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Review

The Adoption of Three-Dimensional Additive Manufacturing from Biomedical Material Design to 3D Organ Printing

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Abstract: Three-dimensional (3D) bioprinting promises to change future lifestyle and the way we think about aging, the field of medicine, and the way clinicians treat ailing patients. In this brief review, we attempt to give a glimpse into how recent developments in 3D bioprinting are going to impact vast research ranging from complex and functional organ transplant to future toxicology studies and printed organ-like 3D spheroids. The techniques were successfully applied to reconstructed complex 3D functional tissue for implantation, application-based high-throughput (HTP) platforms for absorption, distribution, metabolism, and excretion (ADME) profiling to understand the cellular basis of toxicity. We also provide an overview of merits/demerits of various bioprinting techniques and the physicochemical basis of bioink for tissue engineering. We briefly discuss the importance of universal bioink technology, and of time as the fourth dimension. Some examples of bioprinted tissue are shown, followed by a brief discussion on future biomedical applications.

Keywords: bottom-up engineering; 3D bioprinting; additive manufacturing; nanotoxicology; tissue engineering

1. Introduction

Human organs are highly specialized tissue structures performing particular distinctive functions. In the case of dysfunctional organs, clinical treatments are often limited by a scarcity of available donors and by immune rejection of donated tissue [1]. To overcome the lack of available transplantable organs, tissue-engineering approaches are used, which face some challenges [2]. Borrowing the concept of three-dimensional (3D) printing from additive manufacturing technologies, whereby a digital design for a 3D structure is fabricated layer by layer following the bottom-up approach, 3D bioprinting is now being pursued as a potential solution to some of the challenges faced in tissue-engineering methods [3–8]. Layer-by-layer precise positioning of biological materials, and biochemical and living cells, with spatial control of the placement of functional components, is used to fabricate 3D tissue structures [9].

A typical bioprinting process consists of three major steps, namely pre-processing, processing, and post-processing (Figure 1). Pre-processing involves imaging of the tissue or organ and the reconstruction of 3D models from the imaging (Figure 1a–d). Multidetector computed tomography (MDCT) is widely used for rapid prototyping because of its simpler image-processing requirements. Cone-beam computed tomography (CBCT), positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and ultrasonography (US) are other non-invasive imaging modalities [10]. The processing step involves the bioprinting process using an appropriate bioink [11–14] (Figure 1e–g). The bioprinting process can be classified into four different categories, including laser-based bioprinting [15–23], droplet-based bioprinting [24–31], extrusion-based bioprinting [28,29,32–42], and stereolithography-based bioprinting [13,43–50]. Post-processing involves maturation of the bioprinted tissue before its intended use [9,51] (Figure 1h–i).

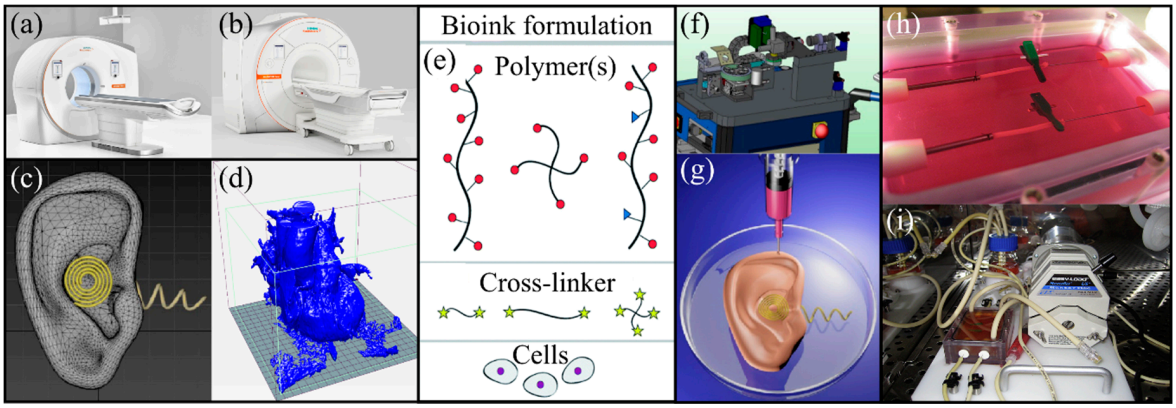

Figure 1. A typical process for bioprinting 3D tissues. Imaging of the tissue or organ using (**a**) CT scanner (shown here is a Siemens SOMATOM Force © Siemens Healthcare GmbH, 2018), or (**b**) MRI machine (shown here is a Siemens MAGNETOM Sola © Siemens Healthcare GmbH, 2018) and (**c**,**d**) reconstruction of 3D models from the imaging (shown here are models of a near and a heart). (**e**) The composition of bioink depends on the intended tissue form and function. Using such inks, methods like (**f**) laser-based bioprinting, or (**g**) extrusion-based bioprinting can be employed to print the intended tissue. (**h**,**i**) Some form of post-processing or maturation may be needed before the 3-d bioprinted tissue can be used (shown here is the maturation of bioprinted tubes composed of porcine aortic smooth muscle cells in a perfusion bioreactor). 3D model of ear is reprinted from Mannoor et.al. [35], with permission from American Chemical Society; Bioink formulation schematic is taken from Gungor-Ozkerin et.al. [12], with permission from Royal Society of Chemistry; Schematic of laser-based bioprinting is taken from Guillemot et.al. [17], with permission from Elsevier; Schematic of extrusion-based bioprinting is taken from Mannoor et.al. [35], with permission from American Chemical Society; Post-processing images are taken from Norotte et.al. [52], with permission from Elsevier.

2. Laser-Based Bioprinting

The main components of a laser-based bioprinter are the laser source, a laser transparent print ribbon coated with a layer of cell-laden bioink, and a substrate or collector slide on a motorized stage. The energy from the laser is utilized to pattern cell-laden bioinks in a three-dimensional spatial arrangement with the aid of computer-aided design and manufacturing (CAD/CAM). The high resolution and reproducibility of this process makes it a viable option for use in biomedical applications [53]. Some of the variations of this method based on the type of laser source and laser transparent print ribbon are shown in Table 1. Stem-cell grafts, skin tissue, multicellular arrays, and biopapers were reported to be printed using this method [23,54]. The major advantage of laser printing is the non-contact process. This eliminates nozzle clogging and also results in high cell viabilities [55]. However, there are several disadvantages of laser-based bioprinting, which outweigh the advantages. Laser exposure on the cells is not without risk, and the use of metal to absorb the laser energy can induce cytotoxicity [53].

Table 1. The comparison among different laser-based bioprinting techniques. CCD—charge-coupled device.

Category		Laser-Based Bioprinting				
		Laser-Induced Forward Transfer (LFT)	**Absorbing Film-Assisted Laser-Induced Forward Transfer (AFA-LIFT)**	**Biological Laser Processing (BioLP)**	**Matrix-Assisted Pulsed Laser Evaporation Direct Writing (MAPLE-DW)**	**Laser-Guided Direct Writing (LG DW)**
Difference	Laser transparent print ribbon	With thin metal layer	With thick metal layer		With biopolymer layer	/
	Laser pulses	High power	High power	High power	Low power	/
	CCD camera	/	/	Included	/	/
	Optical fiber	/	/	/	/	Included or not included
Advantages	Overall	1. High cell viability 2. High resolution 3. High cell densities 4. Low-viscosity cell suspensions				
	Individual	/	Thick metal layer reducing the risk of laser energy on cells damage		Biopolymer facilitating initial cell attachment	/
Disadvantages		1. A risk of photonic cell damage 2. Scalability limitation 3. Fabrication of the laser print ribbon 4. High cost of laser system 5. Complexity of controlling the laser pulses				

3. Droplet-Based Bioprinting

Such a process ejects cell-laden bioink out of the nozzle onto a substrate in the form of droplets [51]. Inkjet printers are one of the most commonly used type for both non-biological and biological applications [9]. Inkjet printers can use thermal [27] or acoustic [56] forces, among others, to eject drops of liquid onto a substrate [9], as seen in Table 2. One of the major advantages of droplet-based bioprinting is its compatibility with a wide variety of biological materials. Furthermore, such bioprinters provide high resolution (20–100 μm) and speed (1–10,000 droplets/s) while being a low-cost alternative [29]. For example, a high-throughput cell printing system was demonstrated for drug screening [30,57–61]. At the same time, a major drawback of this technique is the requirement for the biological material to be in a liquid and less viscous form [29], which may not always be the case.

Table 2. The comparison among droplet-based bioprinting techniques.

Category	Droplet-based Bioprinting				
	Inkjet Bioprinting		**Electro-Hydrodynamic Jetting-Based Bioprinting**	**Acoustic Bioprinting**	**Microvalve Bioprinting**
	Continuous Inkjet	**Drop-on-Demand**			
Trigger Difference	Pneumatic actuator	Thermal, piezo-electric, electrostatic actuator	Electric field	Acoustic actuator	Pneumatic actuator
Advantages	1. High resolution 2. High printing speed 3. Affordability 4. Cell concentration gradient		1. High resolution 2. High-viscosity bioink	1. Without detrimental stressors 2. High resolution 3. High printing speed	1. Synchronized ejection from different print heads
Disadvantages	1. Low-viscosity bioink 2. Nozzle clogging 3. Droplets cannot be controlled precisely	1. Low-viscosity bioink 2. Nozzle clogging	1. Electric field might affect the long-term cell viability 2. Precise spatial placement of cells is onerous	1. Not too high a viscosity of bioink 2. Not too high a cell concentration	1. Nozzle clogging 2. Low resolution 3. Damage of cells

4. Extrusion-Based Bioprinting

In extrusion-based bioprinting, the bioink is extruded out of the nozzle using pneumatic pressure or mechanical force. The biggest advantage of extrusion-based bioprinting is the scalability due to the continuous flow of bioink and large deposition rate (Table 3). At the same time, the resolution of this method is lower than other methods [62,63]. While the printability of high-viscosity bioinks and high cell concentrations is an advantage [37], the inherent nozzle-clogging problem is a disadvantage [64]. Due to their low cost and simple-to-use nature, extrusion-based bioprinters are the most widely used of all bioprinters [51] (Table 4). Cell-laden constructs with tunable 3D microenvironments were constructed by bioprinting gelatin methacryloyl (GelMA)/alginate core/sheath microfibers using extrusion-based bioprinting and subsequent ultraviolet (UV) cross-linking [40]. Further stabilization strategies in extrusion-based bioprinting were also reported, in order to successfully complete the printing of intact, accurate, and biologically relevant constructs with desirable properties [65].

Table 3. The advantages and disadvantages of extrusion-based bioprinting and stereolithography-based bioprinting. UV—ultraviolet.

Category	Extrusion-Based Bioprinting	Stereolithography-Based Bioprinting
Trigger difference	Pneumatic pressure or mechanical force	Light (usually UV) irradiation
Advantages	1. Scalability 2. High-viscosity bioink 3. High cell concentration	1. Highest resolution 2. Reduced printing time
Disadvantages	1. Lowest resolution 2. Nozzle clogging 3. Shear-thinning bioink	1. Nozzle clogging 2. Photopolymerizable bioinks or bioinks containing UV-activated photo initiated damage of cells 3. UV irradiation damage of DNA and promotion of cell lysis

Table 4. A comparison of various bioprinting techniques as tabulated by Vijayvenkataraman et al. [51].

Properties	Laser-Based Bioprinting	Inkjet Bioprinting	EHD Jetting-Based Bioprinting	Acoustic Bioprinting	Microvalve Bioprinting	Extrusion-Based Bioprinting	Stereolithography-Based Bioprinting
Bioink viscosity	1–300 mPa·s	3–12 mPa·s	1–1000 mPa·s	NA	1–200 mPa·s	~600 kPa·s	~5 Pa·s
Cell density	10^8 cells/mL	10^6 cells/mL	10^6 cells/mL	10^6 cells/mL	10^6 cells/mL	10^8 cells/mL	>10^6 cells/mL
Speed	200–1600 mm/s	10,000 droplets per second	10–500 mm/s	10,000 droplets per second	1000 droplets per second	10–50 µm/s	High
Resolution	50 µm	50 µm	100 nm	37 µm	–	100 µm	200 nm–6 µm
Accuracy	High	Medium	Low	Medium	Medium	Low	High
Cell viability	>95%	>80%	>80%	>90%	>80%	40–95%	25–85%
Structural integrity	Low	Low	High	Low	Low–medium	High	Medium–high
Scalability	Low	High	High	Medium	High	High	Medium–high
Cost	High	Low	High	Medium–high	Medium	Low–medium	Medium

5. Stereolithography-Based Bioprinting

In stereolithography-based bioprinters, UV light is used to cure layers of photopolymer, stacks of which form the 3D object (Table 3). The biggest advantage of stereolithography in general and stereolithography-based bioprinting in particular is its very high resolution. Other advantages include high cell concentrations and no problem of nozzle clogging. The preparation of three-dimensional biodegradable poly(ethylene glycol)/poly(D,L-lactide) hydrogel structures using stereolithography at high resolutions was shown [46]. Cell-encapsulated hydrogels were also shown to be 3D-printed using stereolithography [48]. Cell-attachable and visible-light cross-linkable bioinks, based on gelatin methacryloyl (GelMA) with eosin Y (EY) photoinitiation, for stereolithography three-dimensional (3D) bioprinting were developed and used to print cell-laden hydrogels [13]. However, there are many disadvantages of this method. The biggest disadvantage is that only photocurable bioinks can be used. Another disadvantage is that the cells will get exposed to harmful UV light, which affects the cell viability [44].

6. 3D-Printed Tissues and Organs

Bioprinting was used to generate two-dimensional (2D) and 3D structures for various purposes, including fabrication of scaffolds and tissue constructs for tissue regeneration (Table 5). Some examples of printed tissues are shown in Figure 2. Markstedt et al. [66] printed shapes resembling human ear and sheep mensci using a bioink containing alginate and nanofibrillated cellulose and an inkjet-based 3D bioprinter which is largely applied for fibrous nanomaterials packaging [67]. Duan et al. [62] printed aortic valve conduits using hydrogel-based bioinks laden with aortic root sinus smooth-muscle cells and aortic valve leaflet interstitial cells and an extrusion-based 3D bioprinter. Table 5 summarizes the applications of 3D bioprinting in tissue engineering.

Table 5. Tissue-engineering applications using 3D bioprinting, adapted from Seol et al. [8] (with permission from Oxford University Press). BMP-2—; FGF-2—; TGF-β—; CNTF—; VEGF—; EGF—; GelMA—gelatin methacryloyl.

Tissue	Techniques	Cell Types	Growth Factors	Materials	References
Heart valve	Extrusion-based bioprinting	Aortic valve interstitial cell Aortic root sinus smooth-muscle cell	–	Hyaluronic acid Gelatin Alginate	[62,68]
Myocardial tissue	Extrusion-based bioprinting	Cardiomyocyte progenitor cell	–	Alginate	[69,70]
Blood vessel	Jetting-based bioprinting	Endothelial cell Smooth-muscle cell Mesenchymal stem cell	–	Fibrin	[71,72]
	Extrusion-based bioprinting	Endothelial cell Cardiac cell Smooth-muscle cell Fibroblast	–	Collagen Agarose Alginate	[52,73,74]
Musculo-skeletal tissue	Jetting-based bioprinting	Muscle-derived stem cells Myoblast Mesenchymal fibroblast	BMP-2 FGF-2	Fibrin	[75–77]
	Extrusion-based bioprinting	Bone marrow stromal cell Endothelial progenitor cell Endogenous stem cell	TGF-β	Agarose Alginate Hydroxyapatite Polycaprolactone	[78,79]
Nerve	Jetting-based bioprinting	Embryonic motor neuron cell Hippocampal cell Cortical cell Neuronal precursor cell Neural stem cells	CNTF VEGF	Soy agar Collagen Fibrin	[80,81]
	Extrusion-based bioprinting	Bone-marrow stem cell Schwann cells	–	Agarose	[82,83]
Skin	Jetting-based bioprinting	Dermal fibroblast Epidermal keratinocyte	–	Collagen	[84]
	Extrusion-based bioprinting	Epitheleal progenitors	EGF BMP-4	Gelatin	[85]
Bone	Extrusion-based bioprinting	Human mesenchymal stem cells	–	GelMA	[86]

Therefore, it can be said that bioprinting holds tremendous potential and is fast moving toward fully functional 3D-printed organs. For example, in the future, chronic toxicological diseases that are majorly due to industrial particulate pollutants such as pulmonary fibrosis could be cured by transplanting 3D-printed lungs from patients' own programmed cells. Life expectancy can be increased because patients will not be left waiting until a suitable organ is available from an organ donor. Body cells taken from patient blood or from a skin biopsy will be transported to a laboratory [87]. Here, cells will be programmed into routine culture to be transformed into diseased organ cells (e.g., lung cells) and will be expanded in volume/number for the 3D bioprinting to resemble a lung after a few weeks. After maturation into sterile culture conditions, the artificial lung will be ready to be implanted inside the patient (Figure 3) to replace the dysfunctional organ [88]. This whole process will take just a few months and will also produce personalized organs for the patient from their own

cells. This will reduce the possibility of rejection by the body, and the patient will not have to spend the rest of his/her life on anti-rejection drugs and having to deal with all of the associated side effects. Petersen et al. [89] (Figure 3) proposed using scaffolds of extracellular matrix from lungs of adult rat that retain the hierarchical branching with cellular components removed. A bioreactor was used to culture pulmonary epithelium and vascular endothelium on the acellular lung matrix, resulting in hierarchical organization within the matrix and efficient repopulation of the vascular compartment. When implanted into rats in vivo for short time intervals (45 to 120 minutes) the engineered lungs participated in gas exchange, although the inflation of engineered lung was found to be less than that of the native lung, and some bleeding and clotting was observed. While this represents a step toward developing a viable strategy for generating fully functional lungs in vitro, there remains the issue of extracting scaffolds from lungs, among others. This is where 3D bioprinting can help by developing patient-specific, on-demand biological scaffolds.

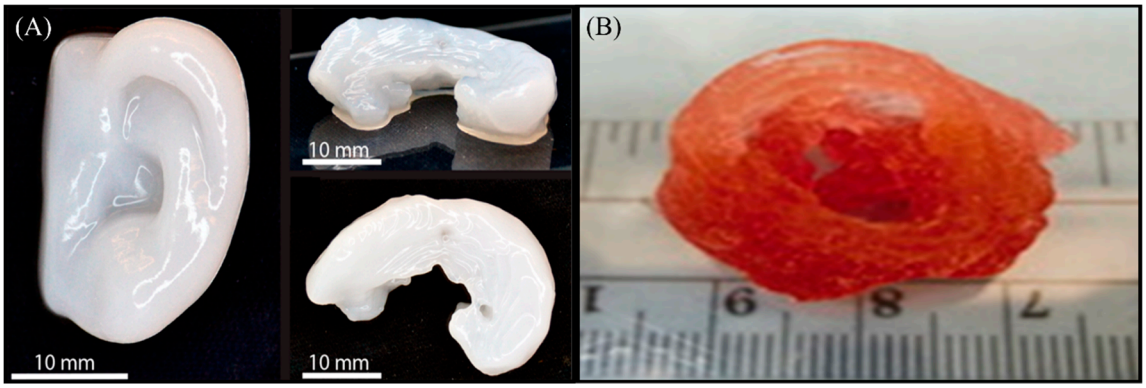

Figure 2. Examples of 3D-bioprinted tissue: (**A**) 3D-printed human ear and sheep menisci [66] (reprinted with permission from American Chemical Society Publications); (**B**) as-printed aortic valve conduit [62] (reprinted with permission from Wiley).

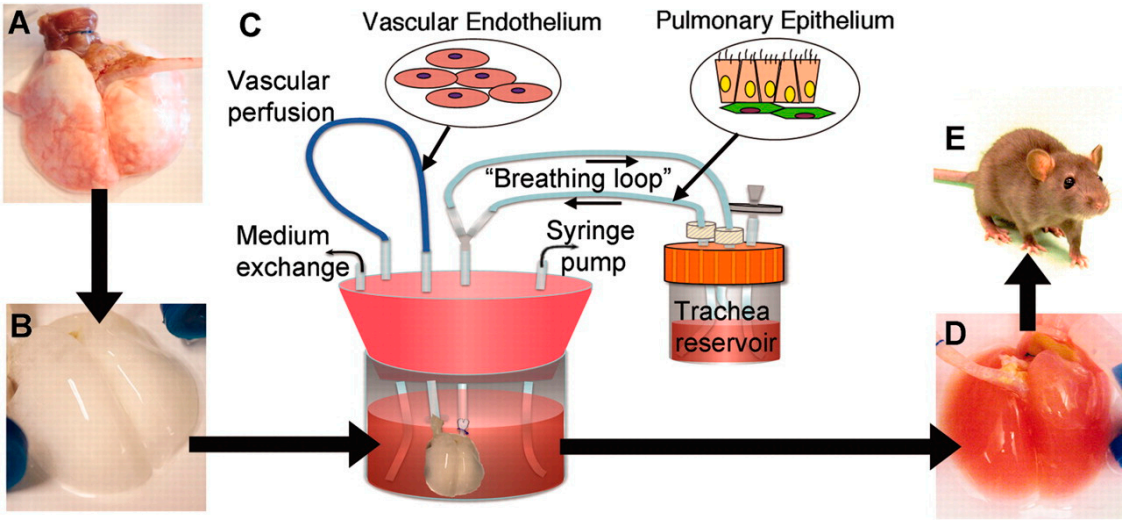

Figure 3. Scheme for lung tissue engineering [89]. (**A**) Native adult rat lung is cannulated in the pulmonary artery and trachea for infusion of decellularization solutions. (**B**) Acellular lung matrix is devoid of cells after 2 to 3 h of treatment. (**C**) Acellular matrix is mounted inside a biomimetic bioreactor that allows seeding of vascular endothelium into the pulmonary artery and pulmonary epithelium into the trachea. (**D**) After four to eight days of culture, the engineered lung is removed from the bioreactor and is suitable for implantation into (**E**) the syngeneic rat recipient. (Reprinted with permission from the American Association for the Advancement of Science (AAAS)).

The entire process involves nano- and micro-to macroscale bottom-up engineering [90,91] using a simple desktop 3D printer. This opens up the possibility that, one day, we will be able to bioprint amputated sub-organs, missing organs, and digitally designed cosmetic body parts. Instead of using plastics to print structures, researchers will use living cells mixed with biocompatible scaffolds to build living tissue inside a sterile safety cabinet to keep the cells protected from harmful foreign substances. In this context, it is also important to discuss the relevance of four-dimensional (4D) bioprinting, the fourth dimension being time [92]. While 3D bioprinting is set to make our lives easier by printing required living tissue on demand, in some cases, it may lose relevance if it is too time-consuming. Therefore, time taken to create the end-product is an important parameter to consider when judging the effectiveness of bioprinting processes. Furthermore, it was suggested that a universal bioink would be a significant technological advancement that could standardize the bioprinting field and accelerate the realization of human tissue product biomanufacturing [93]. With advancement in 3D printing, parallel advancements in 3D bioprinting can also be expected in the future. For example, dip-pen nanolithography is being developed, combining advantages of electron beam lithography, inkjet printing, and microcontact printing [94]. Such methods also allow the parallel application of different inks, which may be useful for printing complex tissue structures when integrated with in silico modeling [95,96].

7. Future Outlook: 3D Bioprinting Air–Liquid Interface (ALI) as an Artificial Material for Nanotoxicity Assessment of Particulate Matter

With the huge potential that 3D bioprinting holds, other applications apart from tissue/organ regeneration can be realized [97], for example, printing a lattice-like membrane, which can act as a biological tape. Such a membrane when placed in a culture microincubator could be used to recreate the microenvironment of the human body. To mimic the air–liquid interface in vivo, 3D bioprinting can build living lung-like tissues via printing the inside of the incredibly intricate branching network of tubes [98,99]. Each of these tubes ends in a tiny structure of air sacs/pouches where oxygen and carbon dioxide are exchanged, which gives an idea of just how complex this structure is. There are 300 million of these tiny air sacs in each lung [100], which makes it a very challenging structure to bioprint. However, a part of it can be printed to be used as a very useful model for toxicological research [101]. Another example is an asthma attack, where patients breathe in certain particulate allergens (micro- and nano- to macroscale particles/airborne spores) which aggravate muscle contraction of reduced-diameter airways [102]. In the future, starting from those airway muscle cells, one can recreate them in the lab and print them into tubular structures [103]. Further incubating these constructs in a suitable microenvironment to mature into similar functional airway muscles is possible via adding a stimulatory compound like histamine. It is released in asthmatic patient airways during an asthma attack, causing muscle contraction. We will be able to 3D print an airway muscle tissue mimicking the biological lifelike contraction and, thus, test advance therapeutics to reverse the contraction, relaxing the air tube as an anti-asthma drug does in patients. The fact that drug tests can be performed in these tissues is a very important point, because the drug development industry faces a big challenge of human trials after testing in vitro cells grown in petri dishes and preclinical tests in in vivo rodents. Rodents such as mice and rats respond very differently to test therapeutic compounds than humans do [104]. There is a huge chasm between the preclinical tools that we test the drugs on and the humans for whom the drugs are designed to help. Therefore, 90% of drugs that show promise in animals actually fail to work in humans, usually because they are just not effective at fighting disease or, sometimes, they are downright toxic [105]. These 3D-printed tissues can help the drug development process by enabling pharmaceutical companies to test these compounds in tissues that reproduce the complexity of the human body [106]. This will save lives by providing better drugs to patients faster and for less expense. It also has an ethical and moral impact, because research can drastically reduce the number of animals that are used for drug development. By the year 2050, it is estimated that the meat and leather industry combined will need around one hundred

billion farm animals to supply us with our animal-based needs such as meat, leather, milk, etc. [107]. To supplement those kinds of needs, animal cells can now be grown in the laboratory in just the same way as human cells; thus, there is potential here to replace a large proportion of these animals using bioprinted cells. We can differentiate them into muscle-like cells and then print those cells into meat products. The first bioprinted beef burger was revealed back in 2013, although incurring high costs (approximately $300,000) [108]. As technology moves forward rapidly, bioprinted leather is also a potential use for this technology. Skin cells can be grown, and the industry could generate customized leather products with specific thicknesses or textures or colors, making it feasible for the potential replacement of animal products by even better bioprinted animal products [109].

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