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TERM

Current Status of Three-Dimensional Printing Inks for Soft Tissue Regeneration

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Recently, three-dimensional (3D) printing technologies have become an attractive manufacturing process, which is called additive manufacturing or rapid prototyping. A 3D printing system can design and fabricate 3D shapes and geometries resulting in custom 3D scaffolds in tissue engineering. In tissue regeneration and replacement, 3D printing systems have been frequently used with various biomaterials such as natural and synthetic polymers. In tissue engineering, soft tissue regeneration is very difficult because soft tissue has the properties of high elasticity, flexibility and viscosity which act as an obstacle when creating a 3D structure by stacking layer after layer of biomaterials compared to hard tissue regeneration. To overcome these limitations, many studies are trying to fabricate constructs with a very similar native micro-environmental property for a complex biofunctional scaffold with suitable biological and mechanical parameters by optimizing the biomaterials, for example, control the concentration and diversification of materials. In this review, we describe the characteristics of printing biomaterials such as hydrogel, synthetic polymer and composite type as well as recent advances in soft tissue regeneration. It is expected that 3D printed constructs will be able to replace as well as regenerate defective tissues or injured functional tissues and organs.

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Key Words: Three-dimensional printing material; Soft tissue regeneration; Tissue engineering; Hydrogel; Three-dimensional bioprinting

INTRODUCTION

Tissue engineering technology has recently expanded into various areas due to increased demand for biocompatibility, that is, tissue repair and regeneration using natural biomaterials [1-3]. Successful tissue engineering approaches have been based on three-dimensional (3D) structures with complex geometries which are similar to native tissues or organs [4-6]. Despite demand for artificial tissues and organs, organ donors that can provide suitable replacements to patients are limited [7,8]. In tissue engineering, traditional scaffold fabrication methods, including electrospinning [9,10], salt-leaching [11,12], and gas foaming techniques [13,14], are very simple and sufficient to regenerate single tissues. However, these methods are limited for the fabrication of complex-shaped structures and multicellular tissues. In particular, owing to the characteristics of soft tissues, e.g., large volumes and flexible structures, they are difficult to reconstruct using methods in tissue engineering and regenerative medicine [15-17]. This limitation led to the concept of applying 3D printing technology to build a viable, similar organ or tissue structure in 3 dimensions (Fig. 1) [18,19].

In 3D printing, recent research has focused on hard tissues for the development of suitable transplants because of the easy discharge and lamination of biomaterials [20]. 3D printing has the potential to deposit various materials in a 3D matrix; therefore, it is possible to achieve great precision and control when 3D printing internal structures and outer resin molds as well as fabricate complex shapes that closely mimic biological organs or tissues through bioprinting [21-24]. Bioprinting is the additive process of creating cell patterns by layer-by-layer deposition using 3D printing technologies. Unlike 3D printing, bioprinting requires optimization of living cell type, biochemical factors, and multi-cellular designs. Most importantly, bioprinting requires the integration of these complexities using methods from various fields of engineering to develop biological organs and tissues that can be applied to living cells [19]. 3D bioprinting is one of the important advances in 3D printing technology; thus, it can fabricate 3D functional living human constructs with bio-

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logical and mechanical properties for the restoration of biomedical scaffolds, tissues and organs [19,25].

For successful tissue regeneration using the 3D bioprinting process, several factors will have to provide the proper environment. These factors should possess similar mechanical strengths as natural tissues including elasticity, flexibility and recovery rate [26]. Furthermore, complex conditions are required for well-fabricated tissues or organs such as the selection of materials, cell types, growth/differentiation factors, and sensitivity of the living cells using 3D bioprinting [27,28]. Mostly, bioprinting has

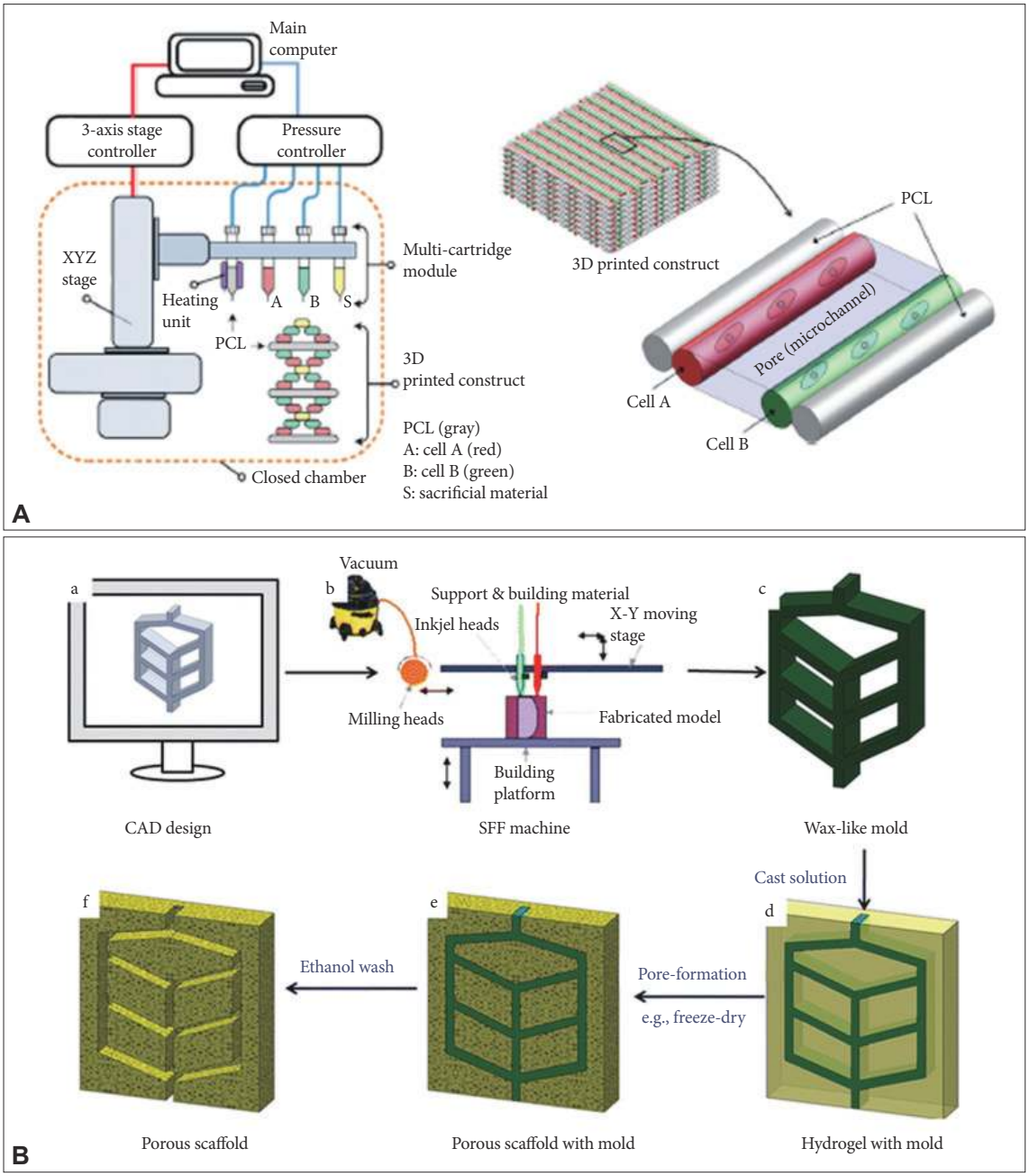

Figure 1. Examples of technique and schematic for 3D printing applications using various hydrogel. (A) Extrusion printing; the system consists of multiple cartridges which can be patterning of 3D architecture with hydrogel, cell and polymer. Adapted from Kang et al. Nat Biotechnol 2016;34:312-319, with permission of Nature publishing group [37]. (B) Inkjet printing; to make the Biomimetic channels in liver scaffold, thermal-plastic mold is printed using indirect solid freeform fabrication (SFF) process.After collagen and chitosan hydrogel is casted in the mold, porous scaffold is formed containing internal channel via freeze-drying and washing process. Adapted from Gong et al. J Mater Sci Mater Med 2014;25:113-120, with permission of Springer [21]. 3D: three-dimensional, PCL: poly(ε-caprolactone), CAD: computer-aided design, UV: ultraviolet, PDMS: polydimethylsiloxane, HAMA: methacrylated hyaluronan, HA: hyaluronic acid, pNIPAAM: poly(N-isopropylacrylamide). (Continued to the next page)

been used for biological hydrogels which can be achieved with layer-by-layer positioning of biomaterials in soft tissues such as skin [29,30], vessels [31-36], muscles [37,38], and adipose tissues [17].

Hydrogels are based on natural or synthetic polymers, which are crosslinked to form a complex network. They have high water content so that the polymer chain can swell in a hydrophilic environment [39]. The key advantages of hydrogel materials are their excellent bio compatibility and bio degradability and their 3D encapsulation of cells within the hydrogel networks when the hydrogel solidifies known as gelation [28,40-42]. A 3D environment facilitates the encapsulation of viable cells as well as maintains the cells without affecting cell-cell interactions [28]. For these reasons, hydrogels have been actively studied for 3D bioprinting; however, there still remain problems which need significant improvement. Generally, most hydrogels have a weak mechanical property making it difficult for them to retain a shape in a predesigned geometry [43,44].

Furthermore, suitable properties for printing materials such as structural and mechanical properties are required for successful 3D printing and for maintaining the function of printed constructs. In order to overcome the limitations of materials including the size, shape, and structural integrity of the structure arising from the mechanical and structural strength, many approaches have been attempted to combine various hydrogels, synthetic and natural polymers and/or cross linking agents resulting in enhancing the integrity of constructs (Table 1). When selecting appropriate materials for use in 3D printing, immunogenicity and inflammatory responses to implanted materials should be considered. Natural biomaterials from allogenic or xenogenic sources may passively or actively result in undesirable responses. Moreover, synthetic material biocompatibility is a major issue [45]. Collagen is especially useful as a biomaterial because it is biodegradable and non-toxic; exogenous collagen is more biocompatible than other natural polymers and has very weak antigenic properties [46]. For these reasons, many researchers have attempted to improve the biocompatibility or non-immunogenicity of biomaterials. For example, one group examined the removal of telopeptides in procollagen for the reduction of immunogenicity [47]. Furthermore, Darnell et al. [48] studied the immunogenicity of a hydrogel consisting of alginate. They reported that alginate-based hydrogels show minimal inflammation after transplantation *in vivo*. This result is consistent with previous analyses of the *in vivo* biocompatibility of alginate hydrogels, which support the non-immunogenicity of alginate.

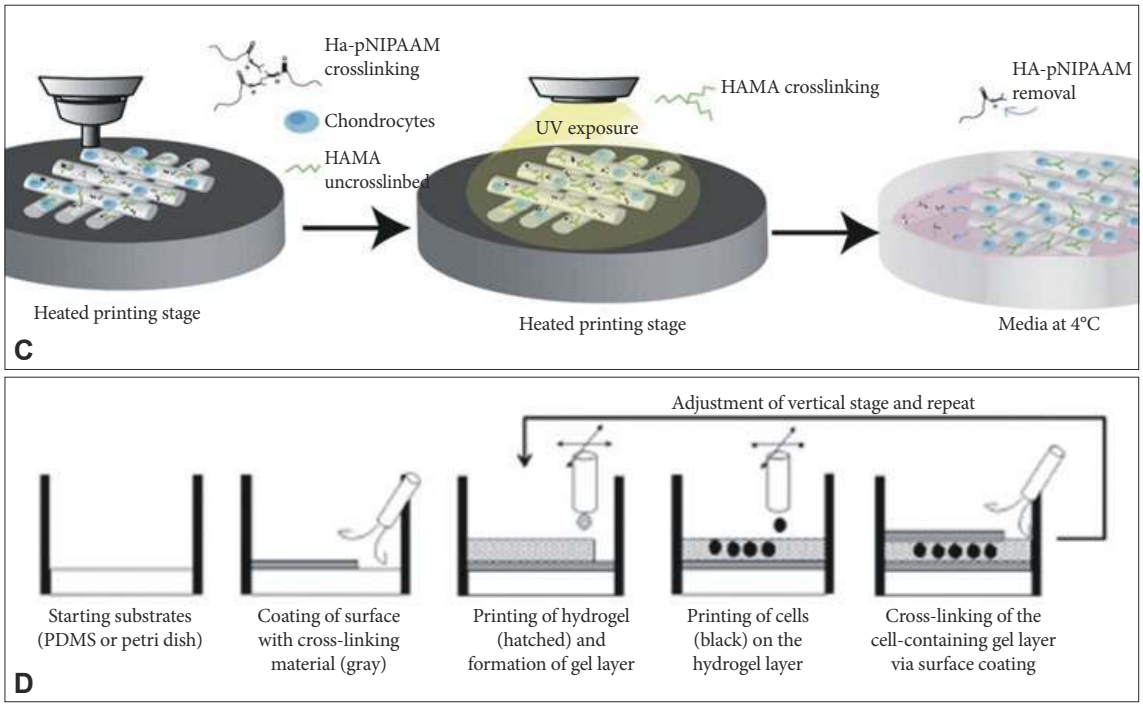

Figure 1. (Continued from the previous page) (C) Extrusion printing; poly(N-isopropylacrylamide) (pNIPAAM) conjugated to hyaluronic acid (HA) were printed onto the heated printing stage for thermal crosslinking (35−38°C). The printed structure was established for mechanically stable secondary network via UV crosslinking, and HA-pNIPAAM was eluted from the scaffold. Adapted from Kesti et al. Acta Biomater 2015;11:162-172, with permission of Elsevier [25]. (D) To construct multi-layered cell and hydrogel composites, the substrate surface was coated using cross-linking agent. Adapted from Lee et al. Biomaterials 2009;30:1587-1595, with permission of Elsevier [70]. 3D: three-dimensional, UV: ultraviolet, PCL: poly(ε-caprolactone), SFF: solid freeform fabrication, PDMS: polydimethylsiloxane, HAMA: methacrylated hyaluronan, CAD: computer-aided design.

Table 1. Tissue engineering applications using various 3D printing inks

Target tissue	Printing type	Materials	Cell types	References
Vessel	Ink jet	Collagen, gelatin	Human umbilical vein endothelial cells	33
	Ink jet	Fibrin	Human microvascular endothelial cells	34
	Ink jet	Alginate, collagen	-	36
Heart	Extrusion	Methacrylated hyaluronic acid, methacrylated gelatin	Human aortic valvular interstitial cells	35
Skeletal tissue	Extrusion	Fibrin, hyaluronic acid, glycerol, gelatin, PCL, Pluronic F-127	C2C12 myoblasts	37
	Extrusion	Polyurethane, PCL, fibrin	C2C12 myoblasts, 3T3 fibroblasts	75
Oesophagus	Extrusion	Fibrin, PCL	Rabbit mesenchymal stem cells	84
Skin	Laser	Collagen, alginate, blood plasma	3T3 fibroblasts, keratinocytes	27
	Electromechanical jetting	Collagen	Human dermal fibroblasts, keratinocyte	67
	Ink jet	Collagen	Human dermal fibroblasts, keratinocyte	66
Adipose tissue	Extrusion	PCL, decellularized adipose tissue	Human adipose derived stem cells	17

3D: three-dimensional, PCL: poly(ε-caprolactone)

In this review, we focus on the current state of 3D printing technology for the regeneration of soft tissues using specific types of materials. Moreover, we describe the characteristics of various materials and their applications according to manufacturing technology in tissue engineering.

3D PRINTING MATERIALS FOR SOFT TISSUE REGENERATION

Alginate

Alginate is a naturally occurring anionic and hydrophilic polysaccharide exhibiting excellent biocompatibility and biodegradability and low toxicity. Moreover, various alginate derivatives, such as amphiphilic and cell-interactive alginate, have been widely used in a range of biomedical applications, particularly in the fields of regenerative medicine, tissue engineering, and drug delivery [49-52]. Alginate is commonly used in various applicable components of three dimensional scaffolding materials because it can easily form a hydrogel through an ion exchange process involving the loss of divalent ions into the surrounding medium [53-55]. In order to make an alginate hydrogel, various methods have been studied including ionic interactions, phase transition (thermal gelation), cell-crosslinking and free radical polymerization [56,57].

Generally, alginate enables easy modifications by chemical and physical reactions resulting in the selection of popular and suitable materials in tissue engineering [49,58]. One group fabricated a 3D printed structure representing the tubular structure of a blood vessel comprised of alginate and HeLa cells with a 3D inkjet bioprinter by using a type of droplet bio-ink [59]. The printed structure was fabricated by the gelation technique with sodium alginate and $CaCl_2$ solutions. The structure has a 1 mm diameter and was more than 1 cm in length containing HeLa cells in an alginate hydrogel. Another group also fabricated 3D printed constructs containing branched vascular structures [55]. They emphasized a new extrusion technique. This approach is for complex 3D alginate hydrogel structures. The printed alginate hydrogels have a sufficiently high viscosity resulting in a well-formed interface layer achieved by dividing the alginate cross-linking process. When sodium alginate and $CaCl_2$ are mixed using two steps, in the first step, a cross-linking reaction occurs between the primary calcium ion and the secondary calcium. The second step leads to the penetration of hallow sections in the first printed structure by the diffusion of the $CaCl_2$ solution. After the bioprinting of the alginate hydrogel, the constructs are exposed to tertiary barium ions using $BaCl_2$ which increase the long-term stability of the constructs by cross-linking. The addition of barium chloride improves the property of printed constructs, and it extends not only the stability of the alginate hydrogel but also increases the viability of the encapsulated cells. Therefore, this technique can be used to fabricate clinically sized soft tissues and may enable the printing of complex and diverse multi-cellular structures.

Fibrin

Fibrin gel consists of fibrinogen and thrombin separated from blood plasma proteins which are produced in the liver of mammals [60]. The fibrin network enables blood clotting, which is specifically a cross-linking process that prevents bleeding and promotes wound healing through the actions of fibrinogen and thrombin [54,61]. It is widely used as a hemostatic agent and sealant in surgery because it exhibits excellent biocompatibility

and sufficient cell attachment. It also induces only minimal inflammation and foreign body reactions, which is desirable for tissue engineering applications. However, fibrin has a limitation in terms of long-term stability. Nevertheless, it is possible to control the mechanical properties, gelation time and stability of fibrin gels because they are formed by the polymerization of fibrinogen monomers catalyzed by a thrombin solution. Its degradation time could also be tuned according to the mixing concentration and ionic strength [62].

For efficient tissue bioprinting, living cells and a suitable polymeric scaffold are well-combined to regenerate functional tissues or organs [19]. Fibrin is suitable due to its high cell seeding efficiency and uniform cell distribution as tissue engineered material. In addition, the component of fibrin, fibrinogen, provides stability to the gel and a microenvironment conducive to cell adhesion and proliferation [63]. One group used an automated and direct inkjet printing technique to fabricate complex cellular patterns and structures composed of NT2 neuronal precursor cells and fibrin gel for 3D functional neural tissues [64]. After the structure was cultured for 15 days, the NT2 neurons were attached to the fibrin fibers in a neural sheet. The authors showed that the ability of fibrin gel to build 3D neural constructs is from the affinity of neurons for the gel because neurons are anchorage-dependent as well as influenced by their attachment onto scaffolds. In addition to using fibrin in gels, printed fibrin-based constructs may provide suitable clinical treatments for neural injuries as well as potentially treatments for degenerative diseases such as spinal cord diseases and Parkinson's disease. Cui and Boland [34] fabricated a human microvasculature with suitable bio-ink composed of human microvascular endothelial cells and fibrin gel. A fibrinogen solution as a bio-paper was deposited onto a microscope cover slip. Thrombin with a cell suspension as the bio-ink was printed onto a substrate scaffold so that a fibrin channel was formed by the polymerization of fibrinogen and thrombin. The printed fibrin channel was well aligned and straight, and the microvasculature had excellent integrity after being cultured for 21 days.

Collagen

Collagen is a natural protein in the body. Collagen type I, II and III are found in soft and hard connective tissues including cartilage and bone tissues [65]. Collagen molecules are comprised of three polypeptide chains, and each chain is composed of a thousand or so amino acid residues [66]. Additionally, collagen forms a triple helix structure, and the arginine-glycine-aspartic (RGD) amino acid residues are an intrinsic and important sequence which forms a motif that enables cells to adhere and proliferate via integrin-RGD binding. Collagen fibrils and their networks are involved in the abundant extracellular matrix (ECM) and result in a 3D scaffold that surrounds cells [67,68]. For these reasons, collagen is a useful material and has yielded many important biological applications. Moreover, it has biodegradable and nontoxic properties, and exogenous collagen is more biocompatible than other natural polymers [54].

In skin therapy and regeneration, collagen has been used as an injectable hydrogel because it is the main component of the ECM in the skin. However, the skin is the largest organ in the body and primarily serves as a protective barrier against the environment [69]. Furthermore, skin has a flexible property; thus, the geometrical 3D environment needs to be controlled when fabricating artificial skin tissues. Various studies have suggested 3D organotypic reconstruction of multiple skin layers for skin repair because skin consists of a dermal and epidermal layer. Lee et al. [70] constructed stratified skin cell layers in an *in vitro* human dermal/epidermal skin model using 3D via robotic cell printing technique using collagen hydrogel and cell suspension. They were used dermal fibroblasts and keratinocytes as cell source for artificially construct stratified layers of skin. Collagen composed hydrogel constructs offer a biodegradable structure with sufficient diffusion of oxygen and other nutrients into the cell layer, which results in successfully cell proliferation *in vitro* skin model. In a similar way, another study reported on 3D bioprinting to engineer human skin in a layer by layer assembly process using two kinds of cells, fibroblasts and keratinocytes.

Collagen hydrogel precursor was used as a scaffold material for printing. According to property of collagen material, the parameters were determined based on the viscosity of the biomaterials being dispensed including air pressure, printing droplet volume, resolution, pattern size and concentration [71]. Furthermore, several studies have shown a development of vessel in the 3D based bioprinting system in order to a great potential in engineering vascularized tissues. For successful artificial tissues and organs, main approach have to be serve as well-organized blood vessel system for nutrients and oxygen supply to the cell. 3D-fabricated vascular network system has been studied via various materials such as natural and synthetic or other complex polymers [72,73]. Above all, collagen hydrogel have been shown to create multi-scale vascular network is relatively straightforward. Collagen gel was reported that it led to supports angiogenic sprouting of vascular cells and the structure integrity required for 3D bioprinting [74]. Due to the advantages of 3D printing technology, it can be create multi-scale vascular network compared to other approaches to micro-fabricate vascular networks such as sophisticated fabrication and assembling steps. Similarly, another research is to develop the 3D biopinting method to construct a perfused vascular channel within thick collagen scaffold. As a consequence, collagen may serve the formation of vascular network and facilitation of soft tissue regen-

eration as well since it allows transportation of nutrient and oxygen.

Decellularized extracellular matrix-derived bioink

The ECM surrounds cells in tissue and provides them with a variety of physical, chemical, structural, and biological cues. Studies on potential therapeutic effects of ECM from tissues and organs have emphasized the necessity of tissue specificity for preserving original functionalities of them. The decellularized extracellular matrix (dECM) is harvested from various tissues, including skin, adipose tissue, cartilage, bone, and heart [17,75-78]. The material is a complex of glycosaminoglycan, collagen, and elastin that reflects the native tissue microenvironment, so it supports the fabrication of native structure mimics. The ECM microenvironment also has the ability to direct and mediate the differentiation of stem cells in culture. These features have prompted the development of dECM bioink for bioprinting 3D functional constructs for clinical restoration of tissue and organ functions. Pati et al., [79] who focused on bioinks for 3D tissue printing, fabricated 3D constructs using a dECM-containing bioink with encapsulated living mesenchymal stem cells (MSCs) and a layer-by-layer technique. They achieved functionality and versatility by providing an ECM microenvironment for specific tissue constructs, including adipose, cartilage, and heart tissues. Jang et al. [77] fabricated a 3D cell-laden construct using a dECM bioink and vitamin B2 and UVA irradiation to improve its printability and the cellular function of encapsulated cardiomyocytes. The resulting bioconstruct had a stiffness similar to that of the target tissue. This strategy using stiffening and bioink printing will have a multitude of applications in tissue engineering and regenerative medicine. The dECM bioink is therefore an attractive material that may prove useful for bioprinting applications.

Synthetic material

As the development of 3D printing technology, various materials were used for regenerative medicine including naturally derived polymers and synthetic materials [19]. Natural polymer has advantages that biocompatibility and less toxicity, while synthetic polymer can be tailored with specific physical properties to suitable clinical application. However, they are limited in their ability to clinical application that is poor biocompatibility, toxic degradation products and loss of mechanical properties during degradation [51,80]. Nevertheless, due to the robust mechanical properties and possibility of control the degradation time when fully regenerated and cured, they have been studied for 3D printing materials as the biological construct or alternative tissue and organ [7].

Poly(ε-caprolactone) (PCL) is a common synthetic polymer material that is frequently used in 3D bioprinting in recent [81-84]. PCL is a polyester based material that is biocompatible, flexible, and has relatively low melting temperature of 60°C which is possible to permit extrusion through a fine nozzle [37,85]. Furthermore, PCL also has a property of long term structural stability, so it has been established for long term (~1.5 to 2 years) as implantable material of construct [55]. Most importantly, PCL is the most suitable polymer for 3D printing inks because it has been approved by the Food and Drug Administration for specific applications in the human body. PCL has been used for making structure with fused deposition modeling type due to low melting temperatures [86]. However, PCL has possibility that non-specific binding with cells due to its hydrophobic property, so it has been co-printing to combine with other functionalized materials or naturally derived materials including hydrogel and derived extra-cellular matrix of each tissue or organ [87]. Pati et al. [17] fabricated decellularized adipose tissue (DAT) with PCL hybrid structure for customized soft tissue regeneration. PCL was used as framework because it is important that maintain the structure during the tissue remodeling time. The advantages of DAT-PCL hybrid structure in their study are that keep the open porous structure thereby maintain the mass transport during the remodeling process. Through the 3D printed dome-shaped adipose tissue constructs of hybrid structure using PCL polymer, they evaluated the cell viability test, adipo-inductive potential and expression of adipogenic genes within *in vitro* and *in vivo* model. It was concluded that the constructs not induced chronic inflammation as well as facilitated positive tissue infiltration, conductive tissue remodeling and adipose tissue formation with vascularized tissue structure. In addition, Park et al. [88] fabricated that 3D printed artificial oesphagus patch using PCL polymers. The 3D printed oesophageal scaffold was constructed by extrusion system of PCL through the layer-by-layer plotting manner. In addition the printed PCL scaffold was coated with the MSCs and fibrin gels. In their study, the hybrid multiple layered scaffolds provided the successful reconstruction of the rabbit oesphagus. The effects are result from mechanical stable environment as well as biochemically favorable cellular environment for tissue regeneration by using PCL hybrid scaffolds.

Composites

Most composites are commonly used for 3D printing to have increased mechanical strength and more intricately designed scaffolds [7]. In soft tissue regeneration, composites have been utilized to control the mechanical properties of hydrogels or synthetic polymers as implantable scaffolds which having suitable strength according to property of tissue or organ. Hong et al. [89] made various shapes 3D-printed structures by using

Poly(ethyleneglycol) (PEG)-alginate-nanoclay hydrogels. After loading of the mixture into the extrusion cartridges of the 3D printer, pre-gel solution was extruded from 15 G-20 G flat tip needle. The printed layers in glass slides were placed in the UV chamber to crosslinking of the PEGDA polymer chain through the covalent bonding. PEG has been utilized in biomaterials for biomedical applications, including surface modification, bioconjugation, drug delivery, and tissue engineering, as an important type of hydrophilic polymer. It has critical properties in terms of biocompatibility, non-immunogenicity, and resistance to protein adsorption, and accordingly it has been widely used in a variety of applications [90]. Nanoclay can be used to control the viscosity of hydrogels; therefore, it is necessary to optimize the concentration needed for extrusion-based 3D printing for tissue engineering. Highly stretchable and tough hydrogels have been fabricated using the reversible Ca^{2+} crosslinking of alginate, which dissipates mechanical energy, while the covalent crosslinking of PEG maintains elasticity under large deformations [89].

In other study, Kang et al. [37] printed cell-laden hydrogels together with biodegradable polymers and anchored on sacrificial hydrogels for stable mechanical structure. The multi-cartridge module and pneumatic pressure controller system based 3D printer was used to generate PCL-based composite scaffolds consisting cell and hydrogel including gelatin, HA, fibrinogen, and glycerol for skeletal muscle reconstruction. To mimic the soft tissue and increasing the stability, 3D muscle construct was fabricated from PCL as supporting pillar and Pluronic F-127 hydrogel as a temporary sacrificial material. HA and glycerol provide for uniformly dispensing condition and blocking nozzle clogging. In addition, the printed 3D structures contained muscle bundle fiber (~400 um width). The each concentration of hydrogel component may be the key to fabrication of individual tissue constructs through 3D bioprinting. Thus, it should be considered various factor such as printing resolution, dispensing uniformity and mechanical properties thereby gelatin (35 mg/mL), fibrinogen (20 mg/mL), HA (3 mg/mL), and glycerol (10% v/v) were used in their study. Furthermore, the concentration of individual hydrogel may be affected to physical stress to encapsulated cells within bioprinted constructs. Merceron et al. [91] fabricated tissue constructs that can mimic the muscle-tendon unit by using 3D bioprinting system, and it is called 3D integrated organ printing (IOP) technology. In their study, Polyurethane (PU) for elasticity and muscle development and PCL for stiffness and tendon development were used as cell-laden hydrogel based bioink. The cells were mixed with hydrogel including gelatin, HA, fibrinogen and glycerol. The IOP system has four-multi dispensing modules that can print four components of materials and cells. And then thrombin was added to cell-laden composite construct for cross-linking with fibrinogen during 30 min. This research was able to fabricate made up of three distinct regions : muscle part which is composed of PU and muscle cells, tendon part which is composed of PCL and tendon cells and muscle-tendon junction is made up of over-lapped PU-PCL and apposed both cells. For muscle tissue part fabrication, PU is a synthetic polymer and an attractive material owing to various properties, such as its biocompatibility, excellent mechanical properties, and mechanical flexibility. The authors implement different mechanical property via young's modulus, tensile strength and elongation break test in three distinct regions. Further, they identified the good viability of cells, highly-aligned morphology of muscle and tendon and increased MTJ related gene expression. Another group made 3D printed tissue construct containing patterned vascular architectures and living cells [92]. The printed construct were fabricated carbohydrate-glass lattice owing to use the sacrificial element for creating fluidic channel. After the lattice is filled to natural and synthetic ECM materials (agarose, alginate, PEG, fibrin, Matrigel), the glass filaments are dissolved to form vessels without damage to nearby cells. This study was provided that 3D printed constructs play a role in increasing mass transport to specific tissues and vascular architecture. Moreover, the constructs having patterned vascular channel are proved sustained the metabolic function of encapsulated living cells, these system could be contribute to building for vasculature mimic tissue by designing the space including selection of arbitrary cell types, matrices, and their patterning in tissue engineering.

CONCLUSION AND FUTURE PERSPECTIVES

3D printing technology for tissue engineering is still far from reaching maturity, which has been undergoing rapid advances. The goal of this field should be achieved to the manufacturing of tissue or organ in tissue regeneration and medicine. In this process, bio-paper was arranged for maintain the structure through bio-ink such as natural, synthetic polymer and composites, and then printed bio-paper can be stacked by layer-by-layer for creating complex composite tissue construct (Fig. 2). In this paper, we review various biomaterials and their applications as bio-ink or bio-paper for 3D printing to regenerate tissues and organs. 3D printing systems have an advantage over other techniques because they can be used to fabricate constructs that are well-defined on the micro-scale. Furthermore, the constructs can be easily designed, making it is possible to control micro-geometries and develop complex cellular constructs. Various materials as previously referred have been challenged, it is still remained the limitation for soft tissue regeneration. Soft tissues

have the characteristic such as weak mechanical property, flexibility and high elastic viscosity compared to hard tissues, thus the development is required to fast printing technique, biomaterial for fast crosslinking, exquisite output resolution and improvement of nozzle and cartridge design in order to laminate the hybrid 3D architecture. Moreover, although 3D printing has potentially advantages for application that control the structure size, shape, pore and orientation of a variety of component such as cells, growth factors, dECM, it is difficult to fabrication for large sized tissue structure. In the recent various research groups

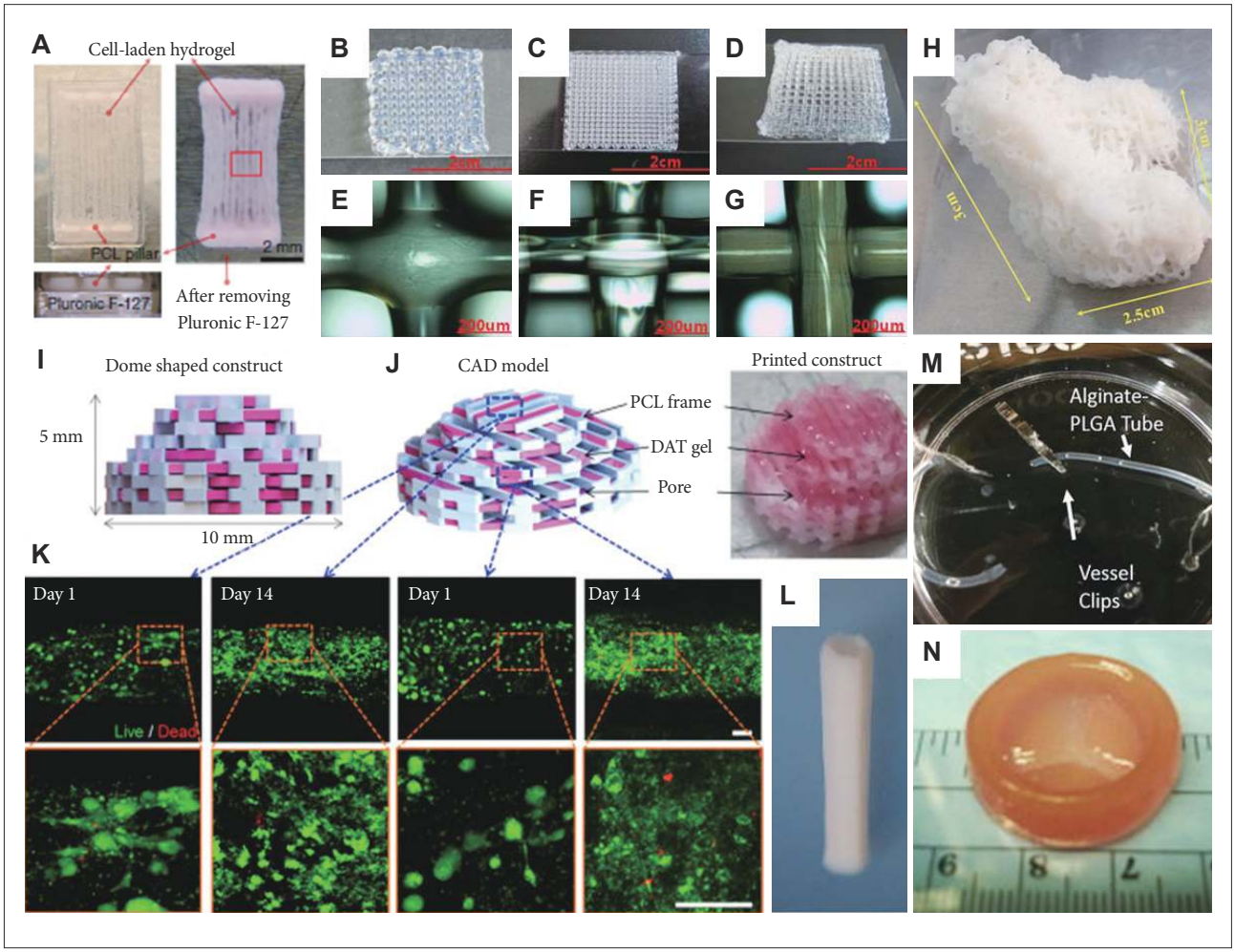

Figure 2. Images of fabricated construct using the 3D printing technology in tissue engineering. (A) 3D printed muscle construct using cell-laden hydrogel, PCL and Pluronic F-127. The right image is represented the designed muscle organized pattern after removing the sacrificial material. Adapted from Kang et al. Nat Biotechnol 2016;34:312-319, with permission of Nature publishing group [37]. (B-G) Photographs of 3D printed scaffolds using various hydrogel such as agarose (B and E), Pluronic F-127 (C and F) and alginate (D and G). Adapted from Park et al. Macromol Res 2011;19:694-698, with permission of Springer [58]. (H) Image of complex ECM-C scaffolds through 3D printing system. Rabbit femur was scanned using micro-CT, and then ECM-C was printed for 3D structure. The ECM-C materials include decellularized articular cartilage powder and viscous collagen solution. Adapted from Song et al. Tissue Eng Regen Med 2015;12:172-180, with permission of Springer [27]. (I, J, and K) 3D printed adipose tissue constructs for soft tissue regeneration. For successful regeneration of adipose tissue, dome-shaped constructs was designed and printed using human decellularized adiposed tissue (DAT) with human adipose tissue derived stem cells (hASCs) and PCL polymer (I and J). Confocal images showing high cell viability of the encapsulated hASCs within printed tissue constructs; scale bars, 100 μm (K). Adapted from Pati et al. Biomaterials 2015;62:164-175, with permission of Elsevier [17]. (L) The image of tubular structure through a rapid prototyping bioprinting method for small diameter vascular reconstruction. Porcine smooth muscle cells were printed within agarose cylinder mold according to the designed tubular structure, and then supporting molds were removed (Diameter: 2.5 mm). Adapted from Norotte et al. Biomaterials 2009;30:5910-5197, with permission of Elsevier [32]. (M) Photographs of 3D printed alginate-PLGA tubes through extrusion printing technique for controlled delivery of drug. Adapted from Do et al. Ann Biomed Eng 2016;Epub, with permission of Springer [31]. (N) The bioprinted heart valve conduits using acellular root and human aortic valvular interstitial cells encapsulated leaflets, with 4% Me-HA/10% Me-Gel hydrogels. Adapted from Duan et al. Acta Biomater 2014;10:1836-1846, with permission of Elsevier [35]. 3D: three-dimensional, PCL: poly(ε-caprolactone), ECM-C: ECM powder blended collagen, PLGA: poly(lactic-co-glycolic acid).

have been studied 3D bioprinting using hydrogel; however, the type of composite structure using two or more than three of individual material as mixture have been generally used for improvement of structure integrity and long term stability. Although hydrogels possess the low mechanical parameter, they have an advantage which can be biocompatible and maintain cellular viability and function, thereby providing suitable environment for soft tissue regeneration. In addition, another important key factor is reconstruction of vascularized tissue that needs to be fully addressed in order for the long-term viability of bioprinted tissue construct. Vascularization is essential to supply of oxygen and nutrients so that many investigators have researched branched vascular matrix or channel using biomaterials such as fibrin, collagen via 3D printing system. However, it is still remain the problem to solve which is not exist fully vascularized entire tissue construct for clinically application. Although various materials are being developed and fabricated, there are still many challenges to optimization, especially in achieving structural integrity through its proper concentration.

Most of all, we emphasized that the fabrication of 3D printed constructs should be sufficient to not only customized delicate design according to complex structure of human organ but also adequate mechanical property including porosity, pore interconnectivity, pore distribution, flexibility and recovery rate in soft tissue regeneration. Therefore, advanced approaches for 3D printing technology will be needs to meet these challenges thereby making the multi-layered structures. In conclusion, development of optimum condition of biomaterials will be enabled to giving the chance from organ failure and dysfunction as well as transplantation of tissues or organ.

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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.

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