Effect of Nanofiber Alignment on Human Periodontal Ligament Fibroblast Response

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Statement of Purpose: The Periodontal Ligament (PDL) is a connective tissue mediating tooth attachment to alveolar bone. The PDL is comprised of organized collagen fibres, embedded in the tooth root cementum at one end and to bone on the other. The importance of these tissue interfaces is evident as periodontal diseases which result in PDL degeneration leads to tooth loosening and often times complete tooth loss. Periodontal disease poses a significant health burden in the US and worldwide. In 2005 it was diagnosed in over 20% of American between 35-40.[1] Furthermore in 2011 nearly 25% of adults over 65 were reported to have lost all their teeth.[2] Current clinical periodontal therapies are variable and do not promote the regeneration of the periodontium complex[3]. A biomimetic scaffold with physiologically relevant architecture is advantageous for tissues such as the PDL, which exhibit matrix anisotropy or well-defined collagenous matrix alignment[4]. The **objective of this study** is to compare the response (morphology, growth, differentiation) of human PDL-derived cells cultured on aligned and unaligned polycaprolactone (PCL) nanofiber scaffolds. It is **hypothesized** that modulation of substrate architecture can be utilized to guide the growth and differentiation of PDL-fibroblasts towards the regeneration of the PDL and the associated periodontium complex.

Methods: *Scaffold Fabrication:* Aligned PCL scaffolds were fabricated by electrospinning an 18%(w/v) PCL in 3:2 dichloromethane (DCM, Sigma):N,N-dimethylformamide (DMF, Sigma) solution at 8-10kV. Nanofibers were collected on a rotating mandrel (2500rpm). Unaligned scaffolds were fabricated by electrospinning a 16% PCL in 1:1 DCM:DMF solution at 8-9kV. Nanofibers were collected on a stationary plate. *Cells & Cell Culture:* Human PDL cells were derived from explant cultures of healthy PDL (19y.o. F) obtained after tooth extraction. Prior to seeding scaffolds were incubated in DMEM+20% FBS to promote cell attachment. Cells (passage 4, 30,000 cells/cm^2) were cultured on the PCL aligned and unaligned scaffolds in DMEM+10% FBS, with monolayer culture on tissue culture polystyrene serving as a control. Cell viability (n=3) was assessed using Live/Dead. Live-stained images were analyzed for cell alignment (n=3) using circular statistics software[4]. Total DNA (PicoGreen), alkaline phosphatase (ALP) activity and collagen (hydroxyproline) were quantified (n=5). *Statistical Analyses:* A two-way ANOVA was performed and the Tukey-Kramer test was used for all pair-wise comparison at $p<0.05$.

Results: Well-organized elongated cells were observed on aligned nanofibers, while cells are disorganized on the unaligned substrate (Fig1). Overtime cells become more elongated and aligned, as indicated by a mean angle between cells and horizontal approaching 0, and a mean vector length approaching 1 (Fig1). Additionally multiple regions of well organized cells are noted on the unaligned nanofibers at Day 28. Cells proliferated on the scaffolds over time and growth was similar between groups (Fig1). Interestingly ALP activity was lower for both scaffold groups compared to monolayer. While collagen deposition was noted on scaffold groups at Day 28 (Fig2), no differences were observed between the groups.

Discussion & Conclusion: The architecture of a substrate (aligned versus unaligned) can impact PDL cell response. Cell spreading, alignment and morphology appear to be guided by the orientation of nanofibers, with an aligned substrate promoting cell spreading and organization. Nanofiber alignment did not affect cell proliferation or collagen deposition. Differences were noted in ALP activity at later culture times. As the PDL is composed of a well organized extracellular matrix it is anticipated that an aligned culture substrate is more biomimetic and may thus be superior for PDL regeneration. Future work will focus on elucidating mechanism of PDL cell-nanofiber interaction.

References: (CDC 2005, CDC 2011, Chen FM. Biomat. 2010;31:7892, Costa KD. Tissue Eng 2003;9:567, Ho SP. Biomat. 2007;28:5238)

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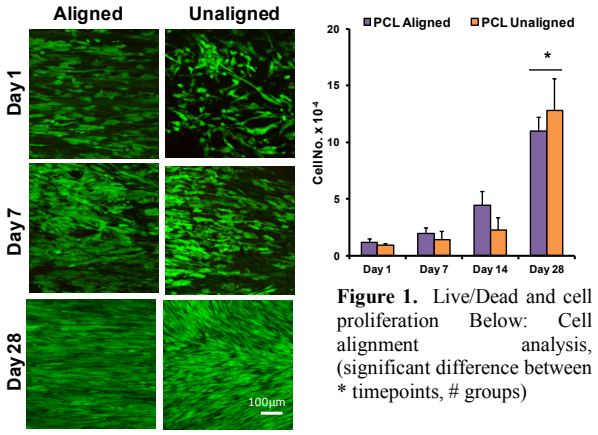

Figure 1. Live/Dead and cell proliferation Below: Cell alignment analysis, (significant difference between * timepoints, # groups)

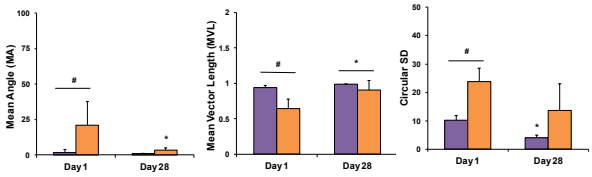

Figure 2. ALP activity and collagen deposition