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3D Printing of Scaffolds for Tissue Engineering

Jingyu Liu and Cheng Yan

Abstract

Three-dimensional (3D) printing has demonstrated its great potential in producing functional scaffolds for biomedical applications. To facilitate tissue regeneration, scaffolds need to be designed to provide a suitable environment for cell growth, which generally depends on the selection of materials and geometrical features such as internal structures and pore size distribution. The mechanical property match with the original tissue to be repaired is also critical. In this chapter, the specific request of materials and structure for tissue engineering is briefly reviewed, and then an overview of the recent research in 3D printing technologies for tissue engineering will be provided, together with a discussion of possible future directions in this area.

Keywords: 3D printing, tissue engineering, scaffolds, growth factor, cell culture

1. Introduction

Tissue engineering is a newly developing field of a combination of biology, materials method and engineering to develop functional substitutes for damaged tissues [1]. According to the broad range of application on cell types, it can be divided into skin, bone, vascular, kidney, and liver tissue engineering. After years of powerful progress, a set of novel tissue culture [2], replacement [3] and implantation technologies have been developed, allowing fabricating artificial extracellular matrices, namely scaffolds, to bear stem cells, growth factors, or other biological nutrients aiming at repair of tissue function. Scaffolds are bulk bioactive materials with specific porosity and structure to contribute to the formation new tissues for completing the medical task. In 2009, first artificial tissue was implanted successfully into a patient who suffered from the tracheoesophageal defect [4]. This case confirmed that artificial organs stand
a chance to substitute the insufficient supply of standard organ in transplantation, which can drastically decrease the demand for living tissue. Now challenges for tissue engineering are the requirements for certain special structures, mechanical property, biocompatibility, and vascularization of tissues for implantation. In efforts to address these issues, it is important to employ an advanced manufacturing technology, which is flexible enough to build the three-dimensional (3D) structure with complex inside feature.

Reform in materials processing methods arose from the pressing needs for high-performance and multi-functional materials for broad applications in energy storage, transportation, lightweight structures, and biomedical engineering, among which 3D printing are in the highest interest by the community of material science research [5–8]. In conventional processing methods, waste is cutting off from the raw material by milling, planning or grinding, and thus desired structure is obtained by these subtractive methods [9]. On the contrary, 3D printing is known as an additive manufacturing method, building the required structure layer by layer, or even pixel by pixel. The terminology “3D printing” firstly emerged was used to refer the work done at MIT in 1993, modifying a standard inkjet printer to a custom processing equipment [10]. Over last thirty years, a variety of innovative 3D printing technologies have been developed, which can be categorized into three groups including powder-based 3D printing, ink-based 3D printing, and polymerization-based printing. In all these cases, the printed structure is firstly modeled using a computer-aided design software packages, such as UG, CATIA, ProE, or other customized software. Then a ST-format file contained all the model information is exported to the 3D printing system to control the moving track of printing device and constructing the structure layer by layer.

Early use of 3D printing focused on its raid manufacture process, which is suitable for pilot production in lab or factory. Now, 3D printing is one of the most flexible technique enables direct manufacturing complex shape with high resolution, as well as processing highly customized medical products combined with image reconstitution technique. The advancement of 3D printing technologies has provided researchers and doctor’s abundant tools to promote the functional scaffolds, which meet the strict criterion of tissue engineering. In addition, broadening choices in materials that can be processed by 3D printing offers researchers “recipe” to tune the biology performance of scaffolds. The ideal role of 3D printing in tissue engineering is to provide the suitable microenvironment for cells to induce cell proliferation and differentiation toward the functional tissue. There are two main modes of 3D printing using for tissue engineering currently. One is creating 3D cell-laden scaffolds that the cells are contained within the bioink. Another is fabricating molds or scaffolds, which can be cultured with cells in-vitro after fabrication [11, 12].

The main objective of this chapter is to provide a comprehensive review of the advanced 3D printing methods for tissue engineering. This chapter is structured as follows: Section 2 describes the basic need for tissue engineering. Then, a variety of advanced 3D printing methods for tissue engineering are introduced in Section 3. Finally, current issues for 3D printing methods applied in tissue engineering and potential investigations in the future are discussed.
2. Key considerations for tissue engineering

To extend the application of 3D printing into the area of tissue engineering, it is a prerequisite to have detailed knowledge of the biomaterial that is suitable for tissue engineering and can be processed by 3D printing meanwhile. The key questions to be considered for tissue engineering are components selection and mechanical features of the scaffold, which are discussed in the following sections.

2.1. Components consideration for tissue engineering

The choice of materials for tissue engineering makes up a significant portion of influence on the performance of scaffolds. Not only do the material properties should be considered, but the cellular or tissue response from the specific position should be optimizing. For all of these selected materials, nontoxicity is just the basic requirement for printing materials. In order to facilitate the cell proliferation while considering the printability from an engineering perspective, a wide range of factors should be taken into consideration when selecting printing materials for a scaffold, such as biocompatibility, bioactivity, biodegradability, and non-immunogenicity. A myriad of biomaterials suitable for scaffolds has been developed, including polymers, ceramics, metals, and even more are created each year. A range of are applied for tissue engineering.

Polymer materials have a long history in the medical industry [13]. Over last 40 years, a variety of biodegradable polymers have been developed, including synthetic and natural polymer materials. The benefits that synthetic polymers prevail over natural are that synthetic polymers can tune their initial mechanical properties and they have an abundant source of raw materials. Saturated aliphatic polyesters, such as poly (lactic acid) (PLA), polycaprolactone (PCL), poly (glycolic acid) (PGA), or their copolymers, are most frequently used tissue materials, as well as can be used as 3D printing materials [14–16]. Moreover, polymeric composites that doped with reinforcement materials, such as bioactive ceramics or carbon fibers, are allowed to be processed by 3D printing [17, 18]. The incorporation of bioactive hard phase into polymers not only enhances the mechanical property of scaffolds but also the biological performance [19].

Ceramics and bioactive glasses have been widely investigated for replacement and repair of hard tissues, such as bone tissue and teeth [20]. Traditional non-degradable bio-ceramics, such as alumina and zirconia, have high hardness and resistance to wear, making those excellent candidates in the area of joint replacement. However, their biological inertness limits the success of tissue engineering, more or less. Therefore, further efforts made by researchers were to find a ceramic with both high mechanical property and bioactivity. It is found that synthesized hydroxyapatite has close chemical components to the inorganic phase in human bone [21]. When implanted into human body, the development of the interface between HA and host tissue involves complex interactions. Solubilization of HA provides adequate beneficial ions for forming collagen and new bone tissue. Another material family used for
Bone regeneration is bioactive glass (45S5) whose main components are silicon dioxide and calcium oxide [22]. Both of these biocompatible ceramics and glasses have the ability to form a hydroxyl carbonate apatite (HCA) layer, which is thought to be the mechanism for their bioactive behavior.

Except for titanium and its alloys [23], which have a high bioactivity and biocompatibility to human tissue, not too much progress has been gotten for metals used in tissue engineering due to their low biocompatibility. Because of the intrinsic high strength and toughness of titanium alloys [24, 25], they are mainly used in the area of bone tissue engineering implants.

2.2. Mechanical features consideration for tissue engineering

Among the many factors need to be considered, mechanical properties of scaffolds should be tailored according to the specific site in host tissue. For example, the critical compressive strength of scaffolds used for cortical bone tissue is completely different with that for cancellous bone tissue. For the application of segmental bone defects of cortical bone, scaffolds require compressive strength comparable to its prototype, ranging from 100 to 150 MPa along the axial direction [26, 27]. In contrast, cancellous bone has a comparatively loosen structure, which is in the range of 2.5–6.5 MPa [28]. Other mechanical properties, such as elastic stiffness, fracture toughness, and relaxation rate should also be modulated to keep consistent with original tissue [29, 30]. Because mechanical property mismatch between scaffolds and host tissue may cause stress shielding [31], which eventually results in osteoporosis.

Except for mechanical property, to achieve the goal of tissue reconstruction, scaffolds must meet some specific requirement for its architecture and internal structure. It is crucial to have interconnected pore within the bulk scaffolds transferring nutrients and oxygen for cell vascularization and proliferation. Considering the tradeoff between printing cost and biological performance ideal pore size for scaffolds ranges from 200 to 500 with a porosity between 60 and 90% [32]. However, it should be kept in mind that large pore size can facilitate cell vascularization [33]. In addition, graded channel structure can significantly promote cell migration by a capillary effect [34]. Another relevant factor is surface morphology of scaffolds, which affects the cell adhesion, can be modified plasma etching to improve its bioactivity, as well as reformed via other deposition methods [35, 36].

3. 3D printing technologies for tissue engineering

A range of 3D printing methods has been developed in the recent years. According to their technique characteristic, printing methods are classified into four categories, which are reviewed in the following sections, respectively.

3.1. Powder-based 3D printing

Powder-based 3D printing is characterized by using a powder bed to provide raw material, and binding powders together by polymer glue or other thermal fusion methods. It is
invented in 1993 by MIT, an extra z-axis was introduced into a commercial printer by adding a height-adjustable platform, allowing printing 3D structures. In addition, the printer cartridge stored binder solution substituting original pigment. When this binder deposited on the powder bed, it can glue material together and form the desired shape. After decades of development, newer powder-based 3D printing methods, selective laser sintering (SLS), and binder jetting (BJ), are all based on this basic concept (Figure 1).

In SLS, particles are locally fused together to form a solid structure by a high-powered laser. During the printing process, the motion of laser beam is controlled by a computer-aided platform according to the input computer-aided design (CAD) file. After one layer sintered, a scroll will spread a new layer of power on the top of the previous layer, and the cycle repeats itself until the whole structure is completed. Unused particles away from heat affect zone can recycle after removing the 3D object from the powder bed, which decrease the cost of this method. Abundant processing parameter of SLS, for example, particle size, laser power, scan speed, and binder fraction, can be used to control the final structure and mechanical property of products [38]. Types of biocompatible materials that can be processed by SLS are broadening recently, from polymers and ceramics to metals. This diversity of material choice makes it possible to synthesize artificial organ matching the mechanical property of human tissue from different positions. The advantage of SLS method comes from the fact that high resolution of the laser beam. The feature size in SLS is decided both by the diameter of the laser beam and particle size, ranging from 10 to 500 μm [37, 39]. In addition, unfused powders on powder bed act as supporting materials to hold the unconnected part, decreasing minor deformation during processing. Furthermore, SLS is a one-step method that post-processing procedure, such as thermal treatment or solvent evaporation, is unnecessary when printing ceramics and metals. Polymers are the most common materials used in SLS for tissue engineering owing to its low synthesizing temperature. As for ceramics and metals, high processing heat may deteriorate the cell or drug embedded inside the printing material. For these reasons, drugs or growth factors are introduced into SLS printed scaffolds after the printing process [40].

Binder jetting is another powder-based method, which employs liquid binder to glue particles together forming the desired structure. The printer head uses either a thermal or a
piezoelectric actuator to deposit binder onto the powder bed. With respect to thermal actuator, a heating element vaporizes fluid to the gas inside of the reservoir, and the increasing volume squeeze droplet out of the nozzle. The thermal method has a high-efficiency at low cost. However, its accuracy is limited due to the difficulty to control the size of the droplet, and residue thermal stress inside the binder may damage the local structure of the printed material. In the piezoelectric system, a high-accuracy piezoceramic is employed to generate pressure to the fluid reservoir. The shape and volume of the jetting droplet are more uniform compared with that in a thermal system.

Choosing suitable materials, including particle and binder, is crucial to both the mechanical property and biological property of the printed scaffolds [41]. Biocompatible ceramics and metals can be used in the binder jetting, such as hydroxyapatite and titanium dioxide. Particle size is a key factor in binder jetting. Finer particles have a smaller pore size distribution in the powder bed, which dramatically decrease the drop penetration time. However, Fine particles have higher mass transfer velocity, which contributes to the sintering efficiency. Therefore, choosing suitable particle size is a trade-off process between processing stage and thermal treatment stage. After choosing the appropriate particle materials, binder materials that used to stick particles together need to be selected as well. For the application in the medical area, the binder should not leave toxic residue when burning out, or it is nontoxic itself. Water-based binder system [42] (a water solution of an acrylic polymer) and water-soluble binder system [43] (polyvinyl acetate (PVA) or polyethylene glycol (PEG)) are two kinds of binder commonly used in ceramic casting as well as binder jetting. Binder material should have a suitable viscosity property to keep spreading from nozzles while having enough penetration ability [44]. The shaping principle of binder jetting is more relying on physical process rather than chemical reaction, which gives rise to the flexibility in the material choice of particle used in binder jetting. Compared with SLS method, binder jetting need an extra post-processing to densify the loosen green body, because polymer binder cannot provide enough strength for the scaffolds in most cases.

3.2. Ink-based 3D printing

The ink-based method is a process that deposits fluidic materials continually or discretely out of a nozzle to a 3D platform layer by layer. It is one of the most suitable ways for processing tissue materials since it can directly print bioinks, which mixture living cells or growth factors with the liquefied material. Several 3D printing methods use this approach, including direct ink writing and fused deposition modeling (FDM).

Indirect ink writing (DIW) method, viscoelastic inks are squeezing out of the nozzle by the pressure from a piston, a screw, or pneumatic force as shown in Figure 2. Utilizing an easy setup, pneumatic force system has the ability to adjust the pressure in a wide range making it the most applied method in DIW. Screw system has a complicated feed module compared with other methods; however, it can provide the largest driving force that suitable for high-viscosity materials [45]. Critical to this technology is the design of the fluid property of inks. They should possess an obvious shear thinning property that allows passing through micro-size nozzle easily while recovering adequate shear strength to maintain the desired shape after inks dispensing onto the substrates. If cells are introduced into the inks as part of
composition, an ideal rheology property of inks is more difficult to achieve. Inhomogeneous cell distribution may result in an increase in viscosity locally, which causes nozzle jamming.

Cell-laden hydrogels, including chemical cross-linking [47], and molecular physical gels [48], are preferred when printing by DIW method. In bio fabrication, the selection for hydrogels is limited to the biocompatible and biodegradable. A wide range of biopolymers has been examined their viability in the medical application, such as alginate, chitosan, collagen, gelatin, and silk. Among these materials, alginate is one of the most frequently used natural biopolymers for tissue repair, wound healing and drug delivery due to its prominent biocompatibility, and the ability to differentiate cells in culture. Controllable degradation property of alginate was achieved by varying oxidation percentages of alginate hydrogel [48]. In this research, cells behavior was investigated under different concentration ranging from 1 to 20%, as well as different oxidation percentages ranging from 0 to 10%. A certain combination of these two parameters (5% of oxidation and 15% of concentration) was favored by cells since they can form a hydrogel with suitable density to hold cells homogeneously. Silk is another kind of natural polymers produced by insects such as spiders or Lepidoptera. Being highly biocompatible and degradable, silk is of interest for a number of industrial applications as well as biomedical applications. Group of Lewis leads the research in DIW area. They designed a high-resolution scaffolds, which can be used for cartilage employing silk fibroin as bioink [49]. Cell compatibility of this scaffold benefited from the mild processing temperature and avoidance of toxic polymer binders. A two-level hierarchical silk structure was created using a template method by removing micro PCL particles after printed [50]. The morphology of resulting pores and its corresponding porosity were both determined by the sacrificial PCL particles.

Fused deposition modeling (FDM) also relies on nozzle and moving platform to construct the 3D structure. However, unlike DIW, of which raw material is under liquid state, FDM need an extra heater to soften material firstly. In addition, a fan is located at the end of the nozzle,
which controls the solidifying velocity of the molten material. Biocompatible polymers such as Poly(lactic acid) (PLA), PCL, and poly(lactic-co-glycolic acid) (PLGA) are most frequently used materials in FDM method. PCL has been widely used in dental devices and wound repair because it has high printability and excellent interactive ability with tissue.

To improve the biomedical performance of the polymer scaffolds printed by FDM, both coating [14], and doping [52] were developed for scaffolds. A better cell proliferation was obtained after surface modification by plasma treating since the improved surface roughness can adhere more cells [53]. In addition, mechanical and bioactivity properties of biopolymers are tunable by doping biocompatible reinforced particles such as HA and β-tricalcium phosphate (β-TCP) [54]. Moreover, the high concentration of reinforced phase is beneficial to the cell growth and differentiation. Generally, it is hard to attain desired mechanical property and biomedical property simultaneously. Recently, multi-material printer is developed to overcome this limitation by using a dual-printer in a single construct, as displayed in Figure 3 [51, 55]. Through this multi-material printing system, optimized carrier materials that embedded different nutrients and cell types are dispensed on discrete location in the 3D structure in one step.

However, limitation of FDM lies in the poor choice of printing materials. Only thermoplastic materials can be fabricated by this method. Moreover, the high-temperature, ranging from 120 to 300°C, is not suitable for embedding cells or drugs inside filament when preparing scaffolds.

3.3. Polymerization-based 3D printing

The polymerization-based method starts with a process that exposing liquid photopolymer to a laser beam, then this specific exposing area would be solidified through polymer chain reaction. After repeating this process layer by layer, the final complex 3D structure can be constructed. The earliest version of this technique is stereolithography (SLA), which utilizes a low-power

![Figure 3.](image-url)
UV light curing photocurable polymers. Recent decades, new techniques such as two-photon polymerization (2PP) and projection micro-stereolithography (PμSL) (Figure 4), also called digital projection lithography (DPL), are developed toward a more precise and effective direction.

In two-photon polymerization (2PP), a long wavelength near-infrared laser beam can be focused inside of the transparent resin rather than being restricted on the surface of resin [57]. Therefore, a real 3D structure can be constructed by controlling the focal point of the laser beam. The advantage of this method is the excitation volume in 2PP is far less than other laser methods, which gives it the best resolution beyond polymerization-based 3D printing. However, the continuous processing character of 2PP confines it to be a micro-size manufacture method. Gelatin modified with methacrylamide moieties (GelMA) shows a wide range of benefits for application in tissue engineering, such as low toxicity, non-immunogenic, and tunable physicochemical properties [58], which can be used as a polymeric precursor in the 2PP method. Laura Brigo et al. successfully processed scaffolds with feature size at submicron level [59] targeting biological use. They synthesized a highly effective reaction initiator, benzylidene cycloketone-based two-photon initiator (P2CK), providing a wide processing window for photon excitation. Larger post-deformation was observed in the woodpile structure synthesized by lower laser power, which derived from the low crosslinking degree. This loose structure property is more suitable for human BJ (hBJ) foreskin fibroblasts accommodation since these cells are easily penetrating into its bulk structure.

Figure 4. Hierarchical structure printed by micro-stereolithography method (a) polymer metamaterial template (b) large-area, high-resolution additive manufacturing of hierarchical metamaterials (c)-(e) optical microscope images of bulk hierarchical lattice material with a network of hierarchical stretch-dominated octet unit cells [56].
PμSL utilizes a digital micromirror device (DMD) substituting the physical masks used in lithography [61] or liquid crystal mask used in liquid crystal display (LCD) [62] method. The basic theory used in PμSL is similar with SLA and 2PP, but the dynamic mask generator can manipulate millions of pixels at the same time rather than just one focus point, which endows PμSL the ability to process a high-resolution, large-scalability material within several minutes. A real 3D extracellular matrix (ECM) was built by DMD method to assess the difference between two-dimensional (2D) and 3D cell culture system [63]. In this research, poly (ethylene glycol) diacrylate (PEGDA), a commonly used biomaterial, was selected as lithography material to synthesize microwell-array structure. The opening space of microwell is changing along the z-position, from 250 μm at the top to 160 μm at the middle. This exquisite structure design with varying feature size was believed to have the potential to manipulate cell proliferation and cell–cell interactions. Similarly, in the 2PP method, GelMA is also a popular biomaterial employed in PμSL method [64]. Considering the different optical source in these two methods, the selection of chemicals for hydrogel preparation, such as a photoinitiator, was changing from P2CK to Irgacure 2959.

Natural structural materials, as in the case of man bone and tooth, are generally lightweight and possess a balanced combination of strength and toughness. However, synthesized bone graft materials for wound repair are relatively brittle and thus cannot match the performance of the natural part [65]. To address this challenge, a spectacular meta-structure with high tensile elasticity (>50%) was built by Xiaoyu Zheng et al. using the PμSL method [56]. This metamaterial has seven level of the hierarchy, ranging from 10 to 50 nm, and thus the mechanical property of it can match the natural materials. The high elasticity getting from the graded structure gives us the foresight to improve the mechanical property, especially the crack resistance of the synthesized biomaterials applied in the bone graft.

3.4. Four-dimensional (4D) printing

Four-dimensional (4D printing) is a recently appeared terminology in 2013 [66] and immediately attracts wide attention in different areas. 4D printing adds a new dimension, time, to ordinary 3D printed products, which allows materials responding to suitable stimuli or self-transform after possessing. It is not a totally new technique but derives from shape-morphing systems [67–69] and relies on the original 3D printing techniques. The definition of 4D printing is still in a controversy that whether the structure degradable effect can be classified into 4D process [70]. In this context, the degradation of printed material will not be discussed as 4D printing. Transformation code of 4D printed materials is hidden in the exquisite design of its structure and constituents. It offers great potential for customized medical devices given that the dynamic mechanical property of printed material accords with the behavior of living tissues [71]. In addition, the time-dependent property of 4D printing makes it suitable for long-term application embedded in human body.

One efficacious application of 4D printing is for the self-folding system [69, 70]. Two or more different kinds of materials with diverse response to outside stimuli are incorporated into an integrated structure by dual-head printers. Under the same external stimuli, the deformation difference aroused from each component will cause the structure bending or swelling toward
the designed direction. This method is especially useful in cell-laden scaffolds [68]. First, a 2D thin microplate with flexible hinge was built by chemical vapor deposition (CVD) together with lithography, as presented in Figure 5. After that, cells were cultured on the thin parylene plate and thus cell traction force drove the plates folding automatically. As the lattice scaffolds can hold the cells firmly by its closed microstructure, issues with respect to how to adhere cells onto scaffolds can be avoided by this method.

Another successful application of 4D printing in tissue engineering is making tracheobronchial scaffolds for patients who suffered from tracheobronchomalacia (TBM) [72–74]. The processing procedure including three parts. Firstly, a digital 3D model of tracheobronchial tree of patients was constructed by image software using the MRI scan data. Then the patient-specific scaffold was processed by one of the previously introduced 3D printing technologies according to the constructed 3D model. After implanted, this airway splint expanded automatically under the thermal stimuli from the internal warm organ, which leaves growing space for Malacia airway.

Figure 5. 4D printing for self-folding cell-laden scaffolds (a) the cells adhere and stretch across two microplates (b) the cells are cultured on micro-fabricated parylene microplates (c) various 3D cell-laden microstructures (d) schematic of the parylene microplates without a flexible joint (e) a fluorescent image merged with phase contrast image of NIH/3T3 cells patterned only on the microplates (f) schematic of the parylene microplates with a flexible joint to achieve precise 3D configurations after folding (g) a SEM image of the microplates with the flexible joint [68].
4. Conclusion

In conclusion, it is clear from the results discussed in this review that there is a huge potential for applying 3D printing in tissue engineering. 3D printing offers unique advantages toward flexible manufacturing, which can be employed to fabricate scaffolds with complex shape and internal porous structure. To improve the biological performance of printed scaffolds, it is crucial to choose suitable biomaterials introduced in Section 2, and it is equally important to select an appropriate printing technology discussed in Section 3. Although we have got great progress in the processing technique, we are still a long way from printing functional artificial tissue to completely substitute human tissue. To the best of our knowledge, 3D printing cannot build a bulk scaffold over one centimeter while possessing feature size at nanoscale. The precise control of scaffold structure, surface morphology and pore size is still a huge challenge for current 3D printing methods. In addition, post-processing is inevitable for most 3D printing methods, which limit the development of in-situ printing method. Moreover, there is a need for a significant amount of research to be carried out in order to understand the bioactive reaction between host tissue and biomaterials. With increasing research efforts in this field, we believe that future developments of novel biomaterials and processing techniques will lead us to a biocompatible artificial tissue that is smart enough to detect an event and respond to it.

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Author details

Jingyu Liu and Cheng Yan*

*Address all correspondence to: c2.yan@qut.edu.au

School of Chemistry, Physics and Mechanical Engineering, Science and Engineering Faculty, Queensland University of Technology (QUT), Brisbane, Queensland, Australia

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