Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting for stimulating epithelial growth in salivary glands

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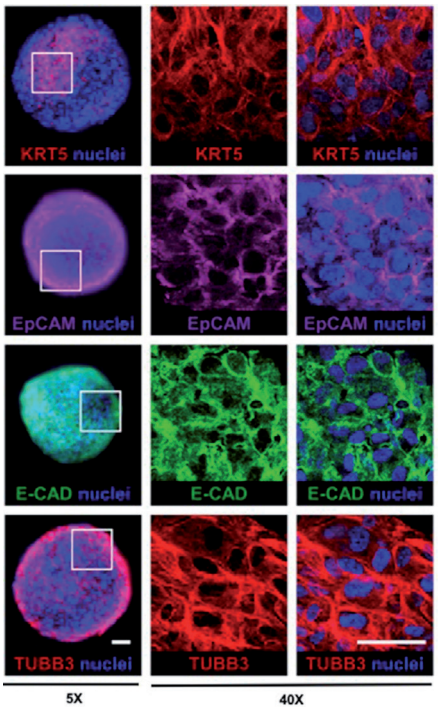
Abstract

Current saliva-based stimulation therapies for radiotherapy-induced xerostomia are not fully effective due to the presence of damaged secretory epithelia and nerves in the salivary gland (SG). Hence, three-dimensional bio-engineered organoids are essential to regenerate the damaged SG. Herein, a recently validated three-dimensional (3D) biofabrication system, the magnetic 3D bioprinting (M3DB), is tested to generate innervated secretory epithelial organoids from a neural crest derived mesenchymal stem cell, the human dental pulp stem cell (hDPSC). Cells are tagged with magnetic nanoparticles (MNP) and spatially arranged with magnet dots to generate 3D spheroids. Next, a SG epithelial differentiation stage was completed with fibroblast growth factor 10 (4e400 ng/ml) to recapitulate SG epithelial morphogenesis and neurogenesis. The SG organoids were then transplanted into ex vivo model to evaluate their epithelial growth and innervation. M3DB-formed spheroids exhibited both high cell viability rate (>90%) and stable ATP intracellular activity compared to MNP-free spheroids. After differentiation, spheroids expressed SG epithelial compartments including secretory epithelial, ductal, myoepithelial, and neuronal. Fabricated organoids also produced salivary a-amylase upon FGF10 stimulation, and intracellular calcium mobilization and transepithelial resistance was elicited upon neuro stimulation with different neurotransmitters. After transplantation, the SG-like organoids significantly stimulated epithelial and neuronal growth in damaged SG. It is the first-time bio functional innervated SG-like organoids are bioprinted. Thus, this is an important step towards SG regeneration and the treatment of radiotherapy-induced xerostomia.

Key features

- First report of a transplanted functional, innervated, salivary gland-like organoid produced through bioprinting from neural crest-derived, mesenchymal stem cells.
- Bioprinted organoids were transplanted into animals where they significantly stimulated epithelial and neuronal growth in damaged salivary glands.
- Demonstrate flexibility of applying magnetic 3D bioprinting for transplantation of SG-like organoids into the excretory ductal area of developing SG glands *ex-vivo.*
- Organoids significantly rescued epithelial growth on this acute SG fetal models of irradiation exposure.
- SG-like organoid model can be further used as a tool for cytotoxicity screening and mechanistic studies.
- A significant step in using magnetic 3D bioprinted cells to help advance tissue engineering towards translational applications in regenerative medicine.
- Bioprinting tool that can consistently control the size of the spheroids by tuning the concentration of MNP, cell number, and the size of the magnet dots.

Results



Whole-mount immunofluorescence staining of Salivary Gland (SG) spheroids

Quote

"Nanoshuttle was instrumental for our 3D bioprinting techniques to effectively produce spheroids on a reproducible manner with our adult stem cell cultures" said **Joao N. Ferreira, DDM, PhD** (Principal Investigator, BioprintME 3D Lab, Excellence Center in Regenerative Dentistry, Chulalongkorn University, Bangkok, Thailandand Clinical Research Fellow, NIDCR, National Institute of Health (NIH))

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