

Natural and phytochemical-based nano scaffolds in odonto-osteogenic regeneration: Structural features and clinical translation

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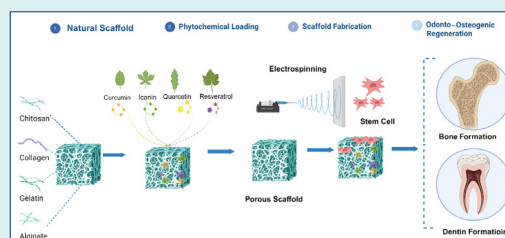
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Abstract

The design of scaffolds is crucial in bone and dental tissue engineering, especially for promoting odontogenic and osteogenic differentiation. Here, we review natural-based scaffolds derived from biological macromolecules such as hyaluronic acid, collagen, chitosan, gelatin, and cellulose, which present excellent biocompatibility and structural similarity to that observed in the extracellular matrix. The incorporation of bioactives of plant origin, such as curcumin, rutin, icariin, and propolis, enhances the bioactivity of the scaffolds through their antioxidant, anti-inflammatory, and pro-regenerative effects. These hybrid systems are capable of overcoming the limitations in mechanical resistance and efficacy of natural scaffolds. This review summarizes recent advances in natural and phytochemical-based nanoscaffolds for odonto-osteogenic regeneration, focusing on their structural characteristics, biological functions, and mechanisms that promote tissue differentiation. In addition, current strategies for scaffold functionalization with phytochemicals and their potential to improve regenerative outcomes are discussed. Particular attention is given to emerging approaches such as controlled drug release systems and three-dimensional (3D), as well as the challenges and prospects for clinical translation in dental and bone tissue regeneration.



Introduction

Tissue engineering provides innovative solutions for regenerating hard tissues like bone and teeth, which often encounter significant challenges in natural repair due to trauma, disease, or congenital defects. Bone tissue engineering (BTE) and dental tissue engineering (DTE) are two closely related distinct fields that aim to restore form and function through the use of biomaterials, cells, and signaling molecules. A central component in both fields is the scaffold, which serves not only as structural support but also as a biologically active platform that influences cell behavior and tissue development.¹⁻³

In this context, scaffolds with odontogenic and osteogenic potential have gained increasing attention, particularly for applications in craniofacial and maxillofacial regeneration, where simultaneous repair of dental and bone tissues is often

required. Natural biological macromolecules, such as those derived from collagen, chitosan, or alginate are favored for their biocompatibility and resemblance to the extracellular matrix (ECM).⁴⁻⁶ despite growing advancements, developing scaffolds capable of supporting odontogenic and osteogenic differentiation remains a critical challenge in both bone and dental tissue engineering. While natural materials offer excellent biological properties, they often lack the mechanical strength or targeted bioactivity needed for efficient regeneration. Synthetic scaffolds, in contrast, provide tunable mechanical properties but require bio-functionalization to support cellular processes.^{7,8}

To address these limitations, recent research has focused on phytochemical functionalization—the incorporation of bioactive compounds from medicinal plants, such as curcumin, rutin, icariin, and propolis, into scaffold systems.



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These agents exhibit antioxidant, anti-inflammatory, and pro-regenerative effects, enhancing cellular responses and promoting both osteogenic and odontogenic differentiation.^{9–11}

The integration of phytochemicals into scaffold systems presents a novel solution by enhancing cellular responses and promoting differentiation.¹⁰

Despite the growing number of reviews on biomaterial scaffolds for bone and dental tissue engineering, most studies focus either on conventional biomaterials or on synthetic nanostructures, with limited attention to the integration of natural macromolecular scaffolds with plant origin bioactive compounds. In particular, a comprehensive analysis linking the structural properties of natural scaffolds with the biological activity of phytochemicals in odonto-osteogenic regeneration remains insufficiently addressed. Therefore, this review aims to provide a comprehensive overview of natural and phytochemical-based nanoscaffolds used for odonto-osteogenic regeneration. We discuss the structural characteristics of natural polymeric scaffolds such as chitosan, collagen, gelatin, and alginate, as well as the regenerative potential of phytochemicals including curcumin, rutin, icariin, and propolis. Furthermore, we highlight how the incorporation of phytochemicals can enhance antioxidant, anti-inflammatory, and osteo/odontogenic differentiation properties of these scaffolds. Importantly, this review also examines current limitations, translational challenges, and emerging strategies such as controlled drug-release systems and 3D bioprinting approaches, aiming to bridge the gap between experimental scaffold design and future clinical applications in dental and bone regeneration.

The literature search was conducted up to Dec 2025, and articles published between 2005 and 2025 were included in this review. Among the 40 studies included in this review, 23 articles were published between 2018 and 2025, indicating increasing research interest in this field. The highest publication frequency was observed in 2020, with a total of 7 studies.

Bone and dental tissue engineering

BTE operates as a multidisciplinary field that integrates engineering principles to restore, maintain, or improve the structure and function of bone at biological and defective sites.^{12, 13} Conventional bone grafting methods face substantial obstacles; autografts are constrained by limited donor tissue availability, while allografts carry potential risks concerning disease transmission and immune rejection.¹⁴ To bypass these limitations, BTE employs a synergistic triad of bioactive factors, cells, and scaffolds to establish an osteogenic microenvironment capable of promoting three-dimensional bone formation.^{15–25} Successful regeneration of complex hard tissues requires biomaterials possessing superior biological and physicochemical attributes, ensuring they are non-allergenic, non-toxic, non-inflammatory, non-immunogenic, and functionally effective upon in vivo implantation.^{26–31} DTE utilizes analogous principles, focusing specifically on the

restoration of tooth-related structures. Dental stem cells (DSCs)—though presently underutilized—represent a viable cellular source exhibiting mesenchymal stem cell-like properties, such as multilineage differentiation potential, fibroblast-like character, immunomodulatory capabilities, and specific surface antigen expression.³² Various DSC types, including dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from the apical papilla, periodontal ligament stem cells (PDLSCs), and dental follicle stem cells, have been effectively retrieved from diverse tooth region.² These cells demonstrate significant promise for regenerating dentin and pulp tissues, offering a biological alternative to traditional endodontic procedures.^{33,34} Dental tissues are categorized into three primary types: enamel, cementum, and dentin. Enamel, which constitutes the crown, lacks living cells and cannot biologically recover once formed. Located beneath the cementum and enamel, dentin comprises the majority of the tooth structure. Unlike enamel, dentin houses living cells known as odontoblasts, situated at the junction between the dentin and the pulp depth. Odontoblasts are essential for dentinogenesis and secrete dentin in response to various stimuli, such as injury or tooth decay.^{35,36}

Cellular processes like migration, differentiation, and proliferation are regulated by an array of signaling molecules, including transforming growth factor beta 1 (TGF- β 1), bone morphogenetic proteins (BMPs), dentin matrix acidic phosphoprotein 1 (DMP1), fibroblast growth factors (FGFs), and tumor necrosis factor alpha (TNF- α).^{37–39} Scaffolds are critical in regenerative dentistry, as they facilitate tissue recovery and rebuild harmed or lost dental structures. These biomimetic and biocompatible architectures serve as temporary systems that permit cell adhesion, growth, and specialization into functional tissue.⁴⁰

Regeneration of dentin has involved both natural polymers—such as alginate, collagen, gelatin, chitosan, and hyaluronic acid—and synthetic polymers, including polycaprolactone (PCL), poly(glycolic acid) (PGA), and poly(lactic acid) (PLA). Composite scaffolds that integrate natural and synthetic polymers or incorporate hydroxyapatite nanoparticles can significantly bolster bioactivity and mechanical stability, thereby enhancing outcomes in dentin regeneration. BMPs play a pivotal role in the success of dental pulp and dentin regeneration, both ex vivo and in vivo.³⁴ For example, Chakka et al illustrated that DPSCs cultivated on collagen scaffolds augmented with pFGF-2 and/or PEI-pBMP-2 nanoplexes exhibit higher BMP-2 and FGF-2 expression, increased proliferative capacity, and superior mineralization properties compared to mineral trioxide aggregate (MTA), findings which were corroborated by ex vivo observations. Similarly, Cordeiro et al conducted the inaugural study transplanting poly-L-lactide scaffolds seeded with SHED into the teeth of immunodeficient mice. The resulting bioengineered tissues exhibited cellular structures closely resembling physiologic dental pulps. Various studies have

investigated pulp and dentine tissue engineering using stem cells (SCs) and scaffolds.^{41,42} Various studies on pulp and dentine tissue engineering utilizing scaffolds and SCs have been conducted. For instance, the in vivo root development of non-vital permanent teeth with apical periodontitis can be bolstered by incorporating GFs and DPSCs into a CS hydrogel, creating dentin-pulp-like tissue.⁴³ Komichi et al's findings demonstrate that protein S100-A7 released from dentin by MMP20 might play a key role in dentin pulp regeneration.⁴⁴ Moreover, Zhang et al found that with the induction of dentin matrix extracellular matrix, human umbilical cord blood MSCs (hUCMSCs) expressed specific odontoblast markers (DSP, DMP-1, and DSPP), and cocultured hUCMSCs and VEGF-induced hUCMSCs (V-hUCMSCs) formed vessel-like structures. Subsequently, hUCMSCs can be utilized as an alternative seed cell source for angiogenesis and dental pulp regeneration.⁴⁵ The recovery of dental pulp and dentin, and restoration of tooth vitality, may be possible with the use of scaffolds and SCs, which can be both safe and effective.⁴⁶

The regeneration of mineralized tissues, such as dentin and bone, remains challenging due to their complex architecture and the biological demands placed on them. Odonto/osteogenic scaffolds are a promising means of inducing stem cell differentiation toward odontogenic (forming dentin) and osteogenic (forming bone) lineages.⁴⁷ These scaffolds often comprise bioactive materials, signaling molecules, or treatment gradients on the surface that mimic the normal microenvironment of dental pulp and bone tissue.⁴⁸ Such dual-functional scaffolds hold excellent promise for treating maxillofacial defects, pulp wounds, and periodontal tissue loss, due to their ability to stimulate concurrent dentin and bone formation. Continuous advances are being made to optimize scaffold design, mechanics, and biological activity, thereby improving clinical translation in regenerative dentistry.

Naturally derived scaffolds

Scaffolds derived from natural sources have recently attracted much attention because of their structural similarities to the natural extracellular matrix (ECM) and ability to support the cellular functions essential for tissue regeneration.⁴⁹ These biomaterials derived from natural sources such as plants, animals, and marine organisms possess many of the desired properties, such as biocompatibility, biodegradability, and bioactivity, and are therefore candidates for bone and dental tissue engineering.^{50,51} Natural scaffolds provide biological cues to cells that bring about adhesion, proliferation, and differentiation of the cells without the cytotoxic and inflammatory reactions caused by synthetic polymers.^{50,52} However, their clinical use is limited as a result of poor mechanical strength, rapid degradation, and variability of materials from batch to batch. For these reasons, much work has gone into the chemical modification, cross-linking, and incorporation of bioactive compounds such as phytochemicals and nanomaterials in order to increase

their stability and functions.^{50,53}

Chitosan

Chitosan is a cationic natural polysaccharide derived from chitin and composed mainly of glucosamine and N-acetylglucosamine units linked by β -(1 $\rightarrow$ 4) glycosidic bonds.⁵⁴ Chitosan has a poor water solubility due to the presence of strong hydrogen bonds between its molecules, primary amino groups, with a pK_a value of 6.3; thus, it is regarded as a strong base. Chemical changes that are probable at two hydroxyl functional groups in the polymer chain can increase the solubility of chitosan.^{55,56}

The initial process after implantation is protein adsorption. Just upon the insertion of the scaffold, protein adsorption takes place, and the cells that reach the external layer of the biomaterial adhere to the adsorbed protein layer rather than the biomaterial itself. Cells recognize bioactive binding sites on the protein surface through receptors or ligands attached to the cell membrane, and they respond to external stimuli accordingly.⁵⁷ Chitosan is a positively-charged polysaccharide, has protonable amino groups on its backbone, which allows the electrostatic interaction with different negatively charged proteins.⁵⁸ Protein charges and the surface of the biomaterials are dependent on pH and the ionic composition of the solution, which in turn affects the electrostatic interactions between proteins and biomaterials. Proteins are typically negatively charged at higher pH levels and positively charged at acidic pH.⁵⁹ Since it is highly stable, readily available at high purity, and soluble in water, bovine serum albumin protein (BSA) is frequently utilized as a typical protein for characterization of biomaterials concerning protein adsorption capacity.⁶⁰ The pH affects how the chitosan chain and BSA protein interact, and the process is quite intricate. According to Kim et al, the electrostatic interaction between BSA and chitosan is controlled by two factors: (1) the interaction between the dissociated carboxyl groups of BSA and the protonated amino groups of chitosan, and (2) the repulsion of the protonated amino groups of BSA and chitosan. At neutral pH, the BSA protein is negatively charged.⁶¹ Since protein adsorption is the initial and most important stage following implantation of the scaffold, it is necessary to ascertain the capacity of biomaterials for protein adsorption.^{62,63} Nonetheless, several conditions must be met to create and design an applicable scaffold for the regeneration of bone tissue. Chitosan has been used in several types as scaffolds for dental and bone tissue engineering, which will be addressed below.^{64,65}

The synthesis of patient-specific scaffolds with intricate geometries has been made possible by 3D-printed chitosan-incorporated scaffolds, which have garnered significant attention in recent years. Recently, a small number of excellent review articles on chitosan 3D printing, comprising bioprinting, have been released.^{65,66}

Chitosan nanofibers

An electric field is used in the electrospinning process to regulate the deposition of polymer fibers onto target substrates.⁶⁷ Natural polymers have a higher molecular

weight, are less spinnable than synthetic polymers because of their restricted solubility in the majority of organic solvents, polycationic feature in solution, and 3D networks of strong hydrogen bonds.⁶⁸ In the 2000s, there was a lot of research done on fiber-shaped chitosan scaffolds made by electrospinning. The high viscosity of chitosan was successfully addressed by Homayoni et al⁶⁹ which imposes limitations on its spinnability, by implementing an alkali treatment that enzymatically breaks down the chitosan chains, leading to a reduction in its molecular weight. Consequently, when these treated chitosan solutions are combined with aqueous acetic acid, the resultant nanofibers exhibit desirable characteristics and stability throughout the processing stage. It is noteworthy that the most optimal nanofibers are attained when the chitosan is hydrolyzed for 48 hours, resulting in a nanofiber diameter of 140 nm. It should be emphasized that the fiber diameter is significantly influenced by the electrospinning situations as well as the concentration of the solvent. Moreover, there have been recent comprehensive assessments of the existing literature on the electrospinning of chitosan-based solutions for applications in tissue engineering.^{64,70}

Ho et al highlighted the pivotal role osteoblasts play in bone tissue formation. In earlier research, they demonstrated that chitosan nanofibers could enhance both proliferation and differentiation of osteoblasts. Building upon those findings, the present translational study employed an animal model to investigate how chitosan nanofiber scaffolds influence bone regeneration and to explore potential underlying mechanisms. Uniform chitosan nanofibers were fabricated, with an average diameter close to 200 nm. Researchers created bone defects surgically in the proximal femurs of male C57LB/6 mice. Each mouse received a scaffold implant in the left femur for 21 days, while the right femur remained untreated as a control. Histopathological evaluations confirmed that the chitosan nanofiber implants did not induce liver or kidney toxicity. Radiographic imaging and micro-computed tomography (micro-CT) revealed enhanced bone repair in scaffold-implanted femurs compared to controls. Further analysis using micro-CT and bone histomorphometry confirmed increased formation of new trabecular bone in the scaffold-treated group. Additionally, chitosan nanofiber implantation significantly increased trabecular thickness and decreased the trabecular parameter factor. To investigate the molecular mechanisms, confocal microscopy showed elevated expression of Runt-related transcription factor 2 (Runx2), a key regulator of osteogenic differentiation, at the bone defect sites treated with scaffolds. Consistent with this, the levels of alkaline phosphatase (ALP) and osteocalcin (OCN)—two hallmark markers of bone maturation—were also elevated. Overall, the findings suggest that chitosan nanofiber scaffolds enhance bone regeneration by promoting trabecular bone formation through Runx2-driven upregulation of ALP and OCN expression. These outcomes support the therapeutic potential of chitosan nanofiber scaffolds in treating bone-related conditions such as fractures and structural defects (Fig. 1).⁷¹

Chitosan sponges

Sponges are typically described as structures composed of gas, most often air, dispersed within a solid matrix. In recent years, there has been growing interest in their application across pharmaceutical and biomedical fields. Their utility spans various functions, including acting as carriers for controlled drug delivery, serving as wound dressings, and providing scaffolds for cell proliferation in tissue engineering. Among the different types, sponges derived from polysaccharides such as alginate and chitosan have garnered attention due to their low cytotoxicity, desirable mechanical properties, and their capacity for bioresorption.⁷²⁻⁷⁴ Particularly in the context of bone regeneration, chitosan-based sponges have attracted significant research interest. These biodegradable and biocompatible sponges, especially when combined with active agents, have been demonstrated to promote bone tissue formation both *in vitro* and *in vivo*. Beyond supporting bone regeneration, chitosan also functions as a delivery vehicle capable of modulating the release of bioactive compounds, such as growth factors.^{72,74,75} In one study by Tsuchiya et al, the efficacy of different carrier systems on guided bone augmentation (GBA) was examined using a rat calvarial model. Researchers implanted plastic caps containing either a chitosan sponge alone or a chitosan sponge loaded with 0.03% platelet-derived growth factor (PDGF) onto the calvariae of 14 rats. Bone regeneration was evaluated using microcomputed tomography (micro-CT) and histological analysis. The results indicated that the group treated with PDGF-loaded chitosan sponges exhibited significantly greater bone volume and augmentation beyond the natural bone boundary compared to the control group containing sponge alone.⁷⁴ Similarly, Seol et al cultured rat calvarial osteoblasts within chitosan sponges that had been fabricated through freeze-drying, resulting in a porous matrix with pore diameters ranging from 100 to 200 μm —ideal for facilitating cell proliferation. Throughout a 56-day culture period, various parameters were measured, including cell density, alkaline phosphatase activity, and calcium deposition. Cell counts within the sponges were recorded as 4×10^6 on day 1, 11×10^6 on day 28, and 12×10^6 on day 56 per gram of sponge. Calcium deposition increased over time, measured at 9 μg on day 1, 40 μg on day 28, and 48 μg on day 56 per sponge. Histological assessments confirmed that bone tissue had formed within the chitosan scaffolds. These findings underscore the effectiveness of chitosan sponges as supportive scaffolding for *in vitro* bone tissue engineering.⁷³

The regenerative potentials of chitosan can be significantly enhanced through the incorporation of bioactive phytochemicals. Propolis contains several flavonoids and phenolic compounds, such as caffeic acid phenethyl ester (CAPE), apigenin, and quercetin, with strong antioxidant and anti-inflammatory properties. These compounds stimulate osteoblast differentiation by activating signaling pathways (e.g., BMP, FGF, and TGF- β), leading to increased expression of RUNX2

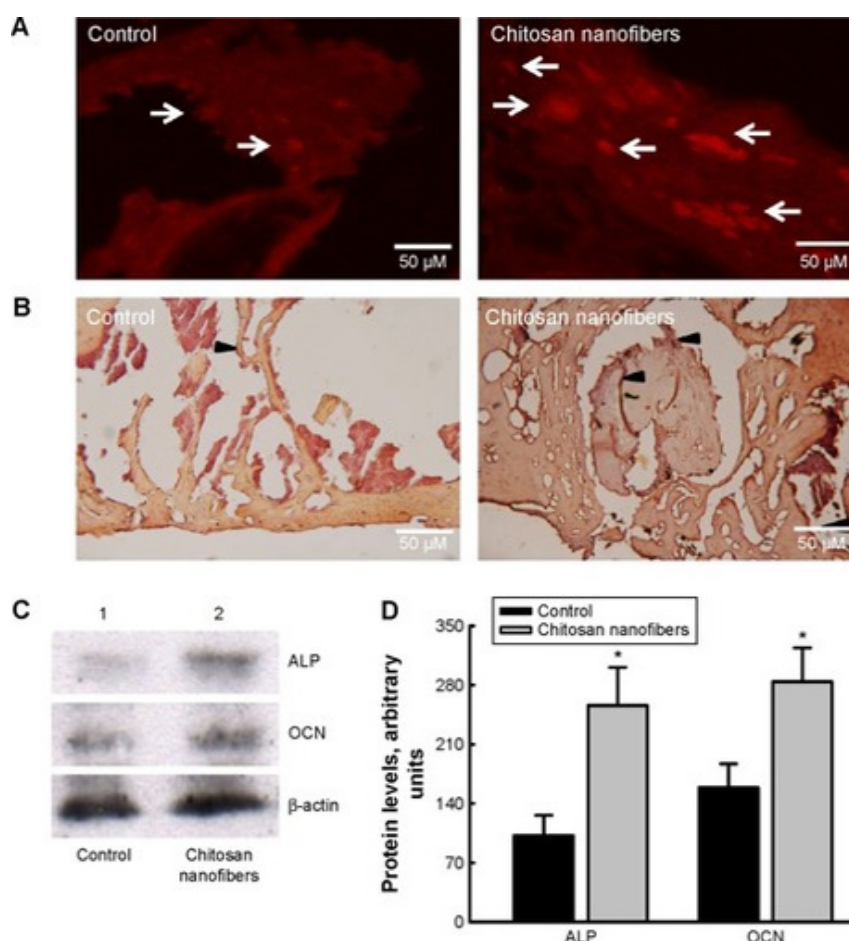


Fig. 1. Bone defects were induced in the proximal femurs of male C57LB/6 mice, with chitosan nanofiber scaffolds implanted in one femur per animal for 21 days. Post-treatment, animals were euthanized, and femurs were harvested for immunohistochemical staining of ALP (A) and OCN (B), as indicated by arrows and arrowheads. Proteins from both treated and control femurs underwent immunoblotting to detect ALP and OCN levels (C, top panels), with β -actin serving as the loading control (bottom panel). Quantitative analysis of these bands was conducted (D), with results expressed as mean $\pm$ SEM ($n=6$). Asterisks denote statistically significant differences from controls (* $P < 0.05$, magnification 200 $\times$). Abbreviations: ALP, alkaline phosphatase; OCN, osteocalcin; SEM, standard error of the mean. Reproduced from Ho et al,⁷¹ which is licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0; <https://creativecommons.org/licenses/by/4.0/>).

and other osteogenic markers. One study demonstrated that scaffolds coated with propolis enhanced calcium deposition, ALP activity, and collagen type I expression—key indicators of osteogenesis. In distraction osteogenesis models, propolis supplementation also accelerated bone consolidation, further confirming its clinical relevance.⁷⁶ A study showed that propolis-coated scaffolds improved calcium deposition, ALP activity, and osteogenic markers such as RUNX-2 and type I collagen, making propolis a promising material for bone tissue engineering.⁷⁷ Additionally, propolis supports bone consolidation in distraction osteogenesis, indicating its potential for clinical bone repair.⁷⁸ A comparative *in vitro* study evaluated the effects of propolis and Biodentine™ on SHEDs. Propolis demonstrated favorable outcomes in cell viability, mineralization (via Alizarin Red staining), and anti-inflammatory activity. Although Biodentine showed slightly higher levels of proliferation and differentiation, propolis exhibited stronger inhibition of matrix metalloproteinases (MMP-2 and MMP-9), highlighting its therapeutic promise in pulp tissue regeneration.⁴⁸ Meimandi-Parizi et al in an animal study further supported the regenerative potential

of chitosan-phytochemical composites. In a critical-sized radial bone defect model in rats, scaffolds composed of chitosan or demineralized bone matrix (DBM) combined with propolis extract showed significantly improved structural, histological, and biomechanical outcomes. The DBM-propolis group, in particular, exhibited comparable healing to autografts, with extensive formation of woven bone and hyaline cartilage by day 56 post-operation.⁷⁹ Meimandi et al showed that royal jelly also exhibits strong regenerative potential by upregulating osteogenic markers such as osteocalcin, osteopontin, and osterix, while downregulating inflammatory cytokines like IL-6. *In vivo* studies involving chitosan scaffolds supplemented with royal jelly (CS-RJ) or bee venom (CS-BV) demonstrated significantly improved bone density, osteoblast/osteocyte count, and mechanical strength compared to both chitosan-alone and autograft controls. Notably, the CS-RJ group outperformed autografts in biomechanical performance at day 56, indicating a promising alternative in critical defect healing. These findings underscore the synergistic potential of combining chitosan scaffolds with bee-derived phytochemicals to promote osteogenic and odontogenic

regeneration. While preliminary outcomes are promising, further studies are warranted to validate their long-term safety, efficacy, and clinical translatability.⁸⁰

Chitosan hydrogels

Hydrogel scaffolds represent a distinct class of three-dimensional crosslinked polymer networks characterized by their substantial water-retention capacity,^{81,82} their hydrophilic nature, cytocompatibility, adjustable degradation behavior, and modifiable mechanical performance make them attractive platforms for regenerative applications. In addition, these systems can serve as carriers for a wide range of bioactive agents.⁸¹ Owing to their softness, elasticity, and structural resemblance to the ECM, hydrogels are particularly suitable for mimicking the microenvironment of dental pulp tissue.⁸³ Natural polymer-based hydrogels further offer inherent bioactivity, excellent biocompatibility, and enzymatic or hydrolytic degradability. Moreover, hydrogels can be functionalized with cell-adhesive motifs, such as arginine–glycine–aspartic acid (RGD), incorporated with signaling molecules, or combined with stem cells to support dentin–pulp complex regeneration. Despite these advantages, the development of hydrogel scaffolds that fully meet the biological and mechanical requirements of regenerative endodontics remains under investigation and holds considerable promise as an alternative to conventional endodontic therapies.⁸¹

Traditional approaches to tissue engineering, such as pre-formed hydrogels and scaffolds, encounter challenges related to surgical implantation, which can lead to heightened susceptibility to infections and difficulties in achieving the desired scaffold shape and size.⁸⁴ Recently, there has been growing attention on smart injectable hydrogels due to their potential applications in minimally invasive therapies. Smart injectable gelling systems exhibit a liquid state at 25 °C, but transform into gels upon injection into the fractured site. This unique property allows them to effectively fill the intricate contours of the defect, ensuring optimal coverage and support.⁸⁵ Hydrogels of this nature have the potential to expedite surgical procedures, mitigate the adverse consequences of extensive muscle retraction, diminish scar size, and alleviate post-operative discomfort. Consequently, patients can experience a swift and economic recovery. Hydrogels have the potential to be employed in non-load-bearing applications to convey and safeguard cells, proteins, growth factors, or drugs. Moreover, they play a critical role in facilitating the appropriate permeability required for the transportation of essential nutrients and metabolites within cells.⁶⁸ The gelation time of injectable hydrogels is a crucial characteristic, as a delayed gelation process can lead to the formation of gel in an uncontrolled manner due to the diffusion of the gel precursor.⁸⁵ Hydrogels synthesized from polysaccharides found in nature exhibit numerous characteristics resembling those of the extracellular matrix. As a result, they possess the capability to guide the movement, growth, and arrangement of enclosed cells in the process of tissue regeneration. Chitosan, being a

polymer that responds to changes in pH, exhibits solubility in gentle acidic conditions. However, when neutralized, it transforms into a hydrogel. This transformation is attributed to the elimination of repulsive electrostatic interactions that take place throughout the neutralization step. Consequently, the amino sites within chitosan can engage in intermolecular hydrogen bonding, facilitating the formation of the hydrogel.⁶⁶

Samiei et al investigated strategies to enhance the proliferation and differentiation potential of DPSCs through the use of injectable, sol-gel-based hydrogels. Two chitosan-based hydrogel formulations were developed using optimized preparation protocols. One utilized β -glycerophosphate (CS/GP) as an ionic cross-linker, while the other employed genipin (CS/GN) as a chemical cross-linker, with sodium hydrogen carbonate serving as a co-gelling agent in both systems. After conducting comprehensive physicochemical analyses, both hydrogels were assessed for their biological performance in promoting DPSC survival, proliferation, and differentiation, with or without the presence of MTA, commonly used in endodontics, and differentiation-inducing medium (D). Cell viability and DNA quantification assays demonstrated improved cytocompatibility of the CS/GN hydrogel, which also exhibited a superior 3D network structure compared to CS/GP. Further evaluations using alizarin red staining, ALP activity assays, and real-time PCR indicated enhanced mineralization, increased alkaline phosphatase activity, and upregulation of osteogenic and odontogenic gene expression in DPSCs cultured on CS/GN hydrogels, particularly when supplemented with both MTA and differentiation medium (D + MTA). These findings suggest that the CS/GN hydrogel, in combination with D + MTA, can significantly drive the osteogenic differentiation of DPSCs.⁸⁶

Wang et al focused on stimuli-responsive biomaterials, particularly for drug delivery systems that allow precise spatial and temporal control. Teriparatide, a drug used in systemic therapy for osteoporosis, has recently been adapted for local biomimetic delivery in bone defect repair due to its anabolic effects, enhanced local concentration, and minimized systemic side effects. In this study, a novel delivery strategy was proposed involving Teriparatide-loaded chitosan/reduced graphene oxide (CS/rGO) hydrogel films, fabricated via electrodeposition and activated by near-infrared (NIR) light. This system aimed to mimic the body's natural pulsatile secretion of Teriparatide through photothermal-triggered release. Bone marrow mesenchymal stem cells derived from ovariectomized rats (OVX-BMSCs) were used to determine the optimal delivery parameters. The most effective dosing schedule was found to be between $1 \text{ hour} \times 24 \times 10^{-9} \text{ mol} \cdot \text{L}^{-1} / \text{day}$ and $2 \text{ hours} \times 12 \times 10^{-9} \text{ mol} \cdot \text{L}^{-1} / \text{day}$. Evaluations in calvarial defect models revealed that the NIR-responsive CS/rGO films promoted robust bone regeneration. Micro-CT and histological analyses confirmed enhanced new bone formation, and vascularization was markedly increased both at the interface and within the central

defect area. This study introduces a novel therapeutic platform that integrates pulsatile hormone delivery with a light-responsive biomaterial to effectively support bone regeneration in osteoporotic conditions.⁸⁷

Collagen

Collagen is a fibrous protein characterized by a triple-helical structure formed from three polypeptide chains. The utilization of collagen in the biomedical domain can be traced back to the 19th century, as indicated by the earliest documented accounts.⁸⁸ At present, collagens obtained directly from animal tissues or synthesized as recombinant proteins have been extensively utilized in laboratory settings as standardized 3D materials. These collagens may undergo additional modifications, such as crosslinking, polymerization, or fibrillization. The purpose of using these materials is to study the impact of microstructural and mechanical characteristics on various cell behaviors.⁸⁹ Collagen-incorporating scaffolds are extensively utilized *in vivo* for diverse clinical applications to facilitate bone tissue regeneration. These materials are available in different forms, such as membranes, sponges or matrices, hydrogel, and composite scaffolds, as described below.⁹⁰

Collagen membranes and nanofibers

Different types of membrane materials are utilized in clinical settings, including the dental field, to accomplish guided bone regeneration (GBR).⁹¹ The initial triumph in achieving GBR and controlling the movement of soft tissue, particularly epithelial cells, was attributed to expanded polytetrafluoroethylene (ePTFE), commonly known as Teflon.⁹² Nevertheless, the extensive utilization of ePTFE has resulted in a significant occurrence of wound dehiscence and infections, thereby necessitating the removal of this non-resorbable membrane through an additional surgical procedure. Consequently, this secondary operation inflicts further harm upon the wound.⁹³ An encouraging solution to address these apprehensions involves the utilization of resorbable collagen membranes. These membranes present numerous benefits, including a streamlined surgical process, enhanced healing of soft tissues, integration with the host tissues, and rapid absorption in the event of exposure.⁹⁴ Nevertheless, collagen membranes exhibit certain drawbacks associated with their unfavorable mechanical characteristics and unpredictable rates of resorption, despite the utilization of materials derived from native dermis.⁹⁵ Various strategies, including the utilization of alternative tissue sources and the exploration of different manufacturing techniques, have been examined in order to address these challenges.⁹⁶ It is worth noting that the utilization of ribose for cross-linking has been demonstrated to be a beneficial substitute in the manufacturing of GBR membranes that are collagen-based.⁹⁷ Additional strategies to address these challenges involve implementing alternative manufacturing techniques, including electrophoretic deposition, electrospinning, and 3D printing, in order to enhance the biomechanical characteristics of collagen membranes.^{98,99} A novel material option has emerged in recent times for guided bone regeneration procedures. This

alternative involves utilizing a resorbable magnesium grid to maintain volume stability. This magnesium grid can be used in combination with either a collagen membrane or a pure magnesium membrane. These advancements in material technology offer promising prospects for GBR processes.¹⁰⁰

Curcumin-containing collagen membranes exhibit a dual role in bone metabolism by modulating various molecular pathways. It promotes osteogenesis by regulating NF- κ B and MAPK pathways, reducing oxidative stress, and supporting ALP activity, calcium deposition, and pre-osteoblast viability—key markers for bone formation.¹⁰¹ In diabetic osteoporosis, curcumin improves bone health by coupling osteogenesis with angiogenesis through suppression of NF- κ B signaling, thereby reducing inflammation and oxidative stress.¹⁰¹ It also promotes PDLSC differentiation via the PI3K/AKT/Nrf2 pathway, enhancing cellular resistance to oxidative damage.¹⁰² However, curcumin can also act as an inhibitor of osteogenesis by inducing miR-126a-3p, which suppresses LRP6 and thereby inhibits WNT signaling¹⁰³—a critical pathway for osteogenic differentiation.¹⁰⁴ Furthermore, it prevents bone resorption by inhibiting RANKL-induced autophagy in osteoclast precursors, interfering with the JNK-BCL2-Bec1 pathway.¹⁰⁵ Curcumin has also been integrated into collagen-hydroxyapatite scaffolds for bone tissue engineering. Khodabandeh et al demonstrated that curcumin, along with ginger extract, improved mechanical strength, osteogenesis, and biocompatibility both *in vitro* and *in vivo*.¹⁰⁶ Additionally, an electrospun nanofibrous GBR membrane by Ghavimi et al, incorporating curcumin and aspirin, showed promising antibacterial and osteogenic activity, enhancing bone regeneration in dog jawbone defects more effectively than commercial membranes (Fig. 2).¹⁰⁷

Acemannan, a polysaccharide from *Aloe vera*, plays a critical role in bone regeneration by stimulating osteoblast differentiation, modulating immune responses, and promoting angiogenesis. Coated nanoparticles containing acemannan enhanced ALP activity and calcium deposition while maintaining high cell viability and fostering an immune profile conducive to healing by promoting M2 macrophage polarization.^{108,109} Clinically, acemannan has shown promise in sinus lift procedures and vital pulp therapy, improving alveolar bone height and supporting continued root formation.¹⁰⁸⁻¹¹¹ It promotes BMSC proliferation, boosts VEGF and BMP-2 levels, and enhances expression of osteopontin and bone sialoprotein, crucial for ECM mineralization.¹¹⁰ In a recent study by Thant et al, a collagen-acemannan (AceCol) membrane scaffold demonstrated superior biophysical and biological properties compared to native collagen. The scaffold enhanced cell proliferation, dentin matrix protein expression (including BSP, OPN, and DSPP), and mineralization, making it a promising tool for dental pulp and bone tissue regeneration.¹¹¹

Collagen sponges

Collagen sponge is widely recognized as a highly efficient

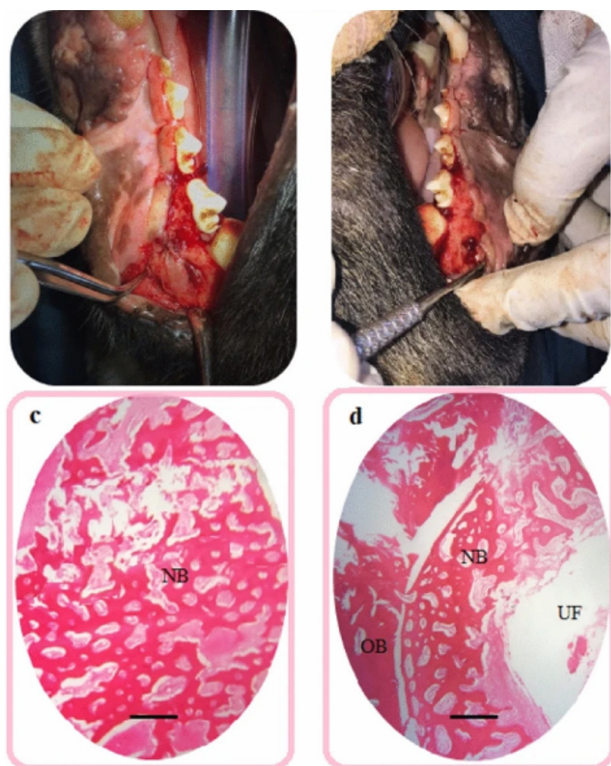


Fig. 2. In histological evaluations of bone regeneration, notable differences were observed between the test and control regions. The area treated with the engineered asymmetric membrane was fully infiltrated with newly formed bone (a), whereas the control region, which was covered using a commercially available membrane, remained devoid of bone tissue (b). Detailed histological sections further illustrated this contrast: the test region (c) displayed substantial new bone (NB) presence adjacent to old bone (OB), while the control region (d) showed large unfilled areas (UF) with limited or no bone regeneration. These sections were visualized at 40× magnification, with a scale bar of 500 μm. Reproduced from Ghavimi et al¹⁰⁷ under the Creative Commons Attribution-NonCommercial 3.0 Unported License (CC BY-NC 3.0; <https://creativecommons.org/licenses/by-nc/3.0/>).

material because of its excellent functionality and specifications, alongside its simple processing, sterilization, and preservation capabilities.¹¹² These biomaterials are typically created through a freeze-drying technique.¹¹³ The customization of collagen sponge's pore size and shape can be achieved by modifying the freezing conditions, including the freezing temperature, duration, and molds utilized. To achieve the most effective tissue regeneration, the pores must have a size that allows for cell migration and nutrient diffusion, while also promoting cell attachment.¹¹⁴ In various clinical scenarios, collagen sponge-based devices that have been approved by the FDA are primarily utilized as absorbable hemostatic. In the field of bone tissue engineering, collagen sponges serve as fundamental scaffolds for delivering bioactive molecules such as growth factors, cells, and chemicals. In a recent study, the use of a collagen sponge, in combination with BMP-2 and a bridge protein, resulted in enhanced bone regeneration.¹¹⁵ Furthermore, collagen sponges hold significant significance in the realm of *in vitro* systems. To replicate the intricate hierarchical setting of the natural ECM, the utilization of experimental design tools and optimized freeze-casting systems presents captivating prospects for customizing the

architectural structure of collagen sponge scaffolds created through ice-templating.¹¹⁶

Dayi et al conducted a comparative study to evaluate the effectiveness of two scaffolding formulations, a collagen Bio-aggregate mixture (CBA-M) and a collagen Bio-aggregate composite sponge (CBA-C), in promoting reparative dentin formation. The CBA-C sponge, prepared at a 10:1 weight ratio, was structurally characterized via scanning electron microscopy (SEM) and mercury porosimetry. Cytotoxicity testing using the L929 mouse fibroblast line confirmed biocompatibility. DPSCs were harvested from sheep teeth and validated for mesenchymal stem cell identity through CD44 marker expression by flow cytometry. These cells demonstrated osteogenic differentiation potential as confirmed by Alizarin Red staining. Subsequently, DPSCs were seeded onto CBA-C sponges to assess cell proliferation and calcium phosphate deposition. *In vivo* testing involved applying both CBA-M and CBA-C scaffolds to perforated sheep teeth and monitoring regenerative outcomes over eight weeks. The CBA-C sponge group exhibited early reparative dentin formation by the second week, culminating in a well-developed dentin structure by the eighth week. In contrast, the CBA-M group showed necrotic tissue remnants. These findings indicate that the CBA-C sponge offers a more favorable microenvironment, supporting DPSC survival and facilitating osteogenic differentiation and mineral deposition.¹¹⁷

Collagen hydrogels

Naturally sourced collagen hydrogels exhibit superior biocompatibility and biological functionality in comparison to synthetic polymers or hybrid polymers. In a similar vein, their low stiffness and rapid degradation pose obstacles for the *in vivo* implementation of these scaffolds.¹¹⁸ To address these limitations, a potential solution involves leveraging the inherent self-assembly capability of collagen hydrogels.¹¹⁹ An alternative effective approach involves creating composite hydrogels by combining collagen hydrogels with synthetic elements, such as functionalized polymers, organic/inorganic nanoparticles, and ions.¹²⁰ In the rabbit model, the hydrogel collagen nanocomposite, when combined with strontium and seeded MSCs, exhibited superior radiographical and histological scores in comparison to both the collagen hydrogel alone and the control groups. This outcome highlights the effectiveness of the hydrogel collagen nanocomposite in promoting full-thickness bone defect regeneration.¹²¹ The utilization of nanoparticles to enhance the mechanical characteristics of hydrogel has demonstrated an augmentation in stem cell adhesion, thereby offering significant insights for the development of hydrogel-shaped materials.¹²² In the current stage of science, the utilization of novel composite collagen hydrogels is extensive in various applications such as injectable hydrogel scaffolds for repairing bone tissue in its original location, flexible drug delivery systems in the form of nanogels or microgels, and implanted scaffolds for bone tissue created through 3D-printing, electrospinning, or alternative methodologies.¹²³

Coyac et al explored unresolved issues in biomineralization, particularly regarding the differentiation of bone and dental cells, the role of extracellular matrix signaling, and the relationship between protein expression and mineral formation. To investigate these areas, they employed a dense, bioengineered three-dimensional collagen hydrogel as a culture scaffold. This hydrogel, designed to mimic the extracellular matrix, was mechanically compressed to increase fibrillar density. Dental pulp SHEDs were seeded into the scaffold and cultured for 24 days. Throughout the culture period, assessments of cell viability, morphology, metabolic activity, and scaffold mineralization were conducted. Osteo/odontogenic differentiation within the dense scaffolds was confirmed by elevated alkaline phosphatase (ALPL) activity and expression of key marker proteins such as dentin matrix protein 1 (DMP1) and osteopontin (OPN), as determined by immunoblotting. Advanced characterization of the mineralized matrix—using electron microscopy, diffraction, energy-dispersive x-ray spectroscopy, and Fourier-transform infrared spectroscopy, verified the formation of apatitic mineral, frequently aligned with collagen fibers. Overall, the study demonstrated that the dense collagen 3D scaffold effectively supported both mineralization and differentiation of SHEDs into osteo/odontogenic lineages.¹²⁴

Rutin, a flavonoid with antioxidant and anti-inflammatory properties, supports osteoblast survival and differentiation. It downregulates p53, facilitates ECM formation via caspase-3 and p65 phosphorylation, and enhances Runx2 expression and ALP activity—key indicators of osteogenesis.^{125, 126} Rutin also reduces reactive oxygen species (ROS) and increases antioxidant enzymes like superoxide dismutase (SOD) and glutathione (GSH), mainly through PI3K/AKT activation. Beyond osteogenesis, rutin alleviates osteoarthritis progression by modulating the CBS-mediated RhoA/ROCK pathway, decreasing inflammatory markers, and increasing cartilage-specific proteins.¹²⁶ *In vivo*, rutin gel combined with allografts accelerated bone healing in rabbit models by inhibiting MMP expression and enhancing collagen type III deposition, suggesting it as a cost-effective alternative to expensive growth factors in dental bone grafts (Fig. 3).¹²⁵ Furthermore, rutin significantly improved PDLSC sheet formation, resulting in thicker, more ECM-rich sheets with enhanced expression of COL1, ALP, RUNX2, and osteopontin, pointing to its potential in periodontal regeneration.¹²⁶

Collagen-based composites

Despite the presence of various biological cues within collagen type I fibers that have the potential to directly influence cell adhesion, proliferation, and differentiation, it is important to note that collagen alone does not possess osteoinductive properties. Nonetheless, the incorporation of collagen into bone substitute materials can significantly enhance their biocompatibility and osteoconductivity, thereby improving their overall performance. In order to attain successful bone regeneration and adequate tissue vascularization, collagen-containing scaffolds can be altered

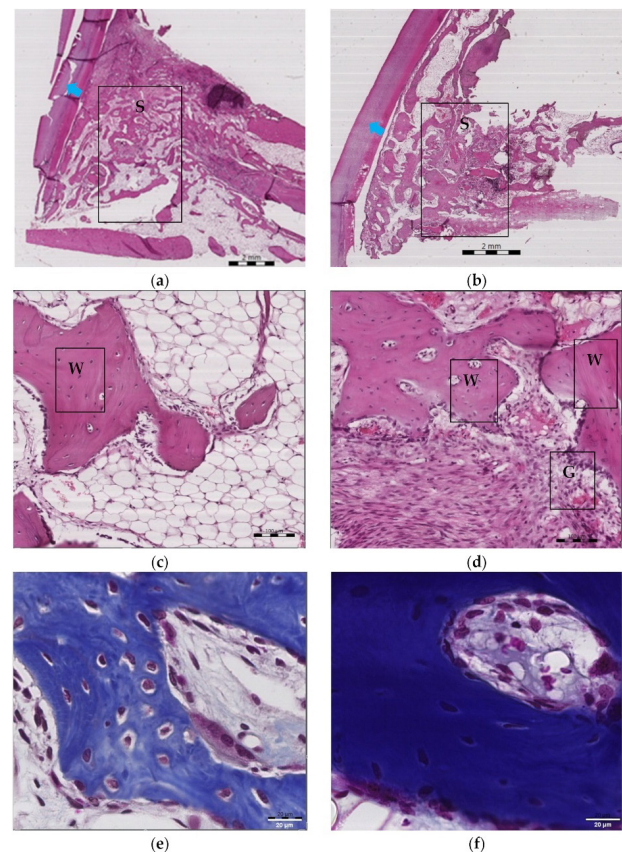


Fig. 3. The study assessed the effects of rutin gel on bone regeneration, vascularization, bone marrow development, and collagen fiber deposition at the grafted site using Hematoxylin–Eosin and Masson's trichrome staining on jawbone samples from control (a, c, e) and rutin-treated (b, d, f) groups. Bone formation and marrow content were analyzed in the lower first premolar socket (S), with the lower second premolar tooth indicated by a blue arrow in the control (a) and rutin-treated (b) samples (magnification 40×, scale bar = 2 mm). Additionally, bone formation (W) and angiogenesis (G) were examined at the blood cell collection site in the control (c) and rutin-treated (d) samples (magnification 100×, scale bar = 100 μm). Collagen fibers were visualized in the control (e) and rutin-treated (f) samples (magnification 400×, scale bar = 20 μm). Reprinted from Albaqami et al,¹²⁵ distributed under the terms and conditions of the Creative Commons Attribution 4.0 International License (CC BY 4.0).

to incorporate additional bioactive molecules, including growth factors, cells, and drugs. The FDA approved the initial collagen bone graft that was loaded with recombinant human bone morphogenetic protein-2 (rhBMP-2) in 2002.¹²⁷ Despite a promising start, concerns have been raised regarding the possible toxicity and expense of BMPs. One potential solution could involve optimizing the delivery system of BMPs in order to reduce the requirement for high doses and avoid unnecessary implementations.¹²⁸ Furthermore, aside from enhancing the loading efficiency through these alterations, alternative endeavors have been undertaken to impede the BMP antagonists and employ a blend of various growth factors.^{129,130} Furthermore, the incorporation of drugs into collagen materials offers the potential for antibacterial, osteogenesis, and angiogenesis effects.

A study conducted by Nabavi et al established that the utilization of collagen combined with tacrolimus, a substance known to activate BMP receptors and promote

osteogenic differentiation, resulted in enhanced bone formation when compared to groups without tacrolimus.¹³⁰ Due to the interactions between the RGD sequence and integrin receptors found on stem cells, collagen-based materials can promote the adhesion, proliferation, and differentiation of these cells. This characteristic makes collagen-containing materials an optimal platform for the delivery of stem cells. Recent research has documented several novel approaches to enhancing the properties of materials through surface modification and stiffness adjustment using nanomaterials. These techniques aim to finely tune the mobility of tethers and achieve an anisotropic nanoscale display of RGD.¹²³ The reconstructed defects exhibited a greater degree of calcification when a collagen scaffold was utilized in conjunction with DPSCs.¹³¹ Nevertheless, an alternative investigation revealed that the acellular scaffolds, as confirmed by *in vivo* micro-CT analysis, exhibited greater bone volumes compared to the scaffolds seeded with DPSCs.¹³² Furthermore, there are ongoing endeavors to develop collagen-based composites that specifically emphasize the amalgamation of collagen with peptide delivery and gene therapy.^{133,134} These investigations continue to be a subject of debate and have not been thoroughly investigated. Another area of potential is the combined impact of various bioactive cations on the process of vascularization and the mending of bone defects, offering a significantly safer and more straightforward alternative.¹³⁵

Gelatin

Gelatin is a partially hydrolyzed collagen biopolymer containing peptide sequences that promote cell adhesion and proliferation. It is a naturally derived, water-soluble protein obtained through the hydrolysis of collagen, wherein the triple-helix structure is disrupted to yield single-chain macromolecules. Owing to the presence of both acidic and basic amino acid residues, gelatin exhibits amphoteric behavior. For decades, mammalian-derived gelatin has served as a widely used biomaterial in regenerative medicine due to its excellent biocompatibility and biodegradability.¹³⁶

Gelatin porous scaffolds

Porous scaffolds based on gelatin have garnered significant attention in bone tissue engineering due to their biodegradability, biocompatibility, and high water absorption capacity. Their three-dimensional porous architecture not only provides mechanical support but also facilitates cell infiltration, nutrient transport, and drug delivery. Incorporation of therapeutic agents such as bisphosphonates within these scaffolds allows for sustained release, potentially reducing the adverse effects associated with systemic administration and enhancing local bone regeneration.¹³⁷⁻¹³⁹ Panzavolta et al investigated glutaraldehyde cross-linked gelatin scaffolds as localized delivery platforms for calcium alendronate monohydrate (CaAL: H₂O), a bisphosphonate (BP) often used to treat bone loss disorders. These highly porous 3D scaffolds exhibited an interconnected porosity exceeding 94%,

regardless of the BP loading (0%, 3%, or 6% by weight), although the pore size decreased with higher drug content. This densification coincided with improvements in compressive modulus and collapse strength. Sustained drug release was observed over 14 days, with significant retention of CaAL·H₂O. Co-culture experiments with osteoblasts and osteoclasts revealed that the drug-laden scaffolds promoted osteoblast activity and suppressed osteoclast function, indicating their potential to support bone healing while mitigating the adverse effects of systemic BP therapy.¹⁴⁰

Gelatin nanofibrous scaffolds

Gelatin-based nanofiber scaffolds, especially those fabricated via electrospinning, closely mimic the native ECM, providing a conducive environment for cellular attachment, proliferation, and differentiation. The incorporation of antibacterial agents or bioactive herbal compounds into these nanofibers not only helps in combating infection but also promotes osteogenic activity. This dual-functional approach is particularly beneficial for treating bone-related infections such as osteomyelitis while simultaneously supporting bone tissue regeneration.^{138,141} Yekani et al developed gelatin-based nanofiber scaffolds integrated with curcumin and vancomycin to simultaneously treat osteomyelitis and support bone regeneration. Fabricated through electrospinning, these nanocomposites demonstrated favorable mechanical integrity, drug-loading efficiency, and sustained release behavior. The curcumin/vancomycin combination significantly enhanced antibacterial and antibiofilm effects against *Staphylococcus aureus*, including methicillin-resistant strains. *In vitro* assays confirmed excellent biocompatibility and increased proliferation and adhesion of mesenchymal stem cells (MSCs), with curcumin contributing significantly to antimicrobial activity and cellular responses. These multifunctional scaffolds hold promise for treating infected bone defects.¹⁴¹

Abadi et al focused on designing a gelatin-based electrospun scaffold enriched with calcium phosphate and *Punica granatum* (pomegranate) extract to promote early osteogenic differentiation. Human dental pulp stem cells (HDPSCs) and SHEDs were used to assess biocompatibility and osteoinductive effects. Structural evaluation confirmed the formation of bead-free, uniform nanofibers. Spectroscopy analyses validated the successful incorporation of scaffold components without undesirable interactions. Both cell lines exhibited significantly increased viability and ALP activity when cultured with the nanocomposite, supporting its suitability for early-stage bone and dental tissue regeneration.¹⁴²

Sharifi et al explored gelatin-hydroxyapatite nanofibers fabricated via electrospinning for use in guided tissue regeneration (GTR). These scaffolds had a consistent nanoscale diameter (~102 nm), a negative surface charge, and a well-formed fiber network. When cultured with human dental pulp-derived mesenchymal stem cells (hDPSCs), the scaffolds demonstrated enhanced proliferation without cytotoxicity. The authors suggested

that modifying scaffold degradation rates to match tissue healing speed, alongside polymer blending, could further optimize their function as GTR membranes.¹³⁹

Gelatin hydrogels

Gelatin-based hydrogels have emerged as promising biomaterials in regenerative medicine due to their ECM-like structure, excellent biocompatibility, and capacity to retain and release bioactive molecules. Their application in bone repair is particularly enhanced when combined with biological additives such as platelet-rich plasma (PRP) or modified gelatin derivatives like GelMA. These hydrogels can serve as effective carriers for growth factors, supporting sustained release and facilitating the osteogenic differentiation of stem cells, ultimately contributing to enhanced bone regeneration. Hokugo et al investigated a gelatin-based hydrogel system incorporating PRP to facilitate bone regeneration in rabbit ulna defect models. Prepared by combining centrifuged PRP with freeze-dried gelatin, this hydrogel allowed controlled release of growth factors such as PDGF and TGF- β 1 through physicochemical interactions with gelatin. *In vivo* studies demonstrated superior bone formation in the PRP-gelatin group compared to controls treated with PRP alone, fibrin-PRP composites, or empty hydrogels. The degradation-synchronized release of bioactive factors appears to underpin the scaffold's therapeutic efficacy.¹⁴³

Fang et al developed biomimetic gelatin methacrylamide (Bio-GelMA) hydrogels using thermally induced phase separation (TIPS) to emulate the structure and composition of native bone extracellular matrix. Adipose-derived stem cells (ADSCs) cultured in these 3D scaffolds showed marked improvements in attachment, proliferation, ALP activity, and osteogenic gene expression. Both subcutaneous and critical-size calvarial defect models in rats confirmed significant bone regeneration when Bio-GelMA was combined with ADSCs. These findings underscore the hydrogels' potential as scaffolds for bone repair through enhanced stem cell-mediated osteogenesis.¹⁴⁴

Cellulose

Cellulose is a natural linear polysaccharide composed of β -1,4-linked glucose units with high mechanical stability.¹⁴⁵ It is a polymer that exhibits hydrophilic properties, rendering it soluble in water. Additionally, it possesses chirality and is capable of undergoing biodegradation. This polymer holds great importance as a fundamental component of the primary cell wall found in various organisms such as algae, plants, cotton, and wood.¹⁴⁶ Cellulose is a biomaterial that is favored in bone tissue regenerations due to its superior mechanical features and biocompatibility. However, one drawback of cellulose is its limited bioactivity. In order to enhance the bioactivity of cellulose, numerous hypotheses have been tested. One such effort was proposed by Kim et al, who suggested the use of a scaffold composed of cellulose/calcium-deficient-HA created through the electrohydrodynamic printing process. The calcium-deficient HA was fabricated by hydrolyzing α -Tri calcium phosphate. The resulting scaffold exhibited

a high fraction of α -TCP in its fiber structure. Additionally, the presence of a higher amount of HA led to a gradual decrease in the pore size of the scaffold. This developed scaffold was found to be an effective platform for promoting cell attachment and proliferation.¹⁴⁷ Cellulose possesses an optimal blend of mechanical robustness, surface properties, and degradation tendencies, rendering it a promising candidate for the triumph of bone tissue regeneration. In their study, He et al ingeniously integrated the advantageous attributes of cellulose into their work by fabricating a scaffold composed of microfibrillated cellulose, collagen, and hydroxyapatite for bone regeneration. The resultant scaffold exhibits commendable physical attributes and exhibits notable biological activity.¹⁴⁸ Several recent studies have demonstrated the potential of various scaffolds for tissue engineering. For instance, one study utilized CMC blended with polyethylene glycol scaffold,¹⁴⁹ while another study explored the use of GO/n-HAp/bacterial cellulose/ β -glucan biocomposite scaffold.¹⁵⁰ Additionally, a cellulose/HA/Ag NPs scaffold and a PLA/cellulose nanocrystals scaffold were investigated.^{151,152} Furthermore, a carboxymethylated cellulose/chitosan scaffold also presented hopeful results.¹⁵³ These studies highlight the diverse range of scaffold materials that can be utilized in tissue engineering research.

Cellulose nanofibrous hydrogels

Injectable calcium phosphate silicate cement (CPSC) has recently gained attention for its suitability in bone repair, especially in elderly patients with age-related bone degeneration. However, the mechanical limitations of CPSC restrict its application in load-bearing scenarios. To overcome this challenge, Gong et al developed CPSC composites reinforced with different types of cellulose nanofibers (CNFs), including unmodified CNFs, carboxylated CNFs (CNF-C), and silanized CNFs (CNF-SH). Among these, CNF-SH demonstrated the greatest reinforcing effect, improving the compressive strength and Young's modulus of the cement, which approached the stiffness of natural trabecular bone. Unlike many modified cements that lose workability upon reinforcement, these composites retained good injectability. Moreover, *in vitro* tests confirmed that the CNF-modified CPSC supports osteoblast proliferation and differentiation, highlighting its potential as a mechanically robust and biologically compatible bone substitute material.¹⁵⁴

Hydrogels are attractive for bone tissue regeneration due to their injectability and tissue-mimicking properties. However, traditional hydrogels often suffer from inadequate mechanical strength. Cui et al addressed this by synthesizing a semi-interpenetrating network hydrogel based on oxidized alginate (OSA), gelatin (Gel), and CNFs. This hydrogel exhibited excellent compressive strength, rapid gelation time ($\sim$ 150 seconds), self-healing capability (92%), and superior injectability. Furthermore, it promoted preosteoblast viability, biomineralization, and osteogenic differentiation. In a complementary approach,¹⁵⁵ Maharjan et al fabricated a porous composite hydrogel using regenerated cellulose nanofibers

embedded within a chitosan matrix. The incorporation of cellulose fibers significantly improved scaffold stiffness, bioactivity, and mineral deposition. Alkaline phosphatase and alizarin red assays confirmed enhanced osteogenic differentiation. These findings suggest that cellulose nanofibers serve as multifunctional reinforcement agents that can simultaneously address structural and biological requirements of hydrogel-based bone scaffolds.¹⁵⁶

Cellulose sponges

Cellulose sponges have been employed as porous frameworks for tissue regeneration due to their high surface area and biocompatibility. Tommila et al demonstrated that coating these sponges with hydroxyapatite (HA) enhances their ability to recruit circulating stem cells, including hematopoietic and mesenchymal progenitors, likely through calcium ion release and calcium-sensing receptor activation. These HA-coated sponges promoted granulation tissue formation with rich vascularization, suggesting their utility in complex wound environments.¹⁵⁷ Conversely, Ekholm et al showed that biomimetically mineralized cellulose sponges, while initially promising, induced a strong inflammatory response and resulted in limited bone formation when implanted in cortical bone defects in rats. These sponges bound more TGF- β 1, potentially contributing to fibrosis rather than ossification. Thus, although cellulose-based sponges show promise in guiding cellular behavior, their performance heavily depends on surface chemistry and implantation context.¹⁵⁸

Hyaluronic acid hydrogels

HA is a linear anionic polysaccharide composed of repeating disaccharide units of glucuronic acid and N-acetylglucosamine. HA has garnered significant attention in regenerative medicine due to its excellent biocompatibility, biodegradability, and ability to modulate cellular behavior.¹⁵⁹ In the context of dental and bone tissue engineering, HA and its derivatives have been used for fabricating dual-function odonto-osteogenic scaffolds biomaterials designed to simultaneously support dentin and bone regeneration. HA plays a critical role in ECM organization, cell proliferation, and differentiation. Its hydrophilic nature promotes water retention, creating a favorable microenvironment for stem cells and progenitor cells. Additionally, HA interacts with cell surface receptors (e.g., CD44 and RHAMM) to regulate osteogenesis and odontogenesis.¹⁶⁰ HA hydrogels are widely used for injectable and 3D-printed scaffolds due to their tunable mechanical properties and ability to encapsulate cells and bioactive molecules. Recent studies have shown that HA hydrogel composites with gelatin, fibrin, or nanohydroxyapatite significantly enhance dentin and bone regeneration. To improve mechanical strength and biological functionality, HA is often combined with bioactive ceramics such as calcium phosphate, hydroxyapatite, and bioactive glass. These composites provide both osteoconductive and odontoconductive properties, essential for dual-tissue regeneration.^{85,161} A study by Niloy et al introduced HA-based hydrogels

modified with 2-aminoethyl methacrylate (AEMA), enabling fine-tuning of their swelling, degradation, and mechanical properties. These hydrogels, synthesized using two different molecular weights of HA (18 and 270 kDa), were found to be non-cytotoxic to human DPSCs and supported the maintenance of their native spherical morphology and stemness. Particularly, when the hydrogel's storage modulus ranged between 486 and 681 Pa, DPSCs retained high expression levels of stemness markers such as NANOG and SOX2, in contrast to cells grown on conventional 2D substrates. These results suggest that AEMA-modified HA hydrogels can effectively mimic the 3D *in vivo* microenvironment, offering significant potential for dental tissue regeneration.¹⁶¹

Alginate hydrogels

Alginate is an anionic polysaccharide composed of β -D-mannuronic acid and α -L-guluronic acid residues. It has gained significant attention in tissue engineering and regenerative medicine due to its biocompatibility, biodegradability, and ability to form hydrogels under mild conditions.^{162,163} In dental and bone tissue engineering, alginate and its derivatives are widely used for fabricating dual-function odonto-osteogenic scaffolds, which are designed to support both dentin and bone regeneration. Alginate is composed of mannuronic acid (M) and guluronic acid (G) units, which contribute to its gelation properties in the presence of divalent cations like Ca^{2+} . These characteristics make alginate an ideal biomaterial for scaffold fabrication, cell encapsulation, and drug delivery in regenerative medicine.

Alginate is often chemically modified to enhance its biological activity and mechanical strength.^{162,163} Common modifications include: Marwa et al Dentin–pulp complex is a term that refers to the dental pulp (DP) surrounded by dentin along its peripheries.⁸¹ Dental pulp and dentin are highly specialized structures susceptible to damage from various pathological insults, chief among which is dental caries.^{81,82} Reconstituting the dentin–pulp complex remains essential for restoring and maintaining dental vitality. To achieve this, regenerative endodontic procedures (REPs) have emerged as advanced therapeutic strategies designed to rebuild the functional dentin–pulp complex by driving the differentiation of either native or transplanted stem and progenitor cells.

Alginate-based hydrogels have emerged as promising candidates for bone tissue engineering due to their injectability, biocompatibility, and tunable degradation properties. Priddy et al investigated the use of oxidized, irradiated RGD-alginate hydrogels for the controlled delivery of bone morphogenetic protein-2 (BMP-2) in a critical-sized femoral defect model. Oxidation was employed to enhance hydrogel degradation, thereby facilitating more efficient integration of the regenerated bone with the host tissue. The oxidized-irradiated alginate hydrogels demonstrated accelerated BMP-2 release *in vitro* and supported osteogenic activity, as confirmed by ALP assays and Alizarin Red S staining. *In vivo*, both

standard and oxidized hydrogels promoted substantial bone regeneration, but the oxidized variant showed significantly greater bone mineral density at 8 weeks and more mature bone architecture by 12 weeks, as assessed via imaging, histology, and biomechanical testing. These findings underscore the potential of fine-tuning alginate degradation to optimize bone healing outcomes.¹⁶⁴

In another study, Barralet et al examined how the architectural design of alginate hydrogels influences cell behavior. Using calcium cross-linked sodium alginate in both disc and tubular forms, they evaluated the proliferation and differentiation of rat bone marrow cells (BMCs). While increased alginate concentration suppressed proliferation in flat disc constructs, tubular hydrogels with the same composition supported robust cell growth. Gel thickness was identified as a critical parameter affecting cell proliferation, though it did not significantly alter differentiation profiles. These results highlight the importance of geometric and structural considerations in the design of alginate-based scaffolds for bone regeneration.¹⁶⁵

Silk scaffolds

Silk fibroin is a protein-based biopolymer with β -sheet structures that provide excellent mechanical strength and biocompatibility. Silk, particularly silk fibroin (SF) derived from the cocoons of *Bombyx mori* silkworms, has gained increasing attention in tissue engineering and regenerative medicine due to its biocompatibility, tunable biodegradability, and excellent mechanical properties. In

dental and bone tissue engineering, silk-based biomaterials can be used for fabricating dual-function odonto-osteogenic scaffolds. SF is composed of hydrophobic β -sheet crystalline domains and hydrophilic amorphous regions, providing it with high mechanical strength, flexibility, and biodegradability.⁴⁷ In a recent *in vitro* study, López-García et al evaluated silk fibroin-based scaffolds, both alone and in combination with graphene oxide (GO) or reduced graphene oxide (rGO), for their potential use in regenerative endodontics. The fabricated scaffolds exhibited favorable porosity, structural integrity, and water absorption characteristics. Notably, scaffolds containing GO or rGO enhanced the expression of key odontogenic and osteogenic markers such as ALP, osteonectin (ON), and dentin sialophosphoprotein (DSPP) in human dental pulp stem cells (hDPSCs), and significantly promoted extracellular matrix mineralization over 21 days (Fig. 4). These findings suggest that SF/GO and SF/rGO composites provide a bioactive environment conducive to dual odontogenic and osteogenic differentiation, though further *in vivo* validation remains necessary.⁴⁷ The comparative summary of naturally derived scaffolds used in odonto-osteogenic regeneration is presented in Table 1.

Phytochemical functionalization of natural scaffolds

One interesting method to improve the biological performance of biomaterials used in odonto-osteogenic regeneration is the functionalization of natural polymeric scaffolds with phytochemicals. Polyphenols, flavonoids, and bioactive compounds derived from plants are examples

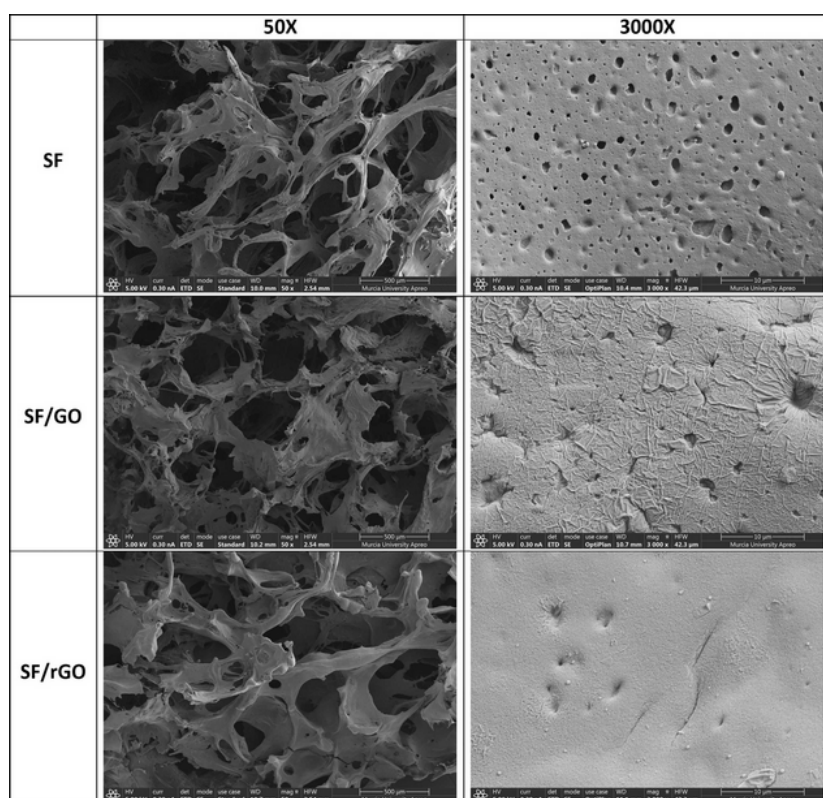


Fig. 4. Representative field emission scanning electron microscopy images to characterize the porous structure of the different scaffolds' microarchitecture. Magnifications: 50x (first column), 300x (second column). Reproduced from López-García et al⁴⁷ under the Creative Commons Attribution 4.0 International License (CC BY 4.0; <https://creativecommons.org/licenses/by/4.0/>).

Table 1. Comparative summary of naturally derived scaffolds used in odonto-osteogenic regeneration

Natural polymer	Phytochemical	Fabrication method	Key properties	Mechanical properties	In vitro findings	In vivo findings	Clinical evidence	Limitations	Advantage	References
Chitosan	Curcumin	Electrospinning, freeze-drying	Cationic polysaccharide, bioadhesive, modifiable, 3D-printable	Moderate compressive strength; improved when crosslinked	↑ ALP, RUNX2, OCN; enhanced proliferation	Improved bone closure in rat calvarial defects	Preclinical	Low mechanical strength; rapid degradation	High protein adsorption, promotes osteoblast attachment, supports mineralization	107, 166, 167
Collagen	Icariin	3D bioprinting, lyophilization	ECM component, RGD motifs, highly biocompatible	Biomimetic; weak unless reinforced	↑ DSPP; odontoblastic differentiation	Improved dentin bridge formation	Used in GTR (limited data)	Fast degradation; variability	Enhances osteoconductivity, supports DPSC adhesion/differentiation, GF delivery (BMP-2)	168, 169
Gelatin	Rutin	Electrospinning, hydrogel formation	Denatured collagen, forms nanofibers/hydrogels, modifiable as GelMA	Tunable elasticity; weak without crosslinkers	↑ mineralized nodules; improved adhesion	Accelerated bone formation (animal models)	No robust trials	Rapid enzymatic degradation	Excellent biocompatibility, controlled GF release, enhances osteogenesis	170-172
Cellulose	Quercetin / Plant Polyphenols	Nanocrystal assembly, electrospinning	Strong, hydrophilic, nanofiber-forming	Low compressive modulus	Anti-inflammatory; ↑ osteoblast proliferation	Enhanced early bone regeneration	No confirmed clinical data	Poor cell adhesion	Reinforces composites, improves stiffness and osteogenic response	173-176
Hyaluronic Acid	Curcumin / Resveratrol	Chemical crosslinking, hydrogel formation	Hydrophilic GAG, injectable, ECM-mimicking	High tensile strength; stable structure	Improved osteoblast adhesion	Sustained bone ingrowth	Limited dental use	Slow biodegradation	Maintains DPSC stemness, tunable mechanics, promotes dentin/bone regeneration	177-179
Alginate	Propolis extract	Ionic crosslinking, injectable hydrogels	Ionic gelation, biocompatible, 3D-printable	Viscoelastic; ECM-like	↑ MSC migration; odontogenic signaling	Improved angiogenesis and mineralization	Adjunct in periodontal therapy	Rapid enzymatic degradation	Supports odontogenic/osteogenic differentiation; tunable degradation	180-183
Silk Fibroin	EGCG	Solvent casting, salt leaching, 3D printing	β-sheet protein, high tensile strength, slow degradation	High toughness; load-bearing capacity	Enhanced hydroxyapatite nucleation	Significant bone formation in cranial defects	Clinical history as sutures	Hydrophobic surface modification needed	Enhances osteogenic/odontogenic markers; good porosity	184, 185

Abbreviations: ALP, alkaline phosphatase; RUNX2, Runt-related transcription factor 2; OCN, osteocalcin; DSPP, dentin sialophosphoprotein; MSC, mesenchymal stem cell; GTR, guided tissue regeneration; EGCG, Epigallocatechin gallate.

of phytochemicals that have inherent antioxidant, anti-inflammatory, and osteoinductive qualities that can work in concert to enhance scaffold-mediated tissue repair.¹⁷³ Natural scaffolds like chitosan, collagen, gelatin, alginate, cellulose, and silk fibroin create an environment that helps cells attach and grow. However, they often lack sufficient bioactivity for bone formation. Adding phytochemicals can enhance the ability of these scaffolds to support bone and tooth formation. For example, curcumin boosts bone cell development by increasing specific markers, while icariin and resveratrol support bone cell activity and blood vessel growth.¹⁶⁶ Several methods, like electrospinning and freeze-drying, are used to integrate phytochemicals

into scaffolds, allowing for controlled release of these beneficial compounds. Research shows that these enhanced scaffolds can improve bone and dentin healing significantly compared to unmodified ones. Still, more studies are needed to standardize fabrication methods and ensure long-term safety in clinical applications.¹⁷³

Scaffold-like materials

Calcium silicate

Although calcium silicate is not a traditional polymeric scaffold, it is often used in a scaffold-like manner due to its inherent bioactivity, osteoconductivity, and compatibility with tissue regeneration processes.^{186,187} When fabricated

into porous or composite forms, calcium silicate-based materials can support cell adhesion and differentiation, effectively functioning as a structural platform in both bone and dental tissue engineering.¹⁸⁸ In addition to calcium phosphates and silicates, calcium silicates exhibit great potential as biocompatible ceramic resources that offer a conducive niche for the regeneration of bone tissue. Silicate ions, along with Ca^{2+} ions, play a decisive role in the process of bone regeneration by effectively regulating the MEK and PKC pathways.¹⁸⁷ Zhou et al¹⁸⁹ conducted a recent study that revealed that calcium silicate exhibits a considerably higher osteoinductive potential in both laboratory settings (*in vitro*) and living organisms (*in vivo*) when compared to the conventional β -tricalcium phosphate bioceramics commonly used in clinical practice (Fig. 5).

Moreover, the investigation of ionic substitutions extends beyond calcium phosphates alone. In recent times, there has been a growing focus on substituted calcium silicates, particularly those involving Sr^{2+} ions.¹⁹⁰ Efforts have been focused on the acquisition of scaffolds composed of chitosan and calcium silicates, owing to their beneficial impact on bone regeneration. In their study, Peng et al successfully created a mesoporous calcium silicate/chitosan scaffold doped with lanthanum¹⁸⁷ and gadolinium.¹⁸⁶ This scaffold demonstrated the ability to facilitate the adhesion, proliferation, and differentiation of MSCs, thereby promoting their growth and development. Lin et al¹⁹¹ improved the characteristics of calcium silicate by developing composite scaffolds using chitosan, thereby ensuring the antimicrobial features of the scaffolds. Early studies in 1994 have documented the enhanced performance of osteoblasts on titanium implant surfaces coated with a nanocomposite comprising apatite, wollastonite (CaSiO_3), and chitosan. The researchers observed a notable improvement in osteocalcin expression and mineralization, indicating the biocompatibility of

the composite coating and its potential to facilitate early osseointegration.¹⁹² Additionally, it has been verified that the incorporation of calcium silicate at a concentration of 40% w/w in gellan-chitosan scaffolds stimulates the differentiation of MSCs into the osteogenic lineage.¹⁹³ This was evidenced by the significant accumulation of minerals and the upregulation of osteogenic genes.

Blood clots and platelet-rich plasma

A blood clot is a natural fibrin-based matrix formed during injury, acting as a temporary scaffold. It contains platelets, fibrin, and cytokines that support tissue repair through angiogenesis, inflammation modulation, and cell migration.¹⁹⁴ In procedures such as tooth extraction, bone grafting, or implant placement, the blood clot initiates the healing process and complements other regenerative materials like scaffolds or PRP.⁶²

While a blood clot does not represent a conventional scaffold, it can temporarily act as a biological matrix in the early stages of tissue repair.¹⁹⁵ Its fibrin-rich, three-dimensional structure facilitates cellular migration, nutrient diffusion, and local release of growth factors, mimicking scaffold-like properties that support initial tissue regeneration, particularly in dental wound healing. PRP and platelet-rich fibrin (PRF) are also not engineered scaffolds in the classical sense, but their fibrin matrix and high concentration of bioactive molecules allow them to function as scaffold-like systems.¹⁹⁶ They create a supportive environment that enhances cellular recruitment, angiogenesis, and tissue regeneration, often being used as biologically active carriers or fillers in regenerative procedures.¹⁹⁷

PRP is an autologous concentrate derived from the patient's blood, rich in growth factors such as PDGF, vascular endothelial growth factor (VEGF), and TGF- β . PRP enhances angiogenesis, promotes soft and hard

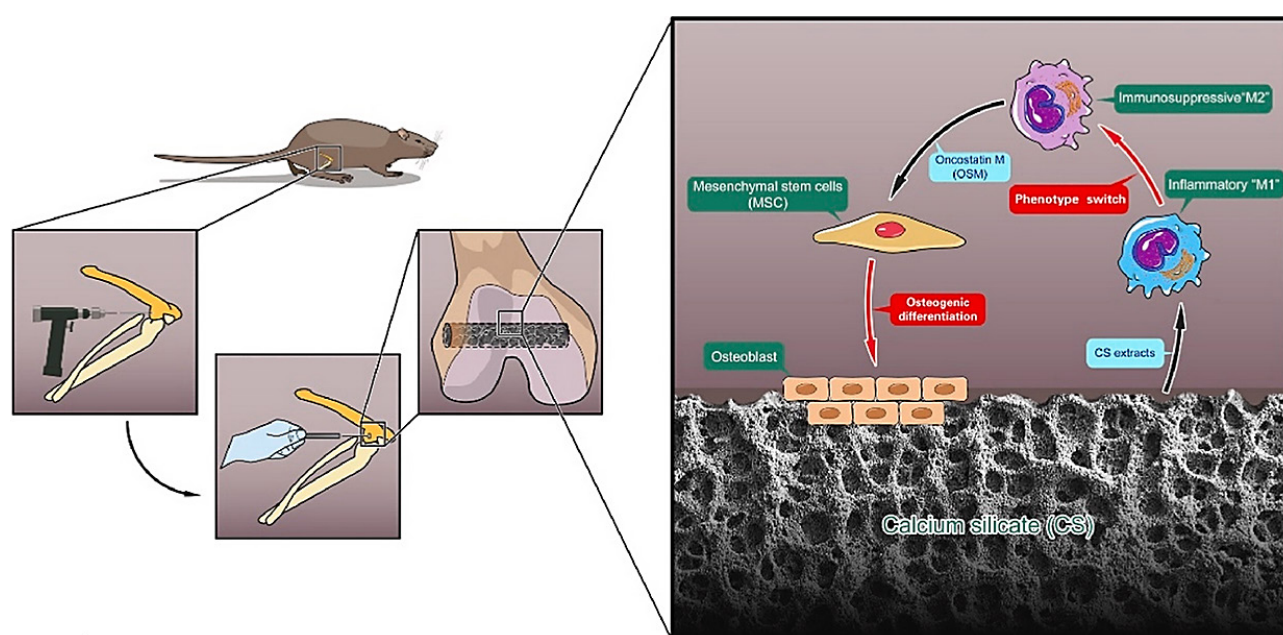


Fig. 5. Calcium silicate exhibits a higher osteoinductive potential *in vitro* and *in vivo* compared with β -tricalcium phosphate bioceramics. Reproduced from Zhou et al¹⁸⁹ under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (CC BY-NC-ND 4.0).

tissue healing, and accelerates cell differentiation. When combined with scaffolds, PRP ensures rapid and efficient tissue regeneration.

Blood clots and PRP have garnered significant attention in regenerative dentistry and medicine for their ability to act as natural scaffolds. These biological materials, without the need for synthetic or engineered scaffolds, create a conducive environment for tissue repair and regeneration through their intrinsic structural and biochemical properties.¹⁹⁸

Blood clots naturally form at injury sites, creating a matrix that facilitates angiogenesis by releasing VEGF from platelets.¹⁹⁹ Acts as a reservoir for growth factors and cytokines, supporting osteogenesis and odontogenesis. Provides a temporary framework for extracellular matrix deposition and cellular infiltration. In tooth extraction sites or alveolar bone defects, blood clots have been shown to support bone and soft tissue regeneration effectively. Studies suggest that preserving the clot in the socket enhances natural bone healing.¹⁹⁹

PRP is not only a reservoir for bioactive molecules but also forms a fibrin-based gel upon activation. This gel mimics a scaffold by providing structural support for cell attachment. Sustaining the localized release of growth factors such as PDGF, TGF- β , and VEGF.^{195-197,199} Enhancing cell-cell communication through the fibrin matrix. Compared to traditional scaffolds, PRP-based scaffolds are biocompatible and fully autologous, reducing immunogenicity. Dynamic, as they degrade naturally while promoting tissue regeneration. Rich in growth factors, enhancing osteogenesis and odontogenesis without the need for additional biomaterials.^{195,197} PRP has been successfully used as a standalone scaffold in procedures like periodontal regeneration. Bone healing after cyst enucleation. Socket preservation and ridge augmentation. The integration of scaffolds, blood clots, and PRP in odonto-osteogenic regeneration creates a synergistic effect: Blood clot provides the initial matrix for cell migration. PRP supplies bioactive molecules for enhanced healing. Scaffolds maintain structural integrity and sustain cellular activity.^{195,197,198} Table 2 shows a comparison between blood clot and PRP for use in odonto-osteogenic regeneration.

Saeed et al reported the successful use of a combination of PRF and induced blood clot as scaffold-like materials for pulp revascularization in three cases involving immature, necrotic permanent teeth with incomplete root formation. Over an 18-month follow-up, all treated teeth remained asymptomatic, and cone beam computed tomography (CBCT) demonstrated evidence of continued root development, thickening of dentinal walls, and healing of

periapical lesions. These findings suggest that PRF, when combined with an induced blood clot, can function as an effective biological scaffold-like matrix in endodontic regeneration, supporting both odontogenic differentiation and hard tissue.¹⁹⁴

Clinical translation and current challenges

Although natural polymer-based nano-scaffolds have shown encouraging regeneration results in odonto-osteogenic applications, their effective clinical translation is still a challenge. Despite the exceptional biocompatibility and bioactivity of biopolymers such as chitosan, collagen, gelatin, cellulose, hyaluronic acid, alginate, and silk fibroin, a number of biological, industrial, and regulatory obstacles still prevent their broad clinical use.⁵⁰

As summarized in Table 3 multiple scientific and translational barriers need to be overcome for natural polymer-based nano-scaffolds to be used in clinical practice. A key issue is the mismatch between lab results and real-world performance, where factors like enzyme breakdown, immune responses, and physical stress can impact scaffold performance.²⁰¹ Biosafety is crucial, especially with the use of phytochemicals or nano-carriers, as harmful byproducts may arise. The challenge of producing natural polymers consistently at scale also exists. Many natural polymers lack mechanical strength, limiting their use in weight-bearing applications unless improved with composites.²⁰² Lastly, managing the release of bioactive compounds is important, as quick releases can lessen their effectiveness. Addressing these issues through better scaffold design, standardized production, and thorough testing is vital for using these materials in dental and bone healing.²⁰³

Conclusion

Both naturally derived scaffolds and those functionalized with phytochemicals demonstrate significant potential in supporting odontogenic and osteogenic differentiation. Natural materials, especially in the form of nanofibers, owing to their ECM-mimicking properties, offer an ideal microenvironment for tissue regeneration, while phytochemical integration introduces additional therapeutic functionalities. However, each approach has limitations, such as the mechanical weakness of some natural polymers or the instability and dosage sensitivity of phytochemical agents. A balanced scaffold design that leverages the biological benefits of natural materials and the functional enhancement of phytochemicals may present a promising route toward multifunctional constructs in regenerative dentistry and orthopedics.

Table 2. A comparison between blood clot and PRP for use in odontogenic and osteogenic regeneration

Feature	Blood clot	Platelet-rich plasma (PRP)
Source	Naturally forms at injury sites ¹⁹⁴	Autologous blood, processed via centrifugation ⁶²
Growth factor release	Moderate, from endogenous platelets ²⁰⁰	Enhanced and concentrated growth factors ¹⁹⁷
Structural support	Fibrin matrix with variable density ¹⁹⁴	Dense fibrin gel with controlled release ¹⁹⁶
Clinical use	Simple, cost-effective ¹⁹⁷	Requires preparation but highly effective ^{49,197}

Table 3. Major challenges in clinical translation of natural and phytochemical-based nano-scaffolds

Challenge	Description	Examples in natural polymer-based nano-scaffolds	Clinical impact	References
In vivo limitations	Differences between in vitro and physiological environments affect scaffold performance.	Enzymatic degradation of gelatin and collagen, rapid clearance of HA-based materials, immune recognition of chitosan particles.	Reduced regenerative efficacy and unpredictable clinical outcomes.	204, 205
Toxicity and biosafety	Potential adverse effects arising from degradation products, residual crosslinkers, nanoparticles, or phytochemical overdosing.	Genipin, glutaraldehyde residues; accumulation of nano-carriers; dose-dependent cytotoxicity of certain phytochemicals.	Safety concerns and restricted therapeutic dosage.	206
Scalability and manufacturing	Difficulty maintaining reproducibility, physicochemical properties, and bioactivity during large-scale production.	Batch-to-batch variation in collagen, alginate, and silk fibroin extraction.	Increased production costs and limited commercialization.	207, 208
Mechanical instability	Natural polymers often exhibit insufficient mechanical strength for load-bearing applications.	Rapid collapse of gelatin or HA scaffolds under physiological stress.	Impaired bone regeneration in critical-sized defects.	201
Controlled phytochemical release	Challenges in achieving sustained and predictable release profiles.	Burst release of curcumin, resveratrol, quercetin, or EGCG.	Reduced therapeutic efficacy and shorter bioactive duration.	166, 209
Regulatory issues	Lack of standardized guidelines for combination products containing biomaterials and bioactive phytochemicals.	Variable regulatory classification across countries.	Delayed approval and increased development costs.	210, 211

HA, hyaluronic acid; EGCG, epigallocatechin gallate.

Future directions

Future research will focus on creating advanced scaffold systems that mimic the natural extracellular matrix to improve regeneration. These smart scaffolds will respond to environmental signals like pH and inflammation, enhancing the delivery of phytochemicals and therapeutic molecules.^{212,213} Another promising direction involves the development of nano-composite biomaterials combines natural polymers with bioactive nanomaterials to improve strength, bioactivity, and osteogenic potential while keeping their compatibility and breakdown features.^{53,214} Emerging evidence shows that extracellular vehicles (EVs), including exosomes, can be effective cell-free therapeutic agents. Using EVs in natural polymer scaffolds may enhance cell communication and tissue growth without the limitations of stem cell use.^{215,216} In addition Advances in AI and modeling can help design biomaterials by predicting the best scaffold compositions and structural parameters, speeding up tailored regenerative applications.²¹⁶⁻²¹⁸

Finally using modern manufacturing technologies, such as microfluidic fabrication systems, four-dimensional (4D) printing, and three-dimensional (3D) bioprinting, to create patient-specific scaffolds with exact architectural control is one potential approach. These methods might make it possible to create biomimetic structures that more closely resemble the hierarchical structure of natural bone and tooth tissues.²¹⁹

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Competing Interests

The authors have no declaration of interest in this study.

Data Availability Statement

The raw/processed data required to reproduce these findings can be shared after publication by request from the corresponding author.

Declaration of AI-assisted Tools in the Writing Procedure

We used AI tools (ChatGBT 5) only for English Grammar corrections.

Ethical Approval

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