Chapter

Importance of Alginate Bioink for 3D Bioprinting in Tissue Engineering and Regenerative Medicine

Sudipto Datta, Ranjit Barua and Jonali Das

Abstract

Among many bioinks used for extrusion 3D bioprinting, the most commonly used bioink is the polysaccharide alginate because of its various cellular-friendly property like gelation. Erratic degradation and cell-binding motifs are not present in alginate which are the limitations of alginate bioinks, which can be improved by blending various low concentrations of natural or artificial polymers. Here in this chapter, we will discuss the various important properties of the alginate which make it as the bioink for almost all bioprinting scaffold designs as well as how improve the cellular properties like its cell-material interaction by blending it with other polymer solutions.

Keywords: 3D bioprinting, alginate, bioink, hydrogel

1. Introduction

The main objective of three-dimensional (3D) printing is to print a living cell or to create a three-dimensional biomaterial's scaffold. This innovative technology allows the reproducible and also the automated fabrication of three-dimensional useful living tissues by depositing biomaterials layer by layer with an accurate positioning of cells. This method allows to make a three-dimensional object and an accurate as well as scalable geometries that are not suggested by any approaches like two-dimensional cell cultures [1].

The choice of applying these 3D functional living tissues comes from fundamental research [2]. Learning about the cell-biomaterial interface at the nanoscale stage is vital in accommodating flaws in tissues, nanoparticle-cell connections and organ defects [3], toxicological analysis or drug investigation [4], and transplantation in living objects [5]. Because of the rising complexity required for these tissues, 3D bioprinting is facing a lot of challenges in all of manufacturing areas. For example, the cell-encapsulated materials are commonly observable to chemical cross linkers for extended periods of time during storage prior to printing, which can harm the cells. At the time of deposition, the mechanical stress generated by the printing itself can result in damage and injury to cell functioning by cell shearing or extrusion [6]. The instant new printing tissue is fabricated, because of the small vascularity of printed material; limited nutrients are supplied in 3D construct [7].

Usually, the requirements for a suitable cell-containing dispensable biomaterial or bioink are generally biocompatibility, exhaustive, biomimicry, printability, and essential mechanical properties. This is the main cause for the huge number of the manufacturers of commercially accessible 3D bioprinters—particularly extrusion-based 3D bioprinter, where hydrogel bioinks are recommended [8]. Particularly, hydrogels are unquestionably the most comprehensive biomaterials applied as cell matrix in bioinks because they can be engaged as cell matrix and be modified to replace or mimic local tissue [9]. The physical and chemical characteristics of the hydrogels will verify the performance of the cells. Normally hydrogels are like as jelly-type materials, where the liquid component is water. Actually, hydrogels are just like water by weight, but practically any flow will not occur in the steady state because of the three-dimensional cross-linked polymer network inside the fluid, which provides them unique properties comparable to those of living tissues. Due to their different biocompatibility and printability, various hydrogels that support cell growth are associated with bioink fabrication, i.e., gelatin, agarose, polyethylene glycol (PEG)-diacrylate, and alginate that are commonly used as bioinks. While alginate is an anionic polysaccharide derived from brown seaweed and generally consists of two polymer blocks, (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, basically all are covalently linked. The main elements in the alginic acid polymer chain are the carboxylic acid group which allows cross-linking. This converts alginate from its liquid state to a semisolid gel state. Sodium alginate is mostly used as bioink in tissue engineering and cell culture because of biocompatibility, low-cost, and fast gelation. In **Figure 1**, the presence of calcium ions and ionic interactions between Ca^{2+} and COO^{-} occur, and cross-linking of alginate polymers results.

Ionic cross-linking is a method where cells cause minimum damage. The cross-linking process happens moderately rapidly. Alginate has structural similarity to natural extracellular matrices that is why it has been applied widely in various biomedical applications as well as in the delivery of bioactive agents and wound healing. For cell encapsulation, alginate hydrogels are generally applied. The whole procedure is prepared by mixing cells in alginate solution, and after the mixing process, the alginate-cell mixture drops into a bath of calcium chloride solution. But in low concentrations (1–2%), due to low viscosity, the alginate solution is not printable. For increasing the viscosity, other materials like methylcellulose or gelatin can be mixed with alginate for preparing the printability. The structural correspondence of alginate to extracellular matrices creates a perfect biomaterial. Matrix stiffness is a functional determinant of stem cell differentiation, and alginate makes a potential material to manage stem cell growth. Alginate helps support the cell growth and also has a high versatility, extending to both in vivo and in vitro differentiation. For 3D bioprinting applications, for example, extrusion printing needs quick gelation. In this case alginate proposes high gelation procedures when combined with a

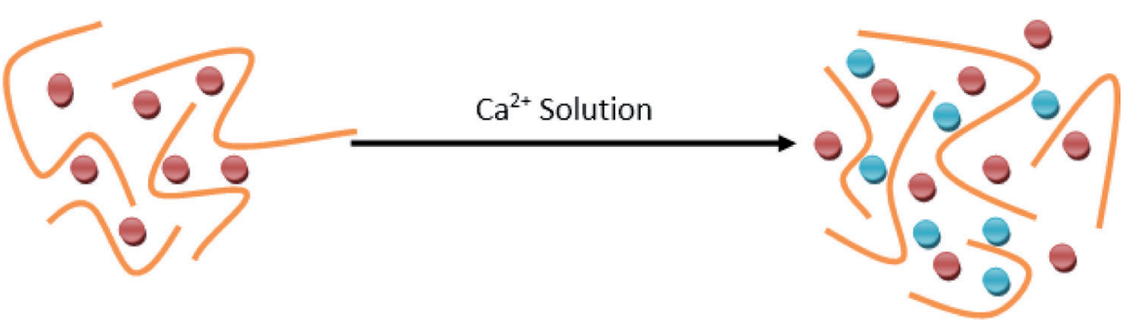

Figure 1.
Cross-linking process of alginate.

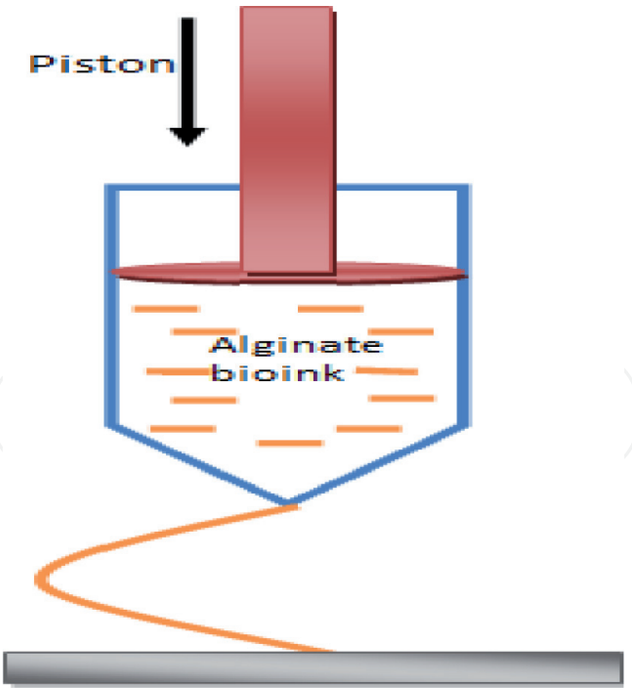

Figure 2.
Extrusion-based 3D bioprinting process.

multivalent cation, permitting gels to build up and deposit at constant temperature. It is also applied to encapsulate cells. This allows it to be an effective tool in varying the release rate of drug and growth factor delivery. While alginate degradation rate can be somewhat controlled by altering the MW of the alginate, it is still slow and difficult to control. The stiffness and composition properties of alginate bioink can be tuned to direct the differentiation of stem cells. Sodium alginate is available naturally which is biodegradable, non-immunogenic linear, and nontoxic polysaccharide polymer; it consists of mannuronic and guluronic acids [10]. The cost is also low being a marine material which can be extracted from the brown algae cell walls, forming hydrogel in certain conditions. Because of these advantages, bioengineers and material scientists use alginate for the preparation of bioinks in tissue engineering and regenerative medicines. The tissue fabrication by 3D bioprinting [11] and sodium alginate applications and properties [12] is currently separately reviewed. In this study we discussed the applications of alginate (**Figure 2**) in 3D bioprinting and blending alginate with other polymers to improve the biomaterial interaction of the cells attached to it [13].

2. Application of alginate

The requirement for alginate-based biomaterials in drug delivery and tissue engineering is huge. As stem cells play a progressively more major function in the area of regenerative medicine [14, 15], the arrangement and relation between alginate-based materials and stem cells have been exclusively emphasized. Investigated by in vitro implantation and in vitro cytotoxicity assay, alginate-based scaffolds and microcapsules have shown minimum or minor cytotoxicity [16, 17]. These in vitro results recommended tunable connections between the bio-composites and the multiple platelet releasate-derived bioagents for improving hematoma-like fracture repair. Also, a simple invasive performance for in situ remedial of the implant structures through injection was established in rat tail vertebrae applying microcomputed tomography. These results confirmed that alginate-based scaffolds

were capable of degrading, permitted the vascularization, and obtained minimum inflammatory responses after transplantation. Consequently, alginate-based scaffolds can present suitable characteristics as probable cell and drug carriers for tissue regeneration. The next sections explain the clinical and preclinical analysis of alginate-based biomaterials and applications.

2.1 3D bioprinting

Sodium alginate which is also known as sodium alginate or algin is a naturally extracted less costly polymer from the brown algae cell walls which have intracellular spaces [10]. Alginate is composed of (1-4)-linked β-D-mannuronic (M) and α-L-guluronic acids (G). Alginate is a polyanionic linear block copolymer made of longer M or G blocks, separated by MG regions. Sodium alginate is a kind of polysaccharide which is charged negatively because it is known that materials which are positively charged produce inflammatory response; this allows the biopolymer to support high biocompatibility and cell growth. G blocks enhance the gel structure and M and MG blocks enhance the elasticity, though a high quantity of M blocks could be the reason of immunogenicity [18]. In alginate matrix, with the help of capillary forces, water and other molecules can be trapped. This feature makes alginate hydrogel suitable for bioink designs. The cell density within the bioink will be very high, whereas the shear stress through the extrusion process decreases the cell viability (80–90%). In the case of inkjet bioprinting, the bioinks have lower cell densities (<16 × 106 cells/mL) and are less viscous (<10 mPa s). This technique suggests 90% cell viabilities but, in laser-assisted bioprinting, needs bioinks with viscosities of 1 and 300 mPa s and also requires medium cell densities (108 cells/mL). In this method, cell viability is very high (>95%). The alginate-based bioink viscosity rests on the alginate molecular weight, alginate concentration, density of cells, and cell phenotype. These are the variables that scientist must consider to optimize the viscosity of the alginate-based bioinks. An additional significant rheological characteristic of aqueous alginate solutions is the shear-thinning, where the shear rate increases while decreasing the viscosity. The viscosity also depends on the performed printing temperature; when the temperature increases, the viscosity gradually decreases. In comparison with other polymers, alginate is convincingly easy to handle and to print and is easy to extrude (printing) while defending the encapsulated cells. Even if it is also a non-cell-adhesive [12], in case of cell encapsulation, alginate is currently one of the most applied biomaterials. After the printing performance, the hydrogel should degrade suitably, allowing the cells to make their specific extra cellular matrix (ECM). The alginate also generates durable insistent cell-laden hydrogels; however oxidation can be performed by slow degradation, for example, sodium peroxide [11]. The main issue of alginate for using it as a biomaterial in bioprinting is slow degradation rate. The release of the hydrogels through the bioprinter nozzle in bioprinting (extrusion) limits the usage to low weight of alginate, which has a major role in the application of reduced mechanical properties. Though the alginate mechanical and structural characteristics are needed for all printed tissue, the biomimicry characteristics required in every instance can be changed by combining new biomaterials in the scaffold or by applying different types of hydrogel fabrication technique. For example, CELLINK is a commercial bioink which is already available for bioprinting; it combines with alginate hydrogel and nanocellulose and presents fast cross-linking and shear-thinning properties; this bioink is appreciated for soft tissue engineering for bioprinting [8]. The formation of blood vessel-like channels is able to transport different materials like nutrients and oxygen via the bioprinted material, which is needed in order to print organs or tissues. To succeed in this aim, Zhang et al. [19] made vessel-like printable

microfluidic channels where a coaxial nozzle strategy is used for transporting the nutrient into printed material, and the printer was pressure-assisted bioprinter and the coaxial needle was applied for printing the hollow alginate filaments that contain cartilage progenitor cells. In the same way, a triaxial nozzle assembly was used to fabricate biocompatible cartilage-like tissues containing tubular channels, where the alginate was encapsulated by cartilage progenitor cells, which is the main element of the bioink. Hydrogels of sodium alginate having high strength and having inner microchannels were found out by Gao et al. [20]. Also, constructs like perfusable vascular-like constructs were also obtained through coaxial multilayered nozzle along with the concentric extrusion channel by 3D printing in one step [21] by mixing 4-arm poly(ethylene glycol)-tetra-acrylate (PEGTA) and gelatin methacryloyl (GelMA) with the sodium alginate. Calcium ions were used to cross-link the alginate, and photo-cross-linking was used for covalent cross-linking for PEGTA and GelMA for setting the rheological and mechanical properties that was reported in this work. Also in another study, Christensen et al. [12] printed vascular structures along with bifurcations (vertical and horizontal) in alginate and fibroblast of mouse bioinks. Blending alginate with other polymers (honey, gelatin) [22], amino acids like polyglutamic acid and poly-L-lysine [13, 23], and some drugs like N-acetylcysteine (NAC) [24] was studied for improving the erratic degradation, cell-material interaction, cell viability, etc. The printer (inkjet) used $CaCl_2$ cross-linking agent supporting material for cross-linking the alginate bioink. To back up the buoyant force in the regions overhanging in both vertical and horizontal printing and also supporting the regions spanning in the horizontal printing, their modified solution was used. Blending alginate with other polymers (honey, gelatin) [22], amino acids like (polyglutamic acid and poly-L-lysine) [13, 23], and some drugs like N-acetylcysteine (NAC) [24] was studied for improving the erratic degradation, cell-material interaction, cell viability, etc. Jia et al. [6] in their study showed the controlled degradation of oxidized alginate in 3D bioprinting.

Varying biodegradability of solution of sodium alginate along with human adipose stem cells was printed with accurate definition. These kinds of bioinks have the capacity to modulate proliferation and stem cell spreading and withstand uniform cell suspension but are imperfect in the case of stem cell diffusion. Wu et al. [25] showed the procedure of slow degradation of the alginate by tissue incubation in a sodium citrate medium. The sodium citrate amount helped the optimization of the alginate degradation time. Chung et al. [18] improved the printing resolution and printability of pre-crosslinked printed constructs by adding alginate with gelatin, keeping the mechanical property and the growth of cells, and keeping pore diameter constant. In **Figure 3** the procedure of alginate cross-linking with the added cells is shown.

2.2 Wound healing

Alginate has been used for dressing of the wounds due to its of good conformability, absorptivity, and mild antiseptic properties coupled with biodegradability and nontoxicity and optimal water vapor transmission rate. Alginate-based products like electrospun mat hydrogels and sponges in dressing of wounds are very good substrates for healing of wounds, which include gel-foaming capability as soon as the absorption of the wound exudates and hemostatic capabilities [26]. It is already been mentioned that dressing wounds with alginate improves healing of wounds through monocyte stimulation to harvest higher cytokine levels like tumor necrosis factor-α and interleukin-6 [27]. Near the wound locations, cytokine production creates pro-inflammatory factors that are helpful for wound healing. Because of the existence of endotoxin in the alginate, a huge level of bioactivity

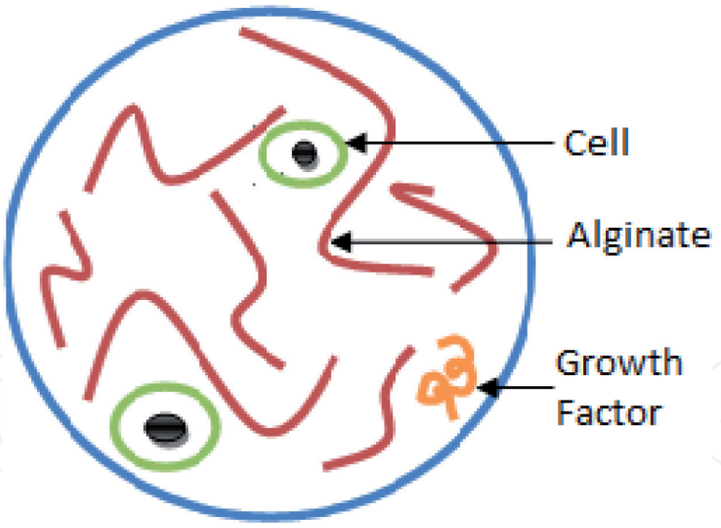

Figure 3.
Alginate with cell cross-linking process.

is present in these dressings. In situ-forming wound dressing hydrogel can be produced by oxidized alginate and gelatin in low borax concentration as shown by Balakrishnan and Jayakrishnan [28]. The homeostatic gelatin effect is present in the mixed matrix and wound healing property of alginate, and the antiseptic borax property makes alginate the appropriate wound dressing material. Tissue-engineered cartilage requirement is immense and has a huge clinical importance. The main causes of disability of the articular cartilage are degenerative and traumatic lesions [29]. Nearly 100 million Chinese people suffer from osteoarthritis. Because of this reason, regeneration and repair of the cartilage have huge impact. The pros of the cartilage repair injectable therapies are that implant within the defect is not only maintained, but it also allows quick bearing of weight because of strength and stiffness which is attained quickly [30, 31]. For bringing close the mechanical properties of the native tissues with the scaffolds, the alginate physical properties are matched with the articular cartilage. Ge and solid alginate injectable hydrogel microspheres are used for cartilage regeneration. Many researchers have studied the growth factor in tissue engineering by using alginate hydrogels and alginate-based microsphere combinations [32, 33]. In one study the demonstration of immobilization of the positive effect of RGD to an alginate porous scaffold for endorsing TGF-β-induced human MSC differentiation is shown [34]. Bian et al. studied the co-encapsulation of the TGF-β including the microsphere of the alginate with the human MSCs in the hyaluronic acid (HA) hydrogels with respect to the design of the constructs implantable for the cartilage repair [35]. The immobilized RGD peptide facilitated the cell-matrix interaction which is proven to be an important feature for the microenvironment of the cells, allowing good cell availability for the chondrogenic-inducing molecule TGF-β. TGF-β-laden alginate microspheres in combination with alginate hydrogels forms a compound carrier which may retain TGF-β bioactivity in the construct and encourages hondrogenesis of MSCs when inserted. The animal experiment displayed that chondrocytes planted into the microsphere scaffold lived habitually in SCID mice and cartilage-like constructions were created after 4 weeks of imbedding.

2.3 Drug delivery

In the past years, drug delivery carriers draw huge interest because of large biomacromolecules like genes and proteins as well as low-molecule weight drugs

which can be delivered in a targeted or a localized manner [36, 37]. Because of its biodegradable and biocompatible nature, alginate is used as a carrier for encapsulating and immobilizing drugs, cells, proteins, and bioactive molecules [38, 39]. Currently, alginate-based carriers like colloidal particles, polyelectrolyte, and hydrogels are under examination; few of them are practically used. Many researchers have examined the alginate-based hydrogel blends, microspheres, and porous scaffolds for precise drug delivery in various tissue engineering fields [40, 41]. Hollow microsphere of alginate-based hollow microsphere has huge applications as drug delivery carrier, micro-reactor, and biosensor [42]. The construction of the hollow microcapsules can be created by successive self-assembly of positively and negatively charged polyelectrolytes by layer-by-layer (LbL) technique. The alginate microcapsule is studied well with respect to precise releasing and loading parameters. The attempt to fabricate microcapsule biopolymer has been made by dropping alginate/chitosan in a decomposable colloid particle after removal of its core is done in an appropriate pathway. For the production of hollow microcapsule, chitosan and alginate are interchangeably deposited in $CaCO_3$ with electrostatic biocompatibility [43]. The chitosan/alginate microcapsule functionalities and properties can be preciously adjusted by changing the microcapsule composition, exterior stimuli introduction, and thickness. Immersing alginate microcapsule in various pH solutions helps in the degradation of the microcapsules which also determines the material role and encapsulation layers for keeping microcapsule stability in various pH conditions. The addition of PEG to the microcapsule allows protection against acidic conditions, whereas the coating layer number only affects the swelling properties, not the microcapsule Young's modulus which was revealed by Wong's study [9]. For surface micro-patternings and microarray systems, 3D platform alginate hydrogels are used. For in situ gelation, a few aliquots of solution of gelatin were trapped selectively on hydrophilic area by a process called dipping process. Cells with various adhesion properties were captured by gel pattern alginate on the hydrogel structures.

Various CYP450 enzymes like vascular endothelial growth factor (VEGF) and β1-integrin upregulation showed that the stage gave many in vitro conditions that result in allowing cells in their natural phenotypes.

2.4 Bone regeneration

For the reconstructive surgery, bone regeneration is an important challenge. It occurs due to tumor removal and trauma. To repair the bone, a good initiative is to induce osteogenesis in situ. To complete this process, one method is by using stem cell differentiation to form bone tissue and then seeding them in an injectable scaffold [44, 45]. As of now there are numerous investigations and studies on alginate-based injectable scaffolds for the bone regeneration. By using MSCs and alginate scaffolds, satisfactory bone tissue formation was noticed [46, 47]. For this reason the application of alginate for gel tissue generation is commonly used which displays angiogenic and osteogenic properties. Many researchers showed bone regeneration by means of injectable constructs by joining microspheres or alginate-based hydrogels that were combined with interchangeable ASCs or MSCs [48]. These studies demonstrated the potential of bone morphogenetic protein (BMP) and TGF-β delivery to induce osteogenic differentiation to mature osteocytes from MSCs and ASCs. Kolambkar et al. presented a growth factor hybrid system of delivery that comprises of a nanofiber mesh tube *which is electrospun* for directing regeneration of bone along with alginate hydrogel peptide modified in the tube for fixed recombination BMP-2 (rhBMP-2) release [49]. The discharge of fixed transport of rhBMP-2 through alginate hydrogel was important for significant regeneration to

take place. The mixed technology can be used clinically for the regeneration of bone in cases as huge bone defect and nonunion fractures.

3. Conclusions

The naturally available biopolymer alginate is cheaper which forms hydrogel by cross-linking with various salts like $BaCl_2$, $CaCl_2$, and $ZnCl_2$ which showed good biocompatibility and printability.

This is broadly applied for cartilage, bone, and vascular tissue printing. Few drawbacks of alginate are slow degradation and poor cell adhesion; in many research, it is shown that alginate has poor cell differentiation and cell proliferation, and for this reason, it is used as a blend with other polymers. To improve these limitations, blending alginate with other polymers like honey, gelatin, and Arg-Gly-Asp adhesions is done. Furthermore, for faster normal degradation in regenerative medicine, oxidized alginate and/or sodium citrate is found to be useful. The combination of 3D printing alginate for cartilage and electrospinning is used positively in various tissue engineering fields. Furthermore, mixing alginate with biopolymers like polycaprolactone and nanocellulose has shown positive results. In bioprinting using coaxial or triaxial nozzles is found out to be promising and provided brilliant results. To improve the mechanical properties of the alginate-based structures used in bone tissue engineering, mixing alginate with other polymers like bio-silica, polyphosphate, polycaprolactone hydroxyapatite, and gelatin is found to produce an excellent result. We think this review will allow researchers to investigate more advanced and improved bioink for 3D printing and also help to invent suitable and more appropriate bioink for various tissue engineering applications

Author details

Sudipto Datta[1*], Ranjit Barua[1] and Jonali Das[2]

1 Centre for Healthcare Science and Technology, Indian Institute of Engineering Science and Technology Shibpur, Howrah, W.B., India

2 Department of Chemistry, Raja Peary Mohan Collage, Calcutta University, Uttarpara, W.B., India

*Address all correspondence to: dattadip440v@gmail.com

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