Three-dimensional Bio-printing Technique: Trend and Potential for High Volume Implantable Tissue Generation

Van-Thuy Duong¹, Jong Pal Kim², Kwangsoo Kim³, Hyoungho Ko⁴, Chang Ho Hwang⁵ and Kyo-in Koo¹

¹Department of Biomedical Engineering, University of Ulsan
²Mobile Healthcare Laboratory, Samsung Advanced Institute Technology
³Department of Electronics and Control Engineering, Hanbat National University
⁴Department of Electronics, Chungnam National University
⁵Department of Physical Medicine and Rehabilitation, Ulsan University Hospital, University of Ulsan College of Medicine

(Manuscript received 1 June 2018 ; revised 22 August 2018 ; accepted 10 October 2018)

Abstract: Recently, three-dimensional (3D) printing of biological tissues and organ has become an attractive interdisciplinary research topic that combines a broad range of fields including engineering, biomaterials science, cell biology, physics, and medicine. The 3D bioprinting can be used to produce complex tissue engineering scaffolds based on computer designs obtained from patient-specific anatomical data. It is a powerful tool for building structures by printing cells together with matrix materials and biochemical factors in spatially predefined positions within confined 3D structures. In the field of the 3D bioprinting, three major categories of the 3D bioprinting include the stereolithography-based, inkjet-based, and dispensing-based bioprinting. Some of them have made significant progress. Each technique has its own advantages and limitations. Compared with non-biological printing, the 3D bioprinting should consider additional complexities: biocompatibility, degradability of printing materials, cell types, cell growth, cell viability, and cell proliferation factors. Numerous 3D bioprinting technologies have been proposed, and some of them have been making great progress in printing several tissues including multilayered skin, cartilaginous structures, bone, vasculature even heart and liver. This review summarizes basic principles and key aspects of some frequently utilized printing technologies, and introduces current challenges, and prospects in the 3D bioprinting.

Key words: 3D Bioprinting, Stereolithography-based, Inkjet-based, Dispensing-based, Microfluidic Nozzle

I. Introduction

The early roots of three-dimensional (3D) printing was known in photo-sculpture and topography almost 16 decades ago [1]. The direct root of the contemporary concept of the 3D printing is known that Charles W. Hull invented stereolithography, a printing method that uses ultraviolet (UV) light to create a 3D object by building up layer by layer at 1984 [2].

The development of solvent-free, aqueous-based systems were able to perform direct printing of biological materials as 3D structures these 3D-printed structures were used for transplantation with or without seeded cells [3]. True 3D bioprinting becomes possible after much more recent advances in printing technology, cell biology and materials science [4].

In the tissue printing, living cells and biological materials are dispensed on a substrate layer-by-layer based on computer-aided design (CAD) [5]. The precise spatial control of the functional materials allows for the fabrication of 3D tissue such as skin, cartilage, tendon, cardiac muscle, and bone. There are various approaches in 3D bioprinting, including biomimicry, autonomous self-assembly, and mini-tissue building blocks. In order to use 3D printed tissues in clinical transplantation, research groups have been trying to print living tissues with biological and mechanical properties suitable for gen-
However, the biggest challenge is to replicate the complex micro-architecture of the extracellular matrix (ECM) components and the organization of multi-type cell organization to develop functional tissues [7].

The 3D bioprinting with stem cell could deliver a long-term clinical solution for serious implant organ shortage [8]. Recently, various research of stem cells, such as human bone marrow stem cells (BMSCs), adipose-derived stem cells (ASCs), and even highly sensitive embryonic stem cells (ESCs) have been reported [9,11,12]. In addition, 3D organ printing has potentials to control microenvironments within a structure by spatial gradient generation of immobilized macromolecules to direct the fate of stem cells [13,14].

The main 3D printing technologies used for deposition and patterning of biological materials are inkjet-based technology, stereolithography-based technology, and dispensing-based technology. These technologies should be evaluated according to surface resolution, cell viability, and materials as shown in Table 1.

In this review, principles and processes of several bioprinting methods, various synthetic materials and hydrogels, as well as types of printing nozzle are described. We also introduce the 3D bioprinting’s achievements, challenges, and then give our prospect.

II. Stereolithography-based 3D Bioprinting

1. Principle

Stereolithography (SL) is known as stereolithography apparatus, optical fabrication, photo-solidification, or resin printing. Like most solid fabrication techniques, SL uses a CAD file as a basic platform for printing [2]. The SL shows considerable performance in the fabrication of 3D structures with very high resolution and accuracy [22,23]. By using UV light to induce a liquid photopolymer (LP) containing UV light-activated initiator, monomer, and other additives into photo-polymerization, the SL could reache a fast performance in printing.

To coagulate LP, UV light, a laser beam or a digital light projector is computer-controlled to illuminate onto the surface of the LP solution (resin). The resin solidifies wherever the light exposed as a sliced part of a 3D structure adhering to a support platform [2]. In detail, during the photo-polymerizing process, small molecules (monomers) links to larger molecules (oligomer) composed of many monomer...
units by triggering free radicals that are produced from the photoinitiator when exposed to UV light of a specific wavelength range [24] (Fig. 1). After the photo-polymerization of the first layer, the platform is moved away from the surface and the solidified layer is recoated by resin [23]. The second layer will be similarly cured with its certain pattern. This process is repeated, layer-by-layer, until the 3D object is completely built (Fig. 2). The SL apparatus accepts a standard triangle language (STL) format translated from solid 3D CAD data and slices it into two-dimensional (2D) cross sections for laser photocuring (Fig. 2(a)). The unpolymerized resin is then drained and washed off, a solid 3D object is obtained. In this object, conversion of reactive groups is usually incomplete. To improve mechanical properties of the structures, post-treating with ultraviolet light is often handled.

Fig. 2(b) and (c) show conceptual diagrams of two types of stereolithography configurations [25-27]. In both systems, objects are built in a layer-by-layer manner by spatially controlled photo-polymerization of LP solution. The differences are in the build orientation and in the illumination method; bottom-up and top-down direction [23]. In the bottom-up system, the first layer is solidified at top of LP and adhered onto the platform by controlled UV light (Fig. 2(b)). The platform then lowers into the LP reservoir to allow the LP to stratify the surface of the solidified layer with a defined thickness. Then the next layer is cured and attached to the upper surface of the previously solidified layer. This process is repeated to complete the 3D object formation on the contrary light direct of the top-down system is opposite to the bottom up system. The top-down system is increasingly being applied in stereolithography. In such setups, light is illuminated through a transparent, non-adhering window which is the bottom of the LP reservoir (Fig. 2(c)). The first layer is solidified LP between the transparent window and the bottom surface of the platform and then separated from the surface of the transparent window by raising the platform with a defined thickness. The next pattern is then solidified in a layer of LP between the transparent window and bottom surface of the previously solidified pattern layer. These steps are repeated as well. This top-down method has several advantages over the bottom-up method. The solidified object does not need to be recoated. Therefore, the surface being illuminated is always smooth. This method can save resin. The illuminated layer is not exposed to the atmosphere.

Following the SL, Micro-stereolithography (MSTL) and Nano-stereolithography (NSTL) have been also proposed and developed with specific light systems, to construct 3D micro/nano-structures [28-32]. Some research groups applied MSTL/NSTL technologies to biomaterial scaffold-forming for tissue engineering [33-36].

2. Applications in creating 3D tissue constructs

The stereolithography supports significant freedom of design and is capable of submicron structures fabricating. Although some stereolithography systems can print structures with $\leq 5 \mu m$ features, most commercial systems fabricate structures with $\geq 50 \mu m$ size [37]. Due to the versatility in design and precise nature of stereolithography, scaffolds with complex overhangs, undercuts, and physiologically relevant microstructures are easily fabricated. These scaffolds exhibit structural and mechanical properties that can mimic native tissue and may permit cellular ingrowth and vascularization to form vascular channels for nutrients and waste transport. Some techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are used to enhance the utility of the stereolithography in scaffold production for tissue regeneration. Developments in resin materials and incorporation of bioactive materials and fillers have also improved the applicability of stereolithography in tissue engineering.

Vincent Chan group fabricated cell-encapsulated hydrogels with complex 3D structures from photopolymerizable poly(ethylene glycol) diacrylate (PEGDA) using modified ‘top-down’ and ‘bottoms-up’ versions of a commercially available stereolithography apparatus (SLA) [38] (Fig. 6(g-h)). Spatial 3D layer-by-layer cell printing was successfully demonstrated, and the feasibility of depositing multiple cell types and material compositions into distinct layers was
established. Arcaute et al. used laser-based SLA to pattern an array of uniaxial channels in PEG-dimethacrylate (PEG-DMA) hydrogels in an early example of photopatterned hydrogels (Fig. 6(i)) [39]. When human dermal fibroblasts were added to the PEG-DMA pre-polymer solution, considerable viability was observed after 24 hours of encapsulation in the polymerized gel. Raman and colleagues recently presented a projection MSTL technique using greyscale projected patterns to 3D-print positive and negative features in PEG with feature resolution <10 μm [40]. Robert Gauvin group demonstrated the capability of the projection stereolithography (PSL) apparatus to engineer microscale 3D structures, supporting cell growth on precisely defined geometries [19]. This fully automated bottom-up approach produced 3D constructs and enabled cell seeding, attachment, and proliferation. The versatility of this technique used in combination with gelatin methacrylate (GelMA) has resulted in both single layer (2D) and multilayer (3D) structures with precise internal architectures.

Most recently, in 2017, Thomas M. Valentin et al. reported a stereolithographic printing method of hydrogels using noncovalent (ionic) crosslinking, which enables reversible patterning with controlled degradation [41]. They demonstrated this approach using sodium alginate, photoacid generators and various combinations of divalent cation salts, which can be used to tune the hydrogel degradation kinetics, pattern fidelity, and mechanical properties. Stereolithographic patterning of alginate microstructures with variable height and patterning and degradation of alginate templates for microfluidic hydrogels are present in Fig. 6(a-f). In this year, Rujing Zhang group reported the fabrication of SLA printed perfusion chip where a confined cell culture volume is traversed and surrounded by perfusable vascular-like networks (Fig. 6(j)) [42]. This printed network further allowed the introduction of cell-laden matrices with independently

---

**Fig. 2.** Process of the stereolithography. (a) Computer-based process is used to design expected 3D structures and convert to STL data before printing. (b) A concept diagram of a bottom-up system for stereolithography-based 3D bioprinting. (c) A concept diagram of a top-down system for stereolithography-based 3D bioprinting.
defined vascular microchannel networks.

III. Inkjet-based 3D Printing

1. Principle

Inkjet printing technology was first developed by Richard G. Sweet in the 1960s to 1970s [43,44]. Currently, inkjet printing has been used in a lot of applications such as product coding and graphic art printing to more advanced applications such as digital fabrication and additive manufacturing. In two recent decades, the number of publications related with the inkjet printing technology has been dramatically increased.

The 3D Inkjet printing is a non-contact method because of droplet printing nozzle. Hence, this printing method can decrease contamination to living cells and biomaterials. On another hand, 3D inkjet printing was applied to construct 3D structures layer-by-layer by printing a binder onto a powder bed or jetting a photopolymer which is subsequently cross-linked [45,46]. Because this technique is based on a digital method that has a great versatility in terms of patterning through droplet deposition. 3D Inkjet printing is compatible with different ink types including polymer solutions, particle suspensions, and biomolecules. Table 2 summarizes some common types of inkjet fluids and their applications [47].

For organ reconstructing, 3D inkjet printing allows the powerful direct-patternning of cells at desired

---

**Table 2.** Common materials and application examples of inkjet printing and inkjet-based 3D printing[47]

<table>
<thead>
<tr>
<th>Categories</th>
<th>Represented materials</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer, monomers and oligomers</td>
<td>Conjugated polymers [e.g. poly(3,4-ethylenedioxythiophene), poly (pyrrole), polyaniline, and poly (p-phenylene vinylene)]</td>
<td>Transistors [50], displays [51], polymer light-emitting devices (PLED)[52], sensors [53], solar cells [54], 3D structures [55], radio-frequency identification (RFID) [56], flexible electronics [57]</td>
</tr>
<tr>
<td>Metal and metal oxide,</td>
<td>Silver and gold nanoparticle dispersions; Silver and gold precursor solutions; Graphene</td>
<td>3D structures[58]</td>
</tr>
<tr>
<td>Carbon materials</td>
<td>Carbon nanotubes and carbon black</td>
<td>Capacitors, 3D structures[59], [60]</td>
</tr>
<tr>
<td>ceramic</td>
<td>Alumina, zinc oxide and silicon nitride/oxide</td>
<td>Smooth muscle cells patterning [21], dissociated neurons patterning [62], Biochips, biomarkers [64,65], biosensors and immunoassay tests [65], regenerative medicine[7,66]</td>
</tr>
<tr>
<td>Biomaterials/cells</td>
<td>Biomolecules (e.g. proteins and DNA) and cells, fibronectin [61], collagen [21], collagen/ poly-d-lysine (PDL) mixture [62], Ca-alginate [63]</td>
<td></td>
</tr>
</tbody>
</table>
locations because the printing droplet size can be regulated at the single-cell level [48]. Thus, direct cell patterning could reach the high similarity with real tissues. In addition, 3D inkjet printing not only allows multiple type of cell production of a construct, but also it enables gradients in cell composition for interface tissue engineering [49].

There are two types of two-dimensional (2D) inkjet printing technique including drop-on-demand (DOD) and continuous inkjet printing (CIJ) [63]. In the CIJ, a continuous stream of ink is forced under a high pressure through a small diameter nozzle and the resulting jet spontaneously breaks up into a stream of droplets (Fig. 3(a)). These droplets then pass through an electrostatic field for electrical charging and are driven in flight by an electric field. Drops not-required for printing will move straight towards a gutter and recirculate to reuse. The CIJ printing is applied mainly in the industry because of its complexity in configuration, droplet path control as well as ink recycling. The DOD inkjet printing uses a digital-based technique to locate droplets directly at an exact target point. Thanks to digital signal control, the droplet can be generated selectively by a pressure change in the ink reservoir, resulting from a piezoelectric effect (piezoelectric DOD inkjet printing) or temporal bubble formation (thermal DOD inkjet printing) (Fig. 3(b) and (c)) [67]. The DOD-based inkjet printing equipment is usually lighter, smaller and cheaper than that for CIJ. Therefore, it is used to print patterns onto objects in households, offices, and industry.

3D inkjet printing techniques are classified into powder-bed-based and direct inkjet printing techniques, known as direct writing (Fig. 4) [27]. The powder-bed-based printing method uses two main materials; a powder-bed-based material and a binder. The binder acts as an adhesive between powder layers. The binder is usually in liquid form and the build material in powder form. A print nozzle moves horizontally along the x and y-axes of the machine and deposits alternating layers of the build material and the binder material. After each layer, the object being printed is lowered on its build platform (Fig. 4(a)). This process is repeated layer-by-layer and the unprinted powder bed plays a role as a self-supporting part to create an overhang, undercut or other complex shapes for the object [68]. The 3D object is completed by detaching the residual non-bonded powder at the end of the printing process. Conversely, the direct inkjet printing method uses solvent evaporation, polymer crosslinking, sintering, crystallization or vitrification to solidify dispensed material droplets (from printer’s nozzle) as a pattern of layers (Fig. 4(b)) [69,70].

![Fig. 4. Schematics of 3D inkjet printing. (a) Powder-bed-based inkjet printing and (b) direct inkjet printing.](image-url)
2. Applications in creating 3D tissue constructs

The inkjet-based printing technique can be applied to pattern surfaces with proteins and other biochemicals to control cell behaviour. To produce fully biomimetic living-cell-laden structures, it is required to fabricate 3D scaffolds on or in which the cells can attach, then proliferate and differentiate. However, the inkjet printing uses materials in liquid form. To generate a solidified structure, there need to be some forms of phase transition. The requirements of an ink material for inkjet delivery have been reported by some groups [59,71]. The key is viscosity property of fluids, which depends on the printing system but is typically around 30 MPa. To date, most workers have used sodium alginate solutions gelled in the presence of Ca\(^{2+}\) ions normally from CaCl\(_2\) solutions.

In 2006, Thomas Boland et al. generated 3D structures by printing less viscous CaCl\(_2\) solution into a tank of sodium alginate solution [72]. The tank containing the Na alginate has a moving table that is initially positioned so that a thin film under 100 µm thickness of the liquid is exposed to the printer. By selectively patterning this film with a CaCl\(_2\) solution, a defined region of the liquid film is solidified. After this, the platform is lowered a defined distance and the second sequence of printing is used to solidify a second layer. This process is repeated until a final desired structure is achieved (Fig. 5(a-c)). Endothelial cells are then attached to these printed structures. Three years later, Nakamura et al. have succeeded in printing living cells in a gelling medium [73]. In this case, Na alginate solution containing HeLa cells in suspension is printed into the CaCl\(_2\) solution to form bead, ring, and tube (Fig. 5(d-g)).

In recent studies, both the powder-bed-based and the direct 3D inkjet printing techniques can be used to create 3D structures using various types of biocompatible materials [72,74]. By controlling 3D coordinates of the printing nozzle, cells and biological
hydrogels can be placed precisely in its positions. Because many tissues and organs are generally composed of heterogeneous cells, it may be necessary to situate biomaterials and living cells in desired positions for effective regeneration of a functional tissue. This 3D inkjet printing technology has the potential to position individual droplets containing single cell and is applicable for positioning multiple bio-components with living cells under digital data for a target tissue. In addition, the 3D inkjet printing process can be applied to building 3D pre-tissues or pre-organs derived from biomaterials and living cells.

IV. Dispensing-based 3D Printing

1. Principle

Dispensing-based 3D printing is a modification of the inkjet-based printing to deposit high viscous materials. This technique is based on fused deposition modeling (FDM), a rapid prototyping technology. The FDM was developed at the end of the 1980s and commercialized in the early 1990s as a 3D printer [34,75].

The dispensing-based 3D printers use either an air-force pump, a piston or a mechanical screw plunger to dispense bio-inks, as shown in Fig. 7(a) [34,76]. By applying a continuous force, the
dispensing-based printing can print uninterrupted cylindrical lines rather than a single bio-ink droplet. Almost all types of hydrogel pre-polymer solutions with various viscosity as well as aggregates with high cell density can be printed with the dispensing-based bioprinters [77].

The process of the dispensing-based 3D printing includes: (1) A material line, like a filament, is ejected from a nozzle with a dispensing system; (2) a 2D pattern is simultaneously fabricated by movement of the nozzle, which ejects the line or a bed where the structure is stacked up; and (3) through a layer-by-layer process, the expected 3D structure is finally fabricated [27,78].

3D structures are fabricated directly with material extruded through the nozzle without any illumination. Therefore, seeded cells can survive and keep their own original characteristics. This process consists of three primary steps: material preparation, code generation and fabrication with the 3D printing system. First, hydrogel filament or pellet-formed material is put into the syringe or dispensing equipment (Fig. 7(a)). Then beam path information for the 3D structure’s shape is generated using a scanning system. Finally, the prepared material in the syringe is extruded from the nozzle by the dispenser, and the dispensed line makes the desired 2D pattern with an encoded beam path. The 2D pattern is stacked up at a sub-hundred-micrometer height, in a layer-by-layer process, to fabricate the expected 3D structure. The dispensing-based 3D system is composed of a 3D motion stage, enabling precise control at the sub-micrometer scale, and a dispensing system controlling the volume of biomaterial in micro- and nanoliters. The 3D motion stage enables the head movement to effect precise and accurate positioning along the x, y and z-axes in the dispensing of the molten material.

2. Applications in creating 3D tissue constructs

Thanks to the simplicity and efficacy, the dispensing-based 3D printing has been widely used to fabricate engineered tissues with living cells. However, this method requires preformed fibers with multiple sizes and various material properties. In addition, this method is relatively slower in fabricating than the inkjet-based printing technique. Many researchers in the tissue engineering field have reformed polycaprolactone (PCL) materials to filament shape or modified the typical FDM system. Hutmacher et al. extruded PCL in a filament shape in preparing the material, because the PCL is a flexible and biodegradable polymer with good fabrication fidelity. They used the PCL filaments in a commercial FDM system (Stratasys Inc., MN, USA) [79,80]. They fabricated a porous scaffold with the PCL via fused deposition modeling and conducted performance tests in terms of mechanical properties and cell cultural response of polycaprolactone scaffolds for bone and cartilage regeneration.

From 2008 to 2010, Karoly Jakab group utilized high concentrations of cells and cell spheroids (i.e., cellular aggregates) as bio-ink for dispensing-based printers, relying on the biophysics of cellular self-assembly [80,81]. This group fabricated scaffold-free cell constructs and geometrically complex multicellular constructs with their bio-ink (Fig. 8).

However, these printed lumen constructs had a big size, which is not suitable to embed to small tissues like blood capillaries. While the ability to create tiny vascular features in bio-printed tissues is often limited, novel bioprinting techniques may resolve
this problem. For example, Dolati et al. utilized a coaxial nozzle system to fabricate bio-printable vascular conduits more than a meter long [82] (Fig. 8(c)). These carbon nanotubes strengthened alginate conduits were perfusable and supported the growth of human coronary artery smooth muscle cells within the ECM. Using this technique, the authors could form sub-millimeter conduits but did not show an ability to print closer to capillary diameters. Mironov reviewed some new methods in which magnetically controlled nanoparticles were added to bio-inks prior to vessel printing [83]. This technique allowed to control the position of the vessels within tissues by applying a magnetic field. However, further research is needed to determine the efficiency and the potential effects of magnetic particles on cells and ECM. To decrease the size of vascular channels and to embed them directly into printed tissues, some research groups have used sacrificial inks to some success. David B. Kolesky et al. used a fugitive Pluronic F127 ink to print 1D, 2D and 3D microchannel arrays with diameters increasing from 45 µm to 500 µm using a single 30 µm nozzle. They subsequently endothelialized these channels with human umbilical vein endothelial cells (HUVECs) [84]. This approach combined with printing fibroblasts encapsulated in a gelatin methacrylate bio-ink yielded multicellular bio-printed constructs (Fig. 8(d)). The fugitive ink composed of Pluronic F127 could be easily removed under mild conditions (4°C).
Table 3. Recent achievements of 3D bioprinting

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell sources</th>
<th>Materials</th>
<th>Printing method</th>
<th>Image</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth muscle cells</td>
<td>Carbon nanotube encapsulated alginate</td>
<td>Dispensing-based</td>
<td></td>
<td>[82]</td>
<td></td>
</tr>
<tr>
<td>Rat heart endothelial</td>
<td>Alginate</td>
<td>Dispensing-based</td>
<td></td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td>cells (RHEC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel</td>
<td>Chinese Hamster Ovary (CHO), Human umbilical vein smooth muscle cells (HUVSMCs) and Human skin fibroblasts (HSFs)</td>
<td>Agarose Gel</td>
<td>Dispensing-based</td>
<td>[87]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUVECs</td>
<td>Poly (ethylene glycol) diacrylate (PEGDA, MW 700) to</td>
<td>Stereolithography-based</td>
<td></td>
<td>[88]</td>
<td></td>
</tr>
<tr>
<td>Aortic valve conduit</td>
<td>Smooth muscle cells and aortic valve leaflet interstitial cells</td>
<td>Gelatin and alginate</td>
<td>Dispensing-based</td>
<td>[89]</td>
<td></td>
</tr>
<tr>
<td>Mouse osteoblastic cells</td>
<td>n-HA</td>
<td>Inkjet-based</td>
<td></td>
<td>[90]</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>MG-63 cells</td>
<td>Alginate</td>
<td>Dispensing-based</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td>Human osteoprogenitor cells</td>
<td>n-HA</td>
<td>Stereolithography-based</td>
<td></td>
<td>[92]</td>
<td></td>
</tr>
<tr>
<td>Cartilage</td>
<td>Patient’s cartilage</td>
<td>Poly (ethylene glycol) dimethacrylates (PEGDMA)</td>
<td>Inkjet-based</td>
<td>[93]</td>
<td></td>
</tr>
<tr>
<td>Minced cartilage cells</td>
<td>Poly (ε-caprolactone) (PCL), and fibrin-collagen hydrogels</td>
<td>Inkjet-based</td>
<td></td>
<td>[94]</td>
<td></td>
</tr>
</tbody>
</table>
Cartilaginous organs such as ear or nose have also been an area of interest in tissue engineering. Kundu et al. utilized advantages of the dispensing-based 3D cell printing technology to create pre-tissue using

**V. Common issues in the three types of the 3D bioprinting**

**1. Materials**

Materials play a key role in 3D bio-printing. A good material can faithfully represent the functions of complex tissue with ECM. The use of a single material type is not enough to produce a suitable tissue environment for more than one functional cell type. Therefore, the incorporating of multiple materials in a printed pattern with associated cells would bring more effects. The ECM is a novel biomaterial derived from living tissue components. The ECM is produced by the resident cells in tissues and secreted into the surrounding medium to provide

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell sources</th>
<th>Materials</th>
<th>Printing method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartilage</td>
<td>Equine chondrocytes and mesenchymal stromal cells (MSCs)</td>
<td>PCL, GelMA, and GelMA-gellan hydrogels</td>
<td>Dispensing-based</td>
<td>[95]</td>
</tr>
<tr>
<td>Human meniscus cells</td>
<td>GelMA</td>
<td>Stereolithography-based</td>
<td></td>
<td>[96]</td>
</tr>
<tr>
<td>Skin</td>
<td>IH3T3 fibroblast, HaCaT keratinocytes</td>
<td>Collagen</td>
<td>Stereolithography-based</td>
<td>[97]</td>
</tr>
<tr>
<td>Neuronal tissue</td>
<td>Mouse bone marrow stem cells</td>
<td>Collagen, and agarose</td>
<td>Dispensing-based</td>
<td>[98]</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>C2C12 mouse myoblasts</td>
<td>Alginate, and gelatin</td>
<td>Dispensing-based</td>
<td>[99]</td>
</tr>
<tr>
<td>Tumor</td>
<td>Hela cells</td>
<td>Gelatin-alginate-fibrinogen hydrogel</td>
<td>Dispensing-based</td>
<td>[100]</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Adipose derived stem cells</td>
<td>Alginate</td>
<td>Stereolithography-based</td>
<td>[101]</td>
</tr>
</tbody>
</table>
biophysical and biochemical support to the surrounding cells due to its content of diverse bioactive molecules. Recently, the ECM material has been used as a promising approach for tissue engineering [102]. ECM has great potential to serve as a bioprinting material in tissue scaffold fabrication because it naturally satisfies the biocompatible requirement [15]. With the application of other bio-printable materials, a stable tissue scaffold can be created using decellularized ECM [8,103]. In recent years, the implementation of programmable self-assembly materials also known as 4D printing, which can be stimulated by cell growth or other specific cellular signals to transform into the desired pattern is another exciting option in tissue scaffold bio-printing [104]. In response to physiological cues or other external stimuli, the materials transform their morphology and functionality to adapt their physiological requirements according to preprogrammed properties. The development of such materials would significantly simplify the current bio-printing process, and thus greatly enhance printing efficiency [105].

2. Microfluidic nozzle

In order to print complex materials for cells, better dispensing tools are requested in the 3D bio-printing field [106,109-110]. A few applications of such smart dispensing tools have been already reported in the literature, such as print heads made from needles used for manufacturing perfusable vascular constructs [111]. The dispensing-based 3D printing can benefit from more complex microfluidic systems which can implement a number of fluidic manipulation functions at the micro-scale. The microfluidics has developed rapidly in recent years, and has significantly contributed to the concept of “Lab on a chip” by allowing the implementation of many fluidic functions such as micro-mixers [112-114], switching valve [115,116], flow focusing [117], particles focusing [118,119], and so on.

Typically, a base microfluidic channel has a Y-shape layout. The fluid of interest feeds from the middle input channel into the main common channel through an integrated nozzle-this forms the “core”. The two input arm side channels converge at the nozzle to form the sheath that surrounds the core (Fig. 9). In 2018, Ludovic Serex et al. proposed to exploit the potential of microfluidics to fabricate a novel nozzle that could perform various operations on the dispensing solution directly [108]. Using this device, they demonstrated multiple smart printing heads that allow the use of new materials, enhance the print resolution, or allow the printing of composite parts or multi-material parts that were only possible using expensive 3D printing techniques (Fig. 10(a-b)). Minghao Nie and his group presented a rapid and high-resolution bioprinter based on a capillary coaxial microfluidic printer head and vacuum substrate [109]. The authors demonstrated the printing of porous (400 µm pitch) 3D bio-scaffolds made from Ca-alginate microfibers with a printing resolution of < 150 µm, at a speed of 40 mm/s, which is approximately 10x faster than existing systems with comparable resolution (Fig. 10(c)). This technique could be further applied to print core-shell type alginate jacketed cell-laden fibers since...
printhead has potential to be extended to multiple axis printheads. Such capability might broaden the application of microfluidic-based bottom-up tissue engineering. Alex T. and Sean J. Hart presented the assemblage and operation of a microfluidic nozzle created using some standard fluidic parts [120]. By elegantly assembling some pieces from a standard assembly, a capillary and a few other standard parts, they were able to fabricate a novel microfluidic device. Precise axisymmetric flow focusing of particles was obtained and observed at the end of the nozzle and within a connected microfluidic device several centimeters away by using this device.

3. vascular networks with bio-printed scaffolds

Ensuring sufficient vascularization of the engineered construct is essential for the long-term viability of any artificially-printed tissue. Several studies have demonstrated generation of a branched vascular tree for bioprinted organ constructs [87], [121,122]. Although their achievements, printing vascularized, and metabolically active thick tissues such as lung, liver or cartilage tissues still faces challenges. One approach to solve this problem is to employ a fully
biological scaffold-free tissue engineering technology, and apply it to fabricate small-diameter multi-layered tubular vascular grafts that are readily perfusable for further maturation [87]. With the assistance of microfluidic techniques, organized microchannels can be created in scaffolds to form initial vascular networks. To avoid the collapse of bio-printed vasculatures, stiffer materials can be dispensed as a shell to protect soft inner channels. Growth factors are important regulators for angiogenesis. With the precise distribution of temporal or sequential growth factors in the tissue scaffolds, the formation of vasculatures including blood capillaries can be guided and facilitated. The maturation of vasculatures through-out the scaffold normally takes a long time, which introduces challenges with respect to maintaining the survival of other functional cells. One attempt to address this issue is using bioreactors. Bioreactors are engineered devices or systems that can provide a physiological environment to support the viability of cells while facilitating biological structural fusion, remodeling, and maturation within a shorter time frame [123]. With the help of bioreactors, connecters the viability of the encapsulated functional cells can be maintained during vascularization [124,125].

VI. Challenges and Perspectives

1. Challenges for bioprinting

Although these three common bioprinting techniques have different printing principles and features, there are a few limitations to the typical bioprinting process related to specific technical, material and cellular aspects of the bioprinting process. The common principle is layer-by-layer

Table 4. Perspective for the 3D bioprinting [7]

<table>
<thead>
<tr>
<th>Area</th>
<th>Research trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioprinter</td>
<td>Simple configuration</td>
</tr>
<tr>
<td></td>
<td>Compatible with materials and living cells</td>
</tr>
<tr>
<td></td>
<td>Applying microfluidics in nozzle developing</td>
</tr>
<tr>
<td></td>
<td>Higher resolution and speed</td>
</tr>
<tr>
<td></td>
<td>Scale up for commercial applications</td>
</tr>
<tr>
<td></td>
<td>Combining bioprinter technologies to overcome technical challenges</td>
</tr>
<tr>
<td></td>
<td>Dynamic printing software</td>
</tr>
<tr>
<td></td>
<td>Combining bioprinter with object scanning with camera, X-ray, MRI systems</td>
</tr>
<tr>
<td>Biomaterials</td>
<td>Complex combinations or gradients to achieve desired functional, mechanical and supportive properties</td>
</tr>
<tr>
<td></td>
<td>Modified or designed to facilitate bioprinter deposition, while also exhibiting desired post-printing properties</td>
</tr>
<tr>
<td></td>
<td>Use of decellularized tissue-specific ECM/biomimetic material scaffolds to study ECM compositions, and/or as printable material</td>
</tr>
<tr>
<td>Cell type</td>
<td>Well-characterized and reproducible source of cells required</td>
</tr>
<tr>
<td></td>
<td>Combinations of cell phenotypes with specific functions</td>
</tr>
<tr>
<td></td>
<td>Greater understanding required of the heterogeneous cell types present in the tissues</td>
</tr>
<tr>
<td></td>
<td>Direct control over cell proliferation and differentiation with small molecules or other factors</td>
</tr>
<tr>
<td>Vascularization</td>
<td>Well-developed vascular tree required for large tissues</td>
</tr>
<tr>
<td></td>
<td>Embedding vascular channels into engineered tissues/organs</td>
</tr>
<tr>
<td></td>
<td>Suitable mechanical properties for physiological pressures and for surgical connection</td>
</tr>
<tr>
<td>Innervation</td>
<td>Innervation is required for normal tissue function</td>
</tr>
<tr>
<td></td>
<td>May be inducible after transplantation using pharmacologic or growth factor signaling</td>
</tr>
<tr>
<td></td>
<td>Simulation before transplantation could be achieved using bioreactors</td>
</tr>
<tr>
<td></td>
<td>Time required for assembly and maturation</td>
</tr>
<tr>
<td></td>
<td>Bioreactors may be used to maintain tissues in vitro</td>
</tr>
<tr>
<td>Maturation</td>
<td>Provide maturation factors as well as physiological stressors</td>
</tr>
<tr>
<td></td>
<td>Potential for preimplantation testing of constructs</td>
</tr>
<tr>
<td>Contamination</td>
<td>Controlling the amount of antibiotic to prevent bacterial and fungi during bioprinting</td>
</tr>
</tbody>
</table>
printing which is applied to all three techniques that generally have difficulty in printing complex hollow structures. When a lumen-shaped structure needs to be formed, the first layer must be printed with a void, then subsequent layers that deposit material over that void with almost same patterns. Also, each printed layer must be connected and mechanically supported to next layer. In this way, the void structure may collapse causing a cascade of offset features and inaccurate geometries. To address this problem sacrificial materials are used, which is a method widely employed in the fabrication of suspended structures. The sacrificial materials support a mechanical connection between printed layers during fabrication. Then they will be removed by putting the solidified objects in chemicals or mild conditions postprocessing step. Some studying groups applied this method including carbohydrate glass [126], Pluronic F-127 [84], gelatin microparticles [127]. However, this type of material requires a printing system with multiple nozzles causing complexity in the printing process. Sacrificial materials must be printable and compatible with non-sacrificial biomaterials and living cells [34]. On another hand, the method of removal and breakdown sacrificial materials must be cytocompatible. These difficulties have limited the development and adoption of new sacrificial materials.

The inkjet-based 3D printing has been widely applied because it enables numerous materials to be built into a 3D scaffold. Also, this technique allows highly precise cell patterning within a 3D hydrogel construct. However, there are some drawbacks including resolution, speed, and mechanical properties. The printed structure of printed objects shows low strength and weak bonding between powder particles and trapped powders inside the printed structure [74]. On another hand, the SL is well known for its very high resolution [41], but has some limitations, such as the low biocompatibility of the printing materials, mainly photoinitiators, and the difficulty in multi-material printing. The biggest problem of SL is to use UV light to illuminate materials for solidification. This step affects directly to living cells which could change the original characteristics of cells, and even kill the illuminated cells.

2. Perspectives for the bioprinting

The 3D bioprinting is at its early stage, researchers achieved creating several tissues at human scale for transplantation (Table 3). However, there still exist many challenges that prevent the realization of artificial tissue constructs. Therefore, more studies are required in the future to address these challenges. Table 4 summarizes some perspectives for 3D printing research.

VII. Conclusion

The scientific community has already obtained some remarkable achievements in the 3D bioprinting. This technique showed great versatility and flexibility for artificial tissues formation. The interdisciplinary development of engineering, biomaterials science, cell biology, physics, and medicine are promoting to realize production of artificial organs replacing organs from donors. The 3D bioprinting is directing to become a strong fabrication technique to fabricate complex in micro- and macro-scale biomedical systems.

Acknowledgements

This paper is the result of research project of Basic Science Research Program, through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future, Planning, Republic of Korea (NRF-2017R1D1A1B03034982, NRF-2017R1A2B4011478, and NRF-2017M3A9E2062707).

References

Three-dimensional Bio-printing Technique: Trend and Potential for... - Van-Thuy Duong et al.


Three-dimensional Bio-printing Technique: Trend and Potential for... - Van-Thuy Duong et al.


[99] “A 3D bioprinted complex structure for engineering the muscle– tendon unit.pdf.”


