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A translational approach in using cell sheet fragments of autologous bone marrow-derived mesenchymal stem cells for cellular cardiomyoplasty in a porcine model

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ABSTRACT

Based on a porcine model with surgically created myocardial infarction (MI) as a pre-clinical scheme, this study investigates the clinical translation of cell sheet fragments of autologous mesenchymal stem cells (MSCs) for cellular cardiomyoplasty. MSC sheet fragments retaining endogenous extracellular matrices are fabricated using a thermo-responsive methylcellulose hydrogel system. Echocardiographic observations indicate that transplantation of MSC sheet fragments in infarcted hearts can markedly attenuate the adverse ventricular dilation and preserve the cardiac function post MI, which is in contrast to the controlled groups receiving saline or dissociated MSCs. Additionally, histological analyses suggest that administering MSC sheet fragments significantly prevents the scar expansion and left ventricle remodeling after MI. Immunohistochemistry results demonstrate that the engrafted MSCs can differentiate into endothelial cells and smooth muscle cells, implying that angiogenesis and the subsequent regional perfusion improvement is a promising mechanism for ameliorating post-infarcted cardiac function. However, according to the data recorded by an implantable loop recorder, the transplanted MSCs may provoke arrhythmia. Nevertheless, the proposed approach may potentially lead to the eventual translation of MSC-based therapy into practical and effective clinical treatments.

1. Introduction

Myocardial infarction (MI) incurs a loss of cardiac tissue and damages heart function [1]. Various cell types that are transplanted into the infarcted heart restore the cardiac function [2–5]. Of the cell types studied, mesenchymal stem cells (MSCs) are regarded as the most practical cell source for clinical applications, owing to their ready isolation from the bone marrows of patients [6,7]. MSCs are applicable for cellular cardiomyoplasty, either through their direct contribution to the formation of vasculatures or via the indirect paracrine secretion of proangiogenic and cardio-protective factors [8–10].

Prior to cell transplantation, the desired cell type must be largely expanded *in vitro* and then dissociated for suspension in saline by using proteolytic enzymes [11]. However, using proteolytic enzymes often disrupts the cell–cell and cell–extracellular matrix (ECM) interactions that profoundly impact cellular functions such as survival, adherence, proliferation and differentiation [12–14]. Additionally, given their inadequate physical size and lack of ECM adhesion molecules, a significant portion of the transplanted cells may leak out from the site of injection upon implantation [15,16]. Consequently, conventional procedures for cell

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transplantation through direct injection of dissociated cells are frequently associated with a low retention rate of engrafted cells and a suboptimal consequence of cell survival and function [8,11].

In light of the above developments, our previous work developed a method using a thermo-responsive methylcellulose (MC) hydrogel system to fabricate cell sheet fragments of MSCs without applying proteolytic enzymes [17–19]. Our results demonstrated that the harvested MSC sheet fragments retained their endogenous ECM together with adhesive molecules and offered a significantly higher cell retention rate following engraftment in a rat model of homologous transplantation, demonstrating a better functional benefit for the infarcted heart than with dissociated MSCs [10,17].

Although MSC sheet fragments are highly promising for restoring the impaired cardiac function in rats, further studies in large animal models are warranted to advance their clinical translation. Swine are considered an ideal large animal model for the clinical translation of cardiovascular research, owing to their resemblance to human organ size and physiology [20,21]. By using a porcine model with surgically induced MI, this study evaluates the effectiveness of engrafting cell sheet fragments of autologous bone marrow-derived MSCs on its post-infarcted cardiac function. The stem cells isolated from autologous source have the advantage of being immune-compatible [22]. Additionally, autologous MSCs can be safely harvested from patients' bone marrows and expanded *in vitro*, making them highly promising for clinical use as a personalized therapeutic approach [6,23]. The groups treated with saline or dissociated MSCs are used as controls. These translational studies in large animal models are necessary to achieve the tremendous potential suggested by rat studies in clinical practice.

2. Materials and methods

The ideal paradigm of MSC-based cellular cardiomyoplasty involves isolating autologous cells, expanding to clinical pragmatic scale and re-introducing cells to the patient. This animal study was designed to replicate clinically relevant treatments. Each studied pig was subjected to surgical induction of MI and extraction of bone marrow simultaneously. The purified bone marrow MSCs were then amplified to fabricate cell sheet fragments for the subsequent intramyocardial transplantation autologously (Fig. 1).

2.1. Induction of MI

Animals were cared for and used in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, published by the National Academy Press in 1996, and approved by the IRB of the Veterans General Hospital, Taichung, Taiwan. Lanyu mini-pigs (approximately 5 months old with a body weight of 25 kg) acquired from the National Taitung Animal Propagation Station (Taitung, Taiwan) were used in the study. To induce MI, pigs were first anesthetized with Zoletil 50 (8 mg/kg, Virbac, Carros, France), followed by intubation. A respirator for periodic positive pressure ventilation with 2% isoflurane was attached. Next, left thoracotomy was performed, and the mid-left anterior descending coronary artery was ligated permanently.

2.2. Isolation, purification and characterization of bone marrow MSCs

Immediately after inducing MI, bone marrow of the infarcted pig was isolated from its iliac crest. Mononuclear cells were collected from the isolated bone marrow by density gradient centrifugation (Ficoll–Paque Premium, Amersham, Uppsala, Sweden) and plated at a concentration of 10^6 cells/cm^2 in MEM Alpha Medium (Invitrogen, Carlsbad, CA, USA) containing 20% fetal bovine serum (FBS, HyClone, Logan, UT, USA) and 1% penicillin-streptomycin (Invitrogen), and maintained at 37 °C in 5% humidified CO_2. Nonadherent cells were removed during medium changes; the remaining purified MSC population was expanded in the culture.

The specific cell surface antigens of the obtained MSCs in culture of passage 4 to 8 were characterized by flow cytometric analyzes. Cells were trypsinized and immunolabeled with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibodies against CD29, CD44, CD45, CD105 (Novus Biologicals, Littleton,

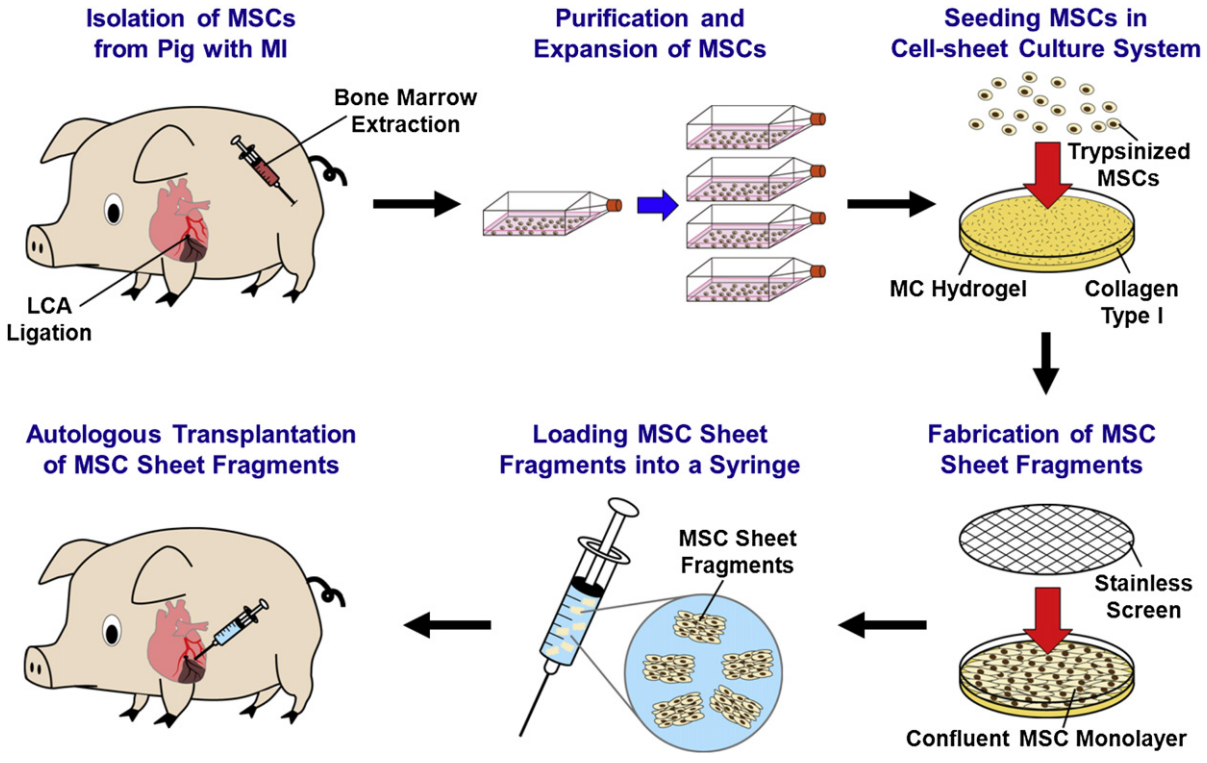

Fig. 1. Schematic illustrations of the procedures used for the fabrication of cell sheet fragments of bone marrow-derived mesenchymal stem cells (MSCs) for autologous cellular cardiomyoplasty. Each pig was subjected to surgically induced myocardial infarction (MI), and its bone marrow was extracted simultaneously. The MSCs were then isolated, amplified and seeded on the surface of methylcellulose (MC) hydrogel system. After reaching confluence, a sterilized stainless screen was employed to compress and fragment the grown MSC monolayer at room temperature, resulting in fragmented MSC sheets. Finally, the obtained MSC sheet fragments were collected for autologous intramyocardial transplantation. LCA: left coronary artery.

CO, USA), CD34 (R&D Systems, Minneapolis, MN, USA) or CD90 (BD Pharmingen, San Jose, CA, USA). The cells were then analyzed by using a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA, USA). Mouse IgG-FITC or IgG-PE (BD Pharmingen) was used as an isotype control.

The multilineage capacity of MSCs was examined by preparing cells for differentiating into osteocytes, chondrocytes or adipocytes. For osteogenesis and adipogenesis, MSCs were seeded into 6-well plates and grown to confluence. Next, differentiation was induced by incubating the cells for 3 weeks in MEM Alpha Medium supplemented with 10% FBS, 0.1 μM dexamethasone, 10 mM β-glycerolphosphate and 50 μM ascorbic acid (Sigma–Aldrich, St. Louis, MO, USA) for osteogenesis or MEM Alpha Medium containing 10% FBS, 1 μM dexamethasone, 0.5 mM methyl-isobutylxanthine, 100 μM indomethacin and 10 μg/mL insulin for adipogenesis. For chondrogenesis, pellets were prepared by centrifugation of 10^6 cells and grown in MEM Alpha Medium with 10 ng/mL transforming growth factor-β1 (PeproTech, Rocky Hill, NJ, USA). Finally, the osteogenic, adipogenic and chondrogenic potentials of MSCs were evaluated by Alizarin Red S staining, Oil Red O staining and Alcian blue staining (Sigma–Aldrich), respectively.

2.3. Construction and characterization of MSC sheet fragments

The MSCs, labeled by 5-bromo-2′-deoxyuridine (BrdU, Sigma–Aldrich) for later identification, were used for the fabrication of cell sheet fragments in a thermo-responsive MC hydrogel system reported previously by our group [10,17,18]. Their mesenchymal marker and endogenous ECM molecules were then examined by fixing the fabricated cell sheet fragments in 4% phosphate-buffered paraformaldehyde, staining them with Alexa Fluor 488 phalloidin (Invitrogen) or a monoclonal antibody against vimentin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), collagen type III (Sigma–Aldrich), fibronectin, laminin or vitronectin (Abcam, Cambridge, MA, USA) and, finally, incubating them with an appropriate Alexa Fluor secondary antibody (Invitrogen). The stained cell sheet fragments were further counterstained to visualize their nuclei using Sytox blue (Invitrogen), followed by observations under an inverted confocal laser scanning microscope (CLSM, TCS SL, Leica, Wetzlar, Germany).

2.4. Transplantation of MSC sheet fragments

Four weeks after induction of MI, surviving animals were divided randomly into 3 groups for treatment. The heart was first exposed by left thoracotomy, as described earlier. A direct intramyocardial injection of saline, dissociated MSCs (1×10^7 cells) or MSC sheet fragments (1×10^7 cells in total) into the peri-infarct zones was then performed with a 27-gauge needle. Following treatment, the pigs recovered under close observation.

2.5. Detection of arrhythmia

As is well known, transplantation of cells into the heart may be arrhythmogenic [24,25]. In this study, the risk of arrhythmia was evaluated by using an implantable loop recorder (Reveal Plus 9526, Medtronic, Minneapolis, MN, USA) for electrocardiogram monitoring. An implantable loop recorder was implanted subcutaneously into the left pectoral region during coronary ligation. Data was stored automatically when arrhythmic events corresponded to the following preprogrammed criteria: sinus arrest as pauses ≥3 s, ventricular tachycardia as a heart rate ≥300 bpm and lasting <30 s, and sustained ventricular tachycardia as lasting ≥30 s [26,27].

2.6. Echocardiography

Cardiac functions were evaluated by echocardiography before MI, at 4 weeks after MI induction and at 4 weeks post cell transplantation, using a SONOS 2500 system equipped with a 2.5-MHz broadband sector transducer (Hewlett–Packard, Palo Alto, CA, USA). Animals ($n = 5$ surviving pigs per group) were anesthetized

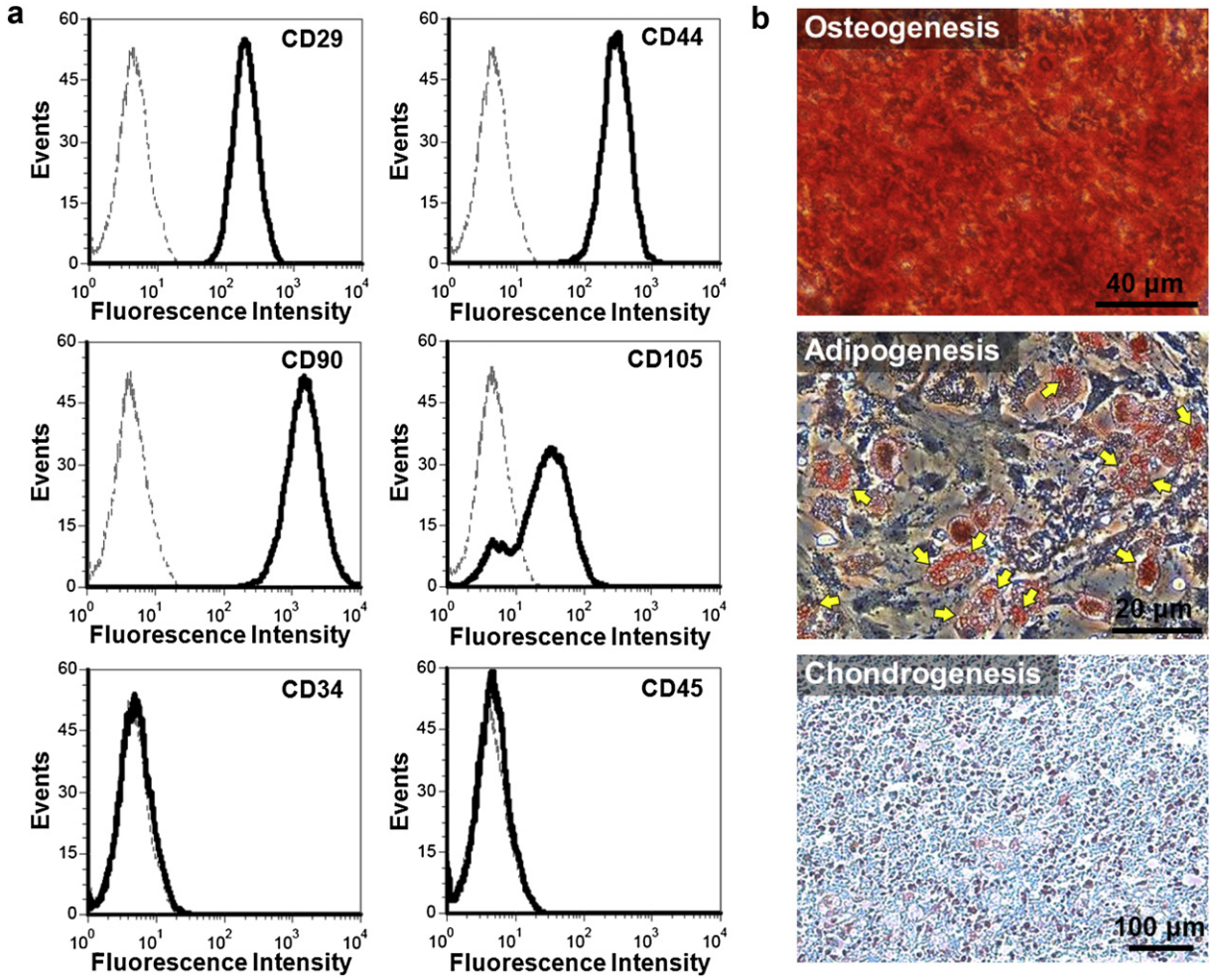

Fig. 2. Multilineage potential of the isolated MSCs. (a) Immunophenotypic characteristics obtained from flow cytometric analyzes indicate that the isolated MSCs expressed mesenchymal markers such as CD29, CD44, CD90 and CD105 but negative for hematopoietic lineage markers CD34 and CD45; (b) The differentiation capability of MSCs was confirmed by Alizarin Red S staining for osteogenesis, Oil Red O staining for adipogenesis and Alcian blue staining for chondrogenesis.

using 2% isoflurane with oxygen. The left ventricular end diastolic dimension (LVEDD) and end systolic dimension (LVESD) were then determined from M-mode tracings at the midpapillary level. Next, the LV ejection fraction (LVEF) was calculated automatically by the echocardiography system as LVEF (%) = [(LVEDV−LVESV)/LVEDV] × 100%, where LVEDV denotes the LV end diastolic volume approximated as $7.0 \times LVEDD^3/(2.4 + LVEDD)$, and LVESV represents the LV end systolic volume estimated by $7.0 \times LVESD^3/(2.4 + LVESD)$ [28].

2.7. Morphological and histological analyzes

Four weeks after cell transplantation, animals were sacrificed, with their hearts subsequently retrieved, cut into slices and grossly examined. Images were then collected, and the morphometric parameters (i.e. the area of LV cavity, total LV area, scar area and thickness of LV wall in the infarct and non-infarcted zones) were quantified using the US National Institutes of Health ImageJ software [3,29]. Next, the degrees of LV dilation and infarct-wall thinning were evaluated by determining the LV expansion index as (area of LV cavity/total LV area) × (thickness of wall in non-infarcted region/thickness of wall in risk region) [30]. Specimens were then fixed in 10% phosphate-buffered formalin for paraffin embedding and processed for immunohistochemistry staining.

Additionally, the capillary and arteriole densities in the infarct and border zones were quantified by staining myocardial sections for either an endothelial marker (von Willebrand factor, vWF, Dako, Denmark) or a smooth muscle marker (smooth muscle actin, SMA, Abcam) using 3,3′-diaminobenzidine as a chromogen. The images were taken from five randomly selected areas, and the vascular densities were counted manually within each section.

Moreover, the fate of transplanted cells in the myocardium was investigated by incubating sections with the FITC-conjugated anti-BrdU antibody (Abcam) and then double-staining with antibody against vWF or SMA. Furthermore, the fluorescent colors were obtained by using appropriate Alexa Fluor-conjugated secondary antibodies (Invitrogen). Finally, the stained sections were counterstained to visualize the nuclei by propidium iodide (Sigma–Aldrich) and examined by CLSM.

2.8. Statistical analysis

All results are presented as means ± SD. The two groups were compared using the one-tailed Student's *t*-test (SPSS, Chicago, IL, USA). In the multiple group comparison, significance was determined using one-way analysis of variation (ANOVA), followed by the Bonferroni *post hoc* test. Differences were considered statistically significant when *P* values were under 0.05.

3. Results and discussion

Despite the promising modality of cell transplantation via direct intramyocardial injection for therapeutic treatment of MI, its therapeutic effect is limited in terms of retention and survival of the transplanted cells [8,11]. Therefore, our earlier study developed a strategy in which infarcted rat hearts were treated using cell sheet fragments of homologous MSCs [10,17]. According to those results, the heart function improved significantly after cell transplantation. However, comparing the hearts of rats with those of humans reveals substantial differences with respect to cardiac characteristics such as anatomy, physiology and function [21]. Therefore, large animal models recapitulating the clinical MI phenotype are necessary for translating the discoveries from rat models into clinical applications [20,21]. This study investigates the clinical translation of autologous MSC sheet fragments for cardiac repair by using a porcine model with surgically created MI as a pre-clinical approach.

3.1. Characteristics of cells isolated from porcine bone marrows

Surface antigens expressed by the isolated cells were analyzed by flow cytometry. According to Fig. 2a, the isolated cells were positive for mesenchymal markers CD29, CD44, CD90 and CD105, yet negative for hematopoietic markers CD34 and CD45. This finding demonstrates that the isolated cells exhibited the same immunophenotype characteristics as those of the porcine bone marrow-derived MSCs reported in the literature [31,32].

As is well known, MSCs are multipotent and capable of differentiating into osteogenic, adipogenic and chondrogenic lineages [31,33]. Here, their multilineage potential was evaluated by preparing the isolated MSCs for osteogenic, adipogenic and

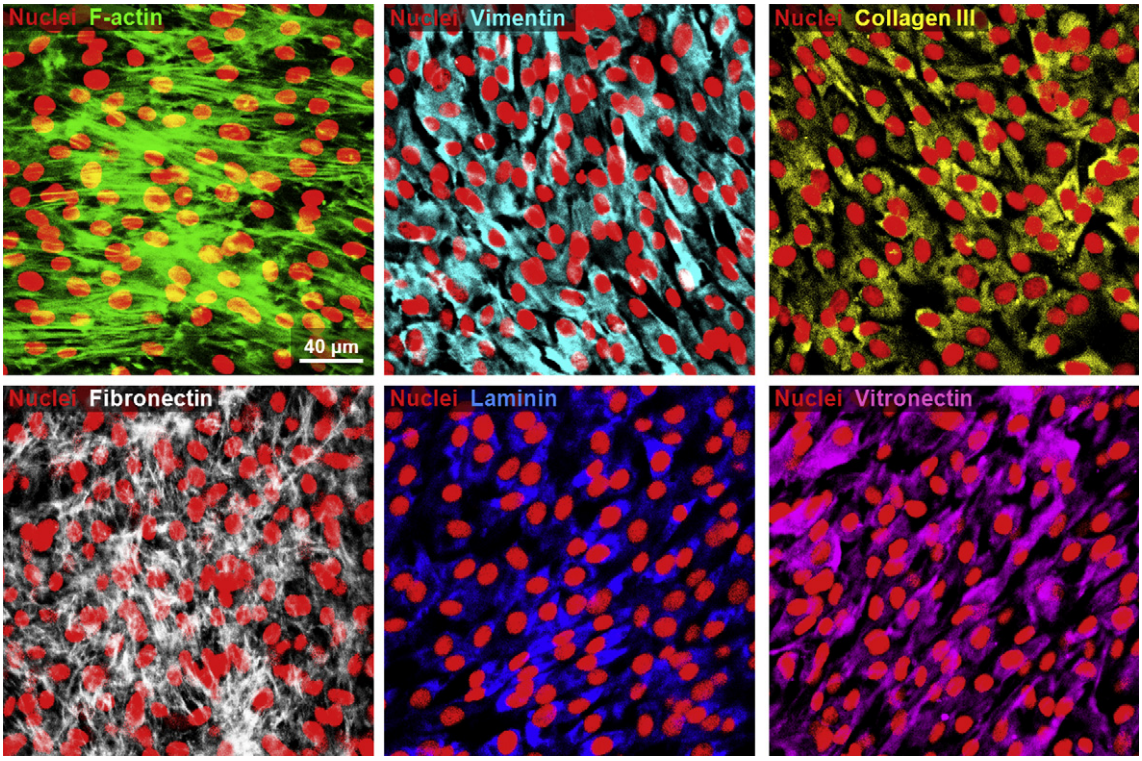

Fig. 3. Immunofluorescent staining for mesenchymal marker and endogenous extracellular matrix (ECM) molecules expressed in the obtained MSC sheet fragments. The fabricated MSC sheet fragments consistently expressed vimentin, suggesting their mesenchymal phenotypes. Additionally, ECM compositions such as collagen type III, fibronectin, laminin and vitronectin that can regulate numerous cellular functions were well-preserved in the harvested cell sheet fragments.

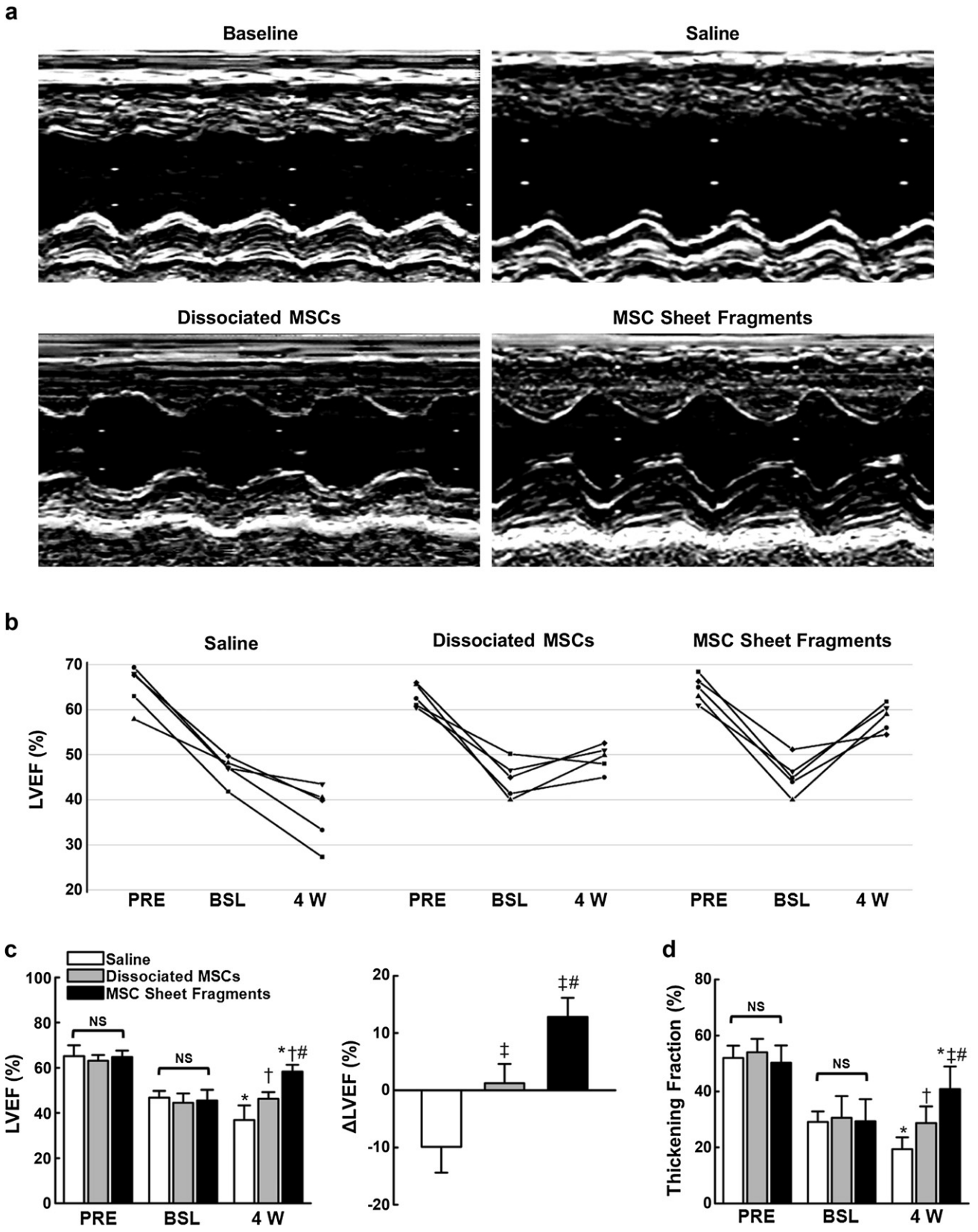

Fig. 4. Echocardiographic evaluation of heart function. (a) Representative traces of left ventricle (LV) short-axis M-mode echocardiograms obtained at baseline (at 4 weeks after MI induction) and at 4 weeks after cell transplantation; (b) LV ejection fraction (LVEF) for each studied pig before induction of MI (PRE), at 4 weeks post-infarcted baseline (BSL) and 4 weeks after treatment (4 W); (c) LVEF and changes of LVEF (ΔLVEF) after cell transplantation, and (d) anterior wall thickening fraction measured by echocardiography. NS: not significant; $^{*}P < 0.05$ vs. corresponding-treatment group at baseline; †$P < 0.05$ vs. saline group; #$P < 0.05$ vs. dissociated MSCs group; ‡$P < 0.001$ vs. saline group.

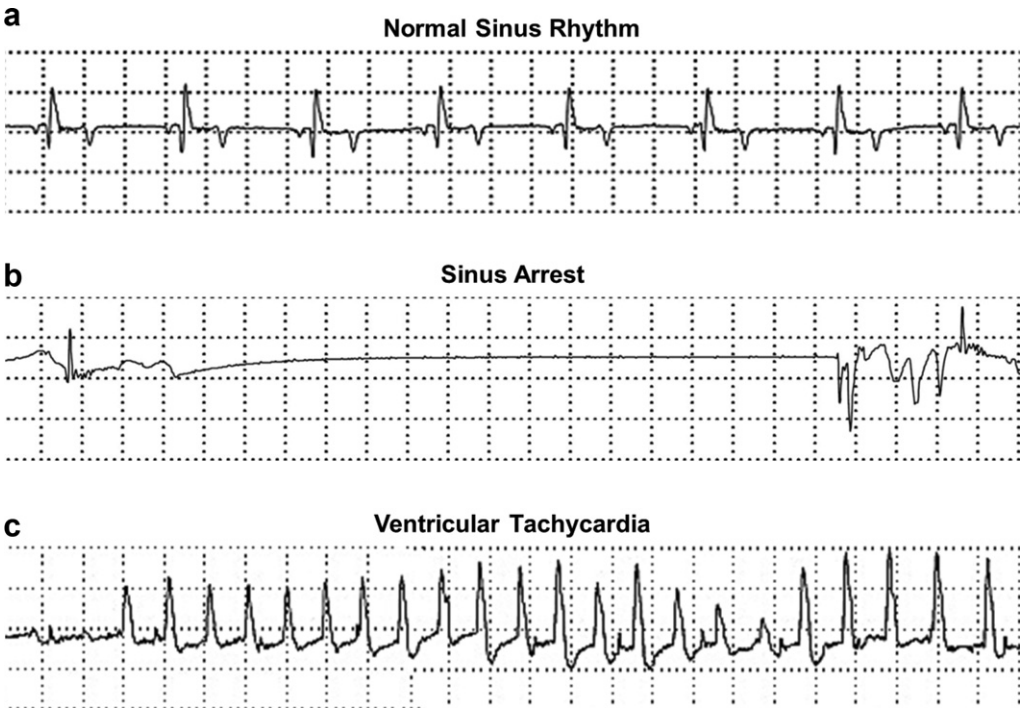

Fig. 5. Representative electrocardiograms obtained by an implantable loop recorder: (a) normal sinus rhythm, (b) sinus arrest, and (c) ventricular tachycardia before/after transplantation of MSC sheet fragments.

chondrogenic differentiations. Under controlled *in vitro* environments, the cells described here can differentiate into osteocytes, adipocytes and chondrocytes consistently, as confirmed by Alizarin Red S staining for mineral deposition, Oil Red O staining for lipid droplets (as indicated by the yellow arrows) and Alcian blue staining for sulfated proteoglycan, respectively (Fig. 2b). The success in differentiation of the isolated MSCs suggests their multipotency. Above results correspond to those of other studies [31,33].

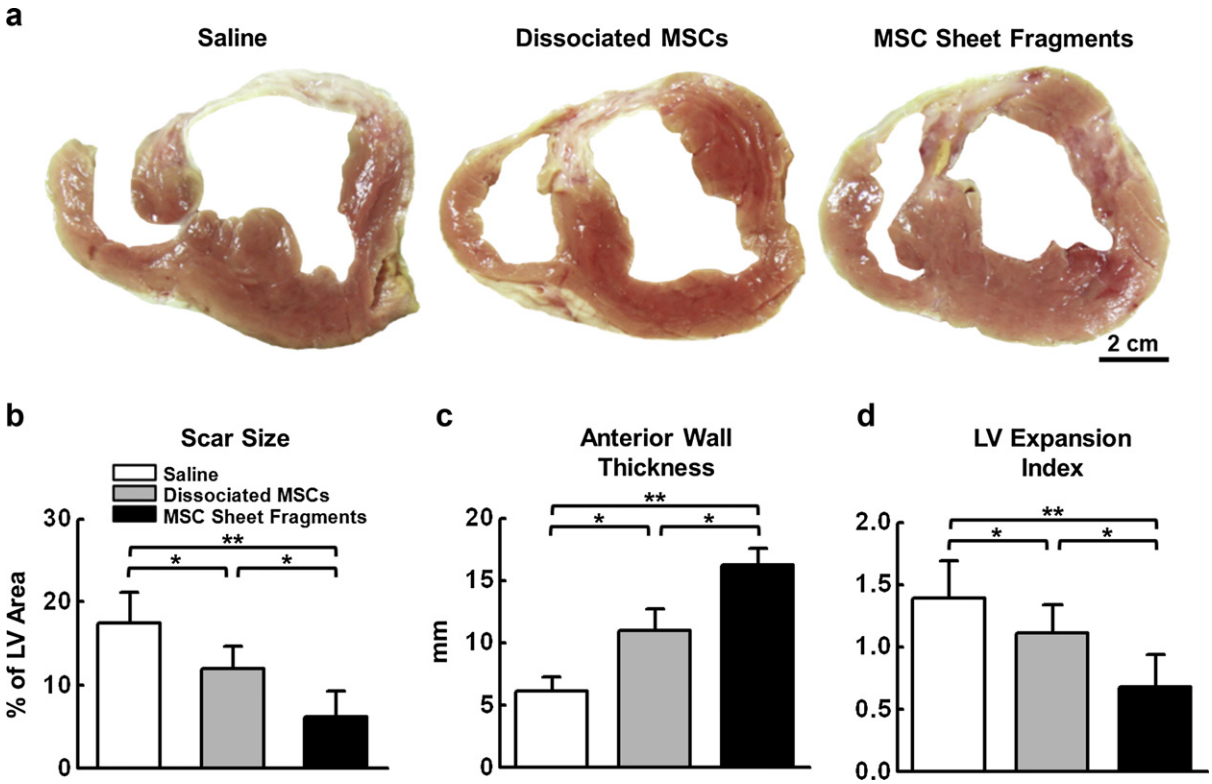

Fig. 6. Morphometric assessment of the retrieved myocardium. (a) Representative histological cross-sections that depicting the infarct zones of the hearts for all studied groups; (b) Percentages of scar size, (c) anterior wall thickness and (d) LV expansion index were quantified using a computer software. NS: not significant; $^{*}P < 0.05$; $^{**}P < 0.001$.

3.2. Characteristics of MSC sheet fragments

A thermo-responsive MC hydrogel system coated on culture dishes was used for harvesting living cell sheet fragments [19]. The hydrogel system was prepared by simply pouring an aqueous MC solution blended with phosphate-buffered saline, which had a lower critical solution temperature of 32 °C [19], on dishes at room temperature. The solution was then left in an incubator at 37 °C to form hydrogel. Cell attachment was improved by further coating a thin layer of collagen on top of the MC hydrogel system. Upon confluence, the grown cell sheet was taken out from the incubator with media present. The MSC sheet grown on the hydrogel system at room temperature was subsequently compressed and fragmented using a sterilized stainless screen. Immediately after compressing the stainless screen, fragmented cell sheets (which were detached from the surface of the culture system, due to sol–gel transition) were obtained and then collected via centrifugation (Fig. 1). The obtained MSC sheet fragments were in a square shape with a width corresponding to the size of the mesh opening (380 μm) and had 120 cells per cell sheet fragment [18]. Finally, the obtained MSC sheet fragments were suspended in saline and loaded in a syringe for subsequent intramuscular injection.

The fabricated MSC sheet fragments were fixed and immunolabeled with antibodies against mesenchymal marker and ECM molecules. According to Fig. 3, MSCs grown on cell sheet fragments were positive for vimentin, implying their mesenchymal origin [34]. Notably, ECM molecules such as collagen type III, fibronectin, laminin and vitronectin that were produced by MSCs during culture remained intact since no proteolytic enzymes were used in this study when harvesting cell sheet fragments. In addition to facilitating the adhesion and survival of the engrafted cells, the retained endogenous ECM can also provide the essential environmental cues to control cellular functions such as migration, proliferation and differentiation [12–14].

3.3. Echocardiography

The therapeutic efficacy of MSC sheet fragments transplanted intramuscularly in a porcine MI model was determined by echocardiography before MI, at 4 weeks post MI (as the baseline) and at

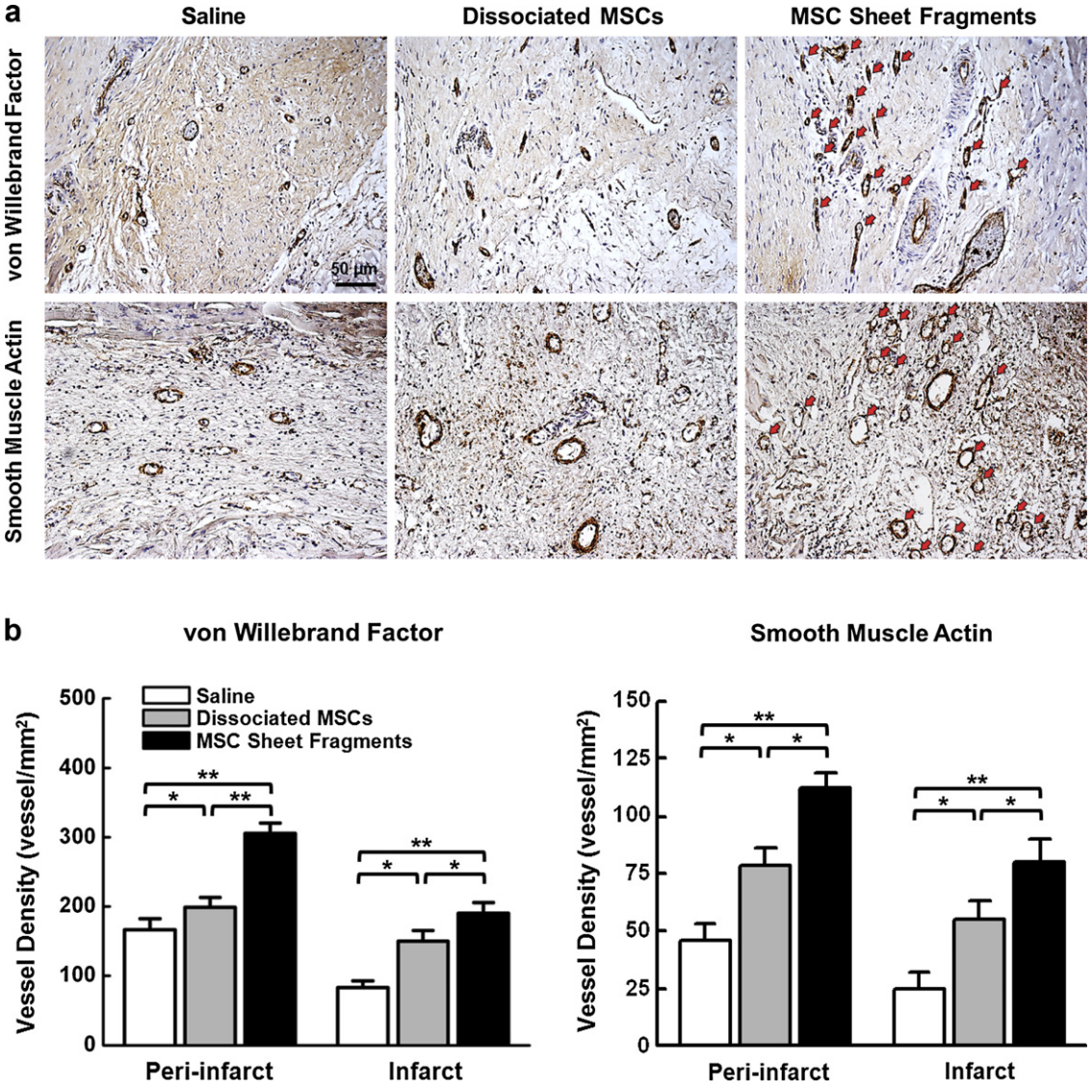

Fig. 7. Analysis of vascular densities. (a) Representative immunohistological images of the capillaries and arterioles observed in the peri-infarct areas. (b) Vessel densities were determined in infarct and peri-infarct zones by vWF or SMA staining and normalized to area. NS: not significant; $^{*}P < 0.05$; $^{**}P < 0.001$.

4 weeks after cell transplantation. According to the M-mode echocardiograms in Fig. 4a, the dilation of LV was more significantly attenuated in the heart that received MSC sheet fragments than those treated with saline or dissociated MSCs (control groups). Moreover, the hearts receiving MSC sheet fragments displayed better contractility than their control counterparts (Supplementary Video).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.03.003.

All studied groups had a similar LVEF at baseline (at 4 weeks after MI, $P > 0.05$, Fig. 4b and c), implying the development of similar post MI dysfunction. During the following 4 weeks, the LVEF progressively decreased in the saline group ($P < 0.05$), remained nearly constant in the group receiving dissociated MSCs ($P > 0.05$), and significantly increased for the heart treated with MSC sheet fragments ($P < 0.05$, Fig. 4c). Additionally, changes in LVEF (ΔLVEF) were calculated to compare the treatment effects. Functional benefit in response to the MSC-sheet-fragment injection was validated by the substantial positive outcome (12.8 ± 3.3% augmentation in LVEF, Fig. 4c), in contrast to a negative response in the saline-treated heart (9.9 ± 4.5% reduction in LVEF, $P < 0.001$). Comparing with the group treated with dissociated cells at 4 weeks postoperatively, the thickening fraction of the anterior wall was significantly enhanced in the heart receiving MSC sheet fragments (Fig. 4d, $P < 0.05$). This finding further demonstrates that cell sheet fragments have an efficacy advantage over dissociated MSCs. Above results suggest that transplantation of MSC sheet fragments in infarcted hearts can markedly attenuate the adverse ventricular dilation, thus preserving the cardiac function post MI. The echocardiography data demonstrate that the proposed approach is highly promising for use as a translational applicable strategy for autologous stem cell-based cardiac repair.

3.4. Electrocardiogram

This study also assesses the risk of arrhythmogenesis of cell transplantation by using an implantable loop recorder to monitor the electrocardiograms of the pigs receiving MSC-sheet-fragment treatment. Before ligation of the coronary artery, no arrhythmic event was observed in all studied pigs (Fig. 5a). Following the induction of MI, one pig died within 3 days due to sinus arrest (Fig. 5b). During the following 4 weeks, ventricular tachycardia occurred in two of the five pigs that survived from MI (Fig. 5c). At 4 weeks of post treatment with MSC sheet fragments, the incidence of ventricular tachycardia increased, suggesting that the transplanted MSCs may provoke arrhythmia. According to a previous study, arrhythmia remains a major concern of cellular cardiomyoplasty, despite its encouraging therapeutic effect for cardiac repair [24,25]. This is owing to the inability of the transplanted cells to couple with the host cardiomyocytes via functional gap junctions and, thus, their inability to propagate electrical impulses competently [11]. Since direct administration of cells into the infarcted myocardium can lead to the development of ventricular arrhythmias, further studies are necessary to reduce the susceptibility to life-threatening rhythm abnormalities before the reach of clinical applications.

3.5. Histological analyzes

Four weeks after cell transplantation, animals were sacrificed and processed for histological assessment. Gross pathological examination revealed that for the heart receiving saline treatment, severe transmural infarction occurred with significantly anterior wall thinning and LV chamber enlargement (Fig. 6a). Conversely, in addition to substantially reducing the ratio of LV occupied by fibrotic scar tissues, transplantation of dissociated MSCs or MSC sheet fragments prevented the global cardiac morphology from post-infarcted dilation. Notably, the LV wall appeared thicker in the heart receiving MSC sheet fragments than its counterpart treated with dissociated MSCs. This finding demonstrates that the former was superior to the latter in terms of prohibiting ventricular remodeling.

Quantitative morphometric analysis results also confirmed the protective effect of MSC-sheet-fragment transplantation. That is, the infarcted heart had more viable myocardium (Fig. 6b) and a thicker infarcted wall (Fig. 6c), but smaller scars and less LV expansion (i.e. lower LV expansion index, Fig. 6d) than those of the control groups. While consistent with our echocardiographic

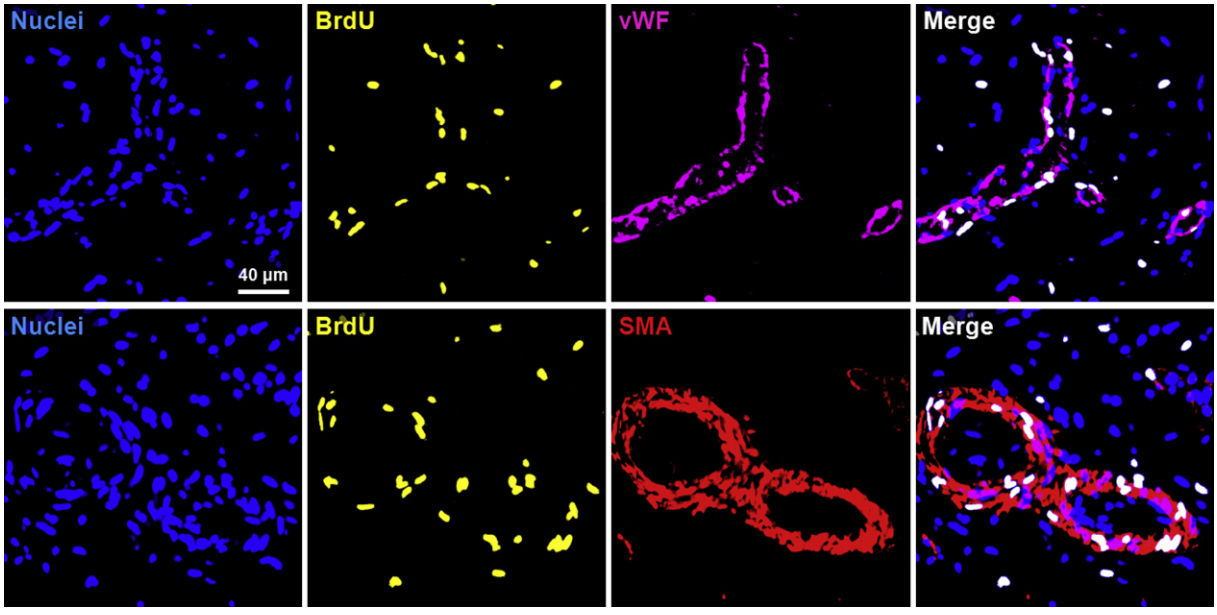

Fig. 8. Expression of vascular structural proteins in engrafted MSCs. Representative confocal microscopic images indicating colocalization of BrdU with vWF or SMA in the peri-infarct zones, suggesting the differentiation of MSCs into endothelial or smooth muscle cells, respectively.

observations (Fig. 4a and Supplementary Video), these findings suggest that administering MSC sheet fragments significantly prevented scar expansion and LV remodeling after MI.

According to previous studies, MSCs can advantage post-infarcted cardiac function by improving the regional perfusion through paracrine-induced angiogenesis [8,9]. Therefore, this study quantified the densities of capillaries and arterioles in infarct and border zones by using vWF and SMA staining (as indicated by the red arrows in Fig. 7a). Immunohistochemistry results indicate that a significantly higher number of capillaries and arterioles per field in the heart received MSC sheet fragments than those treated with saline or dissociated MSCs (Fig. 7b, $P < 0.05$). This finding implies that transplantation of MSC sheet fragments could lead to neovascularization and concomitant cardiac functional benefits.

This study also attempted to confirm the fate of transplanted MSC sheet fragments by using immunofluorescence staining with the antibody against BrdU to trace the implanted cells. According to Fig. 8, the engrafted MSCs could differentiate into endothelial cells and smooth muscle cells, as indicated by the vWF and SMA staining, respectively. Previous investigations have confirmed that MSCs can lead to neovascularization in infarcted zones by providing angiogenic growth factors or differentiating into endothelial and smooth muscle cells that form vessels in mouse, rat, and swine models [8–10]. Therefore, angiogenesis and the resulting improvement in regional perfusion may be the potential mechanism to ameliorate post-infarcted cardiac function.

4. Conclusions

This study demonstrates that autologous transplantation of MSC sheet fragments can benefit post-infarcted cardiac function via transdifferentiation of MSCs into vascular lineages and improvement of regional blood perfusion. As swine have a similar anatomy, physiology and function as those of humans, results of this study have significant implications for the clinical translation of MSC-based therapy. Furthermore, results of this study provide a valuable reference for efforts to design more relevant experiments that will lead to the translation of the proposed approach into preliminary and eventual clinical applications.

Acknowledgments

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