Bioprinting of three-dimensional culture models and organ-on-a-chip systems

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Abstract

Multimaterial bioprinting technologies offer promising avenues to create mini-organ models with enhanced tissue heterogeneity and complexity. This article focuses on the application of three-dimensional bioprinting to fabricate organ-on-chip systems for *in vitro* drug testing and screening. We illustrate the capabilities and limitations of a bioprinting approach compared to microfabrication in constructing an organ-chip device. Further, we propose strategies in multimaterial integration for printing microphysiological tissue models. With these analyses, key challenges and future directions are highlighted.

Keywords: Biomedical; Tissue; Fluidics; Biological synthesis (assembly); biomimetic (assembly).

Introduction

In the last decade, we have seen tremendous progress in three-dimensional (3D) culture technologies, and the development of culture systems exhibiting more complex cellular interfaces than a conventional flat petri dish.[1,2] These culture systems have been used for landmark developments in mini-organ models, such as organoids with realistic microanatomy,[3] and organ-on-a-chip systems in simulating tissue/organ-level physiology.[4] Combined with advances in stem cell technologies, these culture systems are envisaged to fulfill roles in bridging gaps between preclinical and clinical models in the drug development pipeline, and ultimately, reducing costly failures in clinical trials.[5,6]

While it is important to harness *in vitro* biological models to address specific questions, simple two-dimensional cultures cannot capture many of the key microenvironmental factors (**Table I**) known to influence cell fates. Culturing cells in a complex configuration, however, is labor-intensive and costly. Recent advances in bioprinting and biofabrication technologies offer promising new strategies to create tissue scaffolds and tissue models.

Bioprinting can support the ability to repeatedly build small-scale tissue systems, minimizing human intervention and improving standardization and accuracy.[7,8] Second, due to its ability to automate and program the deposition of cells and materials in 3D,[9,10] bioprinting also provides new possibilities to construct a cell niche with prescribed complexity and physiological resemblance. These two general attributes may address some of the critical steps toward the adaption of complex culture systems. These include, for example, the necessity for standardization, validation, and reproducibility, and meeting investigators' desires to create complex co-culture systems with more than four cell types, in a predefined spatial configuration (please see reference [2] for survey results). The latter attribute may be regarded as the key advantage not easily facilitated by microfabrication and lithography-based approaches.

In this article, direct comparisons between 3D bioprinted and microfabricated organ-on-a-chip models are presented. Further, we propose how a fit-for-purpose bioprinting process can be designed to construct cell niches for *in vitro* tissue and organ models.

Comparison between bioprinted and microfabricated models

A number of organ- and disease-on-a-chip models have been developed as a result of advanced microfluidic technologies.[1,11] Mini-organ models have been established for various organs

including the lung,[4] heart,[12] kidney[13] and liver.[14] Simultaneously, disease models such as local cancer invasion and cancer transendothelial migration have also been demonstrated.[15] Among these, there are simple systems comprising a simple cell type within a microfluidic channel,[16] to complex designs containing multicellular components coupled with 3D gels and circulation.[17] D. Huh et al.[18] and E. K. Sackmann et al.[19] have summarized the progress to date.

In comparison to the microfabricated platforms, the capability of 3D bioprinting has yet to be extensively exploited, though select functional tissue and organ models on a chip were recently demonstrated.[8] With the main purpose of recreating aspects of microenvironmental cues, it is important to identify how a bioprinting approach resembles or differs from a microfabrication approach. Table I lists the main operating mechanisms associated with the respective technique in achieving the desired microenvironmental cues. We further present a side-by-side comparison between similar organ-chips and screening models generated by the two techniques in **Table II**. We highlight the key results shown in the two tables here.

Although both microfluidics and bioprinting allow spatial organization of cells and extracellular matrix gels, the mechanisms facilitating these differ greatly. In microfluidic chips, materials are organized by flow paths in the channels, and therefore, compartmentalization and special flow geometries need to be designed to allow localized cell/ material seeding.[32, 33] On the other hand, 3D printing can provide a more direct deposition approach through control of the nozzle position.[34] However, in order to create vessel and tubule-like features, both techniques require flow-directed cell-seeding within a channel geometry.[35]

Nonetheless, the convenience of bioprinting to integrate multiple cells, hydrogels, and even sensors has enabled the creation of liver,[23, 24] heart,[31] and skin[21, 22] models with better biological functions. Lind et al.[31] in a recent heart model reported that soft strain gauge sensors could be directly printed by a multimaterial printer and embedded in a printed cardiac tissue, enabling long-term and facile digital readouts of contractile stresses in the engineered tissue. Such one-step integration of on-chip sensors in tissue constructs is difficult to achieve by a microfabrication-based approach. Agarwal et al.[12] developed a heart-on-a-chip system and showed that multistep assembly procedures were required to form the muscular thin-film (MTF) within the chip, which is also restricted by having a simple layered configuration; the system has benefits of being an autoclaveable and reusable device.

As shown in Table II, current bioprinting approaches offer lower planar resolution than microfluidics. For example, the creation of channel features is important for many purposes, including improving long-term cell viability by providing nutrient/waste exchange, imposing flow-induced shear, and incorporating dynamic concentration gradients. Lithography-based templating offers established protocols that facilitate the creation of these microchannel features.[19] In contrast, achieving channels in bioprinting involves the use of fugitive inks and intricate ink removal procedures, also accompanied by poorer feature resolution of ~100 μm at the present.[29]

For screening applications, one important practical consideration is the amount of cells and extracellular matrix materials required for forming a tissue model, and also the quantity of compounds needed for a drug test. So far, processes inherent in low-resolution cell printing lead to larger tissue sizes compared to the microfabricated chip systems; thus, larger quantities of cells and testing compounds, which can often be expensive or sparse, are required. On the other hand, if materials restrictions are not imposed, bioprinting provides the unique capability to create thick tissues by incorporating 3D interconnected channels or vessels for nutrient/waste exchange.[30, 36]

As shown in Table II, a recent 3D vasculature printed from fugitive inks by Kolesky et al.[30] was able to support a large bioprinted multicellular tissue (>1 cm in thickness) under long-term perfusion (>6 weeks). In comparison, in established microfluidic chips, although the channel resolution and the resultant vasculature are finer, cell layout is usually much thinner in the planar fashion (<1 cm in thickness).[28] Therefore, bioprinted thick tissue has advantages in capturing a higher degree of tissue heterogeneity and complexity[37] and possibly providing closer physiological relevance in simulating realistic drug transport from circulation to the targeted tissues.

Finally, nonflow related mechanical deformation can be seen as one of the most demanding microenvironmental factors to be integrated within a microphysiology device. In soft-lithography based microfluidics, the majority of the published work has harnessed the deformability of poly(dimethylsiloxane) (PDMS) to form pillars, valves, and stretchable membranes.[12] Huh et al.[4] provide a well-known example in the membrane-based double-layer lung-on-a-chip system. To mimic the mechanical deformation of the lung alveoli during the breathing motion, a vacuum pump was connected to two empty side channels to generate a cyclic

stretching motion on the PDMS membrane sandwiched between two layers of microchannels. Such a deformation mechanism imposes stringent demands on the robustness of the materials and device packaging, not yet achievable in a bioprinted construct at the microarchitectural level. Hölzl et al.[38] and Arslan-Yildiz et al.[39] show in their studies how mechanical forces were exerted on the bulk of bioprinted tissues utilizing a separate mechanical rig, which, however, is only viable for larger tissue constructs at a reduced test throughput. Nonetheless, continued improvements in print technology and the potential combination of microfabrication and bioprinting approaches can help address some of these technical challenges.

Material integration for printing microenvironmental cues

The integration among printed cells, extracellular matrices, and a miniature bioreactor can potentially lead to more precise and flexible recreation of the microenvironmental cues *in vitro.* To facilitate such a "bioprinted organ-chip" device, materials with diverse properties, from natural to synthetic in origin, need to be combined together to perform different roles in the device. **Figure 1** summarizes different materials that have been used in bioprinting (not specific to organ-chips). The proposed materials functionality can be largely defined as being biologically focused, or structurally focused. For example, biologically active hydrogels can provide a matrix for cell encapsulation, cell binding, and a reservoir for growth factor release. The mechanical weakness of the biologically active hydrogels can be potentially overcome by combining with other robust hydrogels, or by designing mechanical supports from thermoplastic or thermoset structures.[40] Examples include the creation of core–shell hydrogel structures between alginate and cell-embedded collagen,[41] and the incorporation of nano- and microfibers[42] within hydrogels. The design of multiwell compartments using PDMS can also be seen as a strategy to provide structural support to the printed bioinks.[37]

The diverse materials properties inevitably require different processing techniques for these materials. This means that print techniques based on direct a material dispensing mechanism may find broader applications with their cross-technique compatibility. Based on this concept, a number of commercial bioprinters have already established multinozzle deposition platforms. Ozbolat et al.[9] and Gudapati et al.[10] provide overviews of the different direct deposition printing mechanisms and their material suitability. Regardless of the technique, specific attention needs to be paid to the mismatched processing parameters between different materials. For example, thermoplastics are normally printed under elevated temperatures in a

molten form.[34] However, this processing condition may affect the hydrated biological system if the solidified thermoplastic structure cannot be cooled rapidly. Any solvents or excess cross-linking reagents contained in the thermoset material can also disturb biological behaviors.[8] Further, it is well known that process-induced thermal, mechanical, or photo stresses can dramatically decrease cellular viability.[43] The processing time (i.e., the duration for which cells are taken out of the incubator environment) needs to be minimized.

Finally, it is worth noting that many of the bioprinted constructs demonstrated so far consist of simple components, aimed at musculoskeletal applications, that can create robust, macroscopic tissues for implantation.[36] The bioprinting techniques established from these experiences may need to be adjusted for printing soft, complex tissues for organ-chip devices. Considerations should be given to the self-assembly capability of different cell types; the matrix synthesis and remodeling ability of cells; and also the phase segregation phenomena of different material mixtures. These factors should be accounted for in designing a chip device to accommodate the tissue dynamics over long-term culture.

Conclusion

Advances in bioprinting are accelerating progress toward organ-on-a-chip devices for modeling tissue behaviors with enhanced physiological relevance. Integrating multiple printing techniques and material/cell libraries will provide new opportunities for making complex tissue models supporting long-term cultures; but one should be cautious in designing the fabrication strategy to accommodate the processing tolerances for different materials/cells.

Continued developments in this area could bring higher print resolutions, incorporate dynamic mechanical stimulation, and integrate optical and electronic materials for *in situ* sensing and activation. Harnessing these functionalities will lead to smart organ-chip devices for high-content pharmaceutical screening with low-reagent usage. The examples illustrated in this article stem from an engineering technology development prospective.

The successful creation and implementation of an *in vitro* model relies heavily on in-depth understanding of the biological pathways and physiological systems; and this will require significant collaborative efforts with biomedical researchers to fine-tune and optimize the culture conditions. As an intermediate step, 3D bioprinting can be seen as an invaluable toolkit to facilitate easy customization of the culture conditions, enabling systematic evaluation of the different microenvironmental cues (and their combinations) to realize a particular biological

phenotype. Like all *in vitro* model systems, for bioprinted organ-chips to have a real impact on drug development, one should design the level of biological model complexity, corresponding to the stage of the preclinical testing required. Ongoing system validation, *in vivo–in vitro* correlation, and regulatory approvals should be sought.

Acknowledgments

D.Z. gratefully acknowledges support from the China Scholarship Council. Y.L. acknowledges the Schlumberger Foundation and Trinity College Cambridge. Y.Y.S.H. thanks EPSRC, the Royal Society London, and the Isaac Newton Trust for funding support.

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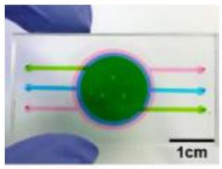
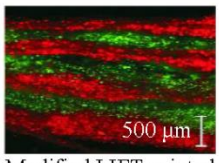
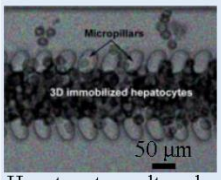
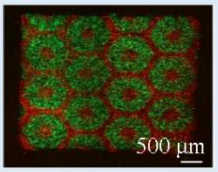
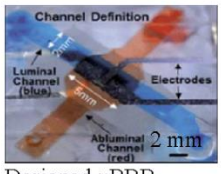
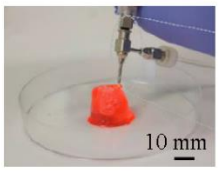
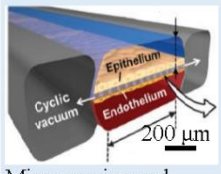
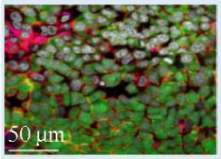
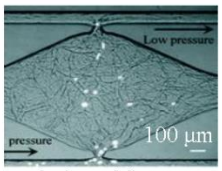
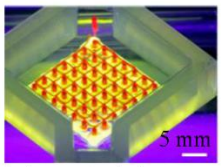
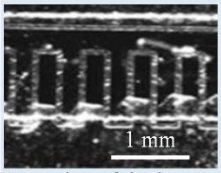
Figure and Figure Captions

Table I. Reproducing microenvironmental cues using established lithography-based microfluidics and three-dimensional (3D) bioprinting. Engineering strategies, capabilities, and limitations of both techniques are compared.

Microenvironment cues to mimic	Strategies		Capabilities		Limitations	
	Conventional microfluidics	3D printing	Conventional microfluidics	3D printing	Conventional microfluidics	3D printing
Spatial organization of cells/ECMs	• Encapsulation of cells/ECMs in compartments • Porous membranes between adjacent channels	• Encapsulation of cells in ECM-mimicking hydrogels • 3D layer-by-layer deposition	• Reduced material usage	• Image-guided deposition • Spatial combination of multiple cell types and materials	• Surface treatment required to improve PDMS compatibility • Cells/ECMs positioning restricted to specific designs	• Limited resolution and shape fidelity • Difficult to form vasculatures
Chemical gradient and soluble factors	• Fluid stream carrying chemicals of different concentrations	• Gels of varying chemical levels in microfluidic nozzle • Incorporating channel structures	• Precise, spatiotemporal gradients within high-resolution channels	• Chemical gradients in ECM hydrogels	• Gradients restricted to planar configuration	• Multi-steps required to form channel structures
Non-flow-related mechanical stimuli	• Utilizing PDMS elastic properties • Vacuum-controlled mechanical strain	• Applying global force stimuli on post-printed tissues	• PDMS Membrane deformation • In situ micro-pumps and valves	• Global deformation in whole tissue	• Limited to PDMS elastic properties	• Difficult to generate local deformation
ECM properties	• Channel coating with ECM components • Inserting hydrogels in micro-compartments • 2D micropatterned ECM	• Tuning hydrogel design and composition in 3D	• Reduced material usage • In situ crosslinking of gels	• Synthetic hydrogels • Decellularized matrix • Micro-carriers	• Surface treatment required to stabilize gel position • Hydrogel localization restricted to specific designs	• Trade-off between hydrogel robustness and cell viability
Topography	• Computer-aided planar design • Established design elements	• Computer-aided design for layer-by-layer deposition	• Versatile design • Micrometer- to nano-scale resolution	• Combining a wide range of materials with varying biomechanical properties	• Limited to planar structures • Difficult to achieve micro-features with varying heights and curvature	• Feature size limited by mechanical properties of the substrate • Low resolution
Fluidic circulation	• Established protocols for channel network formation	• Using fugitive inks for channel formation	• Perfusable planar vasculature • Flow-induced shear stress • Long-term culture	• Perfusable 3D vasculature • Flow-induced shear stress • Long-term culture • Facilitation of thick tissue formation	• Difficult to form 3D channel networks	• Biocompatibility and ease of operation of fugitive inks • Demonstrated resolution ~100 μm

Note: ECM, extracellular matrix; PDMS, poly(dimethylsiloxane); 2D, two-dimensional.

Table II. Side-by-side comparison of microengineered tissue/organ models via established microfabrication versus three-dimensional (3D) bioprinting techniques.

Tissue & Organ model	Established microfluidics culture			3D bioprinting		
	Method	Component	Sample construct	Method	Component	Sample construct
Skin	• Membrance-based 3-layer microfluidic structure[20]	• Keratinocytes, fibroblasts, endothelial cells[20]	 The skin-on-a-chip device with three PDMS layers[20].	• Microvalve DBB[21] • Modified LIFT[22]	• Collagen, fibroblasts, keratinocytes[21, 22]	 Modified LIFT printed color-layers of keratinocytes[22].
Liver	• Microfluidic gradient generator; compartmentation by pillar arrays[14]	• Rat hepatocytes[14]	 Hepatocytes cultured in chip forming 3D aggregates[14].	• Piezo-electric inkjet DBB[23] • DLP[24]	• Fibronectin, gelatin, hepatocytes[23] • hiPSCs, endothelial and mesenchymal cells, GelMA, GMHA[24]	 Patterns of printed hydrogel based hepatic construct[24].
Brain	• Membrane-based co-culture; embedded electrodes[25]	• Rat endothelial cells, astrocytic cells[25]	 Designed μBBB channels model the neurovascular unit[25].	• Piezo-electric inkjet DBB[26]	• Primary cortical neurons, glial cells, RGD modified gellan gum[26]	 Hand-held reactive printed structure with cortical neurons[26].
Lung	• Membrane-based co-culture; cyclic mechanical strain; air-liquid flow[4]	• Human pulmonary endothelial and alveolar epithelial cells[4]	 Micro-engineered model of human pulmonary edema[4].	• Microvalve DBB[27]	• Alveolar epithelial type II cells, endothelial cells, Matrigel[27]	 Two-layer bioprinted co-culture of epithelial cells at day 3[27].
Vasculature	• Lining of predefined micro-channels; angiogenesis[28]	• Human endothelial cells[28]	 Perfusion of fluorescent microparticles inside vessel lumen[28].	• Microvalve DBB[29] • Pneumatic EBB[30]	• Human lung fibroblasts and endothelial cells, fibrinogen[29] • Human dermal fibroblasts and endothelial cells, hMSCs, gelatin, fibrin[30]	 Interpenetrated sacrificial and cell inks as printed on chip[30].
Heart	• Embedded electrodes, thin film cantilever for culturing of cells/ECMs[12]	• Rat cardiac myocytes[12]	 Operation of the heart-on-a-chip during peak systole[12].	• Pneumatic EBB[31]	• Rat ventricular myocyte, human stem cell-derived cardiomyocyte hiPS-CMs, dextran, PU, Ag, pentanol, PDMS[31]	 Automated printing of the device on a glass slide substrate[31].

Note: hMSC, human mesenchymal stem cell; hiPS-CM, human-induced pluripotent stem cell-derived cardiomyocytes; PU, polyurethane; ECM, extracellular matrix; HiPSC, human-induced pluripotent stem cell; μBBB, microfluidic blood–brain barrier; DBB, droplet-based bioprinting; EBB, extrusion-based bioprinting; DLP, digital light processing; LIFT, laser-induced forward transfer; GelMA, gelatin methacryloyl; GMHA, glycidyl methacrylate-hyaluronic acid; RGD, arginine-glycine-aspartic acid; PDMS, poly(dimethylsiloxane).

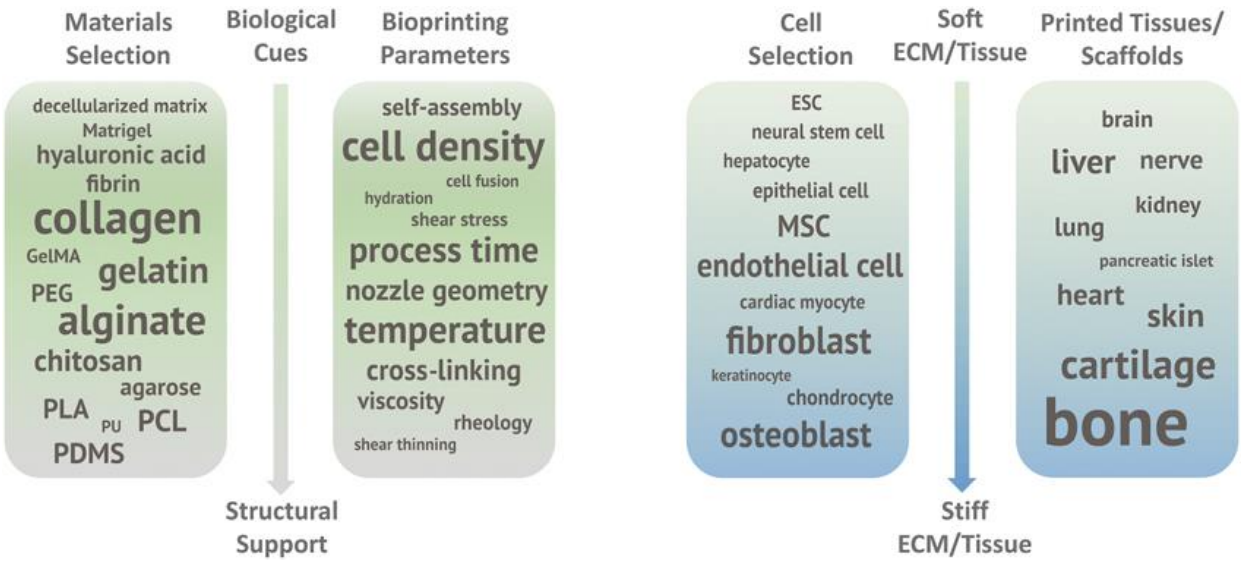


Figure 1. Word cloud diagrams illustrating the statistics of literature reports on materials selection, bioprinting parameters, and the bioprinted cells and tissues. The relative size of each word/phrase is an indication of the relative abundance of the reported subject from Scopus and Web of Science (February 2017), in comparison to the other subjects in the same category. The stiffness ranking of the cells as well as tissue and organs are based on their reported *in vivo* native ECM/tissue stiffness. Note: GelMA, gelatin methacryloyl; GMHA, glycidal methacrylate-hyaluronic acid; PLA, polylactic acid; PCL, polycaprolactone; PEGDA, polyethylene glycol diacrylate; PU, polyurethane; PDMS, poly(dimethylsiloxane); ESC, embryonic stem cell; MSC, mesenchymal stem cell; PEG, polyethylene glycol.