REVIEW ARTICLE

Recent cell printing systems for tissue engineering

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Abstract: Three-dimensional (3D) printing in tissue engineering has been studied for the bio mimicry of the structures of human tissues and organs. Now, it is being applied to 3D cell printing, which can position cells and biomaterials, such as growth factors, at desired positions in the 3D space. However, there are some challenges of 3D cell printing, such as cell damage during the printing process and the inability to produce a porous 3D shape owing to the embedding of cells in the hydrogel-based printing ink, which should be biocompatible, biodegradable, and non-toxic, etc. Therefore, researchers have been studying ways to balance or enhance the post-print cell viability and the print-ability of 3D cell printing technologies by accommodating several mechanical, electrical, and chemical based systems. In this mini-review, several common 3D cell printing methods and their modified applications are introduced for overcoming deficiencies of the cell printing process.

Keywords: bioink, cell-printing, tissue engineering

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1. Introduction

Since the stereolithographic 3D printer (SLA) was invented by Chuck Hull (the co-founder of 3D Systems Co.), 3D printing has been applied to various fields of industry, including tissue engineering application, namely, 3D bioprinting technique[1]. This technique involves printing bioink, which consisted of various biomaterials with and without live cells, in a layer-by-layer fabrication for human tissue regeneration[2–6]. One of the bioprinting processes, the cell printing system, which can position cells in a desired region, has been accomplished via numerous studies of 3D structure fabrication using natural and synthetic hydrogel polymers. Recently, W. Sun proposed computer-aided tissue engineering; the concept involves printing of 3D interconnected porous structures of anatomically modeled patient tissues and organs from CT or MRI image data[7]. Based on this concept, printing of artificial tissues, such as the ear, blood vessels, skin, bladder[8–12], and organs like the heart or liver will be expected soon.

The conventional 3D printing technology has printed porous tissue-engineered scaffolds with natural or synthetic polymers, which are biocompatible and biodegradable, and seeded cells on the designed structures. However, this technique has been quite passive owing to its dependence on the cell viability of the scaffolds, while the new 3D cell printing method can be more active by controlling the amount and position of various cell-types within the scaffolds. This process was well introduced in the work of Wilson and Boland[13]. They succeeded in printing bioinks that contained live cells instead of the conservative

printing materials at a size of 50 μm using an ink-jet printer. The printed cell-laden bioinks showed self-assembling characteristics between the cell-aggregates and formed tissue-like structures during culturing time. The results provided the basis for the fabrication of desired tissues or organs by printing and culturing cells at the required sites.

Owing to the strengths of 3D cell printing technology for tissue regeneration, many studies have been devoted to developing the technology using numerous trials and innovative methods. Primarily, they have been modified from conventional 3D-printing methods, and adapting them for cell culture. The 3D cell printing techniques are mainly classified into three techniques: (1) laser-assisted, (2) inkjet, and (3) extrusion cell printing[14–16]. However, unfortunately, the current cell printing processes have not successfully designed or fabricated 3D porous cell-laden structures (**Table 1**).

Table 1. Advantages and disadvantages of basic 3D cell printing techniques.

Techniques	Laser-assisted	Inkjet	Microextrusion
Advantages	Single cell manipulation Nozzle free Usage of high viscosity bioink High resolution High accuracy High gelation speed	High cell viability Noncontact nozzle Printed cell patterns using different cell types Multicell heterogeneous constructs High throughput High gelation speed	High mechanical properties Short fabrication time Printing of various types and viscosities of bioink Wide range of biocompatible materials
Disadvantages	Low mechanical properties Long fabrication time Damage cells due to heat-generated from laser energy Aggregate in the final tissue construct	Low mechanical and structural integrity Long fabrication time Low upper limit for viscosity of bioink Low reproducibility Cell aggregation Clogging of the nozzle orifice	Low cell viability due to nozzle wall shear stress and mechanical stress Low accuracy Cell death due to changes in dispensing pressure and bioink concentration
References	[18–21]	[23–27]	[29–33]

In this mini-review, we present the basic cell printing technologies and show several modified cell printing systems, which can overcome the limitations of the current cell printing processes, with a focus on a mechanically modified 3D cell printing process (**Table 2**). In addition, since, in many cases, modified cell printing systems are closely related to hydrogel-based bioinks, we mention various bioinks.

2. Basic Techniques of 3D Cell Printing

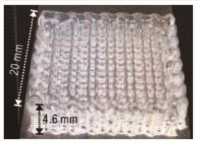
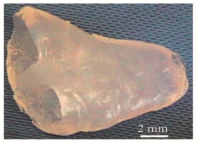
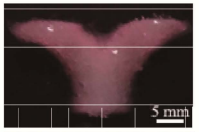
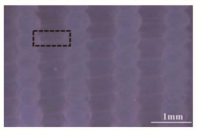
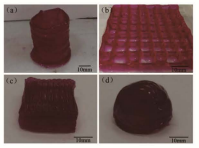
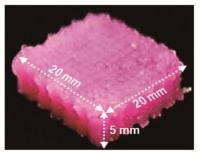
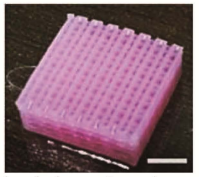
2.1 Laser-assisted 3D Cell Printing Technique

Laser-assisted cell printing is a 3D printing method to pattern and assemble bioinks by direct writing using laser. It has been rise to be an automated system that prints the cell-laden bioinks with a high resolution, accuracy, and precision[17]. As the lasers is beamed on the absorbing layer, the bioink is deposited in micro-sizes by controlling scanning mirrors and focusing lens in x and y-axis (**Figure 1a**)[18–21]. This nozzle-free fabrication prevents cell or material clogging often found in extrusion-based 3D cell printing techniques[16]. However, despite of these advantages, it is difficult to print macroscale 3D porous structures using laser-assisted cell printing. Owing to a relatively low flow rate, the vaporization of cell-laden bioink and possibility of cell contamination can significantly increase if a scaffold is built in larger scale. In addition, the potential cell damages caused by the thermal energy of the laser is another factor to be concerned[17,22]. Therefore, the integration of techniques, such as fast gelation of droplets or bio-papers, are actively attempted to overcome the existing limitations.

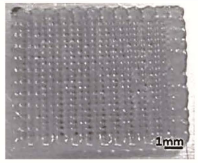
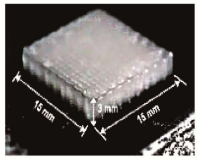
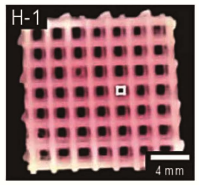
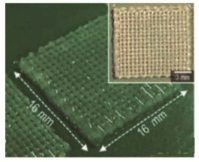
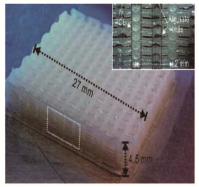
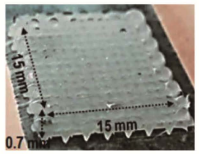
2.2 Inkjet 3D Cell Printing Technique

In the early generation of 3D cell printing, the inkjet cell printing technique was devised to print biomaterials in a 3D structure by remodeling the existing inkjet printers. Inkjet cell printers were designed to use three general methods: thermal, piezoelectric, and acoustic inkjet printers using heat, piezoelectric, and acoustic wave actuators, respectively, to dispense cell-embedded microdroplets (**Figure 1b**)[23–27]. This technique is widely used for its high cell viability and resolution in microscale structures. In addition, it is easily accessible and inexpensive. However, the inkjet printers can only use comparatively low viscosity materials with a low cell density. This is a critical drawback for a stable 3D cell printing process[25,27]. To overcome this problem, many approaches, such as developing a crosslinking method, are being studied and examined. Although inkjet 3D cell printing has

Table 2. Modified 3D cell printing processes

Modified cell printing	Methods		Bioink materials	Crosslinking conditions	Cell types	Cell viability	Geometry of printed structures	Pros and Cons	References
3D cell printing with modified crosslinking process	Aerosol crosslinking		3.5 wt% alginate + 0.5 wt% $CaCl_2$ mixture (7:3)	1. aerosol: 2~10 wt% $CaCl_2$ (fuming rate: 14.5 ml/min) 2. 2nd cross-linking: 1~3 wt% $CaCl_2$ (immersion for 1 min)	MC3T3-E1 (mouse pre-osteoblasts)	Compatible (~86%)		Enhancement of 3D printability and coherence between struts Use of high viscid bioink Possible cell damage by cross-slinking	[36–38]
	Sub-merged crossly-nking (d-rop-on-d emand)	Inkjet	2.3% alginate + 0.1% gelatin	0.25M $CaCl_2$	Mouse endothelial cells	Compatible		High 3D printability with low viscid bioink Low porosity, mechanical properties	[39,40]
		La-ser-assisted	2% (w/v) alginate	1~2 wt% $CaCl_2$	NIH 3T3 mouse fibroblasts	Low (63~71%)		High 3D printability with low viscid bioink Low porosity, mechanical properties, and cell viability	[41]
		Extrusion	2 wt% alginate	50~200 mM $CaCl_2$	ATDC5 mouse teratocarcinoma cells Primary chick chondrocytes	Compatible (76~84%)		Higher 3D printability (sideway porosity) Low coherence between layers Need for additional surface treatment	[42]
		Extrusion with core/shell nozzle	2-5% (w/v) alginate	2-5% (w/v) $CaCl_2$	L929 mouse fibroblasts	Compatible (67~93%)		Printability of micro-size hollow structure with use of core/shell nozzle Need of hysiological analysis of nutrient delivery	[44]
	Absorption crosslinking		3~5 wt% alginate	0.8~1.4 wt% $CaCl_2$	MC3T3-E1 (mouse pre-osteoblasts) Human adipose stem cells (hASCs)	High (92.3~93%)		High 3D printability and coherence of layers Simple modification of printing stage Need of core/shell nozzle (non-adaptable to inkjet/laser-assisted printing)	[46]
Temperature-controlled 3D cell printing	High-temperature stage		3 wt% dECM mixed with pepsin (10:1) in 0.5 M acetic acid solution	Incubation at 37 °C for 30 min	Human adipose-derived stem cells (hASCs) Human inferioir turbinate-tissue derived mesenchymal stromal cells (hTMSCs)	High (90~95%)	 (scale bar = 2 mm)	Capability of printing hASCs or hTMSCs with adequate cell viability and proliferation Enhanced structural maturation of myoblasts with dECM Further applications *in vivo* drug administrations are expected	[48]

Contiued

Modified cell printing	Methods	Bioink materials	Crosslinking conditions	Cell types	Cell viability	Geometry of printed structures	Pros and Cons	References
		10 wt% type I collagen dissolved in 0.05 M acetic acid (pH 3.2) + 10 enriched DMEM in deionized water (1:1)	-	Human primary skin cells (keratinocyte and fibroblast)	Compatible (~85%)		Outstanding biocompatibility and scaffold's structure mimicking native human skin Lower stiffness preventing from commercializing as an engineered skin substitute (ESS)	[49]
	Low-temperature stage	3 wt% alginate + 0.5 wt% $CaCl_2$ mixture (7:3)	0.5 wt% $CaCl_2$ (aerosol) for dispensing with tentative aerosol cross-linking method (DTCM)	Osteoblast-like cell (MG63) Mesenchymal stem cell (MSC)	Compatible (~84%)		Highly porous cell-laden alginate scaffolds with a thickness over 7 mm Homogeneous pore size and 100% pore interconnectivity Relatively low initial cell viability of MSCs (about 70%)	[50]
Electric field assisted 3D cell printing	Electrohydrodynamic jet (EHDJ)	2 wt% alginate + 2 wt% poly(ethylene oxide) + 0.7 wt% lecithin	5 wt% CaCl2 in phosphate buffer saline (PBS) (aerosol) 2.5 wt% CaCl2 in PBS (secondary crosslinking)	Osteoblast-like cells (MG63)	Compatible (~80%)		Homogenous cell distribution Improved mechanical properties Need on *in-vivo* test Need on osteogenic test	[51]
	Extrusion-based	4 wt% alginate + 0.5 wt% $CaCl^2$ mixture (7:3)	5% (w/v) $CaCl_2$ in tris-buffered saline (TBS) (aerosol) 2% (w/v) $CaCl_2$ (secondary crosslinking)	Osteoblast-like cells (MG63) Human adipose stem cells (hASCs)	Compatible (~87%)		Enhanced printing stability and resolution Increased coherence between layers Potential cell damages by electric field (voltage limitation: 2 kV)	[52]
Hybrid 3D cell printing	Conventional 3D printing (melt-plotting) + 3D cell printing (extrusion/ aerosol crosslinking)	PCL (M_W 70,000-90,000) for melt-plotting 4 wt% alginate, alginate and $CaCl_2$, collagen/ BMP-2, or gelatin/alginate mixture for cell printing	$CaCl_2$ (100mM) + NaCl (145mM) solution 2.5 wt% $CaCl_2$ (aerosol crosslinking) 2 wt% $CaCl_2$ solution (post-print crosslinking)	Human chondrocytes Osteoblast-like cells (MG63) MC3T3-E1 (mouse pre-osteoblasts) Mesenchymal dental pulp derived stem cells (DPSCs)	High (85~99%)		Highly improvement of 3D printability and mechanical properties with high cell viability Need of use of non-natural material and dual-nozzle Potential cell damages by melted polymer while printing	[53,54]
	Conventional 3D printing (melt-plotting, electrospinning) + 3D cell printing (extrusion)	10% of PCL (M_W 90,000) in MC/DMF solution for electrospinning PCL (M_W 45,000) for melt-plotting 3 wt% alginate and 2 wt% PEO (M_W 90,000) in PBS were mixed with 0.05% $CaCl_2$ at a 7:3 ratio	–	C2C12 myoblasts	High (94~96%)		Enhanced formation of myotubes evaluated by MHC expression and the expression levels of various myogenic genes Complicating process to build multi-layer structures (3 methods in one printing process)	[56]

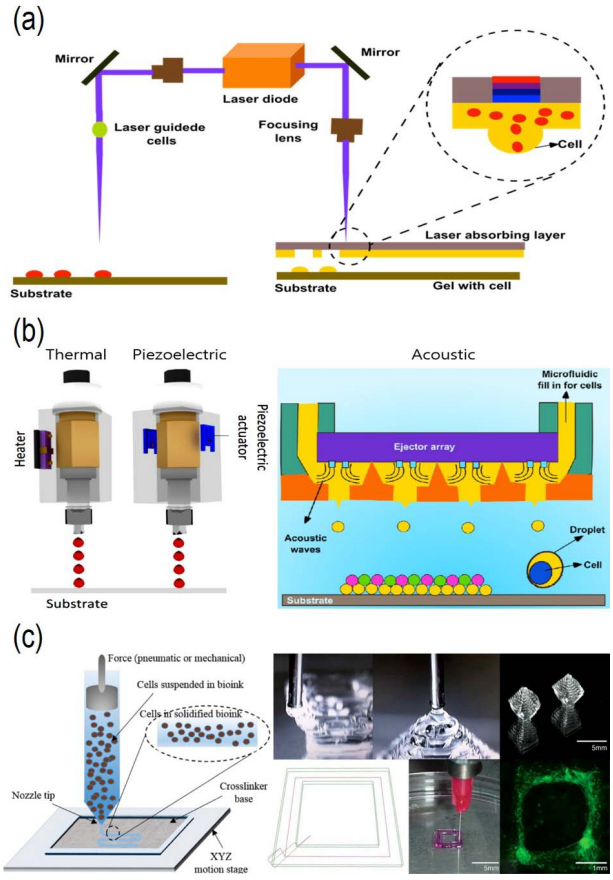


Figure 1. Basic techniques of 3D cell printing, (a) laser-assisted 3D cell printing techniques with and without an absorbing layer,[17,22] (b) thermal, piezoelectric, and acoustic inkjet 3D cell printing systems,[22,28] and (c) microextrusion 3D cell printing systems and products[14,35].

unsolved issues, it is expected to be a versatile tool in broad tissue engineering application[22,28].

2.3 Microextrusion-based Cell Printing

Cell-embedded 3D printing with microextrusion includes a dispensing system that uses pneumatic or mechanical forces to extrude bioink in a line (**Figure 1c**)[29–33]. It is one of the most common cell printing methods owing to its accessibility and versatility in printing 3D structures. Microextrusion can be performed using various bioinks with a broad property range, and especially the viscosity of the bioink in microextrusion is usually much higher than in other 3D cell printing methods. This allows for the fabrication of a complicated 3D structure. Another main advantage of the microextrusion process is its capacity for loading cells at a high density. Using dense cells in the 3D structure can be more effective in the formation of engineered tissues. However, this process also has limitations, such as a relatively low printing resolution owing to the microsized extruding nozzle and comparatively low cell viability caused by severe wall shear stresses within the nozzle using viscous bioink. Therefore, researchers using microextrusion-printing systems are striving for an advanced microextrusion printing technology that creates a precise print with a high cell viability[14,16,34,35].

3. Modified Cell Printing Processes

3.1 3D Cell Printing with Modified Crosslinking Processes

The 3D cell printing process with natural-polymer-based bioink usually contains a crosslinking process owing to low mechanical properties or low viscosity of the bioink. In this section, a few applications of modified crosslinking processes during printing are introduced.

In recent, Ahn *et al.*[36–38] developed a modified 3D cell printing technology with an aerosol crosslinking process (**Figure 2a**) that finely sprayed the crosslinked solution creating a coagulation of the bioink to fabricate the desired form and structure. They reported that the fabrication of a 3D cell-laden porous mesh structure using an alginate bioink can produce adequate cell growth, and it was successfully achieved by spraying aerosols of calcium chloride ($CaCl_2$) solution during the printing process. Spraying the aerosol cross-linked solution induced a high printability of the bioink owing to the hardening of the structure surfaces during the crosslinking process and increased the coherence between the printed cell-laden struts. Throughout the process, the amount and position of the cells were controlled within the scaffold.

The submerged-in-crosslinker cell printing process, referred to as drop-on-demand printing, has been applied to the inkjet[39,40], laser-assisted[41], and extrusion-based[42,43] cell printing processes to build 3D structures with relatively low-viscosity bioinks. Xu *et al.*[39] and Boland *et al.*[40] built the drop-on-demand printing apparatus shown in **Figure 2b**, which uses a layer-by-layer-sinking plate in the crosslinker-filled chamber, and the alginate-based bioink was printed on the surface of the crosslinking liquid. Through their modified method, they overcame one of the limitations of the inkjet printing process, the low 3D printability, and fabricated a 3D structure with a height of approximately 12 mm[40]. In 2015, Xiong *et al.*[41]

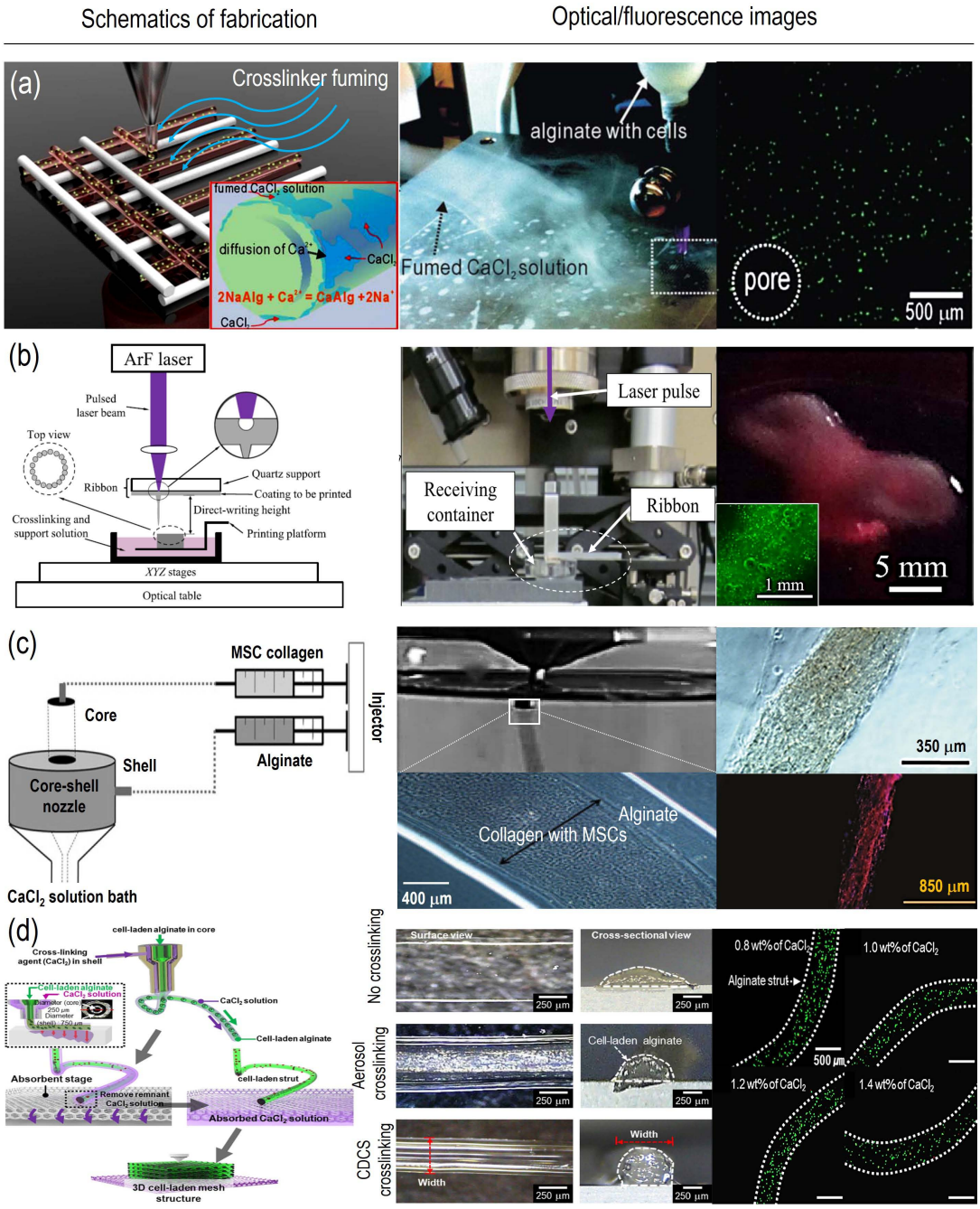


Figure 2. 3D cell printing with modified crosslinking processes, (a) aerosol crosslinking process with calcium chloride using an alginate-based bioink[36–38], (b) drop-on-demand (submerged) crosslinking with a laser-assisted printing process[41], (c) submerged printing with a core (MSC-laden collagen) /shell (2–5 wt% alginate) nozzle[44], and (d) cell printing process with a crosslinked solution and absorbing stage using a core (3 wt% alginate-based cell-laden bioink)/shell (1.2 wt% $CaCl_2$)[46].

applied a similar method to the laser-assisted cell printing process, which contains the same limitations in printing 3D structures, and they were able to fabricate a 3D structure with a height of 9.5 mm. Conversely, You *et al.*[42] fabricated a 3D lattice structure with cell-laden alginate hydrogel via an extrusion-based cell printing process with submerged crosslinking. They coated the surface of a printing plate, instead of using a lifting stage, and printed the bioink in a $CaCl_2$ solution to build a biaxially porous 3D scaffold, which created pores between the deposited layers. Gao *et al.*[43] modified the submerged crosslinking cell print-

ing process using a core/shell nozzle. They extruded the crosslinked solution through the core and the bioink through the shell to create a hollow tube-shaped 3D structure. By applying the drop-on-demand printing method, they were also able to fabricate various 3D cell-laden structures.

For applications in extrusion-based cell printing, a dual or core/shell nozzle is occasionally used as an alternative crosslinking method, as in the study described above[43–46]. The core/shell fibrous collagen–alginate hydrogel was proposed by Perez *et al.*[44] (**Figure 2c**). They placed mesenchymal stem cells (MSCs) into the inner cell-collagen encapsulated with a 2 ~ 5 wt% alginate (the outer portion). The collagen–alginate hydrogel was extruded into a bath filled with a 50-mM $CaCl_2$ solution, and the outer alginate contacted the $CaCl_2$ solution for 5 min and crosslinked. Using this process, sufficient stability of the collagen–alginate hydrogel was maintained and represented by storage moduli as 30 kPa, 40 kPa, and 50 kPa at 2 wt%, 3 wt%, and 5 wt% alginate, respectively. The cell viability was approximately 70 to 80% for the collagen–alginate (3 wt%) sample and pure collagen sample. In addition, Ahn *et al.*[46] developed a simple and innovative cell printing method using a core/shell nozzle and an absorbing printing stage (**Figure 2d**). In their process, the alginate-based bioink was extruded through the core nozzle, and the $CaCl_2$ solution was extruded through the shell nozzle to crosslink the printed bioink simultaneously. The crosslinking solution then immediately absorbed into the absorbing stage to prevent the crosslinked solution from ruining the 3D shape of the alginate struts. On a non-absorbing stage, the crosslinked structure can collapse during printing owing to the weakened coherence between struts by the remaining crosslinked material. The surfaces of the struts can be constantly indurated, and the stability of the scaffold increases through the continuous crosslinking of the previously printed layers. This method formed a 3D structure easy and more consistently than the submerged crosslinking technique, since the submerged process contained a high possibility of the bioink floating in the crosslinking solution during the printing process and required additional treatment, such as polyethylenimine (PEI) surface coating[42,47] or a layer-by-layer interactively moving stage[39–41,43].

3.2 Temperature-controlled 3D Cell Printing Process

For the scaffold printed with formless materials, the temperature was controlled to enhance printability in the 3D structure while the damage to cells was minimized. The rheological property of dECM (decellularized extracellular matrix) bioink was controlled by increasing the temperature to construct a 3D structure (**Figure 3a**)[48]. As the temperature increased beyond 15°C, the storage modulus was increased, and a crosslinked gel was observed at 37°C. In this process, increasing temperature is prerequisite to retain 3D structure, which subsequently makes storage modulus greater than loss modulus at the certain temperature. A high cell viability (> 90%) was maintained over 14 days of culture for the *in vitro* and *in vivo* tests. Furthermore, Yoon *et al.*[49] varied temperature for optimizing the fabrication of a collagen scaffold. In this study, the stage containing a circulating pump, water chamber, and temperature controller was used to maintain the cell-printing plate from 25°C to 60°C. The collagen struts were adequately fabricated between 36°C to 39°C with a cell viability of 85%. Conversely, the strut formation was rather amorphous and not applicable below 35°C or over 42°C, with a significant decrease in cell viability. This phenomenon suggests that the temperature and collagen gelation/crosslinking are correlated, and controlling the temperature allows the 3D structure to be formed by rapid gelation of the bioink. However, the printed collagen scaffold lacks sufficient strength and stiffness (0.01 ± 0.001 kPa of Young's modulus); therefore, further exploration of a non-toxic chemical reagent or crosslinking process is required.

Low-temperature cell printing is a printing method that plots struts by instantly freezing the bioink extruded from the nozzle (**Figure 3b**). The conventional 3D cell printing has revealed the conversion of dispensed nearby struts, which eventually disturbs the layer-by-layer stacking process. To overcome this problem, Ahn *et al.*[50] applied a low temperature from −2°C to−40 °C to fabricate the biaxially porous 3D lattice scaffold in solid structure. Throughout the cell printing process, the alginate bioink with cells was maintained at 4°C to minimize the cell damage by a rapid decrease in temperature. As the temperature was close to 0°C, the cell viability increased up to 84%, but the shaping ability decreased. Conversely, as the temperature decreased to −40°C, the cell viability dropped below 10%, but the shaping ability was enhanced with high fabricating efficiency of 85%. The scaffolds were printed at 487°C with the reasonable initial cell viability (70~84%) and high

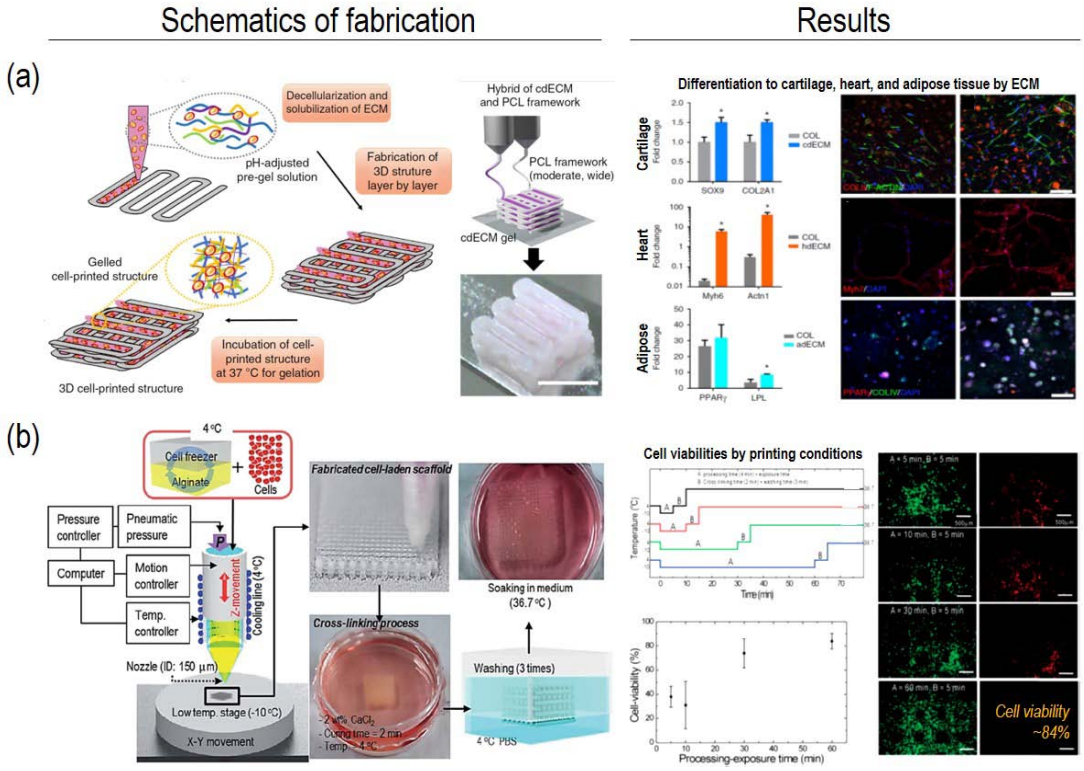


Figure 3. Temperature-controlled 3D cell printing process, (a) increasing temperature-controlled (from 4 to 37°C) printing using ECM-based bioinks [48, 49] and (b) low-temperature (−10°C) cell printing process [50].

shaping ability. It also revealed the successful fabrication of multi-layered scaffold with significantly enhanced mechanical properties (10 ± 2.2 MPa of Young's modulus). For further development, initial cell viability can be improved, and various types of bioink can be used for low-temperature cell printing.

3.3 Electric-field Assisted 3D Cell Printing

Recently, the application of an electric field in cell printing was proposed. Yeo *et al.*[51] combined electric-filed assisted 3D cell printing and aerosol cross-slinking process to fabricate a 3D hybrid cell-laden scaffold. The osteoblast-like cell-laden fibers were deposited with 0.16 kV on 3D lattice PCL struts (**Figure 4a**). The initial cell viability was reasonable (above 80%), and the cells could proliferate for prolonged culture period. The fibers maintained their shape without dispersion on the hybrid scaffold with a significant increase in tensile modulus (4.9 ± 0.6 MPa) compared to alginate mat. Also, Yeo *et al.*[51] applied an electric field to the extrusion-based cell p rinting that pneumatically printed alginate-based bioink with human adipose stem cells with the electrical field (**Figure 4b**). This reduced the wall shear stress in the nozzle and reduced the damage of the cells in the printed bioink[52]. Moreover, the electric field enhanced the printing stability and resolution of the dispensed struts since the electric force pulled down the bioink and resulted in an increase in the coherence between the layers and a decreased strut size. However, there was potential cell damage when the high electric field was used, and they reported that the limitation of the applied voltage with their experimental conditions was less than 2 kV.

3.4 Hybrid Systems for Mechanically Stable 3D Cell-laden Structures

As the 3D cell printing was derived from the conventional 3D printing technology, some researchers have tried to apply the conventional 3D printing methods to the 3D cell printing process. Several papers reported that the melt-plotting method, one of the most common methods among non-cell printing processes, was combined with the cell printing techniques to fabricate and strengthen a ce ll-laden 3D structure by providing a firm frame or support for the soft cell-laden bioinks[48,53–56]. In 2012, Shim *et al.*[53] used the melt-plotting method with a s ynthetic polymer, poly (ε-

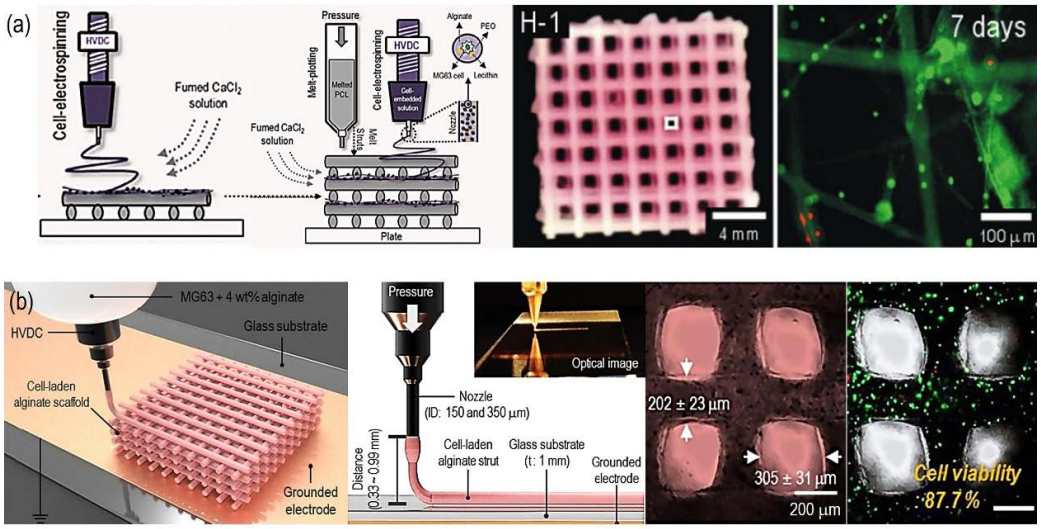


Figure 4. (a) Electrically operated cell printing modification supplemented with the aerosol crosslinking process[51] and (b) extrusion-based cell printing with an electric field (1 to 3 kV in 0.33 to 0.99 mm)[52].

caprolactone) (PCL), to fabricate a frame wall for hydrogel fillings extruded with air pressure between the synthetic polymer walls (**Figure 5a**). Their method enabled the printing of multi-type cells on the desired locations in the 3D spaces. However, the fabricated structures showed necrosis of encapsulated cells in the center of the hydrogel owing to the lack of pores.

In an attempt to overcome this limitation, Lee *et al.*[54] suggested a hybrid 3D cell printing method combined with a crosslinking aerosol process and melt-plotting method to fabricate a highly porous 3D cell-laden structure (**Figure 5b**). This hybrid scaffold was fabricated by printing bioink between the synthetic polymer struts to overcome the low mechanical properties of the struts owing to the low viscosity of the bioink. This printing system contained a high coherence between the strut layers since the two different types of struts had the same diameter and interval. In addition, because of the release of cells inside the bioink struts, the structure contained a uniform cell distribution and a good supply of nutrients to the cells, and it secured the cell transfer, which is important for cell growth. Moreover, hybrid fabrication of the synthetic polymer with high mechanical properties and

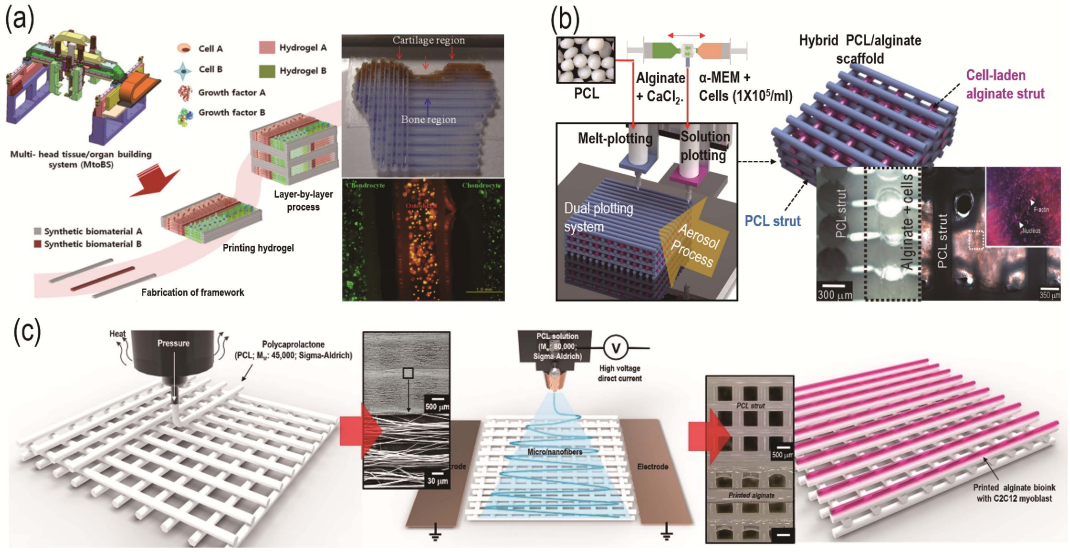


Figure 5. Hybrid modifications of the 3D cell printing process with (a, b) a multi-nozzle system using natural and synthetic polymers ((a) Shim *et al.*[53], (b) Lee *et al.*[54]) and (c) an additional electrospinning process for surface alignment (Yeo *et al.*[56]).

cell-laden bioink enables the production of accurate 3D shapes and can be applied to the regeneration of tissues demanding high mechanical strength because the enhanced mechanical properties and high cellular activity of the structure. Most importantly, a 3D structure like the natural tissue structure can be designed by placing bioink with different cells on each layer of the structure. However, the cells that contacted the PCL struts showed dramatically low viability owing to the hot temperature of the melted PCL during printing. This remains an issue to overcome.

In an additional hybrid application, Yeo *et al*.[22,57] developed a hybrid fabrication of a hierarchical scaffold using an electrospinning method to align fine PCL nano-fibers on the micro-sized PCL struts created from a melt-plotting process as shown in (**Figure 5c**)[56]. Then, they printed alginate-based bioink with myoblasts on the electrospun fibers to examine the alignment and stretching of the myoblast cells. Using this method, they successfully fabricated muscle mimetic scaffold with sufficient mechanical properties. In addition, the myoblasts in the scaffold were well aligned on the nano-fibers as the aspect ratio of F-actin with aligned fibers was over 2 folds of the aspect ratio with random fibers or without fibers, which indicates the elongation of the cell in one direction. These results showed that the fabricated scaffold was suitable and applicable for the regeneration of muscle tissues.

4. Bioink

4.1 Definition of Bioink

In cell printing, hydrogels made of natural and synthetic polymer materials are mainly used in the fabrication of a 3D cell structure. The bioink is defined as a mixture of hydrogel and live cells and is the most important requisite for the successful production of an artificial tissue. The bioink requires several characteristics: (1) 3D printability with uniform viscosity, (2) physical and chemical crosslink ability that enables 3D shape maintenance after printing, (3) cyto-compatibility that supports favorable cell viability and assists cell proliferation and differentiation, and (4) biodegradability after transplantation into a host for the emission of decomposed wastes[58,59]. Currently, the most widely used bioink materials in cell printing are alginate, collagen, hyaluronic acid, gelatin, pluronic F127, polyethylene glycol dimethacrylate[60–62], etc.

4.2 Bioink Viscosity and Crosslink Ability

One of the important variables in producing bioinks with the materials above is viscosity. Viscosity is defined by the concentration of the materials, and the printability and print resolution can be enhanced as the viscosity of the bioink increases. However, it is reported that cell viability can decrease from the severe nozzle wall shear stress generated in a narrow nozzle by high pressure, and this is required to print high-viscosity bioink[63]. In addition, it is known that the ability to crosslink effects the strength and stiffness of the scaffolds and the oxygen and nutrient supply for the cells. However, excessive cross-linking can reduce the cell viability and disturb the formation of new tissue[36]. Therefore, it is essential to develop a biodynamic bioink with appropriate viscosity and crosslink-ability that can be printed in a 3D layered structure with sufficient printing resolution.

4.3 Applications of Bioink in 3D Cell Printing Techniques

The studies of bioink focus on the process conditions to control the viscosity and the bioink's ability to crosslink depending on the compositions of the materials. The printability in 3D printing is an important factor as well as good biocompatibility that promotes and maintains high initial cell viability over 90%, cell proliferation, and differentiation. Therefore, investigations in bi oink composed of different hydrogels have been actively performed and applied to regenerate various cells with 3D cell printing techniques (**Table 3**). For laser assisted 3D cell printing and inkjet 3D cell printing, fibrin bioink is widely used because of its degradability, enhancement of cellular activities, and, most importantly, high printability by fast-gelling and tunable viscosity[64]. In addition, the fibrin bioink can be easily obtained from blood by purification and provides binding affinities that help initial cell attachment[64,65]. For micro-extrusion based 3D cell printing, alginate is the most widely used material because it is inexpensive, and its viscosity can be easily controlled[17,44,46]. In addition, it contains excellent biocompatibility, low toxicity, and a stable 3D structure by simply mixing with the carboxyl of L-guluronic acid in a ca lcium ion solution[66,67]. De spite those advantages, alginate itself lacks bioactive factors inducing cell attachment or activities. There-

fore, collagen bioink has been investigated to improve the 3D cell printing process. To date, the development of collagen bioink has been hindered because of its unstable 3D structure and low process ability; however, modified 3D cell printing techniques, such as aerosol system and crosslinking reagents, are being actively investigated to apply collagen bioink into the 3D cell printing process.

Table 3. Studies and endeavors for the development of bioink in 3D cell printing

Techniques	Materials	Cell types	Crosslinking reagents	References
Laser-assisted 3D cell printing	Fibrin	Endothelial cell/Mesenchymal stem cell	-	[68]
		Smooth muscle cell	-	[69]
Inkjet 3D cell printing	Fibrin	Muscle-derived stem cell	-	[65]
		Mesenchymal fibroblast/Myoblast	-	[70]
		Neuronal precursor cell/Cortical cell	Proteolytic	[64]
		Neural stem cells	-	[71]
	Collagen	Epidermal keratinocyte/Dermal fibro blast	Sodium bicarbonate	[72]
Microextrusion based 3D cell printing	Hyaluronic acid	Aortic valve interstitial cell	Methacrylated gelatin	[73]
	Gelatin/Alginate	Aortic root sinus smooth muscle cell	$CaCl_2$	[74]
	RGD-modified alginate	Cardiomyocyte progenitor cell	$CaCl_2$	[75]
	Alginate/PEO	Myoblast	$CaCl_2$	[56]
		Osteoblast-like cell	$CaCl_2$	[76]
	Alginate	Bone marrow stromal cell	-	[77]
	Fibrin	Endothelial cell	$CaCl_2$	[78]
	Agarose	Smooth muscle cell/Fibroblast	-	[79]
		Schwann cell	-	[80]
	Collagen	Cardiac cell/Endothelial cell	-	[81]
		Adipose stem cell	$CaCl_2$	[82]

5. Conclusion

Since the introduction of 3D cell printing technologies, studies and applications of 3D cell printing have been focused on or striving for the fabrication of 3D tissue-engineered structures that can firmly replace or repair damaged tissues in the human body in a short period of time. If this is possible, 3D cell printing technology may provide patients an instant medical treatment individually by rapidly manufacturing customized tissue-engineered constructs, and, therefore, creating a totally new medical course. This integrated medical course may include the scanning of injured parts, extracting a patient's cells, culturing and printing the cells through 3D cell printing, and implanting the engineered scaffold into the patient's body. However, to implement this new generation of clinical practices several challenges, such as low mechanical properties of natural polymer scaffolds; improvement of crosslink ability without cell damage; materialization of complex 3D structures; development of 3D multi-culturing; and joint works with material sciences, mechatronics, computer engineering, or medicine; etc., need to be surmounted. Especially the fabrication and culturing of 3D multicellular complex organ structure are indispensable steps to achieve the ultimate goal of tissue engineering. This can be reached, however, when the former steps are accomplished, such as the generation of 3D vascular structures in bigger multicell-printed tissue or organ structure. The major challenges of the realization and vascularization of multicellular structure would be the complexity and exquisiteness of the natural tissues and organs that we are striving to mimic. Despite these assignments ahead, we believe that the completion of whole-organ fabrication technology can be occurred in the nearer future than expected, as the studies and collaborations for tissue engineering is now being actively performed.

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