A new technique for Development of perfusable multilayered blood vessel-like structures on Microfluidic Chip

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Abstract: Vascularization of large tissues is crucial for the survival of engineered tissues [1], and is one of the biggest challenges in tissue engineering [2-4]. Despite the significant progress over the last few decades, the current approaches of tissue engineering still lack the ability to make perfusable blood vessels with native blood vessel like tri-layer structure. Recently we have made significant progress toward the development of multilayered vascular structures. In the current work we aim to present a new technique for the development of perfusable multilayered vascular structures on a microfluidic chip using a two-step pre-vascularization method.

Methods: The method used in this study for making multilayered vascular structures is a simple and easy method that can be used for making perfusable single cellular layer micro-capillary like vessels as well as larger blood vessel-like structures with physiological multi-layer cellular wall. The method uses two concentric hypodermic needles, e.g. 800 micron outer diameter and 400 micron outer diameter respectively where the inner diameter of the larger needle was larger than the outer diameter of the inner needle.

By pre-inserting the two concentric needles in a microfluidic device from the two opposite ends a fibroblast cell-laden hydrogel pre-polymer solution was introduced into the device and cross-linked with ultra violet (UV) light. The larger needle was removed leaving behind a tubular hole with the smaller needle at the center. A second gel solution laden with smooth muscle cells (SMC) was injected into the channel followed by a second-step of UV-cross linking. Finally the small needle was removed, and HUVECs were surface seeded into the lumen, generating a tri layer blood vessel like structure. Optimization of the physical and mechanical properties of the gel matrix was performed. The viability, attachment, and spreading of the cells were investigated using Live-Dead and Actin-DAPI staining. The cell-cell junctions were examined using CD31 and VE Cadherin staining.

Results: The vascular structures developed using the new technique were grown for several days with and without the continuous perfusion of media through the lumen of the vessels. The cells remained viable under this condition. The HUVECs aligned along the direction of flow under continuous perfusion. Figure 1 shows a

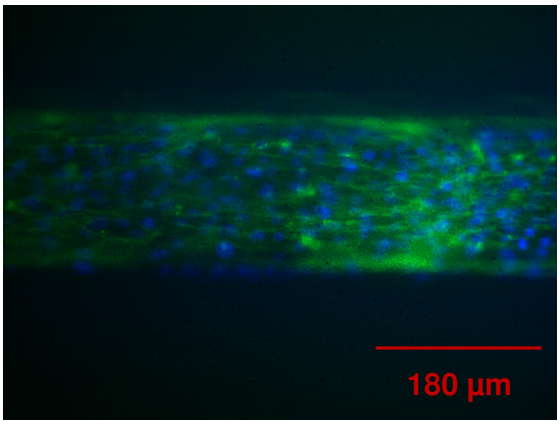

Figure 1. A representative Phalloidin-DAPI staining image showing the attachment, and spreading of cells in the channel at 15% GelMa concentration after 3 days.

phalloidin-DAPI staining image of a representative single layered vessel after three days of culture. The cells spread well on the luminal surface forming a cell monolayer as is seen from the actin skeleton (green) and the nucleus of the cells (blue). The CD 31 and VE cadherin staining revealed tight cell-celll junctions in the HUVECs monolayer.

Conclusions: A new technique has been developed for making perfusable multilayered blood vessel-like structures on a microfluidic platform. The technique can be used for making a single perfusable vessel as well as a mesh of vessels for continuous perfusion. The physical and mechanical properties of GelMa hydrogel have been optimized for formation of the blood vessel like structure. Using the newly developed technique formation of a tri-layered perfusable vessel on a microfluidic chip has been demonstrated. The multilayer vessel will have multifaceted applications including development of in vitro models of various cardiovascular diseases such as atherosclerosis and hypertension.

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