Tissue engineering using vascular organoids from human pluripotent stem cell derived endothelial cells and mural cell phenotypes

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ABSTRACT

Regenerating large tissues requires an intimate supply of the host vascular network, a slow process leading to low viability of the regenerating cells. A solution to this obstacle is the generation of prevascularized tissue engineered constructs. Since Endothelial (ECs) and Mural (MCs) cells, such as smooth muscle cells (SMCs), and pericytes (PCs), are the cellular components of blood vessels and their interactions are crucial for neovascularization, both cell types and their arrangement into correct spatial organization are required in order to rescue tissue engineered constructs from critical ischemia and to form a functional vascular network *in vivo*.

Based on this context and in order to overcome the limitations concerning the isolation and expansion of human SMCs, we developed a protocol to differentiate human pluripotent stem cells (hPSCs) to defined SMC populations (contractile and synthetic hPSC-SMCs) using feeder-free and low serum conditions. hPSC-SMCs phenotypes and hPSC-ECs were extensively characterized concerning their phenotype and function. In addition, we generated ECs from the differentiation of hPSCS (hPSC-ECs). hPSCs-SMCs and ECs (hPSC-ECs or primary ECs), using a methylcellulose-based hydrogel system, were then used to generate 3D vascular organoids, which rapidly give rise to a complex three-dimensional vascular network. Vascular organoids (ECs and hPSC-SMCs) were extensively characterized regarding their phenotype, cell-cell interactions and their ability to form a three-dimensional capillary network *in vitro*. Finally, we investigated the vascularization potential of these vascular organoids, when embedded in hydrogels composed of defined extracellular components (collagen/fibrinogen/fibronectin) that can be used as scaffolds in tissue engineering applications.

To sum up, we developed a robust method for the generation of defined hSMCs phenotypes from hPSCs. In addition, we differentiated hPSCs to ECs. Fabrication of hECs/hPSC-SMC vascular organoids embedded in chemically defined matrices is a significant step forward in tissue engineering and regenerative medicine.

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