. This could be attributed to a certain significant drop in the MI velocity post
Ischemia that happened to rebound with perfusion and the injection of MSCs in the mouse.

Figure 5A: Mean Radial velocity values for posterior wall (Left) and anterior (Right) septum: for post wall Sham (1.25±0.19 cm/s), Sham+MSC (1.18±0.08 cm/s), MI (0.77±0.12 cm/s) and MI+MSC (0.99±0.13 cm/s) respectively. 5B: Anterior septum for Sham (0.74±0.13 cm/s), Sham+MSC (0.85±0.13 cm/s), MI (0.73±0.13 cm/s) and MI+MSC (0.89±0.10 cm/s) respectively. Figure 5C: Mean Circumferential velocity values for posterior wall (Left) and anterior (Right) septum for Sham (251.1±35.13 cm/s), Sham+MSC (273±95.03 cm/s), MI (166±31.88 cm/s) and MI+MSC (160.4±16.95 cm/s) respectively. 5D: Ant septum for Sham (175.4±38.04cm/s), sham+MSC (173.0±52.84cm/s), MI (149.7±22.11cm/s) and MI+MSC (163.4±29.46) respectively.
Circumferential Velocity:

Similarly cine loops were recorded and analyzed for the regional longitudinal velocity measurements in the posterior apex and anterior apex of the heart in the short axis view. It was observed that the longitudinal velocity for the post. Wall for all the groups were in the range between 160.39- 272.96 cm/s with MI+MSC being the least followed by the MI. Here Sham and Sham+MSC groups remained greater than that of the MI and MI+MSC groups. This could be attributed to the degree of injury caused by a sham operation being partial compared to a whole apex ischemia followed by reperfusion after 90 minutes.

4.6 Regional displacement measurements in the short axis:

We recorded cine loops and analyzed the regional radial displacement measurements in the posterior Wall and anterior septum of the heart in the short axis view. The means of the four groups were measured and displayed as a bar graph with their respective standard error to mean. It was observed that the radial displacement for the post. wall for all the groups were in the range between 0.15-0.40 mm. The Ant. septum was observed to be in the range between 0.15-0.26 mm. There was a certain trend that was observed with respect to the displacement measurements where in the MI displacement was
the least and Sham group observed to be the greatest. The Sham+MSC followed & then MI+MSC, displayed up to the second decimal. This could be attributed to the magnitude of displacement being comparable in the respective hearts based on the surgical procedure. Statistically, there was significant difference (p<0.05) between the sham, MI and Sham+MSC groups for the displacement in the post.wall to suggest a steady decrease in displacement-from sham to MI due to ischemia followed by reperfusion.
Figure 6A: Mean Radial displacement values for posterior wall (top) (Left) and anterior (Right) septum for Sham (0.40±0.06 mm), Sham+MSC (0.36±0.04 mm), MI (0.15±0.05 mm) and MI+MSC (0.30±0.03 mm), 6B: Anterior septum, for Sham (0.23±0.05 mm), Sham+MSC (0.25±0.03 mm), MI (0.15±0.03 mm) and MI+MSC (0.26±0.03 mm) respectively. Figure 6C: Mean Circumferential displacement values for posterior wall (bottom) (Left) and anterior (Right) septum for Sham (7.95±1.51 mm), Sham+MSC (6.15±1.89 mm), MI (2.41±0.51 mm) and MI+MSC (1.8±0.46 mm), 6D: Sham (2.82±0.89 mm), Sham+MSC (2.86±1.5 mm), MI (1.889±0.48 mm) and MI+MSC (2.49±0.88 mm) respectively.
Circumferential displacement:

A linearly decreasing trend was observed in the circumferential displacement measurements of the post.wall. This ranged from 7.95-1.8 mm. Sham group recorded the highest followed by them Sham+MSC and MI+MSC respectively. There was statistical significance (p<0.05) between sham, MI and MI+MSC groups. On the other hand, the ant.wall of the heart registered values, which were similar to the radial displacement with Sham+MSC and MI+MSC being comparable and MI being the least. The values ranged between 1.88-2.86 mm.

4.7 Regional strain measurements in the short axis:

Radial short axis strain for the post.wall and ant.septum was measured after the data was analyzed using the cine loops recorded from the B-mode images. The strain values ranged from 12.32-36.30%. The least strain was from the MI due to the loss of contractile function due to ischemia. The sham recorded the highest contractile function recorded in terms of strain at 36.30 %. The introduction of stem cells in the sham did not affect an increase in contractility. At the same time the strain recovered partially in the MI+MSC group at 19.76 %. Statistically, there was a significant difference (p<0.05)
between groups sham and MI and MI+MSC respectively in the post wall with sham.
Circumferential strain:

Figure 7A: Mean Radial strain values for posterior wall (top) (Left) and anterior septum (Right) for Sham (36.31±6.57 %), Sham+MSC (32.59±5.27 %), MI (12.32±4.92 %) and MI+MSC (19.76±2.67 %) & Fig 7B: Sham (10.60±2.35 %), Sham+MSC (11.29±3.72 %), MI (9.00±2.3%) and MI+MSC (19.24±4.17%) respectively. Figure 7C: Mean Circumferential strain values for posterior wall (bottom) (Left) and anterior septum (Right) apex for sham (-19.13±2.66 %), Sham+MSC (-20.39±2.54 %), MI(-8.39±2.21 %) and MI+MSC (-16.57±1.33 %) & Fig 7D: Sham (-19.13±2.66 %), Sham+MSC(-20.39±2.54 %), MI (-8.39±2.21 %) and MI+MSC (-16.57±1.33 %) respectively.
The post wall and ant.septum for the circumferential strain ranged from -20.38 to -8.39 % and -12.89 to -7.46 %. There was a tremendous increase in the strain for MI+MSC in the post.wall and the sham and MI had comparable strain values. In the ant.septum there was a significant difference (p<0.05) between groups sham, sham+MSC and MI as shown in the graph.

4.8 Regional strain rate measurements in the short axis:

The radial strain rate for the post.wall and ant.septum ranged between 6.29-10.02 1/s. The MI+MSC recorded the lowest followed by the MI with Sham group recording the highest strain rate. This was seen to be a linear decrease in the strain rate values starting from sham to MI+MSC. There were significant differences (p<0.05) between the sham, sham+MSC and MI with MI+MSC. On the other hand, the ant.septum strain rate values ranged between 3.6-6.79 1/s. The sham+MSC group had the highest strain rate with sham+MSC being the least at 3.62 1/s. There were significant differences between the sham, MI and MI+MSC groups with MI+MSC.

Circumferential strain rate:

The post.wall strain rate ranged from -4.72 to -8.67 1/s and the ant.septum strain rate ranged from -3.94 to -5.67 1/s. The sham+MSC group had the highest strain rate. The injection of stem cells did help in the strain rate
recovering in the MI+MSC in comparison with the MI. The ant septum on the other hand recorded the highest strain rate for the MI+MSC well above the sham+MSC group to indicate a good recovery in comparison to the MI and sham groups. However, there wasn’t a significant difference between the different groups in the post.wall and ant.septum for the circumferential strain.
Figure 8A: Mean Radial strain rate values for posterior wall (Left) and anterior (Right) septum for Sham (10.02±1.93 1/s), Sham+MSC (8.03±0.60 1/s), MI (6.42±1.05 1/s) and MI+MSC (6.29±0.69 1/s) respectively. 8B: Anterior septum for Sham (4.07±0.64 1/s), Sham+MSC (3.29±0.65 1/s), MI (4.24±0.93 1/s) and MI+MSC (6.15±1.18 1/s) respectively. Figure 8C: Mean Circumferential strain rate values for posterior wall (Left) and anterior (Right) Septum for Sham (-7.32±0.83 1/s), Sham+MSC (-8.67±2.18 1/s), MI (-4.72±0.61 1/s) and MI+MSC (-6.57±0.911/s) respectively. Fig 8D: Sham (-4.90±1.06 1/s), Sham+MSC (-3.97±0.89 1/s), MI (-4.79±0.57 1/s) and MI+MSC (-5.67±1.29 1/s) respectively.
5. Discussion:

The purpose of this study was to demonstrate the ability to quantify the regional strain and strain rate of the LV as they are a well-known clinical index of myocardial contractile function. Ours, was the first study to quantify regional strain and strain rate changes in the LV using STE 24 hrs post MI followed by injection of the MSCs directly into the apex, the site of ischemic injury. The need for measuring regional strain values arises from the lack of evidence to predict progressive heart failure from measuring global left ventricular ejection fraction LVEF and left ventricular end-systolic volumes as they tend to be normal (Thor Edvardsen 2006). Though global LVEF is comparable to the sum of all regional shortening, impairment of regional function does not translate to the reduction in global EF unless there are several regions that are impaired due to injury.

The animal model, C57BL6 mice used for the study was the most widely used inbred strain in cardiovascular biology research due to their advantages. They are either used as a general-purpose strain or a background strain for the generation of cogenics carrying both spontaneous and induced mutations. They are very resistant to tumors and feature other characteristics such as diet-induced obesity, type 2 diabetes and atherosclerosis and hence can be used for a wide variety of research interests (Hoit, Kiatchoosakun et al. 2002). A
cardiovascular system phenotype possessed decreased cardiac muscle contractility with an average LV shortening at 39.1 compared to an A/J mice and a significantly longer aortic ejection time (Salto-Tellez, Yung Lim et al. 2004). They have a decreased heart rate at about 433 beats per minute vs. A/J mice at about 524 beats per minute. There is an increased average LV weight of 46.2 mg, which is significantly higher than control A/J mice, and this results due to an increased end-systolic dimension and a proportional increase in wall thickness (Hoit, Kiatchoosakun et al. 2002). The above stated characteristics helped us choose C57BL6 as our choice of animal for conducting the study.

Generally, after an injury to the myocardium changes occur in the form of left ventricular remodeling as the heart tries to maintain blood outflow with the help of neighboring regions compensating for any loss of function arising from the area of injury. Replacement fibrosis leads to a condition that causes a drastic reduction in strain. This in turn increases the stress on neighboring regions surrounding injured region and impairs contractile function in the heart (Yu Peng 2009). This reduction in strain values was observed in our MI models in both the long axis and short axis strain measurements.
The MI strain and strain rate values were consistently lower compared to MI+MSC and sham+MSC groups, which were comparable to each other. The observed results followed a order in which the introduction of MSCs in the heart post MI or a sham injury caused a change in strain in the apex where both the injury and the cells were introduced. The presence of stem cells altered the measurements in a positive manner, which could potentially lead to an improvement in cardiac function.

Based on our statistical analysis we can state that a significant drop in contractile function could be quantified non-invasively at the regional level with the help of STE. Bauer et. al. observed a significant drop in contractile function measured as strain was using STE in a C57BL6 MI model (Micheal Bauer, 2013) that are consistent with our findings. Also, the introduction of MSCs in the injured heart did have a positive effect in improving the contractile function measured by strain and strain rate to comparable levels of that of a sham+MSC group. This cannot be taken into account as a complete and comprehensive improvement in cardiac function that can be observed in a healthy myocardium. However, this does pave the way for a clearer understanding about the effects of MSCs as a potential tool for repairing damaged myocardium.
Speckle tracking echocardiography identified regional deficits in myocardial strain within 24 hours following ischemia-reperfusion compared to strain measurements in non-ischemic hearts. Direct cardiac muscle injection of bone marrow-derived stem cells following ischemia-reperfusion resulted in increased radial strain measurements in the apical anterior and posterior regions of the left ventricle. Improved radial wall thickening in the ischemic region injected with exogenous stem cells demonstrates that early stem cell therapy results in increased systolic shortening of cardiac muscle. These findings are consistent with improved global cardiac function previously reported by our laboratory.
6. Conclusion:

Our study demonstrated a novel approach to quantify regional changes in the LV that occur during LV remodeling as a result of a myocardial infarction. We demonstrated the need to measure regional strain changes due to the lack of evidence of an inherent cardiac failure just with the measurements of global LVEF and end-systolic LV volume. Furthermore, we demonstrated that STE can identify positive effects of MSC introduction in the ischemic and reperfused myocardium and quantify the improvements in cardiac function within specific regions of the heart as opposed to changes that may occur in global function.
7. CITED LITERATURE:


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**ABSTRACTS**

Subramanian, S., Geenen D.L. Speckle Tracking Ultrasound Detects Strain Changes in the Murine Heart during Acute Ischemia and Right Ventricular Pressure Overload, Center for Cardiovascular Research, Chicago, IL, July 2012