Matrix Degradability Contributes to the Development of Salivary Gland Progenitor Cells with Secretory Functions

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Statement of Purpose: Radiation therapy (RT) for head and neck cancer patients causes damage to the salivary gland acinar cells, resulting in xerostomia or dry mouth that does not have curative solutions. Salivary gland tissue engineering offers an alternative long-term treatment strategy. To this end, we cultured primary human salivary stem/progenitor cells (hS/PC) in hyaluronic acid (HA)-based hydrogels containing integrin-binding peptide RGDSP and varying proteolytic degradability (100% degradable, 50% degradable, and non-degradable). We observed a significant increase in the expression of stem cell markers *c-KIT* (a receptor tyrosine kinase) and keratin 5 (*KRT5*) and acinar markers in 50% and 100% degradable gels. We demonstrate that cell-mediated matrix degradability is necessary for the maintenance of stem and acinar phenotypes.

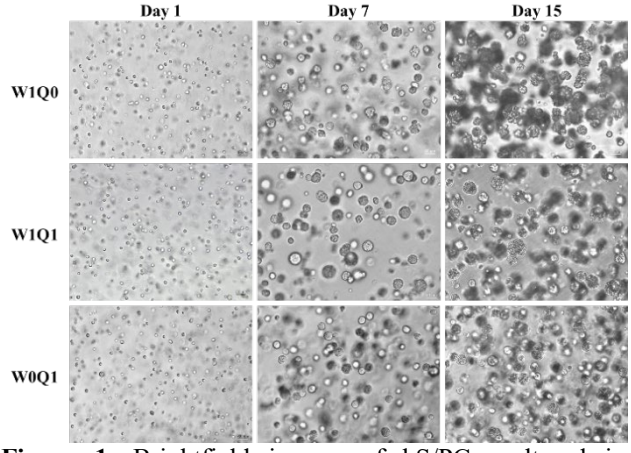

Figure 1. Brightfield images of hS/PCs cultured in hydrogels with different degradability.

Methods: hS/PCs were isolated from human salivary parotid gland tissues obtained from consented patients prior to RT[1]. Thiolated HA (HA-SH), maleimide conjugated RGDSP (MI-RGDSP), and maleimide-tagged protease degradable (PQGIWGQ) or non-degradable (GIQQWGG) crosslinkers (GIW-bisMI or GIQ-bisMI) were synthesized following our reported procedures[2]. Hydrogels were prepared by mixing ice cold HA-SH, RGDSP-MI, and bisMI crosslinker solutions at a SH/MI ratio of 1/1. Hydrogel degradability was tuned by varying the molar ratio of GIW and GIQ crosslinkers while maintaining the total bisMI concentration constant. The resultant gels are referred to as W1Q0, W1Q1, and W0Q1, corresponding to gels containing 100%, 50%, and 0% GIW-bisMI crosslinker, respectively. hS/PCs were encapsulated in the hydrogels at a concentration of 3×10^6 cells/mL and the constructs were cultured in HepatoStim media for 15 days. Constructs were subjected to real time reverse transcription-polymerase chain reaction (RT-PCR), immunofluorescence and ELISA analyses.

Results: The HA-based hydrogels exhibited an average storage modulus of 200 Pa, irrespective of the gel composition. Conjugation of RGDSP peptide through MI-SH reaction is highly efficient, with an estimated 95% conjugation efficiency[3]. hS/PCs cultured in these hydrogels remained viable and formed multicellular spheroids (Figure 1). As the matrix degradability increased, proliferation increased, the circularity of the spheroids decreased, the size of the spheroids increased, the spheroid forming efficiency increased, and cellular secretion of MMP1 and MMP2 increased. To investigate how hS/PCs secreted enzymes degrade the hydrogel over the culture period, we conjugated maleimide functionalized Cyanine 5 (Cy5-MI) to HA-SH and quantified the release of this fluorescent dye in the cell culture media using a plate reader. Our data shows that after 15 days of culture, 26wt% of the W1Q0 gel had been degraded by the resident hS/PCs. ELISA analyses further confirmed that hS/PCs released MMP1 and MMP2 proteins, which contributed to the hS/PC-mediated degradation of the matrix. Finally, we examined the cellular expression of stem cell and differentiation markers. We observed a significant increase in the expression of stem cell markers *c-KIT*, *KRT5*, and *CD44* in degradable hydrogels at the gene level. The degradable hydrogels promoted the secretary phenotype as observed by an increase in acinar markers amylase (AMY1A), aquaporin-5 (AQP5), aquaporin-3 (AQP3), and Na-K-Cl cotransporter (NKCC1).

Conclusions: In this work, we investigated the effect of matrix degradation on the expression of stem and differentiation markers in primary human salivary stem/progenitor cells. We demonstrate that cell-mediated matrix degradation is essential for the maintenance of stem/progenitor and acinar phenotype. This is a major step forward towards engineering an implantable gland with the potential to restore salivary gland function for patients suffering from xerostomia.

Acknowledgement: This work was supported by NIH (R01DC014461, R01 DE029655).

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3. Fowler E. W., et al, *ACS Biomat. Sci. & Eng.*, **2021**, under review.