Migration-Permissive Hydrogels As Biomimetic Matrices for Human Salivary Gland Engineering

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Statement of Purpose: For patients with head and neck cancer, standard care includes radiation treatments (RT), which are effective in improving patient survival. A side effect of RT is the collateral loss of the secretory units in the salivary glands. Thus, for these ~50,000 new patients/year in the United States, the loss of saliva and resultant dry mouth (xerostomia) significantly affects quality of life, and impacts oral cavity defenses from caries and other disease conditions.[1]

Tissue engineered therapies strive to recreate the structure and/or function of the salivary gland. Native salivary glands are highly branched, with differentiated cell function along the organs' length. Distal acinar cells generate fluid and salivary enzymes, and are selectively destroyed by RT. Primary human salivary stem/progenitor cells (hS/PCs) may be isolated from patient biopsies and/or discarded healthy tissue from tumor excision surgeries, but need low modulus substrates for 3D cuture.

We have demonstrated that hyaluronic acid (HA) hydrogel derivatives offer an optimally soft and supportive matrix for hS/PC culture.[2-4] Thiolated HA (HA-SH), crosslinked with poly(ethyleneglycol) diacrylate (PEGDA), enables hS/PC culture as encapsulated spheroids over weeks or months with high viability and progressive spheroid growth. However, despite these successes, HA/PEGDA matrices do not contain sufficient functionality, through labile matrix linkages or integrin-directed adhesion sites, to enable and promote hS/PC branching. Our work here describes the incorporation of matrix metalloproteinase (MMP)-labile crosslinkers and RGD-based adhesion peptides, at multiple hydrogel crosslink densities, to affect spheroid morphologies and enable co-cultures with other salivary-derived cell types that require migration-permissive matrices. We hypothesize that these elements may enable cell-directed remodeling of the HA matrix and reorganization into asymmetric multicellular clusters, and ultimately enable branched morphologies.

Methods: Primary hS/PCs were isolated from discarded surgical specimens of normal non-cancerous tissues outside of the margins of tumor resections, as we have described. HA-SH (>200kDa) and PEGDA (3.4kDa) were purchased from BioTime. The integrin ligand GRGDS (RGD) and the MMP-labile sequence KGGGPQG↓IWGQGK (PQ) were synthesized commercially, and reacted with amine-reactive SVA-PEG-acrylate to produce mono-functional RGD-PEG-Ac, and bi-functional Ac-PEG-PQ-PEG-Ac. hS/PCs were expanded in 2D culture, and resuspended in 1% (w/v) HA-SH in buffered aqueous solution. PEGDA or PQ-based crosslinker, and the RGD adhesion ligand, were added to the cell/HA suspension in varying molar concentrations to generate variations in hydrogel crosslink density and composition. hS/PC cultures were maintained to 21 days, and assessed via confocal microscopy for viability, proliferative capacity, morphology, and phenotype retention.

Results: hSPCs readily formed multicellular spheroids in both HA/PEGDA and migration-permissive variants (MMP-RGD-HA), and retained very high viability in both matrix systems. In systems with high crosslink density (6:1 SH:Ac ratio), hS/PCs were least likely to expand in 3D, or to form mature structures over time. Spheroid size increased as SH:Ac ratio increased (i.e. varied inversely with crosslink density). hS/PCs in all matrices expressed phenotypic markers including salivary enzyme α-amylase and progenitor marker keratin 5, and did not express ductal cell marker keratin 19. A fraction (<10%) of hS/PCs within each multicellular spheroid expressed proliferation marker Ki67.

The primary impact of matrix composition was observed in the morphology of hS/PC spheroids. Spheroids encapsulated in MMP-RGD-HA matrices were 3-4X larger over time than those in HA/PEGDA matrices. At the cell-ECM interface, hS/PC spheroids in MMP-RGD-HA matrices demonstrated a wrinkled morphology, with protrusions along the spheroid boundary. These observations suggest that the matrix modifications specifically enable cell invasion mechanisms at the spheroid periphery, and these can lead to increased hS/PC spheroid size and maturity.

Conclusions: Migration-permissive HA-based hydrogels support the survival and proliferation of hS/PCs in 3D culture. Installation of recognizable peptidic fragments within HA hydrogels can influence aggregate cell behavior, even after cells deposit their own peripheral basement membrane. Ongoing work is analyzing other cell phenotype markers to determine if, for example, cells at the periphery of each aggregate structure express partial differentiation markers, or a significant shift in the balance of E:N-cadherin expression in these cells.

References:

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