REVIEW ARTICLE

Recent Innovations in Biodegradable Alginate-Based Hydrogel Systems: Polysaccharide-Mediated Drug Delivery Matrices for Osteogenic Regeneration and Bone Tissue Engineering

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ABSTRACT

Alginate is a versatile natural polysaccharide widely used as an extracellular matrix component in on-site bone regeneration therapies. Its ability to mimic key physicochemical features of the native matrix enables efficient cell proliferation and tissue repair. Through advanced engineering approaches, the structural and functional properties of alginate can be finely tuned, strengthening its position as a leading biomaterial among natural polymers. With the growing global burden of bone deformities, alginate-based systems have been successfully applied in bone defect repair, cartilage regeneration, and as scaffolds integrated with drug delivery platforms. This review outlines the fundamentals of hydrogels and their biomedical applications, with a focus on the distinctive characteristics of alginate hydrogels. It also highlights strategies for their modification, design considerations, and diverse drug delivery modes in bone regenerative medicine. Overall, alginate-derived hydrogels show substantial promise as effective and adaptable drug delivery systems for bone repair.
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INTRODUCTION

Bones are highly dynamic, vascularized connective tissues composed of collagen, hydroxyapatite, and water. Structurally, bone exhibits distinct organization at the macro, micro, and nano levels, with ratios that are not constant and continuously remodel throughout life. According to Wolff's law, when the load on a particular bone increases, it is inherently capable of regeneration and will remodel over time to become stronger and more resistant to loading (1). Bone damage can result from various factors, and approximately 15% of elderly individuals exhibit clinical symptoms of such damage. There is a growing global demand for bone grafts, which is limited by risks of infection and high non-union rates, particularly in cases of extensive bone loss (2). Due to the limited number of donors, medical restoration of bone tissue remains a significant challenge. Consequently, alternative approaches for permanent repair of bone defects are being actively explored through tissue engineering (3).

Tissue engineering has enabled the in vitro reconstruction and regeneration of bone. Significant progress has been made in developing biocompatible and biodegradable scaffolds that enhance osteogenic priming and facilitate the delivery of cells and growth factors to damaged tissues, providing support until new bone forms (4). The biomechanical complexity of bone necessitates stable treatment systems capable of promoting cell differentiation and maintaining mechanical integrity through slow degradation until bone regeneration is complete (5).

Various biomaterials have been evaluated as scaffolds for bone regeneration. Hydrogels—composed of homophilic or heterophilic polymers—are ideal candidates due to their three-dimensional porous structures. They swell in aqueous solutions and closely mimic the extracellular matrix, improving vascularization and nutrient delivery more efficiently than other biomaterials, which often have limitations in cell adhesion, migration, and proliferation (6). Numerous natural and synthetic polymers have been used to synthesize hydrogels, including chitosan, alginate, chondroitin sulfate, hyaluronic acid, collagen, polyvinyl alcohol, and polyethylene glycol (7). Among these, alginate hydrogels stand out for their superior blendability with other polymers, favorable swelling ratios, and controlled degradation profiles, making them

highly suitable for tissue-engineering applications (8).

ALGINATE HYDROGELS

Alginates are biologically agreeable and non-immunogenic polysaccharides derived from algae (Class: Phaeophyceae) and bacterial sources. They contain consecutive blocks of gluconate and a mannuronate chain which varies depending upon the type of source (9). Depending on their composition, the alginate has been classified into 3 categories. For example, the alginate extracted from *Laminaria hyperborea* contains high G content able to form strong hydrogels. The one which is obtained from *Macrocystis pyrifera* comes under medium-type hydrogels due to the minimal amount of G:M content and the one which is obtained from Ascophyllum nodosum comes under weak hydrogels containing a high ratio of M blocks (10). Alginate hydrogels have specific gelation properties that help to encapsulate the drugs which prominently play a role in regenerative medicine. The cross-linking of ions with divalent ions improves the mechanical characteristics of the alginate hydrogels (11). The hydrophilicity and the gelation mechanisms influence the alginate hydrogels' drug loading ability to render them for biomedical applications. Consequently, their poor mechanical strength limits their potential for bone regeneration due to its unpredictable biodegradation. Efforts have been made to increase the ability of hydrogel matrices to ameliorate defects in the bone by introducing variant fabrication techniques as they are modified by either physical, chemical and biological methods to form controllable materials that find applications towards tissue engineering (12).

Traditionally, the polysaccharide-based hydrogels are reinforced by either of the mentioned methods to improve their mechanical properties that enabled the alginate scaffolds with adequate properties for tissue regeneration applications. This was achieved through interactions such as hydrogen bonding, Vander Waals and electrostatic mechanisms (13). Alginate acts as an excellent temporary matrix for regenerative therapy aimed at restoring the structure and function of the damaged bone tissue. As they are natural in composition, they possess biocompatibility with the bone extracellular matrix, making them attractive materials in regenerative medicine. Vascularization will occur if the alginate hydrogels do not possess enough mechanical stiffness due to the disruption of the matrix which is required for tissue growth. The main strategy to use hydrogels in regenerative medicine is to maintain the structural integrity of the tissues. The alginate hydrogel should be modified by combining biomaterials to improve its properties (14).

Nano-engineering offers the possibility of creating self-assembling strategies that gained interest among researchers for the synthesis of scaffolds that mimic the native extracellular matrix molecules under mild reaction conditions (15). Generally, the properties of the alginate hydrogels are categorized based on the fabrication method employed and the cross-linking agents (16). In earlier times, the traditional alginate hydrogels were synthesized by free dry-lyophilization methods followed by ionic cross-linking (17), phase transition (18), free radical polymerization (19) and click reaction (20) have been elaborately studied for the fabrication of alginate scaffolds. The cross-linking agent used in regenerative medicine should be nontoxic, compatible with cells and tissues and the methods employed for synthesis should be done easily under less stringent operating conditions (21).

PROPERTIES OF ALGINATE HYDROGELS

Molecular weight and solubility

The mechanical characteristics and degradation rate of alginate hydrogels are influenced by the molecular weight (MW) of alginate. Essentially, higher MW diminishes the responsive positions accessible for deterioration, thus encouraging a low rate of degradation (22). Moreover, degradation additionally impacts the mechanical characteristics attributable to the changes in structure at the atomic levels as well as molecular levels. The tuning capability of alginate helps in cell differentiation, proliferation and migration for its application in tissue engineering. The requirement of the gel strength varies depending on the tissue type requiring the regeneration and the mechano-responsive ability of gels can be altered according to the type of native tissues (23).

Surface properties

The great importance of alginate hydrogels application in tissue regeneration engineering is due to the surface properties. The alginate network is made up of hydrophilic, and water-soluble networks that resemble the same as that of the extracellular matrix material found in the living tissues. The surface also possesses carboxyl groups at their backbone which is responsible for the pH responsiveness properties, and it influences the swelling capability of the hydrogels. The surface properties have been enhanced by the addition of cross-linking agents during the gelation process (24) and numerous microscopic (scanning electron, scanning tunneling, atomic force) and spectroscopic (Fourier transform infrared) techniques are available to characterize the surface properties of the hydrogels (25), (26). Alginates can be formulated into different forms like beads, fiber, sponge and each of which promotes the growth of cells in culture and produces a response making it suitable for tissue regeneration applications.

Rheological properties

As the hydrogels will endure massive strain deformation, which can end in structural damage after breakage. when the flowability of the gels can be assessed by using the rheological studies alginate hydrogel showed

nonlinear properties and failure behavior, this showed the thixotropic behavior of the hydrogels. This behavior makes them indispensable for tissue engineering applications (27). Researchers put an effort to modify the rheological properties by devising techniques to modify them with bio-functional moieties and to adjust their biophysical properties which further permit their fitting to the regeneration applications. A lot of reports have been done using injectable hydrogels as they provide an agreeable cell atmosphere, while the hydrogels encounter fewer shears and more supplements nearby during the infusion (28). Additionally, the gels can be formed and take up the state of the cavity or on the other hand space where the tissue is required to recover.

Biocompatible properties

Alginate has high water content and enables the super hydrophilic diffusing surface to make it applied in the biomedical field (29). Alginate has appeared to advance the angiogenesis process, a significant application in the engineering of tissues, where the continued existence of cells in huge implants relies upon the development of fresh blood vessels (30). Also, great osteogenesis has been seen when utilizing alginate/gelatin frameworks in bone tissue designing (31). As the biocompatibility of alginate hydrogels has been broadly assessed within as well as outside the living organisms, the effect of the alginate structure plays an important role in their properties.

Degradation properties

Alginate hydrogels serve as excellent assistance material for the growth of tissue, then undergo degradation once the proliferation of the cells occurs. The hydrogel degradation profile should be balanced with the regeneration of the cells so that it allows cells to absorb nutrients properly and gives enough space to grow (32). The design of the hydrogel in such a way that it should increase the chances of immunogenic reaction, thereby controlling the rate of degradation rate (33). By structural modification and composition, monitored degradation of gels can be achieved (34). Recently, laser-controlled hydrogel degradation methods have emerged which appear to be promising in this process of synthesis of biomimetic scaffolds (35).

CONVENTIONAL METHODS FOR ALGINATE HYDROGEL PREPARATION

Currently, the preparation of hydrogels from natural polymers has shown intrinsic benefits when compared with synthetic polymers owing to natural biocompatibility, especially for biomedical applications (36). Among the various natural polymers (eg.: chitosan, collagen, and hyaluronic acid), the preparation of alginate hydrogels is given an increasing interest due to its unique nature of gelation and lack of use of crosslinkers which results in low-cost products (37). Alginate hydrogels are commonly prepared by two modes by changing the pH of the solution, either acid gels or ionotropic gels. Later, the advancements were at molecular levels using engineering approaches that involve the protonation of polymer chains using thawing or lyophilization techniques. Phase-induced separation technologies in combination with supercritical drying enable the formation of lyogels or aerogels. Because of their hydrocolloid nature, the alginate hydrogels can be regulated to a broad spectrum of gel networks. As a consequence, the alginate hydrogels found their marked potential as tissue engineering materials (38).

Physical cross-linking methods

The physical crosslinking methods are normally used for the preparation of hydrogels as it requires very mild conditions for preparation. This method does not include the exploitation of chemical cross-linking agents, while the resulting hydrogel properties are mainly dependent on type and density of cross-linking, in addition to the concentration of the polymer used for formulation. The hydrogels which are cross-linked by physical means are more effective for tissue regeneration as it does not use any crosslinking agent which may affect the cells and their biological activities. Ionic cross-linking is the most employed method of cross-linking for fabricating hydrogels (39), apart from guest host chemistry (40), cell cross-linking (41), covalent cross-linking (42) and thermo-gelation methods (43).

Ionic cross-linking

This is the most frequently employed method for the formulation of alginate hydrogels by means of sodium ion exchange from the units of glucuronic acid using the divalent cations. Chloride, sulfate and carbonates of calcium are generally utilized intermediaries for the cross-linking the alginates ionically. Despite that, it regularly prompts fast and inadequately controlled gelation because of its high dissolvability in an aqueous solution. Such cross-linked alginate hydrogel has indicated a biocompatible property as well as shown self-healing and reversible dissociation properties under physiological milieu. The mechanical feature of the alginate hydrogels which are cross-linked physically can be controlled by optimizing the process parameters such as the concentration of G residues in the alginate, gelation rate, gelation temperature. From the reports, it was found that the ionically cross-linked hydrogel exhibits many excellent characteristic features such as reversible nature and self-recovering capabilities by modulating the pH conditions (44). Alginate hydrogels are designed as stimuli-responsive biomaterials through ionic cross-linking methods resulting in the configuration of stimuli-responsive 3D printable materials that possess minimal toxicity on epithelial cells utilized for a broad range of applications (45).

Photo-activated ionic gelation

Normally, ionic gelation occurs through in situ gelation, diffusion gelation and gelation through cooling.

The major limitation of ionic gelation is due to the development of calcium gradient which resulted in a high crosslinking rate leading to the uneven distribution of cations (46). Photoactivated gelation is a type of ionic gelation that has been broadly used for the gelation of alginate utilizing ultraviolet radiation or visible light for cross-linking. The process involves the reaction between alginate and calcium carbonate solutions with a photo-acid generator as a cross-linking agent. These photo initiators undergo photolysis upon UV radiation and do the process of ionic cross-linking. The mechanical property associated with alginate is variable according to the amount of irradiation, time of exposure and concentration of the alginate. As the concentration of photo-initiators and irradiation intensity increase, gelation time increases which results in static, as well as dynamic scaffolds with native properties of the extra-cellular matrix, applied for tissue regeneration applications (47).

Thermo-gelation

Thermal gelation includes the polymeric cross-linking which has been widely investigated in the applications of delivering the drugs (48). In this method, the swelling properties changed with the changes in the temperature. This type of fabrication can be done by using repeated cycles of freezing and thawing. As the alginate is not thermosensitive, the growing proportion of the gels is expanded through the grouping of sodium-based alginates by a steady temperature which diminishes at expanded temperature. Hence, the alginate comes under temperature-responsive polymers that can change stages of sol-gel close to the temperature of physiological activity, which empowers the injectable property of hydrogels, and they are reported to have high mechanical strength (49). Alginate gels are effectively copolymerized with synthetic polymers that possess sensitivity near body temperature and leads to the potential carrier in drug delivery applications.

Host – guest interactions

One of the simplest methods of obtaining supramolecular hydrogels can be obtained host-guest interaction, hydrogen bonding interaction, electrostatic interaction, ionic interactions and most importantly the non-covalent interaction such as host-guest interaction phenomenon (50). With this phenomenon, multi responsive hydrogels are yielded using transient and reverse cross-linking which facilitates the yield of dual cross-linked polymers (51). Alginate hydrogels prepared by these methods have served to mimic stress and strain responsive scaffolds which are currently considered for the engineering of bone tissues, tendon and cartilage due to their bio-adhesive properties

Covalent cross-linking

These kinds of chemical methods have been broadly researched in favour of overcoming the limitations that occur in previous methods. The covalent chemical methods of cross-linking have been applied to improve network stability with improved mechanical properties (52). This type of covalent cross-linking enhances the properties of alginate hydrogels so that the cells can grow without any disturbance in the cleavage of the polymer chains. Alginate has likewise been covalently cross-connected and oxidized trying to enhance the properties of alginate hydrogels. An impediment to this methodology is the restricted debasement of covalently crosslinked alginate gels since cells do not discharge the vital compounds for polymer cleavage. The significant objective of these methods was to plan and create a framework with the accompanying qualities: shape-memory properties for the help of insignificant intrusive implantation; a debasement rate with a similar period as new tissue arrangement; and a surface structure to help ideal partiality of cultivated cells. Likewise, the framework ought to have the ability to deliver consolidated development elements to help in tissue fix (53).

Cell cross-linking

Although a series of strategies have been evolved over years to produce alginate hydrogels, the capacity of cells to add to gel development has been to a great extent disregarded. Cell-mediated crosslinking to the multiple polymeric structures of the gels can be done as long as the polymer chains possess explicit ligands to bind with the receptors on the cells outer surface. As the alginates have no such bioactive ligands for the receptors present on the cells to bind, it has been modified with cell adhesion peptide molecules resulting in a cross-linked network structure. This initiated the cell's ability to bind to the chains through receptor-ligand interactions. Conversely, the non-modified alginates added with cells resulted in unevenly structure, because of the predominance of cell-to-cell interactions in the framework. Peptide-modified alginate hydrogels have resulted in uniform distribution of cells within the solution and enzyme possesses high mechanical integrity due to specific receptor-ligand interactions (54), (55).

Enzyme enabled cross-linking

Enzymes are of the greatest importance in biochemical reactions due to their enantioselectivity, chemo selectivity and regio-selectivity. The best significance of enzyme compounds lies in their capacity to catalyze the synthesis of a series of optically purified products. These preferences of enzymes are regularly restricted by their short life span and their recuperation and reuse. However, enzymes have additionally been widely read for cross-linking of various biopolymers, especially alginates and alginate-derived bioproducts (56). Enzymatically cross-linked hydrogels are prepared using oxidative coupling techniques which have emerged as an innovative method designed to prepare the injectable hydrogels (57; 58). Reports suggested that the crosslinking of polymer conjugate was done in presence of enzymes, namely, transglutaminase,

horseradish peroxidase, peroxidases, phosphatases and tyrosinase. Other than selectivity, these compounds intervened with a cross-linking approach likewise displays quick gelation, in addition to the effectively adjustable mechanical properties through changing the centralization of horseradish peroxidase and hydrogen peroxide (59). Injectable hydrogels made of alginate-phenol are formed by in situ gelling procedures involve the use of horseradish peroxidase enzymes. A similar research procedure was followed for polymer –catechol and it was found that the later one has significantly adhesive properties than the former conjugates (60).

Chemical cross-linking methods

Covalent linking occurs between the polymer chains, and their connections are strong with excellent mechanical properties when contrasted with that of other modes of cross-linked hydrogels. This chemical cross-linking occurs through growth of chain polymerization and by solution polymerization besides suspension polymerization with cross-linking agents. Hydrogels of high molecular weight can be possible by means of chemical cross-linking of biologically derived polymers and chemically synthesized materials. This approach allows the formation of high molecular weight hydrogels with the help of natural and synthetic polymers (61). Functional cross-linkers like Bis-compounds are used to cross-link with the hydrophilic polymers' operable groups employing reactions such as addition. A chemical reaction that comes under "click chemistry" is highly favorable for the fabrication of functional hydrogels that have advantages such as exceptional returns under gentle conditions, fewer by-products, high explicitness and selectivity (62), (63). A wide range of functional groups is available as attractive materials in the polymer components for cross-linking to occur. Here, we represent a few old-style chemical cross-linking techniques, including (1) Diels–Alder (64), (2) Schiff base (65), (3) Oxime reaction (66) and (4) Michael-type expansion (67). The properties pertaining to physicochemical nature of "click" alginate hydrogels exhibit a high utility for cell encapsulation and long-term implantation, especially in tissue engineering applications.

MODERN METHODS FOR ALGINATE HYDROGEL SYNTHESIS

A wide range of procedures is accessible to the synthesis of hydrogel-supported scaffold networks. The modern preparation strategies are being utilized to assist scientists for incorporating new age permeable scaffolds.

Emulsification-diffusion methods

Emulsification techniques involve the use of hydrophilic surfactants for the polymers to emulsify and diffuse into the system. The effect of surfactant concentration determines the swellability and it can be controlled by either increasing the alginate to surfactant ratio or by decreasing the agitation speed (68). The aerodynamic properties of the alginate microgels can be improved by using these emulsion polymerization techniques which make them act as an excellent drug carrier (69). High energy and low energy stirring mechanisms are employed to produce nano emulsions of alginate through micro-fluidic technologies which result in the nanoparticles of controlled size and polydispersity (70). Small diameter beads can be synthesized using this method which is mainly based on certain process parameters such as the concentration of the precursors used, the ratio of the emulsion, mixing speed, reaction pH, and temperature. This technique has the potential for enhancing the encapsulation of the drug molecules which is more applicable in the biomedical field (71).

Photolithography techniques

Electrochemical principles are used for the preparation of biomaterials which allows controlling the shape and structure of hydrogels by changing the position of electrodes Figure. 1. 3D alginate hydrogels produced by this technique and used for culturing of cells promote cell attachment and cell proliferation (72). There are 2 different methods under this category mask and mask less photolithography has been reported for the fabrication of tissue constructs. This technology generates designs by using computer tomographic images and magnetic resonance images (73). Lithography based techniques offer intelligent therapeutic solutions that offer high precision dosing of drugs with good dissolution rates (74). Tissue scaffolds which have been fabricated using advanced microfabrication technologies such as soft lithography and nanoimprint lithography, help us to study the cell culture and cell behavioral research (75).

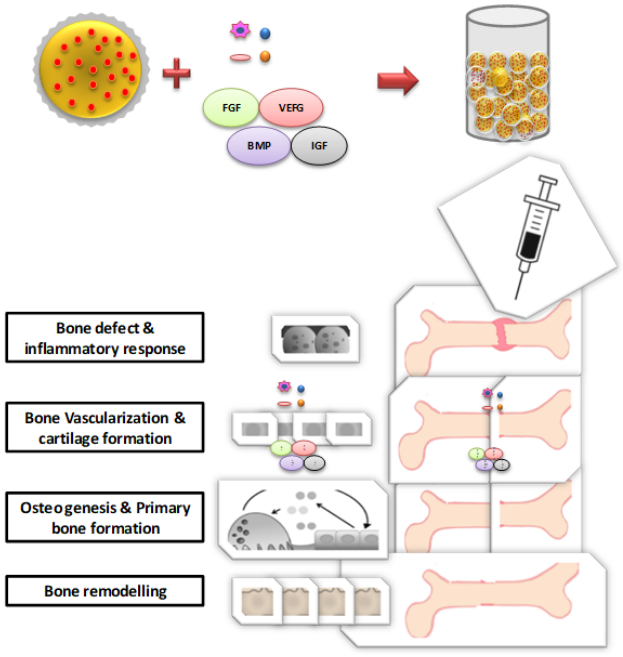

Figure 1: Drug delivery in bone regeneration using alginate hydrogels

These techniques are substrate-specific and able to produce complex 3D constructs with high precision, but they face limitations due to the use of photo initiators. cell-laden microgels produce shape-controlled hydrogels for governing cellular interactions (76). With the aid of computer technology, multi-phase 3D tissue models have been fabricated using techniques such as stereolithography, selective laser sintering, powder-assisted and extrusion-built 3D printing (77). Jual jet electrospinning systems are used for the preparation of polyelectrolyte nanofibers which facilitate the cell adhesion properties by varying the feed rate (78).

Gas foaming technique

Gas-in-liquid foam templates involve the use of inert gas as templates and biocompatible polymers for the preparation of interconnected scaffolds. The inert gases such as carbon dioxide or nitrogen have been bubbled at high pressure into the polymer solution. The porosity has been controlled while designing the 3D scaffolds. Alginate scaffolds, which are highly porous with tunable morphology and excellent mechanical properties were designed using gas foaming techniques (79). This technique eliminates the use of surfactants and due to this porosity, an increase in the mass transfer of oxygen and nutrients into the scaffolds takes place (80). Based upon the concentration of alginate used for the synthesis, the alginate foams can be designed for cell attachment, proliferation and retrieval (81). The major drawback of this method is that it fails to control the morphology of the structures. To overcome these limitations, micro-fluidic technologies have been introduced.

Electro-spinning technique

Electro-spinning is a solution-based approach that involves the preparation of fibers formed from natural/ synthetic polymeric solutions by applying a high voltage (82). The process parameters such as concentration, conductivity and viscosity along with surface tension could be optimized and the environmental conditions were maintained to produce fibers with tunable properties (83). Electro-spinning procedures have high control over the diameter, composition and porosity of the nanofibers that leads to wide applications in the health care and biomedical field (84). As the sodium alginate is polyelectrolyte in nature, it possesses high conductivity and surface tension which is the key factor for the formation of fibers using electro-spinning procedures and it is also found that the composition affects morphology as well as the drug release behavior (85). Sodium alginate electro spinning with polyacrylic acid using a single needle resulted in fibers with a diameter of 278 nm and porosity around 42 nm employed successfully in bone tissue engineering (78).

DESIGN STRATEGIES OF ALGINATE HYDROGELS FOR BONE REGENERATION

Biocompatibility

Alginate has been broadly evaluated as a biomaterial in the field of medicinal applications and the main requirement for therapeutic applications is to design the hydrogels with a high order of purity. However, the alginate is being extracted from natural sources so there is a possible occurrence of metals and toxins. Thus, extensive research has been done for the purification of alginate hydrogels in vitro as well as in vivo (86). The results revealed that biocompatibility corresponded to the varying levels of purity, and it is also found that alginate hydrogel with high gluconate content resulted in poor biocompatible hydrogels. So, it was confirmed that physicochemical as well as mechanical properties influence the biocompatibility properties of alginate hydrogels (87).

Vascularization

Initially, more efforts have been initiated for the bone defects therapy using engineered grafts. The progress has been raised with the use of suitable biomaterials, but it faces limitations in the vascularization process. Currently, the effort has been moved on to the tissue engineering methods which are emerging as a potent alternative to the grafts. The stimulation of lymph angiogenesis for the treatment of cardiovascular disorders has been by using injectable alginate hydrogels. It helps to reverse the disorders which occur due to the lymphatic dysfunction by sustained delivery of angiogenic factors to establish new blood vasculature (88). Alginate-aker injectable composite hydrogels effectively promote the new bone formation by inducing osteogenesis in a concentration dependent manner by initiating the movement and osteogenic specialization of mesenchymal stem cells originated from human bone-marrow to the injured area (89).

Degradation

Alginate hydrogels seem to be the promising polymeric material that promotes the controlled delivery of therapeutics, thereby limiting the biological agents from degradation while the release of drugs (90). Gluronic and mannuronic residues present in the alginate hydrogels undergo partial oxidation which makes them susceptible to hydrolytic degradation. The differing degradation rates can be accomplished by changing the proportion of the alginate without bringing any alterations in their mechanical properties (91). The photo-crosslinked alginate hydrogels loaded with osteogenic materials able to deliver their materials to the sporadic measured bone imperfections accordingly. It has control over material degradation when contrasted with ionically cross-linked hydrogels (92).

MODULATION STRATEGIES FOR TISSUE REGENERATION

Physical modulation mechanisms

Conventional ionic crosslinking is commonly used to prepare alginate hydrogels, but it offers limited control over gelation kinetics. Studies show that their mechanical

characteristics can be modulated by adjusting parameters such as pH, temperature, and alginate concentration (93). Optimizing these factors enhances the suitability of alginate for injectable scaffold applications. However, ionically crosslinked hydrogels often exhibit low drug-loading capacity, restricting their use in regeneration therapies. To overcome these limitations, structural properties can be tailored by increasing the molecular weight (MW) of alginate, improving its suitability for hard-tissue engineering.

Composite scaffolds composed of alginate and hydroxyapatite, fabricated through phase-separation techniques, exhibit porous interconnected structures that promote osteosarcoma cell adhesion and enhance bone tissue formation. Higher alginate MW also improves mechanical strength compared with nanohydrogel counterparts (94). Chemical modifications of the alginate backbone can further refine its properties, enabling the formation of porous scaffolding matrices that support 3D cellular organization and new tissue development. Advances in microscale engineering techniques now allow the design of complex structural and cellular gradients within hydrogels. Various analytical methods have been employed to evaluate and optimize the mechanical properties of these scaffolds, as summarized in Table I and Table II.

Chemical modulation mechanisms

Alginate hydrogels exhibit high swelling and slow

Table I: Different formulations of hydrogels

Hydrogel type	Alginate hydrogel formulations	Type of cross-linking	Physical aspects	Applications	Remarks	References
Homo-polymeric	Alginate hydrogels	Physical, chemical, enzymatic and irradiation techniques	Microspheres and beads	Encapsulation carrier	80% drug release within 4 hrs	(95)
Hetero-polymeric	Oxidized alginate gelatin hydrogel	Covalent cross-linking	Scaffolds	Osteogenic differentiation of murine bone marrow	Support osteogenic differentiation without using any supplements	(96)
Co-polymeric	Methacrylic acid and sodium-2-acrylamido-2-methyl-1-propane sulfonate on to sodium alginate (SA) hydrogels	Free radical graft polymerization	Graft polymers	System for controlled delivery of drugs	pH reversibility favors controlled drug delivery	(97)
Hybrid composites	Hydroxyapatite- alginate nanocomposite	Electrospinning and biomimetic *in situ* synthesis	Nanofibers	Bone tissue regeneration	Increased tensile strength and elastic modulus	(98)
Interpenetrating polymer networks	Alginate /gelatin semi IPN	Double cross-linking	IPN hydrogels	3D bioprinting	Tunable hydrogels with improved physiochemical and biochemical properties	(99)

Table II: Different methods for formulations of alginate hydrogels in bone repair

Type of material	Highlights	Proposed applications	References
Alginate as injectable scaffolds	Reversible chemistry to enhance controlled degradation Native to extracellular matrix Permeability to nutrients	Regeneration of bones Cartilage and spinal cord repair Cardiac regeneration	(100)
Alginate Electrospun nanofibers	The ratio of large surface area to volume Enhanced porosity Stability	Wound healing Tissue engineering Cancer therapy Sensors	(78)
Alginate Bioink	Improved mechanical properties on blending with other polymers	3D tissue printing Wound healing Drug delivery	(101)
Alginate hydrogel laden paper scaffolds	Enhanced *in vivo* tissue regeneration	Osteogenic differentiation	(102)
Alginate/ dialdehyde/ gelatin –bioactive glass	Good cell adherence and proliferation No inflammatory reactions	Bone tissue regeneration	(103)
Alginate nanocomposites	Electrical conductivity, antibacterial, antioxidation, magnetic responsiveness	Tissue engineering Antimicrobial biomaterials	(104)
Alginate microspheres	Improved viability and proliferation of cells Increased expression of cartilage-specific gene	Cell drug delivery Cartilage tissue engineering	(105)

degradation under physiological conditions, which limits their direct use in bone and tissue regeneration. Chemical modification of alginate functional groups offers a practical strategy to control swelling behavior and drug release in acidic or basic environments. Various modification approaches have been explored, including esterification, amidation, reductive amination, copolymerization, phosphorylation, and graft polymerization (106).

One of the most common techniques is oxidation, in which acetal groups in alginate are converted into oxidized forms that degrade more readily under physiological conditions. Another widely studied strategy is sulfation using chlorosulfuric acid, which imparts heparin-like characteristics and enhances growth-factor release (107). Researchers have also developed injectable hydrogels by combining alginate with gelatin and borax as a cross-linking agent. Composite hydrogel formulations are increasingly used as bioinks for 3D printing of bone tissue constructs, supported by chemical reactions such as Michael addition, chain polymerization, and radical polymerization.

Early alginate hydrogel modifications involved Michael-type crosslinking with dithiothreitol to tune mechanical stiffness. Unmodified methacrylated polymers have also been adapted using photoinitiators. Alginate methacrylation with methacrylic anhydride introduces esterified hydroxyl groups that undergo photo-crosslinking under long-wave UV light, enabling fluid-to-solid transitions under physiological conditions. This method has been effectively used to encapsulate bovine nucleus pulposus cells, making the resulting hydrogels suitable as biomaterial scaffolds for NP repair (108).

Schiff-base crosslinked hydrogels, formed through dynamic covalent interactions, provide tunable mechanical and biological properties that meet diverse tissue requirements. These hydrogels can be generated in situ with embedded cells, tissues, or bioactive molecules under physiological conditions, forming reversible, pH-sensitive, and biocompatible polymer networks. Such properties make Schiff-base alginate hydrogels highly promising for biomedical applications (109).

Bio-functional modulation mechanisms

The biosynthesis of alginate relies on inexpensive algal sources, and understanding these pathways enables the production of tailored polymers. Variations in structural composition, acetylation levels, and molecular modifications significantly influence polymer properties and potential applications. The need to enhance the material characteristics of naturally derived algal alginates has driven interest in microbial production. Microbial biosynthesis proceeds through four major phases: (i) precursor formation, (ii) polymerization and transfer across the cytoplasmic membrane, (iii) periplasmic modification, and (iv) export of the processed alginate across the outer membrane (110).

With clearer insight into these mechanisms, biosynthetic steps have been optimized, and alginate-modifying enzymes are now engineered through genetic and protein engineering approaches. These advances have enabled the production of microbial alginates with user-defined and application-specific properties (111).

The ability of alginate hydrogels to mimic extracellular matrix (ECM) characteristics offers significant potential for supporting cell proliferation and tissue regeneration. Efforts have focused on improving the biological performance of alginate hydrogels to achieve compatibility with various cell types in vitro. Controlled degradation properties, achieved through irradiation techniques, have enhanced cell adhesion and proliferation in bone grafting applications. Additionally, encapsulating chondrocytes in oxidized alginate hydrogel matrices has demonstrated excellent cell migration and integration, particularly in cartilage tissue engineering (112).

DRUG DELIVERY FORMULATIONS OF ALGINATE HYDROGELS FOR BONE REGENERATION

Drug delivery through alginate hydrogels improves patient compliance, as they can deliver both low–molecular weight drugs and large macromolecules in a controlled manner, often requiring only minimal doses to achieve therapeutic effects (113). Owing to their unique absorption properties, alginates are extensively used in oral drug formulations. Among polysaccharides, alginate is one of the most widely used biomaterials for bone tissue regeneration because it supports cell adhesion, migration, and proliferation. Its inherent porosity and mechanical stability further enhance bone repair.

Alginate hydrogels are biodegradable and therefore suitable as carriers for a variety of therapeutic agents at both micro- and nanoscale levels (114). Their 3D network structure enables effective encapsulation and delivery of growth factors, bioactive molecules, and proteins (115). Current research explores multiple alginate-based systems—including hydrogels, microspheres, scaffolds, and composite carriers—for bone regenerative medicine. Layer-by-layer engineered microcapsules for controlled loading and sustained release have gained particular attention recently.

Since native alginate lacks active functional groups, it is often chemically modified or blended with polymers or natural bioactive materials to improve cell–cell communication and biological performance, making it an effective platform for growth factor–mediated and stem cell–based therapies.

Alginate microbeads

Alginate microbeads are promising candidates for bone regeneration due to their excellent biocompatibility and suitability as hydrogel-based microspheres. Alginate readily forms microspheres through ionic cross-linking and emulsion–evaporation methods (116, 117). These techniques produce stable, small-sized beads, and the addition of 2% surfactant supports cell growth during encapsulation. Alginate–CPC microbeads containing mesenchymal stem cells demonstrated a 2.4-fold increase in alkaline phosphatase activity in osteogenic medium, indicating strong potential for orthopedic bone regeneration (118, 119).

The high surface area of microbeads enhances cell viability, osteogenic differentiation, and proliferation. Alginate microspheres have also been synthesized for sustained delivery of hydroxyapatite, showing encouraging effects in healing bone defects (120). Hybrid alginate microspheres loaded with bone morphogenetic proteins and transforming growth factor-β (TGF-β) have been developed to induce osteogenic differentiation of mesenchymal stem cells, though large-scale production remains challenging (121, 122). Additionally, alginate microbeads sized 550–650 μm encapsulating Wharton's jelly–derived MSCs have been used for targeted growth factor release in bone tissue (123, 124).

Alginate nanoparticles

Conventional therapies often struggle to deliver drugs effectively to bone tissue, increasing the risk of infections and unwanted side effects in other organs. Nanoparticle-based systems overcome these limitations by providing improved drug targeting; their small size and high surface-area-to-volume ratio enable controlled and site-specific delivery.

Alginate–chitosan nanocarriers encapsulated with iron/bovine lactoferrin have shown promising therapeutic effects in osteoarthritis, with significant improvements observed in arthritis-induced mice models (125). Alginate–octacalcium phosphate–nanobioactive glass composites produced through layer-by-layer deposition have also been evaluated for mesenchymal stem cell–mediated regeneration. These nanocomposite hydrogels demonstrated excellent tensile strength and enhanced cell proliferation due to their strong interaction with physiological fluids and bone tissue, leading to rapid bone formation (126).

Using lower concentrations of nanobioactive glass (12.5% w/w) with biopolysaccharides further supports efficient bone regeneration, representing a key advantage of nanostructured formulations. Additionally, incorporating MG-63 cells into alginate–dialdehyde gelatin nanocomposites-maintained cell viability while enhancing osteogenic activity, as confirmed by EDC analysis (127).

Alginate fibers

Alginate nanofibers serve as effective cell scaffolds by providing a matrix suitable for tissue development, overcoming the limited cellular interaction seen in porous scaffolds. To better mimic extracellular matrix properties, alginate has been nanoscaled using electrospinning techniques. Recent studies have optimized solution properties to enhance the applicability of alginate nanofibers in regenerative medicine (128). Electrospun alginate nanofibers have also been used for the sustained release of recombinant bone morphogenetic protein-2 (BMP-2) (129, 130). These studies showed that the degree of alginate sulfation significantly improves growth factor entrapment, which in turn influences release kinetics and ultimately the outcome of tissue regeneration.

Growth factor–mediated delivery using nanofibers has proven clinically effective in treating bone fractures and defects. In another approach, peptide-modified alginate hydrogels were incorporated into nanofiber mesh tubes to release recombinant human BMP-2 (rhBMP-2), demonstrating strong potential for repairing large bone defects (131).

Injectable scaffolds

Scaffolds exhibit good hydrophilicity and bioactivity, yet they still face challenges in bone tissue engineering due to compositional limitations. Mesenchymal stem cells have been incorporated into injectable alginate scaffolds, enabling in-situ tissue formation and subsequent bone generation. Nanocomposite scaffolds combining graphene oxide with alginate have been tested for their bone-healing potential using MG-63 osteoblast cells. In vitro results showed that graphene oxide–enhanced scaffolds increased swelling capacity by nearly 700% and reduced degradation rates by approximately 30% over 28 days (132).

Injectable alginate hydrogels synthesized through ionic cross-linking have also been used for the sustained release of vascular endothelial growth factors (VEGF-C and VEGF-D), promoting effective lymphangiogenesis (133). High levels of endothelial sprouting were achieved when growth factors were released consistently. Alginate hydrogels are particularly well-suited for localized drug delivery because of their ability to control both the site and rate of release. Additionally, alginate-based delivery systems require comparatively lower doses to produce therapeutic effects (134).

ALGINATE MEDIATED DRUG DELIVERY MODES IN BONE REGENERATIVE MEDICINE

Stem cell-mediated therapy

Stem cells are specialized human cells capable of self-renewal and differentiation into various cell types that perform distinct functions in the body. Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs)

derived from bone marrow can differentiate into osteogenic, myocardial, chondrogenic, and adipogenic lineages. In recent years, MSCs isolated from individual patients have been successfully used in treatments, significantly reducing the risk of immunological rejection (135–137). Several studies on bone repair have demonstrated the therapeutic potential of MSCs in tissue regeneration. Compared with HSCs, MSCs exhibit superior proliferation and differentiation capacity toward mature osteoblasts, making them the most widely used stem cell type in regenerative applications.

However, this approach faces limitations, including difficulty in obtaining high-quality cells for in-vitro expansion, challenges in selecting appropriate biological microenvironments, and inadequate oxygen supply during culture (138). To address these issues, in-vivo tissue engineering strategies use scaffolds—with or without cells—to promote tissue regeneration based on patient needs (139).

Researchers have explored alginate scaffolds to deliver biomolecules required for tissue regeneration. Because alginate lacks intrinsic bioactive sites, it must be functionalized for cell-based applications. Protein functionalization has improved cell adhesion, as demonstrated in composite chitosan/alginate hydrogels photo-encapsulated with MSCs and BMP-2, which promoted osteogenic differentiation in vitro (140). In addition, alginate hydrogels with higher matrix elasticity enhanced MSC-mediated osteogenesis. Alginate hydrogel beads containing MSCs and fibronectin implanted in calvarial bone defects prevented cell loss and supported rapid bone tissue formation (141).

Growth-factor–mediated drug delivery

Natural bone repair relies on a signaling cascade in which growth factors recruit osteoprogenitor cells and stimulate their migration, proliferation, and differentiation into osteoblasts (142). To mimic this biology, many studies have encapsulated growth factors such as BMPs (143), IGF (144), FGF (145), VEGF (146), and SDF-1α (147) within hydrogel matrices to induce osteogenesis (Table III).

Growth factors exert their effects by binding to specific receptors on target cells, triggering downstream events such as phosphorylation and gene expression that drive tissue formation (156, 157). Scaffolds further support these processes by facilitating cellular attachment and colonization. Mineralization within hydrogel systems can also be enhanced by incorporating chondrogenic growth factors (158).

Alginate-based hydrogels have been engineered for controlled, spatiotemporal release of growth factors. For instance, degradable alginate hydrogels loaded with bone progenitor cells and a combination of BMP-2 and TGF-β3 accelerated bone formation in SCID mice. Another study demonstrated that alginate scaffolds loaded with VEGF, PDGF-BB, and TGF-β1 showed differential binding interactions, with fibroblast growth factor displaying the strongest affinity. These interactions directly influence the release rate of each growth factor (159).

Table III: Role of different growth factors in alginate mediated drug delivery in bone regeneration

Alginate formulation	Formulation method	Type of growth factors	Biomedical applications	Merits	References
Alginate- collagen microsphere	Entrapment	FGF family	Bioengineering of tissues	High uniformity microspheres with enhanced cell viability	(148)
Alginate hydrogel	Gamma irradiation	VEGF family	Lymph angiogenesis	Sustained bioactive release	(133)
Alginate Nano scaffolds	Electro-spinning	rhBMP-2	Bone repair	Improved infiltration of osteo-progenitor cells	(149)
Alginate –heparin hydrogel	Photo-cross linking	BMP-2	Osteogenesis	Enhanced peripheral bone formation about 1.9 fold	(150)
Alginate micro-beads	Eight nozzle flow-focusing device method	VEGF, IGF-1, FGF-1, PDGF, HGF and NGF	Revascularization, cell therapy	Prolong the cell survival and stimulation of resident cells	(151)
Alginate- PLGA microcapsule	Electro-dropping	PDGF and VEGF	Angiogenesis	Angiogenic sprouting is more active and controlled release	(152)
Alginate hydrogel	Gamma-irradiation and oxidized irradiation	BMP-2 and BMP-7	Tissue engineering	Bone mineral density is high	(153)
Alginate hydrogel bioink	Entrapment and crosslinking	Platelet-rich plasma	Stem cell recruitment and tissue regeneration	Compatible with commercial bioprinters and patient specific	(154)
Alginate/gelatin/SiO_2 (AGS) hydrogels	Freeze casting	Growth factors	Bone tissue regeneration, drug delivery	Patient specific evaluation of virtual bone defects	(155)

Gene delivery

Different strategies were performed by the researchers for performing bone regeneration using growth factors. As they require larger doses and their half-life is very short, they go for an alternative mode of administration. Gene therapy has evolved to improve healing using recombinant proteins. This has been performed in vivo as well as ex vivo. Most commonly, the adenoviruses are used for transfection of the genes for aiding the delivery of growth factors for a particular duration which promotes tissue regeneration (160). The inclusion of genes alone in certain cases does not activate the cells instead of genetically engineered stem cells that were required to produce the therapeutic activity. This is accomplished by using non-viral methods in which mode of administration can be done through injection or with the help of biomaterial under the in vivo approach (161).

In the case of ex vivo, it does not require the cells to be transfected instead, the stem cells are harvested and transduced outside the body and then placed in bone fracture gaps (162). Alginate hydrogels loaded with BMP-2 CDNA have been tested in vivo as well as in vitro for osteogenic differentiation. It appears to be promising in delivering the growth factors and it is confirmed by elevation of alkaline phosphatase in in-vitro conditions, and it is established by collagen and osteocalcin production in the mineralized alginate matrix in vivo (163). Similarly, in another study, the encapsulation of BMP-2 genes complexed with nanohydroxyapatite/MSC inside the networks of alginate was shown excellent differentiation in the chondrogenic lineages (164).

CHALLENGES AND PROSPECTS

Successful bone regeneration therapy has been achieved by creating feasible interaction among the antigenic factors, cells and hydrogel matrices. Among the various characteristic polymers, alginates are viewed as the ideal material for encapsulation of proteins or cells and their delivery to the target site is also at a controlled rate. Alginate hydrogels are biocompatible scaffolds and so effectively applied in the bone regeneration processes. Although the alginate hydrogels showed advantages in extracellular matrix generation, drug encapsulation and degradation, but still it faces challenges in the healing of bone repair as it lacks blood vessels that must be resolved. Designing methods also concentrate more on the biocompatibility nature of the hydrogels but it fails to meet the mechanical stability properties that sometimes may lead to disruption while doing the process of bone healing. The main aim of the hydrogel matrices is to improve the formation of bone by mediating the beneficial interrelationship with that of surrounding tissues. The optimization could be done to enhance bone repair, regeneration done by changing the polymer concentration, loading of bioactive growth factors. Efforts are taken to optimize the properties of the hydrogels by modulating their mechanism concerning the external stimuli. Injectable alginate hydrogels have been designed with improved cell adhesion properties and have been used in regenerative applications, but it has its limitations in mechanical properties. Efforts have also been undertaken to enhance the mechanical features by adding calcium phosphate to the alginate hydrogel beads along with the encapsulation of umbilical derived MSC. These results in a stem cell construct of injectable type with load-bearing capability showed enhanced mechanical properties during bone regeneration in a minimally invasive manner. As the alginate hydrogel lacks cell adhesion capabilities, it has been blended with other polymers, and it acts as an integral asset for the clinical treatment of bone deformities later.

CONCLUSION

Despite the significant existing challenges, the development of alginate hydrogel with a high swelling ratio, poor degradation profile, comparatively high compressive strength than other natural polymers, and elastic modulus undoubtedly holds assurance for bone-related illnesses therapy. Alginate can cointegrate with other polymeric materials that help in improving the diffusive properties of the hydrogels. This helps in maintaining the controlled delivery of high molecular weight as well as low molecular weight drugs effectively. The advancements in technologies support the generation of alginate hydrogels and their derivatives have shown greater potential in osteoinduction and osteo-generation. The researchers have to put efforts into improving the scale-up, regulatory studies and clinical trials of alginate hydrogel matrices will make it the ideal alternatives for bone regenerative applications in near future.

REFERENCES

1. Hart, N. H.; Nimphius, S.; Rantalainen, T.; Ireland, A.; Siafarikas, A.; Newton, R. U. Mechanical basis of bone strength: influence of bone material, bone structure and muscle action. Journal of musculoskeletal & neuronal interactions 2017, 17(3), 114–139.
2. Fernandez de Grado, G.; Keller, L.; Idoux-Gillet, Y.; Wagner, Q.; Musset, A.-M.; Benkirane-Jessel, N.; et al. Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. Journal of tissue engineering 2018, 9, 2041731418776819–2041731418776819. doi:10.1177/2041731418776819.
3. Harris, J. P.; Burrell, J. C.; Struzyna, L. A.; Chen, H. I.; Serruya, M. D.; Wolf, J. A.; et al. Emerging regenerative medicine and tissue engineering strategies for Parkinson's disease. npj Parkinson's Disease 2020, 6(1). doi:10.1038/s41531-019-0105-5.
4. Mahanta, A. K.; Patel, D. K.; Maiti, P. Nanohybrid

Scaffold of Chitosan and Functionalized Graphene Oxide for Controlled Drug Delivery and Bone Regeneration. ACS Biomaterials Science & Engineering 2019, 5(10), 5139–5149. doi:10.1021/acsbiomaterials.9b00829.
5. Leng, Y.; Yang, F.; Wang, Q.; Li, Z.; Yuan, B.; Peng, C.; et al. Material-based therapy for bone nonunion. Materials & Design 2019, 183, 108161. doi:https://doi.org/10.1016/j.matdes.2019.108161.
6. Yue, S.; He, H.; Li, B.; Hou, T. Hydrogel as a Biomaterial for Bone Tissue Engineering: A Review. Nanomaterials (Basel, Switzerland) 2020, 10(8). doi:10.3390/nano10081511.
7. Li, L.; Yu, F.; Zheng, L.; Wang, R.; Yan, W.; Wang, Z.; et al. Natural hydrogels for cartilage regeneration: Modification, preparation and application. Journal of orthopaedic translation 2019, 17, 26–41. doi:10.1016/j.jot.2018.09.003.
8. Kaczmarek-Pawelska, A. Alginate-Based Hydrogels in Regenerative Medicine. Alginates - Recent Uses of This Natural Polymer 2020, No. July. doi:10.5772/intechopen.88258.
9. Pereira, L. Introductory Chapter: Alginates - A General Overview. Pereira, J. C. E.-L., Ed.; IntechOpen: Rijeka, 2020; p Ch. 1. doi:10.5772/intechopen.88381.
10. Bendtsen, S. T. Alginate Hydrogels for Bone Tissue Regeneration. University of Connecticut 2017, 132.
11. Zhou, Q.; Kang, H.; Bielec, M.; Wu, X.; Cheng, Q.; Wei, W.; et al. Influence of different divalent ions cross-linking sodium alginate-polyacrylamide hydrogels on antibacterial properties and wound healing. Carbohydrate Polymers 2018, 197, 292–304. doi:https://doi.org/10.1016/j.carbpol.2018.05.078.
12. Turnbull, G.; Clarke, J.; Picard, F.; Riches, P.; Jia, L.; Han, F.; et al. 3D bioactive composite scaffolds for bone tissue engineering. Bioactive materials 2018, 3(3), 278–314. doi:10.1016/j.bioactmat.2017.10.001.
13. Zhu, T.; Mao, J.; Cheng, Y.; Liu, H.; Lv, L.; Ge, M.; et al. Recent progress of polysaccharide-based hydrogel interfaces for wound healing and tissue engineering. Advanced Materials Interfaces 2019, 6(17), 1–22. doi:10.1002/admi.201900761.
14. Li, X.; Sun, Q.; Li, Q.; Kawazoe, N.; Chen, G. Functional Hydrogels With Tunable Structures and Properties for Tissue Engineering Applications. Frontiers in Chemistry 2018, 6, 499. doi:10.3389/fchem.2018.00499.
15. Yao, T.; Baker, M. B.; Moroni, L. Strategies to improve nanofibrous scaffolds for vascular tissue engineering. Nanomaterials (Basel, Switzerland) 2020, 10(5), 887. doi:10.3390/nano10050887.
16. Yao, T.; Baker, M. B.; Moroni, L. Strategies to improve nanofibrous scaffolds for vascular tissue engineering. Nanomaterials (Basel, Switzerland) 2020, 10(5), 887. doi:10.3390/nano10050887.
17. Gao, Y.; Jin, X. Dual Crosslinked Methacrylated Alginate Hydrogel Micron Fibers and Tissue Constructs for Cell Biology. Marine drugs. Key Laboratory of Textile Science and Technology of the Ministry of Education, College of Textiles, Donghua University, Shanghai 201620, China. gaoyingj@hotmail.com. 2019. doi:10.3390/md17100557.
18. Hatton, J.; Davis, G. R.; Mourad, A. H. I.; Cherupurakal, N.; Hill, R. G.; Mohsin, S. Fabrication of porous bone scaffolds using alginate and bioactive glass. Journal of Functional Biomaterials 2019, 10(1). doi:10.3390/jfb10010015.
19. Farokhi, M.; Jonidi Shariatzadeh, F.; Solouk, A.; Mirzadeh, H. Alginate Based Scaffolds for Cartilage Tissue Engineering: A Review. International Journal of Polymeric Materials and Polymeric Biomaterials 2020, 69(4), 230–247. doi:10.1080/00914037.2018.1562924.
20. Gopinathan, J.; Noh, I. Click Chemistry-Based Injectable Hydrogels and Bioprinting Inks for Tissue Engineering Applications. Tissue engineering and regenerative medicine 2018, 15(5), 531–546. doi:10.1007/s13770-018-0152-8.
21. Thakur, G.; Rodrigues, F. C.; Singh, K. Crosslinking Biopolymers for Advanced Drug Delivery and Tissue Engineering Applications. Advances in Experimental Medicine and Biology 2018, 1078(January), 213–231. doi:10.1007/978-981-13-0950-2_11.
22. Park, S. Y.; Kim, W.-J.; Choi, J. B.; Kim, S. Physical and mechanical properties of alginate-based hydrogel film as carrier for release of acetylthiocholine. International Journal of Precision Engineering and Manufacturing 2018, 19(1), 129–135. doi:10.1007/s12541-018-0015-1.
23. Mantha, S.; Pillai, S.; Khayambashi, P.; Upadhyay, A.; Zhang, Y.; Tao, O.; et al. Smart Hydrogels in Tissue Engineering and Regenerative Medicine. Materials (Basel, Switzerland) 2019, 12(20). doi:10.3390/ma12203323.
24. Li, J.; He, J.; Huang, Y.; Li, D.; Chen, X. Improving surface and mechanical properties of alginate films by using ethanol as a co-solvent during external gelation. Carbohydrate Polymers 2015, 123(June), 208–216. doi:10.1016/j.carbpol.2015.01.040.
25. Mondal, A.; Gebeyehu, A.; Miranda, M.; Bahadur, D.; Patel, N.; Ramakrishnan, S.; et al. Characterization and printability of Sodium alginate -Gelatin hydrogel for bioprinting NSCLC co-culture. Scientific Reports 2019, 9(1), 19914. doi:10.1038/s41598-019-55034-9.
26. Aston, R.; Sewell, K.; Klein, T.; Lawrie, G.; Grɯndahl, L. Evaluation of the impact of freezing preparation techniques on the characterisation of alginate hydrogels by cryo-SEM. European Polymer Journal 2016, 82, 1–15. doi:https://doi.org/10.1016/j.eurpolymj.2016.06.025.
27. Cuomo, F.; Cofelice, M.; Lopez, F. Rheological

Characterization of Hydrogels from Alginate-Based Nanodispersion. Polymers 2019, 11(2). doi:10.3390/polym11020259.
28. Ghosh, M.; Halperin-Sternfeld, M.; Grinberg, I.; Adler-Abramovich, L. Injectable Alginate-Peptide Composite Hydrogel as a Scaffold for Bone Tissue Regeneration. Nanomaterials (Basel, Switzerland) 2019, 9(4). doi:10.3390/nano9040497.
29. Li, L.; Yu, F.; Zheng, L.; Wang, R.; Yan, W.; Wang, Z.; et al. Natural hydrogels for cartilage regeneration: Modification, preparation and application. Journal of Orthopaedic Translation 2019, 17(2), 26–41. doi:10.1016/j.jot.2018.09.003.
30. Mastrullo, V.; Cathery, W.; Velliou, E.; Madeddu, P.; Campagnolo, P. Angiogenesis in Tissue Engineering: As Nature Intended? Frontiers in bioengineering and biotechnology 2020, 8, 188. doi:10.3389/fbioe.2020.00188.
31. Saleth, L.; Vairamani, M.; Selvamurugan, N. Alginate/Gelatin scaffolds incorporated with Silibinin-loaded Chitosan nanoparticles for bone formation in vitro. Colloids and Surfaces B: Biointerfaces 2017, 158, 308–318. doi:10.1016/j.colsurfb.2017.06.048.
32. Guarino, V.; Caputo, T.; Altobelli, R.; Ambrosio, L. Degradation properties and metabolic activity of alginate and chitosan polyelectrolytes for drug delivery and tissue engineering applications. AIMS Materials Science 2015, 2(4), 497–502. doi:http://dx.doi.org/10.3934/matersci.2015.4.497.
33. Kharkar, P. M.; Kiick, K. L.; Kloxin, A. M. Designing degradable hydrogels for orthogonal control of cell microenvironments. Chemical Society Reviews 2013, 42(17), 7335–7372. doi:10.1039/c3cs60040h.
34. Li, J.; Mooney, D. J. Designing hydrogels for controlled drug delivery. Nature reviews. Materials 2016, 1(12). doi:10.1038/natrevmats.2016.71.
35. Pradhan, S.; Keller, K. A.; Sperduto, J. L.; Slater, J. H. Fundamentals of Laser-Based Hydrogel Degradation and Applications in Cell and Tissue Engineering. Advanced Healthcare Materials 2017, 6(24), 1–28. doi:10.1002/adhm.201700681.
36. Tang, S.; Zhao, L.; Yuan, J.; Chen, Y.; Leng, Y. Chapter 3 - Physical hydrogels based on natural polymers. Chen, Y. B. T.-H. B. on N. P., Ed.; Elsevier, 2020; pp 51–89. doi:https://doi.org/10.1016/B978-0-12-816421-1.00003-3.
37. Kaczmarek, B.; Nadolna, K.; Owczarek, A. Chapter 6 - The physical and chemical properties of hydrogels based on natural polymers. Chen, Y. B. T.-H. B. on N. P., Ed.; Elsevier, 2020; pp 151–172. doi:https://doi.org/10.1016/B978-0-12-816421-1.00006-9.
38. Gurikov, P.; Smirnova, I. Non-Conventional Methods for Gelation of Alginate. Gels 2018, 4(1), 14. doi:10.3390/gels4010014.
39. Mikula, K.; Skrzypczak, D.; Ligas, B.; Witek-Krowiak, A. Preparation of hydrogel composites using Ca2+ and Cu2+ ions as crosslinking agents. SN Applied Sciences 2019, 1(6), 643. doi:10.1007/s42452-019-0657-3.
40. Ooi, H. W.; Kocken, J. M. M.; Morgan, F. L. C.; Malheiro, A.; Zoetebier, B.; Karperien, M.; et al. Multivalency Enables Dynamic Supramolecular Host–Guest Hydrogel Formation. Biomacromolecules 2020, 21(6), 2208–2217. doi:10.1021/acs.biomac.0c00148.
41. Freeman, F. E.; Kelly, D. J. Tuning Alginate Bioink Stiffness and Composition for Controlled Growth Factor Delivery and to Spatially Direct MSC Fate within Bioprinted Tissues. Scientific Reports 2017, 7(1), 17042. doi:10.1038/s41598-017-17286-1.
42. Somo, S. I.; Langert, K.; Yang, C.-Y.; Vaicik, M. K.; Ibarra, V.; Appel, A. A.; et al. Synthesis and evaluation of dual crosslinked alginate microbeads. Acta biomaterialia 2018, 65, 53–65. doi:10.1016/j.actbio.2017.10.046.
43. Don, T.-M.; Huang, M.-L.; Chiu, A.-C.; Kuo, K.-H.; Chiu, W.-Y.; Chiu, L.-H. Preparation of thermo-responsive acrylic hydrogels useful for the application in transdermal drug delivery systems. Materials Chemistry and Physics 2008, 107(2), 266–273. doi:https://doi.org/10.1016/j.matchemphys.2007.07.009.
44. Li, X.; Wang, H.; Li, D.; Long, S.; Zhang, G.; Wu, Z. Dual Ionically Cross-linked Double-Network Hydrogels with High Strength, Toughness, Swelling Resistance, and Improved 3D Printing Processability. ACS Applied Materials & Interfaces 2018, 10(37), 31198–31207. doi:10.1021/acsami.8b13038.
45. Valentin, T. M.; Leggett, S. E.; Chen, P.-Y.; Sodhi, J. K.; Stephens, L. H.; McClintock, H. D.; et al. Stereolithographic printing of ionically-crosslinked alginate hydrogels for degradable biomaterials and microfluidics. Lab on a Chip 2017, 17(20), 3474–3488. doi:10.1039/C7LC00694B.
46. Sergeeva, A.; Vikulina, A. S.; Volodkin, D. Porous alginate scaffolds assembled using vaterite CaCO3 crystals. Micromachines 2019, 10(6), 1–21. doi:10.3390/mi10060357.
47. Leijten, J.; Seo, J.; Yue, K.; Santiago, G. T.; Ruiz-esparza, G. U.; Shin, S. R.; et al. HHS Public Access. 2018, 1–35. doi:10.1016/j.mser.2017.07.001. Spatially.
48. Huang, H.; Qi, X.; Chen, Y.; Wu, Z. Thermo-sensitive hydrogels for delivering biotherapeutic molecules: A review. Saudi Pharmaceutical Journal 2019, 27(7), 990–999. doi:https://doi.org/10.1016/j.jsps.2019.08.001.
49. Kondiah, P. J.; Choonara, Y. E.; Kondiah, P. P. D.; Marimuthu, T.; Kumar, P.; Du Toit, L. C.; et al. A review of injectable polymeric hydrogel systems for application in bone tissue engineering. Molecules 2016, 21(11). doi:10.3390/molecules21111580.
50. Wei, K.; Zhu, M.; Sun, Y.; Xu, J.; Feng, Q.; Lin, S.; et al. Robust Biopolymeric Supramolecular "Host-

Guest Macromer" Hydrogels Reinforced by in Situ Formed Multivalent Nanoclusters for Cartilage Regeneration. Macromolecules 2016, 49(3), 866–875. doi:10.1021/acs.macromol.5b02527.
51. Karoyo, A.; Wilson, L. Physicochemical Properties and the Gelation Process of Supramolecular Hydrogels: A Review. Gels 2017, 3(1), 1. doi:10.3390/gels3010001.
52. Somo, S. I.; Langert, K.; Yang, C.-Y.; Vaicik, M. K.; Ibarra, V.; Appel, A. A.; et al. Synthesis and evaluation of dual crosslinked alginate microbeads. Acta biomaterialia 2018, 65, 53–65. doi:10.1016/j.actbio.2017.10.046.
53. Echalier, C.; Valot, L.; Martinez, J.; Mehdi, A.; Subra, G. Chemical cross-linking methods for cell encapsulation in hydrogels. Materials Today Communications 2019, 20. doi:10.1016/j.mtcomm.2019.05.012.
54. Ren, K.; He, C.; Xiao, C.; Li, G.; Chen, X. Injectable glycopolypeptide hydrogels as biomimetic scaffolds for cartilage tissue engineering. Biomaterials 2015, 51, 238–249. doi:10.1016/j.biomaterials.2015.02.026.
55. Li, X.; Sun, Q.; Li, Q.; Kawazoe, N.; Chen, G. Functional Hydrogels With Tunable Structures and Properties for Tissue Engineering Applications. Frontiers in Chemistry 2018, 6, 499. doi:10.3389/fchem.2018.00499.
56. Labus, K.; Wolanin, K.; Radosiński, Ł. Comparative study on enzyme immobilization using natural hydrogel matrices—experimental studies supported by molecular models analysis. Catalysts 2020, 10(5). doi:10.3390/catal10050489.
57. Lee, J. H. Injectable hydrogels delivering therapeutic agents for disease treatment and tissue engineering. Biomaterials Research 2018, 22, 1–14. doi:10.1186/s40824-018-0138-6.
58. Wu, J.; Chen, Q.; Deng, C.; Xu, B.; Zhang, Z.; Yang, Y.; et al. Exquisite design of injectable Hydrogels in Cartilage Repair. Theranostics. Key Laboratory for Space Bioscience and Biotechnology, Northwestern Polytechnical University School of Life Sciences. 2020, pp 9843–9864. doi:10.7150/thno.46450.
59. Lee, F.; Bae, K. H.; Kurisawa, M. Injectable hydrogel systems crosslinked by horseradish peroxidase. Biomedical materials (Bristol, England) 2015, 11(1), 14101. doi:10.1088/1748-6041/11/1/014101.
60. Hou, J.; Li, C.; Guan, Y.; Zhang, Y.; Zhu, X. X. Enzymatically Crosslinked Alginate Hydrogels with Improved Adhesion Properties. Polymer Chemistry 2015, 6 (12), 2204–2213. doi:10.1039/C4PY01757A.
61. Hu, W.; Wang, Z.; Xiao, Y.; Zhang, S.; Wang, J. Advances in crosslinking strategies of biomedical hydrogels. Biomater. Sci. 2019, 7(3), 843–855. doi:10.1039/C8BM01246F.
62. Konuray, O.; Fern6ndez-Francos, X.; De la Flor, S.; Ramis, X.; Serra, A. The use of click-type reactions in the preparation of thermosets. Polymers 2020, 12(5). doi:10.3390/POLYM12051084.
63. Xu, Z.; Bratlie, K. M. Click Chemistry and Material Selection for in Situ Fabrication of Hydrogels in Tissue Engineering Applications. ACS Biomaterials Science & Engineering 2018, 4(7), 2276–2291. doi:10.1021/acsbiomaterials.8b00230.
64. Garcна-Astrain, C.; Avйrous, L. Synthesis and evaluation of functional alginate hydrogels based on click chemistry for drug delivery applications. Carbohydrate polymers 2018, 190, 271–280. doi:10.1016/j.carbpol.2018.02.086.
65. Xu, Z.; Bratlie, K. M. Click Chemistry and Material Selection for in Situ Fabrication of Hydrogels in Tissue Engineering Applications. ACS Biomaterials Science & Engineering 2018, 4(7), 2276–2291. doi:10.1021/acsbiomaterials.8b00230.
66. Sánchez-Morán, H.; Ahmadi, A.; Vogler, B.; Roh, K.-H. Oxime Cross-Linked Alginate Hydrogels with Tunable Stress Relaxation. Biomacromolecules 2019, 20(12), 4419–4429. doi:10.1021/acs.biomac.9b01100.
67. Capeling, M. M.; Czerwinski, M.; Huang, S.; Tsai, Y.-H.; Wu, A.; Nagy, M. S.; et al. Nonadhesive Alginate Hydrogels Support Growth of Pluripotent Stem Cell-Derived Intestinal Organoids. Stem Cell Reports 2019, 12(2), 381–394. doi:https://doi.org/10.1016/j.stemcr.2018.12.001.
68. Lee, S. M.; Yoo, E. S.; Ghim, H. Do; Lee, S. J. Alginate nanohydrogels prepared by emulsification-diffusion method. Macromolecular Research 2009, 17(3), 168–173. doi:10.1007/BF03218674.
69. Kaczmarek-Pawelska, A. Alginate-Based Hydrogels in Regenerative Medicine. In Alginates - Recent Uses of This Natural Polymer; IntechOpen, 2020. doi:10.5772/intechopen.88258.
70. Lopes, M.; Abrahim, B.; Veiga, F.; Seiэa, R.; Cabral, L. M.; Arnaud, P.; et al. Preparation methods and applications behind alginate-based particles. Expert Opinion on Drug Delivery 2017, 14(6), 769–782. doi:10.1080/17425247.2016.1214564.
71. Baimark, Y.; Srisuwan, Y. Preparation of alginate microspheres by water-in-oil emulsion method for drug delivery: Effect of Ca2+ post-cross-linking. Advanced Powder Technology 2014, 25(5), 1541–1546. doi:https://doi.org/10.1016/j.apt.2014.05.001.
72. Ozawa, F.; Ino, K.; Shiku, H.; Matsue, T. Electrochemical hydrogel lithography of calcium-alginate hydrogels for cell culture. Materials 2016, 9(9), 1–7. doi:10.3390/ma9090744.
73. Sarker, M. D.; Naghieh, S.; Sharma, N. K.; Chen, X. 3D biofabrication of vascular networks for tissue regeneration: A report on recent advances. Journal of Pharmaceutical Analysis 2018, 8(5), 277–296. doi:10.1016/j.jpha.2018.08.005.
74. Tran, K. T. M.; Nguyen, T. D. Lithography-based methods to manufacture biomaterials at small scales. Journal of Science: Advanced Materials

and Devices 2017, 2(1), 1–14. doi:https://doi.org/10.1016/j.jsamd.2016.12.001.
75. Yao, H.; Wang, J.; Mi, S. Photo processing for biomedical hydrogels design and functionality: A review. Polymers 2017, 10(1), 1–27. doi:10.3390/polym10010011.
76. Sarker, M. D.; Naghieh, S.; Sharma, N. K.; Chen, X. 3D biofabrication of vascular networks for tissue regeneration: A report on recent advances. Journal of Pharmaceutical Analysis 2018, 8(5), 277–296. doi:10.1016/j.jpha.2018.08.005.
77. Parra-Cabrera, C.; Achille, C.; Kuhn, S.; Ameloot, R. 3D printing in chemical engineering and catalytic technology: structured catalysts{,} mixers and reactors. Chem. Soc. Rev. 2018, 47(1), 209–230. doi:10.1039/C7CS00631D.
78. Mokhena, T. C.; Mochane, M. J.; Mtibe, A.; John, M. J.; Sadiku, E. R.; Sefadi, J. S. Electrospun alginate nanofibers toward various applications: A review. Materials 2020, 13(4), 1–24. doi:10.3390/ma13040934.
79. Kim, H. D.; Amirthalingam, S.; Kim, S. L.; Lee, S. S.; Rangasamy, J.; Hwang, N. S. Biomimetic Materials and Fabrication Approaches for Bone Tissue Engineering. Advanced healthcare materials 2017, 6(23). doi:10.1002/adhm.201700612.
80. Dehghani, F.; Annabi, N. Engineering porous scaffolds using gas-based techniques. Current Opinion in Biotechnology 2011, 22(5), 661–666. doi:10.1016/j.copbio.2011.04.005.
81. Caroline, C.; Raya, B.; Daniel, C.; Benjamin, D.; Christophe, T.; Philippe, B.; et al. Elaboration and evaluation of alginate foam scaffolds for soft tissue engineering. International Journal of Pharmaceutics 2017, 524(1–2), 433–442. doi:10.1016/j.ijpharm.2017.02.060.
82. Chen, S.; John, J. V; McCarthy, A.; Xie, J. New forms of electrospun nanofiber materials for biomedical applications. J. Mater. Chem. B 2020, 8(17), 3733–3746. doi:10.1039/D0TB00271B.
83. Xue, J.; Xie, J.; Liu, W.; Xia, Y. Electrospun Nanofibers: New Concepts, Materials, and Applications. Accounts of chemical research 2017, 50(8), 1976–1987. doi:10.1021/acs.accounts.7b00218.
84. Ali, W.; Gadkari, R.; Arora, S.; Somkuwar, V.; Chowdhury, A. Antibacterial Electrospun Nanofibres BT - Advanced Antimicrobial Materials and Applications. Inamuddin, Ahamed, M. I., Prasad, R., Eds.; Springer Singapore: Singapore, 2021; pp 239–255. doi:10.1007/978-981-15-7098-8_10.
85. Wongkanya, R.; Chuysinuan, P.; Pengsuk, C.; Techasakul, S.; Lirdprapamongkol, K.; Svasti, J.; et al. Electrospinning of alginate/soy protein isolated nanofibers and their release characteristics for biomedical applications. Journal of Science: Advanced Materials and Devices 2017, 2(3), 309–316. doi:https://doi.org/10.1016/j.jsamd.2017.05.010.
86. Liu, B.; Li, J.; Lei, X.; Miao, S.; Zhang, S.; Cheng, P.; et al. Cell-loaded injectable gelatin/alginate/LAPONITE® nanocomposite hydrogel promotes bone healing in a critical-size rat calvarial defect model. RSC Advances 2020, 10(43), 25652–25661. doi:10.1039/D0RA03040F.
87. Wang, L.; Wang, C.; Wu, S.; Fan, Y.; Li, X. Influence of the mechanical properties of biomaterials on degradability, cell behaviors and signaling pathways: current progress and challenges. Biomaterials Science 2020, 8(10), 2714–2733. doi:10.1039/D0BM00269K.
88. Campbell, K. T.; Hadley, D. J.; Kukis, D. L.; Silva, E. A. Alginate hydrogels allow for bioactive and sustained release of VEGF-C and VEGF-D for lymphangiogenic therapeutic applications. PloS one 2017, 12(7), e0181484–e0181484. doi:10.1371/journal.pone.0181484.
89. Zhang, X.; Zhu, Y.; Cao, L.; Wang, X.; Zheng, A.; Chang, J.; et al. Alginate-aker injectable composite hydrogels promoted irregular bone regeneration through stem cell recruitment and osteogenic differentiation. Journal of Materials Chemistry B 2018, 6(13), 1951–1964. doi:10.1039/C7TB03315J.
90. Abasalizadeh, F.; Moghaddam, S. V.; Alizadeh, E.; akbari, E.; Kashani, E.; Fazljou, S. M. B.; et al. Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. Journal of Biological Engineering 2020, 14(1), 8. doi:10.1186/s13036-020-0227-7.
91. Kurowiak, J.; Kaczmarek-Pawelska, A.; Mackiewicz, A. G.; Bedzinski, R. Analysis of the degradation process of alginate-based hydrogels in artificial urine for use as a bioresorbable material in the treatment of urethral injuries. Processes 2020, 8(3). doi:10.3390/pr8030304.
92. Maji, K.; Dasgupta, S.; Bhaskar, R.; Gupta, M. K. Photo-crosslinked alginate nano-hydroxyapatite paste for bone tissue engineering. Biomedical Materials 2020, 15(5), 55019. doi:10.1088/1748-605x/ab9551.
93. Divya, N.; Reddy, N. Chemical modifications of alginate and its derivatives. International Journal of Chemistry Research 2019, 4(1), 1–17. doi:10.22159/ijcr.2020v4i1.98.
94. Haider, A.; Haider, S.; Rao Kummara, M.; Kamal, T.; Alghyamah, A.-A. A.; Jan Iftikhar, F.; et al. Advances in the scaffolds fabrication techniques using biocompatible polymers and their biomedical application: A technical and statistical review. Journal of Saudi Chemical Society 2020, 24(2), 186–215. doi:https://doi.org/10.1016/j.jscs.2020.01.002.
95. Voo, W.-P.; Ooi, C.-W.; Islam, A.; Tey, B.-T.; Chan, E.-S. Calcium alginate hydrogel beads with high stiffness and extended dissolution

behaviour. European Polymer Journal 2016, 75, 343–353. doi:https://doi.org/10.1016/j.eurpolymj.2015.12.029.
96. Sarker, B.; Li, W.; Zheng, K.; Detsch, R.; Boccaccini, A. R. Designing Porous Bone Tissue Engineering Scaffolds with Enhanced Mechanical Properties from Composite Hydrogels Composed of Modified Alginate, Gelatin, and Bioactive Glass. ACS Biomaterials Science & Engineering 2016, 2(12), 2240–2254. doi:10.1021/acsbiomaterials.6b00470.
97. Clara, I.; Natchimuthu, N. Hydrogels of sodium alginate based copolymers grafted with sodium-2-acrylamido-2-methyl-1-propane sulfonate and methacrylic acid for controlled drug delivery applications. Journal of Macromolecular Science, Part A: Pure and Applied Chemistry 2018, 55(2), 168–175. doi:10.1080/10601325.2017.1403856.
98. Chae, T.; Yang, H.; Moon, H.; Troczynski, T.; Ko, F. K. Biomimetically Mineralized Alginate Nanocomposite Fibers for Bone Tissue Engineering: Mechanical Properties and in Vitro Cellular Interactions. ACS Applied Bio Materials 2020, 3(10), 6746–6755. doi:10.1021/acsabm.0c00692.
99. Chen, Q.; Tian, X.; Fan, J.; Tong, H.; Ao, Q.; Wang, X. An Interpenetrating Alginate/Gelatin Network for Three-Dimensional (3D) Cell Cultures and Organ Bioprinting. Molecules (Basel, Switzerland) 2020, 25(3), 756. doi:10.3390/molecules25030756.
100. Lee, J. H. Injectable hydrogels delivering therapeutic agents for disease treatment and tissue engineering. Biomaterials research 2018, 22, 27. doi:10.1186/s40824-018-0138-6.
101. Datta, S. Importance of Alginate Bioink for 3D Bioprinting in Tissue Engineering and Regenerative Medicine. Barua, R., Ed.; IntechOpen: Rijeka, 2020; p Ch. 7. doi:10.5772/intechopen.90426.
102. Kim, S. H.; Lee, H. R.; Yu, S. J.; Han, M. E.; Lee, D. Y.; Kim, S. Y.; et al. Hydrogel-laden paper scaffold system for origami-based tissue engineering. Proceedings of the National Academy of Sciences of the United States of America 2015, 112(50), 15426–15431. doi:10.1073/pnas.1504745112.
103. Rottensteiner, U.; Sarker, B.; Heusinger, D.; Dafinova, D.; Rath, S. N.; Beier, J. P.; et al. In vitro and in vivo biocompatibility of alginate dialdehyde/gelatin hydrogels with and without nanoscaled bioactive glass for bone tissue engineering applications. Materials 2014, 7(3), 1957–1974. doi:10.3390/ma7031957.
104. Zhao, H.; Liu, M.; Zhang, Y.; Yin, J.; Pei, R. Nanocomposite hydrogels for tissue engineering applications. Nanoscale 2020, 12(28), 14976–14995. doi:10.1039/D0NR03785K.
105. Fan, C.; Wang, D.-A. Potential use of alginate beads as a chondrocyte delivery vehicle and stepwise dissolving porogen in a hydrogel scaffold for cartilage tissue engineering. RSC Adv. 2015, 5(98), 80688–80697. doi:10.1039/C5RA15376J.
106. Szaby, L.; Gerber-Lemaire, S.; Wandrey, C. Strategies to Functionalize the Anionic Biopolymer Na-Alginate without Restricting Its Polyelectrolyte Properties. Polymers 2020, 12(4), 919. doi:10.3390/polym12040919.
107. Arlov, Ш.; Skjek-Brжk, G. Sulfated Alginates as Heparin Analogues: A Review of Chemical and Functional Properties. Molecules (Basel, Switzerland) 2017, 22(5), 778. doi:10.3390/molecules22050778.
108. Chou, A. I.; Akintoye, S. O.; Nicoll, S. B. Photo-crosslinked alginate hydrogels support enhanced matrix accumulation by nucleus pulposus cells in vivo. Osteoarthritis and Cartilage 2009, 17(10), 1377–1384. doi:https://doi.org/10.1016/j.joca.2009.04.012.
109. Xu, J.; Liu, Y.; Hsu, S. Hydrogels Based on Schi ff Base Linkages for. Molecules 2019, 24(3005), 1–21.
110. Pacheco-Leyva, I.; Guevara Pezoa, F.; Dнaz-Barrera, A. Alginate biosynthesis in azotobacter vinelandii: Overview of molecular mechanisms in connection with the oxygen availability. International Journal of Polymer Science 2016, 2016, 11–13. doi:10.1155/2016/2062360.
111. Dharani, S. R.; Srinivasan, R.; Sarath, R.; Ramya, M. Recent progress on engineering microbial alginate lyases towards their versatile role in biotechnological applications. Folia Microbiologica 2020. doi:10.1007/s12223-020-00802-8.
112. Liu, M.; Zeng, X.; Ma, C.; Yi, H.; Ali, Z.; Mou, X.; et al. Injectable hydrogels for cartilage and bone tissue engineering. Bone Research 2017, 5(1), 17014. doi:10.1038/boneres.2017.14.
113. Li, J.; Mooney, D. J. Designing hydrogels for controlled drug delivery. Nature reviews. Materials 2016, 1(12). doi:10.1038/natrevmats.2016.71.
114. Sun, J.; Tan, H. Alginate-Based Biomaterials for Regenerative Medicine Applications. Materials (Basel, Switzerland) 2013, 6(4), 1285–1309. doi:10.3390/ma6041285.
115. Abasalizadeh, F.; Moghaddam, S. V.; Alizadeh, E.; akbari, E.; Kashani, E.; Fazljou, S. M. B.; et al. Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. Journal of Biological Engineering 2020, 14(1), 8. doi:10.1186/s13036-020-0227-7.
116. Mi, X.; Wang, X.; Xu, C.; Zhang, Y.; Tan, X.; Gao, J.; et al. Alginate microspheres prepared by ionic crosslinking of pickering alginate emulsions. Journal of Biomaterials Science, Polymer Edition 2019, 30(12), 1083–1096. doi:10.1080/09205063.2019.1622185.
117. Chaemsawang, W.; Prasongchean, W.; Papadopoulos, K. I.; Sukrong, S.; Kao, W. J.; Wattanaarsakit, P. Emulsion Cross-Linking Technique for Human Fibroblast Encapsulation.

International Journal of Biomaterials 2018, 2018, 9317878. doi:10.1155/2018/9317878.
118. Tang, M.; Chen, W.; Weir, M. D.; Thein-Han, W.; Xu, H. H. K. Human embryonic stem cell encapsulation in alginate microbeads in macroporous calcium phosphate cement for bone tissue engineering. Acta biomaterialia 2012, 8(9), 3436–3445. doi:10.1016/j.actbio.2012.05.016.
119. Chen, H.; Yang, H.; Weir, M. D.; Schneider, A.; Ren, K.; Homayounfar, N.; et al. An antibacterial and injectable calcium phosphate scaffold delivering human periodontal ligament stem cells for bone tissue engineering. RSC Advances 2020, 10(66), 40157–40170. doi:10.1039/D0RA06873J.
120. Nayak, A. K.; Hasnain, Md. S.; Yi*, S. S. N. and D. K. Hydroxyapatite-alginate Based Matrices for Drug Delivery. Current Pharmaceutical Design. 2019, pp 3406–3416. doi:http://dx.doi.org/10.2174/1381612825666190906164003.
121. Kim, H.; Bae, C.; Kook, Y.-M.; Koh, W.-G.; Lee, K.; Park, M. H. Mesenchymal stem cell 3D encapsulation technologies for biomimetic microenvironment in tissue regeneration. Stem Cell Research & Therapy 2019, 10(1), 51. doi:10.1186/s13287-018-1130-8.
122. Fernandes, G.; Wang, C.; Yuan, X.; Liu, Z.; Dziak, R.; Yang, S. Combination of Controlled Release Platelet-Rich Plasma Alginate Beads and Bone Morphogenetic Protein-2 Genetically Modified Mesenchymal Stem Cells for Bone Regeneration. Journal of periodontology 2016, 87(4), 470–480. doi:10.1902/jop.2016.150487.
123. Ansari, A. S.; Yazid, M. D.; Sainik, N. Q. A. V.; Razali, R. A.; Saim, A. Bin; Idrus, R. B. H. Osteogenic Induction of Wharton's Jelly-Derived Mesenchymal Stem Cell for Bone Regeneration: A Systematic Review. Stem Cells International 2018, 2018, 2406462. doi:10.1155/2018/2406462.
124. Choe, G.; Park, J.; Park, H.; Lee, J. Y. Hydrogel biomaterials for stem cell microencapsulation. Polymers 2018, 10(9), 1–17. doi:10.3390/polym10090997.
125. Kanwar, J. R.; Roy, K.; Patel, Y.; Zhou, S. F.; Singh, M. R.; Singh, D.; et al. Multifunctional iron bound lactoferrin and nanomedicinal approaches to enhance its bioactive functions. Molecules 2015, 20(6), 9703–9731. doi:10.3390/molecules20069703.
126. Erol-Taygun, M.; Unalan, I.; Idris, M. I. B.; Mano, J. F.; Boccaccini, A. R. Bioactive Glass-Polymer Nanocomposites for Bone Tissue Regeneration Applications: A Review. Advanced Engineering Materials 2019, 21(8), 1–24. doi:10.1002/adem.201900287.
127. Leite, Á. J.; Sarker, B.; Zehnder, T.; Silva, R.; Mano, J. F.; Boccaccini, A. R. Bioplotting of a bioactive alginate dialdehyde-gelatin composite hydrogel containing bioactive glass nanoparticles. Biofabrication 2016, 8(3), 35005. doi:10.1088/1758-5090/8/3/035005.
128. Mokhena, T. C.; Mochane, M. J.; Mtibe, A.; John, M. J.; Sadiku, E. R.; Sefadi, J. S. Electrospun alginate nanofibers toward various applications: A review. Materials 2020, 13(4), 1–24. doi:10.3390/ma13040934.
129. Kowalczewski, C. J.; Saul, J. M. Biomaterials for the Delivery of Growth Factors and Other Therapeutic Agents in Tissue Engineering Approaches to Bone Regeneration. Frontiers in pharmacology 2018, 9, 513. doi:10.3389/fphar.2018.00513.
130. Mohammadi, S.; Ramakrishna, S.; Laurent, S.; Shokrgozar, M. A.; Semnani, D.; Sadeghi, D.; et al. Fabrication of Nanofibrous PVA/Alginate-Sulfate Substrates for Growth Factor Delivery. Journal of biomedical materials research. Part A 2019, 107(2), 403–413. doi:10.1002/jbm.a.36552.
131. Kolambkar, Y. M.; Dupont, K. M.; Boerckel, J. D.; Huebsch, N.; Mooney, D. J.; Hutmacher, D. W.; et al. An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. Biomaterials 2011, 32(1), 65–74. doi:10.1016/j.biomaterials.2010.08.074.
132. Purohit, S. D.; Bhaskar, R.; Singh, H.; Yadav, I.; Gupta, M. K.; Mishra, N. C. Development of a nanocomposite scaffold of gelatin–alginate–graphene oxide for bone tissue engineering. International Journal of Biological Macromolecules 2019, 133(April), 592–602. doi:10.1016/j.ijbiomac.2019.04.113.
133. Campbell, K. T.; Hadley, D. J.; Kukis, D. L.; Silva, E. A. Alginate hydrogels allow for bioactive and sustained release of VEGF-C and VEGF-D for lymphangiogenic therapeutic applications. PloS one 2017, 12(7), e0181484–e0181484. doi:10.1371/journal.pone.0181484.
134. Bidarra, S. J.; Barrias, C. C.; Granja, P. L. Injectable alginate hydrogels for cell delivery in tissue engineering. Acta Biomaterialia 2014, 10(4), 1646–1662. doi:https://doi.org/10.1016/j.actbio.2013.12.006.
135. Han, Y.; Li, X.; Zhang, Y.; Han, Y.; Chang, F.; Ding, J. Mesenchymal Stem Cells for Regenerative Medicine. Cells 2019, 8(8), 886. doi:10.3390/cells8080886.
136. Oryan, A.; Kamali, A.; Moshiri, A.; Baghaban Eslaminejad, M. Role of Mesenchymal Stem Cells in Bone Regenerative Medicine: What Is the Evidence? Cells Tissues Organs 2017, 204(2), 59–83. doi:10.1159/000469704.
137. Klontzas, M. E. The Use of Alginate Hydrogels for the Culture of Mesenchymal Stem Cells (MSCs): In Vitro and In Vivo Paradigms. Drissi, H., Ed.; IntechOpen: Rijeka, 2020; p Ch. 4. doi:10.5772/intechopen.88020.
138. Upadhyay, R. K. Role of Biological Scaffolds, Hydro Gels and Stem Cells in Tissue Regeneration Therapy. Advances in Tissue Engineering & Regenerative Medicine: Open Access 2017, 2(1),

121–135. doi:10.15406/atroa.2017.02.00020.
139. Han, F.; Wang, J.; Ding, L.; Hu, Y.; Li, W.; Yuan, Z.; et al. Tissue Engineering and Regenerative Medicine: Achievements, Future, and Sustainability in Asia. Frontiers in Bioengineering and Biotechnology 2020, 8, 83. doi:10.3389/fbioe.2020.00083.
140. Marquardt, L. M.; Heilshorn, S. C. Design of Injectable Materials to Improve Stem Cell Transplantation. Current Stem Cell Reports 2016, 2(3), 207–220. doi:10.1007/s40778-016-0058-0.
141. Filippi, M.; Born, G.; Chaaban, M.; Scherberich, A. Natural Polymeric Scaffolds in Bone Regeneration. Frontiers in Bioengineering and Biotechnology 2020, 8, 474. doi:10.3389/fbioe.2020.00474.
142. Ansari, M. Bone tissue regeneration: biology, strategies and interface studies. Progress in Biomaterials 2019, 8(4), 223–237. doi:10.1007/s40204-019-00125-z.
143. Bai, Z.; Guo, X.-H.; Tang, C.; Yue, S.-T.; Shi, L.; Qiang, B. Effects of Artesunate on the Expressions of Insulin-Like Growth Factor-1, Osteopontin and C-Telopeptides of Type II Collagen in a Rat Model of Osteoarthritis. Pharmacology 2018, 101(1–2), 1–8. doi:10.1159/000479160.
144. Zhang, X.; Xing, H.; Qi, F.; Liu, H.; Gao, L.; Wang, X. Local delivery of insulin/IGF-1 for bone regeneration: carriers, strategies, and effects. Nanotheranostics 2020, 4(4), 242–255. doi:10.7150/ntno.46408.
145. Charoenlarp, P.; Rajendran, A. K.; Iseki, S. Role of fibroblast growth factors in bone regeneration. Inflammation and Regeneration 2017, 37(1), 10. doi:10.1186/s41232-017-0043-8.
146. Sharma, S.; Sapkota, D.; Xue, Y.; Rajthala, S.; Yassin, M. A.; Finne-Wistrand, A.; et al. Delivery of VEGFA in bone marrow stromal cells seeded in copolymer scaffold enhances angiogenesis, but is inadequate for osteogenesis as compared with the dual delivery of VEGFA and BMP2 in a subcutaneous mouse model. Stem Cell Research & Therapy 2018, 9(1), 23. doi:10.1186/s13287-018-0778-4.
147. Zhao, W.; Jin, K.; Li, J.; Qiu, X.; Li, S. Delivery of stromal cell-derived factor 1α for in situ tissue regeneration. Journal of biological engineering 2017, 11, 22. doi:10.1186/s13036-017-0058-3.
148. Pradhan, S.; Keller, K. A.; Sperduto, J. L.; Slater, J. H. Fundamentals of Laser-Based Hydrogel Degradation and Applications in Cell and Tissue Engineering. Advanced Healthcare Materials 2017, 6(24), 1–28. doi:10.1002/adhm.201700681.
149. Kolambkar, Y. M.; Dupont, K. M.; Boerckel, J. D.; Huebsch, N.; Mooney, D. J.; Hutmacher, D. W.; et al. An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. Biomaterials 2011, 32(1), 65–74. doi:10.1016/j.biomaterials.2010.08.074.
150. Jeon, O.; Powell, C.; Solorio, L. D.; Krebs, M. D.; Alsberg, E. Affinity-based growth factor delivery using biodegradable, photocrosslinked heparin-alginate hydrogels. Journal of controlled release : official journal of the Controlled Release Society 2011, 154(3), 258–266. doi:10.1016/j.jconrel.2011.06.027.
151. Liu, G.; Pareta, R. A.; Wu, R.; Shi, Y.; Zhou, X.; Liu, H.; et al. Skeletal myogenic differentiation of urine-derived stem cells and angiogenesis using microbeads loaded with growth factors. Biomaterials 2013, 34(4), 1311–1326. doi:https://doi.org/10.1016/j.biomaterials.2012.10.038.
152. Choi, D. H.; Subbiah, R.; Kim, I. H.; Han, D. K.; Park, K. Dual growth factor delivery using biocompatible core-shell microcapsules for angiogenesis. Small (Weinheim an der Bergstrasse, Germany) 2013, 9(20), 3468–3476. doi:10.1002/smll.201300427.
153. Priddy, L. B.; Chaudhuri, O.; Stevens, H. Y.; Krishnan, L.; Uhrig, B. A.; Willett, N. J.; et al. Oxidized alginate hydrogels for bone morphogenetic protein-2 delivery in long bone defects. Acta biomaterialia 2014, 10(10), 4390–4399. doi:10.1016/j.actbio.2014.06.015.
154. Faramarzi, N.; Yazdi, I. K.; Nabavinia, M.; Gemma, A.; Fanelli, A.; Caizzone, A.; et al. Patient-Specific Bioinks for 3D Bioprinting of Tissue Engineering Scaffolds. Advanced healthcare materials 2018, 7(11), e1701347. doi:10.1002/adhm.201701347.
155. Roopavath, U. K.; Soni, R.; Mahanta, U.; Deshpande, A. S.; Rath, S. N. 3D printable SiO2 nanoparticle ink for patient specific bone regeneration. RSC Adv. 2019, 9(41), 23832–23842. doi:10.1039/C9RA03641E.
156. Monteiro, A. I.; Kollmetz, T.; Malmstrum, J. Engineered systems to study the synergistic signaling between integrin-mediated mechanotransduction and growth factors (Review). Biointerphases 2018, 13(6), 06D302. doi:10.1116/1.5045231.
157. Coffin, J. D.; Homer-Bouthiette, C.; Hurley, M. M. Fibroblast Growth Factor 2 and Its Receptors in Bone Biology and Disease. Journal of the Endocrine Society 2018, 2(7), 657–671. doi:10.1210/js.2018-00105.
158. Maisani, M.; Pezzoli, D.; Chassande, O.; Mantovani, D. Cellularizing hydrogel-based scaffolds to repair bone tissue: How to create a physiologically relevant micro-environment? Journal of tissue engineering 2017, 8, 2041731417712073. doi:10.1177/2041731417712073.
159. Gothard, D.; Smith, E. L.; Kanczler, J. M.; Black, C. R.; Wells, J. A.; Roberts, C. A.; et al. In Vivo Assessment of Bone Regeneration in Alginate/Bone ECM Hydrogels with Incorporated Skeletal Stem Cells and Single Growth Factors. PloS one 2015, 10(12), e0145080–e0145080. doi:10.1371/journal.pone.0145080.
160. Lee, C. S.; Bishop, E. S.; Zhang, R.; Yu, X.; Farina, E. M.; Yan, S.; et al. Adenovirus-Mediated Gene Delivery: Potential Applications for Gene and Cell-

Based Therapies in the New Era of Personalized Medicine. Genes & diseases 2017, 4(2), 43–63. doi:10.1016/j.gendis.2017.04.001.
161. Uludag, H.; Ubeda, A.; Ansari, A. At the Intersection of Biomaterials and Gene Therapy: Progress in Non-viral Delivery of Nucleic Acids. Frontiers in bioengineering and biotechnology 2019, 7, 131. doi:10.3389/fbioe.2019.00131.
162. Bougioukli, S.; Saitta, B.; Sugiyama, O.; Tang, A. H.; Elphingstone, J.; Evseenko, D.; et al. Lentiviral Gene Therapy for Bone Repair Using Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells. Human gene therapy 2019, 30(7), 906–917. doi:10.1089/hum.2018.054.
163. Ghosh, M.; Halperin-Sternfeld, M.; Grinberg, I.; Adler-Abramovich, L. Injectable Alginate-Peptide Composite Hydrogel as a Scaffold for Bone Tissue Regeneration. Nanomaterials (Basel, Switzerland) 2019, 9(4). doi:10.3390/nano9040497.
164. Bone, E.; Engineering, T.; Gonzalez-fernandez, T.; Tierney, E. G.; Cunniffe, G. M.; Kelly, D. J. Gene Delivery of TGF- b 3 and BMP2 in an MSC-Laden. Tissue Engineering 2016, 22(1), 776–787.