Microfluidics-Based Coaxial 3D Bioprinting of Hydrogels for Salivary Tissue Engineering

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Objectives: Xerostomia can result from injury or disease to the SG, e.g., acinar death caused by radiation therapy (RT) for head and neck cancer. Only palliative treatments exist for xerostomia. Tissue engineering could offer a permanent solution for SG replacement by isolating healthy SG tissues prior to RT, expanding its cells *in vitro*, and recreating a functional neogland for implantation. 3D hydrogel encapsulation of primary human salivary stem/progenitor cells (hS/PCs) promotes self-assembly into organized acini-like spheroids with coordinated, functional response. 3D bioprinting potentiates spatial cell deposition into defined architectures and generation of extremely thin structures. Our goal is to bioengineer thin salivary epithelium with complex structure and functional responses using a microfluidics-based 3D bioprinter and customized hydrogels.
Experimental Methods: 3D structures were designed using CAD software, imported into *AspectStudio* to optimize printing pathways, and printed using a RX1 bioprinter with a DUO or a CENTRA printhead. On-printhead crosslinking was realized by 1.5% w/v sodium alginate, crosslinked using 20 mM $CaCl_2$. 6% w/v PVA served as the interior sacrificial core. Tube wall thickness was visualized by FITC-dextran using confocal imaging and measured by ImageJ. hS/PCs were mixed in hydrogel to investigate the effect of cell density on viability by live/dead assays and proliferation by Ki67 immunocytochemistry.
Results: Adjusting absolute and relative pressures for the alginate and crosslinker enabled control over fiber diameters, down to <100μm. Tube wall thickness ranging from 45-80 μm could be printed by matching the pressures of the hydrogel, the crosslinker and the core. Printing was down within minutes. Cell-laden, self-adherent tubes could be stacked in honeycomb-like patterns, mimicking salivary ducts. Bioprinted structures preserved structural integrity. hS/PCs remained viable and proliferation days after bioprinting.
Conclusions: Leveraging 3D microfluidics-based coaxial bioprinting, we established innovative hydrogels printing strategies, and fabricated thin, reproducible, and biocompatible hydrogel features that recapitulate characteristics of SG with high hS/PC viability.

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