“Engineered human organs” hold promises for predicting the effectiveness and accuracy of drug responses while reducing cost, time, and failure rates in clinical trials. Multigenerational human models utilize many aspects of currently available technologies including self-organized spherical 3D human organoids, microfabricated 3D human organ chips, and 3D bioprinted human organ constructs to mimic key structural and functional properties of human organs. They enable precise control of multicellular activities, extracellular matrix (ECM) compositions, spatial distributions of cells, architectural organizations of ECM, and environmental cues. Thus, engineered human organs can provide the microstructures and biological functions of target organs and advantageously substitute multiscaled drug-testing platforms including the current in vitro molecular assays, cell platforms, and in vivo models. This review provides an overview of advanced innovative designs based on the three main technologies used for organ construction leading to single and multigenerational systems usable for drug development. Current technological challenges and future perspectives are also discussed.

1. Introduction

Drug discovery remains an unprecedented challenge: Current pharmaceutical approaches to the discovery of a single drug out of 1 million compounds can take 15+ years and cost US$ 900M+ using a series of intensive tests on multiple platforms (Figure 1).[1-5] Failures in clinical trials are in part due to the absence of relevant human models and inaccurate predictions derived from preclinical animal models.[6,7] Therefore, there is a critical need for accurate human-representative models. Such models should provide reliable predictions of drug efficacy and safety in the clinical trials and reduce time and cost for drug testing.

Since Rose G. Harrison developed 2D culture techniques in 1907,[8] 2D cell culturing protocols have been established for human/animal/primary/stem/iPS cells and cell lines. Using 2D cell cultures, basic drug testing with readouts of a wide range of biochemical assays and optical imaging technologies have been implemented. However, 2D environments lack key physiological features observed in human tissues/organs and thus recorded toxicological responses to drugs in those 2D conditions may not be representative.[9-11] In 2D conditions, cells are grown on solid-phased substrates while other surface is exposed to synthetic liquid-phased media. In contrast, a 3D extracellular matrix (ECM) provides mechanical and biochemical clues in vivo. Indeed, the cells grown in 2D conditions show markedly different motility and morphogenesis when compared to those grown in a 3D environment. Moreover, in 2D culture conditions the drug metabolism is limited and lessened likely because multicellular and orchestral interplays present in 3D cultures, tissues or organs are missing. Thus, 2D culture conditions are not appropriate or effective tools for drug discovery.

Currently, animal models serve as gold standards for preclinical trials. However, animal models have limitations including time-consuming development, high costs for maintenance and experiments, not to mention technical challenges in interpreting the results due to the complex in vivo environments.[12] Moreover, some animal models may not be relevant to drug assessment in specific human conditions as animals often metabolize chemical compounds differently from humans. Indeed, species-specific differences in the physiological and pathological biology can result in incorrect prediction of human responses.

Advances in biomedical technologies now enable scientists to miniaturize human organisms in 3D (Table 1). 3D cultures combine multiple types of human cells in ECM-like natural/synthesized gels engineering naturally arranged cell-free and/or cell-laden structures. Further, within those engineered 3D cultures, environments can be precisely organized in arrayed forms to allow high-throughput drug screening.[13-22] Such 3D engineered relevant human models may advantageously replace the basic 2D screening platforms and current preclinical animal models. Indeed, 3D model advantages include involvement of high-ordered regulation of environmental clues, short period/low-cost for model development, reliable tuning of multiple parameters and wide range of analyses as 2D models (Figure 1). Furthermore, engineered 3D cultures abilities to model human organs extends to the integration of multiple human organs. Indeed, engineered 3D cultures have already been used to model how humans to metabolize drugs through the interactions among multiple organs.[23-30]

In this review, we provide a perspective on the three-major emerging 3D culturing technologies: 3D organoids, 3D microfabrication, and 3D bioprinting. As those technologies enable the modeling of the crucial features of human 3D organs essential to advance drug discovery, this review is based on the
technologies used rather than on the organ mimicked. Indeed, construction technologies define the major critical limiting factors including shapes, spatial resolutions, structures, and functions of the organs developed. Therefore, we first introduce the general principles, the technological backgrounds, and the main organs recently successfully modeled. Those 3D organ models are detailed to highlight how 3D culturing technologies overcome the major technical and biological challenges to implement drug discovery processes with low cost, up-scalable, high throughput, and high controllability. Next, we further discuss the current challenges and perspectives of 3D organ modeling technologies integrating multiple organs to achieve the miniaturization of the key human body functions toward applications in fundamental research, translational medicine, and personalized precision medicine.

2. Engineering Technologies

In vivo, cells reside within the complex microenvironments of tissues and organs where they receive cues from other cells, ECM, local soluble environments, and mechanical environments. These interactions play essential roles in maintaining and modulating cellular phenotypes and processes. In engineering model tissue and organ systems, the goal is to mimic many of these interactions to generate models with the key features of tissue organization and function. There are the two general strategies in generating organ models: bottom-up and top-down approaches. In a top-down approach, the strategy employed is to engineer individual components of a tissue environment that, together, mimic and recreate aspects of the system. For example, cellular components can be integrated by co-culturing multiple cell types in defined physical arrangements, 3D organization can be mimicked with biomaterial scaffolds and microfluidic channels, mechanical cues can be presented by biomaterials and fluid flow, and soluble stimuli can be delivered via perfusion. Top-down approaches include organ-on-a-chip models, which aim to generate key aspects of an organ structure and function in a microfluidic device. Bottom-up approaches rely on the emergent behavior of biological systems to generate complex tissue- and organ-like constructs. Pluripotent stem cells (PSCs) are primarily used as the cell source due to their capacity for self-organization through processes of self-assembly, self-patterning, and self-driven morphogenesis. Independent of the approach used to develop 3D printing models, accurately recapitulating key aspects of the in vivo environment remains a major challenge. A fundamental understanding of native tissues and organs is first needed before mimicking these in vitro cultures. Continued progress of these areas will improve the relevance of 3D cultures for the biological investigations of human development and diseased states including drug assessment. Here we highlight the three main technological approaches developed to address these challenges.

2.1. Organoids

Organoids derived from human PSCs have been established for brain, gut, kidney, brain, retina, and cancer. Organoid (i.e.,
spheroid) cultures offer a tissue-like environment for testing drug toxicity. Organoids are 3D microtissues that overcome the major constraints of 2D tissue models and provide prolonged cell viability and functionality.[41] Organoid-based techniques are compatible with a variety of different pipetting methods and coculturing techniques allowing the development of multorgan models.[38] In these multicellular aggregates, the need for supporting gels or matrices is eliminated, the adverse effects

Table 1. Synopsis of miniaturized 3D organs by construction technologies and major achievements.

<table>
<thead>
<tr>
<th>Organ(s)</th>
<th>Technique(s)</th>
<th>Cell type(s)</th>
<th>Major achievement(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Self-assembled</td>
<td>iPSCs[4]</td>
<td>3D culture approach for generating a laminated cerebral cortex like structure from pluripotent stem cells.</td>
<td>[57,58]</td>
</tr>
<tr>
<td></td>
<td>Microfabrication</td>
<td>Neuroprogenitor cells</td>
<td>Microfluidic culture platform containing a relief pattern of soma and axonal compartments connected by microgrooves to direct, isolate, lesion, and biochemically analyze CNS axons</td>
<td>[67,68]</td>
</tr>
<tr>
<td>Intestine (Gut)</td>
<td>Self-assembled</td>
<td>Primary human cortical neurons</td>
<td>Discrete layers of primary neurons in a RGD peptide-modified gellan gum</td>
<td>[118–120]</td>
</tr>
<tr>
<td></td>
<td>Microfabrication</td>
<td>Stem cells</td>
<td>Identified intestinal stem cells and differentiated cells in vitro</td>
<td>[59,60]</td>
</tr>
<tr>
<td></td>
<td>Microfabrication</td>
<td>Human epithelial cells</td>
<td>Mimic contractility by using mecanochemical actuator</td>
<td>[11,19,27,72]</td>
</tr>
<tr>
<td>Liver</td>
<td>Self-assembled</td>
<td>Human stem cells</td>
<td>3D culture of self-renewing human liver tissue</td>
<td>[61,62]</td>
</tr>
<tr>
<td></td>
<td>Microfabrication</td>
<td>Hepatocytes and fibroblasts</td>
<td>Microengineered hepatic microtissues containing hepatocytes and fibroblasts</td>
<td>[73–77]</td>
</tr>
<tr>
<td>Vessel</td>
<td>Microfabrication</td>
<td>Rat brain endothelial cells</td>
<td>3D culture in microfluidic device</td>
<td>[63–66]</td>
</tr>
<tr>
<td>Heart</td>
<td>Microfabrication</td>
<td>Human cardiac differentiated cells</td>
<td>High-throughput pharmacological study</td>
<td>[36,78]</td>
</tr>
<tr>
<td>Lung</td>
<td>Microfabrication</td>
<td>Epithelial cells</td>
<td>Use of porous membrane to mimic lung functions</td>
<td>[31,37]</td>
</tr>
<tr>
<td>Bone</td>
<td>3D bioprinting</td>
<td>AS49 cells and EA hy926 cells</td>
<td>World’s first 3D bioprinted lung tissue</td>
<td>[101]</td>
</tr>
<tr>
<td>Cancer</td>
<td>Self-assembled</td>
<td>Intestinal stem cells</td>
<td>Discovery of LGR5+ intestinal stem cells</td>
<td>[52,62]</td>
</tr>
<tr>
<td></td>
<td>Microfabrication</td>
<td>Breast cancer cells</td>
<td>Perfusible human microvascularized bone-mimicking (BMI) microenvironment</td>
<td>[81,168]</td>
</tr>
<tr>
<td></td>
<td>3D bioprinting</td>
<td>OVCAR-5 and MRC-5 cells</td>
<td>Insight into complex cell–cell communication in 3D</td>
<td>[113–117]</td>
</tr>
<tr>
<td>Multi</td>
<td>Self-assembled</td>
<td>Liver, gut, vessel cells</td>
<td>High throughput hanging drop</td>
<td>[30,49–52]</td>
</tr>
<tr>
<td></td>
<td>Microfabrication</td>
<td>Liver, heart, and vessel cells</td>
<td>Automated control of perfusion</td>
<td>[11,19,27,32]</td>
</tr>
<tr>
<td></td>
<td>3D bioprinting</td>
<td>NPC and HCT-116 cells</td>
<td>Multiorgan bioprinted model</td>
<td>[30,122]</td>
</tr>
</tbody>
</table>

Table 1. Synopsis of miniaturized 3D organs by construction technologies and major achievements.

Figure 1. Current and improved pipeline for drug development with miniaturized human models.
generated by the adhesion to artificial surfaces is removed, and therapeutic agents are more reliably tested. Organoids have been formed via several different methods, e.g., spinner flask cultures,[11] utilizing rotating cultures,[54] stationary cultures in hanging drops with well-known 96 or 384 well plates,[55] and cell growth on nonadherent surfaces (Figure 2A).[50,56,132] These organoids form through self-assembly and their generation remains reasonably reproducible. Limiting dilution assays (LDAs) have been widely used to study the effectiveness of therapeutic drugs on the survival and proliferation of cancer stem cells as well as other types of tumor cells. As the use monolayer of adherent cell lines is not suitable, for high-throughput screening of LDAs, miniaturized 3D cell culture platforms were developed using micropillar/microwell chip allowing highthroughput drug screening and automatic cell colony identifying method (Figure 2B).[55,133] Among the many available 3D cell culture platforms, the micropillar/microwell chip platform may ideally support rapid LDA assays as it permits to easily cycle through many different media or drugs. This technique enables the controllable spheroid formation using a high-performance automated system compared to conventional techniques using 96 well plates. Below we detail recent organoids.

2.1.1. Brain Organoid

The central nervous system derives from the neural ectoderm. This tissue gives rise to the neural plate, which folds and fuses to form the neural tube, an epithelium with apical-basal polarity radially organized around a fluid-filled lumen that eventually forms the brain. Recently, 2D neural tube like structures called neural rosettes were established from isolated neuroepithelium or the directed differentiation of PSCs. Because neural rosettes recapitulate apical-basal polarity and exhibit spontaneous radial organization similar to that of the neural tube, they more
appropriately recapitulate many aspects of brain development. However, the 2D nature of the method prevents the modeling of the overall organization of the developing brain. Alternative 3D culture methods with the potential to recapitulate brain tissue organization have been used extensively recently. In particular, Paşca and co-workers has focused on developing 3D brain-like tissues using human induced PSCs (Figure 3A).\cite{57,58}

Their simple and reproducible 3D culture approach allowed the generation of laminated cerebral cortex like structures, i.e., human cortical spheroids (hCSs). hCSs contain neurons from both deep and superficial cortical layers and transcriptionally mimicked the in vivo fetal development. These neurons are electrophysiologically mature, display spontaneous activities, are surrounded by nonreactive astrocytes, and form functional synapses. These 3D cultures should permit detailed analyses of human cortical development, function and disease, and may prove a versatile platform for generating other neuronal and glial subtypes in vitro. This method was further improved and allowed generations of either the dorsal or ventral forebrain containing cortical glutamatergic or GABAergic neurons. These subdomain-specific forebrain spheroids can be assembled in vitro to recapitulate the saltatory migration of interneurons observed in the fetal forebrain. Using this approach, Paşca and co-workers demonstrated that in Timothy syndrome—a neurodevelopmental disorder that is caused by mutations in the CaV1.2 calcium channel—interneurons display abnormal migratory saltations.

2.1.2. Intestine Organoid

Clevers and co-workers had previously shown that adult intestinal stem cells could form organoids when cultured in 3D in Matrigel.\cite{59,60} Adult-derived organoids self-organized to form 3D crypt-villus structures, that mimicked the physiology and organization of the intestine, and could even be transplanted into mice. Similarly, hindgut spheroids generated from human PSCs can be grown in Matrigel 3D conditions to generate intestinal organoids. Intestinal organoids develop crypt-villus structures with stratified epithelium consisting of all the major cell lineages of the gut. These include columnar epithelial enterocytes with a brush border of apical microvilli. Finally, these organoids displayed intestinal functions including absorptive and secretory activities. Although the intestine is the only gut region so far generated from PSCs, other regions of the digestive tract have been developed into organoids from adult stem cells. These approaches similarly use the 3D Matrigel environment, which suggests that the presence of extracellular matrix components (e.g., Matrigel) is a general requirement in intestine organoid formation.

2.1.3. Liver Organoid

Recently, a progenitor population identified in adult mouse liver and activated after injury, was shown to generate 3D liver organoids when grown in Matrigel. These adult-derived liver organoids can be differentiated to form a network of mature, functional hepatocytes. Furthermore, liver organoids can be transplanted into mice to partially rescue mortality in a mouse model of liver disease, highlighting their functionality. Similar human liver organoids have not yet been generated, but a different approach was recently established to generate tissues reminiscent of human liver buds. First, human PSCs were differentiated into hepatic endodermal cells in 2D culture conditions. Next, three cell populations: the human PSC derived hepatic cells, human mesenchymal stem cells, and human endothelial cells were mixed. This mixed-cell population mimics the early cell lineages of the developing liver. When mixed at high density onto a layer of Matrigel, the cells spontaneously form 3D aggregates. These liver bud like aggregates display vascularization and when ectopically transplanted into mice get supplied with blood. Perhaps most promising is the finding that mice transplanted with these liver bud tissues secreted human-specific liver metabolites in the blood. Furthermore, survival of mice subjected to liver injury increased following liver buds’ transplantation.

2.1.4. Retinal Organoid

The retina has been used as an in vitro model of retinal layer formation for decades. Sasai and co-workers had developed optic cup organoids using mouse and human embryonic stem cells (ESCs) (Figure 3B).\cite{61} These retinal organoids show many of the characteristics common to mouse and human retina; they also display several human specific features. In particular, the human retinal organoids are larger than mouse organoids: they take longer to develop and they present tissue morphological differences such as apical nuclear positioning. Subsequent studies have used retinal organoids to examine the relations between the different cell types and how their differentiation and organization mimic the development of retina tissue. Matrigel dissolved in the medium at an early stage allows the formation of more rigid neuroepithelial tissues, a prerequisite of retinal pigmented epithelium formation. These buds are then cut away from the rest of the neuroepithelial tissues and maintained in a medium that supports retinal tissue differentiation. The resulting optic cup organoids very closely mimic early retina. They display proper markers of neural retina and a pigmented retinal epithelium. They also display retinal stratification with proper apical-basal polarity, and they undergo morphological tissue shape changes that mimic the stepwise evagination and invagination of the optic cup in vivo. These tissues may be useful for the study of the human-specific aspects of eye development.

2.1.5. Cancer Organoid

Tumor organoid models have been developed for breast, colon, and prostate cancers (Figure 3C). Most recently, pancreas and pancreatic cancer organoids have also been developed. Grabin-Botton and co-workers, and Clevers and co-workers have both developed methods for culturing normal murine pancreas in Matrigel as organoids.\cite{62} Grabin-Botton and co-workers cultured murine embryonic pancreas cells inside Matrigel to study pancreatic duct development. These embryonic pancreas
progenitor organoids proliferated in culture and underwent branching morphogenesis. In contrast, Clevers and co-workers, building on their prior work, developed methods to propagate adult murine pancreatic duct cells as organoids. When embedded in serum-free Matrigel and provided a cocktail of defined growth factors, including the Lgr5 agonist R-spondin I to stimulate Wnt signaling, pancreatic duct cells formed proliferating, cystic spheres. Flow-sorting experiments have demonstrated that these conditions allow the propagation of pancreatic duct but not of acinar or islet cells. While these organoids retained ductal characteristics in culture, they could form both duct-like, cytokeratin 19-positive structures and endocrine-like, insulin-positive cells following transplantation into the mouse renal capsule, demonstrating that cells cultured with this method are bipotent. In contrast to transplants of monolayer cell lines which rapidly form aggressive adenocarcinomas, orthotopic transplants of these murine or human pancreatic adenocarcinoma (PDA) organoids initially give rise to structures resembling precancerous lesions (such as pancreatic intraepithelial neoplasia, PanIN), which only later progressed to PDA resembling the tumor of origin. A number of reasons could explain this transplant phenotype. The organoid culture system may “reprogram” more aggressive cancer cells to a less aggressive state. Alternatively, organoid culture may select for less aggressive cancer cells more likely to give rise to PanIN when transplanted. Monolayer cultures may also select for more aggressive cancer cells, which can readily form tumors following transplantation. It is also possible that less aggressive subclones present in the organoid cultures or normal cells present in the host mouse have a “neighbor suppressive” effect on PDA formation initially after transplant of the organoids. Indeed, in vitro studies using monolayer cultures have shown

that fibroblasts can inhibit the growth of transformed cells, although the role of neighbor suppression in PDA remains unclear. More studies are needed to determine the biological basis of this transplant phenotype, and the time (2–8 months depending on the organoid culture) for transplanted tumors to form PDA. Nonetheless, the ability of PDA organoids to form PanIN-like structures following transplant can be exploited to study early pancreas cancer and investigate new diagnostic tools. In addition, murine organoids derived from normal duct cells, PanINs, PDAs, and metastases provide a progression series to examine changes associated with each stage of pancreatic tumorigenesis. The ability to rapidly and efficiently genetically engineer organoids using the CRISPR/Cas9 system also enables further cancer research. Two laboratories independently generated isogenic human intestinal organoids harboring both tumor suppressor and oncogenic mutations. Using these genetically engineered human organoids, researchers studied tumor development and invasive potential.\textsuperscript{[168]}

2.1.6. Lung Organoid

Lungs are composed of a system of highly branched tubes that bring air into the alveoli, where gas exchange takes place. The proximal and distal regions of the lung contain specialized epithelial cells: basal, secretory, and ciliated cells in the conducting airways, and type II and type I cells lining the alveoli. Basal, secretory and type II cells can be grown in 3D cultures, with or without supporting stromal cells. Under these conditions, they form self-organizing structures, i.e., lung organoids. Human lung organoids are well-suited to CRISPR/Cas9 gene editing technology allowing the identification of genes that regulate airway functions including barrier formation, selective permeability, fluid transport, innate immunity, and ciliogenesis.\textsuperscript{[139]} Indeed, recent studies identified the central role of the transcription factor grainyhead-like 2 (GRHL2) in coordinating barrier function and differentiation, and the transcription factor ZNF750 as a new component of the ciliogenesis pathway in the human lung.\textsuperscript{[140]} In these studies, since basal cells were not cloned post-transfection, the used cell populations carried a mixture of mutant GRHL2 alleles. While conditions have been developed in which single basal cells can be cloned in 2D culture,\textsuperscript{[160]} whether post-expansion each clone can form organoids remains to be tested. Additionally, nasospheres, organoids derived from nasal cells, potentially could allow the screening of small molecules and drugs that may regulate or compensate for the activity of mutant forms of the cystic fibrosis transmembrane conductance regulator, as measured by fluid transport and sphere diameter. As nasal basal cells can be isolated with minimal invasion, such an approach might be used to individualize the treatment of patients suffering from CF.

2.1.7. Kidney Organoid

The human kidney contains up to 2 million epithelial nephrons responsible for blood filtration. Regenerating the kidney requires the induction of the more than 20 distinct cell types essential for excretion and the regulation of pH, and electrolyte and fluid balance. Minoru Takasato has described the simultaneous induction of progenitor cells for both collecting duct and nephrons via the directed differentiation of human pluripotent stem cells.\textsuperscript{[141]} Paradoxically, although both are of intermediate mesoderm in origin, collecting duct and nephrons have distinct temporospatial origins. He also identified the developmental mechanism regulating the preferential induction of collecting duct cells versus kidney mesenchyme progenitor cells. On this basis, his group has generated kidney organoids that contain nephrons associated with a collecting duct network surrounded by renal interstitium and endothelial cells.\textsuperscript{[142]} Within these organoids, individual nephrons segment into distal and proximal tubules, early loops of Henle, and glomeruli containing podocytes elaborating foot processes and undergoing vascularization. Transcription profiles of kidney organoids closely mimicked first trimester human kidneys. Furthermore, functionally, the proximal tubules within the organoid properly endocytosed dextran and showed specific apoptosis in response to the nephrotoxic cisplatin. Such kidney organoids represent powerful models for nephrotoxicity screening, disease modeling and as a source of cells for therapy.

2.1.8. Stomach Organoid

The stomach is one of the most structurally diverse organs in mammals. In humans, the gastric mucosa generally consists of two types of epithelial glands. Located in the more proximal anatomical domains—the corpus and fundus—of the stomach, oxyntic glands comprise acid-secreting parietal cells, protease-producing chief cells, mucus-producing cells, and endocrine cells. Antral-type glands, located in the more distal antrum and pylorus, contain mostly mucous and endocrine cells. To simplify the anatomy- and species-specific structures, Kyle W. McCracken used the terms “fundus” and “antrum” to broadly describe these two histologic types of gastric epithelia.\textsuperscript{[143]} His group previously developed a method to direct the differentiation of hPSCs into 3D gastric tissue (human gastric organoids; hGOs) that contained a pure antral epithelium with normal antral cell types. Although these antral hGOs (hAGO) are a robust system to study antral lineage allocation and host–microbe interactions in the stomach, they do not allow studies of fundic biology and disease. More recently, Taka-aki Noguchi successfully differentiated mouse ESCs into organoids comprising various types of mouse gastric tissue.\textsuperscript{[144]} In both mouse and human, Wnt-mediated fundus signaling was required for the subsequent formation of parietal cells. The fundus-specific interventions at each stage of this directed differentiation protocol were essential for a robust parietal cell induction, emphasizing the importance of methodically reproducing the embryonic signaling environment. In combination with animal models, human gastric organoid systems may prove useful, in the elucidation of the signaling pathways between mesenchyme and epithelium coordinating early embryonic gastrointestinal development.

2.1.9. Multiorganoid

Self-assembled organoid technologies have gained significant momentum in recent years showing promise for applications
in research and development. Over the past few years, systems of increased biological complexity have begun to emerge that feature more than one organoid. These two-organoid or multiorganoid devices, sometimes referred to as “body-on-a-chip” systems, have immense potential beyond that of single organoid platforms, but, until recently, were primarily generated using cell lines or animal cells, rather than fully functional human primary cells or fully differentiated cells derived from stem or progenitor populations. These systems still required further improvements to more accurately mimic human physiology and responses to factors such as drugs and toxins. Recently, a reconfigurable microfluidic hanging drop network platform was generated to facilitate the tracking of the bioactivation of tumor cells from a bioengineered colon organoid to a bioengineered liver organoid, in parallel, on the same platform with a recirculating fluid flow (Figure 4). That model represents a highly versatile analytical platform formed of multicellular spheroids. This approach advances the frequently used hanging drop method toward fluidically interconnected hanging drop networks. The concept of interconnecting hanging drops in an array format and the capability to reconfigure these connections offer the following unique features: (i) controllable and reproducible spheroid formation from different cell types in parallel on the same platform, (ii) subsequent functional liquid interconnection of spheroids through microfluidic networks, (iii) controlled, continuous nutrient supply for developmental studies, (iv) microfluidic dosage of defined substances to the spheroids, and (v) continuous fluidic communication between different spheroid types to enable complex multiorgan models, or so-called “body-on-a-chip” experimental set-ups. These examples highlight key steps toward systems that mimic complex responses and interactions between tissues in drug and toxicology screens.

2.2. Microfabricated Organ on Chips

Advances in microscale technologies have greatly facilitated the development of 3D cell culture systems. With fabrication techniques, microfluidic devices can be rapidly designed and prototyped. In a common method of fabrication known as soft lithography, a master is first made in a cleanroom, using

---

**Figure 4.** Examples of multiorgan interactions that cannot be modeled with single organoid systems. A) Three handling steps are required during an experiment (close-up views show key areas): First, four different cell solutions are applied at the loading ports. Second, the isolated columns are connected via liquid introduced through the connecting ports so that the horizontal perfusion channels are open and usable. Third, perfusion is started and generates a gradient fluid flow through the array (highest concentration in bottom row). B) Layout of the device indicating the cell types in the respective drop columns: Columns 1–3 have been loaded with hepatocytes, column 4 contains HCT-116 cancer cells. After reconfiguration, rows are horizontally perfused in a closed-loop format; pairs of channels receive the same cyclophosphamide (CP) concentrations. C) Time protocol of the bioactivation experiment and simplified pathway of the CP activation. D) Phase contrast (Ph1) and fluorescence (GFP) micrographs of drop A3 and A4 in (B). Scale bars, 100 µm. Reproduced with permission. Published under CC-BY 4.0 license 2014. Copyrighted by the authors.
photolithography to pattern features on a silicon wafer. Then, individual devices are casted from the mold in a material such as polydimethylsiloxane (PDMS) and are bonded to a glass slide to generate enclosed channels. PDMS is commonly used for biological samples because it is biocompatible and optically transparent, facilitating imaging (Figure 5). Inherent features of microfluidics, including the small size scale, on the order of biological samples, and the existence of low Reynolds number, laminar flow together enable enhanced control over soluble and physical aspects of cellular microenvironments. Additionally, microfluidics can increase experimental throughput through assay integration, parallelization, and automation. Together, these capabilities make microfluidics well suited for the engineering of many types of organ culture on a chip.

2.2.1. Vessels on a Chip

Functional microvascular structures provide an in vitro model for study of complex vascular phenomena, including healthy angiogenesis and pathological thrombosis. Various methods have been established to generate microvessels on a chip in vitro utilizing the angiogenic/vasculogenic properties of endothelial cells (ECs). Yeon et al. established an array of 3D tubular capillaries of various dimensions inside a microfluidic chip by utilizing the angiogenic properties of human umbilical vein endothelial cells (HUVECs) (Figure 6A).[63] They first selectively patterned the fibrin gel in the middle of microchannel as a provisional matrix for angiogenic sprouts. HUVECs were then attached to both sides of the fibrin gel wall and stimulated to form sprouts by co-culture with fibroblasts. After 3–4 d, perfusable microvessels were constructed within the connection between endothelial sprouts on both sides and their dimensions or networks varied with different chip geometries. Kim et al. also developed a novel microfluidic platform and robust approach to form microvascular networks and establish perfusable lumens in 3D ECM constructs (Figure 6B).[64] The chip contains a central channel flanked by two fluidic channels and two outside stromal cell culture channels. To mimic the vasculogenic process, they filled the central channel with a fibrin gel and EC mixture with fibroblasts co-cultured on the two outside channels. For the angiogenic process, they filled the central channel with fibrin gel and attaching ECs on the gel wall with fibroblasts co-cultured on the opposite outside channels. ECs were stimulated to form a blood vessel network replicating essential features of the 3D vascular architecture: intact barrier function and lumina supported by the factors secreted by fibroblasts.

Figure 5. Fabrication methods for microfluidic chips. A) Replica molding generates stamps with shapes complementary to patterns etched in silicon chips by photolithography. A thin uniform film of a photosensitive material (photoresist) is spin-coated on a silicon chip, which is then overlaid with photomask (e.g., a transparent glass plate patterned with opaque chrome layers) bearing a microscale pattern generated with computer-assisted design software. The photomask protects some regions of the photoresist and exposes others during exposure to high-intensity ultraviolet (UV) light. The UV-exposed material dissolves in a developer solution, leaving the microscale pattern etched into the photoresist. Elastomeric stamps with a surface topography complementary to the etched surface are generated by a replica-molding technique in which liquid prepolymer of PDMS is casted on top of the etched photoresist pattern, polymerized and peeled off. The PDMS stamp can be used for microcontact printing of ECM molecules on any substrate, including those within microfluidic devices (not shown). B) A single-channel microfluidic device is fabricated by making a PDMS stamp with two inlets, a single main channel and one outlet and conformally sealing it to a flat glass substrate. A photograph of a two-chamber microfluidic culture device, in which red and blue dye are perfused through the upper and lower channels, is shown (right). The clear side channels are used to apply cyclic suction to rhythmically distort the flexible central membrane and adherent cells. Reproduced with permission.[27] Copyright 2014, Annual Reviews.
Zheng et al. replicated a network of endothelial microves-
sels within a native 3D collagen matrix and demonstrated
their biofunctionality, i.e., appropriate endothelial mor-
phology and barrier function. They most striking finding
was the demonstration that endothelium-secreted von Wille-
brand factor (VWF) assembled into thick bundles or complex
meshes, depending on the vessel geometry and flow character-
istics (Figure 6C). Cho et al. reported, for the first time, the
generation of 3D blood–brain barrier (BBB) in shape of vessel
tube in a microfluidic device. The 3D BBB chip quality was
confirmed through the localization at endothelial cell bounda-
ries of ZO-1 and VE-Cadherin, two components of tight and
adherens junctions. They also verified the tightness of that 3D
BBB by assessing its ability to reduce the dye leakage and to
block the transmigration of immune cells toward chemot-
tractants (Figure 6D). Moreover, to validate the functionality
of the BBB model, they probed its disruption by neuroinflam-
mation mediators and ischemic conditions and measured the
protective function of antioxidant and ROCK-inhibitor treat-
ments. Overall, the vessel model provided a robust platform,
adequate for detailed functional studies of vessels and for the
screening of BBB-targeting drugs in neurological diseases.
These chips show promising improvements when compared
to conventional Transwell models. Similar complex models are
also needed to reproduce the function and architecture and
match the complexity of the brain.
2.2. Brain on a Chip

Brain is a complex organ based on its structural and functional hierarchy, high specialization, and constant metabolic demand. It is difficult to identify the smallest structural and functional unit as individual neurotransmitters, ion channels, or synapses each play key roles. In vitro systems are unlikely to encompass the entire physiological function of the central nervous system (CNS) and the complexity of the brain. Neural tissue in the CNS has a tight control on the neuronal micromilieu for effective synaptic transmission. The neural parenchyma contains numerous cell types such as neurons (primary effectors of synapses), oligodendrocytes, astrocytes, and microglia. Further, neural tissue through interactions with parenchymal cells, vascular cells and the ECM elicit a variety of actions, such as angiogenesis, remodeling, and immune functions. The brain is comprised of many different interconnected neuronal areas providing specific connections or wirings to guide neuronal development and direct formation of axons, synapses, and dendrites. Various experimental models of circular microfluidic compartmentalized co-culture platforms with highly ordered physiological neuron connection architecture are used to study brain development and degeneration. Taylor et al. described a microfluidic culture platform containing a relief pattern of soma and axonal compartments connected by microgrooves to direct, isolate, lesion, and biochemically analyze CNS axons (Figure 7A).[67,68] Cho et al. reported another key accessory cells in brain, a microglia chip. Progressive microglial accumulation at amyloid-β (Aβ) plaques is a well-established signature of the pathology of Alzheimer’s disease, but how and why microglia accumulate in the vicinity of Aβ plaques is unknown. To understand the distinct roles of Aβ on microglial accumulation, they quantified microglial responses to week-long lasting gradients of soluble Aβ and patterns of surface-bound Aβ in microfluidic chemotaxis platforms. Better understanding of microglial migration can provide insights into the pathophysiology of senile plaques in Alzheimer’s disease (AD) (Figure 7B).[69] Nervous tissues from different regions of brain are composed of different cell types. Wevers et al. demonstrated a method for culturing 3D, ECM-embedded neuronal-glial networks in a microfluidic platform, called the OrganoPlate. The OrganoPlate has a microtiter plate format comprising 96 tissue chips that can be used for 3D cell culture and co-culture. Human induced pluripotent stem cell (iPSC)-derived neural stem cells or iPSC-derived mature neurons and astrocytes from various sources are mixed with Matrigel and seeded in these microfluidic chips. The potential neurotoxic effects of various compounds were studied by assessing the electrophysiological activity of neurons in the network, the extent of neurite outgrowth, and the cell viability in response to drug treatment (Figure 7C).[70] Takeda et al. also developed a novel neural network culture technique to mimic the layered structure of cerebral cortex by controlling the positions of somata and the direction of neurite outgrowth on the basis of the groove guide connected through three compartment chambers (Figure 7D).[71] This 3D heterogeneous neural component allows the visualization of the network of two different neural connections and was used to assess tau propagation in Alzheimer’s disease. Tau spreads in a hierarchical pattern in AD brain during disease progression, likely by trans-synaptic tau transfer between neurons. They examined the uptake and propagation properties of different tau species derived from post-mortem cortical extracts and brain interstitial fluid of tau transgenic mice, and from human AD cortices. The use of microfluidic devices in brain on a chip models to mimic neural circuits associated with different genetic backgrounds may provide a better understanding of the mechanisms involved in multiple neurological diseases and allow the unraveling of brain complexity. Thus, brain on a chip may likely be a useful substitute for animal models in high-throughput drug screening.

2.2.3. Gut on a Chip

Gut functionality relies on the successful maintenance of the commensal relationships between mammalian epithelial cells and gut microbiota. While heterogeneous co-cultures of eukaryotic cells have been demonstrated, the co-cultures of eukaryotic and prokaryotic cells are challenging due to dominant prokaryotic cell growth rates compared to those of eukaryotic cells. This is an intricate challenge, and thus solving it is critical to the development of devices that mimic organs like the gut. Recently, mammalian cells and Escherichia coli were cultured in a compartmentalized fashion demonstrating the growth of these two cell species separately with nutrients delivered through disconnected microfluidic channels before the subsequent removal of an intermediary barrier.[72] The cells were grown on the same substrate with pneumatically actuated PDMS barriers separating the two cultures. The barrier was necessary for both cultures to grow due to the transient host–pathogen interactions rather than symbiotic growth, resulting in the mammalian cell layer deteriorating in the continual presence of E. coli. Indeed, a cyclic dynamic environment led to growing intestinal microbes onto an intact enterocyte monolayer whereas co-cultures in a static environment resulted in reduced viability of the enterocytes (epithelial cells found in the small intestine). Controlling the competitive nature of many interspecies relationships and maintaining a multiorganism symbiosis at the cellular level remain a prominent challenge; however, these studies indicate the potential for great enhancement of the effectiveness of future gut mimicking systems.

2.2.4. Liver on a Chip

The liver is a key organ with a complex structure responsible for detoxification, glycogen storage, and plasma protein synthesis. The functional liver unit is the hepatic lobule, consisting of sinusoids, i.e., blood vessels lined with a permeable endothelium and surrounded by polarized hepatocytes and several mesenchymal cells (Kupffer cells, stellate cells, macrophages, and lymphocytes). Hepatocyte functions are key in the metabolism of xenobiotics and to evaluate hepatotoxic effects in pharmacology. However, it is difficult to maintain the liver-specific function of classically cultured (2D conditions) hepatocytes in vitro because they are nonproliferative differentiated cell that rely on appropriate biological interactions with stroma and other cells of the liver. To enhance the activity of hepatocytes,
co-culture with fibroblasts—which enhances cell-to-cell interactions—is the most widely used method. Kane et al. fabricated a microfluidic array with wells capable of supporting micropatterned primary rat hepatocytes.[73] The co-culture models with two or three cell types demonstrated more effective hepatic function than models containing hepatocytes alone. The co-culture models exhibited better hepatic function under flow loading than in static cultures. Kane et al. concluded that coculture and flow loading were in large part responsible for the improvement in hepatic function in these models. Future microarchitectural modeling should also mimic the basolateral villi and the space of Disse between hepatocytes and blood sinusoids with fenestrated endothelium. A very interesting approach to model the complex sinusoid-like structures using 3D perfused hepatic co-cultures in a microfluidic system has been developed by Griffith and co-workers.[74–77] This microfluidic model includes key features such as adjustable flow rates based on oxygen consumption and long-term steady maintenance of the oxygen gradient. This comprehensive system, using co-cultures of diverse heterogeneous cells, forms liver
sinusoid-like structures in which hepatocytes are not sheltered from shear stress. This in vitro model captures the complexity of liver sinusoid behavior in a scalable and easy-to-use format and is an interesting alternative with drug toxicity and liver metabolism applications.

### 2.2.5. Heart on a Chip

Successful mimicry of muscle structure and function has exciting applications in regenerative medicine. Development of heart muscle-like tissue is heavily dependent on myotube alignment and on cellular organization. Microcontact printing in combination with extracellular matrix glycoproteins improved cellular alignment thus the differentiation of myotubes as well as muscular contraction following electrical stimulation. In addition, muscular thin films (MTFs) have shown promise to assays muscle contractility. Following seeding of muscle cells on a thermally sensitive polymer MTFs can be released through heat application to the substrate. Specifically, MTFs have been generated by spin-coating poly(N-isopropylacrylamide) (PIPAAm) onto a glass substrate masked and patterned with a disposable film. The deflection of a MTF seeded with cardiomyocytes resulting from cell contractions could be measured in response to electrical signals paced at 1 Hz. In contrast to other in-plane 2D membrane-based approaches for applying stress to adherent cells in other organ-mimicking systems, this MTF approach measured out-of-plane deflection. MTF systems can also be incorporated into 2D skeletal tissue-engineering systems with reduced complexity but still allowing measurement of twitch stresses comparable to those observed in 3D skeletal muscle systems. Furthermore, this approach allowed contractility analyses of multicellular muscle units rather than single-cell unit, commonly conducted in earlier muscle function investigations, or muscle-on-a-chip systems. Achievements in the engineered muscle system field have led to the development of multiple platforms available to study muscle systems.

### 2.2.6. Lung on a Chip

Time-varying mechanical deformation is essential in the respiratory and circulatory systems with the lungs and heart pumping air and blood, respectively. The difficulty associated with monitoring and controlling these mechanical cues in vivo prompted the development of systems that accurately replicate these conditions in vitro. For example, microporous elastic membranes made of PDMS have been used to mimic mechanical perturbation including the cyclic expansion and contraction in the lungs. This device uses two pneumatic channels as actuators powered by a vacuum pump to allow cyclic stretching and relaxation of the elastic membrane and device wall of PDMS. This novel design replicates dynamic mechanical distortion of the alveolar–capillary interface caused by respiratory movements. In this lung model, the complex responses to bacteria and inflammatory cytokines introduced to the epithelial compartment were shown. Also, cyclic mechanical strain accentuated toxic and inflammatory responses of the lung to silica nanoparticles. Indeed, mechanical strain enhanced epithelial and endothelial cells absorption of nanoparticles and stimulated their transport across the alveolar–capillary interface into the underlying microvascular channel. This bioinspired microdevice simultaneously mimics the mechanically active and tissue–tissue interfaces critical to lung function. This lung tissue-like device may act as a surrogate for in vivo models and may allow drug screening and toxicology applications.

### 2.2.7. Placenta on a Chip

Studying the biology of the human placenta represents a major experimental challenge. Although conventional cell culture techniques have been used to investigate the different placenta-derived cells, current in vitro models have severe limitations in recapitulating organ-specific structure and key physiological functions of the placenta. Blundell et al. leveraged microfluidic and microfabrication technologies to develop a microengineered biomimetic model that replicates the architecture and function of the placenta. The “Placenta-on-a-Chip” microdevice was generated using a set of soft elastomer-based microfabrication techniques, i.e., soft lithography. This microsystem consisted of two PDMS microfluidic channels separated by a thin ECM membrane. To reproduce the placental barrier in this model, human trophoblasts (JEG-3) and HUVECs were seeded onto the opposite sides of the ECM membrane and cultured under dynamic flow conditions to form confluent epithelial and endothelial layers in close apposition. The physiological relevance of the microengineered placental barrier was assessed through the measure of glucose transport across the trophoblast-endothelial interface over time. The permeability of the trophoblast–endothelial interface was analyzed and compared to that obtained from cell devices and from epithelial or endothelial layers alone. The trophoblast–endothelial microfluidic cell culture system provided a tightly controlled fluidic environment conducive to the proliferation and maintenance of JEG-3 trophoblasts and HUVECs on the ECM scaffold. Prolonged culture in this model produced confluent cell monolayers on the intervening membrane that together formed the placental barrier. This in vivo like microarchitecture was also critical for the generation of a physiologically relevant effective barrier to glucose transport. The “placenta-on-a-chip” platform represents an important advance to model and study the physiological complexity of the human placenta and has a variety of applications including drug assessment.

### 2.2.8. Bone Marrow on a Chip

Current in vitro hematopoiesis models fail to mimic the cell diversity and complex functions of living bone marrow; hence, most translational studies relevant to the hematologic system are conducted in live animals. A method for fabricating “bone marrow-on-a-chip” that permits culture of living marrow with a functional hematopoietic niche in vitro was developed by first engineering new bone in vivo, removing it whole and perfusing it with culture medium onto a microfluidic device. The engineered bone marrow (eBM) retains hematopoietic stem and progenitor cells in normal in vivo like proportions...
for at least one week in culture. eBM models bone marrow toxicity responses and the protective effects of radiation countermeasure drugs, whereas conventional bone marrow culture methods do not. This biomimetic microdevice offers a new approach for the analysis of drug responses and toxicities in bone marrow as well as for the study of hematopoiesis and hematologic diseases in vitro.

2.2.9. Cancer on a Chip

Microfluidic systems are promising platforms to investigate cancer metastases under biochemically and biophysically controlled 3D microenvironments coupled with high-resolution real-time imaging. Various microfluidic models have been developed for studying tumor angiogenesis, intravasation, the role of interstitial flow, and matrix stiffness on cancer cell migration with adhesion and extravasation. Effects of interstitial flow on tumor migration were examined to better understand how cancer cells extravasate from the blood vessels. This technique allowed the precise quantification and tracking of individual migrating cancer cell: in 3D microfluidic assays replicating in vivo conditions of extravasation, mechanically constrained cancer cell movements were determined. Kamm and co-workers developed a microfluidic model to investigate the specificity of breast cancer metastasis to bone, providing quantitative data on cancer cell extravasation rate and reproducing the effects of the CXCL5–CXCR2 chemokine interactions between bone cells and static breast cancer cells observed in vivo. In that system, the vascular wall was represented by an endothelial monolayer on the side of a central gel region. Next, they generated an organ-specific human 3D microfluidic model that enables the study of human metastatic breast cancer cell extravasation within a perfusable human microvascularized bone-mimicking (BMi) microenvironment. The resulting model represents a functional human quad-culture, organotypic model, in which breast cancer cells flow into, adhere to, and metastasize through human microvascular networks. Extravasation rates and microvasculature permeabilities were significantly different in the bone-mimicking microenvironment compared with unconditioned or myoblast containing matrices. Furthermore, blocking breast cancer cell A3 adenosine receptors resulted in higher extravasation rates of cancer cells into the myoblast-containing matrices compared with untreated cells, suggesting a role for adenosine in reducing extravasation. These results demonstrate the efficacy of cancer on a chip model as a drug screening platform and a promising tool to investigate specific molecular pathways involved in cancer biology, with potential applications to personalized medicine.

2.2.10. Multiorgan on a Chip

Advances in bioengineered materials, microfluidic technology, and the availability of human primary, immortalized, and iPSC-derived cells are enabling development of human microphysiological systems, sometimes called “human-on-a-chip,” that combine multiple organ-specific human cells to recapitulate many functional and structural properties of human organs. Microfluidic perfusion further improves model performance by providing a flow of nutrients and oxygen and the removal of waste products from the cell cultures. Physiologically relevant flow increases oxygen consumption, Krebs cycle activity and secretions of synthesized proteins, and decreases the expression of the hypoxia related genes including HIF1α. Flow also improves the absorption and metabolism of compounds like benzo[a]pyrene. The human on-a-chip integrated with functional organ mimetics (lung, heart, gut, liver, kidney, and bone) provides an improved approach to explore the different routes of drug delivery (oral, aerosol, and transdermal), as well as their relevant efficacy and toxicity on human organs.

2.3. 3D Bioprinted Organ Models

In recent years, 3D bioprinting has emerged as a suitable replacement for in vivo and 2D models due to its cost effectiveness and ability to closely mimic the cellular arrangement observed in in vivo tissues. In general, a 3D bioprinter uses various types of cells suspended in a hydrogel, which can be tailored to match the protein composition and mechanical properties of the native extracellular matrix. The ability to tailor the key properties of the tissue environment, allow the generation of 3D in vitro tissue/organ systems that mimic key cell and extracellular tissue functions. The hydrogel is patterned layer-by-layer into a, computer directed, 3D organ scaffold. Computer aided technology is used to deliver patient specific organs or tissues based on scanned organs. During and after deposition of the 3D scaffold the hydrogel is gelled by thermal-, photo-, or chemical-based approaches. The use of toxic materials and high energies requires special care to leave the cells viable and intact. After printing, the tissue/organ construct is matured, either by seeding of cells onto the artificial scaffold or cultivation of cell-laden scaffolds, until proper function can be assessed. In general, 3D bioprinting technology can be categorized into three core techniques: microextrusion, inkjet, and laser-guided. Microextrusion based bioprinting systems involve dispensing hydrogel or polymer based filaments through a microneedle print head via computer controlled motion in a layer-by-layer fashion. The polymer or hydrogel is
A low viscosity polymer or hydrogel is loaded into the printing head via computer-controlled motion and droplet control. The droplets into a 3D construct through a droplet generating print technique require the patterning of polymer or hydrogel-based resolutions. Other technologies have also been adapted to 3D printing that patterns small droplets on the target substrate at cellular characteristics such as yield stress, viscosity, and chemical composition, which induce a microjet to the substrate. A pulse laser is used to induce transfer from the source ribbon to the substrate. The laser-assisted bioprinting of biological materials does not depend on hydrogel, but rather operates on a laser induced forward transferring of cells and biomaterials. A pulse laser is used to induce transfer from the source ribbon to the substrate. The laser pulse strikes the back of the ribbon, typically containing a sacrificial metal, which induces a microjet that patterns small droplets on the target substrate at cellular resolutions. Other technologies have also been adapted to 3D bioprinting including stereolithography.

2.3.1. Inkjet Bio Printing

Inkjet bioprinter operates similarly as conventional inkjet printers, by placing droplets into patterned substrate (Figure 9B). The droplets are formed when pressure pulses are introduced in the fluid chamber through a thermal, a piezoelectric or an electrostatic actuator such that a droplet is ejected when the bioink overcomes the surface tension. The resulting droplet can either be cell-laden or be seeded later. In comparison with other technologies, inkjet printing uses small amounts of reagents and can achieve higher resolution (10 μm) compared to microextrusion. For cell-laden constructs, the solvent of the hydrogels needs to be water, and pH, osmolarity, and ion concentrations of the hydrogel must mimic physiological conditions. Furthermore, the viscosity of the hydrogel must be low (<10 mPa s) to ensure droplet forming and reduce nozzle clogging. The significant limitation of this technology is the need for low viscosity ink. The low viscosity ink causes the printed structures to be weak, thus affecting their durability to external stresses after implantation. In addition, the viscosity limitation affects the homogeneity of the ejected hydrogel. Typically, it is difficult to keep the cells suspended in low viscosity ink, which causes aggregation and nozzle clogging.

2.3.2. Microextrusion

Microextrusion based bioprinters are the most commonly commercially available printer types. Indeed, microextrusion-based bioprinters are practical, easy to setup, and straightforward to operate allowing rapid patterning and building of large constructs (Figure 9C). In comparison with other printing techniques, microextrusion based bioprinting does not require any harmful solvents and allow flexibility in preparation and handling of materials. For cell-laden constructs, hydrogel characteristics such as yield stress, viscosity, and chemical composition, are important for the successful printing of highly viable tissues. The printing environment needs to be carefully controlled to minimize the construct desiccation. In addition, the feed rate, dispensing speed, and nozzle diameter specific to...
the hydrogel used need to be optimized. In seeding constructs, the material used needs to be chemically stable and biocompatible with the cell type. The spatial resolution of microextrusion based bioprinters can be as high as 1–2 µm, depending on the spatial resolution of the filament. Typically, the highest resolution achieved with highly viable cell laden constructs is ≈100 µm. This is due to the shear stresses induced onto the cells during printing. Resolutions on the order of 10 µm can be achieved for no cell-laden constructs seeded after printing. Another significant limitation of this technology is the deformation of the printing constructs. Highly viscous materials are typically used during cell lading printing to overcome this risk ((30–60) × 10^3 mPa s),[145] but the pressure needed to deposit higher viscosity filaments can affect cell viability.[83]

2.3.3. Laser Assisted

Laser assisted bioprinting (LAB) has recently emerged from other fabrication fields as a nozzle free, high-resolution alternative to conventional bioprinting techniques. A source ribbon, containing cells or biological materials, is placed over a substrate. A supporting layer, typically of gold or titanium, is placed on the backside of the ribbon to absorb the laser pulse. The laser pulse absorbed by the supporting material generates a small, high-pressure bubble, which propels the patterning material onto the substrate (Figure 9A). The picoliter sized patterning material polymerizes onto the substrate. This nozzlesstress approach allows for a large range of viscosities (1–300 mPa s).[145] The major limitation of this technology is the time-consuming preparation of the ribbon, especially when cells are present.[83]

2.3.4. Bioprinted Vessels

The vessel-like structures are crucial for the supply of key nutrients to nurture engineered thick tissues to maturity and have applications including as vascular grafts.[153,154] Prospectively, 3D bioprinting may provide vessels with tailorable diameters and branching geometries made of biocompatible materials facilitating the generation of patient specific vascularized tissues, vessels grafts, and rapid wound repair. The fabrication of tubular hydrogel structures by drop-on-demand inkjet printing was first attempted by Kesari and co-workers in 2005.[84] Next, Nakamura et al. pioneered a direct, alginate droplet based, bioprinting technique to pattern 3D vessels.[85,86] They formed fibers and tubes by the addition of alginate droplets to a calcium chloride (CaCl_2) solution. Following ejection from the print head, well-defined hydrogel spheroids were formed by diffusion of Ca^{2+} ions into the alginate ink droplets. This technique optimally prevented bleeding of the semi-solid alginate hydro-spheroids allowing for vertically oriented vessels to be printed without further scaffold. The wall thickness and the inner diameters of tubular structures could be adjusted from 35 to 40 µm and from 30 to 200 µm, respectively, through varying the diameter of the microgel beads used from 10 to 40 µm.[85] The vessel structure was further optimized by altering the viscosity of the CaCl_2 substrate through addition of hyaluronan or polyvinylalcohol. This group also generated cell-laden tubular structures using HeLa cells.[87] Using their method, linear tubular structures with lengths in the centimeter range were achieved.

Norotte and co-workers fabricated scaffoldless self-assembling 3D vessels using an extrusion-based bioprinter. Various vascular cell types, including endothelial cells, smooth muscle cells, and fibroblasts, were aggregated into discrete units, either multicellular spheroids or cylinders of controllable diameter (300–500 µm) (Figure 10A–C). These were printed layer by layer in agarose rods. After printing, the spheroids fused resulting in single and double-layered vascular tubes (outer diameters ranging from 0.9 to 2.5 mm (Figure 10). A unique aspect of the method is the ability to engineer vessels of distinct shapes and hierarchical trees that combine tubes of distinct diameters.[88] These vessel generation methods promoted the development of physiologically relevant vascularization and perfusion models.[89,90]

Using biological laser printing (BioLP), branch/stem structures of HUVEC and human umbilical vein smooth muscle cells (HUVSMC) were fabricated.[91] The printed structure mimicked vascular networks in tissue and allowed angiogenesis, i.e., the sprouting of new, finer vessels away from the stem.
and branches. In that model, co-culture structures were printed by first depositing only HUVECs, followed by a 24 h incubation to allow for adequate cell–cell communication and differentiation into lumina. These cell-printed scaffold layers were further printed through BioLP deposition of HUVSMCs on top and around the previously printed HUVEC structures. The growth and differentiation of these co-culture structures was then compared to the growth of printed structures using either HUVECs or HUVSMCs alone. In the structures using both HUVECs or HUVSMCs, lumen formation was found to closely mimic the original branch and stem structure and a network structure was observed. HUVSMCs acted to limit HUVEC over-growth and migration when compared to printed HUVEC structures alone. HUVSMCs and HUVECS, when printed in close contact, appeared to form cell–cell junctions around lumen-like structures similar to those observed during angiogenesis. The model highlights that the formation and growth of lumen and lumen network can be directed using laser assisted bioprinting. \[91\]

### 2.3.5. Bioprinted Heart Tissue

Typical 2D cultures do not adequately replicate the shape or function of the human heart. Recently, successful bioprinted models that mimic cardiac muscle structure and function have shown great potential for applications in regenerative medicine.\[92\]

Xu et al. used inkjet based bioprinting technique to pattern alternating layers of biocompatible hydrogels and mammalian cardiac cells to generate 3D contractile cardiac hybrids that mimicked “half hearts.” Primary feline adult H1 cardiomyocytes were used as the main cell type. Controlled microshells of alginate hydrogel were generated by spraying crosslinkers in microdrops onto ungelled alginic acid. They could achieve viable constructs up to 1 cm thick due to the programmed porosity in their 3D printed model. Contractile function was demonstrated in vitro. These results suggest that the inkjet bioprototyping method could be used for hierarchical design of functional cardiac pseudo-tissues.\[93\]

Hockaday and co-workers implemented 3D bioprinting to fabricate living alginate/gelatin hydrogel valve conduits with anatomical architecture and directly incorporated dual cell types in a regionally constrained manner.\[94\] Acellular 3D printed hydrogels exhibited reduced modulus, ultimate strength, and peak strain reducing slightly over 7 d culture, while the tensile biomechanics of cell-laden hydrogels were maintained. Aortic valve conduits were successfully bioprinted with direct encapsulation of smooth muscle cells in the valve root and aortic valve leaflet interstitial cells in the leaflets. Encapsulated smooth muscle cells expressed elevated alpha-smooth muscle actin, while aortic valve leaflet interstitial cells expressed elevated vimentin. These results demonstrate that anatomically complex, heterogeneously encapsulated aortic valve hydrogel conduits can be fabricated with 3D bioprinting.\[94\]

Gaebel et al. utilized the laser assisted bioprinting technique and prepared a cardiac patch seeded with HUVECs and human MSCs (hMSCs) in a defined pattern for cardiac regeneration (Figure 11). HUVECs and hMSC were seeded in a defined pattern on a polyester urethane urea cardiac patch. On control patches, equal amounts of cells were randomly seeded without LAB. Patches were cultivated in vitro or transplanted in vivo to the infarcted zone of rat hearts after left anterior descending ligation. Cardiac performance was measured by left ventricular catheterization 8 weeks' post-infarction. Thereafter, hearts were perfused with fluorescein tomato lectin for the assessment of functional blood vessels and stored for histology analyses. Results indicate that LAB-derived cell seeding pattern modified growth characteristics of co-cultured HUVECs and hMSCs leading to increased vessel formation and significant functional
improvement of infarcted hearts following transplantation of a LAB-tissue engineered cardiac patch.

2.3.6. Bioprinted Liver Tissue

The liver plays a key role in detoxification, glycogen storage, and plasma protein synthesis. It has a complex structure with the functional unit being the hepatic lobule, consisting of sinusoids, i.e., blood vessels lined with a permeable endothelium and surrounded by polarized hepatocytes and several mesenchymal cells (Küpffer cells, stellate cells, macrophages, and lymphocytes). The complexity of this organ makes 2D models inadequate for high throughput drug testing due to their lack of physiological relevance and 2D structure. In contrast, the 3D architecture generated by bioprinting supports the differentiation and nurturing of hepatocytes, which play an essential role in liver functions.\textsuperscript{155–157}

Akashi and co-workers,\textsuperscript{96} and Shu and co-workers\textsuperscript{97} both fabricated jet-based livers through layer by layer patterning of hepatocytes, and several mesenchymal cells. The complexity of this organ makes 2D models inadequate for high throughput drug testing due to their lack of physiological relevance and 2D structure. In contrast, the 3D architecture generated by bioprinting supports the differentiation and nurturing of hepatocytes, which play an essential role in liver functions.\textsuperscript{155–157}

Akashi and co-workers,\textsuperscript{96} and Shu and co-workers\textsuperscript{97} both fabricated jet-based livers through layer by layer patterning of hepatocytes, and several mesenchymal cells. The complexity of this organ makes 2D models inadequate for high throughput drug testing due to their lack of physiological relevance and 2D structure. In contrast, the 3D architecture generated by bioprinting supports the differentiation and nurturing of hepatocytes, which play an essential role in liver functions.\textsuperscript{155–157}

Recently, Lee and co-workers utilized a 3D bioprinting system to generate 3D printed hepatic structures using alginate. HepG2 cells were cultured on these 3D structures for 3 weeks and examined by fluorescence microscopy, histology, and immunohistochemistry. The expression of liver specific markers was quantified on days 1, 7, 14, and 21. The cells grew well on the alginate scaffold, and liver-specific gene expression was increased. The cells grew more extensively in 3D culture than 2D culture and exhibited structures close to those of the liver, indicating that the 3D bioprinting method recapitulates the liver architecture confirming the feasibility of hepatic structures 3D bioprinting. This technology may become a major tool and provide a bridge between basic science and the clinical challenges of liver regenerative medicine.\textsuperscript{99} In fact, recently a transplantable liver tissue block consisting of human adipose cells differentiated into hepatocyte-like cells was developed using microextrusion technology.\textsuperscript{100}

2.3.7. Bioprinted Lung Tissue

The lung is the main entry for aerosols and, despite the existence of lung tissue barriers, respiratory diseases are frequent
and increasing. As lung functions require a flexible substrate along with an air–blood barrier 3D bioprinting is a more suitable option to 2D models.

Horváth et al. generated the world first 3D bioprinted in vitro alveolar model. They biofabricated a human air–blood tissue barrier analogue composed of endothelial cells, basement membrane and epithelial cell layer using a jet-based bioprinting technology. In contrast to the manual approach, the bioprinted tissue had reproducible thinner and more homogeneous cell layers, a requirement for an optimal air–blood tissue barrier. This bioprinting platform offers an excellent base to engineer advanced 3D lung models for high-throughput screening, safety assessment, and drug efficacy testing. [101]

2.3.8. Bioprinted Skin

Due to the complexity of skin tissue, 3D bioprinting represents a flexible, automated, and on-demand platform for free-form fabrication of complex living skin architectures. Unlike 2D structures, 3D models can facilitate the development of tissue supporting vascularization, which is essential for thick tissue maturation and survival. Clinically, 3D bioprinted skin applications range from direct printing of skin grafts for patient care to high throughput testing of cosmetics and drugs. [102] Skin tissues were engineered recently. [103,104] A US-based research institute generated skin tissue in vitro with comparable biological and morphological characteristics to the native human skin. [105] The tissue consisted of alternating layers of collagen, fibroblasts, and keratinocytes. Two layers of collagen precursor were bioprinted and then crosslinked with NaHCO₃ vapor. Subsequently, a layer of fibroblasts was bioprinted on top of the resulting structure. The layered pattern of collagen and fibroblasts was continued for six layers of collagen and three layers of fibroblasts. Additionally, two layers of collagen followed by two layers of keratinocytes were bioprinted to complete the tissue fabrication. To mimic the in vivo environment and to facilitate the keratinocytes differentiation into corneocytes and formation of stratum corneum, the dishes were cultured for 4–8 d in a media bath with the keratinocytes exposed to an air–liquid interface. Subsequent histological characterization of the mature tissue cultures revealed that fibroblast density in the dermis was somewhat lower and the ordered stratification of keratinocytes was incomplete when compared to native human skin. [106]

Jorcano and co-workers bioprinted a human plasma-based bilayered skin using bioinks containing human plasma as well as primary human fibroblasts and keratinocytes that were obtained from skin biopsies. They generated 100 cm², a standard P100 tissue culture plate, of printed skin in less than 35 min (including the 30 min required for fibrin gelation). The function and structure of these skin-like constructs was analyzed by histology and immunohistochemistry, both following 3D in vitro cultures and after long-term transplantation to immunodeficient mice. In both cases, the generated skin was very similar to human skin and indistinguishable from bilayered dermo-epidermal equivalents. These results demonstrate that 3D bioprinting is a suitable technology to generate bioengineered skin for therapeutical and industrial applications in an automated manner. [106]

Koch et al. selected different skin cells (fibroblasts, keratinocytes) and hMSC based on their high potential in regeneration of human skin and possible applications in stem cell therapy for their laser printing experiments. [107] This group generated a viable skin tissue free of any DNA damage. The cells survived the transfer procedure with a rate of 98 ± 1% (SEM; skin cells) and 90 ± 10% (SEM; hMSC), respectively. All used cell types maintained their ability to proliferate after LAB. Further, no increase of apoptosis or DNA fragmentation was observed in skin cells and hMSC. In addition, the hMSC kept their phenotype as indicated by fluorescence activated cell-sorting analysis. This study demonstrates that LAB is a suitable technique for computer-controlled positioning of different cell types and a promising tool for future applications in the ex vivo generation of tissue replacements. [107]

2.3.9. Bioprinted Bone

Current in vitro hematopoiesis models fail to mimic the cellular diversity and complex functions of living bone marrow; hence, most translational studies relevant to the hematologic system are conducted in live animals. Nevertheless, 3D bioprinted bone systems have been developed. [108,109,110]

Campbells and co-workers fabricated bone tissue by stimulating growth factor dose-dependent differentiation of MDSC’s toward osteogenic phenotype using jet-based bioprinting of BMP-2 patterns. [109] Primary muscle-derived stem cells were cultured on BMP-2 patterns bioprinted on fibrin substrates. Utilizing the specificity of BMP-2 micropatterns allowed the control of the differentiation of MDSCs. Similarly, the Cui and co-workers engineered bone-like tissue with increased compressive modulus using a jet-based bioprinter. HMSCs, photopolymerizable PEGDMA, and HA nanoparticles were bioprinted in the same bioink at predefined 3D locations. The HA nanoparticles closely mimicked the native tissue microenvironments and stimulated the differentiation of stem cells toward osteogenic lineage with improved mechanical characteristics. [108]

Dhert and co-workers characterized the applicability of 3D fiber deposition with the plotting device Bioplotter, for the fabrication of spatially organized, cell-laden hydrogel constructs. The viability of printed BMSCs was studied over time, in several hydrogels, and following extrusion through different needle diameters. The findings indicate that cells survive the extrusion and that their subsequent viability was not different from that of unprinted cells. The applied extrusion conditions did not affect cell survival, and BMSCs could subsequently differentiate along the osteoblast lineage. Furthermore, they combined two distinct cell populations within a single scaffold by exchanging the printing syringe during deposition, indicating that this 3D fiber deposition system is suited for the development of bone grafts containing multiple cell types. [111]

Catros et al. successfully generated bone tissue using LAB. They used a LAB workstation comprising an infrared laser focused on a quartz ribbon that was coated with a thin absorbing layer of titanium and a layer of bioink. The scanning system, quartz ribbon and substrate were piloted by a dedicated software, allowing the sequential printing of different biological materials into 2D and/or 3D. The cells were characterized after...
printing using a live/dead assay and osteoblastic phenotype markers (alkaline phosphatase and osteocalcin). The results demonstrate that that LAB allowed the print and organization of nano-hydroxyapatite and human osteoprogenitors in two and three dimensions. The LAB used did not alter the physicochemical properties of nano-hydroxyapatite (nHA), nor the viability, proliferation and phenotype of human osteoprogenitors over time (up to 15 d). This study demonstrated that LAB is a relevant method for patterning nHA and osteoblastic cells in 2D, and is also adapted to the biofabrication of 3D composite materials.[112]

2.3.10. Bioprinted Cancer Models

Bioprinted cancer models represent a significant improvement over previous 2D models as 3D models mimic 3D tumor complexity and facilitate physiologically relevant cell–cell and cell–matrix interactions. Bioprinted models can mimic the tumor microenvironment, providing a platform to further understand cancer pathology, screen anticancer drugs, and assess cancer treatment.[113]

Demirci and co-workers bioprinted tumor tissue models for in vitro assays, using OVCAR-5 ovarian cancer cells and MRC-5 fibroblasts jetted using a dual-ejector system focusing on the same point.[114] These two cell types were bioprinted on Matrigel to generate multicellular acini in a high throughput and reproducible manner with a spatially mediated microenvironment, controlled cell density and biologically relevant cell–cell distances. The high post-bioprinting cell viability suggests that this technology may be useful for in vitro cancer modeling and investigations of complex cell-to-cell communications including unknown regulatory mechanisms among various cell types.

Dai et al. fabricated a glioma cancer model. They established a 3D bioprinted glioma stem cell model, using modified porous gelatin/alginate/fibrinogen hydrogel that mimics the extracellular matrix. Glioma stem cells achieved a survival rate of 87%, and proliferated with high cellular activity immediately following 3D bioprinting. During the in vitro culture period, the printed glioma stem cells not only maintained their inherent characteristics of cancer stem cells (expressing Nestin), but also showed differentiation potential (expressing glial fibrillary acidic protein and β-tubulin III). To verify the vascularization potential of glioma stem cells, the expression of the angiogenesis biomarker, vascular endothelial growth factor was assessed, and found to increase from week one to week three during the culture period. Drug-sensitivity results indicate that the 3D printed glioma cells were more resistant to temozolomide (400–1600 µg mL⁻¹) than the 2D monolayer model.[115] In summary, 3D bioprinted glioma model provides a novel alternative tool for studying glioma genesis, glioma stem cell biology, drug resistance, and glioma anticancer drug susceptibility in vitro.[115] Other groups have generated different cancer 3D in vitro models including models for bone and breast cancers using 3D bioprinting.[116,117]

2.3.11. Bioprinted Neural Tissue

The brain is an extremely complex organ structured into various regions of layered tissues. Researchers have attempted to study the brain by modeling the architecture using 2D in vitro cell culture methods. While 2D platforms attempt to mimic the in vivo environment, they fail to resemble the 3D microstructure of neuronal tissues. Development of a more accurate in vitro model of the brain remains essential to our understanding of the functioning of the brain at the tissue and organ scale. 3D bioprinting can address these limitations by patterning more physiologically relevant 3D neural networks.

Neural tissues were engineered by Yoo and co-workers using a micro-valve bioprinter. That neural tissue was fabricated by 3D bioprinting eight alternating layers of either collagen precursor and rat embryonic neurons or collagen precursor and astrocytes with rat embryonic neurons.[118] Each bioprinted cell layer was separated by two bioprinted collagen precursor solution layers. In addition, each bioprinted layer of collagen precursor solution was crosslinked with a sodium bicarbonate (NaHCO₃) mist (droplets of diameter < 2 µm) before bioprinting the subsequent layers. The same group also fabricated neural tissue by stimulating the differentiation of neural stem cells (NSCs) using vascular endothelial growth factor (VEGF).[119] This bioprinted tissue included a single layer of NSCs along with an adjacent circular layer of fibrin laced with VEGF sandwiched between two layers of collagen.

Lozano et al. generated a brain model using an extrusion-based bioprinting technique. The resulting bioprinted 3D brain-like structures consisted of discrete layers of primary neural cells encapsulated in hydrogels. Those brain-like structures were constructed using a bioink composed of a novel peptide-modified biopolymer, gellan gum-RGD, combined with primary cortical neurons. The ink was optimized for a modified reactive printing process and developed for use in traditional cell culturing facilities without the need for extensive bioprinting equipment (Figure 12A,B). Furthermore, the peptide modification of the gellan gum hydrogel promoted primary cell proliferation and network formation (Figure 12C,D). The neural cell viability combined with the support of neural network formation highlighted the cell supportive nature of the matrix. The ability to easily form discrete cell-containing layers validates this novel printing technique to form complex, layered and viable 3D cell structures (Figure 12E,F). These brain-like structures permit the generation of more accurate 3D in vitro microstructures with applications ranging from cell behavior studies to improving our understanding of brain injuries and neurodegenerative diseases.[120]

Othon et al. printed neuronal tissues using LAB with over 95% cell viability. Their results have been extended to primary cultured olfactory ensheathing cells (OECs), harvested from adult Sprague–Dawley rats. OECs were found to provide stimulating environments for neurite outgrowth in spinal cord injury models. LAB is unique in that small load volumes (µLs) are sufficient to achieve printing, requiring only the harvesting of low numbers of OECs, their concentration and printing. LAB was used to form several 8 mm lines of OECs throughout a multi-layer hydrogel scaffold. The line width was as low as 20 µm, with most lines formed of aligned single cells. High-resolution printing of low cell count of harvested OECs is an important advancement for in vitro studies of cell interactions and functionalities. In addition, these cell-printed scaffolds may provide an alternative for spinal cord repair studies, as the single-cell
patterns formed here are on relevant size scales for neurite outgrowth.[121]

2.3.12. Multiorgan Printed Models

Human tissue in vitro models that adequately replicate the in vivo environment are limited. The integration of these models into multiorgan systems with physiological relevance is even more rare. In a comprehensive physiologically meaningful tissue model, it is essential that tissues receive signals and support, such as vascular, neural, metabolic, and hormonal cues from other tissues, for normal tissue viability and function. When investigating drugs’ effects, side effects especially toxicity in secondary tissues can be as critical as effects at the target site. Indeed, side effects can have limited therapeutic benefits and even determine drug failure, drug commercial withdraw, and halt clinical use. During metastasis, cancer cell migration and the colonization of multiple tissues through intravasation of lymphatic and vascular vessels are key events. For such complex, multiorgan biological events, single organoid models are of limited use to model tissue to tissue interactions.

Skardal et al. fabricated a robust three organoid system including liver, endothelium, and cardiac organoids (Figure 13A,B). Those 3D organoids within the system were integrated into a microfluidic platform using an extrusion-based bioprinting of tissue-supportive hydrogels to generate 3D ECM-derived environments for organoid maintenance. Liver organoids fabricated using liver ECM-derived hydrogels maintained viability and function in vitro for 4 weeks,[122] and the presence of key liver markers (e.g., albumin, multiple cytochrome P450 proteins, epithelial cell–cell adhesion markers, dipeptidyl peptidase IV, and organic solute transporter-α) was demonstrated. These liver organoids produced albumin and urea, responded to toxins, such as acetaminophen (APAP) in a dose-dependent manner, and were rescued from APAP insults with N-acetyl-L-cysteine, a common clinical treatment for APAP overdose (Figure 13C). Cardiac organoids also remained viable beyond 4 weeks, supporting the transport of fluorescent dye molecules throughout the organoids, suggesting high levels of cell–cell communications. Moreover, cardiac organoids beat spontaneously and changed their beating rates in response to a variety of drugs (Figure 13D). This kinetics was captured using an onboard camera system and custom software for analysis. Additionally, a blood vessel endothelium device was incorporated in this three-organoid system. The endothelium device responded to agents such as histamine through disruption of its cell monolayer increasing cross-endothelium leakiness demonstrated by the transfer of large molecular weight molecules present in the vessel lumen.[30]

3. Current Challenge and Future Perspectives

Multiorgan systems or miniaturized human tissues and/or organs have promising potentials. However, several challenges remain before their routine use in drug discovery pipelines. The challenges include the matching of environmental cues with in vivo cues, the development of universal culture media that allow multiorgan survival and functions, the use of decellularized extracellular matrix as a source of bioink, the generation of functional vasculatures with thick organs, the scaling-up for high-throughput assays, and the allowance of easy, real-time, on-chip assessment. Addressing those challenges will improve the robustness in the 3D organ construction, the organ-like maturation, the organ function and the relevance of such models to assess drugs in development.

Individual organs grow and function under their own optimized physicochemical cues, such as oxygen concentration, shear stress, pressure, pH, and temperature.[123,124] In contrast,
currently engineered organs are typically grown under similar culturing environments. The use of individual culture conditions for multiorgans may also be complicated by interferences among adjacent organs inside small chambers or the requirement for complicated valves and tubing systems. To address these limitations, further knowledge of the external cues will allow the tailoring of the physicochemical cellular environments for each organ system.

The biochemical cues are another concern: many culture media are cell-specific and are supplemented with specific soluble factors. Furthermore, media compositions are tailored to individual cells for delicate cells, such as human primary cells, stem cells, and iPSC-derived cells. In contrast, organs cultures comprising multiple cell types require a common supplemented culture media itself tailored to a specific organ. With growing evidence that cells constantly remodel their own environments by releasing their own “supplements,” more versatile and basic media, i.e., “universal media” or serum-free media\textsuperscript{[122]} are under development to reduce the media shocks and the overall cost for screening multiple media.

Bioinks must provide adhesion and nutrient transport, contain reagents for growth and viability, and be biodegradable. Most of current bioinks lack the natural components present in tissues and/or organ cellular microenvironments. In contrast, decellularized ECMs (dECMs) are tissue-specific, providing crucial cues for cells engraftment, survival and long-term functions.\textsuperscript{[160–163]} dECMs are obtained by removing the cellular and nuclear content from xenogenic or homogenic tissues or organs. The key challenge in the generation of dECMs is the efficient cell removal to prevent any related immunogenic reactions. Pati and co-workers prepared several tissue-specific dECM bioinks, including from adipose, cartilage, and heart tissues. These dECM bioinks provided essential cues, in part through their collagen and glycosaminoglycans contents, when used in 3D tissue bioprinting.\textsuperscript{[162]} dECMs have been generated to serve as scaffold for the reconstruction of various tissues and organs including skin,\textsuperscript{[164]} small intestinal submucosa,\textsuperscript{[165]} vessels, heart tissue,\textsuperscript{[166]} and a bioartificial heart.\textsuperscript{[167]}

Large tissue-like and organ-like constructs require adequate vascularization. Indeed, the diffusion-based supply of oxygen and soluble factors is limited to only a few hundred microns.
in depth and insufficient supply leads to tissue necrosis and increased morbidity. Vascularization remains a key challenge in organ construction. In addition, active angiogenesis following tissue vascularization should be considered. More sophisticated vasculature designs are required to balance appropriate flow rates, pressures, shear stress among and within organs without multiplex pumps and tubing systems.

For relevant model validation and effective drug assessment, the readouts of miniaturized human organs must correlate with standard clinical end points. Such correlation will allow the interpretation and direct translation of results from the miniature human organs to the clinic and their application for drug screening, and the investigation drug mechanisms. However, mapping the organ readouts to their clinical counterparts is currently still challenging as the low culture volumes and cell numbers in the 3D organ models is often associated with barriers including detection sensitivity and the use of different techniques.[125] Observations of cellular and molecular phenotypes, including gene and protein expressions in intact 3D model tissues is becoming easier in part through technological improvements such as live cell imaging and super resolution microscopy.

4. Outlook

Despite the limitations described above, the miniaturization of human organs has the potential to be transformative in drug discovery and in regenerative medicine. With the rapid advances of related technologies, 3D human multiorgans may, in the near future, replace or supplement the current 2D cell and animal models, as they remain either of limited relevance or high-cost, and time-consuming for the studies of complex human conditions, such as neurodegenerative diseases. Future 3D-engineered human organs will also have significant applications in the modeling of key aspects of physiological and pathological human conditions. They will allow a better understanding of the pathogenic mechanisms, the testing new therapy regimens (dose and schedule), and the reconstruction of personalized human organs using patient-derived tissues or cells in regenerative and/or personalized medicine approaches.[126–131]

Currently, drug development is based on specific patient subsets or targeting broad pathogenic pathways. Such approach often fails in clinical trials in part due to individual’s genetic variations and/or low specificity of the drug. Such outcomes greatly increase the risk of clinical failure and the commercialization expenses. The successful use of patient-derived tissue/cells, stem cells or iPSC cells to generate 3D human multiorgan platforms hold great promises in the development of patient-tailored drugs specifically targeting each individual patient’s biomarkers. Furthermore, based on 3D specific multiorgan platforms, optimal dose and schedule for individual patient therapy could be determined. Similarly, multidrug treatments could also be optimized in such platform. We envision that drug companies could use personalized human organs to screen drug candidates at low cost on a large scale and evaluate both the effectiveness and adverse side effects specific for each patient or patient cohorts using physiologically- and genetically relevant patient-personalized human 3D multiorgan systems instead of animal models.

Acknowledgements

This research was supported by Pioneering Funding Award funded by Cure Alzheimer’s Fund (CAF).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

3D bioprinting, drug development, human organs, microfabrication, organoids

Received: May 1, 2017
Revised: July 14, 2017
Published online:
