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Bioinks for jet-based bioprinting

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ABSTRACT

Due to its customizability and versatility, three-dimensional (3D) bioprinting technologies have gained great popularity in tissue engineering and regenerative medicine. Known as a bioprinting technology, Early researches have demonstrated that jet-based bioprinting has unique advantages over extrusion bioprinting on properties including non-contact, agility, high resolution and precise control of the deposition pattern. It is an accurate and reproduceable method for depositing biomaterials or cells into defined different patterns for various uses. One of the most important components of jet-based bioprinting is the bioinks, which is a mixture of cells, biomaterials and bioactive molecules. To provide readers with a comprehensive understanding, we provide an overview of bioinks for jet-based bioprinting and discuss their classifications, characteristics and applications, as well as present current limitations of existing bioinks and promising solutions to these limitations.

1. Introduction

Also referred to as additive manufacturing technology (AMT) and rapid prototyping technology (RPT), 3D printing was originally proposed by Charles Hull in 1986 [1]. 3D printing technology has now been broadly used in many fields, such as mechanical manufacturing, electronics, education, medicine and biology. Specifically, 3D printing gets great popularity in medical engineering and tissue engineering [1–4] due to its ability to process precise layering of diverse biomaterials. Referred to as bioprinting, it provides a feasible way to distribute the materials at target locations to form planar or spatial construction by changing the relative position between the nozzle and the platform. Furthermore, with proper strategies, bioprinting is capable of depositing cells and materials simultaneously. The relevant materials used in these bioprinting systems are known as bioinks. Each type of bioink has advantages and limitations. Consequently, it is essential to choose proper bioinks according to the printing methods and intended purposes (e.g., microcapsule, scaffold, array pattern). Meanwhile, in order to take full advantage of bioprinting, it is necessary to understand the characteristics of bioinks in distinct bioprinting technologies. To date, there are three typical categories of bioprinting styles: extrusion bioprinting, jet-based bioprinting and stereolithography. Among these technologies, jet-based bioprinting has unique advantages (e.g., agility high, throughput and precise control) over the others.

For the above reasons, the purpose of this paper is to provide an overview of the bioinks used in jet-based bioprinting technology. In this review, first, bioinks are classified as synthetic ink or natural ink. And cells are discussed individually according to the printing methods. Next, their properties and applications are presented. Finally, existing limitations are summarized and future prospects are provided to the reader.

2. Jet-based bioprinting technology

3D bioprinting technologies used in biofabrication can classified into extrusion bioprinting, jet-based bioprinting as stereolithography. Compared with the other categories, jet-based bioprinting has unique and desirable advantage, including agility, high throughput, single-cell printing and droplet-continuous dual mode. In this section, we will introduce jet-based bioprinting technology including inkjet printing, micro-value printing, acoustic printing, laser-assisted printing, electrospun and electrohydrodynamic jet printing (Fig. 1). The Characteristic of jet-based bioprinting are listed in Table 1(see below).

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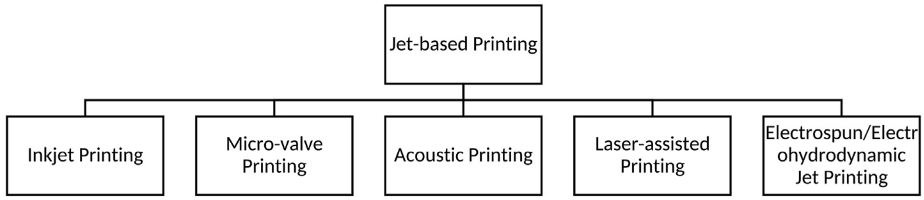

Fig. 1. Jet-based bioprinting technology map.

Table 1
Characteristics of jet-based bioprinting.

	Advantages	Drawback	Resolution [6,21,22] (μm)	Ink Viscosity [5,6,16,21,22] (mPa·s)	Cost [3,5]	Cell Viability [5,6]	Nozzle Size [5] (μm)	Ease of operation [3,5,13,14,23,24]
Inkjet printing	fast fabrication speed high resolution high throughput	poor printability of high viscosity ink, easy clogging, high shear stress, thermal damage (thermal printing)	10–50	<10	low	>85%	electrostatic: not specified thermal: 50 piezoelectric: 21.5–120	easy
Micro-valve printing	high throughput high printing stability, available in single nozzle control	Droplet diameters larger than orifice diameters, high shear stress	>150	1–70	low	>90%	100–300	easy
Acoustic printing	orifice-free, no clogging and mechanical stress problems	indispensability of surface tension and viscosity of inks,	3–200	1–10000	medium	≈90%	not specified	medium
Laser-assisted 3D	orifice-free, no clogging and mechanical stress problems, high resolution, ability to deposit biomaterials in solid or liquid state	indispensability of surface tension and viscosity of inks, long preparation time and process, thermal damage due to laser beam	10–100	1–300	high	>95%	orifice-free	complex
Electrospun/ Electrohydrodynamic jet printing	enable to print droplets or nanofibers enable to print higher viscous inks, droplets diameters smaller than nozzle diameters, simple to assemble high voltage may harm the operator, hard to precisely manipulate the Taylor-cone,	2–100	>2000	low	>90%	2–1000	medium	

2.1. Inkjet printing

According to the classification of driving force, inkjet printers are mainly divided into three types: electrostatic, thermal and piezoelectric forces [8].

In electrostatic inkjet printing, the electrostatic printer generates droplets by manipulating the volume of the fluid chamber [9]. Through increasing the voltage between the pressure plate and the electrode, the volume of the ink chamber increases briefly, causing the ink in the reservoir to flow into the ink chamber (Fig. 2 a1). When charge is off, the deflected pressure plate recovers to its original shape. Subsequently droplets were driven out by the increased pressure. Electrostatic printing is low cost due to its electrostatic driven force, but it tends to clog because of the small orifice diameters.

In thermal inkjet printing, the thermal actuator heats, and then small air bubbles are created to drive the ink out of the nozzle to eject droplets (Fig. 2 a2). First, a short current pulse lasts in the thermal actuator for a few microseconds and raises its temperature to 300 °C [10]. Next the thermal actuator evaporates the ink to create a bubble to force the ink out. The droplet formation can be manipulated by adjusting the printing parameters such as current pulse and ink viscosity [8].

In piezoelectric inkjet printing, a voltage pulse is applied to change the shape of the piezoelectric actuator to deform the fluid chamber (Fig. 2 a3). After applying the voltage pulse, a sudden variation in the fluid chamber volume causes a pressure change. Then a droplet is driven out due to the overcoming the surface tension at the nozzle orifice [5]. There are four types of printheads used in piezoelectric printing including squeeze mode, bend mode, push mode and shear mode printhead [8].

Thermal and piezoelectric inkjet printing methods for biofabrication are most widely used in inkjet printing. Thermal inkjet printing is popular due to its easy and feasible modification of commercial printers. However, its application is limited by the bubble process which is only generated in a limited range of inks. Contrary to thermal inkjet printing, piezoelectric inkjet printing can avoid the influences (e.g., degeneration and viscosity) of inks caused by heat. Similar to electrostatic inkjet printing, thermal inkjet printing and piezoelectric inkjet printing can also tend to clog.

2.2. Micro-valve printing

The micro-valve is mainly comprised of a solenoid coil and a plunger that blocks the orifice. In micro-valve printing, droplets are generated

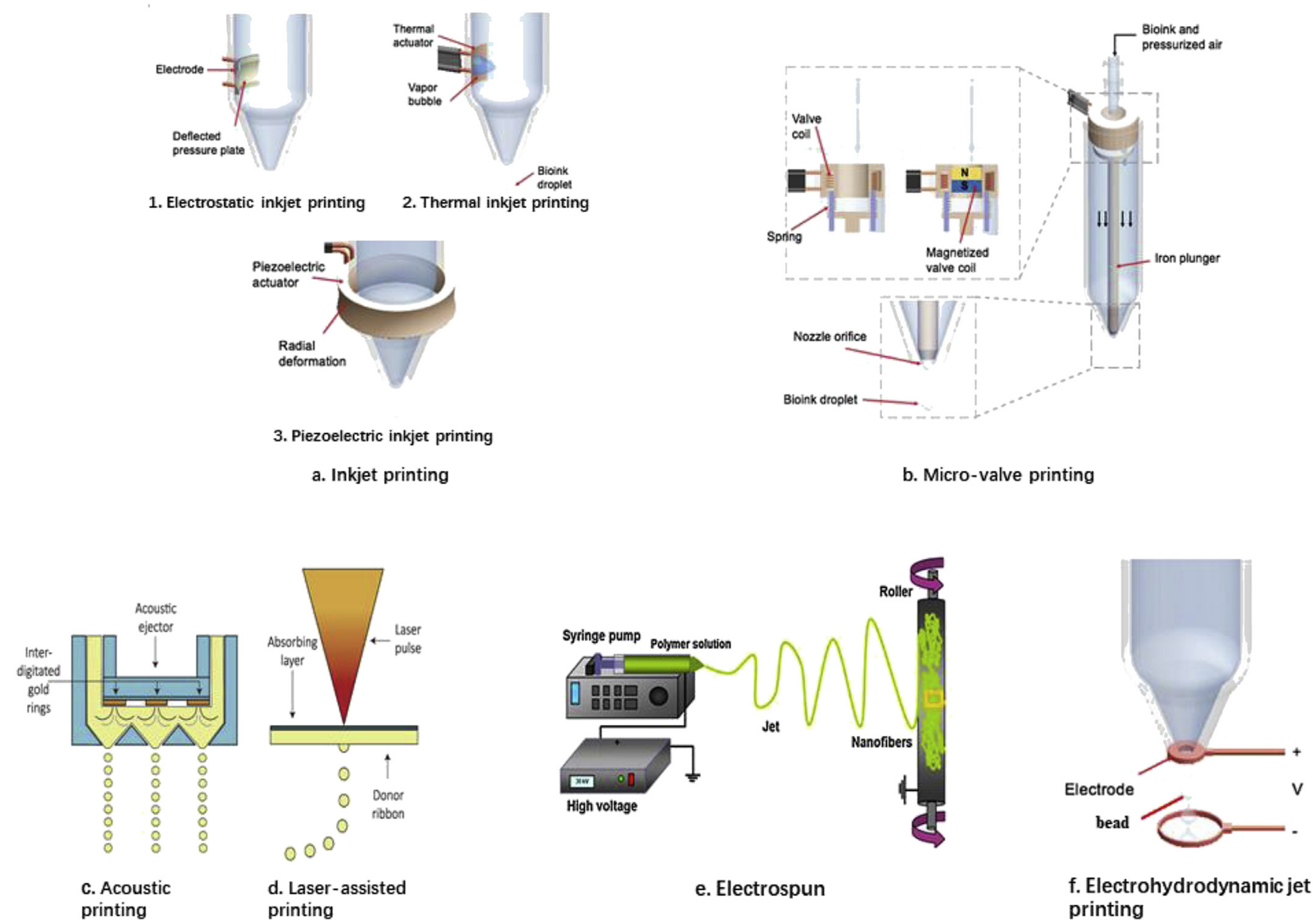

Fig. 2. Mechanisms of jet-based bioprinting. (a) Inkjet printing including:1. Electrostatic inkjet printing, 2. Thermal inkjet printing, 3. Piezoelectric inkjet printing. (b) Micro-valve printing. (c) Acoustic printing. (d) Laser-assisted printing. (e) Electrospun. (f) Electrohydrodynamic Jet Printing (Figure (a) (b) (f) reprinted from Ref. [5] with permission from Elsevier, 2019. Figure (c) (d) reprinted from Ref. [6] with permission from IOPscience, 2019. Figure (e) reprinted from Ref. [7] with permission from Elsevier, 2019.).

through applying the electromechanical valve to control a pneumatic switch [11]. When the printer is working, a voltage pulse is applied to form a magnetic field to pull the plunger upwards. Simultaneously, the nozzle is unblocked and then the back pressure pushes the ink out when the surface tension is less than the back pressure [12] (Fig. 2 b). The nozzle diameter of micro-valve printing is larger than the size of cells so that the shear stress can be reduced when the cells pass through the nozzle [11]. Due to its independent nozzle control, micro-valve printing can easily achieve multiple inks simultaneously printing by adjusting the parameters and manipulating multi-printhead. The pneumatic pressure, the nozzle geometry, the cell concentration, and the ink constituents influence the droplet volume and cell viability [5,13].

2.3. Acoustic printing

Acoustic printing is an orifice-free printing method which utilizes a surface acoustic wave to generate droplets [14]. This printing system consists of a surface acoustic wave piezoelectric substrate and interdigitated gold rings placed on the substrate (Fig. 2 c). During the acoustic printing process, the surface acoustic waves which are circular in geometry form an acoustic focal plane at the interface between air and ink in the fluidic channel exit. When the force of acoustic radiation exceeds the surface tension, the droplets are driven out [5]. As it is free of orifices, acoustic printing can eject droplets without negative factors such as heat, voltage and high shear stress. Interestingly, the size of droplets can also be manipulated to print single cell by adjusting the period between the pulses together with the cell concentration [15]. Stability of the acoustic waves are critical for the quality of ejection so that any movement by print head can interfere with the printing process. Therefore, a mobile substrate maybe a solution of 3D printing. According to the current study, the acoustic printing can achieve high ink viscosity printing [16].

2.4. Laser-assisted printing

In laser-assisted printing, a droplet is ejected by laser beam-induced jet formation [17]. This laser-assisted printing system mainly comprises a laser source and a special substrate. The substrate, also known as donor substrate or intermediate substrate, is typically covered with an absorbing layer (e.g., titanium, silver or gold) on the upper and an adhesive layer of ink on the lower. When a laser beam focuses on a point in the substrate, it generates in situ evaporation of the upper layer, leading to bubble formation (Fig. 2 d). Subsequently, the high-pressure bubble drives a droplet toward a collector when the surface tension is less than the pressure. This printing method is an orifice-free method so that it has no influences of clogging and shear stress caused by the ink passing through a nozzle or a needle. The size of the droplets and the resolution can be affected by different factors, such as the laser energy, the substrate thickness, the ink properties and the diameter of the focal point on the upper layer [18].

2.5. Electrospun

Electrospun, which is also named electrospinning, has gained great popularity due to the potential applications of generating nanofibers in nanoscience and nanostructures [19]. Electrospun is the continuous stream mode of electrohydrodynamic jet printing. The conventional electrospun system is mainly composed of a high voltage power supply, an ink store device containing a syringe and a syringe pump, a metallic needle, and a grounded collector with a variable morphology (e.g., a metal plate or a rotating mandrel) (Fig. 2 e). Before electrospun begins, a prepared solution is loaded into the ink storage device. When a high voltage is applied between the metallic needle and the collector, the electrostatic repulsion interacts with the surface tension of a droplet. As the interaction grows stronger, the polymer droplet begins to deform into a conical structure (Taylor cone). When the applied voltage exceeds the critical value, the jet is ejected from the tip of the Taylor cone and then elongated and whipped continuously until it contacts the grounded collector [20]. There are several factors that affect the electrospun process and the quality of electrospun fibers. These factors can be categorized as electrospun parameters, solution and environmental parameters [19].

2.6. Electrohydrodynamic jet printing

Electrohydrodynamic jet printing has two different jetting modes: pulsating droplets and continuous stream [21]. The former is utilized to generate droplets and the latter is utilized for electrospun. Similar to electrospun, electrohydrodynamic jet printing applies high voltage pressure between the metallic needle and the collector in order to generate a droplet (Fig. 2 f). Through adjusting the parameters including the strength of the electric field and the flow rate, dripping can be achieved at a low electric field and a slow flow rate. As the electric field and flow rate become higher, the frequency and speed of the dripping become faster. When the voltage reaches the critical voltage which depends on the properties of the ink and the applied back pressure, it ejects continuous stream from the nozzle [21]. Due to its strong driving force, electrohydrodynamic jet printing can eject a droplet which is smaller than the nozzle orifice so that it is possible to process single-cell printing [21]. In general, the droplets volume decreases when the applied voltage increases and the cell concentration affects the number of cells encapsulated in each droplet [5].

2.7. Comparison

Each printing methods have unique and unavoidable properties (see Table 1). The printers including inkjet printing and micro-valve printing which need orifice to deliver the droplets may suffer from clogging issues due to the high cell concentration and high viscosity. With the increment of driving force, these printers can eject the droplet with high viscosity ink. However, the increasing force bring stronger mechanical stress on cell so that it decreases the viability of cells. Hence, inkjet printing and micro-valve printing are not suitable for high cell concentration printing. As for acoustic printing and laser-assisted printing, there is no clogging problems due to the orifice-free printers. Moreover, the orifice-free printers can avoid the shear stress caused by the material passing through a nozzle. Thus, these orifice-free printers can process high concentrated inks printing. But they require surface tension and viscosity of inks to eject droplets. Due to its electric field to pull the droplet through the nozzle, electrohydrodynamic jet printing can also print high concentrated inks with low mechanical stress on cell [5]. Another advantage of electrohydrodynamic jet printing is no viscous ink limitation. But it requires precise control of Taylor-cone for precisely printing.

3. Bioinks

The biomaterials used for jet-based bioprinting can be considered as bioinks. With the advances of bioprinting technologies, the materials utilized as bioinks have become diversified. According to the applications, bioinks can be divided into two types: supporting bioink and functional bioink (see Table 2 above). A commonly supporting bioink is hydrogel, which is used to encapsulate various biologics (e.g., cells, growth factors or drugs). Typically, hydrogels are hydrated networks of crosslinked natural (e.g., alginate) or synthetic (e.g., poly(ethylene glycol)) polymers which can absorb plenty of water while retaining a 3D porous structure and simulate the natural cellular environment. Unlike the hydrogel, the functional bioink (e.g., DNA and factor) is mainly used to study intracellular delivery, gene diagnose and cell behaviors. In this section, cells were also discussed individually according to distinct bioprinting methods.

3.1. Supporting bioink

3.1.1. Sol-gel transition/crosslink mechanism

Crosslinked hydrogels can be generally divided into physically crosslinked hydrogels and chemically crosslinked hydrogels. Physically crosslinked hydrogels draw growing interest because they can be effectively crosslinked without introducing any chemical groups or exogenous agents, thus minimizing the risk of chemical contamination and toxicity. Gel forming process is usually reversible and affected by the changing conditions of temperature, pH, ionic concentration, water content and so on. Different physically crosslinked hydrogels can form gels by ionic interactions, crystallization, hydrogen bonds, metal coordination or protein interaction. It's worth that the host–guest interaction is also an important crosslinking strategy to form physically crosslinked hydrogels. Usually, the hosts are porous materials while guests with complementary shapes can interact with the hosts. The host–guest interactions are mainly achieved by hydrogen bonding, electrostatics, van der Waals, or hydrophobic interactions. Physically crosslinked hydrogels are usually poor in mechanical stability and to solve this problem, chemical functionalities are introduced. Chemically crosslinked hydrogels are formed by covalent crosslinking of polymers which often show better mechanical stability with the use of exogenous crosslinking agents [77]. Condensation reactions, enzyme catalyzed reactions, and photo-crosslinking are the most common ways for chemical crosslinking [78]. Some kinds of hydrogels allow cell encapsulation in a highly hydrated, mechanical supporting 3D environment which mimics the native tissue environment for cell.

Adhesion, proliferation and migration [79]. However, natural hydrogels generally have poor performance in mechanical properties while synthetic hydrogels lack ability of cell adhesion or migration without being modified [80]. Thus, a kind of hydrogels known as double network (DN) hydrogels may be one promising solution. DN hydrogels with interpenetrating polymer network (IPN) structure are composed of two networks with totally different properties of density, rigidity, molecular weight, cross-linking density [81]. In this IPN structure, the first network is tightly crosslinked, stiff and brittle and it protects the ductile and soft secondary network [79,82]. To date, DN hydrogels has been developed for cell encapsulation. For example, Shin et al. gellan developed a DN hydrogel with gum methacrylate (GGMA) as the rigid and brittle first network, and gelatin methacrylamide (GelMA) as the soft and ductile second network by using two-step photocrosslinking [79]. In this study, the strength of DN hydrogels approaches closer to the strength of cartilage. However longer crosslinking time and high mechanical properties of the DN hydrogels are likely to result in a lower cell viability. Thus, crosslinking conditions should be optimized to increase cell viability. In another study, Zhang et al. created a printable DN hydrogel with alginate and f-gelatin methacryloyl (f-GelMA) networks via physical and chemical crosslinking methods. And compared to the pure alginate/f-GelMA hydrogels, the DN hydrogel showed higher mechanical strength as well as satisfied cell viability. These results demonstrate that DN hydrogel is an alternative bioink for cell 3D printing due to its combined advantage of natural and synthetic hydrogel such as biocompatibility and easy modification.

Table 2
Typical materials for Jet-based Bioprinting.

	Material		Printing methods	Advantages	Drawbacks	Application
Supporting bioink	PEG		inkjet printing electrospun	excellent hydrophilicity, non-toxic, FDA-approved, easy to modify, adaptable mechanical and degradation properties, able to encapsulate cells	bad oxidation stability, UV leads to cell death during photocrosslinking	human Cartilage Repair [25], prevention of intra-abdominal adhesion [26] antibacterial membrane [27], promote bone and cartilage formation [28]
	Collagen		electrospun inkjet printing micro-valve printing laser-assisted printing acoustic printing	high porosity, facilitate attachment and proliferation of cell, absorbability, low immunogenicity	poor mechanical properties, slow gelation time, fibrous, easily clog the nozzle	fibrous scaffoldings for tissue engineering [29]; substrate patterning for cell research [30]; bilayer skin graft for wound healing [31]; 3D corneal structures [24] quantify cell viability [16]
	Alginate		inkjet printing laser-assisted printing micro-valve printing	easy and fast gelation, abundant and cheap	low mechanical properties, poor cell attachment	cardiac pseudo tissues [32] calcium alginate microcapsules [33], complex heterogeneous tissue constructs [34] encapsulation of human pluripotent stem cells [13]
	Gelatin		inkjet printing electrospun	low cost, biodegradability, biocompatibility, low antigenicity, reversible	weak strength, unstable to heat, modification required	skin regeneration [35], wound dressing, cartilage repair [36], and cell function research [37]
	HA		inkjet printing laser-assisted printing	excellent moisture retention, promotes proliferation	viscous and slow gelation rate, rapid degradation	drug-loading tablets [38], cell–cell and cell–environment interactions [39].
	Chitosan		inkjet printing electrospun	abundant, mild gelation conditions, antibacterial	weak mechanical strength, slow geation	cell behavior research [40], nanoparticle drug-carriers [41,42]
	Silk		electrospun inkjet printing	high mechanical strength, feasible structural modification, controllable degradation, low immunogenicity	require mixture with other polymer for optimal rheology and printability; easily clog the nozzle	core-shell structure for enhancing osteogenesis [43]; nanocomposite scaffold for bone tissue engineering [44]; nanofibers for Wound-dressing [45] microscopic arrays printing [46]
Functional bioink	DNA		inkjet printing laser-assisted printing	high throughput probe,	not easy to prepare, low sensitivity	production of DNA microarrays [47] oligonucleotide arrays printing [48]; patterned paper sensors [49]; digital polymerase chain reaction system [50]
	Factor	FGF-2	inkjet printing	retainment of bioactivity after printing, potential to influence the cell behavior	costly, not easy to preserve	engineered spatial biology factor patterns for cell study [51,52]
		BMP-2;	inkjet printing			multilineage differentiation of adult stem cells [53]
		BMP-2; FGF-2	inkjet printing			combinatorial array of immobilized growth factors for cell fate influence [54]; simultaneous control of cell differentiation and alignment [55]
		HB-EGF	inkjet printing			stem cell population migration [56]
		BMP-2 FGF-2 FGF-4 GDF-7	inkjet printing			direct a stem cell population toward multiple fates [57]

3.1.2. PEG

PEG, usually expressed as H−(O–CH2–CH2)n−OH, is a linear polyether compound with excellent hydrophilicity and a high water absorbing capacity. PEGs are non-toxic and FDA-approved, and are widely used to modify proteins, enzymes, liposomes and other biomolecules. PEG in aqueous solution possesses low viscosity which is free of nozzle clogging issues during jet-based processes and can be modified to achieve simultaneous polymerization during printing. Diacrylate (DA) and methacrylate (MA) are usually highly recommended to be added into PEG for photopolymerization. The structural, functional and mechanical properties of fabricated PEG-based hydrogels can be controlled by adjusting the composition via photocrosslinking [5]. Cui et al. modified a thermal inkjet-based printer adding a simultaneous photopolymerization function for PEG dimethacrylate printing with human chondrocytes to repair cartilage defect [25]. The compressive modulus of the printed cartilage implant was 395.73±80.40 kPa, which was similar to native human articular cartilage. What's more, they increased the viability of human chondrocytes by introducing simultaneous photopolymerization to 89.2±3.6% (n = 3), compared to cell viability of 63.2±9.0% (n = 3) when exposed to the same UV light source continuously for 10 min in PEGDMA after printing. PEG has already become one of the main biomaterials in electrospun. Li et al. fabricated PEG nanofibrous membrane by the electrospun method to prevent abdomen adhesion [26]. They found that the composite with 5% PEG showed the most homogenous morphology, the narrowest diameter distribution, and the largest ultimate stress and strain, as well as the lowest degree of cell attachment. In another study, Toncheva, et al. investigated the difference between the physical blending and the chemical grafting of PEG with PLA [27]. The wettability of the mats had no obvious change after these two processes. After loading a kind of antibacterial drug, the addition of PEG greatly improved the slow-release effect in both incorporation methods.

3.1.3. Collagen type I

Collagen is the most abundant protein present in mammals, constituting approximately 25%–30% of the weight of the mammals [83,84]. There are over 20 known types of collagen and all of them have a structure based on three helix polypeptide chains [83]. Collagen is biodegradable, biocompatible and has low immunogenicity, which is broadly used in tissue engineering [83–85], but mainly collagen type I is utilized [86,87]. Due to its biocompatibility and the properties of cell adhesion, collagen type I has been used for cell-cell or cell-materials studies. Boland et al. used collagen type I in electrospun to develop a three-layered construct of fibrous scaffoldings which meets the requirements of a viable vascular prosthetic [29]. Boland's team successfully used an inkjet printer to print a collagen type I substrate and investigated the cellular attachment and proliferation of cells on it [30]. Collagen has shown great potential for bioprinting. However, collagen remains liquid at low temperatures and forms fibrous structures with neutral PH or higher temperatures, which is its limitation [88]. More importantly, its low mechanical properties are not enough to build a porous structure with the desired mechanical strength for structural support and cell protection. Therefore, besides altering the parameters (e.g., temperature, concentration or PH), a new method has been developed for improving the collagen. By compositing it with other materials, Lee, et al. managed to improve the mechanical properties of collagen [89]. In their study, collagen was blended with decellularized extracellular matrix to induce high cellular activities, and silk-fibroin was added for attaining the proper mechanical strength. This composited ink was printed to form a scaffold. After treating with methanol to induce β-sheet formation, its compressive strength increased by 9-fold over that of the collagen scaffold.

3.1.4. Alginate

Alginates are unbranched polysaccharides consisting of 1/4 linked b-D-mannuronic acid (M) and its C-5 epimer a-L-guluronic acid (G) [90]. Alginates have been popular in the family of bioinks due to their natural attributes, mild gel-forming conditions, high biocompatibility, stability, adjustable porosity and possibilities of modifications. Alginates could be extracted from both algal and bacterial sources and commercially alginates are only produced from algae [91]. Alginic acid was discovered, extracted from brown seaweeds, and patented by the British chemist Stanford for the first time [92]. Alginate undergoes ionic crosslinking with divalent cations to form gels. Calcium chloride ($CaCl_2$) is frequently used as an additive to induce gel formation. The interactions between G-blocks forming tightly held junctions are the main drivers for gel formation with the help of divalent cations. The concentration of alginate can affect both the mechanical properties and printability of alginate hydrogel. Owing to meeting the suitable viscosity, alginate has a narrow mechanical properties range. Low mechanical properties have always obstructed the application of bioprinting alginate, but the mechanical properties of bioprinted structures can be improved by combining alginate with other high-strength biomaterials, such as gelatin and collagen.

Xu et al. introduced inkjet printing method to fabricated heart-like tissue constructs. $CaCl_2$ solution (0.25 M) was ejected into a sodium alginic acid (2.3%) and gelatin (0.1%) pool with cardiomyocytes using a modified HP inkjet pinter. The cells remained viable in constructs as thick as 1 cm due to the programmed porosity [32]. What's more, Xu, et al. created viable three-dimensional heterogeneous constructs with multiple cell types by printing the cells with $CaCl_2$ solution into alginate-collagen composites layer-by-layer [34]. Laser-based bioprinting with alginate has also been widely studied. Gudapati et al. investigated the effects of alginate gelation, gelation time, alginate concentration, and laser fluence on the post-transfer cell viability of NIH 3T3 fibroblasts. They proved that both overlong gelation time and exposure of encapsulated cells to calcium chloride lead to cell injury and death. In addition, the cell viability after 24 h incubation decreased as the laser fluence or alginate concentration increases. Yan et al. first investigated the 3D printing performance of laser-assisted printing of alginate tubes [93]. Successful laser-based bioprinting of alginate depended upon sodium alginate concentration and laser fluence.

3.1.5. Gelatin

Gelatin is a natural biopolymer derived from collagen protein in bones, skin, and connective tissues. Gelatin is widely used in food, cosmetic and medical fields due to its low cost, biodegradability, biocompatibility, low antigenicity and high possibility for modification [94]. Gelatin in aqueous solution is able to respond to the temperature that sets at 15 °C and melts at 25°C–40 °C. The pH also makes a difference: the gelatin is negatively charged at higher pH and positively charged at lower pH. However, gelatin is easy to dissolve when incubated at 37 °C without any modification. Gelatin is rarely bioprinted alone, due to its weak strength. Glutaraldehyde is the most used agent for chemically crosslinking gelatin. Alginate is a good partner of gelatin due to its interaction for enhancement. Gelatin-based hydrogels fabricated by jet-based printing are widely applied in skin regeneration, wound dressing, cartilage repair, and cell function research [35–37,95,96]. Electrospun is a common method to fabricate gelatin-based scaffolds composed of nanofibers as well as introducing many microspores. Bridge et al. fabricated electrospun scaffolds composed by crosslinked gelatin and methacrylated gelatin (GelMA) [37]. The average fiber diameters of these highly aligned scaffolds range from 200 nm to several micrometres with Young's moduli ranging from 1×10^5 to 1×10^7 Pa. These scaffolds can be used for smooth muscle cell culture and enable the contractile forces generated by the aligned three-dimensional sheet of cells to be directly measured. In some special applications, the mechanical properties of the nanofibers could be enhanced by heat treatment [97]. Zhang et al. fabricated a gelatin/antibody layer by inkjet printing to achieve temperature-switched antibody release [98]. They can release the immunostaining reagent on demand, rapidly and completely, by taking advantage of the temperature responds of gelatin.

3.1.6. Hyaluronic acid (HA)

HA, a linear non-sulfated glycosaminoglycan, is distributed widely in neural, connective and epithelial tissues. HA is regarded as the best natural moisturizing factor and there is plenty of HA in our skin. The molecular weights of HA range from 100 000 Da in serum to 8 000 000 Da in the vitreous [99]. There are few studies related to HA used in jet-based printing, due to its viscous nature and slow gelation rate. However, it is possible to improve the printability of HA by chemical modification as well as facilitate faster crosslinking. Acosta-Vélez et al. developed a biocompatible photocurable pharmaceutical polymer for inkjet printing [38]. In that study, HA was modified by norbornene moieties and then underwent a rapid step-growth polymerization reaction through thiol-ene chemistry with the help of PEG, Eosin Y and visible light. The engineered HA bioink could be inkjet printed for the rapid production of tablets loading with hydrophilic active pharmaceutical ingredients. Use of HA in laser-assisted printing has also been demonstrated. Gruene et al. took HA to construct multilayer hydrogel-cell planar structure by laser printing [39]. Human adipose-derived stem cells and endothelial colony-forming cells, were printed respectively as individual droplets onto each hyaluronic acid fibrinogen hydrogel layer and were then covered with another hydrogel layer to study cell–cell and cell–environment interactions.

3.1.7. Chitosan

Chitosan is a linear polysaccharide biomolecule obtained from chitin which is abundant in nature. Commercial chitosan is acquired from the shells of shrimp and other sea crustaceans by inexpensive and easy ways. The structure of chitosan is very similar to that of cellulose (made up of β (1–4)-linked D-glucose units), in which there are hydroxyl groups at C2 positions of the glucose rings [100]. The molecular weights (Mw) of typical chitosan is between 10 and 1000 kDa and the degrees of deacetylation are usually between 70% and 95%. Chitosan are biodegradable and can be metabolized by certain human enzymes, especially lysozyme. Chitosan exhibits a pH-sensitive behavior, being soluble in aqueous solutions when $pH < 6$ and poorly soluble when $pH > 7$. Chitosan possess the ability to form gel itself by neutralizing the amino groups. Chitosan is also able to form ionic crosslinked hydrogel in the presence of anionic components under relatively mild gelation conditions. Covalently cross-linked chitosan hydrogels can be prepared by treating chitosan with various chemical reagents, such as glyoxal, glutaraldehyde, and genipin. Chitosan has been widely applied in wound dressing, cartilage tissue repair and drug delivery. As for wound dressing, chitosan facilitates the haemostatic process by interacting with red blood cells with negatively charged membrane because of its cationic nature [101]. Chitosan is usually joint used with gelatin or alginate due to its weak mechanical strength [102,103]. Chitosan-based hydrogels are frequently used with an extrusion bio-printer but there are very few studies of printing chitosan by jet-based bioprinting methods. Gu et al. patterned chitosan on glass by inkjet printing to investigate the behavior of macrophages [40]. Their study showed that chitosan can enhance the mobility, cytoplasm spreading, and phagocytosis of macrophages. Chitosan shows great potential as a coating material on biomedical implants in promoting the macrophage response to bacteria. Chitosan has received a great deal of attention as an excellent drug carrier in the form of nanoparticles. Zhang et al. developed a one-step electrospray procedure to prepared chitosan solid nanoparticles with an average diameter of 124 nm [41]. In this study, it was determined that stable electrospray was achieved only when viscosity was high and/or conductivity was low. With the decrease of acetic acid concentration, chitosan concentration, and/or flow rate, smaller particles were obtained. Under an optimized concentration of chitosan, a minimum value for the flow rate and highest value for applied voltage are suggested to obtain the minimum size and narrow size distribution of chitosan nanoparticles. Abyadeh et al. prepared solid chitosan nanoparticles successfully with a size of 110.6 nm, size distribution of 32 and zeta potential of 59.3 by the electrospray method [104]. In summary, chitosan is not an appropriate material for large scale scaffolds but could be printed for a good anti-microbial coating film.

3.1.8. Silk

Silks, natural fibrous protein polymers, are produced in nature by arthropods and spiders. Among the various types of silk protein existing in nature, the silkworm silk of *B. mori* is the most frequently investigated material for biomedical research and biotechnology applications, due to its easy availability, along with its desirable material properties [105]. *B. mori* silk fibers consist of two proteins: sericin and fibroins (a heavy and a light chain with a glycoprotein, P25), the fibroin fibers are covered with sericin [105,106]. For biomedical applications, the sericin is removed by degumming, which typically consists of boiling in $NaHCO_3$ or Na_2CO_3 solution to remove the peptide bonds of sericin, followed by rinsing and drying [101,107]. Silk protein can be processed in aqueous environments and its solution displays nearly newtonian behavior with low shear viscosity and low shear elastic modulus and hence it gains popularity in jet-based bioprinting for various applications (see Table 2). Moreover, silk is an ideal additive biomaterial for composite to improve mechanical strength [89].

3.2. Functional bioink

3.2.1. DNA and factor

Jet-based bioprinting can also be used to print DNA, and it benefits fields including but not limited to, genomics and medical diagnostics. Microarray is a technology which is directed at gene expression analysis for diagnostics and identification [108,109]. Due to their non-contacting nature and their compatibility with DNA, as well as the high throughput and high resolution, jet-based bioprinting was investigated for DNA microarray construction 20 years ago [94,108,110]. In recent years, diverse applications of DNA and jet-based printing has been developed, such as paper sensors printing [49] and a digital polymerase chain reaction system [50].

Gene transfection is a strong technology allowing for research of genes and genes function. Several reports have indicated that ink-jet printing can be applied to intracellular delivery and transfection [97, 111,112]. Xu et al. first achieved cell delivery and transfection by co-printing plasmids encoding green fluorescent protein (GFP) and porcine aortic endothelial (PAE) cells into a fibrin-gel substrate [111]. In further study, Cui *et al* evaluated the printing influences of cell viability and the size of cell membrane pores and their results demonstrated the potential of intracellular delivery through transient membrane pores during the printing process [97]. The mentioned research demonstrated that inkjet printing for biology factors deliver a strong and functional tool for cell behavior investigation.

Jet-based bioprinting is a powerful tool to investigate the incorporation of biology factors in Spatial patterns. Campbell et al. initiated the study of the influence of cell behavior on spatially modulated growth factors by inkjet printing and the printed patterns can sustain cell culture for 10 days [51]. In another study, Miller, et al. successfully controlled cell proliferation through precisely depositing fibroblast growth factor-2 (FGF-2) with inkjet printing [52]. Based on inkjet printing, Miller, et al. did a further study to investigate the effect of combinatorial growth factors on cell fate [54]. Phillippi et al. utilized the bone morphogenic protein-2 (BMP-2) as the spatial growth factors gradients pattern to direct the differentiation of muscle-derived stem cells and their result verified its feasibility [53]. Several studies of cell behavior were based on inkjet printing, including controlled cell alignment [55] and cell migration [56].

During laser-assisted printing research, laser-assisted printing has also been applied to print diverse proteins, such as bovine serum albumin, enzymes, antigens, streptavidin, biotin-avidin, immunoglobulin G, and titin [113].

3.3. Cell

The cell printing technique has been studied for different applications, such as cell microarray for drug screening [114], and creation of biomimetic microenvironments for cells to build disease models or tissue constructs [115,116]. With the advantages of high resolution and high cell viability, jet-based bioprinting is an effective method for cell printing. Currently, a study demonstrated that thermal inkjet printing can trigger the activation of the VEGF pathway in human microvascular endothelial cells (HMVECS) in vitro [117]. In this study, the analysis of six cytokines (HSP70, IL-1α, VEGF-A, IL-8, FGF-1, Ang-2) which have potent angiogenic effects showed that the angiogenic signals of HMVECS were activated or promoted after printing. According to this result, jet-based bioprinting may be utilized to develop angiogenic scaffolds [117]. Hence, jet-based bioprinting is also a potential technique for cell modification. In this part, cells printing with jet-based bioprinting was introduced and the relevant parameters were shown in Table 4.

3.3.1. Inkjet printing of cells

Xu and his co-workers used a modified commercial thermal inkjet printer to print bacterial colony arrays for the first time [118]. This was also the first time cells were inkjet-printed into defined patterns. Mammalian cells were then inkjet printed by Xu et al. It is crucial for cell bioinks that cells are not hurt by the printing process. In a typical inkjet printer, there is high temperature at the nozzle and Xu's work demonstrated that this transient high temperature did negligible harm to cells [119]. High cell viability and basic cellular properties were demonstrated to be maintained after printing [119,120]. In these studies, cells were suspended in water or saline and directly printed as bioink and the cell concentration was not allowed to be too high. The bioink had low viscosity, to prevent clogging. The printing was performed at room temperature in a high-throughput manner. Further work performed by Xu et al. demonstrated that the inkjet printing procedure had an effect on cell membranes and could transfect genes into living cells [111]. This discovery indicated that the printing procedure might have an effect on cell bioink. Since the final bioinks generally had low viscosity, the resolution might highly depend on the printer parameters. Without heat generation, piezoelectric inkjet printing technology was also used to print cell bioink and cell viability could be adjusted by varying the pulse amplitude [121]. Besides, electrostatically driven inkjet system was implemented to efficiently print cells [122]. During the printing process, cell bioink might clog and studies on avoiding cell clogging was reported [123]. There was also research indicating that cells might change their morphologies if experiencing inkjet printing [124]. Hydrogel with low concentration and viscosity could be blended with cells to form cell-gel bioink and were printed by inkjet printing [125]. In an inversed manner, cell-crosslinker bioink can also be printed into uncrosslinked gels to form hydrogel structures [116]. The potential applications of inkjet printing cells include printing cells for regeneration [120,126,127], achieving single cell printing [17,122] and gene transfection or cell modification [111,124].

3.3.2. Laser-assisted printing of cells

Laser-assisted printing was demonstrated to print cells at microscale resolution and high speed (5 kHz) [17]. In this manner, cells were suspended in medium supplemented with wide range or extracellular matrices (glycerol, alginate or Matrigel™). The printing process was conducted at room temperature. Cell density had an effect on the viscosity of the final bioink. Koch and co-workers further demonstrated that laser-assisted printing had the capability to fabricate multicellular grafts to mimic tissue functions [128]. In this study, cells were laser-assisted printed along with a sheet of Matriderm™. It might be concluded from the above research that cells were always printed along with gels in laser-assisted printing since the bioink should be attached on the laser-absorb layer. Therefore, the final bioink could be a cell-gel blend rather than a pure cell suspension. The printing resolution was highly determined by the characteristics of the gel added. Another study presented by Sorkio and co-workers showed the printability of cell-laminin-collagen bioinks [24]. Cell viability and function were preserved after printing. Laser-assisted printing with cell bioinks have shown potential in various tissue engineering applications as the above studies mention. Most advanced studies also showed the vasculature application of laser-assisted printing of cell bioink [129,130].

3.3.3. Micro-valve printing of cells

The bioink viscosity in microvalve printing (1–70 m Pa) can be higher than that in inkjet printing (3–30 m Pa) though the nozzle sizes are similar in these two printing modes [22]. With the precondition that higher cell density will cause an increase of bioink viscosity, the cell bioink used in micro-valve printing might have higher cell concentration than inkjet printing [22]. Faulkner-Jones and co-workers presented a work on the development of a valve-based printer and the printing of human embryonic stem cells [11]. In their work, cells were suspended in medium to obtain the cell bioink with low viscosity. High cell viability was maintained after printing. Their further work demonstrated the pluripotency maintenance of stem cell bioink in micro-valve printing [13]. An advanced work conducted by Filardo and co-workers showed the printability of cell-collagen bioink by micro-valve printing [131]. These collected works demonstrated the application of micro-valve printing in tissue engineering.

3.3.4. Acoustic printing of cells

The bioinks used in acoustic printing requires certain surface tension and viscosity. In addition, it has no limitations of clogging, laser-induced damage and mechanical stress so it is a greatly favorable method for generating cell encapsulation in low viscosity ink. Demirci and co-workers developed an acoustic picolitre droplet generation system for cell encapsulation [14]. The droplet size (3 μm–200μm) could be tuned by varying the acoustic wave frequency. Various cell types were printed and high cell viability (>89.8%) as well as cell functions were maintained after printing. Acoustic printing is also useful in single-cell printing [15,132].

3.3.5. Electrohydrodynamic jet printing of cells

Due to its ability to eject high viscosity inks, electrohydrodynamic jet printing can be used to encapsulate cells in viscous ink which may cause clogging in other jet-based bioprinting methods (e.g., inkjet printing and micro-valve printing). In order to investigate the compatibility of the printing procedure with cells, some research has systematically evaluated the main parameters, including nozzle size, ink flow rate, distance of the target and voltage [133,134]. Gasperini and his co-workers used 2% alginate to encapsulate B50 neuroblastoma rat cells, and the cells inside the beads (200–400 μm) showed high viability on day 7 [135]. In another study, Workman, et al. demonstrated that voltage had no significant effect on the viability by jetting THP-1 (a human monocytic cell line), both in no voltage and ≈7 kV applied voltage [133]. Electrohydrodynamic jet printing can also process single cell printing. Kim and co-workers successfully optimized the parameters in order to generate 10 -μm diameter spots which contained a single bacterial cell [136]. Compared with acoustic printing, electrohydrodynamic jet printing has some inconvenience in printing single cell printing due to the difficulty of precision control of the Taylor cone.

4. Further development

Ideal bioinks should have proper biodegradability, good biocompatibility, favorable mechanical properties and desired printability. Natural materials are generally derived from animals and plants. Thus, they possess a native biocompatibility and show great performance in cell interaction and modulation [137]. But they have limitations of poor mechanical properties, shrinking and deformation before printing. Alternatively, most of the synthetic materials have a better performance in mechanical properties but perform poorly in cell adhesion and cell encapsulation (see Table 3 above). Therefore, there is a great demand for novel biomaterials for bioprinting. Composite materials provide the ability to combine different properties of various materials and thus is currently a promising direction .

4.1. Composite materials

Compositing materials is an effective method for improving material properties. Compositing methods are typically classified as chemical or physical. With regards to chemical methods. Roberta et al. produced a printable polymer hydrogel which has an A–B–A architecture, composed of poly(N-(2-hydroxypropyl)methacrylamide lactate) A-blocks and hydrophilic poly(ethylene glycol) B-blocks [137]. The studied polymer not only showed both good mechanical properties and a controllable degradation rate but also had similarities with many natural polymers (e.g., collagen). Additionally, a high chondrocyte viability in the hydrogel demonstrated the potential of bone tissue engineering. Natural

Table 3
Typical synthetic materials.

Material	Printing methods	Advantages	Drawbacks	Application
PCL	electrospun	proper mechanical strength and biodegradation rate	poor hydrophilic, unable to encapsulate cells	regenerate periodontium [58], critical-sized cranial bone defect [59] diabetic ulcers healing [60], annulus fibrosus (AF) defect closure [61]
PLLA				dura repair [62],chronic wound [63], brain injury [64]
PGA		quickly degradation rate		suture [65], engineering intestine [66, 67]
PLGA		controllable mechanical strength and biodegradable rate		sutures [68], neural tissue repair [69], bone tissue regeneration [70,71],wound dressing [72]
PBS		excellent processability, physical property and biodegradability, good hydrophilicity	unable to encapsulate cells, low cell affinity	rapid hemostatic [73], tubular vessel [74], bone scaffold [75], antimicrobial [76]

Table 4
Cells printing.

Cell	Printing methods	Cell concentration	Cell viability	Reference
Neuron	Inkjet printing	2 × 106/ml	>90%	[119]
Keratinocyte epithelial stem cells	Laser assisted printing	3 × 107/ml	≈100%	[128]
Embryonic stem cell	Micro-valve printing	2 × 106/mL	>95%	[11]
Embryonic stem cell	Acoustic printing	4 × 106/mL	>90%	[14]
Neuronal cell	Electrohydrodynamic jet printing	2 × 106/mL	Not specified	[135]

materials can be modified. Li et al. produced a polypeptide–DNA hydrogel which showed a favorable property of mechanical strength, non-swelling/shrinking, biodegradability and biocompatibility [138]. In this study, the researchers synthesized a polypeptide–DNA conjugate by grafting multiple single-stranded DNAs onto a polypeptide backbone. Therefore, hybridization of polypeptide–DNA conjugate and a complementary DNA linker will cause crosslinking, leading to hydrogel formation. As mentioned in section 3.1.1. DN hydrogels with interpenetrating polymer network (IPN) structure is also a potential composite bioink.

Considering the complexity and long-term process of synthetic materials development, physical composite is a quick and effective method to seek the positive solution of medical and tissue engineering, Contrary to chemical composite, physical composite can maintain the material properties of each component and simultaneously achieve some comprehensive properties that cannot be achieved by a single constituent material. Besides evaluating the material characteristics, these studies also investigated the cell behaviors on them. Yao et al. demonstrated PCL/PLA-3D blend scaffolds exceeded PCL-3D scaffolds in their mechanical properties and bioactivity [59]. Similarly, Deng, et al. developed a novel substitute comprised of PLLA and gelatin and their results showed that this composite substitute was superior to collagen substitute in cytocompatibility, tissue ingrowth, and neoangiogenesis [63]. Physical composite also provides an effective method to enable materials with poor printability to be 3D bioprinted. Currently, LEE et al. presented a method to 3D-bioprint collagen to engineer components of the human [139]. In this study, freeform reversible embedding of suspended hydrogels (FRESH) was used as a support material to enable collagen as a bioink for high fidelity bioprinting and ventricles were printed by this method maintain their intended geometry after culturing for 28 days. This study demonstrates that physical composite can simultaneously provide a desirable support during 3D bioprinting process and post-printing. Thus, physical composite is a potential method that can build advanced tissue scaffolds.

4.2. Multi-nozzle hybrid bioprinting

Due to the organic dissolving properties and relatively high melting temperature, most synthetic polymers have difficulty encapsulating cells directly during printing. Thus, a common method is seeding cells on the synthetic polymers' substrate or scaffold via manual operation or 3D printing. But this method is time consuming and easy contamination. On the other hand, the hydrogels provide a practical means of cell encapsulation. Hydrogel structures, however, have poorer mechanical properties than polymers structures have. To address these problems, a multi-nozzle printing system with independent cartridges which can deposit various bioinks layer-by-layer or by precisely staggered positions, is of benefit for bioprinting. More importantly, the technique of multiple material printing provides a method to achieve multi-layer and multi-structure structural integrity, to mimic the native tissue. Xu and co-workers demonstrated the feasibility of constructing an inkjet printing/electrospun system [140]. In this printing process, a PCL fibrous scaffold layer was first printed via electrospun. Subsequently, the inkjet printer ejected the cell-collagen solution onto the scaffold layer to generate a hybrid scaffold. This hybrid bioprinting technique provides a method for the layered structure to mimic cartilage constructs by alternating electrospun and inkjet printing in a multi-nozzle system. Wei et al. built a hydrogel construct with hierarchical pores, which emulated the native tissue [141]. They used micro-valve printers to print cross-linkers ($NaHCO_3$) and polyvinylpyrrolidone (PVP) over each layer of collagen in a bottom-up layer-by-layer method. Simultaneously, the number of printed PVP droplets are manipulated to generate a varied concentration in each layer so that the collagen fibrillogenesis process can be altered to control the microstructure. Considering the intricate structure of real tissues and organs, an integrated tissue–organ printer (ITOP) containing four cartridges was designed by Kang *et al* [142]. With this printer, Kang and co-workers successfully printed human-scale tissues such as ear, bone, and muscle structure which used PCL as a structural support, and cells-hydrogels as fillers. Hence, 3D hybrid multi-nozzle bioprinting for multiple materials deposition can facilitate a promising future in jet-based bioprinting.

5. Conclusion

Jet-based bioprinting shows great superiorities in medical and tissue engineering, due to its characteristics of non-contact, agility, high resolution, and precise control on the deposition pattern. In jet-based bioprinting, the bioinks should have good performances in printability, mechanical strength and cell protection. Additionally, abundant resources and low price are crucial to the practical application of jet-based bioprinting. However, available materials for jet-based printing bioinks are limited because of the strict requirements of printability, biodegradability, biocompatibility, cell adhesion and favorable mechanical properties. One the other hand, there is an imperative need for more intricate and integrated structures in order to achieve tissue and organ printing. Therefore, as a future perspective, we believe that composite materials and multi-nozzle hybrid bioprinting are promising ways to address these urgent demands. To date, researchers are still on their way to achieving fully functional organ printing. Nevertheless, there is no doubt that jet-based bioprinting is one desirable technology for medical and tissue engineering.

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