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Chapter

3D Printed Bioscaffolds for Developing Tissue-Engineered Constructs

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Abstract

Tissue engineering techniques enable the fabrication of tissue substitutes integrating cells, biomaterials, and bioactive compounds to replace or repair damaged or diseased tissues. Despite the early success, current technology is unable to fabricate reproducible tissue-engineered constructs with the structural and functional similarity of the native tissue. The recent development of 3D printing technology empowers the opportunities of developing biofunctional complex tissue substitutes via layer-by-layer fabrication of cell(s), biomaterial(s), and bioactive compound(s) in precision. In this chapter, the current development of fabricating tissue-engineered constructs using 3D bioprinting technology for potential biomedical applications such as tissue replacement therapy, personalized therapy, and in vitro 3D modeling for drug discovery will be discussed. The current challenges, limitations, and role of stakeholders to grasp the future success also will be highlighted.

Keywords: 3D printing, scaffold, drug delivery, regenerative medicine, tissue engineering

1. Introduction

3D printing is a process whereby a real object is created starting with a virtual 3D digital model. It was first developed in 1986 by Hull and Lewis which is an improved stereolithography system using photochemical processes in which light causes chemical monomers to link together to form polymers and generate a solid object [1]. This technology is capable to fabricate a super complex geometry or features by accurately follow the computer-aided design (CAD) model. The fabrication requires appropriate materials that gradually released and overlapped in layer-by-layer fashion by 3D printer. The type of material chosen is crucial to ensure the printed object that can be used for further settings and applications. Various types of metals, polymers, ceramics, and composites such as polycaprolactone (PCL), polyethylene glycol (PEG), polylactic acid (PLA), acrylonitrile butadiene styrene (ABS) plastic, stainless steel, titanium, calcium phosphate, and silica can be used as starting materials in 3D printing [2–4].

Generally, there are four main applications of 3D printing in the medical field, which are as follows: (a) drug delivery, (b) surgical devices/implants, (c) operative planning, and (d) tissue engineering [5–12]. The 3D printing application for drug delivery is extensively used in the pharmaceutical industry to develop sustained release medication [5]. Modulation of the shell thickness as well as the shape of the 3D printed capsule allows precise control of the drug release rate [13]. 3D printing enables a fast and cost-effective way of fabricating personalized medical implants. The capability of producing custom implants gets rid of the need for adjustments during surgery that saves time as well as reduces the cost of operation and the risk of medical complications. This is particularly beneficial where metal implant interfaces with living bone and tissue. The electron-beam melting (EBM) and direct metal laser sintering (DMLS) technologies are both now used in the production of standard and customized implants. Surgical tools are generally designed to work with many patients. However, by fabricating patient-specific tools, it would decrease the risk of complications during surgery [13, 14]. Patient anatomy will be imaged using imager and transferred into the 3D design in CAD to create suitable tools that can be easily controlled during operation. In operative planning, the 3D printing also would provide surgeons with a visualization of the complex injuries. They can plan and strategize their work and choose specific tools required. Some of the common applications that require a 3D model are complex pelvic trauma [15], pediatric deformities [16], and osteotomies [17]. Furthermore, advances in 3D printing technology enable the possibilities of constructing living human tissues in the lab hoping to demonstrate structural and functional similarities as native tissue in the human body [12]. The biggest challenge is to construct thick tissue and to ensure the diffusion of oxygen and nutrients for cellular viability [14].

2. 3D bioprinting for designing bioscaffold

The conventional method to develop an engineered-tissue product involved the initial fabrication of specific native tissue design followed by the provision of cells and biomolecules. However, this approach could contribute to two major drawbacks including limitation in cell distribution and reduction in cell growth due to low nutrient concentration at the core area [10]. Very commonly used techniques for fabricating 3D scaffolds include freeze casting, solvent casting, gas foaming, and salt leaching [18]. The technology advancement in tissue engineering has been contributed to the current approach through computer-aided layered manufacturing technique, which is also known as 3D bioprinting. Briefly, the 3D bioprinting technology involves the combination of the primary ingredients known as "bio-ink" that functions as a biological framework and various types of cells with the presence of chemical factors, and biomolecules to form a solid and functional *in situ* 3D living structure [19].

There are four different techniques under 3D bioprinting including inkjet printing, extrusion-based methods, light-induced (photopolymerization) methods and particle fusion-based methods [7, 20–27]. The first three abovementioned techniques have been widely used to fabricate biomaterial designs [7].

The inkjet-based 3D bioprinting (**Figure 1**), first developed by Thomas Boland from Clemson University in 2003, is a low-cost manufacturing process that performs high-speed printing for 3D structure [21]. Besides, it provides high-resolution printing output up to 50 μm and widely proven to support cell viability and growth [22]. However, the main drawbacks are dealing with a low concentration of printing ink could hamper the reliability of cell encapsulation and significantly affect print fidelity [23]. Besides, this approach potentially could damage the printed

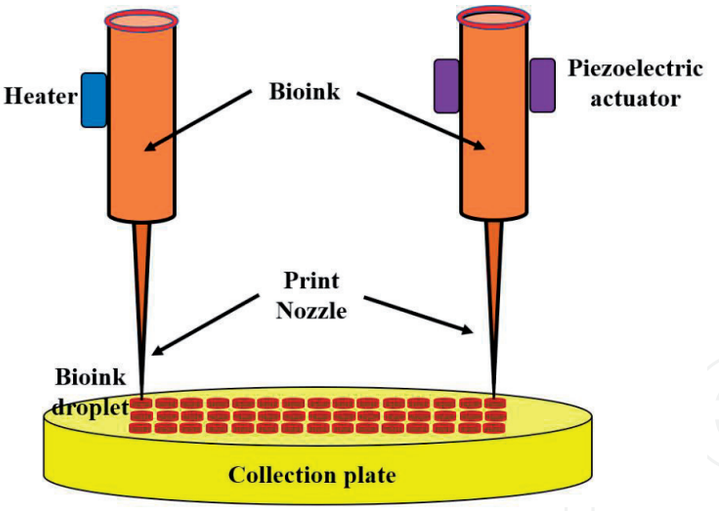

Figure 1.
The inkjet-based 3D bioprinting provides high resolution of printing output around 50 μm.

cells on the plantar under shear stress created through the inkjet-based printing but no concrete evidence is reported so far [21, 24]. Three main stages could affect the printable ink such as the production of the droplet, droplet/substrate close-interaction, and polymerization of the droplet. The two mechanisms, which have been involved under droplet generation through inkjet-based 3D bioprinting, are drop-on-demand and continuous inkjet [25]. The size of ink droplets produced via drop-on-demand and continuous injection is in the range of 25–50 and 100 μm, respectively [19]. Drop-on-demand inkjet has been conveniently used for tissue engineering applications.

The inkjet-based technology can be categorized into three as follows: thermal-based, piezoelectric-based, and magnetic-based inkjet printing [26]. The thermal induction can reach until 100–300°C that is required to nucleate a bubble and directly increase the appropriate pressure in the printhead lead to droplet expulsion [28]. There is no dead effect on the cells due to the presence of high temperature only for a microsecond and the previous study demonstrated consistency in cell viability post-inkjet-based 3D bioprinting [29]. Besides, the ink drop production can be induced by a piezoelectric method that focuses on the pulse pressure or acoustic waves generated from a piezoelectric actuator to expel printing ink drop. Another method to generate the drop expulsion is by using the electromagnetic approach depending on the Lorentz force and permanent magnet-based configurations. However, it produces a larger size of ink droplets as compared to thermal-based and piezoelectric-based approaches [28].

The second approach of 3D bioprinting is the extrusion-based method (**Figure 2**) that can be divided into two types consisting of fused deposition modeling and direct ink writing [19]. It is easy to handle, customized-based design bioprinter, and versatile with the developed current system. The principle of this 3D bioprinting method is that the printed ink extruded from the nozzle in liquid or molten state forms a particular line on the platform before polymerizing [30]. The bioprinting ink is commonly in the form of solid coil or filament that goes through the hot nozzle (temperature of around 200°C) before extrusion onto the platform. The extrusion from the printing nozzle is controlled by a specific system using various interventions including pressure-based control, pneumatic or mechanical control, or solenoid control before forming layered printed ink as required by the computerized set up to build up the 3D biomaterial designs [7]. The biopolymer should have an excellent solid-to-melt transition property to produce high-resolution 3D cell-laden on the printer platform [31]. However, the extrusion-based 3D bioprinting potentially could generate high mechanical force

and shear stress together with high viscous of substrate lead to cellular apoptosis [5]. Further adjustment and optimization of this extrusion-based bioprinting can mitigate the drawbacks but it reduces the bioprinter resolution and speed [32]. Besides, the low concentration of ink viscosity supported cell proliferation and sustained the cell viability by introduced a composite-modified printing ink [33].

Light or laser-assisted 3D bioprinting, also known as stereolithography (SLA) (**Figure 3**), focuses on polymer resins manufacturing [19]. There are many variations of light or laser printing approaches for 3D fabrication. The advantages of these approaches are that they provide excellent accuracy, and good resolution between 10 and 50 μm [21]. This technique involves the patterning of a laser beam toward photo-based polymer to generate physical hardened polymer. This

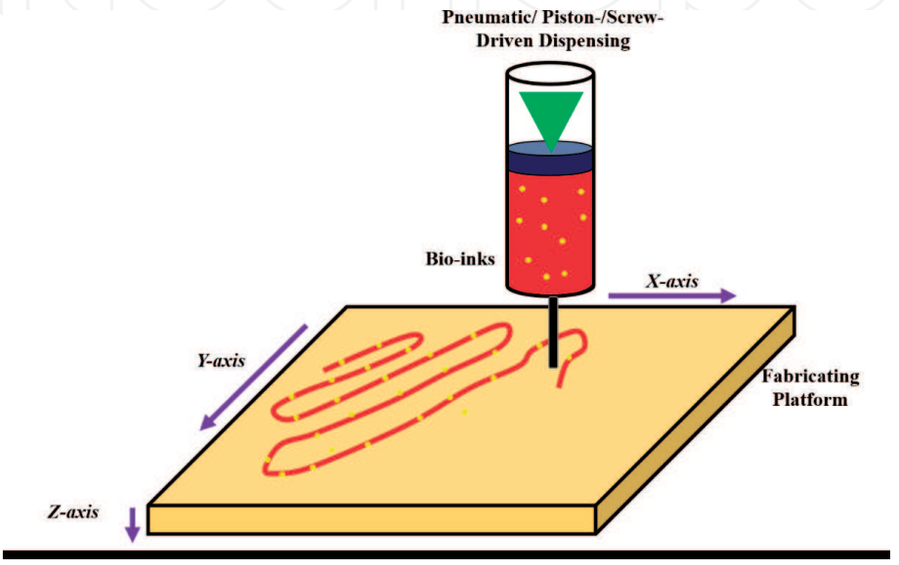

Figure 2.
The extrusion-based 3D bioprinting is easy to handle, customized-based design bioprinter, and versatile with the developed current system. It can be categorized into the fused deposition modeling and direct ink writing.

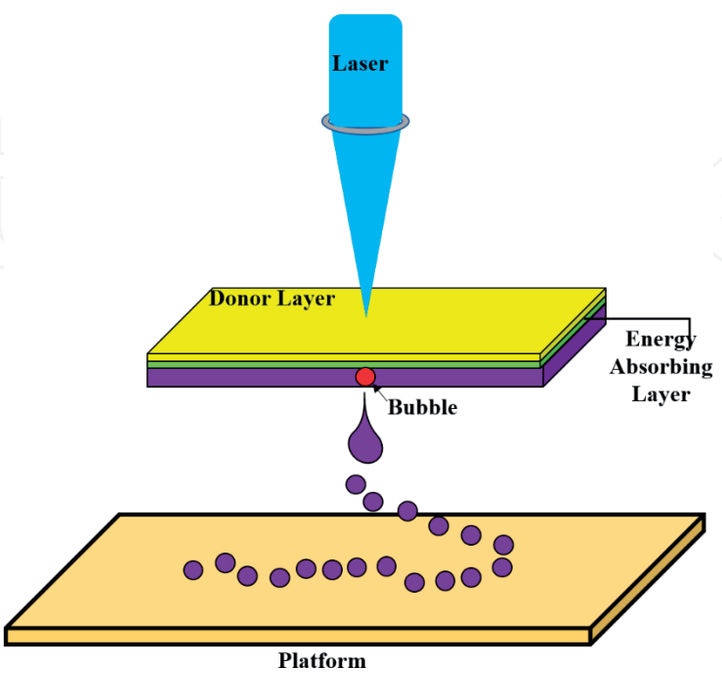

Figure 3.
Light- or laser-assisted 3D bioprinting approaches supported the high cell viability, accuracy, and good resolution between 10 and 50 μm. Two types consist of digital light processing-based bioprinting (DLP) and the two-photon polymerization-based bioprinting (TPP).

procedure is repeatedly applied to fabricate multi-layered polymer in the build-up stage. The other two types of laser-assisted 3D bioprinting techniques that are primarily applied in tissue engineering are digital light processing-based bioprinting (DLP) and the two-photon polymerization-based bioprinting (TPP) [34, 35]. The DLP technology uses a digitalized micro-mirror device chip (DMD) that contains around 2 million micro-mirrors. It functions to ensure light projection patterning precisely and is easy to modulate either on or off while the printing process is running on the platform. This technology consists of two 3D printing platform systems, namely, dynamic optical projection stereolithography (DOPS) and microscale continuous optical printing (μCOP) that support dynamic printing and continuous printing, respectively [7]. The TPP printing mechanism depends on the absorption of two photons by a molecule associated with light intensity square [36]. This phenomenon contributed to the printing of voxel dimension below 1 μm^3. Thus, this printing approach is an ideal method to generate nanoscale and microscale printing 3D output. However, high-resolution printing limits the construct size and printing speed. Nonetheless, the TPP printing speed is still faster than that in extrusion-based bioprinting and at a similar rate with inkjet-based bioprinting [37].

3. 3D bioprinting for developing tissue substitutes for therapeutic applications

Due to the limitation in technology to support the formation of the adequate and functional vascular network *in vitro*, currently, 3D bioprinting is more successful in the bioprinting of avascular tissue such as skin and cartilage. A complex tissue or organ with an extensive vascular network is still very challenging to prepare using the 3D bioprinting technology. To date, researchers are yet to succeed in preparing transplantable complex tissue or organ due to the difficulty in creating the circulatory system, especially the capillaries. However, several strategies have been used to improve the vascularization of 3D printed tissues, including printing of human umbilical vein endothelial cells (HUVECs) and vascular endothelial growth factors [38, 39] as well as seeding of endothelial cells and smooth muscle cells to the 3D printed tissues [40].

3.1 Bone

Bone tissue is one of the earliest tissues that were 3D printed and clinically used due to the ability of this technique to fabricate scaffolds according to the required shape, strength, and porosity. 3D printing enables fabrication of scaffold in any shape, which is not possible with many conventional fabrication techniques [41]. Furthermore, the materials commonly used for bone substitute production, such as hydroxylapatite (HA), synthetic calcium phosphate ceramics, polymethylmethacrylate, polylactides/polyglycolide and copolymer ceramics, tricalcium phosphate (TCP), bioglass, titanium, and other composite materials, are very compatible with the 3D printing technology [42]. The bone 3D printing had started as early as the 1990s, which utilized a powder-based freeform fabrication method [43]. Today, the bone substitute can be fabricated using the 3D plotting/direct ink writing, laser-assisted bioprinting (LAB), selective laser sintering (SLS), stereolithography (SLA), and fused deposition modeling (FDM) [42]. For example, Goriainov et al. prototyped hip joint implants using computer-aided design-computer-assisted manufacturing (CAD-CAM) and fabricated the scaffold using direct metal laser sintering from titanium alloy [44]. The custom-designed implants were seeded with autologous bone marrow aspirate before the implantation to 11 patients who

were unsuitable for standard revision hip surgery. The postoperative results showed extensive new bone formation in the patients and a certain level of load-bearing function at the hip joint. The *in vitro* studies demonstrated the osteogenesis of the skeletal stem cells and osseointegration of the cells with the titanium alloy [44].

3.2 Skin

The other tissue that has a high potential to utilize 3D printing technology to repair and regenerate is skin. Although skin substitutes made by conventional tissue engineering techniques such as Matriderm®, Integra®, Dermagraft®, and OrCel® have been commercialized and been used in clinics for wound treatment, there are still challenges that are yet to be resolved by these skin substitutes. These skin substitutes are expensive, require long production time with prolonged healing time, have limited tissue functionality, and resulted in scarring in some cases [45]. Besides, these skin substitutes lack hairs, sweat glands, sebaceous glands, and other skin appendages as well as pigmentation. The 3D bioprinting technology has led to the paradigm shift in the skin substitute production where this transformative technology enables simultaneous and accurate deposition of multiple types of skin cells, the formation of scaffolds with complex macro- and micro-architecture, creation of vascular networks, and construction of stratified layer [46].

The commonly used skin 3D bioprinting techniques are microextrusion, inkjet, stereolithography, and laser-assisted bioprinting [47]. The materials commonly used in skin 3D bioprinting are mainly natural polymers such as alginate, gelatine, collagen, fibrin, and hyaluronic acid. However, biocompatible synthetic materials such as polycaprolactone (PCL), polyglycolide (PGA), polyethylene glycol (PEG), poly(lactic-co-glycolic acid) (PLGA), and polylactide (PLA) are commonly combined with natural polymers to increase the mechanical strength of the skin substitute [46, 47]. The bio-inks serve either as the cell carrier or sacrificial support that is removed after the printing, or both as a carrier and mechanical support material that provides greater strength and microarchitecture that supports the function of the skin even after the implantation on to the patients [46]. The on-site bioprinting of either autologous or allogeneic dermal fibroblasts and epidermal keratinocytes directly into a wound area is the latest development in skin 3D bioprinting. The direct deposition of the cells in fibrinogen/collagen solution in a layer-by-layer method onto porcine full-thickness wound has shown to promote the wound closure, reduce contraction, and enhance the re-epithelialization, and the regenerated skin tissue had the composition similar to healthy skin [48].

3.3 Vasculature

The other important and potential use of 3D bioprinting technology is the fabrication of vascularized tissues for passage of blood, air, lymph, and other vital fluids in the human body. The cells in dense tissue need to be within 200 mm from a vessel supplying oxygen and nutrients to survive [49]. The conventional technologies faced a major hindrance in fabricating vascular network structure in the dense engineered tissues, which is very crucial for the functioning of the implanted tissue or organ substitute, due to the technological limitation [50]. However, 3D bioprinting technology had enabled the fabrication of complex tissues with an integrated vasculature system, which in turn enabled the integration of the implant vasculature system with that of the host and long-term exchange of air, nutrient, and waste between the native and the implanted tissues [51].

The construction of the vasculature network throughout the tissue is achieved through the design and fabrication of the hollow tube structure in the micrometer scale. This hollow structure is also seeded with vascular cell types or angiogenic factors to promote the formation of functional microvascular networks structure, especially the branching that can size-up to the nanoscale range and also permeation capability [51]. The two main additive manufacturing concepts used for vascularized tissue formation are indirect and direct printing. In the indirect printing, a sacrificial material or negative mold is printed using thermo-reversible hydrogels such as Pluronic F-127 in combination with another material as the permanent scaffold. Upon completion of the 3D printing, the sacrificial mold is removed to form the vascular network that was cellularized with vascular cells [52]. In the direct printing method, the vascular structure is actively printed either with cell-loaded biomaterial or cell-compatible bio-ink. The bio-ink utilized in this process normally has quick gelation/cross-linking ability, or extrinsically induced to crosslink/cured, to maintain a stable hollow structure [52].

3D bioprinting has been utilized to prepare vascular networks in several studies. Miller et al. printed a 3D carbohydrate-glass lattice that was late embedded within an engineered tissue with living cells. Then, the 3D carbohydrate-glass lattice is removed, leaving interconnected hollow structures that can be seeded with endothelial cells to form the vasculature [53]. Later on, the same group of researchers proved that the vascular patch prepared using this technique can guide angiogenesis *in vivo* and rescue the ischemic tissues [54].

3.4 Other tissues

Besides the tissues discussed above, various other tissues have been and being fabricated with the still-evolving 3D bioprinting techniques. Many of these 3D printed tissues had also been implanted on patients as part of a clinical study [55–57] and systematic clinical trials are also being conducted for many of these products, which have been reviewed by Mehrotra et al. [58]. The 3D printed implants are in the clinical trial phase mostly as implants for an ankle injury, bone fracture, disease and deformation, and breast reconstruction. Among the other tissues that are in lab-scale fabrication and optimization but have a high potential for therapeutic use are liver tissue [59, 60], cardiac tissue [61, 62], kidney tissue [63, 64], pancreas tissue [65, 66], cartilage [67, 68], and neural tissues [69, 70].

Although the 3D bioprinting is a new technology, a few types of tissues produced by this technology are already utilized for therapeutic use. However, for the other tissues that have complex microarchitecture, and regulated by multiple signaling factors and cues from surrounding host tissues, it might need a longer time for the 3D printed tissue substitutes to be used in the clinical setting. The 3D printing of complex tissues needs more synergistic research from researches in various fields and various angles before it could fully mimic the native tissue's function. Another aspect to be considered will be the scaling up of the production using the clinical-grade materials and commercial-scale 3D printers as most of the current studies are being done with experimental materials and lab-scale 3D printer technologies.

4. 3D bioprinting for personalized therapy

Personalized medicine, also known as precision medicine, is a concept in medicine that emphasizes that each patient should be managed differently based on an individual's condition. This tailored therapy shall be able to provide the

best treatment plan for the patients to improve their prognosis. In personalized medicine, all the patient's specific characteristics such as age, gender environment, height, weight, diet, environment, and genetics are being considered during the prevention, diagnosis, and treatment phase. Personalized medicine can improve the quality of patient care and reduce the cost by avoiding unnecessary diagnostic testing and treatments [71–73]. Personalized medicine is not only limited to drugs but also for tissue engineering and regenerative medicine. Tissue engineering is highly personalized as a specific tissue-engineered substitute is needed for each patient. For example, different burn patients are presented with different degrees of injury and varied wound location, size, and dimension. Thus, a unique engineered skin needs to be prepared in the current good manufacturing practice (cGMP) facility for each patient.

3D bioprinting is one of the techniques that allow the preparation of personalized tissue-engineered substitutes. One of the major advantages of 3D bioprinting in the field of tissue engineering is the possibility of producing personalized living tissue comprising of stem cells, cell-friendly matrix, and bioactive compound in the dimension uniquely suited for different patients. 3D bioprinting can be used to print simple living tissues like skin to a more complex hollow structure like a trachea and very complex organ like heart and kidney. This is something other living tissue fabrication techniques cannot achieve as these techniques do not allow precise deposition of cells at the space wanted. With the advances in the 3D bioprinting technology, nowadays, it is possible to print multiple types of cells, biomaterials, and bioactive compounds at different spaces to create a complex tissue that mimics the native tissue cellular arrangement and mechanical properties. Maturation of the 3D printed tissues can be achieved using a bioreactor.

To prepare the personalized 3D bioprinted living tissue, the image of the targeted tissue in specific patients needs to be taken and reconstructed into 3D, which will be used to guide the 3D printer to print the tissue in the dimension wanted layer-by-layer to form the 3D tissue [74]. Initially, 3D bioprinting is used to prepare engineered tissue *in vitro*, which can be transplanted *in vivo* afterward. However, it is difficult to maintain the shape and size of the engineered tissue *in vitro*. Thus, researchers come out with the idea of 3D bioprinting the tissue *in situ*, directly on the defect site (**Figure 4**). *In situ* 3D bioprinting allows the precise fitting of the printed tissue to the defect site, which is unique for every patient. *In situ* 3D bioprinting might be more efficient compared to the conventional technique as it allows more accurate reconstruction of defect sites and harnesses the natural healing capacity of the body to mature the printed tissue on time. An *in situ* 3D bioprinters can be as simple as a portable handheld spray gun to a complex robotic arm-assisted 3D bioprinter. Di Bella et al. developed an *in situ* handheld 3D bioprinter that printed mesenchymal stem cells encapsulated within the hyaluronic acid methacrylate-gelatin methacrylamide hydrogel surrounded by the hyaluronic acid methacrylate-gelatin methacrylamide hydrogel + photoinitiator VA-086 shell, which can be photocured using the ultraviolet right for the treatment of cartilage defect [75]. Keriquel et al. used 3D bioprinted mesenchymal stem cells in collagen with hydroxyapatite for bone tissue engineering in a mice model [76]. Cohen et al. used a robot-assisted method of *in situ* 3D bioprinting for the deposition of alginate hydrogel and demineralized bone matrix-gelatin hydrogel for the regeneration of cartilage and bone defects, respectively [77].

Apart from personalized engineered tissue substitutes, 3D bioprinting also can be utilized for the preparation of personalized drug delivery systems and functional tissue models for personalized drug screening and disease modeling. Various models have been developed, including the liver [78], heart [79], blood vessel [80], skin [81],

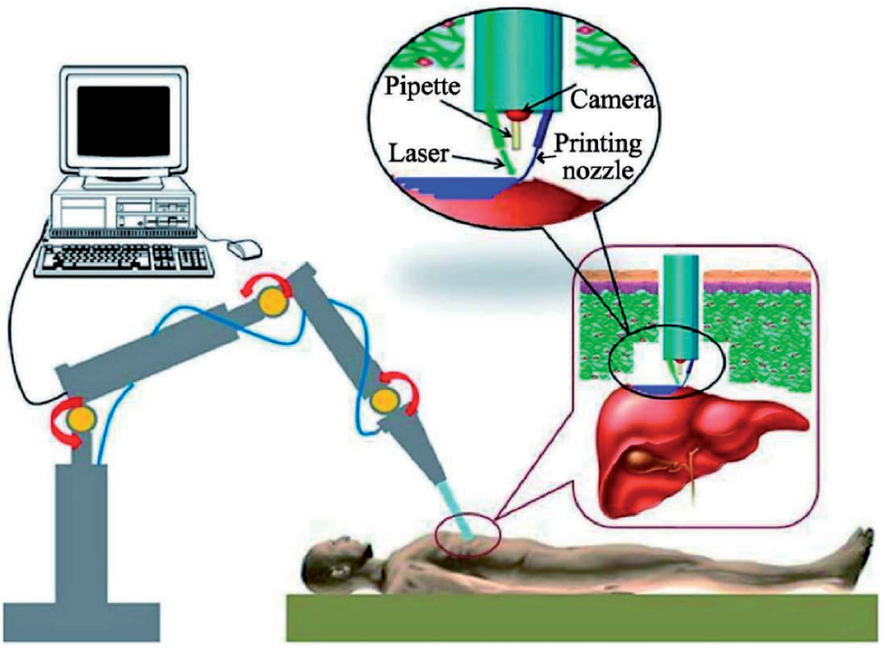

Figure 4.
The personalized 3D bioprinted living tissue has been printed layer-by-layer to form the 3D tissue.

skeletal muscle [82], and cancer [83]. The development of these models can greatly improve the medical care the patients will receive as distinctive prevention and treatment strategies can be designed individually.

5. 3D bioprinting for developing in vitro tissue/organoid models for drug discovery

The invention of 3D bioprinting has revolutionized biomedical research and significant development in translational research closing the gap from bench to bedside. In the pharmaceutical industry, the value of 3D bioprinting is expected in lowering the attrition rate of a new drug since 3D bioprinting has the potential to precisely position multiple cell types as needed according to the tissue of interest (**Figure 5**). Thus, 3D bioprinting enables a more robust design of drug screening, drug delivery, high-throughput drug testing, and ADME assays. The application of 3D bioprinting in the development of in vitro tissue or organoid models for drug discovery is discussed in this section.

5.1 Tumor or cancer model

The ability of 3D bioprinting in replicating tumor microenvironment (TME) provides a better model to assess drug response, tumor proliferation, and metastasis. By 3D bioprinting, a tumor model with hypoxic core and necrosis could be recreated similar to the *in vivo* environment [84, 85]. The 3D-printed glioma model comprising of glioma stem cells incorporated in alginate/gelatin/fibrinogen bioink is an example, and it showed higher resistance to temozolomide than in a 2D culture model [86]. Another case in point, fabrication of breast cancer model was achieved via the Organovo 3D NoveGen Bioprinter system where cancer cells are bordered with a stromal milieu of endothelial cells, fibroblast, and adipocytes. The said breast cancer model was viable for up to 14 days and possesses distinct internal compartmentalization. The model has been used to test hormonal drug response

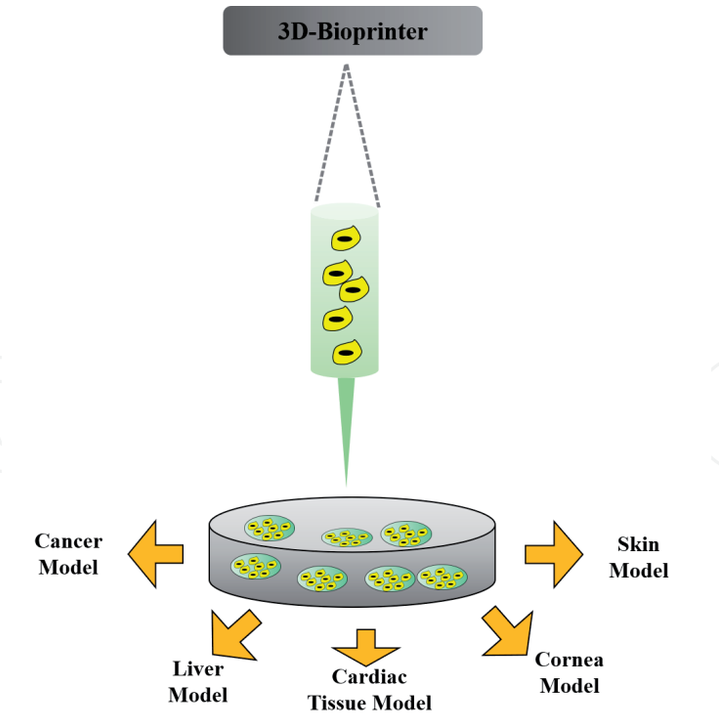

Figure 5.
The potential development of organoid models for drug discovery such as for cancer model, skin, cornea, intestines, muscle, cardiac tissue, and liver.

and chemotherapeutic agents [87]. Most reports conclude that 3D bioprinting gave a higher effect be it tolerance or resistance to the drug tested as compared to the 2D model of the disease, thus proving the value of 3D bioprinting in cancer drug screening.

5.2 Skin

The human skin's inherent multi-layered, multicellular composition is in demand commercially for pharmaceutical and dermatological testing. Dermal skin equivalent has been successfully created using 3D bioprinting through several approaches. One of them is via direct cell printing of fibroblasts and keratinocytes in the collagen-based hydrogel to recreate the skin stratification [88]. The incorporation of melanocytes and fibroblasts in collagen/fibroblast bio-ink was also reported [89]. Maturation and stratification of 3D bioprinted skin construct could be achieved via exposure to the air-liquid interface as shown by Lee et al. with skin construct expressing skin-specific markers [90]. These skin-like constructs are of value in drug toxicity screening as shown by Tseng et al. where five different drugs, i.e. all-trans retinoic acid, dexamethasone, doxorubicin, 5′-fluorouracil, and forskolin, use their 3D bioprinted fibroblasts [91].

5.3 Cornea

Corneal in vitro/ex vivo model is desperately needed as cornea function as major barrier in penetration of drugs into eye; thus, drug absorption thru cornea need to be optimized for topical ocular drug application. Hence, many studies were done in an animal model which is not cost-effective. The complex arrangement of collagen lamellae could be recapitulated using a 3D bioprinting system. Such a corneal model has been successfully produced utilizing extrusion-based bioprinting (EBB) of collagen/alginate/keratinocyte bio-ink [92]. Similar studies utilizing 3D bioprinting with

a promising outcome have also been reported such as the generation of 3D multi-lamellar silk film incorporated with human corneal stromal stem cells (hCSSCs). The silk film architecture supports the growth and differentiation of hCSSCs in producing matured corneal stroma with the desired optical and mechanical properties close to the native cornea [93].

5.4 Intestines

Drugs are commonly absorbed in the intestine; hence, an in vitro intestinal tissue model is of value in the early phase of drug screening. Such a model was fabricated successfully using the Organovo 3D NovoGen bioprinter system with epithelial cells and myofibroblast that has a polarized columnar epithelium with tight junctions and specialized cells that express cytochromes P450 (CYP450). The above said model is a good model for Crohn's disease and internal bowel disease (IBD) that could be used in early-phase drug screening or toxicology study [94].

5.5 Muscle

Development of drugs that are delivered through intramuscular injection or for muscle injuries and muscular dystrophy require an in vitro muscle model for screening and testing. Alginate and Pluronic mixed with murine C2C12 cells have been successfully printed using the EBB method to create a 3D muscle construct that is used to screen several drugs and observe the cell viability, myogenic differentiation, and tissue contractile force against the drug [95, 96].

5.6 Cardiac tissue

Cardiovascular disease (CVD) is the leading cause of death in the world. Cardiotoxicity is the primary cause of CVD drug retraction from the market and is often done in 2D cell cultures. Therefore, the development of cardiovascular disease modeling and drug screening platform is a necessity. Most work focuses on recreating the left ventricular myocardium where cardiac pathologies occur. A spontaneously and synchronously contracting tissue was successfully developed with aligning endothelial cells that are used for cardiotoxicity screening [97]. In another study, Lind et al. fabricated self-assembled rat-derived cardiac cells by direct printing of six functional bio-inks that are highly conductance, piezoresistive, and biocompatible material. This model exhibits inotropic responses similar to isolated post-natal whole rat heart to several CVD drugs, i.e., L-type calcium channel blocker, verapamil, and β adrenergic agonist isoproterenol [98].

5.7 Liver

3D bioprinting approaches have been utilized in creating a liver disease model and liver tissue. Hepatotoxicity study of any drug introduced in the market is essential in any preclinical drug development. The establishment of in vitro liver models includes the incorporation of primary hepatocytes, hepatic cell lines, and stem cell-derived hepatic cells [99–101]. Kang et al. created a five-layer 3D hepatic structure using alginate and mouse induced hepatocyte-like cells that express albumin, ASGR1, and HNF4a [102]. Biomimetic liver tissue builds by Ma et al. showed better liver-specific function and drug metabolism potential compared to 2D monolayer culture [103].

The application of 3D bioprinting technology in the development of in vitro tissue or organoid models for drug discovery has fruitfully shown a better model in mitigating the risk associated with drug development. A 3D environment provides a

better representation of an in vivo model in addition to reducing or eliminating the use of animal model early in the drug development process. All in all, the reliable prediction of safety and efficacy means a significant reduction of time and financial investment of a particular drug in question.

6. Challenges of 3D printing in tissue engineering

Although tissue engineering emerged with this glory for a few decades, the initial attempts took way long [104], whereas 3D printing of complex biomaterials is a promising means of scaffold designing.

There are different types of 3D printers: laser-, inkjet-, and extrusion-based. However, inkjet-based is more popular in tissue engineering, where cells or biomaterials are incorporated into the substrate, as per digitally set instruction, to recreate a functional organ or tissue. Multiple printheads can be used in the case of organs/tissue containing different types of cells. However, there are several challenges to address while designing a 3D printed engineered tissue [105].

6.1 Materials

6.1.1 Choice and processability of materials

The form of material input is important for this specialized process of 3D printing. Hence, it is important to think through before choosing a material, whether it is compatible to form a filament or powder or pellet or solution, that is required for that process. Another important feature to be considered while choosing the material is the expected mechanical strength of the scaffold and their biocompatibility and biodegradability.

6.1.2 Rate of biodegradation

The sole intent of engineered tissue is to replace and regenerate damaged tissue or organ. To comply with this requirement, the scaffold material of the transplanted tissue should be subject to remodeling and absorption. They should be able to degrade in equal or similar pace with the regeneration of extracellular matrix and differentiation of cells. This phenomenon depends on several factors, including hydrophilicity of the scaffold, surface area, porosity, degree of crystallinity, presence or absence of certain enzymes, etc. The most critical part here is harmonization in these factors, so that the degradation of biomaterial and stress release to the surrounding tissue is well synchronized, to ensure healing of the damaged tissue.

6.1.3 Biodegradation of product

Biodegradation rate affects the cell viability and mobility, despite the general concept of this biodegradation being non-cytotoxic. The study finds that the fast degradation of the polymer may affect the cells negatively due to the formation of acidic byproduct. However, more research is required to support these data and to develop the degradation profile of the materials.

6.1.4 Mechanical strength

Cells are described to be sensitive toward the mechanical strength of the polymer scaffold. Rigid and non-flexible material may hinder the cytoskeleton

assembly, cell organization, and receptor recruitment into "focal adhesion plaques," which is crucial for cell signaling and anchoring. On the other hand, highly pliable material may not be able to provide the mechanical strength for anchoring or cytoskeleton assembly and thus affecting the cellular function as well.

6.2 Designing the polymer scaffold structure

6.2.1 Porosity

Different tissues require different porosities for the optimum effect. However, little knowledge is available. A general range of pore size is suggested to be considered for any type of cell, based on observations, rather than the established theory of optimum pore size for each cell type.

6.2.2 Morphology of the polymer scaffold

A study by Yin et al. describes that the microgrooves on the scaffold surface directly affect the cardiac function and susceptibility to arrhythmias [106]. This indicates the importance of the scaffold surface microenvironment, which positively or negatively affects the success of the tissue transplant.

6.2.3 Surface topography

It is stated that surface roughness may enhance adhesion between cell and extracellular matrix. At the same time, too rough surface of the scaffold may exhaust the cell adhesion capability. On the other side, if the scaffold material is too sharp, the cells may get damaged. However, choosing a smooth surfaced scaffold material may require consideration of further modification or coating, as this feature does not facilitate cell adhesion.

6.3 Vascularization

Small and simpler organ printing has been successful, without much difficulty. However, it is not simple when comes to bigger and complex organ, due to difficulty in vascularization. Small tissues are avascular, and most of the time, aneural, alymphatic, and thin or hollow. They can receive nutrition from host vasculature. But when the transplanted tissue is thicker than 150–200 μm, oxygen cannot be diffused from host tissue to it. As such, to create a functional bigger and complex tissue or organ, an integrated vascular system is to be created, which is still not in place [105].

6.4 Cell seeding

The homogenous distribution of the cells throughout the scaffold is important for the effectivity of the tissue. The conventional usage of Petri dish may not be adequate to ensure the uniform seeding of these cells. The bioreactor technology can influence a successful cell seeding, throughout the depth of the scaffold, evenly.

6.5 Future prospect of 3D printing

Despite all the challenges, 3D bioprinting offers great potential and diverse applications for the medical and healthcare sector.

6.5.1 Complex organ engineering

Although few technical aspects are still to be figured out, rapid prototyping creates possibilities to generate complex organs like kidney, liver or even heart, despite having a heterogeneous cellular composition. With the fast pace of advancement in technology and the number of researches going on in this field, the current challenges are expected to be resolved eventually. It has been foreseen that within 20 years, 3D printed organs will be commercially available for transplantation [105].

6.5.2 In vivo test models

The animal study is a mandatory part of drug designing, which applies to tissue engineering and cell therapy. It has been estimated that about 115 million animals are being used in the biomedical industry per year [107]. The printed organs can replace these animal tests of safety, efficacy, and toxicology, saving a number of animals, and resolving the ethical conflict in this issue. At the same time, these printed organs can be more "close to the human subject" model than the animals.

6.5.3 New drug design

In an optimistic vision, it may be possible to have a printed piece of patient's tissue to test which drug is suitable and effective for that particular patient, before applying on them, using this technology.

6.5.4 Mass production

Conventional tissue engineering involves customized scaffold preparation and manual cell seeding. Hence, the success rate is not consistent and the production cost is high, thereby resulting in very costly tissue that many people cannot afford. With the automation and advancement of bioreactor technique in conjunction with rapid prototyping, mass production of the complete organ is a very likely prospect [104]. This will increase the efficiency of the procedure of organ formation, and mass production capability will be economic and more affordable.

6.5.5 Less dependency on organ donation

The organ donation rate has always been far less than the requirement in a given period. On top of that, immunogenicity, rejection, and graft-versus-host disease make the transplantation process further difficult. With rapid prototyping, the scarcity of human organs can be resolved, with less immune rejection and higher effectivity.

6.5.6 In situ tissue printing

In situ generation of skin has already been achieved. With the progress of this technology, it is deemed that in future, a small piece of any tissue can be bioprinted in situ, during surgery, in no time, with precision [105].

7. Conclusions

Regenerative medicine is the new big thing in the medical and healthcare areas. Due to the promising outcome and compatibility for the human body, this

alternative treatment method might compete and/or take over conventional medicine soon. Rapid prototyping has a very wide prospect in regenerative medicine, medical device, and pharmaceuticals. Incorporating the knowledge of cellular biology, biomaterial design, tissue engineering, bioreactors, and so on, organ regeneration will be much more precise and effective. With this speed of progression of science, the remaining challenges will be resolved soon, thereby opening a new era of healthcare and a better life for human beings.

Conflict of interest

The authors declare no conflict of interest.

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