Co-culture of Human Gingival Fibroblasts and Vascular Endothelial Cells in a Perfused Degradable/Polar/Hydrophobic/Ionic Polyurethane (D-PHI)

Jane, Wing Chi, Cheung[a], Christopher A.G. McCulloch[b], J. Paul Santerre[a,b].
[a]Inst of Biomat and Biomed Eng, [b]Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada.

Statement of Purpose: Gingivitis and destructive periodontal disease affect 41% and 14% of Americans aged 35–44 years respectively[1]. In treatments aimed at gingival tissue regeneration, human gingival fibroblasts (HGF) contribute to the reconstruction of the lamina propria and mediate epithelial cell morphogenesis. Gingival tissues are well-perfused for metabolic exchange. Accordingly, perfusion systems and promotion of angiogenesis are required for tissue-engineered constructs. Recent reports describe reconstruction of the lamina propria[2] but because of lack of a perfusion system or capillary-like structures, most cells could not migrate into the core of the constructs or remain viable. Previous studies using a novel polyurethane hydrogel (D-PHI) showed that this material inhibits inflammation post-implantation[3] and facilitates the growth of HGF in a perfused system, showing continuous growth and significant collagen production[4]. Co-cultures of endothelial cells with fibroblasts in synthetic materials have been shown to support capillary formation[5]. Hence, our objective was to co-culture HGF with human umbilical vein endothelial cells (HUVEC) in a perfused system to promote capillary formation in the synthetic D-PHI scaffolds.

Methods: D-PHI scaffolds were fabricated via the free radical polymerization as outlined by Sharifpoor *et al*[6]. The cylindrical scaffolds were 2mm thick and 6mm in diameter; the pore size[6] ranged from 30–250μm. HGF-1 cells and HUVEC (from ATCC) were cultured in Dulbecco's Modified Eagle Medium (with 10% FBS and 1% antibiotics) and in F-12 K Medium (with 10% FBS, 1% antibiotics, 0.1 mg/mL heparin, and 0.05 mg/mL ECGS) respectively. A mixture of both cell types (1:1 ratio) was seeded into D-PHI scaffolds for 24 hrs. The seeded constructs were cultured in a custom-designed perfusion bioreactor for dynamic culture for 28 days with media changed every other day. DNA content was quantified using Hoechst 33258. Histology (H&E and picrosirius red staining) and immunohistochemistry (CD31) were also carried out to monitor for possible capillary formation at various time points. Results in the dynamic culture were compared with those in the static culture (no flow) and HGF monoculture with flow.

Results: Over a culture period of 4 weeks, the DNA mass showed a 1.5±0.4 fold increase for the co-culture population on D-PHI scaffolds under media perfusion between day 1 to day 28 (**Fig. 1a**), whereas slower growth (0.9±0.2 fold) was observed in the static culture during the same culture period. These data indicate that perfused D-PHI scaffolds supported and enhanced the growth of the HGF-HUVEC co-culture. It is unclear why the co-culture populations decreased in both types of cultures at day 14. Conceivably, physical cellular interactions within the confines of a 3D culture or secretion of certain cytokines (e.g. heparin from HUVEC[7]) have been induced when the two cell types are co-cultured. The growth profile in the co-culture was different from that in the HGF monoculture (**Fig. 1b**), which indicates that HGF alone in dynamic culture retained the capacity to grow throughout the culture period but when they are co-cultured with HUVEC, intercellular interactions between HGF and HUVEC may have regulated cell growth to allow other events such as capillary formation to occur. Indeed, H&E staining showed early formation of lumen-like structures in the dynamic co-culture at day 28 (**Fig. 1c**) but not in the static culture (image not shown). Further, these lumenal structures were positive for CD31 (**Fig. 1d)**, indicating that they were likely formed by HUVEC.

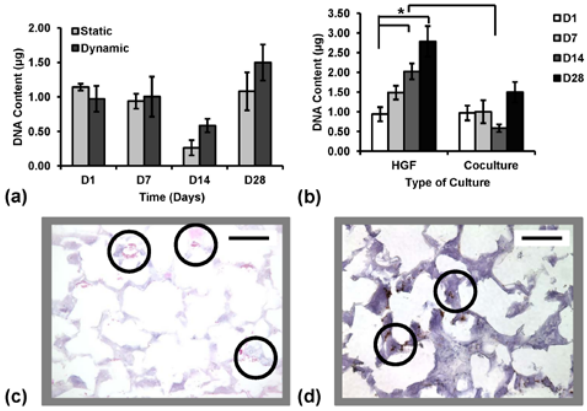

Figure 1. DNA mass from (a) static and dynamic co-cultures of 4 weeks. (n=2, ±standard error (SE)) and (b) dynamic HGF monoculture vs. dynamic co-culture. *Significantly different from each other ($p<0.05$, n=9 for monoculture, n=2 for co-culture, ±SE). (c) H&E and (d) CD31 staining of D28 dynamic co-culture sample. Early lumenal structures are shown with circles. Scale = 200 μm.

Conclusions: HGF-HUVEC co-cultures in perfused D-PHI scaffolds exhibit enhanced growth and capillary formation, demonstrating the potential of this construct for tissue engineering gingival tissues in highly perfused conditions. Ongoing studies include characterizing both cell types, including measuring the production of collagen and angiogenic factors (e.g. TGF-β1, VEGF), as well as optimizing the culture conditions and seeding ratio to maximize cell growth and capillary formation.

References: 1. US CDC. The Burden of Oral Disease. 2011. 2. Moscato S. Micron. 2008;39:569–79. 3. McBane JE. Biomaterials. 2011;32:6034–44. 4. Cheung JWC. Acta Biomaterialia. 2012. (Submitted). 5. Sorrell JM. Cells Tissues Organs. 2007;186:157–68. 6. Sharifpoor S. Biomacromolecules. 2009;10:2729-39. 7. Castellot Jr JJ. J Cell Biol. 1981;90:372–9.

Acknowledgements: NSERC Discovery Grant (360520), NSERC CGS D3, CellSignals Training Fellowship (TGF-53877).