Micropatterned co-cultures of endothelial cells and mesenchymal stem cells within gelatin methacrylate hydrogels

Mehdi Nikkhah[1,2], Mahshid Kharaziha[1,2], Alireza Dolatshahi-Pirouz[1,2,3], Nasim Annabi[1,2,3], Su-Ryon Shin[1,2,3], Yunzhi Yang[4] and Ali Khadmhosseini[1,2,3]

[1]Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA.

[2]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

[3]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA.

[4]Department of Orthopaedic Surgery, School of Medicine, Stanford University, Stanford, California 94305, USA.

Statement of Purpose: The development of biomimetic and functional vasculature remains one of the major challenges of tissue engineering (1). In the past few years, numerous strategies have been developed to engineer vascularized networks within tissue constructs by mimicking the *in vivo* vasculature. The majority of these approaches have relied on immobilization of angiogenic growth factors within the biomaterials resulting in endothelial cell (EC) invasion, migration and tubule formation upon implantation inside the host body (2). Other strategies have exploited cell-based approaches in which the vascular cells have been embedded within the biomaterial structure to coalesce and form capillary networks (3,4). In this regard, it has been shown that the co-culture of ECs with pericyte-like cells significantly accelerates blood vessel formation within the biomaterial matrix. Alternatively, the selection of appropriate biomaterials plays a significant role in formation of functional vasculature. Hydrogels have attracted significant attention as new scaffolding materials for tissue engineering because of their numerous advantages, such as permeability, high water content, biocompatibility and the ability to uniformly encapsulate the cells. In this paper, we present micropatterned co-cultures of ECs and human mesenchymal stem cells (hMSCs) within gelatin methacrylate hydrogel (GelMA) with variable cell:cell ratios to develop highly organized cord-like structures mimicking the native microvasculature.

Methods: Gelatin methacrylation was performed as described previously (4). Green fluorescent protein (GFP)-expressing human umbilical vein ECs (HUVECs) and bone marrow derived hMSCs were used in this work. The cells were encapsulated within GelMA hydrogel with 1:2 and 1:4 (hMSC:EC) ratios while the cell-laden micropatterned hydrogel constructs were developed using standard photolithography technique. The microconstructs height and width were selected to be 100 μm and 50 μm respectively. Following cell encapsulation, the hydrogel constructs were cultured in endothelial growth media for 3, 5 and 7 days prior to biological analyses. After the desired culture time, the samples were fixed in 4% paraformaldehyde solution in PBS for immunofluorescence staining as well as nuclei alignment analysis according to the previously published procedures (4).

Results: Encapsulation of ECs and hMSCs within micropatterned hydrogels resulted in high cellular viability and orientation after 3, 5 and 7 days of culture. Nuclei alignment measurements were grouped in 10° increments to compare the alignment distribution in different conditions. As shown in Figure 1(A), increasing the ratio of the hMSCs to ECs significantly enhanced the overall cell alignment within the microconstructs compared to the EC-only condition. Furthermore, the presence of the hMSCs within GelMA hydrogel accelerated the process of cellular alignment, in which 60% of the cells aligned along the major axis of the microconstructs within the 0-10° preferred angle as early as 3 days. Fluorescence images clearly indicated heterotypic interactions between ECs and hMSCs within micropatterned hydrogel constructs. Alternatively, immunostaining results demonstrated that hMSCs underwent α-SMA expression in co-culture with ECs and organized toward the periphery of the microconstructs.

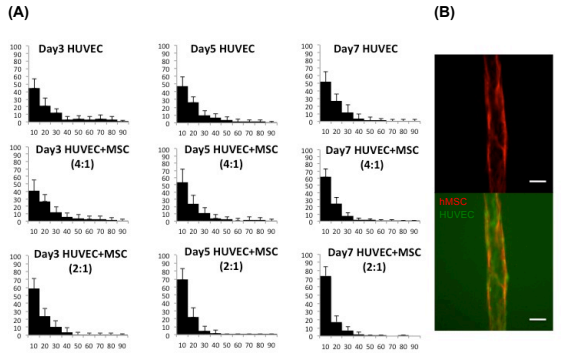

Figure 1: (A) Cells nuclei alignment within the microconstructs after 3, 5 and 7 days of culture. (B) Representative fluorescence images of the α-SMA expressing hMSCs in co-culture with HUVECs within the patterned hydrogel constructs. Scale bars represent 100 μm.

Our findings demonstrate a promising approach to develop 3D organized and biomimetic vascularized networks for tissue engineering applications through integration of microfabrication technologies and advanced biomaterials.

References:

(1) Lovett M, et. al., Tissue Eng Part B Rev. 2009, 15, 353-70; (2) Lee K.Y., et. al. Nature. 2000, 408:998, 1000; (3) Chen Y., et. al., Adv Funct Mater. 2012, 22, 2027-39. (4) Nikkhah M., et. al., Biomaterials , 2012, 33, 9009-18.