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

Lan Luo,* Junnan Tang,* Kodai Nishi, Chen Yan, Phuong-Uyen Dinh, Jhon Cores, Takashi Kudo, Jinying Zhang, Tao-Sheng Li, Ke Cheng

Rationale: Stem cell therapy faces several 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) 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 in vitro and 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. (Circ Res. 2017;120:1768-1775. DOI: 10.1161/CIRCRESAHA.116.310374.)

Key Words: artificial cells • mesenchymal stem cells • myocardial infarction • regeneration • stem cells • tissue engineering

A growing body of studies have demonstrated the therapeutic potential of different stem cell types such as skeletal myoblasts, bone marrow–derived mesenchymal stem cells (MSCs), embryonic stem cells, and endogenous cardiac stem cells in cardiovascular diseases. Among the cell types under investigation, MSCs have attracted great attention owing to their ability to differentiate into mesoderm and nonmesoderm tissues, their immunomodulatory properties, and their broad spectrum release of trophic factors. Preclinical and clinical studies on MSC have shown promise for repair and regeneration of cardiac tissues. 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, they postulated other mechanisms of action promoting the recovery in cardiac function and structure other than the stem cells’ 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. More recently, they found that MSC-secreted exosomes exhibited functions similar to MSC for repairing heart injury. Inspired by this, scientists are considering alternative strategies to stem cell transplantation, namely, the direct delivery of MSC secretome to repair injured tissues. Indeed, in vivo and in vitro studies have demonstrated the therapeutic effect of MSC-conditioned media for treatment of cardiovascular diseases. Moreover, the delivery of individual growth factors, such as vascular endothelial growth factor...
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 mesenchymal stem cells (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 used 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 cryostability and lyostability compared with 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 with living MSCs.

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>MI</td>
<td>myocardial infarction</td>
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<tr>
<td>MSC</td>
<td>mesenchymal stem cells</td>
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<tr>
<td>NRCM</td>
<td>neonatal rat cardiomyocytes</td>
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<tr>
<td>PLGA</td>
<td>poly(lactic-co-glycolic acid)</td>
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<tr>
<td>synMSC</td>
<td>synthetic mesenchymal stem cells</td>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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factor and insulin-like growth factor-1, has also been tested for their cardiac therapeutic effects in clinical trials. Unfortunately, neither has met our expectations. The reasons may be the short half-life of protein factors in vivo, the uncertainty of effective/safe dosages, and the possibility that multiple administration may be necessary to act synergistically to achieve therapeutic effect. It is noteworthy that exosomes could circumvent many of these challenges. The bilipid 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. In addition, exosomes contain proteins and RNAs that may have adequate potential for cardiac repair. However, exosome-based therapeutics also face challenges such as the lack of a standard isolation protocol, rapid clearance, and wash-away because of 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 because of its capability of protecting cytokines from degradation while allowing for the sustained release of factors that target in specific organs or cells. Furthermore, Fang et al. reported cancer cell membrane–coated nanoparticles formed by coating cancer cell membranes onto PLGA-loaded immunologic particles. The membrane-bound tumor-associated antigens permit cancer cell membrane–coated nanoparticles to be efficiently delivered to antigen-presenting cells to promote anti-cancer immune response.

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 (MI). 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 is summarized in Figure 1A. In brief, MSC-conditioned media was incorporated in PLGA to form microparticles and then the microparticles were coated with MSC cell membrane to form synthetic MSCs (synMSC). Scanning electron microscopy and fluorescent imaging (Figure 1B) confirmed the successful MSC cell membrane coating on microparticles. synMSC had a size ≈20 μm, similar to those of microparticles and real MSC (Figure 1C). Flow cytometry analysis showed that synMSC exhibited similar expressions of CD105, CD90, CD45, CD31, and CD34 compared with MSC, whereas microparticles did not (Figure 1D). Furthermore, synMSC could sustain the release of growth factors like vascular endothelial growth factor.
Figure 1. Fabrication and characterization of synthetic mesenchymal stem cells (synMSC).

A, Schematic illustration of the fabrication process of synMSC. Microparticles (MP) were fabricated by encapsulating mesenchymal stem cells conditioned media in a poly (lactic-co-glycolic acid) (PLGA) shell. synMSCs were formed by coating the MPs 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) showing the structure of MP and synMSC. MP was labeled with Texas red succinimidyl ester (red), and synMSC shown as red particle with a green coat, as the MSC membranes were labeled with green fluorescent DiO. Scale bar, 10 μm.

C and D, Quantitative analyses on the diameter and expressions of MSC markers in the MP, synMSC, and MSC. E–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 synthetic mesenchymal stem cells (synMSC) in vitro. A, Fluorescent images of neonatal rat cardiomyocytes (NRCM) stained with α sarcomeric actin (green) and cocultured with microparticles (MP), synMSC, and MSC (red). Scale bar, 100 μm. B and C, Quantitative analyses of NRCM numbers and contractility when cocultured 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 and G, Quantitative analyses on the size and surface antigen expressions of synMSC before and after 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. Nuclei were counter-stained with DAPI. Scale bar, 100 μm. 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 and C, *P<0.05 when compared with control, (D) *P<0.05 when compared with MP, (I) *P<0.05 when compared with synMSC.
Farct wall thickness was increased (Figure 3D) and infarct size and MSC treatment on heart morphology (Figure 3C). The intrichrome staining revealed the protective effects of synMSC (Figure 3B). Left ventricle morphometry imaged by Masson reduction of infarct area (Figure 3B) showed a significant left ventricular volume (Figure 3A). synMSC injection showed a significant reduction of infarct area (Figure 3B) 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 because of its significantly higher number existed on 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 that freeze/thaw treatment did not 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). Furthermore, we tested the lyophilization stability of synMSC and found that the lyophilization process did not alter the structure, size, surface antigen expressions, or sustained vascular endothelial growth factor 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, MSCs were targeted by macrophages while synMSCs were not (Figure 2H and 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 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 (CT) was performed at 1 (baseline) and 14 (end point) days after infarction to measure the infarct area (Figure 3A). 99mTc-tetrofosmin single photon emission computed tomography/CT was performed at 2 (baseline) and 15 (end point) days 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 2 groups (Figure 3B). Left ventricle morphometry imaged by Masson 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 when compared with 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 with 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 with 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 when compared with 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 MI 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 remains 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 vascular endothelial growth factor, stromal cell–derived factor-1, and insulin-like growth factor 1, binding to cardiomyocytes in vitro. In addition, the expression of MHC class I molecules, but not of MHC class II molecules or costimulatory molecules, in MSC cell membranes allows it to escape alloreognition 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 coculturing 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 microparticles could be because of 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.21

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 MSCs (with dead MSCs caused by harsh freezing/thawing) were injected into a mouse heart, they were targeted by macrophages (initiating the phagocytosis of dead MSC) whereas synMSCs were not. This suggested the superior cryopreservation stability of synMSC over MSC.

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

Previous reports have demonstrated that MSC provide cardioprotection by paracrine actions that activate cardiac stem cells, angiogenesis, and cell proliferation. 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 that would provide sufficient oxygen and nutrients to the surrounded cardiomyocytes.

Conclusions

Taken together, we successfully fabricate synMSC and demonstrate their prominent therapeutic effects in an acute MI 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. After all, our results suggest that 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.

Acknowledgments

K. Cheng and T. Li designed the overall experiments. L. Luo, J. Tang, K. Nishi, C. Yan, P.U. Dinh, J. Cores, T. Kudo, and J. Zhang performed the experiments and analyzed the data. L. Luo, K. Cheng, and T. Li wrote the article. All authors read and approved the final article. All authors have provided the corresponding author with written permission to be named in the article.

Sources of Funding

This study was sponsored by the National Institute of Health (RO1 HL123920 and HL137093 to K.C.), a Grant-in-Aid from the Ministry of Education, Science, Sports, Culture and Technology of Japan (to T.L.), the Network-Type Joint Usage/Research Center for Radiation Disaster Medical Science of Hiroshima University, Nagasaki University, and Fukushima Medical University (to K.C. and T.L.), and a UNC General Assembly Research Opportunities Initiative award (to K.C.). The funders had no role in study design, data collection, analysis, and interpretation, or the decision to submit the work for publication.

Disclosures

None.

References

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_Circ Res._ 2017;120:1768-1775; originally published online March 15, 2017; doi: 10.1161/CIRCRESAHA.116.310374

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/120/11/1768

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Supplemental Material

Materials and Methods

Fabrication of PLGA microparticles and synMSC

Human bone marrow-derived mesenchymal stem cells (MSC) were directly obtained from Lonza. The cells were cultured per vendor’s instructions. To harvest conditioned media, the MSC were cultured in serum-free media for 3 days and after that the supernatant was collected. Conditioned media was concentrated by lyophilization and reconstitution. Briefly, conditioned media was collected and filtered through a 0.22μM filter into a sterile 15 or 50 mL conical, as appropriate. The filtered conditioned media was then stored at -80 ºC for at least 24 hours or until solid and then lyophilized by a LABCONCO FreeZone 2.5 Liter Freeze Dry System. MSC conditioned medium-loaded poly(lactic-co-glycolic acid) (PLGA) microparticles (MP) were fabricated by a water/oil/water (w/o/w) emulsion technique. Briefly, human MSC conditioned media as the internal aqueous phase with polyvinyal alcohol (PVA) (0.1% w/v) was mixed in methylene chloride (DCM) containing PLGA as the oil phase. The mixture was then sonicated on ice for 30 s using a sonicator (Misonix, XL2020, Farmingdale, NY, USA). Subsequently, the primary emulsion was immediately introduced into water with PVA (0.7% w/v) to produce a w₁/o/w₂ emulsion. The secondary emulsion was emulsified for 5 min on a high-speed homogenizer. The w₁/o/w₂ emulsion was continuously stirred overnight at room temperature to promote solvent evaporation. The solidified microparticles, were then centrifuged, washed three times with water, lyophilized and stored at -80 ºC. To prepare synMSC, DiO (Invitrogen)-labeled MSC went through three freeze/thaw cycles. After which, the disrupted MSC were sonicated for approximately 5 minutes at room temperature along with the MP. After that, the particles were washed three times in PBS by centrifugation. Successful membrane coating was confirmed using fluorescent microscopy.

Scanning electron microscopy

Scanning electron microscopy (SEM, Philips XL30 scanning microscope, Philips, The Netherlands) were used for study of microparticles and synMSC morphology. Freeze-dried microparticles were mounted on aluminum stubs with double-sided tape and coated with gold particles. The coated specimen was then scanned and photographed at an acceleration voltage of 15 kV.

Flow cytometry

To characterize the surface antigen expressions of MP, synMSC and MSC, flow cytometry was performed using a CytoFLEX Flow Cytometer (Beckman Coulter, Brea, CA) and analyzed using FCS Express software (De Novo Software, Los Angeles, CA). In brief, MP, synMSC, and MSC
were incubated with FITC, PE, or APC-conjugated antibodies against CD105 (3µl per 100 µl of sample, R&D Systems), CD90 (4µl per 100 µl of sample, BD Biosciences), CD45 (5µl per 100 µl of sample, BD Biosciences), CD34 (5µl per 100 µl of sample, BD Biosciences), and CD31 (5µl per 100 µl of sample, BD Biosciences) for 60 min. Isotype-identical antibodies from BD Company served as negative controls (3µl per 100 µl of sample).

**Growth factors release study**

Growth factor release from synMSC were determined using the following method. Approximately 1mg/ml microparticles in PBS buffer (pH 7.4) were sonicated for on ice for 2mins using a sonicator (Misonix, XL2020, Farmingdale, NY, USA) with power set at 23 W. Total protein and growth factor amount were determined. After that, microparticles were incubated in PBS at 37 ºC. Supernatant was collected at various time points and the concentrations of growth factors were determined by commercially available ELISA kits (R & D Systems, Minneapolis, MN, USA) and expressed as cumulative release % of the total amount encapsulated.

**Immunocytochemistry**

MP, synMSC, and MSC were pre-labeled with red-fluorescent Texas red succinimidyl ester (1 mg/ml [Invitrogen, Carlsbad, California]). NRCM or NRCM co-cultured with pre-labeled MP, synMSC, and MSC were plated onto fibronectin-coated chamber slides (BD Biosciences) for 3 days and subsequently fixed with 4% paraformaldehyde (PFA) before immunocytochemistry (ICC) staining. Slides were stained with the antibodies against α-SA (1:100, a7811, Sigma) or ki67 (1:100, ab15580, Abcam) and detected by FITC- or Texas Red-conjugated secondary antibodies (1:100). Nuclei were stained with 4, 6-diamidino-2-phenylin-dole (DAPI). Images were taken with an epi-fluorescent microscope (Olympus IX81).

**Myocardial infarction model**

All animal experiments were performed in accordance to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes or the NIH guidelines. Acute myocardial infarction (AMI) model was created in 9-11-weeks-old male C57BL/6 mice (CLEA Japan, Inc.) based on our previous studies. Briefly, after general anesthesia with intraperitoneal injection of 160 mg/kg pentobarbital and endotracheal intubation, mice were artificially ventilated with room air. A left thoracotomy was performed through the fourth intercostal space, and left anterior descending artery was ligated with 8-0 prolene under microscopy. Immediately after AMI induction, the heart was randomized to receive one of the following treatments: 1) Control group: closing chest cavity with no treatment; 2) synMSC group: intramyocardial injection of 1x10^5 synMSC particles in 50 μl PBS into the heart immediately after AMI; 3) MSC group: intramyocardial injection of 1x10^5 cells in 50 μl PBS into the heart immediately after AMI.
**PET-CT and SPECT-CT evaluation**

Control and synMSC treated mice were underwent PET-CT at day 1 (baseline) and day 14 (endpoint), and SPECT-CT at day 2 (baseline) and day 15 (endpoint) under anesthesia with 2.0% to 2.5% isoflurane based on our previous study. For PET-CT, mice were intravenously injected with 10 MBq of $^{18}$F-FDG (Nihon Medi-Physics Co., Kurume, Japan) via tail vein. Approximately 30 min later, the mice were scanned for 15 min. For SPECT-CT, mice were intravenously injected with 80 MBq of $^{99m}$Tc-tetrofosmin (Nihon Medi-Physics Co., Chiba, Japan) via tail vein. Approximately 5 min later, the mice were scanned for 21 min using imaging parameters as follows; 20 sec/projection, 64 projection/360° and radius of rotation 40 mm. All acquisitions were performed at Triumph LabPET4/SPECT4/CT (TriFoil, Imaging Inc., Chatsworth, CA, USA), a small animal PET/SPECT/CT scanner. Single pinhole collimator (pinhole diameter 1mm, focal-length 90mm) was attached on the SPECT detectors for SPECT imaging. For PET imaging data, 3D-Maximum-Likelihood Expectation Maximization (3D-MLEM) algorithm was applied using 30 iterations. For SPECT imaging data, 3D-MLEM algorithm was applied using 50 iterations. The acquired PET and SPECT data were analyzed using OsiriX MD (FDA cleared, Pixmeo), Dr.View (Infocom Co., Tokyo, Japan).

**Histological analyses**

At 15 days after MI, all mice were euthanized by severing the aorta under general anesthesia with intraperitoneal injection of 160 mg/kg pentobarbital. The heart was injected with 5 ml PBS to remove the blood cells and then embedded in OCT compound for histological analysis. The heart tissue was sectioned at 7 μm thickness from the base to the apex level with 100 μm intervals after the internal radionuclide decayed. Masson’s trichrome staining was performed according to the manufacturer’s protocol (Sigma-Aldrich) and then mounted and imaged by Keyence BZ-9000 fluorescence microscope. Morphometric analyses were performed using the NIH ImageJ software. Infarct size measurement was obtained at the base, mid-papillary and apical regions of the infarcted heart by mid-length measurement. Left ventricle infarct wall thickness measurement was obtained from the infarcted heart.

**Immunohistochemistry**

Heart cryosections were fixed with 4% paraformaldehyde, permeabilized and blocked with protein block serum-free solution (Dako), and then incubated with the following antibodies at room temperature: goat polyclonal anti-mouse c-kit antibody (10 μg/ml, R&D Systems), rat anti-mouse Ki-67 monoclonal antibody (1:400, Dako) and anti-mouse CD34 FITC (5 μg/ml, eBioscience). Donkey anti-Goat IgG(H+L) secondary antibody, Alexa Flour 546 conjugate (1:400, Thermo scientific) and Donkey anti-Rat IgG(H+L) secondary antibody, Alexa Flour 488 conjugate (1:800, Thermo scientific) were used for the conjunction with these primary antibodies.
Nuclei were then stained with DAPI. Images were taken by a fluorescent microscopy (Olympus IX83, Olympus).

**Statistical analysis**

All data were presented as the mean ± SD. Comparisons between two groups were performed with unpaired two-tailed Student’s t-test. Comparisons among more than two groups were performed using one-way ANOVA followed by post-hoc Bonferroni test. Differences were considered statistically significant when \( P < 0.05 \).

**References**


Online Figure I. Characterization of mesenchymal stem cells (MSC)

(A) Representative images of the MSC morphology. (B, C, D, E, F) Flow cytometry analysis on the expressions of CD105, CD90, CD45, CD31 and CD34 on MSCs.
Online Figure II. Lyophilization stability of synthetic mesenchymal stem cells (synMSCs)

(A) Schematic of the lyophilization process on synMSC. (B) Representative white light microscopy images of synMSCs before and after lyophilization. Scale bar: 100μm. (C) Quantitative analyses on the sizes of synMSCs before and after lyophilization. (D) Flow cytometry analysis on the surface antigen expressions. (E) Quantitative analyses on the release of VEGF from synMSC before lyophilization and after lyophilization.