Creation of a 3D-bioprinted neuronal spinal cord scaffold

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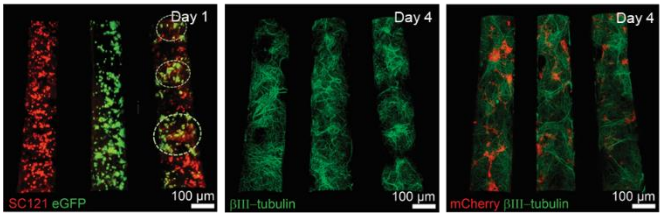
Introduction: The ability to utilize a 3D bioprinting platform to spatially control the placement of living cells and distribution of biomaterials could allow the construction of biologically complex microstructures beyond what is possible with conventional fabrication technologies. In 3D bioprinting, 3D structures are fabricated in a layer-by-layer manner to allow various combinations of cells, hydrogels, and biomolecules for 3D tissue models. Hence, the printed structures can faithfully fabricate skin, bone, cartilage, muscle, and peripheral nerve for disease modeling, drug discovery, and regenerative transplantation. Here, a bioengineered spinal cord-like tissue model is fabricated via extrusion-based multi-material 3D bioprinting, in which clusters of induced pluripotent stem cell (iPSC)-derived spinal neuronal progenitor cells (sNPCs) and oligodendrocyte progenitor cells (OPCs) are placed in precise positions within 3D printed biocompatible scaffolds during assembly. The bioprinted sNPCs differentiate and extend axons throughout microscale scaffold channels. Bioprinting OPCs in combination with sNPCs is a multicellular neural tissue engineering approach, where the ability to direct the position and growth of transplanted neural cells could be beneficial in rebuilding functional axonal connections across areas of tissue damage.

Methods: *(1) 3D printing process:* The 3D scaffold was printed by sequentially depositing scaffold ink and multiple cell-laden bioinks in a layer-by-layer manner to create multiple channels. First, the base layer of the scaffold was created via a continuous printing method, creating a flat surface upon which channels were subsequently printed. The volume of a printed single channel was ~ 150×300×5,000 (w×h×l) μm^3. Then, a cluster of cells – of a single type or multiple types – was deposited using a point-dispensing printing method with 200 μm center-to-center spacing within a channel. This process was repeated for the desired 3D scaffold architecture.
(2) *Cell-laden inks*: Human iPSC-derived sNPCs and mouse iPSC-derived OPCs were suspended at a concentration of 10^7 cells/ml in Matrigel™. Using a custom-made cooling system, we maintained the printing temperature at 4 °C and the printing pressure lower than 1 psi.
(3) *Scaffolds inks:* Acetoxy-based RTV silicone and alginate admixed with methylcellulose (AG/MC) were tested. The AG mixture consisted of 6% (w/v) of low viscosity alginate (Sigma-Aldrich) and 18% (w/v) of medium viscosity MC (4000 cP; Sigma) in DPBS. For cross-linking of alginate, $CaCl_2$ was dissolved in DPBS (20 mg/mL). The printing pressure of the inks was ~ 30 psi at room temperature.

Results: The precise spatial distribution of cell types in specific channels was demonstrated by separately dispensing sNPCs and OPCs. The left channel contained only sNPCs, the middle channel contained only OPCs, and the right channel contained both sNPCs and OPCs via separate dispensing of two different cell types within a channel of a scaffold (Figure 1a). Printed cells were detected along the channels. In the right channel, groups of sNPCs and OPCs were interspersed with a point-dispensing distribution resolution of ~ 200 μm. The ability to control cell type positioning in precise locations allowed for the modeling of native tissue architecture with multiple cell types. Figures 1b show representative scaffolds, where sNPCs were printed in different channels and cultured for 4 days. We observed that the progenitor cells proliferated rapidly and generated axons in the 3D space over a period of 4 days. These observations demonstrated that 3D bioprinted sNPCs have differentiated into neurons with extended axons propagating in a designed scaffold with ~ 150 μm (width) sized channels. Finally, a mixture of sNPCs and OPCs was printed onto the scaffold and cultured for 4 days (Figure 1c). The outgrowth of axons with the presence of associated OPCs was detected within the printed microchannels.



[**Figure 1.** (a) Distribution of cell types in specific channels: sNPCs only (left), OPCs only (middle), and sNPCs and OPCs (right). Groups of sNPCs and OPCs are interspersed with a distribution resolution of ~ 200 μm in a single channel (highlighted on the right channel). sNPCs are detected with human-specific antibody SC121 (red), and OPCs express eGFP (green). The image was taken 1 day post-printing. (b) sNPCs printed in a scaffold after 4 days of culture. Antibody to β3III-tubulin detects axonal projections in the channels. (c) sNPCs (green) and OPCs (red) co-printed in a scaffold after 4 days of culture. β3III-tubulin shows axonal projections down the channels, and the OPCs express mCherry.]

Conclusions: The platform introduced here can be used to prepare novel biomimetic scaffolds modeling complex spinal cord tissue architecture in vitro and could be harnessed to develop new clinical treatments to restore function after spinal cord injury.

References:

(1) D. Joung et al. Adv. Funct. Mater. 30, 1906237 (2020).
(2) D. Joung et al. Adv. Funct. Mater. 28, 1801850 (2018).