

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 μ m) in CaCl₂ 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.

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