Biofabrication of Vascularized Skin Tissue Models with DLP and Extrusion Bioprinters

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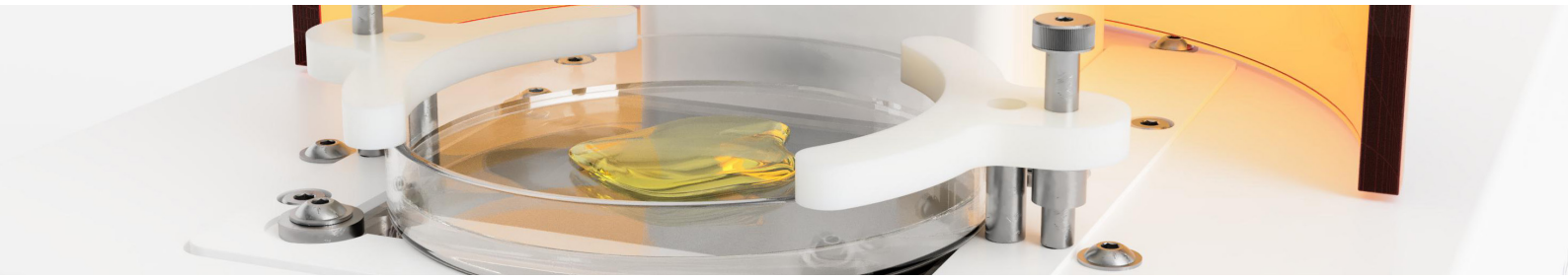

Abstract

With the capabilities of 3D bioprinting, scientists can begin to recreate the human body's microenvironments and enable novel discoveries that will provide a complete physiological understanding when developing clinical treatments. Extrusion-based bioprinting is a prevalent method for bioprinting given its low cost, wide variety of commercially available materials and ease of use. Digital light processing (DLP) printers are currently used industrially to print intricate objects, like jewelry or dental appliances, with high-precision and small feature sizes using photosensitive materials called photoresins. Here, an optimized workflow is presented that combines light-based bioprinting on the Lumen X+™ powered by Volumetric DLP bioprinter with the BIO X6™ extrusion-based bioprinter. By taking advantage of both techniques, researchers are able to include microscale feature sizes and multiple materials in more complex 3D bioconstructs. This technical note demonstrates the capabilities of the proposed workflow to 3D bioprint a vascularized skin tissue model. The Lumen X+ is used to create the vascular bed and the BIO X6 to bioprint the dermal and epidermal layers, in order to produce the niched microenvironments for each part of the vascularized skin model.

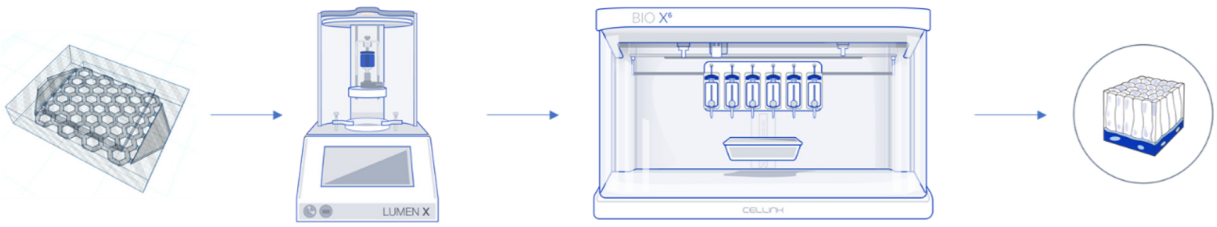

Figure 1. *Suggested product workflow to combine Lumen X+ DLP 3D bioprinter and the BIO X6 extrusion-based bioprinter to fabricate 3D tissue models with fine details as well as multimaterial components.*

Introduction

Growing objections to animal testing in the cosmetics and pharmaceutical industries have fueled research for safer, more ethical and more accurate alternatives (Kim, 2019). Commercially available human skin models are typically produced manually and consist of keratinocytes alone, or keratinocytes and fibroblasts (Kim). While these conventional models have enabled numerous discoveries (Niehues, 2018), key differences in structure and physiology impede them from supplanting animal testing (Kim, Niehues). The absence of sweat glands, hair follicles, and melanocytes, nerves and vasculature prevent a comprehensive *in vitro* assessment of the skin's response when exposed to chemicals, pathogens and environmental stimuli or disease (Kim; Maniță, 2020; Baltazar, 2020).

Bioprinted full skin tissue models could help lower the $27 billion spent annually on burn and wound care worldwide (Han, 2017). To develop the most effective skin treatments, these models have to closely replicate the *in vivo* environments of skin tissue, including vascularization. Lack of vasculature contributes to commercial skin grafts being rejected and falling off (Maniță; Baltazar; Varkey, 2019). 3D bioprinting is a means by which vascularization can be introduced to vastly improve skin models for *in vitro* and *in vivo* applications (Kim; Maniță; Baltazar).

Biological 3D printing enables scientists to develop more complex skin models and grafts with multiple cell types and vascularity to mimic several native processes, including cell-to-cell crosstalking and morphogenesis (Kim). These complex bioprinted skin models can reduce the costs of drug discovery and development, while providing results that better translate to patient treatments (Kim).

Presented here is a workflow that demonstrates how the advantages of two bioprinting techniques, extrusion and DLP, can be combined to create a vascularized skin model. The light-based Lumen X+ fabricates the more intricate vascular bed, while the extrusion-based BIO X6 dispenses the subsequent layers of cells to make up the dermis and epidermis.

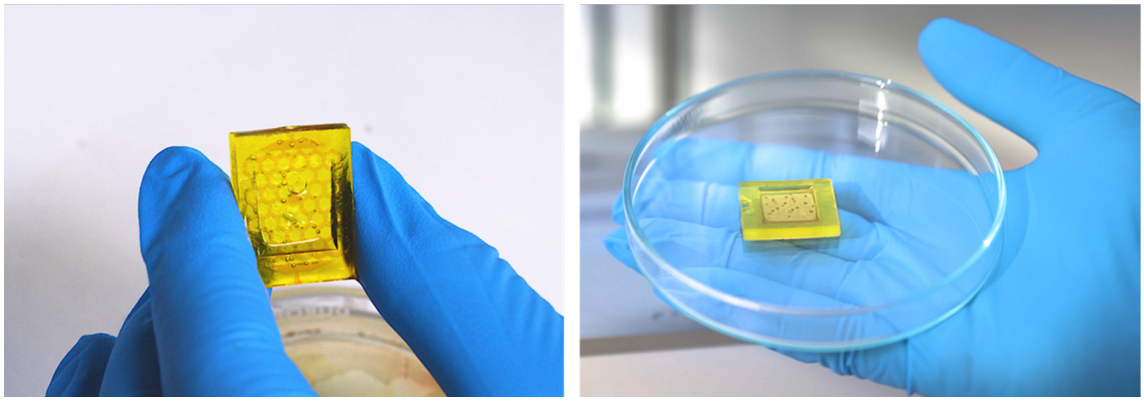

Figure 2. *Vascular bed (left) with channel network visualized in red and final construct (right) of skin model with dermal and epidermal layers.*

Materials and methods

Model design

The vascular bed was designed using commonly available CAD (computer-aided design) software, such as OpenSCAD. To this bed, an outlet was added at each end using TinkerCAD to merge all inlets and outlets to one. A cavity was also added at the top of the vasculature bed to allow for extrusion bioprinting of the dermal and epidermal layers. The cavity on top of the vasculature had the dimensions of 12.8 x 11.2 mm, and the outer dimensions of the vascular bed were 21.8 x 16 mm. The vascular network was designed to have a honeycomb geometry with channels of 800 µm in diameter. Although the Lumen X+ can generate vascular channels with diameters as small as 200 µm, the 800 µm diameter was selected to enhance construct visibility.

Fabrication

The vascular bed was bioprinted using the Lumen X+ DLP bioprinter and polyethylene glycol diacrylate PEGDA500 PhotoInk™ (a biocompatible photoresin). The Lumen X+ was selected because the intricate features of the microvasculature pattern required its precision. PEGDA500 PhotoInk is an advanced biocompatible and nondegradable photoresin designed specifically for use with the Lumen X+. Its robust mechanical properties allow for the creation of thin walls, microfluidic devices and advanced lattice structures with details down to 200 µm resolution, making it an ideal PhotoInk for the creation of drug delivery devices.

For a cell-laden vascular bed with enhanced biological resemblance, the recommended bioink would be GelMA PhotoInk, which contains methacrylated gelatin and provides native cellular cues for cellular proliferation and migration. In addition, GelMA PhotoInk provides a softer and more permeable bed for molecular exchange between compartments. This tech note demonstrates how the Lumen X+ is used to create a vascular bed that the dermis and epidermis are bioprinted onto. Using GelMA PhotoInk, the vascular bed can be cell laden with dermal fibroblasts and serve as vascularized dermis.

Table 1. *Printing parameters for BIO X6.*

	DERMAL LAYER	EPIDERMAL LAYER
PRINTHEAD	Standard pneumatic	EMD
NOZZLE/NEEDLE	22G 0.5 inch needle	Needleless nozzle
VALVE SIZE (µM)	-	300
PRESSURE (KPA)	65	15
SPEED (MM S-¹)	6	10
PRE-FLOW DELAY (MS)	200	-
OPENING/CYCLE TIME (MS)	-	10/500

A 3D CAD model in the form of an STL (standard tessellation language) file of the vascular bed was imported into the Lumen X+ from a USB drive and sliced using the Lumen X+'s integrated LightField software at the lower resolution of 100 µm. The higher resolution capacity of 50 µm was not necessary to use for this design. The Lumen X+ was loaded with 2.2 mL of the PEGDA500 PhotoInk and the power settings were set according to the protocol for PEGDA500 PhotoInk.

After printing, the construct was hydrated and carefully removed from the build platform using a sterile plastic razor blade. To clean the microvasculature of uncured resin, channels were flushed using a 12 mL syringe filled with water and equipped with a 22G needle. The vascular bed was then washed to remove the photoabsorbing dye and stored in water for a few days before the dermal and epidermal layers were bioprinted on top. The photoabsorbing dye is a nontoxic composition, and the storage step preformed within this technical note is optional. One can proceed immediately to the extrusion-based bioprinting step after a wash to remove uncured PhotoInk. However, to achieve high transparency for microscopy, the multiday post wash is strongly recommended. In addition, for cell-laden constructs, balanced buffers or cell media is recommended as washing solutions.

For bioprinting of the dermal and epidermal skin layers, the BIO X6 was equipped with a standard pneumatic printhead and an electromagnetic droplet (EMD) printhead. A two-layer rectangular patch, STL file with the dimensions of 12 x 10.4 mm, was processed using DNA Studio for the BIO X6 to set up a bioprint with rectilinear infill pattern, 40% infill density and 0.41 mm layer height. The first printhead was selected to bioprint the first layer, representing the dermal layer, and was set up with CELLINK Bioink and a standard pneumatic printhead. For the second layer, the second printhead, equipped with a EMD printhead, was selected to inkjet an epidermal cell suspension based bioink on top of the dermal layer.

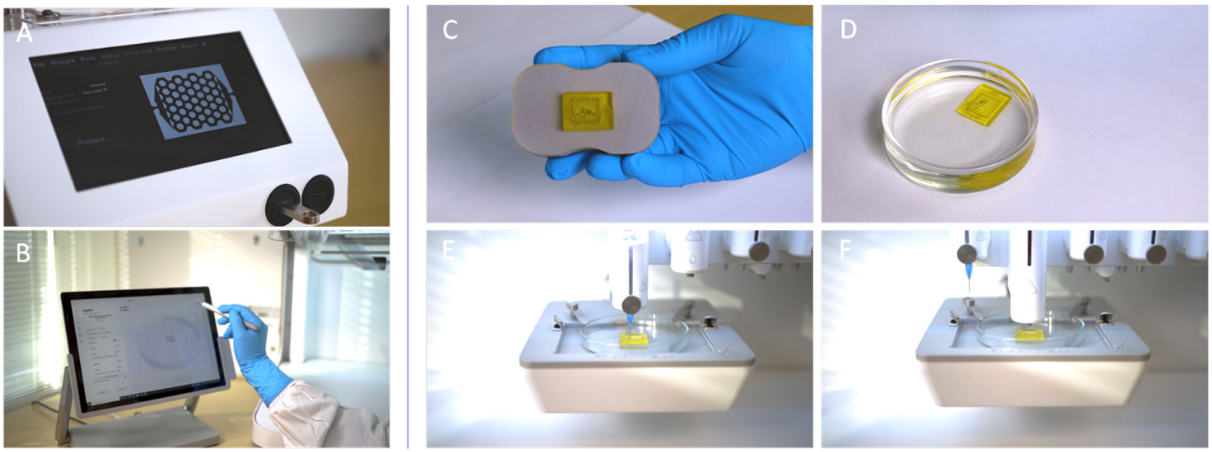

Figure 3. *Overview of the production of the vascularized skin tissue model. Visualization and setup of the model on the Lumen X+* ***(A)*** *and BIO X6* ***(B)****. Vascular bed after print* ***(C)*** *and wash* ***(D)****. Bioprinting of dermal layer using BIO X6 and pneumatic printhead* ***(E)*** *and epidermal layer using EMD printhead* ***(F)****.*

Having software that enabled easy selection of different printheads, and printing positions for these, was crucial for the model creation. The dermal environment was rich in extracellular matrix (ECM) proteins and benefited from having a sturdy but soft 3D environment. CELLINK SKIN bioink allowed for easy creation of the dermal microenvironment and was enhanced with fibrinogen to activate the native stimuli of skin regeneration. The epidermal layer mainly consisted of keratinocytes, and close cell-to-cell contact was essential for epithelial formation. Here, using a low viscosity bioink with high cell and protein content was critical. CELLINK's EMD printhead allowed for even, noncontact dispensing of liquid and low viscosity bioinks, using inkjet technology and was ideal for epithelial layer formation.

Summary

To make 3D *in vitro* models more representative of human biological systems, greater complexity and intricacy are required. For example, adding a vascular layer can shed light on the permeability of skin to compounds entering the blood stream. The closer the vasculature is to capillary size, the better the representation of oxygen, nutrient and compound exchange. This technical note demonstrates how the high resolution of DLP-based bioprinting can be combined with the versatile extrusion-based bioprinting technique to create a vascularized skin tissue model with a rich, dermal fibroblast niched hydrogel-based dermal compartment, a low viscosity, high cell concentration epidermal compartment and a highly complex vascular bed.

Introduction of dynamic culture conditions, vasculature and multiple matrices within one model has the capacity to better recapitulate the *in vivo* complexity of tissues. These advanced models can replace animal tests as well as assess skin irritation induced by chemicals, skin-cream-based drugs and cosmetics. These advanced skin models can also help avoid the misclassification of chemicals and skin corrosion observed in animal systems. Other applications include phototoxicity, percutaneous absorption and penetration, wound healing and metabolism.

References

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