Digital Analysis of Tissue-Assisted Salivary Cell Cultures in Biocompatible Hydrogels

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Objectives: Salivary glands (SG) experience progressive injury when exposed to repeat radiotherapy treatment for head-and-neck cancers, and chronic inflammation from conditions like Sjogren's syndrome. Regenerative engineering seeks to restore functional SG through encapsulation of healthy human SG stem/progenitor cells (hS/PCs) within hyaluronan (HA)-based hydrogels, and return of these composite structures to the site of injury. This process of ex vivo epithelial hS/PC isolation and expansion may be limited by the rate of cell proliferation in 3D hydrogels, and the ability to drive phenotypic organization without the inherent cues of the native SG microenvironment. Driven by our knowledge of epithelial-stromal interactions, and by the composition of the target site for reimplantation, we were inspired to co-encapsulate hS/PCs alongside SG microtissues within HA hydrogels, to determine if this hybrid culture system could impact positively the growth, organization, or function of hS/PCs via paracrine and/or juxtacrine signaling. We hypothesized that co-culture with these microtissues could accelerate desired features for neogland development.

Experimental Methods: hS/PCs (2-3 x10^6 cells/mL) were suspended within HA-SH solutions and crosslinked into hydrogels alongside small SG microtissues. Cell+tissue+gel composites (and cell+gel controls) were cultured over time (2-3 weeks) in humanized salivary media and assessed via fluorescent viability staining and confocal microscopy imaging as z-stacks. The captured z-stacks then were reconstructed within IMARIS software, and quantified digitally for changes in hS/PC cluster size, cell number per cluster, and cluster morphology.

Results: hS/PCs consistently formed larger structures when co-encapsulated with microtissues. IMARIS allowed for more detailed measurement of hS/PC cluster composition and 3D morphology than possible with conventional 2D imaging methods.

Conclusion: Tissue-assisted co-cultures promoted hS/PC growth and confirmed prior results of hS/PC co-culture with fibroblasts. These observations may assist in modeling *in vivo* biointegration performance or in *ex vivo* acceleration of cell growth, organization, and function prior to implantation.

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