Chapter

Bio-Inspired Hydrogels via 3D Bioprinting

Lei Nie, Can Wang, Yaling Deng and Amin Shavandi

Abstract

Many soft tissues of the human body such as cartilages, muscles, and ligaments are mainly composed of biological hydrogels possessing excellent mechanical properties and delicate structures. Nowadays, bio-inspired hydrogels have been intensively explored due to their promising potential applications in tissue engineering. However, the traditional manufacturing technology is challenging to produce the bio-inspired hydrogels, and the typical biological composite topologies of bio-inspired hydrogels are accessible completed using 3D bioprinting at micrometer resolution. In this chapter, the 3D bioprinting techniques used for the fabrication of bio-inspired hydrogels were summarized, and the materials used were outlined. This chapter also focuses on the applications of bio-inspired hydrogels fabricated using available 3D bioprinting technologies. The development of 3D bioprinting techniques in the future would bring us closer to the fabrication capabilities of living organisms, which would be widely used in biomedical applications.

Keywords: 3D bioprinting, hydrogels, biopolymers, tissue engineering, biomaterials

1. Introduction

The design of scaffold materials that can guide tissue regeneration is a very challenging goal [1]. In addition, to support and promote the growth and differentiation of specific cells, an ideal scaffold requires careful control of the material's structure in the range of nanometers to centimeters, and some natural materials with complex structure exist in nature, which provides ideas for the design of ideal scaffolds [2]. These natural materials, such as mammal bones, abalone pearl layers and fish scales, which are composed of multi-layer biominerals and biopolymers, have complex microstructure, which can control the crack growth and fracture in three-dimensional (3D) direction, producing much more strength and toughness than their constituent materials [3–5]. Jellyfish and sea anemones, with a water content of up to 90%, show that their gelatinous bodies exhibit exciting mechanical properties and are able to respond quickly to various environmental stimuli [6–8]. There are also some soft support tissues (such as tendons, ligaments, meniscus, and cartilage), showing softness, toughness and impact resistance [9]. Because of the beneficial properties of natural composite materials, the design of bionic materials has attracted significant attention. Bio-inspired material is considered as a kind of material inspired by nature or biology and then developed by simulating some characteristics [10], and usually, the bio-inspired materials provide better functions than synthetic materials [11].

However, there are still many limitations on the fabrication of bio-inspired materials using traditional material manufacturing technology because they cannot accurately control the distribution and spatial trend of micro-holes inside the materials, and it is challenging to produce the contour matching with natural materials [12, 13]. Recently, 3D bioprinting technology has become a promising tool for manufacturing materials with high-precision, which can overcome the limitations compare with the traditional methods, and finally can eventually produce complex and delicate biomimetic 3D structures. Also, 3D bioprinting technology realizes the automatic biological preparation of cell-laden structure through the layered deposition of bio-inks *in vitro* and *in vivo* [14]. In addition, 3D bioprinting technologies are controlled by computers and can be combined with medical imaging systems, such as computed tomography and magnetic resonance imaging (MRI), also combined with computer-aided design (CAD) and computer-aided manufacturing (CAM), to generate personalized structures organized in different length proportions [15, 16]. Compared with the classical tissue engineering methods, 3D bioprinting allows the direct manufacture of complex 3D structures containing spatial variations of biological materials, cells and biochemical substances with the same structure, which significantly improves the biological simulation level of the composition, structure and biochemical characteristics of cell niche in the human body [17]. The complexity of the resulting structure is not only related to the application of tissue regeneration but also to the development of cell biology, drug development, and disease research *in vitro* models [18].

In recent years, in tissue engineering development, many materials have been developed to meet the needs of 3D bioprinting. The most common 3D bioprinting materials are metals, engineering plastics, photosensitive resins, bioplastics and polymer hydrogels. The bio-inspired hydrogels are very similar to natural extracellular matrix (ECM) and display potential advantages in tissue engineering [19]. Bio-inspired hydrogel provides an adequate and porous microenvironment that allows good nutrition and oxygen to diffuse into the encapsulated cells and can be modified to guide cellular processes with various physical, chemical, and biological cues [20]. Besides, these hydrogels are usually non-toxic or low toxic and have good reproducibility. Next, the 3D bioprinting techniques used for the fabrication of bio-inspired hydrogels were summarized, and the materials used for 3D bioprinting were outlined. This chapter also focuses on the applications of bio-inspired hydrogels.

2. 3D bioprinting techniques

There are several available 3D bioprinting techniques for fabricating bio-inspired hydrogels, including inkjet bioprinting, laser-assisted bioprinting, extrusion bioprinting, and stereolithography, as shown in **Figure 1** [21].

2.1 Inkjet bioprinting

During the inkjet bioprinting process, biomaterials are selectively placed on the construction platform layer by layer until the required structure is formed. The first inkjet printers for bioprinting applications were improved versions of commercial two-dimensional ink printers [22]. For the inkjet bioprinting, the ink in the ink cartridge is replaced by biomaterials, and the paper is replaced by an electronically controlled lifting table to provide the control of the third dimension Z-axis in addition to the X-and Y-axes. The bioprinter based on inkjet printing technology is customized to process and print biomaterials with higher resolution, accuracy and speed [16]. Inkjet bioprinters use thermal or acoustic forces to spray droplets

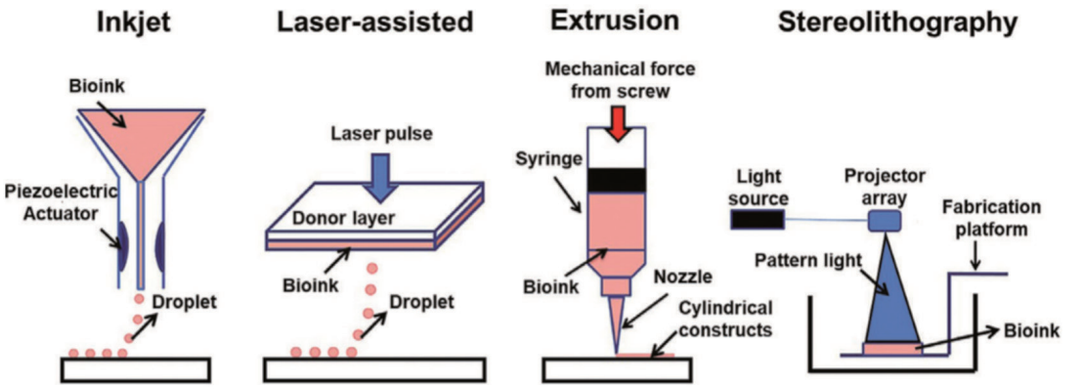

Figure 1.
Bioprinting techniques mainly include inkjet, laser-assisted, extrusion and stereolithography [21].

onto the substrate, which can support or form part of the final structure [23]. Thermal inkjet uses a heating element to induce the evaporation of a small volume of bioink in a reservoir, thereby forming and ejecting a small droplet. Therefore, in the printing process, this method keeps the cells at high temperature (300°C) for several microseconds (about 2 microseconds), which may lead to the formation of transient pores in the cell membrane [16]. Using the thermal inkjet printer, Solis et al., studied the effect of heat generated by the thermal ink-jet bio printer and found that the survival rate of Chinese hamster ovary (CHO) cells was 89% [24]. Such survival rate of cells could be greatly improved by using a piezoelectric inkjet printer, the generation and injection of droplets are realized by applying external voltage to control the mechanical deformation of piezoelectric transducer, which prevents the temperature from rising to the super physiological level [25]. Compaan et al. used alginate as the sacrificial material to prepare cell-supported silk fibroin hydrogels with a clear structure based on the piezoelectric inkjet 3D bioprinting system. The printed tubular structure has a diameter of 5 mm, a height of 2.5 or 5.0 mm and a thickness of about 400 microns. Moreover, the effect of citrate treatment on the printing was compared. The results showed that alginate removal and alginate removal could enable cells to extend and contact each other and form a cell network in the whole hydrogel [26].

The advantages of inkjet bioprinting mainly include: low cost due to its similar structure to commercial printers, high printing speed due to the ability of the print head to support parallel operation mode, and relatively high unit survival rate (usually from 80–90%) determined by many experimental results. However, the risks of cells and materials exposed to thermal and mechanical stresses, low droplet directionality, uneven droplet size, frequent nozzle plugging, and unreliable cell encapsulation have brought considerable limitations to the application in tissue engineering [27].

2.2 Laser-assisted bioprinting

The typical laser-assisted biological printing device include pulsed laser beams, focusing systems, and donor bands that respond to laser stimuli, consisting of glass covered with laser energy absorbing layers, and biomaterial layers (such as cells/hydrogel composite) prepared in liquid and receiving substrates for ribbons. The principle of laser-assisted bioprinting is to apply high-energy pulse laser (usually near-infrared laser) to the donor color band coated with bioink. This laser pulse evaporates a part of the donor layer, forms a high-pressure bubble on the interface of the bioink layer, and pushes the materials containing cells to the receiving

substrate [16, 25, 28]. Compared with inkjet bioprinting, laser-assisted bioprinting can avoid the problem of jamming cell or material, also can avoid direct contact with the printer and biological ink at the same time. The non-contact biological printing method can choose much more types of ink, resulting in printing materials with wider range of viscosity [28].

The laser pulse energy, ECM thickness, and bioink viscosity can influence cell viability. The higher the laser energy is, the higher the cell death rate is, but the increase of membrane thickness and bioink viscosity will lead to an increase of cell viability. Guillotin et al. studied the effects of bioink viscosity, laser energy and printing speed on printing resolution. The microscale resolution and 5 kHz printing speed could be achieved, and the laser-assisted bioprinting could combine cells with ECM to produce soft tissue with high cell density *in vivo* [29]. Laser-assisted biological printing is considered as one of the most promising methods to fabricate engineered tissue because of its unique resolution, high throughput, high resolution, and high resolution, as well as the ability to produce heterogeneous tissue structures with high cell density [25]. However, compared with other bioprinting methods, the laser diode with high resolution and high intensity are expensive, and the control of the laser printing system is complex, which limit the application of this technology [28].

2.3 Extrusion bioprinting

The extrusion bioprinting can fabricate 3D cell carriers for tissue regeneration. The prepolymer solutions need to be prepared first, and almost all types of prepolymer solutions with different viscosities and aggregates with high cell density can be printed with extruded bioprinters [28]. Different from printing small droplets onto the platform, the extrusion bioprinting continuously deposit hydrogel filaments within a diameter of 150–300 microns to generate 3D structures. Common extrusion bioprinting method includes pneumatic, piston-driven, and screw-driven dispensing. In pneumatic dispensing, air pressure provides the required driving force, while in piston and screw-driven dispensing, vertical and rotating mechanical forces start printing respectively [30]. There are three main factors that decide the printability of extrusion bioprinting, mainly including the adjustability of viscosity, the bioink phase before extrusion, and the material-specific bio-manufacturing window [31]. Extrusion bioprinters have been used to produce various tissue types, such as aortic valves, branching vascular trees, in vitro drug movement and tumor models [32]. Although the manufacturing time may be prolonged for high-resolution complex structures, the structures have been manufactured from the clinically related tissue size to the microtissue in the microfluidic chamber. Furthermore, it is convenient to combine cells with bioactive agents, because that the heating process is not involved [33]. Compared with inkjet 3D bioprinting, extrusion bioprinters can achieve a continuous flow of biomaterials, thus achieving the simplicity of operation and a broader selection of biomaterials, including polymers, acellular matrices, cellular hydrogels, spheres and aggregates [34].

2.4 Stereolithography

Among all the bioprinting technologies, stereolithography (SLA) 3D bioprinting display much more advantages over extrusion or ink-jet bioprinting technology [28]. SLA is based on the polymerization of photosensitive polymers, and the digital mirror array controls the light band in the projection field to achieve selective crosslinking of each layer of the hydrogel prepolymer solution [35]. No matter how intricate a layer's pattern is, the printing time is the same because the whole pattern is projected on the printing plane. Therefore, the printer only needs a movable table

in the vertical direction, which significantly simplifies the control of the printer. The cell encapsulated scaffold fabricated by the SLA system can achieve 100 μm resolution with printing time less than 1 hour, also maintain very high cell viability (90%) [36]. The above properties make SLA practical for fabricating delicate construct for tissue engineering. Arcaute et al. used composite lithography technology and two different molecular weight of polyethylene glycol (PEG) to prepare composite multilayer 3D structure of PEG hydrogel, and the properties of prepared hydrogel were influenced by photo-initiator and photosensitive polymer concentration. Besides, the prepared PEG hydrogel supports attachment, proliferation and differentiation of bovine chondrocytes, providing evidence for the applicability of resins for cartilage tissue engineering [37]. Valentin et al. prepared the sodium alginate precursor solution based on ion crosslinking, and different concentrations of cationic sources, such as barium carbonate, magnesium carbonate and calcium carbonate, and photo acid generator (PAG), diphenyliodonium nitrate were used, and the sodium alginate hydrogel was printed by SLA. The printed alginate hydrogel exhibited different mechanical and physical properties when crosslinked with two kinds of cations. The microstructures with variable height could be printed with optimized precursor formulations. Due to the high resolution, the 3D fabrication of natural and synthetic polyelectrolyte hydrogels via SLA enables lab-on-a-chip devices, soft sensors and actuators, and other biologically-inspired devices [38].

3. Polymers used for bio-inspired hydrogels

Hydrogels are considered as the gold standard materials for 3D bioprinting because they can provide a flexible and hydrated cross-linked network, similar to the natural extracellular matrix, in which cells can survive [39]. The polymers prepared for hydrogels can be classified into natural and synthetic polymers [40]. The natural polymers include alginate, chitosan, hyaluronic acid, gelatin, and so on, and the synthetic polymers mainly include polyacrylamide (PAAm), polyvinyl alcohol (PVA), polyethylene glycol (PEG), polylactic acid (PLA), and so on [41, 42].

3.1 Natural polymers

Most hydrogels prepared by natural polymers have the advantages of good hydrophilicity, good biocompatibility, specific enzymatic degradation, and contain various active functional groups and structural domains, and display better interaction with cells to promote cell proliferation and differentiation.

Alginate is extracted from alginate plants, is a kind of natural high molecular, composing of β-d-mannuronate (M) and α-l-guluronate (G). Alginate has been widely used in tissue engineering because of its advantages of abundant production, low price, good biocompatibility, and abundant functional groups, which are suitable for the preparation of bioink for 3D bioprinting [43, 44]. Alginate can react with $CaCO_3$ to release bivalent Ca^{2+} and then form an ionic crosslinking hydrogel bonded with -COO- on G unit of alginate G unit, to achieve the controllability of alginate ion crosslinking. The alginate hydrogel has high toughness and good mechanical properties, but the degradation rate of the alginate hydrogel is not controllable [45].

Chitosan is the product of deacetylation of chitin, which has a straight-chain structure and positive charge due to the presence of amino groups. Because of the useful biological function and biocompatibility, the degradation by microorganisms, chitosan has been widely concerned and applied in various industries [46]. The chitosan ink can be directly printed in air, and then the chitosan scaffold is refined by physical gelation. A chitosan hydrogel that satisfies both biocompatibility

and mechanical properties has been obtained, and it has been confirmed that chitosan hydrogel can guide cell growth [47].

Gelatin is the hydrolysate of collagen, which contains many arginine-glycine-aspartic-acid (RGD) sequences and matrix metalloproteinase (MMP) target sequences, which enhance cell adhesion and cellular microenvironment remodeling respectively [48]. Because of biodegradability, biocompatibility, and low antigenicity, gelatin is attractive for bio-inspired hydrogel [49]. Lewis et al. used gelatin as a bioink to print into a specific 3D geometry using 3D bioprinting, which can regulate the biological processes of hepatocytes, enhance protein function, and facilitate cell proliferation and differentiation [50]. Another commonly used gelatin derivative is to acylate gelatin to form gelatin methacrylamide (GelMA) [51]. Zhou et al. used GelMA, N-(2-aminoethyl)-4-(4-(hydroxymethyl)-2-methoxy-5-nitrosophenoxy) butanamide linked hyaluronic acid (HA-NB) and photo-initiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as biomimetic bioink to fabricated a bio-inspired 3D tissue construct via the digital light process (DLP)-based 3D bioprinting technology for ski regeneration (**Figure 2**) [52]. Bhise et al. used GelMA to carry out Hep G2/C3A cells to prepare biomimetic 3D liver structure hydrogel through bioprinting technology. A bionic human body chip of liver tissue was prepared by bioreactor. The toxicity response test of this chip in the test of acetaminophen is similar to that reported *in vivo* and other *in vitro* models, so this provides conceptual proof that the liver biomimetic human chip can be used in vitro drug toxicity screening experiments [53].

Hyaluronic acid (HA) is a kind of biocompatible non-sulfated glycosaminoglycan composed of N-acetylglucosamine and D-glucuronic acid repeated disaccharide units [54]. It is abundant in tissues including cartilage, neurons and skin. HA is of intrinsic biological importance because it binds to receptors such as CD44, can be degraded by oxidative species and hyaluronidase, and is related to the function and structure of development, wound healing and adult tissues. Because of biocompatibility, biodegradability, and natural biological function, HA hydrogels are widely used in various application fields [55]. Besides, the HA hydrogel can energize cell viability and promote osteoblasts to differentiate into cartilage. Unlike collagen and other proteins, the sequence of HA is different from species and its antigenicity is low, so it is especially promising as an injectable hydrogel.

Several other natural polymers, such as collagen, agarose, carrageenan, fibrin, heparin, chondroitin sulfate, cellulose, hemicellulose, lignin, and so on, could be used for hydrogels using 3D bioprinting [21]. However, natural hydrogels lack adequate mechanical properties, especially when implanted *in vivo* for a long time. Because of the uncontrollable swelling in physiological water environment, the mechanical stability of scaffolds tends to decrease. Thus, the chemical modification on natural polymers would be necessary to improve their printability as bioink, and the pending chemical groups after medication will improve the mechanical properties of construct after 3D bioprinting.

3.2 Synthetic polymers

The hydrogels fabricated using synthetic polymers have the advantages of long service life, strong water absorption, and high gel strength [41]. Polyacrylamide (PA) is a general designation of acrylamide homopolymer and copolymer. PA is a kind of water-soluble polymer, which has many amide groups in its structure and is easy to form hydrogen bond, so it has good stability and flocculation and is easy to be chemically modified. Ahn et al. grafted poly (N-isopropylacrylamide) (PNIPAAm) onto the framework of sodium alginate and synthesized sodium alginate PNIPAAm polymer micelles by self-assembly in aqueous solution, and the micelles could be used for the encapsulation of anticancer drug adriamycin [56]. Polyethylene glycol

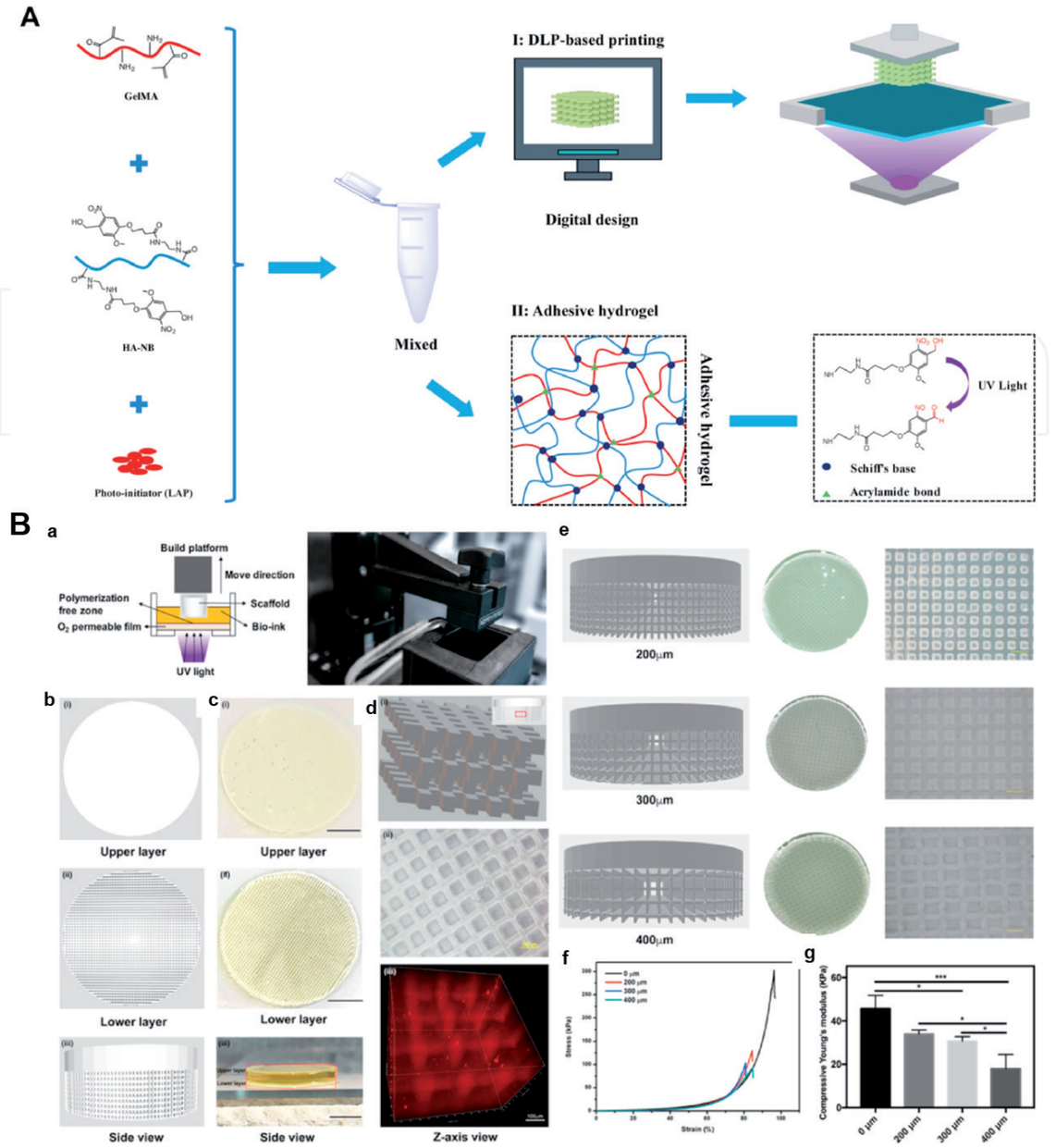

Figure 2.
(A) Fabrication of rapid gelation and tough GelMA/HA-NB/LAP hydrogel for DLP-based printing. (B) the skin analogous with sophisticated two-layer gel structure was fabricated via 3D bioprinting. (a) the bioink was printed with a layer-by-layer style using a DLP-based 3D printer. (b, c) the structure of native skin was displayed in CAD images. (d) the lower layer view of the scaffold was shown. (e) CAD images of different designed microchannel size and the printed products. (f) the elastic compressibility of products. (g) Compressive Young's modulus [52].

(PEG) is another synthetic polymer, and it has no toxicity and irritation, has good biocompatibility, and can be discharged from the body through the kidney. It has been widely used in the field of biomedicine [57]. Gao et al. constructed the polyethylene glycol diacrylate (PEGDA) hydrogel with uniform distribution of human mesenchymal stem cells (hMSCs) inside by simultaneous photopolymerization with commercial thermojet printers. hMSCs filled in 3D PEGDA hydrogel showed no deposition during culture and showed a chondrogenic phenotype [58]. Wang et al. prepared an injectable hydrogel through *in situ* Michael addition reaction between tetraniline-polyethylene glycol diacrylate (TA-PEG) and thiol hyaluronic acid (HA-SH), which was used to carry adipose-derived stem cells (ADSCs) [59].

Polylactic acid (PLA) is a kind of polymer, which is made of lactic acid as the primary raw material, and through polymerization, in which the performance can be adjusted by the structure [60]. Senatov et al. prepared PLA/hyaluronic acid

(HA) interconnected porous scaffold via a melt-wire method; the 3D printing technique avoided thermal degradation of PLA, the porosity and pore size of the scaffold could be well controlled. The porous PLA/HA scaffold with 15% HA has a considerable crack resistance and can work for a long time under the stress of 21 MPa, which was potential for bone tissue engineering applications [61].

Polyvinyl alcohol (PVA) is a synthetic water-soluble polymer, it has good biodegradation, biocompatibility, and no side effects on the human body [62]. PVA has been widely used in ophthalmology, wound dressing, artificial joint, and so on [42, 63]. Shi et al. prepared an injectable dynamic hydrogel using HA grafted with PVA and phenyl boric acid (PBA). The synthesized HA-PBA-PVA dynamic hydrogel has the reactive oxygen species reactivity and the scavenging activity of active oxygen. Furthermore, the hydrogel had good biocompatibility to the encapsulated neural precursor cells (NPC), and its ability to scavenge reactive oxygen species could protect the NPC cells from the damage of reactive oxygen species. The HA-PBA-PVA hydrogel could be used as bioink for 3D biological printing to prepare multilayer and cell loaded structures. The NPC cells showed good viability (85 ± 2% of living cells) after extrusion and maintained the excellent viability of 81 ± 2% of living cells after 3 days of culture. The results indicated that multifunctional injectable and ROS responsive self-healing HA-PBA-PVA dynamic hydrogels were expected to be candidates for 3D culture and 3D bioprinting [64].

Besides, there are also many other synthetic polymers for the fabrication of bio-inspired hydrogels, such as Pluronic and derivatives, PEG or polyethylene oxide (PEO) based block copolymers, poly(L-glutamic acid), poly(propylene fumarate), methoxy polyethylene glycol, and so on. Though, the synthetic polymers can precisely control their gel structure and properties and have better physical and chemical stability and more raw materials to prepare bio-inspired hydrogels. However, it is necessary to pay attention to the possible biocompatibility of unreacted monomers and residual initiators during the preparation of synthetic polymer materials, and the biocompatibility could be greatly improved via compositing or liking with natural polymers [65–67].

4. Applications of bio-inspired hydrogels using 3D bioprinting

4.1 Tissue regeneration

Tissue regeneration research is aim to develop substitute for damaged or diseased tissues or organs using principles of life science, engineering and medicine synergistically. It is crucial to fabricate the substitute as scaffolds, which is inspired by the natural 3D structure of tissue. The natural ECM regulates essential cellular functions, such as adhesion, migration, proliferation, differentiation and morphogenesis [68]. It is important of mimicking the ECM with dynamic nature using 3D bioprinting techniques, and the bio-inspired hydrogels via such techniques displayed potential applications in tissue regeneration, such as cartilage tissue, vascularized engineered tissue, bone tissue, skin regeneration, heart tissue, aortic valve conduits, muscle-tendon, and so on [69]. For example, Alexander et al. displayed a chemically and mechanically biomimetic filler-free bioink for 3D bioprinting of soft neural tissues, as shown in **Figure 3**. The thiolated Pluronic F-127, dopamine-conjugated (DC) gelatin, and DC hyaluronic acid were used as bioinks via a thiol-catechol reaction and photocuring; the storage modulus of the cured bioinks ranged from 6.7 to 11.7 kPa. The micro-extrusion 3D bioprinting was used to fabricate free-standing cell-laden tissue constructs. The Rodent Schwann

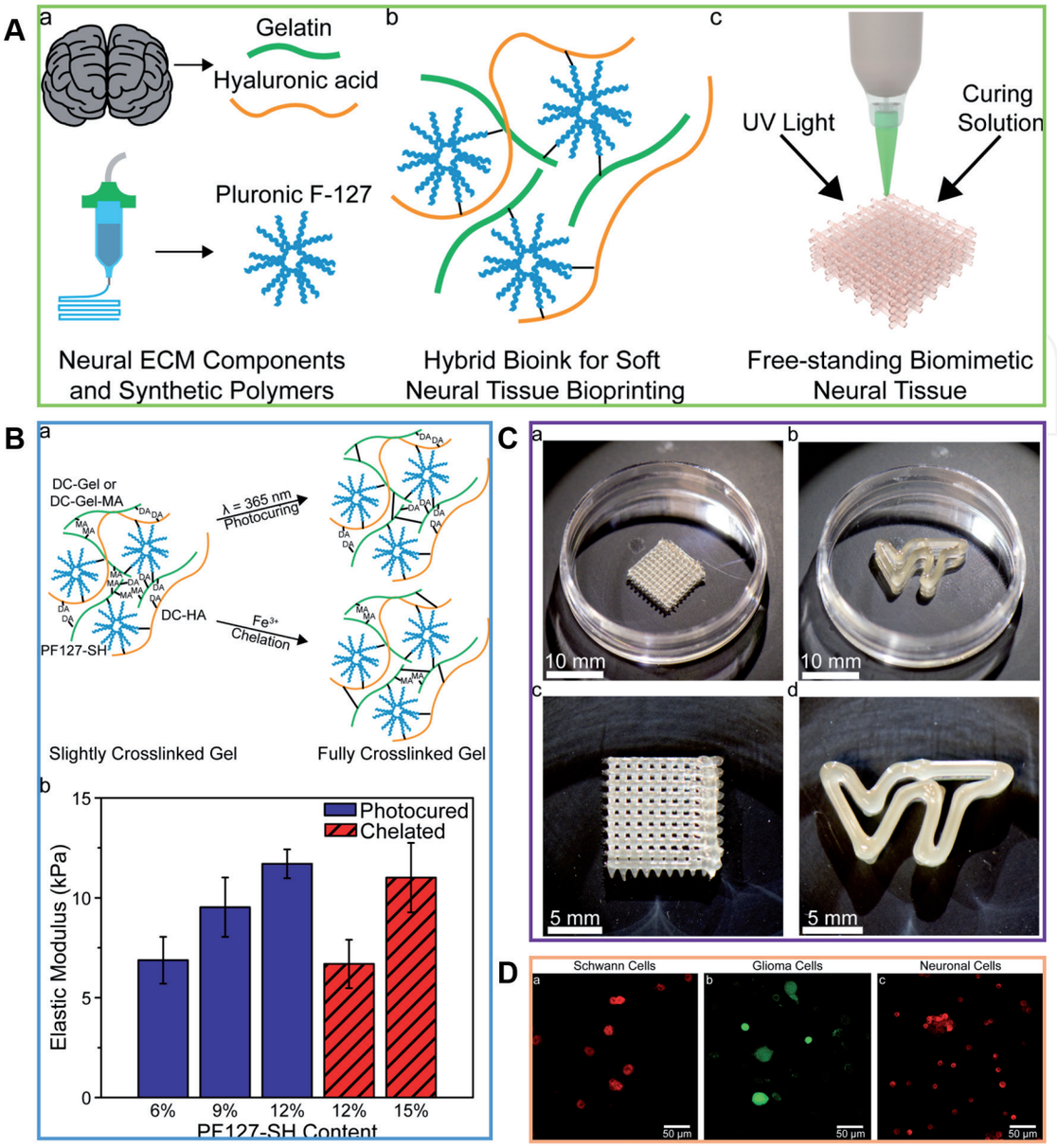

Figure 3.
(A) Native ECM components of neural tissue were combined with a synthetic polymer for microextrusion 3D bioprinting of soft, free-standing neural tissues. (B) Two curing pathways, including UV light exposure, and chelation of dopamine groups with iron (III), are shown to the formulation of photocuring containing methacrylated dopamine-conjugated gelatin. With the increase of PF127-SH content, the compressive properties of inks cured through UV exposure or chelation increased. **(C)** *Printed bioinks are shown. (D) Fluorescence micrographs of 3D bioprinted neural and glial tissue bioink containing rodent Schwann cells (a), human glioma cells (b), and rodent model neuronal cells (c) are shown at day 7 [70].*

cells, rodent neuronal cells, and human glioma cell-laden tissue constructs were printed and cultured over seven days and exhibited excellent viability, which has implications in micro physiological neural systems for neural tissue regenerative medicine [70]. Several works could be found in a recent study that focuses on the specific properties of bio-inspired hydrogels for tissue regeneration, such as high strength structures [30]. Also, the enhancement of printing resolution and versatility is vital for tissue regeneration. For example, the self-healing hydrogels were used to support the direct 3D bioprinting with high resolution by utilizing shear-thinning hydrogels, then the constructs could be printed in any direction [71]. The bio-inspired hydrogels could be accomplished via *in vitro* and *in vivo* 3D bioprinting as for tissue constructs, which are potential and convenient for clinic operation.

4.2 Wound dressing and wearable devices

The bio-inspired hydrogels via 3D bioprinting can be applied for wound dressing and wearable devices, which are considered as important applications, especially in recent years. Skin plays an essential role in protecting the body from external damages, such as abrasions, lacerations, and burns, and so on. The full-thickness defects of the dermis layers are the most challenging wounds to heal because of the limitation of self-repairing capability; thus, the skin regeneration of skin with skin appendages still remains a tough challenge [72]. 3D bioprinting is being applied to fabricate skin constructs using biomaterial scaffolds with or without cells, to address the need for skin tissues suitable for transplantation for wound healing therapy. The natural polymers, including cellulose, collagen and chitin, alginate, and hyaluronic acids are employed to synthesis skin constructs due to the favorable biocompatibility, biodegradation, low-toxicity or nontoxicity, high moisture content, high availability and mechanical stability [73]. Feifei et al. fabricated gelatin methacrylate (GelMA) based bioink to print functional living skin using DLP-based 3D printing (**Figure 2**), while the printed skin could promote skin regeneration and neovascularization via mimicking the physiological structure of natural skin [52].

Furthermore, the bio-inspired hydrogels could not only be functionalized on skin regeneration but also as medical wearable devices. The conductive hydrogels could be designed and fabricated to acquire electronic devices with conductive, capacitive, switching properties, image displaying, and motion sensing [74]. Meihong et al. developed conductive, healable, and self-adhesive hybrid network hydrogels based on conductive functionalized single-wall carbon nanotube (FSWCNT), PVA and polydopamine. The prepared hydrogel exhibits fast self-healing ability around 2 s, high self-healing efficiency of about 99%, and robust adhesiveness, which could be used for healable, adhesive, and soft human-motion sensors [75]. Zijian et al. synthesized a stretchable, self-healing and conductive hydrogel based on gelatin-enhanced hydrophobic association poly(acrylamide-*co*-dopamine) with lithium chloride via physical crosslinking including hydrogen bonding, hydrophobic association, and complexation effect. The hydrogels displayed the stretchability of 1150%, tensile strength of 112 kPa, flexibility and puncture resistance. Also, the hydrogels possess extraordinary conductive property and stable changes in resistance signals [76]. Furthermore, the organogel-hydrogel hybrids have been limelight due to that such kind of hybrids could mimic biological organisms with exceptional freezing tolerance, and thus could provide an advantageous skill to fabricate robust ionic skins [77]. Zhixing developed a series of lauryl acrylate-based polymeric organogels with high transparency, mechanical adaptability, adhesive capability, and self-healing properties; the prepared organogels were expected to provide insights to design the artificial human-like skins with unprecedented functionalities [78]. Due to the delicate structure can be accomplished using 3D bioprinting, bio-inspired hydrogel shows potential applications in medical wearable devices.

4.3 Pharmaceutical applications

The bio-inspired hydrogels could also be used in drug delivery system, such as protein carriers, anti-inflammatory drug carriers, in the pharmaceutical industry [79]. Rana et al. designed a magnetic natural hydrogel based on alginate, gelatin, and iron oxide magnetic nanoparticles as an efficient drug delivery system, the drug doxorubicin hydrochloride (DOX) was loaded, the anticancer activity against Hela cells could be regulated by the release of DOX from hydrogels [80]. Maling et al. provided a proof-of-concept of detoxification using a 3D-printed biomimetic

nanocomposite construct in the hydrogel. A bio-inspired 3D detoxification device by installing polydiacetylene (PDA) nanoparticles in a 3D matrix was fabricated using dynamic optical projection stereolithography (DOPsL) technology; the nanoparticles could attract, capture and sense toxins, while the 3D matrix with a modified liver lobule microstructure allows toxins to be trapped efficiently [36]. The bio-inspired hydrogels via multi-materials 3D bioprinting can easy regulate the loading and release profiles of drugs, which show potentials as biomedicines.

5. Future outlook

The design paradigms shift from 2D to 3D has revolutionized the way of bio-inspired hydrogels for materials components, engineered constructs, *in vitro* disease modeling, medical wearable devices, and precision medicine. 3D bioprinting technology realizes to fabricate the delicate bio-inspired hydrogels with excellent properties and necessary signals to promote healing, tissue regeneration, therapeutics delivery, and health monitor in real-time. However, there are still some issues that need to be addressed in the near future (**Figure 4**). As the researchers begin to scale-up the production of bio-inspired hydrogels, new parameters during the fabrication need be met, such as the bioprinting speeds and resolutions, such parameters need to be simultaneously be increased to create constructs of clinic size. In the near future, it will be essential to develop microscale organ-on-a-chip, such as liver- and heart-on-a-chip, tumor-on-a-chip, etc., that integrate bio-inspired microenvironments with fluid flow inside hydrogels, also other dynamic physiological processes were well regulated by controlling the 3D bioprinting process. For example, the bio-inspired 3D culture in hydrogels could be employed to produce an *in vitro* model of Alzheimer's disease, providing a useful tool for the development of new therapeutics [82]. Future fabrication of bio-inspired hydrogels would be involved with multi-material 3D bioprinting, which provides the ability

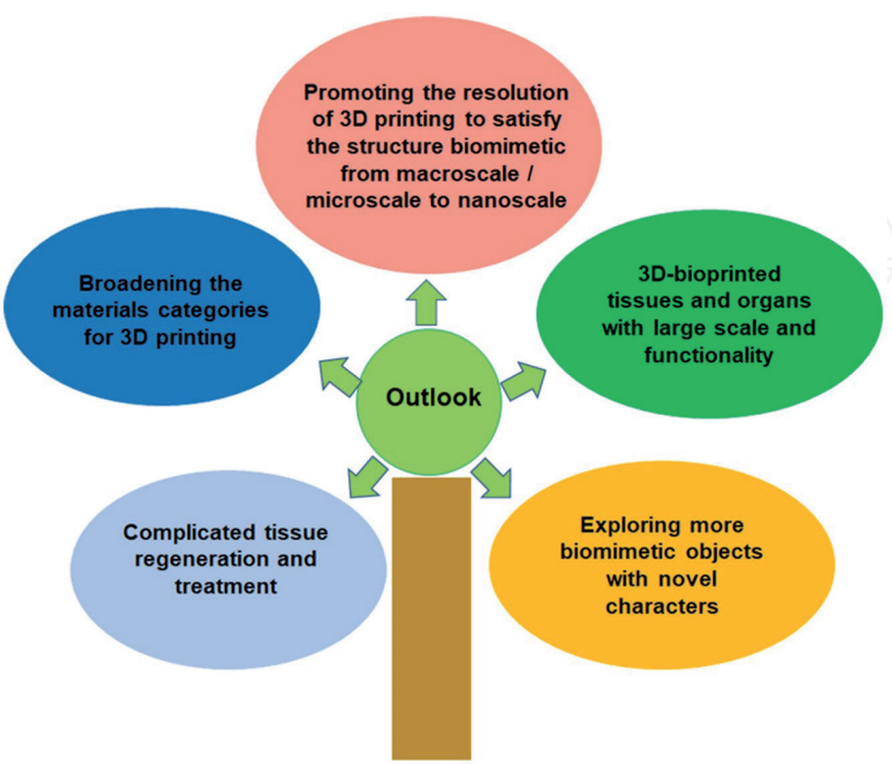

Figure 4.
The future outlook of 3D bioprinting for fabrication of bio-inspired tissues for tissue engineering applications [81].

to deliver growth factors, control cell adhesion, as well as the degradation rate in different regions of the printed constructs. In addition, 3D bioprinting technology needs to overcome vascularization challenge, which is considered a crucial factor in the synthesis of engineered constructs in tissue engineering.

6. Conclusions

The 3D bioprinting has changed the way bio-inspired hydrogels fabricated, and expanded the applications of bio-inspired hydrogels, including tissue regeneration, wound dressing, wearable devices, and pharmaceutical applications, and so on. In this chapter, the available 3D bioprinting techniques were described, the advantages and disadvantages of each printing technology were outlined. Then, the natural and synthetic polymers used for the fabrication of bio-inspired hydrogels via 3D bioprinting were introduced. The applications of bio-inspired hydrogels were focused. At last, the future outlook of bio-inspired hydrogels for tissue engineering were summarized. The bio-inspired hydrogels produced from 3D bioprinting still lacking sufficient clinical evidence, as more clinical trials evaluating bio-inspired hydrogels are still required.

Acknowledgements

This research was funded by the National Natural Science Foundation of China No. 31700840.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ADSCs	adipose derived stem cells
CAD	computer-aided design
CAM	computer-aided manufacturing
CHO	Chinese hamster ovary
DLP	digital light process
DOPsL	dynamic optical projection stereolithography
DOX	doxorubicin hydrochloride
ECM	extracellular matrix
FSWCNT	functionalized single-wall carbon nanotube
GelMA	gelatin methacrylamide
HA	hyaluronic acid
HA-SH	thiol hyaluronic acid
hMSCs	human mesenchymal stem cells
LAP	lithium phenyl-2,4,6-trimethylbenzoylphosphinate
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
NB	N-(2-aminoethyl)-4-(4-(hydroxymethyl)-2-methoxy-5-nitrosophenoxy) butanamide
NPC	neural precursor cells

PA	polyacrylamide
PAAm	polyacrylamide
PAG	photo acid generator
PBA	phenyl boric acid
PDA	polydiacetylene
PEG	polyethylene glycol
PEGDA	polyethylene glycol diacrylate
PEO	polyethylene oxide
PLA	polylactic acid
PNIPAAm	poly (N-isopropylacrylamide)
PVA	polyvinyl alcohol
RGD	arginine-glycine-aspartic-acid
SLA	stereolithography
TA-PEGDA	tetraniline polyethylene glycol diacrylate

Author details

Lei Nie[1*], Can Wang[1], Yaling Deng[2] and Amin Shavandi[3*]

1 College of Life Sciences, Xinyang Normal University, Xinyang, China

2 College of Intelligent Science and Control Engineering, Jinling Institute of Technology, Nanjing, China

3 BioMatter-Biomass Transformation Lab (BTL), Université Libre de Bruxelles, Avenue F.D. Roosevelt, Brussels, Belgium

*Address all correspondence to: nieleifu@yahoo.com, nielei@xynu.edu.cn and amin.shavandi@ulb.ac.be

References

[1] Guarino V, Gloria A, Raucci MG, De Santis R, Ambrosio L. Bio-inspired composite and cell instructive platforms for bone regeneration. International Materials Reviews. 2012;**57**(5):256-275

[2] Feilden E, Ferraro C, Zhang QH, Garcia-Tunon E, D'Elia E, Giuliani F, et al. 3D printing bioinspired ceramic composites. Scientific Reports. 2017;7

[3] Porter MM, Ravikumar N, Barthelat F, Martini R. 3D-printing and mechanics of bio-inspired articulated and multi-material structures. Journal of the Mechanical Behavior of Biomedical Materials. 2017;**73**:114-126

[4] Nie L, Li XC, Wang Z, Hu KH, Cai RH, Li P, et al. In vitro biomineralization on poly(vinyl alcohol)/biphasic calcium phosphate hydrogels. Bioinspired, Biomimetic and Nanobiomaterials. 2020;**9**(2):122-128

[5] Nie L, Deng YL, Li P, Hou RX, Shavandi A, Yang SF. Hydroxyethyl chitosan-reinforced polyvinyl alcohol/biphasic calcium phosphate hydrogels for bone regeneration. ACS Omega. 2020;**5**(19):10948-10957

[6] Green JJ, Elisseeff JH. Mimicking biological functionality with polymers for biomedical applications. Nature. 2016;**540**(7633):386-394

[7] Wegst UGK, Bai H, Saiz E, Tomsia AP, Ritchie RO. Bioinspired structural materials. Nature Materials. 2015;**14**(1):23-36

[8] Ortiz C, Boyce MC. Materials science - bioinspired structural materials. Science. 2008;**319**(5866):1053-1054

[9] Hou RX, Wang YX, Han JL, Zhu YB, Zhang H, Zhang LB, et al. Structure and properties of PVA/silk fibroin hydrogels and their effects on growth behavior of various cell types. Materials Research Express. 2020;7(1).

[10] Munch E, Launey ME, Alsem DH, Saiz E, Tomsia AP, Ritchie RO. Tough, bio-inspired hybrid materials. Science. 2008;**322**(5907):1516-1520

[11] Wang W, Zhang YY, Liu WG. Bioinspired fabrication of high strength hydrogels from non-covalent interactions. Progress in Polymer Science. 2017;**71**:1-25

[12] Feng S, Liu JM, Ramalingam M. 3D printing of stem cell responsive ionically-crosslinked polyethylene glycol diacrylate/alginate composite hydrogels loaded with basic fibroblast growth factor for dental pulp tissue engineering: A preclinical evaluation in animal model. Journal of Biomaterials and Tissue Engineering. 2019;**9**(12):1635-1643

[13] Kim BS, Kwon YW, Kong JS, Park GT, Gao G, Han W, et al. 3D cell printing of in vitro stabilized skin model and in vivo pre-vascularized skin patch using tissue-specific extracellular matrix bioink: A step towards advanced skin tissue engineering. Biomaterials. 2018;**168**:38-53

[14] Xu T, Zhao WX, Zhu JM, Albanna MZ, Yoo JJ, Atala A. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. Biomaterials. 2013;**34**(1):130-139

[15] Vijayavenkataraman S, Yan WC, Lu WF, Wang CH, Fuh JYH. 3D bioprinting of tissues and organs for regenerative medicine. Advanced Drug Delivery Reviews. 2018;**132**:296-332

[16] Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nature Biotechnology. 2014;**32**(8):773-785

[17] Dolatshahi-Pirouz A, Nikkhah M, Gaharwar AK, Hashmi B, Guermani E, Aliabadi H, et al. A combinatorial

cell-laden gel microarray for inducing osteogenic differentiation of human mesenchymal stem cells. Scientific Reports. 2014;**4**

[18] Horvath L, Umehara Y, Jud C, Blank F, Petri-Fink A, Rothen-Rutishauser B. Engineering an in vitro air-blood barrier by 3D bioprinting. Scientific Reports. 2015;**5**

[19] Liaw CY, Ji S, Guvendiren M. Engineering 3D Hydrogels for Personalized In Vitro Human Tissue Models. Advanced Healthcare Materials. 2018;7(4).

[20] Ng WL, Lee JM, Zhou MM, Yeong WY. Hydrogels for 3-D bioprinting-based tissue engineering. Woodh Publ Ser Biom. 2020:183-204

[21] Shavandi A, Hosseini S, Okoro OV, Nie L, Eghbali Babadi F, Melchels F. 3D Bioprinting of Lignocellulosic Biomaterials. Advanced Healthcare Materials.n/a(n/a):2001472.

[22] Xu T, Olson J, Zhao WX, Atala A, Zhu JM, Yoo JJ. Characterization of cell constructs generated with inkjet printing technology using in vivo magnetic resonance imaging. J Manuf Sci E-T Asme. 2008;130(2).

[23] Kim JI, Kim CS. Nanoscale resolution 3D printing with pin-modified electrified inkjets for tailorable Nano/macrohybrid constructs for tissue engineering. ACS Applied Materials & Interfaces. 2018;**10**(15):12390-12405

[24] Derby B. Bioprinting: Inkjet printing proteins and hybrid cell-containing materials and structures. Journal of Materials Chemistry. 2008;**18**(47):5717-5721

[25] Pereira RF, Bartolo PJ. 3D bioprinting of photocrosslinkable hydrogel constructs. Journal of Applied Polymer Science. 2015;132(48).

[26] Compaan AM, Christensen K, Huang Y. Inkjet bioprinting of 3D silk fibroin cellular constructs using sacrificial alginate. Acs Biomaterials Science & Engineering. 2017;**3**(8): 1519-1526

[27] Cui XF, Gao GF, Qiu YJ. Accelerated myotube formation using bioprinting technology for biosensor applications. Biotechnology Letters. 2013;**35**(3):315-321

[28] Mandrycky C, Wang ZJ, Kim K, Kim DH. 3D bioprinting for engineering complex tissues. Biotechnology Advances. 2016;**34**(4):422-434

[29] Guillotin B, Souquet A, Catros S, Duocastella M, Pippenger B, Bellance S, et al. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomaterials. 2010;**31**(28): 7250-7256

[30] Derakhshanfar S, Mbeleck R, Xu KG, Zhang XY, Zhong W, Xing M. 3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances. Bioactive Materials. 2018;**3**(2):144-156

[31] He Y, Yang FF, Zhao HM, Gao Q, Xia B, Fu JZ. Research on the printability of hydrogels in 3D bioprinting. Scientific Reports. 2016;**6**

[32] Duan B, Hockaday LA, Kang KH, Butcher JT. 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. Journal of Biomedical Materials Research. Part A. 2013;**101**(5):1255-1264

[33] Jammalamadaka U, Tappa K. Recent Advances in Biomaterials for 3D Printing and Tissue Engineering. Journal of Functional Biomaterials. 2018;9(1).

[34] Cui HT, Nowicki M, Fisher JP, Zhang LG. 3D Bioprinting for Organ Regeneration. Advanced Healthcare Materials. 2017;6(1).

[35] Wang ZJ, Kumar H, Tian ZL, Jin X, Holzman JF, Menard F, et al. Visible light Photoinitiation of cell-adhesive gelatin Methacryloyl hydrogels for stereolithography 3D bioprinting. ACS Applied Materials & Interfaces. 2018;**10**(32):26859-26869

[36] Gou ML, Qu X, Zhu W, Xiang ML, Yang J, Zhang K, et al. Bio-inspired detoxification using 3D-printed hydrogel nanocomposites. Nature Communications. 2014;**5**

[37] Arcaute K, Mann BK, Wicker RB. Practical use of hydrogels in stereolithography for tissue engineering applications. Stereolithography: Materials, Processes and Applications. 2011:299-331

[38] Valentin TM, Leggett SE, Chen PY, Sodhi JK, Stephens LH, McClintock HD, et al. Stereolithographic printing of ionically-crosslinked alginate hydrogels for degradable biomaterials and microfluidics. Lab on a Chip. 2017;**17**(20):3474-3488

[39] Skardal A, Atala A. Biomaterials for integration with 3-D bioprinting. Annals of Biomedical Engineering. 2015;**43**(3):730-746

[40] Ahmed EM, Aggor FS, Awad AM, El-Aref AT. An innovative method for preparation of nanometal hydroxide superabsorbent hydrogel. Carbohydrate Polymers. 2013;**91**(2):693-698

[41] Ahmed EM. Hydrogel: Preparation, characterization, and applications: A review. Journal of Advanced Research. 2015;**6**(2):105-121

[42] Nie L, Zhang GH, Hou RX, Xu HP, Li YP, Fu J. Controllable promotion of chondrocyte adhesion and growth on PVA hydrogels by controlled release of TGF-beta 1 from porous PLGA microspheres. Colloids and Surfaces B: Biointerfaces. 2015;**125**:51-57

[43] Zhou Q J, Wang TW, Wang C, Wang Z, Yang YA, Li P, et al. Synthesis and characterization of silver nanoparticles-doped hydroxyapatite/alginate microparticles with promising cytocompatibility and antibacterial properties. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2020;**585**

[44] Rastogi P, Kandasubramanian B. Review of alginate-based hydrogel bioprinting for application in tissue engineering. Biofabrication. 2019;11(4).

[45] Nie L, Wang C, Hou RX, Li XY, Sun M, Suo JP, et al. Preparation and characterization of dithiol-modified graphene oxide nanosheets reinforced alginate nanocomposite as bone scaffold. Sn Applied Sciences. 2019;1(6).

[46] Nie L, Wu QY, Long HY, Hu KH, Li P, Wang C, et al. Development of chitosan/gelatin hydrogels incorporation of biphasic calcium phosphate nanoparticles for bone tissue engineering. Journal of Biomaterials Science-Polymer Edition. 2019;**30**(17):1636-1657

[47] Wu QH, Maire M, Lerouge S, Therriault D, Heuzey MC. 3D Printing of Microstructured and Stretchable Chitosan Hydrogel for Guided Cell Growth. Advanced Biosystems. 2017;1(6).

[48] Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N, Khademhosseini A. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials. 2015;**73**:254-271

[49] Klotz BJ, Gawlitta D, Rosenberg AJWP, Malda J, Melchels FPW. Gelatin-Methacryloyl hydrogels: Towards biofabrication-based tissue repair. Trends in Biotechnology. 2016;**34**(5):394-407

[50] Lewis PL, Green RM, Shah RN. 3D-printed gelatin scaffolds of differing pore geometry modulate hepatocyte

function and gene expression. Acta Biomaterialia. 2018;**69**:63-70

[51] Lin RZ, Chen YC, Moreno-Luna R, Khademhosseini A, Melero-Martin JM. Transdermal regulation of vascular network bioengineering using a photopolymerizable methacrylated gelatin hydrogel. Biomaterials. 2013; **34**(28):6785-6796

[52] Zhou FF, Hong Y, Liang RJ, Zhang XZ, Liao YG, Jiang DM, et al. Rapid printing of bio-inspired 3D tissue constructs for skin regeneration. Biomaterials. 2020;**258**

[53] Bhise NS, Manoharan V, Massa S, Tamayol A, Ghaderi M, Miscuglio M, et al. A liver-on-a-chip platform with bioprinted hepatic spheroids. Biofabrication. 2016;8(1).

[54] Larraneta E, Henry M, Irwin NJ, Trotter J, Perminova AA, Donnelly RF. Synthesis and characterization of hyaluronic acid hydrogels crosslinked using a solvent-free process for potential biomedical applications. Carbohydrate Polymers. 2018;**181**:1194-1205

[55] Highley CB, Prestwich GD, Burdick JA. Recent advances in hyaluronic acid hydrogels for biomedical applications. Current Opinion in Biotechnology. 2016;**40**:35-40

[56] Ahn DG, Lee J, Park SY, Kwark YJ, Lee KY. Doxorubicin-loaded alginate-g-poly(N-isopropylacrylamide) micelles for cancer imaging and therapy. ACS Applied Materials & Interfaces. 2014;**6**(24):22069-22077

[57] Zou P, Suo JP, Nie L, Feng SB. Temperature-responsive biodegradable star-shaped block copolymers for vaginal gels. Journal of Materials Chemistry. 2012;**22**(13):6316-6326

[58] Gao GF, Hubbell K, Schilling AF, Dai GH, Cui XF. Bioprinting Cartilage Tissue from Mesenchymal Stem Cells and PEG Hydrogel. 3d Cell Culture: Methods and Protocols. 2017;1612:391-8.

[59] Wang W, Tan BY, Chen JR, Bao R, Zhang XR, Liang S, et al. An injectable conductive hydrogel encapsulating plasmid DNA-eNOs and ADSCs for treating myocardial infarction. Biomaterials. 2018;**160**:69-81

[60] Inkinen S, Hakkarainen M, Albertsson AC, Sodergard A. From lactic acid to poly(lactic acid) (PLA): Characterization and analysis of PLA and its precursors. Biomacromolecules. 2011;**12**(3):523-532

[61] Senatov FS, Niaza KV, Stepashkin AA, Kaloshkin SD. Low-cycle fatigue behavior of 3d-printed PLA-based porous scaffolds. Compos Part B-Eng. 2016;**97**:193-200

[62] Wang TW, Zhang F, Zhao R, Wang C, Hu KH, Sun Y, et al. Polyvinyl alcohol/sodium alginate hydrogels incorporated with silver nanoclusters via Green tea extract for antibacterial applications. Designed Monomers and Polymers. 2020;**23**(1):118-133

[63] Nie L, Chen D, Suo JP, Zou P, Feng SB, Yang Q , et al. Physicochemical characterization and biocompatibility in vitro of biphasic calcium phosphate/ polyvinyl alcohol scaffolds prepared by freeze-drying method for bone tissue engineering applications. Colloids and Surfaces B: Biointerfaces. 2012;**100**:169-176

[64] Shi W, Hass B, Kuss MA, Zhang HP, Ryu SJ, Zhang DZ, et al. Fabrication of versatile dynamic hyaluronic acid-based hydrogels. Carbohydrate Polymers. 2020;**233**

[65] Li HJ, Tan C, Li L. Review of 3D printable hydrogels and constructs. Materials & Design. 2018;**159**:20-38

[66] Zou P, Suo JP, Nie L, Feng SB. Temperature-sensitive biodegradable

mixed star-shaped block copolymers hydrogels for an injection application. Polymer. 2012;**53**(6):1245-1257

[67] Nie L, Zou P, Feng SB, Suo JP. Temperature-sensitive star-shaped block copolymers hydrogels for an injection application: Phase transition behavior and biocompatibility. Journal of Materials Science-Materials in Medicine. 2013;**24**(3):689-700

[68] Dvir T, Timko BP, Kohane DS, Langer R. Nanotechnological strategies for engineering complex tissues. Nature Nanotechnology. 2011;**6**(1):13-22

[69] Antich C, de Vicente J, Jimenez G, Chocarro C, Carrillo E, Montanez E, et al. Bio-inspired hydrogel composed of hyaluronic acid and alginate as a potential bioink for 3D bioprinting of articular cartilage engineering constructs. Acta Biomaterialia. 2020;**106**:114-123

[70] Haring AP, Thompson EG, Tong YX, Laheri S, Cesewski E, Sontheimer H, et al. Process- and bio-inspired hydrogels for 3D bioprinting of soft free-standing neural and glial tissues. Biofabrication. 2019;11(2).

[71] Highley CB, Rodell CB, Burdick JA. Direct 3D Printing of Shear-Thinning Hydrogels into Self-Healing Hydrogels. Advanced Materials. 2015;27(34):5075–+.

[72] Shi LY, Zhao YN, Xie QF, Fan CX, Hilborn J, Dai JW, et al. Moldable Hyaluronan Hydrogel Enabled by Dynamic Metal-Bisphosphonate Coordination Chemistry for Wound Healing. Advanced Healthcare Materials. 2018;7(5).

[73] He P, Zhao JN, Zhang JM, Li B, Gou ZY, Gou ML, et al. Bioprinting of skin constructs for wound healing. Burns & Trauma. 2018;**6**

[74] Tai YL, Mulle M, Ventura IA, Lubineau G. A highly sensitive, low-cost, wearable pressure sensor based on conductive hydrogel spheres. Nanoscale. 2015;**7**(35):14766-14773

[75] Liao MH, Wan PB, Wen JR, Gong M, Wu XX, Wang YG, et al. Wearable, Healable, and Adhesive Epidermal Sensors Assembled from Mussel-Inspired Conductive Hybrid Hydrogel Framework. Advanced Functional Materials. 2017;27(48).

[76] Gao ZJ, Li YF, Shang XL, Hu W, Gao GH, Duan LJ. Bio-inspired adhesive and self-healing hydrogels as flexible strain sensors for monitoring human activities. Materials Science and Engineering: C. 2020;**106**

[77] Gao HN, Zhao ZG, Cai YD, Zhou JJ, Hua WD, Chen L, et al. Adaptive and freeze-tolerant heteronetwork organohydrogels with enhanced mechanical stability over a wide temperature range. Nature Communications. 2017;**8**

[78] Zhang ZX, Wang L, Yu HT, Zhang F, Tang L, Feng YY, et al. Highly transparent, self-healable, and adhesive Organogels for bio-inspired intelligent ionic skins. ACS Applied Materials & Interfaces. 2020;**12**(13):15657-15666

[79] Kim S, Park CB. Bio-inspired synthesis of minerals for energy, environment, and medicinal applications. Advanced Functional Materials. 2013;**23**(1):10-25

[80] Jahanban-Esfahlan R, Derakhshankhah H, Haghshenas B, Massoumi B, Abbasian M, Jaymand M. A bio-inspired magnetic natural hydrogel containing gelatin and alginate as a drug delivery system for cancer chemotherapy. International Journal of Biological Macromolecules. 2020;**156**:438-445

[81] Li T, Chang J, Zhu YF, Wu CT. 3D printing of bioinspired biomaterials

for tissue regeneration. Advanced Healthcare Materials. 2020

[82] Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, et al. A three-dimensional human neural cell culture model of Alzheimer's disease. Nature. 2014;**515**(7526):274-U93