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Review

Towards single-cell bioprinting: micropatterning tools for organ-on-chip development

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Organs-on-chips (OoCs) hold promise to engineer progressively more human-relevant *in vitro* models for pharmaceutical purposes. Recent developments have delivered increasingly sophisticated designs, yet OoCs still lack in reproducing the inner tissue physiology required to fully resemble the native human body. This review emphasizes the need to include microarchitectural and microstructural features, and discusses promising avenues to incorporate well-defined micro-architectures down to the single-cell level. We highlight how their integration will significantly contribute to the advancement of the field towards highly organized structural and hierarchical tissues-on-chip. We discuss the combination of state-of-the-art micropatterning technologies to achieve OoCs resembling human-intrinsic complexity. It is anticipated that these innovations will yield significant advances in realization of the next generation of OoC models.

Highlights

While the organ-on-chip (OoC) field has rapidly evolved with the integration of sophisticated microengineered designs, development of microenvironmental control and sensing capabilities, it is still lagging behind in terms of achieving natural tissue microarchitecture, which is imperative for its actual function.

3D (bio)printing techniques are increasingly used for the fabrication of OoCs, due to either the rapid-prototyping capabilities for chip fabrication or the ability to integrate tissue microarchitecture.

Laser-induced forward transfer is a versatile micropatterning technique and was particularly successful for the precise and reproducible deposition of cells, even with single-cell resolution.

The promise of organ-on-chip models

OoCs are microengineered biomimetic systems that reproduce functional units of human tissues and organs (Box 1). Consisting of perfused channels and cell-laden culture chambers, these models replicate key aspects of human organ/tissue function, such as tissue microarchitecture in 3D via the spatial positioning of multiple cells and tissue types; inclusion of cell microenvironmental cues in a native-like organization to mimic the physiological environment of the cell; and the effect of tissue interfaces such as tissue–tissue, organ–organ interactions, and barrier systems on systemic homeostasis [1,2]. Their applications range from drug discovery and toxicity testing to molecular biology and **personalized medicine** (see Glossary). Therefore, distinct OoC models will benefit from distinct levels of complexity, and have a clear fit-for-purpose design tailored to answer specific biological questions [2].

The first OoC designs were based on modeling barrier systems and liquid–air interfaces. Some of these early designs had similarities with Transwell cultures with added mechanical actuation. The lung-on-a-chip is an example of such a model, where cells are seeded on both sides of a stretchable membrane separating a vascular and an alveolar epithelial compartment [3]. Further advances in barrier models also included those for replication of bacterial infection and tumor progression [4,5]. From the first, most simplistic models, the field has rapidly progressed towards a broader array of models for health, disease, and drug screening with varying degrees of complexity. Currently, the OoC field is moving in the direction of increasing architectural complexity and linking diverse tissues together via, for example, vascular networks, to study systemic responses. In 2022, Ronaldson-Bouchard and colleagues reported on a multiorgan chip containing heart, bone, liver, and skin where mature tissue compartments were linked via vascular-like flow [6].

By having stand-alone OoCs or multiple OoC models linked together, these systems avoid some of the limitations encountered in traditional cell cultures and animal models. In particular, OoC

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Box 1. Organs-on-chips

Microphysiological systems, otherwise known as OoCs, are advanced microfluidic-based cell culture models that replicate the function of a tissue or organ, with diverse levels of complexity on a miniaturized and perfusable platform. Their development leverages advances in the fields of tissue engineering (TE), microfluidics, biomaterials, and (stem) cell biology [79]. Their footprint ranges from USB-sized devices to larger multiplexed systems with a microplate format [80]. Typically, OoCs are fabricated from optically transparent materials, including glass, plastic, or clear silicon-based resins such as polydimethylsiloxane (PDMS), to enable imaging and monitoring. There have been many approaches to the fabrication of OoCs; they often contain microchannels, typically ranging in diameter from a few to hundreds of micrometers, through which media, blood, or air analogs flow [80]. Channels can be fabricated via molding, patterning, or 3D bioprinting [81]. They are typically seeded with cells or contain a tissue-engineered construct (e.g., a cell-laden hydrogel), commonly with adjacent perfusable channels. These models can thus be continuously perfused, ensuring that cells constantly receive nutrients, and shear stress can be adjusted to physiological rates. OoCs aim to replicate and study cell–cell and cell–ECM interactions in both healthy and diseased conditions. Thus, these systems can integrate diverse cell types, which can be combined with ECM-like materials, to reproduce key functional organ and tissue-level structures. To achieve physiological relevance, some devices incorporate a semipermeable membrane to, for example, mimic the barrier function, such as the lung and blood–brain barrier [82,83]. Other devices include hydrogel scaffolds that facilitate cell migration, differentiation, and/or vascularization [84]. Cell source is of paramount importance for increased complexity in OoCs, from 2D cultures to 3D scaffolds, as well as for 3D-bioprinting of complex architectures. Personalized medicine approaches are attainable by using patient-derived stem cells, iPSC-derived cells, and/or organoids [80]. Further complexity can be achieved by including actuation to mimic multiple types of mechanical stimuli and current findings attest the importance of adding physiological parameters to more closely resemble disease progression in humans [1,11,85]. The addition of mechanical cues allows inclusion of physiological characteristics in the models, such as stretching of the airway during breathing, cardiac and musculoskeletal contractility during physical exercise, load bearing of joints during locomotion, and motions of the gastrointestinal tract during peristalsis [4,86–89]. Finally, body-on-a-chip or system-on-chip can be created by linking two or more tissues or organs. These complex systems mimic key systemic connections and are typically used in the pharmaceutical industry to study the absorption, distribution, metabolism, and excretion (ADME) of candidate drugs [90].

models allow the study of human tissues in a more physiologically relevant manner than traditional cell culture models. Moreover, there is ever-growing academic, regulatory, and industrial effort to establish OoC models as new gold standards [7]. In this review, we focus on the advantages of microarchitecture to improve OoC models functionality and how **biofabrication** technologies are anticipated to contribute to this feat. We identify laser-induced forward transfer (LIFT) as a new key technology to engineer microstructures on chip and thus focus on the technical aspects and promise of LIFT for OoC technologies.

On the importance of microarchitectural features for improved tissue functionality

While OoC applications and functional designs have diversified and greatly improved over the past few years, the field is still in its infancy. The requirements for design, sampling, and imaging are still being defined to realize representative platforms that will eventually rival state-of-the-art screening models. Tissue function correlates intimately with its unique microarchitectural structures, here defined as the minute structures, compositions, and arrangements. Hence, the faithful incorporation of such microarchitectures in OoC is anticipated to increase their physiological relevance. Several studies over the past few years have highlighted the significance of microarchitecture in these advanced models [8,9].

Microarchitecture has been proven to be a key factor when replicating relevant biological processes *in vitro*. For example, Kang and colleagues, demonstrated the importance of microarchitecture in a three-layered alveolar barrier model with increased physiological barrier properties (e.g., surfactant secretion and increased ion transport). It reproduced physiological influenza A virus infection processes compared with both a 2D model and a disorganized 3D model, where disease progression was not accurately resolved [8]. Additionally, microarchitecture confirmed its significance in the engineering of a trabecular bone-on-chip. The influence of a calcium-phosphate-rich material coating onto scaffolds was investigated. These constructs were 3D-printed with multi-photon lithography after a computed tomography (CT) scan of the trabecular mesh, seeded with human mesenchymal stromal cells (hMSCs) and

Glossary

4D bioprinting: 3D printing of biological tissues with predictive and controlled remodeling and maturation over time as the fourth dimension. These changes can be the result of specific structural designs but also induced by an external trigger such as temperature, light, electricity, or magnetic fields.
Biofabrication: engineering of living systems using relevant biological materials and techniques such as bioprinting or bioassembly.
(Cell) differentiation: the process during which a pluripotent or multipotent cell acquires a specific phenotype and functionality via lineage commitment.
Cell line: isolated cell, cloned to form a homogeneous population of cells.
Extracellular matrix (ECM): a network of macromolecules providing key characteristics of tissue structure and functionality.
Human-induced pluripotent stem cells (hiPSCs): adult somatic cells are transfused with the Yamanaka factors to revert their differentiation to pluripotency.
Personalized medicine: refers to the study of patient-specific disease to gain specific understanding of individuals and provide a targeted therapy at the individual's level.
Tissue engineering (TE): biofabrication of living functional constructs for reassembly or therapeutical purposes, using relevant cell sourcing and biomaterials, proper architecture, and biochemical and biophysical cues.
Voxel: small element of volume, also defined as 3D pixel.

differentiated to bone under dynamic conditions on chip. The coated scaffold, replicating more precisely the microarchitecture and **extracellular matrix (ECM)** composition of bone, showed higher ECM deposition rich in collagen type I than the uncoated scaffolds [9].

Alterations in the intrinsic composition and microarchitecture of the ECM have a critical role in the development of numerous diseases, resulting in distinct cell behavior. For example, several studies have demonstrated the effect matrix remodeling has on tumor invasion, showing how changes in ECM composition and concentration, pore network, interconnectivity, and fiber plasticity allow tumorigenic cells to migrate and invade neighboring tissues [10,11]. The thickness of collagen type I fibrils affected myofibroblast **differentiation** of adipose-derived MSCs independently of the bulk stiffness of the matrix, and this network architecture also revealed to play a role in wound healing and tumor development [12].

Changes in microarchitecture are predictive of disease, thus a fundamental feature of tissue physiology. The great majority of studies have observed large influences of microarchitecture on bone neoformation and angiogenesis. It has been investigated that more interconnected pore networks with thinner fibers and a higher internal volume favored bone ingrowth and differentiation [13,14]. Sun and colleagues developed a polydimethylsiloxane (PDMS) chip where arrays of 3D tumors were patterned in their relevant microenvironment in a highly reproducible manner for drug screening of chemotherapy agents. Tumors patterned with complex ECM-like gradients as microenvironments showed higher sensitivity to chemotherapy agents compared with dispersed tumor cells without the environmental cues. Together these studies highlight how the microenvironment and microarchitecture play a key role in the response of cells to drugs [15].

Cells and tissues typically fail to exert their native role without proper 3D organization, which is closely related with the functions they perform in the body. To the best of our knowledge, there are only a few reports of microarchitectural features being reproduced in OoC. Nevertheless, we foresee a great opportunity to increase biological complexity and human translation by combining both.

Towards engineering microarchitectures in OoCs

In the past few decades, research efforts have moved from 2D cultures of one single-cell type to 3D cocultures, encompassing scaffolds and ECM-like hydrogel materials. On the one hand, OoCs consist of miniaturized and microperfused cell culture platforms that aim to replicate tissue function with high freedom of design, actuation, and monitoring. The incorporation of microarchitectural features and microstructure on chip such as organized and polarized microfibers, directed microporosity and connected pore networks is troublesome without using assisting technology. On the other hand, 3D-bioprinting has developed the technology and knowledge base to reproducibly incorporate minute microarchitectural features into complex and organized tissue constructs through automatized processes. Therefore, the merging of both fields is expected to allow an increase in the complexity of OoC platforms, which, in turn, will yield highly relevant human and predictive models that more effectively recapitulate human pathophysiology *in vitro,* validated against gold standard models and screening techniques. Combination of both fields may aid in the pace and reproducible production of OoC devices.

Enabling technologies for micropatterning applications

Various micropatterning technologies have been explored as biofabrication or 3D bioprinting tools, we have grouped them into those based on nozzle-based (Box 2) or light-based (Box 3) techniques. We describe their principle of action and how they can hold promise to converge with OoCs for microfabricating architecture on chips. We illustrate representative examples of

Box 2. Nozzle-based bioprinting

To this day, nozzle-based technologies are the most-used biofabrication or 3D-printing method. They are most commonly used to print, for example, hydrogels, bioactive materials, and cells in 3D configurations, also termed scaffolds. Listed technologies are illustrated in Figure I.

EB is based on the continuous extrusion of polymer from a cartridge through a nozzle and its subsequent deposition in the form of a filament. Ideal printable materials are shear thinning and present elastic recovery, which means their viscosity decreases when subjected to shear stress and they subsequently recover shape when the stress is relieved [91]. There are diverse modes of extrusion for EB. Hydrogels can be extruded through a nozzle via pneumatic or mechanical actuation. For the latter, the hydrogel is laden in a corkscrew-based cartridge that rotates or within a piston forced cartridge to dispense the hydrogel. Pneumatic actuation operates through compressed air [92]. The parameters that govern the process of EB are applied pressure on the material, nozzle diameter and nozzle /substrate displacement speed, temperature, distance between the nozzle and the substrate, and printing time. The technology allows the printing of filament diameters of 100–500 μm with high cell densities [91,93].

DoD of biopolymers shares the same mechanism as EB. DoD introduces breaks on the jet strand via the introduction of heat bubbles, piezoelectric actuation, electrostatic forces, and electrodynamic jetting. To introduce strand breaks in the extruding jet, thermal DoD heats the jet locally and introduces vapor bubbles, piezoelectric DoD uses vibrations of an actuator, electrostatic DoD deforms the pressure plate, and finally, electrodynamic DoD uses a high voltage electric field [94]. The printing methods used in DoD are compatible with low-viscosity inks (3.5–12 mPa/s) [22,95].

CEW builds on the principles of melt electrowriting. There, a polymer is placed in a syringe under pneumatic pressure and temperature control. At the same time, the nozzle is subjected to an electric field. The ejected filament enters the electric field which forms a stable jet from the nozzle to a moving collector plate through the electric field [25]. Via this mechanism, CEW can generate fiber diameters between 5 and 50 μm.

Finally, these technologies have limitations in the shear stress cells receive as they are passing through a nozzle. The effect of shear stress depends on the cell concentration, size of suspended particles and affects cell viability and cell morphology [93].

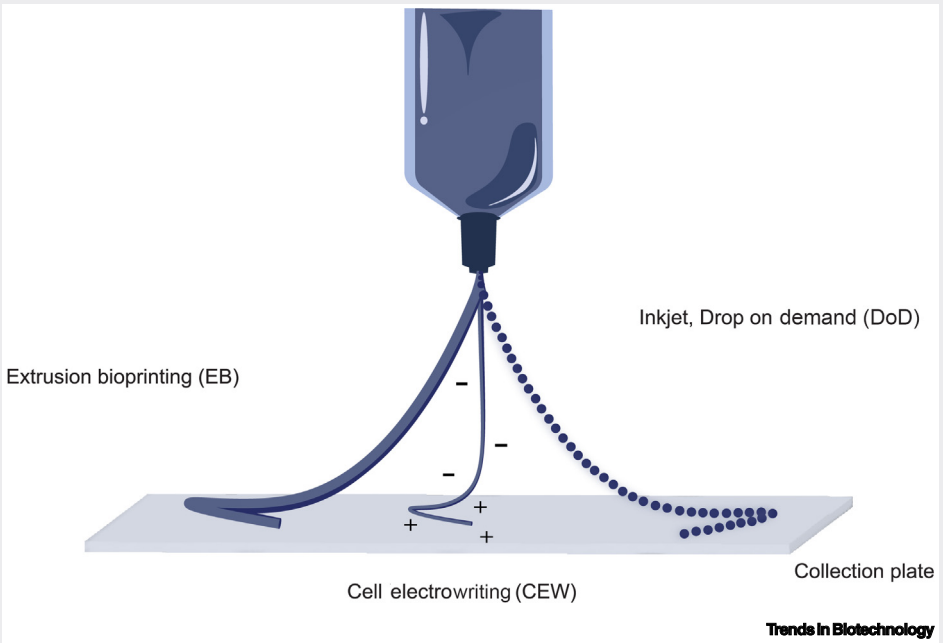

Figure I. Nozzle-based bioprinting. This category of bioprinting can be divided into three main types: extrusion (extrusion bioprinting, EB), inkjet (drop on demand, DoD), and cell electrowriting (CEW).

these technologies in Figure 1. We then compared and annotated them with a relative performance ranking (e.g., suitable/intermediate/limited) based on the desired and optimal characteristics of a printing system: resolution to achieve minute features, which will greatly depend on each example application; printing speed, which accounts for the time cells will be out of their ideal conditions and the negative impact the process can have on the cells [16]; cell viability which will ideally be as high as possible; implementation rates, which designate the level of difficulty expected to adopt the technology in a different laboratory regarding available specific equipment, materials, published knowledge base, economic cost, and commercial systems available.

Nozzle-based 3D printing

Extrusion bioprinting (EB) has been used to fabricate early-stage OoCs using sacrificial materials in a bath suspension. When the supporting material is dissolved, the channels remain open and can be used to seed cells, to perfuse media, for vascularization, or to act as the channels of the OoC. Open perfusable channels can be fabricated in this manner [17]. EB has been used to precisely dispense the cells inside an OoC platform [17,18]. Bhise and colleagues bioprinted hepatic spheroids inside a microfluidic chip with an open-top configuration. The spheroids were printed in concrete spaces of the chip and maintained both viability and metabolic processing of drug compounds over a 30-day culture period [19]. The process of EB is slow and because of viscosity-dependent shear stress, the use of low-viscosity liquids is challenging, while a compromise between cell viability and mechanical properties of the material needs to be achieved to increase fabrication throughput while lowering cellular stress. One solution to printing low-viscosity inks was recently proposed via a novel embedded bioprinting approach that leveraged aqueous two-phase stabilization of the sacrificial ink–bath interface. Advantageously, this allowed for high extrusion speeds (up to 1.8 m/s) without impacting cell survival. Moreover, this approach also grants control over fiber diameter via strand thinning as well as seamless fusion of prints, which offers the ability to create intricate microarchitectures such as complex, interconnected, and multidiameter vascular networks [20]. Conventionally, each material is loaded into a different cartridge, which might affect the efficiency of multimaterial printing. Nevertheless, by combining EB with a microfluidic mixer in the printing nozzle, the composition of the extruded filament can be controlled to create complex living architectures with up to seven different bioinks. Such adaptations have the potential to drive the field forward, towards more complex and directed printing [21].

Drop on demand (DoD) has been successfully used to print in open-top OoCs for various tissue applications. For example, alveolar specialized cells were bioprinted on microwell inserts that

Box 3. Light-based bioprinting

DLP (Figure I) is based on the projection of light **voxels** onto a resin reservoir to crosslink a computer-aided design (CAD)-generated model in a layer-by-layer manner. In DLP, the collector plate makes contact with the surface of the resin and drives the print upwards as each layer is simultaneously crosslinked. Minimum feature resolution is dictated by the layer height, which can be as small as 20 μm [96,97].

VBP is developed on the principle of CT. Polymerization takes place in a vat of optically transparent photo-crosslinkable material. The vat rotates as a laser shines filtered back-projections of a CAD-generated file. The projections coupled with the vat rotation generate a holographic-like pattern in the material, which crosslinks in tens of seconds. VBP has shown to print positive features of 40 μm, and negative features of 100 μm. VBP allows the crosslinking of centimeter-sized constructs in tells of seconds without a significant negative impact on the viability of cells and even organoids [33,98].

MPL is a laser-based technique where two galvanometric scanners scan the laser focus in the *xy* plane and scan the moving *xyz* stage. A femto-laser is focused on an area with a photoresponsive resin where two or more photons are simultaneously absorbed. The photon absorption leads to voxel polymerization in a layer-by-layer manner. MPL has a lateral resolution of 100 nm and an axial resolution of 300 nm [99]. The drawbacks of MPL include the limited range of printable materials, the toxicity of most commonly used photoinitiators, the high cost of the system, and the long time to fabricate small-sized constructs. For printing speed indication, a 450 × 450 μm^2 grid can be fabricated in 56 min [36].

Figure I. Light-based bioprinting. This category of bioprinting can be divided into three main types: digital light printing (DLP), volumetric bioprinting (VBP), and multi-photon lithography (MPL).

were subsequently inserted into OoC platforms, effectively mimicking the alveolar barrier under dynamic perfusion. The OoC with 3D structured cells and materials outperformed traditional 2D Transwell cultures composed of the same cell types without the spatial localization of the cells. OoC with microarchitectural features showed higher barrier integrity and expression of transporters, closer to physiological levels [22,23]. DoD was also used to study the interaction between osteoblasts and osteocytes by printing them in close proximity and applying several

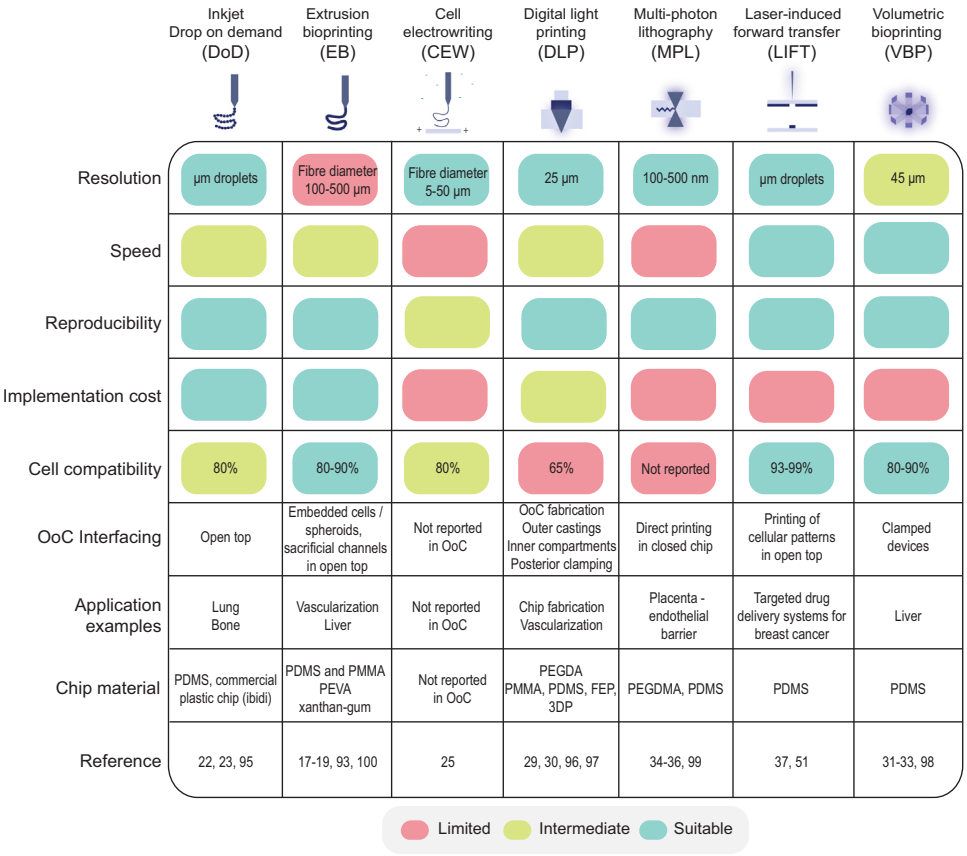

Figure 1. 3D bioprinting technologies used for micropatterning and their use in OoCs. Here, technologies are ranked with a color scale representing beneficial to not beneficial for the application, based on their print resolution, speed to print, reproducibility, implementation cost of the technology as well as reported cell viabilities postprinting. The ways of interfacing with OoCs are indicated with which types of designs have been combined with the technology. Some applications of the printing technique used for OoCs are stated with their composition and corresponding reference: DoD [22,23,95]; EB [17–19,93,100]; CEW [25]; DLP [29,30,96,97]; MPL [34–36,99]; LIFT [37,51]; VBP [31–33,98]. Abbreviations: 3DP, commercial UV-curable resin; CEW, cell electrowriting; DLP, digital light printing; DoD, drop on demand; EB, extrusion bioprinting; FEP, fluorinated ethylene propylene; LIFT, laser-induced forward transfer; MPL, multi-photon lithography; OoC, organ-on-chip; PDMS, polydimethylsiloxane; PEGDA, poly(ethylene glycol)-diacrylate; PEGDMA, poly-(ethylene glycol)-dimethacrylate; PEVA, poly-(ethylene/vinyl acetate); PMMA, poly(methyl methacrylate); VBP, volumetric bioprinting.

regimens of mechanostimulation and fluid flow. This novel approach allowed the study of cell behavior at different boundary conditions and under mechanical load [24].

Cell electrowriting (CEW) also has limitations in material printability and cell viability. With CEW, cell viability typically is around 80%, similar to EB, yet with added adverse effects due to the exposure of cells to an electric field with high voltage and very small diameter nozzles [25]. There are no reports of CEW being used in combination with OoCs at present.

All the aforementioned technologies are promising to combine with OoCs and are widely available with a multitude of printable materials. Traditional versions of these systems have moderate cell viability due to shear stress and long times to print completion, particularly when printing larger structures as organoids. Novel modifications of the systems are being developed to enable

rapid multimaterial printing and the printing of low-viscosity inks. All reported applications for DoD and EB are on open-top OoCs, as direct access is needed for printing.

Light-based 3D printing

Light-based techniques bypass the limitations associated with nozzle-based techniques, increase resolution, allow printing within closed and clamped OoC and allow the patterning of moieties in already present hydrogels and pre-made structures.

Digital light printing (DLP) has historically been used to print hard resins for the fabrication of chips in hard resins or silicon materials [26,27]. ECM-like hydrogels have been used for the printing of cellular microenvironments in high resolution with embedded convoluted networks of human mimetic microchannels that remained open and perfusable, with 50-μm resolution. The developed hydrogels supported embedded MSCs differentiation as proof of concept to use DLP as a high-resolution technique for micropatterning minute structures and microvessels on chip [16,28]. DLP has also been used to directly fabricate cell-laden OoCs with features of 50 μm [29]. Multimaterial printing in commercial DLP printers is restricted by the presence of a single reservoir, but some laboratories use a custom-built stage with several rotating reservoirs with necessary print washes in between. However, reported cell viabilities are low, at 50–65% with elongated printing times [29]. Another strategy uses microfluidic mixing devices in the reservoir to achieve a smooth transition in multimaterial prints, allowing for the incorporation of continuous gradients in porosity, chemicals, mechanical properties, and material gradients [30]. Finally, DLP is compatible with open-top configurations of OoCs as well as chip fabrication. Therefore, such multimaterial approaches could potentially facilitate the printing of both inner and outer structures of cell-laden OoCs.

Volumetric bioprinting (VBP) has been used to fabricate liver biofactories using liver organoids that can be perfused and metabolize urea [31]. In this example, diverse inner structures with varying degrees of porosity and interconnectivity were printed to study the effect of microarchitecture on organoid metabolism. The study showed that more convoluted inner architectures with small, highly interconnected pores increased metabolic activity and urea processing in the organoids. VBP allows for the micropatterning of growth factors in 3D and has been combined with other bioprinting techniques that hold promise for multimaterial printing and the fabrication of reinforced scaffolds as arterial grafts or models for vascular branching [31–33]. VBP has increased cell compatibility and printing speed compared with DLP. Nevertheless, DLP can currently achieve higher resolution than VBP, which allows for easier printing of multimaterial constructs and building up printing on existing surfaces, such as the inner parts of a chip.

Multi-photon lithography (MPL) is a laser-based technique with capabilities for unmatched resolution, offering specific advantages that could be leveraged for high resolution micropatterning OoCs, despite its current inability to print large volumes. MPL has been used to fabricate the inner compartment of a placenta-on-a-chip model through a barrier with thicknesses of 25 and 100 μm, directly inside a closed chip. The barriers were then cell-seeded and studied for their transport ability of both small (10 Da) and large (20 kDa) molecules [34]. These achievements support the fabrication of OoC inner compartments with minute resolutions in an unprecedented manner. MPL was also used to fabricate a trabecular bone scaffold, which was subsequently coated with calcium phosphate and seeded with hMSCs for incorporation in an OoC for bone differentiation over a 21-day period [9]. Consequently, MPL can be considered a promising technology for high resolution with high reproducibility structures to be printed directly inside closed OoCs, in addition to producing scaffolds for open-top or clamped devices. However, a current hurdle to the wider use of this technology is the limited availability of biocompatible photoinitiators.

To address this, increasing efforts are directed toward developing a larger spectrum of biocompatible resins and photoinitiators. Additionally, novel photoinitiator-free approaches have also been developed for MPL. In a study by Nakayama and colleagues, polyethylene glycol diacrylate (PEGDA) and collagen type I were printed without photoinitiator and achieved resolutions of 100 nm [35]. Breakthroughs in biocompatible MPL will bring this technology closer to OoCs, where it holds promise for high reproducibility and minute features printed directly in closed OoCs [34,36].

The last technique to be discussed in this section is LIFT, which is a laser-based technique (Box 2 and Figure 2A). It is mostly discussed in the next part of this review. Unlike DoD, LIFT can micropattern small droplet volume within 2 s per print, with high freedom in material choice as well as notably higher viability than those reported for other light-based printing techniques. In addition, it has the added advantage of printing at single-cell resolution [37]. Selection of the cells to transfer to discrete spaces is unprecedented and anticipated to yield a breakthrough in the creation of highly compartmentalized and structured OoCs. Each print requires the coating of a new donor slide, enabling facile and rapid multimaterial printing compared with other droplet techniques as DoD. Even though the technique has been described for decades, its use in bioapplications is still in its infancy. Therefore, although promising, the adoption cost of LIFT is still high, as most systems are laboratory-made with few systems being commercially available.

We have discussed the need for reliable and relevant models, the importance of microarchitectural features to increase complexity and human relevance to advance OoC functionality, as well as highlighted the potential technologies to enable this feat. Altogether, micropatterning complexity on chip relies on the ability to replicate the cell microenvironment and tissue organization via controlled spatial positioning of hydrogels, bioactive molecules, and cells. The key to tissue complexity resides in replicating a highly heterogeneous environment, which can potentially be achieved by rapid multimaterial printing of high cell densities in precise, micrometer-sized locations. Here, we have focused on 3D bioprinting as a means to rapid and reliable fabrication of microarchitectures on chip. There are microfabrication techniques other than printing technologies that allow the creation of microarchitectures, which have been extensively reviewed elsewhere [38,39]. Here, we focus on LIFT, since this technique is uniquely suitable for use in spatially complex on-chip applications.

Opportunities for complex OoCs with cell-scale resolution: applications of LIFT

The unique capabilities of LIFT in combination with its compatibility with living systems makes it a promising tool for increasing complexity, relevance, and consistent throughput of OoC models. Overall, its reduced material constraints permit the reproducible and exact placement of a wide variety of materials, from solid metals to conductors, and from bioactive molecules to cells (Figure 2B). Owing to this versatility and fast mode of action, LIFT has found applications in numerous and diverse fields, from electronics to life sciences [40].

LIFT for single-cell printing in hierarchical living tissues-on-chip

The laser-printing process induces no DNA damage nor heat-shock to cells (Box 4), rendering LIFT as a biocompatible direct-writing method. In addition, it supports the activity of differentiated cells from primary cultures, **cell lines**, stem cells, and **human-induced pluripotent stem cells (hiPSCs)**. Koch and colleagues successfully printed hiPSCs derived neural stem cells, either before differentiation or post-differentiation at different stages [41]. They leveraged the versatility of LIFT technology for 3D-bioprinting of neuronal networks, which demonstrated how LIFT can use patient-derived cells for personalized therapies or for the biofabrication of personalized OoC models, which is one of the milestones that the field strives to achieve. Therefore, the use of such a material source is of great interest to include the human adaptive immune response or

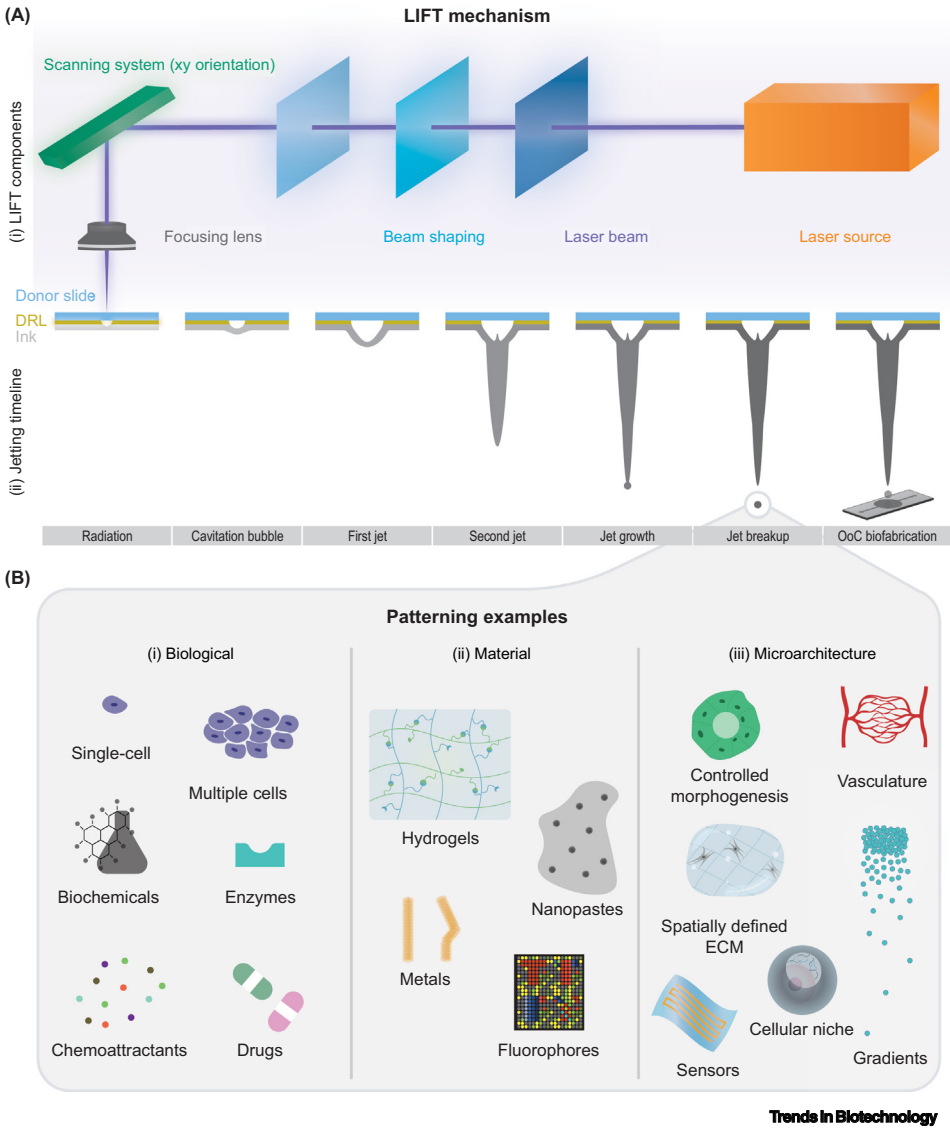

Figure 2. LIFT mechanism and notable patterning examples. (Ai)For : laser source – defined by its wavelength, fluence, pulse duration, and pulse repetition rate – emits a laser pulse that goes through a set of mirrors to shape the beam [101,102]. The latter then goes through a scanning system, which will recreate the designed pattern created via computer-aided design (*xy* position orientation of the beam). Thanks to a lens, the laser beam focuses on the ribbon. (ii) a cavitation bubble (a high-pressure, high-temperature gas bubble) forms, initiating the transfer of the material towards the substrate of the receiver, placed at a given donor–receiver distance [101]. The jet formation occurs at the expansion of the bubble. First, a thin jet rises but it is not sufficient to eject the material. The bubble then deflates and recoils into the bulk fluid. A second bulge then emerges from the liquid–air interface and a thicker jet rises [40,58,101–103]. Once the jet impacts the receiver, the droplet spreads onto the surface and its diameter increases until reaching its final dimension. (B) Patterning possibilities are (i) living matter, up to the single-cell level; (ii) biochemicals; LIFT is also suitable for the printing of (bio)materials such as hydrogels, nanopastes, (3D) metals, and fluorophores; and (iii) the technology is a versatile tool that can build up architectural features in a controlled manner, for the deterministic morphogenesis of tissues, integration of vasculature networks, spatially defined extracellular matrices, defined cellular niches, tissue gradients, or even in printed electronics for sensors that can be embedded within organotypic cultures. Abbreviations: DRL, dynamic release layer; ECM, extracellular matrix; LIFT, laser-induced forward transfer; OoC, organ-on-chip.

Box 4. LIFT mechanism

There is increasing interest in the development of 3D-printing-based tools that enable the fabrication of complex structures with great precision, reproducibility, and resolution. LIFT is one such technique with the ability to print materials in both solid and liquid forms, allowing the deposition of a wide range of viscosities (from 10^{-2} to 10^{3} poise) [104,105]. The technology is based on the focus of a high-power pulsed laser beam on a substrate covered with a thin film of ink. Upon laser irradiation, the material hit by the laser spot is ejected, and a discrete voxel is transferred on the surface of a receiver's substrate placed in front (forward transfer) with great accuracy [101,106]. Figure 2A in the main text depicts the overall mechanism and major components of a LIFT printer, which are the laser source emitting the laser beam, shaped through sets of mirrors, oriented by the scanning system, focused by the lens on the donor, the donor loaded with the ink, and the receiver.

LIFT can be used as a pixelated (2D) and voxelated (3D) micropatterning technique. The final printing resolution depends on several parameters concerning the process, such as laser fluence (e.g., laser energy per area unit), ink layer thickness, distance between donor and receiver, as well as the composition of the ink and the surface properties of the receiver [40,103]. Reported droplet volumes range from femto- to nanoliters. While LIFT stands as an additive manufacturing tool in the *xy* axis (micropatterning and layering), the impingement of a donor material upon landing can bridge the micropatterning in the *z* direction. By optimizing the surface properties of the receiver (its softness) and the transfer speed, a donor material could be injected into a surface at a fixed depth. This technique has been successfully demonstrated by Delrot and colleagues, using gelatin [107].

LIFT is a multimodal technique that often uses a sacrificial layer to avoid ink modification steps for the material's absorption at a specific laser wavelength. This dynamic release layer acts as an intermediary between the donor material and the laser beam and will absorb most of the laser energy during the process [40,58,101–103]. This feature extends the printing possibilities since a single wavelength is now compatible with various donor materials, paving the way for the printing of intact structures [40]. In addition, it may be necessary to preserve the integrity of the structure and therefore avoid direct irradiation of the donor ink. In this case, the dynamic release layer will act as a protective layer against harm from the laser wavelength, although it does not offer protection from possible heating damage. As an elegant example demonstrating the compatibility of LIFT with living systems, Karakaidos and colleagues performed a study on the DNA damage in breast cancer cell lines after LIFT printing on gelatin. This demonstrated that LIFT induces only neglectable amounts of apoptotic cells and minimal amounts of DNA double-strand breaks at the single nucleus level [108]. A potential concern when using LIFT is the brief heating of the donor material, which can potentially have an adverse impact (e.g., heat shock) on living tissues. In this context, Gruene and colleagues, conducted a DNA damage assessment and studied the expression levels of hsp-70 protein (heat-shock protein), which demonstrated negligible differences between printed and non-printed porcine bone-marrow-derived MSCs, providing further confirmation of the biocompatibility of LIFT [109].

when studying a specific profile and choosing the best targeting treatment. In this context, LIFT can potentially offer the precise positioning of such valuable materials.

Biomaterials have been used in cellular constructs to mimic the microenvironment and spatial organization of tissues, natively scaffolded by the ECM. They are therefore key to study and reproduce *in vitro* models. The feasibility of LIFT for micropatterning droplets of biomaterials at high resolution was demonstrated in various studies. Yusupov and colleagues studied the LIFT printing of hyaluronic acid, methylcellulose, and alginate, commonly used bioinks in TE, at high resolution. The authors proposed a strategy to control the LIFT writing process in a decision tree perspective and offer a starting point for the replication of tissue niches, key for unraveling the principles of tissue function, as testified from transcriptomics to tumor research [42–44].

While OoCs provide a platform for *in vitro* complexity and dynamicity, the patterning at the single-cell resolution has not yet been explored. However, the study of biological systems at such high resolution is anticipated to offer deeper insight into cells' behavior within complex systems and under various stimuli. Several studies report the direct writing of single cells via LIFT. Many of these studies, which are listed in Table 1, prove that it is feasible to selectively target single cells or cell clusters within complex and heterogeneous populations, and transfer them without affecting their viability or functionality. These groundbreaking contributions could help advance the OoC field by ensuring single-cell printing with high efficiency and selectively choosing the best cell targets. It also allows for better understanding of organs and tissues at the cellular level by directly observing the influence of a single-written cell onto a complex tissue and at a precise

Table 1. Single-cell bioprinting using LIFT[a]

Context of study	Cell type	Bioink composition	Donor and receiver substrates	Results	Refs
Proof-of-concept studies					
Single-cell printing efficiency, cell viability, and damage	Human osteosarcoma cells (MG-63)	Culture medium	Donor: ink layer onto a quartz disk coated with a metallic DRL Receiver: microscope slide coated with 50 μm Matrigel	Number of cells per printed droplet followed a Poisson distribution and was therefore deterministic Single-cell deposition successfully achieved within reproducible patterns Viability at 100% at 6 days postprinting Cell damage investigated through heat shock protein (HSP) expression, which was minimal.	[110]
Track and precise deposition of single to multiple cells with a femtosecond laser	B16F1 mouse melanoma cells, hTSPCs, hMSCs, human papillary thyroid carcinoma cells (TPC1)	Cells in histopaque 1083 hydrogel	Donor: μ-Dish as a reservoir for the cell laden hydrogel, without DRL Receiver: gelatin-coated substrate for TPC1 cells and hMSCs, collagen-coated substrate for hTSPCs, Matrigel-coated substrate for B16F1 cells, BSA scaffold for hMSCs	Based on cell size, morphology, or fluorescence, cells could be manually selected to print within a homogeneous or heterogeneous population via an inverted optical microscope. Transfer occurred within a 25-μm radius to the laser focus, meaning, for all the cell types, both single and multiple cells could be transferred depending on the distance between each other. Fluorescence imaging of the jetting transfer gives additional proof for single-cell transfer. Transfer precision assessed and hMSCs deposited ± 14–32 μm from their target position. Viability was at 93–99% for all cell types 66 h postprinting with observed migration and proliferation.	[37]
Follow-up study with a longer pulse duration laser (from fs to ps laser) e.g., slower jet	hMSCs (SCP1)	Cells in histopaque 1083 hydrogel	Donor: μ-Dish as a reservoir for the cell laden hydrogel, without DRL Receiver: gelatin-coated substrate	Single-cells could again be transferred over the pulse duration range explored. Viability 15 min postprinting was at 95%. Longer-pulse-duration lasers are cheaper and more compact than fs lasers.	[111]
Track and precise deposition of single to multiple cells within complex population	Human B- lymphocytes expressing GFP (C1R-N1-85), Human T lymphocytes (Jurkat cells), Mouse HPSCs and a human NK cell line	Cells within alginate or methylcellulose	Donor: ink onto a polyimide coated slide (BA-LIFT) Receiver: polyimide coated substrate or 96 well plate containing media for NK cells	Based on cell size, morphology, or fluorescence, they were able to track cells to print within a homogeneous or heterogeneous population. Tested discrimination criteria on the donor were: hematopoietic ± expression (HPSCs), live/dead cells, cell types based on antibody staining (B and T lymphocytes). Cells, both single and multiple, could be transferred depending on the distance between each other, and 50 μm between cells was needed for single-cell printing. Viability was dependent of the printed fluence and was 85–98% for the lowest fluence.	[112]

Table 1. (continued)

Context of study	Cell type	Bioink composition	Donor and receiver substrates	Results	Refs
				Assessment of colony-forming units was done for transferred HSPCs until 14 days postprinting, confirming maintenance of proliferation and differentiation potential of the stem cells. Activation and metabolic activities of NK cells were assessed and proven to be maintained.	
AI tool for single cell and cell cluster isolation within a fully automated device	Epithelial cells (HeLa)	Adherent cells in culture medium	Donor: adherent cells immersed in a 150-µm layer of cell medium within a microtiter plate without DRL Receiver: glass plate	Detection of single-cells positions within an adherent HeLa culture after AI software performs the analysis of the plate scan. A high-speed microscope with a moving stage then follows a continuous path to focus on the exact positions of the single cells and the transfer of the cells via a LIFT printer occurs at this focal point. The AI software was able to discriminate undifferentiated IPSCs, differentiated, detached and dead cells from an IPSC culture but cell transfer was not shown in the study.	[113]
Application studies					
Nerve regeneration for spinal cord injury	Primary rat OECs	Cell suspension in methylcellulose and culture medium	Donor: ink layer onto a quartz disk coated with a metallic DRL Receiver: poly(L-lysine) coated substrate with culture medium or a multilayer Matrigel scaffold (60–100 µm thick)	Because of the low amount rat yields for OEC, LIFT bypass the issue through the use of a small amount of material required for donor loading and single-cell printing. Cells express marker of functionality 3 days postprinting. Viability 24 h postprinting was the same within 5% than initial ink viability. Cell–cell interaction and cell migration were observed. Cells migrated up to 400 µm at 24 h postprinting within the 3D scaffold.	[114]
Angiogenesis dynamics in cancer, using the intact microvascular network (blood and lymphatic) of an explant	Breast cancer cells (MDA-MB-231 and MCF-7) and human dermal fibroblasts (BJ5ta)	Cell suspension	Donor: ink layer onto a quartz disk coated with a gelatin DRL Receiver: rat mesentery tissue explants on Transwell inserts	Single, multiple cells or heterogeneous cell types were transferred on targeted explant locations (blood and lymphatic vasculature). Cancer cell dynamics were studied at single-cell resolution using a biologically relevant and complex *ex vivo* platform, by assessing migration of different cancerous cells, their influence on angiogenic sprouts and vasculature. Pure and heterogeneous populations of fibroblasts and MDA-MB-231 cells migrated within the mesentery tissue while MCF-7 cells remained in their original printed position at day 2 postprinting.	[115]

(continued on next page)

Table 1. (continued)

Context of study	Cell type	Bioink composition	Donor and receiver substrates	Results	Refs
				Angiogenesis was observed over a 5-day period with a twofold increase of capillary sprouting in the vicinity of printed cancer cells.	
Isolation of circulating tumor cells in blood for single cell sequencing	Breast cancer cells (MDA-MB-231)	Cancer cells spiked into peripheral blood from healthy donors, simulating cancer samples	Donor: ink onto a polyimide coated glass slide (BA-LIFT) Receiver: collector tube or culture well	Cancer cells were discriminated as circulating tumor cells from the rest of the blood sample via antibody staining. Cells were then manually selected and individually transferred to be collected.	[116]
Non-LIFT studies					
Micropipette suction technique for single-cell positioning	Mouse fibroblasts (NIH 3T3), canine kidney epithelial cells (MDCK)	Cell suspension in microgels support bath	Donor: cells within the support bath Receiver: cells within the same support bath	Single-cell microgels were manually suctioned and redeposited via a micromanipulator connected to a confocal microscope. This technique exposes the cells to shear stress (hence the protective microgel shield) and is limited by manual deposition. While authors outlook an automation of the process for cell tracking, suction, and deposition, it does not allow for as precise positioning and as damageless single-cell writing as LIFT in its current form.	[117]
Microfluidic sorter to deposit single-cell droplets onto a substrate	NIH 3T3 cells, Jurkat cells, human liver epithelial cell line (HepG2), hiPSCs and primary hepatic stellate cells	Aqueous cell suspension	Donor: microfluidic sorting platform Receiver: no substrate restrictions mentioned	This platform allows for FACS-like sorting of cells with high-precision deposition through a dripping nozzle (10 µm precision) and requires fluorescent-staining of cells prior to printing. Cells are thus either discarded or deposited onto a 3-mm distanced substrate at a desired (*x,y*) coordinate, allowing the selection of specific cell types within a heterogeneous population but also more effective printing, out ruling deterministic prevision of Poisson distribution for single-cell droplets. This technology is interesting; however, it does not have the versatility of LIFT to print more complex structures with various inks, and less importantly, necessitates cell-staining (which could be advantageous but also requires additional experimental step).	[118]
Magnetic positioning of cells via paramagnetic agent and magnetic field	NIH 3T3 cells, hMSCs, HUVECs, MDA-MB-231 cells, brain cancer cells (SH-SY5Y)	Cell suspension in gadolinium-based, nonionic paramagnetic agent, cell medium	Substrate free: capillary between two magnets	No use of magnetic particles which are often cytotoxic. Cell magnetization is removed with a washing step. Guide cell organization in 3D (substrate free). Live cells levitate within the magnetic field and aggregate over time.	[119]

Table 1. (continued)

Context of study	Cell type	Bioink composition	Donor and receiver substrates	Results	Refs
				Controlled cell interaction: cell–spheroid or spheroid–spheroid interaction were analyzed. The strategy developed by the authors is interesting as it allows the 3D assembly of heterogeneous materials into biofunctional units and biological interactions, up to the single-cell level. However, this system does not itself allow the spatial positioning of these bioassembled units, which would be of interest. In addition, it is not clear if it is limited to organoid/spheroid application.	

[a]Abbreviations: HPSC, hematopoietic progenitor stem cell; hTSPC, human tendon stem/ progenitor cells; NK cell, natural killer cell; OEC, olfactory ensheathing cell.

position. Single-cell deposition of various cell types at different stages of differentiation would provide additional reproducibility and fabrication control over the morphogenesis of complex structures such as organoids. Organoids are disease models of interest and various studies combine them with OoCs for vasculature or perfusion purposes [45,46]. Finally, other single-cell positioning strategies are reported in literature and we have stated a few technologies in Table 1 with a brief comparison with LIFT.

LIFT has been increasingly used in **tissue engineering (TE)** for the construction of cardiac patches, corneal tissue or even *in situ* for bone tissue fabrication in a mouse [47–49]. Using the advantage of LIFT in building spatial complexity, Hakobyan and colleagues established a model to study the development of pancreatic ductal adenocarcinoma (PDAC) using a rat pancreatic acinar cell line as well as derived ductal cells. Cell suspensions with defined concentrations were printed in a microarray pattern, and the cells within those microarrays self-assembled into spheroids that reproduce the structure of pancreatic cancer. The LIFT-printed tissue behaved similarly to native PDAC and resembled disease progression more than the analogous 2D cell culture model [50]. This study is an example of how defined spatial positioning, as opposed to random positioning and nondeterministic cell number deposition, can guide and help reproduce the intrinsic architecture necessary to replicate complex biological processes.

Spatial complexity often implies local heterogeneity; for example, the precise spatial patterning of multiple cell types as well as molecular, material, or mechanical gradients. For these applications and due to its versatility, LIFT is a suitable writing tool that can pattern highly controlled heterogeneity within organotypic systems. In a proof-of-concept study, Xiong and colleagues used LIFT for the micropatterning of cells within different matrices on chip [51]. Douillet and colleagues conducted a **4D bioprinting** study of human dermal fibroblasts micropatterned in a collagen matrix and analyzed the self-reorganization and remodeling of the tissue construct over time based on diverse patterns [52]. After writing myofibroblasts and fibroblasts on collagen in isotropic and anisotropic patterns, spatiotemporal remodeling dynamics were tracked of both cell populations on their matrices. They observed differences in tension exerted by cells on the matrix and the cells self-reorganization in clusters or layers based on the initial pattern and, thereby, isotropic/anisotropic contraction of the matrix. Importantly, they demonstrate how we can optimize the biofabrication process by leveraging modifications in cell morphology, cell–cell, and cell–ECM interactions and ultimately, tissue functionalization.

Another important challenge is the engineering of nutrient access in organotypic cultures by promoting vascularization, which can contribute to the prevention of the formation of necrotic cores in large, cell-dense constructs. Strategies have been reported on using LIFT with endothelial cells and endothelial growth factors for the self-assembly of capillary-like structures. Kérourédan and co-workers achieved the formation of vascularized networks by layering patterns of high densities of human endothelial progenitor cells on top of collagen I, containing hMSCs and growth factors [53]. Kawecki and colleagues achieved tubule-like structures of endothelial cells that were printed on top of osseous and stromal cell sheets. In this study, it was hypothesized that such a construct could support *in vitro* perfusion as well as heal autologous bone defects [54]. Later on, Kérouédan and colleagues applied this prevascularization strategy *in situ*, printing endothelial cells on a filled mouse calvarial bone defect with collagen containing MSCs and vascular endothelial growth factor [55]. The TE construct displayed bone regeneration and neovascularization. Importantly, it was demonstrated that following a designed pattern is necessary to achieve control over the organization of the vascularization network, as opposed to the random seeding of cells (as controls). These cases, however, do not yet attest to perfusable networks, although lumen formation has been achieved. Follow-up work with these OoC models are likely to yield perfusable vascular networks.

To conclude, recent studies have confirmed the feasibility to print relevant materials with high spatial positioning. We have highlighted examples that demonstrate the ability to precisely, rapidly, and reproducibly deposit and transfer individual cells or groups of cells with high viabilities. Writing of living matter can also be used to guide the self-organization of tissues via the precise placement of biochemical cues (e.g., molecular gradients, ECM-like biomaterials) and relevant cell types; enhancing cell differentiation, polarization, alignment, tissue organization, and functionality. Several biofabrication techniques have proven significant value for micropatterning hierarchical OoCs, and due to these discussed unique features, it is anticipated that LIFT can expand this toolbox by offering additional technological solutions.

LIFT for close monitoring of complex humanized models

The integration of sensors and actuators within microfluidic systems is studied extensively as it is critical to create multiplexed and readable biological platforms. As LIFT can print a wide range of materials – including bioactive materials and metals – with small volumes and at high resolution, it has been proven a suitable technique for the fabrication of functional devices including biosensors, chemosensors, and 3D structures like metallic micropillars (which could pave the way for the LIFT-manufacturing of micropillar electrode microarrays) [40,56–67].

The integration of oxygen, glucose, lactate, or extracellular field potential sensors have been investigated in electrical, electrochemical, or optical forms to allow for real-time measurements in OoCs to determine tissue behavior. For instance, transepithelial electrical resistance (TEER), is frequently used for the study of cell barrier integrity, which is essential for tissue homeostasis, guaranteeing exchanges and protection [68]. It is therefore important to monitor the integrity of this barrier when replicated in OoCs, as either a characteristic of the relevance of the model or to demonstrate what can cause it to weaken. To our knowledge, no study has reported the use of an *in situ,* reliable TEER sensor that can withstand the stretching of the cell compartment while this would be of high interest to continuously monitor actuated biosystems [69,70]. In that prospect, the field of OoCs could benefit from stretchable electronics. Fabrication of such flexible sensors can be achieved via LIFT [66,71]. Zacharatos and colleagues showed nanoarray printing with embedded gold nanoparticles within planar and non-planar PDMS surfaces as a deformable resonance monochromatic reflector, proving possible the LIFT fabrication of such high-demand devices [72]. PDMS is a material predominantly used in OoCs and the embedding of gold

Key figure

Bioprinting modalities for next generation organs-on-chips (OoCs)

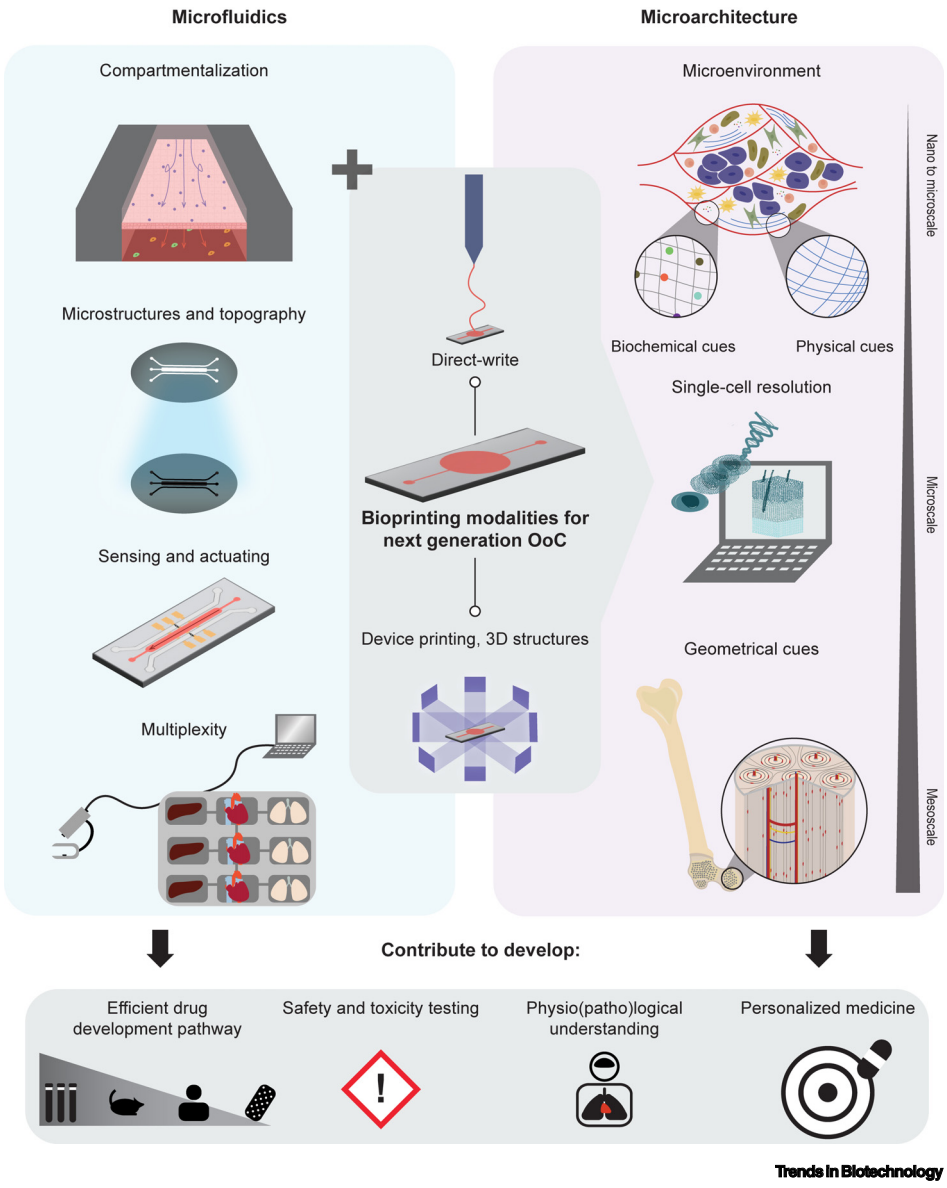

Figure 3. OoC current possibilities rely on their state-of-the-art fabrication via sophisticated techniques such as photolithography or 3D printing (e.g., digital light processing, stereolithography), to achieve compartmentalization and fine topographical features. Due to the possibility of adding microfluidic perfusion and the use of various flexible membrane or actuators, OoCs are defined as dynamic models. It is possible to engineer multiplexed platforms with increased readability via (bio)sensors or microscopical imaging and where various organs or tissues-on-chips can be parallelized for tissue crosstalk studies. Next-generation OoC fabrication strategies include, through the combination with enabling micropatterning tools, manufacturing at the single-cell resolution, at the tissue scale. Importantly, geometrical cues should be designed for better mimicry of tissue function, as seen in their native counterparts. In addition to relevant cells and biomaterials, the micropatterning of biochemical or physical cues can help achieve architectural control over the tissue microenvironment. Overall, the use of bioprinting to incorporate

(Figure legend continued at the bottom of the next page.)

(nanoarrays) in a structured surface such as pillars or channels is of high interest for sensing or actuating purposes. We therefore anticipate that LIFT could aid in the fabrication of flexible and stretchable electronics, which could answer a great demand for reliable sensing in dynamic and complex microfluidic systems.

Among optical chemical sensors, peptide microarrays are often used for multiple purposes, such as antibody profiling. Notably, Paris and colleagues automated a LIFT process to create high-throughput peptide microarrays to *in situ* synthesize residue peptides to screen for Ebola virus, and with higher quality than commercially available peptide microarrays. Owing to the versatility of their process, the researchers also achieved the synthesis of fluorophore arrays via Schiff base condensation reaction which produces fluorescent dyes [73]. Schiff bases can be used as label free optical sensors – for metal ions for instance (which are important in biological processes) – and are compatible with biological environments [74,75]. These demonstrators validate the ability of LIFT to create such sensors, which can be leveraged to also be included within OoC.

A critical aspect for OoC models is microscopic access for imaging, Sopeña and colleagues demonstrated the LIFT printing of conductive and transparent silver nanowire networks as alternatives to highly constraining indium tin oxide that is used in various devices [76]. Therefore, we hypothesize that such conductive networks could be incorporated in OoCs without compromising microscopy access.

The concept of 'Lase and Place' has emerged where LIFT is used to transfer intact structures onto a substrate, which cannot be supported by traditional manufacturing processes in electronic systems [77,78]. With increased efforts in the field, we could envision the direct writing of functional devices (or even functional biostructures such as explants, microgels, or organoids) or their *in situ* assembly within organotypic cultures. Combined with single-cell printing, this could allow for increased monitoring and actuating at cellular level within a complex tissue range.

To summarize, LIFT has the unique ability to both print living matter as well as functional devices, even with added benefits in comparison to traditional techniques, for the field of OoCs (e.g., stretchable and transparent devices).

Concluding remarks

Achieving true physiological complexity on chip remains an unmet challenge. We foresee that the inclusion of microarchitectural features as a future trend that will achieve on-chip organ functionality, behavior, and responsiveness that will rival their native physiological counterpart. However, the engineering of fine, minute microstructures on chip is challenging. We have reviewed solutions to enable the engineering of complex and more predictive *in vitro* models towards the ultimate goal of achieving precision placement of single cells and/or relevant biochemical cues and biomaterials, in 3D or 4D. After comparing several micropatterning techniques that allow the biofabrication of complex tissue structures, we identified LIFT as a raising technique that suits this purpose. Specifically, LIFT allows for the printing of a wide range of (bio)inks in solid or liquid form, which enables micropatterning at single-cell resolution and is suited to print flexible electronics. The combination of LIFT and microfluidics holds potential to create increasingly relevant and effective humanized models that include microarchitecture, microenvironment (biophysical and biochemical

microarchitectural features within microphysiological systems will bring us one step closer to the fabrication of complex, hierarchical OoCs. This will contribute to answering the need for more reliable and relevant platforms for safety, toxicity, and drug testing, increased biological knowledge as well as for targeted and personalized medicine.

Outstanding questions

What microarchitectures are essential for which OoC applications?

To what level do tissue architectures need to be emulated to relevantly improve OoC function?

Will micropatterning of tissue structures aid in the commercial adoption of OoCs via standardized tissue behavior?

Which micropatterning techniques will prove suitable and scalable to create microstructured OoCs?

In which part of the drug discovery pipeline are advanced OoCs containing sophisticated tissue microarchitecture most valuable?

cues), and *in situ* monitoring (e.g., sensors). While several challenges remain to be addressed, such as the introduction of native-like, free-form 3D geometries within OoC systems (see Outstanding questions), we conclude that the control over microarchitectural features within dynamic microphysiological systems can lead to a closer mimicry of characteristic morphological features and functional properties of organs and tissues. Incorporating micropatterning techniques will likely lead to the next generation of OoC models, better supporting their clinical and pharmacological translation (Figure 3, Key figure).

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Declaration of interests

The authors declare no conflict of interests.

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