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Review article

Bioinks and bioprinting: A focused review

Mohammadmahdi Mobaraki [a], Maryam Ghaffari [a], Abolfazl Yazdanpanah [a], Yangyang Luo [b], D.K. Mills [c,*]

[a] Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
[b] Molecular Science and Nanotechnology Interdisciplinary Program, Louisiana Tech University, Ruston, LA, 71272, USA
[c] Center for Biomedical Engineering and School of Biological Sciences, Louisiana Tech University, Ruston, LA, 71272, USA

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ABSTRACT

Bioprinting is a process based on additive manufacturing that uses biomaterials as the microenvironment for living cells. These materials often referred to as bioinks, are based on cytocompatible hydrogel precursors, which gel in a manner compatible with different bioprinting approaches. Hydrogels are highly hydrated three-dimensional (3D) networks of cross-linked hydrophilic polymer chains and have been widely explored for use as bioactive delivery agents, cell carriers, consumer products, tissue engineering scaffolds and for wound healing. Hydrogels can be tailored for different chemical, electrical, mechanical, and thermal properties and can even be made to conduct electricity. Recent trends in bioink development have focused on incorporating carbon-based nanomaterials, clay nanomaterials, ceramics, fibers, and growth factors to create hybrid and multi-composite hydrogels. The goal of this review paper is to examine the synergies resulting from the combination of these materials. This review will feature recent advances in this field, focusing on bioink developments and bioprinting applications, and the use of natural and synthetic additives that impart unique, novel, or critical functionalities.

1. Introduction

Three-dimensional (3D) bioprinting is a multidisciplinary and rapidly growing technology that aims to combine engineering principles and life sciences for the fabrication of tissue and organ constructs by selectively depositing biological materials, biochemical and living cells, typically in a layer by layer fashion. In this way, computational science, material science, and biology are integrated with the design and construction of bioengineered tissues. Groll et al. redefined bioprinting as a bio-fabrication method in which cells, bioactive molecules, biomaterials, or cell-aggregates are printed to fabricate a construct [1]. Before the advent of bioprinting, classical tissue engineering, in situ tissue engineering and cell therapy pioneered research in regenerative medicine. 3D printing makes it possible to create 3D functioning tissues from personalized, specific computer-aided designs. However, there are limitations to this approach, and these are related to the process parameters, cell viability concerns, and long-term functionality (see Table 1).

Bioprintable materials used in the 3D bioprinting process are known as bioinks. A wide range of biomaterials have been used in regenerating damaged or diseased tissues, but the vast majority of them are not compatible with existing bioprinting technologies. For instance, biomaterials, which needs high temperature or organic solvents in their printing process, are not suitable for live-cell printing [2]. Bioinks can be categorized into two main types: scaffold-free bioinks and scaffold-based bioinks. In scaffold-free bioinks, embryonic development mimics the formation of a neo-tissue. Tissue spheroids, cell pellets, and tissue strands are used in this approach for the fabrication of large-scale functional tissue [3]. Scaffold-based bioinks contain cells loaded in hydrogels, microcarriers or decellularized matrix compounds. Various bioprinting strategies have been used to achieve print fidelity and resolution, and these are summarized in Fig. 1.

This paper will summarize and critically assess current bioprinting methods with a specific focus on bioinks and also provide commentary on the current state of the art in scaffold-based bioinks.

1.1. Inkjet-based bioprinting

One of the oldest printing methods that still retains great promise for use in 3D printing of biological and non-biological applications are inkjet printers (also known as drop-on-demand printing, drop-by-drop or drop-on-demand bioprinting) [4]. Inkjet printing is a noncontact reprographic strategy, and it is based on the deposition of bioink drops [5]. Bioinks are

* Corresponding author.
E-mail address: dkmills@latech.edu (D.K. Mills).

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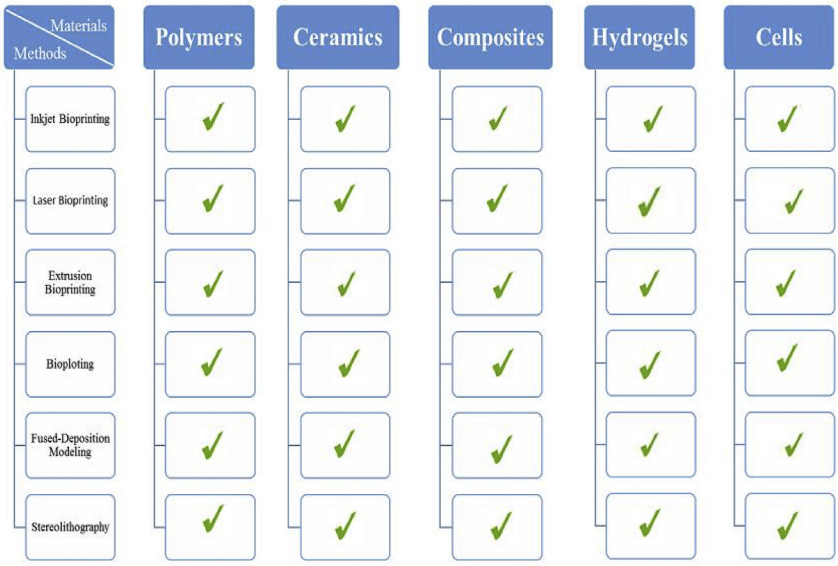
Table 1
Overview of bioprinting methods in regard to choice of materials.



natural or human-made materials that mimic an extracellular matrix environment and support the adhesion, proliferation, and differentiation of mammalian cells.

Fabrication strategies for generating ink droplets rely on three different methods. These include piezoelectric inkjet (acoustic) [6,7], thermal inkjet [8–11], and electrostatic bioprinting [12]. Beside non-living materials, droplets of encapsulated cells can also be printed and assembled into a construct layer by layer [13]. Piezoelectric bioprinters generate acoustic waves within the bioink chamber using a piezoelectric actuator that ejects droplets through the printer nozzle [1]. The thermal method contains a fluid chamber and single or multiple nozzles. Within the bioink chamber, heat is generated and induces pulses of pressure [2]. The pressure results in the ejection of picoliter volume of droplets at the nozzle orifice [3]. In electrostatic bioprinters, droplets are generated by voltage pulses, which are applied between a pressure plate and an electrode [4].

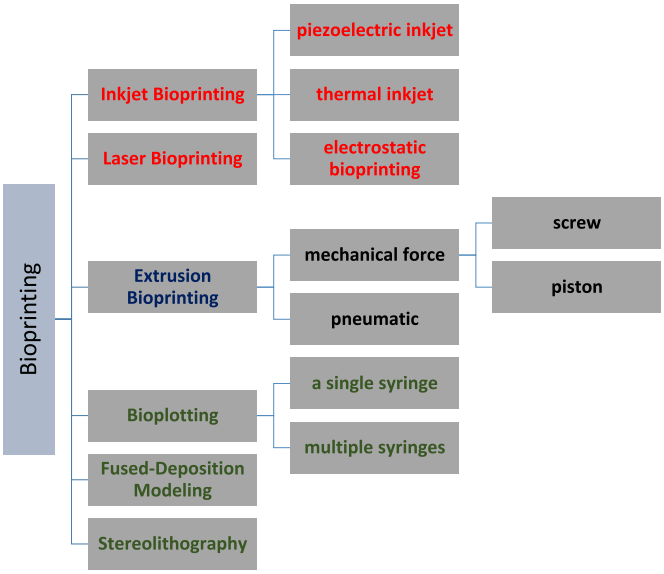


Fig. 1. Classification of bioprinting methods with targeted biomedical application.

In recent years, there has been considerable interest in using droplet bioprinters because of ease in replacing bioinks for the traditional inks, an acceptable speed, high cell viability, low expense, and compatibility with nonliving and living materials [5]. These strategies also some limitations in tissue biofabrication, especially the use of high viscosity materials (>10 cP) because the print heads have very small nozzle openings through which small droplets are ejected. Additionally, a high cell density can cause clogging of the nozzle that creates unwanted pressure issues [6,7]. Furthermore, as droplet bioprinters typically use low viscosity bioinks, the mechanical strength of printed structures are generally inferior when compared to the target tissue [8].

1.2. *Laser-based bioprinting*

Laser-based bioprinting, also known as Biological Laser Printing, is a method for patterning live cells in the fabrication of tissue constructs. This strategy of printing is based on a long-wavelength laser or high energy light source [18,19]. In this method, cells are printed by a laser beam pulsating at controlled rates [20] onto a receiving substrate. A typical laser bioprinting device consists of a pulsed laser beam, a focusing system, a laser absorbing ribbon, a receiving substrate, and a cell-containing material [21,22]. Several parameters influence the resolution of laser bioprinting including surface tension, the air gap between the substrate and the ribbon, the wettability of the substrate, the thickness and viscosity of the organic layer, and the laser type and configuration [23]. Unlike inkjet printing limitations, laser-based bioprinting can print a range of viscosities (1–300 MPa/s) materials [24]. Besides, laser bioprinting is nozzle-free, and therefore, a high density of cells can be loaded high without clogging. Also, laser bioprinting has the ability to printing mammalian cells without significant adverse effects on cell functions and viability. It is essential to the point that this approach is not a standard method for inkjet printing and laser-based bioprinting [4].

1.3. *Extrusion-based bioprinting*

Extrusion bioprinting, also known as direct writing, is one of the 3D printing methods which has received significant interest in the world of tissue engineering and biofabrication [9]. The ink used in extrusion bioprinting is dispensed by mechanical force (screw or piston), or a pneumatically (via a gas or pressurized air), and it is extruded

continuously as a strand. The viscosity of bioinks for extrusion-based bioprinting ranges from 30 to 6×10^7 mPas [5]. Extrusion bioprinting generally consists of a dispenser (single ejector or multiple ejectors) systems that are placed on an automated robotic stage and controlled by a stage controller. The robotic scene has three-axes (x–y–z) [25]. In this method, a bioink (with encapsulated cells) are deposited on a building substrate that is placed directly below the dispenser [10]. The significant advantages of this type of printing method are its compatibility for printing materials with an extensive range of viscosities, bioinks with high cell densities, specialty hydrogels and biodegradable thermoplastics such as polycaprolactone [26]. Furthermore, it is a fast method with an acceptable cell viability after printing [27]. Extrusion bioprinting also decreases the risk of clogging in comparison with inkjet printing, but the structure also has a low fabrication resolution (~200 μm) [25]. Moreover, during ink deposition, shear stresses and bioink deformation may reduce cell viability [5].

1.4. Bioplotting

Bioplotting is another 3D printing method that is used for in tissue engineering and biofabrication. Because of bioplotting's versatility, it offers many exciting and new opportunities for biofunctional rapid prototyping [10]. Bioplotting employs a syringe to extrude either tubes or spheroids of materials. These kinds of printers usually have several syringes and can employ multiple cell types and fabrication conditions. The result of using several syringes enables the construction of multiple tissue types in the final construct, which is ideal for producing bioengineered soft tissues. In this system, layers are arranged on top of each other and cured through a chemical reaction or UV radiation [11].

Despite these advantages, the major challenge in bioplotting is choosing the materials for extrusion because the materials have to be viscous, cell supportive, and provide a functional cellular microenvironment. Often, materials like thermoset resins, polymer melts, pastes with high filler contents, cement, polymer solutions, and even macromolecules such as proteins can be used in the bioplotting approach [10]. According to some research, bioplotting is the best methods for generating co-cultured scaffolds and tissues which do not require high-resolution details [11].

1.5. Fused-deposition modeling (FDM)

FDM is the oldest 3D additive manufacturing technology used in rapid prototyping, modeling, and production applications [12]. This approach plays a significant role in improving dimensional precision, the quality of products, reduced production times, and cost of biofabrication [13]. This method of printing deposits a melted thermoplastic in thin layers that provides support for hydrogel-based bioinks. This process begins with a CAD model. The model produces by melting materials into a liquid state in a liquefier head to form layers of selectively deposited materials through a nozzle [12].

Several parameters can affect the resolution of FDM constructs like nozzle diameter and kind of materials. For having thick filament have to work into raw materials, and there are a small minority of materials can be employed for FDM technology [14]. Optimization of process parameters is one of the vital essential design tasks in FDM. Usually, for obtaining high-quality structure, the main research area is directed towards improving surface roughness, mechanical properties, material behavior, building time, and dimensional accuracy [12]. One of the significant limitations of this method is the lack of mechanical strength of molten thermoplastic because it cannot support itself during slow cooling and hardening [31,32].

1.6. Stereolithography (SL)

Stereolithography was introduced in the late 1980s. It is a reliable freeform technique which uses light to cross-link polymeric materials [33,34]. Most stereolithography techniques use a laser (commonly UV light) and a directed mirror array to project a light beam onto the surface of the liquid photocurable resin. After the resin is cured, the fabrication platform moves in the z-direction, and then a fresh layer of resin is added, and the process repeated to build up a 3D construct. Stereolithography has become an accepted method because of its ability to prepare structures with high resolution and the ability to remove uncured resin from the final product. Despite these advantages, this approach is slow, and the resins used are non-biomimetic. Materials scientists are actively working to develop resins that are appropriate for use in tissue engineering applications [11]. Visible light-based stereolithography is a rapidly developing approach in bioprinting in which visible light crosslinks bioinks have been introduced for stabilizing structure and other applications [15–17].

1.7. Limitations of existing 3D printers

Although different additive manufacturing techniques (inkjet, laser, extrusion, bioplotting, FDM, and SL) have tremendous improvement in tissue engineering, they have many weak points, as a result; research on this subject has continued to explore 3D print techniques that would have the ability to print 3D networks and show great promise for tissue regeneration. One of the biggest challenges of additive manufacturing techniques is the speed of printing because they can create a 1.5-inch cube in an hour [39]. Another significant limitation of 3D printing is the requirement of a perfusable and highly efficient vascular network. When tissues and implants are printing need nutrient transportation and waste removal because it is essential for cell survival [40].

In all the technologies mentioned above, resolution, vascularization, perfusion, automation, cost, precision, ideal bioinks requirement additional modifications before they can contribute significantly towards bioengineering tissues [38].

2. Bioinks

2.1. Consideration of bioinks for organ fabrication

Originally, additive manufacturing techniques were used for non-biological applications. The most popular materials used as bioinks include ceramics, metals, and thermoplastic polymers. These materials generally require high temperature, organic solvents, or crosslinking agents, and they are not compatible with biological materials and cells. The biggest obstacle to the fabrication of organs or tissues through using 3D printing technology is to achieve mechanical, chemical, and morphological properties similar to real organs and tissues. Therefore, bioinks have the leading role in addressing these properties, and they should protect the cells against the fabrication processes like extrusion, inappropriate environment [18]. An ideal bioink material must have the following properties: printability, high mechanical integrity, and stability, insolubility in the culture medium, similar biodegradability rate with the regeneration of tissue, non-toxicity, and non-immunogenicity, and also cell adhesion promotion properties. Furthermore, bioink materials should be produced quickly and commercially feasible [19] (see Table 2).

2.2. Currently available bioinks

Over the past 16 years, 3D printing technologies have developed rapidly. 3D printing has emerged as a major technology in tissue engineering [20]. Several material types are used as bioinks to form 3D structures including ceramics, polymers, elastomer, hydrogels, and lipids. In order to show the variety of bioinks under investigation, major studies on bioinks are summarized in Table 3. In this section, we will focus on the specific advantages of currently employed biomaterials for bioprinting and also the most commonly used printing methods.

Table 2
Comparison of bioprinting methods.

Materials / Methods	Cost	Speed	Resolution	Mechanical properties	Porosity	Processing modes
Inkjet Bioprinting	Inexpensive	Fast	100 mm	Poor	33-60%	Mechanical , thermal
Laser Bioprinting	Expensive	Medium-fast	30 mm	High	<90%	Optical
Extrusion Bioprinting	Resalable	Relatively fast	141-300 mm	Poor	>40%	Mechanical , thermal, chemical
Bioploting	Resalable	Slow	45-2000μm	Poor	< 45-60%	Mechanical
Fused-Deposition Modeling	Inexpensive	Relatively fast	50-762 μm	Good	40-75%	Mechanical
Stereolithography	Expensive	Relatively slow	75 μm	High	50-80%	Optical

2.2.1. Polymers for bioinks

One of the most common materials used as bioink is polymers. Polymer bioinks are used in bioprinting due to their low cost, biocompatibility, degradation, and secure processing. One of the other advantages of polymer bioinks is the ability to change their form. For example, they can be used as filaments for fused-deposition modeling or powders for laser-bioprinting. Water-soluble polymers known as hydrogels are also used as bioinks for bioprinting, due to their chemical configuration and promising environments for cell growth in 3D [35]. Hydrogels are a group of three-dimensional polymeric networks that can hold a large amount of water. As a result, they are known as biocompatible materials and provide a cell-friendly environment due to high water content and low polymer content [36,37]. Recently, hydrogels have to achieved significant use in a diverse array of biomedical applications, including 3D printing, biosensors, cell encapsulation, and wound dressings [38].

In tissue engineering applications, the fabrication of 2D and 3D hydrogel scaffolds form the critical component in many applications as hydrogels as they are permeable to nutrients, oxygen, and other water-soluble compounds [39]. There are three kinds of hydrogels most commonly used for tissue engineering: natural hydrogels, synthetic hydrogels, and blends of synthetic and natural polymers [40]. Generally, mixing a cell suspension with a precursor solution and crosslinking or polymerizing of the resulting mixture causes cell encapsulation within the hydrogel and can include multiple cell types as well as high-cell-density tissue spheroids and at least one polymer capable of forming a hydrogel. Cell-laden hydrogels are widely used for bioinks as they can be formulated for inkjet, extrusion, laser, and stereolithography methods. Natural polymers consist of short repeating units with reversible and directional secondary interactions that the non-covalent bonding (like hydrogen bonding, π-π, and metal coordination) are the most interactions in these kinds of hydrogels [41]. Under high stress, these non-covalent bonds are reversibly broken to dissipate energy. The reversibility of these bonds also leads to shear-thinning properties that facilitate their application in bioprinting [42]. The essential natural polymers which are used in 3D printing are collagen, fibrin, silk, chitosan, alginate, gelatin, hyaluronic acid, and gelatin methacrylate [43,44].

2.2.1.1. Collagen. Collagen is one of the more attractive polymers and has been extensively studied in tissue engineering applications. It is the main component of musculoskeletal tissue and forms the ECM of most tissues. Indeed, collagen is a triple-helical biocompatible protein with a natural source. Thus collagen scaffolds have minimal immunological reactions [20]. Moreover, collagen can help to increase cells growth, adhesion, and attachment [19] (see Fig. 2).

Though collagen type I has been used in bioprinting, it has limitations. Collagen type I remains in a liquid state at low temperatures and forms a fibrous structure as temperature increases. Complete gelation can take up to 30 min at 37 °C. This slow gelation rate is an obstacle for bioprinting of 3D structures. In another study in 2017, the effect of riboflavin photo-crosslinking and pH on rheological properties and printability of collagen was investigated by Nicole Diamantides et al. [45]. Their results from their pH study showed that the shape fidelity of printed constructs during gelation of collagen bioinks was highly dependent on pH; although, the rate of gelation of collagen bioinks did not affect printability (Fig. 3).

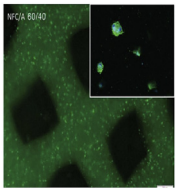
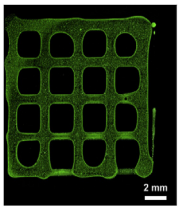
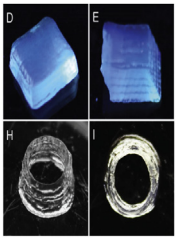
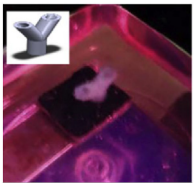
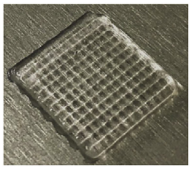
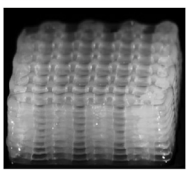
2.2.1.2. Fibrin. Fibrin is one of the essential proteins involved in blood clotting and wound healing [46]. These proteins also have an essential role in neoplasia, cell-matrix interactions, and inflammatory reactions. Fibrinogen is a glycoprotein produced in the liver and its production rate increases after trauma. In the presence of the serine protease thrombin, which released after vascular injuries, fibrinogen is hydrolyzed and polymerizes into fibrin [47].

Usually, fibrin is used as a carrier for growth factors and drugs in drug delivery systems. They are also used in a range of tissue engineering applications as scaffolding materials, particularly vascular grafts [48]. Fibrin gels (gelation time is swift) have attractive features like differentiation, biodegradability, promoting cell growth, angiogenesis, biocompatibility, and tissue regeneration. Other advantages of fibrin are that it has excellent mechanical properties and binding sites for native extracellular matrix proteins and heparin-binding domains with affinity to growth factors [49]. However, fibrin is an expensive material [50].

Hakam et al. utilized fibrin-gelatin hybrid hydrogel as biopaper for application in skin bioprinting [50]. They used this hydrogel an in vivo study, and they could see excellent mechanical properties, cell attachment, and improving biological properties of the structure. Because of presence fibrin, the biopaper helped to reduce wound contrast, wound repair, and wound healing of the rat skin. In another study, England et al. used a novel method for bioprinting fibrin-factor XIII-hyaluronate hydrogel scaffolds [51]. They utilized these scaffolds an in vitro study and encapsulated Schwann cells and could improve nerve regeneration and develop of tissue repair.

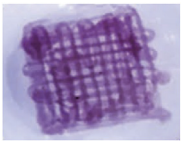
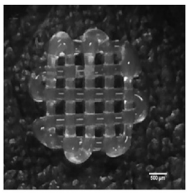
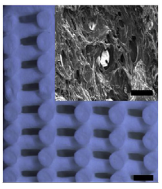
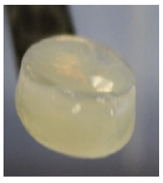
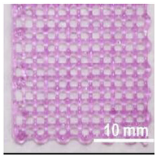
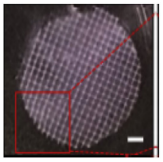
2.2.1.3. Silk. There are many kinds of silk fibers produced by *Bombyx mori* (B. more) and used in tissue engineering, cartilage regeneration, biosensors, small scale catalytic motors, optical waveguides, and strain-gauges for biological applications [52] but in 2017, DeSimone et al. used spider recombinant silk as bioink (41). B. more silk composed of fibroin and sericin that only silk fibroin used for biomedical application

Table 3
Major studies on bioinks are summarized.

Bioink Type	Novelty	Tissue Type(s)	Cells/Growth Factor Type Bioprinted	Key Result (s)	Illustration	REF
Alginate or hyaluronic acid	Nanocellulose hydrogels (NH)	Cartilage	iPS TGFβ1, TGFβ3, GDF5, and BMP2	NH bioink suitable for bioprinting iPSCs leading to cartilage matrix production in a co-culture system		[21]
Methyl-P[nPrOzi50-b-MeOx50]-piperidine-4-carboxylic acid ethyl ester	A Thermogelling Supramolecular Hydrogel with Sponge-Like Morphology hydrogels	-	-	Excellent cytocompatibility NIH 3T3 fibroblasts bioplotted into structured 3D hydrogels without significant loss of cell viability		[22]
Gelatin methacryloyl (GelMA)	Hydrogels	-	-	Showed structural fidelity through bioprinting GPG bioinks into various 3D geometric Constructs showed high porosity, low stiffness could that supported cell survival and enhance cell proliferation		[23]
Hydrogen bonded monomer (N-acryloyl glycinamide) (NAGA)	Nanoclay hydrogels	Bone	–	Promoted osteogenic differentiation of rat osteoblasts. In vitro and in vivo experiments demonstrate that PNAGA-clay scaffolds can promote osteogenic differentiation and bone tissue synthesis.		[24]
Sodium alginate	Gelatin hydrogels	-	Y-shaped tubes immediately	Printing quality can be improved by EAL-assisted laser printing when using various alginate solutions (1%, 2%, and 4%) and cell-laden bioinks		[25]
Hyaluronic acid (HA)	Hydroxyapatite (HAp) hydrogels	Cartilage	Adipose derived mesenchymal stem cells (ADMSC)	HAp content and hypoxia mediate chondrogenesis, hypertrophy, and endochondral ossification of ADMSC. 3D bioprinted multizonal grafts with hard tissue and soft tissue interfaces can be realized.		[26]
Collagen	Polyphenol (tannic acid)/ using core-Shell struts	NA	Human adipose stem cells (hASCs)	Results showed novel bioink and cell-laden structure for regeneration of various tissues		[27]
Thiolene	allyl glycidyl ether-co-glycidoyl and hyaluronic acid hydrogels	NA	NA	Thiolene chemistry permits rapid gelation after printing with good shape fidelity and high reproducibility under identical printing parameters		[28]
Gold nanorod	GelMA hydrogels	Cardiac	Cardiac fibroblasts and			[29]

(*continued on next page*)

Table 3 (*continued*)

Bioink Type	Novelty	Tissue Type(s)	Cells/Growth Factor Type Bioprinted	Key Result (s)	Illustration	REF
				Rapid deposition of cell-laden fibers at a high resolution is possible, while reducing shear stress on encapsulated cells		
Peptide	Self-assembling hydrogels		EpH4 (mammary epithelial cells)	Results demonstrate that well-defined cell-laden 3D constructs, with variable stiffness and improved material properties, can produce a cell-friendly ECM "like" microenvironment.		[30]
Alginate/Polyvinyl alcohol	Bovine serum albumin and bone morphogenetic protein 2/using scaffolds	-	Rat bone marrow	Micropores enhanced water adsorption while decreasing scaffold mechanical properties. BSA and BMP-2 introduced into the bioinks release profiles are strongly dependent micropores.		[31]
Silk	Gelatin and glycerol hydrogels	Soft tissue		Demonstrated in vivo that the material was biocompatible and could be tuned to maintain shape and volume up to three months while promoting cellular infiltration and tissue integration		[32]
Alginate (Alg)	High molecular weight Alg composite with low molecular weight Alg hydrogels	Soft tissue	NIH 3T3 fibroblast	Alg bioink (3 wt% Alg with a mixture of low- and high Alg in a 1:2 ratio showed good printability, enhanced cell growth, proliferation, and distribution.		[33]
Pre-vascularized stem cell	Polycaprolactone hydrogels	Cardiac	Human c-kit + cardiac progenitor cells (hCPCs)	Patterned patch showed enhanced cardiac functions, reduced cardiac hypertrophy and fibrosis, cell migration from patch to the damage area, neomuscle and capillary.		[34]

[46]. Fibroin consist of light and heavy chains, and these chains attached by disulfide bonds [53].

Silk fibroin scaffolds, in comparison with other scaffolds, have several advantages like high biocompatibility perfect mechanical stability, luster, non-toxicity, and low bacterial adherence. Because of these properties, silk protein-based bioinks have recently been studied, and they are a popular choice for using as bioink, but these kinds of natural polymers have some disadvantage like need to mix with another polymer and need to optimize rheology of the bioink [54]. Jose et al. used polyol-silk bioink for controlled delivery, biomedical implants, and tissue engineering application [55]. The printed structures have some properties like optically transparent and flexible properties. Additionally, they have been used in two-part formulations with self-curing features at room temperature, and the reason was due to consist of polyols into silk. In another study, Compaan et al. proposed a two-step gelation process for printing cell-laden silk fibroin hydrogel [56]. They have printed 3D silk fibroin cellular constructs using sacrificial alginate by inkjet bioprinting in order to long term culture. By using these structures, cells continue proliferating and remain metabolically active. Recently, Costa et al. in order to bioprinting of specific memory shape implant used silk fibroin bioink [57]. The structures have notice properties like reproducibility and proper resolution because of consist of silk fibroin.

2.2.1.4. Chitosan. Chitosan is a popular natural polymer for tissue engineering applications. Chitosan has been used for tissue engineering because of its biocompatibility, antibacterial properties, biodegradability, and low price [58]. In 2017, Wu et al. used chitosan hydrogel for guided cell growth [59]. Cheng et al., in 2017, synthesized a composite of chitosan and poly (caprolactone)-diacrylate/poly (ethylene glycol)-diacrylate for photopolymerization-type 3D printing [60]. Lee et al. fabricate scaffolds based on chitosan/gelatin/hydroxyapatite for bone tissue engineering in 2017 [61]. Morris et al. used chitosan and polyethylene glycol diacrylate as bioink for printing scaffolds via stereolithography method [62]. In 2017, Elviri et al. used chitosan scaffold in order to improve cell growth [63].

2.2.1.5. Alginate. Although there are many different materials that have been used as bioink, one of the most popular natural hydrogels used is alginate. It is a natural polysaccharide that has special properties like biocompatibility, low price, different choices of crosslinking, and compatibility with various methods of printing [2]. Another interesting property of alginate are easy forming so; alginate is a useful material for tissue engineering, drug and cell delivery, and cell encapsulation, including skeletal myoblasts [64,65]. Usually, alginate used as bioink for

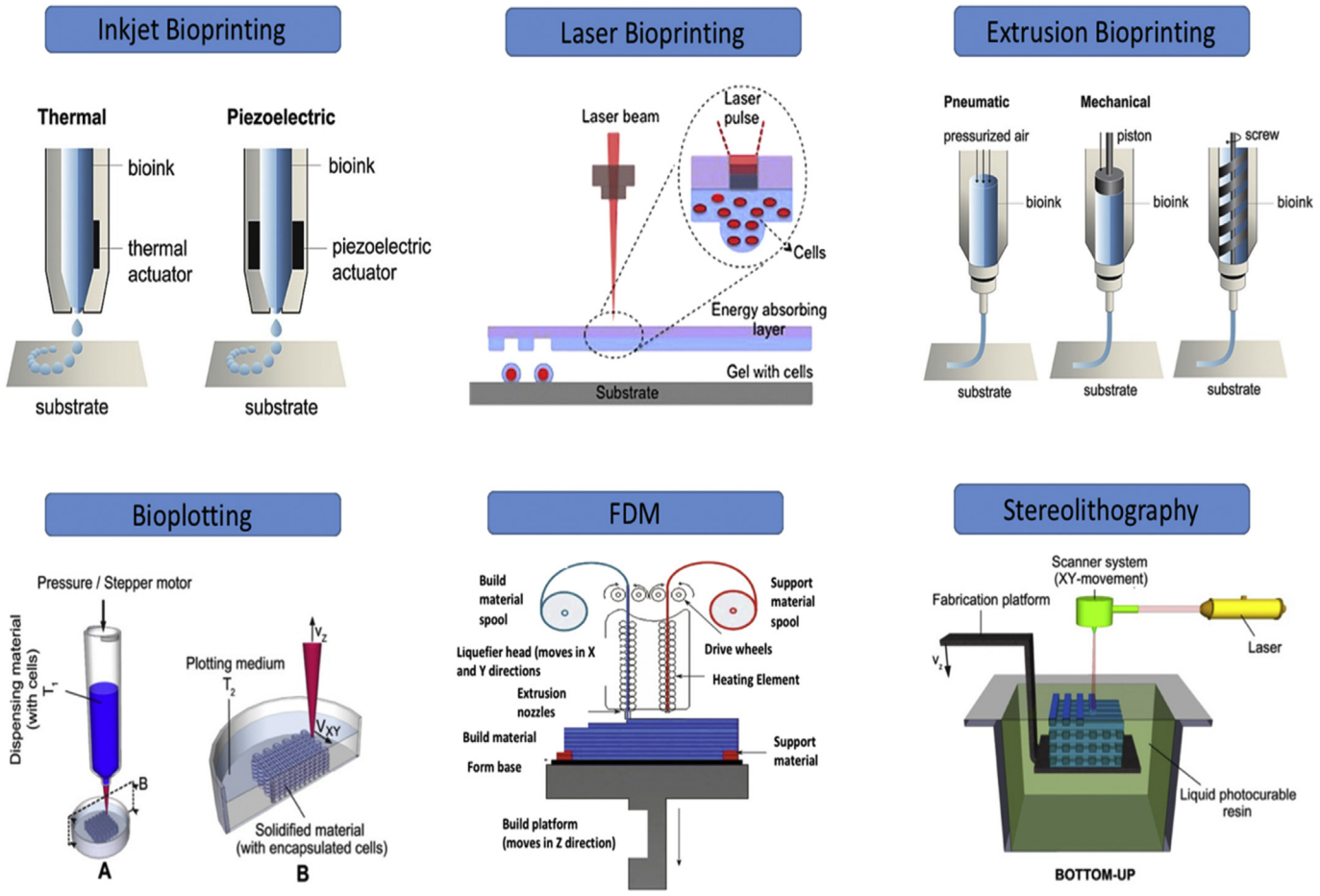


Fig. 2. Components of Inkjet, Laser Bioprinting, Extrusion Bioprinting, Bioplotting, FDM, Stereolithography. Figure adapted from Refs. [2,6,8,10,11].

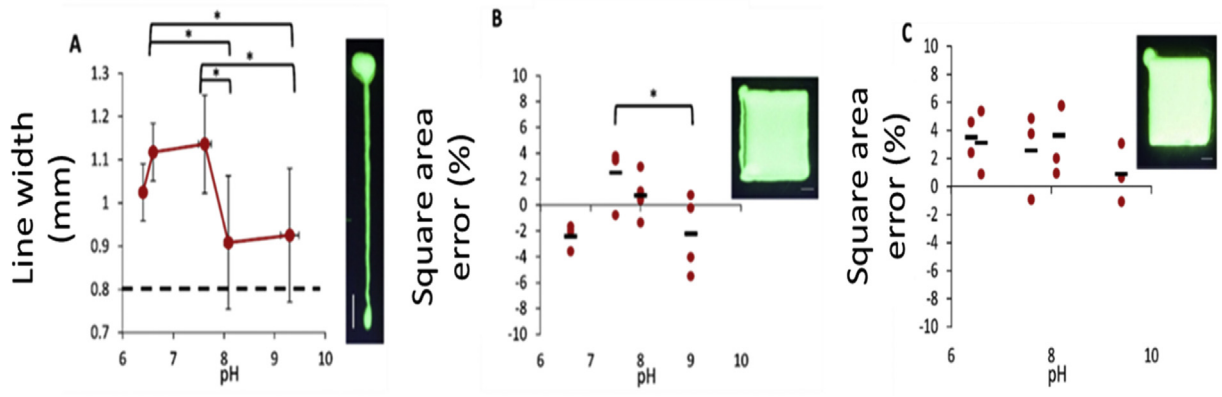


Fig. 3. Shape fidelity measures of 8 mg ml−1 collagen bioinks at various pH. (A) Average width of printed lines. Dashed line indicates intended line width. n = 3–13 for each group. Inset shows representative image of printed line. Scale bar = 5 mm. (B) Error in area of printed 1-layer squares. n = 3–4 for each pH group. Inset shows representative image of printed 1-layer square. Scale bar = 5 mm. (C) Error in area of printed 3-layer squares. n = 2–4 for each pH group. Inset shows representative image of 3 layer printed square. Scale bar = 5 mm * indicates significant difference ($p < 0.05$). Reproduced with permission from Ref. [52].

inkjet and DIW printing [66]. However, alginate, in comparison to other natural polymers has low cell adhesion. To solve this problem, some researchers used a blend of alginate with other natural polymers like gelatin and fibrinogen [67]. In 2017, Li et al. used gelatin-alginate as a bioactive scaffold for skin wound healing application. They added mice bone marrow mesenchymal stem cells before printing the scaffold [68]. Shang et al. used hybrid 3D printing and electrodeposition method to produce 3D calcium alginate hydrogels in a controllable manner [69]. In 2017, Do et al. used a new novel drug delivery device that was fabricated by 3D printing technology. They used alginate and polylactic-co-glycolic acid [70]. In another study, Heo et al. printed alginate– bone formation peptide-1 hybrid scaffolds for increase bone regeneration [71]. In 2017, Bizkaia S. et al. by a new strategy, BioMEDβeta (composed of three different fabrication modules thermoplastic micro-extrusion, multi-head deposition of hydrogels and electrospinning), printed a heterogeneous scaffold with alginate-polycaprolactone [72]. In 2017, Shi et al. seeded mouse fibroblast cells (L929) on different types of alginate hydrogels, and the result showed good cell viability and cell migration [72].

Kosik-Kozioł et al. used alginate and short sub-micron polylactide (PLA) fibers for cartilage tissue engineering [73]. In 2017, Leppiniemi used nano cellulose-alginate hydrogels to increase mechanical properties [74]. Using of interpenetrating networks bioinks is another suggested way to enhance mechanical properties of bioprinted structures. Recently Bakarich et al. fabricated ionic-covalent entanglement hydrogels containing alginate and PNIPAAm interpenetration networks bioinks gel with various concentrations of NIPAAm and used it for extrusion printing [75]. This experiment proved that the application of alginate/PNIPAAm interpenetration networks bioinks gel is shown appropriate mechanical properties which can be achieved with the addition of a biocompatible secondary network. In another study, Hong et al. showed the application of elastomeric interpenetration networks bioinks hydrogels that consist of poly (ethylene glycol) diacrylate and alginate for increasing strength is useful [76].

Moreover, this study demonstrates that without significant plastic deformation, these hydrogel networks could sustain mechanical stress. This experiment illustrated interpenetration networks bioinks can be used to fabricate mechanically robust 3D-printed structures for regenerative engineering. Recently Jiang et al. [77] were proposed an extrusion bioprintable composite hydrogel formulation composed of ionically cross-linked alginate and gelatin hydrogels which can be rapidly cross-linked upon extrusion to form a hard shell while forming a more loosely cross-linked core allowing cell migration in 3D. The composite hydrogels can provide significant improvements and a potential alternative to 3D cell culture models by enabling long culture periods (>30 days) of more than one cell type within a biomimetic environment (Fig. 4).

2.2.1.6. Gelatin. Gelatin is a natural protein that has an amphoteric behavior due to alkaline and acidic amino acids functional groups, and it is derived from collagen hydrolysis [78]. Gelatin has been extracted from several sources (like connective tissues of animals). Gelatin, of mammalian origin, has been used as a biomaterial for regenerative purposes [79]. Gelatin is non-cytotoxic, promotes cell adhesion, water-soluble, biocompatible, biodegradable properties, and have low Immunogenicity. Because of these properties, usually gelatin hydrogel used for DIW printing in the form of gelatin methacryloyl (GelMA) [80]. Recently, Lee et al. compare two different kinds of gelatin methacryloyl (GelMA) for cell-laden bioprinting [81]. They reported up to 75% cell viability in the printed structures of A and B GelMA. Before them, Liu et al. proposed a novel strategy for printing cell-laden gelatin methacryloyl (GelMA) constructs. These structures have high porosity and low stiffness, which could effectively support cell survival and enhance cell proliferation [23].

2.2.1.7. Hyaluronic acid. In 1934, Karl Meyer and John Palmer could extract a glycosaminoglycan from bovine eyes named hyaluronic acid (HA) [80]. HA or hyaluronan is an anionic biopolymer that is based on D-glucuronic acid and D-N-acetylglucosamine repeating units and behaves similar to collagen type 1 [82]. HA is a high molecular weight polysaccharide and it is the main component of the ECM [83]. This material play a crucial role in tissue engineering due to its excellent biodegradability, bioresorbability, biocompatibility and it's also non-adhesive, non-thrombogenic, non-immunogenic [84] and has the potential to form flexible hydrogels [85]. HA has been used for 3D printing in hydrogel forms. In one study in 2015, Highley et al. suggested

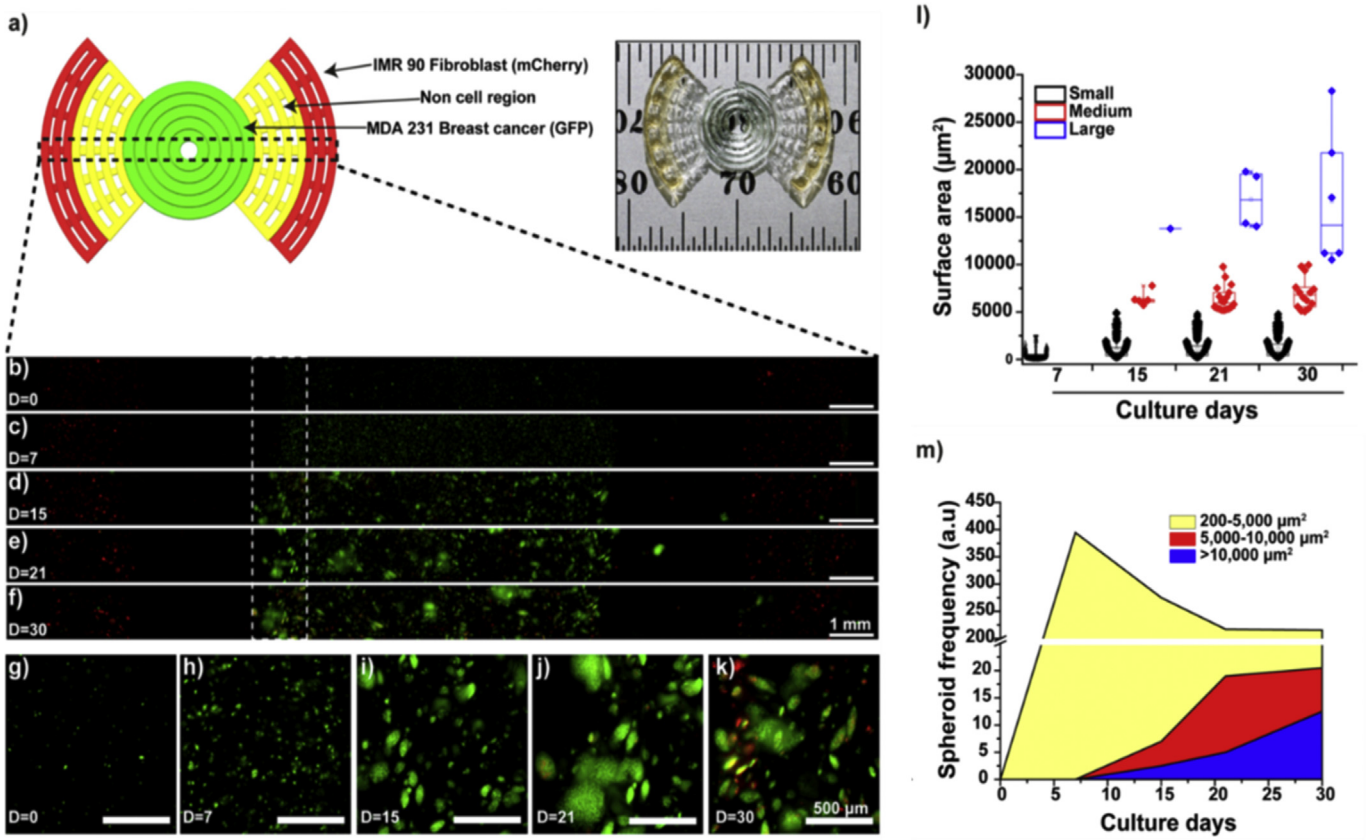


Fig. 4. MCTS formation within a 3D bioprinted in vitro model consisting of IMR-90 fibroblasts and MDA-MB-231 triple-negative breast cancer cells. (a) CAD model and photograph of the bioprinted in vitro sample. (b) Confocal time-lapse image of MDA-MB-231-GFP (green) and IMR-90-mCherry (red) cells bioprinted within the model (b–f) and their zoom-in (g–k). Scale bar is 1 mm (b–f) and 500 µm for selected areas (dotted line (g–h)), magnification x10. (l) MCTS formation and size quantification during a 30 day period: 500–10,000, 10,000–20,000 and > 20,000 µm2 for small, medium and large spheroids, respectively. (m) Frequency of MCTS distribution as a function of time cultured. Box plot graphs were plotted using a box limit of 25th and 75th percentiles and a minimum-maximum whisker's range. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

an approach for the fabrication of a supramolecular hydrogel used in extrusion-based 3D bioprinting applications based on HA. Because of the existence of non-covalent bonds that have reversible properties, this gel has low viscosity when under mechanical deformation [86].

2.3. Advanced bioinks

Some parameters such as chemical, physical, and biological characteristics have direct influence on strategy of printing and properties of bioinks. These properties include viscoelasticity, biodegradation, biocompatibility, cytocompatibility, viscosity, hydration degree, gelation kinetics, and shear-thinning. To have advanced bioink, we must use different methods to increase printability and cytocompatibility. For instance, during extrusion, bioinks designed with shear thinning have different properties like lower viscosities at the high shear rates generated [42]. Advanced bioinks are able to exhibit shear thinning properties and they are categorized in five groups, 1) multimateral bioinks 2) stimuli-responsive bioinks 3) self-assembly bioinks 4) biomolecular bioinks 5) nanoengineered bioinks. In the following sections, we describe these bioink types (Fig. 5).

2.3.1. Multimaterial bioinks for 3D printing

As mentioned above, designing advanced bioinks needs to address two main challenges that improves the functionality of printed scaffolds: printability while maintaining the desired mechanical strength and the ability to incorporate encapsulated cells. Single component bioinks are unlikely to meet these requirements. Multimaterial bioinks is one of the currently under developed strategies [87]. One of the most important hydrogels used as bioink is alginate, but it has some disadvantages. To improve alginate properties, several researchers have added calcium ions to increase the mechanical properties and crosslinking ability [88]. Recently, Yanga et al. utilized collagen-alginate bioinks for cartilage tissue engineering [89]. They used collagen type I (COL) or agarose (AG) mixed with sodium alginate (SA). All specimens showed favorable mechanical strength and biological functionality in compared to SA alone. In another study, Markstedt et al. added alginate for increasing speed of crosslinking ability to nanofibrillated cellulose for printing soft tissue [90]. Li et al. used A robust alginate/methylcellulose (Alg/MC) blend hydrogel for the first time as bioinks for 3D printing [91]. These multimateral showed a highly thixotropic property, great extrudability, and stackability. In a similar experiment, in order to prepare tri-leaflet heart valves, gelatin methacrylate and methacrylated hyaluronan were used by Duan et al. [92]. they used gelatin methacrylate to increase cell adhesion properties of the composite, viscosity and maintenance of a fibroblastic phenotype of encapsulated human aortic valve interstitial cells (HAVIC). . Chitosan is another widely used biopolymer which is superior to alginate. In this way, Demirtaş et al. for the first time used cell laden chitosan hydrogel and hydroxyapatite for bone tissue engineering [93]. Due to the presentation of nanostructure hydroxyapatite, cell viability, proliferation and osteogenic differentiation of the hydrogels improved. Using multiple materials also remains a challenge because of technical considerations to achieve high resolution and controlled cell deposition besides mechanical properties. There are some well-reviewed articles in this filed [94–97]. Fig. 6 illustrates a scheme for the design of a 3D printed bone scaffold. Many bioinks including many of those discussed above could be used in such a design. Here a 'waffle-like' scaffold is designed, modified as needed, and the 3D printed. The scaffold could be printed with many biopolymers or calcium phosphate and would serve as the support structure. The 'syrup' (or bioink) is the printed into the scaffold's wells.

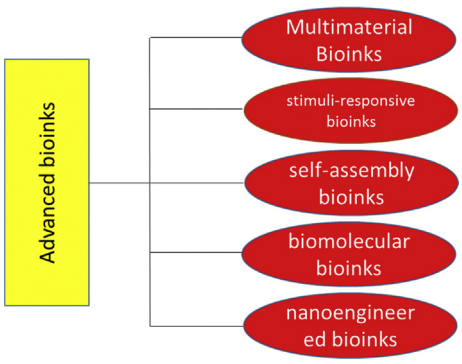


Fig. 5. Overview of five group of advanced bioink.

2.3.2. Stimuli-responsive bioinks

Mimicking the native tissue is one of the remaining challenges in the biofabrication research; as a result, introducing dynamic and stimuli-responsive bioinks opens new sight in Bioprinting methods. another strategy for printing native tissue is 4D bioprinting. 4D bioprinting has developed in recent times and in this method time as fourth dimension consider and time is a parameter that determined. The materials that utilize for 4D bioprinting are responsive biocompatible materials. These materials can change their function according to external stimuli, like magnetic field [98], water [99], temperature [100], and etc. [75,101].

Hydrogels are three-dimensional (3D) materials containing physically or chemically cross-linkers with the potential to absorb large amounts of water while maintaining their dimensional stability. Because of their structural similarities to the natural extracellular matrix, they have been widely utilized as cell carriers and scaffolds in tissue engineering. Different kind of hydrogels exist but the most important species group of hydrogels are "smart" hydrogels that are responsive to various external stimuli such as temperature, light, pH, electric, and magnetic field. Currently, Using smart hydrogels as bioink are one of the important issues that science faced with it [102]. Mouser et al. used thermosensitive hydrogels composed of methacrylated poly[N-(2-hydroxypropyl) methacrylamide mono/dilactate] (pHPMA-lac)/polyethylene glycol (PEG) for printing cartilage constructs [103]. Additionally, they added methacrylated hyaluronic acid (HAMA) to thermosensitive hydrogels for producing novel composite and increase printability. In another study in Apsite et al. fabricated thermoresponsive polymer that consist of polycaprolactone and poly(N-isopropylacrylamide) [104]. They found these kinds of polymers is suitable for cell encapsulation and design of biomaterials. Cochis et al. extruded a 3D thermo-responsive structure based on methylcellulose hydrogels for cell-sheet engineering [105]. 2017, Lorson et al. synthesized a novel thermogelling block copolymer comprising hydrophilic poly(2-methly-2-oxazoline) (PMeOx) and thermoresponsive poly(2-n-propyl-2-oxazine) (PnPrOzi) [22]. They have also investigated the rheological properties of its aqueous solution and claimed that this kind of copolymer is excellent bioink candidates. pH sensitive hydrogels are another smart hydrogel which have been studied as a bioink. Larush et al. used acrylic acid monomer, cross-linker (polyethylene glycol diacrylate) and photoinitiator (2,4,6-trimethylbenzoyl-diphenylphosphine oxide [TPO] nanoparticles) as a bioink to fabricate drug-loaded systems with special designs and unique drug-release characteristics [106]. These structures have pH-responsive swelling properties, thus offer faster drug release at a higher pH. Dutta et al. synthesized a novel material for 3D printed reverse thermo-responsive and pH-sensitive structures [107]. They used the stereolithography (SLA) technique for printing. Methacrylated poly(-ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) hydrogels. The hydrogels have shown fast and reversible swelling–deswelling response. In 2018, Shen et al. used nanocomposite hydrogels that constructed for detection of multiple stimuli-responsive properties like various chemical redox reactions, pH values, biomolecules, enzymes, and cations [108]. Here, 'time' does not refer to how long it takes to print a part, but rather the fact that printed 3D biocompatible materials or living cellular constructs continue to evolve over time after being printed.

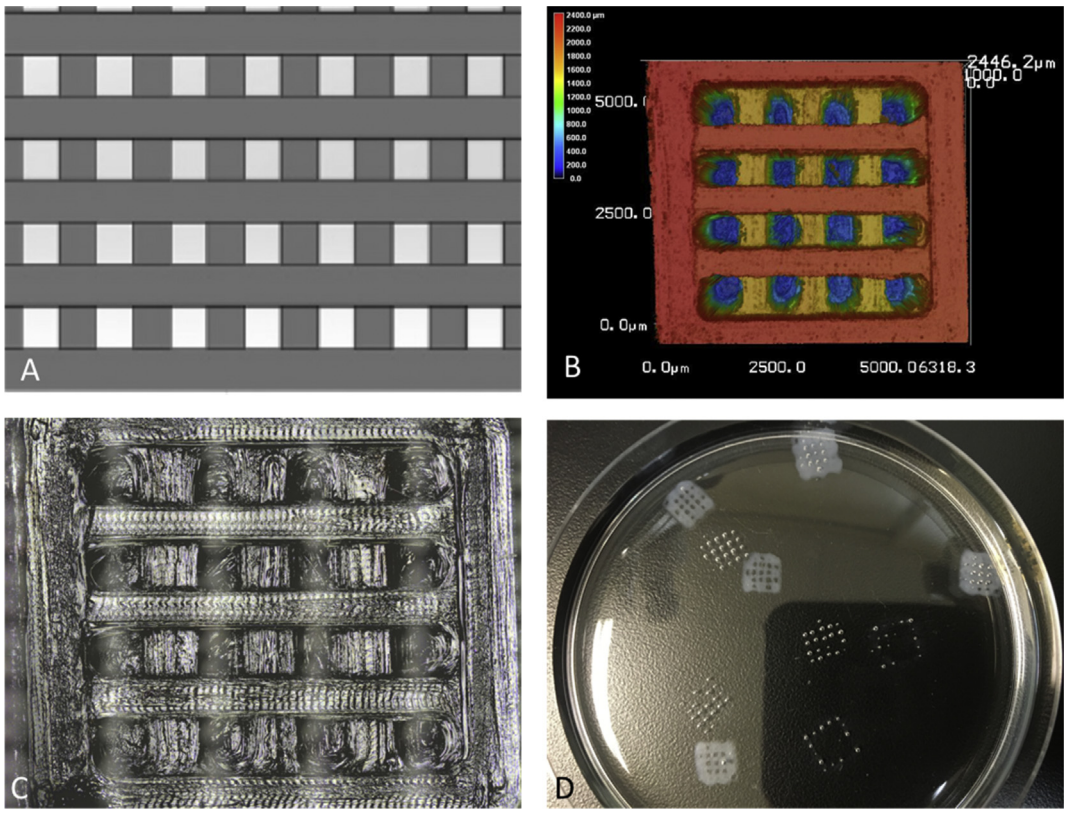


Fig. 6. 3D printing strategy for bone tissue regeneration. (A) 3D CAD Graphic of a 'waffle-like' scaffold. (B) Laser confocal image of the first two layers of the 3D scaffold. (C) 3D printed scaffolds. (D) Optical and laser combined image. (From the senior author's collection) and cells are then seeded into the bioink. Cells could also be seeded added to the bioink and then extruded into the wells.

2.3.3. *Self-assembling bioinks*

The use of self-assembling elements is a new concept in bioprinting. These elements act as building blocks for the fabrication of larger constructs in anatomical shapes [109]. Up to now, self-assembling peptides and tissue strands have been studied as a bioink for scaffold-free bioprinting. Self-assembling peptide shown a good potential to fabricate nanofibrous hydrogels which facilitate the formation of 3D network similar to ECM [110]. Recently, Hedegaard et al. propose a new material fabrication for a self-assembly based supramolecular bioink to create hydrogels of complex geometry, structural hierarchy and tunable chemical composition [111]. They utilized co-assembly of peptide amphiphiles (PAs) with biomolecules and/or proteins found in the ECM. In another study, Raphael et al. used a novel group of self-assembling peptide-based hydrogels for printing cell-laden structure [30]. They have tried to optimize the printing process parameters to achieved well-designed geometries and structural integrity. They have also reported increasing in printability and cell viability. Yu et al. studies have tried to fabricate scaffold-free scalable tissues by deploying cartilage strands which were capable to undergo self-assembly by fusing to each other. They claimed that this scaffold-free approach will facilitates cellular interactions and enable closer tissue biomimicry. Their real-time PCR gene expression revealed the potential of strands for fabricating of functional cartilage tissue [112,113]. Self-fouled hydrogel was a novel approached was proposed by Kirillova et al. [114] in order to fabricate hollow tubular structures by bioprinting. Their cell viability analysis revealed that the printing process does not negatively affect the viability of the printed cells. Although this shape-morphing hydrogel support celled survival but for only 7 days.

2.3.4. *Biomolecular bioinks for 3D printing*

Even though the considerable efforts of scientists in developing cell-printed structures, the interactions between cells and materials is the major challenge in this field. A complication of cells–material interactions have encouraged researchers to provide similar conditions to the natural microenvironment. As a result, they tried to deployment living tissues elements as a bioink [115].

Decellularized extracellular matrix (dECM) is the best approach for solving this problem because it can reproduce nearly all requirements of ECM [116]. DECM-based bioinks include decellularization of tissue by removing the cells while maintaining the ECM. The ECM is then deformed into a powder and dissolved in a cell-friendly solution to produce the bioink [9]. These kinds of bioinks do not need crosslinkers, and they can degrade the surrounding gel [117]. Although these kinds of materials have different advantages, often have some limitation like mechanical strength. So for solving these problems commonly used tunable bioinks with a wide range of material properties [97] Furthermore, for increasing viscosity and solubility, usually a polymer added to bioink [9]. For example, in 2014, Patti et al. added polycaprolactone to dECM-based bioinks (obtained from cartilage, fat, and heart) for printing cartilage and adipose tissue construct [116]. In another study in 2017, Jang et al. for printing complex tissue, used stem cell-laden decellularized extracellular matrix bioinks [34]. In this research, dual stem cells were used, and cell-to-cell interactions improved and differentiation capability and promote functionality for tissue regeneration.

Deoxyribonucleic acid (DNA) based hydrogel is a novel bioink proposed by Chuang Li et al., in 2015 [118]. They fabricated biomolecular hydrogels for rapid in situ multilayer 3D bioprinting. The main valuable property of these kinds of DNA-based hydrogels is to provide excellent healing due to dynamic cross-linking by DNA hybridization, which provides uniform constructs. Other crucial and desirable properties of these hydrogels are their biocompatibility, biodegradability, and permeability to nutrients.

2.3.5. Nanoengineered bioinks for 3D printing

Although polymer materials for 3D printed structure have different kinds of advantage (low weight, low melting point, less cost, and processing flexibility), the significant problem of these structures is mechanical strength and functionality. Thus, to overcome this challenge, some researchers added several materials to the polymer matrix for better mechanical and functional properties. Consequently, in the past decade, composite materials have been used due to their compatibility with the available printers. Many promising results in developing new printable composite materials reinforced by particles, fibers, or nanomaterials have been achieved [119]. Among them nanoengineered materials shown tremendous potential in biotechnology, biomedical and tissue engineering among them nanocomposites have emerged in the last three decades as new applications, "nanoengineered hydrogels," and they are mainly utilized for 3D printing [120].

Today, considerable academic and industrial research efforts have been focused on deploying them in biomedical applications and tissue engineering due to its remarkable properties and various fabrication methods. In this way, nanocomposite bioinks have been widely studied for biomedical applications. These hydrogels contain one or more constituent materials added to polymeric hydrogels that led to significant changes in different chemical, mechanical and physical properties like resistance to degradation under physiological conditions, stiffness, and shear-thinning characteristics [121]. Several parameters, like properties of the matrix, properties, and distribution of the fillers and synthesis or processing methods, affect the mechanical properties of nanocomposites. On the other hand, surface modification of nanostructures shows a crucial role to increase the interfacial adhesion between the nanophase and the matrix and promote better dispersion of fillers [122]. Even though various nanocomposite hydrogels introduced for tissue engineering, a small number of investigations focused on their capacity for 3D bioprinting. In the next section, new approaches in the use of nanocomposites as bioinks will be discuses.

2.4. Nanoparticle reinforced polymer composites

Nanoparticles in nanocomposite have a significant role in improving structural properties of hydrogel network. They can modulate mechanical properties like stiffness or Young's modulus, fracture toughness and creep resistance [123]. Furthermore, several intended properties like bioactivity, electrical conductivity, photoresponsiveness, magnetism, and controlled drug release can be achieved by adding special nanoparticles [124]. On the other hand, nanoparticles mix more easily with polymer and distribute well in a composite matrix so for example powder particles is suitable for laser-based bioprinting [125]. It is worth mentioning that, different parameters of nanoparticles like aspect ratio, shape, distribution, and size have different effects on various properties of the composite [126].

2.5. Ceramics nanocomposites

Bioceramic materials are widely used in biomedical applications because of their biocompatibility and material strength, and many are similar to the mineral phase of natural bone [20]. Also, their osteoinductivity is a significant reason for their applications in dental and orthopedics applications [127,128]. On the other hand, despite the beneficial properties of bioceramics, available 3D printing method for printing of ceramics are limited. For example, ceramic materials have a high melting temperature, so FDM proses are not appropriate for printing ceramics, or they are not responsive to light, and thus SLA is not an option for direct printing of these materials. Inkjet printing is promising methods for direct printing of ceramic materials. Although the direct printing of ceramics is difficult, they can be used as an additive in a composite system. Thus, this approach helps in their use in FDM and SLA methods for printing ceramic composites [129].

In bioprinting, the challenges are more complicated than those highlighted above, because of issues related to cell loading in the bioceramics. Therefore, at the time of writing, bioceramics are limited as a bioink and using them is limited to deploying them as a second phase in composite bioinks. One of the most commonly used ceramics in ceramic-based ink materials in tissue engineering applications is hydroxyapatite (HA). Its powder is extensively used in 3D printing [130]. Wenz et al. used crosslinkable gelatin-based bioink modified by the addition of hydroxyapatite (HAp) particles for increasing bioink viscosity and mechanical properties [131]. In 2017, Wenz et al. utilized polymer solutions based on methacrylated gelatin and methacrylated hyaluronic acid for bioprinting bone tissue [132]. This polymer was modified with hydroxyapatite (HAp) nanoparticles (5 wt%) and the nanoparticles not only modulated mechanical properties and printability of the hydrogels but also enhance osteoconductivity of the matrix.

Recently, the influence of adding HA nanoparticles as an osteogenic agent for pre-osteoblastic cells encapsulated in a PEG hydrogel containing cell adhesion peptide was studied by Carner et al. [133]. Another class of ceramics that are added to bioink is calcium phosphates. α- and β-tricalcium phosphates are found in human skeleton besides hydroxyapatite [134]. Because of the faster resorption rate of β-TCP in comparison to HA, β-TCP is used more than other phosphate phases. Particle size, scaffold geometry, de-powdering efficiency, and binder properties have effected on porosity, strength, and resolution of printed TCP scaffolds. Tarafder et al. performed in vitro and in vivo study to demonstrate the effect of PCL coated 3D printed tri-calcium phosphate scaffolds to increase interlayer binding and scaffold mechanical properties [135]. In another attempt, β-TCP was used for increasing the binding properties of the final scaffold [136]. They were using a 3D mini-screw extrusion printing (Fab@CTI 3D printer). The mechanical and hydrophilic behavior of PCL/b-TCP improved in comparison with PCL scaffolds, and all samples had the potential for bone tissue applications due to their mechanical properties. On the other hand, the hydrophilic properties of the scaffolds increased slightly. Ceramic-halloysite composites have been used to 3D print scaffolds that support pre-osteoblast cell growth and differentiation (Fig. 7). A 'waffle-like' scaffold was designed 3D printed with a calcium phosphate/halloysite bone cement. Pre-osteoblasts were then seeded onto the scaffold which supported cell growth and differentiation (unpublished data).

2.6. Ceramics nanocomposites

One of the oldest materials used in traditional medicine are clay minerals [137]. Due to their availability in nature, clay mineral is an interesting material that have a wide range of applications [138]. In the 1980s, Toyota et al. used clay in nylon-6 for composite fabrication and they saw an improvement in physical and engineering properties. At present, the main aim of engineering is to fabricate clay/polymer nanocomposites in which clay layers in a polymer are separate and well-dispersed. Due to particular physicochemical characteristics, like high water dispersibility, colloidal and swelling capacity, high surface reactivity, and optimal rheological behavior, clay minerals are extensively used in bioprinting applications, pharmaceutics, biosensors, cosmetics, and veterinary medicine [138]. Usually, clay/polymer nanocomposites application showed remarkable changes (improvements) in a wide range of physical and engineering properties. The main role of clay in polymer based composites is improving mechanical properties of the structure by reinforcing polymers with clay on the nanometer scale [139]. Zhai et al. fabricated high strength supramolecular polymer/clay nanocomposite hydrogel scaffolds that was polymerized under UV light for bone regeneration [24]. They could develop a hybrid bioink that composed of nanoclay and (N-acryloyl glycinamide) (NAGA). They sought to promote the osteogenic differentiation of primary rat osteoblasts because of embedded Mg^{2+} and Si^{4+} that released from the PNAGA-clay scaffold. Additionally, when they implant this scaffold in the tibia defect of rats, efficiently elicit the new bone formation. In another study in 2017, Ahlfeld et al. synthesized and

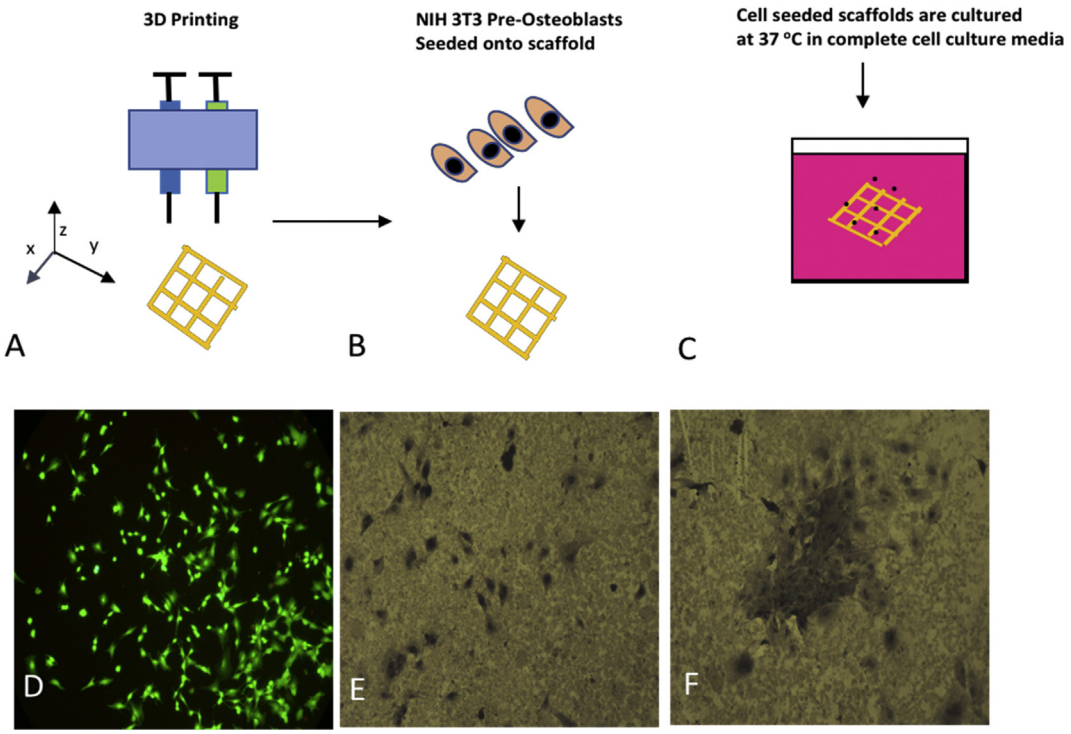


Fig. 7. 3D printing a ceramic-halloysite nanotube composite for bone tissue regeneration. (A) Robocasting of a 3D 'waffle-like' scaffold. (B) NIH 3T3 cells are then seeded onto the 3D scaffold and cell seeded scaffolds are then incubated in vitro (C). (D) Live-Dead cytotoxicity assay reveals little cell death. (E). Cell grow on the 3D composite scaffolds. (F) Classic cellular condensation of 3T3 cells that occurs prior to osteoblast differentiation. (From the senior author's collection).

characterized a bioink based on a synthetic nanosilicate clay, alginate and methylcellulose by an extrusion-based method. They showed high cell viability of hTERT-MSC after extrusion which was maintained over 21 days [140].

2.7. Metallic nanoparticles

Different metals and alloys are being used in biomedicine, such as gold, tantalum, Co–Cr, NiTi, stainless steel, and Ti alloys [135,141]. Metals and their alloys are used because of their high strength, resistance to wear, and ductility. While in many biomedical applications, the lack of biodegradability, weak corrosion resistance, high density, and high stiffness are limitations [142].

In 3D printing, the main reason for using metal composites is to increase bioink material properties such as stiffness, processability, and printability [20]. Although reaching the complex and 3D architectures structures by traditional and existing manufacturing methods have some limitations. Innovation in additive manufacturing and 3D-printing approaches offer the potential to deploy metallic powders in bioprinting. Wu et al. fabricated hierarchical structure that based on microporous nickel-titanium composite (NiTi) which fabricated by powder metallurgy [143]. They treated the scaffold's surface with sodium hydroxide. This modification led to enhanced surface hydrophilicity and deposition of hydroxyapatite, as a result, cell attachment and proliferation improved. A problem, however, was that the nanostructures created by the chemical process was not controllable. In another study, Liu et al. created titanium-silica composite scaffolds by selective laser sintering (SLS) technique for bone tissue engineering [144]. In the fabrication of their scaffold, they used layer-upon-layer deposition of a titanium powder and silica sol slurry. The pre-fabricated scaffold was sintered to achieve the desired final strength. Chou et al. fabricated iron-magnesium (FeMg) composite scaffolds by 3D inkjet printing method [145]. The tensile properties of scaffold were similar to cancellous bone and their samples showed good cell viability on exposure to the scaffolds and a further important property of the scaffold was cell infiltration into the formed pores. A new class of 3D bioinks for complex metallic architecture fabrication was introduced by Jakus et al. The 3D-printed structures were finally oxidized and reduced cyclically to reach the desired structural integrity [146].

2.8. Fiber reinforced polymer composites

Fiber reinforcement composites are one of the suggested structures for tissue engineering. Generally, using fibers in hydrogels enhance hydrogels properties and can control cell binding and determine cell fate. In one hand, hydrogels are mechanically week. On the other hand, fibers are structurally compact. Thus, using two-phase (fibrous and a gelatinous) can offer different features like mechanical properties, biomimetic aspect, and cellular migration for optimizing scaffold functions [147]. 3D fiber deposition with a bioplotter was a suggested method for printing of cell-laden spatially organized hydrogel scaffold with appropriate mechanical strength [148]. Other methods like photopolymerization, microfluidics, etc. have been suggested to combine hydrogels and fibers in a single scaffold. More details about these methods were reviewed by Bosworth et al. [149]. One of the accepted strategies to produce and develop fiber-reinforced polymer composites scaffolds is 3D-printing because, in this method, the spatial injection of materials can be controlled [150]. FDM is one of the more favorable methods to produce fiber-reinforced polymer composites [151]. In FDM, polymer pellets and fibers are first mixed in a blender and then sent to the extruder to fabricate desired structures. FDM provides the opportunity to control the architecture and defined cellular placement. Recently, Narayanan et al. were proposed a new method to the reinforced 3D bioprinted hydrogel. They added short submicron polylactide acid fibers into alginate bioink. By distributing and alignment of nanofibers, they have tried to stimulate the formation of anisotropically oriented ECM to adjust mechanical and biological properties of the printed scaffold [152]. Investigation on deploying PLA fiber to reinforce bioink was extended by another group [73].

2.9. Incorporating carbon-based nanomaterials

After hydrogen and oxygen, carbon is the most abundant element in the universe. One of the properties of carbon is its potential to bind itself and most elements. Because of the structural diversity of carbon, it has a broad range of physical and chemical properties. Nano-carbon such as graphene, carbon nanotube usually exhibit unique thermal, electrical, and mechanical properties [153]. As a result, adding them to printed composite can modulate physical, chemical, and mechanical properties of the composite [154]. Consequently, the fabrication of high-performance functional composites using these nanomaterials and polymers for printing could be useful. This section gives an overview of these nanomaterials.

2.9.1. Graphene-related materials

One of the most widespread forms of carbon is graphite, which is composed of stacked sheets of carbon with a hexagonal structure. Although by physical and chemical modification, its two-dimensional honeycomb lattice can be transformed into graphene-related materials like single and multi-layered graphene, graphene oxide, and reduced graphene oxide [155]. Because of the full application of graphene, researchers have been focused on graphene as building blocks to create unique macroscopic materials [156]. Over the last few decades, various nanomaterials have been used to enhance the performance of polymeric composites, but recent studies Illustrated graphene can promote mechanical properties, thermal properties, electromagnetic interference shielding, optical properties, barrier properties, and electrical properties of polymers [157,158]. Neural tissue engineering is one of the areas that benefited from the electrical properties of graphene. Zhu et al. presented a novel “nano-bioink” composed of gelatin methacrylamide, neural stem cells, and bioactive graphene nanoplatelets. They employed stereolithography to fabricate complex cell-laden scaffolds. Their results revealed that graphene nanoplatelets promoted neuronal differentiation during neural regeneration [159]. In 2017, Zhou et al. used graphene oxide -gelatin methacrylate (GelMA)–poly (ethylene glycol) diacrylate (PEGDA) composite for promoting chondrogenic differentiation of human bone marrow mesenchymal stem cells [160]. In another study, Cheng et al. used collagen–chitosan hydrogel and graphene oxide nanoparticles for cartilage tissue engineering [161]. They added graphene oxide as an inorganic delivery carrier for nanoscale molecule drugs. Study on graphene or graphene oxide bioinks for neural tissue engineering is a growing field [162,163].

2.9.2. Carbon nanotubes

Since the discovery of carbon nanotubes (CNTs), scientists have focused on techniques for exploiting their extraordinary properties. Because of their unique physical and chemical properties, CNTs are new materials for engineering. No previous material has shown the combination of excellent mechanical, thermal, and electronic properties attributed to them [164,165]. Due to this combination of properties, CNTs have become perfect materials for adding in composites. Because of the high porosity of CNTs, they have similar behavior with collagen fibers of the extracellular matrix. Thus, it can influence cell proliferation, adhesion, and differentiation [166]. Generally, CNTs are categorized based on their size. If their size is between 0.8 and 2 nm, they called single-walled carbon nanotubes (SWCNT), and if their size is between 2 and 100 nm, they called multiple walled carbon nanotubes (MWCNT) [167,168].

CNTs are chemically inert and dispense unevenly in many polymers. In biomedical applications, functionalization is required to enhance biocompatibility and to incorporate carbon nanotubes into polymers evenly [169]. Usually carboxyl or alcohol groups were used in functionalization of CNTs are chemically inert and dispense unevenly in many polymers. In biomedical applications, functionalization is required to enhance biocompatibility and to incorporate carbon nanotubes into polymers evenly [169]. Usually, carboxyl or alcohol groups were used in functionalization of CNTs; these groups are added into the ends of the nanotubes. They formed bonds between carbon nanotubes and functional groups are useful in tissue engineering cause they are weak enough to allow different reactions to taking place within the biochemical environment, but not too weak to inhibit rapid chemical decay [170]. Ho et al. printed scaffolds that consist of polycaprolactone carbon nanotube composite for cardiac tissue engineering [171]. They found this scaffold show cell proliferation and have the potential to be used in cardiac tissue engineering. In 2018, Hohimer et al. explored effects of fabrication parameters of through FDM like layer height, nozzle temperature, and bed temperature on the electrical conductivity and piezoresistive response of printed thermoplastic polyurethane/multiwall carbon nanotubes [172]. They did find conductivity, and piezoresistive response is only slightly affected by the print parameters.

3. Perspective

3D printing techniques for biological applications have seen rapid growth during the last decade, and these methods have opened up new avenues and directions in regenerative research. Even though this technique is a relatively early stage of development, 3D bioprinting tissue with this method has shown great promise. 3D printing strategies have the potential to be widely used in tissue engineering applications, including analysis of chemical and biological agents, drug delivery, organ-on-a-chip devices, and other biomedical applications. Although 3D printing has robust features to improve regenerative strategies, there are several limitations which need more attempts to address them. To sustain the surrounding organ tissue, methods have been studied to create thick, complex tissues with full vascularization containing lumens of several sizes, large vascular structures to microstructures.

Additionally, the cost of the product must be considered [173,174]. Another aspect of bioprinting is the fabrication process. Choosing appropriate design criteria such as speed, resolution, and compatibility with cells will have a direct effect on the final result [8]. Moreover, biomaterials and polymers used for printing scaffolds must have and inherent ability for supporting cell growth, sufficiently retain the printed constructs shape, biocompatibility, and a means for initiating cell differentiation or preserving cellular phonotype [175]. We have described a range of materials currently being investigated as bioinks including gelatin, alginate, collagen, modified copolymers, clay and metal nanoparticles, and hyaluronic acid. Additional research is required to develop bioinks with added or multifunctionalities that will provide order printed scaffolds with the requisite structure and inherent biological cues [176].

Declaration of competing interest

The authors declare that they have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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