Layered smooth muscle cell–endothelial progenitor cell sheets derived from the bone marrow augment postinfarction ventricular function

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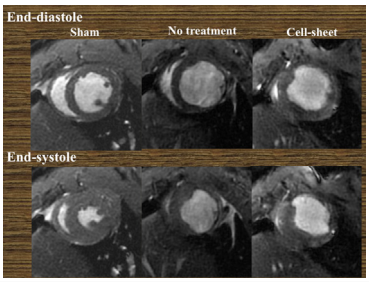
ABSTRACT

Objective: The angiogenic potential of endothelial progenitor cells (EPCs) may be limited by the absence of their natural biologic foundation, namely smooth muscle pericytes. We hypothesized that joint delivery of EPCs and smooth muscle cells (SMCs) in a novel, totally bone marrow–derived cell sheet will mimic the native architecture of a mature blood vessel and act as an angiogenic construct to limit post infarction ventricular remodeling.

Methods: Primary EPCs and mesenchymal stem cells were isolated from bone marrow of Wistar rats. Mesenchymal stem cells were transdifferentiated into SMCs by culture on fibronectin-coated culture dishes. Confluent SMCs topped with confluent EPCs were detached from an Upcell dish to create a SMC-EPC bi-level cell sheet. A rodent model of ischemic cardiomyopathy was then created by ligating the left anterior descending artery. Rats were randomized into 3 groups: cell sheet transplantation (n = 9), no treatment (n = 12), or sham surgery control (n = 7).

Results: Four weeks postinfarction, mature vessel density tended to increase in cell sheet-treated animals compared with controls. Cell sheet therapy significantly attenuated the extent of cardiac fibrosis compared with that of the untreated group (untreated vs cell sheet, 198 degrees [interquartile range (IQR), 151-246 degrees] vs 103 degrees [IQR, 92-113 degrees], $P = .04$). Furthermore, EPC-SMC cell sheet transplantation attenuated myocardial dysfunction, as evidenced by an increase in left ventricular ejection fraction (untreated vs cell sheet vs sham, 33.5% [IQR, 27.8%-35.7%] vs 45.9% [IQR, 43.6%-48.4%] vs 59.3% [IQR, 58.8%-63.5%], $P = .001$) and decreases in left ventricular dimensions.

Conclusions: The bone marrow-derived, spatially arranged SMC-EPC bi-level cell sheet is a novel, multilineage cellular therapy obtained from a translationally practical source. Interactions between SMCs and EPCs augment mature neovascularization, limit adverse remodeling, and improve ventricular function after myocardial infarction. (J Thorac Cardiovasc Surg 2017;■:1-9)



Endothelial progenitor cell–smooth muscle cell cell sheets enhance postinfarction ventricular function.

Central Message

An endothelial progenitor cell–smooth muscle cell construct—derived entirely from bone marrow cells—augments neovascularization and attenuates postinfarction myocardial remodeling.

Perspective

Endogenous effectors of myocardial repair are likely not limited to a single cell type. Tissue engineered cell sheets offer the potential to exploit natural interactions between various cell types. Here, we demonstrate that co-administration of endothelial progenitor cells and smooth muscle cells as an intact construct significantly improves endogenous mechanisms of myocardial repair, and these cells are obtainable from a translationally relevant source.

See Editorial Commentary page XXX.

Despite medical and surgical advances, heart disease is the leading cause of death in the United States. Better characterization of stem cell lineages and endogenous effectors of myocardial repair have burgeoned interest in the use of cell therapies to improve left ventricular (LV) function in patients with advanced heart disease.

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Abbreviations and Acronyms

DMEM = Dulbecco's Modified Eagle's Medium
ECM = extracellular matrix
EPC = endothelial progenitor cells
FBS = fetal bovine serum
LAD = left anterior descending coronary artery
LV = left ventricular
MSC = mesenchymal stem cells
MRI = magnetic resonance imaging
SMA = smooth muscle actin
SMC = smooth muscle cells

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Tissue engineering is an essential component of developing effective regenerative therapies.[5] In the past decade, tissue-engineered cell and scaffold therapies have been investigated widely, and several products are now commercially available. Scaffold-based tissue engineering is particularly popular and includes technologies such as biodegradable scaffolds,[6] decellularized tissues,[7] hydrogel and cell mixtures,[8] bioprinting,[9] and fiber-based tissue engineering.[10] Our group has also used scaffold-free technology for cell-sheet engineering.[11] The cell sheet is created on and removed from a specialized dish that is grafted covalently with a temperature-responsive polymer—poly (*N*-isopropylacrylamide)—which undergoes an enzyme-free transformation from hydrophobic to hydrophilic by simply lowering the temperature.[12] Thus, the specialized dishes permit fabrication of 3-dimensional tissues from densely adherent cells, without an artificial scaffold or enzymatic digestion. Cell sheets are manipulated easily and have a unique ability to integrate within native tissue; they retain cell–cell junctions as well as the extracellular matrix (ECM) deposited on the basal surface of cell sheet.[4]

We previously demonstrated that engineered cell sheets with smooth muscle cells (SMCs) and endothelial progenitor cells (EPCs) harnessed natural interactions between EPCs and SMCs, created structurally mature, functional microvasculature, and induced functional recovery of distressed myocardium.[1] However, cell-sheet SMCs were obtained from the thoracic aorta and therefore precluded clinical translation.[1] To resolve this problem, we have noted that bone marrow–derived mesenchymal stem cells (MSCs) have shown potential to differentiate into various cell types, including SMCs.[2] Given that the ECM is a powerful regulator of SMC phenotypic modulation,[3] we demonstrated that fibronectin helped guide differentiation of MSCs into SMCs while simultaneously preserving cellular proliferative capacity.[4]

In this study, we hypothesized that joint delivery of EPCs and SMCs in a novel, totally bone marrow–derived cell sheet will mimic the native architecture of a mature blood vessel and act as a supratherapeutic angiogenic construct to limit post infarction ventricular remodeling.

MATERIALS AND METHODS

Isolation of MSCs and EPCs

Bone marrow mononuclear cells were isolated from the long bones of male Wistar rats (8 weeks old, 250-300 g; Charles River Laboratories, Worchester, Mass), filtered through a 40-μm Falcon cell strainer (Corning Inc, Corning, NY), and centrifuged at 300*g* for 7 minutes. Red blood cells were excluded by the use of 1× RBC lysis buffer (#00-4337-57; eBioscience/Thermo Fisher Scientific, Waltham, Mass) for 10 minutes at 4°C. Remaining cells were cultured in a medium with Dulbecco's Modified Eagle's Medium (DMEM; #11995-040; Gibco Laboratories, Gaithersburg, Md) containing 10% fetal bovine serum (FBS; Sigma-Aldrich, ST Louis, Mo) and gentamicin on noncoated culture dishes for 24 hours at 37°C. After incubation, the adherent cells were washed and then cultured in a medium with DMEM containing 10% FBS and gentamicin. A purified population of MSCs was obtained 10 to 14 days after the initiation of culture. MSCs were identified in accordance with the criteria of the International Society for Cellular Therapy[2]; specifically, expression of CD 105, CD73, and CD 90, as well as absent expression of CD45 and CD34 were required.[4]

EPCs were isolated and cultured as previously described.[1] In summary, bone marrow mononuclear cells were isolated from the long bones of Wistar rats by density gradient centrifugation with Histopaque 1083 (Sigma-Aldrich) and cultured in endothelial basal medium-2 supplemented with EGM-2 SingleQuots (Lonza, Basel, Switzerland) containing human epidermal growth factor, 5% FBS, vascular endothelial growth factor, basic human fibroblast growth factor, recombinant human long R3 insulin-like growth factor-1, ascorbic acid, and gentamicin on vitronectin (V0132-50VG; Sigma-Aldrich)-coated dishes. The combination of endothelium-specific media and the removal of nonadherent bone marrow mononuclear cells were intended to select for the EPC phenotype. The EPC phenotype was confirmed by expression of CD 31, as previously described.[4]

Transdifferentiation of MSCs Into SMCs

Primary rodent MSCs were transferred and cultured in a medium with DMEM and 10% FBS on 60-mm culture dishes coated with fibronectin (FN group; BD Biosciences, San Jose, Calif) at 37°C in a humidified atmosphere of 5% CO_2 in air. Primary MSCs also were included in this study (control group). The cell number of the primary seeded MSCs was 4 to 6 × $10^3/cm^2$ for each plate. MSC growth medium was used as the nutrient medium, and all media were exchanged every 48 to 72 hours.[4]

Nanovolume Capillary Electrophoresis–Based Protein Analysis of Cultured Cells

Nanovolume capillary electrophoresis–based protein analysis was performed on the cultured MSCs and SMCs with Wes (ProteinSimple Inc, San Jose, Calif). Tissue homogenates from cultured cells were prepared with Halt Protease Inhibitor Single-Use Cocktail (Thermo Fisher Scientific) diluted in T-PER Tissue Protein Extraction Reagent (Thermo Fisher