Development of an *In Vitro* Model for Skin Substitutes with Endothelialized Microvasculature

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Statement of Purpose: It is now widely accepted that tissue-engineered products with their own microvasculature could overcome limitations of nutrient supply due to inadequate mass transfer, by convective delivery of nutrients and removal of waste products. Previously we designed micro-scale flow networks with optimal transport characteristics and fabricated the networks in natural collagen-glycosaminoglycan (CG) scaffolds as a first step to constitute the microvasculature[1]. In this work, we develop an *in vitro* model of tissue-engineered (TE) skin substitute with built-in endothelialized microvasculature. Additionally, the efficacy of TE constructs with integrated flow networks is evaluated by comparing the growth of composite skin substitutes consisting of CG scaffolds with and without built-in flow networks as the dermal analog and differentiated human keratinocytes as the epidermal analog. We also discuss results of endothelialization of closed channel CG flow networks and assays to characterize endothelial cell adhesion and proliferation on the CG matrix. A cytotoxicity assay and a platelet aggregation assay were performed to quantify cell attachment and integrity of cell-cell junctions respectively. This research will have applications in tissue engineering of vascularized 3D organs such as liver, heart and kidney.

Methods: CG membranes were fabricated as described elsewhere[1]. A standard protocol was used for the static culture of keratinocytes on CG membranes[2]. Skin substitutes with built-in flow networks were grown in the same way with convective delivery of nutrients from micropatterned CG networks. These networks were obtained by casting microvascular network patterns onto CG membrane scaffolds using a pressure casting technique developed in our lab[1]. The open CG flow channels were sealed with an –OH terminated PDMS-based adhesive to form closed composite CG flow networks. Microfluidics was established using a syringe pump and the biomaterial network was used as the dermal analog for the skin substitute. Additionally, endothelial cells were seeded within closed CG flow networks and their growth and viability was monitored in a perfusion bioreactor. Medium was perfused at physiological flow rates for up to 30 hours. The constructs were fixed with 4% formaldehyde and processed for SEM image analysis. An MTT assay kit (Invitrogen, CA) was used to compare cell attachment on CG matrix with that on tissue culture plastic for cytotoxicity testing. The formation of tight endothelial cell to cell junctions was quantified by perfusing rat blood through endothelialized networks and testing for platelet aggregation using SEM imaging.

Results and Discussion: **Figure 1A** shows a cross-section of skin substitute stained with H&E showing differentiated epidermal keratinocytes on a CG membrane. This was obtained in a static system with no flow networks, where nutrient supply is by passive diffusion. It is compared with skin substitutes with flow networks embedded in the dermal scaffold with convective delivery of nutrients. The number of cell layers and the density of cells were used as indicators of the efficacy of the skin substitute. **Figure 1B** shows a digital image of microfluidics established in closed composite CG flow networks of complex bifurcating geometries. Flow rates of up to 600 μl/min were sustained by the CG networks. Flow rates used in the growth of skin substitutes with built-in flow networks were on the order of 50 μl/min. Endothelial cells seeded within the flow channels adhered to the CG matrix and showed signs of growth and proliferation. **Figure 1C** shows an SEM image of endothelial cells in CG flow networks after 30 hours of medium perfusion.

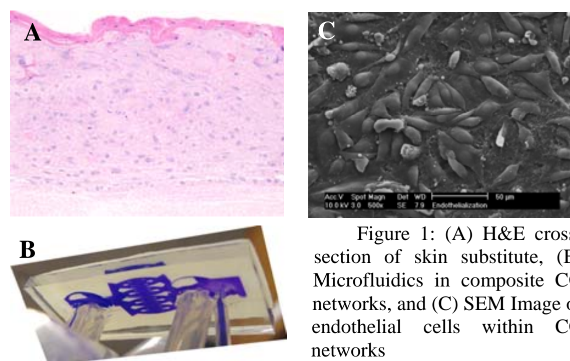

Figure 1: (A) H&E cross-section of skin substitute, (B) Microfluidics in composite CG networks, and (C) SEM Image of endothelial cells within CG networks

Conclusions: In this work, we demonstrate the establishment of microfluidics in composite CG biomaterial networks and further use this system for the development of an *in vitro* model for TE constructs with built-in flow networks. Specifically, in this study, the efficacy of skin substitutes with built-in flow networks for convective delivery of nutrients is compared with that of skin substitutes without flow networks. Furthermore, the flow networks are endothelialized to create "vascularized" networks. The cytotoxicity and platelet aggregation results indicate that the CG biomaterial is conducive to the adhesion and growth of endothelial cells under perfusion. Ultimately, formation of a monolayer of endothelial cells in complex CG microchannel networks will lead to the development of an *in vivo* model to investigate the uptake and function of a skin substitute with built-in microvasculature.

References:

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