Fabrication of Synthetic Mesenchymal Stem Cells for the Treatment of Acute Myocardial Infarction in Mice

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Abstract

Rationale—Stem cell therapy faces a number of challenges. It is difficult to grow, preserve, and transport stem cells before they are administered to the patient. Synthetic analogs for stem cells represent a new approach to overcome these hurdles and hold the potential to revolutionize regenerative medicine.

Objective—We aim to fabricate synthetic analogs of stem cells and test their therapeutic potential for treatment of acute myocardial infarction in mice.

Methods and Results—We packaged secreted factors from human bone marrow-derived mesenchymal stem cells (MSC) into Poly(lactic-co-glycolic acid) PLGA microparticles and then coated them with MSC membranes. We named these therapeutic particles “synthetic MSC” (or...
synMSC). synMSC exhibited a factor release profile and surface antigens similar to those of genuine MSC. synMSC promoted cardiomyocyte functions and displayed cryopreservation and lyophilization stability \textit{in vitro} and \textit{in vivo}. In a mouse model of acute myocardial infarction, direct injection of synMSC promoted angiogenesis and mitigated left ventricle remodeling.

**Conclusions**—We successfully fabricated a synMSC therapeutic particle and demonstrated its regenerative potential in mice with acute myocardial infarction. The synMSC strategy may provide novel insight into tissue engineering for treating multiple diseases.

**Keywords**
Mesenchymal stem cells; paracrine factors; myocardial infarction; synthetic cells; regeneration; stem cell; tissue engineering

**Subject Terms**
Stem Cells; Myocardial Infarction; Myocardial Regeneration

**INTRODUCTION**

A growing body of studies have demonstrated the therapeutic potential of different stem cells types such as skeletal myoblasts, bone marrow-derived mesenchymal stem cells, embryonic stem cells, and endogenous cardiac stem cells in cardiovascular diseases\(^1\). Among the cell types under investigation, mesenchymal stem cells (MSC) have attracted great attention owing to their ability to differentiate into mesoderm and non-mesoderm tissues, their immunomodulatory properties, and their broad spectrum release of trophic factors\(^2\). Preclinical and clinical studies on MSC have shown promise for repair and regeneration of cardiac tissues\(^3\). In an effort to understand the mechanisms responsible for the therapeutic effect of MSC, scientists investigated their retention rates in the myocardium after transplantation. As a result of the low retention rates observed\(^4\), they postulated other mechanisms of action promoting the recovery in cardiac function and structure other than the stem cells’ \textit{in situ} differentiation. Soon, they realized that the broad spectrum release of soluble factors by MSC may be the primary mechanism for their therapeutic effects\(^5\). More recently, they found that MSC-secreted exosomes exhibited functions similar to MSC for repairing heart injury\(^6\). Inspired by this, scientists are considering alternative strategies to stem cell transplantation, namely the direct delivery of MSC secretome to repair injured tissues. Indeed, \textit{in vivo} and \textit{in vitro} studies have demonstrated the therapeutic effect of MSC-conditioned media for treatment of cardiovascular diseases\(^7, 8\). Moreover, the single delivery of cytokines such as vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) have also been tested for their cardiac therapeutic effects in clinical trials\(^9\). Unfortunately, neither has met our expectations. The reasons may be the short half-life of cytokines \textit{in vitro}, the uncertainty of effective/safe dosages, and the possibility that multiple administration of cytokines may be necessary to act synergistically to achieve therapeutic effect\(^10\). It is noteworthy that exosomes could circumvent a number of these challenges. The bi-lipid membrane of exosomes could protect their contents from degradative enzymes or chemicals and the membrane bound molecules might home the exosomes to a specific tissue or microenvironment\(^11\). In addition, exosomes contain proteins.
and RNAs that may have adequate potential for cardiac repair\textsuperscript{12–16}. However, exosome-based therapeutics also face challenges such as the lack of a standard isolation protocol, rapid clearance and wash-away due to their extremely small sizes. Poly(lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer, is emerging as a prominent element in drug delivery system due to its capability of protecting cytokines from degradation while allowing for the sustained release of factors that target in specific organs or cells\textsuperscript{17,18}. Further, Fang et al. reported cancer-cell-membrane coated nanoparticles (CCNPs) formed by coating cancer cell membranes onto PLGA-loaded immunological particles. The membrane-bound tumor-associated antigens permit CCNPs to be efficiently delivered to antigen presenting cells to promote anticancer immune response\textsuperscript{19}.

In the present study, based on the polymer encapsulation and membrane cloaking approaches, we fabricated a therapeutic particle, namely synthetic MSC (synMSC), by coating MSC cell membranes onto MSC-secretome-loaded PLGA particles. We then characterized its physiochemical and biological properties in vitro, and tested its regenerative potential in mice with acute myocardial infarction. The scientific premise of our study is that the synMSC idea overcomes several major challenges of the status quo of cell therapy practice, namely cryopreservation stability, standardization, and “off the shelf” feasibility. In addition, because of the MSC membrane coating, synMSC will likely avoid the tumorigenicity and immunogenicity risks associated with stem cell transplantation. Although the present study targets the heart, the synMSC technology represents a platform technology that is generalizable to other stem cell types.

**METHODS**

A detailed methods section is provided in the Online Data Supplement.

**RESULTS**

**synMSC fabrication and biological properties**

The schematic design of synMSC fabrication was summarized in Figure 1A. In brief, MSC conditioned media was incorporated in PLGA to form microparticles (MP), then the MP were coated with MSC cell membrane to form synthetic mesenchymal stem cells (synMSC). Scanning electron microscopy and fluorescent imaging (Figure 1B) confirmed the successful MSC cell membrane coating on MP. synMSC had a size around 20 μm, similar to those of MP and real MSC (Figure 1C). Flow cytometry analysis showed that synMSC exhibited similar expressions of CD105, CD90, CD45, CD31, and CD34 compared to MSC, while MP didn’t (Figure 1D). Furthermore, synMSC could sustain the release of growth factors like vascular endothelial growth factor (VEGF) (Figure 1E), stromal cell-derived factor-1 (SDF-1) (Figure 1F), and insulin-like growth factor-1 (IGF-1) (Figure 1G). These results demonstrated that synMSC and MSC were comparable in terms of secretome and surface antigen expressions.

**synMSC promotes cardiomyocyte functions in vitro**

To test the cardiomyocyte protective capability of synMSC in vitro, neonatal rat cardiomyocytes (NRCM, stained by alpha-sarcomeric actin, green in Figure 2A) were co-
cultured with MP, synMSC and MSC (red in Figure 2A). Solitary NRCM culture was included as negative control. synMSC significantly increased NRCM number (Figure 2B) and promoted NRCM contractility (Figure 2C). Such beneficial effects were comparable to those from MSC. The promotion of NRCM number and contractility of synMSC might be due to its significantly higher number existed in NRCM (Figure 2D), although the same amount of particles was originally applied to NRCM. These results demonstrated that the MSC membrane on synMSC allow them to bind and interact with cardiomyocytes.

**Cryopreservation and lyophilization stability of synMSC**

Cryopreservation stability is one of the major challenges of cell therapy products. Here, we tested the stability of synMSC after rapid freezing and thawing. Fluorescent and white light microscopy images revealed freeze/thaw treatment didn’t alter the structure (Figure 2E) or size (Figure 2F) of synMSC. Flow cytometry analysis showed no significant difference on the surface antigen expressions of synMSC pre and post freeze (Figure 2G). Further, we tested the lyophilization stability of synMSC, and found that the lyophilization process didn’t alter the structure, size, surface antigen expressions, or sustained VEGF release of synMSC (Online Figure II). MSC, however, could not undergo the harsh freeze/thaw process without inducing cell death. After injecting freeze/thawed synMSC or MSC into a mouse heart, MSC were targeted by macrophages while synMSC were not (Figure 2H, 2I). These results demonstrated the cryopreservation and lyophilization stability and advantages of synMSC over real MSC.

**synMSC injection mitigates left ventricle remodeling of infarcted heart**

To test the therapeutic effect of synMSC, we made an acute myocardial infarction (MI) model in mice by left anterior descending artery ligation, and then synMSC were immediately injected intramyocardially. Negative control mice received no treatment after MI. 18F-fluorodeoxglucose positron emission tomography/computed tomography (18F-FDG PET/CT) was performed at 1 (baseline) and 14 days (endpoint) after infarction to measure the infarct area (Figure 3A). 99mTc-tetrofosmin single photon emission computed tomography/computed tomography (SPECT/CT) was performed at 2 (baseline) and 15 days (endpoint) after infarction to measure left ventricular volume (Figure 3A). synMSC injection showed a significant reduction of infarct area (Figure 3B). The left ventricular volume changes were indistinguishable between the two groups (Figure 3B). Left ventricle morphometry imaged by Masson’s trichrome staining revealed the protective effects of synMSC and MSC treatment on heart morphology (Figure 3C). The infarct wall thickness was increased (Figure 3D) and infarct size was reduced (Figure 3E) both in synMSC and MSC treated mice as compared to the control group.

**synMSC injection promotes endogenous repair in the infarcted heart**

To reveal the mechanisms underlying the therapeutic benefits of synMSC, we investigated whether synMSC injection could recruit more c-kit-positive stem cells, promote angiogenesis, and improve cell proliferation in the infarcted heart. Immunostaining analyses with c-kit (Figure 4A), CD34 (Figure 4B), and ki67 (Figure 4C) were performed in the infarcted hearts of control, synMSC, and MSC treated mice. Compared to control, synMSC and MSC treatments increased the c-kit positive stem cell recruitment (Figure 4D) and
vessel density (Figure 4E) of the infarcted heart. Compared to control, the proliferated cells were slightly increased in the infarcted heart of synMSC treated mice, but significantly increased in the infarcted heart of MSC treated mice (Figure 4F). These results suggested that the therapeutic effects of synMSC may be through activation of c-kit-positive stem cells and promotion of angiogenesis.

**DISCUSSION**

In this study, we fabricated a particle named synMSC by coating MSC cell membranes onto PLGA particles loaded with MSC secretome. This novel particle exhibited similar secretome and surface antigen profiles as compared to real MSC. synMSC promoted cardiomyocyte function, and displayed cryopreservation and lyophilization stability *in vitro*. Intramyocardial injection of synMSC mitigated left ventricle remodeling in a mouse model of acute myocardial infarction at a level comparable to genuine MSC.

Emerging lines of evidences indicate that adult stem cells exert their therapeutic effects mainly through paracrine effects rather than direct differentiation. To that end, scientists have begun to consider the direct delivery of stem cell-released soluble factors as an alternative approach to stem cell transplantation. However, the progress is hindered by the short lived effect of injected soluble factors. The cardiac contraction can quickly “wash away” the injected factors. Approaches that allow controlled release of soluble factors are paramount and urgently needed for the clinical implementation of stem-cell-derived factors for therapeutic heart regeneration. Although, exosomes show great potential in cardiac repair and may overcome the shortcomings associated with cell transplantation, the lack of standardized protocol for exosome isolation and the quick washout of exosomes after injection remain challenges for clinical application. We designed synMSC, which combined the secretome (containing both soluble factors and exosomes) and membranes of MSC. synMSC can release soluble factors such as VEGF, SDF-1, and IGF-1, binding to cardiomyocytes in vitro. Additionally, the expression of MHC class I molecules, but not MHC class II molecules or costimulatory molecules in MSC cell membranes allow it to escape allorecognition by the immune system and may modulate the host immune response. The MSC membrane coating on PLGA particles could effectively protect synMSC from being attacked by host immune and inflammatory cells.

A great number of cardiomyocytes die after the induction of MI. The restoration of cardiomyocyte numbers is one important target for cell-based therapy. By co-culturing the synMSC with NRCM, we observed a significant increase in NRCM number and contractility at a level comparable to MSC, which may be associated with the growth factors released by synMSC. The superiority of synMSC over MP could be due to several reasons. First, the MSC membrane on synMSC allow them to closely attach to cardiomyocytes by cell-cell interactions. Second, it has been reported that the stem cell membranes are not null in the regeneration process: direct contact may trigger downstream signaling in cardiomyocytes to favor survival and function augmentation.

One major challenges of stem cell based therapy is the cryopreservation stability of cells. Here we found that snap freezing in −80 °C and rapid thawing did not alter the structure,
size, or surface antigen expressions of synMSC. Furthermore, lyophilization did not alter the traits of synMSC. Importantly, when the freeze/thawed MSC (with dead MSC caused by harsh freezing/thawing) were injected into a mouse heart, they were targeted by macrophages (initiating the phagocytosis of dead MSC) while synMSC were not. This suggested the superior cryopreservation stability of synMSC over MSC.

Currently, as computed tomography (CT) can provide great detail in anatomic structure, hybrid imaging of PET and SPECT with CT have been adopted in clinical and small animal cardiovascular disease diagnosis\textsuperscript{22, 23}. PET utilizing glucose tracer analog \textsuperscript{18}F- FDG allows the detection of cells with different metabolic activity\textsuperscript{24}, and gated SPECT utilizing \textsuperscript{99}mTc-tetrofosmin makes accurate assessment of ventricular volumes\textsuperscript{25}. So we evaluated the myocardial viability and left ventricle volume of mice heart by \textsuperscript{18}F-FDG PET/CT and \textsuperscript{99}mTc-tetrofosmin SPECT/CT. synMSC significantly mitigated left ventricle remodeling, as indicated by a significant reduction of infarct area, confirming the therapeutic potential of synMSC. Further, the left ventricle morphometry evaluation by Masson’s trichrome staining revealed synMSC exhibited protection of heart morphometry at a level that was comparable to MSC.

Previous reports have demonstrated that MSC provide cardio-protection by paracrine actions that activate cardiac stem cells\textsuperscript{26}, angiogenesis and cell proliferation\textsuperscript{8}. Consistent with these findings, a significant increase of c-kit-positive stem cells was found in synMSC treated mice (similar to MSC treatment), although it is hard to distinguish the origination of these c-kit-positive stem cells (cardiac-derived or bone marrow-derived). In addition, a larger number of vessels were found in synMSC treated mice which would provide sufficient oxygen and nutrients to the surrounded cardiomyocytes.

\textbf{Conclusions}

Taken together, we here successfully fabricate synMSC and demonstrate their prominent therapeutic effects in an acute myocardial infarction mouse model, suggesting the feasibility of this approach in regenerative medicine. Moreover, this synthetic stem cell approach provides novel insight into tissue engineering for treating multiple diseases. All after all, our results suggest synthetic stem cells offer an alternative option to stem cell-mediated regenerative therapies. Future studies should focus on streamlining the handling and manipulations of synthetic stem cells to facilitate clinical translation.

\textbf{Supplementary Material}

Refer to Web version on PubMed Central for supplementary material.

\textbf{Acknowledgments}

\textbf{SOURCES OF FUNDING}

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Nonstandard Abbreviations and Acronyms

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<tr>
<th>Abbreviation</th>
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<tr>
<td>MSC</td>
<td>mesenchymal stem cells</td>
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<tr>
<td>synMSC</td>
<td>synthetic mesenchymal stem cells</td>
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<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic acid)</td>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<td>IGF-1</td>
<td>insulin-like growth factor 1</td>
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<td>CCNPs</td>
<td>cancer cell membrane-coated nanoparticles</td>
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<tr>
<td>MP</td>
<td>microparticles</td>
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<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
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<tr>
<td>SDF-1</td>
<td>stromal cell-derived factor-1</td>
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<tr>
<td>NRCM</td>
<td>neonatal rat cardiomyocytes</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<td>CT</td>
<td>computed tomography</td>
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<td>18F-FDG</td>
<td>18F-fluorodeoxglucose</td>
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<td>PET-CT</td>
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<tr>
<td>SPECT-CT</td>
<td>single photon emission computed tomography/computed tomography</td>
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References


## NOVELTY AND SIGNIFICANCE

### What Is Known?

- Stem cell transplantation for heart repair has shown some benefits in animal studies and clinical trials, but it is difficult to expand, preserve, and transport stem cells before they are administered to the patient.
- Benefits from stem cell therapy, including the injection of mesenchymal stem cells (MSCs), are presumably from the secretion of regenerative factors rather than from direct tissue replacement.
- The stem cell membrane plays an important role in anchoring the injected stem cells to the host tissue and mediating the repair process through cell-cell communication.

### What New Information Does This Article Contribute?

- We describe a process to fabricate synthetic MSCs (synMSCs) by encapsulating MSC-secreted factors in biodegradable polymer particles and then coating the particles with MSC-derived cell membranes.
- Unlike authentic, living MSCs, synMSCs can undergo harsh cryopreservation and lyophilization processes without changing their properties. In vitro, synMSCs release various growth factors and promote cardiomyocyte functions.
- In a murine model of myocardial infarction, injection of synMSCs leads to reduction of scar and mitigation of ventricular remodeling without triggering inflammatory responses. Such therapeutic benefits are similar to those from MSC therapy.

We employed a core/shell polymer particle design to fabricate synthetic stem cells designed to emulate authentic stem cells. The new product, named as synMSCs, contained the secreted factors and surface antigens similar to genuine MSCs. synMSCs exhibited superior cryo-stability and lyo-stability compared to MSCs while preserving regenerative abilities of MSCs in treating mice with ischemic myocardial injury. The synMSC technology would offer a more uniform treatment strategy from patient to patient, rather than an inherently variable autologous or allogeneic cell-based strategy. The cell-free nature of our synthetic approach is readily translatable to the clinic, with a potentially similar safety profile compared to living MSCs.
Figure 1. Fabrication and characterization of synMSC

(A) Schematic illustration of the fabrication process of synMSC. Microparticles (MP) were fabricated by treating mesenchymal stem cells conditioned-media with Poly (lactic-co-glycolic acid) (PLGA). Synthetic mesenchymal stem cells (synMSC) were formed by coating the MP with MSC membranes. After that, we tested the therapeutic effects of synMSC injection in mice with acute myocardial infarction. (B) Scanning electron microscopy images (left) and fluorescent images (right) on the structure of MP and synMSC. MP was labeled with Texas red succinimidyl ester (red), and synMSC as cell membranes labeled with green fluorescent DiO (red particle with green coat). Scale bar: 10 µm.
μm. (C, D) Quantitative analyses on the diameter and expressions of MSC markers in the MP, synMSC, and MSC. (E, F and G) Quantitative analyses on the release of vascular endothelia growth factor (VEGF), stromal cell-derived factor-1 (SDF-1), and insulin-like growth factor-1 (IGF-1) from synMSC. n=3 for each group. All data are mean ± SD.
Figure 2. Potency and stability of synMSC in vitro

(A) Fluorescent images of neonatal rat cardiomyocytes (NRCM) stained with alpha sarcomeric actin (green) and co-cultured with MP, synMSC, and MSC (red). Scale bar: 100 μm. (B, C) Quantitative analyses of NRCM numbers and contractility when co-cultured with MP (blue bars), synMSC (red bars), and MSC (green bars). (D) Quantitative analyses on the number of MP and synMSC binding to NRCM. (E) Fluorescent images (above) and white light microscopy images (below) on synMSC morphology and aggregation before and after freeze/thaw. Scale bar: above, 10 μm; below, 100 μm. (F, G) Quantitative analyses on the size and surface antigen expressions of synMSC pre and post freeze/thaw. 

(H) Representative fluorescent images and illustration showing macrophage (green) attraction after the injection of freeze/thawed MSC and synMSC (red) into a mouse heart. Scale bar: 100μm. (I) Quantitative analyses of the CD68+ macrophages in freeze/thawed MSC- or synMSC- injected mouse heart. n=4 for each group. All data are mean ± SD. (B, C)* * P < 0.05 when compared to control, (D)* * P < 0.05 when compared to MP, (I)* * P < 0.05 when compared to synMSC.
Figure 3. Benefits of synMSC injection in mice with myocardial infarction

(A) Representative PET/CT images and SPECT/CT images obtained at baseline and endpoint of mice after MI with or without synMSC treatment. (B) Quantitative analyses on the percentage of altered infarct area and left ventricular volume (endpoint vs baseline) in control and synMSC treated mice. (C) Masson’s trichrome staining images from the base, mid-papillary and apical regions of the infarcted heart two weeks after MI of control, synMSC and MSC treated mice. Quantitative analyses of infarct wall thickness (D) and infarct size (E) of left ventricle in control, synMSC and MSC treated mice. n=8 for each group. All data are mean ± SD. * P < 0.05 when compared to control.
Figure 4. Injection of synMSC promoted endogenous repair in the infarcted heart

(A, B, C) Representative fluorescent images showing c-kit-positive, CD34-positive, and ki67-positive cells in the infarcted heart after control, synMSC, or MSC treatment. Arrows indicate the positively stained cells. Scale bar: (A), (C): 20 μm; (B): 50 μm. (D, E, F) Quantitative analyses on c-kit-positive cells, CD34-positive cells, and ki67-positive cells in the infarcted heart after control, synMSC, or MSC treatment. n=6 for each group. All data are mean ± SD. * P < 0.05 when compared to control.