2 3D Bioprinting Technologies for Tissue Engineering Applications

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Abstract

Three-dimensional (3D) printing (rapid prototyping or additive manufacturing) technologies have received significant attention in various fields over the past several decades. Tissue engineering applications of 3D bioprinting, in particular, have attracted the attention of many researchers. 3D scaffolds produced by the 3D bioprinting of biomaterials (bio-inks) enable the regeneration and restoration of various tissues and organs. These 3D bioprinting techniques are useful for fabricating scaffolds for biomedical and regenerative medicine and tissue engineering applications, permitting rapid manufacture with high-precision and control over size, porosity, and shape. In this review, we introduce a variety of tissue engineering applications to create bones, vascular, skin, cartilage, and neural structures using a variety of 3D bioprinting techniques.

Keywords

Bioprinting · Scaffold · Bio-ink · Tissue engineering

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2.1 Introduction

In recent decades, regenerative medicine and tissue engineering research has been directed toward the regeneration, replacement, or restoration of injured functional living tissues and organs, such as bone, vascular, skin, neural, and cartilage [2, 22, 37, 40, 87]. These tissue engineering applications require the insights of researchers from a number of fields, as well as specialized knowledge of biomaterials, cell biology, biocompatibility, imaging, and the characterization of scaffold surfaces [27, 64]. One of the most important aspects of tissue engineering is the fabrication of porous three-dimensional (3D) scaffolds that provide the appropriate environment for regenerating tissues and organs. 3D scaffolds for use in tissue engineering field are fabricated using a various manufacturing methods and biomaterials [41, 79]. Several important characteristics must be considered in these applications [11]: First and most importantly, a tissue engineering scaffold must be biocompatible. Second, fabricated 3D scaffolds should be biodegradable or bioabsorbable so that tissue ultimately replaces the scaffold. Third, the ideal scaffold should have mechanical properties con-

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sistent with the tissue to be implanted. Fourth and finally, the 3D scaffold should be readily manufacturable in a variety of shapes and sizes. Several methods have been developed for fabricating 3D scaffolds using synthetic and natural polymers, including gas foaming, phase separation, electrospinning, and melt molding [20, 40, 55, 65]. These scaffold fabrication methods cannot precisely control the pore size, shape of the scaffold, or the internal channel configuration within the scaffold. Moreover, there are limits on the ability to fabricate scaffolds using cells.

In recent years, 3D bioprinting methods that can readily control the size and shape of a 3D scaffold and the capacity to produce scaffolds together with cells have attracted attention [19, 21]. In this review, we describe 3D bioprinting and its use in adjusting the shape and size of a 3D scaffold. 3D bioprinting was first introduced by Charles W. Hull in 1986 [45]. 3D bioprinting is the process of making 3D solid or gelation objects using 3D modeling software based on computer aided design (CAD) or computer tomography (CT) scan images [7]. 3D bioprinting refers to the technique associated with creating a 3D structure using metals, ceramics, synthetics, or natural polymers [73]. Typically, 3D bioprinting equipment consists of an X-, Y-, Z-axis drive machine, computers, 3D modeling software, and bioprinting materials. After creating a design using CAD or CT images, the 3D bioprinting equipment connected to a computer creates a 3D scaffold structure [91]. 3D bioprinting methods typically consist of stereolithography (SLA) [70], digital light processing (DLP) [25], multi jet modeling (MJM) [89], fused deposition modeling (FDM) [86], selective laser sintering (SLS) [48], and laminated object manufacturing (LOM) [1] components. The SLA and DLP methods use liquid photopolymer resins and ultraviolet (UV) lasers to create 3D structures [25, 70]. The MJM method involves feeding materials through a small diameter nozzle in a material injection process that operates in a manner similar to typical inkjet printers [89]. MJM is an inkjet bioprinting process that uses print head technologies to deposit photo-curable plastic resins or casting wax materials in a layer-by-layer method. The FDM 3D bioprinting method is a common and simple method of using thermoplastic filaments as a bioprinting material [86]. Filaments are melted in the head of a 3D printer by heating and then used to create 3D structures. The SLS bioprinting technology fuses small particles, such as polymers and ceramics, by heating using a high-power laser to form a 3D structure [48]. LOM is a method of creating 3D models by stacking layers of defined sheet materials, such as polymers, plastics, and metals [1]. These methods are used in a variety of fields, including architectural modeling, art, lightweight machinery, as well as in 3D biomaterials used in tissue engineering.

Among the 3D bioprinting methods available SLA and DLP laser-based bioprinting methods, inkjet bioprinting, and the FDM bioprinting of 3D structure used in tissue engineering field have attracted the most attention from researchers. Figure 2.1 illustrates the various 3D bioprinting methods used for tissue engineering applications. 3D bioprinting of biomaterials is a new technology aimed at developing new organs and tissues. 3D bioprinting technologies can adjust the size and shape of a 3D scaffold to control cell proliferation, differentiation, and attachment within the 3D construct. This review introduces a various of tissue engineering field to produce bone, vascular, skin, neuron, and cartilage tissue using 3D bioprinting methods. Table 2.1 summarizes the 3D bioprinting technologies that have been used to produce bio-inks (biomaterials) and cell types.

2.2 Bone Tissue Engineering Using 3D Bioprinting

Bone has a highly specialized organic–inorganic structure that can be classified as a micro- and nano-composite structure that is maintained adjacent to complex cellular components [9]. Researchers have investigated efficient approaches to replacing lost or defective bones and developing good bone substitutes for a very long time [61]. The 3D artificial bone scaffold is used in the clinic for bone regeneration. Bone displays excellent self-healing capabilities if defects are small;

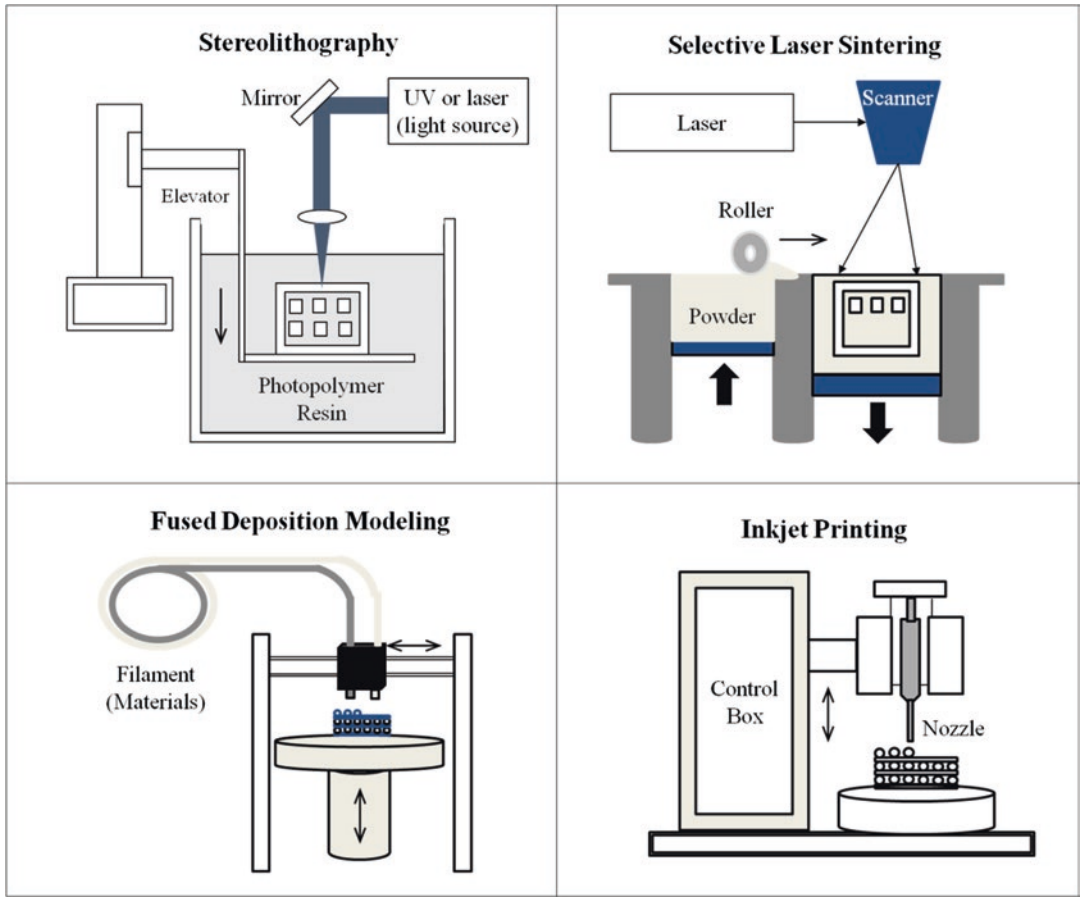

Fig. 2.1 Various 3D printing methods for tissue engineering applications

however, large-scale bone losses or defects cannot be completely healed by the body's innate regeneration systems [82]. In such cases, surgery is needed to replace or repair lost or defective bone. Researchers have attempted to manufacture bone substitutes and to develop restoration methods ([24, 78, 92, 93]). Bones may be classified into two types of structures: cancellous bone (inner part of the bone), which has a spongy structure with a porosity of 50–90%; and cortical bone, which forms a dense outer layer with a porosity of less than 10%. Differences between the internal and external structures of bone require careful consideration for scaffold design for use in bone regeneration. Scaffolds can be used to deliver biomolecules, such as TGF-beta, BMP, IGF, FGF, or VEGF, to promote bone regeneration [49]. Recently, researchers have been interested in 3D bioprinting technologies that can create structures in one step using a various of biomaterials [10, 60].

This chapter examines bone tissue engineering field based on various 3D bioprinting techniques. Rath et al. performed preliminary *in vitro* tests to evaluate the effects of dynamic versus static 3D culture conditions during the seeding of osteogenic cells (bone marrow-derived stromal cells and osteoblasts) onto biphasic calcium phosphate (CaP) 3D scaffolds under osteoinductive and basal culture conditions [71]. Dong et al. integrated poly(ε-caprolactone) (PCL) and chitosan thermogels to form a hybrid 3D scaffold for bone regeneration [17]. The 3D PCL scaffolds were fabricated by FDM bioprinting method. The *in vitro* study shows that the hybrid 3D scaffold could enhance cell proliferation and improve the

Table 2.1 Summarizes of the 3D printing technologies for various tissue engineering applications

Organ	Printing method	Bioink composition	Crosslinking	Cell type	Refs.
Bone	Inkjet-based	Hydroxyapatite/β-TCP Dextrin (Inkjet)	UV	Osteoblasts/bone marrow derived stromal cells	[14, 17, 71, 90]
		Alginate/Collagen (Inkjet)	$CaCl_2$	Human osteogenic sarcoma cells	
	Extrusion based	Chitosan/Polycaprolactone (FDM)	–	Rabbit bone marrow MSC	
		Poly(lactic acid)/Hydroxyapatite (FDM)	~180 °C		
Neural	Inkjet-based	Fibrinogen (Inkjet)	Thrombin	Primary hippocampal Cortical cell	[21, 28, 32, 52, 66, 95]
		Biologically active macromolecules (Inkjet)	–	NSC	
		Collagen Fibrinogen (Inkjet)	Thrombin	NSC	
		Collagen (Inkjet)	37°C	Schwann cell/Bone marrow stem cell	
		Polyurethane (Inkjet)	37°C	NSC	
		Alginate Chitosan/Agarose (Inkjet)	$CaCl_2$	NSC	
Vascular	Inkjet-based	Fibrinogen (Inkjet)	Thrombin	HMVEC	[16, 35, 44, 63, 69, 94]
		Cell spheroid (Inkjet)	–	HUVSMC/Fibroblast	
		Pluranic F127 diacrylate (Inkjet)	UV	–	
		Gelatin methacrylate (Inkjet)	UV	HUVEC/Fibroblast	
		Gelatin methacryloyl/Alginate PEG-tetra-acrylate (Inkjet)	$CaCl_2$UV	HUVEC/hMSC	
	Extrusion-based	Poly(vinyl alcohol)/Gelatin (FDM)	Transgultaminase	Hepatocellular carcinoma	
Skin	laser-based	Blood plasma/Alginate (SLA)	$CaCl_2$	Fibroblast/Keratinocyte hMSC	[15, 26, 42, 43, 50, 59]
		Collagen/Blood plasma Alginate (SLA)	$CaCl_2$	Fibroblast/Keratinocyte	
		Collagen (SLA)	37 °C	Fibroblast/Keratinocyte	
	Inkjet-based	Collagen (Inkjet)	37 °C	Fibroblast/Keratinocyte	
		Collagen (Inkjet)	$NaHCO_3$	Fibroblast	
		Blood plasma/Fibrinogen (Inkjet)	$CaCl_2$	Fibroblast/Keratinocyte	
Cartilage	Extrusion-based/ Inkjet-based	Polycaprolactone/Alginate (Inkjet, FDM)	$CaCl_2$	Chondrocyte	[39, 47, 72]
		Gellan/Alginate/BioCartilage (Inkjet)	$SrCl_2$/4 °C	Chondrocyte	
		Collagen (Inkjet)	37 °C	Chondrocyte	
Cancer	Inkjet-based	Gelatin/Alginate/Fibrin (Inkjet)	$CaCl_2$/Thrombin	Cervical tumor cell	[29, 81, 98]
		Matrigel (Inkjet)	37 °C	Human hepatic carcinoma cell/Human mammary epithelial	
	Laser-based	Poly (ethylene glycol) diacrylate (SLA)	UV	Cervical tumor cell Murin fibroblast	

osteogenesis of rabbit bone marrow mesenchymal stem cells (MSCs). They hypothesized that the PCL/chitosan 3D scaffolds could improve osteoinductivity, cell seeding efficacy, and provide excellent mechanical properties compared to the PCL or chitosan-thermogel 3D scaffold alone. Corcione et al. developed a solvent-free process for producing a hydroxyapatite and poly(lactic acid) composite material suitable for 3D bioprinting processes (using the FDM method) to realize customized scaffolds for bone tissue engineering [14]. In their study, a clinical image of maxillary sinus obtained by cone beam computer tomography were converted into a suitable format and successfully used to fabricate a 3D maxillary sinus model using 3D bioprinting of the composite material. Wang et al. explained that a cell-laden collagen/alginate scaffold could be supplemented with bioglass particles, a well-fabricated, porous, hard material used to fabricate bone replacement scaffolds, to increase the material stiffness and stimulate cell growth and mineralization [90].

2.3 Neural Tissue Engineering Using 3D Bioprinting

More than a billion people around the world are thought to suffer from nervous system disorders [5]. Chronic degenerative diseases or traumatic injury of the nervous system affect central nervous system (CNS) function. Neurodegenerative diseases due to aging are also becoming increasingly important. Despite many studies, treatments that can fully restore neuronal function are not yet available, and our molecular understanding of pathogenic mechanisms is limited. These limitations arise from the lack of models suitable for simulating complex environments *in vivo*. 2D cultures are primarily used for their cost-effectiveness, ease of handling, and applicability to various cell types; however, 2D cultures are not able to support the cell–cell and cell–extracellular matrix (ECM) interactions present *in vivo* [33, 84, 96, 97]. By contrast, 3D tissue engineering is thought to provide a more human-like environment for cells. A variety of 3D tissue engineering systems have been studied for their ability to integrate multiple cell types and create complex neural tissue organization structures [23, 36, 38, 83]. 3D bioprinting can accurately mimic the complexity of our bodies using precisely placed cells and biomaterials based on a desired design [62]. This chapter reviews research into 3D neural tissue models prepared using 3D bioprinting technologies.

Xu et al. used fibrin as a bio-ink to create 3D cellular structures. Whole-cell patch-clamp recordings and immunostaining analysis showed that embryonic hippocampal and cortical neurons maintained their functions and basic cellular properties, including normal, healthy neuronal phenotypes and electrophysiological characteristics, after being printed through thermal inkjet nozzles [95]. Ilkhanizadeh et al. used an inkjet bioprinting system to print biologically active macromolecules onto poly(acrylamide)-based hydrogels that were subsequently seeded with primary fetal neural stem cells (NSCs). They found that the printed macromolecules remained biologically active when printed onto poly(acrylamide)-based hydrogels and influenced the differentiation of multipotent primary fetal NSCs in an efficient and spatially well-controlled manner [32]. Lee et al. reported a tissue engineering scaffold for bioprinting murine NSCs, VEGF-releasing fibrin gels and collagen hydrogels in the construction of an artificial neural tissue. They confirmed the morphological changes displayed by the printed murine NSCs embedded in the collagen and its migration toward the VEGF-releasing fibrin gel. The murine NSCs showed high viability (92.9%) after bioprinting, a viability equivalent to that of manually plated cells. Murine NSCs printed within 1 mm from the border of a VEGF-releasing fibrin gel displayed growth factor-induced morphological changes. The cells printed within this range migrated toward the VEGF-releasing fibrin gel, with a total migration distance of 102 ± 76 μm after 3 days of culture. The results show that 3D bioprinting of VEG-releasing fibrin gel supported sustained release of the growth factor in the collagen scaffold [52]. Owens et al. printed fully biological grafts composed exclusively of cells and cell secreted material. They printed grafts in a rat sciatic nerve injury model of both motor

and sensory function. In particular, they compared the regenerative capacity of the 3D bioprinted grafts with that grafts composed of hollow collagen tubes or autologous grafts by measuring the compound action potential (for motor function) and the change in the mean arterial blood pressure as a consequence of electrically eliciting the somatic pressor reflex [66]. Hsieh et al. used a thermo-responsive hydrogel as a bio-ink and 3D bioprinted NSCs. The stiffness of the hydrogel could be easily fine-tuned based on the solid content of the dispersion. The NSCs in the PCL/poly-DL-lactide (PDLLA) hydrogels displayed differentiation and excellent proliferation but not in the PCL/poly-L-lactide (PLLA) hydrogels. Moreover, the NSCs-laden PCL/PDLLA hydrogels injected into a zebrafish embryo neural injury model rescued the function of the impaired nervous system; however, the NSCs-laden PCL/PLLA hydrogels only displayed minor repair effects in the neural injury model. The function of an adult zebrafish with traumatic brain injury was rescued after implanting the 3D bioprinted NSCs-laden PCL/PDLLA constructs [28]. Gu et al. produced neural tissue by 3D bioprinting human NSCs that were supporting neuroglia and differentiated in situ into functional neurons. The bio-ink incorporated novel clinically relevant polysaccharide-based biomaterials comprising agarose, alginate and carboxymethyl chitosan. Differentiated neurons formed synaptic contacts, established networks, were spontaneously active, showed a bicuculline-induced increased calcium response, and predominantly expressed gamma-aminobutyric acid [19, 21].

2.4 Vascular Tissue Engineering Using 3D Bioprinting

3D tissue engineering to mimic human body functions has been pursued for a long time. As the thickness of a 3D tissue increases, blood vessels become essential components. In a 2D cell culture having a cell population thickness of approximately 20~30 μm, nutrients and oxygen readily diffuse. However, when the 3D tissue thickness exceeds 100 μm, it is difficult for oxygen and nutrients to diffuse to every corner of the tissue [13]. Therefore, vascular tissue guided into the 3D tissue serves to supply nutrients, oxygen and remove waste products. New blood vessel formation in highly vascularized tissues (e.g., liver, kidney, lung, spleen, heart, pancreas, or thyroid) is essential [74, 75]. Thus, there is general consensus that the ability to reconstruct complex vascular networks is crucial to 3D tissue engineering [53]. However, this issue remains a major stumbling block to efforts to create 3D engineering structures with the volume and complexity of human organs [50, 51, 67]. 3D bioprinting technologies that create objects of a desired shape using a variety of bio-inks and cell types have emerged as attractive approaches to designing small-diameter vessels. The advantages of 3D bioprinting method are that researchers can produce heterocellular tissue constructs that readily control the cell density. This provides researchers with finer tools for addressing angiogenic problems in 3D tissue engineering [57]. These techniques can create biomimetic microenvironments in 3D tissues and produce vessels with ideal functions and structures. This chapter introduces research using 3D bioprinting technologies to form blood vessels in 3D tissues.

Cui et al. fabricated fibrin micro-channels using an inkjet-based bioprinting method. When bioprinting human microvascular endothelial cells (HMVEC) laden fibrin hydrogel, they confirmed that the cells aligned themselves within the fibrin channels and proliferated to form confluent linings. A 3D tubular structure was obtained from the printed patterns. They concluded that simultaneously bioprinting both the cells and scaffold promoted HMVEC proliferation and microvasculature formation [16]. Norotte et al. printed small-diameter multilayered tubular vascular grafts that were readily perfused for further maturation. Agarose was used as a bio-ink to print smooth muscle cells and fibroblasts, aggregated into discrete units, either multicellular spheroids or cylinders of a controlled diameter (300–500 μm). The post-bioprinting fusion of the discrete units resulted in single- and double-layered small diameter vascular tubes [63]. Wu et al. used an omnidirectional bioprinting method to fabricate 3D micro-

vascular networks embedded within a pluronic F127 hydrogel scaffold. Using this method, they fabricated 3D microvascular networks using a hierarchical, 3-generation branching topology to form microchannels of diameter 200–600 μm, in which two large parent channels were subdivided into many smaller microchannels [94]. Kolesky et al. reported a new 3D bioprinting method for fabricating 3D tissue constructs replete with vasculature, ECM and multiple types of cells. They confirmed the printability of these structures using two materials, a silicone elastomer and pluronic F127, and confirmed cell viability using a cell-laden gelatin methacryloyl (GelMA) hydrogel. They found that human neonatal dermal fibroblasts and human umbilical vein endothelial cells (HUVEC) proliferated over time [44]. Jia et al. [35] developed perfusable vascular structures with highly ordered arrangements in a single-step process. 4-arm poly(ethylene glycol)-tetra acrylate (PEGTA), GelMA, and alginate were used in combination with a multilayered coaxial extrusion printing system to achieve direct 3D bioprinting. The rheological properties of the bio-ink and the mechanical strengths of the resulting constructs were tuned by introducing PEGTA, which facilitated the precise deposition of complex multilayered 3D perfusable hollow tubes. This blend bio-ink also displayed favorable biological characteristics that supported the proliferation and spread of encapsulated endothelial and stem cells within the 3D bioprinted constructs, leading to the highly organized, formation of biologically relevant, perfusable vessels [35]. In other approaches, perfusable systems and 3D bioprinting have been integrated to achieve 3D tissue vascularization. Pimentel et al. [69] fabricated thick (1 cm) and densely populated tissue constructs using a 3D 4-arm branch network with stiffness comparable to that of soft tissues. This construct could be directly perfused on a fluidic platform over long periods of time (>14 days). They used poly(lactic acid) (PLA) as the support structure and poly(vinyl alcohol) (PVA) as the water-soluble main material. The PLA was selectively removed, and the PVA structure was used to create a artificial 3D vascular network within the ECM that mimicked the stiffness of the liver and encapsulated hepatocellular carcinoma (HepG2) cells. These hybrid constructs were directly perfused with medium to induce the proliferation and formation of HepG2 spheroids. In this study, the highest spheroid density was obtained with perfusion, but overall, the tissue construct displayed two distinct zones: one with a high cell death rate and almost no cell division and one of rapid proliferation. The model, therefore, simulated tissue gradients within necrotic tumor regions [69].

2.5 Skin Tissue Engineering Using 3D Bioprinting

In human body, the skin is the largest organ and protects other tissues from external stimuli. Skin damage leading to infections, or other genetic or physical ailments, can produce chronic ulcers. Skin injuries can expose other tissues to the external environment, including bacteria and viruses. Skin loss can disrupt body temperature regulation. Pathological components of normal skin flora can proliferate in the presence of a broken skin barrier. Skin loss in humans can lead to death in severe cases [12]. Thus, skin damage is a major problem with far-reaching effects on other tissues. Autologous grafts obtained directly from the patient are often used to avoid immune rejection and restore skin function and wound healing after skin damage. Unfortunately, skin damage wounds over large areas or with a significant depth are not adequately healed using autologous grafts [3, 54, 76]. For this reason, there is a need to produce artificial skin substitutes using novel approaches to skin regeneration [34, 58]. These studies have developed sophisticated skin substitutes that interact with human tissues after *in vitro* maturation and transplantation [56, 77, 85]. It has been difficult to mimic the skin of a person while accommodating nerve endings, capillaries, multi-layered 3D structures, and the numerous derivative structures, such as sebaceous glands, sweat glands, and hair follicles. Complex skin structures require accurate signaling systems. Without such systems, the skin structure is lost [80]. In this respect, 3D bioprinting is a very

attractive method that enables the construction of human skin structure mimics using the spatio-temporal patterning of various bio-inks and cells. This chapter introduces research into human skin models using 3D bioprinting technologies.

Koch et al. fabricate a skin model containing dermis and epidermis layers using 3D laser-based bioprinting system. They used an alginate hydrogel as a bio-ink and printed fibroblast, keratinocyte, and hMSC. They then evaluated the influence of the laser-based bioprinting system on the cellular proliferation, survival rate, and apoptotic activity. Modifications of the cell surface markers and DNA damage were assessed and statistically evaluated over several days. The cells survived the transfer procedure with a viability exceeding 98%. All cell types tested maintained their proliferation ability after 3D bioprinting [43]. A collagen bio-ink, keratinocytes, and fibroblasts were printed to form a simple skin structure. The 3D bioprinted cell constructs were assessed after different culture times using immunohistological methods. The presence of cell–cell channels, which indicated tissue formation, was investigated in the vital 3D structures [42]. Michael et al. fabricated a skin substitute using 3D laser-based bioprinting. The skin substitutes were created using fibroblasts and keratinocytes. These 3D structures were subsequently tested *in vivo*. The bioprinted keratinocytes formed a multi-layered epidermis with initial differentiation and stratum corneum after 11 days of culture. Their proliferation was mainly detected in the suprabasal layers. E-cadherin, an indicator of adherens junctions and, therefore, tissue formation, was found in the epidermis *in vivo* as well as *in vitro*. In mice, some blood vessels were found to grow from the wound edges and the wound bed in the bioprinted direction of the cells [59]. Lee et al. demonstrated the potential utility of 3D bioprinting in tissue engineering using skin model as a prototypical human example. They printed collagen as a bio-ink, and fibroblasts and keratinocytes were used as constituent cells to form the dermis and epidermis. Immunohistological characterization revealed that the 3D bioprinted skin tissue was biologically and morphologically representative of human skin tissue *in vivo*. Compared to traditional methods of tissue engineering for skin, 3D bioprinting offers several advantages in terms of form retention and shape, reproducibility, flexibility, and a high culture throughput [50, 51]. Cubo et al. printed bilayer skin using fibrin bio-inks containing human primary keratinocytes and fibroblasts and human plasma. The structure and function of the 3D bioprinted skin was analyzed using immunohistochemical methods, both in 3D cultures *in vitro* and after long-term transplantation into immunodeficient mice. In both cases, the regenerated skin was very similar to human skin and, furthermore, was indistinguishable from the bilayered dermo-epidermal equivalents that were handmade in laboratories [26]. Nanoparticles have recently emerged as a transdermal delivery system. Their surface properties and size determine their efficacy and efficiency in penetrating the skin tissue. Hou et al. utilized 3D bioprinting technologies to generate a simplified artificial skin model useful for rapidly screening nanoparticles for their transdermal penetration capacity. A collagen hydrogel was used as a bio-ink, and fibroblasts were printed into the structure. The effectiveness of this platform was evaluated by using a 3D scaffold using one layer of fibroblasts sandwiched between two layers of a collagen hydrogel to screen silica nanoparticles with different surface charges for their penetration ability. Positively charged nanoparticles demonstrated deeper penetration, consistent with observations from previous studies involving living skin tissue [15].

2.6 Cartilage Tissue Engineering Using 3D Bioprinting

Cartilage, which is only a few millimeters thick, prevents friction between joints and endures extreme load stresses during limb movements. The cartilage defects due to aging, degenerative diseases, trauma or other several factors inevitably lead to arthralgia and chronic disorders [4, 31]. Despite numerous attempts, artificial cartilage that can fully mimic the composition of

the tissue, ECM, and mechanical properties has not yet been developed [30]. 3D bioprinting, which can fabricate products of a desired shape using various materials and cells, presents a great opportunity in cartilage tissue engineering. This chapter discusses research into cartilage tissue engineering using 3D bioprinting.

Kundu et al. fabricated cell-printed 3D scaffolds using the PCL and chondrocyte- encapsulated alginate hydrogel. Cell-based biochemical *in vitro* assays were performed to measure the DNA, total collagen content, and glycosaminoglycans (GAGs) in the different alginate/PCL gel constructs. Alginate/PCL gels containing transforming growth factor-β (TGF-β) displayed greater ECM formation. The cell-printed 3D alginate/PCL gel scaffolds were implanted in the dorsal subcutaneous spaces of female nude mice. Immunohistochemical analyses revealed enhanced cartilage tissue and collagen type II fibril formation in the alginate/PCL gel (TGF- β) hybrid scaffold after 4 weeks [47]. Kesti et al. developed a cartilage-specific bio-ink for use in 3D bioprinting applications based on a blend of alginate and gellan mixed with commercially available BioCartilage particles. They imaged bioprinted scaffolds using magnetic resonance imaging (MRI) to compare the 3D shapes with the original model, and evaluated the utility of MRI in detecting changes in the water relaxation times as they related to ECM production in tissue-engineered grafts. To evaluate cartilage formation, cell-laden Bioink and Bioink/BioCartilage disks were cultured for 8 weeks *in vitro* with and without TGF-β3 supplementation. All of the characteristics of the 3D bioprinted scaffolds were superior to those of native articular cartilage [39]. Ren et al. used collagen hydrogel as a bioink and fabricated 3D cartilage constructs. The chondrocyte density gradient indicated a zonal distribution throughout the ECM. They evaluated the effect of the chondrocyte density gradient on the formation of the regional distribution of ECM in the bioprinted 3D structure. The ECM production was positively correlated with the cell density during the early stages of culture, and the biosynthetic abilities of chondrocytes were affected by both the cell density and the cell distribution in the bioprinted 3D structure [72].

2.7 Cancer Model Using 3D Bioprinting

Cancer is a leading cause of disease and death throughout the world. Despite advances in cancer treatment, many challenges remain, and the characteristics of the tumor microenvironment must be considered [6, 68]. Cancer research commonly relies on 2D cultures and animal models; however, it is difficult to imitate 3D human tissues in 2D cultures, and animal model results cannot necessarily be extrapolated to the human response [8, 18, 46, 88, 96, 97]. These problems may be addressed by furthering our understanding of complex cancers tissue using 3D tumor models that can mimic the microenvironment of native cancer. This chapter introduces research into bioprinted 3D cancer models.

Snyder et al. fabricated a 3D liver cancer model and studied the radiation protection functionalities and drug conversion of living liver tissue analogs. They used matrigel as a bio-ink onto which were printed human hepatic carcinoma cells. Cell-laden bioprinted matrigel and microfluidic chips were used to evaluate the radiation shielding properties of the liver cells using the amifostine pro-drug. The benefits of radiation protection and the conversion of pro-drug by multiple cell types were best realized using a dual-tissue model [81]. Huang et al. created a *in vitro* 3D micro-chip in a hydrogel using 3D projection bioprinting. The micro-chip featured a honeycomb branched structure that mimicked a 3D vascular morphology useful for monitoring, and analyzing differences in the behaviors of cancer cell lines (Hela) versus normal cells (fibroblasts). Fibroblasts exhibited greater morphological changes due to channel width than HeLa cell lines; however, the channel width had a limited influence on fibroblasts migration, whereas HeLa cells migration increased as the channel width decreased [29]. Zhao et al. reported a HeLa cells laden 3D bioprinting alginate/fibrinogen/gelatin hydrogels to construct *in vitro* cervical tumor models. Cell viability was

exceeding 90% using the defined bioprinting technology. Comparisons between the 2D and 3D culture models revealed that the HeLa cells showed a higher proliferation rate in the bioprinted 3D culture model and tended to form HeLa cells spheroids, whereas they formed monolayer cell sheets in the 2D culture system. HeLa cells in the bioprinted 3D models also displayed higher chemoresistance and higher matrix metalloproteinase (MMP) expression than those in the 2D cultures [98].

2.8 Challenges and Future Directions

Thus far, a variety of 3D bioprinting technologies have been used to study tissue engineering applications intended to mimic a variety of tissues and organs. 3D bioprinting has paved the way combining biomaterials, imaging, modeling, and computational technologies in the biomedical and tissue engineering field. The 3D bioprinting technologies permits adjustments to the shape, porosity, and size of the 3D scaffolds, attracting much attention in the tissue engineering field. Several challenges remain, for example the development of biomaterials (bio-inks) for use in bioprinting tissues or organs. Conventional 3D bioprinting focuses on 3D structure creation without cells, whereas recent 3D bioprinting technologies have quickly and accurately produced 3D structures using cells in one step. 3D bioprinting with cells requires materials with excellent biocompatibility and cell affinity. For this reason, the development of bio-inks is very important for bioprinting with cells. In the future, it will be necessary to develop new biomaterials and increase the precision of bioprinting equipment to quickly create accurate 3D structures.

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