European Cells and Materials Vol.52 2025 (pages 58–79) DOI: 10.22203/eCM.v052a05 ISSN 1473-2262

Review

CHITOSAN SCAFFOLDS FOR BONE APPLICATIONS: A DETAILED REVIEW OF MAIN TYPES, FEATURES AND MULTIFACETED USES

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Abstract

Chitosan scaffolds, derived from the exoskeletons of crustaceans, have become a foundational element in bone tissue engineering, celebrated for their exceptional biocompatibility, biodegradability, and inherent osteoconductive properties. This review provides a comprehensive analysis of the various forms of chitosan scaffolds, with a particular focus on their porosity and swelling behaviors, as well as their biological and mechanical characteristics—key factors that underpin their therapeutic efficacy. The distinctive versatility of chitosan scaffolds is highlighted by their expanding applications in dental regeneration, including dental pulp restoration, periodontal repair, and alveolar ridge preservation following tooth extraction. Furthermore, the review underscores the critical role of chitosan scaffolds in advancing bone tissue engineering, encompassing applications such as fracture healing, spinal fusion, joint reconstruction, and craniofacial remodeling. Innovative strategies to enhance osteoblast activity and stimulate new bone formation are showcased, positioning chitosan scaffolds as effective carriers for osteogenic growth factors and genetic material. By integrating current advancements with future prospects, this review offers a thorough overview of the transformative potential of chitosan scaffolds in regenerative medicine, illustrating a promising convergence of research and clinical application that is poised to revolutionize bone regeneration therapies.

Keywords: Chitosan scaffolds, bone tissue engineering, bone regeneration therapy, scaffolds features, scaffolds types.

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Introduction

In the rapidly evolving field of biomedical materials, bone tissue engineering has emerged as a critical area of research. Addressing the complexities of bone defect repair and regeneration necessitates the development of materials that not only provide structural support but also promote bone cell proliferation and differentiation [1–3]. Among various biomaterials, scaffold materials have shown immense promise for bone regeneration, with chitosan (CS) standing out due to its exceptional properties.

CS, a natural polysaccharide derived from the deacetylation of chitin, is favored in biomedical applications for its superior biocompatibility, biodegradability, and low immunogenicity [4]. This review focuses on why authors select CS scaffolds over other materials by examining its unique properties that make it an ideal candidate for bone tissue engineering. CS's abilities to form porous structures, enhance cell adhesion and growth, and degrade into non-toxic oligosaccharides and glucosamine are pivotal advantages that set it apart from other materials.

The impact of this review lies in its comprehensive evaluation of CS scaffolds, offering insights into their performance and applications compared to other scaffold materials. By exploring the differences between CS derived from synthetic sources and those from animal origins, this review provides a nuanced understanding of how these variations affect biomedical applications. Animal-derived CS is prevalent due to its abundant availability and cost-effectiveness. However, differences in deacetylation degree, molecular weight, and purity can influence its effectiveness in bone regeneration.

This review aims to synthesize current research on CS scaffolds in bone tissue engineering, comparing perfor-

mance metrics such as retention and degradation rates, as well as angiogenesis promotion. The structure of this review is illustrated in Fig. 1. Furthermore, we examine the application of CS scaffolds across various bone types, such as skull and long bones, and assess the impact of particle or mesh size on bone regeneration. By highlighting potential defects and suggesting improvements, this review aspires to provide a comprehensive resource for future research and clinical applications in bone tissue engineering.

Principal Chitosan Scaffold Types

Advancements in bone-related medical research are pivotal, with an emphasis on innovative strategies for bone repair and regeneration. CS, a biocompatible and biodegradable polymer sourced from the exoskeletons of crustaceans, has emerged as a promising candidate for bone tissue engineering applications [5,6]. CS sources are not limited to animal-derived materials (such as crustacean exoskeletons); they can also be produced through microbial synthetic pathways. Although most commercial CS materials are derived from marine animal shells, recent studies have shown that synthetic CS offers a viable alternative [7,8]. Comparatively, animal-derived CS involves a complex production process and may contain allergens or impurities, whereas synthetic CS demonstrates advantages in controlled purity and composition in specific applications. However, the higher cost of synthetic CS could limit its widespread use [9]. By comparing these two CS sources, we gain clearer insights into their suitability for various biomedical applications. Its versatility allows for fabrication into various scaffold architectures, including molded macroporous scaffolds, fibrous scaffolds, injectable hydrogels, microspheres, and three-dimensional (3D)-printed structures. These scaffolds are intricately designed to emulate the natural porous matrix of osseous tissue, thereby fostering osteoblast proliferation and differentiation [10]. The ensuing subsection will elucidate the characteristics and potentialities of each scaffold type with an illustrative overview provided in Fig. 2, delineating the structural nuances of the respective stents [11–15].

Molded Macroporous Scaffolds

Macroporous scaffolds fabricated through molding techniques have become a cornerstone in the realm of bone tissue engineering, primarily due to their uniquely porous architecture and superior mechanical attributes [16]. These scaffolds, often referred to in the literature as foams or sponges, are synthesized utilizing a specialized process encompassing phase separation and lyophilization. The end result of this process is a scaffold characterized by an extensively interconnected pore network, which not only facilitates a substantial surface area conducive to cellular attachment and proliferation but also ensures the efficient exchange of nutrients necessary for cell viability. The tailored porosity of these constructs, modifiable during the production phase, renders them particularly amenable to applications in bone tissue engineering [17]. Moreover, the simplicity and economic viability of the manufacturing process further enhance the appeal of these scaffolds for widespread clinical use.

The architecture of these scaffolds, which is inherently porous, is among their most salient features for bone tissue engineering applications. The interconnected pore network is essential as it provides extensive surface area for cellular adherence and growth, as well as the exchange of critical nutrients, all of which are vital for the successful infiltration of cells and subsequent formation of new bone tissue. During the fabrication phase, the porosity can be manipulated with precision by modulating the concentration of biomaterials like CS and altering the molding conditions, thereby allowing customization of the pore size and distribution to meet specific bone tissue requirements [18].

The manufacturing protocol for these molded macroporous scaffolds is notably straightforward and cost-efficient, particularly when contrasted with alternative scaffold types. The process involves the casting of a polymer solution, such as CS, into a predetermined mold, succeeded by a crosslinking step to fortify the scaffold's structure. This methodological approach is conducive to the mass production of scaffolds, thereby making them suitable for extensive bone tissue engineering endeavors [19]. Additionally, the mechanical properties of these scaffolds are commendable; their structural support is provided by the interconnected pore system, while the material properties of CS offer sufficient strength and rigidity appropriate for bone tissue engineering. The mechanical resilience of the scaffold is critical to endure the forces exerted by the surrounding bone tissue while preserving its structural integrity [20].

Expanding beyond the confines of bone tissue engineering, these molded macroporous scaffolds also exhibit significant potential for drug delivery applications, particularly for diseases associated with bone. The scaffold's extensive surface area and porous network facilitate a high payload of therapeutic agents, and the controlled degradation of the scaffold matrix can regulate the drug release kinetics. This characteristic is particularly advantageous for targeted drug delivery, necessitating a high drug concentration at a specific anatomical site [21]. Moreover, the efficacy of these scaffolds in bone repair and regeneration has been substantiated through extensive pre-clinical studies [22]. Animal model research has demonstrated that these scaffolds promote cellular proliferation and bone tissue regeneration, with successful applications in bone repair signaling their promise for future clinical translation [23].

Despite the myriad advantages presented by molded macroporous scaffolds, challenges persist, particularly regarding the attainment of a uniform pore structure, which is pivotal for optimal cell growth and bone tissue regeneration. The fabrication process parameters, such as the concentration of CS and the specific conditions of molding, can

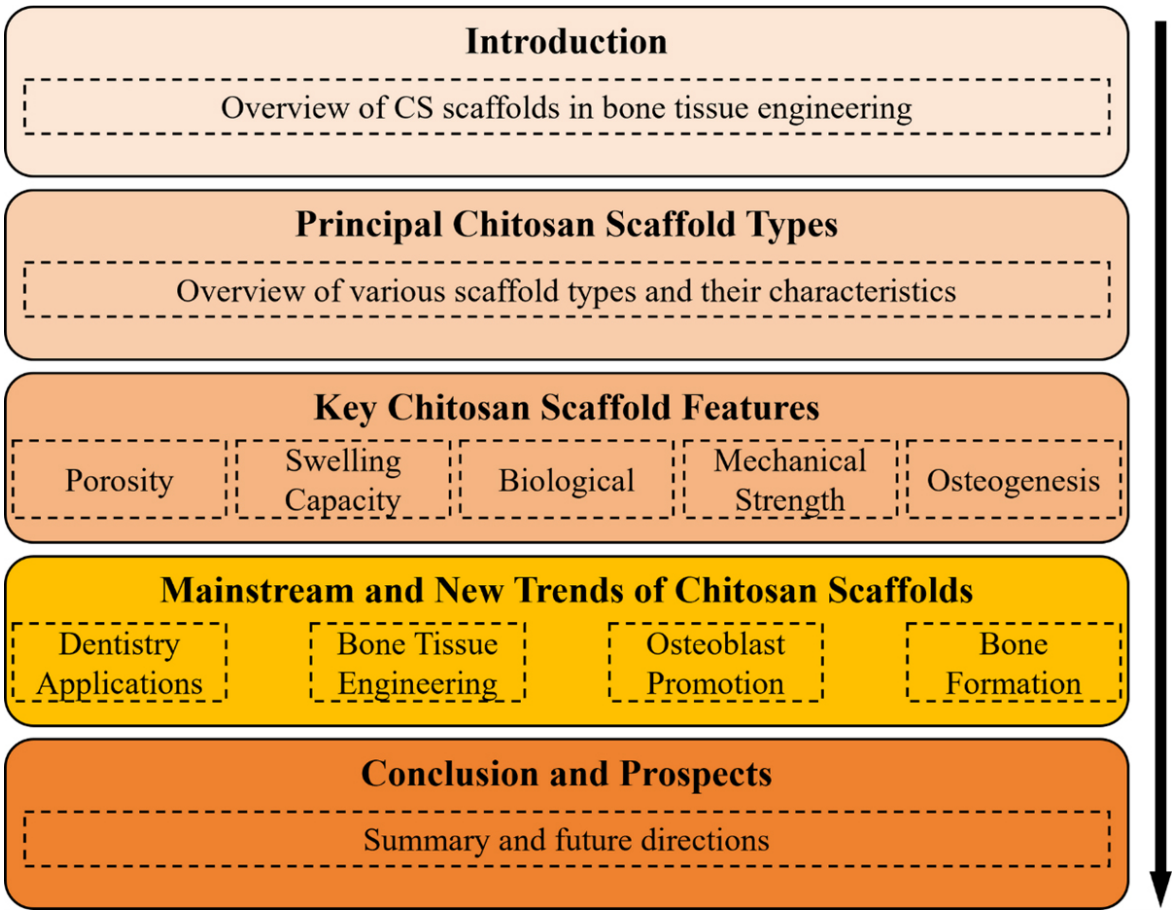

Fig. 1. The structure of this review. This image was created by PowerPoint. CS, chitosan.

significantly influence the pore size and distribution. As such, meticulous control over these parameters is imperative to ensure that the desired properties of the scaffold are achieved. Another limitation pertains to the biodegradation rate of the scaffold [24]; while the biodegradability of materials like CS is generally viewed as beneficial, an accelerated degradation rate could potentially compromise the scaffold's structural integrity prematurely, thereby impeding the process of bone tissue regeneration. Addressing this concern may entail the integration of additional materials or modification of the CS's molecular structure to more precisely control the rate of degradation [25].

Fiber-Based Scaffolds

In the burgeoning field of tissue engineering, electrospun CS fiber-based scaffolds have attracted considerable interest for their potential to facilitate the growth and regeneration of various tissues, including bone. These advanced three-dimensional constructs are generated from fine CS fibers, which are produced using electrospinning—a technique that forms fibers from a CS solution by applying a high voltage to create an electrically charged jet. The fibers are subsequently collected and arranged in a layered fashion to produce a scaffold [26].

The architecture of these scaffolds is characterized by a highly porous network of interconnected fibers, providing an expansive surface area that supports cellular adhesion and proliferation [27]. A critical advantage of these scaffolds lies in their biomimetic properties; they closely resemble the extracellular matrix with fiber diameters ranging from nanometers to micrometers, thus offering a conducive environment for cell attachment, nutrient exchange, and ultimately, tissue regeneration [28].

The utility of fiber-based CS scaffolds extends across diverse tissue engineering applications, including the regeneration of bone, cartilage, and neural tissues. Their structural design not only provides the necessary support for cellular ingrowth but also emulates the intricate architecture of natural tissues, thereby promoting effective tissue integration and function. Furthermore, the fabrication process of these scaffolds offers meticulous control over scaffold properties [29]. By fine-tuning electrospinning parameters such as voltage, flow rate, and collector distance, the fiber diameter, orientation, and overall scaffold porosity can be customized. This adaptability is vital for engineering scaffolds that can replicate specific tissue characteristics, which is paramount for enhanced cell growth and tissue regeneration [30].

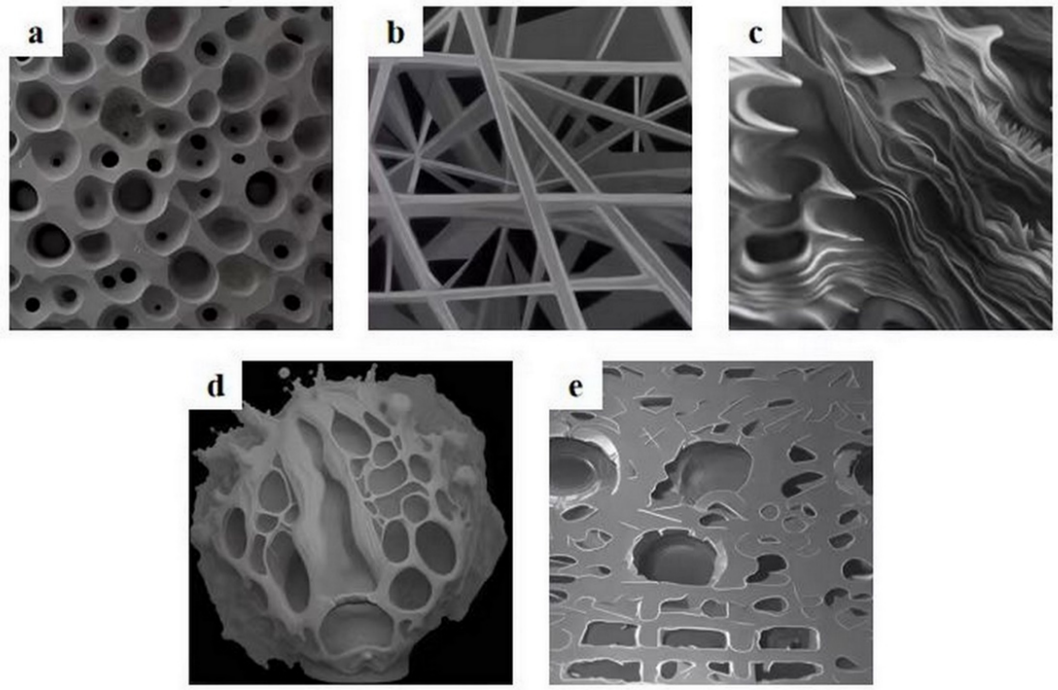

Fig. 2. Schematic diagram of each type of chitosan scaffold. (**a**) Molded macroporous scaffolds. (**b**) Fiber-based scaffolds. (**c**) Injectable hydrogels. (**d**) Microspheres. (**e**) 3D-printed scaffolds. 3D, three-dimensional. This image was generated using an artificial intelligence tool (Baidu Wenxin Yige).

Another notable attribute of fiber-based CS scaffolds is their impressive mechanical robustness. The individual fibers exhibit remarkable strength and stiffness, equipping the scaffolds to endure the mechanical stresses present within the tissue environment. This mechanical integrity is essential in tissue engineering applications, where scaffolds must withstand *in vivo* forces and provide a reliable framework during the tissue formation process.

The high surface area and porous nature of these scaffolds also render them effective platforms for drug delivery applications. They can be loaded with substantial quantities of therapeutic agents, with the controlled degradation of the scaffold matrix dictating the release kinetics of the drugs. Such targeted drug delivery systems are particularly beneficial in scenarios where high local concentrations of drugs are required, as is often the case in bone-related pathologies [31,32].

Despite these strengths, electrospun CS scaffolds are not without their challenges. Achieving consistency in the electrospinning process can be complex, which may lead to variability in the scaffold's structural features and performance. Such inconsistencies can adversely impact the scaffold's ability to support cellular functions and tissue formation [33]. Additionally, managing the degradation rate of the scaffolds can pose difficulties, as it is influenced by various factors, including fiber thickness and organization. An overly rapid degradation could undermine the structural integrity of the scaffold before adequate tissue regeneration, thus impeding the healing process [34]. To address these limitations, strategies such as the incorporation of additional materials or chemical modifications to the CS polymer are employed. These approaches aim to refine the degradation profile and improve the consistency of the scaffold structure, thereby optimizing their performance for tissue regeneration and drug delivery applications. As research in this domain advances, the potential of electrospun CS scaffolds to revolutionize the field of bone tissue engineering and drug delivery continues to grow.

Injectable Hydrogels

Injectable hydrogels, particularly those synthesized from CS, have emerged as a versatile solution in the realm of tissue engineering, with a marked focus on the regeneration of bone tissue. These hydrogels are formulated by crosslinking CS with biocompatible agents such as glycerophosphate or genipin, resulting in a viscoelastic gel that can be applied in a minimally invasive manner directly into the injury site or defect [35]. A standout advantage of these hydrogel systems is their injectability, which distinguishes them from conventional scaffold-based approaches that often necessitate surgical implantation. The ability to administer these gels via syringe not only minimizes the invasiveness of the procedure but also substantially reduces the risk of postoperative complications and enhances the precision

of regenerative material placement. This property is particularly beneficial for areas that are challenging to access surgically [36].

The porous architecture of injectable CS hydrogels, characterized by an intricate network of interconnecting pores, provides an extensive surface area conducive to cellular adhesion and proliferation [37]. Moreover, the porosity facilitates efficient nutrient and waste exchange, which are critical processes in cell survival and proliferation, thereby supporting the ingrowth of new tissue. These characteristics are highly desirable for bone tissue engineering, where scaffold-mediated support is essential for osteogenesis [38,39].

The versatility of injectable hydrogels is further underscored by the tunability of their properties. Through careful manipulation of crosslinking parameters, one can modulate the mechanical properties, degradation kinetics, and drug release profiles of these hydrogels to align with the specific demands of bone tissue regeneration. Such precision in design ensures that the scaffold provides the appropriate mechanical support and biochemical cues to promote bone healing and integration [40].

Despite typically possessing lower stiffness compared to other scaffold materials, the mechanical properties of injectable CS hydrogels can be engineered to mirror the biomechanical characteristics of the surrounding bone tissue. Such mechanical compatibility is critical to ensure that the hydrogel scaffolds provide an adequate mechanical framework to facilitate tissue regeneration, while also allowing for harmonious integration with the host tissue. As the hydrogel degrades, it gradually cedes space to newly formed tissue, resulting in a natural and progressive tissue restoration [41,42]. Nevertheless, the injectable nature of these hydrogels presents certain challenges. The mechanical resilience of the hydrogel must be sufficient to withstand *in vivo* forces; otherwise, the scaffold may collapse under physiological loads, thereby impeding the regeneration of bone tissue. Therefore, the design of the hydrogel's material properties, particularly its stiffness, requires meticulous optimization to strike a balance between injectability and structural integrity [43].

Microspheres

CS microspheres, minute spherical particles fabricated through the crosslinking of CS with biocompatible agents such as glutaraldehyde or genipin, have become a focal point in the advancement of tissue engineering and drug delivery systems, with a strong emphasis on bone regeneration. The process of creating these scaffolds involves meticulously shaping CS into uniform microspheres [44,45].

The appeal of CS microspheres in the domain of bone tissue engineering lies in their exceptional surface area to volume ratio. This attribute is critical as it significantly enhances the available surface for cellular attachment and proliferation, thereby accelerating tissue regeneration. The microspheres' structure, defined by a network of interconnected pores, not only supports cellular infiltration and vascularization but also facilitates the formation of new bone tissue through osteoconduction [46].

The precision with which these microspheres can be tailored is paramount in their utility as scaffolds. Adjustments in crosslinking agents and parameters enable the customization of the microspheres' size, stiffness, and degradation rate. Such meticulous control ensures that the scaffolds meet the intricate requirements of bone tissue, supporting both the initial cellular framework and subsequent bone matrix deposition [47].

In the context of mechanical compatibility, CS microspheres typically exhibit lower stiffness relative to other CS-based scaffolds. This, however, is offset by their microscale size and the high surface area, which collectively promote superior integration with the host bone tissue. The integration is further enhanced as the material's degradation rate can be synchronized with the rate of new bone formation, ensuring that the scaffold provides a transient yet supportive role in tissue regeneration [48,49].

Despite their considerable advantages, CS microspheres are not without their challenges. Primarily, the small size of the particles poses a challenge in achieving a homogeneous distribution within the scaffold matrix, which is essential for uniform cell growth and tissue regeneration. Inhomogeneities could lead to inconsistent regeneration and weak points within the newly formed tissue. Consequently, the design and fabrication process must be finely tuned to ensure a consistent distribution of the microspheres. This involves a combination of advanced manufacturing techniques and rigorous quality control measures to achieve a scaffold that can reliably support the complex process of bone regeneration [50].

3D-Printed Scaffolds

In the burgeoning field of bone tissue engineering, 3D-printed CS scaffolds represent a significant technological advancement. These structures, crafted layer by layer through advanced additive manufacturing techniques, utilize CS-based bioinks to construct highly customizable and complex three-dimensional matrices [51]. The flexibility and precision of this technology are unparalleled, enabling the production of scaffolds that closely emulate the intricate architecture of natural bone [52].

The customizability of 3D-printed scaffolds is a paramount advantage, allowing for the creation of structures tailored to the specific needs of bone tissue engineering. Using computer-aided design (CAD) software, engineers and scientists can devise scaffolds that not only facilitate bone tissue in-growth but also mimic the biomechanical properties of the target tissue. This intricacy in design is vital for the formation of a conducive environment that promotes osteogenesis and supports new bone formation [53].

A distinctive feature of these printed scaffolds is their highly porous architecture, which is essential for replicating the porosity found in natural bone. The interconnecting pore network within the scaffold ensures a substantial surface area for cellular attachment, proliferation, and differentiation. Additionally, it facilitates critical nutrient and waste exchange, which are essential for cell survival and the generation of new tissue. The production process of 3D-printed scaffolds allows for meticulous control over their physical properties. Parameters such as layer thickness, pore size, and infill density can be finely tuned to influence the mechanical strength and degradation rate of the scaffold, ensuring that it provides the optimal support for bone regeneration and integrates seamlessly with the host tissue [54].

Despite these advantages, the field of 3D bioprinting is not without its challenges. The printing process can be time-intensive, and the cost of materials and equipment may be prohibitive. A significant consideration is the resolution of the printed scaffold, which is critical for its structural integrity and the scaffold's functional performance in supporting bone tissue regeneration. To overcome these limitations, ongoing research and development are focused on optimizing the printing process. Innovations in printing technology aim to enhance the resolution and fidelity of printed scaffolds, thereby improving their structural and functional integration with the existing bone tissue. By addressing these challenges, 3D-printed CS scaffolds continue to hold great promise for the future of regenerative medicine and the restoration of bone defects [55,56]. A summary of all the different types of CS scaffolds mentioned above is given in the Table 1 (Ref. [57–82]).

Key Chitosan Scaffold Features

Porosity

The pivotal role of CS scaffold porosity in tissue engineering, particularly in applications pertaining to bone regeneration and drug delivery systems, cannot be overstated. This porosity, carefully crafted within the scaffold's structure, is instrumental in mimicking the intricate extracellular matrix (ECM) of natural tissues, thereby providing an environment conducive to cellular activities essential for tissue growth. High porosity, typically around 80–90 %, is essential for facilitating cellular infiltration, nutrient diffusion, and waste removal [83]. The interconnectivity of pores within CS scaffolds is finely tuned to accommodate the multifaceted needs of bone tissue engineering, where the transport of nutrients, oxygen, and cellular waste is paramount to the survival and proliferation of cells [84]. Specifically, pore sizes exceeding 100 μm are essential to facilitate nutrient diffusion and cell migration, while smaller pores between 50–100 μm promote initial cell attachment and proliferation [85,86].

These interconnected pores enhance vascular infiltration, allowing regenerated tissue to access vital nutrients and seamlessly integrate with the host's circulatory system. This vascularization process not only supplies critical nutrients but also efficiently removes metabolic waste, preventing the accumulation of toxic byproducts that could impede healing [87,88].

The customization of porosity is also significant for mechanical integrity, particularly in bone tissue engineering, where the scaffold must withstand various stresses. The stiffness of the scaffold, modulated by its porosity, balances the need for mechanical support with the necessity for a biomimetic structure that promotes tissue in-growth. Gradients of pore sizes, incorporating both smaller and larger pores, have demonstrated superior outcomes by addressing the diverse requirements of different cell types. For instance, bone scaffolds require a particular pore size, often exceeding 100 μm, to support cell infiltration and the formation of mineralized matrices. Pore sizes in the range of 300–600 μm mimic cancellous bone structures, providing optimal conditions for osteoconduction and osteoinduction, processes imperative for bone healing and integration [89].

In the design of CS scaffolds, pore size plays a crucial role in cell attachment and proliferation. Surface roughness further enhances cell attachment by providing micro- and nanoscale topographies that mimic the natural ECM [90]. These features increase the scaffold's surface area and promote integrin-mediated focal adhesion formation, activating mechanotransduction pathways such as Wnt/β-catenin, which are critical for osteogenesis. For example, scaffolds with pore sizes around 400 μm are most beneficial for the growth of chondrocytes and osteoblasts, while those with pore sizes around 190 μm are more suitable for fibroblast growth.

In addition to providing structural support, the porous nature of CS scaffolds offers a versatile platform for drug delivery, particularly beneficial for bone healing processes that may require localized and sustained release of osteogenic factors or antibiotics. The pores act as microreservoirs from which therapeutic agents can be steadily released, enhancing the healing process while minimizing systemic side effects. For example, scaffolds with a porosity range of 88–97 % and pore sizes between 50–700 μm have been shown to facilitate tissue regeneration by supporting cellular activities while maintaining mechanical stability. Interconnected pores of 100–200 μm can enhance the diffusion of bioactive molecules, while larger pores of 300–400 μm ensure prolonged vascularization and drug retention [91,92].

The production of CS scaffolds with the desired porosity is a complex procedure influenced by various factors, including the source of the CS, the crosslinking agents used, and the method of scaffold fabrication. These factors must be carefully controlled to achieve the optimal porosity that allows for mechanical stability, biocompatibility, and the

Table 1. Summary of different types of chitosan scaffolds.

Scaffold type	Key features	Retention rate and degradation rate in bone	Mechanical properties	Animal model	Evaluation methods
Molded macroporous scaffolds	- Highly porous structure - Excellent mechanical properties - Simple and cost-effective fabrication - Allows for mass production [57], - Porosity can be easily controlled [58], porosity easily controlled for large-scale bone tissue engineering	- Retention: high - Degradation: moderate [59]	- Excellent mechanical properties - Adjustable pore sizes for specific tissue needs [60]	Rodent models [61]	- Micro-CT - Histological analysis - Mechanical testing [62]
Fiber-based scaffolds	- Mimic natural extracellular matrix - Highly porous - Precise control over scaffold properties - Excellent mechanical strength [63] - Versatile for tissue engineering [64]	- Retention: high - Degradation: slow	- High tensile strength - Customizable fiber alignment for directional strength [65]	Rats and rabbits [66]	- SEM for porosity - Mechanical strength testing - Cell proliferation assays
Injectable hydrogels	- Minimally invasive - Highly porous - Lower stiffness for better integration - Precise control over scaffold properties [67–69], - Suitable for bone tissue engineering [70]	- Retention: moderate - Degradation: fast [71]	- Low stiffness - Adaptable to different tissue types	Small mammal models [72]	- Rheological analysis - *In vivo* imaging - Degradation rate analysis [73]
Microspheres	- High surface area - Highly porous - Precise control over scaffold properties - Lower stiffness for enhanced cell interaction [74] - Suitable for bone engineering	- Retention: moderate - Degradation: adjustable	- Low stiffness - Promotes specific cellular interactions	Mice and rats	- *In vitro* cell assays - Surface area and porosity measurements [75]
3D-printed scaffolds	- Highly customizable structures - Mimics natural bone environment - Highly porous - Precise control over properties - Fabrication can be time-consuming and costly [76–79], resolution can affect scaffold integrity [80]	- Retention: high - Degradation: adjustable	- Moderate to high mechanical strength (depending on material) [81]	Large animal models (sheep and pigs) [82]	- Imaging techniques (CT/MRI) - Mechanical testing - Histological analysis [75]

3D, three-dimensional; CT, computed tomography; MRI, magnetic resonance imaging; SEM, scanning electron microscope.

effective delivery of biological agents. For example, the freezing and drying processes significantly impact the pore structure. Slow freezing rates result in larger, more uniform pores suitable for vascular infiltration, while faster rates produce smaller pores ideal for initial cell seeding. Crosslinking treatments, critical for enhancing the mechanical properties of CS scaffolds, must be judiciously applied to prevent the occlusion of pores, which could compromise the scaffold's biofunctionality [93,94]. Interconnectivity must also be optimized, as scaffolds with better pore connectivity facilitate cellular migration and vascular infiltration, ensuring effective integration of the scaffold with the host tissue while maintaining sufficient mechanical integrity. Establishing the correct balance between the crosslinking density and maintaining the requisite porosity is paramount for ensuring the scaffold's performance in the dynamic and complex environment of bone tissue regeneration [95].

In summary, the porosity of CS scaffolds is a foundational feature that underpins their utility in bone tissue engineering and drug delivery. An optimal pore structure, typically within 50–400 μm, enables effective diffusion of nutrients and waste, supports cell adhesion and proliferation, allows for the controlled release of drugs, and provides the necessary mechanical support for bone regeneration. Incorporating regions with smaller pores for initial cell seeding and larger pores for vascularization creates a synergistic environment conducive to comprehensive bone regeneration. The successful application of CS scaffolds in clinical settings hinges on the precise manipulation of their porosity, a task that necessitates a nuanced understanding of the interplay between scaffold design, biological function, and mechanical demands [96].

Swelling Capacity

CS scaffolds possess a unique swelling capacity, a critical property that is integral to their performance in bone repair and regeneration. The hydrophilic groups present within the CS matrix—the -OH (hydroxyl) and -NH2 (amine) functionalities—are primarily responsible for this characteristic. These groups engage in hydrogen bonding with water molecules, facilitating the absorption of fluids into the matrix and thus contributing to the scaffold's swelling [97,98].

The degree to which these scaffolds expand upon hydration is influenced by both intrinsic properties, such as the degree of deacetylation, which correlates with the number of amine groups available for bonding, and molecular weight, where lower weights may enhance solubility and thus swelling. The internal crosslinking density also plays a pivotal role; a denser crosslinking network may inhibit swelling by restricting the polymer chains' ability to absorb water. Additionally, the pH sensitivity of CS is a double-edged sword; it can either augment swelling in acidic conditions by protonating amine groups or curtail it in basic environments due to deprotonation. Extrinsic factors are equally influential. Ionic strength, for instance, can modulate the electrostatic interactions within the scaffold, while the solvent composition dictates the scaffold's affinity for the liquid. Temperature, another external variable, can affect the molecular mobility within the scaffold, thereby impacting its ability to swell [99,100].

Understanding the swelling kinetics is essential for tailoring scaffold properties to specific applications. Models like Fickian diffusion and Schott's second-order kinetics offer insight into the interplay between water diffusion and polymer relaxation within the swelling process. For bone applications, the swelling capacity of CS scaffolds is not merely a matter of fluid uptake; it is a gateway to scaffold functionality. It influences the absorption of body fluids, nutrient transport, and cell infiltration, all of which are vital for fostering an environment conducive to bone regeneration [101].

Biological Properties—Biocompatibility, Biodegradability and Osteoconductivity

CS, a biopolymer derived from the natural process of deacetylation of chitin, holds a pivotal role in the realm of bone tissue engineering due to its exceptional biological properties. These properties encompass biocompatibility, biodegradability, and osteoconductivity—each a cornerstone in the scaffold's ability to support bone regeneration effectively.

At the heart of CS's appeal is its biocompatibility; it is remarkably well-tolerated by the body, causing minimal inflammatory or adverse immune reactions upon implantation. This compatibility with biological systems is a critical attribute, as it ensures that CS scaffolds can be introduced into bone tissues without eliciting harmful responses. Instead, they facilitate a conducive environment where cells can adhere, proliferate, and function optimally to kickstart the healing and regenerative processes [102].

The biodegradability of CS is equally important in bone tissue engineering applications. As CS scaffolds degrade, they generate non-toxic byproducts, which the body can easily resorb or excrete. The degradation kinetics of CS scaffolds can be meticulously controlled to coincide with the timeline of bone healing, ensuring the scaffold serves as a temporary framework that relinquishes its role as the new bone matures and is capable of sustaining mechanical loads independently.

Osteoconductivity is another intrinsic quality of CS scaffolds, enabling the absorption and transport of essential nutrients and expulsion of metabolic wastes from the bone tissue. This property, supplemented by the scaffold's positive charge, fosters the adhesion of proteins and cells, which is integral to initiating and sustaining bone tissue development. The utility of CS scaffolds in bone regeneration is rooted in their ability to support and orchestrate critical biological events such as cellular attraction, proliferation,

differentiation, and extracellular matrix deposition. These scaffolds provide a three-dimensional matrix that not only encourages the growth of osteoblasts but also acts as a template for the formation of new bone tissue [103]. The interaction between the scaffold and the host tissue is a dynamic one, where the scaffold's osteoconductive nature plays a pivotal role in guiding bone tissue formation [104]. To enhance the osteoinductive capability of CS scaffolds, they can be functionalized with bioactive molecules, such as bone morphogenetic proteins (BMPs), to encourage the differentiation of progenitor cells into osteoblasts. This functionality can significantly bolster the body's intrinsic bone-healing mechanisms and lead to improved outcomes in bone regeneration.

The overarching goal within bone tissue engineering is to stimulate osteogenesis—the creation of new, healthy bone. CS scaffolds are designed to offer an environment that mimics natural bone extracellular matrix, promoting the deposition of new bone matrix by osteoblasts and supporting the complex process of bone formation. Seamless integration with the host bone is a crucial determinant of a scaffold's success in bone tissue engineering [105]. CS scaffolds are advantageous because they can be remodeled by the body's natural processes and gradually replaced by new bone. This remodeling ensures that the scaffold integrates effectively with the host bone tissue without leaving any residual foreign materials that could potentially cause complications. Vascularization is a vital aspect of bone healing and regeneration. The successful integration of a scaffold into the host tissue not only depends on osteoconduction and osteoinduction but also on the formation of new blood vessels—a process known as angiogenesis. CS scaffolds encourage this process, ensuring that the newly formed tissue is well-nourished and oxygenated, which is critical for the survival of bone cells and the overall healing process [106].

In summary, the biological properties of CS scaffolds—biocompatibility, biodegradability, and osteoconductivity—are instrumental in their function as aides in bone regeneration. These properties ensure that CS scaffolds can support the complex cellular processes necessary for bone repair, be broken down and removed safely by the body, and facilitate the formation of new bone, ultimately leading to successful integration with the host tissue. The strategic design of CS scaffolds, leveraging these biological properties, heralds a new era in regenerative medicine, particularly in the restoration of bone integrity and function.

Mechanical Strength

The mechanical integrity of CS scaffolds in bone tissue engineering is a pivotal factor that is as critical as their biological compatibility. When engineering scaffolds for bone repair, one must consider the intrinsic mechanical demands placed on bone and the need for the scaffold to withstand these forces without failure. Bone, by its nature, is a composite material endowed with remarkable tensile and compressive strengths, a result of its collagen fibers and mineral content, predominantly hydroxyapatite [107]. A scaffold intended for bone regeneration must meet several mechanical prerequisites. It must be robust enough to support physiological loads during the bone healing period to prevent structural collapse, provide a stable matrix for cellular activities, and enable a gradual transfer of load to the regenerating bone tissue as it acquires mechanical competence [108].

Enhancing the innate mechanical strength of CS scaffolds is achievable through various approaches. Crosslinking with agents like glutaraldehyde or genipin can create robust interchain bonds, bolstering the scaffold's structural integrity. Moreover, blending CS with inorganic constituents such as bioactive glass, hydroxyapatite, or tricalcium phosphate can yield composite scaffolds that not only exhibit superior mechanical properties but also closely resemble the composition of natural bone. For example, high molecular weight chitosan films exhibit superior mechanical strength compared to low molecular weight chitosan films when their degree of acetylation (DA) is similar [109]. The integration of nanostructured materials like carbon nanotubes, graphene oxide, or nanocellulose can further augment the mechanical robustness of CS scaffolds. These nanomaterials act as reinforcements within the scaffold structure, enhancing its load-bearing capacity while also providing a favorable microenvironment for cellular interactions critical for bone tissue formation.

The elastic modulus of CS scaffolds significantly influences osteogenic cell behavior by modulating mechanotransduction pathways such as yes associated protein (YAP)/transcriptional coactivator with a PDZ binding domain (TAZ), Wnt/β-catenin, and integrin-focal adhesion kinase (FAK). Studies have shown that softer matrices, with elastic moduli ranging from 0.6 MPa to 2.7 MPa, enhance the expression of osteogenic markers like collagen type I (*Col I*), osteocalcin (OCN), and osteopontin (*OPN*) [110,111]. Similarly, intermediate stiffness scaffolds mimicking natural bone collagen (25–40 kPa) promote mesenchymal stem cell (MSC) differentiation and upregulate osteogenic genes more effectively than very soft matrices (0.1–17 kPa). On the other hand, high stiffness substrates ($>$100 kPa) activate the Integrin-FAK signaling pathway, enhancing β-catenin accumulation and RUNX family transcription factor 2 (*RUNX2*) expression, but may limit cellular infiltration and increase adverse inflammatory responses [110]. These findings highlight the importance of optimizing scaffold stiffness to balance cellular proliferation, differentiation, and tissue integration.

Fabrication techniques also play a significant role in determining the mechanical properties of scaffolds. Methods like freeze-drying, electrospinning, and 3D printing afford precise control over the scaffold's porosity, inter-

connectivity, and overall architecture—factors that are intimately linked to the scaffold's mechanical behavior. For example, high molecular weight chitosan has been observed to form elongated pores, while medium and low molecular weight chitosan tends to form polygonal pores [112]. These structural differences directly influence mechanical properties, as scaffolds with elongated pores tend to exhibit higher tensile strength due to aligned polymer chains. Furthermore, low DA chitosan scaffolds exhibit superior compressive strength compared to high DA scaffolds, as low acetylation allows for stronger intermolecular interactions within the polymer network [112].

Specifically, scaffold stiffness influences key mechanotransduction pathways that drive osteogenesis. For example, YAP/TAZ signaling is highly sensitive to substrate stiffness, where stiffer scaffolds promote nuclear localization of YAP/TAZ and enhance osteogenic differentiation by upregulating *RUNX2*. Similarly, the Wnt/β-catenin pathway is activated on stiffer substrates, leading to increased β-catenin translocation and further promoting bone matrix formation [113]. In contrast, softer scaffolds may support angiogenesis and cellular migration but require careful tuning to avoid diminished osteogenic responses. Future scaffold designs should focus on achieving stiffness ranges that optimize these pathways for specific bone tissue engineering applications.

The mechanical strength of a CS scaffold is not just a passive property but actively influences the bone regeneration process. Initially, it provides critical support and protection to the healing site. The mechanical cues emanating from the scaffold's structure also play a role in dictating cellular behavior—factors such as stiffness and elasticity can guide cell differentiation, with certain mechanical environments favoring the development of osteoblasts. For example, scaffolds made from squid-derived chitosan with a molecular weight of 10^5 Da and DA of 4–30 % demonstrated uniform pore distribution and superior mechanical performance compared to scaffolds with higher DA, which exhibited heterogeneous pore distribution [112].

As the bone tissue begins to form and mature, the scaffold must allow for a seamless transfer of load, avoiding abrupt changes that could result in stress shielding or reinjury. The scaffold's mechanical properties should complement the natural bone growth, gradually transferring the load to the new tissue as the scaffold itself degrades in a controlled manner. For instance, scaffolds with DA values of 5–12 % have been reported to have smaller pores and enhanced compressive strength compared to scaffolds with DA values above 30 % [112]. These differences underscore the critical role of molecular weight and DA in optimizing the mechanical performance of CS scaffolds for bone tissue engineering applications.

Ultimately, the scaffold's degradation profile must be synchronized with the development of the new bone tissue, ensuring that mechanical support is provided for as long as needed until the regenerating bone can sustain the required loads. This strategic balance between initial mechanical support and eventual degradation to pass the baton to the new bone underscores the sophistication required in designing CS-based scaffolds for bone tissue engineering.

In summary, the mechanical strength of CS scaffolds is a vital attribute that must be meticulously designed to enable the scaffold to meet the multifaceted demands of bone tissue engineering. Through innovative crosslinking, the creation of composites, nanostructuring, advanced fabrication techniques, and mechanical conditioning, CS scaffolds can be tailored to provide the necessary support for bone repair while simultaneously fostering an environment conducive to osteogenesis and eventual integration with host bone tissue. The overall properties of all chitosan scaffolds mentioned above are summarized in Table 2 (Ref. [114–118]).

Mainstream and New Trends of Chitosan Scaffolds in Bone-Related Therapies

Wide Range of Applications in Dentistry

The realm of dentistry has been significantly enriched by the advent of CS scaffolds, which have emerged as a versatile tool in the quest for regenerative solutions. Their utility extends across various sub-disciplines within dentistry, with a notable impact on the treatment and regeneration of dental pulp, periodontal therapy, and alveolar ridge preservation post-tooth extraction.

In the specialized field of endodontics, CS scaffolds have shown promise in dental pulp treatment and regeneration. Their application in pulp capping procedures takes advantage of CS's ability to encourage reparative dentin formation, crucial for maintaining pulp vitality following dental trauma or caries excavation [119]. This is of paramount importance as it allows for the preservation of the tooth's natural vitality, preventing the need for more invasive treatments such as root canals or extractions [120]. Moreover, CS's supportive nature in cell attachment and growth positions it as a favorable candidate for regenerative endodontic procedures, which aim to revitalize the pulp-dentin complex, restoring its normal function and integrity within the tooth structure.

Beyond the confines of the tooth, CS scaffolds play a pivotal role in the regeneration of periodontal tissues. Periodontal disease often leads to the destruction of the supporting structures of teeth, including alveolar bone, periodontal ligament, and cementum. The application of CS within this context aims to not only halt disease progression but to actively regenerate these lost structures, thus reinstating the essential support teeth require. The scaffold provides a three-dimensional matrix that can facilitate the repopulation of periodontal cells and serve as a reservoir for regenerative molecules, ultimately leading to the re-establishment of healthy periodontal tissue.

Table 2. Summary of overall characteristics of chitosan scaffolds.

Characteristic	Description
Porosity	Highly porous 3D structure provides large surface area for cell attachment, nutrient diffusion and waste removal. Interconnected pores support angiogenesis.
Swelling capacity	Hydrophilic groups allow absorption of water molecules, enabling uptake of body fluids, nutrient transport and cell infiltration. Depends on degree of deacetylation, crosslinking density, etc. [114].
Biocompatibility	Minimal inflammatory or immune response upon implantation, provides favorable environment for cell proliferation and function.
Biodegradability	Degrades into non-toxic byproducts that can be easily eliminated by the body. Degradation rate can be controlled [115].
Osteoconductivity	Allows absorption and transport of nutrients, expulsion of wastes, and protein/cell adhesion. Supports osteoblast proliferation and bone matrix deposition [116].
Mechanical strength	Must withstand physiological loads during bone healing. Can be enhanced by crosslinking, composites with inorganic particles, nanostructuring and controlled fabrication [117].
Other osteogenesis properties	Promotes cell adhesion and proliferation , carries and releases osteoinductive molecules [118], supports angiogenesis through its porous structure, providing nutritional support and oxygen to new bone tissue, thereby enhancing the osteogenesis process.

Following tooth extraction, the preservation of the alveolar ridge is of critical concern, particularly in anticipation of future dental implants or other prosthetic restorations. The loss of a tooth can lead to significant resorption of the alveolar bone, compromising the quantity and quality of bone available for future dental rehabilitative procedures [121]. CS scaffolds can be employed effectively in these scenarios to maintain or augment the alveolar ridge dimensions. Their application helps to stabilize the blood clot, support the ingrowth of new bone cells, and minimize the resorption that typically follows tooth extraction, thereby maintaining the ridge's height and width. This application is not only crucial for aesthetic considerations but also for ensuring that patients have the option for dental implants, which require a certain amount of bone for successful osseointegration and long-term stability.

The wide-ranging applications of CS scaffolds in dentistry underscore the material's potential to revolutionize dental tissue engineering and regenerative therapies. By providing a conducive environment for tissue regeneration, CS scaffolds have the potential to restore function and aesthetics, improving patient outcomes and advancing the field of reconstructive dental medicine. Their adaptability and efficacy in promoting healing and regeneration across various dental tissues place CS scaffolds at the forefront of dental biomaterials that are likely to become integral components of future dental treatment protocols.

Application in Bone Tissue Engineering

CS scaffolds have carved a niche in the domain of bone tissue engineering with their versatile applications, mirroring the natural extracellular matrix and offering a propitious terrain for the growth and maturation of osteoblasts. As three-dimensional matrices, these scaffolds not only provide the structural support requisite for the burgeoning bone tissue but also present an environment that is congenial for the attachment, proliferation, and differentiation of cells essential for osteogenesis. The biocompatibility, biodegradability, and modifiable surface properties of CS enhance its appeal as a scaffold material, as it can be engineered to degrade at a rate that coincides with the regeneration of bone, thereby ensuring that the scaffold's role is transient yet pivotal during the critical phase of bone healing [122].

The profound impact of CS scaffolds in bone tissue engineering is exemplified in their application to fracture healing. Particularly in instances of large bone defects, where the body's innate healing mechanisms falter, these scaffolds provide a critical interim support structure that can be colonized by osteoprogenitor cells and in which new bone matrix can be deposited. This accelerates the healing process, bridging gaps that would otherwise impair skeletal integrity and function [122].

In the intricate arena of spinal surgery, CS scaffolds have emerged as valuable tools to facilitate spinal fusion. Here, they serve to encourage the growth of new bone between spinal segments, a process indispensable for the stabilization and unification of the spine following surgical in-

Table 3. Research on the application of different types of chitosan scaffolds in skull and long bones.

Type	Material	Applications	Reasons
Skull	Chitosan-graphene oxide 3D scaffold	Chitosan-graphene oxide 3D scaffolds as promising tools for bone regeneration in critical-size mouse calvarial defects [128]	Enhanced mechanical properties, suitable porosity, structural features, cell proliferation, and vitality
	Hydroxyapatite-silk fibroin-chitosan 3D scaffold regeneration	Effect of bone marrow mesenchymal stem cells on hydroxyapatite/silk fibroin/chitosan composite 3D scaffold for rat skull defects repair [129]	Facilitate completely degrades during repair, generates new bone, remodels scaffold, distributed in trabecular bone structure
	Porous chitosan-alginate scaffold	Evaluation of three-dimensional porous chitosan-alginate scaffolds in rat calvarial defects for bone regeneration applications [130]	Supports mesenchymal stem cell growth, promotes spherical morphology, improves defect closure
	Porous hydroxyapatite/chitosan scaffold	Comparative study of porous hydroxyapatite/chitosan and whitlockite/chitosan scaffolds for bone regeneration in calvarial defects [131]	Large pore diameter (~ 105 μm) conducive to cell settlement within the scaffold
	Whitlockite/chitosan scaffold	Comparative study of porous hydroxyapatite/chitosan and whitlockite/chitosan scaffolds for bone regeneration in calvarial defects [131]	Promotes hBMSCs proliferation, enhances osteoinductivity, significantly improves new bone formation
	Hydroxyapatite-mineralized collagen/chitosan sponge	Use of collagen/chitosan sponges mineralized with hydroxyapatite for the repair of cranial defects in rats [132]	Improves osteogenic effects, but the process is slow and insufficient for complete bone regeneration in a short time
Long bone	Nacre-mimetic cerium-doped layered nano-hydroxyapatite/chitosan scaffold	Nacre-mimetic cerium-doped nano-hydroxyapatite/chitosan layered composite scaffolds regulate bone regeneration via OPG/RANKL signaling pathway [133]	Provides strength and toughness, cerium ions have anti-osteoclastogenic ability, promotes hBMSCs adhesion and proliferation, allows *in-situ* growth of new bone tissue
	Chitosan-gelatin scaffold with decellularized platelet-rich fibrin	Chitosan-gelatin scaffolds incorporating decellularized platelet-rich fibrin promote bone regeneration [134]	Appropriate biocompatibility and mechanical properties, incorporation of platelet-rich fibrin enhances bioactivity
	Polyethyleneimine/BMP2 plasmid/hydroxyapatite/chitosan microspheres	A novel gene-activated matrix composed of PEI/plasmid-BMP2 complexes and hydroxyapatite/chitosan-microspheres promotes bone regeneration [135]	Excellent biocompatibility, provides more cell adhesion sites, controlled plasmid release, effectively promotes bone regeneration
	Multi-channel scaffold based on porous hydroxyapatite	Phosphonate-chitosan functionalization of a multi-channel hydroxyapatite scaffold for interfacial implant-bone tissue integration [136]	Used for segmental bone defects, specific geometry and design, enhances new bone formation, suitable for segmental defects

BMP, bone morphogenetic protein; hBMSCs, human bone marrow mesenchymal stem cells; OPG, osteoclastogenesis inhibitory factor; RANKL, receptor activator of nuclear factor-κ B ligand; PEI, polyethyleneimine.

tervention. Their ability to be moulded to specific shapes and to conform to complex anatomical geometries makes them particularly suitable for such applications, where precision is paramount for successful outcomes [123,124].

Joint reconstruction surgeries also benefit from the application of CS scaffolds. As the interface between an orthopaedic implant and native bone is a critical determinant of the long-term success of the procedure, CS scaffolds can be employed to foster bone regeneration at this juncture. By enhancing osseointegration, they contribute to a more stable and durable union between the implant and the skeletal system, thereby improving patient outcomes and implant longevity [125].

Table 4. Summary of chitosan scaffold application scenarios.

Application field	Specific application	Description
Dentistry	Dental pulp regeneration	Help encourage reparative dentin formation to maintain pulp vitality after dental trauma or caries removal [155]
	Periodontal tissue regeneration	Facilitate repopulation and regeneration of lost structures like alveolar bone, periodontal ligament, etc. to treat periodontal disease [156]
	Alveolar ridge preservation	Help maintain ridge dimensions after tooth extraction to allow future dental implant placement [157]
Bone tissue engineering	Fracture healing	Provide interim structural support and environment for osteoblast growth to heal large bone defects [158]
	Spinal fusion	Encourage bone growth between spinal segments to facilitate fusion after spinal surgery [159]
	Joint reconstruction	Enhance osseointegration between implants and native bone for more stable and durable joint reconstruction [160]
	Craniofacial reconstruction	Aid complex bone regeneration for craniofacial defects from trauma, tumors, congenital conditions, etc. [161]
Growth factor delivery	BMP delivery	Provide sustained localized release of BMPs to stimulate progenitor cell differentiation into osteoblasts [162]
Gene delivery	Osteogenic gene delivery	Deliver genes encoding osteogenic proteins to upregulate bone formation directly at injury site [163]
Bone formation	Critical bone defect repair	Serve as osteoconductive template for ingrowth of new bone tissue to bridge critical-sized defects [30]

Moreover, the realm of craniofacial reconstruction has witnessed the integration of CS scaffolds into its therapeutic arsenal. Given the complex geometry and the unique mechanical demands of craniofacial bones, CS's adaptability becomes particularly beneficial [126]. Its applications extend to the reconstruction of defects arising from trauma, tumor resections, or congenital anomalies. In these scenarios, CS scaffolds not only provide the necessary support for bone regeneration but also contribute to the aesthetic restoration of the craniofacial contour, which is critical from a psychosocial perspective [127].

The efficacy of chitosan scaffolds varies across different bone regions. Table 3 (Ref. [128–136]) summarizes research on various chitosan scaffold types used in the skull and long bones. The findings demonstrate that chitosan scaffolds, when combined with different materials, show notable variations in biocompatibility, mechanical properties, and bone regeneration enhancement. For skull applications, composites of chitosan with oxidized graphene, hydroxyapatite, and silk fibroin exhibit excellent cell proliferation and new bone formation capabilities. In the context of long bones, combinations of chitosan with pearl layers, cerium-doped nano-hydroxyapatite, and gelatin display superior mechanical properties and bone regeneration outcomes. These studies emphasize the importance of selecting appropriate chitosan composite scaffolds to meet the specific requirements of different bone regions.

The diverse applications of CS scaffolds in bone tissue engineering reflect their potential to address a spectrum of clinical challenges. By aligning scaffold properties with the physiological and mechanical requirements of bone regeneration, CS-based materials stand at the forefront of innovations, poised to enhance and perhaps transform the standard of care in orthopaedic and maxillofacial surgery. The ongoing research and development in this field are continuously refining the properties of CS scaffolds, tailoring them to meet specific clinical needs, and expanding their potential in aiding the natural regenerative processes of the human body [137].

Promotion of Osteoblast

One of the most powerful applications of CS scaffolds is in the domain of growth factor delivery. By serving as carriers for BMPs, these scaffolds provide a sustained release of key molecules directly at the site of bone damage or defect [138]. BMPs are one of the most potent groups of growth factors with a proven track record of stimulating the proliferation and differentiation of progenitor cells into osteoblasts. When these proteins are intricately incorporated into the CS scaffold, they create a localized and conducive environment for bone tissue formation, thereby enhancing the natural healing process [139].

The incorporation of BMPs into chitosan scaffolds activates osteoblast-specific molecular pathways, such as the Smad signaling pathway, which is crucial for osteogenesis. BMPs bind to their receptors on the osteoblast surface, leading to the phosphorylation of Smad proteins. These activated Smads translocate to the nucleus, where they

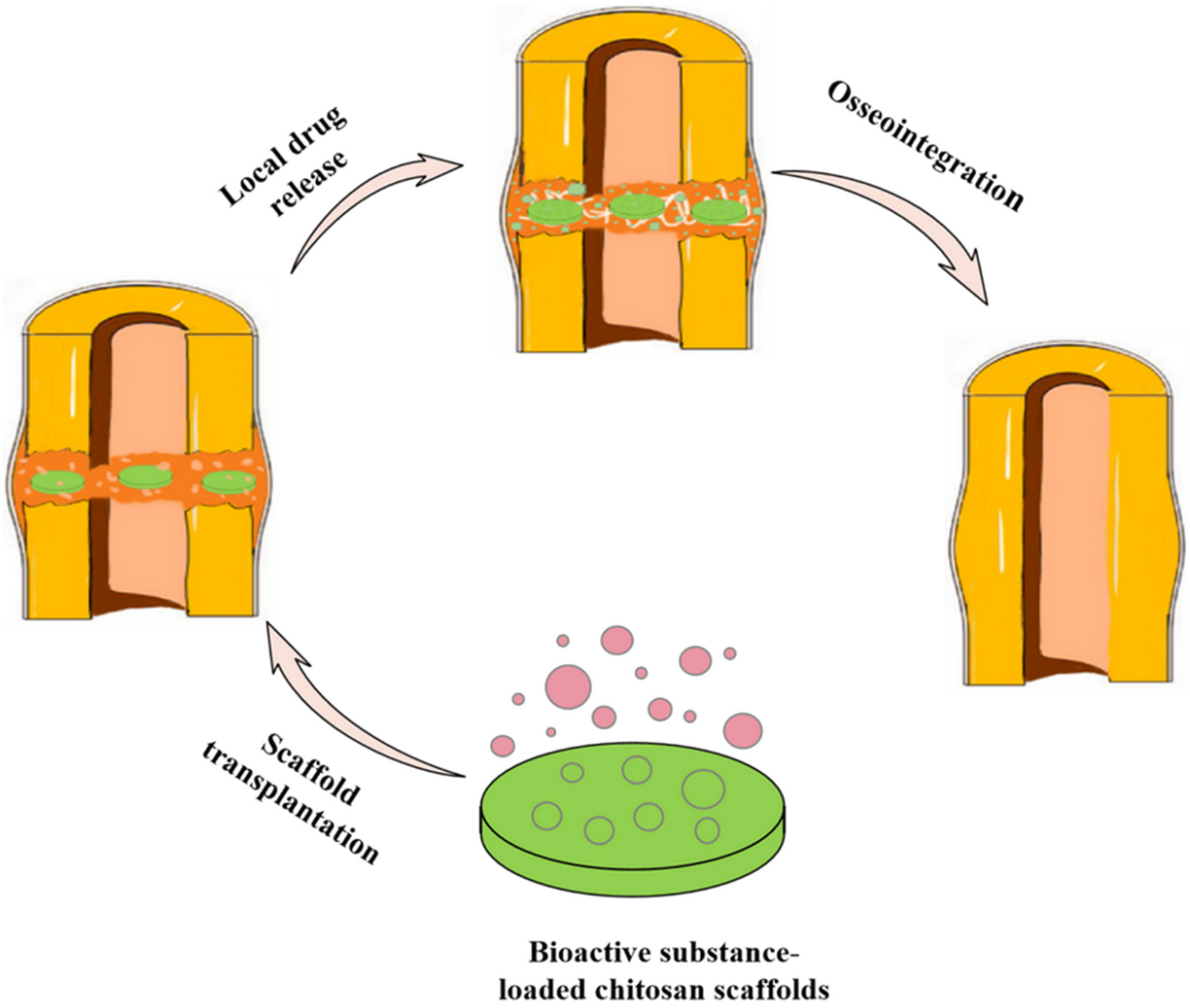

Fig. 3. Bioactive chitosan scaffolds for enhanced bone regeneration. It was created by PowerPoint.

upregulate the expression of osteogenic markers, including *RUNX2*, osteocalcin, and alkaline phosphatase (ALP). This molecular cascade enhances both the proliferation and differentiation of osteoblasts, ultimately promoting matrix mineralization [140].

Furthermore, the versatility of CS scaffolds extends into the realm of gene delivery. This innovative approach involves the delivery of genes that are responsible for encoding osteogenic proteins directly to the injury site. CS's cationic nature allows for the efficient encapsulation and protection of genetic material, which can then be introduced to the target cells, leading to the upregulation of bone-forming proteins. The presence of chitosan scaffolds has been shown to upregulate the expression of key osteoblast-specific genes, such as *RUNX2*, osteopontin, and collagen type I [141]. *RUNX2*, a master transcription factor, plays a central role in osteoblast differentiation by regulating the transcription of genes essential for matrix production and mineralization. This gene regulation mechanism is further supported by the controlled and localized release of genetic material, ensuring a sustained osteogenic effect at the defect site.

Such gene therapy techniques, combined with the biocompatibility of CS scaffolds, hold immense promise in revolutionizing bone repair strategies. They offer a targeted and controlled approach to healing, reducing the likelihood of systemic side effects and concentrating the regenerative efforts where they are most needed [142].

In addition to their role in delivering growth factors and genetic material, chitosan scaffolds also directly support osteoblast proliferation through their nanofibrous structure and bioactive surface properties. Nanofiber-based CS scaffolds, which mimic the extracellular matrix, provide a high surface area for cell attachment and growth. Studies have shown that human osteoblasts cultured on these scaffolds exhibit increased ALP activity, osteocalcin secretion, and calcium deposition over time, highlighting their role in enhancing matrix mineralization [143,144].

Moreover, the mechanical properties of CS scaffolds, which can be tailored through the incorporation of hydroxyapatite or collagen, provide critical mechanical cues to osteoblasts. These cues influence cell behavior via mechanotransduction pathways, such as YAP/TAZ signaling, which are activated in response to scaffold stiffness. For instance, stiffer scaffolds promote osteoblast differentiation by encouraging nuclear localization of YAP/TAZ, further upregulating osteogenic gene expression.

Chitosan scaffolds also play a role in osteoblast response to electrical stimulation. Due to their osteoconductive properties, these scaffolds enhance cellular behavior in dynamic environments where electrical signals are present. Electrical stimulation has been shown to increase the expression of ALP and other osteogenic markers, providing an additional layer of functionality for chitosan scaffolds in bone regeneration applications [145].

In summary, CS scaffolds actively influence osteoblast behavior not only by serving as delivery platforms for growth factors and genes but also through their structural, mechanical, and bioactive properties. These scaffolds enhance cellular proliferation, differentiation, and matrix mineralization by modulating key molecular pathways and providing an environment that mimics the natural ECM. Future studies should focus on optimizing these cellular mechanisms to improve the clinical outcomes of CS-based scaffolds in bone tissue engineering.

New Bone Formation

CS scaffolds have emerged as a cornerstone in the field of regenerative medicine, particularly in the realm of new bone formation. These bio-inspired structures harness the innate ability of CS, a naturally derived polymer, to foster osteogenesis, the process of bone growth. Mimicking the extracellular matrix of bone tissue, CS scaffolds serve as osteoconductive platforms. Additionally, molecular signals (such as growth factors) and mechanical signals (such as stiffness and surface characteristics) play crucial roles in regulating osteoblast behavior and bone regeneration. Studies have shown that the stiffness of CS scaffolds can influence osteoblast proliferation and differentiation, while the optimization of surface properties aids in cell adhesion and bone tissue formation [146,147]. Their unique structure and composition create an ideal environment that not only supports but also guides the proliferation and differentiation of osteoblasts, the cells responsible for bone formation. This guidance is crucial in ensuring that new bone growth follows the intended pathways, thus maintaining the integrity and functionality of the regenerated tissue [148].

In addition to their role in osteoblast differentiation, CS scaffolds also actively modulate inflammation, which is a critical factor in bone regeneration. Chitosan has been shown to promote M2 macrophage polarization, shifting the inflammatory response from a pro-inflammatory (M1) state to an anti-inflammatory (M2) state. This transition, mediated by pro-resolution lipid mediators such as lipoxin A4 (LxA4) and resolvin D1 (RvD1), reduces inflammatory cytokine levels (e.g., tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)) and enhances tissue repair [149]. Such modulation creates a favorable microenvironment for osteogenesis, supporting the recruitment and differentiation of osteoblast precursors.

The significance of CS scaffolds is particularly pronounced when addressing critical-sized bone defects—those which the body cannot heal on its own. In such instances, the scaffold provides a three-dimensional template that facilitates the in-growth of new bone tissue, effectively bridging the gap between separated bone ends [150]. Mechanical signals, in synergy with molecular signals, further promote bone tissue regeneration. For example, appropriate scaffold stiffness can mimic the mechanical environment of natural bone tissue, thereby enhancing osteoblast function [151]. By doing so, it substantially accelerates and improves the healing process. Moreover, CS's biocompatibility ensures minimal inflammatory response, while its biodegradability allows it to be gradually resorbed and replaced by the natural bone, leaving behind no foreign material in the body [152].

Angiogenesis is another critical process supported by chitosan scaffolds during bone regeneration. The porous structure of CS scaffolds facilitates vascular infiltration, ensuring the delivery of oxygen and nutrients to regenerating tissues. Furthermore, the incorporation of bioactive agents, such as hydroxyapatite or VEGF, into the scaffold enhances angiogenesis by promoting endothelial cell migration and capillary formation. These processes activate key signaling pathways, including hypoxia-inducible factor 1 alpha (HIF-1α) and vascular endothelial growth factor to vascular endothelial growth factor receptor (VEGF-VEGFR), which are essential for vascular network development and subsequent bone regeneration [153]. The synergy between angiogenesis and osteogenesis is pivotal for integrating newly formed bone with the host tissue.

Fig. 3 illustrates the ability of chitosan scaffolds, loaded with bioactive substances, to promote bone growth. In addition to their osteogenic and angiogenic properties, CS scaffolds also support the coordinated growth of soft and hard tissues, a crucial factor in successful bone regeneration. By mimicking the extracellular matrix, CS scaffolds provide a conducive environment for the attachment and proliferation of both osteoblasts and fibroblasts [5]. Their mechanical properties and degradation rates can be tuned to ensure that scaffold support aligns with the growth rates of both bone and surrounding soft tissues, facilitating seamless integration. This coordination is further enhanced by the ability of CS scaffolds to modulate the Wnt/β-catenin pathway, which regulates both osteoblast activity and tissue remodeling. The adaptability of chitosan scaffolds lies in their capacity to incorporate bioactive agents, such as growth factors, which can further enhance osteogenesis.

Simultaneously, the regulation of surface properties, such as the introduction of nanostructures, can significantly improve cell adhesion and the integration of bone tissue [154]. Moreover, their physical properties can be precisely tuned to meet the specific requirements of different bone types and defect sites. This versatility holds significant potential not only for bone repair but also for the development of advanced implantable devices and bone tissue engineering strategies aimed at restoring skeletal integrity and function following injury, disease, or congenital defects. As research in this field progresses, chitosan scaffolds continue to affirm their role as a critical tool in the advancement of effective bone regeneration therapies. All the above application scenarios of CS scaffolds are summarized in Table 4 (Ref. [30,155–163]).

Conclusions

This review has comprehensively highlighted the significant advantages and broad clinical potential of CS scaffolds in bone tissue engineering. CS scaffolds possess inherent qualities of biocompatibility, biodegradability, osteoconductivity, and adequate mechanical strength, making them suitable candidates for bone regeneration applications. Various scaffold forms—including molded macroporous scaffolds, fiber-based scaffolds, injectable hydrogels, microspheres, and 3D-printed scaffolds—offer diverse structural features that meet distinct clinical requirements.

Considerable progress has been achieved in applying CS scaffolds to dentistry, spinal fusion, joint reconstruction, and craniofacial repair, demonstrating their transformative capability across bone-related therapies. However, challenges persist in uniformly controlling pore architecture, optimizing degradation rates, and achieving adequate mechanical performance, especially under load-bearing conditions. Furthermore, production costs, manufacturing scalability, and complex regulatory pathways remain significant obstacles to widespread clinical adoption.

Looking forward, innovations such as incorporation of nanocomposite materials, personalized 3D printing for patient-specific scaffolds, and integration of bioactive molecules hold great promise for overcoming existing limitations. Advances in automated fabrication and standardized evaluation protocols will be critical to lowering costs and accelerating regulatory approval. Interdisciplinary collaborations among materials scientists, engineers, biologists, and clinicians will be essential in driving these developments.

Ultimately, CS scaffolds represent a highly versatile and effective platform for bone regeneration, with the potential to significantly improve therapeutic outcomes and quality of life for patients worldwide. Ongoing research and technological advancement are expected to further enhance their functional performance, facilitate clinical translation, and expand their application in regenerative medicine.

List of Abbreviations

BMPs, bone morphogenetic proteins; CAD, computer-aided design; CS, chitosan; *Col I*, collagen type I; DA, degree of acetylation; ECM, extracellular matrix; LxA4, lipoxin A4; M1, pro-inflammatory; M2, anti-inflammatory; MSC, mesenchymal stem cell; OCN, osteocalcin; *OPN*, osteopontin; RvD1, resolvin D1; 3D, three-dimensional; CT, computed tomography; MRI, magnetic resonance imaging; SEM, scanning electron microscope; YAP, yes associated protein; TAZ, transcriptional coactivator with a PDZ binding domain; FAK, focal adhesion kinase; *RUNX2*, RUNX family transcription factor 2; hBMSCs, human bone marrow mesenchymal stem cells; OPG, osteoclastogenesis inhibitory factor; RANKL, receptor activator of nuclear factor-κ B ligand; PEI, polyethyleneimine; ALP, alkaline phosphatase; HIF-1α, hypoxia-inducible factor 1 alpha; VEGF-VEGFR, vascular endothelial growth factor to vascular endothelial growth factor receptor; HIF-1α, hypoxia-inducible factor 1 alpha; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6.

Declaration of AI and AI-assisted Technologies in the Writing Process

The manuscript has been linguistically enhanced by GPT-4o. Furthermore, the schematic illustration of chitosan scaffolds presented in Fig. 1 was generated using the AI-based platform Baidu Wenxin Yige.

Availability of Data and Materials

All data reported in this paper will be shared by the primary contact upon request.

Author Contributions

ZMY, ZGW and GXZ contributed to the conception and design of the work. WMX, YY, JMC, YZ, XDW, YXD, and YSP contributed to data acquisition, analysis, and interpretation. WMX, YY, JMC, YZ, XDW, YXD, and YSP drafted the manuscript. ZMY, ZGW and GXZ revised critically for important intellectual content. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgments

Not applicable.

Funding

This study was supported by Liaoning Province Applied Basic Research Program (Joint Program, Grant number 2022JH2/101500011), as well as Liaoning Province

Minsheng Science and Technology Plan Joint Plan Project (Grant number 2021JH2/10300098).

Conflict of Interest

The authors declare no conflicts of interest.

References

[1] Koons GL, Diba M, Mikos AG. Materials design for bone-tissue engineering. Nature Reviews Materials. 2020; 5: 584–603. https://doi.org/10.1038/s41578-020-0204-2.

[2] Perić Kačarević Ž, Rider P, Alkildani S, Retnasingh S, Pejakić M, Schnettler R, *et al*. An introduction to bone tissue engineering. The International Journal of Artificial Organs. 2020; 43: 69–86. https://doi.org/10.1177/0391398819876286.

[3] Bhushan S, Singh S, Maiti TK, Das A, Barui A, Chaudhari LR, *et al*. Cerium oxide nanoparticles disseminated chitosan gelatin scaffold for bone tissue engineering applications. International Journal of Biological Macromolecules. 2023; 236: 123813. https://doi.org/10.1016/j.ijbiomac.2023.123813.

[4] Elizalde-Cárdenas A, Ribas-Aparicio RM, Rodríguez-Martínez A, Leyva-Gómez G, Ríos-Castañeda C, González-Torres M. Advances in chitosan and chitosan derivatives for biomedical applications in tissue engineering: An updated review. International Journal of Biological Macromolecules. 2024; 262: 129999. https://doi.org/10.1016/j.ijbiomac.2024.129999.

[5] Purohit SD, Bhaskar R, Singh H, Priyadarshi R, Kim H, Son Y, *et al*. Chitosan-based electrospun fibers for bone-tissue engineering: Recent research advancements. International Journal of Biological Macromolecules. 2024; 281: 136530. https://doi.org/10.1016/j.ijbiomac.2024.136530.

[6] Venkatesan J, Kim SK. Chitosan composites for bone tissue engineering–an overview. Marine Drugs. 2010; 8: 2252–2266. https://doi.org/10.3390/md8082252.

[7] Anusha JR, Fleming AT, Valan Arasu M, Chul Kim B, Al-Dhabi NA, Yu KH, *et al*. Mechanochemical synthesis of chitosan submicron particles from the gladius of Todarodes pacificus. Journal of Advanced Research. 2016; 7: 863–871. https://doi.org/10.1016/j.jare.2016.08.006.

[8] Lee H, Jung Y, Lee N, Lee I, Lee JH. Nature-Derived Polysaccharide-Based Composite Hydrogels for Promoting Wound Healing. International Journal of Molecular Sciences. 2023; 24: 16714. https://doi.org/10.3390/ijms242316714.

[9] Azmana M, Mahmood S, Hilles AR, Rahman A, Arifin MAB, Ahmed S. A review on chitosan and chitosan-based bionanocomposites: Promising material for combatting global issues and its applications. International Journal of Biological Macromolecules. 2021; 185: 832–848. https://doi.org/10.1016/j.ijbiomac.2021.07.023.

[10] Damiri F, Gowda BHJ, Andra S, Balu S, Rojekar S, Berrada M. Chitosan Nanocomposites as Scaffolds for Bone Tissue Regeneration. In Swain, S.K., Biswal, A. (eds.) Chitosan Nanocomposites. Biological and Medical Physics, Biomedical Engineering (pp. 377–394). Springer: Singapore. 2023. https://doi.org/10.1007/978-981-19-9646-7_16.

[11] Beheshtizadeh N, Farzin A, Rezvantalab S, Pazhouhnia Z, Lotfibakhshaiesh N, Ai J, *et al*. 3D printing of complicated GelMA-coated Alginate/Tri-calcium silicate scaffold for accelerated bone regeneration. International Journal of Biological Macromolecules. 2023; 229: 636–653. https://doi.org/10.1016/j.ijbiomac.2022.12.267.

[12] Gupte MJ, Swanson WB, Hu J, Jin X, Ma H, Zhang Z, *et al*. Pore size directs bone marrow stromal cell fate and tissue regeneration in nanofibrous macroporous scaffolds by mediating vascularization. Acta Biomaterialia. 2018; 82: 1–11. https://doi.org/10.1016/j.actbio.2018.10.016.

[13] Guzmán-Soria A, Moreno-Serna V, Canales DA, García-Herrera C, Zapata PA, Orihuela PA. Effect of Electrospun PLGA/Collagen Scaffolds on Cell Adhesion, Viability, and Collagen Release: Potential Applications in Tissue Engineering. Polymers. 2023; 15: 1079. https://doi.org/10.3390/polym15051079.

[14] Ma J, Du C, Zhang Y, Zhan J, Lai Y, Zhao M. Important application value of injectable hydrogels loaded with omeprazole Schiff base complex in the treatment of pancreatitis. Open Chemistry. 2023; 21: 20230147. https://doi.org/10.1515/chem-2023-0147.

[15] Ressler A. Chitosan-Based Biomaterials for Bone Tissue Engineering Applications: A Short Review. Polymers. 2022; 14: 3430. https://doi.org/10.3390/polym14163430.

[16] Xun X, Li Y, Ni M, Xu Y, Li J, Zhang D, *et al*. Calcium crosslinked macroporous bacterial cellulose scaffolds with enhanced *in situ* mineralization and osteoinductivity for cranial bone regeneration. Composites Part B: Engineering. 2024; 275: 111277. https://doi.org/10.1016/j.compositesb.2024.111277.

[17] Chu L, Jiang G, Hu X, James TD, He X, Li Y, *et al*. Osteogenesis, vascularization and osseointegration of a bioactive multiphase macroporous scaffold in the treatment of large bone defects. Journal of Materials Chemistry. B. 2018; 6: 4197–4204. https://doi.org/10.1039/c8tb00766g.

[18] Bahraminasab M. Challenges on optimization of 3D-printed bone scaffolds. Biomedical Engineering Online. 2020; 19: 69. https://doi.org/10.1186/s12938-020-00810-2.

[19] Jang JW, Min KE, Kim C, Shin J, Lee J, Yi S. Review: Scaffold Characteristics, Fabrication Methods, and Biomaterials for the Bone Tissue Engineering. International Journal of Precision Engineering and Manufacturing. 2023; 24: 511–529. https://doi.org/10.1007/s12541-022-00755-7.

[20] Kuang T, Chen S, Gu Z, Shen Z, Hejna A, Saeb MR, *et al*. A facile approach to fabricate load-bearing porous polymer scaffolds for bone tissue engineering. Advanced Composites and Hybrid Materials. 2022; 5: 1376–1384. https://doi.org/10.1007/s42114-022-00418-1.

[21] Manzini BM, Machado LMR, Noritomi PY, da Silva JVL. Advances in Bone tissue engineering: A fundamental review. Journal of Biosciences. 2021; 46: 17. https://doi.org/10.1007/s12038-020-00122-6.

[22] Crowley C, Wong JM, Fisher DM, Khan WS. A systematic review on preclinical and clinical studies on the use of scaffolds for bone repair in skeletal defects. Current Stem Cell Research & Therapy. 2013; 8: 243–252. https://doi.org/10.2174/1574888x11308030009.

[23] Wang H, Yang J, Tian W, Peng K, Xue Y, Zhao H, *et al*. A sodium alginate/carboxymethyl chitosan dual-crosslinked injectable hydrogel scaffold with tunable softness/hardness for bone regeneration. International Journal of Biological Macromolecules. 2024; 257: 128700. https://doi.org/10.1016/j.ijbiomac.2023.128700.

[24] Mohammed Mohammed AH, Shariff KA, Wahjuningrum DA, Bakar MHA, Mohamad H. A comprehensive review of the effects of porosity and macro- and micropore formations in porous β-TCP scaffolds on cell responses. Journal of the Australian Ceramic Society. 2023; 59: 865–879. https://doi.org/10.1007/s41779-023-00880-0.

[25] Lu M, Sun L, Yao J, Zhao B, Liu Y, Shao Z, *et al*. Protein-inorganic hybrid porous scaffolds for bone tissue engineering. Journal of Materials Chemistry. B. 2022; 10: 6546–6556. https://doi.org/10.1039/d2tb00853j.

[26] Mohammadalizadeh Z, Bahremandi-Toloue E, Karbasi S. Synthetic-based blended electrospun scaffolds in tissue engineering applications. Journal of Materials Science. 2022; 57: 4020–4079. https://doi.org/10.1007/s10853-021-06826-w.

[27] Cheng A, Schwartz Z, Kahn A, Li X, Shao Z, Sun M, *et al*. Advances in Porous Scaffold Design for Bone and Cartilage Tissue Engineering and Regeneration. Tissue Engineering. Part B, Reviews. 2019; 25: 14–29. https://doi.org/10.1089/ten.TEB.2018.0119.

[28] Chen J, Fan Y, Dong G, Zhou H, Du R, Tang X, *et al*. Designing

biomimetic scaffolds for skin tissue engineering. Biomaterials Science. 2023; 11: 3051–3076. https://doi.org/10.1039/d3bm00046j.

[29] Samrot AV, Sathiyasree M, Rahim SBA, Renitta RE, Kasipandian K, Krithika Shree S, *et al*. Scaffold Using Chitosan, Agarose, Cellulose, Dextran and Protein for Tissue Engineering—A Review. Polymers. 2023; 15: 1525. https://doi.org/10.3390/polym15061525.

[30] Levengood SL, Zhang M. Chitosan-based scaffolds for bone tissue engineering. Journal of Materials Chemistry. B. 2014; 2: 3161–3184. https://doi.org/10.1039/C4TB00027G.

[31] Sapkota S, Chou SF. Electrospun Chitosan-based Fibers for Wound Healing Applications. Journal of Biomaterials. 2020; 4: 51–57.

[32] Ding Y, Li W, Zhang F, Liu Z, Ezazi NZ, Liu D, *et al*. Electrospun Fibrous Architectures for Drug Delivery, Tissue Engineering and Cancer Therapy. Advanced Functional Materials. 2019; 29: 1802852. https://doi.org/10.1002/adfm.201802852.

[33] Antunes JC, Domingues JM, Miranda CS, Silva AFG, Homem NC, Amorim MTP, *et al*. Bioactivity of Chitosan-Based Particles Loaded with Plant-Derived Extracts for Biomedical Applications: Emphasis on Antimicrobial Fiber-Based Systems. Marine Drugs. 2021; 19: 359. https://doi.org/10.3390/md19070359.

[34] Anisiei A, Oancea F, Marin L. Electrospinning of chitosan-based nanofibers: from design to prospective applications. Reviews in Chemical Engineering. 2023; 39: 31–70. https://doi.org/10.1515/revce-2021-0003.

[35] Garshasbi H, Salehi S, Naghib SM, Ghorbanzadeh S, Zhang W. Stimuli-responsive injectable chitosan-based hydrogels for controlled drug delivery systems. Frontiers in Bioengineering and Biotechnology. 2023; 10: 1126774. https://doi.org/10.3389/fbioe.2022.1126774.

[36] Kaur K, Murphy CM. Advances in the Development of Nano-Engineered Mechanically Robust Hydrogels for Minimally Invasive Treatment of Bone Defects. Gels. 2023; 9: 809. https://doi.org/10.3390/gels9100809.

[37] Ding Q, Zhang S, Liu X, Zhao Y, Yang J, Chai G, *et al*. Hydrogel Tissue Bioengineered Scaffolds in Bone Repair: A Review. Molecules: a Journal of Synthetic Chemistry and Natural Product Chemistry. 2023; 28: 7039. https://doi.org/10.3390/molecules28207039.

[38] Gilarska A, Lewandowska-Łańcucka J, Guzdek-Zając K, Karewicz A, Horak W, Lach R, *et al*. Bioactive yet antimicrobial structurally stable collagen/chitosan/lysine functionalized hyaluronic acid-based injectable hydrogels for potential bone tissue engineering applications. International Journal of Biological Macromolecules. 2020; 155: 938–950. https://doi.org/10.1016/j.ijbiomac.2019.11.052.

[39] Dang PA, Palomino-Durand C, Elsafi Mabrouk M, Marquaille P, Odier C, Norvez S, *et al*. Rational formulation design of injectable thermosensitive chitosan-based hydrogels for cell encapsulation and delivery. Carbohydrate Polymers. 2022; 277: 118836. https://doi.org/10.1016/j.carbpol.2021.118836.

[40] Olov N, Bagheri-Khoulenjani S, Mirzadeh H. Injectable hydrogels for bone and cartilage tissue engineering: a review. Progress in Biomaterials. 2022; 11: 113–135. https://doi.org/10.1007/s40204-022-00185-8.

[41] Wasupalli GK, Verma D. Thermosensitive injectable hydrogel based on chitosan-polygalacturonic acid polyelectrolyte complexes for bone tissue engineering. Carbohydrate Polymers. 2022; 294: 119769. https://doi.org/10.1016/j.carbpol.2022.119769.

[42] Jung HY, Le Thi P, HwangBo KH, Bae JW, Park KD. Tunable and high tissue adhesive properties of injectable chitosan based hydrogels through polymer architecture modulation. Carbohydrate Polymers. 2021; 261: 117810. https://doi.org/10.1016/j.carbpol.2021.117810.

[43] Pan S, Zhu C, Wu Y, Tao L. Chitosan-Based Self-Healing Hydrogel: From Fabrication to Biomedical Application. Polymers. 2023; 15: 3768. https://doi.org/10.3390/polym15183768.

[44] Cai L, Lin D, Chai Y, Yuan Y, Liu C. MBG scaffolds containing chitosan microspheres for binary delivery of IL-8 and BMP-2 for bone regeneration. Journal of Materials Chemistry. B. 2018; 6: 4453–4465. https://doi.org/10.1039/c8tb00875b.

[45] Xue Y, Wu M, Liu Z, Song J, Luo S, Li H, *et al*. *In vitro* and *in vivo* evaluation of chitosan scaffolds combined with simvastatin-loaded nanoparticles for guided bone regeneration. Journal of Materials Science. Materials in Medicine. 2019; 30: 47. https://doi.org/10.1007/s10856-019-6249-3.

[46] Fourie J, Taute F, du Preez L, de Beer D. Chitosan Composite Biomaterials for Bone Tissue Engineering—a Review. Regenerative Engineering and Translational Medicine. 2022; 8: 1–21. https://doi.org/10.1007/s40883-020-00187-7.

[47] Khoshroo K, Jafarzadeh Kashi TS, Moztarzadeh F, Tahriri M, Jazayeri HE, Tayebi L. Development of 3D PCL microsphere/TiO_2 nanotube composite scaffolds for bone tissue engineering. Materials Science & Engineering. C, Materials for Biological Applications. 2017; 70: 586–598. https://doi.org/10.1016/j.msec.2016.08.081.

[48] Zhang X, Liu Y, Wen J, Zhao Z, Chen H, Liu X, *et al*. Host–guest interaction-mediated fabrication of a hybrid microsphere-structured supramolecular hydrogel showing high mechanical strength. Soft Matter. 2020; 16: 3416–3424. https://doi.org/10.1039/d0sm00271b.

[49] Hu X, He J, Yong X, Lu J, Xiao J, Liao Y, *et al*. Biodegradable poly (lactic acid-co-trimethylene carbonate)/chitosan microsphere scaffold with shape-memory effect for bone tissue engineering. Colloids and Surfaces. B, Biointerfaces. 2020; 195: 111218. https://doi.org/10.1016/j.colsurfb.2020.111218.

[50] Seol YJ, Lee JY, Park YJ, Lee YM, Young-Ku, Rhyu IC, *et al*. Chitosan sponges as tissue engineering scaffolds for bone formation. Biotechnology Letters. 2004; 26: 1037–1041. https://doi.org/10.1023/B:BILE.0000032962.79531.fd.

[51] Lu H, Pan X, Hu M, Zhang J, Yu Y, Hu X, *et al*. Fabrication of graphene/gelatin/chitosan/tricalcium phosphate 3D printed scaffolds for bone tissue regeneration applications. Applied Nanoscience. 2021; 11: 335–346. https://doi.org/10.1007/s13204-020-01615-4.

[52] Taghizadeh M, Taghizadeh A, Yazdi MK, Zarrintaj P, Stadler FJ, Ramsey JD, *et al*. Chitosan-based inks for 3D printing and bioprinting. Green Chemistry. 2022; 24: 62–101. https://doi.org/10.1039/D1GC01799C.

[53] Harini G, Bharathi R, Sankaranarayanan A, Shanmugavadivu A, Selvamurugan N. Nanoceramics-reinforced chitosan scaffolds in bone tissue engineering. Materials Advances. 2023; 4: 3907–3928. https://doi.org/10.1039/D3MA00422H.

[54] Li Y, Meng Y, Wang Y, Wang Y, Wang Z. Application of Mineralized Chitosan Scaffolds in Bone Tissue Engineering. Coatings. 2023; 13: 1644. https://doi.org/10.3390/coatings13091644.

[55] Cadena M, Ning L, King A, Hwang B, Jin L, Serpooshan V, *et al*. 3D Bioprinting of Neural Tissues. Advanced Healthcare Materials. 2021; 10: e2001600. https://doi.org/10.1002/adhm.202001600.

[56] Choi J, Lee EJ, Jang WB, Kwon SM. Development of Biocompatible 3D-Printed Artificial Blood Vessels through Multidimensional Approaches. Journal of Functional Biomaterials. 2023; 14: 497. https://doi.org/10.3390/jfb14100497.

[57] Jourdin L, Freguia S, Donose BC, Chen J, Wallace GG, Keller J, *et al*. A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis. Journal of Materials Chemistry A. 2014; 2: 13093–13102. https://doi.org/10.1039/c4ta03101f.

[58] Yuan L, Li X, Ge L, Jia X, Lei J, Mu C, *et al*. Emulsion Template Method for the Fabrication of Gelatin-Based Scaffold with a Controllable Pore Structure. ACS Applied Materials & Interfaces. 2019; 11: 269–277. https://doi.org/10.1021/acsami.8b17555.

[59] Zhang K, Yang Z, Seitz MP, Jain E. Macroporous PEG-Alginate Hybrid Double-Network Cryogels with Tunable Degradation Rates Prepared via Radical-Free Cross-Linking for Cartilage Tissue Engineering. ACS Applied Bio Materials. 2024; 7: 5925–5938. https://doi.org/10.1021/acsabm.4c00091.

[60] Xun X, Li Y, Zhu X, Zhang Q, Lu Y, Yang Z, *et al*. Fabrication of

Robust, Shape Recoverable, Macroporous Bacterial Cellulose Scaffolds for Cartilage Tissue Engineering. Macromolecular Bioscience. 2021; 21: e2100167. https://doi.org/10.1002/mabi.202100167.

[61] Liu K, Fan Z, Wang T, Gao Z, Zhong J, Xiang G, *et al*. All-Aqueous-Processed Injectable *In Situ* Forming Macroporous Silk Gel Scaffolds for Minimally Invasive Intracranial and Osteological Therapies. Advanced Healthcare Materials. 2020; 9: e2000879. https://doi.org/10.1002/adhm.202000879.

[62] De Witte TM, Fratila-Apachitei LE, Zadpoor AA, Peppas NA. Bone tissue engineering via growth factor delivery: from scaffolds to complex matrices. Regenerative Biomaterials. 2018; 5: 197–211. https://doi.org/10.1093/rb/rby013.

[63] Chen J, He X, Sun T, Liu K, Chen C, Wen W, *et al*. Highly Elastic and Anisotropic Wood-Derived Composite Scaffold with Antibacterial and Angiogenic Activities for Bone Repair. Advanced Healthcare Materials. 2023; 12: e2300122. https://doi.org/10.1002/adhm.202300122.

[64] Liu J, Cui T, Xu X, Du Y, Wang L, Chen S, *et al*. Robust Alcohol Soluble Polyurethane/Chitosan/Silk Sericin (APU/CS/SS) Nanofiber Scaffolds toward Artificial Skin Extracellular Matrices via Microfluidic Blow-Spinning. Advanced Fiber Materials. 2023; 5: 349–361. https://doi.org/10.1007/s42765-022-00227-7.

[65] Ravishankar P, Balachandran K. Polymer Fiber-reinforced GAG Based Hydrogel Scaffolds for Heart Valve Tissue Engineering. Structural Heart. 2020; 4: 97. https://doi.org/10.1080/24748706.2020.1717204.

[66] Wang XL, Xie XH, Zhang G, Chen SH, Yao D, He K, *et al*. Exogenous phytoestrogenic molecule icaritin incorporated into a porous scaffold for enhancing bone defect repair. Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society. 2013; 31: 164–172. https://doi.org/10.1002/jor.22188.

[67] Furlani F, Montanari M, Sangiorgi N, Saracino E, Campodoni E, Sanson A, *et al*. Electroconductive and injectable hydrogels based on gelatin and PEDOT:PSS for a minimally invasive approach in nervous tissue regeneration. Biomaterials Science. 2022; 10: 2040–2053. https://doi.org/10.1039/d2bm00116k.

[68] Wang M, Du J, Li M, Pierini F, Li X, Yu J, *et al*. *In situ* forming double-crosslinked hydrogels with highly dispersed short fibers for the treatment of irregular wounds. Biomaterials Science. 2023; 11: 2383–2394. https://doi.org/10.1039/d2bm01891h.

[69] de Paiva Narciso N, Navarro RS, Gilchrist AE, Trigo MLM, Aviles Rodriguez G, Heilshorn SC. Design Parameters for Injectable Biopolymeric Hydrogels with Dynamic Covalent Chemistry Crosslinks. Advanced Healthcare Materials. 2023; 12: e2301265. https://doi.org/10.1002/adhm.202301265.

[70] Franca CM, Athirasala A, Subbiah R, Tahayeri A, Selvakumar P, Mansoorifar A, *et al*. High-Throughput Bioprinting of Geometrically-Controlled Pre-Vascularized Injectable Microgels for Accelerated Tissue Regeneration. Advanced Healthcare Materials. 2023; 12: e2202840. https://doi.org/10.1002/adhm.202202840.

[71] Lavanya K, Chandran SV, Balagangadharan K, Selvamurugan N. Temperature- and pH-responsive chitosan-based injectable hydrogels for bone tissue engineering. Materials Science & Engineering. C, Materials for Biological Applications. 2020; 111: 110862. https://doi.org/10.1016/j.msec.2020.110862.

[72] Meng T, Wang X, Jiang S, Chen SR, Zhou S, Zhu Y, *et al*. Delivery of Small-Molecule Drugs and Protein Drugs by Injectable Acid-Responsive Self-Assembled COF Hydrogels for Combinatorial Lung Cancer Treatment. ACS Applied Materials & Interfaces. 2023; 15: 42354–42368. https://doi.org/10.1021/acsami.3c10074.

[73] Zhang M, Wang Z, Huang P, Jiang G, Xu C, Zhang W, *et al*. Real-time and noninvasive tracking of injectable hydrogel degradation using functionalized AIE nanoparticles. Nanophotonics. 2020; 9: 2063–2075. https://doi.org/doi:10.1515/nanoph-2020-0087.

[74] Chen T, Cai Z, Zhao X, Wei G, Wang H, Bo T, *et al*. Dynamic monitoring soft tissue healing via visualized Gd-crosslinked double network MRI microspheres. Journal of Nanobiotechnology. 2024; 22: 289. https://doi.org/10.1186/s12951-024-02549-7.

[75] Dolgin J, Hanumantharao SN, Farias S, Simon CG, Rao S. Mechanical Properties and Morphological Alterations in Fiber-Based Scaffolds Affecting Tissue Engineering Outcomes. Fibers. 2023; 11: 39. https://doi.org/10.3390/fib11050039.

[76] Liu S, Hu Q, Shen Z, Krishnan S, Zhang H, Ramalingam M. 3D printing of self-standing and vascular supportive multimaterial hydrogel structures for organ engineering. Biotechnology and Bioengineering. 2022; 119: 118–133. https://doi.org/10.1002/bit.27954.

[77] Oladapo B, Zahedi A, Ismail S, Fernando W, Ikumapayi O. 3D-printed biomimetic bone implant polymeric composite scaffolds. The International Journal of Advanced Manufacturing Technology. 2023; 126: 4259–4267. https://doi.org/10.1007/s00170-023-11344-x.

[78] Lim HK, Ryu M, Woo SH, Song IS, Choi YJ, Lee UL. Bone Conduction Capacity of Highly Porous 3D-Printed Titanium Scaffolds Based on Different Pore Designs. Materials. 2021; 14: 3892. https://doi.org/10.3390/ma14143892.

[79] Zohoor S, Abolfathi N, Solati-Hashjin M. Accelerated degradation mechanism and mechanical behavior of 3D-printed PLA scaffolds for bone regeneration. Iranian Polymer Journal. 2023; 32: 1209–1227. https://doi.org/10.1007/s13726-023-01191-8.

[80] Kharaghani D, Kaffashsaei E, Haider MK, Kim IS. The Effect of Polymeric Nanofibers Used for 3D-Printed Scaffolds on Cellular Activity in Tissue Engineering: A Review. International Journal of Molecular Sciences. 2023; 24: 9464. https://doi.org/10.3390/ijms24119464.

[81] Kirillova A, Kelly C, Liu S, Francovich J, Gall K. High-Strength Composites Based on 3D Printed Porous Scaffolds Infused with a Bioresorbable Mineral-Organic Bone Adhesive. Advanced Engineering Materials. 2022; 24: 2101367. https://doi.org/10.1002/adem.202101367.

[82] Nagaraj A, Etxeberria AE, Naffa R, Zidan G, Seyfoddin A. 3D-Printed Hybrid Collagen/GelMA Hydrogels for Tissue Engineering Applications. Biology. 2022; 11: 1561. https://doi.org/10.3390/biology11111561.

[83] Mukasheva F, Adilova L, Dyussenbinov A, Yernaimanova B, Abilev M, Akilbekova D. Optimizing scaffold pore size for tissue engineering: insights across various tissue types. Frontiers in Bioengineering and Biotechnology. 2024; 12: 1444986. https://doi.org/10.3389/fbioe.2024.1444986.

[84] Pádua AS, Figueiredo L, Silva JC, Borges JP. Chitosan scaffolds with mesoporous hydroxyapatite and mesoporous bioactive glass. Progress in Biomaterials. 2023; 12: 137–153. https://doi.org/10.1007/s40204-023-00217-x.

[85] Malda J, Klein TJ, Upton Z. The roles of hypoxia in the *in vitro* engineering of tissues. Tissue Engineering. 2007; 13: 2153–2162. https://doi.org/10.1089/ten.2006.0417.

[86] Henkel J, Woodruff MA, Epari DR, Steck R, Glatt V, Dickinson IC, *et al*. Bone Regeneration Based on Tissue Engineering Conceptions—A 21st Century Perspective. Bone Research. 2013; 1: 216–248. https://doi.org/10.4248/BR201303002.

[87] Rojas-Yañez MA, Rodríguez-González CA, Martel-Estrada SA, Valencia-Gómez LE, Vargas-Requena CL, Hernández-Paz JF, *et al*. Composite scaffolds of chitosan/polycaprolactone functionalized with protein of *Mytilus californiensis* for bone tissue regeneration. AIMS Materials Science. 2022; 9: 344–358. https://doi.org/10.3934/matersci.2022021.

[88] Soriente A, Amodio SP, Fasolino I, Raucci MG, Demitri C, Engel E, *et al*. Chitosan/PEGDA based scaffolds as bioinspired materials to control *in vitro* angiogenesis. Materials Science & Engineering. C, Materials for Biological Applications. 2021; 118: 111420. https://doi.org/10.1016/j.msec.2020.111420.

[89] Haghbin M, Esmaeilzadeh J, Kahrobaee S. Freeze Dried Biodegradable Polycaprolactone/Chitosan/Gelatin Porous Scaffolds for Bone

Substitute Applications. Macromolecular Research. 2020; 28: 1232–1240. https://doi.org/10.1007/s13233-020-8170-5.

[90] Gholap AD, Rojekar S, Kapare HS, Vishwakarma N, Raikwar S, Garkal A, *et al*. Chitosan scaffolds: Expanding horizons in biomedical applications. Carbohydrate Polymers. 2024; 323: 121394. https://doi.org/10.1016/j.carbpol.2023.121394.

[91] Pinto RV, Gomes PS, Fernandes MH, Costa MEV, Almeida MM. Glutaraldehyde-crosslinking chitosan scaffolds reinforced with calcium phosphate spray-dried granules for bone tissue applications. Materials Science & Engineering. C, Materials for Biological Applications. 2020; 109: 110557. https://doi.org/10.1016/j.msec.2019.110557.

[92] Nazeer MA, Onder OC, Sevgili I, Yilgor E, Kavakli IH, Yilgor I. 3D printed poly(lactic acid) scaffolds modified with chitosan and hydroxyapatite for bone repair applications. Materials Today Communications. 2020; 25: 101515. https://doi.org/10.1016/j.mtcomm.2020.101515.

[93] Liu S, Zhang H, Ahlfeld T, Kilian D, Liu Y, Gelinsky M, *et al*. Evaluation of different crosslinking methods in altering the properties of extrusion-printed chitosan-based multi-material hydrogel composites. Bio-Design and Manufacturing. 2023; 6: 150–173. https://doi.org/10.1007/s42242-022-00194-3.

[94] Mukasheva F, Moazzam M, Yernaimanova B, Shehzad A, Zhanbassynova A, Berillo D, *et al*. Design and characterization of 3D printed pore gradient hydrogel scaffold for bone tissue engineering. Bioprinting. 2024; 39: e00341. https://doi.org/10.1016/j.bprint.2024.e00341.

[95] Biernat M, Woźniak A, Chraniuk M, Panasiuk M, Tymowicz-Grzyb P, Pagacz J, *et al*. Effect of Selected Crosslinking and Stabilization Methods on the Properties of Porous Chitosan Composites Dedicated for Medical Applications. Polymers. 2023; 15: 2507. https://doi.org/10.3390/polym15112507.

[96] Mak YW, Leung WW. Crosslinking of genipin and autoclaving in chitosan-based nanofibrous scaffolds: structural and physiochemical properties. Journal of Materials Science. 2019; 54: 10941–10962. https://doi.org/10.1007/s10853-019-03649-8.

[97] Raju L, AR SCL, Prakash NKU, Rajkumar E. Chitosan-terephthaldehyde hydrogels–Effect of concentration of cross-linker on structural, swelling, thermal and antimicrobial properties. Materialia. 2021; 16: 101082. https://doi.org/10.1016/j.mtla.2021.101082.

[98] Kamaci M, Kaya İ. Biodegradable and antibacterial poly(azomethine-urethane)-chitosan hydrogels for potential drug delivery application. Polymers for Advanced Technologies. 2020; 31: 898–908. https://doi.org/10.1002/pat.4824.

[99] Jiang Z, Zhang K, Du L, Cheng Z, Zhang T, Ding J, *et al*. Construction of chitosan scaffolds with controllable microchannel for tissue engineering and regenerative medicine. Materials Science & Engineering. C, Materials for Biological Applications. 2021; 126: 112178. https://doi.org/10.1016/j.msec.2021.112178.

[100] Maharjan B, Park J, Kaliannagounder VK, Awasthi GP, Joshi MK, Park CH, *et al*. Regenerated cellulose nanofiber reinforced chitosan hydrogel scaffolds for bone tissue engineering. Carbohydrate Polymers. 2021; 251: 117023. https://doi.org/10.1016/j.carbpol.2020.117023.

[101] Zhang Y, Wu D, Zhao X, Pakvasa M, Tucker AB, Luo H, *et al*. Stem Cell-Friendly Scaffold Biomaterials: Applications for Bone Tissue Engineering and Regenerative Medicine. Frontiers in Bioengineering and Biotechnology. 2020; 8: 598607. https://doi.org/10.3389/fbioe.2020.598607.

[102] Xiao F, Shi J, Zhang X, Hu M, Chen K, Shen C, *et al*. Gadolinium-doped whitlockite/chitosan composite scaffolds with osteogenic activity for bone defect treatment: *In vitro* and *in vivo* evaluations. Frontiers in Bioengineering and Biotechnology. 2023; 11: 1071692. https://doi.org/10.3389/fbioe.2023.1071692.

[103] Signorini L, Marenzi G, Facente A, Marrelli B, Marano RM, Valletta A, *et al*. Critical Overview on Pure Chitosan-based Scaffolds for Bone Tissue Engineering: Clinical insights in Dentistry. International Journal of Medical Sciences. 2023; 20: 1527–1534. https://doi.org/10.7150/ijms.87978.

[104] Ge YW, Chu M, Zhu ZY, Ke QF, Guo YP, Zhang CQ, *et al*. Nacre-inspired magnetically oriented micro-cellulose fibres/nano-hydroxyapatite/chitosan layered scaffold enhances pro-osteogenesis and angiogenesis. Materials Today. Bio. 2022; 16: 100439. https://doi.org/10.1016/j.mtbio.2022.100439.

[105] Tao F, Cheng Y, Shi X, Zheng H, Du Y, Xiang W, *et al*. Applications of chitin and chitosan nanofibers in bone regenerative engineering. Carbohydrate Polymers. 2020; 230: 115658. https://doi.org/10.1016/j.carbpol.2019.115658.

[106] Yadav LR, Chandran SV, Lavanya K, Selvamurugan N. Chitosan-based 3D-printed scaffolds for bone tissue engineering. International Journal of Biological Macromolecules. 2021; 183: 1925–1938. https://doi.org/10.1016/j.ijbiomac.2021.05.215.

[107] Gao X, Xu Z, Li S, Cheng L, Xu D, Li L, *et al*. Chitosan-vancomycin hydrogel incorporated bone repair scaffold based on staggered orthogonal structure: a viable dually controlled drug delivery system. RSC Advances. 2023; 13: 3759–3765. https://doi.org/10.1039/d2ra07828g.

[108] Azaman FA, Daubiné F, Lebatard A, Brennan Fournet ME, Devine DM. Chitosan/hydroxyapatite scaffolds with P28 as a promising osteoinductive scaffold for bone healing applications. Micro. 2023; 3: 118–142. https://doi.org/10.3390/micro3010010.

[109] Hsu SH, Whu SW, Tsai CL, Wu YH, Chen HW, Hsieh KH. Chitosan as Scaffold Materials: Effects of Molecular Weight and Degree of Deacetylation. Journal of Polymer Research. 2004; 11: 141–147. https://doi.org/10.1023/B:JPOL.0000031080.70010.0b.

[110] Zhang Y, Xing Y, Li J, Zhang Z, Luan H, Chu Z, *et al*. Osteogenesis-Related Behavior of MC3T3-E1 Cells on Substrates with Tunable Stiffness. BioMed Research International. 2018; 2018: 4025083. https://doi.org/10.1155/2018/4025083.

[111] Zhou Q, Lyu S, Bertrand AA, Hu AC, Chan CH, Ren X, *et al*. Stiffness of Nanoparticulate Mineralized Collagen Scaffolds Triggers Osteogenesis via Mechanotransduction and Canonical Wnt Signaling. Macromolecular Chemistry and Physics. 2021; 21: e2000370. https://doi.org/10.1002/mabi.202000370.

[112] Thein-Han WW, Kitiyanant Y. Chitosan scaffolds for *in vitro* buffalo embryonic stem-like cell culture: An approach to tissue engineering. Journal of Biomedical Materials Research. Part B, Applied Biomaterials. 2007; 80: 92–101. https://doi.org/10.1002/jbm.b.30573.

[113] Nellinger S, Kluger PJ. How Mechanical and Physicochemical Material Characteristics Influence Adipose-Derived Stem Cell Fate. International Journal of Molecular Sciences. 2023; 24: 3551. https://doi.org/10.3390/ijms24043551.

[114] Abarrategi A, Lópiz-Morales Y, Ramos V, Civantos A, López-Durán L, Marco F, *et al*. Chitosan scaffolds for osteochondral tissue regeneration. Journal of Biomedical Materials Research. Part A. 2010; 95: 1132–1141. https://doi.org/10.1002/jbm.a.32912.

[115] He JM, Zhu PF, Li LH, Wang Z, Li XL, Wang S, *et al*. Silk fibroin/chitosan/TGF-β1-loaded microsphere scaffolds for cartilage reparation. Bio-Medical Materials and Engineering. 2021; 32: 347–358. https://doi.org/10.3233/BME-201178.

[116] Pelin IM, Popescu I, Calin M, Rebleanu D, Voicu G, Ionita D, *et al*. Tri-Component Hydrogel as Template for Nanocrystalline Hydroxyapatite Deposition Using Alternate Soaking Method for Bone Tissue Engineering Applications. Gels. 2023; 9: 905. https://doi.org/10.3390/gels9110905.

[117] Yildizbakan L, Iqbal N, Ganguly P, Kumi-Barimah E, Do T, Jones E, *et al*. Fabrication and Characterisation of the Cytotoxic and Antibacterial Properties of Chitosan-Cerium Oxide Porous Scaffolds. Antibiotics. 2023; 12: 1004. https://doi.org/10.3390/antibiotics12061004.

[118] Guzmán R, Nardecchia S, Gutiérrez MC, Ferrer ML, Ramos V, del

Monte F, *et al*. Chitosan scaffolds containing calcium phosphate salts and rhBMP-2: *in vitro* and *in vivo* testing for bone tissue regeneration. PLoS One. 2014; 9: e87149. https://doi.org/10.1371/journal.pone.0087149.

[119] Fakhri E, Eslami H, Maroufi P, Pakdel F, Taghizadeh S, Ganbarov K, *et al*. Chitosan biomaterials application in dentistry. International Journal of Biological Macromolecules. 2020; 162: 956–974. https://doi.org/10.1016/j.ijbiomac.2020.06.211.

[120] Zhang C, Hui D, Du C, Sun H, Peng W, Pu X, *et al*. Preparation and application of chitosan biomaterials in dentistry. International Journal of Biological Macromolecules. 2021; 167: 1198–1210. https://doi.org/10.1016/j.ijbiomac.2020.11.073.

[121] Tian Y, Chen C, Xu X, Wang J, Hou X, Li K, *et al*. A Review of 3D Printing in Dentistry: Technologies, Affecting Factors, and Applications. Scanning. 2021; 2021: 9950131. https://doi.org/10.1155/2021/9950131.

[122] Vaidhyanathan B, Vincent P, Vadivel S, Karuppiah P, AL-Dhabi NA, Sadhasivam DR, *et al*. Fabrication and Investigation of the Suitability of Chitosan-Silver Composite Scaffolds for Bone Tissue Engineering Applications. Process Biochemistry. 2021; 100: 178–187. https://doi.org/10.1016/j.procbio.2020.10.008.

[123] Yang B, Wang PB, Mu N, Ma K, Wang S, Yang CY, *et al*. Graphene oxide-composited chitosan scaffold contributes to functional recovery of injured spinal cord in rats. Neural Regeneration Research. 2021; 16: 1829–1835. https://doi.org/10.4103/1673-5374.306095.

[124] Ham TR, Pukale DD, Hamrangsekachaee M, Leipzig ND. Subcutaneous priming of protein-functionalized chitosan scaffolds improves function following spinal cord injury. Materials Science & Engineering. C, Materials for Biological Applications. 2020; 110: 110656. https://doi.org/10.1016/j.msec.2020.110656.

[125] Sukpaita T, Chirachanchai S, Pimkhaokham A, Ampornaramveth RS. Chitosan-Based Scaffold for Mineralized Tissues Regeneration. Marine Drugs. 2021; 19: 551. https://doi.org/10.3390/md19100551.

[126] Zafeiris K, Brasinika D, Karatza A, Koumoulos E, Karoussis IK, Kyriakidou K, *et al*. Additive manufacturing of hydroxyapatite-chitosan-genipin composite scaffolds for bone tissue engineering applications. Materials Science & Engineering. C, Materials for Biological Applications. 2021; 119: 111639. https://doi.org/10.1016/j.msec.2020.111639.

[127] Nga NK, Thanh Tam LT, Ha NT, Hung Viet P, Huy TQ. Enhanced biomineralization and protein adsorption capacity of 3D chitosan/hydroxyapatite biomimetic scaffolds applied for bone-tissue engineering. RSC Advances. 2020; 10: 43045–43057. https://doi.org/10.1039/d0ra09432c.

[128] Hermenean A, Codreanu A, Herman H, Balta C, Rosu M, Mihali CV, *et al*. Chitosan-Graphene Oxide 3D scaffolds as Promising Tools for Bone Regeneration in Critical-Size Mouse Calvarial Defects. Scientific Reports. 2017; 7: 16641. https://doi.org/10.1038/s41598-017-16599-5.

[129] Wang QX, Qi XY, Dong ZM, Meng X, Liu HN, Liu WX. Effect of Bone Marrow Mesenchymal Stem Cells on Hydroxyapatite/Silk Fibroin/Chitosan Composite 3D Scaffold for Rat Skull Defects Repair. Journal of Biomaterials and Tissue Engineering. 2017; 7: 978–983. https://doi.org/10.1166/jbt.2017.1647.

[130] Florczyk SJ, Leung M, Li Z, Huang JI, Hopper RA, Zhang M. Evaluation of three-dimensional porous chitosan-alginate scaffolds in rat calvarial defects for bone regeneration applications. Journal of Biomedical Materials Research. Part A. 2013; 101: 2974–2983. https://doi.org/10.1002/jbm.a.34593.

[131] Zhou D, Qi C, Chen YX, Zhu YJ, Sun TW, Chen F, *et al*. Comparative study of porous hydroxyapatite/chitosan and whitlockite/chitosan scaffolds for bone regeneration in calvarial defects. International Journal of Nanomedicine. 2017; 12: 2673–2687. https://doi.org/10.2147/IJN.S131251.

[132] Munhoz MAS, Hirata HH, Plepis AMG, Martins VCA, Cunha MR. Use of collagen/chitosan sponges mineralized with hydroxyapatite for the repair of cranial defects in rats. Injury. 2018; 49: 2154–2160. https://doi.org/10.1016/j.injury.2018.09.018.

[133] Liu XL, Zhang CJ, Shi JJ, Ke QF, Ge YW, Zhu ZA, *et al*. Nacre-mimetic cerium-doped nano-hydroxyapatite/chitosan layered composite scaffolds regulate bone regeneration via OPG/RANKL signaling pathway. Journal of Nanobiotechnology. 2023; 21: 259. https://doi.org/10.1186/s12951-023-01988-y.

[134] Chi H, Song X, Song C, Zhao W, Chen G, Jiang A, *et al*. Chitosan-Gelatin Scaffolds Incorporating Decellularized Platelet-Rich Fibrin Promote Bone Regeneration. ACS Biomaterials Science & Engineering. 2019; 5: 5305–5315. https://doi.org/10.1021/acsbiomaterials.9b00788.

[135] Ding R, Liu Y, Cheng D, Yang G, Wu W, Du H, *et al*. A novel gene-activated matrix composed of PEI/plasmid-BMP2 complexes and hydroxyapatite/chitosan-microspheres promotes bone regeneration. Nano Research. 2022; 15: 6348–6360. https://doi.org/10.1007/s12274-022-4292-8.

[136] Abueva CDG, Jang DW, Padalhin A, Lee BT. Phosphonate-chitosan functionalization of a multi-channel hydroxyapatite scaffold for interfacial implant-bone tissue integration. Journal of Materials Chemistry. B. 2017; 5: 1293–1301. https://doi.org/10.1039/c6tb03228a.

[137] De Witte TM, Wagner AM, Fratila-Apachitei LE, Zadpoor AA, Peppas NA. Immobilization of nanocarriers within a porous chitosan scaffold for the sustained delivery of growth factors in bone tissue engineering applications. Journal of Biomedical Materials Research. Part A. 2020; 108: 1122–1135. https://doi.org/10.1002/jbm.a.36887.

[138] Gao K, Wang X, Wang Z, He L, Lin J, Bai Z, *et al*. Design of novel functionalized collagen-chitosan-MBG scaffolds for enhancing osteoblast differentiation in BMSCs. Biomedical Materials. 2021; 16: 065028. https://doi.org/10.1088/1748-605X/ac3146.

[139] Hu J, Wang Z, Miszuk JM, Zhu M, Lansakara TI, Tivanski AV, *et al*. Vanillin-bioglass cross-linked 3D porous chitosan scaffolds with strong osteopromotive and antibacterial abilities for bone tissue engineering. Carbohydrate Polymers. 2021; 271: 118440. https://doi.org/10.1016/j.carbpol.2021.118440.

[140] Wang X, Wang G, Liu L, Zhang D. The mechanism of a chitosan-collagen composite film used as biomaterial support for MC3T3-E1 cell differentiation. Scientific Reports. 2016; 6: 39322. https://doi.org/10.1038/srep39322.

[141] Nathan KG, Genasan K, Kamarul T. Polyvinyl Alcohol-Chitosan Scaffold for Tissue Engineering and Regenerative Medicine Application: A Review. Marine Drugs. 2023; 21: 304. https://doi.org/10.3390/md21050304.

[142] Lekhavadhani S, Shanmugavadivu A, Selvamurugan N. Role and architectural significance of porous chitosan-based scaffolds in bone tissue engineering. International Journal of Biological Macromolecules. 2023; 251: 126238. https://doi.org/10.1016/j.ijbiomac.2023.126238.

[143] Krieghoff J, Picke AK, Salbach-Hirsch J, Rother S, Heinemann C, Bernhardt R, *et al*. Increased pore size of scaffolds improves coating efficiency with sulfated hyaluronan and mineralization capacity of osteoblasts. Biomaterials Research. 2019; 23: 26. https://doi.org/10.1186/s40824-019-0172-z.

[144] Chim H, Hutmacher DW, Chou AM, Oliveira AL, Reis RL, Lim TC, *et al*. A comparative analysis of scaffold material modifications for load-bearing applications in bone tissue engineering. International Journal of Oral and Maxillofacial Surgery. 2006; 35: 928–934. https://doi.org/10.1016/j.ijom.2006.03.024.

[145] Luo S, Zhang C, Xiong W, Song Y, Wang Q, Zhang H, *et al*. Advances in electroactive biomaterials: through the lens of electrical stimulation promoting bone regeneration strategy. Journal of Orthopaedic Translation. 2024; 47: 191–206. https://doi.org/10.1016/j.jot.2024.06.009.

[146] Robling AG, Turner CH. Mechanical Signaling for Bone Modeling and Remodeling. Critical Reviews in Eukaryotic Gene Expression.

2009; 19: 319–338. https://doi.org/10.1615/critreveukargeneexpr.v19.i4.50.

[147] Tamaddon M, Liu C. Enhancing Biological and Biomechanical Fixation of Osteochondral Scaffold: A Grand Challenge. In Oliveira, J., Pina, S., Reis, R., San Roman, J. (eds.) Osteochondral Tissue Engineering. Advances in Experimental Medicine and Biology (pp. 255–298). Springer: Cham. 2018. https://doi.org/10.1007/978-3-319-76735-2_12.

[148] Ramesh N, Ratnayake JTB, Moratti SC, Dias GJ. Effect of chitosan infiltration on hydroxyapatite scaffolds derived from New Zealand bovine cancellous bones for bone regeneration. International Journal of Biological Macromolecules. 2020; 160: 1009–1020. https://doi.org/10.1016/j.ijbiomac.2020.05.269.

[149] Vasconcelos DP, Costa M, Amaral IF, Barbosa MA, Águas AP, Barbosa JN. Modulation of the inflammatory response to chitosan through M2 macrophage polarization using pro-resolution mediators. Biomaterials. 2015; 37: 116–123. https://doi.org/10.1016/j.biomaterials.2014.10.035.

[150] Chatzipetros E, Damaskos S, Tosios KI, Christopoulos P, Donta C, Kalogirou EM, *et al*. The effect of nano-hydroxyapatite/chitosan scaffolds on rat calvarial defects for bone regeneration. International Journal of Implant Dentistry. 2021; 7: 40. https://doi.org/10.1186/s40729-021-00327-w.

[151] Li W, Zhao J, Sun W, Wang H, Pan Y, Wang L, *et al*. Osteocytes promote osteoclastogenesis via autophagy-mediated RANKL secretion under mechanical compressive force. Archives of Biochemistry and Biophysics. 2020; 694: 108594. https://doi.org/10.1016/j.abb.2020.108594.

[152] Singh BN, Veeresh V, Mallick SP, Sinha S, Rastogi A, Srivastava P. Generation of scaffold incorporated with nanobioglass encapsulated in chitosan/chondroitin sulfate complex for bone tissue engineering. International Journal of Biological Macromolecules. 2020; 153: 1–16. https://doi.org/10.1016/j.ijbiomac.2020.02.173.

[153] Soriente A, Fasolino I, Gomez-Sánchez A, Prokhorov E, Buonocore GG, Luna-Barcenas G, *et al*. Chitosan/hydroxyapatite nanocomposite scaffolds to modulate osteogenic and inflammatory response. Journal of Biomedical Materials Research. Part A. 2022; 110: 266–272. https://doi.org/10.1002/jbm.a.37283.

[154] Qin L, Liu W, Cao H, Xiao G. Molecular mechanosensors in osteocytes. Bone Research. 2020; 8: 23. https://doi.org/10.1038/s41413-020-0099-y.

[155] Sequeira DB, Diogo P, Gomes BPFA, Peça J, Santos JMM. Scaffolds for Dentin-Pulp Complex Regeneration. Medicina. 2023; 60: 7. https://doi.org/10.3390/medicina60010007.

[156] d'Avanzo N, Bruno MC, Giudice A, Mancuso A, Gaetano FD, Cristiano MC, *et al*. Influence of Materials Properties on Bio-Physical Features and Effectiveness of 3D-Scaffolds for Periodontal Regeneration. Molecules: a Journal of Synthetic Chemistry and Natural Product Chemistry. 2021; 26: 1643. https://doi.org/10.3390/molecules26061643.

[157] Wu DT, Munguia-Lopez JG, Cho YW, Ma X, Song V, Zhu Z, *et al*. Polymeric Scaffolds for Dental, Oral, and Craniofacial Regenerative Medicine. Molecules: a Journal of Synthetic Chemistry and Natural Product Chemistry. 2021; 26: 7043. https://doi.org/10.3390/molecules26227043.

[158] Costa-Pinto AR, Reis RL, Neves NM. Scaffolds based bone tissue engineering: the role of chitosan. Tissue Engineering. Part B, Reviews. 2011; 17: 331–347. https://doi.org/10.1089/ten.teb.2010.0704.

[159] Carvalho M, Costa LM, Pereira JE, Shirosaki Y, Hayakawa S, Santos JD, *et al*. The role of hybrid chitosan membranes on scarring process following lumbar surgery: post-laminectomy experimental model. Neurological Research. 2015; 37: 23–29. https://doi.org/10.1179/1743132814Y.0000000414.

[160] Agarwal R, García AJ. Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair. Advanced Drug Delivery Reviews. 2015; 94: 53–62. https://doi.org/10.1016/j.addr.2015.03.013.

[161] Gu L, Huang R, Ni N, Gu P, Fan X. Advances and Prospects in Materials for Craniofacial Bone Reconstruction. ACS Biomaterials Science & Engineering. 2023; 9: 4462–4496. https://doi.org/10.1021/acsbiomaterials.3c00399.

[162] Liu H, Peng H, Wu Y, Zhang C, Cai Y, Xu G, *et al*. The promotion of bone regeneration by nanofibrous hydroxyapatite/chitosan scaffolds by effects on integrin-BMP/Smad signaling pathway in BMSCs. Biomaterials. 2013; 34: 4404–4417. https://doi.org/10.1016/j.biomaterials.2013.02.048.

[163] Raftery RM, Mencía Castaño I, Chen G, Cavanagh B, Quinn B, Curtin CM, *et al*. Translating the role of osteogenic-angiogenic coupling in bone formation: Highly efficient chitosan-pDNA activated scaffolds can accelerate bone regeneration in critical-sized bone defects. Biomaterials. 2017; 149: 116–127. https://doi.org/10.1016/j.biomaterials.2017.09.036.

Editor's note: The Scientific Editor responsible for this paper was Bo Lei.

Received: 1st November 2024; **Accepted**: 28th May 2025; **Published**: 28th August 2025