The Effects of Microenvironment on the Growth and Differentiation of Human Pulpal-Derived Stem Cells

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Statement of Purpose: The overall health of a tooth is heavily influenced by the dental pulp in the root, which contains not only contains the nerves and blood but also undifferentiated pulp cells. These pulpal-derived stem cells (PDSCs) cells can differentiate to replace damaged odontoblasts under the proper conditions.[1,2] However, while there have been many studies focusing on other adult stem cell sources, the effect that changes in the microenvironment can have on PDSCs has not been well studied. For instance, media content and changes in substrate stiffness have been shown to induce differentiation many stem cell sources, however, there has been little work studying these effects on dental cell differentiation. The goal of this study was to determine the effect of changes in substrate mechanical properties on PDSC differentiation in the presences of osteogenic growth factors.

Materials and Methods: PDSCs were extracted from human teeth and maintained in culture with mesenchymal stem cell growth medium (MSCGM). Polyacrylamide (PA) gels of varying mechanical properties were made by varying the ratio of acrylamide to bis-acrylamide. The elastic moduli of the gels were estimated using AFM nanoindentation data. The gels and control glass substrates were coated with fibronectin to promote cell attachment. 10,000 cells were placed onto control coverslips, 100 kPa PA gels, or 30 kPa PA gels coated with fibronectin, in either MSCGM, osteogenic differentiation media (ODM) or ODM with the addition of hydroxyapatite microparticles. An alkaline phosphatase (ALP) assay was performed over the first 3 days in culture. After one week the cells were immunostained for osteopontin, an important early osteogenic differentiation marker.[3]

Results: The PDSCs proliferated better on the coverslips than the PA gels. However, ALP production was greater for the cells on the PA gels (See Figure 1). In contrast, the cells on PA gels in MSCGM had greater ALP production than those in ODM (See Figure 1). In addition, cells on the PA gels began to form osteopontin-filled nodules after one week in culture, while those on coverslips did not (See Figure 2). The addition of HA microparticles did not seem to significantly affect the differentiation of PDSCs for these early time points.

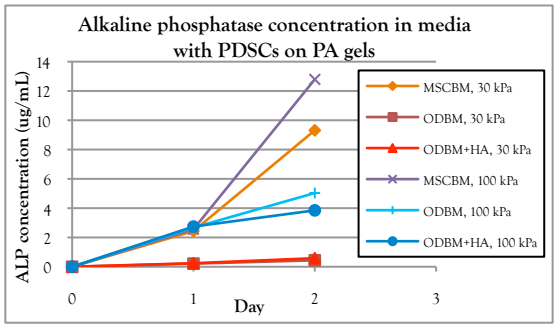

Figure 1: Results of initial ALP assays

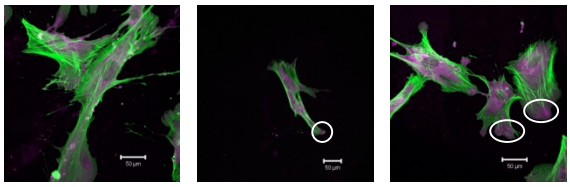

Figure 2: Pulp-derived cells in ODM on a coverslip, 100 kPa PA gel, and 30 kPa PA gel, respectively. Osteopontin is marked by the purple color and osteopontin-filled nodules are circled in white.

Conclusions: The osteopontin-filled nodules found in the cells on the PA gels, as compared to the coverslips, suggest that the decreased stiffness may be accelerating the differentiation process, which has been reported to take up to 6 weeks in literature.[4] In addition, the increased ALP production of cells in the MSCGM on the PA gels may indicate that it is indeed the stiffness of the gel, and not the induction media, which is leading the undifferentiated pulp cells along an odontoblast-like lineage. Future work will include repeating the outlined experiments over a longer time period to observe complete differentiation, along with performing the ALP assay for the full length of the experiments.

References: 1. Murray and Garcia-Godoy. Stem Cells and Development. 2004; 2. Magloire, et al. Journal of Experimental Zoology. 2008; 3. Tambasco de Oliveira, et al. The Journal of Histochemistry & Cytochemistry. 2003; 4. Liu, et al. Archives of Oral Biology. 2005.

Acknowledgements: Funding provided by the SC LIFE/HHMI grant, NSF GRFP, and NIH K25 HL092228