Effect of Seeding Density on Human Dental Pulp Cell Response in Polyethylene Glycol-Fibrinogen Hydrogel

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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 in lieu 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 evaluate the potential of 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 cell density and culturing time. The PEG-F hydrogel system has previously been shown to modulate the migration and morphology of smooth muscle cells[3]. It is anticipated that the PEG-F hydrogel will support pulp cell growth and biosynthesis, and this effect will be enhanced with increasing cell seeding density.

Methods: *Cells & Cell Culture* - Human dental pulp cells (P.6, explant culture) were seeded in PEG-F (10kDa, 7.7mg/ml) and PEG-diacrylate (PEG-DA, 10kDa) at three densities: $1.6x10^6$, $3.2x10^6$, and $4.8x10^6$ cells/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 2, 7, 14, 21, 28, and 35 days for cell viability (n = 2), cell morphology (actin and DAPI staining, n=2), cell proliferation (n=6), collagen content (n=6), and alkaline phosphatase (ALP) activity (n=6). The expression of human dental pulp phenotypic markers (n=3) type I and III collagen, and dentin sialophosphoprotein (DSPP) was determined using RT-PCR. *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: Cells cultured in the PEG-F hydrogel remained viable over time and all cells, which were initially rounded, began to spread within the hydrogel over time. In contrast, a large number of dead cells were found within the PEG-DA hydrogels and, as expected, remained spherical over time (Fig A). Cell number decreased over time ($p<0.05$) in the PEG-DA hydrogel and was significantly lower than that of PEG-F groups. In contrast, no significant change in cell number was measured after day 2 in the PEG-F hydrogel for the 1.6 and 4.8 million density groups. Interestingly, for the 3.2 millions density group, cell number increased significantly by day 35 (Fig B). While no detectable collagen production was found in the PEG-DA hydrogels, a significant increase in collagen content was measured on day 35 for the PEG-F group with the highest seeding density (Fig C). The expression for DSPP, collagen I and III was detected in both PEG-DA and PEG-F hydrogels and DSPP gene expression of cells in PEG-DA was significantly higher on day 28 (Fig D). ALP activity remained at basal levels for all groups.

Discussion: The results of this study suggest that human dental pulp cells are able to maintain their morphology, viability and phenotypic response over time in PEG-F hydrogels. Spreading of pulp cells within the hydrogel matrix is likely facilitated by proteolytic degradation of PEG-F hydrogel which depends on enzyme protease diffusion through the hydrogel network[3]. The highest cell density within the PEG-F hydrogel also promoted the formation of a collagen-rich matrix, suggesting that there is a critical cell density for optimal biosynthesis in these hydrogels. These results demonstrate that the PEG-F hydrogel is a promising matrix for dental pulp tissue engineering. Future studies will focus on further optimizing cell response in this novel system.

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

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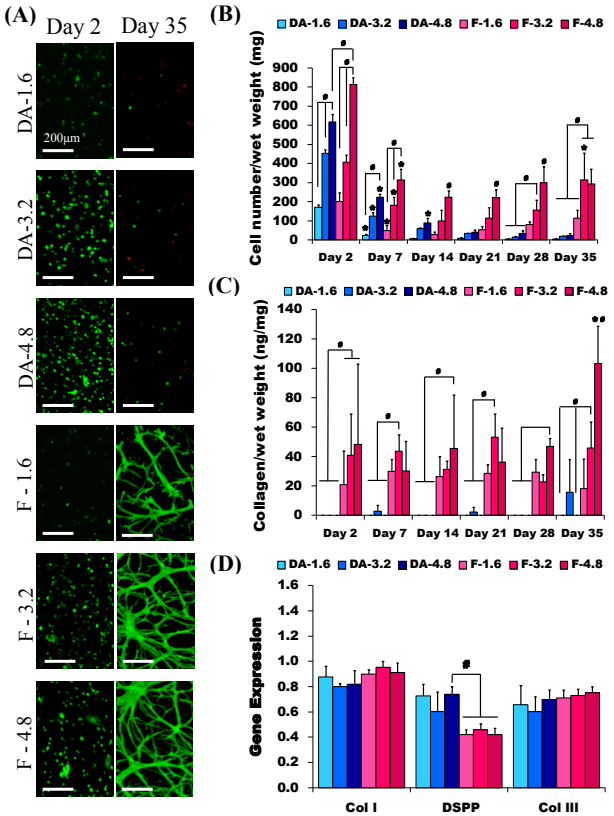

Figure (A) Human dental pulp cell viability and morphology within PEG-F and PEGDA hydrogels over time (10X, scale=200μm) (B) Cell number over time (C) Collagen content over time (D) Collagen types I and III, and DSPP gene expression on day 28 (*, #:$p<0.05$)