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Multimodality noninvasive imaging for assessing therapeutic effects of exogenously transplanted cell aggregates capable of angiogenesis on acute myocardial infarction

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ABSTRACT

Although the induction of neovascularization by cell-based approaches has demonstrated substantial potential in treating myocardial infarction (MI), the process of cell-mediated angiogenesis and its correlation with therapeutic mechanisms of cardiac repair remain elusive. In this work, three-dimensional (3D) aggregates of human umbilical vein endothelial cells (HUVECs) and cord-blood mesenchymal stem cells (cbMSCs) are constructed using a methylcellulose hydrogel system. By maximizing cell–cell and cell–ECM communications and establishing a hypoxic microenvironment in their inner cores, these cell aggregates are capable of forming widespread tubular networks together with the angiogenic marker $\alpha_v\beta_3$ integrin; they secret multiple pro-angiogenic, pro-survival, and mobilizing factors when grown on Matrigel. The aggregates of HUVECs/cbMSCs are exogenously engrafted into the peri-infarct zones of rats with MI *via* direct local injection. Multimodality noninvasive imaging techniques, including positron emission tomography, single photon emission computed tomography, and echocardiography, are employed to monitor serially the beneficial effects of cell therapy on angiogenesis, blood perfusion, and global/regional ventricular function, respectively. The myocardial perfusion is correlated with ventricular contractility, demonstrating that the recovery of blood perfusion helps to restore regional cardiac function, leading to the improvement in global ventricular performance. These experimental data reveal the efficacy of the exogenous transplantation of 3D cell aggregates after MI and elucidate the mechanism of cell-mediated therapeutic angiogenesis for cardiac repair.

1. Introduction

The induction of therapeutic angiogenesis by cell-based transplantation has been shown to have substantial potential for treating limb or myocardial ischemia [1,2]. Before transplantation, cells of the desired types must be grown on a large scale *in vitro* and then dissociated from their culture dishes using proteolytic enzymes. The retention of intramuscularly injected dissociated cells at engrafted sites is reportedly problematic [3]. The poor cell retention

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adversely influences the efficacy of cell-transplantation therapy, suggesting that the cell delivery strategy warrants further refinement [4].

To promote the success of cell engraftment for therapeutic angiogenesis, our group has developed a cell delivery strategy that involves three-dimensional (3D) cell aggregates that are assembled in a thermo-responsive methylcellulose (MC) hydrogel system [5–8]. The cell aggregates that are capable of angiogenesis consisted of human umbilical vein endothelial cells (HUVECs) and cord-blood mesenchymal stem cells (cbMSCs). To ensure vascular maturation and stability, ECs must functionally interact with mural cells such as pericytes or smooth muscle cells (SMCs) [9]. Investigations have shown that MSCs can differentiate into pericytes and SMCs [10]. Assembling cells into 3D aggregates enables the cell–cell and cell–extracellular matrix (ECM) interactions to be re-established, forming a native tissue-mimicking microenvironment [11,12]. Using a mouse model of hindlimb ischemia, the 3D cell aggregates that were transplanted intramuscularly *via* local injection were demonstrated to be entrapped effectively in the interstices of muscular tissues and then to adhere to engraftment sites [7,13]. The engrafted cells subsequently promoted considerable angiogenesis, improving the regional perfusion and salvaging the ischemic limb.

Although the therapeutic efficacy of HUVEC/cbMSC aggregates appears to be favorable, the mechanism of their angiogenesis in repairing ischemic tissues remains elusive. As one of the key cell-surface receptors and adhesion molecules in initiating and regulating angiogenesis, $\alpha_v\beta_3$ integrin is strongly expressed by ECs during neovascular growth [14,15]. The expression of integrins modulates the migration of angiogenic vessels by enabling ECs to adhere to ECM [16]. Furthermore, $\alpha_v\beta_3$ integrin associates with growth-factor receptors, facilitating their activation for angiogenesis [16,17]. The important roles of $\alpha_v\beta_3$ integrin in angiogenesis make it a potential target for the noninvasive imaging of angiogenesis [14].

This study extends our earlier observations by elucidating the process of cell-mediated angiogenesis and its therapeutic effects that are induced by exogenously engrafted HUVEC/cbMSC aggregates in rats with myocardial infarction (MI), using multimodality noninvasive imaging methods. Noninvasive methods for the evaluation of angiogenesis, myocardial perfusion, and cardiac function would be valuable in reducing the number of animals required and limiting inter-subject variability, as each animal could be imaged repeatedly.

To elucidate the beneficial effects of cell aggregates in treating MI, $\alpha_v\beta_3$ integrin was used as an imaging target to track the angiogenic process after cell treatment by positron emission tomography (PET). Blood perfusion recovery, global cardiac function, and regional myocardial strains were evaluated using single photon emission computed tomography (SPECT) and echocardiography, respectively. PET and SPECT are noninvasive molecular imaging modalities for assessing responses to cell therapies that involve the stimulation of angiogenesis. Measurements of myocardial 2D strains by a noninvasive echocardiographic technique offer a sensitive means of detecting changes in regional contraction during ischemia.

2. Materials and methods

2.1. Cell culture

Human cbMSCs and HUVECs were obtained from Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan. The cbMSCs, which were transfected non-virally with red fluorescent protein (RFP; pDsRed2-N1, Clontech, Palo Alto, CA, USA) and human telomerase reverse transcriptase (pGRN145, American Type Culture Collection, Manassas, VA, USA) [18], were cultured in minimum essential medium Alpha (α-MEM; Life Technologies, Carlsbad, CA, USA) that contained 20% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 30 μg/mL hygromycin B, and 200 μg/mL geneticin (Life Technologies). The HUVECs were cultivated in Medium 199 (Life Technologies) that was supplemented with 10% FBS and 1% penicillin–streptomycin (Life Technologies). Cells were grown at 37 °C in a humidified incubator with 5% (v/v) CO_2.

2.2. Construction and characterization of 3D HUVEC/cbMSC aggregates

The 3D aggregates of HUVECs/cbMSCs were constructed in α-MEM that contained 20% FBS and 1% penicillin–streptomycin, using a thermo-responsive MC hydrogel system that was created in 96-well plates [19,20]. Briefly, equal amounts of HUVECs and cbMSCs were suspended in a culture medium, which was then added to each well that contained the MC hydrogel system (5×10^3 cells of each type per well), and then cultivated for 24 h with orbital shaking at 85 rpm (Fig. 1). The cell aggregates thus formed were collected and fixed in 4% paraformaldehyde (Sigma–Aldrich, St. Louis, MO, USA), before being cryosectioned at 10 μm thickness and stained with antibodies against von Willebrand factor (vWF; Dako, Glostrup, Denmark), fibronectin or hypoxia-inducible factor (HIF)-1α (Abcam, Cambridge, MA, USA); they were then incubated with Alexa Fluor 488-conjugated secondary antibodies (Life Technologies), mounted with 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA), and visualized using confocal laser scanning microscopy (CLSM; Carl Zeiss, Jena GmbH, Germany). Cell viability was investigated using a live/dead staining kit (Life Technologies), in which the hydrolysis of calcein-AM in live cells generated green fluorescence, while the ethidium homodimer produced red fluorescence in dead cells.

2.3. Tube formation assay

The grown cell aggregates were transferred onto growth factor-reduced Matrigel (BD Biosciences, San Jose, CA, USA)-coated μ-Dish (ibidi, Munich, Germany). On days 1, 4, 7, 10, and 14, the immunofluorescence staining of the tubular structures that were grown on Matrigel was carried out with anti-vWF and anti-$\alpha_v\beta_3$ integrin antibodies, visualized by fluorophore-conjugated secondary antibodies (Life Technologies), counterstained with DAPI, and observed by CLSM. Concomitantly, the culture media were collected and analyzed by the Procarta Plex Cytokine assay ($n = 6$; Affymetrix, Santa Clara, CA, USA).

2.4. Animal study

All animal experiments in this study conformed to the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council, published by the National Academy Press in 1996. The Institutional Animal Care and Use Committee of Veterans General Hospital (Taichung, Taiwan) reviewed and approved all animal protocols. Lewis rats that weighed 250–300 g underwent permanent ligation of the left coronary artery to induce acute MI [21–23]. Animals that fulfilled the echocardiographic inclusion criterion by exhibiting left ventricular fractional shortening <35% were used in the subsequent experiments. These animals were intramyocardially injected with saline, dissociated HUVECs/cbMSCs (1×10^6 cells each type per rat), or 3D HUVEC/cbMSC aggregates (200 cell aggregates per rat,