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Targeted Delivery of Insulin-Like Growth Factor-1 Improves Stem Cell Therapy in A Rat Myocardial Infarction Model

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Abstract

Background: Stem cell therapy for treating myocardial infarction (MI) has been widely studied but the clinical applications of these studies have been disappointing due to poor stem cell engraftment and survival in the diseased tissue. In this study, insulin-like growth factor 1 (IGF-1) was selectively delivered to the infarcted myocardium prior to stem cell transplantation to improve the local microenvironment.

Methods: Rat MI were induced by left anterior descending artery ligation. One week after the MI surgery, immunoliposomes containing IGF-1 were infused into rats via tail vein and mensenchymal stem cells (MSCs) were transplanted to the MI through intramyocardially injection. Left ventricular fractional shortening (FS) was measured as an index of heart contractility.

Results: The combination of targeted IGF-1 and MSCs treatment significantly improved heart contractility 3 weeks after the treatment (2.5% gain in FS) compared to either no treatment (8% FS loss), targeted IGF-1 alone (4% FS loss), or MSCs treatment alone (8% FS loss). Immunohistochemical staining shows that both IGF-1 alone and IGF-1 + MSCs treatment facilitated vessel regrowth into the MI area. There was also much stronger stem cell fluorescence in the IGF-1 + MSCs treatment group compared to the MSCs alone treatment group, indicating that IGF-1 treatment greatly improved the survival of the transplanted stem cells.

Conclusion: The combination of targeted IGF-1 and stem cell transplantation results in a larger recovery in cardiac function compared to either IGF-1 or stem cell treatment alone. This recovery is probably achieved by improved stem cell survival due to targeted IGF-1treatment and the subsequent stem cell engraftment in the damaged myocardium.

Keywords: Stem cell therapy, Myocardial infarction, IGF-1, Immunoliposomes.

Introduction

MI occurs in 735,000 Americans every year [1]. Survivors of MI are at increased risk of recurrent infarctions and have an annual death rate of 5%, which make it the leading cause of morbidity and mortality in most countries [2]. MI occurs when a part of the heart muscle doesn't receive enough blood flow causing permanent cell death and necrosis after the occlusion of coronary blood vessels. Due to the limited regeneration capability of cardiacmyocytes, the damaged heart muscle cells are eventually replaced with scar tissue such as collagen to prevent further structural damage of the heart tissue. Clinically, MI treatment strategies focus mainly on blood flow restoration and pathological

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remodeling modulations [3]. However, these treatment modalities ameliorate symptoms at best and are not capable of regenerating the lost myocardium of the MI patient [4].

Non-hematopoietic bone-marrow-derived stem cells, frequently referred to as mesenchymal stem cells (MSC), have been shown to differentiate into mesenchymal cell types, including muscle, brain, vascular endothelial cells and cardiac myocytes [5-8]. Several studies in animal models using MSCs showed improvement of impaired heart function resulting from induction of myocardial angiogenesis and/or regeneration in the infarcted tissue [9-11]. However, clinical trials of infusion of MSCs into damaged hearts revealed, at best, marginal improvement of cardiac function in patients with myocardial infarction [12,13]. A likely reason may be that the therapeutic potential of MSCs is limited by their viability and engraftment within the post injury environment [14-18]. A hostile microenvironment flooded with *inflammatory* cells and lack of necessary vasculature for the highly oxygen dependent cardiomyocytes all contribute to the disappointing results of recent attempts at rebuilding myocardium using stem cells [19-23]. Therefore, if novel strategies such as MSC therapy are to succeed, the survival and engraftment of the induced MSCs must be improved.

Recently, investigators have demonstrated that genetically engineered MSCs that overexpress Akt were markedly resistant to death under hypoxic conditions *in vitro* [24], and prevented left ventricular (LV) remodeling and improved cardiac performance in rats with MI after transplantation into the ischemic myocardium [25]. These outcomes may reveal that activation of PI3k/AKT pathway may greatly enhance survival of the transplanted cells via reducing inflammation [26]. However, these genetically modified stem cells may have potential tumorigenicity due to prolonged activation of PI3k/AKT signaling pathway [27]. Therefore, temporary activation of the PI3K/Akt pathway at the time of transplantation is an attractive therapeutic strategy to improve the survival of MSCs. The PI3K/Akt pathway can be activated by various growth factors and cytoprotective cytokines [28], including insulin-like growth factor-1 (IGF-1) [29]. Indeed, a recent study demonstrated that IGF-1 promoted survival of cardiac stem cells and facilitated regeneration of the infarcted myocardium [30]. However, due to the adverse side effects and quick removal of IGF-1 from the circulation [31,32], targeted delivery system is expected to enhance the therapeutic efficacy. Previously, we have reported that we can successfully deliver angiogenic drugs (VEGF) to infarcted myocardium by anti-P-selectin conjugated immunoliposomes resulting in improved cardiac function and a significant increase in the number of both anatomical and perfused vessels in the MI region in rats [33,34]. In this study, we show that selective delivery of IGF-1 to the infarcted tissue can enhance the MSC survival and engraftment and can further significantly improve cardiac function in a rat MI model.

Materials and Methods

Rat MI model

MI was induced by left anterior descending coronary artery (LAD) occlusion technique in 6-wk-old male Sprague Dawley rats (Charles River Laboratories International. Inc, Wilmington, MA, USA) as described previously [33,34]. Briefly, rats were anesthetized with isoflurane, intubated and ventilated with a rodent ventilator (Euthanex Corporation, Allentown, PA, USA). The left anterior descending coronary artery was occluded with silk ligature. Rat bone marrow-derived MSCs expressing GFP were purchased from Cyagen (Santa Clara, CA, USA). Evidence of MI was confirmed by S-T segment elevation and the appearance of Q wave on an electrocardiogram. After surgery, rats were randomly assigned into five experimental groups: I) Sham (chest opened/closed without ligation), n = 8 rats; II) MI without treatment (saline injection), n = 8 rats; III) MSC treatment, n = 8 rats; IV) targeted delivery of IGF-1, n = 8 rats; V) targeted delivery of IGF-1 + MSC combination treatment, n = 10 rats. Systemic delivery of IGF-1 performed in a separate study has shown that systemic treatment does not result in significant improvements in cardiac function (data not shown). All animal protocols were approved by the Temple University Institutional Animal Care and Use Committee. Buprenorphine was given to the animals before surgery to prevent residual pain during surgery and after surgery to relieve pain and stress during the recover from anesthesia. The animals were then placed on a heating pad and observed every 15 minutes until fully recovered from anesthesia as indicated by the ability to maintain sternal recumbency and moving normally, at which point they were moved back to the cage. After recovering from anesthesia, the animals were observed every 30 minutes for the first 8 hours. After 8 hours, they were checked daily until 7 days post-op and once every other day thereafter for 3 more weeks. Animals that appeared to be in distress or pain (muscle spasm, loss of balance, loss of weight, etc.) during the 4-week observation period were euthanized using an overdose of KCl (2 meq/kg) injected via the tail vein under gas anesthesia.

Preparation of immunoliposomes

The targeted drug delivery system was produced by a two-step process as described previously [34,35]. First, IGF-1 (R&D Systems, Minneapolis, MN, USA) encapsulated long circulating liposomes, composed of 50% hydrogenated soy L-α-phosphatidylcholine (HSPC), 45% cholesterol, 3% 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[(polyethylene glycol)2000] (DSPE-PEG2000), and 2% DSPE-PEG-maleimide, were prepared by solvent evaporation and film formation. All lipids were obtained from Avanti Polar Lipids (Alabaster, Alabama, USA). Second, anti-P-selectin monoclonal antibody (courtesy of Dr. Andrew Issekutz) was coupled to the PEG component of the liposome membrane to form immunoliposomes through thiolation of the linker 2-imminothiolane (Sigma-Aldrich Corporation, St. Louis, MO, USA). P-selectin was chosen as the target receptor because it is upregulated on the vasculature of the infarct tissue [35] The antibody was first thiolated with 2-iminothiolane (Sigma-Aldrich Corporation, St. Louis, MO, USA) at pH 8.0. The introduced thiol groups were then coupled with maleimide groups on DSPE-PEG2000 component of the liposomes at pH 6.5. Unconjugated antibodies were removed by tangential flow filtration method using MicroKros hollow fiber filters (Rancho Dominguez, CA, USA). Previously [33,34]

we have shown that this targeted drug delivery system can successfully and selectively deliver encapsulated drug to the MI tissue.

Administration of IGF-1 & MSCs into rat MI model

MSCs at passage 2 were injected intramyocardially one-week post-MI induction as described in our previous studies [33,34]. Briefly, a syringe was used to inject a total of 100 µL of MSCs in saline at a concentration of 10,000 cells/µL equally to 4 different sites (25 µL at each site) around the MI area to achieve a homogenous stem cell distribution. Immediately after MSC injection, immunoliposomes containing IGF-1 (1 mL of liposome/kg of animal weight at 10 mM lipid concentration corresponding to 200 ng of IGF-1/kg of animal weight) were administered via the tail vein. Accordingly, the saline group received saline treatments at same time points. The IGF-1 only group received immunoliposomes containing IGF-1 immediately after saline injection intramyocardially at 1-week post-MI. The MSC only group received saline treatment immediately after MSC injection at 1-week post-MI.

Histology and immunohistochemistry (IHC)

Animals were sacrificed 4 weeks post-MI and hearts were removed for frozen sectioning purpose. Briefly, ventricles were first filled with OCT (Fisher Scientific Co., Houston, TX, USA) and then flash frozen for either immediate sectioning or stored at -80 °C. Samples were sectioned at 6 µm thickness using a -20°C cryostat (Leica CM3050 S, Buffalo Grove, IL, USA) and mounted onto glass slides (Superfrost plus, Fisher Scientific Co., Houston, TX, USA) for staining and imaging purpose.

CD31 protein (BD Biosciences, Franklin Lakes, NJ, USA) immunohistochemical stainings were used to identify anatomical blood vessels. Blood vessels were stained, imaged and processed to count the number of blood vessels. The anatomical blood vessel density was then calculated by dividing the number of vessels by the area. Images were taken, and the number blood vessels were quantified using ImagePro software (Media Cybernetics, Bethesda, MD, USA). Survived GFP expressing MSCs were identified by GFP fluorescent signals. The fate of the engrafted MSCs were identified by immunostaining against cardiac troponin T and α-actinin, markers for cardiomyocytes; and α-smooth muscle actin and vimentin were used to identify newly formed blood vessels [36-38]. All antibodies were purchased from Thermo Fisher Scientific Inc. (Rockford, IL, USA).

Image Processing

Images were obtained using a TE200 inverted microscope (Nikon Instruments, Inc., Melville, NY, USA) equipped with a LEP MAC 5000 motorized stage (Ludl Electronic Products Ltd., Hawthorne, NY, USA) controlled by ImagePro software. Immunofluorescence images of anti-cardiac troponin T, anti-α-actinin, anti-α-smooth muscle actin and anti-vimentin staining were obtained and superimposed with MSC fluorescence images to determine the viability and differentiation of engrafted MSCs. Bright field images of CD31 staining were taken and enhanced in ImagePro to identify AEC-stained blood vessels. For each heart section, the number of vessels in the normal area and of the infarct area were quantified and compared.

Data analysis and statistical analysis

Echocardiography was performed at 1 week and 4 weeks post-MI to evaluate rat heart function. Left ventricular percent fractional shortening (FS), left ventricle end diastolic dimension (LVEDD) and left ventricle end systolic dimension (LVESD) were measured directly from echocardiography images and FS was calculated by:

$$FS = \frac{LVEDD - LVESD}{LVEDD} * 100\%$$

Rats with a FS value of less than 30% at 1-week post-MI were considered having a fully developed MI since normal rats usually have a FS value of greater than 40%. Statistical significance in FS between 1 week and 4 weeks post-MI was tested using paired *t*-test. One-way ANOVA with SNK post-hoc were used to test statistical significance among different treatment groups. Data are presented as mean ± standard error (SEM).

Results

Fractional shortening changes

Left ventricle fractional shortening (FS) is a standard measure of the pumping function of the heart. As the myocytes begin to die after a myocardial infarction, the motion of LV wall is diminished which is reflected by decreasing of Fractional shortening (FS). FS of rats at 1 week and 4 weeks post-MI were measured using echocardiography. For sham operated normal rats (Figure 1A), there is no change in FS (by paired t-test) from 1 week to 4 weeks post-MI (FS of "mean ± SEM": 45.3% ±2% to 46.4% ± 2.2%), and FSs from both 1 week and 4 weeks post-surgery are in the normal range (above 40%, as demonstrated by the pink dash-dot line). However, there was a significantly loss in cardiac function in untreated MI group from 1 to 4 weeks post-MI (FS: 22.0% ± 2.2% to 13.2% ± 1.8%, $p<0.01$) (Figure 1B). Either MSC only treatment (Figure 1C) or targeted IGF-1 alone treatment (Figure 1D) significantly attenuated the loss of cardiac function from first to fourth weeks post-MI (FS in MSC group: 25.8% ± 2.8% to 22.7% ± 2.6%, $p<0.05$; FS in targeted IGF-1 group: 28.0% ± 2.7% to 30.3% ± 3.2%, $p<0.05$). The targeted IGF-1 + MSCs combination treatment not only stopped the deterioration of FS, but also significantly increased FS from first to fourth weeks post-MI (29.0% ± 1.7% to 35.8% ± 1.7%, $p<0.01$) (Figure 1E). After the combination treatment, the cardiac function of three of the MI rats (out of 10 rats) returned to normal as indicated by the improvement of the FSs to the normal level (40% - 60%) at 4 weeks post-MI. Figure 2 shows the overall comparison of FS gain (week 4 FS – week 1 FS) between each treatment group. Likewise, the IGF-1 + MSC combination treatment significantly ($p<0.01$) increased the heart FS from 1 week to 4 week post-MI compared with untreated group. Single treatment of targeted IGF-1 also increased the FS significantly ($p<0.01$) compared with untreated group, but not as much as the combination treatment did. Single treatment of MSCs delayed FS deterioration compared with untreated MI. From previous studies, independent

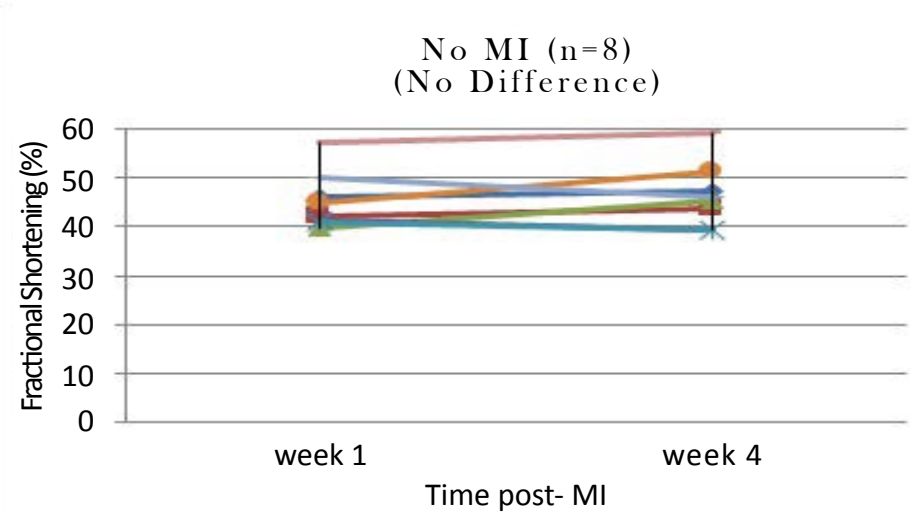

Figure 1A: A) sham. The pink dash-dot line illustrates the normal FS cut off value (40%). Each solid line represents the FS changes between 1 week and 4 weeks post-MI of one rat.

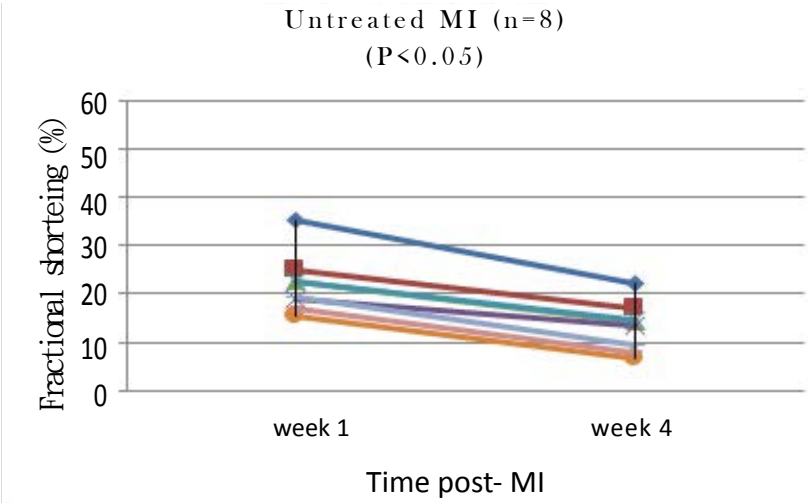

Figure 1B: B) untreated. The pink dash-dot line illustrates the normal FS cut off value (40%). Each solid line represents the FS changes between 1 week and 4 weeks post-MI of one rat.

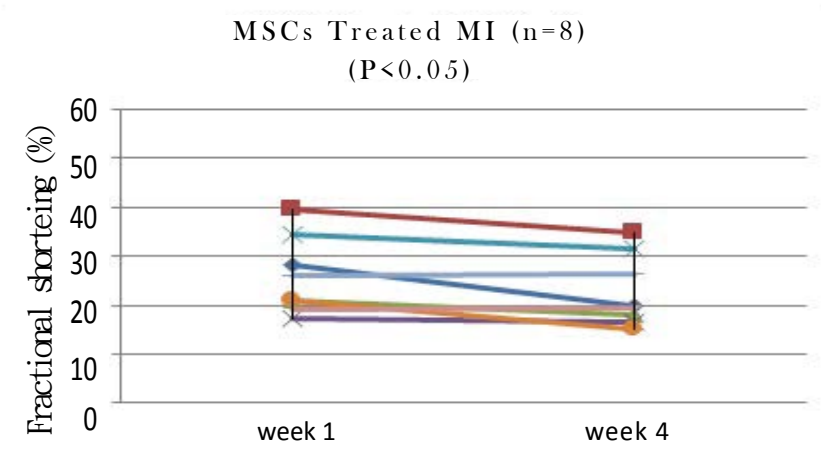

Figure 1C: C) MSCs. The pink dash-dot line illustrates the normal FS cut off value (40%). Each solid line represents the FS changes between 1 week and 4 weeks post-MI of one rat.

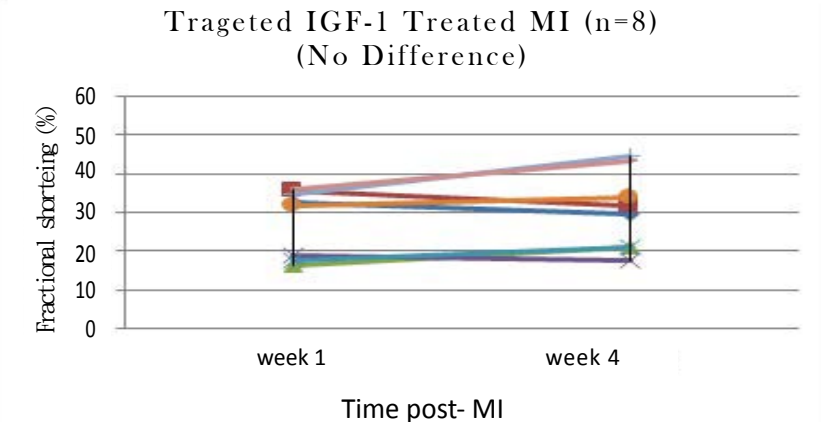

Figure 1D: D) targeted IGF-1 treated. The pink dash-dot line illustrates the normal FS cut off value (40%). Each solid line represents the FS changes between 1 week and 4 weeks post-MI of one rat.

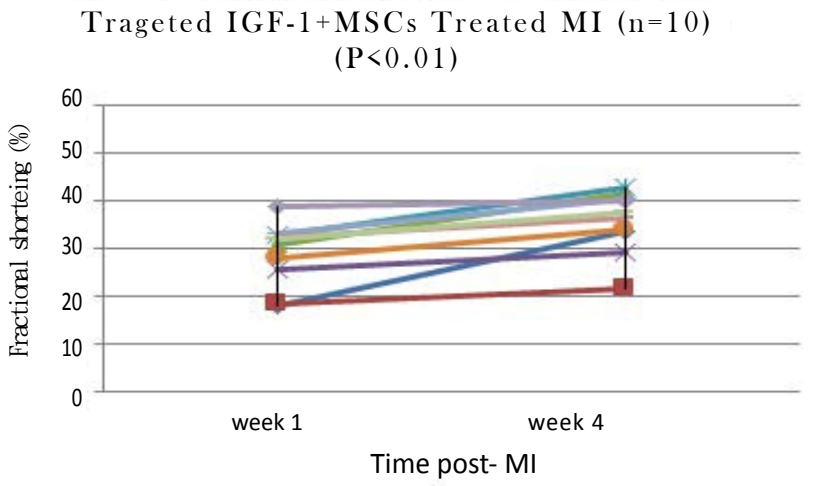

Figure 1E: E) combination treatment, from 1 week post-MI to 4 weeks post-MI in different treatment groups. The pink dash-dot line illustrates the normal FS cut off value (40%). Each solid line represents the FS changes between 1 week and 4 weeks post-MI of one rat.

histological examination by Dr. Stanley D Kosanke from the University of Oklahoma Health Sciences Center confirmed that there was no inflammatory response in major organs after targeted delivery of immunoliposomes (34).

Blood vessel regeneration in MI area

Anatomical blood vessel density was quantified using CD31/AEC staining. The CD31 staining pictures directly reflected the density and size of anatomical blood vessels at MI area. As the myocytes begin to die after a myocardial infarction, the left ventricular (LV) wall becomes thinner, the anatomical vessel density also decreased significantly (Figure 3). Due to the fact that anatomical vessel density varies in different hearts, but stays the same in the same heart [33,34], we normalized the vessel density by calculating the ratio of vessel density in the MI region of each heart sample to that in the non-MI region of the same sample (Figure 4). For the non-MI rat (Figure 4A), vessels with a very high density were evenly distributed at left ventricle, and the size of each vessel was small. The density became extremely low in the untreated MI group (Figure 4B), while the size of each vessel became bigger as compared with normal rats. MSCs treatment (Figure 4C) slightly increased the blood vessel density as compared with the untreated MI group. Targeted IGF-1 treatment (Figure 4D) slightly increased the blood vessel density as compared with the untreated MI group, but enlarged the size of each vessel. The IGF-1 + MSC combination treatment (Figure 4E) significantly increased vessel density, as well as maintained the size of each vessel in a reasonable manner (closer to that of normal). Figure 5 shows the quantitative measurements of the number of anatomical blood vessels for each treatment group, the value was normalized by dividing the number of anatomical vessels in the MI region by the number of anatomical vessels

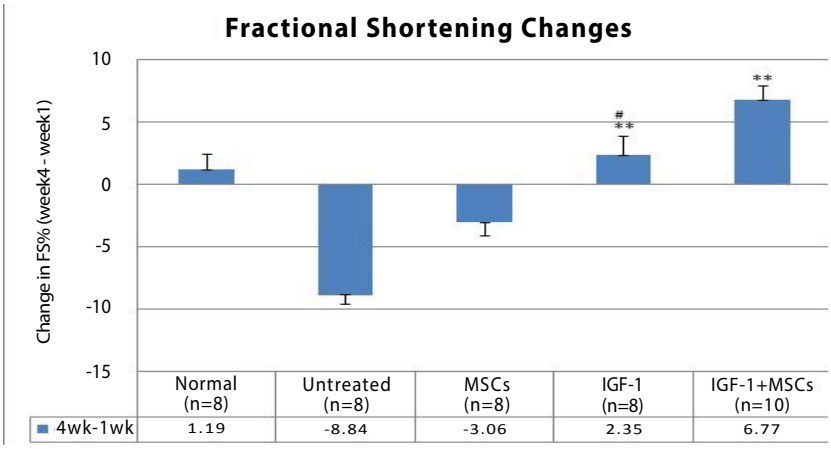

Figure 2: Cardiac function loss over time represented by FS changes between 1 and 4 weeks Post-MI. Y-axis is calculated by subtracting the FS at 1 week post-MI from the FS at 4 weeks post-MI. * ($p<0.05$) and ** ($p<0.01$) compared to untreated group; # ($p<0.05$) and # # ($p<0.01$) compared to MSCs treated group; + ($p<0.05$) compared to IGF-1 treated group by XXX test.

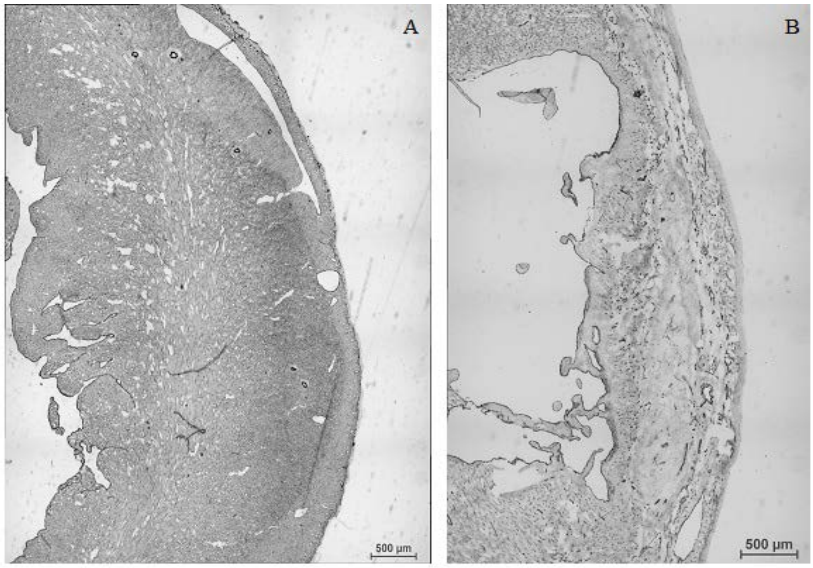

Figure 3: Anatomical vessel changes after MI. normal rat heart (A), and MI rat heart (B). Heart sections were stained with CD31. The scale bars represent 500 µm.

in the right ventricle of the same heart at 4 weeks post-MI, to eliminate the animal to animal variation. As seen in Figure 5, both targeted IGF-1 treatment and the targeted IGF-1 + MSC combination treatment significantly increased vessel density. Compared to the untreated MI group, transplantation of MSCs alone resulted in slight but not a significant increase in the density of anatomical vessels. The targeted delivery of IGF-1 treatment promoted angiogenesis in the MI area, and the combination treatment of targeted IGF-1 and MSCs resulted in a further increase in angiogenesis in the MI area as indicated by the highest density of anatomical blood vessels in MI area.

MSC engraftment

The total number and density of EGFP expressing MSCs in the MI area were determined from their fluorescent signature by measuring the area and intensity of green fluorescence using the fluorescent images taken immediately after tissue sectioning. Figure 6 shows GFP fluorescence images for MSCs in the MI area 4 weeks post-MI from (A) MSCs treatment group, and (B) IGF-1 + MSCs treatment group. As shown in Figure 6, IGF-1 + MSCs treated MI group greatly increased the total number and density of engrafted MSCs compared with MSCs only group.

Fate of MSC

The differentiation of MSCs after the combination treatment was determined by immunohistochemistry 3 weeks after transplantation. As shown in Figure 7, both (A) anti-cardiac troponin T and (B) anti-alpha-actinin immunoreactivity were presented in EGFP positive cells indicating the number of the implanted MSCs that were differentiated into cardiac muscle in the MI region 3 weeks after MSC transplantation. There are a lot MSCs in the MI

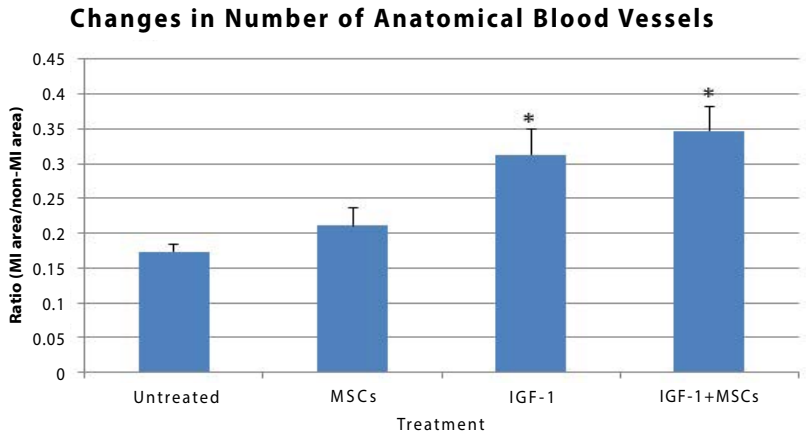

Figure 4: (bottom panel) The anatomical blood vessel density, quantified from the top panel. The Y axis "ratio" was calculated by normalizing the vessel density in the MI region of each heart sample to that in the non-MI region of the same sample.

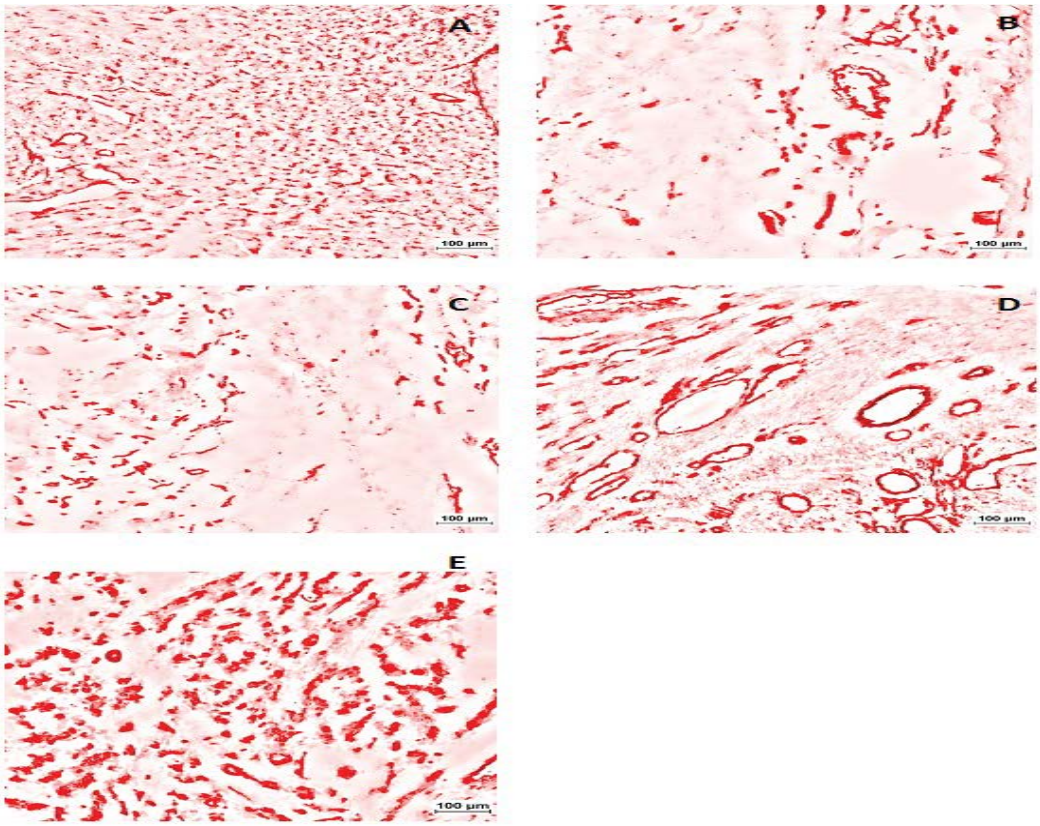

Figure 4 (A to E): (top panel) Anatomical blood vessel regeneration after each treatment. The scale bars represent 100 µm. Data are presented as "mean + standard error" (n = 5 samples for each group). *Significant difference compared to the "untreated" group, by ANOVA, $P < 0.01$. Top panel: anatomical vessels (CD31 staining) in sham operated (no MI) (A), untreated (B), MSC treated (C), IGF-1 treated (D), and IGF-1 + MSC treated (E) rats measured 4 weeks after MI induction.

area positively stained for (C) anti-vimentin and (D) anti-alpha-smooth muscle actin indicating that a lot of implanted MSCs differentiated into blood vessels 3 weeks after MSC transplantation.

Discussion

Despite advances in stem cell therapy in treating myocardial infarction over the last decade, effective cell delivery remains a major obstacle for successful myocardial repairs due to the hostile microenvironment generated by ischemic myocardium [39], such as inadequate angiogenesis, inflammation, and presence of reactive oxygen species (ROS). These hostile factors would impair stem cell survival, engraftment, and reduce the success of cell-based myocardial repairs. In fact, the survival and engraftment rate of implanted MSCs is very low, less than 11%. It also depends

on the route of infusion. The survival and engraftment rate of MSCs is ~11% with the route of intramyocardial injection, ~3% with the routes of intravenous infusion, ~3-6% using intracoronary infusion [40-43]. Therefore, the regulation of MI microenvironment to increase stem cell engraftment, survival and homing should be a major goal in the field of stem cell therapy. Several strategies have been used to enhance transplanted cell survival, e.g. pretreating stem cells with growth factors to improve implantation efficacy [44], preconditioning stem cells with hypoxia to activate the survival pathway [45], genetically modifying stem cells to overexpress anti-death signals [24], using *in situ* cardiac tissue engineering [46,47], etc. Although these strategies have proven to be successful to some extent, they have all failed to improve the hostile microenvironment, and may have undesirable side effects. In the present study, we demonstrated for the first time that the combination therapy using targeted IGF-1 delivery and MSC transplantation to MI area in a rat model resulted in the most significant improvement in cardiac function as measured by the change in heart fractional shortening 4 weeks post-MI as compared to either IGF-1 only treatment or MSC only treatment. Our data show that targeted delivery of IGF-1 to the MI area greatly improves MSC survival 4 week after MI and suggests that the local microenvironment, preconditioned by targeted delivery of IGF-1 treatment, correlates with the engraftment of stem cells in the MI area and more robust formation of blood vessels after MI. However, despite the significant improvements in cardiac function, we observed that very few stem cells underwent cardiac myocyte differentiation which may have limited the overall treatment efficacy.

Although combining IGF-1 therapy with MSC transplantation has not been performed previously, several studies have shown that IGF-1 is a potential therapeutic agent that can improve the MSC survival and engraftment in the hostile microenvironment such as myocardial infarction [24-26]. These studies have demonstrated that activation of PI3k/AKT pathway results in a better homing of MSCs into the ischemic microenvironment, thus enhancing the cardiac functional recovery after acute myocardial infarction. In order to minimize the side effects of systemic delivery of IGF-1, while extending the residence time of IGF-1 in the MI area, we have applied targeted delivery approach through biocompatible drug carriers. Our group has developed long circulating immunoliposomes [34,35, 48-51] for delivering another peptide agent, VEGF, to MI tissue and antivascular drugs to irradiated tumors.

In this study, we combined the targeted IGF-1 delivery using immunoliposomes and intramyocardial implantation of MSCs in MI tissue to treat MI. We chose intramyocardial injection of MSCs due to the fact that this route provides the highest cell survival and engraftment rate (~11%) as compared to other routes (intravenously ~3%,

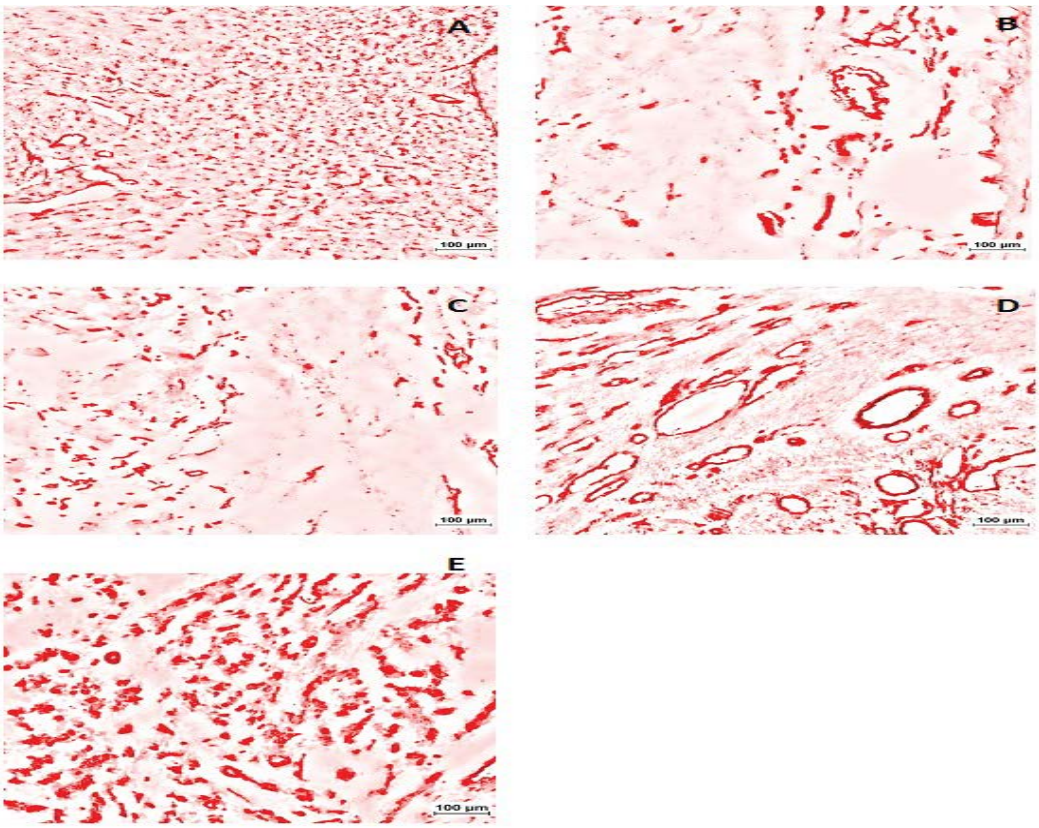

Figure 4 (A to E): (top panel) Anatomical blood vessel regeneration after each treatment. The scale bars represent 100 µm. Data are presented as "mean + standard error" (n = 5 samples for each group). *Significant difference compared to the "untreated" group, by ANOVA, $P < 0.01$. Top panel: anatomical vessels (CD31 staining) in sham operated (no MI) (A), untreated (B), MSC treated (C), IGF-1 treated (D), and IGF-1 + MSC treated (E) rats measured 4 weeks after MI induction.

intracoronary ~3%–6%) [52].Our previous studies have shown that the optimal window for stem cell therapy may be within the two weeks of MI [53]. Here we took advantage of the inflammatory response after MSC transplantation to selectively deliver IGF-1 to the infarcted site using anti-P-selectin coated immunoliposomes.

Various *in vitro* and *in vivo* studies have shown that stem cell therapy partly restores heart function by MSC's paracrine effect [54,55]. Therefore, increase the survival and engraftment of the implanted MSCs will directly enhance the MSC's paracrine effect for improving the cardiac function of the MI heart. Our results (Figure 5) have shown that targeted delivery of IGF-1 to MI heart significantly improved

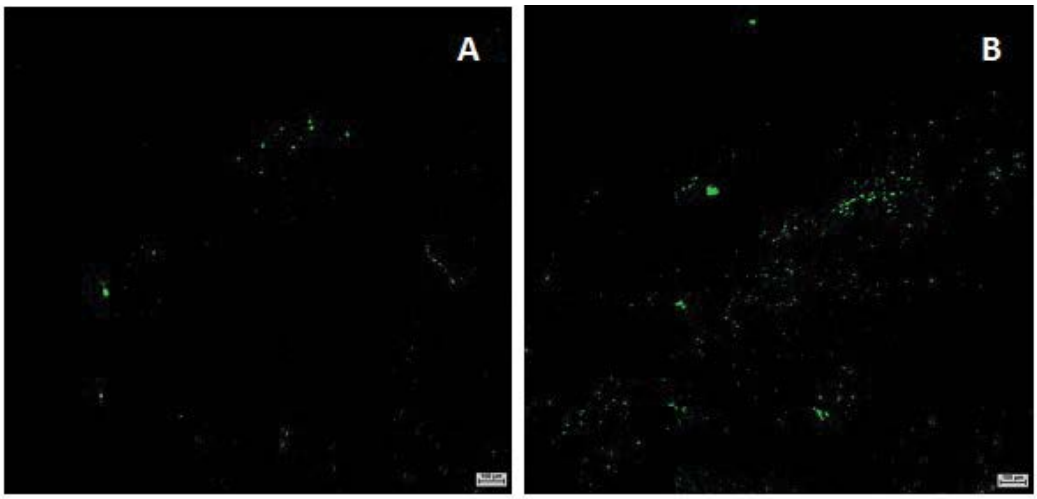

Figure 5: MSCs in the MI region as indicated by GFP fluorescence 3 weeks after transplantation. Images taken from (A) MSCs treated, or (B) IGF-1 + MSCs combination treated MI rats. The scale bars represent 100 μm.

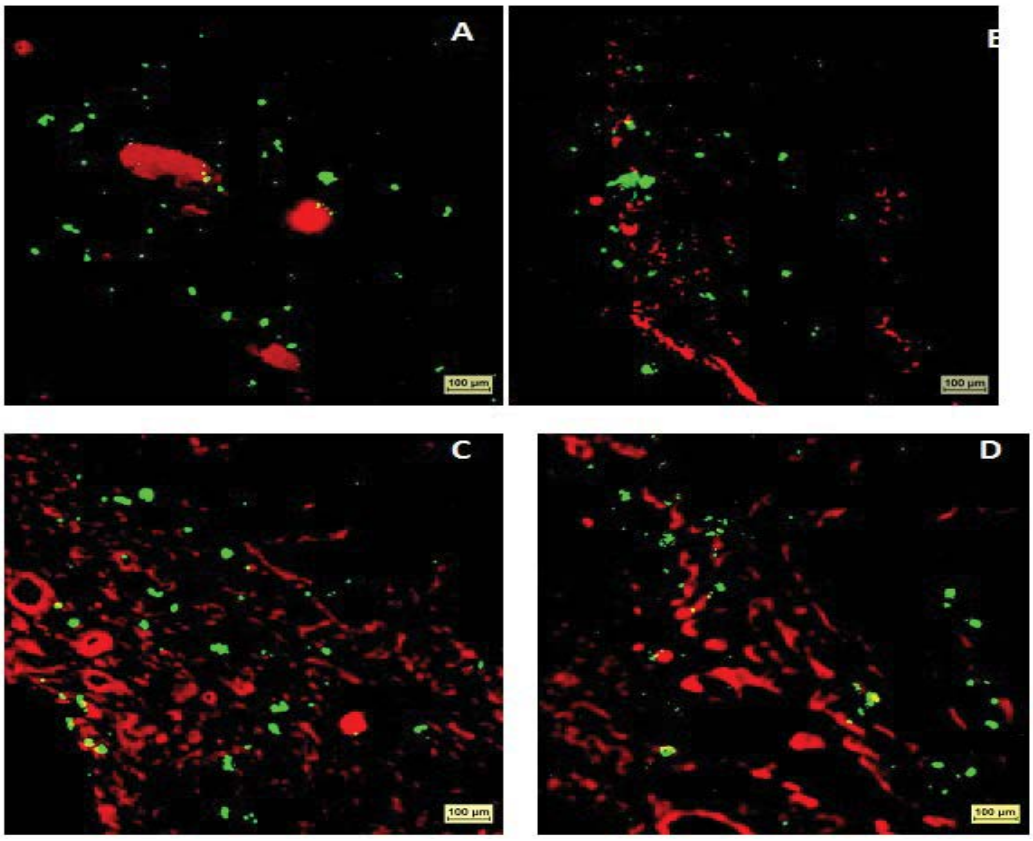

Figure 6: MSC differentiation after IGF-1 + MSC combination treatment. Images were taken from the border zone of the MI region. Samples were stained with different antibodies: (A) anti-cardiac troponin T, or (B) anti-alpha-actinin, or (C) anti-vimentin, or (D) anti-alpha-smooth muscle actin. Trhasplanted GFP expressing MSCs are shown in green. Cy3 labeled antibodies are shown in red. The scale bars represent 100 μm.

the survival and engraftment of the implanted MSCs into the host cardiac tissue after MI. To look at the paracrine effect of the engrafted MSC, we have indirectly evaluated one of the most important growth factors expressed by MSC's paracrine effect, angiogenic growth factor VEGF, by measuring the changes of the number of anatomical blood vessel. Our results (Figure 4) shown that the IGF-1 + MSC combination treatment significantly increased the number of the anatomical vessel compared to the MSC treatment alone. To further study the fate of the engrafted MSCs, we also looked at the differentiation of the MSCs in MI region after transplantation. Our results (Figure 6) indicate that portion of the MSCs was differentiated into vascular cells and cardiomyocytes. Taken all together, the improvement of the cardiac function after IGF-1+MSC treatment may due to both the paracrine effect of the MSCs and the differentiation of the MSCs into blood vessels and cardiomyocytes, which is consistent with the literature [56,57].

Conclusion

In conclusion, the current study demonstrates the low survival and engraftment of the implanted MSCs due to the hostile microenvironment in MI region can be improved by targeted delivery of IGF-1 to the MI region immediately after MSC transplantation. Furthermore, the survived MSCs promoted angiogenesis in the MI region, which further enhanced the survival and engraftment of the implanted MSCs. The engraftment of the MSCs results in a significantly larger recovery in cardiac function compared to either IGF-1 only or MSC only treatment. This recovery was achieved partially from the differentiation of the MSCs into cardiac myocytes and blood vessel cells. Overall, our results indicated that our targeted drug delivery system significantly improved the cardiac function by the differentiation of MSCs into both vascular cells and cardiomyocytes and by paracrine effect of the survived and engrafted MSCs. To further improve the cardiac function, other therapeutic agents such as angiogenesis agents that further improve the local microenvironment and/or growth factors that assist cardiac myocyte differentiation can be incorporated into our targeted delivery strategy.

Acknowledgement

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