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# Injecting hope: chitosan hydrogels as bone regeneration innovators

Gayatri Vaidya[a], Sheersha Pramanik[b], Ammar Kadi[c], Ahmed Raheem Rayshan[d], Bassam M. Abualsoud[e], Mohammad Javed Ansari[f], Rehana Masood[g] and Jacob Michaelson[h]

[a]Department of Studies and Research in Food Technology, Davangere University, Davangere, India; [b]Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras, Chennai, India; [c]Department of Food and Biotechnology, South Ural State University, Chelyabinsk, Russia; [d]Department of Physiology, Pharmacology, and Biochemistry, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq; [e]Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan; [f]Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia; [g]Department of Biochemistry, Shaheed Benazir Bhutto Women University, Peshawar, Pakistan; [h]Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai, India

**ABSTRACT**

Spontaneous bone regeneration encounters substantial restrictions in cases of bone defects, demanding external intervention to improve the repair and regeneration procedure. The field of bone tissue engineering (BTE), which embraces a range of disciplines, offers compelling replacements for conventional strategies like autografts, allografts, and xenografts. Among the diverse scaffolding materials utilized in BTE applications, hydrogels have demonstrated great promise as templates for the regeneration of bone owing to their resemblance to the innate extracellular matrix. In spite of the advancement of several biomaterials, chitosan (CS), a natural biopolymer, has garnered significant attention in recent years as a beneficial graft material for producing injectable hydrogels. Injectable hydrogels based on CS formulations provide numerous advantages, including their capacity to absorb and preserve a significant amount of water, their minimally invasive character, the existence of porous structures, and their capability to adapt accurately to irregular defects. Moreover, combining CS with other naturally derived or synthetic polymers and bioactive materials has displayed its effectiveness as a feasible substitute for traditional grafts. We aim to spotlight the composition, production, and physicochemical characteristics and practical utilization of CS-based injectable hydrogels, explicitly focusing on their potential implementations in bone regeneration. We consider this review a fundamental resource and a source of inspiration for future research attempts to pioneer the next era of tissue-engineering scaffold materials.

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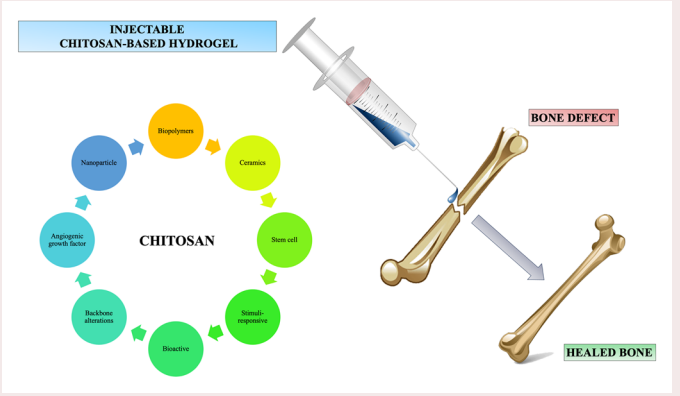
**CONTACT** Sheersha Pramanik sheershopramanik24@gmail.com Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras, Chennai 600036, Tamil Nadu, India; Gayatri Vaidya gay3_pk@yahoo.co.in Department of Studies and Research in Food Technology Davangere University, Karnataka, 577007, India.

**GRAPHICAL ABSTRACT**




## 1. Introduction

The bone holds an exceptional ability to regenerate when injured, and in many examples, fractures can heal inherently when immobilized. Nevertheless, in cases where defects of bone are adequately significant or critical in size, they cannot undergo natural healing *via* normal physiological procedures. These circumstances require intervention in the form of bone grafts. This is specifically true for circumstances like traumatic fractures, fracture nonunions, and segmental defects where there is minimal residual integrity of tissue at the area of the defect. The established/gold standard clinical standards for bone grafting necessitate the employment of autografts, which are derived from another site within the patient's body, and allografts, which are acquired from cadavers and sterilized before utilization. Frequently, additional materials like pins, plates, and bone fillers are necessary to stabilize and assist the grafts in the course of the healing procedure. Although conventional strategies for the repair of bone defects are generally efficient, they come with certain complexities. Morbidity of donor sites and the risk of transmission of disease are usual issues allied with these approaches [1–3]. Therefore, there is a pressing need to explore and research suitable alternatives to address these concerns, thus emphasizing the critical role of bone tissue engineering (BTE). Strategies concerning BTE offer hopeful replacements for autografts and allografts by employing synthetic grafts, usually known as scaffolds, to promote the regeneration of tissue. The vital attributes of an effective synthetic bone scaffold include being osteoconductive, fostering the formation of bone on its surface, and having high porosity to assist nutrient and waste transport, neovascularization, and ingrowth of bone [4,5]. Furthermore, the scaffold should hold adequate mechanical strength to endorse bone ingrowth and preserve structural integrity during tissue remodeling, gradually degrading as bone regeneration advances.

In recent years, there has been substantial exploration of injectable biomaterials-based scaffolds to mimic the natural extracellular matrix (ECM) closely. This procedure is intended to create an environment that encapsulated cells can easily recognize, improving their optimal growth and differentiation. Moreover, these scaffolds are

designed to efficiently retain the encapsulated cells within their intended target areas within the body [6]. Injectable hydrogels, developed as specialized biomaterials, are becoming progressively beneficial in the context of restoration of bone tissue. Their exceptional sol–gel characteristics make them especially suitable for this purpose. There is an increasing demand for the employment of injectable hydrogels as both bulk fillers and as carriers for encapsulated cells/active delivery [7]. Fundamentally, injectable hydrogels can assist as a less invasive or minimally invasive substitute to conventional implant surgery. Furthermore, they possess the ability to fill defect areas that often have unevenly shaped geometries, improving their versatility in tissue repair applications [8].

Over the past few years, several hydrogels using polymers have been prepared and presented as viable options for injectable therapies. Among them, chitosan (CS), a naturally sourced polymer, has found comprehensive utilization as a biomaterial. CS, obtained from the partial deacetylation of chitin, is a natural polycationic polysaccharide with a linear polymeric backbone. Chitin is a structural component found in the exoskeleton of crustacean species such as lobsters, crabs, and shrimps. CS comprises β-(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine repeating units. Its cationic nature allows the formation of multilayer structures or electrostatic complexes with other cationic natural or synthetic polymers [9]. Unlike many synthetic polymers, its hydrophilic surface enables its future use in cell adhesion, differentiation, and proliferation of my tissues [10]. Several fascinating characteristics of CS make it popular in biomedical applications, such as being biocompatible, which avoids any adverse reaction when in contact with living tissue. This is essential in tissue engineering, where hydrogel-based scaffolds must be compatible with the encompassing tissue to enhance the growth and differentiation of cells. It is comfortably degraded inside the body into natural substances, diminishing the chances of long-term toxicity, following which they can be substituted by new tissues over time [11]. CS also displays antimicrobial characteristics, making it an efficient barrier against infections caused by bacteria and fungi. This is especially significant in BTE, where infections can generate substantial problems, for example, implant failure [12]. CS can be utilized to develop hydrogels, which are gel-like frameworks that can be molded or injected into shape to offer a supportive structure for attachment and growth of cells. Hydrogels are usually employed as scaffolds in BTE, and the capability of CS to develop hydrogels makes it a favorable choice in this field. CS has displayed good mechanical characteristics, promoting its utilization as a scaffold material [13,14].

CS is chosen as a biomaterial for hydrogel formation in bone, cartilage, and other tissue engineering purposes because of its unique advantages. CS interacts with the cell surface receptors of injured tissues and encourages cell migration and the extracellular production of proliferating signals. Its significant advantages as a tissue regeneration scaffold stem from its low toxicity, good biocompatibility, biodegradability, and controlled degradation by particular enzymes such as lysozyme. Additionally, CS shares structural similarities to glycosaminoglycans (GAGs), the principal component of bone ECM, and together with its tendency for the formation of soft gels, which can last up to one week in mobile chondral defect sites, CS has emerged as a suitable choice of biomaterial for bone and cartilage repair. However, its main limitation lies in its high crystallinity because of its numerous amino groups, rendering a poorly

aqueous solution of CS. Nevertheless, combining other biopolymers can improve CS's characteristics and increase its clinical utility [15]. CS hydrogel composites are a promising material for CS owing to their combination of biocompatibility, biodegradability, and capability to support the growth and differentiation of cells [10]. The addition of hydroxyapatite or other material can substantially improve the stiffness and strength of the hydrogel, making it more favorable for utilization in repairing and regenerating tissues of bone. Additionally, CS hydrogel composites can also furnish a 3D framework and improve the growth and differentiation of cells that mimic the innate ECM and support its mechanical strength and biochemical signaling to the cells [16].

Though recently published reviews [17,18] discussed about the application of injectable CS hydrogels in BTE, the novelty of our article lies in our unique approach to enlightening the enhanced functionalities of CS hydrogel-based composites in tissue engineering. We distinguish our work by providing a thorough overview of how various components have been strategically incorporated into CS composites to augment their effectiveness in BTE applications. This deliberate integration of diverse elements serves as a unique and innovative aspect of our review, showcasing a delicate understanding of the synergistic effects of these constituents within the context of bone tissue regeneration. Our emphasis on detailing the precise contributions of these added components contributes to the progress of knowledge in the field and offers valuable perceptions for investigators and practitioners engaged in BTE endeavors. The principal objective of this review is to comprehensively examine the current state-of-the-art applications of CS injectable hydrogels in the field of BTE. Additionally, the review concludes with a forward-looking perspective, discussing the developmental prospects and challenges that lie ahead in the realm of CS injectable hydrogels.

## 2. Production of CS

The production of CS includes the deacetylation of chitin the procedure of elimination of acetyl groups from the chitin molecule. The initial stage in the production of CS is the extraction of chitin from its natural origin, for example, the shells of crustaceans like shrimp and crabs [19,20]. These shells are cleaned, ground, and subjected to a chain of physical and chemical treatments to eliminate impurities, for instance, proteins and lipids. The resulting substance is chitin, which becomes the starting material for the manufacture of CS. The second stage is the deacetylation of chitin, which is generally done by treating chitin with an alkaline solution, for example, sodium hydroxide (NaOH), at a controlled temperature and pH. The degree of deacetylation of CS can be governed by modifying the reaction environments, for example, the concentration of the alkali, time for reaction, and temperature. For example, the concentration of alkali, often NaOH, may vary between 40% and 50%, with higher concentrations fostering increased deacetylation. Reaction times can vary from several hours to overnight or longer, depending on the desired degree of deacetylation. Temperatures typically fall within the range of 90–120 °C [21–23]. Also, a higher degree of

deacetylation (generally above 85%) results in a CS with a higher molecular weight and enhanced mechanical characteristics [19,20]. Following deacetylation, excess alkali and other impurities are removed from the CS solution *via* purification. This is commonly done employing dialysis, wherein a semipermeable membrane isolates the CS from the excess impurities and alkali. The purified CS solution is further lyophilized to remove the residual water to obtain a solid CS powder [24]. Figure 1 demonstrates the conventional approach to chitosan production from crustacean shells. In addition to chitosan extraction methods reliant on chemicals [25–27], diverse alternative approaches exist, encompassing biological and enzyme-based methods [28–33], the utilization of ionic solvents [34,35], deep eutectic solvents [36,37], and ultrasound-assisted techniques [38–40].

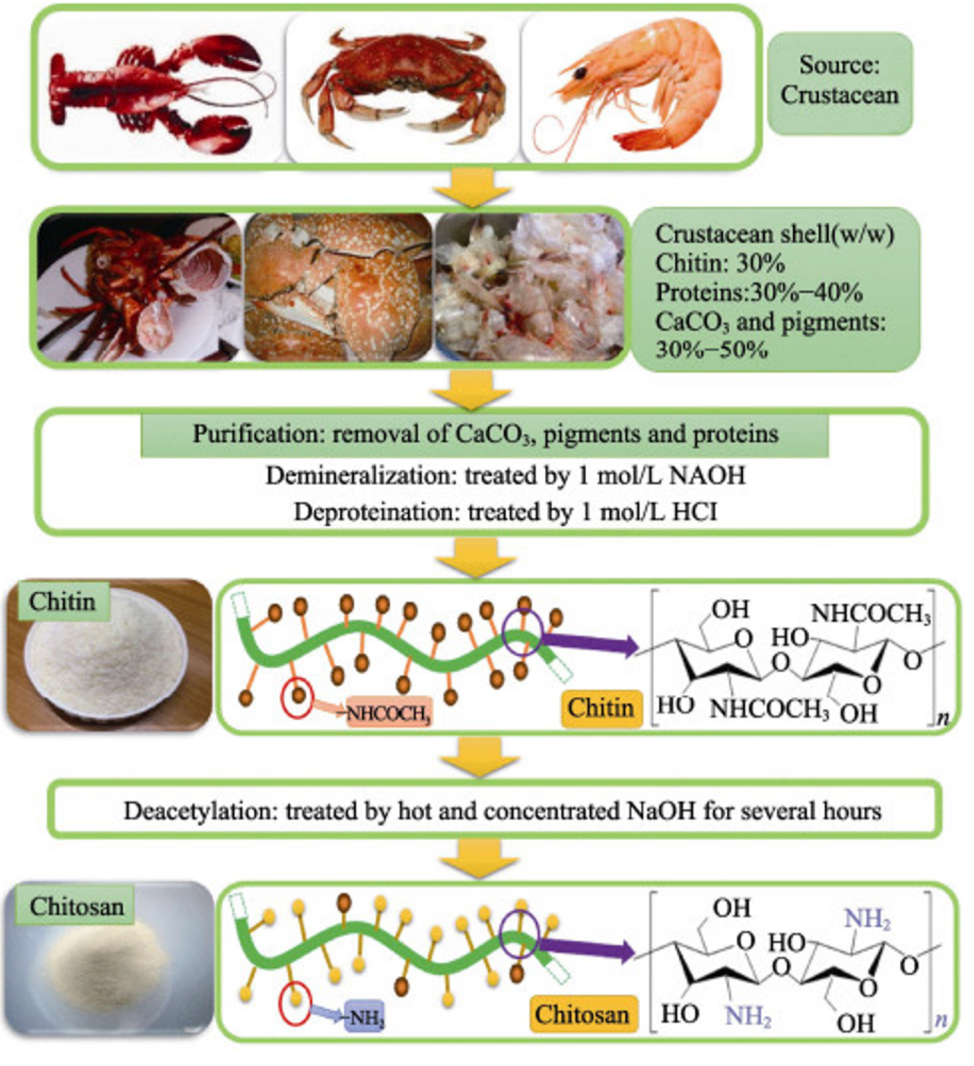



**Figure 1.** Illustration describing the traditional procedure for producing chitosan from shellfish [41].

## 3. Properties of chitosan

CS molecule, derived from chitin, is a linear polymer composed of β-(1,4)-linked D-glucosamine (Deacetylated units) and N-acetyl-D-glucosamine (Acetylated units) units (as shown in Figure 2). The degree of deacetylation (the proportion of deacetylated units) of CS can be controlled through the deacetylation process and varies from 50% to almost 100% [42]. CS shares a chemical structure with cellulose, a polysaccharide frequently found in plants. CS, on the other hand, varies from cellulose in that it contains a variable quantity and orientation of hydroxyl groups. CS has a different chemical makeup than cellulose, making it a cationic polysaccharide, which means it has a positive charge on its surface. The molecule of CS has primary amine groups, which can be protonated (accept hydrogen ions) at a specific pH (less than 6.5), which accounts for its positive charge. Basically, in an aqueous solution, as the pH of the environment becomes acidic, the amine groups on the chitosan molecule tend to readily accept protons, leading to their protonation [43]. The capability of these amine groups to undergo protonation is closely associated with the concept of pKa, which portrays the acid dissociation constant of a substance. In this context, the pKa values are associated with the equilibrium between the protonated form ($NH_3^+$) and the unprotonated form ($NH_2$). The primary amine groups on chitosan become protonated when the pH of the surrounding environment is below the pKa of these amine groups [44]. The chemical properties of chitosan suggest that it demonstrates immiscibility in water due to the absence of protonation under neutral and alkaline pH environments [45]. CS's molecular weight has a significant impact on both its characteristics and potential uses. Commercial CS with a Daltons (DA) of about 80–100% has a molecular weight that ranges from 50–2000 kDa. Through the deacetylation procedure and the kind of CS employed, the molecular weight of CS can be managed (low molecular weight or high molecular weight). CS with a low molecular weight has a mass of fewer than 50,000 DA, whereas CS with a high molecular weight has a mass beyond 50,000 DA [46].

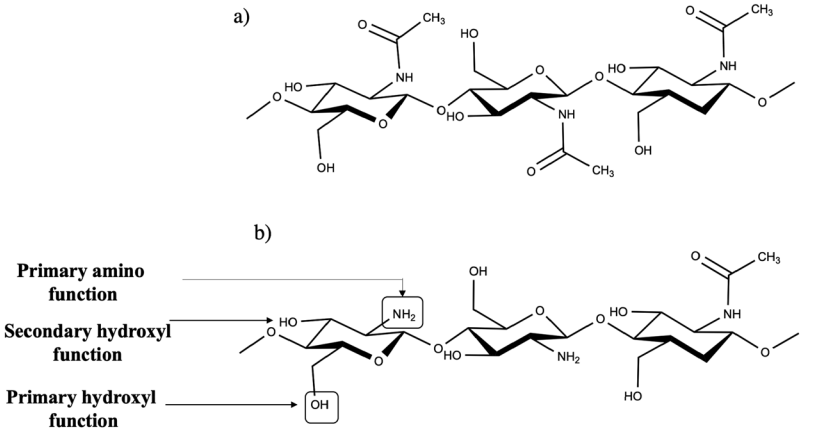



Figure 2. The chemical structure of (a) chitin (b) chitosan.

Another crucial element that influences CS's characteristics and applications is its solubility. CS may dissolve in acidic solutions (acetic acid, lactic acid, hydrochloric acid, propionic acid, phosphoric acid, formic acid, tartaric acid, and citric acid) [47] but not in neutral or basic conditions. This solubility is mediated by the CS molecule's abundance of ionizable amino groups, which can be protonated at a pKa of 6.5, where the polysaccharide gets converted to a polyelectrolyte complex in aqueous acidic media. Beyond a pH of 6.2, neutralization of CS solutions results in hydrogen bonds with other CS molecules and the formation of a gel-like structure that is not water-soluble [48]. The pH of the solution can be changed to alter the solubility of CS, switching it between soluble and insoluble forms. CS has both crystalline and amorphous portions in its structure, making it a partly crystalline polymer. While the amorphous parts of CS are disorganized and less stable, the crystalline regions are highly ordered and stable [49,50].

The properties of CS can be altered by chemical modifications, which can enhance its potential applications in various fields, including biomedical engineering, agriculture, and water treatment [51]. Enhancing the DA of CS to roughly 50% improves its solubility in acidic solvents (for instance, oxalic, acetic, lactic acids, etc.) while reducing the DA decreases its solubility [52]. Deacetylation can be accomplished by treating chitin with an alkaline solution (for example, sodium hydroxide). Quaternization involves the inclusion of groups of quaternary ammonium into CS. Quaternized CS has enhanced water solubility and displayed antimicrobial characteristics. Quaternization can be reached by processing CS with alkyl halides, for instance, methyl chloride. Hydroxylated CS has increased water solubility and can be employed as a wound-healing material. Hydroxylation can be accomplished by treating CS with hydrogen peroxide. Crosslinking includes the generation of chemical bonds between CS chains. Crosslinked CS has enhanced mechanical strength and can be utilized as a scaffold in tissue regeneration. Crosslinking can be attained by treating CS with crosslinking agents, for example, glutaraldehyde or genipin [16]. Mechanical characteristics are the physical features that establish a material's reaction to external pressures, such as stress and strain. The tensile strength of CS relies on the deacetylation degree, molecular weight, and the existence of additives. Usually, CS with a higher degree of deacetylation and molecular weight presents increased tensile strength than CS with a low molecular weight and degree of deacetylation [42]. Moreover, the positive charge on the surface of CS draws cells with a negative charge, those found in the human body. CS's positive charge also aids in safely forming a stable material with negatively charged elements, such as DNA and proteins [16]. CS is biodegradable and has the ability to degrade in various environments, which involves the human body [16].

This material can be used as is or modified to enhance its characteristics for specific applications. For instance, the molecular weight of CS can be improved by crosslinking the CS or with the addition of a co-polymer to the CS solution prior to the purification and lyophilization stages [53]. Additionally, CS can be functionalized by adding functional groups to its molecular structure, such as carboxyl, amino, or hydroxyl groups, to improve its biocompatibility and interactions with other materials [54]. The unique properties of chitosan are mentioned in Figure 3.

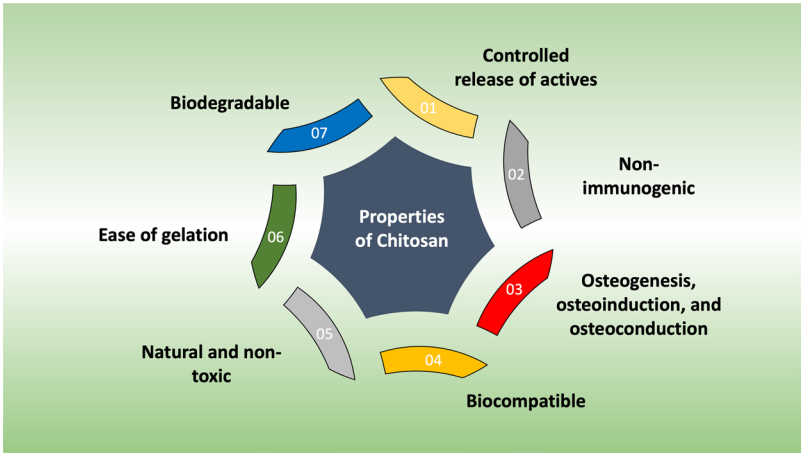



**Figure 3.** The unique characteristics of chitosan.

## 4. Injectable hydrogels

Hydrogels refer to the natural or synthetic, three-dimensional, physically or chemically crosslinked polymeric matrices with inherent hydrophilicity because of their functional groups. They can absorb water from 20% to a thousand times their dry weight. Their distinctive qualities include flexibility, porosity, smoothness, high water content, permeability, biocompatibility, and a high affinity for water and bodily fluids. Hence, hydrogels offer numerous prospects in biomedical and pharmacological applications because these properties are similar to those of many soft biological tissues [55–57]. They may be classified as "physical" and "chemical" hydrogels. Physical hydrogels involve molecular-level arrangements, which can be dissolved by changing the physical conditions such as pH. Whereas, when covalent interactions hold the polymeric chains together with the aid of a crosslinking agent, it is known as a chemical hydrogel.

Injectable hydrogels represent the hydrogels with physicochemical properties compatible with injecting *in situ* in the body, which then converts to an *in situ* solid hydrogel after injection. Hence, shear-thinning biomaterials are suitable for this purpose because they can be injected in the gel state [58].

*In situ* hydrogels are formed by the reaction of two or more components, such as a polymer precursor and a cross-linker, that are mixed together just prior to application. The reaction between these components results in forming a gel-like material, which can be tailored to have specific mechanical, chemical, and biological properties. Different techniques (as shown in Figure 4), both chemical and physical, are utilized to develop injectable hydrogels. Physical cross-linking mechanisms encompass hydrogen bonding, ionic cross-linking, and thermal gelation. Chemical cross-linking procedures for injectable hydrogel development *in situ* are composed of click chemistry, Micheal addition, enzymatic reaction, photo-initiated cross-linking, and Schiff-base cross-linking [59]. Injectable hydrogels display numerous advantages over conventional hydrogels in tissue engineering, which include enhanced injectability, controlled release of therapeutic actives, and improved migration and proliferation of cells. They also diminish the hazard of contamination of bacteria and increase the stability of the hydrogel, making them a

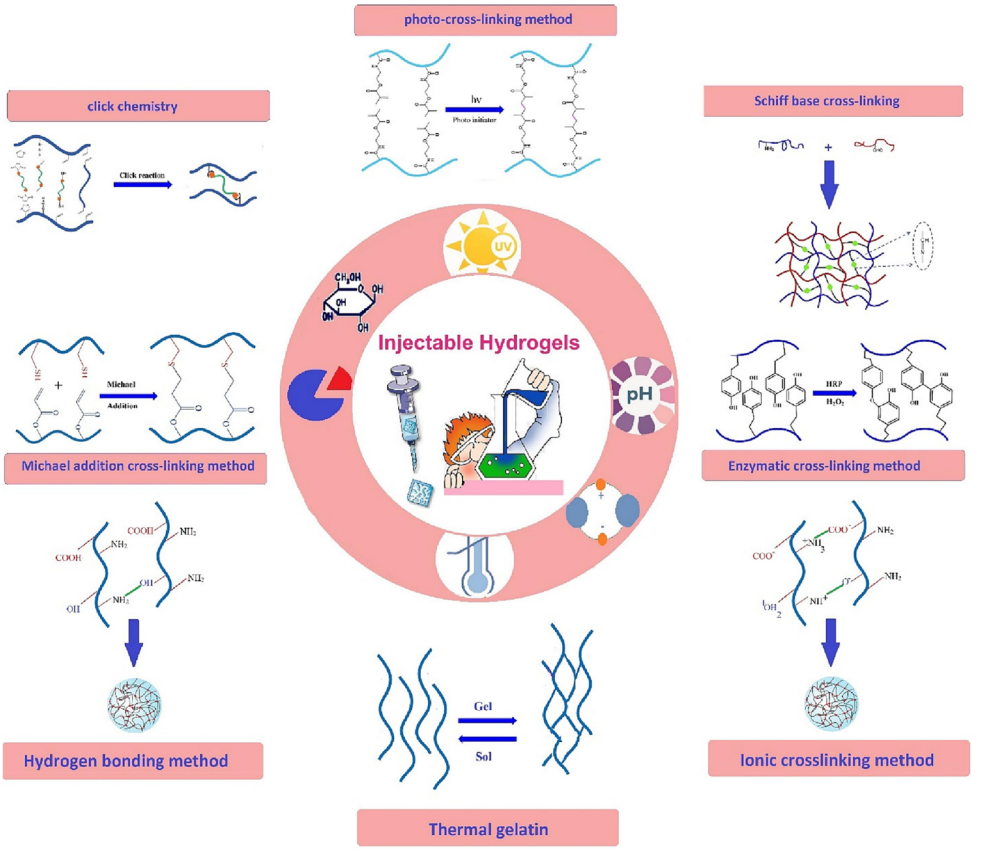



**Figure 4.** Different methods, both chemical and physical, are employed to produce injectable hydrogels, reproduced from [59], copyright 2023, Elsevier.

more feasible alternative for tissue engineering implementations. The mechanical characteristics, swelling behavior, and rate of degradation of injectable hydrogels can be tailored by governing the density of crosslinking, constituents, and kind of polymers. This permits the development of hydrogels with particular characteristics that are favorable for various applications [60,61]. The hydrogel's viscosity impacts diverse aspects of its performance, which includes migration, proliferation, and differentiation of cells. Hydrogels possessing higher viscosity can restrict the migration and proliferation of cells and may hinder cells from reaching their target site. On the other hand, hydrogels with low viscosity can allow for easy cell migration and promote cell proliferation and differentiation. This is particularly important for CS, where cells need to reach and integrate with the surrounding tissue [62].

## 5. Bone tissue engineering

Bone, a dynamic tissue that continuously remodels over the course of a person's life span, protects the body's fragile essential organs while engaging in locomotion. Bone defects can arise from congenital abnormalities in pathological conditions such as

osteogenesis imperfecta, osteopetrosis, cysts or tumors, traumas, and age-related bone disorders [5, 63].

Despite having a great potential for regeneration, severe or critically large bone defects brought on by tumor removal, nonunion fractures, and the correction of congenital deformities frequently require extensive corrective surgery. Clinically, bone grafts are commonly used to fill critical-sized bone lesions and stimulate bone regrowth. The accessibility of donor tissues and donor-site morbidity are the two main issues with autografting, the recommended treatment for addressing bone regrowth [10]. The unavailability of graft materials and surgical sites for autografts in bone tissue injuries has prompted researchers to investigate novel biomaterial composites for tissue regeneration.

BTE is a rapidly developing field that aims to prepare functional and biological substitutes for repairing or substituting injured or diseased bones. The objective of BTE is to create a favorable microenvironment that sustains the growth and differentiation of cells, guiding the establishment of functional and durable tissues of bone. BTE can revolutionize the treatment of injuries and diseases of bone and decrease the dependency on conventional techniques, for example, autografts and allografts. BTE typically involves utilizing scaffold materials, cell sources, and bioactive factors. Scaffold materials furnish mechanical support and a suitable microenvironment for proliferation and differentiation. Cell sources, for example, mesenchymal stem cells (MSCs), are vital for tissue regeneration and repair. Bioactive factors, for instance, growth factors and drugs, can improve the therapeutic effectiveness and promote the proliferation and differentiation of cells [16].

Scaffold materials perform an essential role in CS. They provide mechanical support for tissue regeneration and assist as a matrix for the attachment and growth of cells. Scaffold materials can be categorized into three groups: natural, synthetic, and hybrid materials. Natural materials, for example, collagen, hydroxyapatite, and CS, are biocompatible and biodegradable and have been extensively utilized in CS because they mimic the characteristics of the ECM. Synthetic materials, for example, polylactide (PLA) and polyglycolide (PGA), are also biodegradable but have restricted biocompatibility and poor mechanical characteristics. Hybrid materials, like composite materials from natural or synthetic sources, can overcome the challenges of natural and synthetic materials and furnish better mechanical characteristics and biocompatibility [64].

The critical requirements of scaffolds in BTE are porosity, biocompatibility, mechanical characteristics, biodegradability, and the possibility of surface alterations. Scaffolds must have a porous framework to permit the infiltration of cells, nutrients, and oxygen. Pore size and interconnectivity are substantial factors that impact the development of cells and tissue. The chosen scaffolds should resemble the target tissue's mechanical strength. Scaffold surfaces can be altered to govern the behavior of cells, for example, cell adhesion, differentiation, and proliferation. This is substantial for maintaining the process of tissue engineering and guiding the development of tissue [65].

Scaffolds possessing high porosity can furnish an enhanced surface area for the attachment of cells, fostering cell proliferation and differentiation. On the other hand, scaffolds possessing low porosity can restrict cell attachment, impacting cell growth

and survival. This is specifically essential in CS, where cells are required to attach to the scaffold and generate new tissue. Porosity also influences the supply of nutrients and oxygen to cells, which is vital for the survival and growth of cells [42].

MSCs have been extensively used in CS because they have the capability to proliferate, migrate, and differentiate into bone-forming cells, forming functional and durable bone tissue [42]. Bioactive factors, for example, growth factors and drugs, can improve the therapeutic impacts and enhance the proliferation and differentiation of cells. Growth factors, for example, bone morphogenetic proteins (BMPs), can stimulate the differentiation of MSCs into bone-forming cells and promote the formation of bone. Drugs, like bisphosphonates, can hinder bone resorption and prevent the loss of bone mass. Bioactive factors can be incorporated into scaffold materials or delivered directly to the site of bone injury or disease to enhance the therapeutic effects of CS [66].

The lack of mechanical strength of many scaffold materials, the slow rate of tissue regeneration, and the poor vascularization compromise the tissue's long-term stability and durability [18]. Current bone defect healing techniques lack monitoring features. Hence, smart scaffold materials that can assess the effectiveness of new bone growth are essential. Carbon nanotubes (CNTs) show promise regarding biocompatibility and electrical conductivity. Carbon-based materials such as CNT, graphene oxide, and graphene are increasingly being used along with CS-based hydrogels as reinforcing organic-inorganic hybrid scaffolds owing to their excellent mechanical properties [67]. In a study by Huang et al. by incorporating carboxylated CNT into chemically cross-linked carboxymethyl CS hydrogel, a painless and smart monitoring scaffold for BTE was created. Carboxymethyl CS is one of the most widely used CS derivatives, and it can interact with cells, leading to cell growth and tissue regeneration. It also exhibits enhanced bioactivity and higher solubility in water than pure CS [52]. CNT scaffold (0.5% w/v) displayed better mechanical characteristics while being biocompatible and electrochemically sensitive. Remarkably, the CNT scaffold may compensate for BMP-2 facile deactivation by persistently increasing stem cell osteogenesis and new bone tissue synthesis *via* CNT activities. This study advanced the development of non-invasive and electrochemically sensitive bioinspired scaffolds, an essential step toward developing smart tissue regeneration [68].

## 6. Recent advances in CS hydrogels in BTE

CS-based three-dimensional (3D) scaffolds can be of various types, such as hydrogels, fibers, microspheres, molded macroporous scaffolds, and 3D printed scaffolds [61]. Compared to pre-formed scaffolds and hydrogels, *in situ* hydrogels are advantageous because they fill the complex shapes of defects, reducing surgical operation time and the damaging effects of short muscular retractions [69]. Injectable hydrogels serve a dual purpose: they provide structural support to damaged bone tissue and function as a delivery system for therapeutic agents and cells (as shown in Figure 5). These hydrogels are administered to the site of the defect, where they release the encapsulated biomolecules or cells. Subsequently, they undergo a gelation process triggered by changes in physical and/or chemical properties in response to a stimulus.

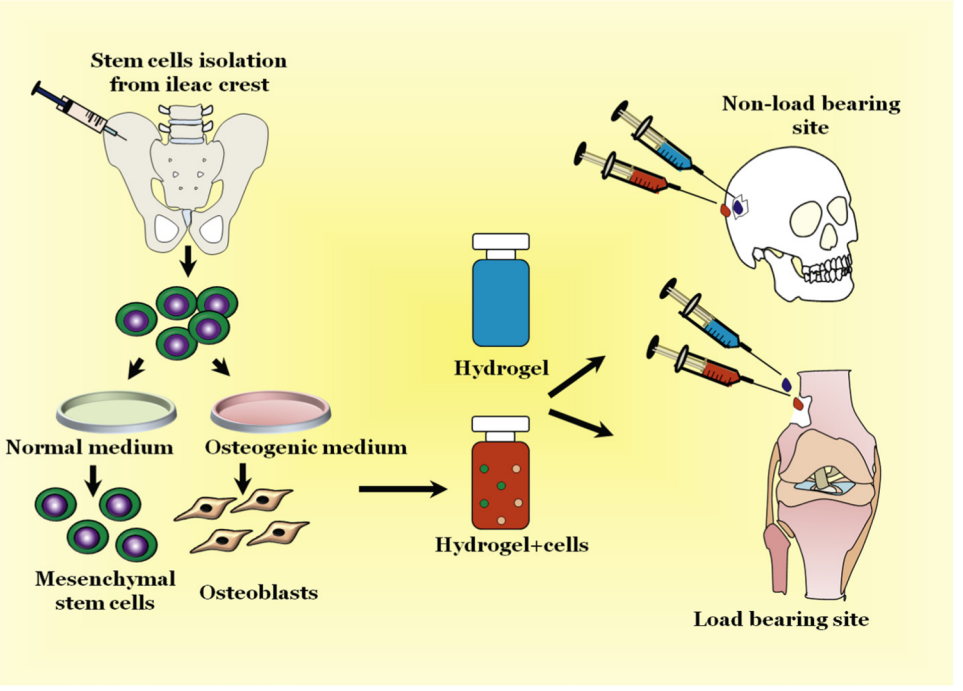



**Figure 5.** Schematic illustration of injectable hydrogel-based strategies for repairing bone defects. Bone marrow MSCs separated from the patient are preserved under a standard medium or under osteogenic stimulants for osteodifferentiation. Both cell-free scaffolds and cell-based scaffolds are employed for treating loss of bone at both non-load-bearing and load-bearing areas, reproduced with permission from [70], copyright 2019, elsevier.

One of the main benefits of CS-based injectable hydrogels is their ability to be efficiently delivered to the location of injury *via* minimally invasive injection. This is especially promising for sites in the body that are complicated to access with conventional surgical procedures [71]. Studies have displayed that CS-based injectable hydrogels can enhance the formation of bone, improve bone density, and advance healing in contrast to traditional therapies, like bone grafts or synthetic materials [61]. For instance, CS hydrogels have been found to stimulate the proliferation of bone cells and accelerate osteogenesis *in vivo* [72]. In spite of these promising outcomes, there are still some hurdles to employing CS-based injectable hydrogels in BTE. One issue is that the mechanical characteristics of CS hydrogels may not be favorable for specific applications, such as large weight-bearing bones. Additionally, it would be advantageous if further studies were conducted to establish the long-term biocompatibility and stability of CS hydrogels in the body.

The latest research on the utilization of chitosan hydrogel in BTE (as shown in Figure 6) has been described in the latter sections.

### 6.1. Composites with other biopolymers

Composites involving chitosan, a versatile biopolymer, and other biopolymers have gained substantial attention in biomaterials research. These composites capitalize on

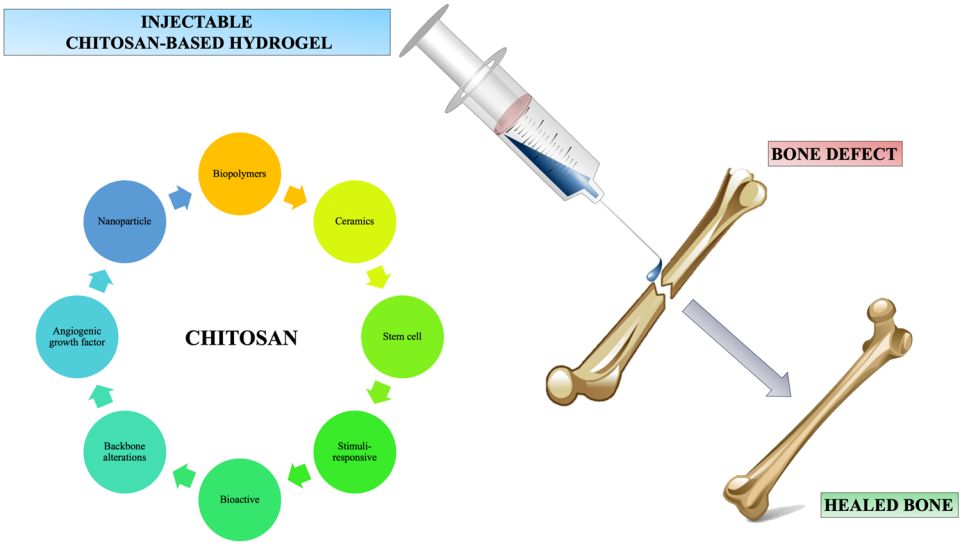



**Figure 6.** Recent progress in the utilization of chitosan-based injectable hydrogel for BTE.

the unique attributes of chitosan and complementary characteristics of other biopolymers to develop materials with enhanced functionalities. Biopolymers such as collagen, alginate, hyaluronic acid, gelatin, and others are often combined with chitosan to form composite structures. The synergistic effects of these combinations result in improved mechanical strength, biocompatibility, and tailored release profiles for various applications.

In a study by Klara et al. a novel bioactive collagen/CS/lysine-functionalized chondroitin sulfate injectable hydrogels was presented. The amine-modified chondroitin sulfate was covalently linked with the hydrogel network, as depicted in Figure 7(i). The authors found that the materials became more hydrophobic with the chondroitin sulfate content. *In situ*, rheological tests demonstrated that the resulting systems were injectable. The biological *in vitro* analysis revealed that all proposed materials were biocompatible, enabling MG-63 cell line growth and adherence. Interestingly, in these modified hydrogels, no additional inducers were required to render them bioactive, and the apatite-like structures were observed in modified hydrogels compared to those observed in neat collagen/CS hydrogels [73]. The study principally focuses on *In vitro* evaluations, and the transition to *in vivo* models is crucial to better understand the hydrogel behavior within living organisms.

CS-based hybrid hydrogels have demonstrated significant promise for bone tissue synthesis and repair. It has been shown that adding polyhedral oligomeric silsesquioxanes (POSS) to the biocomposite matrix enhances the hybrid materials' viscoelastic and biological characteristics. In a study by Celesti et al., a combination of CS and POSS nanoparticles was used to create new nanostructures for repairing bone tissue. The model drug ketoprofen was incorporated into the scaffold during gelling. Figure 7 (ii) showed that the hybrids had more controlled drug releases than CS alone (up to 91% drug release after two weeks). The high biocompatibility of the hybrid materials was confirmed on human fetal osteoblastic cells (hFOB 1.19)

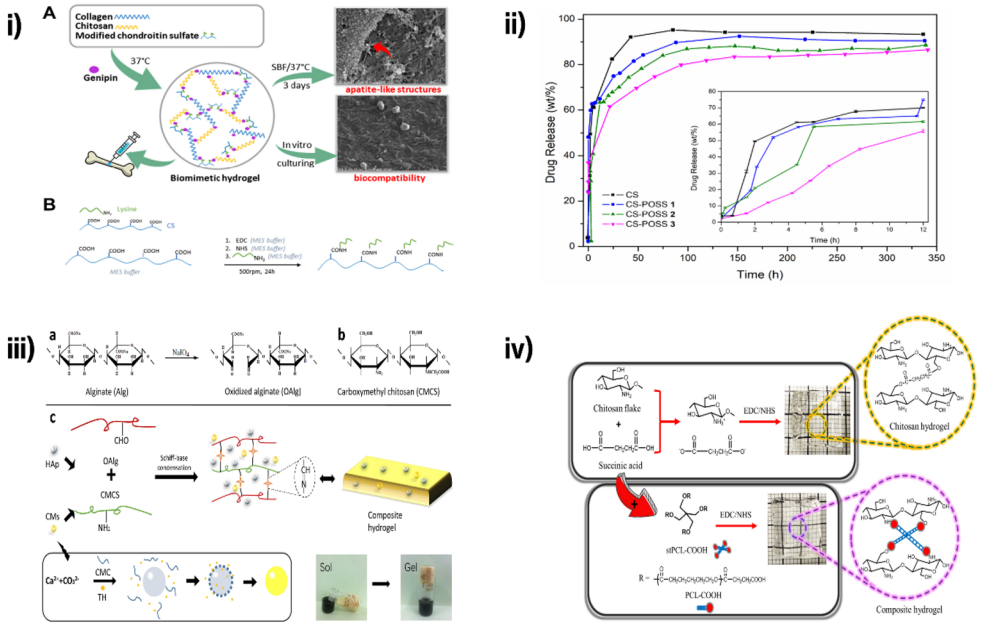



**Figure 7.** i) (A) Detailed schematic of the production of ColChCSmod hydrogels (B) Schematic representation of the lysine-modified chondroitin sulfate production pathway, reproduced with permission from [73], copyright 2022, elsevier. Ii) At varied POSS concentrations, CS and CS-POSS hydrogels released ketamine at 37 °C in PBS (pH 7.4) [74] iii) Chemical compositions of oalg and CMCS, respectively. (c) Graphical view of a composite gel scaffold loaded with tetracycline hydrochloride (TH) using the schiff-base reaction. The scaffold contains hydroxyapatite (hap) and calcium carbonate microspheres (CMs), reproduced from [75], copyright 2018, elsevier iv) The methods involved in making CS hydrogel and (stPCL-COOH/CS) composite hydrogel, reproduced from [76], copyright 2022, elsevier.

cultures. The hybrids revealed cell mortality rates comparable to control cells (11.1 vs. 9.8%) at various POSS contents, indicating that the CS/POSS hydrogels could be used for bone tissue regeneration [74]. The integration of CS and POSS employing diverse conjugation methods and various CS/POSS ratios holds great potential as a prospective avenue for future research. This exploration focuses on advancing the development of biocompatible and biomimetic-engineered scaffolds with applications in drug delivery and the facilitation of bone tissue regeneration.

Injectable hydrogels have gained more popularity in tissue regeneration and local drug delivery applications to suit the evolving needs for bone regeneration. In this context, Ren et al. developed an approach to create an injectable and biodegradable hydrogel based on polysaccharides that are doubly incorporated with calcium carbonate microspheres (CMs) and hydroxyapatite nanoparticles (Hap) at normal physiological conditions. The Schiff-base reaction between the -$NH_2$ and aldehyde groups of carboxymethyl CS and oxidized alginate, respectively, is thought to constitute the route of cross-linking, as shown in Figure 7 (iii). Tetracycline hydrochloride (TH) loaded CMs with a mean diameter of 6.62 µm were simultaneously created by the precipitation procedure. In order to develop injectable gel scaffolds that mimic the bone niche, nano-Hap, and CMs carrying TH were combined with hydrogels made

of polysaccharides to improve their biological and mechanical capabilities. Furthermore, computation of drug release and an assessment of the composite gel scaffolds' antibacterial capabilities indicated excellent sustained drug release. Additionally, the dynamic equilibrium of the Schiff-base reaction imparted the self-healing ability [75].

The search for hybrid CS-based BTE hydrogels with tunable mechanical characteristics and antibacterial properties is ongoing. As shown in Figure 7 (iv), Ekapakul et al. prepared composite hydrogels using star-shaped polycaprolactone and CS with excellent mechanical characteristics and stability by the co-crosslinking of modified stPCL with the carboxylic acid group at the end chain (stPCL-COOH). The findings demonstrated that the composite hydrogels completely disintegrated within two days. Compared to Gram-positive (Staphylococcus epidermidis) bacteria, the hydrogels had stronger antibacterial properties and were more effective against Gram-negative (Escherichia coli) bacteria. After 21 days of testing, calcium and phosphorous preferred to bind to the surfaces of the composite hydrogel to form apatite crystal (Ca/P ratio: 1.86 vs. 1.48 for CS hydrogel) [76].

Besides these studies, numerous other studies involving CS and composite hydrogels that showed promising results were CS/type I collagen/type II collagen/nanohydroxyapatite [77], CS/poly (N-isopropyl acrylamide)/graphene oxide [78], CS/collagen/2-hydroxypropyl γ-cyclodextrin/nanohydroxyapatite/BMP-2/17β-estradiol [79], CS/poly (vinyl alcohol) double-network hydrogel [80], polyaniline/laponite/CS hydrogel [81].

### 6.2. Calcium phosphate (HA, beta HA, alpha tricalcium)/ceramic-based composites

Calcium phosphates such as α-tricalcium phosphate, β-tricalcium phosphate, and hydroxyapatite are usually used as bioactive phases along with CS hydrogels because they mimic the naturally occurring bone minerals (apatite). Several studies demonstrate the improvement in osteogenic induction and differentiation by incorporating calcium phosphates in CS hydrogels [61].

In order to obtain a scaffold mimicking composition found in osteogenic tissue compartments, Reyna-Urrutia et al. prepared a physically stable hydrogel of caprolactone/CS/polyvinyl alcohol/hydroxyapatite (CS/PCL/PVA/HA), followed by lyophilization at −58 °C to obtain a 3D porous scaffold. The highly porous network of these scaffolds promoted the osteogenic differentiation of seeded swine dental pulp stem cells (DPSCs). This two-step procedure ensured that the resultant pore size distribution (50-300 µm) maintained the interconnectivity required to exchange waste and nutrients. Mineralization of calcium deposits was observed in both Dulbecco's Modified Eagle Medium and using the osteogenic MesenCult™ kit, as shown in Figure 8(i) [82].

Despite excellent biocompatibility, the practical applicability of CS-based hydrogels is hindered by the lack of mechanical strength of the hydrogel scaffolds. Kappa-carrageenan is a biopolymer that is biocompatible and capable of forming hydrogels for tissue formation. Developing a bioactive milieu with adequate mechanical characteristics in biopolymer-based composites is difficult in BTE. To this end,

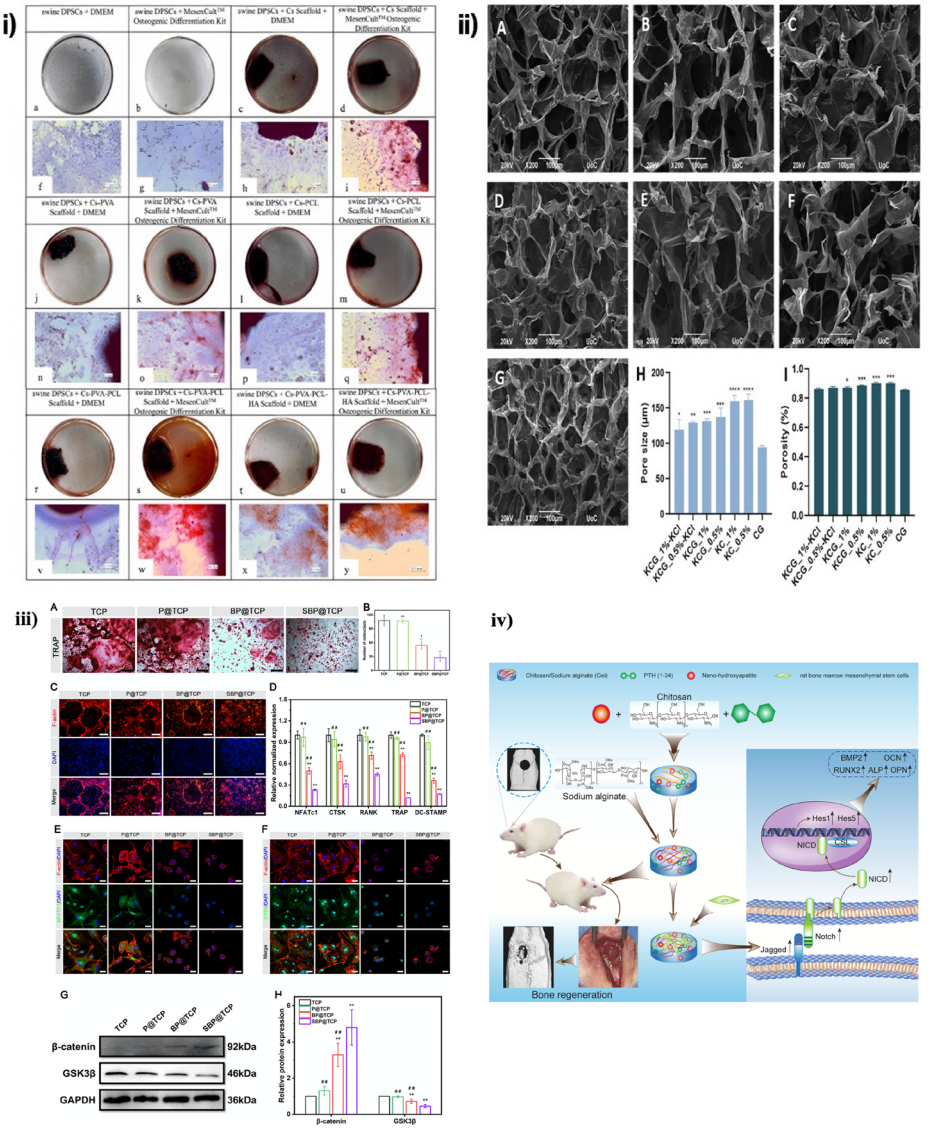



**Figure 8.** i) On day 28, illustrative alizarin red S staining pictures of the mineralization in swine DPSCs (a-d, j-m, and r-u) and microscope images (f-i, n-q, and v-y) [82]. ii) exemplary SEM photos displaying the morphological features scaffolds made of kappa-carrageenan/chitosan/gelatin (KCG) enriched with KCl at (A) 1% and (B) 0.5%, KCG at (C) 1% and (D) 0.5%, KC at (E) 1% and (F) 0.5%, and (G) CG. The scaffolds' porosity and pore size distribution are shown in figs. H and I, respectively, reproduced from [83], copyright 2022, Elsevier iii) The biofunctionalized scaffolds' *in vitro* anti-osteoclastic efficacy. (A–B) Exemplary TRAP staining photos and the outcomes of a quantitative study. (C) Images representing the results of the F-actin ring formation experiment. (D) mRNA expression levels of markers associated with osteoclastogenesis, such as RANK, CTSK, NFATc1, DC-STAMP, and TRAP, following three days of culture. (E, F) Characteristic immunofluorescent staining photos of the osteoclastic CTSK and NFATc1 proteins after three days of culture. Fluorescent green, red, and blue dyes were used to mark the protein of interest, F-actin, and cell nuclei, respectively. (G) Typical GSK3 and -catenin Western blot findings from BMMs co-cultured with different scaffolds. (H) Protein quantification using Western blotting [84] iv) schematic representation of the preparation procedure for the CS/SA hydrogel, loaded simultaneously with parathyroid hormone fragment PTH(1–34) and nano-hydroxyapatite, aimed at promoting cranial bone regeneration through activation of the notch signaling pathway [85].

Loukelis et al. created 3D hydrogel scaffolds out of chemically crosslinked kappa-carrageenan, CS, and gelatin, and these exhibited degradation rates varying from 30% weight loss on day 21, average pore size distribution of 100–160 µm and more than 80% porosity as shown in Figure 8(ii). The *in vitro* biological assessment revealed a substantial proliferation of pre-osteoblasts across all hydrogel scaffold compositions from days 3 to 14. There is a noteworthy increase in ALP activity up to day 14, indicating improved osteogenic potential. Moreover, the consistent rise in calcium production from day 14 to day 28 provided evidence that all hydrogel scaffold compositions efficiently support osteogenic differentiation. Though the *in vitro* study was found promising, the *in vivo* studies are essential to validate the biocompatibility and regenerative potential of these scaffolds within a living organism [83].

In BTE, techniques that concentrate on biofunctionalized implants to accelerate the healing of bone defects through the combined upregulation of angiogenesis and osteogenesis and downregulation of osteoclastogenesis are increasingly receiving interest. The Wnt/-catenin signaling cascade promotes osteogenesis and angiogenesis while decreasing osteoclastogenesis. Unfortunately, literature on producing biofunctionalized bone implants with coordinated osteoclastogenesis, osteogenesis, and angiogenesis by stimulating the Wnt/-catenin system is scarce. In a study by Wu et al., BML-284, a selective Wnt signaling inducer, was loaded on a mussel-like polydopamine surface immobilized on a porous beta-tricalcium calcium phosphate (-TCP) surface and later sandwiched by a biomimetic carboxymethyl CS hydrogel surface. The findings revealed that prolonged BML release from the hybrid surface improved MC3T3-E1 cell adhesion and osteogenic differentiation, in addition to the angiogenesis induced by human umbilical vein endothelial cells and also reduced osteoclastic activity, as depicted in Figure 8(iii). Surprisingly, in a critical-sized cranial defect model, the beta-TCP scaffold triggered M2 macrophage polarization, recruited endothelial cells and endogenous stem cells at the injury site to create a desirable microenvironment for promoting osteogenesis and vascularization along with impeding osteoclastogenesis, consequently enhancing bone restoration [84]. This experiment introduces an environmentally friendly, straightforward, and cost-efficient surface modification approach. The noteworthy biological functions achieved by the intelligently designed cell-instructive TCP implants hold substantial promise for clinical translation.

In a different investigation, Zhou and his team reported on cranial bone regeneration facilitated by hydrogels incorporating parathyroid hormone (PTH) peptide PTH(1–34) and nano-hydroxyapatite (nHAP). The hydrogels, formed from the combination of positively charged chitosan (CS) and negatively charged sodium alginate (as depicted in Figure 8 (iv)), displayed porous structures observed through scanning electron microscopy. Rheological characterizations indicated that the mechanical attributes of the hydrogels remained almost unchanged with the addition of nHAP and PTH(1–34). *In vitro* studies demonstrated good biocompatibility of the hydrogel containing nHAP and PTH(1–34), fostering osteogenic differentiation of rat bone marrow mesenchymal stem cells (rBMSCs) *via* the Notch signaling pathway, as evidenced by increased expression of osteogenic-related proteins. Higher PTH(1–34) content in the hydrogels correlated with enhanced BMSC osteogenic differentiation. Implanting the complex hydrogel into a rat cranial defect model resulted in effective bone regeneration in contrast to rats treated with the hydrogel alone or with nHAP, highlighting

the synergistic therapeutic effect of nHAP and PTH. Both *in vitro* and *in vivo* results suggested the simultaneous incorporation of nHAP and PTH into hydrogels as a promising approach for bone regeneration, offering new avenues for tissue engineering and regeneration [85].

In a study by Min et al., ABG/CH-CY composite hydrogels were created by combining CS-cysteine (CS-CY) conjugate having a desired thiol content with amine-functionalized mesoporous bioglass NPs with radially porous morphology. In addition to the network created by the self-crosslinking of thiols in CY-specific side chains, other connections were developed in the composite gels by crosslinking amino groups on CH-CY or ABG NPs using difunctionalized PEG (DF-P) crosslinkers and varying the lengths of PEG portions. In order to achieve sustainable and regulated quercetin release from the hydrogels, quercetin was encapsulated into ABG NPs prior to integrating these NPs into the hydrogel. It was observed that the elasticity of these hydrogels was significantly impacted by PEG fragment lengths. ABG/CH-CY hydrogels demonstrated mechanically robust and elastic properties by having elastic moduli of about 8.2 kPa. These gels also showed the capacity to release Ca, Si, and/or quercetin ions in controlled manners over time. The best-possible ABG/CH-CY hydrogels could be injected and capable of promoting the expansion of MC3T3-E1 cells and forming a given matrix [86].

In addition, other studies with ceramic-based CS hydrogels for BTE displaying favorable results were CS/carboxymethyl cellulose/hydroxyapatite hydrogels/thyroxine [87], coralline HA/silk fibroin/glycol CS/difunctionalized polyethylene glycol [88], CS/sodium alginate hydrogel/parathyroid hormone/nano HA/peptide PTH (1–34) [85], CS/dextran hydrogel/strontium-doped hydroxyapatite [89], HA/zirconia composite/gelatin/CS hydrogel/BMP-2/bone MSCs [90].

### 6.3. Stem-cell encapsulated CS hydrogels

Stem cells, particularly MSCs, are generally used in regenerative medicine because of their self-renewal and differentiation ability. MSCs can locate inflammatory and damaged tissue with their homing properties. They also secrete certain exosomes carrying growth and differentiation signaling molecules, which further aids in improving healing. MSCs are crucial for bone repair and osteogenic differentiation after bone injury. Inflammatory cytokines such as IL6, IL1B, TNFa, and IL-17 are secreted after a hematoma forms following bone injury, attracting immune cells to the bone lesion and creating an inflammatory microenvironment. Therefore, stem cell-encapsulated hydrogels are often used in CS applications because they can help maintain the viability of cells and support cell growth [91].

One of the leading causes of clinical transplant failure in tissue regeneration is inadequate oxygen supply. Creating spontaneous and persistent oxygen-carrying scaffolds for *in situ* tissue repair can circumvent this restriction. In a study by Huang et al., a flexible hydrogel incorporating fluorine was developed that provided oxygen to MSCs when they needed it most—before they vascularize scaffolds—but also exhibited enhanced antibacterial properties to prevent pathogenic bacteria. By photo-crosslinking, the CS/HAp@PDA-F hydrogels were created by preparing the

HAp@PDA-F nanoparticles and combining them with methacrylated and quaternized CS. The hydrogels had exceptional mechanical properties and the oxygen-carrying capacity to extend the survival of rat bone-derived MSCs under hypoxic conditions, increase the proliferation and osteogenic differentiation potential, and stimulate the expression of genes related to osteogenesis. Furthermore, *in vivo* research showed that the CS/HAp@PDA-F group generated more native bone tissue than the CS/HAp@PDA group in terms of mineral density and bone volume [72]. Hence, CS-based hydrogel scaffolds could be treated as outstanding carriers for repairing bone defects *in vivo via* stem cell transplantation.

Furthermore, several studies have utilized stem cells-loaded injectable CS hydrogels showing promising results for BTE, for example, CS/β-glycerophosphate/MSC-derived small extracellular vesicles [92], CS/hyaluronic acid hydrogel/adipose tissue-derived MSCs/chondrocyte extracellular vesicles [93], CS/gelatin hydrogel/human bone marrow-derived MSCs [94], CS/poly (vinyl alcohol) hydrogel/bone marrow-derived MSCs [95], hyaluronic acid-g-chitosan-g-poly(N-isopropyl acrylamide) hydrogel/biphasic calcium phosphate microparticles/rabbit adipose-derived stem cells [96].

### 6.4. Stimuli-responsive CS hydrogels

Stimuli-responsive hydrogels demonstrate a dynamic class of biomaterials designed to respond to external triggers, enabling controlled drug release and tailored therapeutic interventions. These hydrogels can be engineered to respond to diverse stimuli such as pH, temperature, light, or specific ions, allowing for precise modulation of their properties [97]. pH-responsive CS hydrogels, for instance, undergo conformational changes in response to variations in acidity, facilitating drug release in specific physiological environments.

The high tensile characteristics of bone must be met by an optimal scaffold structure for BTE. The outcome of the cell is determined by the elasticity of the scaffold ECM through cell attachment and differentiation in both *In vitro* and *vivo*. Wasupalli et al. investigated the impact of hydrothermal treatment on polyelectrolyte complex fibrous biopolymers. The capacity of the thermal treatment to alter how HAp interacts with polymeric PEC fibers was discovered by FTIR research. According to FESEM, the interconnectivity and pore size increased with temperature (control: $82.38 \pm 12.92\,\mu m$; at 120 °C: $335.48 \pm 85.10\,\mu m$). The hydrogel scaffolds heated to 90 °C demonstrated the maximum stiffness in both dry and wet conditions (dry condition: $1.82 \pm 0.07$ MPa, wet condition: $122 \pm 1.78$ kPa). Furthermore, the aqueous stability and swelling capability were both increased by the crosslinker-free gelation process. Scaffolds built at 90 °C enhanced higher MG63 cell adhesion, multiplication, and differentiation [98]. Thus, mechanical testing in an *in vivo* environment helps us understand hydrogel scaffolds' mechanical properties when implanted in human patients.

To achieve the desired healing or restoration of injured tissue or organs, an ECM simulating a 3D microenvironment is necessary. In this situation, hydrogels might be able to produce the ideal 3D milieu. Their applicability is restricted by the limitation of mechanical properties. In a study by Wasupalli et al. thermosensitive injectable hydrogels premised on CS and polygalacturonic acid were created. The authors

presented a technique for creating innovative, bioinspired polymeric injectable hydrogels with hydrothermal aided hydrolysis. Combining hydroxyapatite and glycerophosphate (GP) improved the hydrogel's biocompatibility and rate of gel formation. At 37 °C, the artificial liquid composition could gel. The hydrogels containing gelatin-integrated and hydrothermally processed PEC fibers appeared to have better cellular responses to different hydrogels produced during a 14-day culture [65].

In the biomedical industry, particularly for pharmaceutical delivery systems, thermoresponsive nanoparticles with phase transformation capabilities have been deemed appropriate materials. Furthermore, for bone tissue repair, electroactive injectable hydrogels aiding geriatric osteogenesis will be significantly wanted. In a study by Zarrin et al. thermoresponsive nanoparticles were added to CS/poly(N-isopropyl acrylamide) electroactive injectable hydrogels made of aniline pentamer, silk fibroin, and polyacrylamide with vitamin C. Simvastatin acid released at higher rates at 37 °C than at 23 °C without experiencing any notable burst release, indicating that the nanoparticles showed thermosensitive characteristics. The hydrogels also demonstrated good biodegradability, high water absorption, stable mechanical and rheological properties, and an optimum gelation duration and were nontoxic and biocompatible. At 4 and 8 weeks after implanting the hydrogels into the critical calvarial bone fracture of elderly rats, significant improvements in bone healing were seen [64]. Similarly, electroactive CS-based hydrogels have been developed for vascular [99] and muscular tissue regeneration [100].

CS can be modified with other biopolymers to improve its poor mechanical strength. Besides calcium phosphates, bioactive glasses can be used as biofillers in CS-based hydrogel scaffolds because they increase cellular response and induce osteogenesis by chemically interacting *in vivo* and forming a calcium phosphate layer. Their addition to CS-based hydrogels enhances the mineralization and metabolic activities of cells [101]. Wu et al. observed that injectable hydrogels were prepared by loading copper-based bioactive glass nanoparticles into CS/silk fibroin/glycerophosphate composites and underwent rapid gelation when exposed to physiological pH and temperature. These cell-free systems delivered Si, Cu, and Ca ions within their safe doses in a controlled fashion. MC3T3-E1 cells and human umbilical vein endothelial cells showed improved growth in the hydrogel group. *In vivo* studies on a critical-sized rat calvarial bone defect showed that the optimized hydrogels restored bone defects with vascularization and mineralization observed during the eight weeks. These results suggested that the newly developed hydrogels hold substantial potential for clinical translation in the context of bone repair and regeneration applications [102].

Similarly, pH-responsive and thermos-responsive coupled CS hydrogels have also been fabricated [103,104]. The pH of the surrounding environment affects how effectively CS hydrogels respond to the protonation and deprotonation of its primary amine $NH_2$ groups. The protonated -$NH_2$ group in CS results in electrostatic repulsion at lower pH, allowing polymer chains to extend and contact with water molecules more readily, facilitating water solubility. The -$NH_2$ group in CS is deprotonated when the pH exceeds the pKa value, which involves no net charge, and it also weakens the spherical structure of CS and increases its water-insoluble nature. As a result,

the pKa value of CS determines how sensitive to pH it is in water. As a result, a CS hydrogel's swelling and shrinking conduct depends on the pH of its surroundings.

Other thermosensitive and pH-sensitive CS hydrogels developed for BTE showing promising results are carboxymethyl CS/sodium alginate nanoparticles/nanohydroxyapatite/Pluronic F127 [105], quaternary ammonium CS/carboxylated CS NPs/vancomycin [106], CS/collagen/single-walled CNTs [107], CS/gelatin/β-sodium glycerophosphate/aspirin/erythropoietin [108], photoresponsive CS methacrylate/graphene oxide hydrogel [109].

### 6.5. Bioactive-loaded CS hydrogels

These hydrogels are used to deliver drugs or other therapeutic agents directly to the site of bone injury or disease. The drug is either incorporated into the hydrogel or adsorbed onto its surface. Therapies for critical-size bone abnormalities are still challenging to find. Since it contributes to stimulating osteogenic differentiation, the glycogen synthase kinase three inhibitor 6-Bromoindirubin-3′-Oxime (BIO) is a possible alternative for treating these abnormalities. In order to encourage osteogenic differentiation, BIO is added to a novel guanosine diphosphate cross-linked CS hydrogel scaffold in a study by Agnes et al. Using 13C NMR, the incorporation of BIO was verified by a unique, concentration-dependent signal at about 41 ppm. Both the stability of the internal structure and the quick gelation rate were preserved. The control scaffold's microstructure was sustained by the 10 μM BIO dosage, showing appropriate porosity and a low closed pore fraction. Using C2C12 cells as a proof-of-concept, researchers discovered a dose-dependent BIO release on the initial osteogenic differentiation of MC3T3-E1 cells and C2C12 cells, as shown in Figure 9(i) [110]. While this proof-of-concept study has emphasized the positive impacts of the new formulation on early-stage differentiation and osteogenic gene expression, additional investigation is needed to explore the dosage response and confirm BIO's supplementary pro-osteogenic role in BMP signaling. Utilizing a cell source like adipose-derived stem cells in future studies would improve the comprehensiveness of the investigation. Furthermore, it is essential to conduct a release kinetics study for BIO as the scaffold degrades, understanding how its gradual release impacts the differentiation of co-encapsulated cells and osteoprogenitor cells in the surrounding bone environment.

Ongoing efforts to develop drug delivery strategies for creating hydrogel scaffolds are considered potentially favorable for BTE. Several CS-based composite scaffolds demonstrate good biocompatibility as well as osteoconductivity, but to improve the osteoinductivity, either cytokines or trace elements are loaded in the matrix [111]. Zhu et al. created an acetylsalicylic acid (ASA)-loaded double-network (DN) hydrogel with CS and methacrylated gelatin (GelMA). A DN hydrogel with an appropriate porosity and acceptable mechanical characteristics was formed by combining physical entanglement, electrostatic attraction, and chemically cross-linked methacrylated gelatin (GelMA) framework. A sustained ASA release from DN hydrogels contributed to anti-inflammatory and osteoinductive capabilities, as shown in Figure 9(ii), and these matrices could be used for culturing adipose tissue-derived stromal cells (ADSCs) [112]. This discovery may offer a potential avenue for developing translational ASA

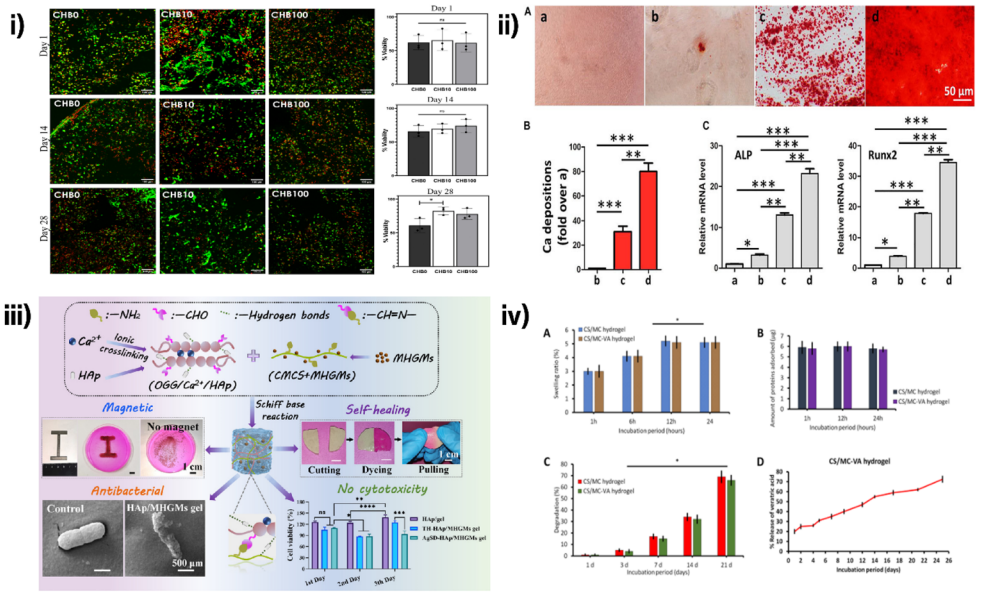



**Figure 9.** i) The cell survival over 28 days was shown by MC3T3 cells stained with live/dead (calcein/ethidium bromide) dye in CS sponges with various BIO contents. Additionally, the percentage of living cells determined from stained images is provided, reproduced from [110], copyright 2023, elsevier. ii) Impact of GelMA-CS@ASADN hydrogels on bone formation. (A) Alizarin red-based osteogenic differentiation analysis. Calculating the amount of calcification deposits. (C) ALP and Runx2 osteogenic markers mRNA analysis. In contrast to (a) untreated cells, (b) GelMA-CS hydrogel, (c) GelMA-CS@ASA hydrogel (ASA, 10 μg/ml), and (d) GelMA-CS@ASA hydrogel (ASA, 100 μg/ml), there are statistically significant changes [112]. iii) Injectable, self-healing, magnetic hydrogels prepared by dual cross-linking for bone regeneration and drug delivery, reproduced from [113], copyright 2022, elsevier iv) physical evaluation of the chitosan/methylcellulose and chitosan/methylcellulose/veratric acid hydrogels. (A) CS/MC and CS/MC-VA hydrogels swelling(B) 1% FBS-derived B protein-mediated adsorption of CS/MC and CS/MC-VA hydrogels (C) Hydrogels' degradation profile (D) VA release profile from CS/MC-VA hydrogels, reproduced from [114], copyright 2023, springer.

formulations and developing multifunctional tissue engineering scaffolds with controlled drug delivery in the future.

In the realm of bone healing, injectable hydrogels centered on functional biomaterials have advanced quickly. In a study by Chen et al. a self-healing, injectable CS hydrogel was created for bone tissue regeneration which was constructed from calcium pre-crosslinked oxidized gellan gum (OGG) and carboxymethyl CS (CMCS), cross-linked *via* the Schiff-base reaction. As a result of emulsion cross-linking, magnetic hydroxyapatite/gelatin microspheres (MHGMs) were generated. Tetracycline hydrochloride and silver sulfadiazine, two antibacterial medications, were incorporated into the MHGMs. Hydroxyapatite (HAp) and drug-loaded MHGMs were combined to create composite hydrogels, which improved the tensile and biological characteristics of the hydrogels, as depicted in Figure 9(iii). The authors found that hydrogels containing 6% (w/v) HAp and 10% (mg/mL) MHGMs had excellent magnetic responsiveness, injectability, and self-healing. It demonstrated 38.8% reduced gelation time (196–120 s), 65.6% reduced swelling (39.4–13.6), 51.9% higher residual mass following degradation (79.5–120.8%), and 143.7% increased maximum compressive stress when compared to

the pure hydrogel (53.6 to 130.6 KPa). It also demonstrated effective antibacterial activities against E. coli and S. aureus and good drug-delaying capabilities. After three days, the CCK-8 experiment revealed that composite hydrogel still preserved significant cell survival (> 87%) and rapid cell multiplication, suggesting that these smart hydrogels could be an alternative strategy for drug delivery [113].

The addition of phytoconstituents has been shown to alter bone metabolism by activation of the osteogenic signaling pathways. In this vein, Durairaj et al. encapsulated veratric acid (VA), a derivative of protocatechuic acid, into thermoresponsive hydrogels composed of CS and methylcellulose. The integration of VA in the system was confirmed by the molecular analysis of the CS-specific MC decamer with VA. They observed a mean degradation rate of 69% and a VA release of 72.5% after 25 days, as shown in Figure 9(iv). The hydrogels were biocompatible with mouse MSCs, and VA induced osteogenic differentiation in the stem cells [114].

The two fundamental factors that contribute to the inability of bone regeneration are a delayed inflammatory response and inadequate osteoblast formation. As a result, aspirin and bone morphogenetic protein 2 (BMP-2) were delivered sequentially in a study by Wan et al. using a dual-responsive hydrogel composite made of near-infrared (NIR)-light-sensitive polydopamine-coated magnesium-calcium carbonate microspheres and a thermoresponsive hydroxybutyl CS hydrogel, and these hydrogels effectively reduced early-stage inflammation and expedited the progress to the regeneration phase by promptly releasing aspirin. Then, BMP-2 release is enabled in a manner responsive to NIR light, maximizing its osteoinductive benefits. The hydrogel's consecutive and regulated release is shown to encourage the development of new bone tissues using an SD rat calvaria-defect model [115].

In addition, several studies reported the promising applications of drug-loaded CS hydrogels in BTE such as 30B nanoclay/layer double hydroxide nanofiller/CS hydrogel/tetracycline hydrochloride [116], CS/glycerophosphate/gelatin hydrogel/rosuvastatin [117], CS/glycerophosphate hydrogel/graphene oxide [118], CS/poly(ethylene glycol) semi-interpenetrating network composite hydrogel/aspirin [119], CS/β-glycerophosphate hydrogel/quercetin [120].

### 6.6. Backbone alterations

To be used as BTE scaffolds, CS must be soluble and show thixotropic behavior. Saekhor et al. modified CS with carboxymethyl chloride, and the resultant carboxymethyl CS was conjugated with α-cyclodextrin. This conjugation improved the aqueous solubility and exposed the crosslinking groups to form an inclusion complex with polyethylene glycol. The sol-to-gel transformation of the modified-CS hydrogel was observed within 450 ± 10 min [121].

Nanolayered silicates have attractive benefits in biomedicine because of their chemical inertness, high specific surface area, and good biocompatibility. Clay minerals such as montmorillonite, halloysite, and kaolinite allow the delayed release of drugs owing to their surface charge. They also have attractive applications in tissue engineering [122]. Hakimi et al. prepared a CS-polyethylene oxide (CS-PEO)/nano clay-alginate (NC-ALG) nanofiber/hydrogel using a layer-by-layer approach of

NC-ALG and CS-PEO. This organic-mineral hybrid scaffold was compatible with MC3T3-E1, and incorporating NC particles enhanced the bone-like apatite formation [123].

Other examples of backbone alterations that demonstrated favorable outcomes are carboxymethyl chitosan/mesoporous silica/clindamycin [124], calcium sulfate hemihydrate/carboxymethyl chitosan/nanohydroxyapatite/vancomycin [125], carboxymethyl chitosan hydrogel/bone marrow-derived stem cells/BMP-2 [126], oxidized alginate hybrid hydroxyapatite nanoparticles/carboxymethyl chitosan hydrogel [127], N, O-carboxymethyl chitosan/fucoidan conjugate [128].

### 6.7. Angiogenic growth factor-encapsulated CS hydrogels

Angiogenic growth factor-encapsulated CS hydrogels represent a specialized class of biomaterials designed to foster angiogenesis, the formation of new blood vessels. By incorporating angiogenic growth factors such as vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF) into CS hydrogels, these materials can stimulate and improve blood vessel formation. The controlled release of angiogenic factors from the hydrogel matrix creates a microenvironment conducive to vascularization, which is essential for tissue regeneration and wound healing.

Modern minimally invasive cell therapy techniques have included the study of biologically active injectable composites to stimulate angiogenesis during bone repair. In a study by Kocak et al. *in situ* injectable composite hydrogels using CS, hydroxyapatite, and heparin were gelled at 37 °C and showed promising pro-angiogenic properties. Adding glycerol and producing them in a pre-sterile environment preserved the homogeneity of the solution and permitted an additional pH increase during neutralization; this significantly increased the mechanical properties of hydrogels by synergistic effects of pH increase and more hydrogen bonding brought on by the addition of glycerol. At 37 °C, incipient gelation periods for the hierarchical interlocking pore topology hydrogel solutions were just 2 to 3 min. These solutions exhibited more elastic flow characteristics. Heparin produced a coating on the top of the hydrogels, contributing to anionic bioactive surface characteristics. In comparison to basic CS hydrogels, CS-matrixed hydrogels containing hydroxyapatite and modest amounts of heparin (33 g/mL) significantly increased angiogenesis, according to the chick chorioallantoic membrane (CAM) experiment [129].

A key issue in clinical settings is the high-efficiency healing of severe bone deformities. Unfortunately, the majority of biological substitute materials do not concurrently meet the demands of biocompatibility and mechanical strength. In a study by Zhang et al., CS methacryloyl (CSMA) and -tricalcium phosphate (-TCP) were photocrosslinked to create a CSMA/-TCP composite hydrogel with significant material characteristics that aid in bone replacement. Additionally, its scaffold can modify the appearance of bone marrow mesenchymal stem cells (BMSCs), encourage growth, boost ALP expression, and increase nodular calcium deposition. ALP, osteocalcin, and osteopontin expression levels were raised simultaneously, and the Hippo signaling pathway's regulation mechanism was confirmed [130]. Nevertheless, as a biomaterial addressing clinical hurdles, current studies are not yet sufficient. Optimization of

parameters like the diameter and porosity of the complexes, which can influence cell morphology, requires further investigation. Additionally, a deeper understanding of *in vivo* regulatory mechanisms of the Hippo signaling pathway to induce osteogenesis is crucial for future research.

In order to fully elucidate the clinical potential of CS hydrogel scaffolds, they must pass through the benchmark of *in vivo* studies. In a study by Jiang et al. sulfated CS (SCS)–based hydrogel scaffolds were prepared to expedite bone tissue regeneration and improve angiogenesis, with enhanced presentation of epithelial progenitor cells and immune cells in this milieu. *In vivo* investigations revealed that SCS, in concert with BMP-2 and MSCs, had a crucial physiologic impact on angiogenesis [131].

Similarly, other studies based on angiogenic growth-factor loaded injectable hydrogels are CS biguanidine/carboxymethylc cellulose/VEGF/r-BMP-2 [132], 2-N, 6-O-sulfated chitosan/mesoporous bioactive glass/rh BMP-2/VEGF [133], CS/nHA hydrogel/PLGA microsphere/BMP-2/VEGF [134], CS biguanide/carboxymethylcellulose/BMP-2/VEGF [132].

### 6.8. Nanoparticle-loaded CS hydrogels

Nanoparticle-loaded CS hydrogels demonstrate a versatile and favorable platform in the field of tissue engineering. These hydrogels, composed of natural polymer chitosan, are designed to encapsulate and deliver nanoparticles [135], offering a tailored and controlled release of therapeutic agents. Socrates et al. reported an increase in tensile strength by 4.6-fold and a 2.2-fold greater surface calcium phosphate mineralization using silver nanoparticles-loaded fibrillar collagen-CS hydrogels with the incorporation of CS. These fibrillar composites exhibited a layer-like native fibrillar arrangement. These hydrophilic hydrogels were found to be hemocompatible and biocompatible and showed antibacterial efficiencies against *E. coli* and *S. aureus* owing to silver nanoparticles [136].

In a separate study, Chen et al. improved the angiogenic, mechanical, and osteogenic properties of injectable CS hydrogels by incorporating MgO nanoparticles into phosphocreatine-functionalized chitosan (CSMP) aqueous solution through the formation of injectable hydrogels *via* supramolecular combinations between magnesium and CSMP's phosphate groups. Lyophilization was used to form the water-soluble CSMP by grafting phosphocreatine and methacrylic anhydride. The pore size range in the lyophilized hydrogels was between 50–100 µm. No brittle fracture was observed for CSMP-MgO hydrogels (compressive strength 195.0 kPa) during compression, even at >85% strain in comparison to the CSMP group (28.0 kPa). Moreover, a controlled release of Mg2+ ions was observed. Osteogenic differentiation of MC3T3-E1 cells was promoted with upregulation of OPN, BSP, and Osterix osteogenic gene expression and mineralization. In addition, critical-sized calvarial bone defects in rat models showed healing when injected with CSMP-MgO hydrogel [137]. As reported, the researchers will further investigate the stability, including the mechanical attributes of this innovative injectable hydrogel, as it is crucial for bone-related cell growth. Despite obtaining the phenotype of the osteogenic and angiogenic properties of this hydrogel, the novel combination of unique physical-co-chemical attributes and Mg ion release

may involve undiscovered osteogenic and angiogenic mechanisms. In their subsequent study, they planned to utilize genomics or proteomics techniques to explore these mechanisms. While the hydrogel is fully biodegradable, complete degradation was not observed. Hence, they intended to incorporate imaging dots into the hydrogel system in their next study to monitor its *in vivo* degradation procedure.

In another investigation by Sareethammanuwat et al. thermosensitive bTCP-chitosan/collagen-quercetin hydrogels were synthesized by combining an organic matrix of 2:1 (w/w) chitosan/collagen composite with an inorganic network of beta-tricalcium phosphate (bTCP) nanoparticles and quercetin. At physiological temperatures and pH levels, beta-glycerophosphate (bGP) and temperature fluctuations induced a sol-gel transition in the hydrogels. Intriguingly, the hydrogels' ability to gel at physiological temperatures and pH levels was unaffected by adding 1%–3% (w/v) bTCP. The bTCP-hydrogels showed interconnected porous geometry and a mean pore size of 100–200 µm. The hydrogels' mechanical properties were improved by the addition of 3% bTCP, but their rates of swelling, degradation, high porosity, permeability, and quercetin release were all reduced. The hydrogels could support cell entrapment and were noncytotoxic. The 3% bTCP-sustained hydrogel's quercetin release profile further indicated that the hydrogel may be used to deliver flavonoids for bone repair [138]. The hydrogel's mechanical strength could be further improved to meet the physiological demands of particular anatomical sites, and additional research is needed to explore the biological functions of the hydrogels in more detail.

Other studies that utilized nanoparticles in CS hydrogels to deliver promising results are CS/gelatin hydrogel/calcium phosphate nanoparticles [139], CS/silk fibroin hydrogel/mesoporous silica nanoparticles [140], CS-poly(dioxanone)/hyaluronic acid polyelectrolyte complex nanoparticles/CS/glycerophosphate hydrogel/BMP-2 [141], CS hydrogel/CS nanoparticles/BMP-2 plasmid DNA [142], aldehyde hyaluronic acid/*N*, *O*-carboxymethyl CS hydrogel/sphingosine 1-phosphate/polyelectrolyte-modified mesoporous silica nanoparticles [143].

### 6.9. CS hydrogels based on 3D printing

Using a 3D-printed scaffold as a potential treatment for individualized bone restoration is beneficial. The scaffold should provide an adequate bone regeneration milieu as well as outstanding mechanical qualities. In reality, natural ECM with liquid crystalline and viscoelastic properties provides the most suitable osteogenic microenvironment [144]. Nevertheless, replicating a 3D structured bone-mimicking ECM milieu with exceptional tensile characteristics is a significant problem. Liu et al. presented a method for creating a bionic scaffold that ideally combines a bone-like ECM microenvironment with superior mechanical characteristics. A 3D-printed poly(l-lactide) (PLLA) scaffold was efficiently reinforced by electrostatic self-assembling chitin whiskers *via* a layer-by-layer approach. A chitin whisker/CS composite hydrogel in a bone ECM-mimicking liquid crystalline state and viscoelasticity was infused into durable PLLA scaffolds to produce a 3D structure, enhancing bone regeneration significantly. Furthermore, an angiogenic agent, deferoxamine, was incorporated into the composite hydrogel and showed sustained release, providing a long-term effect on angiogenesis and encouraging osteogenesis [145].

The current advancements in CS-based scaffolds deal with multifaceted approaches to designing an optimum scaffold for patient needs. Such progress is presented by Zhao et al. who developed pH-responsive, self-assembled, bioprintable hydrogels of carboxymethyl CS and amorphous calcium phosphate. Glucono δ-lactone was added to aqueous dispersion, or alternatively, the freeze-dried nanoparticles were rehydrated in a pH-dependent controlled-assembly method. MSC proliferation and adhesion were improved by the hydrogels. Interestingly, the CMCh-ACP hydrogels themselves acted as osteoinductive and upregulated the expressions of osteoblastic regulatory factors in MSCs and suppressed bone resorption in long-term ectopic osteogenetic conditions. *In vivo* studies indicate the BMP9-dependent bone tissue regeneration ability of these hydrogels [146]. Thus, the 3D-printing landscape of CS-based hydrogels is still under exploration. Combining 3D printing and CS hydrogels holds immense potential in developing advanced biomedical implants [147].

3D printing has been used for fabricating injectable CS hydrogels in several studies such as CS/HA hydrogel/MC3T3-E1 cell-loaded [148], freeze-dried CS hydrogel [149], CS/dicalcium phosphate dihydrate crystals [150], carboxymethyl cellulose-glycol CS hydrogel/lactoferrin [151], poly(ε-caprolactone)/CS hydrogel/rabbit bone marrow MSCs/BMP-2 [152].

Table 1 describes the recent investigations concerning injectable CS-based hydrogels for BTE.

## 7. Conclusion

Chitosan, acquired from the deacetylation of chitin, is a broadly employed natural polymer in BTE due to its cost-efficiency, positive charge, and several benefits. This review offers a concise overview of the different physicochemical characteristics exhibited by CS-based injectable hydrogels for bone repair. It also delves profoundly into the recent developments in CS-based hydrogels, which hold intrinsic benefits for enhancing cell attachment, spreading, proliferation, and differentiation. These attributes have considerably assisted the field of tissue repair over the past two decades. To improve their mechanical, physicochemical, and biological characteristics, CS-based hydrogels are often blended with other natural or synthetic polymers. This tactical integration advances a range of injectable hydrogels tailored for BTE. CS-based injectable hydrogels have demonstrated excellent compatibility with bone tissues. They facilitate cell adhesion and differentiation and promote the vascularization of mineralized bones and a carrier for drug delivery to bone tissues. Although CS-based biomaterials have been reported in the literature, arduous efforts are paramount to bridge the challenges associated with their clinical applications. The scaffolds must be synthesized to be suitable for *in vivo* and human use; adequate testing at the research stage is required to achieve this. A standardized characterization methodology must be established to assess the material properties of the prepared injectable hydrogels thoroughly. The challenge of raw material variability in the case of naturally derived biomaterials is expected to play a huge role in the eventual performance characteristics of CS-based tissue scaffolds. Also, the utilization of high molecular weight chitosan in bone grafts can perhaps lead to inflammation. As a substitute, chitosan

**Table 1.** Recent studies on CS-based injectable hydrogel scaffolds for BTE.

| Hydrogel formulation | Preparation method | Incorporated cells | Effects/results | References |
|---|---|---|---|---|
| CS/gelatin/bioactive glass | | Rat bone marrow mesenchymal stem cells | 4.3% loss in hydrogel mass in simulated body fluid; 27% greater cell viability; nontoxic and when injected subcutaneously in the dorsum of Swiss rats | [153] |
| Methacrylated gelatin/ methacrylated chitosan and polyhedral oligomeric silsesquioxane | Photo-crosslinking | MSCs | Biodegradable guides MSCs to osteogenic differentiation based on calcium deposition and enzyme level; caused bone regeneration in rat calvarial defects. | [154] |
| Collagen/chitosan/hyaluronic acid | Chemical cross-linking | MG-63 cells | Hybrid materials prepared by a one-step procedure avoided phase separation of covalently attached mineral particles. Bioactive particles enhanced MG-63 cell adhesion and upregulated alkaline phosphatase expression. | [155] |
| Methacrylated glycol chitosan/ montmorillonite | Photocrosslinking | MSCs | The inclusion of nanoclays increased Young's modulus and delayed the degradation rate of hydrogels. Promoted calvarial healing in critical-size mouse calvarial defect model | [156] |
| Collagen/chitosan/ lysine-functionalized hyaluronic acid | Genipin crosslinking | MG-63 cells | HA formed covalent bonds with the hydrogel network, resulting in structurally robust hydrogels. HA had a beneficial effect on storage modulus and porosity (up to 95%). The results also showed combined osteoblastic proliferation and antibacterial activities. | [157] |
| CS-graphene oxide hydrogel/ polylactide-hydroxyapatite microfibers | Freezing-thawing-gelling | – | Novel scaffolds were found to be chemically stable and biocompatible and showed calcium phosphate formation in simulated body fluid. | [158] |
| Quantum dots/cyclodextrin/histidine labeled/ chitosan | | Human adipose stem cells | (QD-βCD-His@Dex) induced bone differentiation in labeled hASCs. Dual purpose osteoinductive-stem cell nanocarrier benefit in BTE. | [159] |
| Hydroxyethyl CS/polyvinyl alcohol/ biphasic calcium phosphate | Freeze-thawing | – | The hydrogels showed thicker pore walls and compressive strength in the range of 5–7 MPa. The mechanical properties of CS were improved by modification. | [160] |
| Regenerated cellulose fibers/CS | – | MC3T3-E1 | Higher compressive strength in comparison to neat CS. Osteogenic differentiation and enhanced biomineralization on MC3T3-E1 cells. | [161] |
| Decellularized bone ECM/oleoyl chitosan | – | Human amnion-derived stem cells | Mimicks 3D nano fibrillar microenvironment of bone tissues along with greater mechanical strength, antimicrobial properties, and neovascularization after 21 days. In a rabbit tibial defect model, hydrogels supported bone mineralization. | [162] |
| Chitosan/lysozyme | Photocrosslinking | MSCs | The addition of lysozyme increased the dose-dependent degradation rate of CS hydrogels. The results also demonstrated enhanced osteogenic differentiation of mesenchymal stem cells and bone formation in calvarial defects of mice models. | [163] |

(*Continued*)

**Table 1.** Continued.

| Hydrogel formulation | Preparation method | Incorporated cells | Effects/results | References |
|---|---|---|---|---|
| Icariin-loaded modified halloysite nanotubes/Chitosan/ Glycerophosphate | Sol-gel | Human adipose-derived stromal cells | Icariin was efficiently loaded into mesoporous halloysite nanotubes, creating a sustained drug release system. Subsequently, nanocomposite chitosan/mHNTs hydrogels were synthesized with enhanced mechanical strength. MSCs were encapsulated within these hydrogels, and *In vitro* viability assays demonstrated the biocompatibility of the scaffolds. | [164] |
| Chitosan/β-glycerophosphate/ Hydroxyapatite/Polyelectrolyte complexes | Polyelectrolyte complexation methodology | MG-63 | The assessment of Young modulus exhibited a notable enhancement in stiffness for the hydrogel reinforced with PEC fibers, revealing a threefold increase compared to chitosan-βGP gels. Furthermore, the gelation time was substantially decreased to 3 minutes, and the resulting hydrogels displayed porous structures, gelling at physiological pH and temperature. These hydrogels exhibited excellent biocompatibility, as evidenced by over 80% cell viability in MTT assays using MG63 cells. Confocal imaging of PEC fiber-reinforced hydrogels revealed distinct viability and proliferation. | [165] |
| Chitosan/ poly (N-isopropylacrylamide) (PNIPAAm)-based copolymer/ graphene oxide (GO) | Free-radical copolymerization | human dental pulp stem cells (hDPSCs) | The hydrogel demonstrated the capability to augment mineral deposition and enhance the activity of alkaline phosphatase (ALP), a phenomenon primarily attributed to the presence of oxygen and amine-containing functional groups from GO and chitosan. This engineered hydrogel further exhibited the ability to upregulate the expression of key osteogenic markers, including the Runt-related transcription factor 2 and osteocalcin, in hDPSCs cultivated in both normal and osteogenic media. Additionally, it appeared to facilitate the absorption of osteogenic inducers. | [78] |
| Chitosan/polygalacturonic acid/ β-glycerophosphate (βGP)/ hydroxyapatite | Hydrothermal assisted hydrolysis | MG-63 | The synthesized hydrogels exhibited commendable compressive stiffness. Scanning electron microscopy showcased a uniform distribution of PEC fibers in the hydrogel matrix. Notably, the hydrogels incorporating gelatin and hydrothermally treated PEC fibers demonstrated superior cellular responses at the 14-day mark, suggesting an enhancement in bioactivity and mechanical attributes through hydrothermal treatment and the incorporation of gelatin in the chitosan-βGP hydrogel system. | [65] |

(*Continued*)

**Table 1.** Continued.

| Hydrogel formulation | Preparation method | Incorporated cells | Effects/results | References |
|---|---|---|---|---|
| Crosslinked decellularized bone ECM (DBM)/fatty acid-modified chitosan (oleoyl chitosan, OC) | – | human amnion-derived stem cells (HAMSCs) | Incorporating OC in the hybrid hydrogels offered an optimal combination of viscoelastic strength and controlled degradability in physiological media over an extended period. The inherent growth factors in DBM fostered excellent cellular viability, proliferation, and osteogenic differentiation. Chorioallantoic membrane assay revealed an improved formation of neovascular vessels converging towards the DBM/OC hydrogel, indicating superior angiogenic potential in contrast to the Col-I/OC hydrogel. The outstanding osteoconductive potential of DBM/OC hydrogel, with or without HAMSCs, was evident in improved bone regeneration and the formation of a mineralized bone matrix compared to the sham control when orthotopically implanted. | [162] |
| Genipin crosslinked collagen/ chitosan/lysine-modified hyaluronic acid (ColChHAmod) | Sol-gel | MG-63 | The hydrogels displayed a high level of porosity ranging from 85% to 95%. The hydrogels exhibited injectability, with in situ rheological measurements confirming their structural stability. Increased HAmod content correlated with higher storage modulus values, indicating efficient crosslinking within the biopolymer mixture. *In vitro* studies demonstrated the biocompatibility of the hydrogels, supporting MG-63 cell proliferation, adhesion, and ALP expression. Notably, the hydrogels demonstrated antibacterial activity against Escherichia coli without additional antibiotics, highlighting their multifunctionality and broad potential biological applications. | [157] |
| Collagen/aldehyde modified-nanocrystalline cellulose (CNC)/ chitosan loaded with gold NPs (CS-Au) | Schiff base reaction | NIH 3T3 | The experimental results displayed that different molar ratios of Collagen/CNCs and the inclusion of CS-Au substantially influence the microscopic morphology, equilibrium swelling, *In vitro* degradation, and mechanical properties of the hydrogels. Cytotoxicity analysis conducted on the NIH 3T3 cell line demonstrated the efficiency and non-toxic nature of the developed hydrogels toward cell destruction. | [166] |

(*Continued*)

**Table 1.** Continued.

| Hydrogel formulation | Preparation method | Incorporated cells | Effects/results | References |
|---|---|---|---|---|
| Manganese oxide NPs (MgO)-loaded phosphocreatine-functionalized chitosan (CSMP) (CSMP-MgO) | Supramolecular combination | MC3T3-E1 | The lyophilized CSMP-MgO injectable hydrogels displayed a porous structure with dimensions of 50 to 100 µm, characterized by thick pore walls. They revealed lower swelling in DI water compared to chemically cross-linked hydrogels (CSMP-MgO (0.5) and CSMP). Injectable hydrogels demonstrated higher strength and modulus. *In vitro,* CSMP-MgO hydrogels released Mg ions steadily, undergoing gradual degradation. CSMP-MgO (5) exhibited superior performance in promoting calcium phosphate deposition, cell proliferation, osteogenic differentiation, and mineralization. It also upregulated osteogenic gene expression and fostered tube formation in HUVECs compared to CSMP and CSMP-MgO (0.5). *In vivo*, especially CSMP-MgO (5), substantially promoted bone regeneration in a rat critical-sized calvarial defect compared to CSMP hydrogel and the control group. | [137] |
| Chitosan methacrylate/graphene oxide | – | Normal Human Osteoblast cell line (CC-2538) | Incorporating GO into ChiMA hydrogels improved mechanical strength, which is crucial for sustained bone-to-bone adhesion in a simulated physiological medium for over two weeks. Photo-crosslinking with short blue light exposure correlated with superior textural attributes, pseudoplastic response, and tensile resistance (8.5 N). Cytocompatibility assays displayed cell viability above 80% for concentrations below 6% (v/v). The hydrogels demonstrated intermediate platelet aggregation, favorable for tissue regeneration, and minimal hemolytic tendencies. In silico studies exhibited superior performance in femur fractures compared to commercial cement for oblique and transverse lesions. | [109] |
| N-carboxyethyl chitosan (NCEC) and oxidized dextran (ODex)/ nano-hydroxyapatite | Schiff base crosslinking | – | The hydrogel, displaying biocompatibility, a porous framework, suitable rheological properties, and controlled degradation rates, serves as an ideal scaffold for BTE. The treatment of bone defects with this polysaccharide hydrogel facilitated the integration of newly formed tissue with the host bone. Additionally, incorporating nano-hydroxyapatite for repairing rat cranial bone defects resulted in excellent bone regeneration effects. | [167] |

acquired from fungal and marine sources may furnish greater reliability. Therefore, it is imperative that future research activities centralize on two key aspects: first, the advancement of uniform standards and the progress of cutting-edge technology to widen the scope of potential clinical applications. Second, a principal research objective should be improving the loading capability and controlled release of bioactive materials within CS-based hydrogels to enhance their performance upon implantation in clinical environments.

Advancing, clinical research should investigate the combination of CS-based hydrogels with diverse biofabrication procedures, for example, electrospinning and 3D-printing. This integration targets building a versatile and multilayered bone graft for employment in bone regeneration and fracture management, thereby further progressing the area of tissue engineering.

In the foreseeable future, we can forecast the advent of self-healing hydrogels and biomimetic scaffolds intended for long-term sustainability. These novelties hold the promise of improving the efficiency of regenerative therapies and implants, eventually restoring patients to a higher quality of life.

## Author contributions

Conceptualization and Supervision: Gayatri Vaidya, Sheersha Pramanik; Resources: Gayatri Vaidya, Ammar Kadi, Bassam M. Abualsoud, Sheersha Pramanik, Rehana Masood, Jacob Michaelson; Literature review and writing—original draft preparation: Gayatri Vaidya, Sheersha Pramanik, Ahmed Raheem Rayshan, Ammar Kadi, Bassam M. Abualsoud, Rehana Masood; writing—review and editing: Mohammad Javed Ansari, Jacob Michaelson, Ahmed Raheem Rayshan, Bassam M. Abualsoud, Sheersha Pramanik. All authors have read and agreed to the published version of the manuscript.

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## ORCID

Sheersha Pramanik http://orcid.org/0000-0001-6912-937X

## References

[1] Betz RR. Limitations of autograft and allograft: new synthetic solutions. Orthopedics. 2002;25(5 Suppl): S561–S570. doi: 10.3928/0147-7447-20020502-04.

[2] Khan Y, Yaszemski MJ, Mikos AG, et al. Tissue engineering of bone: material and matrix considerations. JBJS. 2008;90(Supplement_1):36–42.

[3] Soucacos PN, Johnson EO, Babis G. An update on recent advances in bone regeneration. Injury. 2008;39 Suppl 2:S1–S4. doi: 10.1016/S0020-1383(08)70009-3.
[4] Schaffler MB, Cheung W-Y, Majeska R, et al. Osteocytes: master orchestrators of bone. Calcif Tissue Int. 2014;94(1):5–24. doi: 10.1007/s00223-013-9790-y.
[5] Pramanik S, Kharche S, More N, et al. Natural biopolymers for bone tissue engineering: a brief review. Eng Regen. 2023;4(2):193–204. doi: 10.1016/j.engreg.2022.12.002.
[6] Kim B-S, Park I-K, Hoshiba T, et al. Design of artificial extracellular matrices for tissue engineering. Prog Polym Sci. 2011;36(2):238–268. doi: 10.1016/j.progpolymsci.2010.10.001.
[7] Mathew AP, Uthaman S, Cho K-H, et al. Injectable hydrogels for delivering biotherapeutic molecules. Int J Biol Macromol. 2018;110:17–29. doi: 10.1016/j.ijbiomac.2017.11.113.
[8] Deng H, Dong A, Song J, et al. Injectable thermosensitive hydrogel systems based on functional PEG/PCL block polymer for local drug delivery. J Control Release. 2019;297:60–70. doi: 10.1016/j.jconrel.2019.01.026.
[9] Cheung RCF, Ng TB, Wong JH, et al. Chitosan: an update on potential biomedical and pharmaceutical applications. Mar Drugs. 2015;13(8):5156–5186. doi: 10.3390/md13085156.
[10] Saravanan S, Leena RS, Selvamurugan N. Chitosan-based biocomposite scaffolds for bone tissue engineering. Int J Biol Macromol. 2016;93(Pt B):1354–1365. doi: 10.1016/j.ijbiomac.2016.01.112.
[11] Mohebbi S, Nezhad MN, Zarrintaj P, et al. Chitosan in biomedical engineering: a critical review. Curr Stem Cell Res Ther. 2019;14(2):93–116. doi: 10.2174/1574888X13666180912142028.
[12] Liu J, Sun L, Xu W, et al. Current advances and future perspectives of 3D printing natural-derived biopolymers. Carbohydr Polym. 2019;207:297–316. doi: 10.1016/j.carbpol.2018.11.077.
[13] Wang Y, Yuan X, Yu K, et al. Fabrication of nanofibrous microcarriers mimicking extracellular matrix for functional microtissue formation and cartilage regeneration. Biomaterials. 2018;171:118–132. doi: 10.1016/j.biomaterials.2018.04.033.
[14] Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev. 2010;62(1):3–11. doi: 10.1016/j.addr.2009.09.004.
[15] Qi P, Ning Z, Zhang X. Synergistic effects of 3D chitosan-based hybrid scaffolds and mesenchymal stem cells in orthopaedic tissue engineering. IET Nanobiotechnol. 2023;17(2):41–48. doi: 10.1049/nbt2.12103.
[16] Islam MM, Shahruzzaman M, Biswas S, et al. Bioactive materials chitosan based bioactive materials in tissue engineering applications-A review. Bioact Mater. 2020;5(1):164–183. doi: 10.1016/j.bioactmat.2020.01.012.
[17] Lavanya K, Chandran SV, Balagangadharan K, et al. Temperature- and pH-responsive chitosan-based injectable hydrogels for bone tissue engineering. Mater Sci Eng C Mater Biol Appl. 2020;111:110862. doi: 10.1016/j.msec.2020.110862.
[18] Tang G, Tan Z, Zeng W, et al. Recent advances of Chitosan-Based injectable hydrogels for bone and dental tissue regeneration. Front Bioeng Biotechnol. 2020;8:587658. doi: 10.3389/fbioe.2020.587658.
[19] Pramanik S, Sali V. Connecting the dots in drug delivery: a tour d'horizon of chitosan-based nanocarriers system. Int J Biol Macromol. 2021;169:103–121. doi: 10.1016/j.ijbiomac.2020.12.083.
[20] Abourehab MAS, Pramanik S, Abdelgawad MA, et al. Recent advances of chitosan formulations in biomedical applications. Int J Mol Sci. 2022;23(18):10975. doi: 10.3390/ijms231810975.
[21] Tasar OC, Erdal S, Taskin M. Chitosan production by psychrotolerant *Rhizopus oryzae* in non-sterile open fermentation conditions. Int J Biol Macromol. 2016;89:428–433. doi: 10.1016/j.ijbiomac.2016.05.007.

[22] Jia Z, Shen D. Effect of reaction temperature and reaction time on the preparation of low-molecular-weight chitosan using phosphoric acid. Carbohydr Polym. 2002;49(4):393–396. doi: 10.1016/S0144-8617(02)00026-7.
[23] No HK, Meyers SP. Preparation and characterization of chitin and chitosan—a review. J Aquat Food Prod Technol. 1995;4(2):27–52. doi: 10.1300/J030v04n02_03.
[24] Gabriel) Kou S, (Peters LM, Mucalo MR. Chitosan: a review of sources and preparation methods. Int J Biol Macromol. 2021;169:85–94. doi: 10.1016/j.ijbiomac.2020.12.005.
[25] Said Al Hoqani HA, Al-Shaqsi N, Hossain MA, et al. Isolation and optimization of the method for industrial production of chitin and chitosan from omani shrimp shell. Carbohydr Res. 2020;492:108001. doi: 10.1016/j.carres.2020.108001.
[26] Pakizeh M, Moradi A, Ghassemi T. Chemical extraction and modification of chitin and chitosan from shrimp shells. Eur Polym J. 2021;159:110709. doi: 10.1016/j.eurpolymj.2021.110709.
[27] El Knidri H, Belaabed R, Addaou A, et al. Extraction, chemical modification and characterization of chitin and chitosan. Int J Biol Macromol. 2018;120(Pt A):1181–1189. doi: 10.1016/j.ijbiomac.2018.08.139.
[28] Zhang H, Yun S, Song L, et al. The preparation and characterization of chitin and chitosan under large-scale submerged fermentation level using shrimp by-products as substrate. Int J Biol Macromol. 2017;96:334–339. doi: 10.1016/j.ijbiomac.2016.12.017.
[29] Sedaghat F, Yousefzadi M, Toiserkani H, et al. Bioconversion of shrimp waste penaeus merguiensis using lactic acid fermentation: an alternative procedure for chemical extraction of chitin and chitosan. Int J Biol Macromol. 2017;104(Pt A):883–888. doi: 10.1016/j.ijbiomac.2017.06.099.
[30] Manni L, Ghorbel-Bellaaj O, Jellouli K, et al. Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste by treatment with crude protease from Bacillus cereus SV1. Appl Biochem Biotechnol. 2010;162(2):345–357. doi: 10.1007/s12010-009-8846-y.
[31] Vázquez JA, Noriega D, Ramos P, et al. Optimization of high purity chitin and chitosan production from illex argentinus pens by a combination of enzymatic and chemical processes. Carbohydr Polym. 2017;174:262–272. doi: 10.1016/j.carbpol.2017.06.070.
[32] Younes I, Hajji S, Frachet V, et al. Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. Int J Biol Macromol. 2014;69:489–498. doi: 10.1016/j.ijbiomac.2014.06.013.
[33] Jantzen da Silva Lucas A, Quadro Oreste E, Leão Gouveia Costa H, et al. Extraction, physicochemical characterization, and morphological properties of chitin and chitosan from cuticles of edible insects. Food Chem. 2021;343:128550. doi: 10.1016/j.foodchem.2020.128550.
[34] Tolesa LD, Gupta BS, Lee M-J. Chitin and chitosan production from shrimp shells using ammonium-based ionic liquids. Int J Biol Macromol. 2019;130:818–826. doi: 10.1016/j.ijbiomac.2019.03.018.
[35] Silva SS, Mano JF, Reis RL. Ionic liquids in the processing and chemical modification of chitin and chitosan for biomedical applications. Green Chem. 2017;19(5):1208–1220. doi: 10.1039/C6GC02827F.
[36] Galvis-Sánchez AC, Castro MCR, Biernacki K, et al. Natural deep eutectic solvents as green plasticizers for chitosan thermoplastic production with controlled/desired mechanical and barrier properties. Food Hydrocoll. 2018;82:478–489. doi: 10.1016/j.foodhyd.2018.04.026.
[37] Bangde PS, Jain R, Dandekar P. Alternative approach to synthesize methylated chitosan using deep eutectic solvents, biocatalyst and 'green' methylating agents. ACS Sustainable Chem Eng. 2016;4(6):3552–3557. doi: 10.1021/acssuschemeng.6b00653.
[38] Fiamingo A, Delezuk J. A D M, Trombotto S, et al. Extensively deacetylated high molecular weight chitosan from the multistep ultrasound-assisted deacetylation of beta-chitin. Ultrason Sonochem. 2016;32:79–85. doi: 10.1016/j.ultsonch.2016.02.021.

[39] Vallejo-Domínguez D, Rubio-Rosas E, Aguila-Almanza E, et al. Ultrasound in the deproteinization process for chitin and chitosan production. Ultrason Sonochem. 2021;72:105417. doi: 10.1016/j.ultsonch.2020.105417.
[40] Singh A, Benjakul S, Prodpran T. Ultrasound-Assisted extraction of chitosan from squid pen: molecular characterization and fat binding capacity. J Food Sci. 2019;84(2):224–234. doi: 10.1111/1750-3841.14439.
[41] Duan C, Meng X, Meng J, et al. Chitosan as a preservative for fruits and vegetables: a review on chemistry and antimicrobial properties. J Bioresour Bioprod. 2019;4(1):11–21. doi: 10.21967/jbb.v4i1.189.
[42] Tao F, Cheng Y, Shi X, et al. Applications of chitin and chitosan nanofibers in bone regenerative engineering. Carbohydr Polym. 2019;230:115658. doi: 10.1016/j.carbpol.2019.115658.
[43] Roy Choudhury A. Richa, pH mediated rheological modulation of chitosan hydrogels. Int J Biol Macromol. 2020;156:591–597. doi: 10.1016/j.ijbiomac.2020.04.049.
[44] Meng X, Xing R, Liu S, et al. Molecular weight and pH effects of aminoethyl modified chitosan on antibacterial activity in vitro. Int J Biol Macromol. 2012;50(4):918–924. doi: 10.1016/j.ijbiomac.2012.01.018.
[45] Liu J, Pu H, Liu S, et al. Synthesis, characterization, bioactivity and potential application of phenolic acid grafted chitosan: a review. Carbohydr Polym. 2017;174:999–1017. doi: 10.1016/j.carbpol.2017.07.014.
[46] Aranaz I, Mengibar M, Harris R, et al. Functional characterization of chitin and chitosan. CCB. 2009;3(2):203–230. doi: 10.2174/2212796810903020203.
[47] Bakshi PS, Selvakumar D, Kadirvelu K, et al. Chitosan as an environment friendly biomaterial – a review on recent modifications and applications. Int J Biol Macromol. 2020;150:1072–1083. doi: 10.1016/j.ijbiomac.2019.10.113.
[48] Gonçalves C, Ferreira N, Lourenço L. Production of low molecular weight chitosan and chitooligosaccharides (COS): a review. Polymers. 2021;13(15):2466. doi: 10.3390/polym13152466.
[49] Kittur FS, Vishu Kumar AB, Tharanathan RN. Low molecular weight chitosans–preparation by depolymerization with Aspergillus Niger pectinase, and characterization. Carbohydr Res. 2003;338(12):1283–1290. doi: 10.1016/s0008-6215(03)00175-7.
[50] Zargar V, Asghari M, Dashti A. A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications. ChemBioEng Rev. 2015;2(3):204–226. doi: 10.1002/cben.201400025.
[51] Sacco P, Cok M, Scognamiglio F, et al. Glycosylated-chitosan derivatives: a systematic review. Molecules. 2020;25(7):1534.
[52] Upadhyaya L, Singh J, Agarwal V, et al. The implications of recent advances in carboxymethyl chitosan based targeted drug delivery and tissue engineering applications. J Control Release. 2014;186:54–87. doi: 10.1016/j.jconrel.2014.04.043.
[53] Fei Liu X, Lin Guan Y, Zhi Yang D, et al. Antibacterial action of chitosan and carboxymethylated chitosan. J Appl Polym Sci. 2001;79(7):1324–1335. doi: 10.1002/1097-4628(20010214)79:7<1324::AID-APP210>3.0.CO;2-L.
[54] Methacanon P, Prasitsilp M, Pothsree T, et al. Heterogeneous N-deacetylation of squid chitin in alkaline solution. Carbohydr Polym. 2003;52(2):119–123. doi: 10.1016/S0144-8617(02)00300-4.
[55] Caló E, Khutoryanskiy VV. Biomedical applications of hydrogels: a review of patents and commercial products. Eur Polym J. 2015;65:252–267. doi: 10.1016/j.eurpolymj.2014.11.024.
[56] Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliv Rev. 2012;64:18–23. doi: 10.1016/j.addr.2012.09.010.
[57] Alonso JM, Andrade Del Olmo J, Perez Gonzalez R, et al. Injectable hydrogels: from laboratory to industrialization. Polymers. 2021;13(4):650. doi: 10.3390/polym13040650.
[58] Overstreet DJ, Dutta D, Stabenfeldt SE, et al. Injectable hydrogels. J Polym Sci B Polym Phys. 2012;50(13):881–903. doi: 10.1002/polb.23081.

[59] Ghandforoushan P, Alehosseini M, Golafshan N, et al. Injectable hydrogels for cartilage and bone tissue regeneration: a review. Int J Biol Macromol. 2023;246:125674. doi: 10.1016/j.ijbiomac.2023.125674.
[60] Huang L, Abdalla AME, Xiao L. Biopolymer-Based microcarriers for three-dimensional cell culture and engineered tissue formation. Int. J. Mol. Sci. 2020;21(5):1895.
[61] Ressler A. Chitosan-Based biomaterials for bone tissue engineering applications: a short review. Polymers. 2022;14(16):3430. doi: 10.3390/polym14163430.
[62] Dyondi D, Webster TJ, Banerjee R. A nanoparticulate injectable hydrogel as a tissue engineering scaffold for multiple growth factor delivery for bone regeneration. Int J Nanomed. 2013;8:47–59. doi: 10.2147/IJN.S37953.
[63] Li Y, Chen S-K, Li L, et al. Bone defect animal models for testing efficacy of bone substitute biomaterials. J Orthop Translat. 2015;3(3):95–104. doi: 10.1016/j.jot.2015.05.002.
[64] Zarrin NK, Mottaghitalab F, Reis RL, et al. Thermosensitive chitosan/poly(N-isopropyl acrylamide) nanoparticles embedded in aniline pentamer/silk fibroin/polyacrylamide as an electroactive injectable hydrogel for healing critical-sized calvarial bone defect in aging rat model. Int J Biol Macromol. 2022;213:352–368. doi: 10.1016/j.ijbiomac.2022.05.176.
[65] Wasupalli GK, Verma D. Thermosensitive injectable hydrogel based on chitosan-polygalacturonic acid polyelectrolyte complexes for bone tissue engineering. Carbohydr Polym. 2022;294:119769. doi: 10.1016/j.carbpol.2022.119769.
[66] Bharadwaz A, Jayasuriya AC. Materials science & engineering C recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. Mater Sci Eng C Mater Biol Appl. 2020;110(January):110698. doi: 10.1016/j.msec.2020.110698.
[67] Sang S, Cheng R, Cao Y, et al. Biocompatible chitosan/polyethylene glycol/multi-walled carbon nanotube composite scaffolds for neural tissue engineering. J Zhejiang Univ Sci B. 2022;23(1):58–73. doi: 10.1631/jzus.B2100155.
[68] Huang Y, Zhang L, Ji Y, et al. A non-invasive smart scaffold for bone repair and monitoring. Bioact Mater. 2023;19:499–510. doi: 10.1016/j.bioactmat.2022.04.034.
[69] Liu H, Li H, Cheng W, et al. Novel injectable calcium phosphate/chitosan composites for bone substitute materials. Acta Biomater. 2006;2(5):557–565. doi: 10.1016/j.actbio.2006.03.007.
[70] Saravanan S, Vimalraj S, Thanikaivelan P, et al. A review on injectable chitosan/beta glycerophosphate hydrogels for bone tissue regeneration. Int J Biol Macromol. 2019;121:38–54. doi: 10.1016/j.ijbiomac.2018.10.014.
[71] Deng P, Yao L, Chen J, et al. Chitosan-based hydrogels with injectable, self-healing and antibacterial properties for wound healing. Carbohydr Polym. 2022;276:118718. doi: 10.1016/j.carbpol.2021.118718.
[72] Huang B, Chen M, Tian J, et al. Oxygen-Carrying and antibacterial fluorinated Nano-Hydroxyapatite incorporated hydrogels for enhanced bone regeneration. Adv Healthc Mater. 2022;11(12):e2102540. doi: 10.1002/adhm.202102540.
[73] Klara J, Marczak A, Łatkiewicz A, et al. Lysine-functionalized chondroitin sulfate improves the biological properties of collagen/chitosan-based injectable hydrogels. Int J Biol Macromol. 2022;202:318–331. doi: 10.1016/j.ijbiomac.2022.01.026.
[74] Celesti C, Iannazzo D, Espro C, et al. Chitosan/POSS hybrid hydrogels for bone tissue engineering. Materials. 2022;15(22):8208. doi: 10.3390/ma15228208.
[75] Ren B, Chen X, Du S, et al. Injectable polysaccharide hydrogel embedded with hydroxyapatite and calcium carbonate for drug delivery and bone tissue engineering. Int J Biol Macromol. 2018;118(Pt A):1257–1266. doi: 10.1016/j.ijbiomac.2018.06.200.
[76] Ekapakul N, Sinthuvanich C, Ajiro H, et al. Bioactivity of star-shaped polycaprolactone/chitosan composite hydrogels for biomaterials. Int J Biol Macromol. 2022;212:420–431. doi: 10.1016/j.ijbiomac.2022.05.139.
[77] Korpayev S, Kaygusuz G, Şen M, et al. Chitosan/collagen based biomimetic osteochondral tissue constructs: a growth factor-free approach. Int J Biol Macromol. 2020;156:681–690. doi: 10.1016/j.ijbiomac.2020.04.109.

[78] Amiryaghoubi N, Noroozi Pesyan N, Fathi M, et al. Injectable thermosensitive hybrid hydrogel containing graphene oxide and chitosan as dental pulp stem cells scaffold for bone tissue engineering. Int J Biol Macromol. 2020;162:1338–1357. doi: 10.1016/j.ijbiomac.2020.06.138.

[79] García-García P, Reyes R, Segredo-Morales E, et al. PLGA-BMP-2 and PLA-17β-Estradiol microspheres reinforcing a composite hydrogel for bone regeneration in osteoporosis. Pharmaceutics. 2019;11(12):648. doi: 10.3390/pharmaceutics11120648.

[80] Bi S, Wang P, Hu S, et al. Construction of physical-crosslink chitosan/PVA double-network hydrogel with surface mineralization for bone repair. Carbohydr Polym. 2019;224:115176. doi: 10.1016/j.carbpol.2019.115176.

[81] Zhang L, He G, Yu Y, et al. Design of biocompatible chitosan/polyaniline/laponite hydrogel with photothermal conversion capability. Biomolecules. 2022;12(8):1089. doi: 10.3390/biom12081089.

[82] Reyna-Urrutia VA, Estevez M, González-González AM, et al. 3D scaffolds of caprolactone/chitosan/polyvinyl alcohol/hydroxyapatite stabilized by physical bonds seeded with swine dental pulp stem cell for bone tissue engineering. J Mater Sci Mater Med. 2022;33(12):81. doi: 10.1007/s10856-022-06702-2.

[83] Loukelis K, Papadogianni D, Chatzinikolaidou M. Kappa-carrageenan/chitosan/gelatin scaffolds enriched with potassium chloride for bone tissue engineering. Int J Biol Macromol. 2022;209(Pt B):1720–1730. doi: 10.1016/j.ijbiomac.2022.04.129.

[84] Wu M, Chen F, Liu H, et al. Bioinspired sandwich-like hybrid surface functionalized scaffold capable of regulating osteogenesis, angiogenesis, and osteoclastogenesis for robust bone regeneration. Mater Today Bio. 2022;17(September):100458. doi: 10.1016/j.mtbio.2022.100458.

[85] Zou Z, Wang L, Zhou Z, et al. Simultaneous incorporation of PTH(1–34) and nano-hydroxyapatite into chitosan/alginate hydrogels for efficient bone regeneration. Bioact Mater. 2021;6(6):1839–1851. doi: 10.1016/j.bioactmat.2020.11.021.

[86] Min Q, Tan R, Zhang Y, et al. Multi-Crosslinked strong and elastic bioglass/Chitosan-Cysteine hydrogels with controlled quercetin delivery for bone tissue engineering. Pharmaceutics. 2022;14(10):2048. doi: 10.3390/pharmaceutics14102048.

[87] Malik MH, Shahzadi L, Batool R, et al. Thyroxine-loaded chitosan/carboxymethyl cellulose/hydroxyapatite hydrogels enhance angiogenesis in in-ovo experiments. Int J Biol Macromol. 2020;145:1162–1170. doi: 10.1016/j.ijbiomac.2019.10.043.

[88] Wang L, Wang J, Zhou X, et al. A new Self-Healing hydrogel containing hucMSC-Derived exosomes promotes bone regeneration. Front Bioeng Biotechnol. 2020;8:564731. doi: 10.3389/fbioe.2020.564731.

[89] Ding X, Li X, Li C, et al. Chitosan/dextran hydrogel constructs containing Strontium-Doped hydroxyapatite with enhanced osteogenic potential in rat cranium. ACS Biomater Sci Eng. 2019;5(9):4574–4586. doi: 10.1021/acsbiomaterials.9b00584.

[90] Shao R-X, Quan R-F, Wang T, et al. Effects of a bone graft substitute consisting of porous gradient HA/ZrO(2) and gelatin/chitosan slow-release hydrogel containing BMP-2 and BMSCs on lumbar vertebral defect repair in rhesus monkey. J Tissue Eng Regen Med. 2018;12(3):e1813–e1825. doi: 10.1002/term.2601.

[91] Stamnitz S, Klimczak A. Mesenchymal stem cells, bioactive factors, and scaffolds in bone repair: from research perspectives to clinical practice. Cells. 2021;10(8):1925. doi: 10.3390/cells10081925.

[92] Wu D, Qin H, Wang Z, et al. Bone mesenchymal stem Cell-Derived sEV-Encapsulated thermosensitive hydrogels accelerate osteogenesis and angiogenesis by release of exosomal miR-21. Front Bioeng Biotechnol. 2021;9:829136. doi: 10.3389/fbioe.2021.829136.

[93] Heirani-Tabasi A, Hosseinzadeh S, Rabbani S, et al. Cartilage tissue engineering by co-transplantation of chondrocyte extracellular vesicles and mesenchymal stem cells, entrapped in chitosan-hyaluronic acid hydrogel. Biomed Mater. 2021;16(5):055003. doi: 10.1088/1748-605X/ac0cbf.

[94] Olfat Noubari Z, Golchin A, Fathi M, et al. Designing robust chitosan-based hydrogels for stem cell nesting under oxidative stress. Bioimpacts. 2022;12(1):57–64. doi: 10.34172/bi.2021.23831.

[95] Peng L, Zhou Y, Lu W, et al. Characterization of a novel polyvinyl alcohol/chitosan porous hydrogel combined with bone marrow mesenchymal stem cells and its application in articular cartilage repair. BMC Musculoskelet Disord. 2019;20(1):257. doi: 10.1186/s12891-019-2644-7.

[96] Liao HT, Tsai M-J, Brahmayya M, et al. Bone regeneration using adipose-derived stem cells in injectable Thermo-Gelling hydrogel scaffold containing platelet-rich plasma and biphasic calcium phosphate. Int J Mol Sci. 2018;19(9):2537. doi: 10.3390/ijms19092537.

[97] Ansari MJ, Rajendran RR, Mohanto S, et al. Poly(N-isopropylacrylamide)-based hydrogels for biomedical applications: a review of the state-of-the-art. Gels. 2022;8(7):454. doi: 10.3390/gels8070454.

[98] Wasupalli GK, Verma D. Development of chitosan-polygalacturonic acid polyelectrolyte complex fibrous scaffolds using the hydrothermal treatment for bone tissue engineering. J Biomed Mater Res A. 2023;111(3):354–366. doi: 10.1002/jbm.a.37461.

[99] Nourbakhsh M, Zarrintaj P, Jafari SH, et al. Fabricating an electroactive injectable hydrogel based on pluronic-chitosan/aniline-pentamer containing angiogenic factor for functional repair of the hippocampus ischemia rat model. Mater Sci Eng C Mater Biol Appl. 2020;117:111328. doi: 10.1016/j.msec.2020.111328.

[100] Guo B, Qu J, Zhao X, et al. Degradable conductive self-healing hydrogels based on dextran-graft-tetraaniline and N-carboxyethyl chitosan as injectable carriers for myoblast cell therapy and muscle regeneration. Acta Biomater. 2019;84:180–193. doi: 10.1016/j.actbio.2018.12.008.

[101] Mota J, Yu N, Caridade SG, et al. Chitosan/bioactive glass nanoparticle composite membranes for periodontal regeneration. Acta Biomater. 2012;8(11):4173–4180. doi: 10.1016/j.actbio.2012.06.040.

[102] Wu J, Zheng K, Huang X, et al. Thermally triggered injectable chitosan/silk fibroin/bioactive glass nanoparticle hydrogels for in-situ bone formation in rat calvarial bone defects. Acta Biomater. 2019;91:60–71. doi: 10.1016/j.actbio.2019.04.023.

[103] Ding H, Li B, Liu Z, et al. Decoupled pH- and Thermo-Responsive injectable chitosan/PNIPAM hydrogel via Thiol-Ene click chemistry for potential applications in tissue engineering. Adv Healthc Mater. 2020;9(14):e2000454. doi: 10.1002/adhm.202000454.

[104] Kocak FZ, Talari ACS, Yar M, et al. In-situ forming pH and thermosensitive injectable hydrogels to stimulate angiogenesis: potential candidates for fast bone regeneration applications. Int J Mol Sci. 2020;21(5):1633. doi: 10.3390/ijms21051633.

[105] Shi Z, Yang F, Pang Q, et al. The osteogenesis and the biologic mechanism of thermo-responsive injectable hydrogel containing carboxymethyl chitosan/sodium alginate nanoparticles towards promoting osteal wound healing. Int J Biol Macromol. 2023;224:533–543. doi: 10.1016/j.ijbiomac.2022.10.142.

[106] Tao J, Zhang Y, Shen A, et al. Injectable Chitosan-Based thermosensitive hydrogel/Nanoparticle-Loaded system for local delivery of vancomycin in the treatment of osteomyelitis. Int J Nanomedicine. 2020;15:5855–5871. doi: 10.2147/IJN.S247088.

[107] Kaur K, Paiva SS, Caffrey D, et al. Injectable chitosan/collagen hydrogels nano-engineered with functionalized single wall carbon nanotubes for minimally invasive applications in bone. Mater Sci Eng C Mater Biol Appl. 2021;128:112340. doi: 10.1016/j.msec.2021.112340.

[108] Xu X, Gu Z, Chen X, et al. An injectable and thermosensitive hydrogel: promoting periodontal regeneration by controlled-release of aspirin and erythropoietin. Acta Biomater. 2019;86:235–246. doi: 10.1016/j.actbio.2019.01.001.

[109] Céspedes-Valenzuela DN, Sánchez-Rentería S, Cifuentes J, et al. Preparation and characterization of an injectable and Photo-Responsive chitosan methacrylate/graphene oxide hydrogel: potential applications in bone tissue adhesion and repair. Polymers. 2021;14(1):126. doi: 10.3390/polym14010126.

[110] Agnes CJ, Murshed M, Takada A, et al. A 6-bromoindirubin-3′-oxime incorporated chitosan-based hydrogel scaffold for potential osteogenic differentiation: investigation of material properties in vitro. Int J Biol Macromol. 2023;227:71–82. doi: 10.1016/j.ijbiomac.2022.12.130.

[111] Friedlaender GE, Lin S, Solchaga LA, et al. The role of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) in orthopaedic bone repair and regeneration. Curr Pharm Des. 2013;19(19):3384–3390. doi: 10.2174/1381612811319190005.

[112] Zhu W, Chen R, Wang W, et al. Fabrication of naturally derived double-network hydrogels with a sustained aspirin release system for facilitating bone regeneration. Front Chem. 2022;10:874985. doi: 10.3389/fchem.2022.874985.

[113] Chen M, Tan H, Xu W, et al. A self-healing, magnetic and injectable biopolymer hydrogel generated by dual cross-linking for drug delivery and bone repair. Acta Biomater. 2022;153:159–177. doi: 10.1016/j.actbio.2022.09.036.

[114] Durairaj K, Balasubramanian B, Arumugam VA, et al. Biocompatibility of veratric acid–encapsulated chitosan/methylcellulose hydrogel: biological characterization, osteogenic efficiency with in silico molecular modeling. Appl Biochem Biotechnol. 2023;195(7):4429–4446. doi: 10.1007/s12010-023-04311-5.

[115] Wan Z, Dong Q, Guo X, et al. A dual-responsive polydopamine-modified hydroxybutyl chitosan hydrogel for sequential regulation of bone regeneration. Carbohydr Polym. 2022;297:120027. doi: 10.1016/j.carbpol.2022.120027.

[116] Mahanta AK, Senapati S, Paliwal P, et al. Nanoparticle-Induced controlled drug delivery using Chitosan-Based hydrogel and scaffold: application to bone regeneration. Mol Pharm. 2019;16(1):327–338. doi: 10.1021/acs.molpharmaceut.8b00995.

[117] Akbari V, Rezazadeh M, Ebrahimi Z. Comparison the effects of chitosan and hyaluronic acid-based thermally sensitive hydrogels containing rosuvastatin on human osteoblast-like MG-63 cells. Res Pharm Sci. 2020;15(1):97–106. doi: 10.4103/1735-5362.278719.

[118] Saravanan S, Vimalraj S, Anuradha D. Chitosan based thermoresponsive hydrogel containing graphene oxide for bone tissue repair. Biomed Pharmacother. 2018;107:908–917. doi: 10.1016/j.biopha.2018.08.072.

[119] Zhang Y, Dou X, Zhang L, et al. Facile fabrication of a biocompatible composite gel with sustained release of aspirin for bone regeneration. Bioact Mater. 2022;11(October 2021):130–139. doi: 10.1016/j.bioactmat.2021.09.033.

[120] Arpornmaeklong P, Sareethammanuwat M, Apinyauppatham K, et al. Characteristics and biologic effects of thermosensitive quercetin-chitosan/collagen hydrogel on human periodontal ligament stem cells. J Biomed Mater Res B Appl Biomater. 2021;109(10):1656–1670. doi: 10.1002/jbm.b.34823.

[121] Saekhor K, Udomsinprasert W, Honsawek S, et al. Preparation of an injectable modified chitosan-based hydrogel approaching for bone tissue engineering. Int J Biol Macromol. 2019;123:167–173. doi: 10.1016/j.ijbiomac.2018.11.041.

[122] Khatoon N, Chu MQ, Zhou CH. Nanoclay-based drug delivery systems and their therapeutic potentials. J Mater Chem B. 2020;8(33):7335–7351. doi: 10.1039/d0tb01031f.

[123] Hakimi F, Jafari H, Hashemikia S, et al. Chitosan-polyethylene oxide/clay-alginate nanofiber hydrogel scaffold for bone tissue engineering: preparation, physical characterization, and biomimetic mineralization. Int J Biol Macromol. 2023;233:123453. doi: 10.1016/j.ijbiomac.2023.123453.

[124] Sungkhaphan P, Thavornyutikarn B, Kaewkong P, et al. Antibacterial and osteogenic activities of clindamycin-releasing mesoporous silica/carboxymethyl chitosan composite hydrogels. R Soc Open Sci. 2021;8(9):210808. doi: 10.1098/rsos.210808.

[125] Wang Y, Zhao Z, Liu S, et al. Application of vancomycin-impregnated calcium sulfate hemihydrate/nanohydroxyapatite/carboxymethyl chitosan injectable hydrogels combined with BMSC sheets for the treatment of infected bone defects in a rabbit model. BMC Musculoskelet Disord. 2022;23(1):557. doi: 10.1186/s12891-022-05499-z.

[126] Yoon S-J, Kim SH, Choi JW, et al. Guided cortical and cancellous bone formation using a minimally invasive technique of BMSC- and BMP-2-laden visible light-cured carboxymethyl chitosan hydrogels. Int J Biol Macromol. 2023;227:641–653. doi: 10.1016/j.ijbiomac.2022.12.137.
[127] Ma L, Su W, Ran Y, et al. Synthesis and characterization of injectable self-healing hydrogels based on oxidized alginate-hybrid-hydroxyapatite nanoparticles and carboxymethyl chitosan. Int J Biol Macromol. 2020;165(Pt A):1164–1174. doi: 10.1016/j.ijbiomac.2020.10.004.
[128] Lu H-T, Lu T-W, Chen C-H, et al. Development of nanocomposite scaffolds based on biomineralization of N,O-carboxymethyl chitosan/fucoidan conjugates for bone tissue engineering. Int J Biol Macromol. 2018;120(Pt B):2335–2345. doi: 10.1016/j.ijbiomac.2018.08.179.
[129] Kocak FZ, Yar M, Rehman IU. Hydroxyapatite-Integrated, heparin-and Glycerol-Functionalized Chitosan-Based injectable hydrogels with improved mechanical and proangiogenic performance. Int J Mol Sci. 2022;23(10):5370 doi: 10.3390/ijms23105370.
[130] Zhang Y, Li Z, Wang Z, et al. Biomaterials advances mechanically enhanced composite hydrogel scaffold for in situ bone repairs. Biomater Adv. 2022;134(February):112700. doi: 10.1016/j.msec.2022.112700.
[131] Jiang M, Pan Y, Liu Y, et al. Effect of sulfated chitosan hydrogel on vascularization and osteogenesis. Carbohydr Polym. 2022;281:119059. doi: 10.1016/j.carbpol.2021.119059.
[132] Divband B, Aghazadeh M, Al-Qaim ZH, et al. Bioactive chitosan biguanidine-based injectable hydrogels as a novel BMP-2 and VEGF carrier for osteogenesis of dental pulp stem cells. Carbohydr Polym. 2021;273:118589. doi: 10.1016/j.carbpol.2021.118589.
[133] Tang W, Yu Y, Wang J, et al. Enhancement and orchestration of osteogenesis and angiogenesis by a dual-modular design of growth factors delivery scaffolds and 26SCS decoration. Biomaterials. 2020;232:119645. doi: 10.1016/j.biomaterials.2019.119645.
[134] Wang T, Guo S, Zhang H, et al. Injectable hydrogel delivering bone morphogenetic protein-2, vascular endothelial growth factor, and adipose-derived stem cells for vascularized bone tissue engineering. J Drug Deliv Sci Technol. 2020;57:101637. doi: 10.1016/j.jddst.2020.101637.
[135] Pramanik S, Mohanto S, Manne R, et al. Nanoparticle-Based drug delivery system: the magic bullet for the treatment of chronic pulmonary diseases. Mol Pharm. 2021;18(10):3671–3718. doi: 10.1021/acs.molpharmaceut.1c00491.
[136] Socrates R, Prymak O, Loza K, et al. Biomimetic fabrication of mineralized composite films of nanosilver loaded native fibrillar collagen and chitosan. Mater Sci Eng C Mater Biol Appl. 2019;99:357–366. doi: 10.1016/j.msec.2019.01.101.
[137] Chen Y, Sheng W, Lin J, et al. Magnesium oxide nanoparticle coordinated Phosphate-Functionalized chitosan injectable hydrogel for osteogenesis and angiogenesis in bone regeneration. ACS Appl Mater Interf. 2022;14(6):7592–7608. doi: 10.1021/acsami.1c21260.
[138] Sareethammanuwat M, Boonyuen S, Arpornmaeklong P. Effects of beta-tricalcium phosphate nanoparticles on the properties of a thermosensitive chitosan/collagen hydrogel and controlled release of quercetin. J Biomed Mater Res A. 2021;109(7):1147–1159. doi: 10.1002/jbm.a.37107.
[139] Nie L, Wu Q, Long H, et al. Development of chitosan/gelatin hydrogels incorporation of biphasic calcium phosphate nanoparticles for bone tissue engineering. J Biomater Sci Polym Ed. 2019;30(17):1636–1657. doi: 10.1080/09205063.2019.1654210.
[140] Yu Y, Yu X, Tian D, et al. Thermo-responsive chitosan/silk fibroin/amino-functionalized mesoporous silica hydrogels with strong and elastic characteristics for bone tissue engineering. Int J Biol Macromol. 2021;182:1746–1758. doi: 10.1016/j.ijbiomac.2021.05.166.
[141] Min Q, Yu X, Liu J, et al. Chitosan-Based hydrogels embedded with hyaluronic acid complex nanoparticles for controlled delivery of bone morphogenetic protein-2. Pharmaceutics. 2019;11(5):214. doi: 10.3390/pharmaceutics11050214.

[142] Li H, Ji Q, Chen X, et al. Accelerated bony defect healing based on chitosan thermosensitive hydrogel scaffolds embedded with chitosan nanoparticles for the delivery of BMP2 plasmid DNA. J Biomed Mater Res A. 2017;105(1):265–273. doi: 10.1002/jbm.a.35900.
[143] Zhang Q, Pei Q, Yang J, et al. Vascularized nanocomposite hydrogel mechanically reinforced by polyelectrolyte-modified nanoparticles. J Mater Chem B. 2022;10(28):5439–5453. doi: 10.1039/d2tb00735e.
[144] Rajabi M, McConnell M, Cabral J, et al. Chitosan hydrogels in 3D printing for biomedical applications. Carbohydr Polym. 2021;260:117768. doi: 10.1016/j.carbpol.2021.117768.
[145] Liu K, Li L, Chen J, et al. Bone ECM-like 3D printing scaffold with liquid crystalline and viscoelastic microenvironment for bone regeneration. ACS Nano. 2022;16(12):21020–21035. doi: 10.1021/acsnano.2c08699.
[146] Zhao C, Qazvini NT, Sadati M, et al. A pH-Triggered, Self-Assembled, and bioprintable hybrid hydrogel sca ff old for mesenchymal stem cell based bone tissue engineering. ACS Appl Mater Interf. 2019;11(9):8749–8762. doi: 10.1021/acsami.8b19094.
[147] Pahlevanzadeh F, Emadi R, Valiani A, et al. Three-Dimensional printing constructs based on the chitosan for tissue regeneration : State of the art, developing directions and prospect trends. Materials. 2020;13(11):2663.
[148] Demirtaş TT, Irmak G, Gümüşderelioğlu M. A bioprintable form of chitosan hydrogel for bone tissue engineering. Biofabrication. 2017;9(3):035003. doi: 10.1088/1758-5090/aa7b1d.
[149] Liu I-H, Chang S-H, Lin H-Y. Chitosan-based hydrogel tissue scaffolds made by 3D plotting promotes osteoblast proliferation and mineralization. Biomed Mater. 2015;10(3):035004. doi: 10.1088/1748-6041/10/3/035004.
[150] Ramirez Caballero SS, Saiz E, Montembault A, et al. 3-D printing of chitosan-calcium phosphate inks: rheology, interactions and characterization. J Mater Sci Mater Med. 2018;30(1):6. doi: 10.1007/s10856-018-6201-y.
[151] Janarthanan G, Tran HN, Cha E, et al. 3D printable and injectable lactoferrin-loaded carboxymethyl cellulose-glycol chitosan hydrogels for tissue engineering applications. Mater Sci Eng C Mater Biol Appl. 2020;113:111008. doi: 10.1016/j.msec.2020.111008.
[152] Dong L, Wang S-J, Zhao X-R, et al. 3D- Printed poly(ε-caprolactone) scaffold integrated with cell-laden chitosan hydrogels for bone tissue engineering. Sci Rep. 2017;7(1):13412. doi: 10.1038/s41598-017-13838-7.
[153] Moreira CDF, Carvalho SM, Florentino RM, et al. Injectable chitosan/gelatin/bioactive glass nanocomposite hydrogels for potential bone regeneration: in vitro and in vivo analyses. Int J Biol Macromol. 2019;132:811–821. doi: 10.1016/j.ijbiomac.2019.03.237.
[154] Zhang Y, Chen M, Tian J, et al. In situ bone regeneration enabled by a biodegradable hybrid double-network hydrogel. Biomater Sci. 2019;7(8):3266–3276. doi: 10.1039/c9bm00561g.
[155] Lewandowska-Łańcucka J, Gilarska A, Buła A, et al. Genipin crosslinked bioactive collagen/chitosan/hyaluronic acid injectable hydrogels structurally amended via covalent attachment of surface-modified silica particles. Int J Biol Macromol. 2019;136:1196–1208. doi: 10.1016/j.ijbiomac.2019.06.184.
[156] Cui Z-K, Kim S, Baljon JJ, et al. Microporous methacrylated glycol chitosan-montmorillonite nanocomposite hydrogel for bone tissue engineering. Nat Commun. 2019;10(1):3523. doi: 10.1038/s41467-019-11511-3.
[157] Gilarska A, Lewandowska-Łańcucka J, Guzdek-Zając K, et al. Bioactive yet antimicrobial structurally stable collagen/chitosan/lysine functionalized hyaluronic acid–based injectable hydrogels for potential bone tissue engineering applications. Int J Biol Macromol. 2020;155:938–950. doi: 10.1016/j.ijbiomac.2019.11.052.
[158] Kosowska K, Domalik-Pyzik P, Krok-Borkowicz M, et al. Polylactide/hydroxyapatite nonwovens incorporated into chitosan/graphene materials hydrogels to form novel hierarchical scaffolds. Int J Mol Sci. 2020;21(7):2330. doi: 10.3390/ijms21072330.

[159] Jahed V, Vasheghani-Farahani E, Bagheri F, et al. Quantum dots-βcyclodextrin-histidine labeled human adipose stem cells-laden chitosan hydrogel for bone tissue engineering. Nanomedicine. 2020;27:102217. doi: 10.1016/j.nano.2020.102217.
[160] Nie L, Deng Y, Li P, et al. Hydroxyethyl Chitosan-Reinforced polyvinyl alcohol/biphasic calcium phosphate hydrogels for bone regeneration. ACS Omega. 2020;5(19):10948–10957. doi: 10.1021/acsomega.0c00727.
[161] Maharjan B, Park J, Kaliannagounder VK, et al. Regenerated cellulose nanofiber reinforced chitosan hydrogel scaffolds for bone tissue engineering. Carbohydr Polym. 2021;251:117023. doi: 10.1016/j.carbpol.2020.117023.
[162] Datta S, Rameshbabu AP, Bankoti K, et al. Decellularized bone matrix/oleoyl chitosan derived supramolecular injectable hydrogel promotes efficient bone integration. Mater Sci Eng C Mater Biol Appl. 2021;119:111604. doi: 10.1016/j.msec.2020.111604.
[163] Kim S, Cui Z-K, Koo B, et al. Chitosan-Lysozyme conjugates for Enzyme-Triggered hydrogel degradation in tissue engineering applications. ACS Appl Mater Interfaces. 2018;10(48):41138–41145. doi: 10.1021/acsami.8b15591.
[164] Kazemi-Aghdam F, Jahed V, Dehghan-Niri M, et al. Injectable chitosan hydrogel embedding modified halloysite nanotubes for bone tissue engineering. Carbohydr Polym. 2021;269:118311. doi: 10.1016/j.carbpol.2021.118311.
[165] Wasupalli GK, Verma D. Injectable and thermosensitive nanofibrous hydrogel for bone tissue engineering. Mater Sci Eng C Mater Biol Appl. 2020;107:110343. doi: 10.1016/j.msec.2019.110343.
[166] Nezhad-Mokhtari P, Akrami-Hasan-Kohal M, Ghorbani M. An injectable chitosan-based hydrogel scaffold containing gold nanoparticles for tissue engineering applications. Int J Biol Macromol. 2020;154:198–205 doi: 10.1016/j.ijbiomac.2020.03.112.
[167] Cao Z, Bai X, Wang C, et al. A simple polysaccharide based injectable hydrogel compositing nano-hydroxyapatite for bone tissue engineering. Mater Lett. 2021;293:129755. doi: 10.1016/j.matlet.2021.129755.