The Effect of Culture Medium in the Co-culture of Human Gingival Fibroblasts and Vascular Endothelial Cells in a Perfused Polyurethane (D-PHI) Scaffold

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Introduction:

Periodontal diseases affect nearly 50% of Americans 30 years and older[1]. Gingival tissue engineering is a potentially advantageous approach for reconstructing lost gingival tissue and overcomes the problem of graft donor site morbidity. Human gingival fibroblasts (HGF) have been used previously to reconstruct the gingival lamina propria and mediate epithelial cell morphogenesis but gingival tissues need to be well-perfused for metabolic exchange and cell viability. Accordingly, perfusion systems and methods to promote angiogenesis should be considered for tissue-engineered gingival constructs. While the reconstruction of gingival lamina propria with tissue constructs has been described[2], the lack of a suitable perfusion system or the development of capillary-like structures, has limited cell and tissue viability. Polyurethane hydrogels (D-PHI) have been used to inhibit inflammation post-implantation[3] and facilitate the proliferation and production of collagen by HGF[4]. Co-cultures of endothelial cells with fibroblasts in synthetic materials can support capillary formation[5] however the choice of culture medium must be optimized for each unique cell source and culture environment. Our objective was to investigate the effect of culture medium in the co-culture of HGF with human umbilical vein endothelial cells (HUVEC) in a perfused bioreactor system in promoting growth, metabolic activity and capillary formation in D-PHI scaffolds.

Materials and Methods:

D-PHI scaffolds were fabricated via the free radical polymerization as described[6]. The cylindrical scaffolds were 2 mm thick and 6 mm in diameter; the pore size[6] ranged from 30–250 µm. 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 up to 28 days with media changed every other day. The samples were either cultured in a 50/50 mix of DMEM and F-12 K Medium (by volume), or in DMEM only. DNA content was quantified with Hoechst 33258; metabolic activity was measured with a WST-1 assay. Histology (H&E) and immunohistochemistry (CD31) were conducted to assess possible capillary formation. Results in dynamic co-cultures were compared with HGF monoculture with flow.

Results and Discussion:

Over a culture period of 28 days, the DNA mass (**Fig. 1a**) in the two co-culture systems remained relatively constant up to day 14. As the culture period progressed from day 1 to day 28, the co-culture in 50/50 mix media had a 3.6±1.2 fold increase in DNA mass ($p=0.07$) while the co-culture in DMEM only had a 1.8±0.7 fold increase. Interestingly, the co-culture in 50/50 mix media showed a substantial drop in metabolic activity (**Fig. 1b**) after day 1. The metabolic activity for co-culture in DMEM-only showed a 3-fold decrease to a base line value. From **Fig. 2**, the growth profile in the 50/50 co-culture was different from that in the HGF monoculture in DMEM. These data indicate that HGF alone in perfused culture retained the capacity to grow throughout the culture period. However, when co-cultured with HUVEC, intercellular interactions between HGF and HUVEC appear to

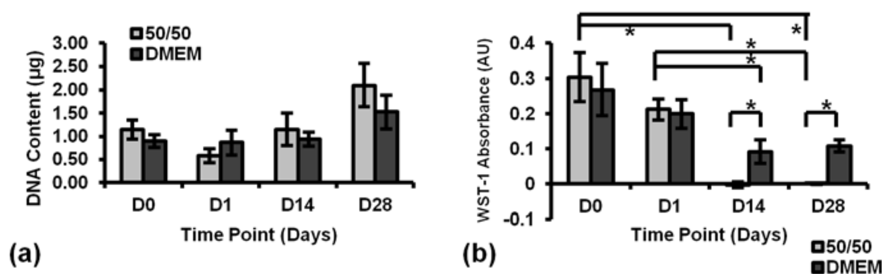

Figure 1. (a) DNA mass and (b) metabolic activity from co-cultures in 50/50 mix media vs. DMEM. *Significantly different from each other (p<0.01, n=6, ±standard error (SE)).

regulate cell growth and may allow for other cellular events. For example, during capillary formation, contact inhibition may have occurred between HUVEC and HGF as well as between HUVECs in the capillary structure via gap junctions[7], which resulted in a slower cell cycle and decreased metabolic activity. H&E staining showed early formation of lumenal structures in the 50/50 co-culture at day 28 (**Fig. 3a**), which were also positive for CD31 (**Fig. 3b**), indicating that such features were likely formed by HUVEC. Current work is being carried out to examine whether similar lumenal structures are observed in the DMEM-only co-culture. In addition, components in the two culture media are being considered for their role in regulating cell growth (e.g. heparin has been shown to regulate fibroblast proliferation[8]).

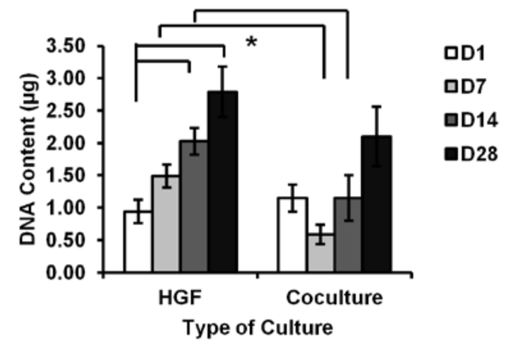

Figure 2. DNA mass from HGF monoculture vs. 50/50 co-culture. *Significantly different from each other (p<0.05, n=9 for monoculture, n=6 for 50/50 co-culture, ±SE).

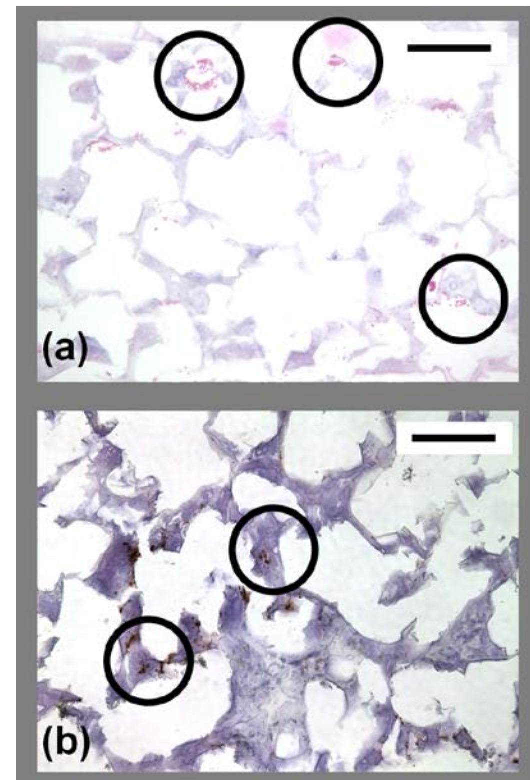

Figure 3. (a) H&E and (b) CD31 staining of D28 50/50 co-culture sample. Early lumenal structures are shown with circles. Scale = 200 µm.

Conclusion:

HGF-HUVEC co-culture in perfused D-PHI scaffolds with a 50/50 mix of media exhibit enhanced growth compared to co-culture in DMEM. Lumenal structures were observed in the 50/50 co-culture, which supports the potential of this co-culture system to produce a tissue-engineered construct for regenerating the gingival connective tissues in highly perfused conditions.

References:

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