Bioprinting Ultrasound-Responsive Particles for Remote-Controlled Cellular Manipulation in Tissue Constructs

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Statement of Purpose: 3D bioprinting has gained considerable attention as a fabrication platform for in vitro models of cancer, due to the ability to create tissue architectures and biophysical parameters which better mimic the tumor microenvironment. Bioprinting techniques can be utilized to create tubular geometries which mimic tumor vasculature or the ductal structures of the mammary gland[1]. In order to model the complex dynamic signaling environment and oncogenic transformation associated with disease progression, techniques are needed to manipulate cells within the printed structures over time, without compromising the structure of the print. Focused ultrasound, in combination with ultrasound-responsive gene delivery microparticles, is a promising trigger for remote manipulation of cells within hydrogel constructs, due to its multi-centimeter penetration depth and capability to be tightly focused to small volumes in tissue[2].

Our study objective is to design a 3D bioprinting technique that allows uncompromised printing of ultrasound-responsive delivery particles within a cell-compatible bioink and enables focused ultrasound-mediated gene delivery to cells in the printed structure. We hypothesize that extrusion printing and subsequent activation of these particles is possible despite the pressure applied during the extrusion process, and can allow for ultrasound-controlled remote manipulation of printed cells.

Methods: A custom extrusion 3D bioprinter was loaded with a solution containing an alginate bioink precursor pre-mixed with lipid-based ultrasound-responsive microparticles. Coaxial printing was utilized via a 21G inner needle and a 17G outer needle with calcium chloride solution to promote crosslinking of the alginate. In a secondary setup, a Cellink Bio-X6 printer was loaded with a nanocellulose-alginate ink (Cellink) pre-mixed with ultrasound-responsive microparticles and used to extrusion print filaments which were crosslinked via calcium post-printing. At approximately 1 hr post-printing, constructs were exposed to focused ultrasound with the 1 mm^3 focal volume of the ultrasound beam centered within the hydrogel construct. For gene delivery experiments, HEK293T cells were incorporated within alginate bioink along with GFP-plasmid and ultrasound-responsive particles. Printed constructs were imaged via brightfield and fluorescence microscopy to evaluate particle integrity and cell transfection at 48 hr post-ultrasound exposure. Viability was assessed via calcein-AM and ethidium homodimer staining.

Results: Successful coaxial bioprinting of solid alginate hydrogel filaments containing the lipid-based microparticles was confirmed via microscopy and enabled fabrication of 3D grid patterns of the particle-containing filaments (Figure 1A)**.** The integrity of the microparticles was found to be uncompromised after extrusion through the printhead and the printed particles were well-dispersed throughout the filaments. Multilayer lattice prints were also achieved using extruded nanocellulose-alginate bioink containing microparticles (Figure 1B). In response to applied focused ultrasound, the particles were observed to rupture only within the area of ultrasound exposure, while leaving the remaining particles intact. Furthermore, HEK293T cells were added to the initial alginate bioink and successfully printed with GFP plasmid-coupled microparticles, with cells showing over 80% viability post-printing. Following ultrasound exposure, cell-laden 3D-printed constructs with the GFP plasmid microparticles showed GFP transfection localized to the ultrasound focal region.

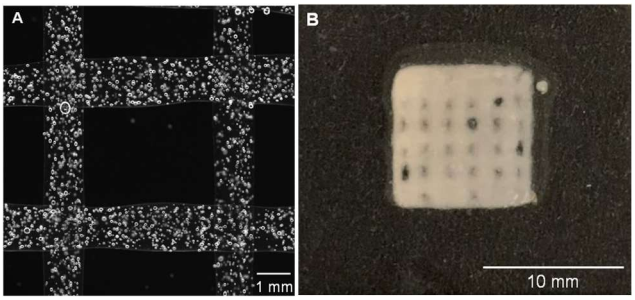

Figure 1. 3D bioprinting of ultrasound-responsive microparticles. **A.** Microscopy image of coaxially-printed grid of alginate filaments containing ultrasound-responsive microparticles (in white). **B.** Macro-scale image of a multilayer nanocellulose-alginate bioink extrusion-printed lattice construct containing responsive particles.

Conclusions: A 3D bioprinting method was developed to extrusion print ultrasound-responsive microparticles within alginate bioinks, allowing for noninvasive focused ultrasound-controlled particle activation and localized gene delivery within the print. This provides the first demonstration, to our knowledge, of a 3D-bioprinted structure designed to be genetically manipulated via ultrasound. Future work will leverage this remote-controlled gene delivery platform to induce site-specific oncogene expression within bioprinted tumor constructs and to investigate behavior and signaling of transformed cells in the 3D context.

References:

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