Trends in Biotechnology

CellPress

Opinion

Bioprinting towards Physiologically Relevant Tissue Models for Pharmaceutics

Weijie Peng,[1,2] Derya Unutmaz,[3] and Ibrahim T. Ozbolat[1,4,5,*]

Improving the ability to predict the efficacy and toxicity of drug candidates earlier in the drug discovery process will speed up the introduction of new drugs into clinics. 3D *in vitro* systems have significantly advanced the drug screening process as 3D tissue models can closely mimic native tissues and, in some cases, the physiological response to drugs. Among various *in vitro* systems, bioprinting is a highly promising technology possessing several advantages such as tailored microarchitecture, high-throughput capability, coculture ability, and low risk of cross-contamination. In this opinion article, we discuss the currently available tissue models in pharmaceutics along with their limitations and highlight the possibilities of bioprinting physiologically relevant tissue models, which hold great potential in drug testing, high-throughput screening, and disease modeling.

The Need for Reliable Models of Biological Activity in Drug Discovery

The conventional path for drug discovery and development entails a time-consuming and costly endeavor. According to a study by the Tufts Center for the Study of Drug Development [1], developing a new drug from target discovery to entering the market takes longer than a decade and is estimated to cost approximately $2.6 billion. It has been reported that only one out of ten drug candidates entering clinical trials gets market approval [2]. The vast majority of the drug candidates fail in clinical trials due to low efficacy, adverse events, and other reasons, such as safety issues [3].

In drug discovery, the conventional procedure of screening drug compounds starts with 2D cell culture tests, followed by animal model tests and finally clinical trials. 2D models are usually nonpredictive and often unrelated to *in vivo* responses as 2D models do not recapitulate the complex nature and organization of native tissues [4]. To accelerate drug discovery and reduce the cost burden, ineffective and/or unacceptable toxic compounds should be dismissed as early as possible. Therefore, it is imperative to develop reliable models that closely mimic *in vivo* conditions for drug testing, **high-throughput screening** (**HTS**, see Glossary) and toxicology analysis before animal trials. Recent advances in 3D *in vitro* assay systems have suggested this technology as an ideal way to satisfy the requirements since 3D tissue constructs can closely recapitulate the native tissue environment and be fabricated in **microarrays** to perform HTS [5]. Unlike 2D models with limited cell–cell and cell–matrix interactions, 3D models enable growth of cells into native-like organizations, which are ideal for drug screening.

3D Models in Pharmaceutics

Pharmaceutical testing on 3D tissue models has been widely implemented using scaffold-based [6,7] or scaffold-free [8] approaches. Scaffold-based 3D models can be generated by seeding cells, including primary or stem cells along with stromal cells, on a prefabricated scaffold or

Trends

Improving the ability to predict the efficacy and toxicity of drug candidates earlier in the drug discovery process will speed up the translation of new drugs into clinics.

Bioprinting for functional tissue fabrication has been a recent interest in pharmaceutics, as bioprinting has advantageous aspects, such as controllable tissue size and microarchitecture, high-throughput capability, coculture ability, and low risk of cross-contamination.

There is currently a growing trend in bioprinting of heterocellular tissue models such as liver, kidney, heart, and tumor models for drug testing and high-throughput screening.

Combining bioprinting technology with other technologies such as microfluidics is needed for fabricating physiologically simulated, complex, and long-term viable target micro-organ array.

[1]Engineering Science and Mechanics, The Pennsylvania State University, State College, PA 16802, USA
[2]Department of Pharmacology, Nanchang University, Nanchang, Jiangxi, China
[3]The Jackson Laboratory of Genomics Medicine, Farmington, CT 06032, USA
[4]The Huck Institutes of the Life Sciences, The Pennsylvania State University, State College, PA 16802, USA
[5]Materials Research Institute, The Pennsylvania State University, State College, PA 16802, USA

*Correspondence: ito1@psu.edu (I.T. Ozbolat).

embedding cells in a hydrogel matrix. Commonly used scaffold materials include decellularized **extracellular matrix** (ECM) components and a myriad of native and synthetic biomaterials [9]. In scaffold-free tissue models, cells self-assemble into neotissues through cadherin-mediated adhesion without using an exogenous scaffold support [10]. 3D models can be fabricated using various methods such as hanging drop [11], a microwell [12], micropatterned matrices [13], **microfluidics**- [14], acoustic force- [15], and magnetic force- [16] based techniques. In addition to physiological models of organs such as skin, heart, liver, kidney, and lung, disease models such as pathological muscles, pulmonary edema, and tumors have been developed for acute or chronic drug testing and HTS of compounds including drugs and cosmetics [17–19].

Current Limitations in 3D Models

Despite their great benefits over 2D models, 3D tissue models still encounter several limitations [20,21]. One of their limitations lies in the requirement of large numbers of cells and cell types integrated into complex configurations. Second, ECM-derived matrices may have batch-to-batch variability in their biological characteristics. Some studies using standardized microfluidics- or microarray-based HTS for drug discovery or toxicity testing reported that specific ECM components or natural scaffolds were often not consistent [22]. Third, 3D culture is generally very expensive for large-scale studies and high-throughput assays. Fourth, vascularization in 3D models remains an unsolved problem but it is the subject of active research [23], which plays a vital role in tissue growth and survival, and drug delivery. The core of **tissue spheroids** may create a hypoxic environment or limit the diffusion of compounds into the core. These shortcomings are mainly due to a low level of biomimetic organization of the heterocellular environment, and instability and low repeatability of the developed 3D models. Biomimetically developed **miniaturized tissue models** that meet these limitations, by contrast, may be fabricated by the accurate deposition of cells and ECM components to recapitulate the native architecture of tissues, which is highly feasible using 3D bioprinting. Examples to specific capabilities of bioprinting will be further discussed in the next section.

Bioprinting: From Basic Science to Pharmaceutics

Bioprinting is a developing field that has gained growing interest worldwide and has great potential to make a revolutionary impact on biomedical sciences and pharmaceutics [24]. It offers very precise spatial and temporal control on placement of cells, proteins, DNA, drugs, growth factors, and other bioactive substances to better guide tissue formation. This powerful technology is a promising method for advancing tissue fabrication towards physiologically relevant tissue constructs, **organoids**, and **organ-on-a-chip** models for pharmaceutics, drug testing, and HTS (see Box 1 for background information on bioprinting). There are three main technological modalities of bioprinting including droplet-, extrusion-, and laser-based bioprinting [25,26]. **Droplet-based bioprinting** utilizes thermal-, piezo-, or acoustic-driven mechanisms to deposit droplets of cell suspension in a high-throughput manner, whereas **extrusion-based bioprinting** uses mechanical- or pneumatic-driven systems to deposit cells in the form of a filament. **Laser-based bioprinting**, by contrast, utilizes laser energy to deposit cells from a donor slide to a receiver slide without the need for a nozzle. Among the three different modalities, droplet-based bioprinting has been the most common for pharmaceutical use due to its simplicity, versatility, and high-throughput capability [27]. Table 1 shows the major strengths and limitations of each modality, within the application domain of pharmaceutics.

Can Bioprinting Outperform Existing Tissue Models?

Among various methods for fabricating 3D *in vitro* systems, such as soft lithography, surface patterning, and microfluidic-based manipulation, 3D bioprinting has numerous advantages, including high precision control over size, microarchitecture and cellular composition, high-throughput capability, coculture and vascularization ability, and low risk of cross-contamination, where multiple tissue types need to be located separately with minimum cross-migration of cells

Glossary

ATP luciferase assay: an assay to measure ATP levels using a fluorescent plate.
Chimeric antigen receptors: engineered receptors grafting an arbitrary specificity onto an immune effector cell.
Droplet-based bioprinting: bioprinting of biologics with the aid of droplet deposition mechanism medicated by electrical, thermal, or acoustic energy.
Extracellular matrix: a network of proteins and carbohydrates secreted by cells that provides structural and biochemical support to cells in three dimensions.
Extrusion-based bioprinting: bioprinting of biologics using an extrusion mechanism.
Fugitive ink: a temporarily printed ink to be liquefied and removed to generate vascular network.
High-throughput screening (HTS): an automated process to quickly assay the biological or biochemical activity of a large sample of compounds during the drug discovery process.
Human-on-a-chip: a 3D microfluidic chip that stimulates the activities, mechanics, and physiological responses of several organ types.
Impedance spectroscopy: a technique to measure the dielectric properties of a medium as a function of frequency.
Laser-based bioprinting: bioprinting of biologics with the aid of laser energy as the major deposition mechanism.
Microarray: a collection of microscopic biological spots attached to a test sample for high-throughput screening.
Microfluidics: the science of manipulating and controlling fluids in the range of microliters to picoliters.
Micro-organ array: a collection of micro-organs on a test sample for high-throughput screening.
Miniaturized tissue model: a microtissue model representing the tissue physiology, anatomy, and function.
Organoid: a 3D organ bud grown *in vitro* recapitulating organ microanatomy closely.
Organ-on-a-chip: a 3D microfluidic chip that stimulates the activities, mechanics, and physiological response of an organ model.

Box 1. Background Information on Bioprinting

Definition of Bioprinting: Bioprinting can be defined as the simultaneous positioning of biomaterials and living cells in a prescribed layer-by-layer stacking organization to fabricate engineered tissues and organs [24].

Emergence of Bioprinting: Bioprinting was first demonstrated by Klebe in 1988 as cytoscribing technology, a method of micropositioning cells and constructing 2D synthetic tissues [62]. In that study, cytoscribing was carried out using a Hewlett–Packard (HP) inkjet printer and a graphics plotter for high positioning of biologics.

Application Areas: Bioprinting has a broad utility in various application areas such as tissue engineering and regenerative medicine, transplantation and clinics, drug screening and high-throughput assays, and cancer research. Bioprinting technology has been used for the fabrication of a wide variety of tissues including bone, brain, cancer, cardiac, cartilage, heart valve, liver, lung, neural, pancreas, retinal, skin, vascular, and composite tissues [27].

Advantages of Bioprinting: As listed below, bioprinting has numerous advantages over the other biofabrication techniques such as molding, magnetic assembly, and microfluidic-based approaches.

- Bioprinting enables fabrication of anatomically correct tissue constructs according to the medical image data obtained from patients.
- Bioprinting allows fabrication of porous structures with controlled architecture.
- Bioprinting has the ability to coculture multiple cell types locally.
- Bioprinting facilitates precise patterning of cells and biologics.
- Bioprinting enables controlled delivery of growth factors and genes.
- Bioprinting allows fabrication of tissue models in a high-throughput manner.
- Bioprinting has the ability to integrate vascularization within engineered tissues.
- Bioink The biomaterial solution used in bioprinting of living cells is referred to as 'bioink'. In bioprinting processes, there are four main types of bioink materials utilized including hydrogels, microcarriers, cell aggregates, and decellularized matrix components [26]. Cell aggregate-based bioink materials can be further classified into three: tissue spheroids, cell pellet, and tissue strands.

Modalities of Bioprinting: Depending on their bioink deposition mechanism, bioprinting modalities can be classified into droplet-, extrusion, and laser-based bioprinting [63].

Droplet-based Bioprinting: It is a bioprinting modality that allows patterning of living cells and other biologics using various energy sources such as sound, heat, and electric to generate droplets in a high-throughput manner. It offers greater advantages due to its simplicity and agility with precise control on deposition of biologics including cells, growth factors, genes, and drugs [64].

Extrusion-based Bioprinting: It is a combination of a fluid dispensing and an automated robotic system for extrusion and bioprinting, respectively [26]. During bioprinting, bioink is dispensed by a deposition system, under the control of a computer, resulting in precise deposition of cells encapsulated in cylindrical filaments of desired 3D custom-shaped structures.

Laser-based Bioprinting: It is a modality of bioprinting allowing high-precision patterning of biologics or fabrication of tissue constructs using laser energy [65]. It offers greater advantages due to its precise control on deposition of biologics including cells, growth factors, genes, drugs, and biomaterials.

Bioprinter: The 3D printer used in deposition of bioink solutions for fabrication of tissue and organ construct is referred to as 'bioprinter'. An ideal bioprinter has specific requirements including but not limited to the ability to dispense various biomaterials simultaneously, high resolution and accuracy, high degree-of-freedom motion capability, sufficient motion speed, user friendliness, full automation capability, ease of sterilization, affordability, versatility, and compactness [25].

Pharmacokinetics: the study of the time course of drug absorption, distribution, metabolism, and elimination from the body.
Polydimethylsiloxane (PDMS) chip: a device made of polydimethylsiloxane silicone for microfluidic studies.
T cell: a subtype of white blood cell that is of key importance to the immune system.
Tamoxifen: a medication to prevent breast cancer in women.
Tissue spheroid: aggregated cells in spheroid form in a scaffold-free manner that has certain measurable and controllable properties.

[28]. Bioprinting facilitates layer-by-layer stacking of cells and compounds in a high-throughput manner. Figure 1A shows a comparison of a manually fabricated multilayer air–blood barrier model versus a bioprinted one [29]. It is clear that bioprinting enables precise stacking of layers of cells and ECM components. Similarly, Xu and his coworkers modified a Hewlett–Packard printer into an inkjet-based bioprinter with a resolution of picoliter-per-droplet [30]. Three layers were bioprinted successively onto the same spot over a glass slide, where the first layer was made of a blend solution of agar and bacteria, the second layer consisted of 0.3% alginate, and the third layer consisted of calcium chloride ($CaCl_2$) and one of three selected antibiotics. Results showed that the cell viability, functionality, and antibacterial effects of antibiotics in inkjet bioprinted samples were similar to those of micropipetted samples, demonstrating that inkjet bioprinting did not compromise the biological performance, but instead facilitated HTS.

Table 1. Bioprinting Modalities and Their Performance Comparison in Pharmaceutical Applications

	Background	Strengths	Limitations	Applications in Pharmaceutics	Refs
Droplet-based Bioprinting (DBB)	First introduced in early 2000s Inkjet printers are the most commonly used type of DBB Driven by thermal, piezoelectric, or acoustic forces Print materials in the form of liquid droplets	Compatibility with small viscosities in the range of 3.5–12 mPa/s High speed (1–10 000 droplets/s), high resolution (1–300 pl in volume) Compatibility with many biological materials including living cells, DNA, RNA, biochemicals Suitable to drop cell populations on microarrays or organ-on-a-chip for HTS Affordable, versatile, and commercially available	No uniformity in droplet size Inconstancy in encapsulating a single cell in each droplet on microarrays for HTS Nozzle clogging in high cell densities and fibrous bioink solutions Cross-contamination when bioprinting of multiple bioink solutions takes place simultaneously	Thermal inkjet bioprinting *Escherichia coli*-laden alginate for high-throughput antibiotics screening Piezoelectric jetting of Sac6–EGFP yeast cells as microarrays for analysis of drug dose–response of latrunculin A	[30,64]
Extrusion-based Bioprinting (EBB)	Introduced in early 2000s The most common and affordable bioprinting modality Driven by pneumatic or mechanical forces Print materials in the form of filaments Compatible with a wide range of bioink properties	Compatibility with viscosities in a wide range (30 mPa/s to >6 × 10^7 mPa/s) Enables bioprinting of scaffold-free bioink such as tissue spheroids, which is not currently feasible using other modalities Facilitates vascularization using direct or indirect (with fugitive ink) bioprinting Suitable to extrude 3D tissue constructs or organ-on-a-chip for drug testing and toxicity analysis Commercially available with moderate cost	Substantial cell damage due to shear stress of highly viscous fluids, small nozzle diameter, and high dispensing pressure Not practical for high-throughput bioprinting of tissue models Limited bioprinting resolution preventing direct fabrication of microcapillary network Limited control on cell–cell and cell–matrix interactions	Liver-on-a-chip on a PDMS bioreactor for testing hepatic toxicity of acetaminophen Valve- and pneumatic-based extrusion of liver micro-organ on a PDMS chamber for assaying drug metabolic properties Extrusion of breast cancer neotissues in a multiwell plate to test antitumor drugs	[34,35,39,41]
Laser-based Bioprinting	First introduced in 1999 Less popular than DBB or EBB Consists of a pulsed laser beam with a focusing system, a donor	Compatibility with viscosities in the range of 1–300 mPa/s Nozzle-free Generating negligibly cell damage	Labor-intensive and time-consuming preparation Difficulty of accurately targeting and depositing cells High cost and no commercial	Laser-based bioprinting has not been applied to pharmaceutical use yet	[27]

Table 1. (continued)

	Background	Strengths	Limitations	Applications in Pharmaceutics	Refs
	slide including two layers (energy absorbing layer and biological material layer), and a collector substrate Stereolithography and its modifications also enable bioprinting of cells Driven by laser generated shock waves	Facilitates deposition of cells in the densities of 10^8 cells/ml with a resolution of one cell per droplet High-resolution feature of stereolithography and its modifications enables integration of vascular channels within tissue constructs	availability Not practical to bioprint heterocellular models		

Compared with other 3D *in vitro* systems, bioprinting enables precise positioning of cells with controlled cell density and cell–cell distance, and facilitates coculture models. For example, the Demirci group demonstrated bioprinting of tumor tissue models for *in vitro* assays [31]. In their study, human ovarian cancer (OVCAR-5) cells and MRC-5 fibroblasts were bioprinted using an inkjet-based bioprinting platform with dual ejectors. Multiple cell types were spontaneously bioprinted (inkjetted onto the same spot simultaneously) on Matrigel™ to form multicellular acini in a high-throughput and reproducible manner with a spatially mediated microenvironment.

Bioprinting paves the way for a biomimetic environment with supported cell–cell and cell–matrix interactions similar to *in vivo* conditions. In a study by the Sun group (Figure 1B), bioprinting of HeLa cells was used to generate cervical tumor models [32], where HeLa cells migrated towards each other and formed tumor spheroids within hydrogel filaments in 5 to 8 days. Cells in 2D culture formed cell sheets (Figure 1B1–B4) with lower chemoresistance and lower level expression of metalloproteinase.

In addition, bioprinting enables high-resolution fabrication of tissue microenvironments with vascularization. Huang *et al.* [33] demonstrated a laser-based 3D projection printing system to bioprint HeLa cells and noncancerous 10T1/2 fibroblasts in poly(ethylene glycol) diacrylate (PEGDA) along with a microvascular network with channel widths of 25, 45, and 120 μm to reflect blood vessel diameters. The results revealed that HeLa cells migrated significantly farther when the channel diameter decreased. The generated vascular network with branches in multiple-scale may be a great platform to facilitate physiologically relevant flow conditions for drug testing purposes.

In addition to its great advantages, bioprinting can also be integrated with other techniques, such as microfluidics, for drug testing. In this respect, the Sun group successively extruded HepG2 encapsulated in Matrigel™ and human epithelial cells M10 encapsulated in Matrigel™ in respective indentations on a **polydimethylsiloxane** (**PDMS**) **chip** for *in vitro* **pharmacokinetics** analysis [34,35]. Subsequently, the printed cellular construct was sealed with a glass cover and connected to form dual-microtissue microfluidic chips for perfusion. In that study, hepatocytes were used as the target cells, and epithelial cells, which line the lumen *in vivo*, were used to mimic drug transference paths. An antiradiation drug, amifostine, was used to evaluate the metabolic efficacy of epithelial cells and the occurrence of binucleated hepatocytes were

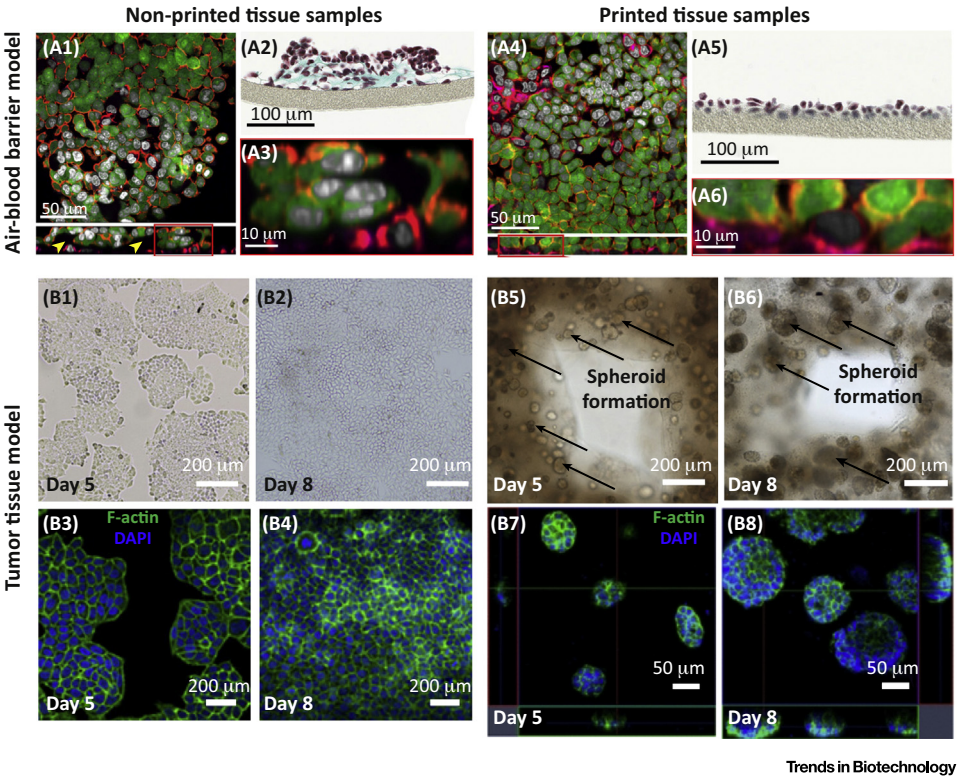

Figure 1. Comparison of Non-Bioprinted versus Bioprinted Tissue Models. An air–blood barrier model composed of a layer of Matrigel™ with endothelial cells on it and a second Matrigel™ layer with epithelial (A549) cells on it (adapted, with permission, from [29]): (A1) the manual seeding approach resulted in a thick Matrigel™ layer between epithelial and endothelial cells shown with yellow arrowheads. Endothelial cells were labeled with VE-cadherin in pink, and F-actin and nuclei was labeled in red and white, respectively. (A2) Nonuniform organization of cell and bioink layers are clear in histological cross-sections stained with Masson–Goldner trichrome. Cytoplasm, collagen fibers, and cell nuclei were stained in red, green, and dark brown, respectively. (A3) Frontal plane view of the immunostaining image showing limited cell–cell contacts. (A4) The bioprinted air–blood barrier model with highly organized distribution of A549 cells (green) and endothelial cells. (A5) A histological cross-section demonstrates a highly uniform thickness of the tissue model. (A6) Frontal cross-section demonstrates a uniform epithelial cell layer on the top and endothelial cell layer at the bottom. A cervical tumor model (adapted, with permission, from [30]): (B1–B2) phase-contrast images showing HeLa cells in 2D planar culture on days 5 and 8. (B3–B4) Under immunofluorescence imaging, F-actin, and 4′,6-diamidino-2-phenylindole (DAPI) (nuclei) of HeLa cells in 2D culture showed a flat and elongated morphology on days 5 and 8. (B5) Phase-contrast images showing bioprinted HeLa cells forming spheroids within gelatin/alginate/fibrinogen filaments on day 5, (B6) where spheroids got larger with further aggregation and proliferation of cells on day 8. (B7–B8) Immunofluorescence images showing F-actin and DAPI of the forming aggregates on days 5 and 8.

observed as a result of the effect of radiation. Table 2 compares 3D bioprinting technology with existing technologies for fabrication of 3D *in vitro* models.

Bioprinting of Physiologically Relevant Tissue Models for Pharmaceutics

The majority of bioprinting research has evolved around homocellular tissue construction; however, native tissues are heterocellular in nature with multiple cell types patterned in a highly complex anatomy [36]. Although simplified models are relatively acceptable in basic research, functional tissue bioprinting for pharmaceutics necessitates inclusion of multiple cell types (Figure 2A1–A3) as some of the functionality of cells can be enabled or enhanced by cell–cell interactions. There is currently a growing trend towards bioprinting of heterocellular tissue models, such as pancreas (Figure 2B1), liver, muscle/tendon, kidney, heart, and tumor models for drug testing and HTS [37–40]. Although a scaffold support may be essential to provide

Table 2. Comparison of Bioprinting with Other 3D *In Vitro* Technologies[a]

Methods	Hanging Drop Method	Microwell-Based Method	Microfluidics	Magnetic Force-Based Patterning	Bioprinting
Mechanisms	Cellular spheroids are formed by gravitational force	Microwells are fabricated by nonadhesive materials to forming cellular spheroids	Micro-flow mediates stacking cells in layers or forming cell spheroids using trapping	Magnetically-labeled cells are compacted in spheroids form under magnetic forces	Cells are deposited in scaffold- based or scaffold-free manner
Size Uniformity	++	+++	+++	+++	+++
Microarchitectural Controllability	+	++	+++	+++	+++
Scalability	++	+	+	++	+++
Coculture Ability	++	++	++	+	+++
High-Throughput Capability	+	+++	+++	+++	+++
Low Risk of Cross-contamination	+	+	++	++	+++

[a]+++, high; ++, medium; +, low.

organizational and biochemical cues in some tissue models, bioprinting in a scaffold-free manner may have a greater capacity to generate physiologically relevant tissue models as cellular interactions are better facilitated when cells are loaded at high densities close to native tissue and not immobilized in a hydrogel network [9]. This enables generation of tissues in a very short period of time with close biomimicry and preserved cell phenotype, gene expression, and functionality for longer periods of times. The Organovo company has recently demonstrated a scaffold-free human breast cancer model for pharmaceutical use [41]. To test the chemotherapeutic effects of **tamoxifen**, a scaffold-free human breast cancer model was bioprinted using a NovoGen Bioprinting™ platform in which cancer cells were surrounded by a biomimetic environment consisting of mesenchymal stem cell-differentiated adipose cells, mammary fibroblasts, and endothelial cells. The chemotherapeutic effects were assessed by an **ATP luciferase assay**. Histomorphological analysis showed that bioprinted tissues formed a clear compartmentalization of adipose, stromal, and epithelial components with microcapillary formation; the tissues maintained their viability for 2 weeks *in vitro*.

To mimic the interplaying of different organs, as the physiology of an organ can be altered by the physiology of another organ, models of various organs should be bioprinted and integrated within a single organ-on-a-chip device, referred to as '**human-on-a-chip**' [42–46]; however, there remain many challenges that need to be addressed. In this regard, bioprinting can be used to simultaneously deposit multiple types of organoids at different locations on a microfluidic chip. Vascular network connection can be generated using currently existing techniques, such as the use of **fugitive ink** for perfusable channel fabrication, or biological sprouting of capillaries and anastomosing them between organoids, as can be seen in Figure 2B2–B3. Depositing cells with different human genetic features can be used in human-on-a-chip models for personalized medicine. As different organoids have different physiological responses over time, advanced monitoring techniques should be developed and utilized to monitor bioprinted human-on-a-chip devices in real time with high resolution and in a noninvasive manner. Two or more biosensing techniques can be combined, such as in a dual-parameter cell analysis

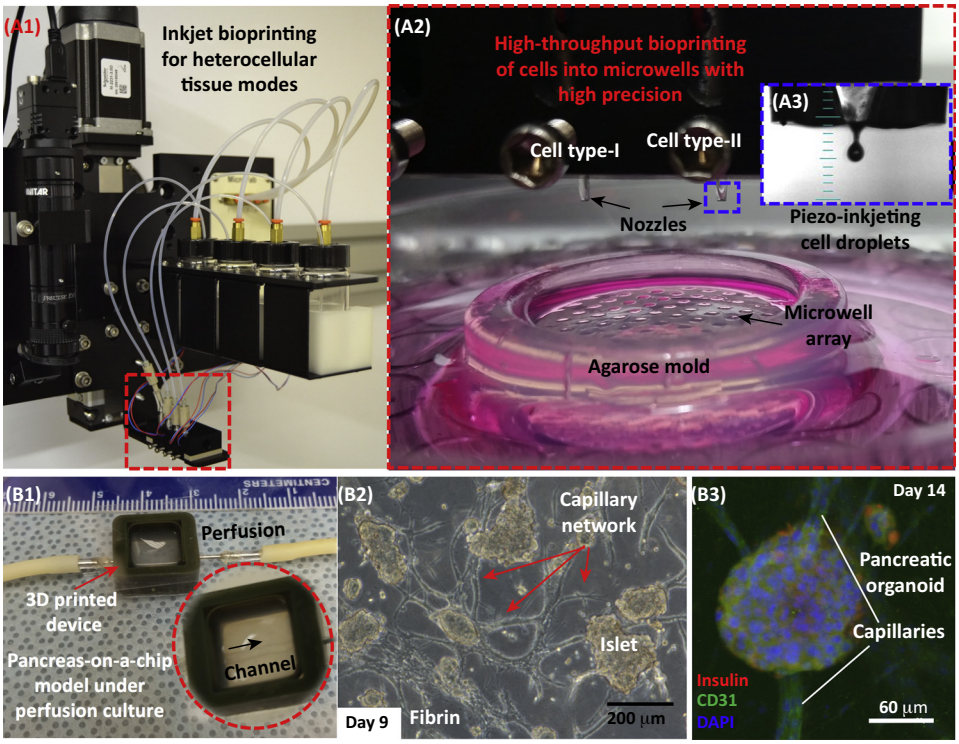

Figure 2. Bioprinting Capillarized Organ-On-A-Chip Models. (A1) A high-resolution inkjet-based bioprinter for fabrication of tissue models, (A2–A3) where droplets of multiple cell suspensions are deposited into an agarose microwell array in a high-throughput manner. Such an approach can generate scaffold-free heterocellular tissue models, such as vascularized pancreatic organoids, with controlled cell composition. (B1) A prototype perfusable pancreas-on-a-chip model with potential to test type 1 diabetes medication. (B2) Capillary network can be generated through anastomosing microcapillaries (B3) sprouted from engineered pancreatic islets, made of rodent cells, that are expressing insulin. Such organ-on-a-chip models can be further advanced by bioprinting various vascularized human organoid models directly into chips towards human-on-a-chip models.

system [47] combining a light scattering technique to determine cell numbers and intracellular granularity with an **impedance spectroscopy** technique to monitor cell-to-cell and cell-to-matrix adhesion. However, further investigations are needed to explore the advantages of bioprinting beyond existing approaches for fabricating organ-on-a-chip and human-on-a-chip devices.

Miniature 3D array platforms, such as tissue/organ-on-a-chip models, are a prominent means of tissue fabrication for drug testing and HTS. To better recapitulate the human physiology, bioprinting technology can be integrated with other technologies such as microfluidics (Figure 2B1), which can facilitate biomechanical and biochemical stimulation (i.e., shear stress, perfusion of nutrients, delivery of compounds, and biochemical cues) of the target **micro-organ array**. Bioprinting mediated controlled drug delivery onto these assays should be carefully considered and implemented.

Future Outlook

Bioprinting tissue models and microarrays represent a promising technology for pharmaceutics, particularly for pharmacokinetics, toxicity, and antitumor testing. 3D bioprinted tissue models and microarrays for pharmaceutical use are not subject to the rigorous safety and ethical issues that are required for implantation into humans and can easily provide valuable relevant preclinical

data. Commercial products such as bioprinted micro-liver and -kidney arrays have been recently of interest to several companies [38,48].

Various methods, including drug patterning, drug stamping, microcontact printing, aerosol sprays, and microfluidic-assisted drug loading, have been investigated for controlled drug delivery onto cell microarrays [49,50]. To screen the chronic effects of different drugs or toxins in a high-throughput manner, every unit in a microarray should be connected to an independent flow, in which drug candidates are separately perfused. Nevertheless, single organ chips or microarrays do not reflect the complexity of a whole human system [46], and multiple individual miniaturized organs or microarrays should be dynamically linked to create more predictive human-on-a-chip platforms to produce a more global assessment of human drug responses. For developing predictive preclinical drug discovery protocols in bioprinted models, mathematical and computational models reflecting complex *in vivo* scenarios are needed to combine multiorgan tissue engineering with HTS modalities.

Although a few bioprinted models have been used for *in vitro* pharmaceutical studies, bioprinting *in vitro* disease models is yet to be demonstrated [51]. 3D *in vitro* disease models, such as pulmonary edema, Alzheimer's disease, or pathological muscles, have been investigated by other methods [52–54]. Bioprinted physiological or pathological models are imperative for different pharmaceutical applications, including but not limited to drug screening, pharmacokinetics, toxicity and antitumor testing, receptor dynamics, dose–effect relationship studies, and identification and optimization of drug candidates. Primary human cell lines should be considered in bioprinting *in vitro* models to closely recapitulate human physiology, in addition to other alternatives such as pluripotent or multipotent stem cells. Genetic transfer of cells [55] and genetic manipulation techniques [56,57] should also be combined with bioprinting technology to fabricate different gene expression models for pharmaceutical research.

An emerging and exciting field for bioprinting applications could be immunotherapy approaches in cancer, which are designed to boost immune defenses to fight tumors. These approaches are to either: (i) use biologics, such as antibodies and antibody-recruiting molecules to enhance the immune response, or (ii) engineer a patient's cytotoxic **T cells** with **chimeric antigen receptors** (CARs), then transfer the cells back to the patient to seek and destroy their tumors [58]. Both of these approaches have shown remarkable success in clinical trials, in some cases resulting in a completely curing cancer that had become resistant to all available treatment options [59]. However, not all patients with the same tumor types respond to immunotherapy approaches. In many solid tissue tumors, there is also the challenge of generating cytotoxic T cells to migrate or infiltrate into the tumor sites and determining how tumor cells evade these cells [60]. In both of these challenges, 3D bioprinting of tumors would provide excellent models to study mechanistic interactions between the immune and tumor cells, testing novel biologics, drugs, or engineered T cells with novel synthetic targeting/activating molecules to develop the next generation of immunotherapy treatments [61]. It is also conceivable that, to identify ideal personalized treatment options for a particular patient, robust bioprinting approaches may fabricate patient-derived tumors to test therapies with their own natural or engineered immune cells.

Concluding Remarks

With its superior capacity for accurate placement of biologics, bioprinting will bring about revolutionary changes in pharmaceutical testing; however, there remain many challenges that need to be addressed (see Outstanding Questions). Along with the development of novel advanced bioprinting techniques, fabrication of physiologically relevant tissue models will become a vital tool in pharmaceutical development in the next decade. By integrating with other 3D biofabrication and supporting techniques, bioprinted organ/human-on-a-chip models

Outstanding Questions

How can models of various organs be bioprinted and integrated into a single human-on-a-chip device in the future?

What are the possible approaches to facilitate vascular network and microcapillarization between different organoids?

How should we stimulate such a complex human-on-a-chip device to recapitulate human physiology better?

What types of advanced monitoring technologies should be implemented to monitor human-on-a-chip devices in real-time high resolution in a noninvasive manner?

and microarrays for HTS will significantly decrease the attrition rate of new therapeutics in preclinical trials and significantly shorten the drug development process.

Acknowledgments

This work has been supported by National Science Foundation CMMI Awards 1349716 and 1462232, Diabetes in Action Research and Education Foundation grant #426, the Osteology Foundation grant #15-042, and the China Scholarship Council 201308360128. The authors are grateful to Monika Hospodiuk and Hemanth Gudupati for subimages in Figure 2 and Donna Sosnoski for her assistance with the typesetting of the manuscript.

References

1. DiMasi, J.A. *et al.* (2016) Innovation in the pharmaceutical industry: new estimates of R&D costs. *J. Health Econ.* 47, 20–33
2. DiMasi, J.A. *et al.* (2015) The cost of drug development. *N. Engl. J. Med.* 372, 1972
3. Waring, M.J. *et al.* (2015) An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nat. Rev. Drug Discov.* 14, 475–486
4. Rimann, M. and Graf-Hausner, U. (2012) Synthetic 3D multicellular systems for drug development. *Curr. Opin. Biotechnol.* 23, 803–809
5. Gaharwar, A.K. *et al.* (2015) 3D biomaterial microarrays for regenerative medicine: current state-of-the-art, emerging directions and future trends. *Adv. Mater.* Published online 26 November, 2015. http://dx.doi.org/10.1002/adma.201503918
6. Centola, M. *et al.* (2013) Scaffold-based delivery of a clinically relevant anti-angiogenic drug promotes the formation of in vivo stable cartilage. *Tiss. Eng. A* 19, 1960–1971
7. Li, X. *et al.* (2014) Micro-scaffold array chip for upgrading cell-based high-throughput drug testing to 3D using benchtop equipment. *Lab Chip* 14, 471–481
8. Beauchamp, P. *et al.* (2015) Development and characterization of a scaffold-free 3D spheroid model of induced pluripotent stem cell-derived human cardiomyocytes. *Tiss. Eng. C Methods* 21, 852–861
9. Ozbolat, I.T. (2015) Scaffold-based or scaffold-free bioprinting: competing or complementing approaches? *J. Nanotechnol. Eng. Med.* 6, 024701
10. Akkouch, A. *et al.* (2015) Microfabrication of scaffold-free tissue strands for three-dimensional tissue engineering. *Biofabrication* 7, 031002
11. Foty, R. (2011) A simple hanging drop cell culture protocol for generation of 3D spheroids. *J. Vis. Exp.* 51, e2720
12. Lee, D.W. *et al.* (2013) High-throughput screening (HTS) of anticancer drug efficacy on a micropillar/microwell chip platform. *Anal. Chem.* 86, 535–542
13. Sakar, M.S. *et al.* (2012) Formation and optogenetic control of engineered 3D skeletal muscle bioactuators. *Lab Chip* 12, 4976–4985
14. Shin, Y. *et al.* (2012) Microfluidic assay for simultaneous culture of multiple cell types on surfaces or within hydrogels. *Nat. Protoc.* 7, 1247–1259
15. Xu, F. *et al.* (2011) The assembly of cell-encapsulating microscale hydrogels using acoustic waves. *Biomaterials* 32, 7847–7855
16. Tasoglu, S. *et al.* (2015) Magnetic levitational assembly for living material fabrication. *Adv. Healthc. Mater.* 4, 1469–1476
17. Benam, K.H. *et al.* (2015) Engineered in vitro disease models. *Annu. Rev. Pathol. Mech. Dis.* 10, 195–262
18. Nam, K.-H. *et al.* (2015) Biomimetic 3D tissue models for advanced high-throughput drug screening. *J. Lab. Autom.* 20, 201–215
19. Wang, L. *et al.* (2014) Engineering three-dimensional cardiac microtissues for potential drug screening applications. *Curr. Med. Chem.* 21, 2497–2509
20. Edmondson, R. *et al.* (2014) Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay Drug Dev. Technol.* 12, 207–218
21. Eglen, R.M. and Randle, D.H. (2015) Drug discovery goes three-dimensional: goodbye to flat high-throughput screening? *Assay Drug Dev. Technol.* 13, 262–265
22. Hoque, M.E. *et al.* (2012) Extrusion based rapid prototyping technique: an advanced platform for tissue engineering scaffold fabrication. *Biopolymers* 97, 83–93
23. Rouwkema, J. and Khademhosseini, A. (2016) Vascularization and angiogenesis in tissue engineering: beyond creating static networks. *Trends Biotechnol.* Published online March 28, 2016. http://dx.doi.org/10.1016/j.tibtech.2016.03.002
24. Ozbolat, I.T. (2015) Bioprinting scale-up tissue and organ constructs for transplantation. *Trends Biotechnol.* 33, 395–400
25. Dababneh, A. and Ozbolat, I.T. (2014) Bioprinting technology: a current state-of-the art review. *J. Manuf. Sci. Eng.* 136, 061016
26. Ozbolat, I.T. and Hospodiuk, M. (2016) Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials* 76, 321–343
27. Ozbolat, I.T. *et al.* (2016) Application areas of 3D bioprinting. *Drug Discov. Today* Published online April 13, 2016. http://dx.doi.org/10.1016/j.drudis.2016.04.006
28. Ozbolat, I.T. and Yu, Y. (2013) Bioprinting toward organ fabrication: challenges and future trends. *IEEE Trans. Biomed. Eng.* 60, 691–699
29. Horvath, L. *et al.* (2015) Engineering an in vitro air–blood barrier by 3D bioprinting. *Sci. Rep.* 5, 7974
30. Rodriguez-Devora, J.I. *et al.* (2012) High throughput miniature drug-screening platform using bioprinting technology. *Biofabrication* 4, 035001
31. Xu, F. *et al.* (2011) A three-dimensional in vitro ovarian cancer coculture model using a high-throughput cell patterning platform. *Biotechnol. J.* 6, 204–212
32. Zhao, Y. *et al.* (2014) Three-dimensional printing of Hela cells for cervical tumor model in vitro. *Biofabrication* 6, 035001
33. Huang, T.Q. *et al.* (2014) 3D printing of biomimetic microstructures for cancer cell migration. *Biomed. Microdevices* 16, 127–132
34. Chang, R. *et al.* (2010) Biofabrication of a three-dimensional liver micro-organ as an in vitro drug metabolism model. *Biofabrication* 2, 045004
35. Snyder, J.E. *et al.* (2011) Bioprinting cell-laden Matrigel for radioprotection study of liver by pro-drug conversion in a dual-tissue microfluidic chip. *Biofabrication* 3, 034112
36. Lee, W. and Park, J. (2012) The design of a heterocellular 3D architecture and its application to monitoring the behavior of cancer cells in response to the spatial distribution of endothelial cells. *Adv. Mater.* 24, 5339–5344
37. Knowlton, S. *et al.* (2015) Bioprinting for cancer research. *Trends Biotechnol.* 33, 504–513
38. Nelson, B. (2015) 3-Dimensional bioprinting makes its mark: new tissue and organ printing methods are yielding critical new tools for the laboratory and clinic. *Cancer Cytopathol.* 123, 203–204
39. Nupura, S.B. *et al.* (2016) A liver-on-a-chip platform with bioprinted hepatic spheroids. *Biofabrication* 8, 014101
40. Rimann, M. *et al.* (2015) 3D Bioprinted muscle and tendon tissues for drug development. *Chimia* 69, 65–67
41. King, S.M. *et al.* (2014) Development of 3D bioprinted human breast cancer for in vitro drug screening. *Cancer Res.* 74, 2034
42. Esch, E.W. *et al.* (2015) Organs-on-chips at the frontiers of drug discovery. *Nat. Rev. Drug Discov.* 14, 248–260
43. Huh, D. *et al.* (2011) From 3D cell culture to organs-on-chips. *Trends Cell Biol.* 21, 745–754

44. Kim, J. *et al.* (2015) Organ-on-a-chip: development and clinical prospects toward toxicity assessment with an emphasis on bone marrow. *Drug Safety* 38, 409–418
45. Wilmer, M.J. *et al.* (2016) Kidney-on-a-chip technology for drug-induced nephrotoxicity screening. *Trends Biotechnol.* 34, 156–170
46. Zhang, Y.S. and Khademhosseini, A. (2015) Seeking the right context for evaluating nanomedicine: from tissue models in Petri dishes to microfluidic organs-on-a-chip. *Nanomedicine* 10, 685–688
47. Charwat, V. *et al.* (2013) Standardization of microfluidic cell cultures using integrated organic photodiodes and electrode arrays. *Lab Chip* 13, 785–797
48. Vaidya, M. (2015) Startups tout commercially 3D-printed tissue for drug screening. *Nat. Med.* 21, 2
49. Xu, F. *et al.* (2011) Microengineering methods for cell-based microarrays and high-throughput drug-screening applications. *Biofabrication* 3, 034101
50. Dias, A.D. *et al.* (2014) Recent advances in bioprinting and applications for biosensing. *Biosensors* 4, 111–136
51. Dai, G. and Lee, V. (2015) Three-dimensional bioprinting and tissue fabrication: prospects for drug discovery and regenerative medicine. *Adv. Health Care Technol.* 1, 23–35
52. Choi, S.H. *et al.* (2014) A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature* 515, 274–278
53. Huh, D. *et al.* (2010) Reconstituting organ-level lung functions on a chip. *Science* 328, 1662–1668
54. Vandenburgh, H. *et al.* (2008) Drug-screening platform based on the contractility of tissue-engineered muscle. *Muscle Nerve* 37, 438–447
55. Loozen, L.D. *et al.* (2013) Porous bioprinted constructs in BMP-2 non-viral gene therapy for bone tissue engineering. *J. Mater. Chem. B* 1, 6619–6626
56. Binder, K.W. *et al.* (2011) Drop-on-demand inkjet bioprinting: a primer. *Gene Ther. Regul.* 6, 33–49
57. Xu, T. *et al.* (2008) Inkjet-mediated gene transfection into living cells combined with targeted delivery. *Tiss. Eng. A* 15, 95–101
58. Khalil, D.N. *et al.* (2016) The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat. Rev. Clin. Oncol.* 13, 273–290
59. Rosenberg, S.A. and Restifo, N.P. (2015) Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 348, 62–68
60. Palucka, A.K. and Coussens, L.M. (2016) The basis of oncoimmunology. *Cell* 164, 1233–1247
61. Feder-Mengus, C. *et al.* (2008) New dimensions in tumor immunology: what does 3D culture reveal? *Trends Mol. Med.* 14, 333–340
62. Klebe, R.J. (1988) Cytoscribing: a method for micropositioning cells and the construction of two- and three-dimensional synthetic tissues. *Exp. Cell Res.* 179, 362–373
63. Ozbolat, I.T. (2015) Special issue on three-dimensional bioprinting. *ASME J. Nanotechnol. Eng. Med.* 6, 020301
64. Saunders, R.E. and Derby, B. (2014) Inkjet printing biomaterials for tissue engineering: bioprinting. *Int. Mater. Rev.* 59, 430–448
65. Riggs, B.C. *et al.* (2011) Matrix-assisted pulsed laser methods for biofabrication. *MRS Bull.* 37, 1043–1050