Assessment of Adhesion and Growth of Human Gingival Fibroblasts on a Degradable-Polar Hydrophobic Ionic Polyurethane (PU)

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Statement of Purpose: Periodontal disease such as periodontitis affected over 40% of Americans over the age of 20 between 1999 and 2004[1]. Gingival tissue engineering is a preferred approach over conventional methods such as grafting and surgical procedures for repairing damaged gingiva in that it addresses the problem of tissue shortage and donor site morbidity. In gingival tissue regeneration, human gingival fibroblasts (HGF) have been used to regenerate the lamina propria and mediate epithelial cell morphogenesis. Among the synthetic scaffolds used for tissue engineering to date, polylactic/ glycolic acid has been explored the most extensively; however its degradation products have been reported to often be pro-inflammatory[2]. Previous studies showed that a degradable-polar hydrophobic ionic (D-PHI) PU was non-cytotoxic, promoted wound healing and improved the wettability of the scaffold for enhancing cell seeding[3]. This study investigated the adhesion and growth of a HGF cell line on the D-PHI PU scaffold. The objective of the study was to evaluate the viability, morphology, proliferation, metabolic activity, and protein secretion of HGF on the scaffold.

Methods: Divinyl oligomer (DVO) was synthesized by reacting polyhexamethylene carbonate diol with lysine diisocyanate and 2-hydroxyethyl methacrylate in a 1:2:2 ratio. D-PHI PU scaffolds were fabricated via free radical polymerization which involved the mixing of DVO with methacrylic acid and methyl methacrylate in a 1:5:15 ratio. Benzoyl peroxide was used as the initiator. Poly(ethylene glycol) and sodium bicarbonate were used as the porogens[3] and the pore size[3] ranged from 30–250μm. The resulting mixture was molded into discs and cured at 110°C for 24 hrs. The scaffolds were subjected to sonication in water for 14 days for porogen leaching. HGF-1 cells (from ATCC) were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% antibiotics. HGF were seeded onto D-PHI PU scaffolds at 40000 cells/scaffold for 24 hrs. The seeded scaffolds were cultured for 14 days with media changed every other day. At days 1, 7 and 14 after seeding, Live/Dead viability staining (confocal microscopy) was done to confirm cell viability and visualize cell morphology. Cell morphology was also investigated using scanning electron microscopy (SEM). DNA mass was quantified at each time point using Hoechst 33258. A water soluble tetrazolium (WST)-1 assay was carried out to evaluate the HGF metabolic activity. Western blotting was used to measure the secretion of α-smooth muscle actin (α-SMA) and vimentin as HGF's phenotypic markers.

Results: Over a culture period of 2 weeks, Live/Dead viability staining showed that HGF remained viable and the cell population increased as time progressed. DNA mass data showed that the HGF population increased by 2.3±0.4 fold in 2 weeks (**Fig. 1a**). The increase in metabolic activity (2.6±0.8 fold, **Fig. 1b**) also agreed with this increase. Both of these values showed that HGF continued to proliferate on the scaffolds. As time progressed, the growth rate of HGF (**Fig. 1a**) decreased, most likely due to limited diffusion of nutrients in static culture. SEM images (**Fig. 2a and b**) showed that the spindle-shaped HGF were well spread across the scaffold surface and cell prostheses were extended into the scaffold's pores, indicating HGF's adhesion to the scaffold. Results from Western blotting (data not shown) demonstrated that HGF residing on the scaffolds secreted α-SMA and vimentin, indicating that HGF did not undergo phenotypic change. These results further demonstrated the capability of the D-PHI PU scaffold to be applied for gingival tissue regeneration.

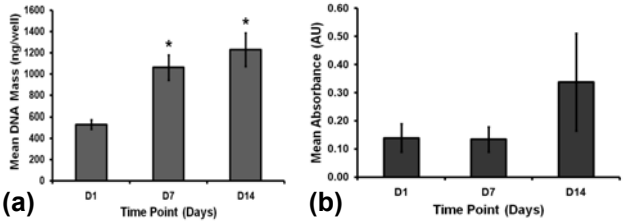

Figure 1. (a) Mean DNA mass reported at days 1, 7, and 14. *Significantly higher DNA mass compared to day 1 (p=0.002 (D14), p=0.004 (D7)). (b) Metabolic activity (WST-1, mean absorbance) at days 1, 7, and 14. n=3, ±standard error.

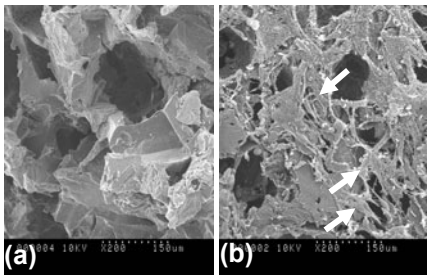

Figure 2. (a) SEM image of the D-PHI PU scaffold without cells (control). (b) SEM image of HGF (white arrows) on D-PHI PU scaffold at day 14.

Conclusions: The results presented here suggest that D-PHI PU scaffolds support the adhesion and growth of HGF. On-going studies include culturing the seeded scaffolds under media perfusion to improve nutrient/waste transport. Tissue-engineered gingival tissue from future work may be used as a test model for studying wound healing therapies, mucotoxicity of various components, and the biocompatibility of new dental materials, in addition to providing a grafting tissue.

References: 1. United States Centers for Disease Control and Prevention. Vital and Health Statistics. 2007;11(248):63-99. 2. Yang, *et al*. Tissue Eng. 2001;7(6):679-689. 3. Sharifpoor, *et al*. Biomacromolecules. 2009;10(10):2729-2739.

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