Biomaterials 32 (2011) 8446–8455

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Core–shell cell bodies composed of human cbMSCs and HUVECs for functional vasculogenesis

Wen-Yu Lee [a,1], Hung-Wen Tsai [b,1], Jen-Hao Chiang [a], Shiaw-Min Hwang [c], Ding-Yuan Chen [a], Li-Wen Hsu [a], Yi-Wen Hung [d,e], Yen Chang [b,**], Hsing-Wen Sung [a,*]

[a] Department of Chemical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan, ROC
[b] Division of Cardiovascular Surgery, Veterans General Hospital-Taichung, and College of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC
[c] Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, ROC
[d] Department of Education and Research, Veterans General Hospital-Taichung, Taichung, Taiwan, ROC
[e] Department of Veterinary Medicine, College of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan, ROC

ARTICLE INFO

Article history:
Received 18 June 2011
Accepted 20 July 2011
Available online 25 August 2011

Keywords:
Vascularization
Tissue regeneration
Cell therapy
Tube formation
Vascular network

ABSTRACT

Rapid induction and creation of functional vascular networks is essential for the success of treating ischemic tissues. The formation of mature and functional vascular networks requires the cooperation of endothelial cells (ECs) and perivascular cells. In the study, we used a thermo-responsive hydrogel system to fabricate core–shell cell bodies composed of cord-blood mesenchymal stem cells (cbMSCs) and human umbilical vascular ECs (HUVECs) for functional vasculogenesis. When seeded on Matrigel, the shelled HUVECs attempted to interact and communicate vigorously with the cored cbMSCs initially. Subsequently, HUVECs migrated out and formed tubular structures; cbMSCs were observed to coalesce around the HUVEC-derived tubes. With time progressing, the tubular networks continued to expand without regression, indicating that cbMSCs might function as perivascular cells to stabilize the nascent networks. In the *in vivo* study, cbMSC/HUVEC bodies were embedded in Matrigel and implanted subcutaneously in nude mice. At day 7, visible blood-filled vessels were clearly identified within the implant containing cbMSC/HUVEC bodies, indicating that the formed vessels anastomosed with the host vasculature. The cored cbMSCs were stained positive for smooth muscle actin, suggesting that they underwent smooth muscle differentiation and formed microvessels with the shelled HUVECs, as the role of perivascular cells. These data confirm that the formation of mature vessels requires heterotypic cooperation of HUVECs and MSCs. This study provides a new strategy for therapeutic vasculogenesis, by showing the feasibility of using cbMSC/HUVEC bodies to create functional vascular networks.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Construction of new vascular networks for therapeutic purposes has been critical but elusive for tissue engineering/regeneration [1]. A prerequisite for preserving the viability of regenerating tissues is that a vascular network is assembled and forms anastomoses with the host vasculature to ensure adequate nutrients, gas exchange, and elimination of waste products [1,2]. Studies have shown that delivery of angiogenic growth factors can promote revascularization [3–6]; however, growth factors alone are insufficient to create a mature and stable vasculature [7].

Cell transplantation for vascularization is a promising strategy for therapeutic angiogenesis and treatment of ischemic diseases [8,9]. It has been reported that endothelial cells (ECs) implanted *in vivo* can form microvessels [10,11]; however, without perivascular cells, the formed vessels quickly regress [12,13]. To establish a functional and robust vascular network, ECs must interact with pericytes or vascular smooth muscle cells (SMCs) that in turn provide for vascular maturation and stability [12,14]. Recent studies indicate that mesenchymal stem cells (MSCs) may provide angiogenic benefits via their potential of being recruited during arteriogenesis and vascular repair [9,15]. Additionally, it is known that MSCs have the potential to differentiate into perivascular cells or SMCs [13,16].

* Corresponding author. Tel.: +886 3 574 2504; fax: +886 3 572 6832.
** Corresponding author.
E-mail addresses: ychang@vghtc.gov.tw (Y. Chang), hwsung@che.nthu.edu.tw (H.-W. Sung).
[1] The first two authors (W.Y. Lee and H.W. Tsai) contributed equally to this work.

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.07.061

MSCs can be isolated from bone marrow, adipose, and other adult tissues [17–19]. However, the differentiating frequency and capacity of MSCs decrease significantly with age [20]. The umbilical cord blood can be obtained without ethical issues and has been considered as an alternative source of MSCs (cbMSCs). When compared to other adult MSCs, cbMSCs are more primitive with a lower immunogenicity and can be stored in advance and therefore rapidly available [19].

Despite the potential advantages in cell-based therapy, low retention of the delivered cells limits its functional benefits, which presents a large obstacle in a clinical setting [21,22]. Typical cell transplantation involves the administration of dissociated cells, which are not abundant in extracellular matrices (ECM) and adhesion molecules [23,24]. We have previously shown that the dissociated cells transplanted intramuscularly are insufficient in physical size to entrap into the muscular interstices, and the transplanted cells do not have a temporary matrix to which they can attach, thus causing the cell loss. To enhance the engraftment of cells, a thermoresponsive methylcellulose (MC) hydrogel system was developed in our laboratory for the formation of multicellular aggregates (cell bodies) with endogenous ECM. Our results demonstrated that cell retention can be significantly enhanced at the site of cell graft when implanted in the form of cell bodies [22,23].

Herein, we develop a method for the fabrication of core–shell cell bodies composed of cbMSCs and human umbilical vascular ECs (HUVECs) for functional vasculogenesis. Their potential in forming tubular structures on Matrigel was investigated *in vitro*, and the ratio of cbMSCs to HUVECs used in forming the core–shell bodies was optimized. In the *in vivo* study, cbMSC/HUVEC bodies were subcutaneously implanted in nude mice, examining whether they can form vascular networks and anastomose with the host vasculature. Matrigel alone, cbMSC bodies, and HUVEC bodies were used as controls.

2. Materials and methods

2.1. Cell culture

HUVECs and human cbMSCs were kindly provided by Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan. HUVECs were cultured in Medium 199 (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA). cbMSCs were transfected non-virally with human telomerase reverse transcriptase and red fluorescent protein (RFP) [25] and cultured in Minimum Essential Medium (MEM) Alpha Medium (Invitrogen) supplemented with 20% FBS. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Cultivation of cell bodies

Preparation of the MC hydrogel system for the cultivation of cell bodies has been previously reported by our group [23]. cbMSCs were dissociated from culture dishes with 0.05% trypsin, seeded in the prepared MC hydrogel system, and cultivated for 24 h to construct the core of cell bodies; subsequently, HUVECs were added in and cultured for another 24 h to form the shell on cell bodies (cbMSC/HUVEC bodies, Fig. 1). In the formation of each core–shell body, a total number of 5×10^3 cells were used at different cbMSCs to HUVECs ratios (2:1, 1:1, 1:2, and 1:4). Cell bodies of cbMSCs and HUVECs were fabricated separately as controls, using the same cell numbers.

2.3. Immunostaining of test cells and cell bodies

Test cells and cell bodies were fixed and stained with rabbit anti-RFP (Invitrogen), mouse anti-von Willebrand factor (vWF), collagen I, collagen III, vascular endothelial (VE)-cadherin, or fibronectin antibodies (Abcam, Cambridge, MA, USA). Different Alexa Fluor secondary antibodies (Invitrogen) were used to obtain fluorescent colors. Cells were costained to visualize the nuclei by SYTOX Blue (Invitrogen) and examined using an inverted confocal laser-scanning microscope (CLSM, TCS SL, Leica, Germany).

2.4. Real-time polymerase chain reaction (real-time PCR)

Total RNA was extracted with TRIzol Reagent (Invitrogen), and cDNA was synthesized from RNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA); all procedures were performed according to the instructions of the manufacturers. Real-time PCR was performed using Power SYBR

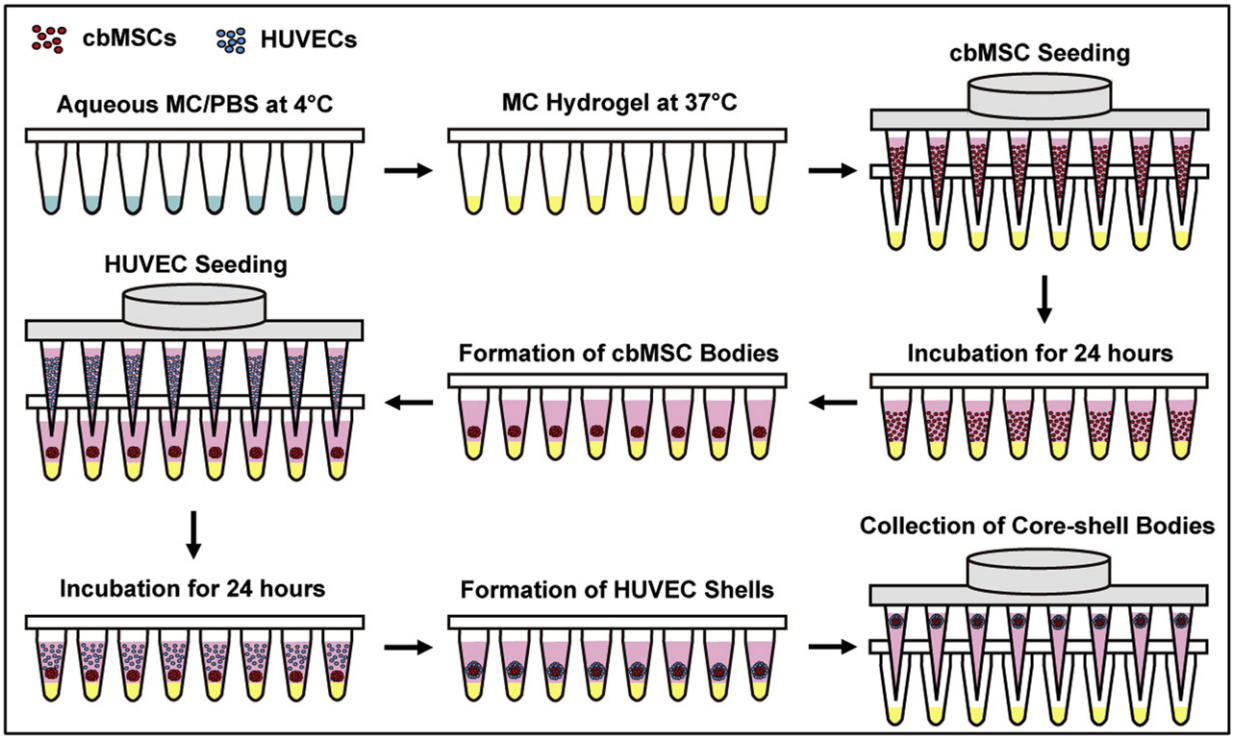

Fig. 1. Schematic illustrations showing the processes used in the cultivation of cbMSC/HUVEC core–shell bodies. Aqueous MC/PBS was poured individually into each well of the 96-well plate at 4 °C and then pre-incubated at 37 °C for gelation. cbMSCs were seeded in the prepared hydrogel system and cultivated for 24 h to form cbMSC bodies. Subsequently, HUVECs were added in to form the shell on cbMSC bodies. The grown core–shell cell bodies were then collected and used for the studies.

Download English Version:

https://daneshyari.com/en/article/7236

Download Persian Version:

https://daneshyari.com/article/7236

Daneshyari.com