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REVIEW

Bioprinting technology and its applications

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Summary

Bioprinting technology has emerged as a powerful tool for building tissue and organ structures in the field of tissue engineering. This technology allows precise placement of cells, biomaterials and biomolecules in spatially predefined locations within confined three-dimensional (3D) structures. Various bioprinting technologies have been developed and utilized for applications in life sciences, ranging from studying cellular mechanisms to constructing tissues and organs for implantation, including heart valve, myocardial tissue, trachea and blood vessels. In this article, we introduce the general principles and limitations of the most widely used bioprinting technologies, including jetting- and extrusion-based systems. Application-based research focused on tissue regeneration is presented, as well as the current challenges that hamper clinical utility of bioprinting technology.

Keywords: Bioprinting • Reconstruction • Regenerative medicine

INTRODUCTION

Recently, bioprinting technology has gained much attention due to its ability to overcome some of the engineering challenges encountered in the field of tissue engineering [1–4]. The key components of tissue engineering include cells, scaffolds and biological molecules. Cells are seeded onto solid and biodegradable scaffolds, and tissue formation is often induced by biomolecules, such as growth factors. Various tissue engineering approaches have been employed to build tissues and organs that could be used in patients, including myocardial tissue, vessel, heart valve and trachea [5–11]. While the conventional tissue engineering approach has shown to be successful in building a number of tissues clinically [12–15], challenges continue to exist in building complex and composite tissues and organs. These include inadequate scaffold fabrication methods that could replicate tissue microarchitecture, limited availability of biomaterials for tissue construction and methods to deliver multiple cell types in their precise locations within the bioscaffold [2, 4, 16].

To fabricate the ideal scaffolds for tissue regeneration, several requirements must be met, including biocompatibility, biodegradability, porosity and structural support [17–20]. Numerous methods have been employed to fabricate scaffolds for tissue engineering applications, including solvent casting, particulate leaching, gas foaming, phase separation and freeze drying. Although these methods are able to produce necessary structures, controlling the size, microarchitecture and interconnectivity of pores, which are needed to transport oxygen and nutrients for cell survival, remains difficult. In addition, organic solvents used to dissolve the biomaterials can remain in the scaffolds, the residues of which can be toxic to cells [17, 20, 21]. Thus, many researchers applied additive manufacturing (AM) technology to fabricate scaffolds to overcome the limitations of conventional methods.

AM is a manufacturing technique that produces complex 3D structures by selectively adding materials and it is categorized into stereolithography, fused deposition manufacturing and selective laser sintering. This technology is able to control the size, shape, distribution and interconnectivity of pores of the scaffold. Moreover, AM technology can be used to fabricate biomimetic-shaped 3D structures from medical images, such as magnetic resonance imaging (MRI) and computerized tomography (CT) using computer-aided design and manufacturing (CAD/CAM) technologies [20–23].

Although scaffolds fabricated using AM technology may yield a controllable architecture, the utility is often limited due to difficulties in placing multiple cell types, biomaterials and bioactive molecules within the scaffold. Bioprinting technology overcomes these limitations by its ability to construct 2D and 3D structures with proper placement of cells, biomaterials and biomolecules in defined locations (Fig. 1). Bioprinting is based on AM technology and it allows direct cell deposition in organotypic architecture. Furthermore, it can be combined with CAD/CAM technology to fabricate a structure that has an accurate anatomical shape [4]. As a result, bioprinting technology is gaining attention as an advanced fabrication method for engineered tissue construction (Fig. 2). In this review article, we introduce the general principles and challenges of the most widely used bioprinting technologies, including jetting- and extrusion-based, and highlight representative research on tissue regeneration, including applications in the field of cardiothoracic surgery.

BIOPRINTING TECHNOLOGY

Jetting-based bioprinting

Jetting-based bioprinting is a non-contact technique in which 2D and 3D structures are generated using picolitre bio-ink droplets

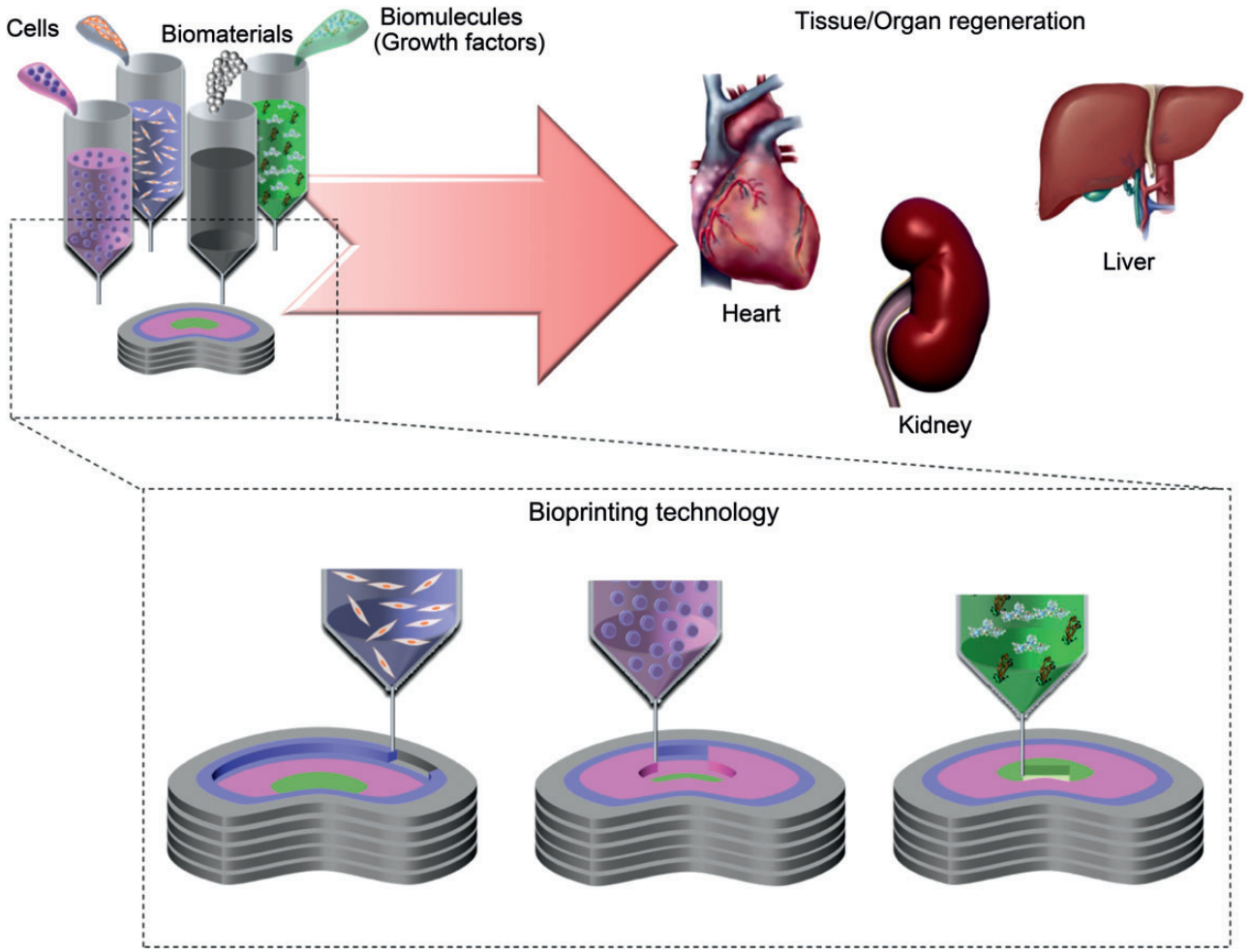

Figure 1: Bioprinting can be defined as additive three-dimensional fabrication of tissues or organs using cells, biomaterials and biological molecules. Various types of tissue constituents are positioned in spatially defined locations to generate tissue and organ constructs.

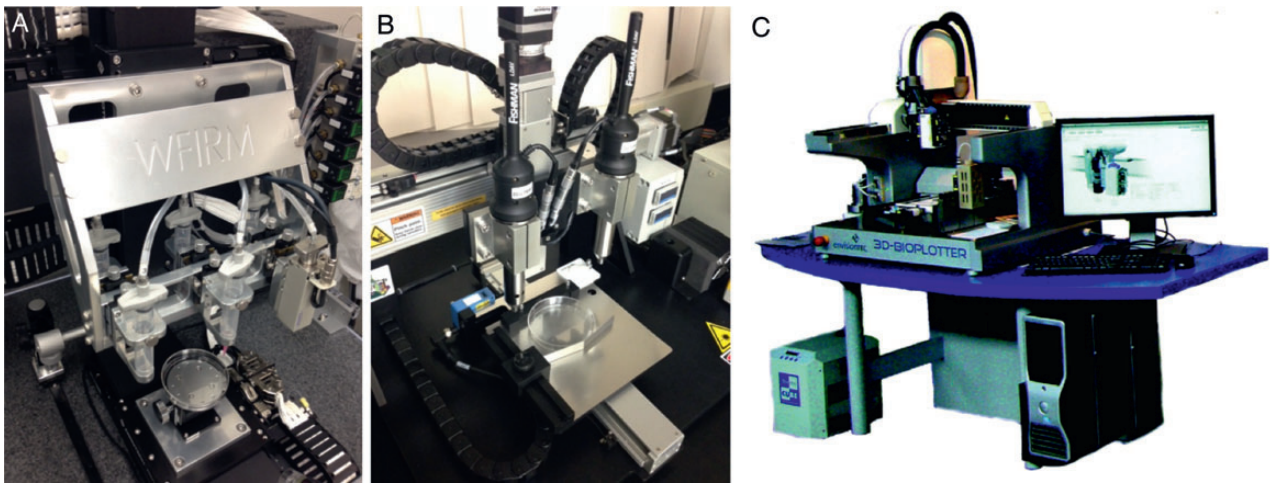

Figure 2: Images of three-dimensional (3D) bioprinting systems. (**A**) 3D Integrated Organ Printer (Designed by Wake Forest Institute for Regenerative Medicine, USA), (**B**) Commercialized Novogen MMX bioprinter™ (Designed by Organovo, USA), (**C**) Commercialized 3D-Biopllotter (Designed by Envisiontec, Germany) [24]. Reproduced with permission from Ref. [24].

layered onto a substrate [16, 25]. This type of bioprinting can be categorized by the mechanism used to generate the bio-ink droplet: thermal method, piezoelectric actuator, laser-induced forward transfer and pneumatic pressure (Fig. 3) [3, 26–29]. The thermal method involves the use of a heat generator, which increases temperature locally within the bio-ink chamber. The

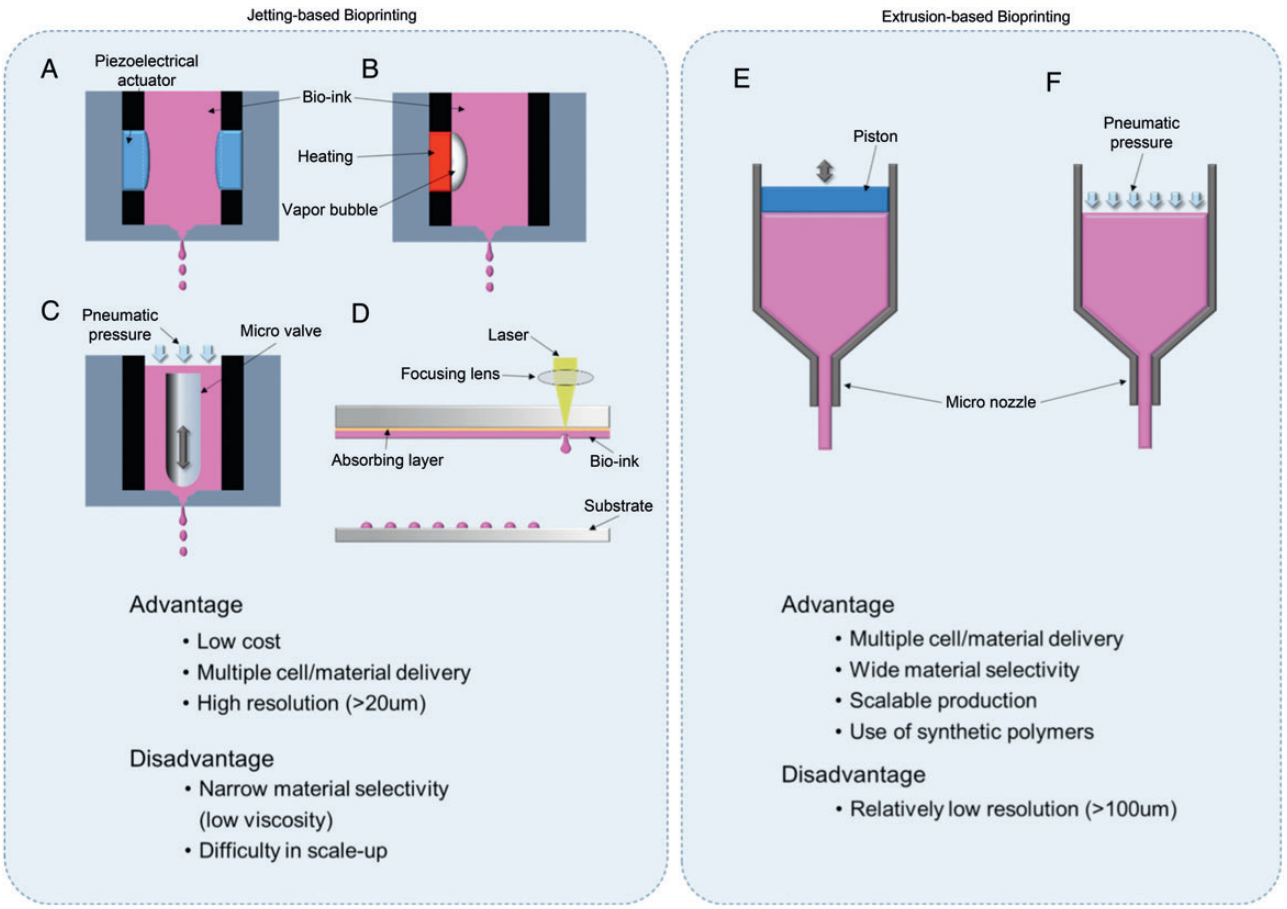

Figure 3: Bioprinting technology is categorized into jetting- and extrusion-based bioprinting. Jetting-based bioprinting generates picolitre droplets containing cells and biological molecules using (**A**) piezoelectrical actuator, (**B**) thermal, (**C**) pneumatic pressure and valve or (**D**) focused laser energy. Extrusion-based bioprinting dispenses continuous filaments of a material through a micro-nozzle using (**E**) piston or (**F**) pneumatic pressure.

local heating generates a bubble and ejects a small droplet. The bioprinter using a piezoelectric actuator applies piezo-crystal pulse actuator mediated by electrical input, and the generated pulse results in ejection of a small droplet. These two methods are the most widely used for jetting-based bioprinting, and commercialized inkjet printers utilize these delivery mechanisms. And the laser-induced forward transfer method generates vaporization by the laser system to produce a small droplet. This technique produces relatively high resolution patterns; however, cell viability in the printed hydrogel is decreased when compared with other delivery mechanisms. For the bioprinter using pneumatic pressure, the droplet is generated by the opening and closing of a microvalve under constant pneumatic pressure. The principle of this method is relatively simple compared with other jetting-based bioprinting methods.

While jetting-based bioprinting methods can eject droplets of picolitre volume and have a high fabrication resolution of 20–100 um, processing time is prolonged due to the size of printed droplets [30, 31]. Moreover, the bio-ink material with high viscosity cannot be used to obtain picolitre droplets. Therefore, low-viscous materials such as thrombin, $CaCl_2$, saline and fibrogen have been used as bio-inks for jetting-based bioprinting [28, 32–35]. In addition, the mechanical properties of printed structures are weak, and fabrication of durable 3D constructs that maintain their shape and withstand external stress after implantation is difficult [25, 35].

Extrusion-based bioprinting

Extrusion-based bioprinting systems dispense continuous filaments of a material consisting of cells mixed with hydrogel through a micro-nozzle to fabricate 2D or 3D structures. Cell-laden hydrogel can be dispensed by using pneumatic pressure or a syringe pump, and the amount dispensed can be adjusted by controlling the pressure level or the displacement of the piston of the pump, respectively (Fig. 3) [32, 36–39]. After printing 2D patterns, hydrogels are solidified physically or chemically, and 3D structures can be fabricated by stacking 2D patterns layer by layer. Extrusion-based bioprinting allows a wider selection of biomaterials since high-viscosity biomaterial can be printed through the micro-nozzle. Consequently, fabrication and scale-up of 3D structures can be achieved by the use of viscous biomaterial [39–42]. Additionally, cell viability in the fabricated structure by extrusion-based bioprinters is reportedly higher than 90%.

Integrated bioprinting

As is generally known, bioprinting technology relies on cell-laden hydrogels and cell aggregates to fabricate structures. However, it is difficult to fabricate 3D structures with sufficient strength, clinically applicable size and shape due to the low mechanical properties and inadequate stability of the hydrogel materials. To overcome

these limitations, we recently developed a hybrid system that can concurrently print a synthetic biopolymer and cell-laden hydrogel to generate tissue constructs with high mechanical strength [43]. The synthetic biopolymer provides physical support of 3D structures and the biological components of the cell-laden hydrogel promote tissue regeneration. As such, the mechanical properties of 3D printed tissue structures can be improved by the use of the hybrid bioprinting system.

BIOMIMETIC SHAPED TISSUE CONSTRUCT USING MEDICAL IMAGING

In addition to delivery of multiple tissue constituents within a 3D space for tissue engineering, bioprinting technology allows the fabrication of biomimetic shaped 3D structures unique to the target tissue or organ, since it can be combined with CAD/CAM technology using patients' medical images (Fig. 4) [20, 44–46]. One could envision obtaining 3D volumetric information of a defected tissue or organ from a patient's medical imaging data (CT or MRI). The scanned 2D segmentation of the human body is collected and stored in a digital imaging and communications in medicine (DICOM) file, then the CAD model is converted from the stored data by a reverse engineering process.

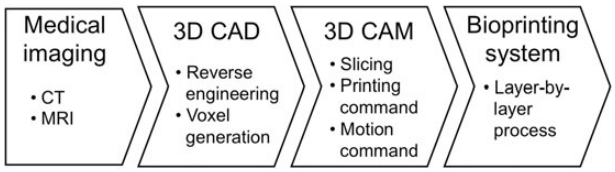

Figure 4: Computer-aided design and manufacturing (CAD/CAM) process for bioprinting technology to fabricate biomimetic-shaped tissue or organ.

A 3D CAD model of the damaged tissue or organ can be created by mirroring the configuration of undamaged or normal tissue anatomy. Using the 3D CAD model created from medical images, a fabrication code in which the printer can recognize and execute can be generated by CAM technology. Because human tissues and organs have complex inner architecture with multiple cellular materials, not only well-defined tool paths, but also proper inner architecture should be generated and constructed, respectively, for efficient tissue/organ regeneration. Therefore, strategies for tool path generation are important to the fabrication of biomimetic shaped 3D structure for highly efficient tissue or organ regeneration.

APPLICATIONS IN TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Recently, bioprinting technology has gained increasing attention due to its ability to spatially control the placement of cells, biomaterials and biological molecules for tissue or organ regeneration (Fig. 5 and Table 1) [52, 54, 56, 57]. Bioprinting has been used to generate 2D and 3D structures for various purposes, including fabrication of scaffolds and tissue constructs for tissue regeneration.

Patients with valvular heart disease often require valve replacement, either with mechanical or with biological prostheses. However, these prosthetic valves are frequently associated with complications, such as mechanical failure and calcification. As such, various approaches have been proposed to improve the

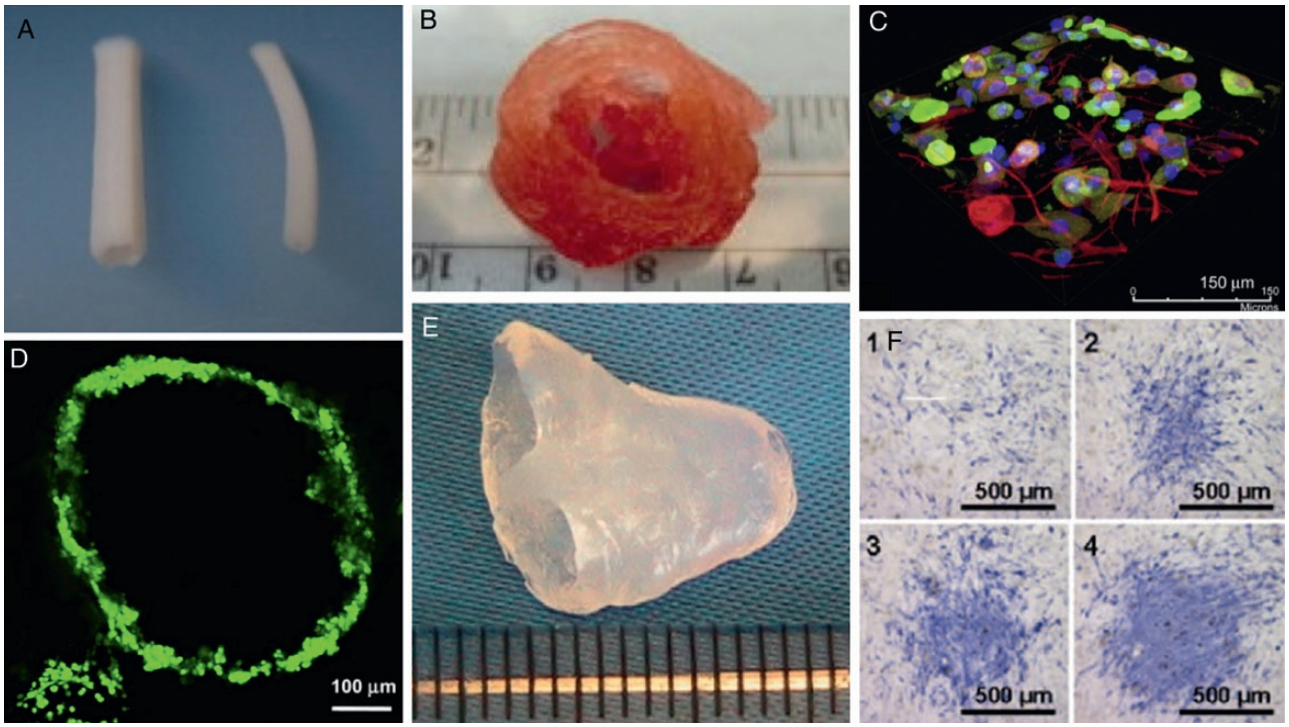

Figure 5: Tissue engineering applications using the bioprinting technology. (**A**) Blood vessel construct fabricated by extrusion-based bioprinting [38]. (**B**) Aortic valve conduit fabricated by extrusion-based bioprinting [47]. (**C**) Confocal microscopic image of multilayered skin structure fabricated by jetting-based bioprinting [28]. (**D**) Fluorescent image of microvascular structure fabricated by jetting based-bioprinting [35]. (**E**) Three-dimensional cardiac tissue with biomimetic shape fabricated by jetting-based bioprinting [25]. (**F**) Alkaline phosphate staining of cultured muscle stem cells on spatially controlled BMP-2 by jetting-based bioprinting [33]. Reproduced with permission from references [25, 28, 33, 35, 38, 47].

Table 1: Tissue engineering applications using bioprinting technology

Tissue	Techniques	Cell types	Growth factors	Materials	References
Heart valve	Extrusion-based bioprinting	Aortic valve interstitial cell Aortic root sinus smooth muscle cell	–	Hyaluronic acid Gelatin Alginate	[47, 48]
Myocardial tissue	Extrusion-based bioprinting	Cardiomyocyte progenitor cell	–	Alginate	[49]
Blood vessel	Jetting-based bioprinting	Endothelial cell Smooth muscle cell Mesenchymal stem cell	–	Fibrin	[50, 51]
	Extrusion-based bioprinting	Endothelial cell Cardiac cell Smooth muscle cell Fibroblast	–	Collagen Agarose Alginate	[35, 37, 38]
Musculo-skeletal tissue	Jetting-based bioprinting	Muscle-derived stem cells Myoblast Mesenchymal fibroblast	BMP-2 FGF-2	Fibrin	[33, 52]
	Extrusion-based bioprinting	Bone marrow stromal cell Endothelial progenitor cell Endogeneous stem cell	TGF-β	Agarose Alginate Hydroxyapatite Polycaprolactone	[40, 53]
Nerve	Jetting-based bioprinting	Embryonic motorneuron cell Hippocampal cell Cortical cell Neuronal precursor cell Neural stem cells	CNTF VEGF	Soy agar Collagen Fibrin	[34, 54]
	Extrusion-based bioprinting	Bone marrow stem cell Schwann cells	–	Agarose	[55]
Skin	Jetting-based bioprinting	Dermal fibroblast Epidermal keratinocyte	–	Collagen	[28]

outcomes, including the use of bioprinting technology. In one study, Duan *et al.* [48] applied an extrusion-based bioprinting technology into the construction of trileaflet heart valve conduit, composed of hybrid hydrogel of hyaluronic acid and gelatin and human aortic valve interstitial cells. This study showed that the printed tri-leaflet heart valve conduit, assessed at 7 days, is highly viable and has great potential for remodelling. Furthermore, a subsequent study showed that it is possible to fabricate an anatomically complex living aortic valve conduit composed of alginate/gelatin hydrogel containing aortic root sinus smooth muscle cells and aortic valve interstitial cells [47]. These studies suggest that cellularized tissue valves can be generated using the bioprinting technology for eventual clinical use.

In another study, Gaebel *et al.* [50] applied the laser-assisted bioprinting technique to deliver human umbilical vein endothelial cells and human mesenchymal stem cells into a defined area on a polyester urethane urea cardiac patch for the regeneration of cardiac tissue. The printed structure was implanted into the infarcted zone of rat heart and then an enhanced vessel formation of bioprinted tissue was demonstrated. To repair myocardial tissue, Gaetani *et al.* [49] fabricated structures composed of a mixture of human cardiomyocyte progenitor cells and alginate hydrogel by bioprinting. In an *in vivo* study, the printed cells retained their commitment for the cardiac lineage and expressed the genes of the early cardiac transcription factors.

Cui *et al.* [35] used thermal inkjet technology to fabricate microvasculature whereby a mixture of human microvascular endothelial cells (HMVEC) and fibrin was printed on a 10 μm scale. After 21 days of culture, the printed HMVEC proliferated to form a confluent lining of cells aligned with the fibrin. In addition to the use of inkjet printer, extrusion-based bioprinting was utilized to fabricate vascular structures with agarose as a sacrificial material, and successful fabrication of vascular structures was also demonstrated using human umbilical vein smooth muscle cells and human skin fibroblasts [38]. The printed cells were aggregated into discrete units with ∼300–500 μm diameters. Furthermore, fusion of these units resulted in a vascular tube having multiple layers and branching geometry. In a recent report, 3D bioprinting was used to create vascularized, heterogeneous tissue constructs [58]. This highly scalable platform allows one to produce engineered tissue constructs in which vasculature and multiple cell types are programmably placed within extracellular matrices. These findings suggest that 3D microengineered environments may open new avenues for drug screening and fundamental studies of wound healing, angiogenesis and stem cell niches, as well as potentially leading to the rapid manufacturing of functional 3D tissues and organs.

Bioprinting has also been used to build musculoskeletal tissues, such as muscle and bone. Delivery of bone marrow stromal cells (BMSC) in Lutrol F127 and alginate hydrogels to regenerate bone and cartilage was previously attempted with an extrusion-based bioprinting system [40, 59]. These cells delivered through nozzles maintained cell viability and the surviving cells displayed osteogenic differentiation. Human multipotent stromal cells (MSC) and chondrocytes, encapsulated in alginate hydrogel, showed the formation of extracellular matrix. In another study, bone morphogenic protein-2 was printed (BMP-2) in 2D pattern on fibrin substrates [33]. Muscle-derived stem cells (MDSC) cultured on fibrin substrates with BMP-2 patterns showed osteogenic differentiation. This result showed the possibility of controlling multilineage stem cell differentiation with precise spatial deposition of cells and growth factors using bioprinting technology.

Extrusion-based bioprinter has been used to fabricate scaffold-free biological nerve grafts for regeneration of peripheral nerve injury [55]. The nerve graft consisting of marrow-derived stem

cells and Schwann cells was implanted into a rat model. This study showed that axons at the proximal stump reached the distal segment of the sciatic nerve in the printed nerve graft.

Another application where bioprinting technology has been utilized is the skin. In one study, biomimetic multilayered skin tissue consisting of human skin fibroblasts and keratinocytes was created using a jetting-based bioprinter [28]. Cells and collagen hydrogel were printed separately, and cell-containing collagen was cross-linked. This study showed the formation of dermal/epidermal-like distinctive layers by printing both fibroblasts and keratinocytes. Similarly, we have developed an *in situ* skin printer to deliver cells directly on the body for repairing extensive burn wounds [60]. Using this skin printer, we were successful in repairing full-thickness wounds of pigs by delivering keratinocytes and fibroblasts, and showed rapid re-epithelialization and accelerated wound healing.

CURRENT LIMITATIONS AND FUTURE *PERSPECTIVES*

Various bioprinting technologies have been developed and utilized for applications in life sciences, ranging from studying cellular mechanisms to constructing tissues and organs for implantation. These technologies have shown to safely deliver cells, biomaterials and biological molecules to target locations in a precise manner. Studies have shown that bioprinting simple tissue structures is possible; however, constructing a more complex and composite tissue structures such as solid organs remains a challenge. While printing fully functioning organs seems to be far fetched at the present time, these technologies show enormous potential and great promise to become an essential tool in the field of medicine in the future. To further develop and harness these technologies for clinical use, many of the technological challenges have to be addressed.

A common problem of the structures fabricated by the current bioprinting technologies is a lack of mechanical strength and integrity in the printed constructs due to the innate properties of hydrogels. The printed structures should have sufficient mechanical strength to maintain their shape and withstand external stress after implantation. Most hydrogels used in bioprinting systems possess low mechanical properties since bio-ink needs to maintain low viscosity to prevent clogging of the delivery nozzles. Thus, fabrication of clinically applicable bio-printed structures has been a challenge. Therefore, future development will need to focus on new biocompatible materials that could maintain structural integrity. Suitable hydrogel materials with appropriate mechanical properties, diffusion coefficient, biocompatibility and compatibility with the printing process are required for the long-term success of bioprinting technologies.

The complex structure of tissues and organs consists of multiple types of cells with a resolution in the micrometre scale and complex inner architecture. Although considerable research has been undertaken to enhance the fabrication resolution of the bioprinter, obstacles to fabrication of 3D structures with high resolution still remain as a challenge. Jetting-based bioprinting methods have been shown with a high resolution of 50 μm; however, this is not sufficient for fabrication of sizable 3D structures due to the low viscosity of hydrogel materials. Alternatively, extrusion-based bioprinting methods are more suitable for the fabrication of 3D structures as this method has shown the highest resolution of ∼100–300 μm. Regardless, fabrication of biomimetic and complex 3D structures with the actual size of inner architecture is not possible due to continuous filament materials for patterning. Therefore, the fabrication resolution of bioprinters needs to be further improved.

Vascularization of engineered tissue is one of the key issues that need to be fully addressed in order for engineered tissue to survive and be translated into the clinic. This is especially true of volumetric tissue constructs requiring adequate supply of oxygen and nutrients. Many investigators have demonstrated that neovascularization created in 2D structures can be used for cardiac patch. In contrast, 3D neo-vascularization still remains as a big obstacle to obtaining clinically relevant-sized tissue or organ [50, 58, 61]. Several researchers have applied the bioprinting technology to form vascularization; however, further development is necessary in order to fabricate fully vascularized tissue constructs for clinical use.

Fabrication time related to the speed of material printing is another limitation. Although an extrusion-based bioprinter has a relatively higher printing speed compared with a jetting-based bioprinter, shear stress generated between the inner surface of the nozzle and the cells induces cell damage [62, 63]. Because the conditions that increase printing speed cause increased shear stress, reduction of processing time for printing is limited and this issue must be addressed before bioprinting technology can be used clinically.

SUMMARY

Bioprinting technology has gained enormous attention as a fabrication methodology for producing 3D structures. Multiple cells, biomaterials and biological molecules can be printed simultaneously in defined spatial locations, yet many challenges remain for building complex tissues consisting of multiple cell types in a confined microarchitecture. More importantly, hydrogel development, resolution enhancement and vascularization are necessary to apply bioprinting technology clinically.

Conflict of interest: none declared.

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