Tuning Engineered Hydrogel Matrix Composition for Salivary Gland Tissue Mimetic Culture

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Statement of Purpose: Approximately 80% of the 700,000 head and neck cancer patients diagnosed globally each year will receive radiation therapy. Chronic dry mouth, or xerostomia occurs in >50% of patients due to off-target salivary gland damage, leading to loss of acinar cell secretory function. Xerostomia causes chronic sore throat, difficulty eating or talking, dry, painful oral and nasal passages, and chronic oral infections. Existing treatments are palliative and there is no cure. Thus, radioprotective drug development is imperative for preventing this highly debilitating condition. Salivary gland cells (SGCs) rapidly lose secretory acinar phenotype *in vitro*, likely due to disruption of the extracellular matrix during dissociation, which eliminates cues required for proper cell function. To address this deficiency, SGCs were encapsulated and cultured in engineered extracellular matrices based on poly(ethylene glycol) (PEG) hydrogels [1]. This system provides tunable control of the matrix microenvironment by alteration of enzymatically-responsive peptide crosslinkers and presentation of small-peptide matrix epitopes [2]. We combined this approach with microbubble (MB) array technology, to develop a high throughput drug screening platform wherein cells are seeded in high density arrays of 15 nL spherical cavities molded in PDMS (polydimethylsiloxane). Within in the gel-MBs, SGCs organize into tissue mimetic structures which morphologically and functionally resemble acinar units of the salivary gland [1].

Methods: SGC clusters in hydrogel precursor solutions (2 mM 20 kDa 4-arm PEG-amide norbornene, dicysteine-functionalized peptide crosslinker, cysteine-functionalized matrix epitopes, and 0.05 wt% lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as the photoinitiator) were seeded into 96-well MB chips, and photopolymerized under 365 nm, ~5 mW/cm^2 (Fig 1A,B). Light microscopy images were taken at 0, 24, and 48 hr in culture for sphere formation analysis. Fluorescent calcium-flux response after treatment with 100 µM ATP or 1 µM carbachol was measured at day 7, and tissue lysates were retained for gene expression analysis by quantitative real-time PCR.

Results: Crosslinkers with lower MMP2 degradation rate promoted more and larger sphere formation (Fig 1C,D), while reducing cell outgrowth after prolonged culture. Gene expression analysis by qPCR showed increased expression of the saliva protein prolactin-induced peptide (Pip) in cultures with slower degrading crosslinkers, while expression of α-smooth muscle actin, a myofibroblast marker (Acta2) was elevated when using the fastest-degrading linkers. Hydrogels with slower degrading peptide crosslinkers increased the maximum and duration of the calcium flux response after stimulation with secretory agonists, carbachol and ATP (Fig 1E,F). Addition of the small-peptide matrix epitopes resulted in reduced sphere size (Fig 1C). Further, incorporation of an RGD-containing peptide reduced sphere formation and blunted calcium-flux response (Fig 1E, F). Examination of tissue structure by calcein-AM staining and confocal microscopy revealed significant differences in structural organization. Matrix epitope incorporation yielded branched, lobular structures reminiscent of salivary gland tissue (Fig 1G). Further, thermal representations of Calbryte-520 fluorescence intensity after stimulation with carbachol revealed highly localized responsive regions in tissues grown in epitope-containing hydrogels, as opposed to the diffuse response observed across tissue spheres grown in hydrogels without epitopes (Fig 1H).

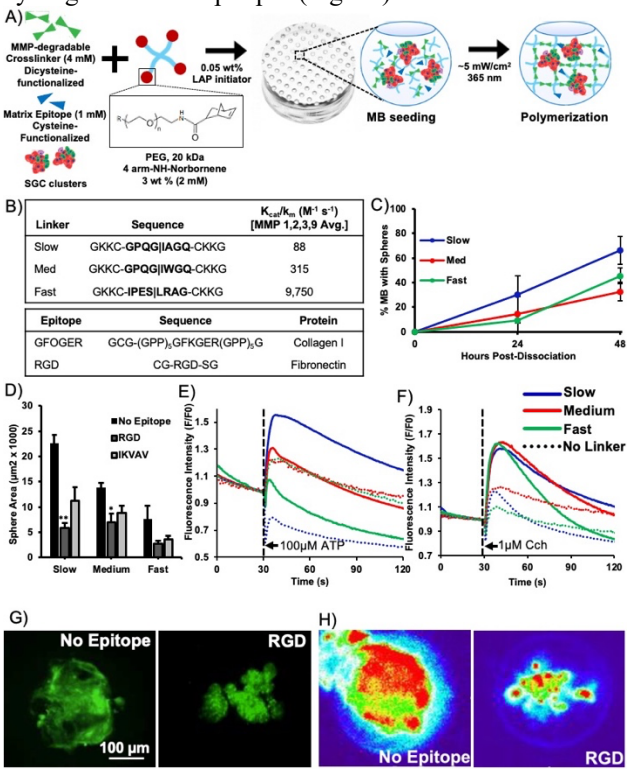

Figure 1: (A) Schematic of SGC-MB-gel assembly and (B) tested crosslinkers and epitopes. (C) Sphere formation and (D) size through 48hr. Normalized Calbryte-520 fluorescence after treatment with (E) carbachol and (F) ATP. (G) representative confocal images of live-cell staining via calcein-AM (H) representative thermal images of Calbryte 520 fluorescence intensity 15 seconds post carbachol stimulation.

Conclusions: These studies indicate that gel degradation rate and matrix epitopes presented within PEG hydrogels play a critical role in the organization and function of SGCs in the SGC-MB-gel system. Further optimization and characterization of responses to these key components is likely to be critical for the maintenance of acinar cell function and long-term culture of SGCs in MB-gel for studies of radiation damage and radioprotective drug screening. **References:** [1] (Y. Song et. al. Commun Biol. 2021. 4:361.) [2] (J. Patterson et. al. Biomaterials, 2010. 31(30):7836-7845).