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Alginate-Based Hydrogels in Regenerative Medicine

Agnieszka Kaczmarek-Pawelska

Abstract

This chapter presents the following multipotential applications of alginate-based hydrogels in tissue healing and drug delivery. It contains state of the art and summary of the literature reports, which demonstrate that alginate-based hydrogels have a great potential in tissue healing. Sodium alginate (SA) is mainly used in medical devices for healing of wounds, scars, injuries of bones, regeneration of joint cartilage, and scaffold for cell growth and in drug delivery systems (DDSs). The latest literature describes the effects of laboratory tests and in vivo, which confirm the validity of its use as a biomaterial. Alginate biodegradable scaffolds can be a template that provides a suitable substrate for cellular growth while matching the physiochemical properties of the native extracellular matrix (ECM). Matching scaffold stiffness to the surrounding tissue and optimising its rate of degradation ensure that the infiltrating cells remain viable, maintain their desired phenotype and coordinate their response over the entirety of the wound healing process.

Keywords: sodium alginate hydrogels, regenerative medicine, artificial organs, bioresorbable hydrogels, tissue healing

1. Introduction

Advances in tissue regeneration are possible due to development in the areas of engineering of the cells, materials and tissue architecture. Cell engineering mainly use genetic tools, mesenchymal stem cells or induced pluripotent stem cells. Engineering of materials developed novel chemistries, growth factors and biomechanical conditions, whereas engineering of tissue architecture proposed novel techniques for manufacturing of scaffolds for cell growth, i.e., by using decellularised organs, 3D printing and self-assembly structures [1]. These three research areas are crucial for progress in tissue healing, and they must be compatible with each other. The stem cells will not grow properly on toxic material or in toxic biochemical and biomechanical conditions. For proper tissue healing, micromovements between the tissue structures—cells and extracellular matrix—are crucial. Like in bone growth, Wolff's law states: *If loading on a particular bone increases, the bone will remodel itself over time to become stronger to resist that sort of loading* [2, 3]. To develop new achievements in material design to improve tissue regeneration, it is necessary to analyse the interactions between the material and tissue on macro-, micro- and nanoscales. It is important to consider cell-matrix interactions and cell-cell interactions in the design and fabrication of hydrogels such as tissue engineering scaffolds because these interactions significantly affect the cell phenotype such as cell growth, adhesion and differentiation [32].

Hydrogels, as cross-linked polymeric networks, contain hydrophilic groups that promote swelling due to interaction with water. They have been in use for clinical applications since the 1960s, initially for use in ocular applications including contact lenses and intraocular lenses due to their favourable oxygen permeability and lack of irritation leading to inflammation and foreign body reaction, which was observed with other plastics. Hydrogels used in regenerative applications can be based on naturally or synthetically derived polymers. By most definitions, native tissues, particularly the extra-cellular matrix, are hydrogels and derivatives of these and other naturally based systems which are in widespread use [4, 5]. In a three-dimensional, cross-linked network of hydrophilic polymer, hydrogels offer properties such as flexibility in mechanical properties, biocompatibility, capacity to retain large ratio of solvent and ability to be prepared in an injectable form among others. These have made them as a potential candidate for use in biomedical and pharmaceutical applications such as tissue engineering [22, 24].

Molecules used for fabricating hydrogels range from natural polymers, such as dextran, gelatine and hyaluronic acid (HA), to synthetic polymers, such as polyethylene glycol (PEG), polyacrylamide (PAAm) and polyvinyl alcohol (PVA). Natural and synthetic polymers have their own advantages such as biocompatibility and high mechanical strength, respectively. Different cross-linking methods for 3D construction of hydrogel scaffolds include chemical cross-linking with covalent bond, electrostatic interaction, hydrogen bond interaction and self-assembly of polymer chains [6].

Hydrogels from natural polymers have a great potential in regenerative medicine because of their biocompatibility, biodegradability, mechanical properties, bioresorption ability and relative low cost. Among them, sodium alginate, a polysaccharide derived from brown seaweed, is widely investigated and used in biomedical applications. Alginate is highly hydrophilic and able to absorb wound exudate maintaining moist microenvironment. Alginate dressings are also useful as delivery platform in order to provide a controlled release of therapeutic substances (e.g., pain relieving and antibacterial and anti-inflammatory agents) [7], which will be discussed in the following sections.

2. Alginate hydrogels in tissue regeneration

Sodium alginate is classified in the group of hydrogels used in regenerative medicine and medical technology applications, and it is the second most abundant polysaccharide on earth, derived from seaweed, and contains β -D-mannuronate (M blocks) and β -L-guluronate subunits (G blocks) bonded with 1,4 linkage. The mechanical properties of these gels can be modulated depending on the divalent cation used to achieve cross-linking. Bivalent and trivalent cations such as Ca^{2+} , Ba^{2+} , Mg^{2+} , Fe^{2+} and Al^{3+} covalently bind alginate G blocks to form a three-dimensional structure called “egg box”. For example, the use of barium or strontium instead of calcium leads to more rigid gels. M and G blocks can be combined in different sequences or alternately ensuring that bivalent cation cross-linked polymer chains form a 3D structure capable of binding large amounts of water, drugs and bioactive substances supporting tissue regeneration. Results of studies revealed that sodium alginate cross-linked with calcium ions stimulates proliferation and differentiation of osteoblasts in vitro [4, 38, 61].

2.1 Wounds and skin

As the largest organ of the human body, the skin plays a pivotal role in maintaining homeostasis as well as protecting the internal organs from the external

environment. Cutaneous injuries, especially chronic wounds, burns and skin wound infections, require long-term treatment. In the United States, chronic wounds affect 6.5 million patients, with about 18% of diabetic patients over the age of 65 suffer from non-healing foot ulcers [36]. Wound healing is a complex and highly regulated physiological process that involves various cell types (i.e., immune cells, endothelial cells, keratinocytes and fibroblasts) and pathways, activated and coordinated in order to restore the tissue integrity and homeostasis [7, 8]. In the proliferation phase of a wound formation, epithelialisation occurs, and newly formed granulation tissue consisting of endothelial cells, macrophages and fibroblasts begins to cover and fill the wound area by producing new extracellular matrix (ECM). The presence of the new extracellular matrix is crucial for proper healing because it provides conditions for sustaining cells and blood vessels, which provide nutrients needed to restore the tissue integrity and homeostasis [9]. The extracellular matrix also serves as a porous and pliable scaffold for supporting the movement of cells, nutrients and growth factor through the wound environment. Studies on chemical composition of ECM during wound healing indicate that the deposition of a number of matrix components is different in chronic and acute wounds. Therefore, drug-incorporated scaffolds are particularly promising for synergistically accelerating the healing process of chronic wounds [36, 37].

Summa et al. [7] examined wound-healing potential of the hydrogel films containing sodium alginate (NaAlg) 3 g/100 ml of H₂O with the addition of 10% of the antiseptic povidone iodine (PVPI). The films were tested on a wound model on male mice, and the results showed significant reduction of the wound area and enhancement of re-epithelialisation in comparison to two groups: control and commercial products. The authors reported a significant reduction of the unhealed area after 3 days, and the wound closure was achieved within 12 days, which was more rapid and efficient than the other two groups. Histological results confirmed that NaAlg/PVPI films cause positive architectural changes on cellular level during the new tissue formation; the distance between tips of migrating epithelial tongues was the smallest in comparison to the other two groups. NaAlg/PVPI hydrogel films and their polysaccharide chains organised in a 3D structure on micro level enhance the wound healing and induce cell proliferation, whereas the presence of antiseptic povidone iodine in the films prevent bacterial infections. The authors did not examine the biomechanical properties of the NaAlg/PVPI films, so the elasticity and strength of these films are unknown, but the obtained results show that these films have biomechanical properties similar to mice skin.

Alginate-based hydrogels for wound healing with antibacterial components were also presented by Kaczmarek-Pawelska et al. [11]. Alginate hydrogel discs were obtained by cross-linking of sodium alginate solution with 0.5 M CaCl₂. Moreover, the researchers examined different alginate concentrations, 0.10, 0.15 and 0.20 mg/ml H₂O, and two antibacterial agents: metronidazole and silver nanoparticles (AgNP). The results showed that the obtained hydrogels have mechanical characteristics similar to the human skin, so the material is biomechanically compatible and proper for wound healing, but the value of Young modulus decreased with increasing sodium alginate concentration from 8 MPa for 0.1 mg/ml to 1.2 MPa for 0.2 mg/ml of sodium alginate. Due to the research of Edwards, the Young modulus value of the skin is from 0.3 to 30 MPa [12]. The high range of this value is caused by the individual features of the samples donor. Mechanical tests revealed that for the hydrogel samples containing silver nanoparticles, the mechanical properties are similar for each sodium alginate concentration. The difference in sodium alginate concentration determines the cross-linking level, when the cross-linking agent (CaCl₂) concentration is the same in every case examined. The cross-linking level influences the sample

elasticity and elongation under loading. Antibacterial tests revealed that hydrogels with the addition of metronidazole and AgNP inhibits the bacterial growth after 18 h in comparison to control pure sodium alginate hydrogel. In the case of Gram-negative *Escherichia coli*, both of the aseptic additives inhibit the bacterial growth, but sodium alginate hydrogel with silver nanoparticles gives better results in tests with Gram-positive *Staphylococcus aureus*. The effectiveness of silver nanoparticles released from sodium alginate hydrogel is twice as great as metronidazole.

In the case of the sodium alginate hydrogels, the antibacterial activity and biocompatibility of the sodium alginate hydrogels also depend on the type of cation used as a cross-linking agent. Zhou et al. carried out in vitro and in vivo studies of the different cross-linking agents and examined copper, zinc, strontium and calcium ions. The results showed that zinc-cross-linked hydrogel had a spectrum of antibacterial activities, cell viability, mechanical strength and the ability of wound closure by promoting fibroblast migration, vascularisation, collagen deposition and the formation of granulation tissue. Wound healing (**Figure 1**) was compared in the six groups of rats during 21 days; it was found that ion-cross-linked hydrogels could accelerate the wound repair on rat model, especially the zinc- and strontium-cross-linked hydrogels [18].

Wichai et al. [10] proposed another wound dressing containing sodium alginate. The authors obtained membranes from bacterial cellulose (BC) incorporated with sodium alginate (AG), chitosan (CS) and copper sulphate (Cu). Moreover, they also examined how different amounts of sodium alginate and chitosan influence the membrane properties. Both of the polymers were used in different concentrations: 0.2, 0.4, 0.6, 0.8 and 1.0% w/v. The results showed that the presence of sodium alginate increased the swelling ratio, but the tensile strength decreased with high alginate concentration in the membrane. Alginate in the BC/AG composite enhanced the molecular motion of BC and perturbed the hydrogen bond of the BC composite. Furthermore, it can be as the cause of the reduced mechanical strength. Cytotoxicity of the membranes was evaluated in tests with mouse fibroblasts (L929) and human dermal fibroblasts (HDFa), in comparison to commercially available wound dressings: Acticoat®, Askina® and BluRibbon®. The results of these tests show that BC/AG/Cs-Cu composite membranes appear to display excellent antibacterial activity against *E. coli* and good biocompatibility when compared with the Acticoat®, Askina® and BluRibbon® commercial wound dressings.

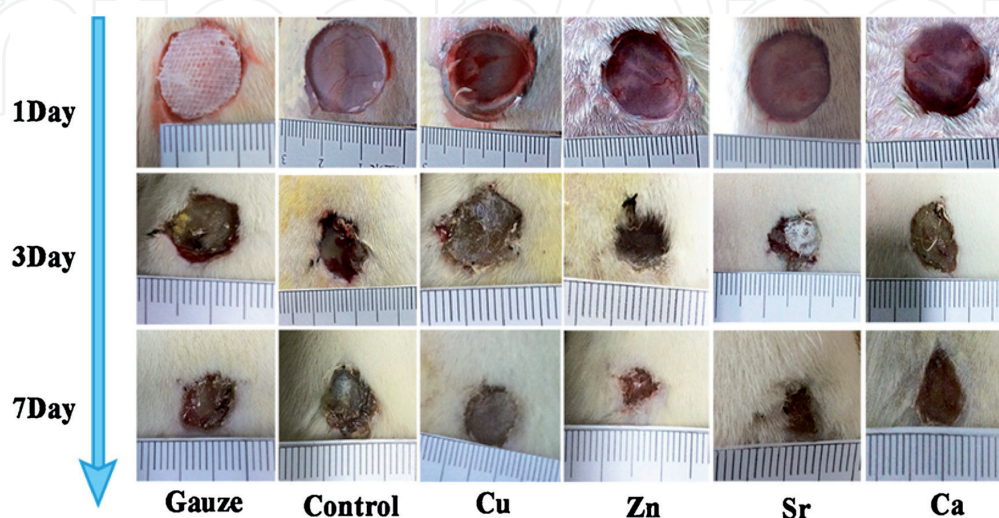


Figure 1. The area of wound healing and general wound observation after treating with the ion-cross-linked hydrogels, nonionically cross-linked sodium alginate hydrogels such as control group and petrolatum gauze group at different times [18].

Novel dressings for skin injury healing contain alginate alone or, much more often, as a component of a multicomponent material, with, e.g., polyvinyl alcohol [13, 14, 19, 20], gelatine [13, 20], silk fibroin [15, 16], hyaluronic acid [17] or even graphene oxide [21]. These components are added to increase the mechanical properties, hydrophilicity and adhesion of the cells to the scaffolds. Moreover, to target cell response, the additives like collagen [13] and hormones such as triiodothyronine may be added [20]. To support the healing process at the cellular level and recreate favourable architecture for the cell proliferation, the hydrogel scaffolds are produced by electrospinning. The high surface-to-volume ratio of nanofibers has been proven to be beneficial for the loading and delivery of drugs for wound recovery. Sobhanian [13] extracted collagen from rat tail and successfully grafted it on a nanofibrous scaffold from polyvinyl alcohol/gelatine/alginate. Modification of the scaffold with collagen increased its hydrophilicity and adhesion of the fibroblast cells. Collagen grafting resulted in the improved cell viability and proliferation on the scaffold. The obtained results confirmed that the as-prepared scaffold is hydrophilic and had acceptable bio-response and tensile properties. Tang et al. [14] produced and examined polyvinyl alcohol/alginate electrospun membrane with the addition of honey as an ancient natural wound-healing agent. The addition of honey in the nanofibers efficiently inhibited the growth of both Gram-positive bacterium (*S. aureus*) and Gram-negative bacterium (*E. coli*) and exhibited better anti-bacterial effect against Gram-positive bacterium than Gram-negative bacterium. Satish et al. [20] proposed a dressing for patients who suffer from chronic wounds caused by diseases such as diabetes. It is formulated in the form of a lyophilised hydrogel comprising alginate, gelatine and polyvinyl alcohol (AGP), with the aim to absorb exudates, maintain a moist environment and enhance interaction with the tissues. To enhance the healing potential of the dressing, they add triiodothyronine (active form of thyroid hormone) which plays a significant role in repair and regeneration of tissues (samples AGPT). The biomechanical properties of obtained hydrogels were proper for skin healing. Animal experiment studies (full-thickness excision wounds created in Wistar rat model) substantiated the explicit potential of the scaffold to encourage faster wound healing.

The advance in 3D bioprinting can offer precise cell patterning in predefined spatial locations, which enables the recapitulation of architectural organisation of native skin. However, the current bio-inks for skin cell printing are mostly alginate, collagen, fibrin or their mixture, which remain suboptimal [36].

2.2 Articular cartilage

Articular cartilage is a highly specialised and organised tissue that lines the ends of long bones and is integral to the functioning of all joints. It provides lubrication and mechanical strength to resist compressive and tensile forces that are essential for weight-bearing and movement. The cartilage has a unique extracellular matrix, which is comprised of an interpenetrating network of collagen and negatively charged proteoglycans and contains only one cell type—chondrocytes. Defects in cartilage do not heal properly because the cartilage has a limited regenerative capability [26]. Regeneration of articular cartilage remains an unmet medical need, which imposes a heavy burden on global economy and on the health-care community. Articular cartilage repair is still a huge challenge for researchers and clinicians. Articular cartilage defects mainly result from mechanical trauma (e.g., sports injuries). Patients with acute traumatic joint injuries have a higher chance of developing posttraumatic osteoarthritis, a degenerative condition that results in severe pain and disability, eventually requiring a total joint replacement [29]. Similar to cartilage, hydrogels retain a large portion of water, which provides lubrication

and decrease of coefficient of friction (COF). The presence of water also plays an important role in the mechanical behaviour of the hydrogel structure [23].

Arjmandi and Ramezani [22] proposed and examined hybrid hydrogel-based on alginate (ALG)-polyacrylamide with the addition of silica nanoparticles (SiNP), as a candidate for cartilage replacement. They proved that its mechanical properties result from the structure of the ALG/PAAm hydrogel. The presence of covalent cross-links between two entangled polymer chains, between amine and carboxyl groups of PAAm and ALG, respectively, implies the load sharing of both networks. Alginate forms a short chain that can dissipate strain energy via recoverable breaking of ionic cross-links, meaning that after the unloading, the ionic cross-linking can be reformed, thus will be healing the damage, while the long-chained polyacrylamide network remains intact, contributing to stabilisation of the structural deformation. Moreover, the silica nanoparticles, located in a three-dimensional hydrogel structure, enhance mechanical properties and also encourage cell proliferation. Hydrogel SiNP-ALG-PAAm showed ultralow COF, coupled with high wear resistance, and tunable elastic and viscoelastic behaviour suggesting these biomaterials as promising candidates for use as a cartilage replacement.

The biomechanical properties of the hydrogels come from a combination of compositions of different factors and cross-linking levels, but they also can be determined by molecular weight and chemical properties of the hydrogel. This relationship was described by Fenbo et al. [25], Lee et al. [27] and Chen et al. [28]. It was found that hydrogels formed from alginate exhibited faster stress relaxation, which facilitated the promotion of cartilage matrix formation by chondrocytes. Moreover, 3D microenvironment, especially the elastic and relaxation moduli of the cell culture matrix, can regulate the metabolic properties of the living cells. Authors improve that molecular weight and chemical properties of the polymers used for the cell culture matrix influence on the three-dimensional cell culture system. Fenbo et al. examined high-molecular-weight alginate/chondroitin sulphate (CS) and low-molecular-weight ALG/CS (B) cross-linked by strontium. The dynamic rheology results suggest that the storage and loss moduli increase with the increase of molecular weight of alginate applied during fabrication. Cell results revealed that chondrocytes encapsulated with alginate hydrogels exhibited the best results on maintaining cell viability and inhibiting cell death [25].

Also the presence of the bioactive proteins placed in hydrogels based on sodium alginate plays a role in regeneration of the articular cartilage. Ruvinon et al. obtained injectable growth factor-loaded affinity-binding alginate hydrogel [29]. They were the first to conduct the evaluation of acellular and injectable growth factor-biomaterial combination therapy for the treatment of articular cartilage defects with a 6-month follow-up period in a large animal model. As growth factors, they use TGF- β 1 and BMP-4, which were conjugated with alginate sulphate obtained from sodium alginate. Macroscopical and histological assessment of the cartilage defects treated with growth factor affinity-bound hydrogel showed effective reconstruction of articular cartilage layer, with major features of hyaline tissue, such as a glossy surface and cellular organisation. Physical nature of the applied hydrogel ensured its shear resistance, seamless integration and topographical matching to the surroundings and opposing articulating surface [29].

A great potential in articular cartilage regeneration has also a mixture of alginate with collagen as a bio-ink for 3D cell printing. Yang et al. [31] printed scaffolds and compared properties and biocompatibility in vitro of three types of the bio-ink: sodium alginate (SA) alone, sodium alginate with collagen type I (COL) and sodium alginate with agarose (AG). The results showed that the mechanical strength was improved in both SA/COL and SA/AG groups compared to SA alone. The addition of COL or AG has little impact on gelling behaviour, demonstrating the advantage

as bio-inks for 3D printing. Among the three scaffolds, SA/COL could distinctly facilitate cell adhesion, accelerate cell proliferation and enhance the expression of cartilage-specific genes and may be a promising 3D bioprinting bio-ink for cartilage tissue engineering [31].

Alginate hydrogels have also a great potential in cartilage healing when mixed with hyaluronic acid [30, 32–35]. What deserves special attention is the research results. An et al. [32] presented hyaluronic acid-alginate hybrid hydrogel modified with biomimetic peptides. They enrich hydrogels with RGD peptide, which is widely used for enhancing cell-matrix interactions, and HAV peptide to increase cell-cell interactions. The authors prove that the precise control of cell-cell interactions using a scaffold is essential to regulate the cell phenotype and chondrogenic differentiation. Furthermore, the combination of these two peptides in hyaluronic acid-alginate hydrogels had a synergistic effect on the chondrogenesis of encapsulated chondrocytes, and the presented biomaterials are potentially useful for the tissue, especially cartilage regeneration [32].

2.3 Bone

The bone is a typical complex tissue with hierarchical structure that consists of approximately 70% of nano-hydroxyapatite ($\text{HA}, \text{Ca}_{10}(\text{PO}_4)_6(\text{OH}_2)$) and 30% of collagen by weight. Water (with some dissolved non-collagenous organic matter) is the third elementary component. The ratio of volume and weight fractions of hydroxyapatite/collagen/water is not constant and is an individual characteristic. Among the natural polysaccharides, alginate is widely used as a biomaterial for bone tissue engineering next to chitin and chitosan. Alginate, due to its scaffold-forming ability, is a promising material for tissue engineering [38].

The biomechanical bone system is complicated; therefore, the requirements for treatment systems are high. The perfect biomaterial for bone healing should be biocompatible, have a sufficient surface area, be nontoxic with three-dimensional structure and have porosity with pore size more than 100 μm . The biomaterial should also support cell adhesion, migration and proliferation. For proper tissue growth, biomaterial should improve vascularisation that is crucial for cell migration and proliferation into the desired direction. Moreover, it should be biodegradable, be a carrier for drugs and growth factors and have mechanical properties equivalent to cortical bone [38].

Current literature report presented by Purohit et al. [39] describes fabrication of a nanocomposite scaffold of graphene oxide (GO), gelatine and alginate (GA). The properties of the obtained scaffold qualify it as a material supporting bone healing. The presence of graphene oxide in the gelatine-alginate scaffold increases its biomechanical strength. Hydrogels without the addition of graphene oxide have a compressive strength of 30 MPa, while those containing GO 44 MPa. Moreover, the presence of graphene oxides enhanced the hydrophilicity of the scaffold and provided slow degradation (~30% in 28 days). In vitro studies confirmed that an osteoblast cell line (MG-63) growth over the nanocomposite scaffolds revealed an enhancement in the cell attachment and proliferation as compared to the gelatine-alginate scaffold.

Scaffolds are crucial for bone tissue engineering since their compositions and properties could significantly affect the seeded cell behaviour. Zheng et al. [43] developed an interpenetrating network hydrogel by utilising Ca^{2+} from calcium silicate (CS) to simultaneously cross-link silk fibroin (SF) and sodium alginate. Obtained scaffolds, with different contents of calcium silicate, were systematically evaluated by physical and in vitro characterisations. Researchers found that calcium silicate inside the porous structure of hydrogel scaffolds enhance hydrophilicity,

degradation, compression resistance and bioactivity. The scaffolds containing higher amount of calcium silicate have better biocompatibility and promote the osteogenic differentiation in vitro.

Sodium alginate can be easily manufactured in microspheres with three-dimensional net structures using cross-linking with calcium ions [40]. Hydrogels formed as microspheres possess some advantages for use in biomedical applications because of the larger specific surface area that can improve cell adhesion, proliferation and drug delivery. Bi et al. [40] obtained sodium alginate microspheres with the addition of chitosan (CS) and hydroxyapatite. Doxorubicin hydrochloride (Dox) was used as a drug model to study the drug loading behaviour of HA nanoparticles and hydrogel composite microspheres. In vitro examinations confirmed that the prepared HA/SA/CS/Dox drug-loaded microspheres support regeneration of the bone defects and also provide drug delivery with control of the drug release. However, the actual process of the material in vivo has not been studied. The results indicate that the hydroxyapatite/alginate hydrogel microsphere has potential in bone healing and as a drug carrier [40].

A combination of hydroxyapatite, in nano-form (nHA), and sodium alginate was also recently examined by Nabavinia et al. [45]. Nano-hydroxyapatite combined with sodium alginate showed a statistically significant impact on swelling reduction and improvement of stability and mechanical strength of hydrogels, respectively. The authors mixed SA/nHA with gelatine, to improve the cell adhesion and proliferation activities. In the research, the microcapsules were formed from solutions with a different content of each ingredient. The results show that the addition of the gelatine significantly increased swelling ratio at initial phase of incubation, similar to the degradation rate. Young modulus of the microcapsules was $0.19 \text{ MPa} \pm 0.02$. Swelling ratio of examined hydrogel was $52\% \pm 8$ for 24 h, and degradation rate of microspheres was $12\% \pm 4$ (96 h). The addition of nano-hydroxyapatite in hydrogel significantly increased proliferation of the microencapsulated osteoblast cells. Examinations of cell surface receptors and protein adsorption onto hydrogel inhibit high cell proliferation and activity. The presence of gelatine in hydrogel microcapsules increases the cell proliferation, and they may be proper to build blocks for modular bone tissue [45].

Another use of the complex of sodium alginate and chitosan as a material for bone regeneration is proposed by Lee et al. [41]. The authors developed an injectable material, new calcium phosphate cement (CPC) system, incorporated with chitosan/alginate complex. Hydrogel biomaterial was produced by the interaction between cationic and anionic polymers: chitosan and alginate. In the studies, the bioactivity of cement without the polymer addition was compared with the cement containing the mixture of alginate and chitosan. Both of the cements were implanted in a rabbit femoral head defect model for 1 and 3 months. After 3 months of implantation, micro-computed tomography revealed better bone formation after implantation of cement that contains polymers than without it. The results indicate the potential value of the CPC system containing alginate-based polymer complex as an injectable bone substitute. The obtained cement system, based on chitosan/alginate mixture, may also serve as a drug carrier for faster bone healing [41].

Also a novel bone cement was presented by Shi et al. [46]. Authors use two calcium-binding agents, citric acid (CA) and sodium alginate. They were mixed with α -tricalcium phosphate (α -TCP) cement to improve the physicochemical properties of a novel biomaterial. The combination of citric acid and sodium alginate accelerated the binding of cement, increased its mechanical properties, delayed the process of hydration and prevented the formation of unclean earlandite phase. The results show that sodium alginate-citric acid hydrogel networks provided a strong cohesive action through the tight chelation with calcium ions during the hydration process of bone cements [46].

Macroporous biomaterials prepared from sodium alginate were proposed by Catanzano et al. [44] and Park et al. [42]. The first team of scientists designed natural bioactive scaffolds mimicking bone tissue. These bioactive scaffolds have to possess physicochemical properties suitable to address biological response towards newly formed bone tissues. Authors improve that the scaffold porosity and pore size play a crucial role in cell migration, adhesion and proliferation; thus they increase the cell-material surface interaction area and induce osteogenic signals transmission between cells. The authors proposed the development of macroporous alginate foams (MAFs) of porous and well-interconnected structure, useful to enhance growth and osteogenic differentiation of human mesenchymal stem cells (hMSCs) [44]. The second team [42] synthesised bio-ink, based on sodium alginate, alginate sulphate and a growth factor—bone morphogenetic protein 2. Authors used alginate sulphate, because it is a structural mimic of heparin and can strongly bind with the growth factors to prolong their activities. The exanimated mixture of alginate/alginate sulphate had good rheological properties that were not changed, after the addition of alginate sulphate, so it is proper to be used in 3D printing to obtain a structure with appropriate porosity. The bio-inks containing alginate sulphate displayed greater and more prolonged BMP-2 activities than the control bio-ink containing alginate only. Moreover, alginate/alginate sulphate bio-inks exhibited an improved retention of bone morphogenetic protein 2 in 3D-printed scaffolds. An optimal composition of alginate/alginate sulphate 3D-printed constructions to stimulate osteoblastic proliferation and differentiation in vitro is 3% alginate and 2% alginate sulphate [42].

2.4 Drug delivery

This paper describes the use of hydrogels based on sodium alginate as a material for the regeneration of specific tissues. Hydrogels were often enriched with an active substance to induce a specific tissue reaction, and they may also be classified as drug delivery systems (DDSs). But the largest group of drugs is not delivered directly to the tissue, but via oral or inhalation routes.

Poor aqueous solubility is a major problem faced in the formulation of active pharmaceutical ingredients, and it causes poor bioavailability. Although several formulation strategies have been proposed to solve this problem, a modest success has been achieved in meeting the requirements of commercially viable drug delivery systems. Therefore, extensive research on the development of optimum DDSs is still necessary. Nanoscale colloidal carrier systems developed from natural compounds such as lipids, proteins and polysaccharides for encapsulation of poorly soluble drugs have been promisingly successful recently [48].

Many drug delivery systems are still in the research phase. **Table 1** shows an overview of the latest achievements and DDSs development on the basis of sodium alginate.

A novel DDSs for pulmonary drug delivery was proposed by Athamneha et al. [47]. As the authors declare, no hybrid formulations based on hyaluronic acid and alginate have been developed for inhalation routes so far. The researchers prepared porous aerogel microspheres consisting of the mixture of alginate and hyaluronic acid. Authors examined the mixture and alginate alone to improve aerodynamic properties of microspheres, to be delivered in the lower respiratory tract. The particles were prepared via the emulsion-gelation process with subsequent supercritical CO₂ drying. Mixture of hyaluronic acid and alginate showed positive effect, prevented particle agglomeration and also improved biodegradation. The microsphere properties result from the fact that there is a hydrogen bond between carboxylate groups of ALG and the amide of the N-acetyl-D-glucosamine, which prevents the

COMPOSITION	DRUG	IN VITRO TESTS ENVIRONMENT	IN VIVO TESTS	REF.
SODIUM ALGinate (SA)	DEXTRAMESONE	PBS	Rats	[50]
	DICLOFENAC	PBS	-	[58]
SA + MESOPOROUS SILICA	PRENDISOLONE	PBS; RWPE-1 (prostatic epithelial cells)	-	[49]
	DOXORUBICIN	PBS HELLA CELL LINE	-	[56]
SA + CHITOSAN	QUERCETIN-	PBS	-	[51]
SA + COCHLEATES	ARTEMISININ	PBS	-	[48]
SA + CHITOSAN + IRON NANOPARTICLES	DOXORUBICIN	PBS HUMAN LIVING CANCER CELLS (A549)	-	[52]
SA + CHITOSAN + CELLULOSE	DEXTRAMESONE	PBS	-	[53]
SA + NANOCELLULOSE	IBUPROFEN	PBS	-	[54]
SA + LIPOSOME	DOXORUBICIN	PBS	-	[55]
		HUMAN TONGUE SQUAMOUS CELLS (CAL-27)	-	
SA + GRAPHENE OXIDE + PROTEAMINE	DOXORUBICIN	MCF-7 CELLS	-	[57]
SA + OLEICACID	VORICONAZOLE	HELA CELL LINE VERO CELL LINE	-	[59]
SA + POLYVINYL ALCOHOL	METFORMIN	HYDROCHLORIC ACID	-	[60]

Table 1.
Examples of tested hydrogels based on sodium alginate as drug delivery systems.

separation of components from the microsphere structure. The physicochemical and aerodynamic properties of the microspheres provided their potential suitability as a drug carrier for the pulmonary tract [47].

3. Conclusions

This chapter summarises the most frequent uses of sodium alginate in regenerative medicine and the studies on tissue regeneration. Sodium alginate is mainly used in medical devices for healing of wounds, scars, injuries of the bones, regeneration of joint cartilage, scaffolds for the cell growth and as a carrier of drug. The latest literature describes the effects of laboratory tests and in vivo, which confirm the validity of its use as a biomaterial. But there are more reports on the use of sodium alginate as a material for healing of other tissues as well. Sodium alginate is rarely used alone; most commonly it can be found in a mixture with other polymers, such as hyaluronic acid and polyvinyl alcohol, even with graphene oxide. Alginate-based hydrogels, due to their 3D polymer structure, support tissue regeneration on macro-, micro- and nanoscales. But due to its unique properties, sodium alginate is a substrate with a great potential as a hydrogel, not only in regenerative medicine but also in many different spheres of life.

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Conflict of interest

The author declares that she has no conflict of interest in this publication.

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