Advancing 3D-Bioprinting with Photopolymerization of Orderly Extruded Multi-Materials (POEM)

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ABSTRACT

Printing functional tissues and organs on demand is a major goal in biofabrication. However, replicating intricate structures resembling cellular arrangements and physical characteristics of human tissues and organs remains the greatest challenge. Up to date, several systems, such as extrusion and light-based bioprinting techniques, have been widely studied. Achieving desired realistic, high-resolution 3D features on multi-material and multi-layer complex structures while simultaneously incorporating cells and maintaining high cell viability is the holy grail of bioprinting and remains to be achieved. Addressing this limitation, we proposed, developed, and fully characterized a novel 3D-bioprinting technique called photopolymerization of orderly extruded multi-materials (POEM). The proposed technique operates by infusing temporarily viscous photo-cross-linkable bioinks layer-by-layer. It is subsequently followed by precise and high-resolution photopatterning of the layers to the desired shapes and configurations. The proposed POEM technique offers a single-step photopolymerization that eliminates the requirement for multiple processing steps, interim cleaning processes, or material exchange throughout the multi-material/multi-layer printing procedure. This also eliminates cross-contamination and the loss of valuable cells and inks during the cleaning process. Herein, we demonstrate the utility of the POEM technique for rapid and high-resolution 3D printing of multi-material, multi-layer, and cell-laden structures. The printed configurations exhibit remarkable cell viability (approximately 80%) and metabolic activity for over 5 days. As proof of concept, we successfully fabricated and characterized a 3D structure representing the esophagus. The development of POEM represents a significant advancement in 3D-bioprinting technology, offering new possibilities for constructing physiologically relevant tissue constructs.

Keywords: Digital light processing, 3D-Bioprinting, Multi-material, Photopolymerization, Additive manufacturing

1. INTRODUCTION

The field of biomanufacturing has seen significant advancements with the introduction of 3D bioprinting technologies in biomedical and tissue engineering due to its capacity to replicate intricate structures that resemble the cellular arrangement and physical characteristics of biological tissues and organs. Notably, engineering artificial tissues and organs from cells significantly advances drug screening[1], disease modeling[2], high throughput assays[3], cancer research[4], and clinical transplantation[5]. Various technologies and methods have been developed to fabricate such complex structures, including extrusion[6], stereolithography[7], two-photon polymerization[8], and digital light processing (DLP)[9]. Each of these techniques presents its own set of advantages and drawbacks concerning material versatility, precision, resolution, and printing speed. However, a critical limitation persists across most of these methods, as they are primarily capable of working with only a singular type of biomaterial. Hence, the 3D bioprinting field still lacks technologies to fabricate intricate and heterogeneous 3D cell-laden architectures using multiple materials in a multilayered arrangement to emulate in-vivo human organs and tissues precisely. In the literature, elegant solutions that involve the use of microfluidic systems for quickly exchanging and blending multiple (bio) inks as well as multi-vat DLP stations, have been suggested[10,11]. However, these methods still need to be improved to address potential issues such as the risk of

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losing valuable cells and inks, cross-contamination, and the potential damage to small features during the cleaning process, given their integration of multiple processing steps during the 3D bioprinting.

To address these limitations, we introduce a multi-material bioprinting technique based on the Photopolymerization of Orderly Extruded Multi-materials (POEM)[12] to print 3D multi-layer, multi-material, and cell-laden tissue structures with high cell viability and good resolution. The core principle of the POEM technique involves the extrusion of biocompatible photo-cross-linkable hydrogels in a layer-by-layer manner, followed by high-resolution patterning of the layers into desired shapes and configurations using photo exposure set up with a 4-*f* lens system. This innovative approach enables single-step photopolymerization by eliminating multiple printing processing steps, including interim cleaning and material exchange. As a result, POEM effectively eliminates concerns related to cross-contamination and minimizes material loss associated with the exchange process. Furthermore, the incorporation of a support bath in the POEM technique ensures stability for 3D bioprinted structures throughout the entire process, eliminating the risk of collisions and preventing any potential structural deformations. Hence, the POEM technique paves the way for the 3D bioprinting of multi-material and multi-layer architectures closer to 3D tissues with high resolution and high cell viability by using custom-made bioink formulations.

2. RESULTS AND DISCUSSIONS

2.1 Development of POEM bioprinter

The proposed POEM technique involves three primary steps: infusion of temporarily-viscosity-increased bioinks, precise and high-resolution photopatterning of the infused bioinks into complex patterns of interest, and subsequent viscosity readjustment with the removal of uncured bioinks and viscous additives, as depicted in Fig. 1(a). During the multi-material vat preparation phase (Step-1), glass slides were first placed against the inner walls of a quartz reservoir to remove the pattern without damaging it. Then, various prepolymers (such as PEGDA-Carbopol or PEGDA+GelMA-Carbopol) with cells are loaded into printer cartridges and precisely extruded into the quartz reservoir. Here, the design process can involve not only different materials but also different cell types, cell concentrations, and a range of viscosities, allowing for a versatile and customized approach to bioprinting. The extrusion process can be tailored to specific needs, adopting configurations like longitudinal and lateral layering, depending on the intended application. Achieving varied material thicknesses is possible by adjusting the printing speed, needle gauge, or G-code according to the ink viscosity during the extrusion process. In the photopatterning phase (Step-2), a customized DLP-printing system is featured by modifying the projection lens and illumination source of the commercial DLP (Texas Instruments, Dallas, TX). Instead of using its illumination module with a visible light source, a UV source operating at 395 nm is integrated for the illumination of the DMD chips. Here, the DMD chipset functioned as a photomask to generate dynamic optical patterns[13]. This DMD configuration, often modeled as a blazed grating with tilted mirrors, introduces diffraction orders in the reflected beam. Hence, to ensure directing sharp, in-focus images to the bioink during the patterning process, the projection optics are replaced with a 4-*f* system for image retrieval. After the bioink + support bath mixture is filled into a quartz reservoir, it is positioned over the stage, and the mask is projected onto the reservoir wall. Upon projecting the DMD-patterned UV light onto the reservoir, the exposed section of the prepolymer underwent crosslinking, transforming into a solid polymer through a free polymerization reaction. As a post-printing step (Step-3), a straightforward washing procedure was performed using phosphate-buffered saline (PBS) solution. In this way, the uncured bioinks that have temporarily increased in viscosity are turned back into a liquid state by adjusting the pH, which reverses the temporary increase in viscosity.

2.2 System characterization and multi-material 3D printing

The performance of the proposed POEM system was assessed by examining its resolution and registration capability concerning exposure time variations (0.5–3 min). To optimize the exposure time for the target thickness, the PEGDA prepolymer solution was patterned, adjusting exposure times for different strip thicknesses (65–2000 μm). Notably, the exposure time was capped at 3 minutes to ensure cell viability during the bioprinting process. A 65 μm printing resolution is achieved by using the proposed POEM technique. In-depth analyses and further details regarding the presented findings can be explored in Ref. 12.

To demonstrate the versatility and applicability of the POEM technique, fluorescent particles of different colors were incorporated into the prepolymer solutions. These solutions were then extruded to create both longitudinal layers and concentric squares (lateral layering) with two/three different colors. Following the extrusion process, various 3D micro-structures were printed, including a mask that has vertical and horizontal symmetry (including circular, strip, and corner

features), a cylindrical channel, a UCI logo, and a bilayer micro-capillary structure with a hollow channel as illustrated in Fig. 1b–e, respectively. The obtained results show the capability of the bioprinter to fabricate multi-material constructs, paving the way for the development of future generations of micro-tissue and organoid models.

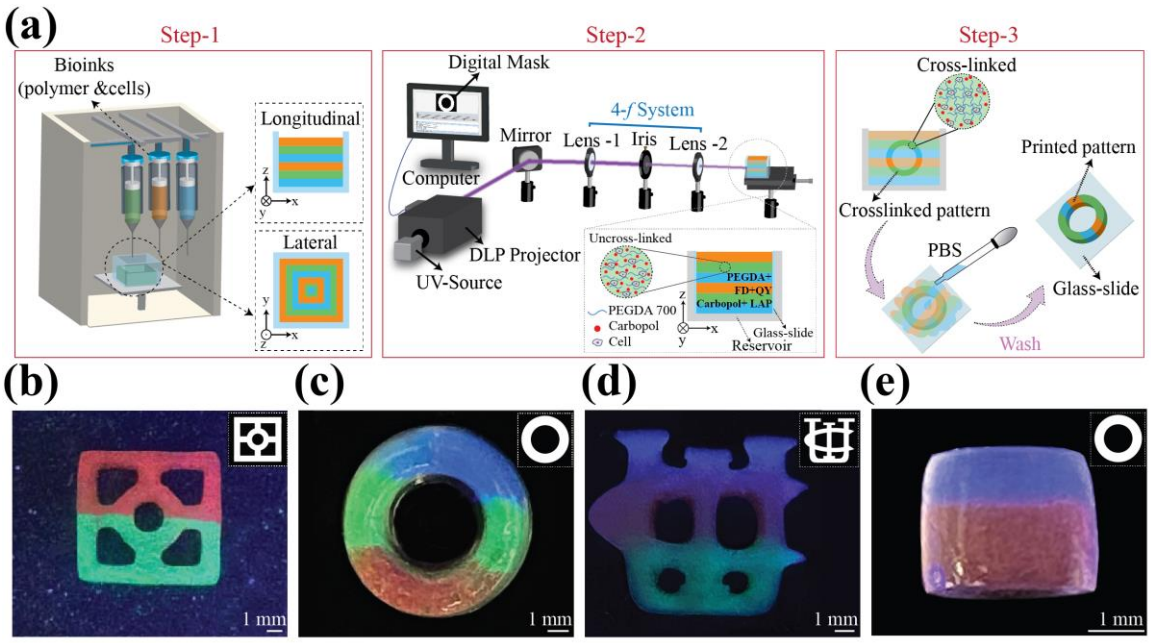

Figure 1. (a) Simplified schematic representation of the printing mechanism of the POEM technique with three main steps: Multilayer vat preparation via cell-laden bioink infiltration, photopatterning, and recovering printed objects. (b-d) Top views of DLP printed structures with two/ three colors longitudinal layering arrangement. (e) Side view of a DLP printed cylinder with lateral layering arrangement. Insets show digital masks with printed patterns.

2.3 Bioprinting

The POEM technique demonstrates promising potential in providing a biocompatible environment for incorporating living cells into the bioprinting process. In this work, as a showcase, Esophagus-like structures were successfully printed by incorporating muscle cells (C2C12) and fibroblast cells (L929) into the PEGDA (i.e., PEGDA 700) and PEGDA+GelMA inks. Several criteria influence the selection of bioink, including functionality, biocompatibility, mechanical properties, and degradation. PEGDA offers advantages in terms of superior fabrication fidelity and stability, attributes that are challenging to achieve in soft hydrogels like GelMA. While GelMA may lack high printing fidelity, it is well-suited for cell encapsulation and growth. In this regard, we have examined the performance of two cases with PEGDA (i.e., PEGDA 700) and PEGDA+GelMA inks. Cell viability was evaluated through the Live/Dead assay (Fig. 2a), revealing a significant population of viable cells on the first day of printing, with 91.2 ± 3.5% for PEGDA+GelMA and 68.5 ± 6.0% for PEGDA (Fig. 2b). As presented in Fig.2cThe preserved cell viability of up to 80% on day 5 for the PEGDA+GelMA blend while 25% reduction observed in PEGDA ink. Metabolic activity on day 5 exhibited a decrease of up to 60% in PEGDA ink, while PEGDA+GelMA displayed a modest variation of approximately 25% compared to day 1. Consequently, combining GelMA with PEGDA in mixtures appears promising as it can potentially provide the necessary stiffness while concurrently preserving improved surface attachment and adhesion for cells. Following this, the ratio of the inks can be systematically optimized to achieve a balance between high cell viability and printing fidelity.

3. CONCLUSION

In conclusion, the POEM technique addresses critical limitations in 3D bioprinting by enabling single-step photopolymerization and eliminating concerns such as cross-contamination and material loss. The bioprinter demonstrates high resolution (up to 65 µm) and versatility, showcasing its potential for micro-tissue and organoid

models. Successfully printing esophagus-like structures with different bioinks, including GelMA and PEGDA blends, highlights POEM's biocompatibility and potential for a balance between cell viability and printing fidelity.

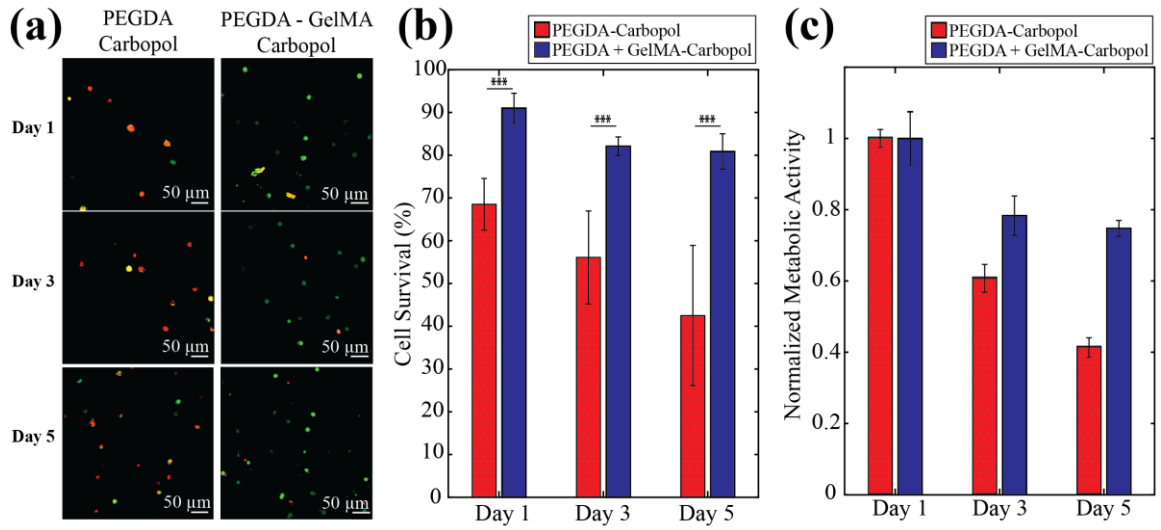

Figure 2. (a) Live/Dead assays of 3D bioprinted constructs of PEGDA-Carbopol and PEGDA+GelMA-Carbopol on days 1, 3, and 5. Living cells are depicted in green and dead cells in red. (b) Quantitative analysis of cell viability assay (N = 3, n = 4, ***$p \leq 0.001$). c) Resazurin assay on cells encapsulated in bioprinted constructs (N = 3).

Demonstrating its capabilities, the POEM technique exhibits rapid and precise 3D-printing of multi-material, multi-layer, and cell-laden structures with sustained high cell viability (approximately 80%) and metabolic activity for over 5 days. Additionally, the incorporation of a support bath ensures stability throughout the printing process, eliminating the risk of collision and structural deformations. Overall, POEM emerges as a promising solution, advancing the field of biomanufacturing with its capability to fabricate complex, multi-material, and cell-laden tissue structures with high resolution and cell viability.

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