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Review Article

Advantage of Alginate Bioinks in Biofabrication for Various Tissue Engineering Applications

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Bioprinting is fast emerging as a viable technique for organ fabrication. Though various types of bioprinting methods have been developed, the most commonly used bioprinting is extrusion-based bioprinting (EBB). Bioinks are extruded layer-by-layer forming a 3D multicellular construct and scaled up to dimensions depending upon the specific tissue to be regenerated. Among various bioinks, alginate, a natural polysaccharide, has been extensively used because of its good printability in physiologically amenable conditions. Though alginate possesses good printability properties, it promotes little cell–material interaction resulting in limited biofunctionality. Therefore, it becomes necessary to blend/modify alginate to improve the biological properties of bioink without compromising printability. This paper presents a review of the various approaches used to optimize bioprinting with alginate bioinks and their limitations.

1. Introduction

The technology of 3D bioprinting is rapidly achieving clinical translation where life sciences and engineering principles are combined to fabricate organ models and tissue constructs using living cells and biochemicals in a layer-by-layer deposition process [206, 207]. In regenerative medicine, several conventional cell-based and scaffold-based techniques had been applied in the last two decades but with limited translation. The advancement of 3D printing enables the fabrication of patient-specific constructs using a computer-aided design process. Although having many advantages, bioprinting also has a few limitations, which need to be addressed for fabricating any desired construct. Major constraints arise due to cell viability loss, a narrow window for optimization of process parameters, and maintaining long-term functionality after bioprinting. The materials used for bioprinting constructs mostly contain cells and biopolymers based on the specific tissue engineering application. These cell–biopolymer blends are called bioinks. Though a wide number of biomaterials are available for tissue engineering and regenerative medicine, many of them are not suitable for bioprinting. For bioprinting, biomaterials used should not require organic solvents or high temperatures [2]. There are two types of bioinks currently available namely (a) scaffold-based bioinks and (b) scaffold-free bioinks. For fabrication of functional tissues on a large scale, scaffold-free cell pellets, tissue strands, and tissue spheroids are used [3]. On the other hand, scaffold-based bioinks include hydrogels, decellularized matrix compounds, or microcarriers loaded with cells. One of the most commonly used biomaterial for bioprinting is alginate, a natural polymer, which is non-immunogenic, biodegradable, and non-toxic and is composed of mannuronic and guluronic acids. The alginate applications and properties and tissue bioprinting have been widely analyzed and are reviewed independently. In this review paper, the present bioprinting techniques, especially extrusion-based bioprinting and alginate bioinks, are presented in detail. Also, the alginate bioinks (scaffold-based bioprinting) are discussed in detail.

2. Types of Bioprinting

2.1. Inkjet-Based Bioprinting. The first printing technique of bioprinting is inkjet-based bioprinting, it is also identified as drop-on-demand bioprinting, drop-by-drop printing [4].

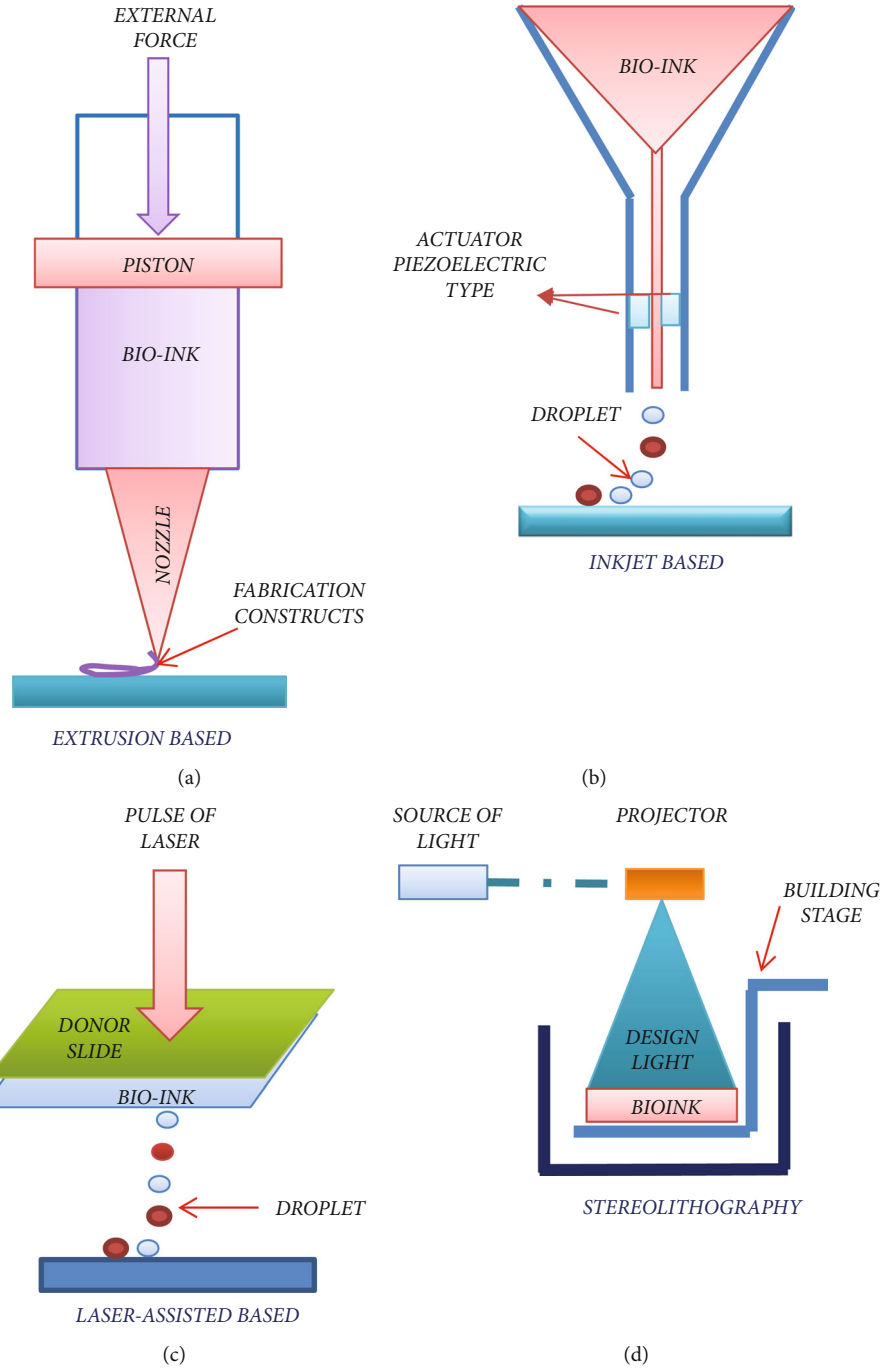

Figure 1: Bioprinting methods: (a) extrusion based, (b) inkjet based, (c) laser-assisted, and (d) vat polymerization (VP).

Figure 1(b) shows the inkjet-based bioprinting procedure. In this process, the reprographic strategy is non-contact type deposition of bioinks in drops [5]. Bioinks mimic the environment of the extracellular matrix (ECM) and help in the differentiation, adhesion, and proliferation of mammalian cells. The generation of the droplets for the fabrication of the constructs depends on three different techniques as follows: (a) thermal inkjet [8–11], (b) electrostatic bioprinting [12], and (c) piezoelectric inkjet (acoustic) [6, 7]. Depending upon the biomaterial, the encapsulated cell droplets can be assembled or printed layer-by-layer into a construct [13]. Wijshoff [1] discussed the dynamics of the piezoinkjet printhead operation. Piezoelectric inkjet-based bioprinters produce acoustic waves in the bioinks using the piezoelectric actuator, which helps in the discharge of the droplets via the bioprinter nozzle. In thermal-based bioprinters, there are single or multiple nozzles and a chamber for containing the fluid. Heat is produced inside the chamber of the bioink, which ultimately results in the induction of pressure pulses [2]. This pressure helps in the ejection of the droplets in the picometer range volume from the orifice nozzle [3]. In the case of bioprinters of electrostatic type, the production

of the droplets occurs by the voltage pulse, which is applied between the electrode and the pressure plate [4]. In current years, the use of inkjet bioprinters has attracted many researchers because of their acceptable speed, compatibility with living materials, low expense, high cell viability, and versatility of bioinks [5]. In the case of tissue biofabrication, however, inkjet-based bioprinters pose an inability to extrude highly viscous bioinks (>10 cP) because the nozzle opening is small. Another problem is unwanted production of pressure at the nozzle occurs during the printing of high-cell density bioinks [6, 7]. In this bioprinting technique, the bioinks used are therefore of low viscosity, which ultimately results in the formation of printed structures having low mechanical strength [8]. Presently, this technology of bioprinting is becoming an important tool in tissue engineering and biomedical applications like the deposition of cells, scaffold building, and the development of drugs. Li et al. [210] reported the various types of inkjet printing, applications, limitations, and advantages of this technology in detail. Log Ng et al. [211] investigated the effect of sub-nanoliter droplet-based bioprinting on the cell viability of printed primary human cells. Droplet evaporation and droplet impact velocity were investigated and reported as the best understanding factors, which affect the cell viability of the nanoliter droplet-printed cells.

2.2. Laser-Based Bioprinting. In the case of laser-based bioprinting, fabrication with the living cells is accomplished by employing a high-energy light source or a long-wavelength laser [14, 15]. Figure 1(c) shows the laser-based bioprinting process. In this technique, cell printing is done using a laser beam, which is pulsating at a controlled rate [16] onto a collecting substrate. In a common laser-based bioprinter, the main components that are present are a focusing system, a receiving substrate, a ribbon that absorbs laser, a pulsed laser beam, and a material containing cells [17, 18]. Various factors that affect the bioprinter resolution of the laser-based bioprinters are laser type and configuration, viscosity, thickness of the organic coating, substrate wettability, an air gap between substrate and ribbon, and surface tension [19]. Compared to inkjet bioprinters, the laser-based bioprinters print bioinks having material viscosities of a wide range (1–300 MPa/s) [20]. Furthermore, laser-based bioprinting does not require a nozzle for printing so it can print a large density of cells without clogging issues. Laser bioprinters also do not affect mammalian cell viability and cell functions to a significant level. It should be noted that this method is not the alternate solution to inkjet bioprinting in tissue engineering applications though having a higher resolution compared to other bioprinting techniques [4]. Dou et al. [109] reviewed various laser-based bioprinters and their applications, advantages, disadvantages, and latest advancements.

2.3. Extrusion-Based Bioprinting. In tissue engineering and regenerative medicine, the most commonly used biofabrication technique is extrusion bioprinting [9]. Figure 1(a) shows the extrusion-based bioprinting process. In this process of bioprinting, bioink is extruded via the extrusion nozzle by the mechanical force produced by a piston or a screw, or a pressurized air/gas. The extrusion occurs as a strand continuously. For extrusion bioprinting, the viscosity range of the bioinks is from 30 to 6×10^7 mPa s [221]. In extrusion bioprinter, there is a dispenser system, i.e., single or multiple ejectors, which are fixed on a robotic stage that is automatic and is controlled using a stage controller. Three-axis are present in the robotic stage, i.e., (x–y–z) [21]. Bioink is extruded onto a substrate that is located beneath the extrusion nozzle of the bioprinter [10]. This type of bioprinting process can print bioinks having a wider range of viscosities and higher cell densities without nozzle clogging, and it can print biodegradable thermoplastics and specialty hydrogels [22]. Additionally, it is a quick technique with suitable viability of cells after bioprinting. The clogging risk is less in this technique though the method produces lower resolution (~200 μm) [21] of fabricated constructs compared to the other methods of bioprinting. Laser-induced forward transfer, stereolithography (SLA), extrusion, and inkjet are the four bioprinting techniques that are generally used in tissue engineering. For extrusion-based bioprinting, the bioink is loaded in the syringe of the bioprinter and is extruded using mechanical and pneumatic actuation. Multinozzle printing is also possible for the same desired structure for fabricating heterogeneous constructs [208]. Zhuang et al. [209] improved the mechanical properties of gelatin methacryloyl (GelMA) hydrogel by using an inbuilt UV source with their extrusion-based bioprinter. They reported a soft bioprinted construct having tunable mechanical properties. However, bioink deformation and shear stress can cause reduce in cellular viability [5]. Fisch et al. [164] assessed a new extrusion technique depending upon a reduced advanced pump of the cavity and studied the precision and accuracy of the system with that of the extrusion pneumatic-based system and verified both for their cell viability effect after the extrusion process.

2.4. Bioplotting. Bioplotting is another biofabrication technique used. In this technique, a syringe is used, which extrudes either spheroids or tubes of materials [10]. Bioprinting with multiple syringes and the capability to employ many cell types are the main attractive features of the bioprinter. Because it can fabricate multiple types of tissues in the output construct, this leads to the creation of bioengineered soft tissues. Here the crosslinking of the post-printed constructs is done either by UV radiation or chemical reaction [11]. Though having many advantages, it suffers from a few disadvantages also, which are as follows: the chosen extrusion material should be viscous, it should provide a functional cellular microenvironment, and it should be cell supportive. For bioplotting, the materials used paste with macromolecules, such as proteins, polymer melts, etc., and high filler contents [10]. It is reported that the bioplotting technique is one of the most acceptable techniques for fabricating co-cultured tissues and scaffolds that do not need high resolution [11].

2.5. Vat Polymerization. Among all the other biofabrication techniques, the emerging biofabrication technique vat

polymerization (VP) has higher accuracy of fabrication. For the fabrication of complex constructs structures, various photo-initiators (PIs) are added with the bioinks for creating crosslinking process for getting the best printing resolution. This printing technology advancement led to a huge revolution for tissue constructs from the fabrication process of non-biocompatible type (seeding cells on post-printed scaffolds) to the fabrication process of biocompatible type (printed scaffolds already containing cells in it in a 3D environment). There are two common types of VPs used in biofabrication namely (a) SLA and digital light processing (DLP) [213]. Figure 1(d) shows the process of VP bioprinting.

2.5.1. Stereolithography. During the 1980s, SLA was introduced. In this technique, crosslinking of polymers is performed using light and this is a freeform reliable technique [26, 27]. In most of these techniques, UV radiation is usually used and is directed to an array of a mirror for projecting the beam of light to the liquid photocurable resin surface. As soon as the resin is crosslinked, the fabrication stage travels on the *z*-axis for introducing a fresh resin layer, and the process is repeated for the fabrication of the 3D structure. This method is highly acceptable for most applications because it can prepare high-resolution structures. Regardless of this, researchers are restlessly working to developing a novel resin that would be biocompatible and maybe be used in regenerative medicine and tissue engineering applications [11]. Grigoryan et al. [23] presented the application, characterization, and development of an SLA bioprinter, which supports multi-material, minimizes bioink mixing, and yields precise regional feature alignment. Kumar and Kim [24] addressed the advancement in SLA 3D bioprinting synchronized with the fabrication of new photo-crosslinkable biomaterials with improved chemical and physical properties. SLA based on visible light is also being developed for bioprinting where normal light can be used for curing bioinks for stabilization of constructs [14].

2.5.2. Digital Light Processing. This type of VP is a robust and fast biofabrication process used in tissue engineering. This technique can fabricate models of tissues that can reproduce the complexity and resolution and complexity of the natural construct and tissues. In this technique, digital masks are used to project 2D images on the bioink for creating the construct. In this technique, a projector is used to fabricate the construct. The DLP bioprinter resolution is dependent on the bioink microenvironment photo-crosslinking response and the projected beam of light. Goodarzi Hosseinabadi et al. [212] addressed a summary of this technology focusing mainly on light characteristics in the resolution of bioprinted constructs, PI selection, and bioink properties. Rashid et al. [213] addressed various opportunities and challenges of this technology in detail. In Table 1, the various parameters required for bioinks for different bioprinting types are mentioned briefly. Alginate mixed with various other polymers can be used for extrusion-based bioprinting [214, 217]. For VP bioprinting, functionalization of alginate bioinks is done to bioprint the construct like GelMA-Alginate bioinks. For the case of inkjet-based bioprinting, low concentrations of alginate bioinks having less viscosity and volume can be used for bioprinting. Table 1 shows the various parameters that are needed to be fulfilled for various bioprinting processes.

3. Limitations of Existing Bioprinting

In tissue engineering, additive manufacturing technology has improved a lot but still, many limitations need to be addressed further. So, research is still going on for all the bioprinting techniques (extrusion, inkjet, fused-deposition modeling, laser, bioplotting, as well as SLA) to improve and rectify the limitations in tissue engineering applications for bioprinting 3D networks, which should show good results for regeneration of tissues. Printing speed is one of the major challenges in bioprinting as it produces lattices of 15 inches in 1 hour [28]. Another disadvantage of bioprinting is the fabrication of vascular networks, which is still not possible in an efficient manner. The removal of wastes and transportation of nutrients are required for the survival of cells during bioprinting [29]. In any tissue engineering application, the mentioned above bioprinting processes are always modified to some extent for getting the desired printed construct [30].

4. Bioinks Commonly Being Used

Earlier, the technology of additive manufacturing was not used in biological applications as they required crosslinking agents, organic solvents, and high temperatures. The commonly used bioinks used were thermoplastic polymers, metals, and ceramics. The most challenging aspect of tissue and organ bioprinting is to achieve chemical, morphological, and mechanical properties resembling original tissues and organs. For these reasons, bioinks were developed to provide a cell-friendly environment and protect cells during fabrication processes [44]. The bioink used for bioprinting should have the properties as follows: (a) cell adhesion promote properties, (b) non-immunogenicity, (c) non-toxicity, (d) rate of biodegradability matching tissue regeneration, (e) insolubility in culture medium, and (f) high mechanical stability and integrity. Moreover, the material used for bioinks should be commercially and quickly feasible [45].

4.1. Collagen. Collagen has been one of the most eye-catching polymers used in tissue engineering. It acts as an ECM for many tissues and it is the musculoskeletal tissue's main component. Collagen is obtained from natural sources and its structure is triple-helical. Because of these reasons, the scaffolds of collagen have fewer immunological reactions [16]. Furthermore, cell growth, attachment, and adhesion are increased with the help of collagen [15]. However, collagen Type-I is used as a bioink in bioprinting, but it also has some drawbacks. The Type-I collagen stays in liquid form at low temperature, and when the temperature is increased, the structure formed is fibrous. Gelation occurs at 37°C and takes approximately 30 minutes. Because of the slow gelation of collagen Type-I, it is a challenge to bioprinting 3D

Table 1: Bioink parameters and bioink printing concentrations for various bioprinting techniques.

Parameters	Inkjet bioprinting	Laser bioprinting	Extrusion bioprinting	SLA	DLP
Resolution	High	High	Medium	High	High
Viscosity of bioink	3.5–12 mPa/s	1–300 mPa/s	Till 6×10^7 mPa/S	No limit	No limit
Printing speed	Fast	Medium	Slow	Fast	Fast
Cell density	Low, $<10^6$ cells/ml	$<10^8$ cells/ml,	No limit	No limit	No limit
Cost	Less	High	Medium	Low	Low

constructs. In 2017, Diamantides et al. [31] investigated the effect of pH and photo-crosslinking of riboflavin on the printability and rheological properties of collagen. From the pH study, it was clear that during the gelation of the collagen bioink printed constructs, the shape fidelity is highly related to the pH. Though, the gelation rate of the collagen bioink did not disturb the printability. Guo et al. [143] synthesized a norbornene-functionalized neutral soluble collagen (NorCol) by the reaction of carbic anhydride and acid-soluble collagen (Col) in the aqueous phase for improving the lower self-assembly speed, which limits the efficiency of highly accurate cell-laden structures bioprinting.

4.2. Agarose. This is a linear polymer having thermos-reversible and heat-reactive characteristics. The low melting point of agarose results in rapid solidification of the agarose as soon as the extruded agarose filaments are deposited on the refrigeration bed. The study of agarose gel with mesenchymal stem cells for bioprinting has been reported by Duarte Campos et al. [39]. The entire was performed using fluorocarbon. After 21 days, the cell deposition and the tubular structure were formed, and approximately 100% of the cells were found to be viable. Because of the property of natural cell attachment of agarose bioinks, it is commonly used in the platform of 3D cell culture.

4.3. Silk. Bombyx mori produces various silk fibrous, which are used in cartilage regeneration, tissue engineering, strain gauges for biological applications, optical waveguides, small-scale catalytic motors, small-scale catalytic motors, biosensors, etc. [32]. In 2017, the bioink of spider recombinant silk was used by DeSimone et al. [220] with many silks having sericin and fibroin for use in biomedical applications [33]. In fibroin, heavy and light chains are present and these two kinds of chains are connected by disulfide bonds [34]. With other scaffolds, the silk fibroin scaffolds are compared, and it has many pros like low bacterial adherence, non-toxicity, luster, high biocompatibility perfect mechanical stability, etc. All these pros make silk an acceptable bioink for use in bioprinting. They also suffer from a few limitations like rheology optimization and they are needed to be mixed with other polymers. For biomedical implants, tissue engineering applications and controlled delivery polyol-silk bioink have been used by Jose et al. [219], and the structures that are printed show good flexibility and optically transparent properties.

4.4. Chitosan. This is a naturally available polymer that shows properties like antibacterial and biodegradation, and because of these reasons, it is mostly used in wound dressing. These hydrogels are also used in skin, bone, and cartilage tissue engineering applications. The inadequate mechanical strength and lower gelation time are the two disadvantages of this bioink. In acid solutions, chitosan dissolves and it can also be crosslinked by covalent and ionic bonds. The gelation of the chitosan occurs fast at higher pH. In neutral pH values, the chitosan is soluble in water and the gelation occurs at 40°C [155]. Rahimnejad et al. [165] developed a rheological method to study the chitosan-based thermosensitive ink printability.

4.5. Gelatin. Properties like non-immunogenicity, hydrophilic property, and biocompatibility are important advantages in gelatin [41]. Gelatin is a thermos-reversible gel, i.e., it is converted to solid at low temperature and under physiological conditions becomes mechanically unstable. For making the gelatin structure stable below 37°C, chemical modifications are needed. The crosslinking of modified gelatin occurs with methacrylamide in the presence of a PI. In bioprinting, GelMA hydrogel extrusion can be easily done by ultraviolet irradiation for molding. GelMA printing properties are dependent on cell density, ultraviolet exposure duration, and gel concentration. The intensity and duration of the ultraviolet irradiation affect cell viability.

4.6. Hyaluronic Acid. Hyaluronic acid is widely used as a joint lubricant and skin filler in clinics [42]. It plays a significant part in the regulation of cell function and cell behavior like angiogenesis, proliferation, and spreading. In the hydrogels of bioprinting, when the hyaluronic acid is sealed in cartilage tissues, the cell viability is more compared to collagen-based hydrogels. Furthermore, hyaluronic acid has lower mechanical properties because of quick degradation. For controlling the degradation rate, chemical modifications are needed. Because of these reasons in the bioprinting research area, hyaluronic acid bioinks are not commonly used. However, hyaluronic acid crosslinking can be improved using functional treatment with methyl acrylate (MA) photocuring for controlling the photopolymerization duration.

4.7. Alginate. Naturally available polymer, i.e., alginate, is obtained from bacteria and brown algae. It is most widely used as a bioinks because of the following properties like rapid gelation, low price, and biocompatibility. The advantages and disadvantages of alginate bioinks are mentioned in Table 2. Also, it is used in other bioprinting applications because of its rapid gelation when comes in contact with

Table 2: Advantages and disadvantages of alginate bioinks.

Advantages	Disadvantages
Bio inertness	Inadequate degradation
Low-cost	Cell-binding motifs
Easily available	Limited cell–material interactions
Easily tunable	Strong hydrophilic nature
Biocompatible	Fast gelation causing nozzle clogging
Biodegradable	Printing inhomogeneities
Tissue-specific mechanical properties	Poor dimensional stability

calcium ions (like calcium sulfate, calcium chloride, and calcium carbonate). Alginate is mixed with other polymers to improve the biological properties as they are good for molding with a suitable biological nature [40]. Furthermore, lower alginate concentration solutions have lower mechanical properties though they have properties to promote cell proliferation and viability. A homogenous pre-crosslinking technique was developed by Hazur et al. [187], which is widely used for all materials based on alginate. Alginate degradation is a major problem in tissue engineering and it can be manipulated by adjusting the crosslinking agent concentration and mixing it with various other polymers such that it does not shift the physiological conditions of the bioink to a non-physiological range [214]. Alginate degrades homogenously at various locations of the printed scaffolds [219]. The printability as well can also be improved by blending it with bioactive, bioinspired polymers, improving the gelation time, viscosity, and rheological properties of the bioink. Extrusion-based bioprinting causes 60% of cell death due to mechanical stress, which causes ROS-induced cell death. Datta et al. [217] improved the ROS-induced cell damage during extrusion-based bioprinting by incorporating N-acetyl cysteine (NAC) at a very low concentration along with MC3T3 cells. They showed that the NAC addition to the cell-laden alginate bioink lowered the Caspase 3 and Cox 2 markers, which are responsible for cell death. Without affecting the physiological conditions and printing parameters, they have developed a simple technique to improve the ROS-induced cell death during and after extrusion bioprinting. Cell binding affinities for alginate constructs were improved by Datta et al. [214, 218] by mixing alginate with low concentrations of bioinspired and bioactive polymers. They have improved cell–material interaction for bone and skin tissue engineering applications.

5. Applications of Alginate Bioink Uses in Tissue Engineering

Alginate is usually a commonly used bioink. It is a natural polymer and can be extruded from brown algae intracellular spaces and cell walls because of its low cost [46]. Alginate consists of β-D-mannuronic (M) along with α-L-guluronic acids (G) as shown in Figure 2. Alginate copolymer has polyanionic linear block completed with M and G blocks, distinguished by GM areas. M and G blocks improve the flexibility though a huge amount of immunogenicity is produced by the manifestation of the M blocks [47]. In the alginate matrix, molecules and water can get trapped because of the capillary forces. Because of all these features, alginate is commonly and widely used as a formulation of bioinks. Many bioprinting techniques like extrusion-based bioprinting need fast gelation. Alginate allows fast gelation when it is blended with multivalent cations like Ca^{2+} by creating ionic interchain bridges. The process of gelation is still not understood well now though many researchers stated that the gelation process occurs because of the binding of cations with M and G blocks [48]. By this method, quick and easy cell encapsulation can be done, and the layer-by-layer process is avoided by cell interlayer adhesion [49]. Regardless of bioprinting bioinks, cell encapsulation is also achieved by alginate, which was first used in the therapeutic application in the form of microencapsulated Langerhans cells, which was transplanted into rats having diabetes [50, 51]. The alginate pore size was observed in the range of 5–200 nm [52, 53], and in the G-block-alginate content, the largest pore was observed. Various bioprinting methods bioinks require specific rheological properties [9]. In the case of extrusion bioprinting, the bioinks viscosity lies between 30 and 6×10^7 mPa s range. The bioinks cell density can be more but because of the shear stress at the time of the process of extrusion bioprinting, the cell viability can be decreased to 80–90%. For inkjet bioprinters, the cell density$<16 \times 10^6$ cells/mL, as well as the viscosity of the bioinks range, is also less, i.e., <10 mPa s. Cell viability of about 90% is available in this method. In the case of laser-assisted bioprinters, the cell density is medium of almost 10^8 cells/mL, and the viscosity ranges between 1 and 300 mPa s. The viability of cells in this method lies >95%. Alginate-based bioinks viscosity is linked with phenotype and cell density, alginate molecular chain lengths, alginate concentration, and the molecular weight (MW) of the cells. These all mentioned parameters should be taken into account by the researchers for tuning and developing bioinks of alginate. Shear-thinning is one of the other important parameters of alginate-based bioinks in the rheological department, where the decrease in viscosity occurs as a result of the increase in the share rate. Temperature is also a determinant of the bioink viscosity, where the decrease in viscosity occurs as the temperature is increased. Compared to other polymers available for bioinks, alginate bioprinting is easier and cell encapsulation is also good during the bioprinting extrusion process where it protects the cells more effectively. Though alginate is non-cell-adhesive [54], for cell encapsulation, it is currently the most employed biomaterial. To improve the printability, there are many problems with the alginate bioinks like bad structural stability, lower cell–material interactions, and bad mechanical characteristics and these are needed to be addressed, and for this, many researchers use other polymers with it (natural or synthetic) also reinforcements are done carbon nanoparticles and ceramics. Datta et al. [214] improved the mechanical and biological properties of the alginate bioinks using natural honey in low concentrations. So that it does not affect the physiological printing conditions and the

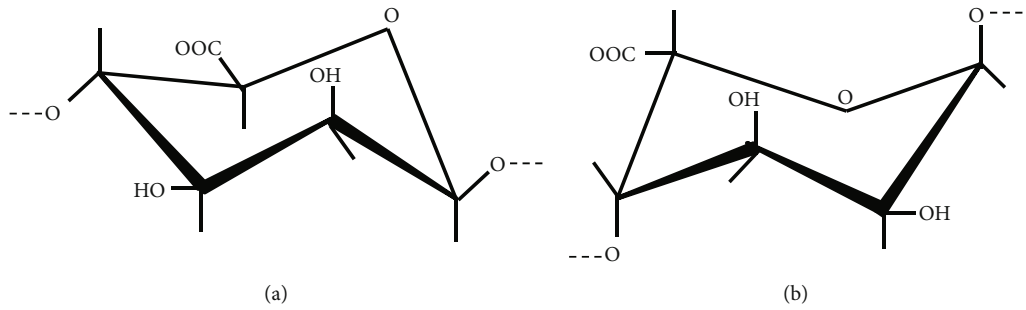

Figure 2: Alginate structural unit. (a) β-D-mannuronic acid and (b) α-L-guluronic acid.

rheological properties of the modified alginate bioink. Alginate material is bioinert with imperfect degradation [54]. The degradation rate of the alginate is very slow and degrades in an uncontrolled way. In mammals, the enzyme that is needed for breaking alginate is not present so its application is limited for in vivo regeneration of tissues [93]. Bouhadir et al. [215] amplified the alginate rate of degradation in a controlled way without changing the gelation capability by Ca^{2+} ions by using oxidized alginate in contact with sodium periodate. Boontheekul et al. [55] reported a simple way to control the alginate rate of degradation by partially oxidizing with a low percentage of sodium periodate and also by changing the ratio of the MW of alginate from high to low Molecular weight alginates. The cell proliferation and differentiation of the alginate can be improved by blending alginate bioinks with various agents like natural polymers, amino acids, growth factors, etc.

As soon as the printing of the material is done, the hydrogel degradation should be present, which helps the cells for producing their ECM. Persistent cell-laden hydrogels of long-term are produced by alginate, but because of the lower kinetics of degradation, it is needed to be tuned using oxidation using sodium peroxide [55] for example, or changing the distribution of the MW of the alginate using gamma rays [56]. Degradation of alginate is also done by alginate lyases catalyse, because of the slow as well as the uncontrolled degradation, researchers face problems while using alginate as a bioink for bioprinting [57]. Because of the slow as well as the uncontrolled degradation, researchers face problems while using alginate as a bioink for bioprinting. The low-weight hydrogel alginates are restricted by the extrusion bioprinting during discharge of the hydrogel, which is application dependent as well as shows bad mechanical properties. In the later examples, we shall see that alginate bioinks are being tuned by other biomaterials incorporation or performing other hydrogel fabrication methods for improving the mechanical, structural as well as biomimicry properties for obtaining the desired scaffolds. For example, CELLINK™ is a commercially modified available bioink made from alginate and nanocellulose, where fast crosslinking and shear-thinning properties are present, which makes it suitable for soft tissue engineering applications [9]. However, bioprinters of extrusion-based like Revolution from Ourobotics and Bioscaffolder® from Gesim endorse using alginate bioinks. Alruwaili et al. [94] printed a crosslinking solution of calcium chloride using gelatin alginate hydrogels and examined the printed hydrogel dimensions concerning the extrusion rate, nozzle diameter effect, effect on surface and nozzle distance, the concentration of the calcium chloride, etc. Hajikhani et al. [98] studied the chemo-mechanical modeling of swelling and crosslinking reaction kinetics in alginate hydrogels. Bertuola et al. [110] studied the properties like printability and the rheology of gelatin–alginate–hyaluronic acid bioinks with 2–8% wt of 45S5 bioglass (BG), which followed a pseudoplastic behavior at the time of the 3D-printing process. Rahman et al. [120] investigated the pulse electric field-assisted electrohydrodynamic working conditions for sodium alginate bioprinting by the plan of the experimental method. Flores-Torres et al. [133] developed bioprinted cancer spheroid models using alginate–gelatin–Matrigel hydrogels. A review of 3D cell cultures material of alginate-based as well as their applications and properties is published by Łabowska et al. [145].

5.1. Alginate Bioink in Vascular Tissues. In spaces of a volume of less than 3 mm^3, the isolated cells die, if bioprinted construct has limited vascularity [58, 59]. For the fabrication of large organs and tissues, the channel of the blood vessel requires the transportation of oxygen and nutrients via the material printed. Zhang et al. [60] reported a new method for delivery of the nutritions by using coaxial nozzle bioprinting by fabricating a vessel-like microfluidic channel. In their study, they printed hollow alginate hydrogel filaments having progenitor cells of cartilage by using a bioprinter of the pressure-assisted type having a coaxial needle. By using the assembly of a triaxial nozzle, Yu et al. [61] fabricated tubular channels inside cartilage-like tissues. In alginate, the progenitor cells of cartilage are encapsulated, which is the main bioink component. Microchannels inside alginate hydrogels having high strength were fabricated by Gao et al. [62]. In the same way, the coaxial nozzle of multilayered type with extrusion in one-step concentric channel 3D bioprinting for the development of perfusable vascular constructs was achieved [63] by blending 4-arm poly(ethylene glycol)-tetra-acrylate (PEGTA) along with GelMA. For this work, the calcium crosslinking was done, and PEGTA and GelMA were photo-crosslinked covalently for improving the rheological and mechanical properties. Christensen et al. [64] printed junctions (vertical and horizontal) in vascular-like structures in fibroblast-based alginate bioinks of mouse and alginate bioinks. They used an inkjet bioprinter armed with crosslinker calcium chloride-like supporting

material. For providing the buoyant force, the calcium chloride crosslinked solution was used in the regions that are overhung in both horizontal and vertical printing, also in the horizontal printing spanning regions. It can be said from all the reports published widely that bioinks of alginate-based were used in vascular tissue bioprinting, which is coaxial needle-assisted because of the fast crosslinking in the presence of ions. The coaxial needle allows modification of the kinetics of gelation in bioinks of alginate-based by providing more precision by changing the alginate concentration as well as the crosslinker.

5.2. Alginate Bioink in Bone Bioprinting. For the bone printing composition of the novel, the hydrogel is made by hydroxyapatite, alginate, and gelatin bioinks [65]. In the process of two-step mixing, chemical crosslinking of alginate and thermosensitive properties of gelatin are used for getting long-term structural integrity and fast crosslinking in the bioprinted structures having mesenchymal stem cells of humans during the bioprinting process. For bone tissue engineering, hydroxyapatite bioinks are widely used. Alginate mixed with polycaprolactone (having good mechanical properties) for fabrication of osteochondral tissue using bioprinting [66]. These two material combinations improved the mechanical properties, which are required for the bioprinted constructs having chondrocytes and osteoblasts in bone tissue engineering applications. Armstrong et al. [67] developed a bioink by poloxamer, a sacrificial material, and alginate for fabricating cartilage and bone bioprinted constructs having porous structures with improved rheological and mechanical properties from the microscopic point of view. In another study, SaOS-2 cells inked to bone were bioprinted using alginate and gelatin and then overlayed using calcium salt of polyphosphate and agarose, which resulted in getting improving cell mineralization and better cell proliferation [68]. Wang et al. [69] performed the same technique where they studied the effects of BG on SaOS-2 cell mineralization and growth using alginate/gelatin hydrogels. Biosilica and polyphosphate improved cell mineralization and proliferation. Jang et al. [70] developed three-dimensional constructs using bioprinting, dip-coating, and centrifugal melt-spinning by blending nanofibers, polycaprolactone microfibers, collagen, and mesenchymal stem cell-laden alginate. These scaffolds encouraged osteogenesis soon after the mastoid obliteration, also in the in vivo experiments ultimately promoting the development of new bones. A new method is reported by Daly et al. [71] where they fabricated templets of cartilages using stem cells that are supported by Arg-Gly-Asp adhesion peptides along with gamma-irradiated alginate bioink. After that, the templets reinforcement was done by the bioprinted polycaprolactone for receiving a ≈350-fold, which increased the modulus of the compression and provided a benefit for bone tissue engineering application. The alginate mechanical properties are very low compared to the bone. For example, the stiffness at the time of elastic deformation of bone lies between 15 and 25 GPa, and for alginate, it lies between 150 and 550 kPa. We can say that alginate combined with other polymers like biosilica, polycaprolactone, and hydroxyapatite improved the 3D printed scaffolds mechanical properties for bone tissue engineering applications. Gonzalez-Fernandez et al. [95] assessed the physicochemical properties, osteogenic potential, and printability of four common alginate bioinks: alginate–nanocellulose (alg–ncel), alginate–gelatin (alg–gel), alginate–CaCl2 (alg–$CaCl_2$), and alginate–$CaSO_4$ (alg–$CaSO_4$) for bioprinting of structurally precise osteogenic grafts. Ghosh and Webster [108] reviewed various mesoporous silica-based nanostructures for bone tissue regeneration. Li et al. [125] developed a porous scaffold by using Sr-doped hydroxyapatite and sodium alginate for bone tissue engineering. Karamchand et al. [126] reported a technique to evaluate the crystalline nanocellulose (CNC) embedded agarose composite biocompatibility, which is developed into a 24-well culture system, with mouse bone marrow-derived mast cells (BMMCs) by flow cytometric assays for biomarker expression and cell viability. Nulty et al. [127] invented a bioprinting approach to engineer pre-vascularized tissues in vitro and to examine the volume of those constructs to improve the regeneration and vascularization of large bone defects in vivo. Raja et al. [134] developed and characterized a unique coiled-structured bioceramic contained in hydrogel beads for cell and drug delivery at the same time by the combination of bioprinting and bone cement chemistry. Guduric et al. [138] tailored a composite bioink, i.e., zinc-substituted mesoporous bioactive glass (BG)/alginate-methylcellulose [78] for bone tissue engineering. Moore et al. [144] used bioprinting to produce a BM construction with wide-ranging alginate (A): methylcellulose (M) ratios, they nominated hydrogels having 2% (w/v) A and 4% (w/v) M, which reviews ultrastructural and rheological features of BM though keeping constancy in culture. Hussin et al. [146] analyzed the global tendency of using hydroxyapatite, alginate, gelatin, and alginate for bone tissue engineering applications. Zhang et al. [151] developed a human mesenchymal stem cell (hMSC)-laden graphene oxide (GO)/alginate/gelatin composite bioink for 3D bone-mimicking scaffolds by 3D bioprinting technique. Zhang et al. [167] used a BG of boron- which was mixed with a (3D) bioprinting technique to fabricate an implantable scaffold that is osteoinductive, depending on the CT scan imaging instructions on the defects of bone. Carabba et al. [174] investigated the hybrid scaffolds which are cell engineered that are implantable near the artery of occluded femoral and have therapeutic benefits by the creation of new collateral arteries. Wu [175] created a hydrogel bioink of nanocomposite [gelatin–alginate–montmorillonite (GT–AT–MMT)] for bioprinting related to GT's thermosensitive properties, sodium AT ionic crosslinking advantages, toughening mechanism, and the shear-thinning of nano-MMT. The bioprinting of the variable AT constituent bioink is optimized, and the properties like compression, tensile, and creep are studied. Ratheesh et al. [176] bioprinted patient-specific bone particles for bone tissue engineering. Yu et al. [186] constructed a hybrid scaffold of polycaprolactone/alginate bipartite. Hernández-González et al. [195] reviewed alginate hydrogels for their application in bone tissue engineering, from injectables to bioprinting. Choe et al. [197] developed novel bioink graphene oxide/

alginate composites for bone regeneration applications and 3D mesenchymal stem cell printing. Beheshtizadeh et al. [198] reviewed 3D bioprinting for bone and skin tissue engineering. Ostrovidov et al. [199] reviewed present bioprinting approaches and a summary of the bioink preparations and properties that are used in 3D bioprinting are provided for skeletal muscle tissue engineering uses. Ojansivu et al. [203] reported bioinks of BG-modified gelatin–alginate and wood-based nanocellulose for bone cell bioprinting.

5.3. Alginate Bioink in Cartilage Bioprinting. Keeping aside the previously mentioned alginate bioinks applications, alginate bioinks are also being used in cartilage bioprinting. In Atala lab, Winston-Salem, NC, USA [42], researchers combined the technique of bioprinting and electrospinning for fabricating cartilages that are layered and have good mechanical properties compared to the alginate bioprinted hydrogels. In vivo, the cells printed showed the formation of the ECM. Polycaprolactone electrospinning fibers were combined with elastic chondrocytes of rabbits, which are encapsulated in fibrin/collagen gel. Kundu et al. [75] investigated the chondrocyte cells encapsulated in alginate and polycaprolactone, which are printed layer by layer for the fabrication of 3D scaffolds. Hydrogels containing transforming growth factor-β (TGF-β) presented a better formation of a cartilage-like ECM. Markstedt et al. [76] bioprinted a human ear and also printed a meniscus of sheep with a bioink mixed with alginate and nanofibrillated cellulose. In a report [77], the combination of bioprinting and digital modeling was used to fabricate a cartilage meniscus having a required pattern, which was printed in a process that was a single step [77]. Mixing epoxy-based adhesive and alginate/acrylamide solution, the extrusion of posteriori was done. The mixture curing was done by UV radiation. Müller et al. [78] modified the alginate sulfate by mixing nanocellulose for printing, which can be used in the applications of cartilage tissue engineering and showed improved printing properties. However, during the bioink extrusion, the proliferation of the chondrocyte cells was affected seriously when nozzles with small diameters were used and valves that reduced their uses to very lower printing resolution. Izadifar et al. [79] currently published a review where the advances in 3D cartilage printing are discussed in detail. To resemble the cartilage properties, bioprinting of constructs using bioinks containing mixtures of alginate impregnated with chick primary cells and polycaprolactone was printed. The biostable hydrogel, i.e., alginate, has suitable mechanical properties and slow biodegradability for use in cartilage bioprinting like methylcellulose, agarose, or PEG [80]. For cartilaginous tissue bioprinting, Wang et al. [100] studied the alginate sulfate, a sulfated glycosaminoglycan (sGAG) mimic bioinks, for functionalizing an alginate GelMA interpenetrating network (IPN) bioink. Nedunchezian et al. [128] reported the construction of bioinks by mixing acid (HA)-based hydrogels and adipose-derived stem cells (ADSCs) and investigated their capability to encourage chondrogenesis using the technology of 3D bioprinting for the application in cartilage tissue engineering. Zhou et al. [129] added fibronectin (FN) and chondrogenic progenitor cells (CPCs) to composite hydrogel, i.e., alginate/gelatin/hyaluronic acid (Alg/Gel/HA), for repairing defects in cartilage. Yang et al. [194] 3D bioprinted osteochondral scaffolds for repairing the defects in articular cartilage of a rabbit knee.

5.4. Alginate Bioink in Skin Bioprinting. Skin printing for skin grafting and wound dressing is also possible as alginate bioinks. For skin printing, the scaffolds must be antibacterial and should possess good mechanical and cell–material interaction properties. Rastin et al. [99] presented a bioink that is cell-laden antibacterial, which is based on methylcellulose/alginate (MC/Alg) hydrogel for excluding the risk of bacterial infection for skin tissue engineering. Barros et al. [106] developed bioprinted skin model by using bioengineered techniques and biomaterial-based approaches having layers of dermal fibroblasts, multilayered keratinocytes, and endothelial cell networks. Manita et al. [131] reviewed the present state of the art of bioprinting of skin substitutes as an effective method to deal with skin injuries. Milojević et al. [132] demonstrated the development of hybrid hydrogel–thermoplastic polymer scaffolds with tunable chemical properties and structural for skin tissue engineering applications. Taymour et al. [135] 3D bioprinted hepatocytes: core–shell structured cocultures with fibroblasts for enhanced functionality using alginate, methylcellulose (algMC), and Matrigel. A bioink is developed by Lee et al. [188] having porcine skin powder (PSP) for determining the cell's ECM formation in PSP-ink, rheological properties, and biocompatibility after bioprinting. A new crosslinked bioink having chitosan (CH)–genipin (GE) laden with human dermal fibroblast cells and keratinocyte was successfully printed by extrusion bioprinter and was reported by Hafezi et al. [190]. Abasalizadeh et al. [192] reviewed alginate hydrogels with their properties that have been presented as well as the procedure of manufacturing alginate hydrogels. In another study, an evaluation of different properties categories of dressing was done by Del Amo et al. [196] where they used gauzes, meshes, films, hydrofibers, foams, hydrocolloids, and alginates in terms of affinity for release management, the kinetics of cytokine release, platelet-rich fibrin (PRF), and combination product influence [PRF + dressing] on the behavior of dermal cells targeting to give data for selecting the best acceptable dressing for particular patients.

5.5. Advances in Alginate-Based Bioprinting. The first applications of alginate bioinks in extrusion bioprinting for printing endothelial cells were reported in 2009 [82]. An inkjet bioprinter was developed in 2010, which was used for printing various cells using fibrin hydrogels and alginate [83]. In the mentioned experiments, they reported that good mechanical properties were shown by alginate and worse properties in the case of cell differentiation, proliferation, and adhesion for the formation of tissues compared to hydrogel of fibrin. After one-year, alginate hydrogel was for fabricating tissues in large volumes because of its fast gelation properties by using bioprinting system mounted with multinozzle [84]. Keeping aside the other breakthrough reports of alginate bioinks in extrusion bioprinting, alginate

was also used for fabricating the first three-dimensional artificial bioprinted neural tissue [85]. The researchers used a mixture of agarose, carboxymethyl-chitosan, and alginate bioink for printing and crosslinked rapidly to form 3D scaffolds having stem cells for in situ differentiation and expansion. The printing of neural stem cells of humans was done by the research group where in situ differentiation was done for forming synaptic contacts and functional neurons for establishing networks. Alginate bioinks were also used for printing human-induced pluripotent stem cells (hiPSC) and human embryonic stem cells [86]. The investigation of differentiation into hepatocyte-like cells after printing and valve-based printing was also done. Recently, in another report [87], alginate-based bioinks were used for fabricating complex anatomical structures embedded with printed hydrogel inside another support hydrogel. From 3D optical models, imaging data of magnetic resonance and computed tomography, bioprinting of trabeculated embryonic hearts, human brains, coronary arteries, and femurs were done. The behavior of the flow of the alginate solution containing live cells was also examined by Ning et al. [88]. Alginate–skeletal muscle cell, alginate–Schwann cell's rheological properties, and alginate–fibroblast cell were determined at the time of the printing process shearing, which showed that flow behavior significantly affected cell proliferation viability. The flow behavior is also affected by cell suspension cell density along with biomaterial concentration and temperature. Also in the case of in vitro 3D biology, 3D bioprinting gives some light for fabricating real-like tissue models. By using bioinks of gelatin/alginate/fibrinogen and HeLa cells, a cervical tumor 3D bioprinted model was fabricated by Zhao et al. [89]. The HeLa cell's biological response in the comparison between 3D and 2D data showed a significant difference. Rodríguez-Dévora et al. [81] developed a platform for rapid drug screening using Escherichia coli cells and alginate, which in picoliter-scale volume showed biochemical reactions in a cheap way and high-speed rate. The problem with alginate-based bioinks is that they need to be bridged between the bench-to-bedside translation gaps having bioprinted material enhancing the biological functions. For solving this problem, the introduction of growth factors in alginate bioinks was done in an interesting work reported recently [90]. A bioink is developed by Lim et al. [117], which fulfills the needs for both biocompatibility and printability by successfully employing hydrocolloid materials by using xanthan gum (XG) and carboxymethyl cellulose (CMC) for maintaining appropriate shear properties in high-pressure as well as to increase the bioink mechanical properties without excessively affecting the bioink viscosity, and thus improve the biocompatibility and printability. Hou et al. [118] printed bioink 3D vagina tissue analogs with vagina-decellularized ECM bioink by 3% sodium alginate and 15% gelatin blended with the solution of acellular vagina matrix. By using GelMA bioink, Wu et al. [121] bioprinted artificial ovaries by an extrusion-based technique. Muthukrishnan [122] reviewed imminent antimicrobial bioink deploying synthetic polymers, exopolysaccharides, alginate, and cellulose for bioprinting of tissue constructs. By using hydroxyethylcellulose-based bioink, Gospodinova et al. [123] developed a cervical tumor model using extrusion bioprinting. Bakhtiiari et al. [140] studied the inspection of different parameters of the bioprinter—using simulation software—for printing a hydrogel so that excludes huge amounts of shear stress, which is harmful to cell proliferation and cell viability using bioinks like gelatin, collagen, gelatin, and alginate. Chopin-Doroteo et al. [153] highlighted the importance of the intricate response between cell biological, materials science, and the rheological properties to get a real bioink design. The hydrogel properties like the crosslinking properties (a) degradation and (b) swelling and the biocompatibility properties (a) printability, (b) mechanical behavior, and (c) rheological properties of alginate–gelatin–whey protein isolate-based hydrogels are used for bioprinting in a layer-by-layer fashion structure, which was reported by Sümbelli et al. [154]. The crosslinking of these structures was done by Light Underpinning Conjugation Approach (ANADOLUCA) method and the Amino Acid (monomer) Decorated. Pedroza-González et al. [158] reviewed two decades of advancements in the extrusion bioinks. Schwab et al. [185] reviewed and discussed the quantitative and qualitative procedures to calculate the printability of bioinks for lithography-based and extrusion-based bioprinting. A protein named bone morphogenic protein 2 (BMP-2) when released continuously from the printed scaffolds causes osteogenicity of the printed tissues. Better releasing properties of BMP-2 were seen in gelatin microparticles loaded BMP-2 when compared to direct loading of BMP-2 in bulk gelatin or alginate. Limited degradation is also another problem of natural alginate. To solve this problem of limited degradation of alginate, Wu et al. [91] showed a useful technique where they used sodium citrate-containing tissue medium mixed with alginate hydrogels. The regulation of the rate of degradation of bioink alginate was done by changing the concentration of sodium citrate. For improving the alginate printability, Chung et al. [92] mixed alginate and gelatin, which improved the printability and the printing resolution of the bioprinted constructs that were alginate pre-crosslinked alginate bioinks. They obtained good cellular and mechanical properties having a definite structure with a similar diameter of the pores, which highlighted storage modulus and higher viscosity. In one study, Freeman and Kelly [93] demonstrated that the mechanical properties of the alginate bioinks can be tuned by changing the alginate ratio to the ionic crosslinker inside the bioink for improving the growth factor release, which improved the fate of the mesenchymal stem cells. Heo et al. [96] bioprinted carbohydrazide-modified gelatin into microparticle-suspended oxidized alginate for the construction of tissue constructs having complicated shapes. Soltan et al. [97] studied the cell viability and the printability of the bioprinted alginate dialdehyde–gelatin scaffolds. Kapr et al. [101] studied the alginate/gellan gum/laminin (ALG/GG/LAM) hydrogel blends for the fabrication of hiPSC-based 3D neural models. Lafuente-Merchan et al. [102] studied the different sterilization procedures by applying on NC–Alg and NC–Alg–HA bioinks and their effect on several parameters was evaluated. Amaral et al. [103] explored a dynamic bioink containing alginate and boronic acid-functionalized laminarin for the fabrication of constructs by 3D bioprinting below physiological conditions for multifunctional bioinks development and donate to the biomimetic 3D scaffolds fabrication with applicability in a huge range of

predictive or exploratory biomedical platforms. Li et al. [104] used 12 nm bioactive nanoparticles (BNPs), which could have released silicon (Si) ions that were used to improve the alginate/gelatin hydrogel bioink properties for maintaining MSC stemness. Bhattacharyya et al. [105] developed a semiautomated twin-screw extruder (TSE) head to confirm the homogenous mixing of bioink and micro/nanomaterials and then 3D-bioprinting. Jin et al. [107] studied the fate and function of human T-cells via 3D bioprinting using alginate bioinks. Ceballos-González et al. [111] bioprinted fine-scale bacterial microcosms using chaotic flows induced by a printhead containing a static mixer for printing fine scale for the development of constructs of hydrogels with intercalated sheets of bacterial strains. Costa et al. [112] reviewed various constructs of 4D-bioprier for regenerative medicine and tissue engineering and also delivered critically discussed in light of foreseeable advances and current challenges. Wu et al. [113] reported a new tunable hollow microfiber bioink-enabled microfluidic printing for the quick creation of blood vessels by compositing biomaterials with sodium alginate, glycidyl-methacrylate silk fibroin, and gelatin methacrylate, a new composite bioink having biocompatibility and good printability. Zhao et al. [114] reported bioprinting of polythiophene materials for promoting stem cell proliferation in a nutritionally deficient environment using integrating poly[3-(3′′-N,N-ntriethylamino-1′ -propyloxy)-4-methyl-2,5-thiophenehydrochloride] in an anionic gelatin/alginate matrix. Chen et al. [115] improved the proliferation, long-term subaqueous fidelity, and cell viability of the printed scaffolds by making bioinks mixture of 1% aldehyde hyaluronic acid (AHA) and 0.375% N-carboxymethyl chitosan (CMC), two polysaccharides with strong water retention and water absorption capacity, into classic gelatin (GEL, 5%)—alginate (ALG, 1%) ink. Mastrorocco et al. [116] re-formed the (3D) construction of the cumulus–oocyte complex (COC) by bioprinting technologies based on alginate microbeads (COC microbeads) for in vitro 3D maturation. Samandari et al. [119] developed a quick, cost-effective, and simple method for nonstop multicompartmental hydrogel fibers printing having intrinsic 3D microfilaments to regulate cellular orientation using alginate/GelMA hydrogel fibers. Kim et al. [124] reported alginate derivatives to make quick gelation, and a bioink was developed by blending silk fibroin with alginate derivatives to improve cellular compatibility. Balasubramanian et al. [130] reported a bioprinting technique using environmentally friendly chemistry to encapsulate microalgae inside an alginate hydrogel matrix. Kim et al. [136] reported a balanced approach for a hydrogel of alginate fibers by mussel-inspired catechol chemistry, which includes crosslinking of inter-catechol in a few minutes under simple circumstances. Klak et al. [137] reported how the 3D bioprinting process affects islet viability as creating a bioactive scaffold with pancreatic islets and presents many challenges. Distler et al. [139] demonstrated the oxidized hydrogel of alginate–gelatin–laminin for simplifying the development of hiPSC neurospheres and neuronal differentiation. Xiong et al. [141] studied a novel cell delivery system, chondroitin sulfate microsphere hydrogel (nCACSMH), and negatively charged alginate, which was developed with biocompatibility and good permeability in electric with high voltage. By using three extrusion bioprinters (REGEMAT3D, BIO X, and INVIVO) and alginate/gelatin (Alg/Gel) hydrogels, Roche et al. [142] estimated 3D-bioprinted double-layer patches for cardiac patches for epicardial transplantation. Barrs et al. [147] developed an alginate hydrogel with peptide-functionalized defined with biochemical, rheological, and mechanical properties for microvascularized tissues direct bioprinting. Jongprasitkul et al. [148] studied methacrylated compositions (i.e., precursors) to examine their processability. They effectively methacrylated alginate, collagen, hyaluronan, hyaluronan, and gelatin to 30% and 60% degree of modification. By using ionic crosslinking using calcium chloride (2% w/v), Temirel et al. [149] improved the shape fidelity of alginate–TEMPO oxidized cellulose nanofibril (T-CNF) (1% w/v T-CNC and 4% w/v alginate) and alginate-cellulose nanocrystal (CNC) (4% w/v CNC and 2% w/v alginate). Spangenberg et al. [150] developed and characterized a magnetic bioink formed by incorporating magnetite microparticles (25% w/w) with alginate (Alg, 3%) and methylcellulose (MC, 9%). The particle shape and size were observed by X-ray micro-computed tomography and scanning electron microscopy. Miranda et al. [152] assessed the chemosensitizing potential of glycoalkaloidic extract (GE) with cisplatin (cDDP) in RT4 and PDX cells using 2D and 3D cell culture models. Zhu et al. [156] reviewed the current condition of bioprinting of the biomimetic structures and materials in biomedical engineering field. The classification, limitations, advantages, applications, use, and process steps of bioprinting, as well as the auxiliary materials and materials, which are used in bioprinting technology, are reviewed by Koçak et al. [161]. The primary human hepatocellular carcinoma used in personalized medicine is bioprinted by Xie et al. [166]. By using model–support bioink interaction, low-viscosity cell-laden hydrogels with high-resolution novel indirect bioprinting are reported by Tan et al. [169]. The review of currently available bioprinter's fundamental characteristics and current bioinks used for bioprinting and their pros and cons is published by Yu et al. [171]. The measurement of the extrusion pressure, bioink composition feasible region, and continuous strand extrusion needle size is reported by Thakare et al. [173]. Ioannidis et al. [177] reported a 3D printer that is converted into an open-source 3D bioprinter as well as developed a modified bioink depending upon available alginate/gelatin precursors for a low-cost printing solution. The investigation of GMP-compliant tenogenic differentiation and ADSCs 3D bioprinting is reported by Stanco et al. [179]. The fully sized human heart model from magnetic resonance imaging (MRI) data sets from a patient is reported by Mirdamadi et al. [181]. Bioprinting of 3D hydrogels using electrodeposition including a pin art device is reported by Taira et al. [201]. The review of the art of cells-laden 3D bioprinting having hydrogel-based biomimetic microenvironments by controlling and the building is published by Luo et al. [202]. The cost-effective and easy method for bacterial bioprinting is shown, and the extension of the technology for bioprinting genetically

engineered *E. coli* biofilms is shown by Balasubramanian et al. [204]. Mao et al. [157] examined the printing stage moving speed on sheath lines and core size inside a printed filament, the feeding rate of collagen and alginate, and sheath lines inside a printed filament. The study of materials of hydrogel having a mixture of methylcellulose (Alg/MC) and alginate as the supporting material along with cell-laden photopolymerizable GelMA used as the primary material was done by Li et al. [159] because of their good thixotropic property and high viscosity. A bioprinted model for the fabrication of a scaffold, which is a patterned, complex, and embryoid body (EB)-laden tubular made of hydrogel (GelMA or alginate) and polycaprolactone (PCL), was reported by Hamid et al. [160]. Shin et al. [162] reported bioinks having poly(ethylene glycol) diacrylate (PEG-DA), Laponite-XLG nanoclay, and porcine cardiac decellularized extracellular matrix (cdECM), which were partially digested. Liu et al. [163] used bioink of alginate-gelatin (Alg-Gel) blends for bioprinting mesenchymal stem cells (MSCs) and investigated the impact of stiffness on MSC differentiation toward sweat glands. Santis et al. [168] reported a bioink which is a tissue-specific hybrid having an ECM derived from decellularized tissue (rECM), alginate, and natural polymer. Cai et al. [170] developed and characterized a new composite hydrogel combining Laponite® nanoclay as an inorganic nanofiller and oxidized alginate–gelatin (ADA–GEL) hydrogel. For laccase immobilization, Liu et al. [172] developed a novel immobilization technology by 3D bioprinting. Wei et al. [178] reported the incorporation of BG nanoparticles (particle size of 12 and 25 nm) into Alg–Gel hydrogel for optimizing the biological and mechanical properties. For extrusion-based bioprinting, Han et al. [180] examined the effects of alginate/gelatin bioinks by mixing nanocellulose in them. Karavasili et al. [182] reported the comprehensive physicomechanical assessment of bioactive components (Manuka honey, *Aloe vera* gel, Eucalyptus essential oil) and 3D printable alginate–methylcellulose hydrogels by the combined experimental–numerical method. Othman et al. [183] reported the bioprinting of HeLa spheroids with hexagon-shaped alginate–gelatin scaffolds laden. Bioprinting of vascularized tumor model was reported by Han et al. [191] for drug testing. Li et al. [184] discovered the new hybrid sodium alginate–Matrigel (SA–MA) hydrogel extruded 3D bioprinting to develop an in vitro scaffold to encourage the growth and differentiation of ectomesenchymal stem cells. A breast tumor model development by bioprinting for structure–activity relationship study was reported by Li et al. [189] Ahearne et al. [193] reviewed various corneal regeneration using bioprinted scaffolds. Chansoria et al. [200] detailed a novel acoustophoretic (3D) biofabrication method that uses radiation forces produced by overlaying ultrasonic bulk acoustic waves (BAW) to differently form cellular arrays inside multilayered and single-layered hydrogel structures. Fantini et al. [205] reported printing parameters and bioink composition for neural tissue 3D modeling using gelatin and sodium alginate, along with three different cell types: neural stem cells, iPSCs, and a neuroblastoma cell line (SH-SY5Y).

6. Conclusions

In biological applications, bioprinting is currently emerging technology and is growing rapidly in the last few decades as it opened doors to various tissue engineering applications and regenerative research. Though this technology is in the developmental stage, it has shown many promising results in the tissue engineering field. Extrusion-based bioprinting has various advantages and can be easily tuned compared to other printing methods. For maintaining the tissue surroundings, this method is well-studied for creating a complex, thick tissue having lumens vascularization of various sizes—microstructures to large structures. Produced constructs are also supposed to be cost-effective [35, 36]. Sodium alginate is a naturally occurring polymer hydrogel with the widest application as bioink due to its low cost, excellent biocompatibility, and printability. It has been applied for cartilage, skin, bone, and vascular tissue printing. Though having many advantages, it also has a few disadvantages like slow degradation, minimal cell adhesion, and poor cell differentiation and proliferation. Growth factors like TGF-β are combined with it to improve cell differentiation and proliferation. Cellular adhesions are greatly improved by mixing alginate with various natural polymers, bioinspired polymers, drugs, Arg-Gly-Asp adhesion peptides, etc. Sodium citrate and/or oxidized alginate have shown promising results in improving the alginate slow degradation in tissue engineering and regenerative medicine applications. These approaches fundamentally alter the conditions in which alginate gelation can take place. For example, bioinks of alginate, which are oxidized, require more amount of Ca^{2+} ions for gelation, which are harmful to certain cell forms. The ideal bioink for cell-laden bioprinting should be blended homogenously with the alginate and should have moderate gelation time and optimum rheological properties.

Data Availability

Data supporting this research article are available from the corresponding author or first author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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