Chapter

Alginate-Based Composite and Its Biomedical Applications

Yaling Deng, Ningning Yang, Oseweuba Valentine Okoro, Amin Shavandi and Lei Nie

Abstract

Alginate has received much attention due to its biocompatibility. However, the properties of pure alginate are limited, such as weak mechanical strength, which limits its application. Alginate-based composite effectively overcomes the defect of pure alginate. The molecular weight and microstructure can be designed. More importantly, the essential properties for clinical application are improved, including mechanical properties, biocompatibility, gelation ability, chondrogenic differentiation and cell proliferation. This chapter will describe development of alginate-based composite in biomedical application. In the fields of wound dressing, drug delivery, and tissue engineering, the impact of structural changes on performance has been stated. To provide readers with understanding of this chapter, the structure and characterization of alginate will be included.

Keywords: Alginate, composite, wound dressing, drug delivery, tissue engineering

1. Introduction

Alginate is a natural heteropolysaccharide extracted from brown seaweed. Due to the abundantly available in nature and less expensive, *L. hyperborea*, *L. digitata*, Laminaria japonica, *A. nodosum*, and *M. pyrifera* are the main choice of alginate [1]. Alginate, without pungent smell, usually has a white or yellowish-brown character. It is water-soluble but insoluble in organic solvents, such as alcohol, chloroform. Carboxylic acid groups in the saccharide residue endow the special anionic nature. Calcium alginate, for instance, is insoluble, while sodium alginate has water-solution [2]. However, the process of water dissolving is slow and usually takes several hours, forming stable and viscous solutions [3]. Due to the ability to form a hydrogel, alginate could be utilized as a gelling agent, which expands its application in the field of biomedical applications.

Alginate and its derivatives show outstanding properties, such as biocompatibility, biodegradation, gel-forming ability, being suitable for sterilization and storage. Owing to its unique characteristics, alginate has been widely used in diverse fields, including wound dressings, drug delivery, tissue regeneration. In this chapter, the characteristics and wide applications of alginate will be introduced.

2. Structure and characterization

2.1 Structure

Alginate with molecular weight between 32,000 to 400,000 g/mol is mainly comprised of a sequence of liner polymers of β-(1–4)-D-mannuronic (M-blocks), α-L-guluronic acid (G-blocks), and inserted MG sequences (MG-blocks), with varying proportions and linear arrangements [4]. That organized in homogenous patterns with repeated G residues, repeated M residues, and heterogenous patterns with alternating G and M residues [5]. Alginate derived from different sources displays different M/G ratios and contents in M and G [6], leading to the change of molecule weight and physicochemical properties. These parameters are related to the characteristics and applications of alginate. Generally, alginate with high M units shows good biocompatibility and more immunogenic [7]. Alginate with high M units has soft and elastic properties, G-rich alginate exhibits hard and brittle characteristics [8, 9]. The rigidity of the chains increases in a sequence, MG < MM < GG, due to the electrostatic repulsion between charged groups. G-rich alginate gels have better mechanical stability (**Figure 1**) [5].

The electrostatic interactions between the carboxylate groups of G units and divalent cations, such as Ca^{2+}, Fe^{2+}, Mg^{2+}, form an "egg-box" structure, which crosslinks to obtain the hydrogels. There are several hydroxy and carboxyl groups in the molecular structure of alginate. As the active site, more groups and side-chain molecules were introduced into the main chain to decorate the structure, which endow more features and expand its applications [10].

2.2 Solubility and viscosity

Sodium alginate exhibits slowly water-soluble and forms viscous and stable solution. At low solvent pH, more heterogeneous MG-blocks contribute to solute

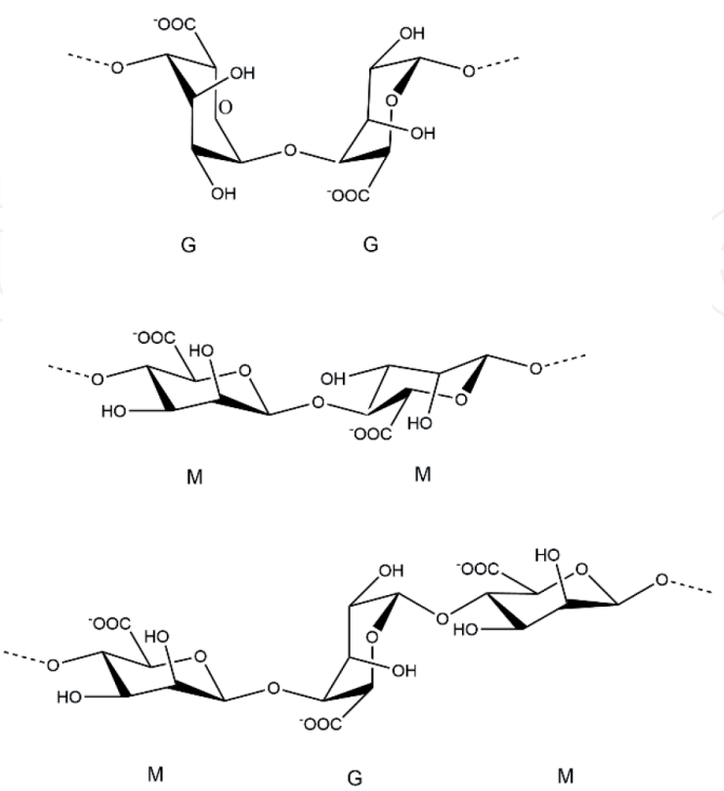

Figure 1.
Chemical structure of alginate [5].

than M-rich and G-rich alginate [11]. With the decrease of pH, the viscosity of alginate solution increase. When the range of pH is 3–3.5, the viscosity has the maximum. In the Mark-Houwink relationship ($[\eta] = KM_v^a$), $[\eta]$ is intrinsic viscosity (mL/g) and M_v is the viscosity-average molecular weight (g/mol) [12]. For sodium alginate in 0.1 M NaCl solution at 25°C, K and a, the parameter of the Mark-Houwink relationship ($[\eta] = KW_v^a$), are 2×10^{-3}, $a = 0.97$ respectively.

The primary structure is associated with different amounts and sequential distribution of M and G, which affects the molecular weight and properties. For example, the viscous behavior is remarkably relevant to molecular weight during the preparation. Alginate with high molecular weight polymer could form an obviously viscous solution [13] and result in a higher elastic modulus in the gels [14]. Meanwhile, the alginate with a long chain shows a higher solution viscosity. For example, G-rich alginate displays more excellent water solubility than M-rich alginate [15].

2.3 Biocompatibility

Alginate has excellent biocompatibility, that has been widely assessed. However, some gaps of biocompatibility between alginate and alginate complex still exist. For purified alginate gels, the relative amounts and distribution of M and G have an effect on biocompatibility [16]. For example, M. Otterlei et al. reported that alginates with low G were approximately 10 times more potent inducing cytokine production compared with high G alginates [7]. S.K. Tam et al. found that gel beads prepared with alginate contenting intermediate guluronate (IntG, 44% G) exhibited better biocompatible than high guluronate content (HiG, 71% G). There are no inflammatory reactions around alginate implants [17]. For the alginate complex, the impurities from alginate-based materials, such as heavy metals, proteins, and polyphenolic compounds, have the potential to cause an immunogenic response. A multi-step extraction procedure reduces the concentration of impurities and will not cause foreign body reactions. Meanwhile, the biocompatibility properties of alginate are attributed to hydrophilicity, chain migration, and water-absorbing [18]. Swelling properties contribute to enhance biocompatibility, that limiting the adsorption of proteins and cells of immune response [19].

2.4 Degradation

Degradability of biomaterials, as a critical property, contributes to providing a biomimetic microenvironment for use in cell delivery, survival, and expansion [20]. Hydrolytic is the main reason for degradation, which will be initiated spontaneously after contacting water-based fluids [21]. The degradation rate is related to the molecular weight of alginate. Generally, with the increase of molecular weight, the amounts of reactive sites for hydrolysis degradation decrease, that reduce the degradation rate [22]. Degradation, accompanied by adjusting the structure and molecular weight distribution, has an effect on the mechanical properties. In the physiological environment, alginate will undergo rapid degradation [23]. For alginate hydrogel, the degradation process will be accomplished by releasing the divalent ions into the surrounding environment. However, some residues of alginate still exist and will not be removed [24]. Many physical and chemical methods have been used to control alginate degradation, including ultrasonic, ultraviolent, gamma irradiation, and partial oxidation.

3. Applications

The various physicochemical properties of alginate are favorable to extensive usages, such as the ability to form a gel and the ability to maintain a moist

environment, which has been confirmed by the literature. Due to the biocompatibility and nontoxic, alginate has been applied in biomedical applications, such as wound dressing, drug delivery, and tissue engineering.

3.1 Wound dressing

Skin, as the largest human organ, plays a vital role in protecting the human body from outside germs [25]. It is also a vulnerable organ. Once the skin is damaged, all kinds of microbes and pathogens will gather around the wound site to affect the wound closure cause bacterial infection. For severe wounds with large, deep, or bleeding, handling and management are essential [26]. Wound healing is a complex process, involving the treatment of infectious, germs. Therefore, wound dressing has been attracted extensive attention and is widely used to accelerate wounding recovery and to control infection. Traditional dressing, consisting of sterile pad and gauze, has the features of preventing the invasion of germ into the wound site and keeping dry [27]. Some disadvantages of these dressings are displayed during the application, such as poor vapor transmission, susceptiblity to bacterial infection, and easy adhesion, which seriously interfere with wound healing. With the rapid development of technology, some biomaterials such as polysaccharides, proteoglycans, and proteins have been investigated for wound healing owing to their ability to accelerate healing and control infection [28, 29]. These polymers have been utilized to develop modern dressing due to its high water-content, biodegradable, and biocompatibility. Therefore, modern wound dressing effectively mimics the features of natural tissue and offers a moist environment, better water vapor permeation, autolytic debridement, which cause to promote re-epithelialization and accelerate wound healing [30].

Alginate, as one of these biomaterials, has been widely concentrated and investigated because of its biocompatibility and water-retaining capacity [31]. It has the ability to carry pro-inflammatory signals and initiate or accelerate the healing of chronic wounds [32]. Currently, there are several forms of alginate dressing on the market, including hydrogel, films, membrane, and sponges. Ionic cross-linking is a representative fabrication method of alginate dressings. Multivalent ions are chosen to fabricate gel, such as calcium [33], magnesium [34], iron [35]. Free-dried porous sheets and fibrous dressing that followed are formed. Alginate dressings are characterized by absorbing wound fluid, physiologically moist environment, maintain the optimal pH, and reduced bacterial infections, that improve the physicochemical stability [36, 37].

Alginate wound dressings with multifunctional properties have been investigated for nearly 40 years. Kaltostat® alginate dressing, as a famous wound dressing, has been designed and applied to absorb exudate and avoid infections, especially calcium sodium alginate dressing. Nevertheless, only 70% wound closure is exhibited [38]. Considering that single alginate dressing could not completely prevent infections and moderate wound healing, the development of effective alginate dressing, based on biopolymers and nanoparticles, draw much attention and expanded research.

Alginate composites combining natural polymer and synthetic polymer are utilized to prepare dressing hydrogel via physical or chemical methods. Chitosan, as a typical natural polymer, has several advantages, such as biocompatibility, biodegradability, ionic character, and hemostatic properties. It could be incorporated with alginate to create bioactive interpolymer (polyelectrolyte) complexes and promising wound dressing. Wound dressing formed with this method exhibited superior water uptake of 4343.4% over 24 h and 78% biodegradation than commercial Pharma-Algi wound dressing [39]. Mudlovu et al. fabricated an

alginate-chitosan bioplatform through a three-step method; partial-crosslinking of polymers, lyophilization, and pulverization (**Figure 2**) [39]. These wound dressings showed a higher degree and rate of fluid uptake (3306.61%, 4343.4%) than Pharma-Algi® (2168.21%; 1612.56%). For chitosan with high (MW ~ 800 000) and low (MW ~ 3000) molecular weight, it was mixture as a coating to develop calcium alginate dressing. The results showed that chitosan promotes angiogenesis by increasing VEGF and exhibits better wound healing than pure calcium alginate dressing [40]. In addition to the natural polymer, poly (vinyl alcohol) (PVA), as a type of common synthetic polymer, has the inherent characteristics of non-toxic, non-carcinogenic, biocompatibility. PVA hydrogel formed with cross-linking method has desirable properties such as high swelling rate, keeping the moist environment. Therefore, PVA has been attracted attention and explored for wound dressing. PVA-alginate hydrogel enhanced swelling properties and protein adsorption. When alginate has a high ratio in the PVA/Calcium alginate wound dressing, high a water vapor transmission rate, 2725.8 g/m^2/2h, was obtained, which contributes to holding a moist environment [41].

In order to endow the additional effects and accelerate wound healing, the antimicrobial agent has been incorporated into the wound dressing. Silver nanoparticles (AgNPs), as a promising wide-spread local antibacterial agent, have demonstrated huge potential and received more attention in the area of microbial resistance. Wound dressing carried with AgNPs is considered to control and treat acute and chronic wounds, accompanied by rapid healing via accelerating reepithelialization [42]. Alginate dressings incorporated with silver have highly absorbent features of alginate and antimicrobial efficacy of silver. Silver-loaded hydrogel has a uniform pore structure, had excellent water absorption and water retention, maintaining a moist wound environment for wounds [43]. The antibacterial performance is also

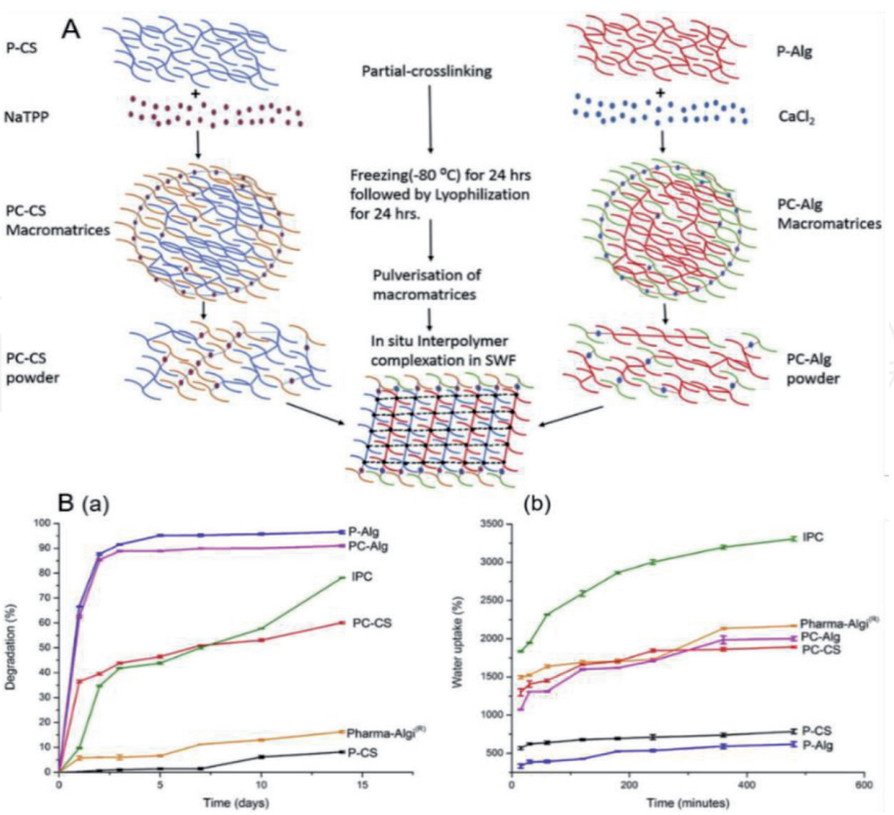

Figure 2.
(A) Schematic representation of the three-step method of partial-crosslinking and interpolymer complexation of the polymers. (B) (a) biodegradation and (b) water uptake behavior of the pristine polymers, bioplatforms and pharma-Algi® in PBS (pH 7.4) solution at 37°C at 50 rpm [39].

improved. Alginate/carboxymethyl cellulose silver dressing was found to have a sustained antimicrobial effect against microorganisms, for up to 21 days [44]. The sustain and stable release of sliver minimize clinical treatment time and relieve the patient's pain. Compared with pure alginate, alginate dressings incorporating ionic silver and nanocrystalline silver were confirmed to enhanced antimicrobial effect, improved the binding for elastase, matrix metalloproteases-2, and proinflammatory cytokines, and boosted the antioxidant capacity [45].

3.2 Drug delivery

Drug delivery systems could transport and release drug molecules to a target position under the microenvironment, especially for poorly water-soluble drug molecules. Many polymers have been explored and investigated for drug delivery. Due to the outstanding biocompatibility and gelation properties, alginate has been received wide attraction and utilized in drug delivery applications. Alginate could be prepared excipient. Three forms of participation are exhibited, such as solid dosage, semisolid dosage, liquid dosage. Solid dosage form contains tablets and capsules, while, gels and buccal patches are the most common semisolid dosage. Liquid dosage usually includes emulsions and suspensions. Alginate could be decorated to adjust the properties for novel drug release. Due to the facility to bond with drug molecules and rapid gelation in a mild environment, alginate could be utilized to modulate the drug release properties. D.J. Mooney et al. found that the pore size of alginate gel is about 5–6 nm [46], which contributes to the diffusion and release of drug molecules.

Alginate could be utilized to design microcapsules for sustained release of the drug, which has attracted extraordinary attention by the reason of outstanding advantages, including high drug loading rate, satisfactory biodegradable, non-immunogenic, and non-toxicity. Stimuli-responsive alginate-based microcapsules had shown a potential for targeted delivery and release drugs. Drug delivery system using magnetic nanoparticles was reported. Magnetic nanoparticles have the ability to control movement and aggregation at the target sites during the process of the external magnetic field. Therefore, the system could obtain a high local concentration at the target sites. It is demonstrated that magnetic reduction-responsive alginate-based microcapsules (MRAMCs) systems exhibited good magnetic targeted ability owing to the superparamagnetism of OA-Fe_3O_4 nanoparticles [47]. In addition, alginate microcapsules encapsulated glucocorticoid is a novel method for the treatment of osteoarthritis. *E. Lengert* et al. found that hollow silver alginate microcapsules could effectively deliver water-insoluble glucocorticoid betamethasone [48]. This system enhanced loading efficiency and sustained release, reducing the injury rate of intraarticular glucocorticoids delivery.

Alginate, as a well-known drug carrier, is utilized to prepare wound dressing. Antimicrobial agents or drugs are encapsulated into the hydrogel. Wound dressing provides a moist environment, and more importantly, drugs release to the wound and facilitate the healing. For example, alginate-based dressing encapsulated with vancomycin has a 44% drug release rate after 24 h, and the antimicrobial activity against various bacteria was confirmed [49]. Recently, double-membrane hydrogel formed with alginate and cellulose nanocrystals was developed. The results showed good release behaviors for complexing antibiotic drugs. The rapid drug release was completed by outer neat alginate hydrogel, meanwhile, the prolonged-release behavior corresponded to the inner hydrogel [50]. Another dual-drug delivery system, poly (D, L-lactic) (PDLLA) microspheres embedded in calcium alginate hydrogel beads, was developed by D.G. Zhong et al. [51]. The microspheres encapsulated glycyrrhetinic acid showed a sustained release, and hydrogel loaded with BSA exhibited a rapid release.

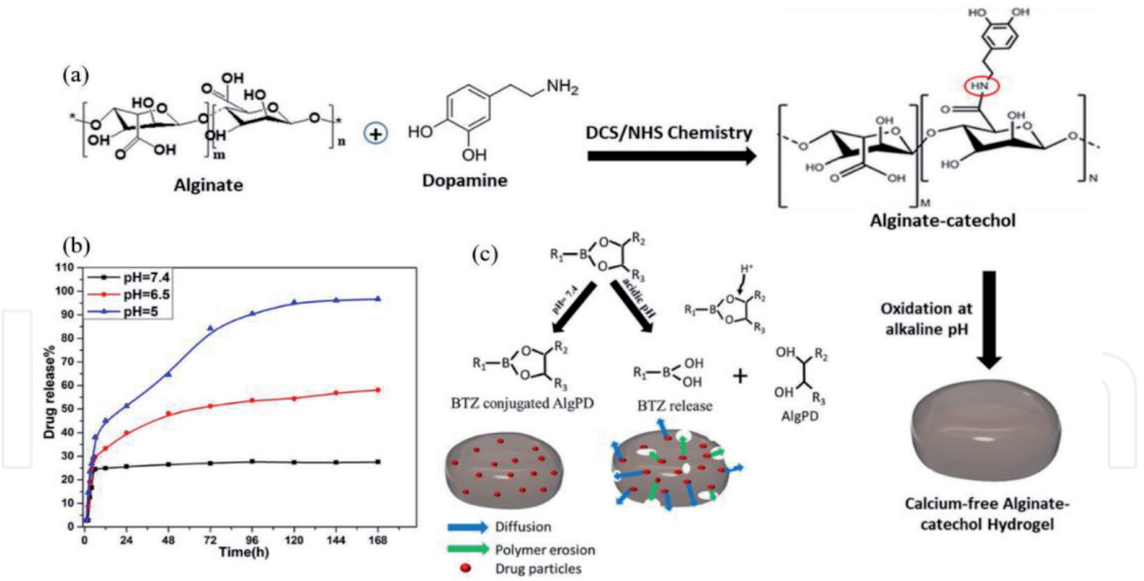

Figure 3.
The synthesis of AlgPD-BTZ hydrogel and drug release properties. (a) Schematic of the synthesis process of AlgPD-BTZ hydrogel by the conjugation of dopamine to the alginate backbone, and subsequent oxidation of catechol groups for cross-linking; (b) pH sensitive cumulative drug release from AlgPD-BTZ hydrogel; (c) scheme shows pH sensitive boronic ester bond of BTZ with the polymeric catechol group and the drug release mechanism [52].

pH-sensitive alginate carrier was investigated to enhance the efficacy, especially for localized drug delivery systems. The pH-sensitive reversible bond can control the drug delivery. C. H. Park and C. S. Kim prepared AlgPD-BTZ hydrogel using alginate-conjugated polydopamine as a building block polymer (**Figure 3**) [52]. The catechol group binds to the boronic acid group of BTZ drug, that covalent bond is a pH-sensitive reversible bond. Therefore, the system showed that BTZ was selectively released in cancer cells with a pH-dependent method. Sodium alginate could be used as a pH-sensitive bilayer coating on iron oxide nanoparticles by combining hydroxyapatite to deliver drug molecules. The higher encapsulation efficiency was detected, 93.03 ± 0.23% for curcumin and 98.78 ± 0.05% for 6-gingerol [53]. The electrostatic interactions of molecules could act as an adjustable gate to hold and release the drug molecules depending on the pH. The pH triggered drug-releasing mechanism play a virtual role in the releasing of tumor drug owing to the leaky vasculature [54].

In addition, alginate hydrogels are an excellent candidate and have been studied for protein drug delivery. Protein incorporated into hydrogels is protected, which reduces the denaturation and avoids degradation. Alginate hydrogels containing adjusting factors of neovascularization, vascular endothelial growth factor (VEGF), exhibited a sustained release, within 7 days approximately 60% of the total VEGF [55]. The protein release rate can be controlled by altering the degradation rate of alginate hydrogel. Also, sodium alginate-bacterial cellulose hydrogels had shown promising potential for carrying protein-based drugs, lysozyme (LYZ), via electrostatic adsorption [56].

3.3 Tissue engineering

Tissue engineering, proposed by National Science Foundation in 1987, belongs to the field of biomedical engineering, which is a valuable approach and can be used to restore, maintain, enhance tissues and organs [57]. Tissue engineering involves the getting of seed cells, biological scaffold materials, preparation of tissue and organs, and its clinical application. The biological scaffold materials were utilized to encapsulate and support the propagation of cells. Afterward, these materials

were transferred into the body and the target tissues were eventually formed. The research principal field contains bone [58], cartilage [59], vascular [60], ocular tissues [61], skin [62], and other tissues [63].

Alginate, as a most commonly known biomaterial with outstanding properties of scaffold-forming, has been extensively investigated and developed to treat the loss or failure of organs in tissue engineering. It usually combines with other substances to form new alginate derivative materials with the methods of physical or chemical. The different features and functions are obtained, accompanied by improved properties for tissue engineering, such as mechanical strength, cell affinity, and gel-forming ability. Therefore, alginate and its composite have received much attention in tissue engineering.

3.3.1 Bone

Bone has the hierarchical structure forming with 70% of nano-hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH_2)$) and 30% of collagen by weight [64]. It is a rigid connective organ and plays a major role in the movement of organs, involving affording structural framework, mechanical support and protection, mineral storage, and homeostasis [65]. There are several ways to cause bone defects or fractures in daily life, involving sports injury, accident damage, osteoporosis, osteoarthritis, bone neoplasm, and so on. Bone tissue engineering, as an effective alternative treatment for restoring bone defects, has addressed much attention. Several therapies have been available to treat bone defects or loss, including autograft (bone from patient's own healthy tissue), allograft (bone from the human donor), and xenograft (from other species) [66]. The final goal of bone tissue engineering is to construct bone tissue that should have the same qualities of structurally, physical, and chemical features as natural bone tissue. Alginate has been extensively investigated to synthetic scaffolding materials for bone reconstruction and transfer cells for bone tissue engineering [67].

Sufficient mechanical strength of scaffold in bone tissue engineering is essential to support bone regeneration. To obtain enough mechanical properties, alginate composite scaffolds were prepared mixing other polymers or inorganic components, such as chitosan, collagen, and hydroxyapatite. Chitosan, as a most abundant cationic polysaccharide, is selected to form alginate-chitosan composites. Chitosan/alginate composite scaffolds are extensively investigated for bone tissue engineering. The rigid strength and structural stability are obtained. S. J. Florczyk et al. prepared a chitosan-alginate scaffold with enhanced compressive strength, 0.79 ~ 1.41 MPa [68]. It has the homogeneous pore structure, and the pore size depends on acetic acid and alginate concentration (**Figure 4**). The cell proliferation potential was also improved when the viscosity was below 300 Pas. The greatest defect closure (71.56 ± 19.74%) was observed at 16 weeks [69]. Chitosan/alginate scaffolds present advantages in stimulating osteogenesis and vascularization [70]. Collagen is usually utilized to prepare scaffolds because of its specific properties, such as inducing cell adhesion and degradation [71]. Collagen I, as the essential component of bone tissue's ECM, contributes to migrant and penetration of osteoblasts and vessels [71, 72]. Therefore collagen/alginate hybrid scaffolds have an effect on the osteogenic ability of osteoblasts. It could promote cell spreading, proliferation, and osteogenic differentiation. S. Sotome et al. demonstrated that hydroxyapatite/collagen-alginate could deliver recombinant human bone morphogenetic protein 2 (rh-BMP2) efficiently [73]. There is bone formation within 5 weeks after implantation, accompanied with no obvious deformation. Hydroxyapatite (HA) is the main component of bone. The structure of alginate combined HA composites is similar to the native extracellular matrix of bone.

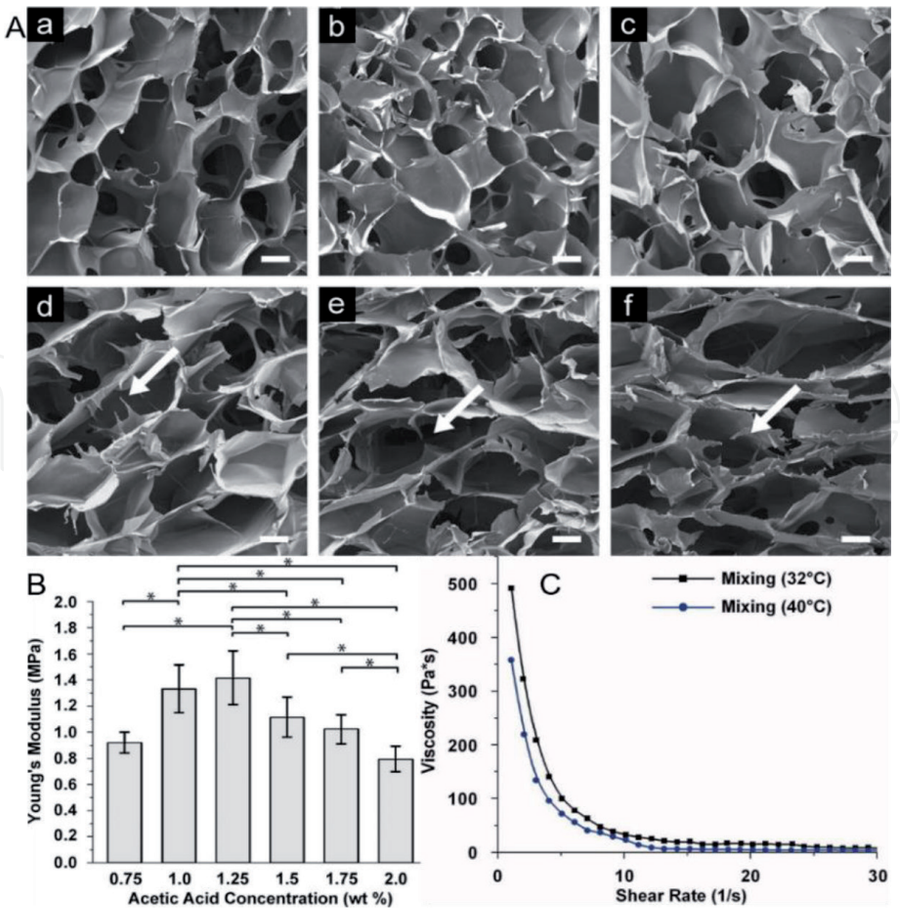

Figure 4.
*(A) SEM images of CA PEC scaffold pore structures made from CA solutions (4 wt % chitosan and 3.75 wt % alginate) with acetic acid concentrations of (a) 0.75 wt %, (b) 1.0 wt %, (c) 1.25 wt %, (d) 1.5 wt %, (e) 1.75 wt %, and (f) 2.0 wt %. The arrows indicate incomplete interconnects and scale bars are 100 μm. (B) Compressive Young's moduli of CA PEC scaffolds prepared from CA solutions (4 wt % chitosan and 3.75 wt % alginate) with varying acetic acid concentrations. *the significant differences between the scaffold groups for the modulus measurements. (C) Influence of mixing temperature on viscosity of CA PEC solution (1.0 wt % acetic acid, 4 wt % chitosan, and 3.75 wt % alginate) at zero shear rate [68].*

The interconnected porous structures and high porosity promote good cell viability, proliferation rate, adhesion, maintenance of osteoblastic phenotype, and bone regeneration [74]. The results of Lin et al. showed that alginate/HA is the better composite porous scaffold with an average pore size of 150 μm and over 82% porosity [75]. In another report, the alginate/HA composite scaffolds have 84% porosity [76]. The uniform pore morphology is favorable to improve compressive strength and elastic modulus, which is proportional to the content of HA. The satisfactory scaffold should have excellent performance to promote bone tissue growth, such as mechanical strength, cell proliferation, and morphology [77]. N. Firouzi et al. found that the addition of HA in alginate-based hydrogel could reduce degradation rate to 41.5%, and significantly improve compressive modulus, reaching 294 ± 2.5 kPa [78]. Meanwhile, microencapsulated osteoblast-like cells showed more proliferation as well as metabolic activities when they were cultured in Alg-Gel Ph-nHA microcapsules during the culture period.

3.3.2 Cartilage

The articular cartilage is an organized and specialized tissue that is highly hydrated (up to 80%), aneural, devoid of blood or lymphatic vessels [79]. It is the connective tissue covering the surface of articulating bones, that provides

the lubrication and mechanical strength for body weight and movement. Other body organs are also made by cartilage, such as the ear, nose, bronchial tubes, and so on. Cartilage has a restricted self-repair and regenerative capacity because of lacking nerves and blood vessels. It cannot heal appropriately after the injury, which eventually causes osteoarthritis and cartilage damage. Therefore, repair or reconstruction of damaged cartilage is still a major challenge. Tissue engineering is the potential approach to solve damaged or degraded cartilage. It could mimic the structure and function of body cartilage tissue, stimulating cartilage growth and restoration at the damaged sites. The tissue engineering scaffold has the three-dimensional structure for cell attachment/proliferation.

Alginate scaffolds have been proved to apply for the regeneration of cartilage tissue. It can induce redifferentiation of 2D culture-expanded dedifferentiated chondrocytes [80], therefore alginate could promote the growth of chondrocytes cells, restore damaged cartilage, and keep chondrocyte properties [81]. Chitosan-alginate scaffold effectively promotes the culture of osteogenic and chondrogenic cells. A.E. Erickson et reported that alginate-chitosan + hydroxyapatite scaffolds displayed a defined, interconnected porous network structure [82]. The compressive modulus and stiffness increased with polymer content. After culturing with

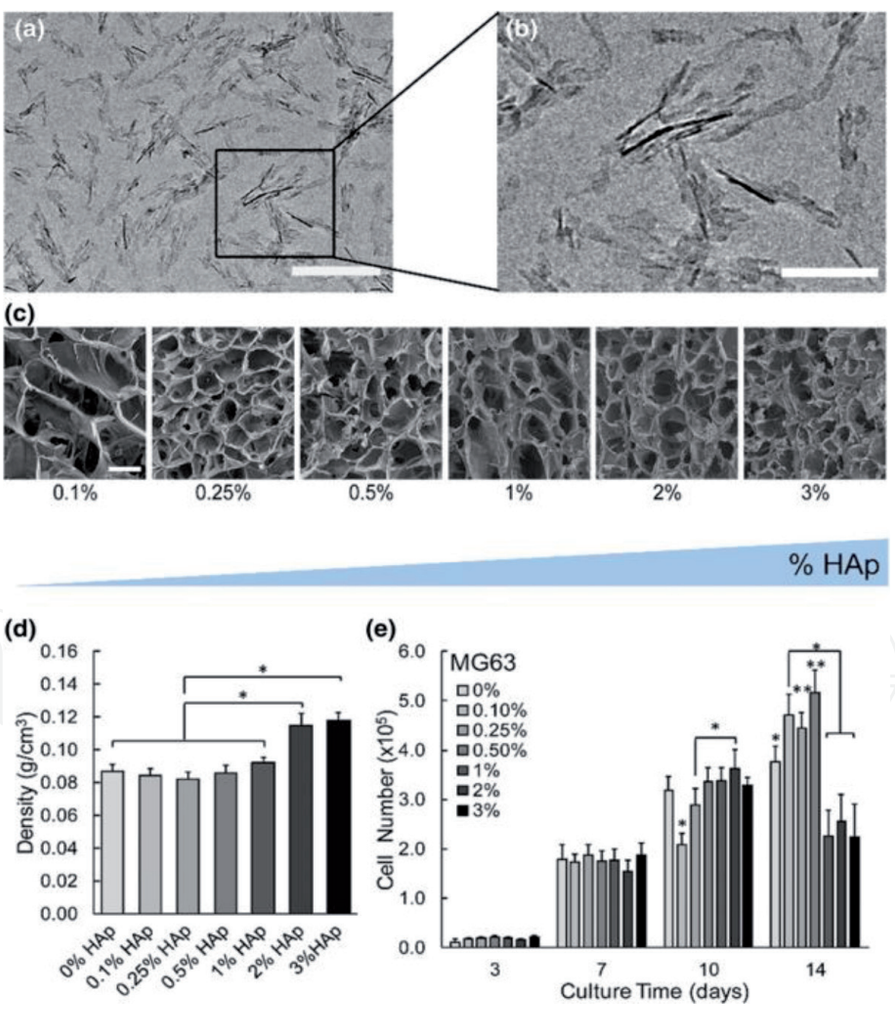

Figure 5.
*Effect of HAp concentration on 6% CA scaffold properties. a, b visualization of HAp nanorods with TEM. Scale bars represent 200 nm, and 100 nm, respectively, in (a) and (b). c SEM images of scaffold pore morphology with varying HAp (HAp concentration increases from left to right). Scale bar represents 200 μm. d density of CA + HAp composite scaffolds (n = 8) ($p \leq 0.05$). (e) MG63 proliferation on CA + HAp composite scaffolds over a 2-week culture period (n = 4). *statistically significant from all or specified conditions ($p \leq 0.05$). **statistically significant from all conditions except 0.1% HAp ($p \leq 0.05$) [80].*

chondrocyte-like (mesenchymal stem cells, MSC), the number of cells on 4% CHA scaffolds was significantly higher than the number of MSCs on 6% CHA scaffolds at day 10 (**Figure 5**) [80]. Hyaluronate, in addition, was explored to reinforce alginate to improve chondrocyte proliferation. It is demonstrated that alginate-hyaluronic acid hydrogel has stable physicochemical properties. C. Mahapatra et reported that alginate-hyaluronic acid-collagen hydrogel provided a binding motif for chondrocytes [83]. These gels effectively protected chondrogenic phenotypes after three weeks of cultivation. The results demonstrated the efficient expression of chondrogenic genes and the formation of cartilage ECMs. Another alginate hybrid gel made combining with polyacrylamide was confirmed to have remarkable mechanical strength, such as high toughness (up to 9000 J/m^2) and stretch ratio [84, 85]. It has

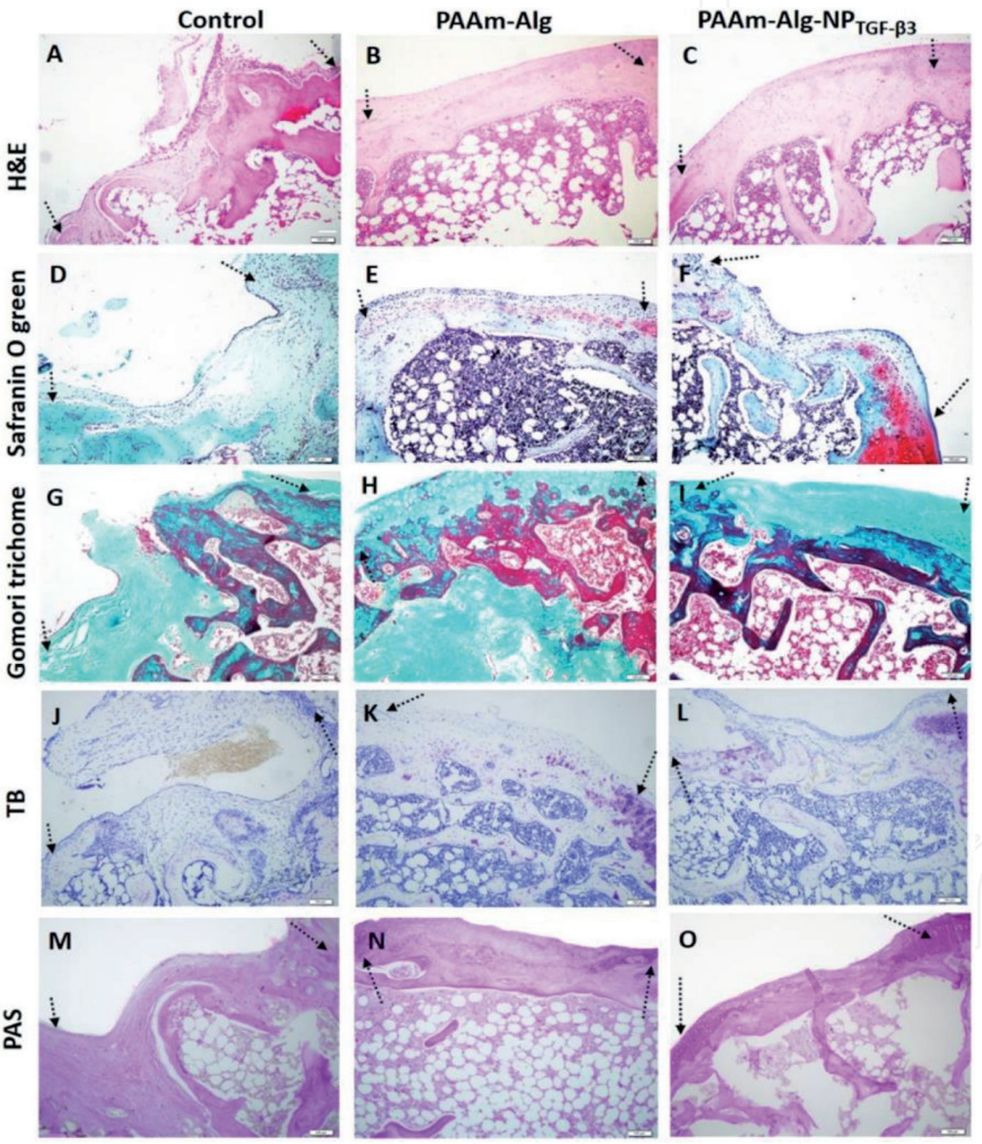

Figure 6.
In vivo representative photomicrographs of tissue sections in the experimental groups stained with (A, B, C) H&E; (D, E, F) safranin O/fast green, (G, H, I) Gomori's trichrome stains, TB (J, K,L), and PAS (M, N,O). (A, D, G) control group demonstrates mostly fibrous tissue with limited tissue repair in the chondral region. (B, E, H) PAAm-Alg group shows fibrous tissue with few fibroblast and chondrocyte-like cells in the joint surface chondral regions. (E) Fibrous tissue in the deeper regions is still observed in some rats. (C, F, I) PAAm-Alg-NP$_{TGF-\beta3}$ group reveals fibrous tissue with some fibrocartilage and hyaline cartilage groups in the chondral regions. PAAm-Alg and PAAm-Alg-NP$_{TGF-\beta3}$ groups demonstrated higher (K, L) glycosaminoglycan deposition and (N, O) glycoprotein and proteoglycan contents compared to control group (the arrows indicate the margins of the defect area; scale bars: 100 μm) [87].

also been proved that alginate-polyacrylamide showed good tribological behavior, ultra-low coefficient, and high wear-resistance [86]. After transplantation, chondrocytes displayed an organized distribution and superior integration with surrounding tissue (**Figure 6**) [87]. Alginate and its composites provide chondrogenic differentiation and cell proliferation, therefore, these hybrid gels can be used as cartilage implants.

3.3.3 Liver tissue engineering

Liver disease is a great threat to human health, and it is one of the major reasons for the increased mortality. There are about 2 million deaths all over the world every year [88]. Liver transplantation is the most effective way to solve this problem. Yet, this is a very difficult process because of lacking suitable liver donors. Liver tissue engineering is considered the best way to provide liver to meet the excessive requirement of the liver. It could improve the function of the liver and form a complete organ.

Alginate composites can be used for hepatocyte growth in liver tissue engineering. For example, R. Rajalekshmi et al. reported that fibrin (FIB) incorporated injectable alginate dialdehyde (ADA) - gelatin (G) hydrogel effectively supports growth, proliferation, and functions of hepatic cells [89]. The reason is that fibrin provides several cell adhesion pockets for cell attachment. Liver tissue engineering scaffold, extracellular matrix (ECM), were synthesized with oxidized alginate and galactosylated chitosan via Schiff base reaction. After being cultured in the scaffolds, the hepatocytes exhibited spheroidal morphology. And the multi-cellular aggregates and perfect integration were observed [90]. In addition, the formation of microcapsules is another method for liver tissue engineering. Microcapsules have the ability to encapsulate cells into microbeads. Subsequently, microbeads were covered by the semipermeable membrane to form microcapsules [91]. Microcapsules provide safety microenvironment for cells and protect the cell from interference. After encapsulated HepG2 cells into alginate-based microbeads, the proliferate and protein were clearly observed for at least 12 d [92]. These researches suggested that alginate is a potential candidate for LTE strategies.

4. Conclusion and future prospects

Alginate, as a potential biomaterial, has been successfully explored in different applications such as wound dressing, drug delivery, bone, cartilage. It could afford a moist microenvironment for wound dressing, serve as the carrier for drug delivery, and act as a scaffold for tissue engineering. The outstanding characteristic of alginate for its applications contains biocompatibility, degradable properties, gelatinization capacity, and effective modification to obtain new performances. However, alginate gel suffers from the lack of cell adhesive and mechanical properties, that cause the structural deformation of the scaffold. Despite some strategies that have been carried out to solve these problems, the disadvantages still exist such as lower mechanical properties compared with nature cartilage and lower drug delivery efficiency. For wound dressing, it lacks enough robust and flexible to allow adherence to the skin for a period of time, which maximizing patient uncomfortable and inconvenience. In the future, more novel alginate composites with controlled properties should be constructed by chemical or physical modification. That will play a vital role in intricating drug or cell-loading. Novel alginate composites also could provide mild and targeted degradation properties.

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