Biofabrication of Bioreactors with DLP and Extrusion Bioprinters

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Abstract

Standardizing tissue and biological manufacturing for cellular grafts, artificial organ replacements and biochemicals requires a controlled and reproducible *ex vivo* tissue growth culture that accurately mimics *in vivo* environments. Bioreactors can create these physiologically relevant environments and can be customized to specific microbes (eg, cell types or bacteria) for optimized 3D microbiological and tissue culturing. But finding a time- and cost-efficient protocol for producing bioreactors has remained a challenge until now. This technical note proposes a workflow solution for designing and fabricating bioreactors using the Lumen X+™ powered by Volumetric and the BIO X6™. First, this technical note details how to fabricate a closed bioreactor on the digital light processing (DLP) Lumen X+ bioprinter. The technical note also demonstrates how the BIO X6 can create precise co- and multicell cultures inside a bioreactor to complete the workflow.

Introduction

Culturing cells and bacteria lets researchers study *in vitro* and *in vivo* behaviors of living materials and synthetic biological systems, a useful experimental method for a plethora of fields, including microbial biology, mechanobiology, disease modeling, drug discovery and biomanufacturing (Kapałczyńska, 2016; Shen, 2020; Vukasovic, 2019). 2D culturing has been carried out since Ross G. Harrison developed the technique in 1907 (Harrison, 1907) and remains one of the most popular methods although it does not accurately mimic natural environments because cells or microbes are grown as monolayers on a flask or petri-dish surface that may or may not have been functionalized (Estermann, 2021; Hirt, 2015; Kapałczyńska; Shen), drastically altering microbial properties, from differentiation to vitality to stimuli-responsive behaviors to drug metabolism (Kapałczyńska). As a result, 2D experimental results then translate poorly to *in vivo* applications, particularly for disease studies in pharmacokinetics and pharmacodynamics (Hirt; Shen). For optimal experimental performance, many researchers today prefer 3D culturing methods.

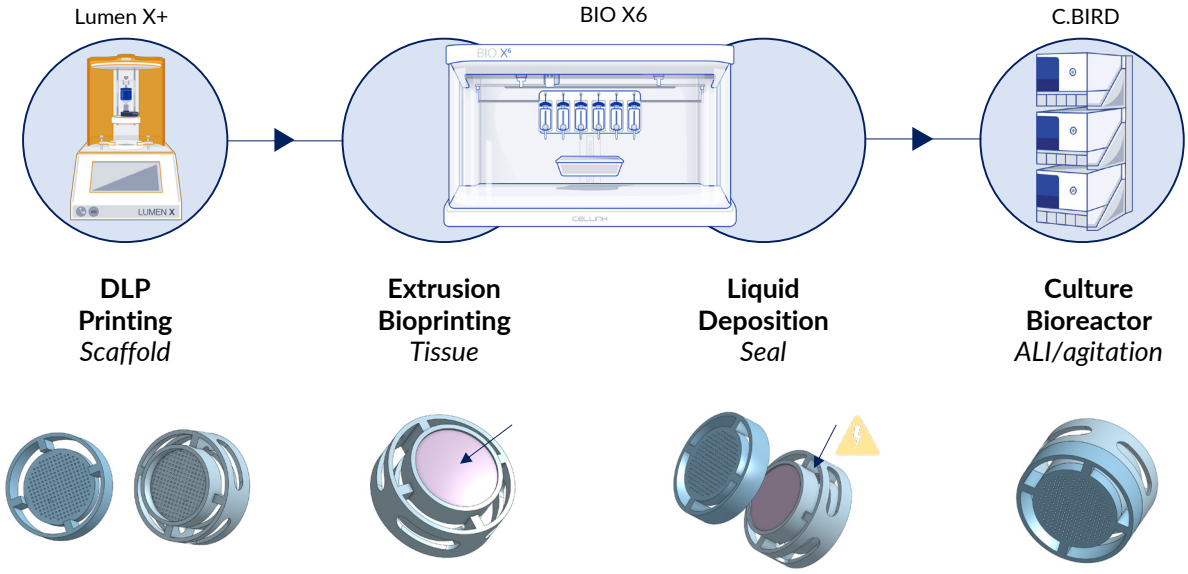

Figure 1. *Bioreactor production workflow.*

Recent technological advances and the commercial availability of bioprinters have made it easier to design, rapidly prototype and reliably produce 3D cultures. One popular method that has emerged involves 3D culturing in bioreactors, or manufactured tissue-engineered devices that mimic physiological environments for live cells. Bioreactors have gained attention for their broad applications, such as implant grafts (Lee, 2021; Notorgiacomo, 2021; Tsimbouri, 2017; Vukasovic), improving spheroid and organoid maturation (Cho, 2021; Qian, 2016; Shen; Velasco, 2020), cultivating stem cells (Rodrigues, 2011), and manufacturing engineered live bacteria therapies (Charbonneau, 2020). Bioreactors also have the potential to increase the repeatability of experiments while lowering costs (Franzen, 2019), enable researchers to translate their discoveries more effectively (Morgan, 2018) into clinically approved therapies (Sarkar, 2015) and living material implants. Given these potentials, the following workflow demonstrates the use of the Lumen X+ and BIO X6 to fabricate a bioreactor with bioprinted, cell-laden biomaterials.

Materials and methods

Model design

The bioreactor used was designed with the help of Onshape CAD (computer-aided design) software and exported in two STL (standard tessellation language) files. The bioreactor's two parts include a bottom part with a compartment for extrusion bioprinting (print chamber, **Figure 2**) and a lid to seal the device and create a closed bioreactor. Both the print chamber and the lid consist of a 10 mm in diameter mesh bottom, allowing for exchanges between the encapsulated matrix and surrounding media.

Fabrication

The bioreactor was bioprinted using the Lumen X+ DLP bioprinter and photo-polymerizable polyethylene glycol diacrylate PEGDA500 PhotoInk™. The Lumen X+ was selected because the bioreactor's micron feature sizes required precise lithographic (light-based curing) fabrication. PEGDA has been widely used in biology, functionalized with cell adhesion domains or in association with a biomaterial like GelMA, to encapsulate cells or to create functionalized scaffolds to guide cell orientation and proliferation. PEGDA500 PhotoInk is an advanced biocompatible and nondegradable PhotoInk designed for 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 ideal for creating drug delivery devices.

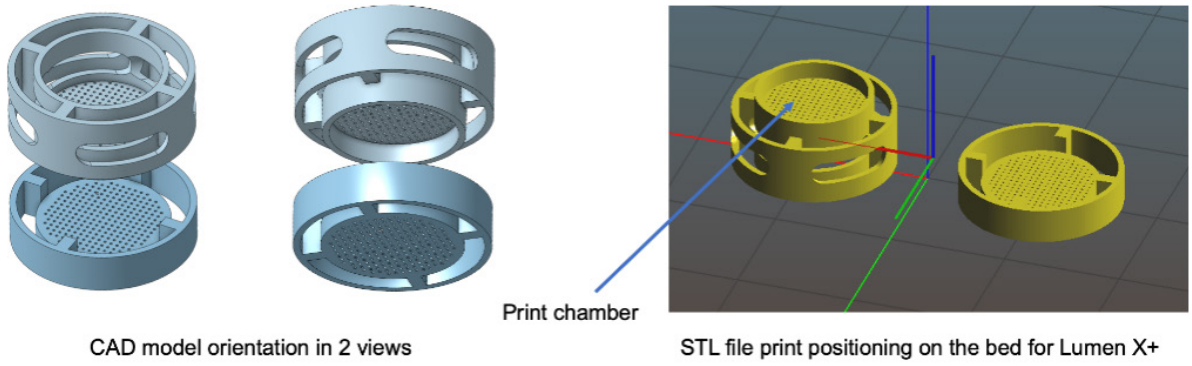

Figure 2. *Illustrations of bioreactor.*

The STL models of the bioreactor were imported into the Lumen X+ and sliced using the Lumen X+ LightField software at the higher resolution setting of 50 µm. The Lumen X+ was loaded with 1 mL of PEGDA500 PhotoInk for each model, and the power settings were set according to the protocol for PEGDA500 PhotoInk. Post-printing, the construct was hydrated then carefully removed from the build platform using a plastic razor blade. The bioreactor was then washed in deionized water to remove photoabsorbing dye and uncured resin. Finally, the construct was stored in a hydrated state for a few days before extrusion bioprinting was used to print cells into the print chamber.

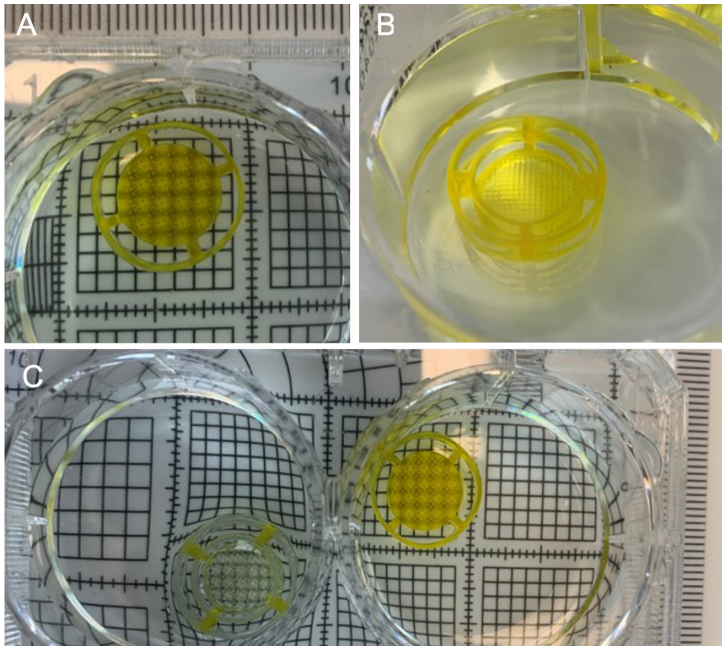

Figure 3. *Post-print wash of bioreactor chamber.*

The photoabsorbing dye has a nontoxic composition and the storage step performed within this technical note is optional. For optimal microscopy conditions, it is recommended to remove excess photoabsorbing dye. If microscopy is not needed, then one can proceed immediately to the extrusion-based bioprinting step after a wash to remove uncured PhotoInk. For cell-laden constructs, balanced buffers or cell media are recommended as washing solutions.

For bioprinting into the print chamber, a BIO X6 equipped with a standard pneumatic printhead and CELLINK Bioink was used. A model with a cylindrical shape, 10 mm in diameter and 1 mm high, was selected and adjusted using DNA Studio software to a final diameter of 8 mm and a total of three layers. After curing the extruded bioink, the edges on the lid of the bioreactor were covered with PEGDA500, then it was placed on top of the print chamber to close it. Finally, the device was sealed using the 405 nm photocuring modules of the BIO X6, positioned at 3 cm to illuminate the scaffold for 15 seconds. Sealing the bioreactor is optional, and the print chamber can be used on its own like a Transwell system or for air-liquid interface (ALI) cultures.

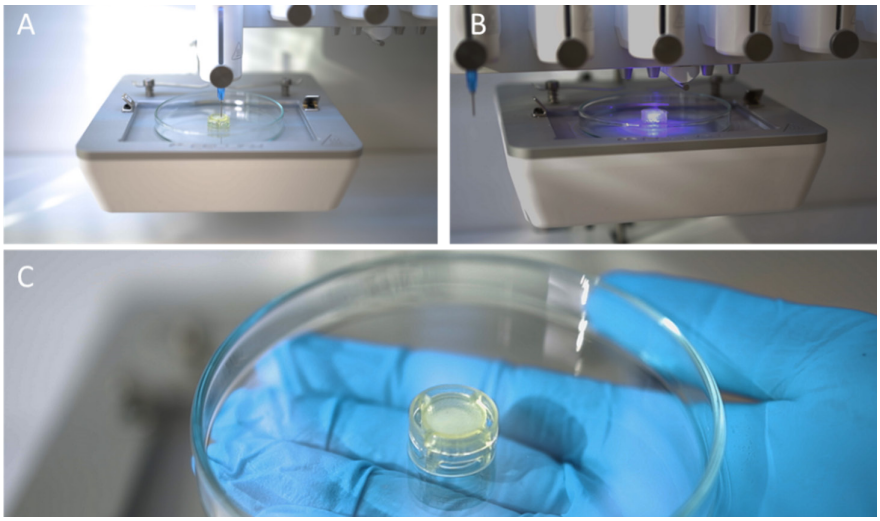

Figure 4. *Overview of bioprinting into bioreactor.*

Summary

In the fields of tissue engineering and cell culture, combining organ-on-a-chip and bioreactor technologies has been a key factor in better recapitulating human physiology for greater accuracy and efficiency of cultures. In this technical note, the high-resolution DLP-based Lumen X+ bioprinter was combined with multimaterial extrusion-based bioprinting to create a living scaffold in a chamber usable as a bioreactor, Transwell system or co-culture chamber.

The bioreactor can be positioned in a classic 24-well plate totally, or partially immersed, in cell culture medium. The porous system allows for hydration and circulation of nutrients inside the print chamber. Moreover, the bioreactor can be used without the lid as an ALI system classically used for skin tissue model growth.

Beyond tissue engineering applications, it is possible to place other elements in the bioreactor's chamber. For example, cells encapsulated in beads can be placed in the chamber to perform suspension cultures by placing the system under agitation with systems such as the C.BIRD™ system (CYTENA). Another approach would be the use of one medium in the chamber and one medium in the well to create a bicompartmental configuration, a gradient system or a dynamic flow based on the exchange between two types of solution.

Co-culture approaches are also interesting to set up by arranging cells on both sides in bioprinted hydrogels, then monitoring exchange, communication and colonization through the pores of the chamber for cell migration/invasion studies. The chamber can also be functionalized with ECM (extracellular matrix) elements to promote cell adhesion, and chemoattractant molecules can be added to promote migration. Moreover, the PEGDA500 chamber can be loaded with pharmacological molecules to form a drug-releasing scaffold.

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