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REVIEW OPEN ACCESS

Recent Advances in Injectable Hydrogel Biotherapeutics for Regenerative Dental Medicine

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ABSTRACT

Dental caries and periodontitis continue to be significant oral health challenges. Traditional therapies typically manage infection and inflammation but seldom restore lost tissue. In this way, injectable hydrogels have emerged as versatile biomaterials that adapt to the complex anatomy of the dentin-pulp complex and periodontal lesions. Generally speaking, by incorporating antimicrobial, anti-inflammatory, and pro-regenerative cues, these hydrogels support localized disinfection, promote angiogenesis, and foster tissue regeneration. More specifically, recent advances in functionalizing hydrogels with growth factors, bioactive compounds, and dentin matrix components have effectively modulated inflammatory responses, stimulated host cell activity, and facilitated the formation of hard and soft tissue for regenerative endodontic therapies. In periodontology, engineered hydrogels have demonstrated their ability to deliver osteogenic, antimicrobial, and immunomodulatory agents while also enabling the encapsulation of stem cells or extracellular vesicles, thereby enhancing cellular activity and supporting the regeneration of alveolar bone and soft tissue. This review summarizes recent advancements in injectable hydrogels for regenerating the dentin-pulp complex and periodontal tissues, discussing their design principles, performance, and future directions. Although not exhaustive, it provides a comprehensive overview to inspire further research and innovation, encouraging the development of novel, easy-to-apply, and highly biocompatible multifunctional hydrogels for regenerative dental medicine.

1 | Introduction

Dental caries is one of the most common global oral health challenges, affecting individuals of all ages. This condition occurs through a complex process where acid-producing bacteria break down fermentable carbohydrates, gradually damaging the tooth structure. Host factors such as enamel, dentin, saliva, and personal dietary habits further influence the onset and progression of the disease. In severe cases, especially when decay penetrates the enamel and dentin layers, the dentin-pulp complex may become inflamed or infected, resulting in intense pain and, if left untreated, eventual necrosis of the pulp tissue. This deterioration

necessitates invasive procedures, such as endodontic treatment or even tooth extraction, highlighting the limitations of current strategies that primarily aim to halt the disease rather than regenerate lost or damaged tissues [1].

Periodontitis, in contrast, is a chronic inflammatory disorder that gradually destroys the tooth-supporting tissues, including the gingiva, periodontal ligament, alveolar bone, and cementum. Recognized as a highly prevalent condition, periodontitis often presents with tissue recession and tooth mobility. Traditional therapies have focused on reducing bacterial biofilms and alleviating inflammation; however, restoring the damaged periodontal architecture remains a challenge. Therefore, advanced biotherapeutic approaches have been proposed to regenerate lost periodontal tissues and enhance long-term clinical outcomes [2].

Among the innovative solutions gaining increasing attention are injectable hydrogels, versatile biomaterials that can adapt to the geometrically complex dental, oral, and craniofacial (DOC) anatomical structures. These hydrogels provide a platform for delivering cells, growth factors, and other biotherapeutics directly to affected areas in the dentin-pulp complex and periodontal lesions [3–5]. Their injectability and ability to fill irregular defects make them particularly suitable for regenerative dental medicine, where precise, minimally invasive interventions are often preferred. Recent advancements have focused on developing hydrogels that support the viability and differentiation of dental stem cells, enhance tissue integration, and facilitate the controlled release of growth factors, such as BMP-2 and VEGF, or antimicrobial agents. Additionally, smart, stimuli-responsive hydrogels that react to pH, enzymes, or light show promise in adapting to the dynamic environment of the oral cavity.

However, several key challenges persist. Many hydrogels lack the mechanical strength needed to withstand forces in the oral environment and may degrade too quickly *in vivo*. Additionally, controlling the spatial distribution and release timing of encapsulated therapeutics remains a significant challenge, and inflammatory conditions in the oral cavity can compromise the performance of hydrogels. Furthermore, there is limited long-term clinical data available, and issues such as manufacturing scalability, sterility, and regulatory compliance must be addressed before these solutions can achieve widespread use. Nevertheless, ongoing innovations suggest that injectable hydrogel systems have significant potential to transform regenerative strategies in dental medicine. This review highlights recent advances in designing injectable hydrogels, exploring their potential to support the functional repair and regeneration of DOC tissues.

2 | The Dentin-Pulp Complex

Dentin is a permeable mineralized connective tissue with a tubular structure that constitutes the bulk of the tooth. While root dentin is covered by cementum, a structure that integrates the tooth with the surrounding bone, coronal dentin is covered by enamel, a highly mineralized structure. Together, these structures protect the dental pulp, a non-mineralized loose connective tissue beneath the dentin. The pulp tissue is rich in nerve endings and features a vascular network that connects with surrounding tissues. Despite their distinct composition and characteristics, dentin and pulp share the same mesenchymal origin [6]. These tissues are interconnected by secretory cells known as odontoblasts, which form a pseudostratified layer along the dentin-pulp interface. This interdependence defines the dentin-pulp complex [6]. This review presents the state-of-the-art injectable hydrogels for regenerating the dentin-pulp complex, considering their antimicrobial, anti-inflammatory, pro-angiogenic, and pro-osteo/odontogenic features.

2.1 | Dynamics and Therapeutic Approaches

Odontoblasts are post-mitotic, terminally differentiated cells derived from the neural crest [7]. During odontogenesis, the epithelial-mesenchymal interaction of the dental lamina mobilizes a subpopulation of mesenchymal cells, which extend cytoplasmic processes toward the basement membrane, differentiating into primary odontoblasts [7]. These elongated cells have eccentrically located nuclei and abundant supranuclear organelles, which are responsible for the production and secretion of predentin, an extracellular matrix primarily composed of type I collagen fibrils [7]. The subsequent mineralization of this matrix results in mineralized dentin through a complex process involving non-collagenous proteins also secreted by odontoblasts [6, 7]. Key proteins, including tissue non-specific alkaline phosphatase (TNAP or ALP), dentin matrix protein-1 (DMP-1), and dentin sialophosphoprotein (DSPP), regulate extracellular calcium and phosphate levels, promote mineral nucleation, and facilitate hydroxyapatite crystal formation within and along the collagen fibrils of the predentin matrix [6–9].

Dentin formation occurs actively during tooth development, resulting in the tubular primary dentin. After tooth eruption, the secretory activity of odontoblasts decreases by approximately tenfold through autophagy, which degrades intracellular components to preserve cell functionality and ensure cell survival [10]. At this stage, odontoblasts produce secondary dentin, deposited slowly and physiologically throughout life, maintaining its tubular structure [10]. As mineralization progresses, odontoblasts migrate toward the innermost pulp, extending their cytoplasmic processes into the predentin and mineralized dentin tubules [7]. These extensions impact the transmission of sensory stimuli and primary cellular defense against toxic agents, dental materials, and injuries affecting the overlying tissues [7, 10, 11].

In response to low-intensity stimuli, odontoblasts increase dentin deposition, resulting in the production of reactionary tertiary dentin. This response reduces the permeability of the dentin-pulp complex and distances the cell bodies from the irritant [11, 12]. However, intense stimuli may result in odontoblast death, triggering the recruitment of undifferentiated (stem) pulp cells to the injury site; these cells proliferate and differentiate into odontoblast-like cells, adopting an elongated morphology and secreting an amorphous mineralized matrix known as reparative tertiary dentin, aiming to restore the structural integrity of the dentin-pulp complex [6, 11, 13].

The dentin-pulp complex's response to injury extends beyond pulp cells. Components embedded in the mineralized dentin extracellular matrix exhibit significant biological activity, challenging the perception of dentin as an inert tissue with a predominantly mechanical role [14]. In addition to collagenous and non-collagenous proteins related to mineralization, the dentin extracellular matrix contains growth factors, neurotrophic factors, and plasma-derived proteins [15]. These molecules can be solubilized during dentin demineralization processes, such as carious lesions, acid etching in restorative procedures, and cavity preparations [11, 15]. Once released, these components mediate various tissue repair processes, including regulating primary odontoblast activity, recruiting stem cells, inducing odontoblast differentiation, modulating the activity of differentiated cells, and stimulating angiogenesis. These events aim to maintain homeostasis and the functional capacity of the dentin-pulp complex, thereby protecting tooth integrity [11, 15].

The structural integrity of mineralized dental tissues can be compromised by various factors, including carious lesions, trauma, or during cavity preparations, which may result in pulp tissue damage [16]. In this context, any treatment aimed at preserving or restoring the structure and function of the vital pulp is referred to as Vital Pulp Therapy (VPT) [17]. Clinically, these treatments are classified as indirect pulp capping, direct pulp capping, or pulpotomy. Indirect pulp capping is performed in very deep cavities, where a biocompatible material (e.g., conventional or resin-modified glass ionomer cement) is placed over a thin dentin layer to protect and stimulate underlying odontoblasts to deposit reactionary tertiary dentin [12, 13, 16]. In contrast, direct pulp capping is a minimally invasive procedure indicated when pulp tissue is exposed, which involves the application of a material to facilitate the formation of reparative tertiary dentin, a protective mineralized barrier produced by odontoblast-like cells [11, 16]. Pulpotomy only differs from direct pulp capping in that a portion of the remaining pulp tissue is removed before applying the capping material [16].

Clinically, vital pulp can present in three conditions depending on the signs and symptoms: normal pulp, reversible pulpitis, and irreversible pulpitis. Normal pulp shows no clinical symptoms; reversible pulpitis is characterized by short-duration thermal sensitivity that ceases immediately after removing the stimulus; irreversible pulpitis typically involves spontaneous, prolonged, and/or referred pain [16]. VPT with direct pulp capping is currently indicated for treating exposed pulp in young teeth with normal or reversible pulpitis conditions [16, 17]. Radiographically, the apical region should show no pathological changes, and responses to percussion, palpation, and periodontal probing should be within normal limits [16]. The exposure site should ideally be less than 1 mm in diameter, and local bleeding must be controlled before placing the capping material [16]. If these conditions cannot be met, direct pulp capping with currently available materials is not recommended, as subsequent endodontic treatment may be required [16, 17].

Ideally, materials for capping exposed pulp should exhibit the following properties: biocompatibility and maintenance of pulp vitality; stimulation of reparative dentin formation; adhesion to dentin and overlying restorative materials; resistance to subsequent restorative procedures and masticatory forces; bactericidal and/or bacteriostatic action; sealing against microbial recontamination; ability to be sterilized; and radiopacity [17, 18]. More recently, immunomodulatory properties have also been highlighted, considering that VPT is performed under inflammatory conditions [19]. Over the past century, calcium hydroxide and calcium silicate-based formulations have been proposed for direct pulp capping [20]. However, none of these materials fully meet all the desired characteristics.

The mineralized tissue formed after applying currently available calcium hydroxide and calcium silicate-based formulations for direct pulp capping results from superficial necrosis followed by dystrophic calcification, representing a tissue repair process. [11, 21] Tissue repair is characterized by restoring lost or damaged tissues by depositing fibrous scar tissue [22]. In contrast, tissue regeneration involves restoring original structures by depositing tissue with characteristics similar to those lost [22]. Recent advances in materials science, cellular, and molecular biology have driven the development of new therapeutic approaches to promote regenerative processes with improved safety and efficacy [23].

Considering dentin regeneration, emerging strategies focus on developing cytocompatible and bioactive biomaterials that stimulate the formation of new dentin tissue, predominantly deposited by newly differentiated odontoblast-like cells in the pulp tissue [24]. This approach enables the continuous formation of dentin with tubular conformation and sensory properties, aligning with the principles of tissue engineering established in the early 1990s, which integrate engineering and biological concepts to create functional substitutes for lost tissues [25]. Tissue engineering relies on three key components: cells capable of generating the lost or damaged tissue, scaffolds that serve as supportive biomaterial frameworks, and bioactive molecules that guide the cells to form new tissue [25]. The identification of stem cells in the pulp tissue of adult permanent teeth has opened new possibilities for craniofacial regenerative therapies, as these cells, located primarily around blood vessels, display the defining features of undifferentiated mesenchymal cells, including high proliferative capacity, clonogenicity, self-renewal potential, and multilineage differentiation capability [26, 27]. Their ability to differentiate into odontoblast-like cells, form mineralization nodules *in vitro* and produce tubular dentin when transplanted ectopically highlights the inherent regenerative capacity of pulp tissue [26]. Consequently, employing materials that induce the mobilization of these cells to injury sites represents a promising and clinically viable application of tissue engineering concepts for VPT.

Considering pulp capping and pulpotomy as regenerative procedures in which the remaining pulp tissue serves as a source of resident stem cells, searching for alternative treatment protocols to optimize the intrinsic regenerative capacity of pulp tissue becomes an alternative to enhance the success rate of these therapies [24]. Even in advanced stages of pulpitis or pulp necrosis where the cellular source is lost, recruiting, housing, and adequately stimulating other stem cells from the periapical region through bioactive scaffolds may be a viable strategy for controlling infection and inflammation and regenerating the dentin-pulp complex in the future, restoring its physiological composition and structure [19, 27].

2.2 | Hydrogels for Dentin-Pulp Complex Regeneration

Scaffolds are critical in tissue engineering as they replicate key features of the ECM, a nanostructured network of proteins, glycosaminoglycans, and glycoproteins that surround cells, provide mechanical support, and mediate signaling, thus creating an environment conducive to cellular function and new tissue formation under physiological conditions [25]. Their physicochemical properties, including composition, morphology, porosity, pore interconnectivity, and biodegradability, are essential for biocompatibility and directly influence cell behavior and tissue regeneration [23, 28, 29]. To achieve these functionalities, various scaffold materials have been explored, encompassing ECM components such as proteins, glycosaminoglycans, and peptides, as well as natural polymers, synthetic polymers, bioceramics, and blends, each conferring distinct bioactive properties suitable for regenerating specific tissues [23, 29].

Hydrogels are among the most prominent scaffolds capable of mimicking the ECM due to their ability to integrate with host tissue, support cell growth, and facilitate nutrient and oxygen diffusion [4]. They are used in various biomedical applications, including tissue regeneration, organoid culturing, and 3D microenvironment reconstruction. Hydrogels are defined as cross-linked, porous, 3D polymeric networks, including both fibrous and non-fibrous components, that exhibit a high affinity for water. These materials can be modified with bioactive molecules to enhance cell-matrix interactions [4, 30, 31]. Their composition orchestrates the biocompatibility, biodegradability, and ease of functionalization. Hydrogels can be classified into three categories: synthetic, natural, and hybrid [4].

Natural components like collagen, chitosan, hyaluronic acid, and gelatin facilitate cell adhesion and interaction. However, they often exhibit considerable variability between batches and can provoke immune responses [32]. Synthetic polymers, such as polyacrylamide and polymethyl methacrylate, are characterized by their robust mechanical properties and minimal immunogenicity [32]. Hybrid hydrogels intend to combine the advantages of natural and synthetic polymers, presenting both elements. Collagen methacryloyl (ColMA) and gelatin methacryloyl (GelMA) are two examples that combine the natural polymers collagen or gelatin with cross-linkable methacrylate groups [33, 34]. This category of polymers can overcome the limitations of natural polymer-based hydrogels, including temperature-dependent solidification and solubility. Moreover, they can be conformed under various conformities and cross-linked with other polymers, molecules, and particles that reinforce the mechanical and biological properties to provide desirable regenerative effects, including odontogenic, osteogenic, and angiogenic responses [4, 32].

Regenerative endodontics has advanced rapidly; however, treating necrotic immature teeth with open apices remains challenging due to incomplete root canal development, thin dentinal walls, and bacterial infiltration within the dentinal tubules of young teeth, with persistent apical infection and clinical symptoms often complicating treatment outcomes [35, 36]. To address these issues, regenerative endodontic procedures (REPs) now incorporate innovative tissue-engineered constructs and drug delivery systems designed to navigate complex canal anatomies, promote continued root maturation, enhance dentin formation, and support apical tissue healing. These strategies employ cell-friendly approaches that preserve stem cell viability, stimulate proliferation and differentiation, and utilize novel disinfecting materials and scaffolds to manage infection, control inflammation, and promote regeneration within the pulp-dentin complex.

Injectable photocrosslinkable hydrogels are particularly attractive for dentin-pulp complex regeneration because their low-viscosity sol state can fill irregular spaces in the pulp chamber and root canals, and a brief exposure to light triggers rapid crosslinking that not only stabilizes the gel but also delivers bioactive signals vital for controlling contamination, inflammation, and promoting mineralization [4, 32]. However, for successful clinical translation, these hydrogels must meet some requirements: a) the capacity for controlled gelation or degradation; b) the absence of toxic reagents in their formulation; c) the prevention of toxic byproduct formation; and d) the ability to retain suitable mechanical properties post-injection [37]. In general, the physicochemical and biological properties of these materials can be modified by the type of cations and the strength of interactions or crosslinking strategies.

Several key aspects of injectable hydrogel synthesis and its functional characteristics, relevant to tissue regeneration, must be carefully considered during both production and assessment (Figure 1). Injectable hydrogels are primarily formed through interactions between polymer chains [38]. Various parameters, such as the concentration of biopolymers and the crosslinking ratio, can influence their physicochemical, mechanical, rheological, and biological properties [38, 39]. Crosslinking can occur via chemical or physical mechanisms. Chemical crosslinking involves dynamic covalent bonds, such as Michael addition and Schiff base reactions, which confer reversible sol-gel transitions to the hydrogel, enabling its injection via syringe and subsequent in situ gelation [39, 40]. In contrast, physically crosslinked hydrogels rely on non-covalent interactions, including hydrogen bonding and hydrophobic interactions [39]. Although physically crosslinked hydrogels are easier to fabricate than chemically crosslinked hydrogels, their weaker interactions may result in reduced mechanical strength and lower stability [38]. Furthermore, depending on the gelation mechanism of the hydrogel, significant heat generation or byproduct formation may occur and must be managed appropriately [40]. A summary of some of the most extensively studied injectable and biodegradable hydrogel materials, highlighting their physicochemical and biological properties, as well as their clinical applications in Periodontics and Endodontics, is available in (Table S1, Supporting Information).

Meanwhile, increasing attention has focused on “smart” hydrogels that respond to external stimuli (such as pH, ionic strength, or temperature) by altering swelling behavior, sol-gel transition, network structure, or mechanical strength [41]. Among these, thermosensitive hydrogels are particularly notable, as they

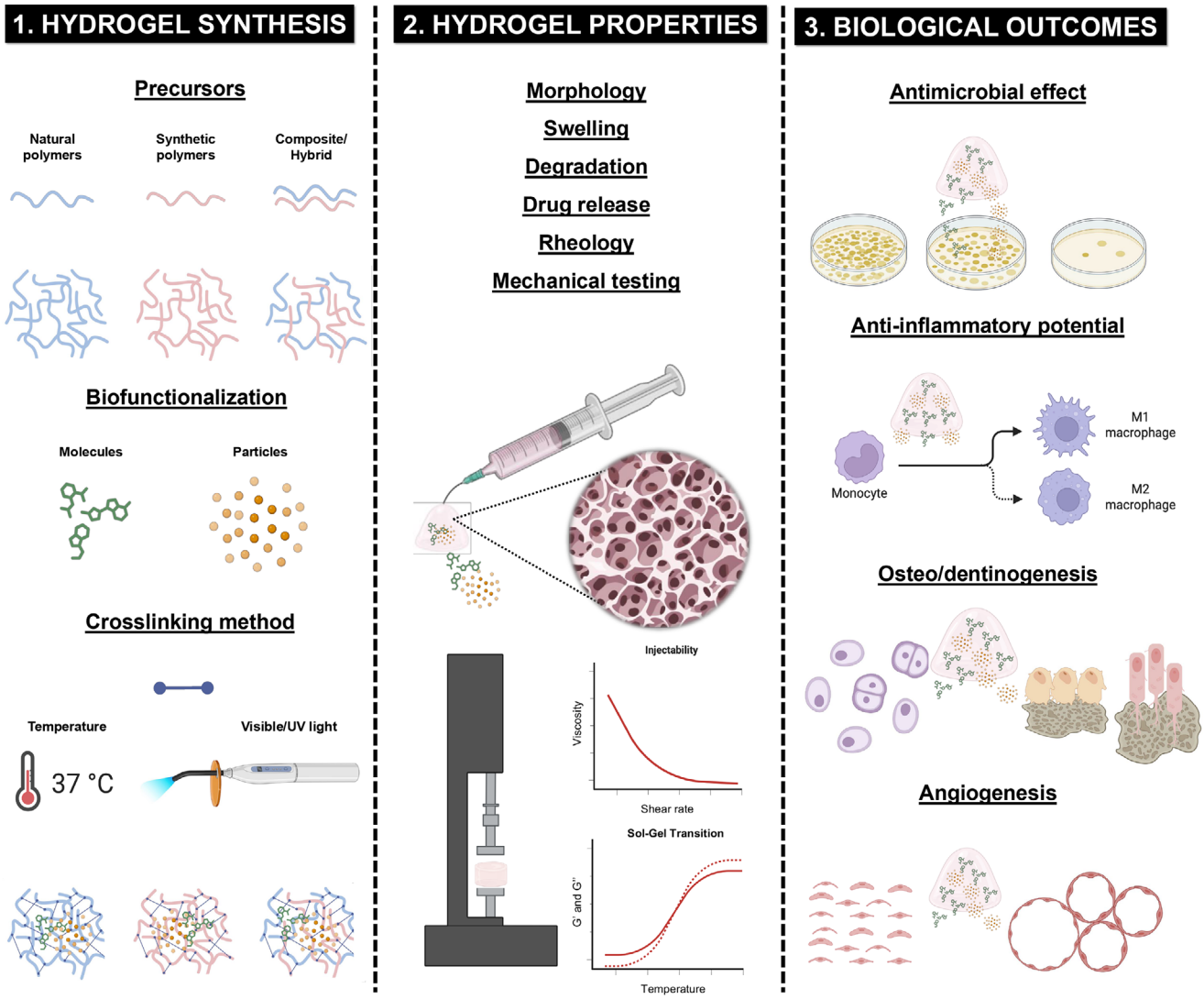

FIGURE 1 | Schematic of injectable hydrogel synthesis and functional characteristics relevant to tissue regeneration. The systems can use natural or synthetic polymers functionalized with bioactive molecules and undergo rapid in situ gelation via photopolymerization or thermal transition. This results in a biomaterial with multiple properties, including antimicrobial, anti-inflammatory, pro-osteo/dentinogenic, and pro-angiogenic effects.

undergo in situ gelling when transitioning from ambient to physiological temperatures, supporting efficient and site-specific drug delivery due to their biodegradability, low toxicity, high drug-loading capacity, and sustained release potential [42]. Examples include chitosan (often combined with weak bases like beta-glycerol phosphate or sodium bicarbonate), and polyethylene glycol (PEG) blended with polylactide-glycolide (PLGA), both of which exhibit temperature-triggered gelation; however, despite their promise for endodontic use by enabling hydrogel solidification within the root canal, their clinical application remains limited [43–51].

In sum, injectable hydrogels are an emerging biomaterial in regenerative endodontics. Their tunable physical and chemical properties enable minimally invasive administration and address the complex root canal anatomy [52]. They show promise for enhancing stem cell function, reducing inflammation, exerting antibacterial effects, and promoting soft tissue regeneration and mineralization in dentin-pulp complex regeneration, whether applied as a REP or not. The following sections discuss recent developments in hydrogels and explore their potential clinical applications.

2.2.1 | Biologically Active Functionalized Hydrogels

Incorporating bioactive molecules, such as growth factors and inorganic particles, along with the dentin matrix, into hydrogels promotes dentin regeneration by enhancing both odontogenic and angiogenic responses [53]. Growth factors like fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) are critical for odontoblast differentiation and mineralization, driving dentin formation and pulp tissue regeneration [54–56]. For instance, studies on the controlled release of FGF-2 from gelatin hydrogels in rat molars have demonstrated its potential in dentin-pulp complex regeneration [54]. Notably, high doses of FGF-2 promoted the formation of DMP-1-positive calcified particles, while moderate doses facilitated the sealing of a mineralized matrix, highlighting the dose-dependent effects of FGF-2 on the structure of regenerated mineralized

tissues [54]. In contrast, VEGF primarily supports angiogenesis, enabling the formation of blood vessels that supply essential nutrients and oxygen to the regenerating dentin-pulp complex [57]. A chitosan/β-glycerophosphate (CS/β-GP) hydrogel was developed as a sustained release system to enhance VEGF delivery. This VEGF/CS/β-GP hydrogel significantly improved the odontogenic differentiation of dental pulp stem cells (DPSCs) compared to VEGF alone, demonstrating its potential as an effective carrier for bioactive molecules in pulp capping therapy [55].

Adding inorganic particles, such as calcium phosphates and bioactive glass, enhances the structural integrity of hydrogels while mimicking the natural dentin matrix, creating an ideal microenvironment for cell attachment and proliferation [58, 59]. These particles also release bioactive ions into the extracellular environment, stimulating cell differentiation and exhibiting antibacterial properties [60]. For example, a SrCuSi4O10/gelatin methacrylate composite hydrogel demonstrated antibacterial activity against *Streptococcus mutans* and *Lactobacillus casei* while promoting odontogenesis and angiogenesis via sustained ion release [60]. In a rat dental pulp infection model, this hydrogel outperformed commercial materials like iRoot BP Plus, highlighting its promise as a pulp-capping material for infected pulp treatment [60]. Similarly, injectable alginate hydrogels loaded with boron-doped mesoporous bioactive glass nanoparticles (BMBG NPs) exhibited enhanced porosity and bioactivity, facilitating hydroxyapatite formation that resembles natural bone composition [59]. Gelatin methacryloyl (GelMA) hydrogels incorporated with 58S bioactive glass doped with strontium showed comparable results, supporting DPSC adhesion, controlled degradation, and increased mineral nodule deposition compared to GelMA hydrogels without inorganic particles [58].

The dentin matrix, abundant in bioactive molecules and proteins, guides tissue regeneration by releasing signaling cues that modulate cellular behavior. These bioactive components work synergistically to stimulate tissue repair, ultimately restoring the functionality and integrity of the dentin-pulp complex [61–63]. For instance, demineralized dentin matrix hydrogel (DDMH) demonstrated superior biocompatibility and antimicrobial activity compared to mineral trioxide aggregate (MTA), promoting DPSC viability, attachment, and COL-I gene expression [64]. Similarly, injectable photo-crosslinkable GelMA hydrogels containing decellularized (BMdc) and deproteinized (BMdp) dentin matrix exhibited a stable, porous structure that supported the viability of human dental pulp cells (HDPCs), enhanced odontoblastic differentiation, and increased mineral deposition [61]. Studies of injectable gelatin-treated dentin matrix light-cured hydrogel (LCG-TDM) have revealed the formation of complete dentin bridges with minimal inflammation, resulting in significantly thicker and more organized tubular dentin bridges compared to alternative materials. In contrast, TheraCal LC induced moderate inflammation, resulting in less favorable dentin formation, and LCG failed to promote hard tissue regeneration [65]. Furthermore, 3D-printed GelMA microgels facilitated the formation of organized pulp tissue, tertiary dentin, and new blood vessels, achieving more significant dentin bridge formation and reduced necrosis compared to MTA [63].

In an orthotopic model of induced periapical lesions in immature dog teeth, chitosan hydrogels used as scaffolds in REPs promoted the formation of highly vascularized loose connective tissue with evidence of ectopic calcification islands [66]. However, after 13 weeks post-treatment, these hydrogels did not appear to enhance mineralization compared to the blood clot group, exhibiting some areas of dentinal formation associated with odontoblast-like cells [66]. A recent study has also explored the regenerative potential of an enzymatically modified chitosan hydrogel with phenol on dogs' teeth undergoing REPs [67]. This modified chitosan hydrogel has been selected due to its documented antioxidant properties, non-toxicity to stem cells, and ability to enhance fibroblast adhesion and proliferation [68]. Unlike the findings of Palma et al. (2017), this study reported the enhanced formation of vital, well-vascularized connective pulp-like tissue, mineralization in the periapical area, and foramen closure after three months of treatment [67]. These outcomes were observed in teeth treated with a combination of blood clots and both native and phenol-modified chitosan hydrogels, compared to treatment with blood clots alone. The observed differences may be attributed to the type of model used (non-contaminated) and the specific properties of the modified chitosan hydrogel.

Bo Wen et al. constructed a composite mineral matrix hydrogel (PAA-CMC-TDM) by integrating amorphous calcium phosphates (ACPs), polyacrylic acid (PAA), carboxymethyl chitosan (CMC), and dentin matrix (TDM). This hydrogel exhibited notable self-repairing and injectable properties. Cytological experiments and animal models of hard tissue defects demonstrated that it effectively promoted mesenchymal stem cells' odontogenic and osteogenic differentiation, adapted well to irregular defect geometries, and supported the in situ regeneration of both dental and bone tissues (Figure 2) [69]. Han et al. hypothesized that varying the stiffness of a collagen-based hydrogel could guide DPSCs toward functional pulp and dentin regeneration. They prepared low-stiffness (3 mg/mL, Col3) and high-stiffness (10 mg/mL, Col10) hydrogels, encapsulated DPSCs, and incorporated growth factors VEGF into Col3 and BMP2 into Col10. When these hydrogels were concentrically injected into dentin slices and implanted in SCID mice, the Col10+Col3+DPSCs+GFs combination significantly enhanced pulp-dentin tissue formation. Notably, the lower-stiffness Col3 facilitated endothelial differentiation of DPSCs. At the same time, the higher-stiffness Col10 promoted odonto/osteogenic differentiation, demonstrating an injectable system capable of directing lineage-specific outcomes and regenerating pulp-dentin tissues *in vivo* (Figure 3) [70].

Functionalized hydrogels offer a versatile platform for achieving controlled and sustained release of bioactive molecules. They ensure localized delivery, prolonged bioactivity, and synergistic effects on cellular signaling pathways critical for tissue regeneration and inflammation modulation [71, 72]. These hydrogels enable the modulation of growth factors, proteins, and ion concentrations in the extracellular environment. These systems combine bioactive molecules and structural cues to create an optimized microenvironment, emphasizing their transformative potential in regenerating the dentin-pulp complex and restoring dental tissue functionality.

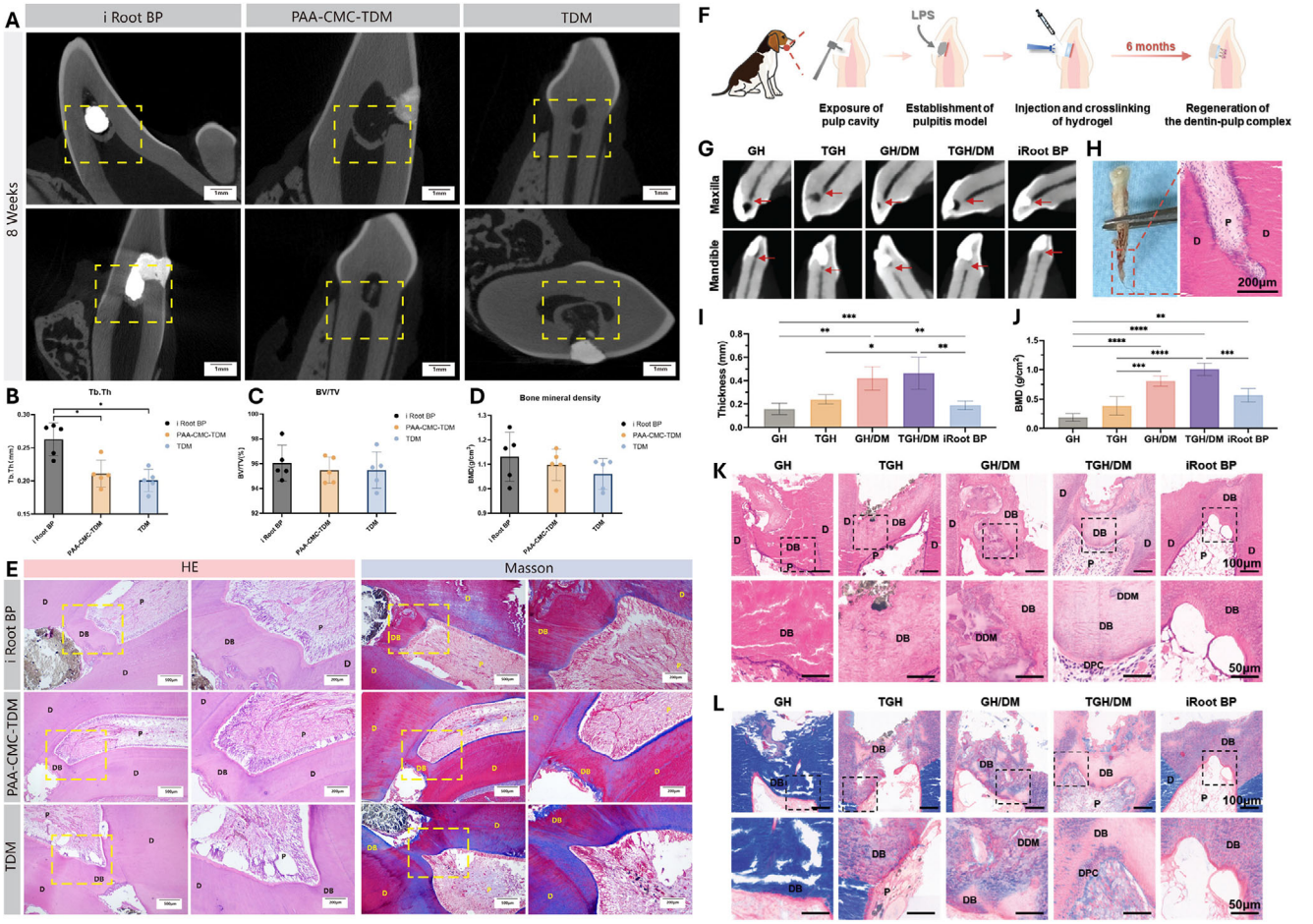

FIGURE 2 | (A) Representative micro-CT images illustrating dentin bridge formation in a dog tooth 8 weeks after pulp capping using iRoot BP plus, PAA-CMC-TDM, and TDM. (B–D) Micro-CT was employed to comprehensively evaluate the newly formed dentin bridge by quantifying its thickness, assessing its mineralization ratio, and determining its bone mineral density. (E) Histological evaluation by H&E and Masson's trichrome staining of dog teeth 8 weeks after pulp capping with PAA-CMC-TDM, iRoot BP, and TDM. (F) Schematic diagram depicting establishing a pulpitis model in beagle dogs for in situ evaluation of the mineralization-promting effects of TGH/DM. (G) Cone-beam computed tomography (CBCT) images showing newly formed dentin bridges (indicated by red arrows) in the anterior teeth of beagle dogs at 6 months after pulp capping with GH, TGH, GH/DM, TGH/DM, and iRoot BP. (H) A representative image of an extracted experimental tooth used for histological analysis, along with its apical region stained with H&E. (I,J) Quantitative analyses of reparative dentin thickness and BMD as determined by CBCT. (K) H&E-stained sections demonstrating newly formed dentin bridges and pulp tissues in dog teeth at 6 months post-pulp capping with GH, TGH, GH/DM, TGH/DM, and iRoot BP. Insets (lower panels) show magnified views of the indicated regions. (L) Masson's trichrome-stained images of newly formed dentin bridges and pulp tissues in dog teeth at 6 months post-pulp capping with GH, TGH, GH/DM, TGH/DM, and iRoot BP. Insets (lower panels) display enlarged views of the marked areas. Abbreviations: P, dental pulp; D, dentin; DB, dentin bridge; DDM, demineralized dentin matrix particles; DPC, dentin-pulp complex. Statistical significance: ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Reprinted (adapted) with permission from [69, 88]. Copyright 2023 - Nature Portfolio. Copyright 2024 - John Wiley and Sons.

2.2.2 | Immunomodulatory and Anti-Inflammatory Hydrogels

Teeth can be compromised by both trauma and caries. The pulp may become infected depending on the extent of hard tissue damage or lesion depth [73]. Bacterial infiltration then triggers inflammation, characterized by the proliferation of inflammatory cells and mediators, which can potentially result in tissue necrosis [74, 75]. Inflammation is vital for tissue repair and regeneration during vital pulp therapy, but can also pose a barrier to healing [76]. In its acute phase, immune cells, such as macrophages and neutrophils, clear debris, pathogens, and damaged cells, thereby creating a microenvironment conducive to regeneration [77]. Pro-inflammatory cytokines, such as IL-1β and TNF-α, stimulate key regenerative pathways, including BMP-2-activated MAPK signaling [78, 79]. However, excessive or prolonged inflammation can hinder healing and lead to chronic tissue damage, emphasizing the need for a balanced inflammatory response [76].

Radical and conservative endodontic treatments require thorough dental pulp or root canal decontamination, typically achieved through physical-chemical preparation, irrigation, medicaments, and capping materials. However, the complexity of these procedures remains challenging for researchers and clinicians [80]. With a growing emphasis on tooth preservation, especially for immature teeth, endodontic therapy now focuses on REPs to form new tissue [81]. Recent scientific advances have led to the development of biomaterials that promote

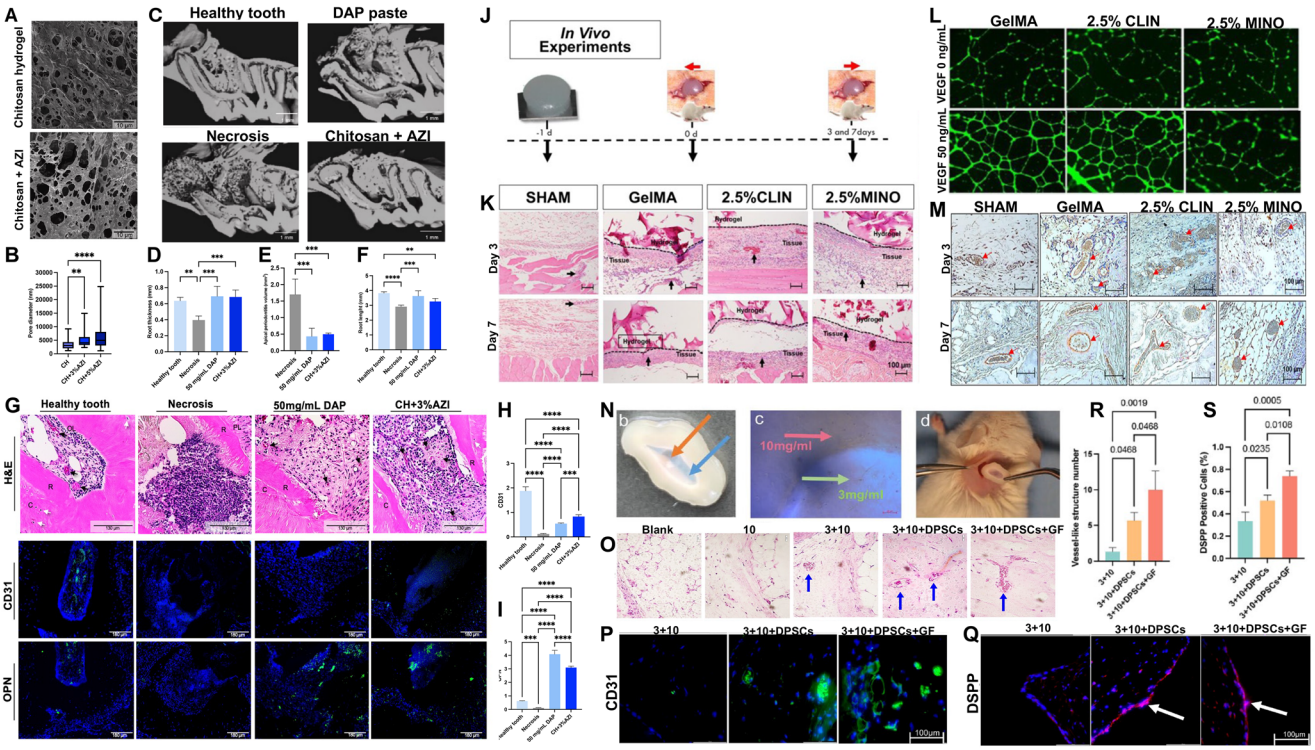

FIGURE 3 | (A) SEM images reveal the microstructure of pure and azithromycin-laden chitosan hydrogels, with pore size diameter among groups (B). (C–F) Micro-CT sagittal images of the rat's upper first molar and morphometric analyses assess root thickness, length, and periapical lesion volume after 28 days of treatment. (G) Histological and immunofluorescence images of CD31 and OPN in the apical region of the mesial root canal in rats after 28 days of regenerative treatment. Black arrows indicate blood vessels, while white arrows indicate cementoblasts/cementocytes. (H,I) Quantification of CD31- and OPN-positive cells across all treatment groups. (J) Schematic diagram illustrating the *in vivo* subcutaneous implantation. (K) Representative H&E-stained images of hybrid hydrogels and surrounding tissues, with arrows indicating blood vessels and dashed lines marking the boundary between the hydrogel and tissue. (L) Fluorescence microscopy images of HUVECs on Matrigel, treated with hybrid hydrogels, showing capillary-like structure formation after 24 h. (M) Immunohistochemical staining for CD31 post-implantation, with arrows indicating blood vessels. (N) Tooth slices are injected with two different concentrations of collagen scaffolds containing cells into the pulp chamber (Ob, Oc), followed by *in vivo* implantation (Od). (O) H&E staining of regenerated pulp/dentin-like tissue after 6 weeks shows perfused vessels (blue arrows). (P–S) Representative immunofluorescence images and quantification of *in vivo* pulp-dentin regeneration for CD31 (P, R) and DSPP (Q, S), with white arrows indicating the newly formed dentin. Reprinted (adapted) with permission from [44, 49, 70] Copyright 2023 - Royal Society of Chemistry. Copyright 2024 - KeAi Publishing. Copyright 2024 - SAGE Publications.

decontamination, homeostasis, vascularization, healing, and bone regeneration [81, 82]. These materials enhance disinfection and modulate inflammation, support mesenchymal stem cell proliferation, regulate pro- and pre-inflammatory mediators, and induce hydroxyapatite formation for dentin mineralization [83, 84]. Among these biomaterials, hydrogels stand out for their ability to release targeted anti-inflammatory agents while preserving the beneficial aspects of the inflammatory cascade, creating an optimal environment for dentin-pulp complex regeneration. Due to their gelling properties, injectable hydrogels can access hard-to-reach areas of the root canal system. Their bioactive nature, drug delivery capabilities, and capacity to incorporate various substances make them highly versatile [74, 85, 86].

Various agents can be incorporated into hydrogels in vital pulp therapy, including established anti-inflammatory drugs and natural compounds with immunomodulatory properties. For example, L-arginine-modified calcium phosphate/zinc phosphate nanoparticle hydrogels have been studied for their ability to combine anti-inflammatory and pro-mineralization effects, enhancing vital pulp therapy. Compared to MTA, this hydrogel demonstrated superior biocompatibility, reduced inflammation, and stimulated reparative dentin formation by activating the p38 signaling pathway, both *in vitro* and in a rat pulpitis model [72]. Similarly, a tannin-containing hydroxypropyl chitin hydrogel (HPCH/TA) exhibited favorable physicochemical properties, suppressed inflammation by inhibiting the NF-κB pathway, and promoted reparative dentin formation *in vitro* [87]. Additionally, a multifunctional hydrogel system combining dexamethasone-loaded halloysite clay nanotubes (HNTs/DEX) with GelMA hydrogel demonstrated improved mechanical strength, controlled drug release, and compatibility with stem cells, promoting anti-inflammatory effects and osteogenic differentiation *in vitro*, as well as enhanced bone formation [71].

Zhuo Xie et al. engineered a multifunctional hydrogel (TGH/DM) that integrates an antimicrobial peptide (AMP) with a demineralized dentin matrix to enable sustained delivery of both AMP and a range of growth factors. *In vitro*, this hydrogel effectively eliminated endodontic pathogens, mitigated inflammatory responses in human dental pulp stem cells, and

promoted their odontogenic differentiation by activating peroxisome proliferator-activated receptor gamma. Moreover, using a preclinical pulpitis model in dogs, the favorable microenvironment created by TGH/DM facilitated the formation of continuous reparative dentin bridges, emphasizing its potential as a promising pulp capping material for vital pulp therapy applications (Figure 2) [88].

The immunomodulatory effects of bioactive hydrogels directly contribute to improved clinical outcomes in vital pulp therapy and dentin regeneration by modulating the inflammatory response. These effects can reduce postoperative pain and discomfort for patients [89]. At the same time, their ability to promote a balanced transition from the pro-inflammatory to the anti-inflammatory phase creates an optimal environment for tissue healing and regeneration. This balance accelerates the repair of the dentin-pulp complex, enhancing the quality and durability of the regenerated tissue [76]. Clinically, this would translate to higher success rates, a reduced need for additional interventions, and improved long-term preservation of tooth vitality, marking a significant advancement in regenerative endodontics.

2.2.3 | Antibacterial Hydrogels

Controlling infection in REPs is challenging due to the complex root canal anatomy and the varying susceptibility of microorganisms to antimicrobial agents. Residual bacteria can hinder root development and cause treatment failure by sustaining intracanal and periapical inflammation [35, 90]. While current guidelines recommend antibiotic pastes or calcium hydroxide-based medications for disinfection [91], there is increasing interest in incorporating natural compounds and alternative antibiotics that provide potent antimicrobial properties in a biocompatible, cell-friendly environment. These alternatives eradicate infections, support pulp-dentin complex regeneration, and minimize adverse effects on stem cell survival [92–96]. Hydrogels loaded with antimicrobial agents, such as antibiotics or herbal extracts, have gained significant attention in REPs due to their biocompatibility, biodegradability, and effective, selective antimicrobial action [43, 44, 97, 98]. Their polymeric structure allows efficient bacterial disinfection through direct contact in tight dental tissue gaps and offers long-term protection against microbial recolonization. Additionally, hydrogels facilitate the controlled release of antimicrobial agents, enhancing infection control while promoting tissue regeneration. Lastly, hydrogels serve as barriers against bacterial colonization. By filling root canal voids, they limit space for bacterial growth and biofilm formation, supporting infection control.

Certain hydrogels possess intrinsic antibacterial properties due to their polymeric composition. For instance, hydrogels derived from chitosan, a biopolymer obtained from chitin, demonstrate natural fungistatic and antimicrobial activity. This is primarily achieved through disrupting bacterial cell membranes, resulting in bacterial lysis. Furthermore, chitosan's positive charge facilitates electrostatic interactions with negatively charged bacterial surfaces, enhancing its bactericidal efficacy. Similarly, hyaluronic acid demonstrates bacteriostatic effects and reduces bacterial adhesion to surfaces. In Gram-positive bacteria, it promotes cell lysis by compromising the structural integrity of the peptidoglycan layer [45].

Hydrogels can be loaded with antimicrobial agents, including antibiotics, nanoparticles (such as silver and zinc oxide), and naturally occurring antimicrobial peptides. These agents are either incorporated into the hydrogel matrix or released in a controlled manner to inhibit bacterial growth, thereby enhancing cell proliferation and differentiation. Hydrogels are increasingly used as an alternative to conventional intracanal medications recommended for REPs, such as triple or double antibiotic pastes and calcium hydroxide-based formulations. The use of these traditional medications has been questioned, mainly due to their detrimental effects on stem cells and their potential to impair angiogenesis, as observed with minocycline [99]. Moreover, the use of antibiotics in REPs presents additional challenges, including the potential development of antibiotic-resistant microorganisms in biofilms, sensitization reactions, coronal discoloration, and a reduction in dentinal strength and fracture resistance.

Studies have investigated loading hydrogels with alternative antimicrobial agents to address these challenges, including combinations of metronidazole, ciprofloxacin, clindamycin [86, 98], diclofenac [86], azithromycin [49], and Augmentin [98]. These agents have been incorporated into chitosan-based hydrogels [49, 97] or other commonly studied hydrogel systems, such as GelMA [44, 60] or methylcellulose [86, 100]. Dubey et al. developed a novel hybrid hydrogel by embedding cryomilled, antibiotic-eluting fibrous microparticles into a GelMA matrix. They incorporated either minocycline (MINO) or clindamycin (CLIN) into electrospun polymer fibers, which were then processed into microparticles and suspended in GelMA at various concentrations. The resulting hydrogels exhibited antibiotic-dependent swelling and degradation, effectively inhibiting bacterial growth and maintaining low toxicity to dental-derived stem cells. *In vivo*, the CLIN-modified hydrogels showed minimal inflammation and a significantly higher presence of endothelial cells compared to the MINO variant, highlighting enhanced biocompatibility and angiogenic potential, suggesting that CLIN-eluting GelMA hydrogels offer a promising scaffold for antimicrobial root canal disinfection and the promotion of host cell recruitment and vascularization in dental pulp tissue engineering (Figure 3) [44].

In addition, other studies have also explored incorporating antibacterial agents into hydrogels to enhance their properties. Examples include chlorhexidine [43, 101], $SrCuSi_4O_{10}$ [60], lactic acid nanoparticles with antibiotics [102], gelatin, silver ions [103], and hyaluronic acid combined with hydroxyapatite [45]. For example, the incorporation of specific metallic ions, such as silver nanoparticles, has been proposed due to their broad-spectrum antibacterial properties against both Gram-negative and Gram-positive bacteria, as well as their lower likelihood of inducing microbial resistance compared to certain antibiotics [45]. These agents have been predominantly incorporated into modified chitosan or GelMA hydrogels, and other alternative hydrogels, such as demineralized dentin matrix and fibrin hydrogels, have been investigated [64, 102]. Therefore, researchers must carefully evaluate the agents to be encapsulated in

hydrogels based on their intended therapeutic goals and clinical applications.

Recently, a novel intracanal medication for REPs has been developed using a chitosan-based hydrogel supplemented with 3% azithromycin. This formulation was injected into the mesial root canals of rat molars with experimentally induced periapical disease. Remarkably, the formulated antibiotic-loaded chitosan-based hydrogel did not hinder root development. Additionally, it significantly reduced periapical lesions and bacterial colonization while promoting increased angiogenesis. It also promoted mineralization, as evidenced by increased osteopontin (OPN) expression compared to untreated groups (Figure 3) [49]. It is worth noting that most studies have evaluated the antibacterial potential of these modified hydrogels against monospecies bacteria, primarily using agar diffusion methods for direct or indirect testing or biofilm models, with a particular focus on *E. faecalis*. While understanding their effects on endodontic pathogens is crucial, especially when addressing multidrug-resistant pathogens, evaluating their performance against polymicrobial endodontic biofilms on dentin samples or in infected root canals is equally important. Such assessments are essential for simulating real-life conditions where biofilm-associated infections are the leading cause of pulp tissue necrosis, periapical disease, and the disruption of continued root development.

3 | The Periodontal Complex

The periodontal complex comprises mineralized and non-mineralized tissues supporting and anchoring the tooth. Root dentin is surrounded by cementum, a calcified layer that facilitates attachment to the surrounding alveolar bone via the periodontal ligament, a fibrous connective tissue abundant in collagen fibers. The alveolar bone forms the tooth socket and offers structural stability, while the gingiva, a specialized mucosal tissue, covers and protects the underlying structures. Together, these components sustain the tooth and enable its functional movement during chewing. The periodontal tissues are richly innervated and vascularized, providing nourishment and sensory feedback. Despite their different compositions, cementum, the periodontal ligament, and alveolar bone originate from the same mesenchymal lineage [104].

Innovative methods for delivering cells and biomolecules have attracted considerable interest in biotechnology and biomedicine. Injectable hydrogels stand out for their versatility and minimally invasive nature, efficiently delivering cells and therapeutics to fill irregularly shaped bone defects while reducing infection risks [105]. Periodontal defects are typically complex, involving multiple tissues and various cell types, with inflammatory cells and bacteria potentially hindering healing [106]. Hydrogels are effective platforms for periodontal regeneration due to their high biocompatibility and adjustable porosity. Several factors discussed in this review are essential for designing functional hydrogels that provide effective therapeutics. These hydrogels must address infections when necessary and promote angiogenesis, osteogenesis, and, ultimately, new bone formation. The following sections will discuss the state-of-the-art injectable hydrogels for periodontal regeneration, focusing on their osteogenic, antimicrobial, anti-inflammatory/immunomodulatory properties, and features related to mesenchymal stem cells and exosome delivery.

3.1 | Osteogenic Hydrogels for Periodontal Regeneration

Injectable hydrogels can form complex 3D platforms with spatiotemporal architectures and unique properties, making them highly promising for bone tissue engineering, particularly in preserving irregular bone and periodontal defect shapes. Since bones are highly vascularized and possess complex structures, promoting angiogenesis and osteogenesis is fundamental for regenerating vascularized bone that closely mimics the composition and structure of natural bone [107, 108]. Both natural and synthetic polymer-based injectable hydrogels are effectively utilized in developing drug delivery systems and regenerative therapies. Naturally occurring polymers such as chitosan, cellulose, gelatin, alginate, hyaluronic acid (HA), and dextran are favored for their structural similarities to the ECM and excellent biocompatibility [109]. The drugs' therapeutic dose and release rate are determined by diffusion, swelling, and chemical degradation. While conventional hydrogels release their contents gradually without precise control, responsive hydrogels can significantly alter their 3D network structure, swelling behavior, permeability, and mechanical strength in response to specific stimuli [110].

The properties of hydrogels are influenced by several parameters that affect their synthesis. For example, alginate has hydrophilic and negatively charged characteristics that can reduce cell attachment and osteoconductivity. Therefore, the combination of alginate with poly(vinyl alcohol) (PVA), hydroxyapatite (HAP), collagen, or chitosan creates an optimal environment for the attachment and proliferation of osteoblasts [7]. Bendtsen et al. developed an injectable hydrogel of alginate and collagen that facilitates *in situ* HAP nucleation on collagen fibers. The hydrogel exhibited higher compressive moduli and predictable ratios of phosphate to calcium (P:Ca) [111]. The enhanced mechanical properties make it a suitable substitute for bone tissue. Additionally, calcium alginate significantly increased the number of mineralized nodules and demonstrated superior osteoinductive properties [112].

Load-bearing regions of craniomaxillofacial (CMF) bone require materials that provide adequate mechanical support. Nano-HAP is widely used for bone regeneration due to its chemical composition and crystalline structure, resembling natural bone [113]. However, HAP has inherent limitations, including insufficient hardness, fragility, and flexibility, which pose challenges for its application [113]. Injectable osteoinductive materials offer a promising solution by enhancing the functionality of CMF bone while maintaining aesthetic appeal. For example, a composite scaffold made of HAP and polysaccharide-based hydrogels can be injected into tooth extraction sites to preserve the alveolar ridge in areas where aesthetics is critical [114]. Additionally, the encapsulated bioactive molecules within these hydrogels benefit from improved delivery, retention at target sites, and controlled release.

The hierarchical structure of hard cranial tissue mainly consists of HAP crystals embedded in a collagen matrix. Hydrogel biomineralization enables the formation of a hydrogel-like biomacromolecule matrix and the mineralization of amorphous calcium phosphate (ACP) nanoclusters [115]. The hydrogel stabilizes the ACP clusters as they penetrate the collagen fibrils, resulting in the intrafibrillar mineralization of the HAP nanocrystals. Ex vivo studies revealed that hydrogels laden with clusters exhibited increased bone affinity, creating a microenvironment conducive to the proliferation and differentiation of bone-forming cells. Furthermore, the effectiveness of both mechanical and biological approaches was demonstrated through experiments involving bone defects [115].

Near-infrared (NIR) light can create thermosensitive hydrogels that release bioactive substances on demand. Multiple irradiation *cycles* caused the hydrogel to break down, resulting in a microporous structure within the gel [116]. The micropores present in the material enhance the recruitment, proliferation, differentiation, and regeneration of bone-forming cells. Concurrently, when NIR light is activated, black phosphorus (BP) rapidly decomposes into phosphate ions. This process attracts calcium ions from the surrounding environment, facilitating HAP formation and accelerating biomineralization [117]. A novel injectable multifunctional calcium phosphate nanoparticle (ICPN)-coordinated poly(dimethylaminoethyl methacrylate-co-2-hydroxyethyl methacrylate) (DHCP) hydrogel loaded with parathyroid hormone (PTH) was developed. This hydrogel allows for the on-demand release of PTH and creates micropores *in situ* by using an NIR laser to induce a gel-to-solution transition. Maintaining an appropriate concentration of PTH achieves a balance between osteoblasts and osteoclasts, facilitating osteoporotic bone repair [118].

The regulation of osteogenesis and the development of new blood vessels relies on producing several key factors, including VEGF, Notch, hypoxia-inducible factor-1 alpha (HIF-1α), and platelet-derived growth factor (PDGF). These factors are produced by various mediators, such as endothelial cells (ECs), osteoblasts, osteoclasts, and chondrocytes. Under normoxic conditions, prolyl hydroxylase (PHD) inhibits the degradation of HIF-1α. Therefore, an injectable hydrogel loaded with the PHD inhibitor 1,4-dihydrophenanthroline-4-one-3-carboxylic acid (1,4-DPCA) has been shown to stimulate alveolar bone formation in a model of ligature-induced periodontitis. Notably, compared with vehicle control-treated mice, mice treated with a subcutaneous injection of the 1,4-DPCA/hydrogel mixture presented significant increases in gingival HIF-1α protein levels and enhanced bone regeneration. Additionally, the treated mice demonstrated a marked reduction in the expression of proinflammatory cytokine genes and a substantial increase in the prevalence of FOXP3+ T regulatory (Treg) cells in their periodontal tissue [119].

Bioactive, thermoresponsive injectable hydrogels simplify manufacturing but may release substances unpredictably or degrade insufficiently, hindering complex bone and cartilage healing. In contrast, ultrasound-triggered thermoresponsive hydrogels enable on-demand delivery, enhancing bone repair effectiveness. The thermally responsive P(Alg-g-NIPAAm) hydrogels maintain their structural integrity while demonstrating the ability to sustain the delivery of sodium fluorescein (NaF) and bone morphogenetic protein 2 (BMP-2) [120]. Yet, Poly(N-isopropyl acrylamide) (PNIPAAm or PN) is a standard example of a thermoresponsive hydrogel with poor mechanical characteristics, monomer toxicity, and nonbiodegradability [121]. Hence, combining it with natural materials such as chitosan improves its mechanical properties, promotes biodegradation, and regulates the gelling temperature to correspond with physiological conditions. As a result, naturally derived chitosan and poly(N-isopropylacrylamide) in conjunction with mesenchymal stem cells obtained from the infrapatellar fat pad (IFPSC) and platelet-rich plasma (PRP) demonstrated adequate mechanical support and biocompatibility for cell development and differentiation into osteochondral lineages [121].

Rapid *in situ* methods for delivering bioactive compounds in bone tissue engineering involve modifying the hydrogel scaffold through enzyme-responsive strategies. Crosslinked chondroitin sulfate-tyramine (TA) and hyaluronic acid-tyramine (HA-TA) are created using hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP). Later, the resulting injectable hydrogel system was loaded with BMP-2 and bone marrow stem cells (BMSCs) [122]. The H_2O_2 and HRP composition affected the hydrogel's gelation rate and mechanical strength. H_2O_2 decomposition produced hydrogen and oxygen, creating porosity that promotes neovascularization and hard tissue formation for bone regeneration. Additionally, controlled BMP-2 release regulates the fate of bone BMSCs [122].

Pathologic microenvironment dynamics create biochemical stimuli that increase gel sensitivity, including low pH, reactive oxygen species (ROS), and elevated matrix metalloproteinase (MMP) levels. Consequently, integrating stimuli-responsive characteristics into injectable formulations represents a promising approach. Additionally, activating biological mechanisms within the host to promote tissue regeneration, mainly through HIF-1α, is essential for effective tissue regeneration strategies [119]. A promising approach utilizing gelatin nanoparticles was employed to construct a dual-logic-based, adaptive hydrogel integrated with crosslinked PVA and a colloidal network, thereby programming the release to align with the immune-osteo cascade for enhanced tissue regeneration in diabetic bone defects. When exposed to ROS or high glucose, the PVA network can be broken down reversibly. In contrast, the colloidal gelatin network provides bioactive patterns that enhance cell affinity and facilitate MMP-induced disintegration, thereby implementing a responsive "diagnostic" logic. This results in multidimensional orchestration combining immunoregulation and osteogenesis for diabetic bone regeneration [123].

Electric stimuli effectively trigger molecule release. Bioelectricity governs cell fate and behavior in electroactive tissues like bone, which are inherently conductive [124]. Consequently, fabricating these tissues requires conductive scaffolds that replicate the architectural, mechanical, and biochemical properties of the ECM [125]. Polypyrrole nanoparticles synthesized via emulsion polymerization encapsulate drug compounds, creating an innovative drug delivery system responsive to temperature and electric fields for targeted and controlled release [125]. These conducting polymer nanoparticles act as drug reservoirs within a temperature-responsive hydrogel that remains liquid at low temperatures and solidifies at body temperature for localized

injection. Due to their large surface area, nanoparticles can load and release drugs with greater sensitivity when exposed to a weak external electric field. Research has demonstrated that conductive nanoparticles can form hybrid "smart" drug delivery systems [125].

Moreover, pH-responsive hydrogels offer new treatments for chronic diseases like tumors, diabetes, and inflammation by targeting their unique pH levels [126]. Injectable and self-healing hydrogels enable flexible, continuous drug release. For instance, polysaccharide-based hydrogels are created through a network of dynamic Schiff bases and crosslinked coordination bonds. The duration required for a gel to transition into a solution can be regulated using a double-dynamic network that reacts to stimuli, causing a change in its molecular structure. This gel-to-solution transition process ensures adaptability and sustained drug release [126]. Additionally, utilizing disulfide bonds to construct interconnected hydrogel networks has emerged as an effective method for producing degradable and self-healing hydrogels. These disulfide-crosslinked hydrogels exhibit redox-responsive gelation and degradation, facilitating cell growth and offering adaptable release profiles. Consequently, they function as promising injectable carriers for tissue regeneration [127].

ECM secretion increases with larger hydrogel pore sizes, enhancing cell growth and penetration within the 3D structure. The microscale features of pores and clusters shape the hydrogel's microarchitecture, affecting its orientation, aggregation, and function [128, 129]. Controlling this microarchitecture and composition is crucial for engineered tissue properties, as it supports MSC attachment, growth, differentiation, and nutrient transfer. Uniformly dispersing MSCs in injectables and combining them with other bioactive factors significantly improves bone regeneration [130]. In addition to the biophysical signals provided by hydrogels, incorporating angiogenic stimulators or features that support angiogenesis is beneficial for bone engineering. For example, magnesium ions (Mg2+) promote the formation of new blood vessels, attract progenitor cells to the bone microenvironment, and stimulate the endogenous expression of VEGF [131]. Hydrogels enhance angiogenesis and encourage bone regeneration by supporting new blood vessel formation, which delivers essential growth factors to defect sites for effective bone repair. However, loading angiogenic factors is challenging, and it is crucial to ensure their effectiveness while minimizing side effects from high concentrations. Chemokines may be better alternatives to growth factors as they indirectly regulate angiogenesis with more controlled effects.

Unlike traditional bone grafts, a thermosensitive hydrogel can be injected into deep periodontal pockets, allowing for *in situ* formation and filling of alveolar bone defects. Jiang et al. developed porous, injectable thermosensitive hydrogels including magnesium ions by adding magnesium particles (MPs) to a glycerophosphate solution and adding a chitosan solution. The incorporation of MPs created interconnected pores within the hydrogel, which exhibited high cytocompatibility, proliferation, spreading, and osteogenesis *in vitro* [132]. In a rat periodontitis model, Mg^{2+}-containing hydrogels reduced inflammation, inhibited osteoclast activity, and prevented alveolar bone loss, as demonstrated by micro-CT and histological analysis (Figure 4). These thermosensitive, porous hydrogels show promise for treating periodontitis. Additionally, hydrogels that release cobalt ions in a controlled manner have been proven to enhance bone formation and new blood vessel growth in a rat calvarial defect model [133].

The delivery of stem cells was also improved via injectable, thermosensitive chitosan/gelatin/glycerol phosphate hydrogels [134], and a complex system of iPSC-BMP-6 hydrogels promoted osteogenesis and PDL-like tissue formation [135]. Unlike new bone synthesis and PDL regeneration, combining iPSCs and BMP-6 resulted in a noticeable increase in bone volume due to their synergistic effects, resulting in significant mineralization, trabecular number, and thickness. An injectable hydrogel, which can form *in situ*, was prepared by covalently immobilizing BMP-2 through the site-specific incorporation of O-propargyltyrosine (OpgY) into BMP-2 and methoxy polyethylene glycol-(polycaprolactone-(N3)) block copolymer (MC-N3) [136]. The quantitative preparation of covalently immobilized BMP-2 on an MC hydrogel was achieved via a Cu(I)-catalyzed click reaction, with a straightforward biorthogonal reaction involving alkyne groups on BMP2-OpgY and azide groups on MC-N3. When injected into animals, the hPLSC-loaded MC-BMP2 produced a hydrogel virtually instantly and induced increased levels of calcium deposition [136]. A bioink hydrogel has been created via shear dilution, closely mimicking the body's microenvironment and enabling self-healing at the cellular level.

The acellular bone matrix (ABM) is gaining popularity as a replacement for recipient bone tissue due to its minimal immunogenicity and osteoinductive characteristics. Nevertheless, the brittle ABM granules may migrate out of the cavity defect, causing significant inflammation and excessive discomfort. Therefore, combining the benefits of intrinsic osteoinductive ABM granules and injectable thermosensitive smart hydrogels, poly(ethylene glycol)-poly(-caprolactone)-poly(ethylene glycol) (PECE) hydrogel ABM/PECE composites may be essential for mending cranial lesions [137].

The extrusion-based bioprinting of hydrogel has gained popularity for producing high-resolution structures while protecting cells from damaging shear forces. Nasim et al. suggested that glycosaminoglycan nanoparticles (GAGNPs) interact with negatively charged groups on laponite (LA), facilitating the rapid development of nanocomposite hydrogels [138]. The shear-thinning properties of the hydrogel help protect encapsulated cells during the bioprinting process [138]. In situ crosslinked and shear-thinning hydrogels are promising as injectable materials because they can adapt to irregular defects and be applied via less invasive techniques. However, hydrogels' poor mechanical and biological properties constrain their clinical applications despite their shear-thinning ability, making them injectable. Liu et al. developed a method to enhance bone regeneration via injectable nanocomposite hydrogels. They accomplished this by growing calcium phosphate (CaP) nanoparticles and nanocomposite particles (ICPNs) in situ through the radical polymerization of dimethylaminoethyl methacrylate (DMAEMA) and 2-hydroxyethyl methacrylate (HEMA) in a poly(dimethylaminoethyl methacrylate) (PDH) matrix. The hydrogel's injectability and mechanical properties were significantly enhanced, demonstrating a tensile strength of 321.1 kPa and a fracture toughness of 29.0 kJ/m^2, which exceed those of

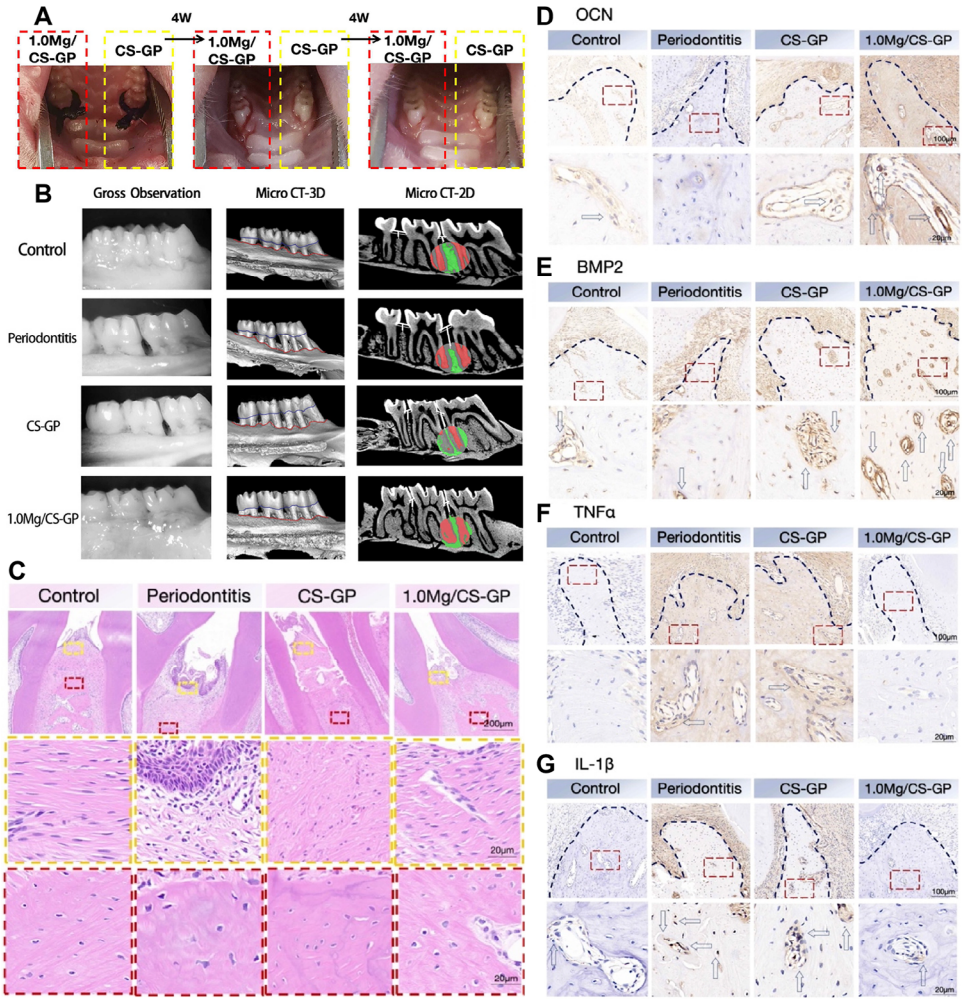

FIGURE 4 | (A) Photographs illustrate the treatment of an experimental periodontitis rat model with either 1.0Mg/CS-GP hydrogel (red boxed) or CS-GP hydrogel (yellow boxed), along with the associated soft tissue recovery. (B) Gross views, three-dimensional reconstructions, and two-dimensional micro-CT slices of the maxillae from all groups are shown; the white vertical line marks the distance between the cementoenamel junction (CEJ) and the alveolar bone crest (ABC). (C) H&E-stained sections reveal the status of the periodontal tissues across the different groups. (D,E) Immunohistochemical staining for osteocalcin (OCN) and BMP2 evaluates bone regeneration, while (F,G) staining for TNF-α and IL-1β assesses the anti-inflammatory response. In these images, the blue line delineates the edge of the alveolar bone, and blue arrows highlight areas of positive staining. Reprinted (adapted) with permission from [132]. Copyright 2024 - KeAi Publishing.

hydrogels made with in situ prefabricated calcium phosphate nanoparticles (ECPNs) [118].

Excessive ROS can damage the periodontium and cause unpredictable inflammatory responses, so using antioxidants to eliminate ROS and their abnormal effects is an effective treatment for periodontitis. Findings have demonstrated that P-113, which consists of 12 amino acids (AKRHHGYKRKFH), is the minimal peptide that retains Histatin 5 activity. Additionally, Nal-P-113, created by substituting the bulky nonnatural amino acid naphthylalanine (Nal) for histidine 4, 5, and 12 in P-113, exhibits greater stability and antibacterial activity than P-113 under body fluid conditions [139]. Moreover, polydopamine nanoparticles (PDNPs) are mussel-inspired organic biopolymers with suitable biocompatibility, low toxicity, and notable antioxidative and anti-inflammatory properties [140]. Taking advantage of these properties, Ma et al. formulated an antibacterial and antioxidant therapeutic by integrating antimicrobial peptides (Nal-P-113) and/or antioxidants, such as polydopamine nanoparticles (PDNPs), into chitosan-based hydrogels [141]. The release kinetics demonstrated that Nal-P-113 and PDNPs released steadily for up to 13 days. The scavenging activity of the hydrogel against DPPH was ≈80%, and its antibacterial ratio against *Streptococcus gordonii* (*S. gordonii*), *Fusobacterium nucleatum* (*F. nucleatum*), and *Porphyromonas gingivalis* (*P. gingivalis*) was ≈99%. Composite, a self-assembling peptide in sodium alginate, resulted in a hydrogel that displayed a nanofibrous structure similar to the ECM of natural bone. The composite hydrogel exhibited thixotropic behavior, biocompatibility, and a high storage modulus of ∼ 10 kPa. In addition, MC3T3-E1 preosteoblast cells demonstrated osteogenic differentiation and bone regeneration properties [142].

Furthermore, because ROS inhibit bone formation, reducing them is essential for periodontal tissue regeneration. To that end, a thermosensitive hydrogel containing dithiothreitol (DTT) and stromal cell-derived factor-1 (SDF-1) was developed. This hydrogel effectively decreases cellular ROS levels and reactivates the Wnt/β-catenin pathway to restore the osteogenic

potential of osteoblasts under inflammatory conditions [143]. Additionally, long-term aspirin use, which reduces immune cell activity during inflammation, has enhanced bone repair, and an injectable thermoresponsive hydrogel combining miR222 and aspirin with mesoporous silica nanoparticles (MSNs) utilizes a two-stage reaction for effective cellular transfection, promoting both innervation and bone repair in a mandibular lesion model [144].

Quercetin effectively reduces oxidative stress in orofacial mesenchymal stem cells (OMSCs) *in vitro* and promotes bone-forming growth by regulating the m6A modification of the Per1 gene. However, systemic medications have limited effectiveness, and local treatments face challenges in the small, moist, and dynamic environments of periodontal tissues. To address this, a biocompatible injectable hydrogel loaded with quercetin was developed to enhance bone repair. Composed of nanoscale bioglass microspheres and a light-cured matrix, this hydrogel ensures effective drug loading and retention in the periodontal area. Additionally, it stimulates the osteogenic differentiation of OMSCs in a Per1-dependent manner, thereby promoting the repair of periodontal bone (Figure 5) [145].

Alternatively, an ultrasound-triggered thermosensitive hydrogel is a method for delivering bone-repairing therapeutic chemicals on demand. This dual stimulation method could supply thermoresponsive hydrogels to the injury site. Using ultrasound could also enable the controlled release of hydrogel therapeutics, such as growth factors and other bioactive molecules [120]. The hydrogel can preserve responsiveness and injectability despite retaining high mechanical strength and bioactivity. HA-based hydrogels containing nHAps have been developed for mandibular bone augmentation in rats through minimally invasive surgery. Furthermore, injectable hydrogels serve as carriers for drugs with low oral absorption or bioavailability, as well as those associated with severe side effects, such as alendronate (ALN), by providing time-controlled release that minimizes immune responses. They exhibit exceptional biocompatibility and offer significant clinical potential for oral implantation [146–148].

Hydrogels can conform to the uneven contours of biological tissue and degrade slowly, supporting bone growth and providing long-term mechanical support at bone defect sites. They degrade in a controlled manner, enabling cell migration and tissue healing [149, 150]. Many hydrogels use peptide modifications to enhance matrix disintegration at specific proteolytic sites. Additionally, incorporating RGD and MMP-sensitive regions into a PEG backbone creates bioactive patterns that promote cell attachment [151]. Recent findings indicated that enhanced cell adhesion and increased expression of osteogenic markers are present in hydrogels. The innovative composite hydrogel developed by Ingavle et al. combines two naturally occurring polymers, alginate and hyaluronate, and includes biomineralized polymer microspheres. This composite hydrogel markedly improved mesenchymal stromal cells' proliferation and osteogenic differentiation (MSCs). Additionally, incorporating the adhesion tripeptide arginine-glycine-aspartic acid (RGD) from these polymers further enhanced osteogenic differentiation *in vitro*. Moreover, studies have revealed significant increases in blood vessel density and osteoid development [152]. Overall, multifunctional hydrogels with various mechanical and physical properties, as well as enhanced self-healing and degradation properties, are substantial factors in the functional success of bone tissue regeneration.

3.2 | Antibacterial Hydrogels for Periodontal Regeneration

Periodontitis, a common condition marked by the host's inflammatory response to plaque bacteria, is challenging to treat because periodontal defects have irregular shapes and involve complex healing processes with infections and inflammation [150, 153]. Current treatments, such as scaling, flap surgery, and guided bone and tissue regeneration, can reduce probing depth and restore periodontal attachment, but not fully regenerate the damaged periodontium [110]. Scaffolds loaded with antibacterial and anti-inflammatory agents show promise for engineering bone and periodontal tissues. However, the rise of drug-resistant strains and the declining effectiveness of these scaffolds over time may require multiple applications and more invasive procedures. Consequently, injectable hydrogels with targeted actions and inherent antibacterial properties have attracted significant attention in biomedicine.

The high viscosity of commonly utilized drug carrier biomaterials often limits their ability to effectively permeate small crevices in deep and irregular periodontal pockets. Furthermore, elevated polymer concentrations may pose cytotoxic risks when applied locally. To address these challenges, Wang et al. developed an innovative antibacterial and anti-inflammatory treatment by combining doxycycline (DOX) and/or lipoxin A4 (LXA4) with a 0.5 wt% thermoreversible polyisocyanopeptide (PIC). This polymer produces hydrogels at low concentrations, and its thermoreversible properties increase its suitability for application within periodontal pockets. This formulation was then tested on dogs suffering from naturally occurring periodontitis. The gels that included LXA4 and/or DOX significantly decreased subgingival bacterial loads and reduced levels of the pro-inflammatory cytokine interleukin-8. Moreover, PIC-DOX and PIC-DOX + LXA4 enhanced gingival clinical attachment by 0.6 mm compared to mechanical debridement alone [154].

In another study, researchers developed an injectable hydrogel system (GPM) comprising gelatin, Ti3C2Tx MXene nanosheets, and poly-l-lysine, a broad-spectrum antimicrobial peptide, through an enzymatic cross-linking method. Incorporating MXene nanosheets provided anti-inflammatory effects and improved the scaffold's osteoconductivity, while poly-l-lysine gave robust antibacterial activity against *Porphyromonas gingivalis*. The GPM hydrogels exhibited favorable biodegradability, wet adhesive properties, and porous structures and showed no toxicity *in vitro*. In a periodontal disease model, they significantly inhibited bacterial growth, scavenged reactive oxygen species, reduced inflammation, and promoted bone regeneration. Remarkably, alveolar bone volume and height, as well as the arrangement of junctional epithelium in the GPM-treated group, approached levels seen in healthy controls, accompanied by increased osteocalcin immunolabeling. This indicates that the features of GPM create an optimal environment for periodontal healing [155–157].

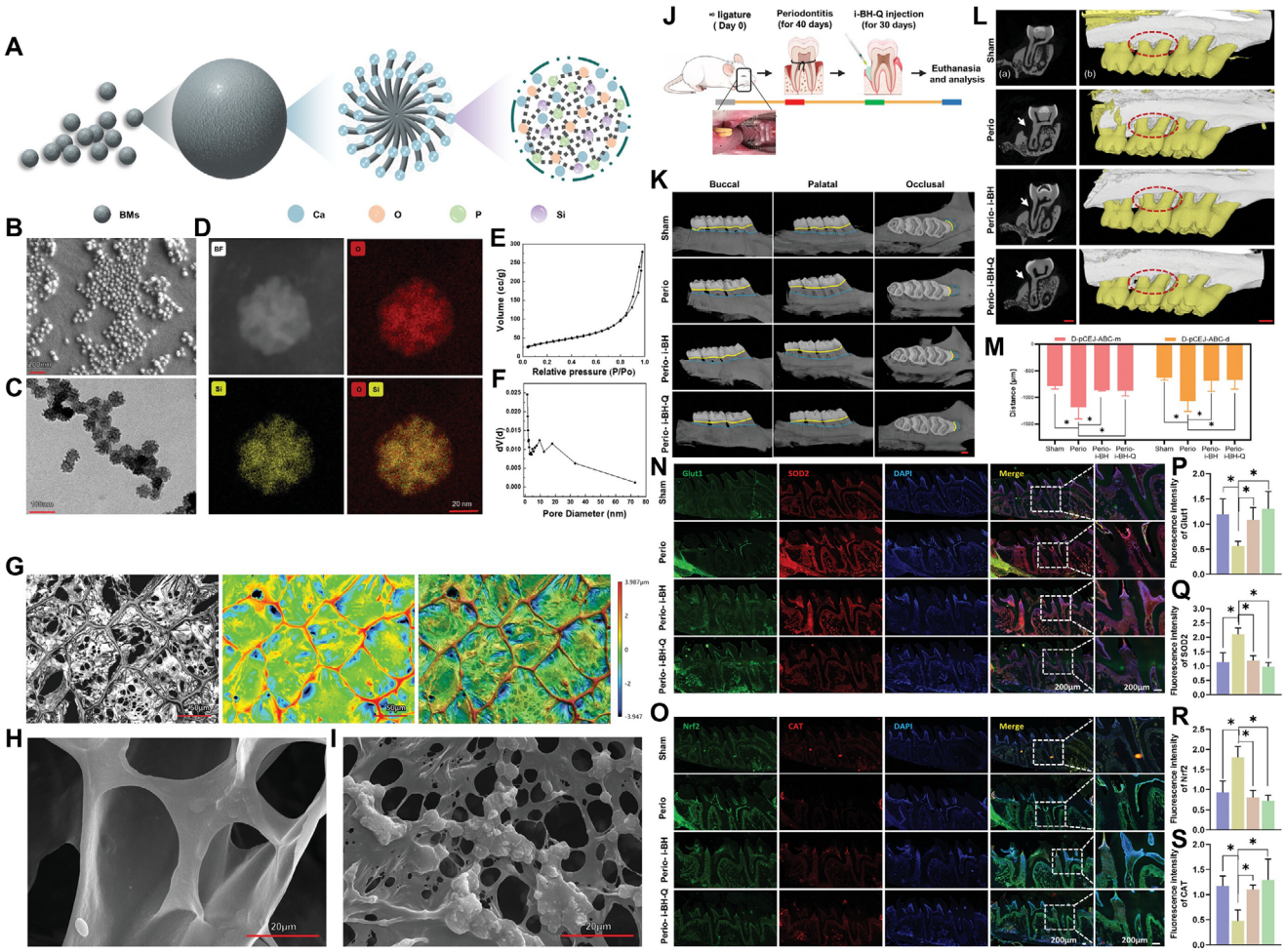

FIGURE 5 | (A) Schematic overview illustrating the synthesis of bioglass microspheres (BMs). (B–D) Representative images of the BMs were obtained by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDS). (E,F) Nitrogen adsorption-desorption isotherms and the corresponding pore size distribution curves for the BMs. (G) A confocal microscopy image displays the topographical features of the hydrogel. (H) SEM image of the hydrogel alone, and (I) SEM image of the hydrogel loaded with bioglass. (J) Diagram outlining the *in vivo* evaluation of the hydrogel's effectiveness in repairing bone defects caused by periodontitis. (K) Images of rat maxillary molars' buccal, palatal, and occlusal surfaces from the control, periodontitis, and treated groups. The blue dashed line indicates the position of the alveolar ridge, while the yellow solid line marks the enamel-dentin boundary. (L) Palatal views of maxillary molars from all groups depicting the distance from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC) on the palatal aspect of the second molar (white arrows), with a red dotted circle highlighting the CEJ-ABC distance in the 3D reconstruction. (M) Micro-CT-based quantitative analysis of the CEJ-ABC distance in the maxillary second molars. (N–S) Immunofluorescence images, corresponding statistical analyses for Glut1 and SOD2, and Nrf2 and CAT expression in the maxillary molars of rats across the control, periodontitis, and treatment groups. Abbreviations: Sham (control group), Perio (periodontitis group), Perio-i-BH (bioglass hydrogel treatment), Perio-i-BH-Q (quercetin-loaded bioglass hydrogel treatment). $^{*}p < 0.05$. Reprinted (adapted) with permission from [145]. Copyright 2024 - John Wiley and Sons.

Yang et al. created a hybrid hydrogel that combines copper sulfide nanoparticles (CuSNPs), which exhibit potent antibacterial action through near-infrared (NIR) light-activated photothermal therapy (PTT), with gelatin methacryloyl (GelMA), known for its injectability and biocompatibility [158]. CuSNPs were precipitated with chitosan (CS) and then modified with methacrylic anhydride (MA) to form CuSNP@CS-MA. This mixture was photocrosslinked with GelMA to create GelMA/CuSNP hydrogels. These hydrogels have adjustable mechanical properties and broad-spectrum antibacterial effects that can be controlled via NIR light. The hybrid hydrogels demonstrated suitable cytocompatibility *in vitro*, superior hemostatic properties, and successfully treated periodontitis in a rat model within four weeks by promoting bone and blood vessel formation [158].

The invasiveness and lack of specificity associated with intravenous antibiotics, antibiotic-releasing bone cement, and collagen sponges have prompted interest in locally delivered treatments. For example, an injectable localized drug delivery system utilizes gentamicin (GEN)-loaded poly(lactide-co-glycolide) (PLGA) nanoparticles embedded in gellan gum hydrogel [159]. The injectable method ensures continuous and consistent release of GEN at ≈60% of the initial dose over 90 days. This system has antibacterial properties and is compatible with osteoblast-like cells. Additionally, a gelatin methacryloyl (GelMA) hydrogel

exhibits beneficial biological interactions and features sites for matrix metalloproteinase (MMP) degradation, which is essential for diseases characterized by high MMP expression, specifically MMP-1, MMP-2, MMP-8, and MMP-9 [160].

Wang et al. utilized a quality-driven (QbD) approach to develop injectable thermoresponsive hydrogels that met safety and efficacy standards by effectively incorporating egg yolk immunoglobulin (IgY), an antibody produced in egg yolks in response to bacterial, viral, or parasitic infections, whose amphipathic properties inhibited periodontitis-causing bacteria and regulated immune responses, resulting in controlled release formulations that significantly reduced bacterial growth, periodontal inflammation, and alveolar bone resorption [161]. Cai et al. introduced a novel treatment for periodontitis by loading Embelin, a plant-derived compound, into an injectable hydrogel system composed of carboxymethyl chitosan-oxidized dextran (CMCS-OD). This hydrogel exhibits self-healing properties and pH responsiveness, enabling the controlled release of Embelin in the oral microenvironment. It effectively prevents bacterial invasion and reduces excessive immune responses. The hydrogel also modulates the macrophage M1/M2 phenotypes and suppresses the expression of inflammatory cytokines, promoting bone regeneration and addressing periodontitis-induced bone loss [162].

In comparison, gelatin-oxidized dextran hydrogel loaded with calcium peroxide and penicillin (CP-P hydrogel) exhibits excellent mechanical properties, low swelling, and good biocompatibility. This formulation helps reverse anaerobic conditions in periodontal pockets, eliminates drug-resistant bacteria, and promotes continuous drug release [163]. A pH-sensitive carrier enables optimal drug release in acidic environments, improving therapeutic dosage accuracy. The *in vitro* release of amoxicillin follows the Korsmeyer–Peppas equation, with chitosan-based hydrogels displaying protonated amino groups under acidic conditions (pKa = 6.2-7.0) [164]. Oxidized dextran (OD) serves as a crosslinking agent, forming a weaker Schiff base in acidic media, which contributes to its pH-sensitive characteristics [144].

In a novel approach, Riberio et al. developed an injectable, photocrosslinkable GelMA hydrogel incorporating chlorhexidine (CHX)-loaded nanotubes, acting as an on-demand CHX reservoir. This composition enables sustained, effective, and cell-friendly localized delivery of antibiotics. Additionally, the hydrogel system effectively suppresses the growth of bacteria such as *A. naeslundii*, *F. nucleatum*, *C. albicans*, and *E. faecalis* [101]. Comparably, to manage the attachment loss associated with periodontitis, Mi et al. utilized tetracycline (TC), which is covalently bonded to polylactic-co-glycolic acid (PLGA) microspheres (MSs), as a bone-affinity group. These TC-modified microspheres were then loaded with berberine (BBR) and incorporated into a thermosensitive self-healing hydrogel delivery system called BBR/TC-MS. Upon injection, the BBR/TC-MS gel quickly formed an in-situ reservoir within the periodontal pocket. The chelation between TC and the cementum in the periodontal pocket enriched the anchoring effect of TC-modified microspheres on the cementum, avoiding the wash-out effect of gingival crevicular fluids. The BBR/TC-MS gel demonstrated impressive bacteriostatic properties against *Fusobacterium necrophorum* and promoted osteogenesis in periodontal and gingival tissues [165].

Both antibacterial therapy and bone regeneration are essential for treating periodontitis. Wang et al. developed injectable, thermosensitive hydrogels with 3D networks to release an osteoinductive agent BMP-2 and near-infrared region II (NIR-II) phototherapy agents (T8IC nanoparticles). These nanoparticles, created through reprecipitation, were photosensitizers under 808 nm laser irradiation [166]. Moreover, H_2O_2 was added to PDT to enhance the antibacterial action without raising the temperature of photothermal therapy (PTT). The combination of hydrogel, T8IC nanoparticles, laser, BMP-2, and H_2O_2 at mild PTT (45°C) resulted in excellent bactericidal properties and promoted safe osteogenic induction *in vitro*, successfully promoting bone regeneration. Yang et al. developed an injectable composite hydrogel (SFD/CS/ZIF-8@QCT) that encapsulates quercetin-modified zeolitic imidazolate framework-8 (ZIF-8@QCT) (Figure 6). This hydrogel exhibits thermosensitivity and adhesion, providing excellent flowability, stability after injection, and strong tissue adhesion. Notably, SFD/CS/ZIF-8@QCT not only acts rapidly to promote hemostasis but also sustains the release of zinc ions and quercetin. These components exhibit antibacterial properties and promote bone and blood vessel regeneration, resulting in effective alveolar bone repair [167].

Short antimicrobial peptides (SAMPs) and stromal cell-derived factor-1 (SDF-1) drastically inhibit bacterial growth while attracting endogenous PDLSCs to periodontal defects. This process stimulates functional cell proliferation, osteogenic differentiation, and bone regeneration [168]. A novel functional peptide module (FPM) featuring a SAMP at its center with two lateral anchor peptides was created to provide gingipain-responsive antibacterial activity. Each anchor peptide contains an Rgp-specific splicing site. The introduction of gingipain, followed by SAMP, has proven highly effective in combating bacteria. Additionally, the hydrogel is infused with SDF-1 to attract and stimulate the development of stem cells in the host following antimicrobial treatment [168]. Collectively, a hydrogel with antibacterial properties is an effective strategy to modulate infection and promote tissue healing.

3.3 | Immunomodulatory and Anti-Inflammatory Hydrogels for Periodontal Regeneration

Subgingival biofilm is recognized as the initial trigger for periodontal inflammation; however, the excessive and dysregulated host immune response in susceptible individuals ultimately drives soft and hard periodontal tissue breakdown. This inflammatory process is quite complex, with one key feature being a significant buildup of neutrophils in the gingival tissues triggered by bacterial biofilm. As bacteria and their byproducts enter deeper tissues, other immune cells, such as macrophages, release inflammatory signals that further escalate the immune reaction [169, 170]. In particular, the proinflammatory cytokines TNF-α and IL-1β increase the production of MMPs and receptor activators of nuclear factor κB ligand (RANKL), contributing to additional tissue breakdown and bone loss. Moreover, activated neutrophils and fibroblasts produce high levels of MMPs and ROS, causing further damage to the gums. Meanwhile, various factors, including immunoregulatory abnormalities, aging, systemic diseases, environmental influences, and epigenetic modifications, can amplify the severity of this inflammatory

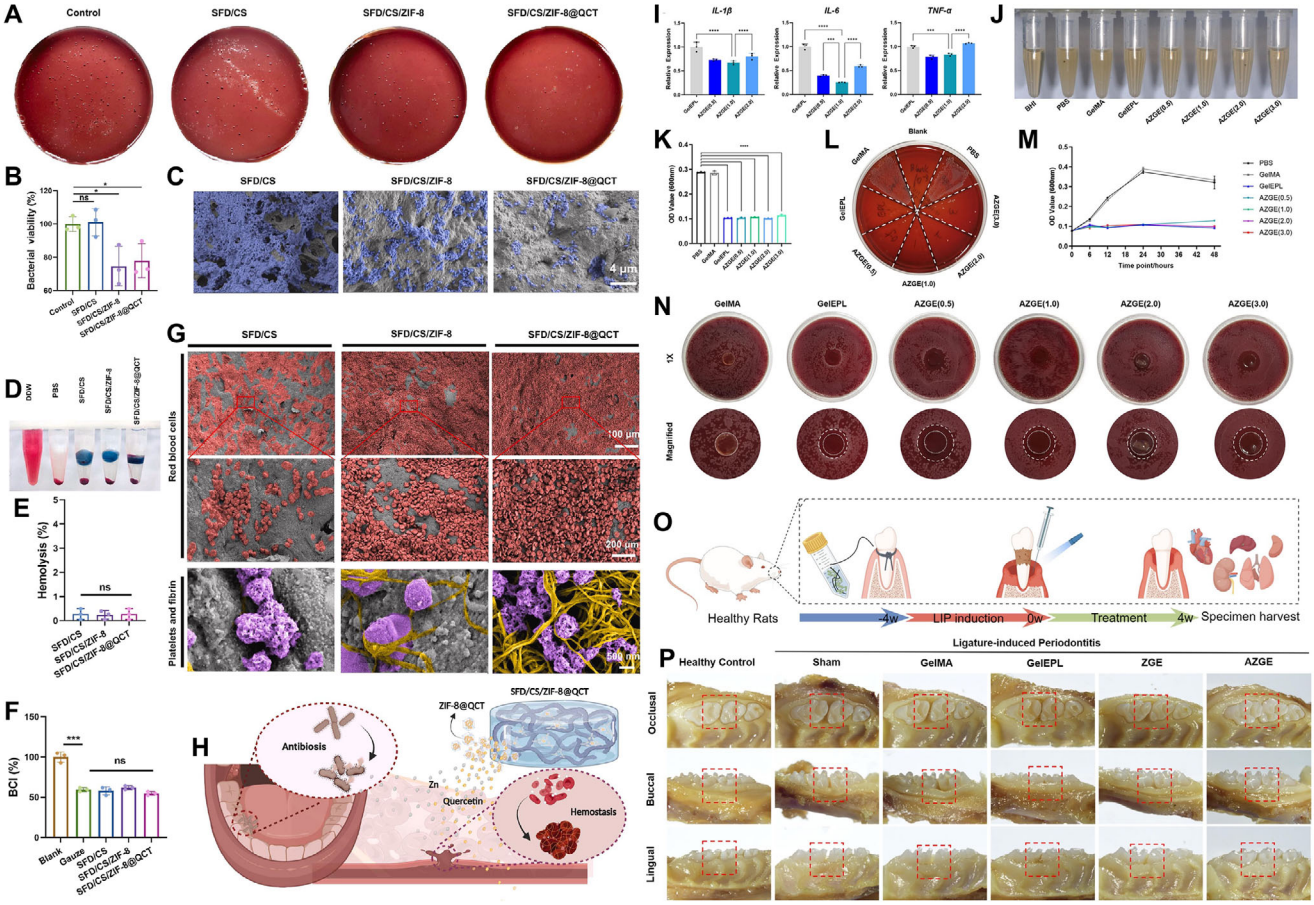

FIGURE 6 | (A) Optical images of P. gingivalis colonies grown on blood agar after 12 h of treatment with silk fibroin/chitosan hydrogel (SFD/CS), SFD/CS combined with ZIF-8, and SFD/CS loaded with quercetin-modified ZIF-8 (SFD/CS/ZIF-8@QCT). (B) Viability assays performed at 24 h revealed the antimicrobial efficacy of these formulations against P. gingivalis. (C) SEM micrographs taken at 48 h illustrate the morphological changes in P. gingivalis following treatment. (D,E) Representative optical images and quantification of hemolysis assess the blood compatibility of SFD/CS, SFD/CS/ZIF-8, and SFD/CS/ZIF-8@QCT. (F) The blood clotting index (BCI) of each formulation was determined to evaluate their hemostatic properties. (G) SEM images display the adhesion of erythrocytes, platelets, and fibrin on the surfaces of the different hydrogels. (H) A schematic diagram summarizes the dual antibacterial and hemostatic mechanisms of SFD/CS/ZIF-8@QCT. (I) Expression levels of M1 polarization-related genes (IL-1β, IL-6, and TNF-α) in RAW264.7 cells were measured to assess inflammatory responses. (J–L) Changes in the turbidity of P. gingivalis suspensions after 24 h of co-culturing with the hydrogels (J), the corresponding quantitative analysis (K), and colony formation on BHI blood agar plates (L) further demonstrate the antimicrobial activity. (M) Growth curves of P. gingivalis co-cultured with the various hydrogels were recorded at 6, 12, 24, and 48 h. (N) Inhibition zones against P. gingivalis observed after 24 h are shown, with the inner circle delineating the hydrogel disc boundaries. (O) A schematic timeline outlines the design of the *in vivo* study. (P) The overall morphology of periodontal tissues in each group is depicted, with red dotted frames highlighting the key areas between the maxillary first and second molars. Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.001$; ns, not significant. Reprinted (adapted) with permission from [167, 176]. Copyright 2024 - Elsevier. Copyright 2024 - KeAi Publishing.

response [171–173]. Because this strong immune reaction harms periodontal tissues, many treatment strategies now center on controlling inflammation to reduce or repair the damage.

Hydrogels' 3D architecture offers an excellent biological environment and, when combined with various growth factors and immune modulators, can regulate pro-inflammatory cytokines, reduce periodontal tissue damage, and foster cell proliferation and differentiation through immunomodulation, improving periodontal tissue regeneration [174]. Hydrogel delivery systems incorporating widely used anti-inflammatory drugs, such as NSAIDs, dexamethasone, statins, metformin, and anti-inflammatory plant extracts, have shown potential in controlling inflammation by regulating pro-inflammatory mediators and cytokines. In contrast, immune-modulatory agents such as cytokines and exosomes help shift macrophages and dendritic cells toward less inflammatory phenotypes [175].

All-trans retinoic acid (ATRA) has shown potential in reducing proinflammatory M1-type macrophage polarization and promoting osteogenesis, but its effectiveness in periodontitis remains uncertain due to its poor solubility and stability [176]. A study by Xiang et al. developed a nanoparticle-loaded hydrogel that combines immunomodulatory effects, osteogenesis promotion, and antibacterial properties. ATRA was integrated into zeolitic imidazolate framework-8 (ZIF-8) nanoparticles (ATRA@ZIF-8) and encapsulated in an ε-poly-l-lysine (EPL)-grafted gelatin methacryloyl (GelMA) hydrogel for local injection (Figure 6). The ATRA@ZIF-8-loaded hydrogel (AZGE) effectively reduced M1-type macrophage polarization. It promoted the osteogenic

differentiation of periodontal ligament cells (PDLCs). Moreover, the AZGE hydrogel demonstrated significant antimicrobial activity against *Porphyromonas gingivalis* and was validated in an experimental model of ligature-induced periodontitis [176].

Acetylsalicylic acid (aspirin) has been shown to enhance bone regeneration, a process often associated with its immunomodulatory effects. These mechanisms include suppressing T cells, extending the lifespan of mesenchymal stem cells (MSCs), and increasing immunomodulatory capacity. Numerous studies have investigated these pathways, highlighting the significance of osteo-immunomodulation in aspirin's role in bone repair [177–179]. Researchers prepared an injectable, thermosensitive hydrogel using chitosan (CS), β-sodium glycerophosphate (β-GP), and gelatin, which continuously released aspirin and erythropoietin (EPO) to provide anti-inflammatory and tissue-regenerative effects; this CS/β-GP/gelatin hydrogel exhibited excellent biocompatibility, and proved effective in reducing inflammation and restoring alveolar bone height (reflected by shortened CEJ-ABC distances) compared with a periodontitis control, while also relieving inflammatory damage (Figure 6). Notably, COX-2 and MMP-9 activity declined significantly in the aspirin-loaded hydrogel group, indicating that aspirin by acetylating serine residues of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibits prostaglandin E2 production and decreases matrix metalloproteinase-9 (MMP-9) activity, in addition to displaying chemotactic properties that accelerate adipose stem cell homing and thereby promote tissue regeneration (Figure 7) [180]. Similarly, Gu et al. created a periodontitis rat model to study the effects of a chitosan/β-sodium glycerophosphate (β-GP)/glycolic acid (GA) hydrogel containing EPO and FK506 (EPO-FK506-CS/β-GP/GA). The EPO-FK506-CS/β-GP/GA hydrogel showed stability and sustained drug release. Additionally, it effectively minimized inflammatory mediators' expression and upregulated the osteoblastic differentiation markers (collagen I, Runx2, OPN, and OCN), alleviating gingival inflammation and promoting periodontal tissue regeneration [181].

In another aspirin investigation, researchers developed a semi-interpenetrating network composite hydrogel by incorporating short-chain chitosan into a covalently crosslinked tetra-armed poly(ethylene glycol) network. This hydrogel-encapsulated aspirin allowed a sustained release through electrostatic interactions and chain entanglement, improving the proliferation and osteogenic differentiation of PDLSCs. In this study, continuous aspirin release from the hydrogel increased MCP-1 levels *in vitro*, while immunohistochemistry showed that macrophages recruited to defect sites were guided toward an M2 phenotype. Specifically, this biomimetic hydrogel enhanced bone repair by regulating macrophage polarization, partly via the monocyte chemoattractant protein-1 (MCP-1 / CCL2), which is secreted by various cell types under inflammatory conditions to attract monocyte-macrophage lineage cells and encourages M2 polarization, thereby reducing bone resorption in periodontitis and aiding bone formation [182].

Another drug frequently used is dexamethasone (DEX), a potent steroidal anti-inflammatory and immunosuppressive agent, which is widely utilized in biomaterial design to mitigate immune responses, promote the differentiation of bone marrow-derived stromal cells and mesenchymal stem cells, and support the proliferation of periodontal membrane stem cells for hydrogel loading and applications in periodontal tissues [183–185]. Li et al. introduced a strategy for creating functional hydrogels using pH-responsive nanoparticles loaded with DEX. In a rat periodontitis model, these nanoparticles served as vehicles for dexamethasone and transitioned into hydrogels within the acidic periodontal environment. By day 21, micro-CT scans revealed significant bone loss on the buccal side in untreated rats, whereas treatment with these nanoparticles notably mitigated this damage (Figure 6). Quantitative analysis of the distance between the CEJ and the ABC confirmed improved outcomes post-treatment, and TNF-α, IL-6, and MCP-1 expressions were significantly downregulated (Figure 7). Histological assessments using H&E, Masson, or tartrate-resistant acid phosphatase (TRAP) staining further demonstrated reduced inflammatory infiltration, lower bone absorption, and fewer periodontal pockets in the treatment group (Figure 7) [186].

Xie et al. created an injectable nanocomposite hydrogel by encapsulating DEX-loaded zeolitic imidazolate frameworks-8 (DZIF) nanoparticles within a photocrosslinked matrix of methacrylic polyphosphoester (PPEMA) and methacrylate gelatin (GelMA) [187]. This hydrogel could be readily injected into deep periodontal pockets, enabling high local DEX concentrations. *In vitro* demonstrated potent antibacterial activity, preserved cell viability, supported proliferation and osteogenesis, and suppressed inflammatory gene expression. In a rat periodontitis model, micro-CT scans and histological assessments confirmed that the nanocomposite hydrogel effectively reduced periodontal inflammation and prevented bone loss (Figure 7).

Statins, a class of drugs typically used to manage hyperlipidemia and cardiovascular diseases, including atorvastatin, rosuvastatin, and simvastatin, have also shown promise in treating inflammatory and immune-mediated conditions such as periodontitis. Their anti-inflammatory properties involve suppressing pro-inflammatory cytokines and enhancing the release of anti-inflammatory and pro-resolution molecules, primarily through modulation of the ERK, MAPK, PI3-Akt, and NF-κB pathways [188]. Chen et al. improved the therapeutic potential of simvastatin, a lipid-lowering medication known for its anti-inflammatory and bone-forming effects, by encapsulating it in a thermoresponsive hydrogel made from pyrophosphorylated Pluronic F127, a triblock copolymer of ethylene oxide and propylene oxide [189]. In a ligature-induced periodontitis rat model, this simvastatin-loaded hydrogel reduced periodontal inflammation and preserved alveolar bone (Figure 8). At the molecular level, statins appear to exert their anti-inflammatory and bone-regenerative benefits through suppression of MMP-9 and TNF-α, along with increased expression of IL-10, IL-1 receptor-like 1 (IL1rl1), IGF-1, and BMP-2 [190].

Moreover, in two randomized controlled trials, Pradeep et al. assessed the clinical outcomes of a methylcellulose-based gel containing 1.2% simvastatin (SIM) as an adjunct to scaling and root planing (SRP) in patients with chronic periodontitis. Their findings showed that SRP plus locally delivered SIM resulted in more significant reductions in the gingival index and probing depth, more pronounced clinical attachment level (CAL) gains, and significant intrabony defect fill, indicating a role for SIM in promoting bone formation. Moreover, the gingival bleeding

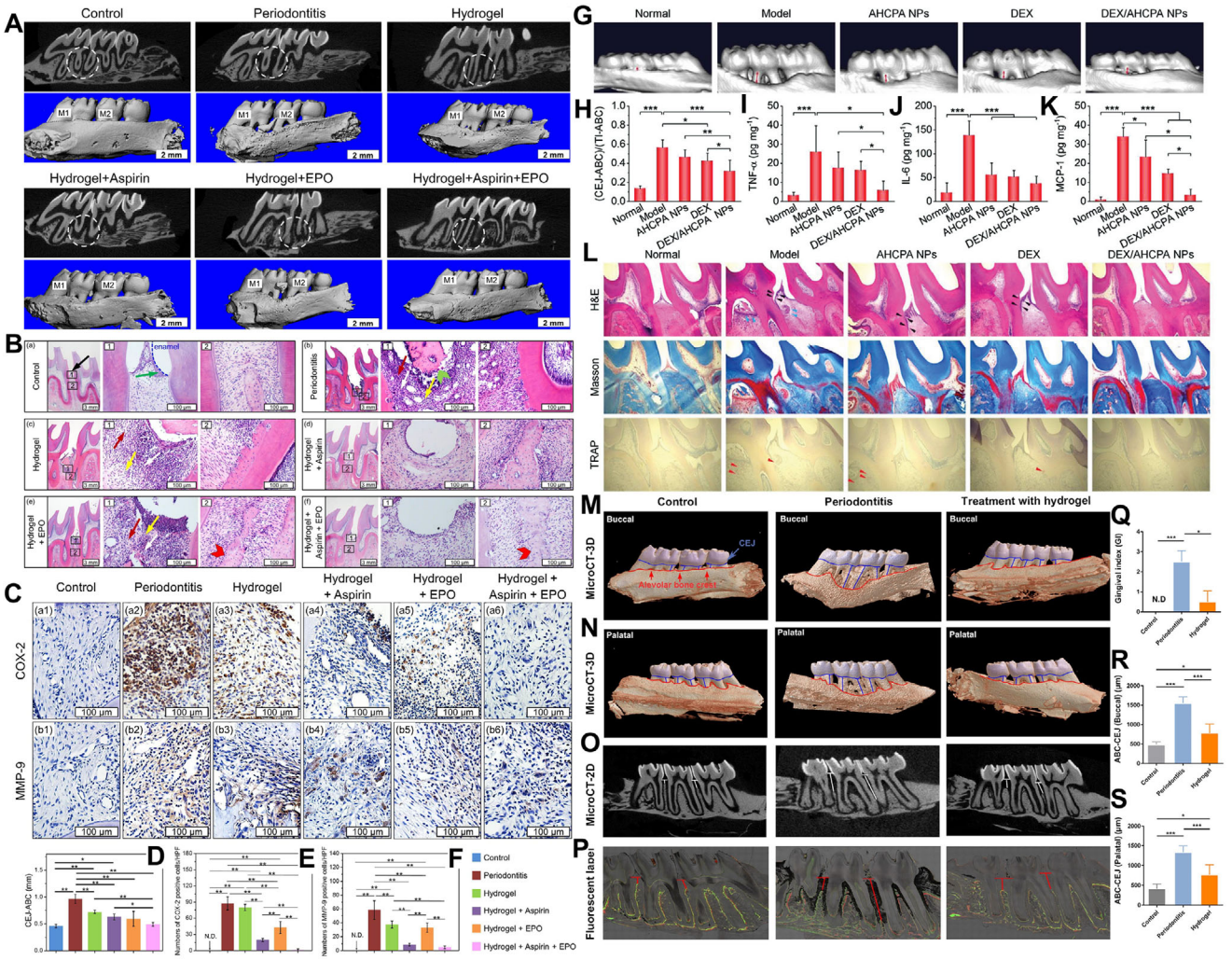

FIGURE 7 | (A) Micro-CT images of the maxillary alveolar bone surrounding the first (M1) and second (M2) maxillary molars. Images against a black background are representative sagittal micro-CT slices, while those with a blue background are 3D reconstructions. (B) H&E-stained sections of the periodontium, where black arrows indicate interdental papillae, green arrows indicate the junctional epithelium, red arrows indicate neutrophils, yellow arrows indicate lymphocytes, white arrows indicate plasma cells, and the green arrowhead identifies an osteoclast. (C) Immunolabeling for COX-2 and MMP-9, with panels (a1-a6) showing COX-2 staining and (b1-b6) showing MMP-9 staining. (D) Quantification of the distance between the cementoenamel junction (CEJ) and the alveolar bone crest (ABC) based on micro-CT data. The number of COX-2 (E) and MMP-9 (F) positive cells immunolabeled between M1 and M2. (G) Treatment of periodontitis via triggerable AHCPA NPs containing an anti-inflammatory drug dexamethasone (DEX). 3D micro-CT images of teeth and periodontal tissues of mice in different groups. The red lines with double arrowheads indicate the distance from the cementoenamel junction to the. (H) Quantification of the ratio of the exposed root's height to the entire tooth's height. The expression levels of TNF-α (I), IL-6 (J), and MCP-1 (K) in the periodontium of rats. (L) histological sections of the periodontal tissues stained with H&E, Masson, or TRAP. Black arrowheads indicate periodontal pockets, blue arrowheads denote inflammatory cells, and red arrowheads show osteoclasts. (G) illustrates the treatment of periodontitis using triggerable AHCPA nanoparticles loaded with the anti-inflammatory drug dexamethasone (DEX), along with 3D micro-CT images; red lines with double arrowheads mark the distance from the CEJ to the ABC. (H) Depicts the ratio of exposed root height to total tooth height. (I–K) Show TNF-α, IL-6, and MCP-1 expression levels in the rat periodontium. L) Displays histological sections of the periodontal tissues stained with H&E, Masson, or TRAP, where black arrowheads denote periodontal pockets, blue arrowheads indicate inflammatory cells and red arrowheads highlight osteoclasts. (M, N) show illustrative 3D reconstructions and (O) 2D cross-sectional images captured by micro-CT; (P) demonstrates sequential fluorescence labeling of bone formation in non-decalcified bone sections, with Alizarin Red S in red and calcein in green; (Q) provides the statistical analysis of gingival index (GI) based on standardized scoring criteria; and (R, S) depicts measurements of the vertical distance between the ABC and CEJ. Reprinted (adapted) with permission from [180, 186, 187] Copyright 2019 and 2022 - Elsevier. Copyright 2022 - John Wiley and Sons.

index decreased from baseline to six months, suggesting an anti-inflammatory effect of SIM [191, 192]. Rosuvastatin (RSV) is another commonly formulated statin used in hydrogels. In one study, a sub-gingivally delivered 1.2% RSV methylcellulose gel resulted in more significant reductions in probing depth and gingival index, as well as higher CAL gains, compared to a placebo [193]. Similarly, an alginate-based RSV hydrogel demonstrated notable improvements in clinical parameters such as the modified sulcus bleeding index (mSBI), PD, and CAL [194].

In other trials, Pradeep et al. evaluated a 1.2% atorvastatin (ATV) in situ methylcellulose-based gel as an adjunct to SRP in patients

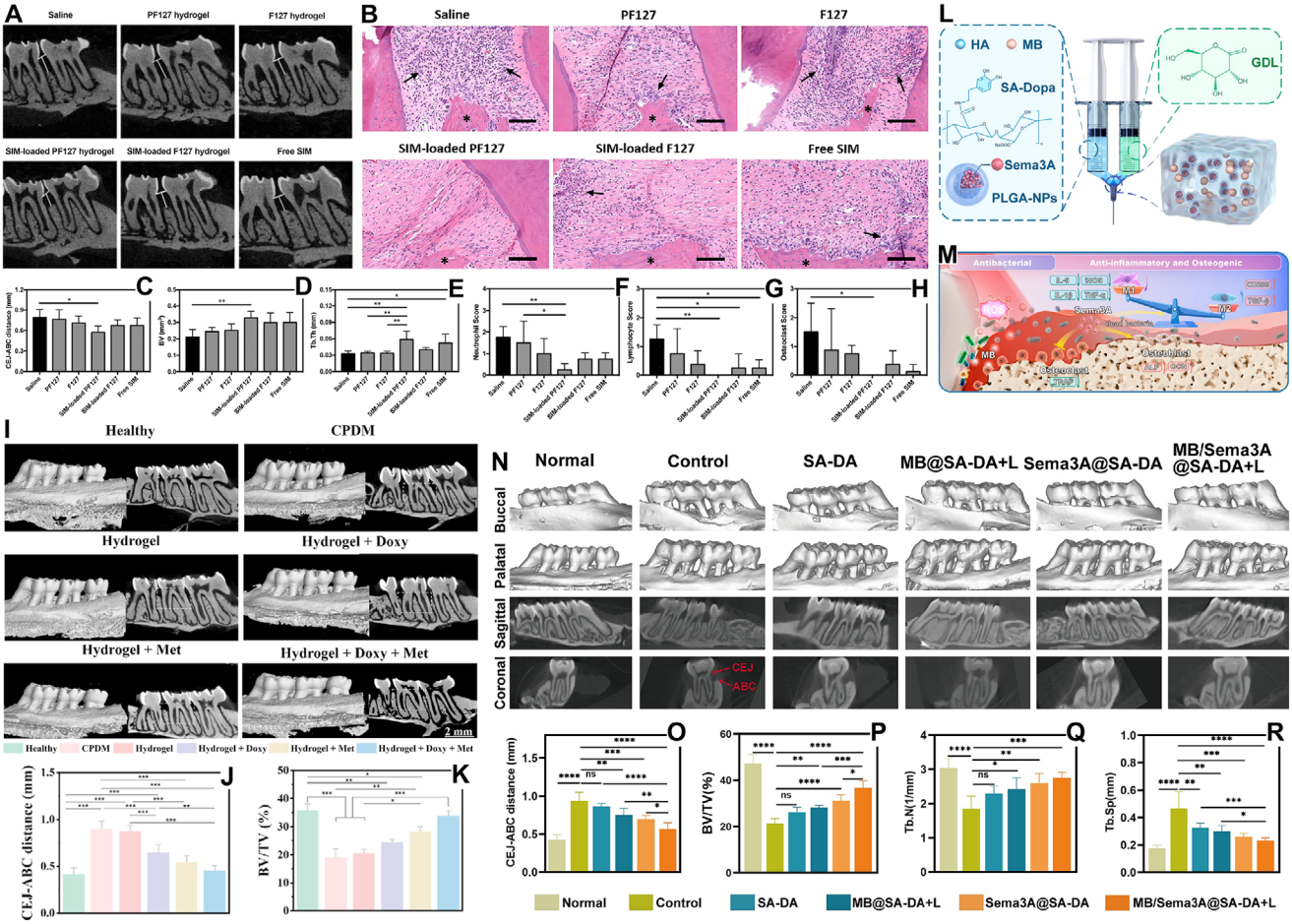

FIGURE 8 | (A) Representative micro-CT sagittal images of the maxilla after three weeks of treatment, with white lines indicating the distance from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC). (B) Histological views of connective tissue and alveolar bone between the first (M1) and second (M2) molars in different treatment groups, where asterisks mark the ABC and black arrows highlight inflammatory infiltrates (scale bar = 100 μm). (C) The CEJ-ABC distance measurement shows that the SIM-loaded PF127 hydrogel group exhibits a significantly shorter distance than the saline control group. (D, E) Micro-CT morphometric analyses of bone volume (BV) and trabecular thickness (Tb.Th). (F–H) Qualitative scores for neutrophils, lymphocytes, and osteoclasts across all treatment groups. (I) Micro-CT images of maxillary alveolar bone around M2, with 3D reconstructions on the left and bucco-palatal sagittal slices on the right. (J) Quantification of the CEJ-ABC distance for M2. (K) Analysis of the bone volume fraction (BV/TV). (L, M) Schematic diagrams illustrating the fabrication, antibacterial, anti-inflammatory, and tissue-regeneration potential of MB/Sema3A@SA-DA. (N) Micro-CT scans of maxillary alveolar bone in each rat group. (O–R) Quantitative assessments of the CEJ-ABC distance, BV/TV, trabecular number (Tb.N), and trabecular separation (Tb.Sp). Reprinted (adapted) with permission from [189, 204, 205]. Copyright 2021 - American Chemical Society. Copyright 2022 and 2023 - Elsevier.

with chronic periodontitis [195, 196]. Compared to a placebo gel, the ATV formulation led to notable clinical and radiographic improvements, including reduced gingival bleeding, more significant reductions in probing depth, and more pronounced gains in clinical attachment levels at three, six, and nine months. Additionally, at nine months, a higher percentage of radiographic bone fill was observed in the ATV group. This suggests that local injection of 1.2% ATV gel into periodontal pockets, in conjunction with SRP, significantly enhances clinical and radiographic outcomes. In a recent study, researchers developed a thermosensitive, chitosan-based hydrogel loaded with ATV and lovastatin nanoemulsions, allowing for the localized release of both statins. When applied to *Porphyromonas gingivalis*-infected oral epithelial cells and gingival fibroblasts, these nanoemulsions significantly reduced the expression of pro-inflammatory markers (TNF-α and IL-1β) and the pro-osteoclastic protein RANKL. In a 2 mm calvarial bone defect model, treatment with this statin-loaded hydrogel led to a marked increase in new bone formation [197].

Metformin (N,N-dimethylbiguanide) is a first-line treatment for type 2 diabetes and metabolic syndrome, with immunomodulatory effects primarily attributed to its activation of AMP-activated protein kinase (AMPK). Inhibiting respiratory-chain complex 1 of the mitochondrial electron transport chain reduces ATP production, elevates the AMP/ATP or ADP/ATP ratio, and subsequently activates AMPK. This leads to suppression of mTOR signaling while also inhibiting the NF-κB pathway [198]. Furthermore, metformin has been shown to block NLRP3 inflammasome activity, thereby preventing IL-1β release in both periodontitis and diabetes [199]. Several studies have incorporated metformin (MF) in concentrations ranging from 0.5% to 1.5% into gels as an adjunct to SRP for moderate to severe chronic periodontitis 200–202. The results consistently indicate that local MF delivery significantly

enhances probing depth reduction, CAL gain, and intrabony defect depth reduction compared to placebo gels, emphasizing its effectiveness when combined with standard periodontal therapy.

Tetracycline antibiotics, such as minocycline and doxycycline, are known for their anti-inflammatory properties and have shown clinical benefits in acute and chronic inflammatory conditions, including gingivitis and periodontitis. For instance, doxycycline significantly reduces TNF-α, IL-1α, and inducible nitric oxide synthase in mouse lipopolysaccharide (LPS)-induced sepsis. It modulates the phosphorylation of NF-κB, p38, and ERK1/2/MAPK pathways to suppress cytokine and chemokine production in LPS-stimulated THP-1 cells. At sub-antimicrobial concentrations, doxycycline inhibits MMPs, further contributing to its anti-inflammatory effects [203]. One study combined oxidized dextran and phenylboronic acid-functionalized poly(ethylene imine) to create a local drug delivery hydrogel system for doxycycline and metformin [204]. Compared to single-drug-loaded hydrogels, the co-delivery system resulted in shorter cementoenamel CEJ-ABC distances, indicating better tissue regeneration. In fact, the double-drug-loaded hydrogel achieved CEJ-ABC distances similar to those of healthy controls, reflecting a substantial restoration of bone mass in an animal periodontitis model (Figure 8).

In a recent investigation, methylene blue (MB) and semaphorin 3A (Sema3A) were co-loaded into a dopamine-grafted sodium alginate (SA-DA) hydrogel [205] Sodium alginate, a naturally abundant polysaccharide known for its mild gelation conditions, excellent biocompatibility, flexibility, and biodegradability, was chemically modified with dopamine to enhance its adhesive properties, making it suitable for periodontal applications. The early-stage release of MB (within 14 h) created an antibacterial environment that suppressed bacterial infiltration, while the sustained release of Sema3A (over 28 days) promoted alveolar bone regeneration, reduced bone resorption, and encouraged the transition of macrophages from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype. In a rat model of periodontitis, the in situ application of the MB/Sema3A@SA-DA hydrogel effectively provided antibacterial, anti-inflammatory, and osteogenic benefits, demonstrating the potential of this dual-delivery system for periodontal therapy (Figure 8).

A multifunctional controlled-release delivery system was developed by encapsulating self-assembled nanoparticles composed of chlorhexidine acetate and epigallocatechin-3-gallate (EGCG), a potent water-soluble polyphenol from green tea that suppresses IL-17 production, within a gelatin methacryloyl-oxidized hyaluronic acid hydrogel. *In vitro*, the hydrogel exhibited strong antimicrobial, antioxidant, and anti-inflammatory properties by scavenging reactive oxygen species and promoting the polarization of macrophages toward the M2 phenotype. Employing a rat periodontitis model showed biocompatibility, effectively preventing alveolar bone loss while reducing periodontal inflammation, inflammatory infiltration, and collagen degradation [206]. In another investigation, researchers developed an injectable, UV-light-photopolymerizable chitosan methacryloyl (CMSA) hydrogel carrying Pep-B, a functional fragment of human β-defensin 1 (HBD1), an endogenous cationic antimicrobial peptide highly effective against Gram-negative bacteria and involved in innate immune defense and inflammation regulation. In a periodontitis rat model, the application of CMSA/Pep-B notably alleviated the local inflammatory response, reduced osteoclast numbers, and minimized collagen destruction, as evidenced by micro-CT and histological staining. The alveolar bone in the CMSA/Pep-B group was less damaged and resembled normal bone more closely than in the untreated periodontitis group [207].

Minocycline hydrochloride (MH)-loaded glycopeptide hydrogel (MH/GRWgel) was engineered to address alveolar bone loss by delivering sequential antibacterial and RANKL-blocking effects. The hydrogel, composed of oxidized glucomannan (GM-CHO) and the self-assembling peptide RADA16 (RADA), was coassembled with W9, a peptide resembling the cysteine-rich domain of TNF receptor type I, to bind RANKL and suppress osteoclastogenesis specifically. In a rat periodontitis model, the treatment significantly reduced disease progression by inhibiting bacterial biofilm formation, reducing alveolar bone resorption, promoting bone regeneration, and alleviating inflammation [208].

Xu et al. developed a TM/BHT/CuTA hydrogel system through the self-assembly of copper-based nanozyme nanosheets (copper tannic acid coordination nanosheets, CuTA NSs) within a triglycerol monostearate/2,6-di-tert-butyl-4-methylphenol (TM/BHT) hydrogel. The released CuTA nanozyme demonstrated antibacterial properties and scavenged multiple reactive oxygen species by mimicking superoxide dismutase (SOD) and catalase (CAT) activity. Furthermore, it modulated macrophage polarization from the M1 phenotype to the M2 phenotype via the Nrf2/NF-κB pathway, thereby reducing pro-inflammatory cytokines, increasing anti-inflammatory cytokines, and promoting osteogenic gene expression, all contributing to alleviating inflammation and accelerating periodontal tissue regeneration [209].

Moreover, researchers created an injectable, self-healing hydrogel by linking aldehyde-modified hyaluronic acid (HA-CHO) and glycol chitosan (GC) through Schiff base (imine) bonds and coordinating HA-CHO's carboxyl groups with Fe^{3+} ions. When loaded with ginsenoside Rg1 and amelogenin, this hydrogel restored alveolar bone in periodontitis, as confirmed by micro-CT, H&E staining, immunohistochemical analysis of IL-1, TNF-α, TGF-β, and TRAP staining [126]. Using a ROS-responsive hydrogel (CC-B-CAPE@CY-NPs) containing caffeic acid phenethyl ester (CAPE) and chrysin (CY) nanoparticles for periodontitis therapy. CAPE, caged by an aryl borate strategy, provided anti-inflammatory effects, while CY promoted osteogenesis. The hydrogel scavenged ROS, shifted macrophages from M1 to M2, and intensified alkaline phosphatase activity in periodontal ligament stem cells by blocking TLR4-dependent NF-κB signaling and activating Wnt/β-catenin. In a rat model, it alleviated alveolar bone loss, downregulated MMP-9, and outperformed single-agent treatments, offering a dual-function solution for inflammation suppression and bone regeneration [210].

3.4 | Delivery of Mesenchymal Stem Cells and Exosomes for Periodontal Regeneration

Stem cell therapy faces significant challenges, prompting recent research to explore innovative regenerative approaches, including cell-free therapies that utilize paracrine signaling via extracellular

vesicles (EVs). However, the body's circulatory system quickly clears EVs, limiting their capacity to remain at targeted sites for effective tissue repair. Hydrogels have been developed as ideal carriers to address this limitation due to their excellent biocompatibility and porous, flexible structures. Encapsulating EVs within hydrogels enables the enhancement of their retention and provides a controlled, sustained release, thereby improving the effectiveness of periodontal treatments [211].

Exosomes are nanosized extracellular vesicles (40–160 nm) that originate from intracellular multivesicular bodies and carry proteins, DNA, and RNA, capable of modulating the behavior of recipient cells. They can be loaded with diverse therapeutic payloads, such as small interfering RNAs, antisense oligonucleotides, chemotherapeutics, and immune modulators, and can be directed to specific targets. Their lipid and protein composition provides favorable pharmacokinetics and may help minimize adverse effects. In periodontal therapy, exosomes offer promising immunoregulatory effects by transferring biologically active molecules, such as microRNAs (miRNAs), that can alter gene expression and signaling pathways, as well as presenting surface molecules to modulate immune responses. Unlike synthetic liposomes, exosomes efficiently enter target cells with minimal immune clearance. This suggests that delivering exosomes via hydrogels could be a potent, biocompatible strategy for treating periodontitis and promoting tissue regeneration [212].

Liu et al. developed a hydrogel incorporating bone marrow mesenchymal stem cell-derived small extracellular vesicles (BMSC-sEVs) and tested it in a rat periodontitis model. Following administration, micro-CT and histological analyses revealed less alveolar bone loss, inflammation, and collagen degradation in the BMSC-sEV-hydrogel group compared to the controls. Immunohistochemical and immunofluorescence staining revealed fewer TRAP-positive cells and a lower osteoprotegerin OPG to RANKL ratio in the BMSC-sEV-hydrogel group. Conversely, transforming growth factor-beta 1 (TGF-β1) expression and the macrophage M2/M1 ratio were elevated, suggesting that BMSC-sEVs support periodontal regeneration [213]. In a recent study by Wang et al., a multifunctional hyaluronic acid-based hydrogel was developed to deliver osteoinductive extracellular vesicles derived from dental pulp stem cells. This bioadhesive, self-healing, and environmentally responsive hydrogel increased BPM-2 expression, stimulated alkaline phosphatase activity, and increased calcium nodule formation compared to hydrogel-only control and non-osteoinductive vesicles. RNA sequencing showed that these vesicles contained elevated levels of microRNA-1246, suggesting they may accelerate bone repair and have potential applications in periodontal tissue regeneration [214].

Shen et al. showed that incorporating exosomes derived from DPSCs into a chitosan hydrogel can promote the healing of alveolar bone and the periodontal epithelium in mice with periodontitis. Gene Ontology analysis revealed that this therapy suppressed inflammation and modulated immune responses by shifting macrophages from a pro-inflammatory to an anti-inflammatory phenotype, a process potentially influenced by microRNA-1246 in DPSC exosomes. These effects reduced alveolar bone loss and improved epithelial healing, emphasizing the promise of exosome-loaded chitosan hydrogel as a potential treatment for periodontitis [215].

Researchers have developed an innovative self-healing hydrogel composed of coralline HAP, silk fibroin, glycol chitosan, and difunctionalized polyethylene glycol (CHA/SF/GCS/DF-PEG), designed to deliver exosomes derived from human umbilical cord mesenchymal stem cells (hucMSCs) for bone regeneration. This novel hydrogel was tested in rat models with bone defects, demonstrating significant therapeutic benefits. Studies have shown that hucMSC-derived exosomes enhance angiogenesis and fracture healing by upregulating VEGF and HIF-1α., evidenced by higher new bone tissue formation, elevated BMP-2 expression, and increased microvessel density [216]. Although not used in a periodontal context, these findings highlight the effectiveness of this self-healing hydrogel as a potent vehicle for exosomes in bone regenerative medicine. This indicates promising applications that may be significant in other biomedical fields, including periodontal therapy.

Shi et al. demonstrated that exosomes derived from MSCs can significantly enhance periodontal tissue regeneration. Building on earlier studies, they found that an inflammatory environment triggered by LPS enhances the proliferation, differentiation, and adhesion of dental follicle cells (DFCs), which are crucial for periodontal regeneration, likely due to the influence of exosomes. They investigated the effects of LPS-preconditioned dental follicle cell-derived small extracellular vesicles (L-D-sEV) on periodontal ligament cells from periodontitis (p-PDLCs) and their potential to regenerate periodontal tissue in a rat model of induced periodontitis. Hydrogels with L-D-sEV reduced alveolar bone loss in rats after 4 and 8 weeks, outperforming D-sEV-infused ones. L-D-sEV-loaded hydrogels promoted collagen, osteogenic, and cementogenic tissue formation by upregulating COL I, OPN, Runx2, CEMP-1, and periostin, while maintaining alveolar bone levels. Additionally, TRAP staining showed a steady decrease in TRAP-positive osteoclasts from weeks 4 to 8, indicating prolonged inhibition of bone resorption [217]. Another study employing preconditioned LPS in MSCs produced extracellular vesicles (L-EVs) with enhanced immunomodulatory and reparative abilities. These L-EVs were incorporated into regenerated silk fibroin (RSF)-based injectable hydrogels and tested in a rat model of alveolar bone defects. The RSF/LAP/L-EV hydrogels effectively combined the bone-promoting effects of (Laponite) LAP with the immunomodulatory properties of L-EVs, targeting key cells such as bone marrow-derived MSCs and macrophages. This combination therapy enhanced bone regeneration, demonstrating the potential of L-EV-loaded hydrogels for advanced periodontal therapy [218].

Zhao et al. evaluated the osteogenic potential of exosomes from periodontal ligament stem cells (PDLSCs-Exos) and their effectiveness in repairing alveolar bone defects in rats using an alginate-gelatin hydrogel. *In vitro*, PDLSCs-Exos significantly enhanced the proliferation and osteogenic differentiation of bone marrow-derived mesenchymal stem cells. Rats treated with the exosome-loaded hydrogel exhibited substantially more new bone formation in alveolar defects than the control and hydrogel-only groups [219]. In a periodontitis model using miniature pigs, one study evaluated the regenerative potential of

gingival margin-derived stem/progenitor cells combined with an IL-1ra short-term releasing hyaluronic acid hydrogel synthetic extracellular matrix. Compared to negative controls, the IL-1ra-loaded and unloaded gingival margin-derived stem/progenitor cell-hydrogel groups showed significant improvements in clinical attachment level, probing depth, and gingival recession. They also had higher periodontal attachment, cementum regeneration, bone regeneration, shorter junctional epithelium, and enhanced bleeding-on-probing outcomes [220].

In another study, PDLSCs were encapsulated in injectable, photocrosslinkable hydrogels composed of gelatin methacrylate and poly(ethylene glycol) dimethacrylate, which were then efficiently fabricated using a 3D bioprinting platform. The optimized formulation was injected into alveolar bone defects in a Sprague-Dawley rat model, revealing that stem-cell-laden hydrogel significantly promoted new bone formation compared to hydrogel-only and saline controls [221]. In a critical-sized rat model, GelMA hydrogels encapsulating hPDLSCs significantly enhanced alveolar bone regeneration compared to controls at 4 and 8 weeks. Micro-CT and histological results showed increased new bone formation and higher alkaline phosphatase activity, indicating robust osteogenic differentiation. GelMA's liquid state allows it to fill irregular defects, providing a supportive microenvironment that protects hPDLSCs and promotes viability, making this approach a promising strategy for alveolar repair [222].

Induced pluripotent stem cells (iPSCs) are adult cells that have been reprogrammed to resemble embryonic stem cells, offering significant potential for tissue regeneration. In an animal model of maxillary molar defects, an injectable, thermosensitive hydrogel composed of chitosan, gelatin, and glycerol phosphate, which delivers iPSCs and BMP-6, significantly enhanced bone and cementum formation, promoted new periodontal ligament development, and decreased inflammation, indicating a promising approach to periodontal repair [135].

4 | Summary and Future Perspectives

Although current therapies for dental pulp and periodontal diseases achieve high success rates, the scientific community continues to explore alternative treatments to further enhance therapeutic outcomes. Root canals, for instance, exhibit a variety of shapes and anatomical variations, which often complicate treatment procedures and reduce success rates [223]. Similarly, periodontal defects are typically irregular, posing additional challenges for effective clinical management. Given these complexities and challenges in endodontics and periodontics, injectable hydrogels have emerged as promising biotherapeutics for regenerative dental medicine. Their versatile and biocompatible properties can support tissue regeneration, significantly improving treatment success [80].

Microorganisms are the primary cause of apical periodontitis, presenting significant challenges for endodontic treatment due to the complex anatomy of root canals and the resilient nature of biofilms. These biofilms, which often include diverse bacteria and fungi, are highly resistant to antimicrobial agents, impeding effective disinfection. Additionally, microbial metabolites can trigger inflammation and suppress immune responses, further complicating treatment outcomes. Consequently, there is an urgent need for advanced therapies and biomaterials that can enhance microbial elimination, prevent biofilm formation, and resolve inflammation [224–227]. Despite advancements in rotary and reciprocating endodontic systems, studies have shown that 59.6-79.9% of dentinal walls remain unprepared after debridement [228, 229]. Moreover, current irrigation techniques, such as passive or continuous ultrasonic irrigation, with or without intracanal dressings, cannot completely eliminate microorganisms [230]. Given that successful endodontic outcomes rely on effective clinical practices and the patient's immune response [231], injectable hydrogels emerge as promising therapeutics to enhance disinfection, support tissue repair, and address the limitations of existing methods.

Similarly, periodontitis is primarily driven by pathogenic microorganisms forming robust biofilm, making effective treatment particularly challenging. These biofilms, composed of diverse bacteria, protect the microbes from antimicrobial agents and the host's immune response, resulting in persistent infections and chronic inflammation. Current treatment modalities, including scaling and root planing, surgical interventions, and antimicrobial therapies, are frequently insufficient in eliminating biofilms and restoring periodontal health. Moreover, factors such as patient compliance, systemic health conditions, and the complex anatomy of periodontal pockets further hinder successful outcomes. Biologically tailored Injectable hydrogels have the potential to significantly improve the management and outcomes of periodontitis, as they can deliver antimicrobial and immunomodulatory agents, growth factors, and regenerative cues directly to the affected sites.

This review brought the latest advancements in injectable hydrogels for dental and periodontal tissue regeneration. While it does not cover every development in the field, it offers a comprehensive overview designed to inspire further research. The goal is to encourage the design of novel injectable hydrogels that are easy to apply, highly biocompatible, and possess antimicrobial, immunomodulatory, and regenerative properties. Such innovations will enhance dental treatments and significantly benefit patients who receive these advanced therapies in the future.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: mabi70027-sup-0001-Table1.docx.