Characterization of Human Gingival Fibroblasts on a Degradable Polar Hydrophobic Ionic Polyurethane (D-PHI)

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Statement of Purpose: Periodontal disease 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 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. HGF mainly produce collagen in the lamina propria and some of their phenotypic markers include type I collagen, α-smooth muscle actin (α-SMA) and vimentin. Among the synthetic scaffolds used for tissue engineering to date, polylactic/glycolic acid has been explored the most extensively; however in some conditions its degradation products have been reported to be pro-inflammatory[2]. Studies with an alternative polycarbonate-based scaffold (D-PHI) showed the material to be non-cytotoxic, promoted non-inflammatory character in monocytes, and improved the wettability of the scaffold for enhancing cell seeding[3]. A recent study also showed that D-PHI scaffolds support the adhesion and growth of an HGF cell line. The objective of the current study was to characterize HGF on D-PHI scaffolds by quantifying soluble collagen production, α-SMA and type I collagen expression, and secretion of vascular endothelial growth factor (VEGF).

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 scaffolds were fabricated via the free radical polymerization 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 (2mm thick, 6mm diameter) and cured at 110°C for 24 hrs. The scaffolds were subjected to sonication in water for porogen leaching. HGF-1 cells (from ATCC) were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% antibiotics and were seeded onto D-PHI 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, soluble collagen production was quantified using picrosirius red staining and the results were normalized to the DNA mass (quantified using Hoechst 33258). Western blotting was used to measure the expression of α-SMA and type I collagen as HGF's specific phenotypic markers. The conditioned medium at each time point was collected and enzyme-linked immunosorbent assay was used to quantify the secretion of VEGF.

Results: Over a culture period of 2 weeks, the DNA mass showed that the HGF population on D-PHI scaffolds increased by 2.3±0.4 fold in 2 weeks (**Fig. 1a**), which provided evidence that D-PHI scaffold supported the growth of HGF. Between days 1 and 7, the soluble collagen production decreased significantly (0.142±0.043 mg/μg vs. 0.025±0.008 mg/μg respectively, **Fig. 1a**). A slight increase was observed at day 14 relative to day 7. The decrease in collagen was possibly the result of the HGF being in a proliferative (**Fig. 1a**) and migratory state (observed in histological analysis, images not shown here). Western blots (data not shown) demonstrated that HGF residing on the scaffolds expressed α-SMA and type I collagen, indicating that HGF remained functional on D-PHI scaffolds. Since the gingival tissue is highly perfused, it is desired to induce capillary formation in the tissue engineered construct. However, the monoculture of HGF will not sustain this as VEGF secretion from HGF monoculture showed a significant decrease after day 1 (**Fig. 1b**). It is possible that the presence of other growth factors produced by HGF such as transforming growth factor-β1[4] may have an autoregulatory effect on VEGF release. Thus further study is planned to examine how these factors affect VEGF release and to explore strategies and the design of a bioreactor to increase its value.

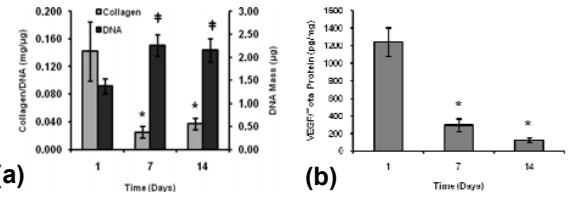

Figure 1. (a) Collagen production from HGF on D-PHI scaffolds reported at days 1, 7, and 14. *Significantly less than day 1 ($p<0.05$, n=3); ‡ significantly greater than day 1 ($p<0.01$, n=9); ±standard error. (b) VEGF secretion from HGF on D-PHI scaffolds. *Significantly less than day 1 (p=0.001); n=9, ±standard error.

Conclusions: The production of collagen and the growth of HGF on D-PHI scaffolds suggest that HGF remain functional. The results also demonstrated that HGF on D-PHI can release VEGF, an important factor for angiogenesis, but additional strategies will be needed to sustain its release. On-going studies include culturing the seeded scaffolds under media perfusion to improve nutrient/waste transport and investigating the effect of media perfusion on the phenotype of these cells.

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