**Simultaneous Deposition of Human Endothelial Cells and Materials for Fabrication of Vascular Channels**

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**Statement of Purpose:**
Considerable effort has been focused on precision fabrication of synthetic biodegradable scaffolds for hard tissue defect repair. The requirements for soft tissue scaffolds, in particular thick tissue are somewhat different since the cellular arrangement is more essential for achieving function. The goal of this study was to develop a technique that allows precision fabrication of channels lined with endothelial cells.

**Materials and Methods:**
*3D thermal inkjet printers*: HP thermal inkjet printers were modified as previously described[1]. An inkjet cartridge was emptied and rinsed thoroughly with double distilled water, sonicated using an ultrasonic sonicator to remove any ink or blockages, sprayed with 100% ethanol and the whole assembly of the printer along with the cartridge was placed overnight in a Class II hood under ultraviolet light prior to use.
*Printing of hydrogels*: A test patterns of parallel lines with 42 microns diameter [0.12 pt] were printed layer-by-layer by spraying a crosslinker onto liquid hydrogels. The hydrogels consisted of 80mg/ml fibrinogen dissolved in 1x phosphate buffered saline (PBS). The crosslinker was 50u/ml thrombin augmented with 40mM calcium chloride in serum free MEM. Resulting patterns were analyzed under a phase contrast microscope.
*Printing and functional characterization of cells in printed hydrogels.* Human Microvascular Endothelial Cells (HMEC) were trypsinized, resuspended in the thrombin/MEM solution to form a bioink and filled into the ink cartridge. A pattern of parallel lines with varying thickness from 40 to 200 microns were printed onto the liquid fibrinogen solution with this ink. As control, a small amount of the bioink was manually pipetted onto the fibrinogen containing solution. Samples were incubated at 37ºC and 5% $CO_2$ for varying amounts of time up to 3 weeks, then analyzed under fluorescence using live/dead assays and lectin stains.

**Results and Discussion:**
Cell viability assays showed that HMEC cells attached and proliferated on fibrin gels. The printer was capable of reliably creating fibrin channels between 30µm and 1mm, though some resolution is lost as the scale decreases. HMEC cells were successfully printed along with the thrombin to form patterned fibrin gels. The presence of cells was confirmed by light microscopy and fluorescent detection. The fibrin patterns were sustained and recognizable after 7 days in culture, and the printed cells remained attached to the printed fibrin.
A live/dead assay at 3 days post-printing showed that printed endothelial cells attached to the fibrin as they were patterned, and early results suggest a tendency toward alignment parallel to the pattern grain (Figure 1).

In an ongoing experiment, the samples will be kept in culture to measure the proliferation and in order to continue to assess the cellular growth patterns and orientation.

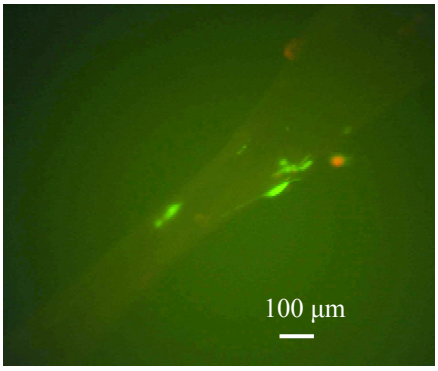


Figure 1. Microscopy images of printed fibrin channels and co-printed HMEC cells in fibrin channels. Image of a live/dead assay after 3 days incubation showing attached cells in a 80 µm channel.

**Conclusions:**
Modified inkjet printers are capable of simultaneous deposition of cells and biomaterials. Control group showed cells were healthy in thrombin solution (bioink) during printing. Proliferation of HMEC in fibrin channels showed fibrin was cell friendly and it has great potential of being a biomaterial scaffold for human vascular fabrication.

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**References:**
1. Xu, T., et al. 2006. Biomaterials. 27(19):3580-8.