Microfluidic Hydrogels for Microvascular Tissue Engineering

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Statement of Purpose: There is interest in fabricating in vitro tissue models, such as microvascular models, for studying physiology and screening new therapies for diseases. Some groups have focused on culturing cells in relevant biological scaffolds, while others have focused on relevant geometries and flow conditions. Here, we combined both design philosophies to create and employ microfluidic networks of defined geometries through relevant extracellular matrix (ECM)-based scaffolds.

Methods: Soft lithography was employed to micropattern poly(dimethylsiloxane) (PDMS), which was sealed to glass substrates to create microfluidic molds. The microfluidic surfaces were pretreated – light UV oxidation followed by pluronic (1-6% solution of F127 pluronic in phosphate buffered saline (PBS); BASF, Florham Park, NJ) - to facilitate the later separation of gelatin from the molds without deformation. Melted gelatin (10% in water) was introduced into the molds and, after gelation at 4°C, gelatin meshes were removed and transferred to a flow chamber. The meshes were encased in a precursor solution of collagen (Type 1 rat tail; 3, 8 or 10 mg/mL) or fibrin (50 mg/mL). After gelation of the precursor at room temperature for 1 h, heating to 37°C melted the gelatin, which was removed by exhaustively flushing the gel with PBS. This procedure yielded a microfluidic gel that was held in place by a PDMS housing. The housing had one hole at each end for introducing a perfusate. A suspension of blood endothelial cells (Cambrex) was introduced into the microfluidic scaffolds and, after cells adhered to the channel walls, the two holes of the housing were connected to reservoirs of media by PE-50 polyethylene tubing (as described in Chrobak et al., 2006). The microvessels were cultured under flow (~1 mL/h) for 3 days and barrier function was quantitatively assessed by examining the leakage of fluorescent macromolecules - bovine serum albumin (BSA; 67 kDa) and dextran (DEX; 10 kDa) - from the lumen of the microvessels (as described in Chrobak et al., 2006). Cross-sections were obtained by fixing samples and sectioning with a Vibrotome. Hoechst 33342 (~1 μg mL) was introduced by adding 100 μL of labeled media to the inlet and allowing the mixture to perfuse the gel for 30 min. Nonlithographic microvessels were fabricated from cylindrical gelatin shapes that were derived from a steel needle with a round cross-section. Statistical comparisons used the Mann-Whitney U test.

Results: First, we developed a general procedure for the formation of hydrogels that contain microfluidic networks. Micromolded meshes of gelatin served as sacrificial materials. Encapsulation of gelatin meshes in a hydrogel and subsequent melting and flushing of the gelatin left behind interconnected channels in the hydrogel. The channels were as narrow as ~6 μm, and faithfully replicated the features in the original gelatin mesh. Fifty-μm-wide microfluidic networks in collagen and fibrin readily enabled delivery of macromolecules and particles into the channels and transport of macromolecules from channels into the bulk of the gels. Microfluidic gels were also suitable as scaffolds for cell culture, and could be seeded by human microvascular endothelial cells to form rudimentary endothelial networks for potential use in tissue engineering. Second, we used these microfluidic gels as scaffolds for microvascular tissue engineering. Microvessels were optimized until their flow rates and geometries were stable for over 6 days. Then, we investigated whether channels with rectangular cross-sections, an artifact of our lithographic procedure, could be incorporated into scaffolds to form microvessels with strong barriers to macromolecules. Barrier function is important for controlling the passage of macromolecules and fluid, and loss of barrier is a hallmark of inflammation. The barrier function of lithographic microvessels was compared to that of nonlithographic microvessels, which are limited to simple cylindrical geometries. Cross-sections of **both** lithographic and nonlithographic microvessels were round at day 3, the day at which barrier function was measured. Also, there was no difference in barrier function between lithographic and nonlithographic microvessels (N=20 samples), as assessed by 1) the permeability of BSA ($p=0.304$), 2) the permeability of DEX ($p=0.787$), 3) the selectivity of the barrier to DEX vs. BSA ($p=0.914$), and 4) the number of focal leaks per mm ($p=1$). Microvessels had permeabilities that were on the order of in vivo venules (~5E-07 cm/s), and they were disrupted by the introduction of thrombin, an inflammatory mediator.

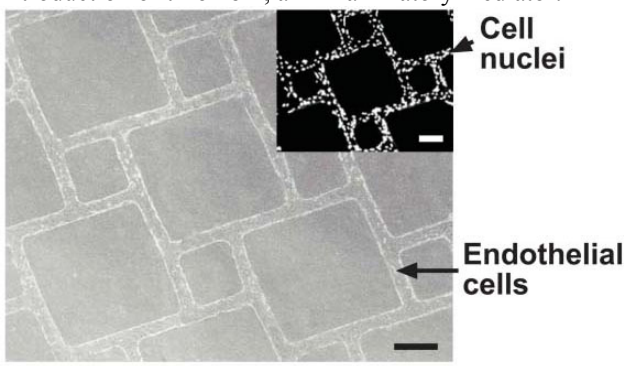

Figure 1. Microvascular endothelial cells cultured inside a microfluidic collagen scaffold (inset is Hoescht-stained microvessel; scale bars = 200 μm)

Conclusions: A general technique is presented to create microfluidic networks inside ECM gels. Microfluidic hydrogels were employed as scaffolds for microvascular tissue engineering. Resultant microvessels appear suitable for in vitro studies of barrier function.

References: Golden AP, Lab Chip 2007;7:720-725. Chrobak KM, Microvasc. Res. 2006;71:185-196.