Salivary Gland Tissue Engineering through Suspension Bath Bioprinting

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Objectives

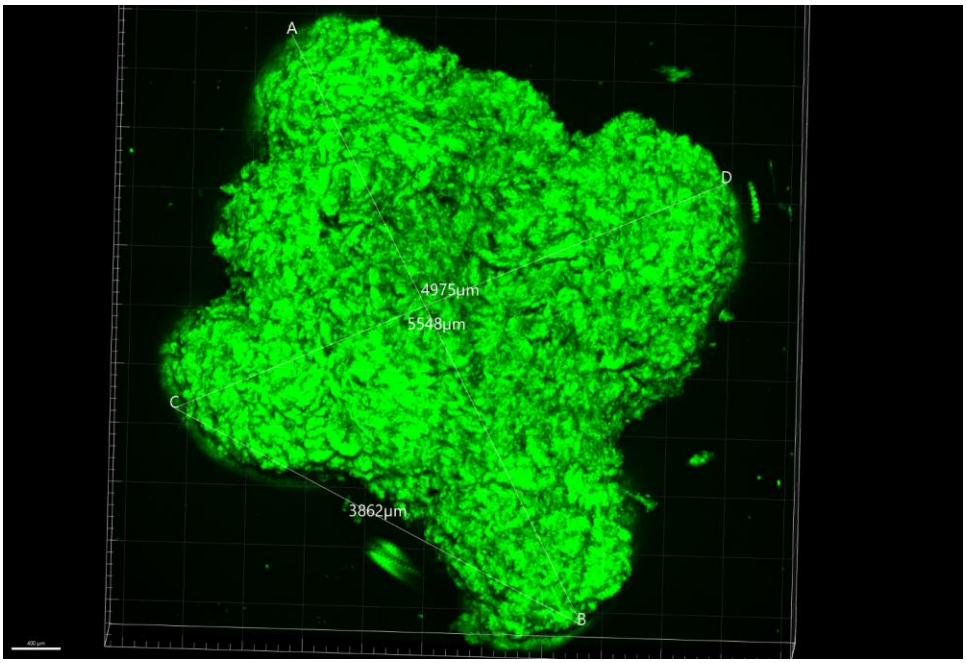
Bioprinted salivary glands (SG) offer a potential treatment for the >900,000 people (about half the population of Idaho) head-and-neck cancer who lose salivary function after standard-of-care radiotherapy treatment. However, hydrogel-forming bioink solutions cannot readily sustain printed shapes. Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting enables precise SG reconstruction, by extruding bioink into a thermoreversible gelatin microparticle support bath. Here we assess print parameters to optimize small structure fidelity.

Experimental Methods

Using Imaris software, we rendered scaled 3D SG models from externally-sourced z-stacks of E13 developing mouse SG. We sliced the models with Prusaslicer software, adjusting preslicing print parameters speed, layer height, overlap, and step motor movements to yield gcode. We doped 5% (w/v) sodium alginate bioink with 25% (v/v) fluorescein-alginate to provide contrast for imaging. We rehydrated commercial LifeSupport gelatin microparticles (20 µm) in $CaCl_2$ solution, and prepared the thick slurry as a support bath. A retrofitted Flashforge Finder extruded bioink along *xyz* coordinates from a flat-end syringe needle immersed in the bath. After sufficient gelation time we heated the bath to 37°C to melt the gelatin and release the alginate model. We imaged and reconstructed the bioprinted mimics via confocal microscopy.

Results

Optimized print parameters promoted interlayer adhesion, enabling production of a minimized 12X SG mimic (x:5.7mm, y:5.7mm, z:1.2mm) with distinctive position and size of each buddings reflecting the rendered 3D model. Elevated print speed, layer height, and overlap reduced model integrity and fine feature fidelity. Vertically-oriented models could be printed with an open interior lumen. Reconstructed fluorescence imaging revealed microparticle-induced surface textures on the model, but consistent reproduction of larger branched features.



Conclusion

Our study revealed the feasibility of bioprinting small, stable hydrogel structures via FRESH printing. Future work will focus on further reducing print size while improving resolution.

Acknowledgements
This study was supported by the UTSD Student Research Program.
K.I.S. was supported by NIH RISE-E-BASE grant R25GM127191. This work was funded by NSF CAREER grant 2240008 to D.A.H.