UTHealth Houston
School of Dentistry

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

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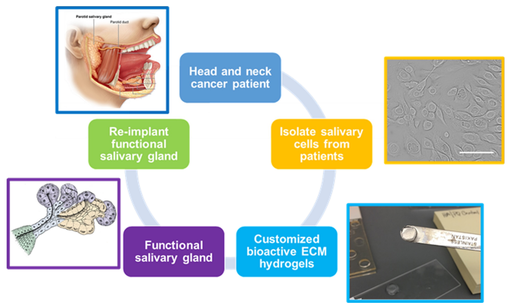
Introduction

- Head and neck cancer affects ~60,000 U.S. patients annually, and is commonly treated with surgery and radiation therapy (RT)
- RT often results in secondary, permanent damage to the adjacent salivary glands, resulting in prolonged xerostomia, hyposalivation, and associated sequelae such as dysphagia and increased oral caries

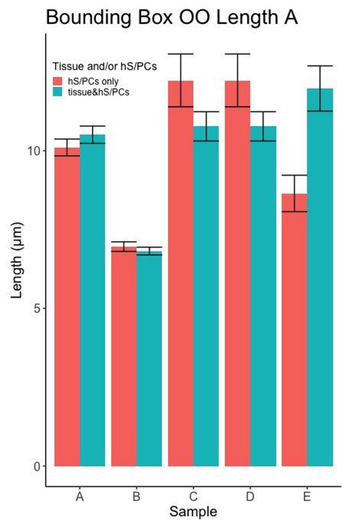
- Tissue engineering offers a potential therapeutic solution, by combining native healthy SG cells with a supportive hydrogel matrix to create a functional neogland

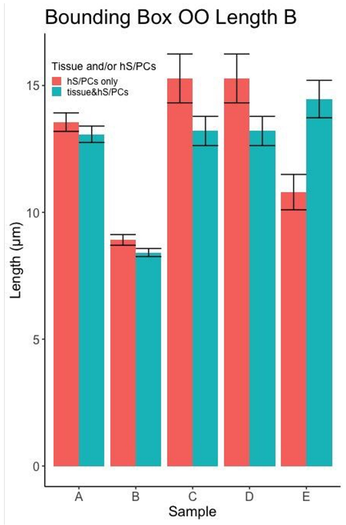


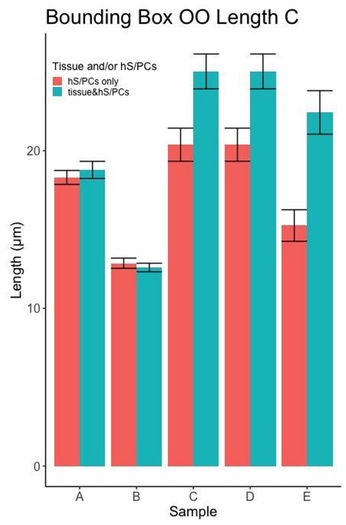
- High numbers of cells are needed to recreate these tissues, and primary tissue-derived cells can be slow to expand *in vitro*, especially in 3D matrices
- Native tissues expand readily during development, through the actions of cytokines produced by adjacent tissues

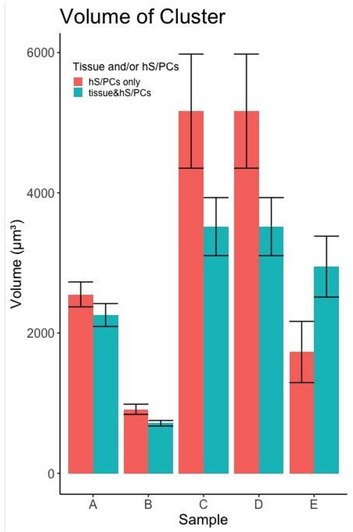
We hypothesized that proliferation of individual salivary cells within hydrogel matrices might be faster if intact tissue fragments were co-cultured nearby, to provide necessary cues

Results





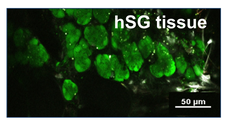


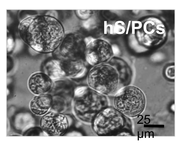


- The bounding box values of A, B, C were sufficiently different in each sample set to confirm that a spheroid model (A=B=C) would not adequately fit the samples
- HS/PC's encapsulated with SG tissue fragments were observed to form clusters with a larger single edge ("C"), but smaller opposing edges ("A", "B")
- Resultant hS/PC cluster volumes varied, without a clear trend or pattern

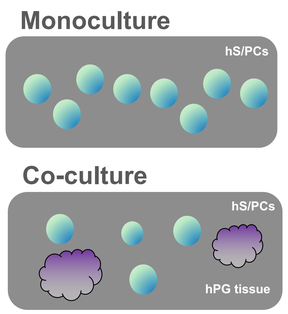
Methods – Cell Culture

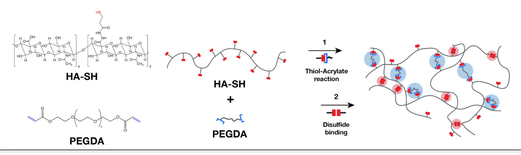
- Healthy human salivary gland (hSG) was obtained as discarded parotid or submandibular surgical tissues under IRB approval
- To isolate individual human salivary stem/progenitor cells (hS/PCs), hSG was minced and plated onto tissue culture plates; explant outgrowth was collected as hS/PCs and confirmed for phenotype
- Additional hSG tissue was minced and used for co-cultures





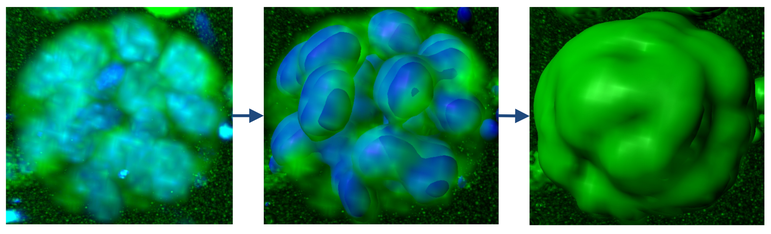
- Cells were encapsulated (2-3x10^6 cells/mL) within 3D hyaluronic acid hydrogels, either as hS/PC monocultures, or as tissue-assisted co-cultures of hS/PCs and hSG tissue fragments
- Cells and tissues within hydrogel "pucks" were incubated in salivary media for 2-3 weeks, and periodically stained for viability reagents, to identify live and dead cells, and cell nuclei
- Hydrogel pucks were imaged as Z-stacks by confocal microscopy and analyzed for changes in cell organization



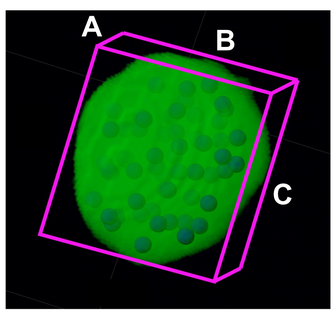


Methods – Image Analysis

- Clusters were stained for nuclei (blue) and cytoplasm (green)
- ImarisCell was designed to count subcellular components within a cell. It was used to quantify larger structures (clusters)



- To evaluate the size of clusters, two sets of parameters were calculated in Imaris:
  - The "**bounding box oo**" lengths were determined when the software constructed a minimum rectangular prism around each cluster (Bounding box OO length A < B < C)
  - Cluster **volumes** were obtained using the images shown above, through automated determination of a cluster surface, and integration of the space inside



Conclusions

- SG tissue fragments can be encapsulated within HA-PEGDA hydrogels, alongside hS/PCs, and sustained over extended *in vitro* culture.
- The irregular shape of multicellular hS/PC clusters implies that sphericity cannot be assumed when measuring cluster sizes.
- Imaris software can render the volume of hS/PC clusters from confocal Z-stacks, to provide the most accurate measure of cluster size.
- Bounding box dimensions provide additional information about the smallest possible bounding box to surround the object, and its dimensions.
- SG co-culture with hS/PCs yielded differing trends under this analysis, despite prior observations that co-cultures would yield larger hS/PC clusters
- Future efforts will include broader datasets, and reviewing in histograms to assess cluster distributions

Acknowledgements

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