Design and Optimization of a Cell-Instructive Hydrogel for Dental Pulp Tissue Engineering

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Statement of Purpose: Upwards of 15 million root canal procedures are performed annually in the United States[1] to treat pulp inflammation. However, this procedure is associated with a high failure rate[2] and results in devitalization of tooth upon extraction of the infected dental pulp. As a result, there is a demand for an alternative endodontic therapy to preserve tooth vitality and ensure long term dental health. To this end, regeneration of the dental pulp instead of a root canal is attractive as it focuses on viable endodontic therapy with the potential to restore normal tooth function. The **objective of this study** is to design and optimize a hydrogel scaffold for dental pulp tissue engineering. Specifically, the response of human dental pulp cells in a composite gel of polyethylene glycol and fibrinogen (PEG-F) will be determined as a function of fibrinogen concentration and culturing time. The PEG-F hydrogel is advantageous as its degradation is cell-mediated[3]. It is also injectable and has been shown to regulate cellular response using structural modifications[3]. It is anticipated that the PEG-F hydrogel will support pulp cell growth and biosynthesis, and cell migration and morphology will be regulated by fibrinogen concentration.

Methods: *Cells & Cell Culture* - Human dental pulp cells (P.6, explant culture) were seeded in PEG-F (10kDa) at three fibrinogen concentrations: 7.7, 8.5 and 9 mg/ml, photo-polymerized with 0.1% (w/v) Irgacure2959 under UV light (365nm), and maintained in fully supplemented medium with ascorbic acid. *Endpoint Analyses* - Samples were analyzed at 1, 7, 21, 28, and 42 days for cell viability (n = 2), cell proliferation (n=6), collagen content (n=6), alkaline phosphatase (ALP) activity (n=6) and corresponding histology including collagen type I and III (n=3). The expression of ALP and dentin sialophosphoprotein (DSPP) was determined using RT-PCR (n=3). *Statistical Analysis* - ANOVA and the Tukey-Kramer *post-hoc* test were used for all pair-wise comparisons ($p<0.05$ *over time, ^between groups).

Results: Changes in cell morphology and spreading was observed for all groups over time. Interestingly, the cell network was densest in the group with the highest fibrinogen on day 42 (Fig A). Cell number in all PEG-F groups decreased significantly on day 7 and stabilized overtime. By day 42, cell number in the 9 mg/ml group was the highest and it increased significantly over time (Fig. B). A significant increase in collagen content was found for all groups over time (Fig C). Interestingly, collagen per cell was significantly higher in the 9 mg/ml group by day 21 (Fig D). Similarly, the highest ALP activity was detected in the 9 mg/ml group on both day 28 and 42 (Fig E). In addition, immunohistochemical staining on day 42 showed that cells produced both collagen I and III in the PEG-F hydrogels.

Discussion: The results of this study suggest that the PEG-F hydrogel supports the phenotypic morphology of dental pulp cells, and the cells form denser interconnected networks with increasing fibrinogen concentration. Higher fibrinogen content also enhanced gel stiffness, likely due to the increased density of bioactive sites for cellular interaction[4]. Specifically, the 9 mg/ml PEG-F group measured the highest cell proliferation, collagen production, and mineralization potential over time. These results demonstrate that the PEG-F hydrogel is a promising scaffold for dental pulp regeneration, and 9 mg/ml is an optimal protein concentration for cell biosynthesis. Future studies will focus on scaffold optimization and *in vivo* evaluation of the scaffold for pulp regeneration.

References: **1)** American Dental Association Survey Center. Chicago: ADA; 2007 **2)** Friedman S. Oxford: Blackwell Science. 1998: 367-401**3)** Dikovsky D. Biomaterials.2006; 27:1496-1506 **4)**Shapira-Schweitzer K. Acta Biomater. 2007; 3(1):33-41

Acknowledgements: Royal Thai Government Scholarship (SP)

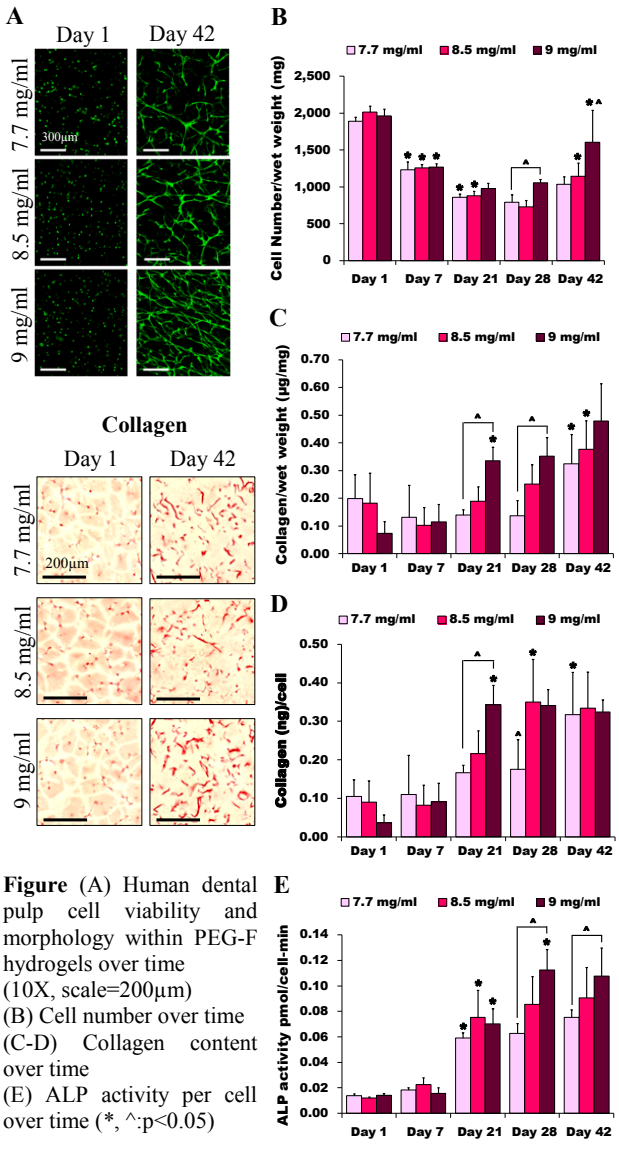

Figure (A) Human dental pulp cell viability and morphology within PEG-F hydrogels over time (10X, scale=200µm)
(B) Cell number over time
(C-D) Collagen content over time
(E) ALP activity per cell over time (*, ^:$p<0.05$)