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Delivering Stem Cells to the Healthy Heart on Biological Sutures: Effects on Regional Mechanical Function

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Abstract

Current cardiac cell therapies cannot effectively target and retain cells in a specific area of the heart. We previously developed cell-seeded biological sutures to overcome this limitation, demonstrating targeted delivery with > 60% cell retention. Herein, we implanted both cell-seeded and non-seeded fibrin based biological sutures into normal functioning rat hearts to determine the effects on mechanical function and fibrotic response. Human mesenchymal stem cells (hMSCs) were used based on our previous work and established cardioprotective effects.

Non-seeded or hMSC-seeded sutures were implanted into healthy athymic rat hearts. Prior to cell seeding, hMSCs were passively loaded with quantum dot (QD) nanoparticles. One week after implantation, regional stroke work index and systolic area of contraction (SAC) were evaluated on the epicardial surface above the suture. Cell delivery and retention were confirmed by QD tracking, and the fibrotic tissue area was evaluated.

Non-seeded biological sutures decreased SAC near the suture from 0.20 ± 0.01 measured in sham hearts to 0.08 ± 0.02 , whereas hMSC-seeded biological sutures dampened the decrease in SAC (0.15 ± 0.02). Non-seeded sutures also displayed a small amount of fibrosis around the sutures ($1.0 \pm 0.1 \text{ mm}^2$). Sutures seeded with hMSCs displayed a significant reduction in fibrosis ($0.5 \pm 0.1 \text{ mm}^2$, $p < 0.001$), with QD-labeled hMSCs found along the suture track. These results show that the addition of hMSCs attenuates the fibrotic response observed with non-seeded sutures, leading to improved regional mechanics of the implantation region.

Keywords

biological sutures; stem cells; tissue regeneration; wound healing; mechanical function; cardiac biomechanics

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Ethics: All animal studies in this manuscript were reviewed and approved by the Worcester Polytechnic Institute IACUC.

1. Introduction

The use of bone-marrow derived stem cells to treat myocardial infarction and heart failure in patients appears to be safe and effective for improving mechanical cardiac function. (Schächinger *et al.*, 2006, Wollert *et al.*, 2004, Yousef *et al.*, 2009) However, the utility of cell therapies for myocardial regeneration is limited by both the low retention of stem cells in the damaged myocardium and the inability of current methods to target delivery to specific regions of the heart. Of methods currently used for cell delivery, intravenous delivery of cells is the least invasive, but most of the delivered cells become trapped in the lungs, with less than 1% of the cells residing in the infarcted heart 4 hours later. (Barbash *et al.*, 2003) Intracoronary infusion directly delivers cells to the heart muscle. However, the majority of cells are washed away upon the restoration of blood flow, leaving only 2.6% of the delivered cells engrafted in the heart. (Hou *et al.*, 2005) Direct intramyocardial injection of cells into the heart wall results in a modest increase in the number of cells delivered to the myocardium, with 11% of the cells engrafting. (Hou *et al.*, 2005) All of the current cell delivery approaches fall short of a feasible engraftment rate necessary to provide significant myocardial regeneration. To overcome the limitations of current cell therapy methods, more cells must be grown and delivered to regenerate the large number of host cells lost, which drives up production costs and detracts from the financial feasibility of the treatment.

Biological sutures can be made from biocompatible, naturally-derived materials such as fibrin, which is easily degraded by host muscle when implanted in animals. Recently, our lab has developed a novel method to produce discrete fibrin microthreads, which can be bundled into biological sutures and seeded with human mesenchymal stem cells (hMSCs). The biological sutures can support hMSC viability and proliferation, while maintaining their ability to differentiate into adipocytes and osteocytes. (Proulx *et al.*, 2011) The biological sutures can be attached to a surgical needle allowing for targeted delivery to the left ventricular wall. We recently demonstrated that cell-seeded biological sutures serve as a platform technology to improve localized delivery and engraftment of viable cells to damaged tissue. (Guyette *et al.*, 2012) However, how the addition of a passive material (biological sutures) affects the regional contractile properties of the healthy heart is unknown. In addition, the effect of how cell-seeded biological sutures may augment resulting function also needs to be determined. In this study we aim to characterize the effects of biological suture implantation on regional cardiac mechanical function in healthy cardiac tissue and to determine how the addition of adult stem cells to these sutures changes their effects.

2. Methods

2.1. hMSC Seeded Biological Sutures

As described previously, (Cornwell and Pins, 2007, Proulx *et al.*, 2011) discrete fibrin microthreads were made by coextruding fibrinogen and thrombin into threads with a diameter of approximately 100 μm . Eight microthreads were then bundled together and passed through the eye of a 22 gauge, $\frac{1}{2}$ circle surgical needle to form a biological suture. Bone-marrow derived hMSCs (Lonza, Walkersville, MD; passage 4-9) were cultured in Mesenchymal Stem Cell Growth Medium (MSCGM, Lonza, Walkersville, MD) according

to the manufacturer's instructions. To prepare hMSCs for tracking, cells were first quantum dot loaded for 24 hours. The quantum dot medium was created by adding Qdot655 ITK™ carboxyl quantum dots (1.025 μ l qdot solution per 1 ml media, Invitrogen Q2132MP, Carlsbad, CA) to MSCGM. Cells were then trypsinized, centrifuged, and resuspended at a concentration of 10^6 cells/ml in MSCGM. To minimize preparation time and maximize cell seeding, the biological suture was placed in a bioreactor (1.98 mm I.D. Silastic tubing), ethylene oxide sterilized, then seeded with 100 μ l of 10^6 hMSCs/ml suspension. The ends of the bioreactors were clamped, with biological sutures enclosed, and placed in a MACSmix tube rotator for 24 hours (Miltenyi Biotec, Bergisch, Germany) to ensure the biological sutures were uniformly seeded. The average cell density on these microthread bundles was 5,750 hMSCs per cm length of suture.(Guyette et al., 2012)

2.2. Cell viability on biological sutures

To characterize cell viability on the biological sutures, 6 sutures were seeded as described above. Sutures were stained with a Live/Dead cell viability assay (L3224, Life technologies, Carlsbad, CA) and Hoechst dye following the manufacturer's instructions. Dead controls were treated with 70% ethanol for 30 minutes prior to staining. Briefly, threads were incubated at 37°C for 30 minutes in a solution of Ethidium bromide (1 μ L/mL), Calcein AM (1 μ L/mL) and Hoechst dye (0.5 μ L/mL) in DMEM. Threads were thoroughly washed in several changes of phosphate buffered saline and then fixed in 4% paraformaldehyde for 10 minutes. Fluorescent images were obtained with a Leica TCS SP5 confocal laser scanning microscope at four evenly spaced locations from the needle attachment to the distal end of the suture.

2.3. Myocardial Implant Model

All animal procedures were conducted in accordance with the NIH guidelines for the care and use of laboratory animals and with institutional approval from the Worcester Polytechnic Institute IACUC. Seventeen male Rowlett Nude Rats (NIH-Foxn1, Charles River Laboratories, Wilmington, MA, USA), weighing 220 to 320 g, were randomly assigned into 3 groups. Animals were anesthetized with isoflurane inhalation (2%), intubated, mechanically ventilated with room air maintaining isoflurane (0.5%) and supplemented with oxygen. As previously described,(Tao *et al.*, 2004) the heart was exposed via a 2-cm incision at the third intercostal space and enlarged by an eye speculum (3.5 $\times$ 8.0 cm). Biological sutures seeded with (n=6) or without (n=6) hMSCs were delivered to the anterior left ventricular wall through surgical needles. The biological sutures were then cut from the suture needle, leaving the sutures implanted in the myocardium. Immediately after thread implantation, the chest was closed and animals were allowed to recover for one week. Sham-operation animals received the same procedure described above with the exception of thread implantation (n=5). At the one week time point, all groups had a 100% survival rate.

2.4. Global Mechanical Function

To assess the global mechanical function one week after biological suture implantation, animals were again anesthetized and maintained on isoflurane gas and the heart exposed as described above. A 3.5 French size high-fidelity, catheter-tipped micromanometer (model SPR-330, Millar Instruments, Houston, TX) was inserted into the left ventricular cavity and

pressure waveforms were recorded at 250 Hz. The resulting pressure loops were used to determine mean maximal developed left ventricular pressure. Additionally, pressure waveforms were smoothed with a 5 point averaging filter and maximal rate of pressure development and decline (maximum and minimum dP/dt) were determined and averaged over at least 10 cardiac cycles. Finally, the diastolic relaxation time constant (τ) was measured and averaged across each beat using the 3 parameter nonlinear least squares fitting method described by De Mey et al.(2012)

2.5. Regional Mechanical Function

To assess the regional mechanical function, the heart wall was then imaged with a CMOS camera (Photron Fastcam 1280 PCI, Photron USA, San Diego, CA) at 250 frames per second, with 8 bit depth. High density mapping (HDM), an optical technique that can determine regional mechanical function with high spatial resolution, was used in conjunction with simultaneous pressure measurements to determine regional stroke work index (RSW) and systolic area contraction (SAC) in the area of biological suture of beating hearts as previously described.(Kelly *et al.*, 2007) Pressure–area loops used to determine RSW and SAC values were individually inspected and verified.

2.6. Fibrosis area

After acquiring data for HDM analysis, 0.2 ml of Beuthanasia-D Special (Schering-Plough Animal Health) was injected into the left atrium to arrest the heart. The heart was then removed and the great vessels, atria, and right ventricle were trimmed away. The left ventricle was cut in half, bisecting the biological suture, and fixed in 4% paraformaldehyde overnight. After fixation, the heart was transferred and stored in 30% sucrose for at least 8 hours. Each half of the heart was placed in a separate Peel-A-Way® Disposable Embedding Mold (Polysciences Inc., Warrington, PA) and immersed in OCT Compound (Sakura Finetek USA Inc., Torrance, CA) and immediately placed in a -80°C freezer. Once the OCT compound was completely frozen, the block containing half of the heart was placed in a Leica CM3050 cryostat (Leica Microsystems, Bannockburn, IL) and cut into 10 μm thick sections across the entire region containing microthreads. Sections were placed on VWR® MicroSlides (VWR International, West Chester, PA). Along the total length of the microthread track in each heart, five evenly spaced sections were selected and stained with Masson's Trichrome staining, which was performed according to manufacturer's instruction. Images were acquired at 100 $\times$ using a Leica DMLB2 upright microscope (Leica Microsystems, Bannockburn, IL). The images were then imported into a custom MatLab program (Mathworks, Natick, MA) and analyzed for non-myocyte area. Nonmyocyte area was defined as a region of the heart near the microthread that had a greater blue intensity than red intensity in a Masson's trichrome image. Non-myocyte area was defined to include the area of the fibrin microthread and any white space enclosed within the blue area. Additionally, the area of the thread itself was measured by manually selecting the thread region using ImageJ (<http://rsbweb.nih.gov/ij/>). Fibrosis area was defined as the non-myocyte area minus the thread area. All fibrotic area measurements were visually verified for accuracy.

Chosen sections were also imaged with a scale using a 16× zoom high definition digital camera (Lumix DMC-ZS10, Panasonic, Secaucus, NJ) to determine the total cross sectional area of the left ventricular myocardium. These images were imported into a custom MatLab program which found the heart section and measured its cross sectional area and subtracted out the ventricular lumen. All measurements were verified visually for accuracy and the resulting area measurements were used to determine percent left ventricular fibrosis area.

2.7. Confirmation of Cell Delivery

To confirm the delivery of hMSCs with microthreads, two Sprague Dawley rats (SD, Charles River Laboratories), weighing 500-600 g, were sacrificed 1 hour and 1 week after implantation of Qdot655 labeled hMSC seeded fibrin microthreads. Heart tissues were immediately frozen in liquid N₂ and then placed in an embedding mold and immersed in OCT compound and immediately placed in a -80°C freezer. Once the OCT compound was completely frozen, the block was placed in a Leica CM3050 cryostat and 6 µm thick heart sections were cut and placed on VWR® MicroSlides. Cryosections were fixed in ice cold acetone for 10 min, and then nonspecific epitope antigens blocked with goat serum at room temperature for 60 min. The sections were incubated with specific mouse anti-α-actinin monoclonal antibody (Sigma, Catalog No A7811) at room temperature for 1 hour, and treated with goat anti-mouse secondary antibody (Alexa Fluor 488 A11029) at room temperature for 1 hour. The nuclei were stained with Hoechst33342 (0.5 µg/ml) for 5 min at room temperature. Fluorescent images were obtained with a Leica TCS SP5 confocal laser scanning microscope.

2.8. Statistics

Results are presented as mean ± SEM. Comparison of fibrosis areas between the two groups was made with the independent-sample t test, and comparisons among the three groups were made with a one-way analysis of variance (ANOVA), followed by the Tukey post hoc comparison test. In all tests, differences were considered statistically significant at a value of $P < 0.05$.

3. Results

3.1. Cell viability on sutures

Cell viability and longitudinal distribution of cells on the sutures was assessed using a Live/Dead viability assay. Qualitatively, cell distribution on the sutures was consistent across all 4 regions imaged on all threads. Figure 1 shows representative images of cell distribution on the sutures. Cell viability appears to be high in all regions on the sutures.

3.2. Global Function

One week after surgery, no significant differences were found in the body weights or heart rates amongst the animals in the three experimental groups (Table 1). One animal in the hMSC seeded suture group died during acquisition of data for mechanical analysis due to technical issues. This animal was excluded from the study results. Maximum developed left ventricular pressure (LVP), defined as the mean difference between maximum and minimum ventricular pressure across at least 10 beats, was lower in animals with microthreads alone

(88.9 ± 9.2 mmHg) and hMSC seeded microthreads (84.5 ± 5.3 mmHg) compared with sham-operation animals (130.7 ± 11.4 mmHg, $P < 0.05$). (Figure 2A).

The diastolic relaxation time constant (τ) was calculated to be 11.1 ± 0.7 ms in sham operated animals, 12.4 ± 0.9 ms in unseeded suture animals and 12.0 ± 0.6 ms in animals with hMSC-seeded sutures. No statistically significant differences were found in any measurements of tau. (Figure 2B)

The maximum rate of pressure development (Maximum dP/dt ; Figure 2C) in sham-operation animals was found to be $5,667 \pm 486$ mmHg/s. The implantation of non-seeded biological sutures appeared to decrease Max dP/dt ($3,867 \pm 592$), but differences were not significant compared to sham-operated animals ($P = 0.055$). The implantation of hMSC-seeded sutures significantly decreased Max dP/dt ($3,604 \pm 347$ mmHg/s, $P < 0.05$) compared to sham-operated animals, but there was no statistical difference found in Max dP/dt for the two suture groups.

Minimum rate of pressure decline (Minimum dP/dt) in sham operated animals was $-5,422 \pm 583$ mmHg/s. Both microthreads alone ($-3,124 \pm 536$ mmHg/s) and hMSC seeded microthreads ($-2,965 \pm 263$ mmHg/s) caused a significant reduction in magnitude of the rate of pressure decline ($P = 0.013$ and $P = 0.015$ respectively; Figure 2D).

3.3. Regional Function

Systolic area contraction (SAC) and regional stroke work (RSW) were determined in the implantation zones within the left ventricle, by calculating and averaging values over 5 cardiac cycles. The average region of interest (ROI) area for HDM analysis was 11.2 ± 1.1 mm². Figure 3 shows representative regional work loops for each of the three groups, from which parameters of mechanical function were determined.

SAC was defined as the difference between the systolic regional area and diastolic regional area, normalized to diastolic regional area. Compared with sham-operation (0.198 ± 0.010), unseeded biological sutures (0.079 ± 0.015) caused a significant decrease in SAC ($P < 0.001$). This decrease was significantly reversed by seeding biological sutures with hMSCs (0.149 ± 0.025 , $P < 0.05$ vs. unseeded sutures) (Figure 4A).

RSW was determined as the integral of LV pressure with respect to regional area of interest and normalized to end-diastolic area and maximum developed pressure. The average RSW in sham-operation was 0.150 ± 0.008 . One week after implantation, RSW in microthreads alone became smaller (0.069 ± 0.02) than sham-operation ($P < 0.001$). However, RSW for hMSC seeded sutures (0.114 ± 0.011) was not significantly different from either sham or unseeded sutures (Figure 4B).

3.4. Tracking of hMSCs

One hour after delivery, Qdot655 labeled hMSCs, with punctate red signal, were seen around the circumference of the microthread bundle; however, the highest concentration of red signal was observed at the entry point of microthreads and the lowest concentration located at the exit. The signal from labeled hMSCs was still present one week after

implantation on biological sutures (Figure 5). These cells appear to be in close proximity to the native myocardium.

3.5. Fibrotic response to suture implant

Figure 6 shows representative images of fibrosis areas with both unseeded and hMSC seeded biological sutures. From the entry to exit of suture implant, an average of 5 slides per heart was selected for Masson's trichrome staining. Implantation of unseeded sutures resulted in a non-myocyte area of $1.11 \pm 0.08 \text{ mm}^2$, corresponding to $2.8 \pm 0.2\%$ of the total left ventricular muscle cross sectional area. With the addition of hMSCs to the sutures, the non-myocyte area was significantly decreased ($0.54 \pm 0.09 \text{ mm}^2$, $P < 0.001$) corresponding to $1.3 \pm 0.1\%$ of the total left ventricle muscle cross section area. Total left ventricular cross sectional area did not vary significantly between the two groups.

To evaluate changes in degradation rate of the fibrin sutures, the cross sectional area of the suture was measured in the unseeded biological suture ($0.07 \pm 0.01 \text{ mm}^2$) and hMSC seeded suture groups ($0.09 \pm 0.01 \text{ mm}^2$). Although there is a trend toward decreased thread area in the unseeded suture group, there is no significant difference between the two groups ($P = 0.065$).

The suture area, non-myocyte area, and fibrosis areas along the length of the thread were also examined. From the entry point to exit of fibrin suture implant, at least 3 sections were chosen in each of 4 regions (entry-25%, 25%-50%, 50%-75% and 75%-exit) for each heart. A significant difference ($P < 0.05$) between suture cross sectional areas in the two groups was found near the exit point but not in any other location (Figure 7A). Significant differences between the suture and seeded suture groups were found in several regions along the suture length for both the fibrosis and non-myocyte areas. Non-myocyte area and fibrosis area are shown in Figure 7B and 7C respectively.

4. Discussion

Contemporary methods used to deliver stem cells to the damaged myocardium fail to provide adequate cell retention for effective cell therapy; with direct myocardial injection only providing a cell retention rate of about 11% (same ref as before). Introduction of exogenous cellular matrix materials into the infarcted heart along with stem cells has been attempted by several groups to improve the cell retention and effectiveness of cellular therapies. Addition of stem cells enriched with extracellular matrix (ECM) has been shown to improve cell retention in the infarcted heart after myocardial injection in the rat (Lee *et al.*, 2011) and to improve cardiac function post infarction (Dai *et al.*, 2013). Others have used an injectable thermosensitive polymer to attenuate adverse cardiac remodeling (Seif-Naraghi *et al.*, 2013, Wang *et al.*, 2009). Fibrin matrix serves as a reservoir for cytokines and acts as a scaffold that directs the recruitment of cells from the wound margin into the injury site. (Clark, 2001) Recently, fibrin gels have been used to deliver cells to the infarcted myocardium, showing increased cell transplant survival, decreased infarct size, increased blood flow to ischemic myocardium (Christman *et al.*, 2004b) and preserved cardiac function (Christman *et al.*, 2004a). Fibrin microthreads have higher tensile strength than other forms of fibrin including fibrin gels and glue, and were shown to allow fibroblast

attachment, proliferation, and alignment,(Cornwell and Pins, 2007) and supported MSC growth while maintaining differentiation potential *in vitro*.(Proulx et al., 2011) The higher tensile strength of fibrin microthreads allows them to be connected to suture needles. The overall strength of the suture can be altered by changing the number of microthreads bundled together. In the present study, biological sutures composed of 8 discrete microthreads, which were made from bovine thrombin and fibrinogen, had sufficient mechanical strength to pull through the rat left ventricular wall.

Implantation of fibrin biological sutures, a passive (non-contracting) material, into the healthy myocardium decreased both regional and global mechanical function. By using a high spatial resolution method to evaluate regional mechanical function, the epicardial region above the implanted fibrin sutures could be evaluated. The replacement of healthy, contracting myocytes with a passive material appeared to decrease global function, as well as regional function in comparison to control animals. However, multiple variables outside of regional mechanical function could have contributed to the changes in developed blood pressure. To better evaluate global function, afterload independent measurements, such as preload recruitable stroke work, could be used.

Implantation of the biological sutures into healthy heart muscle may also result in damage to local muscle tissue. Although the response was minimal, the passing of a needle and the fibrin suture through the wall of a heart likely activated an inflammatory response, and fibrosis followed. The trend for reduced thread cross sectional area in hearts with unseeded microthreads, suggests that the introduction of hMSCs to the heart may reduce the rate at which the biological sutures breakdown by reducing the local inflammatory response. This observation is supported by previous research which suggests that hMSCs reduce fibrosis in the heart (Nagaya *et al.*, 2005) and other organs.(Abdel Aziz *et al.*, 2007, Ninichuk *et al.*, 2006, Ortiz *et al.*, 2003, Oyagi *et al.*, 2006, Rojas *et al.*, 2005) To determine the extent of the inflammatory reaction in the two groups, representative sections from each group were stained using Giemsa. Giemsa stains the cytoplasm of lymphocytes and monocytes as well as their nuclei. As seen in Figure 8, we observed a significant number of cells migrating to the region around the non-seeded thread, while only a limited number of cells were observed around the hMSC seeded thread.

When implanting fibrin sutures into the heart, the sutures displace the contractile myocytes, replacing them with a non-contractile material. Thus, regional mechanical function was likely impaired not only by the implant surgery but also by replacement of contractile tissue with a passive material. As HDM allows for the evaluation of mechanical function in small regions, it is possible to focus on the region of tissue just above the microthreads. While HDM cannot determine function through the thickness of the ventricular wall, changes in mechanical properties throughout the wall will be transferred to the epicardial surface, where HDM is able to detect them. Fibrin serves as a provisional matrix *in vivo* and therefore is completely degraded by host muscle in 1-2 weeks. Qualitative assessment of fibrin sutures in the heart suggest that they will degrade over time. At one hour the fibrin suture can be seen as an intact bundle, but at one week, the bundle is seen to separate, suggesting degradation and breakdown over time. Since, at one week, the fibrin bundle is

still clearly visible in the heart, we expect the entire degradation process will take several weeks or even months.

In our previous studies, we identified quantum-dot signals co-localized with intact nuclei, demonstrating that hMSCs are retained in the heart for at least one hour.(Guyette et al., 2012) Although we have not yet assessed cell viability, we have seen that cell nuclei remain intact in the heart up to one week after hMSC delivery. In the present study, delivering hMSC seeded sutures significantly increased regional mechanical function compared to unseeded sutures, as evident by increases in SAC (Figure 4) and improved RSW. The mechanism by which hMSCs attenuates fibrosis is not currently known, but previous studies have suggested that hMSCs deliver growth and signaling factors that promote cardiomyocyte viability and function. In order to track delivered cells, MSCs were loaded with quantum dots. In a previous study (Rosen et al., 2007), we demonstrated that quantum dots remain stable in cells up to 8 weeks after implant and that the quantum dots are not taken up by myocytes in vivo. One week after implant of seeded fibrin microthreads, Qdot655 labeled hMSCs were found in the border around the suture (Figure 5). There were no signs of quantum dots in striated myocytes to suggest differentiation of MSCs, but this possibility cannot be ruled out as an exhaustive search was not conducted. MSCs are multipotent adult stem cells and are the main type of hematopoiesis-supportive stromal cells in bone marrow. They are readily accessible from bone marrow, adipose tissue and skeletal muscle, and there are minimal ethical considerations and immunological complications when applied in cell therapy.(Jiang *et al.*, 2007) Intramyocardial injection of MSCs into the border zone of myocardial infarction could reduce infarct size, improve cardiac remodeling and improve cardiac function.(Amado *et al.*, 2005, Dai *et al.*, 2005, Tao *et al.*, 2010) The underlying mechanisms of this remodeling can be based on promoting myocardial regeneration and angiogenesis, (Amado et al., 2005, Quevedo *et al.*, 2009, Satija *et al.*, 2009) and modulating allogeneic immune cell responses.(Aggarwal and Pittenger, 2005, El Haddad *et al.*, 2011) Thus, there are multiple potential mechanisms responsible for the improvement in SAC and RSW and changes of fibrosis area due to hMSC delivery by fibrin sutures.

In addition to increased mechanical function, this study demonstrated a decrease in non-myocyte areas and fibrotic expansion with hMSCs. As Figure 7 demonstrates, significant differences were found at all points along the length of the suture except from 50-75% of the suture length when compared between the two suture groups. This probably resulted from uneven distribution of hMSCs, as we have recently reported.(Guyette et al., 2012)

Our previous studies have demonstrated that >60% of cells delivered to the heart using fibrin biological sutures remain in the heart wall one hour after delivery.(Guyette et al., 2012) This engraftment rate is dramatically higher than the ~11% rate that we(Guyette et al., 2012) and others(Hou et al., 2005, Terrovitis *et al.*, 2009) have shown for intramyocardial injection. This improvement in engraftment rate gives fibrin microthreads the potential to reduce the number of cells migrating to non-cardiac tissue and targeted delivery allows for targeted assessment in the research setting. Additionally, we have previously seen that the cells are unevenly distributed throughout the length of thread insertion in the heart with the most cells located at the insertion point.(Guyette et al., 2012) We believe that this spatial variation is due to shear forces on the thread during thread implant. Although the number of cells

needed to create a therapeutic effect in humans remains to be determined, the number, and length of fibrin microthreads delivered to the heart can be modified to increase the number of cells delivered. This would allow clinicians to target cell numbers to meet a patient's specific needs, allowing clinicians to better target infarcted tissue. The number of cells needed for a therapeutic effect in the infarcted rat heart can be estimated based on previous research. Current approaches to hMSC delivery via IM injection can deliver anywhere from 5×10^6 to 50×10^6 hMSCs to the heart. (Barbash et al., 2003, Orlic *et al.*, 2001, Wolf *et al.*, 2009, Zhang *et al.*, 2008) Based on the weight of the animals studied and the reported engraftment rates, the number of cells can be normalized to the rat heart. Based on this calculation, 400-40,000 cells must be delivered to the rat heart to have a therapeutic effect. (Murphy, 2008) In its present state, the fibrin biological suture technology would be most useful to cardiac surgeons during open heart surgeries. In most of these cases, the heart is exposed providing an easy opportunity for the surgeon to quickly suture cell-seeded fibrin microthreads through the infarcted heart. In the future, the clinical utility of this method could be expanded via the development of a catheter that could implant biological sutures with an endovascular approach.

One of the limitations associated with this study is the lack of characterization of cell viability after implantation into the rat heart. Although improvements in regional mechanical function are seen to coincide with the implantation of seeded sutures, implanted hMSCs were not tracked to verify viability in the heart at the one week time point. Future studies should incorporate marker genes (such as luciferase or GFP) into the hMSCs to verify cell count and viability in the heart.

While the results presented here are measured in the healthy heart, the eventual goal of the biological suture technology is to deliver cell-seeded sutures to the damaged myocardium such as in the infarcted heart. The infarcted rat heart has greatly reduced function (Bachner-Hinzen et al., 2012, Helle-Valle *et al.*, 2009, Luo and Konofagou, 2008, Skulstad *et al.*, 2006) and altered regional mechanical properties (Fomovsky *et al.*, 2012) that will likely benefit from the delivery of a compliant material in addition to MSCs. Although the implantation of the biological sutures reduced regional and global mechanical function in the normal heart (likely due to the replacement of contractile tissue with passive material), the introduction of cell-seeded sutures to a damaged region of the myocardium would likely have a more beneficial effect. In this study we showed that, even in the healthy heart, the addition of hMSCs to the biological suture caused a significant reduction in fibrosis formation and increased mechanical function. Use of fibrin biological sutures as a method to deliver cells to the heart may provide an effective method to increase cell therapy retention and improve cardiac mechanical function.

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Figure 1. Live/dead viability stain of human mesenchymal stem cells (hMSCs) on a suture for 24 h. Green (Calcein AM) staining of cytoplasm indicates live cells, red (ethidium homodimer-1) staining of nuclei indicates dead cells and blue (Hoechst dye) indicates nuclei (A). (B) Staining of cells in four evenly spaced regions across the suture. Representative image of cells on a dead (70% ethanol treated) control suture. Scale bar = 100 μ m.

Figure 2.

Effects of microthreads with or without human mesenchymal stem cells (hMSCs) on maximum pressure developed (A), diastolic relaxation time constant (B), maximum rate of development of pressure (C) and maximum rate of decline in pressure (D). *p < 0.05, **p < 0.01; all values reported as mean $\pm$ SEM.

Figure 3. Representative regional stroke work loops from sham operated (A), microthreads alone (B) and microthreads + human mesenchymal stem cells (hMSCs) (C). Arrows indicate the direction of the work loops.

Figure 4.

Effects of microthreads with or without hMSCs on systolic area contraction (A) and regional stroke work (B). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; values reported as mean $\pm$ SEM. LVP, left ventricle.

Figure 5. Microthread delivered human mesenchymal stem cells (hMSCs) labelled by Qdot655 1 week after implantation. Original magnification $\times 20$ (left) and $\times 40$ (right). Arrows indicate Qdot655 labeled hMSCs.

Figure 6.

Representative fibrosis areas from a heart by microthreads with or without human mesenchymal stem cells (hMSCs) at locations of entry (0–25%), 25–50%, 50–75% and exit (75–100%). Scale bar = 500 μ m.

Figure 7.

Effects of microthreads with or without human mesenchymal stem cells (hMSCs) on (A) thread area, (B) non-myocyte area and (C) fibrosis area at locations of entry, 25–50%, 50–75%, exit and overall average. * $p < 0.05$, *** $p < 0.001$; values are mean $\pm$ SEM.

Figure 8.

Giemsa staining of representative tissue sections in the region adjacent to the microthread (A). Fibrin suture alone in the heart reveals that the microthread has broken down into pieces and cells have been recruited to the region (B). In contrast, human mesenchymal stem cell-seeded fibrin suture in the heart remains mostly intact, with a very limited recruitment of cells to the region. Black arrows indicate the fibrin microthread, yellow arrows indicate inflammatory cells in the region and orange arrows indicate the native myocardium. Scale bar = 100 μ m.

Table 1

Experimental Group	Number of rats	Heart Rate (beats per minute)	Body Weight (grams)
Sham	5	372 ± 21	303 ± 11
Un-seeded biological suture	6	302 ± 28	279 ± 23
hMSC seeded biological suture	6	334 ± 27	271 ± 35