Fabrication of a 3D bioprinted perfusable vascularized model of Neuroblastoma

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INTRODUCTION

In biomedical research, 3D cultures and cell-laden hydrogel constructs play a crucial role by providing physiologically relevant models that offer valuable insights into cell behavior and fate. Additive manufacturing techniques, particularly 3D bioprinting, have revolutionized the field by enabling the precise fabrication of constructs and patient-specific patches. 3D bioprinting allows for the replication of the complex *in vivo* environment, which is especially significant in studying the tumor microenvironment (TME) and surpasses the limitations of 2D cultures or scaffold-free 3D approaches. Researchers have already developed various 3D bioprinted tumor models, such as slices, grids, fibers, mini-organs, and custom shapes.
However, to be clinically applicable, tissue constructs sizes must exceed those where diffusion alone is sufficient to support cell growth. To adequately supply nutrients to all cells within thick constructs, the creation of a vascular network within the cell-laden constructs is fundamental. We here propose a fabrication method based on sacrificial bioprinting, where two different bioinks are used to generate hollow structures embedded within a cell-laden matrix. We successfully created customizable 3D constructs that were endothelialized, perfusable, and supported cell culture for more than two weeks under continuous perfusion.

MATERIALS AND METHODS

Our optimized bioinks were two hydrogels, with 8% w/v methacrylate gelatin (GelMA) serving as the cell-laden matrix. GelMA possesses biocompatibility, biodegradability, and can be photo-crosslinked in the presence of agents like Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). Pluronic F-127 (PLU), a synthetic copolymer with different thermoreversible behavior from GelMA, was chosen as sacrificial ink. It liquifies below 15°C and forms a gel at higher temperatures. PLU is known for its excellent printability, particularly at higher concentrations (40% w/v) and is commonly employed as a sacrificial material.
To fabricate the cell-laden vascularized structures, we followed an optimized multi-material bioprinting protocol. This involved layer-by-layer construction of the tissue construct by alternating the printing of the two bioinks. Once the printing phase was completed, GelMA was exposed to UV light for crosslinking, while PLU was liquefied by refrigerating the structure at 4°C for 5 minutes. This liquefaction step created hollow channels within the structure, which would serve as conduits for nutrient delivery throughout the construct.
Subsequently, a co-culture of Human Umbilical Vein Endothelial Cells (HUVEC) and Human Bone-Marrow Mesenchymal Stem Cells (hMSC) (10 million cells/mL, with a ratio of 70% HUVEC and 30% hMSC) was seeded inside the channels to line their surfaces and replicate the endothelial barrier, which separates the flow of culture medium from the cells embedded in the surrounding matrix. Finally, to ensure a continuous supply of nutrients, the structure was connected to a perfusion circuit and cultured for up to 3 weeks.

RESULTS AND DISCUSSION

We have developed and produced a vascularized structure featuring a serpentine-like geometry embedded within a parallelepiped shape. The performance of this construct was validated through long-term perfusion culture and evaluation of vascular channel endothelialization. Specifically, we examined cell viability and morphology of SK-N-AS neuroblastoma cells and hMSCs, observing that perfusion culture significantly improved cell viability throughout the thickness of the construct, making this model an excellent and versatile tool for biomedical studies.
By optimizing the endothelialization protocol, we obtained a fully developed endothelial barrier within a short timeframe of 14 days, while using a relatively low seeding density of 10 million cells/mL. The co-culture of HUVEC and hMSC resulted in the formation of a monolayer that coated the entire surface of the vascular channel. The cells were correctly elongated and aligned along the direction of the flowing medium. Ongoing co-culture experiments aim to recreate an endothelialized tumor niche to investigate early-stage metastatic dissemination.

CONCLUSIONS

In summary, we successfully developed a vascularized 3D construct with a serpentine-like geometry embedded within a parallelepiped shape. This construct holds great potential for drug testing applications. Our study demonstrated improved cell viability under perfusion conditions, making it an ideal platform for studying cell proliferation and cell responses in drug screening experiments. The optimized endothelialization protocol allowed for the formation of a mature endothelial barrier within a short timeframe, enabling investigation into early-stage metastatic dissemination and interactions within an endothelialized tumor niche. Overall, our vascularized 3D construct provides a versatile and reproducible platform for drug testing, offering a physiologically relevant microenvironment for assessing therapeutic responses and advancing personalized medicine approaches.