properties. Therefore, new methods that can tailor the physicochemical properties at both cell and tissue levels independently would be useful. Recently, one study described the engineering of composite fibers with a mechanically strong core from synthetic polymers covered with a layer of cell-laden hydrogel (Figure 1F) [11]. Upon their assembly, both the mechanical properties of the constructs and the distribution of cells could be controlled at the same time. The mechanical properties of these constructs, like tensile stress, tensile strength, Young's modulus, and elongation at break, could be easily tailored across several orders of magnitude to match the values for various tissues. In addition, the presence of a hydrogel layer, which could be tailored independently, created the opportunity to optimize its characteristics without affecting the properties of the entire construct.

The multistep fabrication of these composite fibers creates the opportunity to add functionalities to the engineered tissue. For example, the polymeric fibers can be used for controlled release of biological factors and cues to direct cellular growth and differentiation [12]. To this end, drug-eluting polymer-based sutures have been engineered that could be employed for many tissue engineering applications.

Envisioned Future Opportunities for the Use of Textile Platforms in Tissue Engineering

Overall, fiber-based technologies have emerged as a strong tool for various tissue engineering applications that can address many of the unmet needs in the field. It is expected that the similarity of braided and woven constructs to native muscle, tendon, ligament, and myocardium will eventually generate functional musculoskeletal tissues and cardiac patches. The combination of these technologies with advanced biomaterials will enable the development of more advanced scaffolds and engineered tissues. The possibility of tuning tissue-level properties independently of cell-level properties by using composite fibers and simultaneously controlling and directing cellular distribution, growth, and alignment is a unique capability that is essential for engineering load-bearing and highly organized tissues such as muscle, cardiac tissue, and ligaments.

In addition, along with advances in the field of flexible electronics and in fabricating electrical systems on nonconventional platforms, smart fibers can be engineered that can stimulate better tissue formation or subsequent monitoring of cellular function in culture [13]. Such characteristics could be important for engineering tissues such as cardiac and muscle tissues whose function depends on their electrophysiological activity. These aims can be achieved by engineering composite fibers with multiple independent compartments and their assembly using textile processes.

Fibers and textile technologies can also be used in regenerative medicine and cell therapies as drug and cell carriers. Similarly, textile technologies can be used in regenerative medicine through engineering advances of surgical meshes with regenerative properties.

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Special Issue: Biofabrication

Forum

Towards Single-Step Biofabrication of Organs on a Chip via 3D Printing

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Organ-on-a-chip engineering employs microfabrication of living

tissues within microscale fluid channels to create constructs that closely mimic human organs. With the advent of 3D printing, we predict that single-step fabrication of these devices will enable rapid design and cost-effective iterations in the development stage, facilitating rapid innovation in this field.

Goal of Organ-on-a-Chip Engineering

Organs on a chip are microengineered tissues cultured within microfluidic devices that serve as bioreactors [1]. These systems are specifically designed and fabricated to mimic the structure of human tissues better than current models, which largely rely on traditional static 2D systems. The field has seen recent success in modeling the blood–brain barrier, lung, intestinal ('gut on a chip'), and cancer tissue. These systems can ultimately be used to better understand human organs and disease, screen drugs for safety and efficacy, and generate replacements for damaged or diseased organs.

3D printing has demonstrated a strong promise to revolutionize numerous fields, including microfluidics and tissue engineering research, by enabling rapid, versatile, and customizable fabrication of a multitude of different objects (Box 1). Here we discuss recent progress using 3D printing in two fields relevant to organ-on-a-chip engineering–microfluidics and tissue engineering–as well as the potential to apply 3D printing to single-step fabrication of organ-on-a-chip devices.

3D-Printed Microfluidics

Traditionally, microfluidic devices are fabricated using UV lithography to generate a master of raised structures and soft lithography to create an imprint of those structures in an elastomer such as polydimethylsiloxane (PDMS) followed by a bonding step to seal the imprint to a glass slide, creating microfluidic channels. However, this approach requires several labor-intensive steps and specialized equipment, making the process inaccessible for many research laboratories and preventing rapid iteration of designs.

3D printing for microfluidic device fabrication takes advantage of the capabilities of 3D printing to rapidly generate microscale fluid channels within a few hours using simple, user-friendly equipment such as commercially available 'desktop-style' 3D printers [2]. This approach facilitates a rapid iterative design and fabrication process and improves interdisciplinary accessibility to microfluidics-based research, which may accelerate innovation in organ-on-a-chip engineering.

Further, 3D printing allows fabrication of 'truly 3D' channel geometries; that is, fluid channels with 3D complexity in contrast to the traditional '2½D' devices in which 2D channel designs are simply projected into the third dimension. Added 3D complexity can facilitate additional microfluidic capabilities, such as more efficient micromixing [3].

Several types of 3D printers have been proposed for printing microfluidic chips. Stereolithography uses a liquid resin material that is readily removed from the channels post-printing, but the channel resolution is limited with this method. By contrast, extrusion printing offers high resolution but requires the use of support materials that must be removed post-printing; sacrificial support materials have been employed to address this challenge.

The biocompatibility of 3D-printed microfluidic platforms is a critical challenge when moving toward organ-on-a-chip devices, necessitating the use of printable materials that are nontoxic and, for some applications, facilitate cell attachment on the printed surface. Biocompatible materials are available and have been demonstrated in several studies to facilitate cell culture of 3D micropatterned cellular constructs [4] and dental pulp stem cells [5] for tissue engineering applications.

There has also been a push toward a 'body on a chip' in which multiple organs are organized on a single chip to better model the multiorgan interactions that occur *in vivo*. In this respect, microfluidic circuits may be 3D printed and later seeded with cells or bioprinted with a cell lining to mimic human vasculature.

3D-Printed Living Tissues within Microfluidic Devices

Bioprinting is an extension of 3D printing in which living cells are mixed with scaffold materials to create a 'bioink' that is then deposited into a 3D construct; this has been applied to a range of tissues [6–8]. Bioprinting offers the ability to create a 3D biomimetic tissue by patterning cells and, in some approaches, multiple cell types with precise and reproducible spatial control. Several approaches have been proposed,

Box 1. 3D Printing Technologies

3D printing uses a computer-aided design model to deposit materials layer by layer, generating a 3D structure. Two of the most common types of 3D printing are stereolithography and extrusion-based printing. Stereolithography uses a photocurable liquid resin material that, on exposure to UV light, solidifies into a solid material. UV light is applied using either a laser in a raster pattern or digital light projection to expose each 2D layer, iterated in a layer-by-layer fashion to generate a 3D structure.

Extrusion-based bioprinting involves depositing a material either in a continuous filament (fused deposition modeling) or in droplets (inkjet printing) to generate each layer. Hybrid approaches combine multiple categories of printing. One example is the polyjet 3D printing approach (commercialized by Stratasys Ltd), which involves inkjet printing of a photocurable material that is solidified by UV flood exposure after each layer is deposited. Interesting applications include 3D printing of microfluidic devices as well as living tissues.

3D printing is also referred to as rapid prototyping because it enables quick succession of model generation, testing, and redesign. From this perspective, 3D printing will serve as a powerful tool in the coming years to facilitate rapid innovation in several fields, including organ-on-a-chip engineering.

from extrusion-based to laser-assisted bioprinting, with some bioprinters now commercially available (www.biobots.io) and other companies offering bioprinting services (including www.organovo.com).

Bioprinting living tissues directly into a microfluidic device facilitates the use of microfluidic devices as bioreactors, providing a steady stream of fluid flow supplying nutrients and growth factors and removing waste. In one study, hepatic spheroids were encapsulated in a photocrosslinkable hydrogel and deposited via direct-write bioprinting into a microfluidic device that was later sealed to contain the construct (Figure 1A) [9]. In another study, direct cell writing was used to create reproducible alginate hydrogel-based cellular constructs (Figure 1B) [10].

Remaining challenges associated with bioprinting living tissues include the need for printable bioinks compatible with current techniques to generate 3D vascularized cellular constructs of clinically and physiologically relevant size and geometry. The mechanical properties of the materials post-printing are also a concern: they must offer mechanical integrity to the structure, particularly for multilayered structures, and maintain their mechanical properties over long-term incubation times under culture conditions. The same materials must also be biocompatible to facilitate cell attachment and tissue development and be biomimetic, replicating the cell signaling that is important to elicit the same cell behavior observed *in vivo*. Solutions to these challenges may lie in the use of photocurable hydrogels or scaffold-free bioprinting,

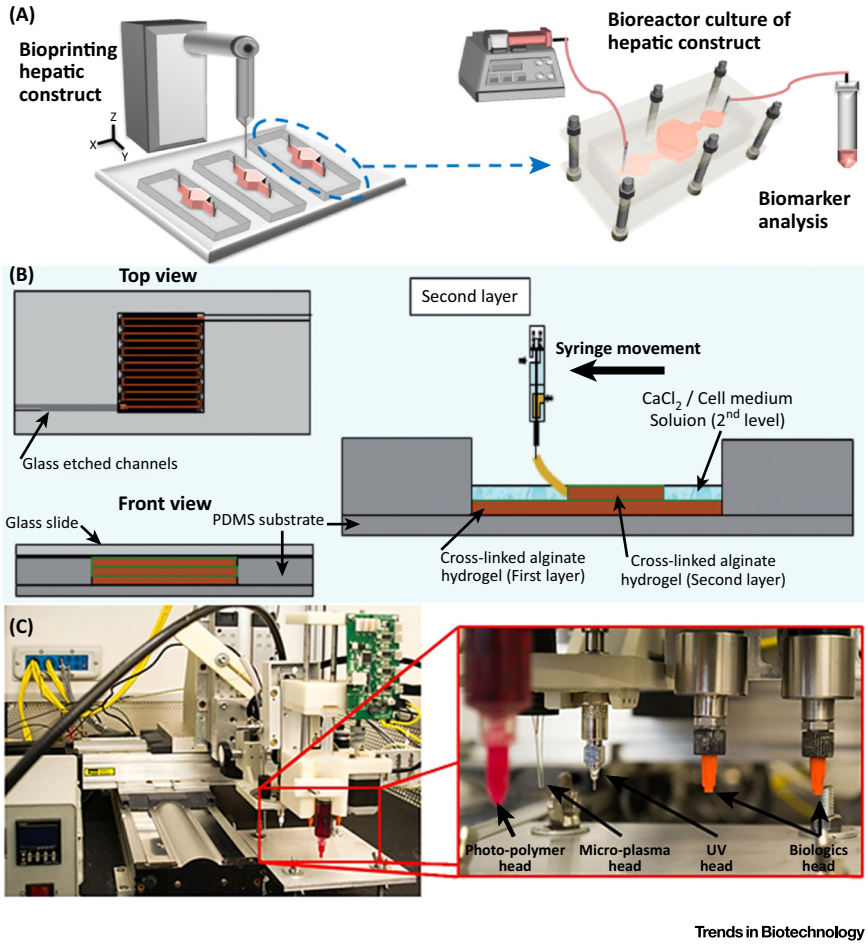

Figure 1. Bioprinting Cellular Constructs within Microfluidic Devices. (A) Direct cell write fabrication of hepatic spheroids encapsulated in GelMA within a microfluidic device. The device may be assembled and disassembled during the course of the experiment to enable access to and monitoring of the cells. Reproduced, with permission, from [9]. (B) Direct cell write fabrication of cells suspended in alginate into a polydimethylsiloxane (PDMS) microfluidic device with calcium chloride to crosslink the structure. The result is a sinusoidal flow pattern with 250-mm-diameter struts to mimic hepatic structures. Reproduced, with permission, from [10]. (C) An integrated solid freeform fabrication system with four print heads: a photopolymer head, which is used to deposit SU8 as a filament; a UV head, which follows the toolpath of the photopolymer head to cure the layer of the microfluidic device structure; a microplasma head to follow the path of the printed channels to treat the surface such that the surface chemistry and topological features are more conducive to cell attachment and proliferation; and a biologics head, which prints cells directly into the channels. Reproduced, with permission, from [12].

which allows the cells to generate their own extracellular matrix.

Towards 3D-Printed Organ-on-a-Chip Models

While most fabrication approaches employ 3D printing for either microfluidic device fabrication or printing 3D tissues, it may be possible to perform these two fabrication processes in parallel. With a combinatorial single-step fabrication approach, organ-on-a-chip platforms would be far more accessible and cost-effective due to enabling faster design iterations and shorter turnaround times.

In a novel approach, an integrated system was developed that uses both a digital micromirror system to generate the internal architecture of the microfluidic device and a cell printer for depositing cells directly into the fabricated construct [11]. Another integrated solid freeform fabrication system has also been developed, with four print heads: a photopolymer head, a UV head, a microplasma head, and a biologics head (Figure 1C) [12].

Of the many fabrication approaches available, extrusion- and stereolithography-based 3D printing approaches have been used more widely in microfluidic chip fabrication and demonstrated potency for the realization of single-step parallel fabrication of living tissues and the microfluidic manifold. Extrusion printing can be used to print multiple materials by employing multiple print heads with different bioinks. In an obvious extension of existing capabilities, some print heads could be dedicated for bioprinting the tissue constructs while others could print the materials to form the microfluidic channels. A challenge will be using a biocompatible support material that may be co-extruded and later removed to form microfluidic channels.

Stereolithography has shown promise for both bioprinting and microfluidic device fabrication but it is not easily adapted to the multimaterial fabrication required to print the device and tissues simultaneously. Instead, it may be possible to combine stereolithography with laser-induced bioprinting, in which cells are contained on a ribbon and transferred to the substrate on application of a pulsed laser source. Alternatively, a hybrid approach using a combination of these techniques or others may be developed to further enable rapid and versatile fabrication of organ-on-a-chip devices.

Concluding Remarks and Discussion

With the demonstrated recent success in 3D printing for microfluidic device fabrication as well as bioprinting, and in light of the rapid innovation in both areas, it is likely that 3D printing will serve as a tool for organ-on-a-chip engineering in the coming years. The intersection of 3D printing for microfluidic fabrication and bioprinting 3D tissues shows great promise in the direction of single-step organ-on-a-chip engineering. The availability of biocompatible printable materials currently limits microfluidic channel and tissue construct dimensions in bioprinted tissues. However, rapid improvements in 3D printing resolution, even in low-cost consumer-grade 3D printers, are likely to resolve this concern in the near future.

Ultimately, organs on a chip may create a more biomimetic tissue model superior to traditional 2D static cultures and allow the use of human cells in contrast to animal testing. This would make it a useful tool to perform high-throughput drug screening and test for safety and efficacy before or in parallel with animal trials and before reaching the costly clinical trial stage. Further, organ-on-a-chip devices may be applied to study human tissues and diseases *in vitro*. With the use of induced pluripotent stem cells derived from patients and differentiated into cell types for a particular tissue, it will eventually be possible to generate organs on a chip with a patient's own cells to better study diseases, test drugs, and move toward personalized medical treatments. Single-step 3D printing of organ-on-a-chip devices will facilitate rapid prototyping and enable agile iterative design, which are likely to accelerate innovation in this area.

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