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TERM

Current Advances in Three-Dimensional Tissue/Organ Printing

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Three-dimensional (3D) tissue/organ printing is a major aspect of recent innovation in the field of tissue engineering and regenerative medicine. 3D tissue/organ printing aims to create 3D living tissue/organ analogues, and have evolved along with advances in 3D printing techniques. A diverse range of computer-aided 3D printing techniques have been applied to dispose living cells together with biomaterials and supporting biochemical factors within pre-designed 3D tissue/organ analogues. Recent developments in printable biomaterials, such as decellularized extracellular matrix bio-inks have enabled improvements in the functionality of the resulting 3D tissue/organ analogues. Here, we provide an overview of the 3D printing techniques and biomaterials that have been used, including the development of 3D tissue/organ analogues. In addition, *in vitro* models are described, and future perspectives in 3D tissue/organ printing are identified.

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Key Words: 3D tissue/organ printing; Bio-ink; 3D tissue/organ analogue; *In vitro* tissue model; Tissue engineering and regenerative medicine

INTRODUCTION

In the field of tissue engineering and regenerative medicine, the application of three-dimensional (3D) printing technology has been believed to be a rational strategy to produce 3D biomaterial-based matrices (known as scaffolds), overcoming the inherent limitations of traditional techniques. During the additive process of 3D printing, thin layers are printed by adding desired materials selectively, and stacked to create complex 3D structures through layer-by-layer process. Direct or indirect 3D printing techniques have been shown to provide precise control over the size, external shape, internal architecture, and pore interconnectivity of 3D scaffolds formed of various biomaterials [1-6]. 3D printing also has been used to create 3D structures with the correct anatomical shape and size of defective part based on medical imaging data, such as magnetic resonance imaging (MRI) and computerized tomography (CT), using computer-aided design and computer-aided manufacturing systems [7-10].

Furthermore, technological advances in 3D printing have enabled recent innovation, named as 3D tissue/organ printing whereby living cells are included in the printing process itself with the aim of creating 3D living tissue/organ analogues [11-15]. The 3D printing techniques with automated computer-aided system, including dispensing, inkjet and stereolithography (SLA) techniques, have been applied to dispose various types of living cells, together with desired biomaterials and supporting biochemical factors within pre-designed 3D tissue/organ analogues. Therefore, 3D tissue/organ printing involves additional considerations of the cell types, biomaterials, supporting biochemical factors and printing strategies; these factors are directly related to the viability of the incorporated living cells and the functionality of printed 3D analogues.

3D tissue/organ printing begins with the design of 3D tissue/organ analogue. Medical imaging data of the defective parts, including anatomical and architectural information, can be used to guide the structural and biological design of 3D tissue/organ analogues to be printed. In addition, variations in the printing process and available biomaterials also affect the design of tissue/organ analogue. With the desired cell types, biomaterials including synthetic or natural polymers are selected to be used to form a framework materials or bio-inks containing living cells with or without supporting biochemical factors. The selected cell types and biomaterials are integrated with the 3D tissue/organ printing systems, such as dispensing, inkjet and SLA, and pre-designed 3D tissue/organ analogue starts to be printed. In

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the 3D tissue/organ printing, one of the most important challenges is to reproduce the complex micro-architecture and components of the extracellular matrix (ECM) with spatial control over multiple cell types in sufficient resolution to recapitulate biological functions of the target tissue or organ [15].

To date, 3D tissue/organ printing has demonstrated remarkable progress by creating 3D tissue/organ analogues with the competent functionality. In particular, recent development of bio-inks derived from decellularized ECM (dECM) has gained much attention as a new printable biomaterial that can accelerate the advance of 3D tissue/organ printing technology [16,17]. Here, we provide an overview of widely used 3D printing techniques and printable biomaterials. We then highlight representative applications, including the development of 3D tissue/organ analogues and *in vitro* models, and go on to identify future perspectives in 3D tissue/organ printng.

TECHNIQUES FOR 3D TISSUE/ORGAN PRINTING

Dispensing technique

Dispensing is the most common technique that has been applied to 3D tissue/organ printing. Dispensing involves direct extruding of the molten or dissolved biomaterials through micro-sized nozzle (Fig. 1A). Biomaterials that are commonly used in dispensing techniques should have the appropriate viscosity, high fidelity and formability to be dispensed [16,18,19]. The biomaterials also should have thermo-plasticity and thermo-stability if they are melted by heating to be dispensed [20,21].

Dispensing based 3D printing systems consist of temperature controller, dispenser and a three-axis (x-, y-, and z-axis of Cartesian coordinate) motion stage. Three-axis motion stages either feature gentry or stacked according to the motion way, and these stage types determine the whole appearance of the system. The three-axis motion stage controls the position of the nozzle through which biomaterials are extruded, and it should have accuracy and repeatability of less than±3 μm in all three axes to obtain sufficiently fine structures for applications to tissue/organ regeneration [22,23]. In addition, the systems with the multiple heads that controlled independently, have been used to facilitate independent control over the dispensing of multiple biomaterials [23,24].

The most common types of dispensing used with biomaterials are pneumatic and mechanical (piston or screw) dispensing systems [15,24,25]. Pneumatic dispensing systems have a simple drive-mechanism that uses air pressure to extrude the biomaterials through a nozzle [15]. This system also has the advantage of small quantities of discarded biomaterial remaining inside the syringe. However, it is relatively difficult to achieve accurate dispensing of very small and accurate quantities of biomaterials due to the residual air pressure following dispensing. Mechanical dispensing systems employ a piston or screw to dispense biomaterials, and can provide relatively accurate control over the dispensed volume, as well as enable the extrusion of highly viscous biomaterials. However, mechanical dispensing systems suffered from leakage of biomaterial through the clearance between the piston (or screw) and the syringe, especially with high-viscosity biomaterials [15,26].

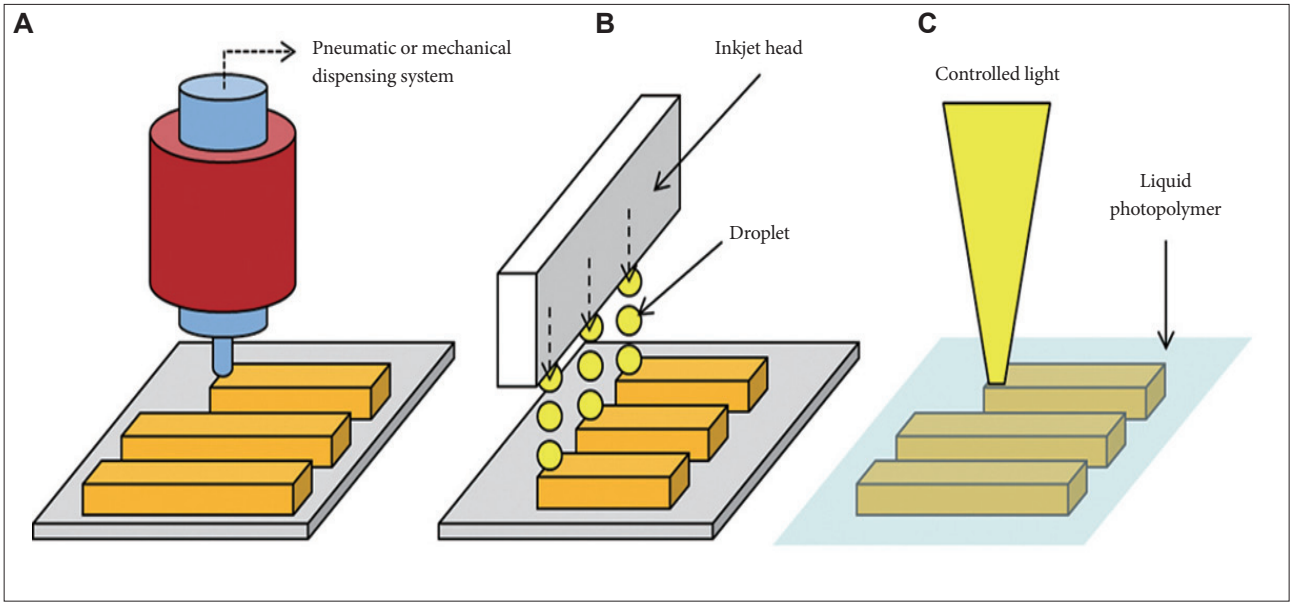

Figure 1. 3D printing techniques to create 3D tissue/organ analogues. (A) dispensing, (B) inkjet, and (C) stereolithography technique. 3D: three-dimensional.

Inkjet technique

Inkjet technique ejects bio-ink droplets from the inkjet-head nozzle so that it can achieve two-dimensional (2D) patterned arrays with a high spatial resolution (Fig. 1B). The volume of the droplet can be regulated to the single-cell level [27], which allows direct printing of cells with spatially well-organized patterns. In addition, the use of multiple inkjet-head in controllable arrays facilitates not only simultaneous printing of multiple bio-inks containing different cell types, but also the creation of the multi-tissue interface with spatial gradients in the cell composition [28]. 3D tissue/organ printing using inkjet technique includes various system setups according to the mechanism used to eject the bio-ink droplets such as thermal expansion, piezoelectric force, laser-induced forward transfer and pneumatic pressure [29-33].

Although this technique can achieve a spatial printing resolution up to 20−100 μm [26,34], this can only use low-viscosity bio-inks because the material in the inkjet-head cartridge must be in liquid form to enable droplet formation. As a result, the inkjet-printed tissue/organ analogues typically have poor mechanical properties cannot maintain their initial shape or withstand external stress after implantation [14]. Furthermore, long printing times caused by the small volume of droplets is another drawback with these techniques.

Stereolithography technique

SLA is the oldest 3D printing technique, and was developed in the 1980s. In general, it exhibits superior performance with higher spatial resolution and accuracy than other 3D printing techniques [35]. SLA uses an optic source, such as an ultraviolet (UV), together with a liquid photopolymer (known as a photo-curable resin) containing a light-activated initiator (photo-initiator), monomers and other additives. This technique creates 2D patterned layers with a well-defined thickness by the irradiation of a controlled light source to induce selective photo-polymerization, resulting in spatial solidification of a liquid photopolymer (Fig. 1C). A 3D structure is then built by stacking up solidified 2D patterned layers through a layer-by-layer process. This technique includes various system setups according to their building orientations; bottom-up and top-down setups, and approaches for 2D patterned layer solidification; beam scanning, image projection, and two-photon based setups. Among these setups, image projection based setups show significant decrease in the printing time, and two-photon based setups have superior accuracy and resolution compared with other system setups.

In the field of tissue engineering and regenerative medicine, micro-stereolithograhpy and nano-stereolithograhpy have been developed from SLA using specific optical systems to realize micro- and nano- scaled architectures, and have been applied to direct or indirect 3D printing of tissue engineering matrices without incorporating living cells [1,3-6,36]. The 3D tissue/organ printing based on SLA has also been enabled by the development of water-soluble photopolymers that are compatible with living cells, as well as visible light which does not damage to the cellular DNA [37-39]. However, SLA remains less well developed than inkjet and dispensing techniques and the printed analogues using SLA are so far only a few layers thick.

BIO-INKS FOR 3D TISSUE/ORGAN PRINTING

Living cells and biomolecules are encapsulated in printable biomaterials to print 3D tissue/organ analogues. This mixture is termed a 'bio-ink' [40]. In particular, hydrogels are widely used as printable biomaterials to form 3D tissue/organ analogues. This is because the composition and structure of hydrogels are similar to those of the native tissue micro-environment [41]. The most important role of bio-inks is to protect cells from the external environment during the printing process, and so they must be biocompatible [42]. Furthermore, the bio-ink enhances cellular functions, including proliferation, differentiation and maturation, by providing a tissue-mimicking micro-environment [15,43]. The rheological properties are relevant to the printability of bio-ink [44]. In particular, the viscosity should be carefully controlled to ensure viability of the cells and to provide the correct yield stress of the bio-ink, which is related to the shape fidelity of the printed analogues [45,46]. Following the printing of 3D analogues using bio-inks, they are then stabilized using thermal, ionic or chemical, or a combination of crosslinking methods [38,47].

Alginates are derived from algae, and consist of two repeating monosaccharides (i.e., L-guluronic and D-mannuronic acids) [48]. This materials form a hydrogel with multivalent cations, such as Ca^{2+}, Ba^{2+}, and Fe^{3+} [49]. Such biomaterials have been widely used for cartilage regeneration because the natural phenotype of chondrocytes can be maintained, which allows the de-differentiated cells that have been cultured in 2D culture plates to re-differentiate [50,51]. Moreover, they can be modified chemically for a variety of specific tissue engineering applications [52-54]. The most important characteristic of alginate as a bio-ink is that it can facilitate rapid gelation (within 1 s), which provides stability to the printed analogues [55]. These properties of alginates have motivated the development of alginate-based bio-inks, for applications in 3D-printed tissue/organ analogues.

Collagen is the most abundant protein in tissues, and has been widely used in biomedical applications [56]. The main

advantage of collagen in 3D printing of tissues is that it facilitates simple crosslinking via self-assembly in physiological conditions, and provides cellular recognition sites to promote cell adhesion and proliferation during and after the printing process [41,57].

Gelatin is a denatured protein by processing of the collagen. Gelatin is commonly used as gelling agent in foods, pharmaceuticals and cosmetic manufacturing [58]. There are abundant proteins in gelatin, including fibronectin, vimentin, vitronectin and RGD peptides, which are known for enhancing cell attachment [59]. The gelation property of gelatin can be simply modified by chemically conjugating methacrylate groups; therefore, it is popular in 3D tissue/organ printing applications. This chemically modified (methacrylated) gelatin forms a gel by UV light-mediated crosslinking [60]. Recently, many researchers have mixed various biocompatible materials for enhancing functionality of the gelatin [61-63]. For example, a mixture of methacrylated gelatin and poly(ethylene-glycol) is used as a tunable and cyto-compatible bio-ink with improved printability [61].

Fibrin can be formed by a mixture of fibrinogen and thrombin and degraded in the body by hydrolytic or enzymatic processes [64]. Fibrin plays important role in wound healing process and is widely used as surgical glue [65]. In tissue engineering field, fibrin is commonly used material for delivery of cells and drugs [66,67]. In addition, it can be applied for modifying chemical properties by conjugating to the other scaffold [68]. The major advantage of using fibrin as a bio-ink is that it can be applied for printing various types of cells without any safety issue. In particular, the freeform reversible embedding of suspended hydrogel method has been recently developed for printing soft, but complex shaped structures using fibrin bio-ink. Hinton et al. [69] printed fibrin construct into the fibrinogen-based medium and then the printed fibrin structure were removed from the bath material by incubation at 37°C after 1 hour printing process.

Hyaluronic acid (HA), which is a widely used anionic polysaccharide material in clinic, has been also applied as a printable bio-ink [70]. HA is an abundant ECM in cartilage tissues and has been used for the treatment of damaged joints or the arthritis [71]. To form a gel, HA commonly modified with photo-crosslinkable methacrylate groups which can be crosslinked by free radical polymerization when exposed to UV light [72].

Recent advances in dECM highlight the requirement for a biomaterial that can provide tissue-specific micro-environment [73,74]. It is well known that proteins in the ECM, such as collagens, proteoglycans and glycoproteins, can provide strength and space-filling functionality, bind to and release growth factors, regulate protein complexes, promote cell adhesion and participate in cellular signaling [15]. Recent advances in methods for tissue or organ decellularization have potential to provide an intact ECM, and various applications have demonstrated enhanced tissue regeneration, as well as the potential for using dECM as a cell-delivery platform in cell therapy [75]. In particular, it has been reported that dECM can be processed into an injectable hydrogel at physiological temperatures for injection *in vivo* [76], and printable tissue-specific dECM bio-ink has recently been developed, which has enabled further advances in 3D printing of tissues and organs [16,17].

APPLICATIONS

3D tissue/organ printing for tissue regeneration

3D printing based on extrusion technique has been widely applied with the aim of creating tissue/organ analogues for tissue regeneration. Shim et al. [57] initially proposed a hybrid type 3D structure consisting of a synthetic polymer and a natural hydrogel as a rational model of 3D tissue/organ analogue. They printed a blended poly ε-caprolactone (PCL) and poly (lactic-coglycolic acid) (PLGA) framework, and intentionally infused bio-inks, such as atelocollagen, HA and gelatin, into every second canal between the lines of a framework (Fig. 2A). In this manner, they could create a fully interconnected porous tissue/organ analogue that can avoid the depth limitations of mass transportation in the bio-ink region. In particular, cells in a printed analogue with atelocollagen bio-ink achieved a viability of up to 94.8±2.4% at day 10.

Shim et al. [24] then have developed a novel 3D tissue/organ printing system based on a dispensing technique, named the multi-head tissue/organ building system (MtoBS), which they used to create mechanically enhanced 3D tissue/organ analogues. The MtoBS uses six dispensing heads, which can be individually controlled. Of the six heads, two heads were connected to a heating system to melt thermoplastic polymers, such as PCL and PLGA, and the molten polymers were extruded by pneumatic pressure. The remnant four heads excluded a heating system to prevent bio-ink damage by temperatures greater than 37°C, and can extrude bio-inks containing living cells and/or biochemical factors. Control over the volume of bio-inks was achieved to within±1 μL using a plunger system.

They investigated the effects of various experimental conditions, including the nozzle size, pneumatic pressure and feed rate, on the line width, position and volume of both PCL and alginate bio-inks, and demonstrated 3D printing of heterogeneous tissue/organ analogue by printing 3D acellular osteochondral-shaped structure consisting of distinctive bone and cartilage regions using the MtoBS (Fig. 2B). In addition, an osteochondral analogue consisting of a PCL framework and two kinds of

alginate bio-inks (containing chondrocytes and osteoblasts, respectively) was successfully printed using the MtoBS with the high viabilities of cells (Fig. 2C).

Kundu et al. [77] also applied 3D tissue/organ printing technology to create a 3D analogue for cartilage tissue engineering. They used a printing process whereby alginate bio-inks containing chondrocytes and transforming growth factor-β (TGF-β) were dispensed without any adverse effect on the cell viability. They then successfully printed a fully interconnected 3D analogue composed of PCL and alginate bio-ink containing chondrocytes and TGF-β (Fig. 2D). The printed 3D analogues were implanted in the dorsal subcutaneous site of female nude mice for 4 weeks, and provided not only mechanical stability, but also significantly formed cartilaginous ECM by chondrocytes.

Lee et al. [78] demonstrated excellent performance in developed 3D tissue/organ printing technology that uses multiple printing heads to realize accurate anatomical composition of heterogeneous tissue. They developed a printing technique that uses a biocompatible sacrificial layer to allow the creation of complex shaped 3D tissue/organ analogues (Fig. 3A), and printed the geometrically and anatomically identifiable ear tissue analogue composed of cartilage and adipose tissue regions (Fig. 3B). The chondrocytes and adipocytes were encapsulated separately into alginate bio-inks, and these two bio-inks were directly localized at the physiologically relevant sites within the ear-shaped PCL framework. Interestingly, the printed analogue effectively exhibited cartilage and adipose tissue formation in different regions of the single structure, which was achieved by spatial control over the printing chondrocytes and adipocytes.

Park et al. [79] developed a pre-vascularized 3D tissue/organ analogue as a strategy to overcome the size limitation of tissue implants. They confirmed formation of hypoxic area in a

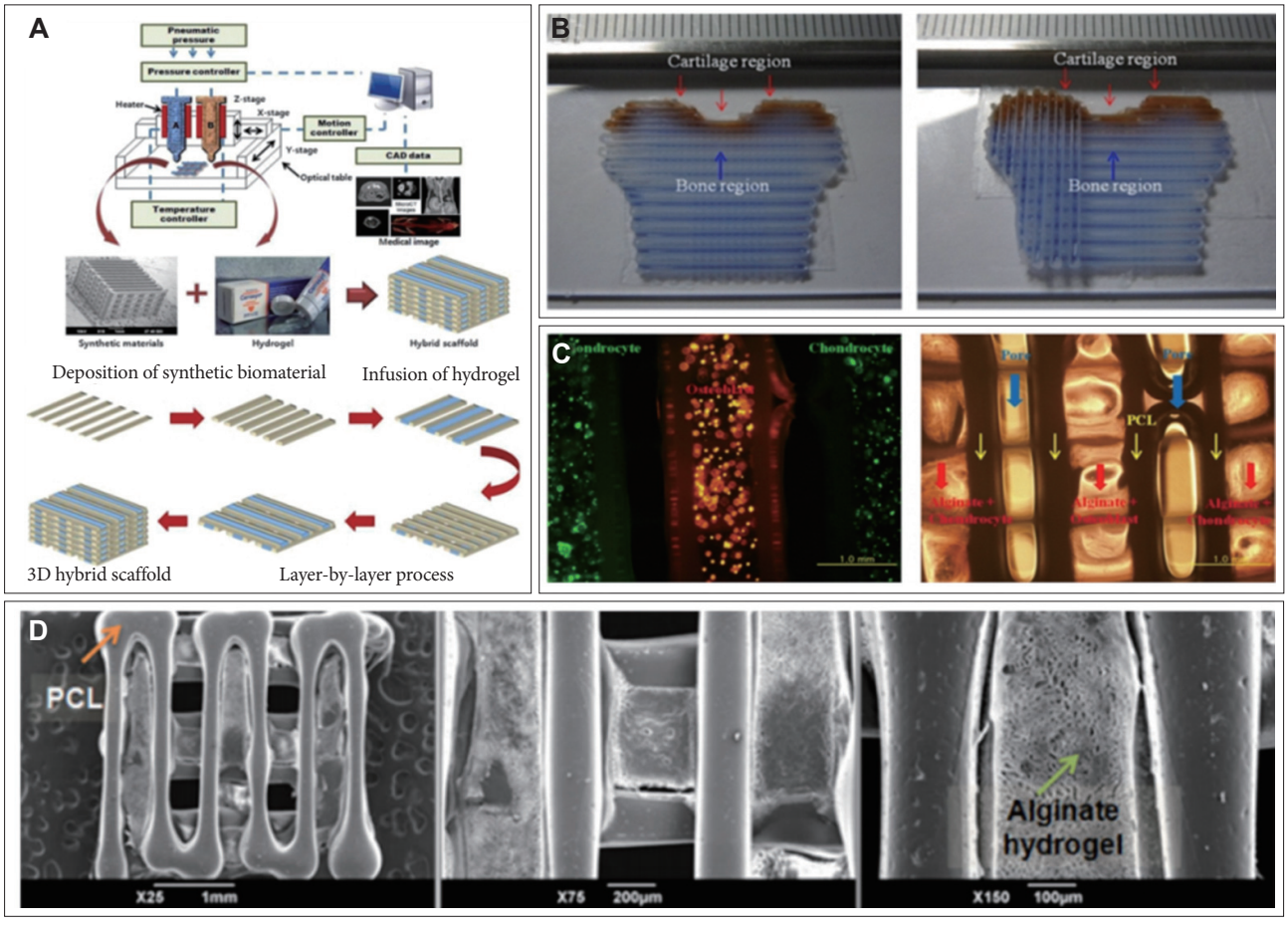

Figure 2. (A) The schematic diagram of the hybrid 3D structure as a model of 3D tissue/organ analogue and its printing process [57]. (B) A conceptual 3D osteochondral structure printed using PCL and two different alginates (cartilage and bone regions are filled with red stained alginate and blue strained alginate, respectively) [24]. (C) Images of dispensed chondrocytes and osteoblasts encapsulated in the alginate bio-inks [24]. (D) A fully interconnected 3D analogue composed of PCL and an alginate bio-ink containing chondrocytes and TGF-β, shown at magnifications of ×25, ×75, and ×150 [77]. 3D: three-dimensional, PCL: poly ε-caprolactone, TGF-β: transforming growth factor-β. Adapted from Shim et al. Biofabrication 2011;3:034102, with permission of IOP Publishing [57]. Adapted from Shim et al. J Micromech Microeng 2012;22:085014, with permission of IOP Publishing [24]. Adapted from Kundu et al. J Tissue Eng Regen Med 2015;9:1286-1297, with permission of Wiley [77].

large-volume structure by evaluating the cell survival rate, and they printed large-volume 3D bone analogue using two kinds of bio-inks; an alginate/gelatin mixture bio-ink containing mesenchymal dental pulp-derived stem cells (DPSCs) and vascular endothelial growth factor (VEGF), and a collagen bio-ink containing DPSCs and bone morphogenetic protein 2 (BMP-2) (Fig. 3C). An alginate/gelatin mixture bio-ink containing DPSCs and VEGF was used in the central zone, which formed the hypoxic area, so that VEGF could be released rapidly due to the rapid swelling of gelatin at physiological temperatures. In this manner, neo-vascularization was accelerated in the central zone following printing (Fig. 3D). A collagen bio-ink containing DPSCs and BMP-2 was used in the peripheral zone, except the central zone, to promote the formation of bone tissue. This strategy achieved sufficient blood vessel formation by early vasculogenesis, and thereby enhanced bone formation even in the

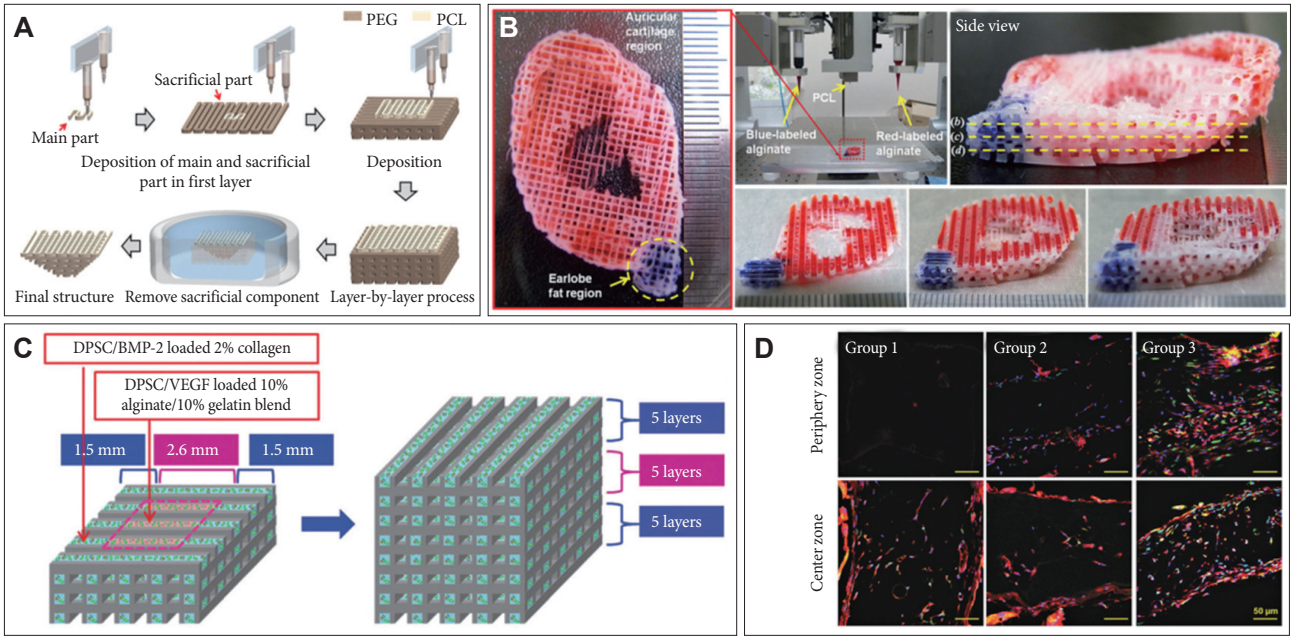

Figure 3. (A) schematic diagram of a 3D printing technique using a biocompatible sacrificial layer [78]. (B) An acellular ear tissue analogue composed of the cartilage and adipose tissue regions [78]. (C) 3D bone analogue using two kinds of bio-inks; a 10% alginate/10% gelatin mixture bio-ink containing DPSCs and VEGF, and a 2% collagen bio-ink containing DPSCs and BMP-2 [79]. (D) Vessel formation in a bone tissue analogue, showing positive staining with BSI-Lectin, VE-cadherin, and DAPI at the center (top) and periphery (bottom) zone of the printed structure [79]. 3D: three-dimensional, DPSCs: dental pulp-derived stem cells, VEGF: vascular endothelial growth factor, BMP-2: bone morphogenetic protein 2, PCL: poly ε-caprolactone. Adapted from Lee et al. Biofabrication 2014;6:024103, with permission of IOP Publishing [78]. Adapted from Park et al. J Mater Chem B 2015;3:5415-5425, with permission of RSC publishing [79].

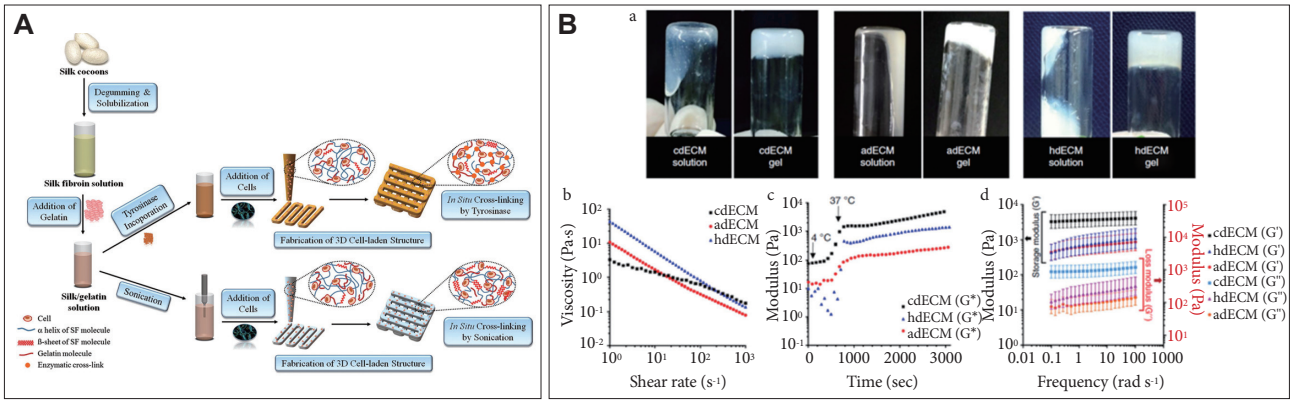

Figure 4. (A) Schematic diagram of the 3D tissue/organ printing using *in situ* cross-linkable SF-G bio-ink [80]. (B) The rheological behaviour of the decellularized extracellular matrix (dECM) bio-inks prepared from cartilage dECM (cdECM), heart dECM (hdECM) and adipose dECM (adECM) [16]. G*: complex modulus, G': storage modulus, G": loss modulus, 3D: three-dimensional, SF-G: silk fibroin-gelatin. Adapted from Das et al. Acta Biomater 2015;11:233-246, with permission of Elsevier [80]. Adapted from Pati et al. Nat Commun 2014;5:3935, with permission of Springer Nature [16].

core of the large-volume structure.

Meanwhile, recent developments in printable and functional bio-inks also have enabled improved capability of 3D tissue/organ printing technology. Das et al. [80] developed a directly printable bio-ink of SF-G, which can be cross-linked using physical means (i.e., conformational change of silk fibroin) as well as chemical means (i.e., the use of tyrosinases) (Fig. 4A). They investigated the optimal ratio of silk fibroin to gelatin for printing, and confirmed that the printed 3D structure using SF-G bio-inks containing human nasal inferior turbinate tissue-derived mesenchymal stromal cells exhibited superior cell viability and better multi-lineage differentiation than the alginate bio-ink that is a widely used for cell encapsulation and printing.

Pati et al. [16] recently developed tissue-specific dECM bio-inks based on cardiac, cartilage and adipose tissues. They prepared dECM-based printable pre-gel solutions by modifying the rheological properties. The processing temperature was below 15°C to facilitate printing, and gelation of the printed dECM construct occurred at temperatures above 37°C (Fig. 4B). They successfully printed 3D tissue/organ analogues using tissue-specific dECM bio-inks, and demonstrated the important feature that 3D tissue/organ analogues based on dECM bio-ink can induce superior differentiation of the encapsulated stem cells toward lineages (identical to that of tissue-specific dECM) compared with collagen and alginate bio-inks.

In vitro model

In recent years, there has been growing interest in the prospect of 3D tissue/organ printing for the development of *in vitro* tissue models for use in the screening of therapeutic compounds [81,82]. Carrying out fundamental investigations using 3D-printed tissues or organs instead of humans can provide more effective modes of prevention and therapeutic intervention [83]. Human-like animals have been developed, including transgenic mice with specific gene alterations; however, the results to date showed that the underlying metabolism differs significantly between these animals and humans [84]. Therefore, alternative models of human disease are urgently necessary.

Some researchers have developed alternative *in vitro* models using cells and biomaterials to mimic the tissue micro-environment [85]. These *in vitro* tissue models can be categorized into 2D and 3D models. The 3D *in vitro* tissue models provide better results compared to 2D cell culture and animal models because of the complex 3D structure, which enables a more accurate biophysical and biochemical micro-environment with respect to human tissues [86]. The most commonly used technique to create 3D *in vitro* tissue models is to encapsulate the cells in hydrogels, and cross-link them into the desired geometry [87-89]. The encapsulated cells are then polarized in the hydrogel, and exert forces on each other. Such models can reproduce spreading of cells in a matrix or clustering of cells in self-assembling cellular architectures known as organoids [90].

In particular, 3D tissue/organ printing technology can provide physiologically relevant 3D constructs by patterning cells, drugs or biochemical factors incorporated in bio-inks in a similar micro-environment to the body [16,91]. Printed organoids have been reported, and the 3D bio-printing company Organovo Holdings Inc. (San Diego, CA, USA) has begun to manufacture 3D-printed liver tissue models to screen drugs for investigating liver toxicity [92]. These biological features of printed *in vitro* tissue models have potential for the study of diseases as well as in investigations of the development of drugs and vaccines.

FUTURE PERSPECTIVES

Although research into 3D tissue/organ printing is at an early stage, remarkable potential has already been demonstrated by creating several 3D tissue-like structures that exhibit competent functionality. However, many technical challenges remain. In particular, 3D tissue/organ printing requires increased spatial resolution, faster printing speeds and better compatibility of relevant biomaterials.

Existing printing techniques have a limited range of biomaterials. In particular, the 3D printing approaches that can achieve high resolution, such as inkjet techniques, can only use bio-inks with a low viscosity, and it limits the mechanical strength of printed analogues, which cannot maintain their own shape and withstand external stress following implantation [14]. Reinforcement of 3D tissue/organ analogues using high-strength synthetic polymer is one strategy to improve the mechanical strength of 3D tissue/organ analogues; however, this is of limited value for soft tissue regeneration due to the mismatch of mechanical properties.

Current layer-by-layer process for 3D printing typically require long printing times, which scale with the number of biomaterials used. The 3D tissue/organ analogues cannot maintain cell viability during a prolonged printing process, and this imposes a limit on the scale and complexity of the printed analogues. Therefore, the printing speed must be increased to achieve high cell viabilities with large and complex structure. This limitation can be addressed by optimizing the printing paths of each printing component with minimal movement, as well as by developing new printing strategies that differ from layer-by-layer process. In addition, integration of different printing techniques for different biomaterials in a single printing system is a strategy that can improve the compatibility and flexibility of the relevant biomaterials in 3D tissue/organ printing process.

CONCLUSIONS

A major milestone in the field of tissue engineering and regenerative medicine is the development of effective 3D tissue/organ printing technology. There remain, however, a number of significant challenges for 3D tissue/organ printing, and further advance in the 3D printing techniques, as well as in the printable bio-ink materials, are required to realize the potentials of 3D tissue/organ printing.

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Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

REFERENCES

1. Sohn YS, Jung JW, Kim JY, Cho DW. Investigation of bi-pore scaffold based on the cell behaviors on 3D scaffold patterns. Tissue Eng Regen Med 2011;8:66-72.
2. Lee JS, Cha HD, Shim JH, Jung JW, Kim JY, Cho DW. Effect of pore architecture and stacking direction on mechanical properties of solid freeform fabrication-based scaffold for bone tissue engineering. J Biomed Mater Res A 2012;100:1846-1853.
3. Kang HW, Cho DW. Development of an indirect stereolithography technology for scaffold fabrication with a wide range of biomaterial selectivity. Tissue Eng Part C Methods 2012;18:719-729.
4. Park JH, Jung JW, Kang HW, Joo YH, Lee JS, Cho DW. Development of a 3D bellows tracheal graft: mechanical behavior analysis, fabrication and an in vivo feasibility study. Biofabrication 2012;4:035004.
5. Jung JW, Park JH, Hong JM, Kang HW, Cho DW. Octahedron pore architecture to enhance flexibility of nasal implant-shaped scaffold for rhinoplasty. Int J Precis Eng Manuf 2014;15:2611-2616.
6. Park JH, Hong JM, Ju YM, Jung JW, Kang HW, Lee SJ, et al. A novel tissue-engineered trachea with a mechanical behavior similar to native trachea. Biomaterials 2015;62:106-115.
7. Kang HW, Park JH, Kang TY, Seol YJ, Cho DW. Unit cell-based computer-aided manufacturing system for tissue engineering. Biofabrication 2012;4:015005.
8. Hutmacher DW, Sittinger M, Risbud MV. Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems. Trends Biotechnol 2004;22:354-362.
9. Seol YJ, Kang TY, Cho DW. Solid freeform fabrication technology applied to tissue engineering with various biomaterials. Soft Matter 2012;8:1730-1735.
10. Park JH, Jang J, Cho DW. Three-dimensional printed 3D structure for tissue engineering. Trans Korean Soc Mech Eng B 2014;38:817-829.
11. Mironov V, Reis N, Derby B. Review: bioprinting: a beginning. Tissue Eng 2006;12:631-634.
12. Billiet T, Vandenhaute M, Schelfhout J, Van Vlierberghe S, Dubruel P. A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. Biomaterials 2012;33:6020-6041.
13. Derby B. Printing and prototyping of tissues and scaffolds. Science 2012;338:921-926.
14. Seol YJ, Kang HW, Lee SJ, Atala A, Yoo JJ. Bioprinting technology and its applications. Eur J Cardiothorac Surg 2014;46:342-348.
15. Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol 2014;32:773-785.
16. Pati F, Jang J, Ha DH, Kim SW, Rhie JW, Shim JH, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun 2014;5:3935.
17. Pati F, Ha DH, Jang J, Han HH, Rhie JW, Cho DW. Biomimetic 3D tissue printing for soft tissue regeneration. Biomaterials 2015;62:164-175.
18. Smith CM, Christian JJ, Warren WL, Williams SK. Characterizing environmental factors that impact the viability of tissue-engineered constructs fabricated by a direct-write bioassembly tool. Tissue Eng 2007;13:373-383.
19. Billiet T, Gevaert E, De Schryver T, Cornelissen M, Dubruel P. The 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability. Biomaterials 2014;35:49-62.
20. Hoque ME, Chuan YL, Pashby I. Extrusion based rapid prototyping technique: an advanced platform for tissue engineering scaffold fabrication. Biopolymers 2012;97:83-93.
21. Shim JH, Kim JY, Park JK, Hahn SK, Rhie JW, Kang SW, et al. Effect of thermal degradation of SFF-based PLGA scaffolds fabricated using a multi-head deposition system followed by change of cell growth rate. J Biomater Sci Polym Ed 2010;21:1069-1080.
22. Leong KF, Cheah CM, Chua CK. Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. Biomaterials 2003;24:2363-2378.
23. Kim JY, Park EK, Kim SY, Shin JW, Cho DW. Fabrication of a SFF-based three-dimensional scaffold using a precision deposition system in tissue engineering. J Micromech Microeng 2008;18:055027.
24. Shim JH, Lee JS, Kim JY, Cho DW. Bioprinting of a mechanically enhanced three-dimensional dual cell-laden construct for osteochondral tissue engineering using a multi-head tissue/organ building system. J Micromech Microeng 2012;22:085014.
25. Khalil S, Nam J, Sun W. Multi-nozzle deposition for construction of 3D biopolymer tissue scaffolds. Rapid Prototyp J 2005;11:9-17.
26. Chang CC, Boland ED, Williams SK, Hoying JB. Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. J Biomed Mater Res B Appl Biomater 2011;98:160-170.
27. Barry RA, Shepherd RF, Hanson JN, Nuzzo RG, Wiltzius P, Lewis JA. Direct-write assembly of 3D hydrogel scaffolds for guided cell growth. Adv Mater 2009;21:2407-2410.
28. Seidi A, Ramalingam M, Elloumi-Hannachi I, Ostrovidov S, Khademhosseini A. Gradient biomaterials for soft-to-hard interface tissue engineering. Acta Biomater 2011;7:1441-1451.
29. Boland T, Xu T, Damon B, Cui X. Application of inkjet printing to tissue engineering. Biotechnol J 2006;1:910-917.
30. Le HP. Progress and trends in ink-jet printing technology. J Imaging Sci Technol 1998;42:49-62.
31. Odde DJ, Renn MJ. Laser-guided direct writing for applications in biotechnology. Trends Biotechnol 1999;17:385-389.
32. Lee W, Debasitis JC, Lee VK, Lee JH, Fischer K, Edminster K, et al. Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional freeform fabrication. Biomaterials 2009;30:1587-1595.
33. Nahmias Y, Schwartz RE, Verfaillie CM, Odde DJ. Laser-guided direct writing for three-dimensional tissue engineering. Biotechnol Bioeng 2005;92:129-136.
34. de Gans BJ, Schubert US. Inkjet printing of well-defined polymer dots and arrays. Langmuir 2004;20:7789-7793.
35. Melchels FP, Feijen J, Grijpma DW. A review on stereolithography and

its applications in biomedical engineering. Biomaterials 2010;31:6121-6130.
36. Joo YH, Park JH, Cho DW, Sun DI. Morphologic assessment of polycaprolactone scaffolds for tracheal transplantation in a rabbit model. Tissue Eng Regen Med 2013;10:65-70.
37. Lu Y, Mapili G, Suhali G, Chen S, Roy K. A digital micro-mirror device-based system for the microfabrication of complex, spatially patterned tissue engineering scaffolds. J Biomed Mater Res A 2006;77:396-405.
38. Dhariwala B, Hunt E, Boland T. Rapid prototyping of tissue-engineering constructs, using photopolymerizable hydrogels and stereolithography. Tissue Eng 2004;10:1316-1322.
39. Arcaute K, Mann BK, Wicker RB. Stereolithography of three-dimensional bioactive poly(ethylene glycol) constructs with encapsulated cells. Ann Biomed Eng 2006;34:1429-1441.
40. Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 2009;30:5910-5917.
41. Song BR, Yang SS, Jin H, Lee SH, Park DY, Lee JH, et al. Three dimensional plotted extracellular matrix scaffolds using a rapid prototyping for tissue engineering application. Tissue Eng Regen Med 2015;12:172-180.
42. Malda J, Visser J, Melchels FP, Jüngst T, Hennink WE, Dhert WJ, et al. 25th anniversary article: engineering hydrogels for biofabrication. Adv Mater 2013;25:5011-5028.
43. Guillotin B, Guillemot F. Cell patterning technologies for organotypic tissue fabrication. Trends Biotechnol 2011;29:183-190.
44. Macario DK, Entersz I, Abboud JP, Nackman GB. Inhibition of apoptosis prevents shear-induced detachment of endothelial cells. J Surg Res 2008;147:282-289.
45. Liu Tsang V, Chen AA, Cho LM, Jadin KD, Sah RL, DeLong S, et al. Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. FASEB J 2007;21:790-801.
46. Pescosolido L, Schuurman W, Malda J, Matricardi P, Alhaique F, Coviello T, et al. Hyaluronic acid and dextran-based semi-IPN hydrogels as biomaterials for bioprinting. Biomacromolecules 2011;12:1831-1838.
47. Zhang S. Fabrication of novel biomaterials through molecular self-assembly. Nat Biotechnol 2003;21:1171-1178.
48. Lee SH, Jo AR, Choi GP, Woo CH, Lee SJ, Kim BS, et al. Fabrication of 3D alginate scaffold with interconnected pores using wire-network molding technique. Tissue Eng Regen Med 2013;10:53-59.
49. Park KE, Kim YY, Ku SY, Baek SM, Huh Y, Kim YJ, et al. Effects of alginate hydrogels on in vitro maturation outcome of mouse preantral follicles. Tissue Eng Regen Med 2012;9:170-174.
50. Domm C, Schünke M, Christesen K, Kurz B. Redifferentiation of dedifferentiated bovine articular chondrocytes in alginate culture under low oxygen tension. Osteoarthritis Cartilage 2002;10:13-22.
51. Wei Y, Zeng W, Wan R, Wang J, Zhou Q, Qiu S, et al. Chondrogenic differentiation of induced pluripotent stem cells from osteoarthritic chondrocytes in alginate matrix. Eur Cell Mater 2012;23:1-12.
52. Laurienzo P, Malinconico M, Motta A, Vicinanza A. Synthesis and characterization of a novel alginate-poly(ethylene glycol) graft copolymer. Carbohydr Polym 2005;62:274-282.
53. Lee SH, Chung HY, Shin HI, Park DJ, Choi JH. Osteogenic activity of chitosan-based hybrid scaffold prepared by polyelectrolyte complex formation with alginate. Tissue Eng Regen Med 2014;11:106-112.
54. Hong JK, Yun J, Kim H, Kwon SM. Three-dimensional culture of mesenchymal stem cells. Tissue Eng Regen Med 2015;12:211-221.
55. Fedorovich NE, Alblas J, de Wijn JR, Hennink WE, Verbout AJ, Dhert WJ. Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. Tissue Eng 2007;13:1905-1925.
56. Kim S, Kim BS. Control of adult stem cell behavior with biomaterials. Tissue Eng Regen Med 2014;11:423-430.
57. Shim JH, Kim JY, Park M, Park J, Cho DW. Development of a hybrid scaffold with synthetic biomaterials and hydrogel using solid freeform fabrication technology. Biofabrication 2011;3:034102.
58. Ikada Y, Tabata Y. Protein release from gelatin matrices. Adv Drug Deliv Rev 1998;31:287-301.
59. Lee KY, Mooney DJ. Hydrogels for tissue engineering. Chem Rev 2001;101:1869-1879.
60. Schuurman W, Levett PA, Pot MW, van Weeren PR, Dhert WJ, Hutmacher DW, et al. Gelatin-methacrylamide hydrogels as potential biomaterials for fabrication of tissue-engineered cartilage constructs. Macromol Biosci 2013;13:551-561.
61. Rutz AL, Hyland KE, Jakus AE, Burghardt WR, Shah RN. A multimaterial bioink method for 3D printing tunable, cell-compatible hydrogels. Adv Mater 2015;27:1607-1614.
62. Yan Y, Wang X, Xiong Z, Liu H, Liu F, Lin F, et al. Direct construction of a three-dimensional structure with cells and hydrogel. J bioact compat polym 2005;20:259-269.
63. Xu W, Wang X, Yan Y, Zheng W, Xiong Z, Lin F, et al. Rapid prototyping three-dimensional cell/gelatin/fibrinogen constructs for medical regeneration. J bioact compat polym 2007;22:363-377.
64. Zhao H, Ma L, Zhou J, Mao Z, Gao C, Shen J. Fabrication and physical and biological properties of fibrin gel derived from human plasma. Biomed Mater 2008;3:015001.
65. Spotnitz WD. Commercial fibrin sealants in surgical care. Am J Surg 2001;182(2 Suppl):8S-14S.
66. Xiong Q, Hill KL, Li Q, Suntharalingam P, Mansoor A, Wang X, et al. A fibrin patch-based enhanced delivery of human embryonic stem cell-derived vascular cell transplantation in a porcine model of postinfarction left ventricular remodeling. Stem Cells 2011;29:367-375.
67. Whelan D, Caplice NM, Clover AJ. Fibrin as a delivery system in wound healing tissue engineering applications. J Control Release 2014;196:1-8.
68. Stabenfeldt SE, Gourley M, Krishnan L, Hoying JB, Barker TH. Engineering fibrin polymers through engagement of alternative polymerization mechanisms. Biomaterials 2012;33:535-544.
69. Hinton TJ, Jallerat Q, Palchesko RN, Park JH, Grodzicki MS, Shue HJ, et al. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. Sci Adv 2015;1:e1500758.
70. Gerecht S, Burdick JA, Ferreira LS, Townsend SA, Langer R, Vunjak-Novakovic G. Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. Proc Natl Acad Sci U S A 2007;104:11298-11303.
71. Collins MN, Birkinshaw C. Hyaluronic acid based scaffolds for tissue engineering--a review. Carbohydr Polym 2013;92:1262-1279.
72. Kim DH, Martin JT, Elliott DM, Smith LJ, Mauck RL. Phenotypic stability, matrix elaboration and functional maturation of nucleus pulposus cells encapsulated in photocrosslinkable hyaluronic acid hydrogels. Acta Biomater 2015;12:21-29.
73. Singh S, Afara IO, Tehrani AH, Oloyede A. Effect of decellularization on the load-bearing characteristics of articular cartilage matrix. Tissue Eng Regen Med 2015;12:294-305.
74. Chae SY, Chun SY, Park M, Jang YJ, Kim JR, Oh SH, et al. Development of renal extracellular matrix (ECM) scaffold for kidney regeneration. Tissue Eng Regen Med 2014;11:1-7.
75. Nguyen MM, Gianneschi NC, Christman KL. Developing injectable nanomaterials to repair the heart. Curr Opin Biotechnol 2015;34:225-231.
76. Seif-Naraghi SB, Singelyn JM, Salvatore MA, Osborn KG, Wang JJ, Sampat U, et al. Safety and efficacy of an injectable extracellular matrix hydrogel for treating myocardial infarction. Sci Transl Med 2013;5:173ra25.
77. Kundu J, Shim JH, Jang J, Kim SW, Cho DW. An additive manufacturing-based PCL-alginate-chondrocyte bioprinted scaffold for cartilage tissue engineering. J Tissue Eng Regen Med 2015;9:1286-1297.
78. Lee JS, Hong JM, Jung JW, Shim JH, Oh JH, Cho DW. 3D printing of composite tissue with complex shape applied to ear regeneration. Bio-

fabrication 2014;6:024103.
79. Park JY, Shim JH, Choi SA, Jang J, Kim M, Lee SH, et al. 3D printing technology to control BMP-2 and VEGF delivery spatially and temporally to promote large-volume bone regeneration. J Mater Chem B 2015; 3:5415-5425.
80. Das S, Pati F, Choi YJ, Rijal G, Shim JH, Kim SW, et al. Bioprintable, cell-laden silk fibroin-gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs. Acta Biomater 2015;11:233-246.
81. Chang R, Nam J, Sun W. Direct cell writing of 3D microorgan for in vitro pharmacokinetic model. Tissue Eng Part C Methods 2008;14:157-166.
82. Zhao Y, Yao R, Ouyang L, Ding H, Zhang T, Zhang K, et al. Three-dimensional printing of Hela cells for cervical tumor model in vitro. Biofabrication 2014;6:035001.
83. Benam KH, Dauth S, Hassell B, Herland A, Jain A, Jang KJ, et al. Engineered in vitro disease models. Annu Rev Pathol 2015;10:195-262.
84. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 2013;110:3507-3512.
85. Kimlin L, Kassis J, Virador V. 3D in vitro tissue models and their potential for drug screening. Expert Opin Drug Discov 2013;8:1455-1466.
86. Griffith LG, Swartz MA. Capturing complex 3D tissue physiology in vitro. Nat Rev Mol Cell Biol 2006;7:211-224.
87. Szot CS, Buchanan CF, Freeman JW, Rylander MN. 3D in vitro bioengineered tumors based on collagen I hydrogels. Biomaterials 2011;32: 7905-7912.
88. Elliott NT, Yuan F. A review of three-dimensional in vitro tissue models for drug discovery and transport studies. J Pharm Sci 2011;100:59-74.
89. Chwalek K, Tang-Schomer MD, Omenetto FG, Kaplan DL. In vitro bioengineered model of cortical brain tissue. Nat Protoc 2015;10:1362-1373.
90. Baptista PM, Siddiqui MM, Lozier G, Rodriguez SR, Atala A, Soker S. The use of whole organ decellularization for the generation of a vascularized liver organoid. Hepatology 2011;53:604-617.
91. Song HH, Park KM, Gerecht S. Hydrogels to model 3D in vitro microenvironment of tumor vascularization. Adv Drug Deliv Rev 2014;79-80: 19-29.
92. Vaidya M. Startups tout commercially 3D-printed tissue for drug screening. Nat Med 2015;21:2.