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Exosome-loaded injectable hydrogel for regeneration of the dentin-pulp complex: An in vivo study

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ABSTRACT
Regeneration of the dentin-pulp complex has emerged as a promising approach in modern regenerative endodontics, aiming to restore the structural integrity and biological function of damaged dental pulp rather than relying solely on conventional reparative procedures. Among the innovative biomaterial-based strategies, exosome-loaded injectable hydrogels have attracted considerable attention because they combine the regenerative potential of extracellular vesicles with the supportive properties of biocompatible hydrogel scaffolds. Exosomes contain a diverse range of bioactive molecules, including proteins, lipids, and nucleic acids, which regulate cellular communication, promote angiogenesis, stimulate odontogenic differentiation, and modulate inflammatory responses. Injectable hydrogels provide a three-dimensional microenvironment that enables localized delivery, sustained release of exosomes, and effective adaptation to irregular pulp chamber anatomy. In vivo investigations have demonstrated encouraging outcomes, including enhanced pulp tissue regeneration, increased vascularization, formation of reparative dentin, reduced inflammatory reactions, and improved tissue integration. These findings suggest that exosome-loaded injectable hydrogels represent a promising cell-free therapeutic strategy capable of overcoming many limitations associated with stem cell-based therapies. Despite these advances, challenges remain regarding the standardization of exosome isolation, optimization of hydrogel formulations, large-scale production, long-term biosafety, and clinical translation. Continued preclinical and clinical investigations are essential to establish their efficacy, safety, and potential for routine application in regenerative endodontic practice.
Keywords: Exosomes; Injectable hydrogel; Dentin-pulp complex; Regenerative endodontics; Tissue engineering; Pulp regeneration; Biomaterials; Cell-free therapy; In vivo study; Odontogenesis.

Introduction

The dentin-pulp complex is a highly specialized biological unit that plays a fundamental role in maintaining tooth vitality, sensory function, nutrition, and defense against microbial invasion. The dental pulp consists of connective tissue, blood vessels, nerves, immune cells, and odontoblasts that are responsible for

the continuous formation and repair of dentin throughout the lifespan of the tooth. Preservation and regeneration of this complex have become major objectives in contemporary restorative dentistry and regenerative endodontics, as traditional treatment approaches frequently remove diseased pulp tissue and replace its function with inert filling materials rather than restoring its biological activity (Singh et al., 2026).

Deep dental caries, traumatic injuries, and bacterial infections can compromise pulp vitality by initiating inflammatory responses that may ultimately result in pulp necrosis if left untreated. Conventional therapies, including direct pulp capping, pulpotomy, and root canal treatment, have demonstrated clinical success in controlling infection and relieving symptoms; however, these procedures often fail to regenerate the native pulp tissue and its associated vascular and neural networks. Recent in vivo evidence has emphasized the importance of preserving pulp vitality whenever possible, as maintaining healthy pulp tissue enhances the long-term prognosis of the tooth and supports continued dentin formation (Singh et al., 2026).

Regenerative endodontics has emerged as a transformative field that seeks to restore the structural and functional integrity of the dentin-pulp complex through biological rather than purely mechanical approaches. This discipline integrates principles of tissue engineering involving stem cells, bioactive signaling molecules, and biomaterial scaffolds to facilitate the regeneration of functional pulp tissue. While stem cell-based therapies have demonstrated promising regenerative capacity, their clinical implementation remains constrained by challenges including donor variability, ethical concerns, immune compatibility, storage requirements, and regulatory limitations. Consequently, increasing attention has shifted toward cell-free therapeutic strategies capable of delivering comparable regenerative outcomes with improved safety and practicality.

Among these innovative strategies, extracellular vesicles, particularly exosomes, have gained significant interest because of their ability to mediate intercellular communication and regulate tissue repair. Exosomes are nanosized membrane-bound vesicles secreted by numerous cell types and contain diverse bioactive molecules, including proteins, messenger RNA, microRNA, lipids, and growth factors. These molecular cargos influence cellular proliferation, migration, angiogenesis, immunomodulation, and odontogenic differentiation, thereby promoting tissue regeneration without the risks associated with direct cell transplantation. Their low immunogenicity, high biological stability, and capacity for targeted molecular signaling make exosomes promising candidates for regenerative applications within the dentin-pulp complex.

Successful regenerative therapy also depends on the availability of an appropriate scaffold capable of supporting cell recruitment, maintaining structural integrity, and enabling controlled delivery of therapeutic molecules. Injectable hydrogels have emerged as highly attractive biomaterials because they can be introduced into irregular pulp spaces through minimally invasive procedures while conforming to the geometry of the root canal system. These hydrogels provide a hydrated three-dimensional extracellular matrix-like environment that supports cellular infiltration, nutrient diffusion, vascular development, and sustained release of encapsulated bioactive agents. When combined with exosomes, injectable hydrogels enhance vesicle retention, protect them from rapid degradation, and prolong their regenerative activity at the site of injury.

The integration of exosomes with injectable hydrogel systems represents a significant advancement in regenerative endodontics by combining biological signaling with structural support in a single therapeutic platform. Experimental in vivo investigations have demonstrated encouraging outcomes, including enhanced angiogenesis, accelerated formation of reparative dentin, reduced inflammatory responses, improved collagen organization, and restoration of pulp-like tissue architecture. Similar regenerative principles have also been demonstrated in other areas of oral tissue engineering, where biologically active agents have successfully promoted bone healing and tissue regeneration, reinforcing the potential applicability of advanced biomaterial-based therapies in dental practice (Latimer et al., 2025).

Inflammation remains a critical determinant of successful tissue regeneration, as excessive inflammatory responses may interfere with healing and extracellular matrix remodeling. Earlier investigations demonstrated that modulation of matrix degradation

and inflammatory mediators plays an essential role in preserving connective tissue integrity during healing, providing an important biological foundation for current regenerative strategies that seek to balance repair and inflammation within the dentin-pulp complex (Golub et al., 1998).

Advances in digital technologies have further strengthened regenerative dental research through improved methods of imaging and quantitative tissue evaluation. Artificial intelligence-based image analysis has enhanced the precision of radiographic assessment and may provide valuable tools for objectively monitoring mineralized tissue formation, restorative outcomes, and regenerative success during experimental and clinical investigations (Rohrer et al., 2022).

Therefore, exosome-loaded injectable hydrogels represent a promising cell-free regenerative platform capable of overcoming many limitations associated with conventional therapies and stem cell transplantation. By combining sustained bioactive signaling with biomimetic scaffold support, this therapeutic approach has the potential to promote predictable regeneration of the dentin-pulp complex while preserving tooth vitality. Continued in vivo research is essential to optimize hydrogel formulations, exosome delivery systems, therapeutic efficacy, and long-term clinical translation, thereby advancing the future of biologically based regenerative endodontic treatment (Singh et al., 2026; Latimer et al., 2025).

Biology of the Dentin-Pulp Complex and Regenerative Strategies

A. Anatomy and Physiology of the Dentin-Pulp Complex

The dentin-pulp complex is a highly specialized biological unit composed of mineralized dentin and the enclosed dental pulp, functioning together to maintain tooth vitality, provide sensory perception, and respond to injury. Dentin, produced by odontoblasts lining the pulp chamber, forms the bulk of the tooth and contains numerous dentinal tubules that facilitate communication between the external environment and the pulp tissue. The dental pulp is a richly vascularized and innervated connective tissue composed of fibroblasts, odontoblasts, mesenchymal stem cells, endothelial cells, immune cells, extracellular matrix proteins, and nerve fibers. This integrated structure supports continuous dentin formation, immune surveillance, nutrient transport, and tissue repair following injury.

The pulp is anatomically divided into four distinct zones: the odontoblastic layer, the cell-free zone of Weil, the cell-rich zone, and the central pulp core. Odontoblasts are responsible for primary, secondary, and tertiary dentin formation, while dental pulp stem cells (DPSCs) serve as a reservoir of progenitor cells capable of differentiating into odontoblast-like cells during tissue repair. The extensive vascular network supplies oxygen and nutrients necessary for cellular metabolism, whereas sensory nerve fibers mediate pain perception and initiate neurogenic inflammatory responses. The structural and functional integrity of these cellular components is essential for maintaining pulp vitality and supporting regenerative processes following pathological insults (Singh et al., 2026).

Beyond its structural role, the dentin-pulp complex functions as an active biological defense system. Odontoblasts recognize bacterial invasion through pattern-recognition receptors and initiate innate immune responses by releasing cytokines, chemokines, and antimicrobial peptides. These mediators recruit immune cells to eliminate pathogens while simultaneously activating reparative mechanisms that promote tertiary dentin formation. The coordinated interaction among odontoblasts, immune cells, endothelial cells, and resident stem cells creates an environment capable of responding dynamically to tissue injury while preserving tooth vitality.

B. Mechanisms of Pulp Injury and Healing

Pulp injury occurs through multiple etiological factors, including deep dental caries, restorative procedures, traumatic injuries, chemical irritation, and bacterial invasion. Among these, dental caries remain the most common cause of pulpal inflammation. As cariogenic microorganisms penetrate dentinal tubules, bacterial toxins diffuse toward the pulp, triggering inflammatory responses characterized by vasodilation, increased vascular permeability, leukocyte infiltration, and cytokine production. If left untreated, persistent inflammation may progress from reversible pulpitis to irreversible pulpitis and eventual pulp necrosis.

The healing response of the dentin-pulp complex depends largely on the severity and duration of the injury. Mild inflammatory stimuli can stimulate odontoblasts and progenitor cells to produce reactionary dentin, whereas more extensive injuries requiring odontoblast destruction necessitate the differentiation

of dental pulp stem cells into odontoblast-like cells responsible for reparative dentin formation. Successful healing requires adequate angiogenesis, extracellular matrix remodeling, controlled inflammation, and effective recruitment of progenitor cells capable of reconstructing damaged tissues.

Matrix metalloproteinases (MMPs) play important roles during tissue remodeling by degrading extracellular matrix components. Although controlled MMP activity is essential for normal healing, excessive activation contributes to connective tissue destruction and chronic inflammation. Golub et al. (1998) demonstrated that tetracyclines possess non-antimicrobial properties capable of inhibiting MMP activity, reducing collagen degradation, and preserving extracellular matrix integrity. These findings have significantly influenced current understanding of host modulation therapy and emphasize the importance of controlling matrix degradation during regenerative endodontic procedures.

Recent in vivo investigations have further demonstrated that preservation of pulpal vitality substantially enhances healing potential. Singh et al. (2026) reported favorable pulpal responses following silver diamine fluoride application in deep carious lesions, indicating that biologically conservative therapeutic approaches can maintain pulp vitality while supporting natural reparative mechanisms. These observations reinforce the concept that successful regenerative therapies should prioritize preservation of viable pulp tissue whenever possible.

C. Current Regenerative Endodontic Approaches

Regenerative endodontics has evolved from traditional apexification procedures toward biologically driven therapies aimed at restoring the normal architecture and function of the dentin-pulp complex. Current regenerative strategies primarily involve stem cell therapy, growth factor delivery, scaffold-based tissue engineering, and emerging cell-free therapeutic approaches utilizing extracellular vesicles such as exosomes.

Stem cell-based regeneration relies predominantly on DPSCs, stem cells from the apical papilla (SCAP), periodontal ligament stem cells, and bone marrow-derived mesenchymal stem cells. These cells possess remarkable self-renewal capacity and multilineage differentiation potential, enabling them to generate odontoblast-like cells, vascular structures, and supporting connective tissues. Nevertheless, clinical translation remains limited by concerns regarding cell harvesting, expansion, immunogenicity, regulatory approval, and manufacturing complexity.

Growth factors including vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF), and fibroblast growth factors (FGFs) regulate cellular proliferation, migration, angiogenesis, and odontogenic differentiation. Controlled delivery of these bioactive molecules enhances tissue regeneration by creating an environment conducive to cellular recruitment and extracellular matrix deposition. Similar regenerative principles have also been demonstrated in other oral tissues, where biologically active agents significantly improved bone healing during experimental in vivo studies (Latimer et al., 2025).

Scaffold-based tissue engineering provides structural support for regenerating tissues by creating three-dimensional microenvironments that facilitate cell attachment, proliferation, differentiation, and nutrient diffusion. Natural biomaterials such as collagen, gelatin, fibrin, hyaluronic acid, and chitosan offer excellent biocompatibility, whereas synthetic polymers provide tunable mechanical properties and degradation rates. Injectable hydrogels have become particularly attractive because they conform to the complex anatomy of the pulp chamber while enabling minimally invasive delivery of therapeutic agents.

More recently, cell-free regenerative strategies employing exosomes have emerged as promising alternatives to stem cell transplantation. Exosomes contain proteins, messenger RNA, microRNA, lipids, and signaling molecules capable of modulating inflammation, promoting angiogenesis, stimulating odontogenic differentiation, and enhancing extracellular matrix remodeling without introducing living cells. When incorporated into injectable hydrogels, exosomes achieve sustained localized release while maintaining biological activity, thereby improving regenerative outcomes and reducing many challenges associated with conventional cell-based therapies.

Advances in imaging technologies and artificial intelligence are also contributing to regenerative

research by improving diagnostic precision and treatment evaluation. Deep learning algorithms have demonstrated considerable potential for automated segmentation and analysis of dental structures, facilitating objective assessment of regenerative outcomes and long-term monitoring of restorative procedures (Rohrer et al., 2022). Although primarily developed for radiographic interpretation, these technologies may increasingly support future regenerative endodontic research through standardized image analysis.

Overall, the biology of the dentin-pulp complex provides the biological foundation for regenerative endodontic therapy. Understanding the interactions among odontoblasts, stem cells, immune mediators, extracellular matrix components, and vascular networks has facilitated the development of advanced regenerative strategies that move beyond conventional repair toward true tissue regeneration. Contemporary approaches integrating biomaterials, bioactive molecules, and cell-free therapeutics offer considerable promise for achieving predictable regeneration of the dentin-pulp complex while preserving long-term tooth vitality (Singh et al., 2026; Golub et al., 1998; Latimer et al., 2025).

Exosome-Loaded Injectable Hydrogel as a Regenerative Platform

A. Exosomes in Dental Tissue Regeneration

Exosomes are nanosized extracellular vesicles, typically ranging from 30 to 150 nm in diameter, that are secreted by numerous cell types and play a central role in intercellular communication. They carry a diverse cargo of proteins, lipids, messenger RNAs (mRNAs), microRNAs (miRNAs), and other signaling molecules capable of regulating cellular activities in target tissues. Unlike stem cell transplantation, exosome-based therapy represents a cell-free regenerative approach that minimizes the risks associated with immune rejection, tumorigenesis, and ethical concerns while preserving many of the therapeutic benefits of stem cells. Consequently, exosomes have become an attractive biological tool in regenerative endodontics for promoting dentin-pulp complex repair.

In dental applications, exosomes may be isolated from dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligament stem cells, bone marrow mesenchymal stem cells, and adipose-derived stem cells. These extracellular vesicles stimulate odontoblastic differentiation, enhance angiogenesis, regulate inflammatory responses, and promote extracellular matrix synthesis. Their molecular cargo activates signaling pathways involved in tissue repair, thereby facilitating the regeneration of functional pulp tissue following injury. Experimental evidence indicates that biological stimulation of dental tissues significantly improves healing responses in vivo, supporting the integration of exosome-based therapies into regenerative strategies (Singh et al., 2026).

The immunomodulatory properties of exosomes are particularly advantageous during pulp regeneration. They suppress excessive inflammatory reactions by regulating cytokine production while simultaneously encouraging the recruitment of reparative cells. This balanced immune response creates a favorable microenvironment for tissue healing and dentin formation. Similar concepts of biologically mediated tissue regeneration have been demonstrated in other dental tissues, where therapeutic agents enhanced bone regeneration and tissue remodeling in experimental animal models (Latimer et al., 2025).

B. Injectable Hydrogels

Injectable hydrogels are three-dimensional polymeric biomaterials capable of absorbing substantial amounts of water while maintaining structural integrity. Their injectable nature enables minimally invasive placement into complex pulp chamber geometries, where they subsequently undergo gelation to form a stable scaffold. Because of their high water content and extracellular matrix-like architecture, hydrogels provide an ideal environment for cell migration, proliferation, differentiation, and nutrient diffusion.

Hydrogels used in regenerative dentistry may be classified as natural, synthetic, or hybrid materials. Natural hydrogels, including collagen, gelatin, hyaluronic acid, fibrin, alginate, and chitosan, exhibit excellent biocompatibility and intrinsic bioactivity. Synthetic hydrogels such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), and poly(N-isopropylacrylamide) (PNIPAAm) provide superior mechanical strength, controllable degradation rates, and reproducible physicochemical properties. Hybrid hydrogels combine the biological advantages of natural polymers with the mechanical stability of synthetic

materials, thereby optimizing regenerative performance.

An ideal injectable hydrogel should possess adequate viscosity before injection, rapid gelation after placement, high biocompatibility, controlled biodegradability, suitable mechanical properties, and the capacity to encapsulate and gradually release therapeutic molecules. These characteristics allow hydrogels to maintain structural support during tissue regeneration while protecting incorporated bioactive agents from rapid degradation.

C. Integration of Exosomes with Injectable Hydrogels

The combination of exosomes with injectable hydrogels represents an advanced regenerative platform that integrates biological signaling with structural support. Hydrogels serve as delivery vehicles that encapsulate exosomes and release them gradually over time, thereby maintaining therapeutic concentrations at the site of injury. This controlled release system overcomes one of the principal limitations of free exosome administration, namely rapid diffusion and clearance from damaged tissues.

Several loading techniques have been developed to incorporate exosomes into hydrogels, including simple physical encapsulation, electrostatic interactions, covalent bonding, and affinity-based immobilization. Physical encapsulation is the most commonly employed technique because it preserves exosome integrity while enabling sustained release. More sophisticated methods seek to regulate release kinetics through modifications of hydrogel composition, pore size, degradation behavior, and cross-linking density.

Once delivered into injured pulp tissue, exosomes continuously release signaling molecules that stimulate odontoblast differentiation, angiogenesis, neurogenesis, and extracellular matrix synthesis. These biological processes collectively facilitate the formation of vascularized pulp tissue and reparative dentin. Additionally, exosome-loaded hydrogels regulate inflammatory mediators, preventing prolonged inflammation that could compromise tissue regeneration. The regulation of connective tissue remodeling is particularly important because excessive matrix degradation impairs successful healing. Matrix preservation through modulation of inflammatory pathways has long been recognized as a critical component of oral tissue regeneration (Golub et al., 1998).

The localized and sustained release provided by injectable hydrogels also enhances treatment efficiency by reducing the frequency of therapeutic interventions. Since the hydrogel gradually degrades as regeneration progresses, it is naturally replaced by newly formed tissue without requiring surgical removal. These properties make exosome-loaded injectable hydrogels highly attractive for clinical applications in regenerative endodontics.

D. Advantages Over Conventional Biomaterials

Exosome-loaded injectable hydrogels provide several advantages compared with conventional pulp capping materials and regenerative scaffolds. First, they offer a cell-free therapeutic strategy that avoids complications associated with stem cell transplantation while retaining potent regenerative capacity. Second, sustained exosome release ensures prolonged biological activity, resulting in enhanced cellular recruitment and tissue remodeling. Third, injectable delivery enables minimally invasive clinical procedures and excellent adaptation to irregular pulp chamber anatomy.

Furthermore, these composite biomaterials effectively promote angiogenesis, improve vascular supply, encourage odontoblast differentiation, reduce inflammatory damage, and accelerate reparative dentin formation. Their biodegradable nature eliminates the need for secondary removal procedures while supporting progressive tissue replacement. Recent in vivo investigations have demonstrated that biologically active regenerative therapies significantly enhance tissue healing and mineralized tissue formation, supporting continued development of advanced biomaterial systems for dental regeneration (Latimer et al., 2025; Singh et al., 2026).

Despite these advantages, several challenges remain before widespread clinical implementation. Standardization of exosome isolation methods, optimization of hydrogel formulations, scalable manufacturing, quality control, long-term biosafety evaluation, and regulatory approval continue to require extensive investigation. Future research should also focus on improving exosome loading efficiency, controlling release kinetics, and evaluating long-term functional outcomes through well-designed preclinical and clinical studies.

In Vivo Evaluation of Exosome-Loaded Injectable Hydrogel

A. Animal Models Used

In vivo animal studies are indispensable for evaluating the biological performance, regenerative potential, and safety of exosome-loaded injectable hydrogels before their translation into human clinical applications. These models provide valuable insights into the interactions between biomaterials and host tissues under physiological conditions that cannot be fully replicated through in vitro experiments. Selection of an appropriate animal model depends on anatomical similarities to human teeth, regenerative capacity, ease of handling, ethical considerations, and study objectives.

Rodent models, particularly rats and mice, are widely employed because of their affordability, rapid breeding cycles, and well-characterized immune systems. Rat molars are commonly used to investigate direct pulp capping and pulp exposure models, allowing researchers to evaluate inflammatory responses, angiogenesis, odontoblast differentiation, and tertiary dentin formation following hydrogel implantation. Murine models also facilitate molecular investigations owing to the availability of genetically modified strains that help elucidate signaling pathways involved in tissue regeneration.

Canine models offer closer anatomical and physiological resemblance to human dentition. Their larger pulp chambers facilitate operative procedures, biomaterial placement, and histological evaluation. Dogs have therefore been extensively utilized in regenerative endodontic research to assess pulp vitality, vascular regeneration, dentin bridge formation, and long-term tissue integration.

Large animal models, including miniature pigs and sheep, provide additional translational value because their tooth morphology, occlusal loading patterns, and pulp dimensions closely resemble those of humans. These models are particularly useful for preclinical evaluation of biomaterials intended for future clinical application, despite higher maintenance costs and ethical considerations.

The use of carefully selected animal models enables comprehensive evaluation of tissue healing, inflammatory modulation, vascularization, and biomaterial degradation. Similar experimental approaches have demonstrated successful regeneration of mineralized tissues in oral structures, highlighting the importance of well-designed in vivo investigations in regenerative dentistry (Latimer et al., 2025).

B. Experimental Design

Successful evaluation of exosome-loaded injectable hydrogels requires standardized experimental protocols that ensure reproducibility and reliable comparison among studies. Animals are typically selected based on age, health status, weight, and absence of systemic diseases that may influence wound healing. Ethical approval and compliance with institutional animal care guidelines are essential prerequisites before experimentation.

Following anesthesia, standardized pulp exposure cavities are prepared under aseptic conditions using high-speed dental burs with continuous water cooling. Hemostasis is achieved before introducing the exosome-loaded hydrogel directly into the exposed pulp chamber. The injectable characteristic of the hydrogel enables adaptation to irregular pulp spaces while minimizing mechanical trauma during application.

Exosomes incorporated into hydrogels are generally isolated from mesenchymal stem cells derived from dental pulp, bone marrow, adipose tissue, or umbilical cord tissue. These extracellular vesicles are characterized before incorporation to ensure appropriate particle size, concentration, and biological activity. Controlled release from the hydrogel matrix facilitates prolonged therapeutic effects by protecting exosomes from rapid degradation within the tissue microenvironment.

Experimental groups commonly include untreated controls, hydrogel-only groups, exosome-only groups, and exosome-loaded hydrogel groups to determine the specific contribution of each component. Observation periods range from one week to several months depending on the regenerative objectives and anticipated healing timeline.

Standardized in vivo methodologies have become increasingly important in dental regenerative research, as demonstrated by investigations evaluating biological responses of pulp tissue following novel therapeutic interventions (Singh et al., 2026).

C. Outcome Assessment

Evaluation of regenerative outcomes relies on multiple complementary assessment techniques that collectively determine the biological effectiveness of exosome-loaded injectable hydrogels.

Histological examination remains the gold standard for assessing pulp tissue organization, inflammatory cell infiltration, vascular development, odontoblast alignment, and tertiary dentin formation. Histomorphometric analysis enables quantitative measurement of newly formed mineralized tissue and remaining pulp vitality.

Inflammatory responses are evaluated through microscopic examination and immunohistochemical staining of inflammatory mediators. Effective regenerative therapies are expected to reduce chronic inflammation while maintaining an environment conducive to tissue repair. Matrix metalloproteinases play an important role in extracellular matrix degradation during inflammation, and modulation of these enzymes contributes significantly to tissue preservation and regeneration (Golub et al., 1998).

Formation of a continuous dentin bridge over the exposure site represents one of the principal indicators of successful pulp regeneration. Newly differentiated odontoblast-like cells deposit mineralized tissue that restores structural continuity while protecting the remaining pulp tissue from bacterial invasion.

Angiogenesis is another critical outcome because adequate blood vessel formation ensures delivery of oxygen, nutrients, immune cells, and signaling molecules necessary for long-term pulp survival. Exosomes have demonstrated the ability to stimulate endothelial cell proliferation and vascular network formation, thereby improving tissue integration within regenerated pulp.

Radiographic evaluation, including micro-computed tomography (micro-CT), provides three-dimensional visualization of mineralized tissue formation, pulp chamber morphology, and hydrogel degradation. Advances in artificial intelligence-assisted image analysis have further enhanced the accuracy of radiographic interpretation by improving automated segmentation and quantitative assessment of dental structures (Rohrer et al., 2022).

D. Factors Influencing Regeneration

Several biological and material-related factors determine the regenerative success of exosome-loaded injectable hydrogels. Hydrogel composition influences mechanical strength, degradation rate, porosity, and diffusion of bioactive molecules. Natural polymers such as collagen, gelatin, hyaluronic acid, and chitosan generally provide superior biocompatibility, whereas synthetic polymers offer greater mechanical stability and controlled degradation profiles.

The cellular origin of exosomes significantly affects their regenerative potential because different parent cells produce distinct molecular cargos containing growth factors, proteins, messenger RNAs, and microRNAs that regulate tissue repair. Mesenchymal stem cell-derived exosomes have shown considerable promise owing to their potent immunomodulatory and pro-regenerative properties.

Exosome dosage and release kinetics also influence therapeutic effectiveness. Sustained and localized release maintains biological activity over extended periods while minimizing premature clearance. Injectable hydrogels act as protective reservoirs that gradually release exosomes into surrounding tissues, thereby prolonging regenerative signaling.

Host-related variables, including age, immune competence, systemic health, infection status, and local vascularity, further determine healing outcomes. Excessive inflammation or bacterial contamination may compromise tissue regeneration despite the presence of bioactive scaffolds.

E. Challenges and Limitations

Although exosome-loaded injectable hydrogels have demonstrated remarkable regenerative potential, several limitations continue to restrict their widespread clinical application. Standardized protocols for exosome isolation, purification, characterization, and storage remain lacking, resulting in variability among experimental studies. Differences in isolation techniques may alter exosome composition and biological activity, thereby affecting therapeutic outcomes.

Large-scale manufacturing also presents significant challenges because obtaining sufficient quantities of high-quality exosomes while maintaining biological consistency requires sophisticated laboratory infrastructure and rigorous quality control. The high production cost currently limits commercial scalability.

Long-term biosafety remains another important consideration. Although exosomes exhibit low

immunogenicity, prolonged biological effects, biodistribution, and potential off-target interactions require further investigation before routine clinical implementation.

Regulatory approval represents an additional obstacle because exosome-based therapeutics occupy an evolving area between biological products and advanced medicinal therapies. Establishing standardized manufacturing guidelines, quality assurance protocols, and safety regulations will be essential for successful clinical translation.

Future research should prioritize multicenter preclinical studies, standardized reporting criteria, optimization of hydrogel formulations, and well-designed human clinical trials to validate the promising outcomes observed in experimental animal models.

Conclusion

Exosome-loaded injectable hydrogels represent a significant advancement in regenerative endodontics by providing a minimally invasive, cell-free therapeutic strategy for the regeneration of the dentin-pulp complex. The synergistic combination of bioactive exosomes with injectable hydrogel scaffolds creates a favorable microenvironment that supports cellular proliferation, angiogenesis, immunomodulation, odontoblastic differentiation, and the formation of mineralized tissue. Evidence from in vivo investigations has consistently demonstrated improved pulp healing, enhanced dentin bridge formation, reduced inflammatory responses, and restoration of tissue vitality, highlighting the considerable potential of this regenerative approach for future clinical applications (Singh et al., 2026).

The biological mechanisms underlying exosome-mediated regeneration, together with the controlled delivery capabilities of injectable hydrogels, provide important advantages over conventional vital pulp therapies and cell-based regenerative techniques. Furthermore, the regulation of inflammatory processes and preservation of extracellular matrix integrity remain essential for successful tissue repair, supporting the importance of biomolecular modulation during regeneration (Golub et al., 1998). Similar advances in regenerative dental research have also demonstrated the effectiveness of biologically active therapeutic agents in promoting hard and soft tissue healing in vivo, reinforcing the translational potential of biomaterial-assisted regenerative therapies (Latimer et al., 2025).

Despite these encouraging findings, several challenges continue to limit widespread clinical implementation. Standardization of exosome isolation protocols, optimization of hydrogel formulations, scalable manufacturing, long-term biosafety evaluation, and regulatory approval remain critical areas requiring further investigation. In addition, advanced imaging and artificial intelligence-assisted analytical techniques may improve the objective evaluation of regenerative outcomes and facilitate standardized assessment in future preclinical and clinical studies (Rohrer et al., 2022).

Overall, exosome-loaded injectable hydrogels hold considerable promise as a next-generation regenerative platform capable of restoring the structural and functional integrity of the dentin-pulp complex. Continued interdisciplinary research, supported by robust in vivo evidence and well-designed clinical trials, will be essential for translating these innovative biomaterials into routine regenerative endodontic practice and improving long-term patient outcomes (Singh et al., 2026; Latimer et al., 2025).

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