



Exosomes In Endodontics: Emerging Perspectives In Pulp–Dentin Complex Regeneration

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Abstract

Regenerative endodontics aims to restore the structural integrity and biological function of the dentin-pulp complex rather than merely eliminating infection. Conventional endodontic treatments, although clinically successful, result in permanent loss of pulp vitality and sensory function. Stem cell-based regenerative strategies have demonstrated promising outcomes in experimental models; however, their clinical translation is limited by ethical, immunological, and technical challenges. Increasing evidence indicates that the therapeutic effects of stem cells are largely mediated through paracrine signaling, particularly via exosomes. Exosomes are nanosized extracellular vesicles capable of transferring bioactive molecules that regulate cell proliferation, migration, differentiation, angiogenesis, neurogenesis, and immune modulation. Recent studies have highlighted the significant role of odontogenic stem cell-derived exosomes in dentin-pulp complex regeneration. This review comprehensively discusses the biological characteristics of exosomes, their sources in endodontics, mechanisms of action, role in dentin-pulp complex regeneration, influencing factors, and potential clinical applications, based exclusively on existing experimental evidence.

I. Introduction

The dental pulp is a specialized connective tissue enclosed within mineralized dentin and plays a crucial role in tooth nutrition, defense, sensation, and dentinogenesis.^[1] Together with dentin, it forms a functional unit known as the dentin-pulp complex. Owing to its confined anatomical environment and

limited collateral circulation, the dental pulp exhibits reduced tolerance to inflammation. Pathological insults such as dental caries, trauma, periodontal disease, and iatrogenic injury frequently result in pulpitis, which may progress to irreversible inflammation or necrosis if untreated.^[2]

Conventional treatment modalities, including vital pulp therapy and root canal treatment, primarily aim to control infection and alleviate symptoms.^[3] Although effective in preserving teeth, these approaches fail to regenerate native pulp tissue or restore physiological function. Root canal treatment, in particular, results in permanent loss of pulp vitality, diminished immune defense, and increased susceptibility to tooth fracture, especially in immature teeth where root development remains incomplete.^[4]

Regenerative endodontic therapy has emerged as a biologically driven alternative focused on restoring pulp vitality and dentin-pulp complex architecture. Dental mesenchymal stem cells such as dental pulp stem cells, stem cells from human exfoliated deciduous teeth, and stem cells from the apical papilla have shown the capacity to generate pulp-like tissue, promote dentin deposition, and support angiogenesis and neurogenesis. Despite promising experimental results, clinical translation of stem cell-based therapies is limited by challenges related to cell sourcing, expansion, storage, immunogenicity, ethical concerns, and unpredictable *in vivo* behaviour.^[4]

Recent research indicates that stem cells exert their regenerative effects predominantly through paracrine mechanisms rather than direct differentiation. Among paracrine mediators,

exosomes have gained increasing attention as critical biological effectors. Exosomes are nanoscale extracellular vesicles capable of transporting functional biomolecules to recipient cells, thereby modulating multiple regenerative processes. Their application in endodontics represents a promising cell-free therapeutic approach that may overcome limitations associated with stem cell transplantation.^[5]

II. Biological Characteristics of Exosomes

2.1 Definition and Biogenesis

In 1983, Pan and Johnstone identified small membrane-bound vesicles released during the

maturation of sheep reticulocytes that facilitated the extracellular export of transferrin receptors. Subsequently, Johnstone and co-workers introduced the term 'exosomes' to describe these vesicles secreted into the extracellular space.^[6] Exosomes are membrane-bound extracellular vesicles with a diameter ranging from approximately 30 to 150 nm. They originate from the endosomal pathway through inward budding of the plasma membrane to form early endosomes, which subsequently mature into multivesicular bodies (MVBs) (Fig.1).^[5] Fusion of MVBs with the cytoplasmic membrane results in the release of intraluminal vesicles (ILVs) into the extracellular environment as exosomes.^[7]

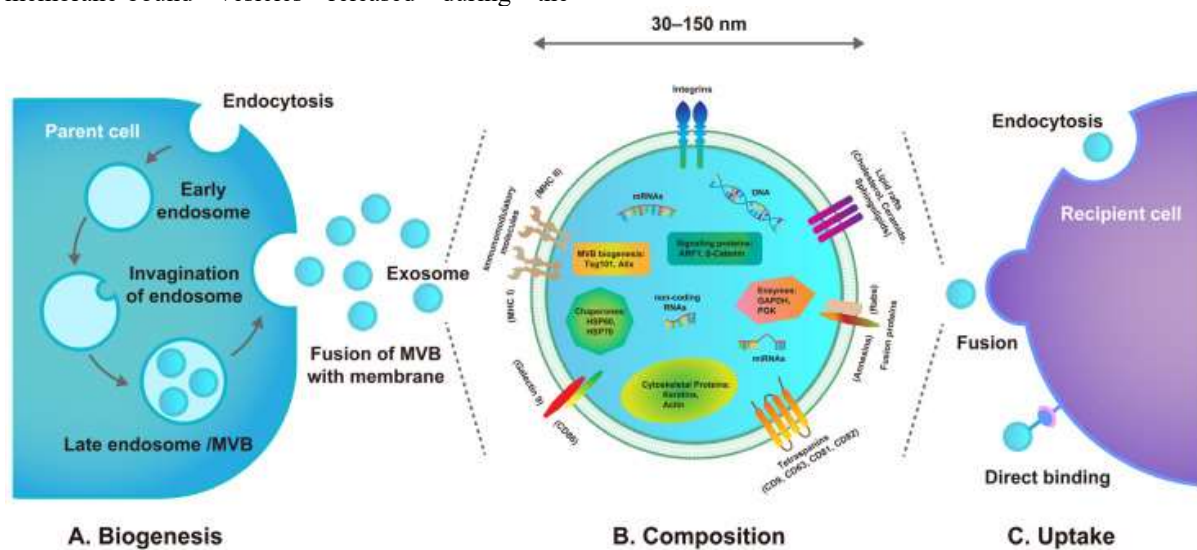


Figure 1- Schematic diagram of exosome biogenesis, composition and uptake. (A) The biogenesis of exosomes start with the invagination of cytoplasmic membrane, resulting in the generation of early endosomes. Subsequently, the invagination of the endosomal membrane promotes the maturation of early endosomes into late endosomes, which contribute to the production of MVBs. Eventually, MVBs fuse with the cytoplasmic membrane and exosomes are thus released into the extracellular environment. (B) Exosomes are lipid bilayer nanoparticles measuring 30-150nm in diameter. They contain a variety of proteins, lipids and nucleic acids, which are reflective of parental cells and can be delivered to recipient cells. (C) Exosomes can be internalized by recipient cells through endocytosis, fusion with the plasma membrane, or direct binding to the surface proteins.

2.2 Composition

Exosomes contain a complex cargo of bioactive molecules, including proteins, lipids, messenger RNA, microRNA, and other nucleic acids.^[8] The molecular composition of exosomes reflects the characteristics and physiological status of their parent cells. This cargo enables exosomes to influence the behavior of recipient cells and regulate diverse biological processes essential for tissue repair and regeneration.^[5]

2.3 Mechanisms of Cellular Uptake

Exosomes interact with recipient cells through multiple mechanisms, including direct binding to surface receptors, fusion with the plasma membrane,

and endocytosis.^[9,10] These interactions allow the transfer of exosomal cargo into target cells, thereby initiating intracellular signaling pathways involved in proliferation, differentiation, angiogenesis, and immunomodulation.^[5]

III. Sources of Exosomes Relevant to Endodontics

Dental stem cell-derived exosomes (DSC-Exos) are categorized according to their parental cell origin (Fig.2).^[11]

- Dental pulp stem cell-derived exosomes (DPSC-Exos)
- Stem cells from apical papilla-derived exosomes (SCAP-Exos)



- Stem cells from human exfoliated deciduous teeth-derived exosomes (SHED-Exos)
- Periodontal ligament stem cell-derived exosomes (PDLSC-Exos)
- Gingival mesenchymal stem cell-derived exosomes (GMSC-Exos)
- Dental follicle stem cell-derived exosomes (DFSC-Exos)



Figure 2- Classification of DSC-Exos

Among these, DPSC-Exos, SHED-Exos, and SCAP-Exos are most directly relevant to pulp-dentin regeneration.^[12]

IV. Isolation and Characterization

Currently, no standardized protocol exists for exosome isolation.^[13] Differential ultracentrifugation remains the most frequently employed method. Other approaches include ultrafiltration, immunoaffinity chromatography, size-exclusion chromatography, and polymer precipitation.^[5]

Characterization typically involves:

- Transmission electron microscopy for morphology.
- Nanoparticle tracking analysis for size distribution.
- Western blotting for exosomal markers such as CD9, CD63, CD81, Alix and Tumor Susceptibility Gene 101.

Accurate characterization is essential to distinguish exosomes from other extracellular vesicles.^[11]

V. Biological Functions of Exosomes in Endodontics

5.1 Promotion of Odontogenic Differentiation and Dentinogenesis

Exosomes derived from odontogenic stem cells enhance odontoblastic differentiation and mineral deposition.^[14] DPSC-Exos promote dentin matrix formation and calcium deposition by mimicking the microenvironment of dentin development (Fig.3).^[11] SCAP-Exos stimulate dentin sialophosphoprotein expression and mineralized nodule formation, contributing to pulp-dentin like tissue regeneration. In vivo studies further demonstrate that exosomes can

induce regeneration of pulp-like tissue and tertiary dentin formation.^[4]

5.2 Angiogenesis

Successful pulp regeneration requires revascularization. Exosomes exhibit strong pro-angiogenic properties by promoting endothelial cell proliferation and migration.^[4] SHED-Exos and GMSC-Exos have demonstrated regulatory effects on angiogenesis, supporting neovascularization during tissue repair. Exosome-mediated angiogenesis is critical for sustaining regenerated pulp tissue.^[11]

5.3 Neuroprotection and Neuroregeneration

Pulp tissue is highly innervated and thus, neural regeneration is vital. Odontogenic stem cell-derived exosomes regulate Schwann cell (SC) migration and differentiation, contributing to neuroprotection and axonal regeneration.^[15,16] Exosomes participate in neurovascular regeneration processes and support craniofacial tissue repair.^[11] Studies have shown that GMSCs-Exo, after being engulfed by SCs, can significantly promote SCs proliferation, axon growth, and promote myelination to repair peripheral nerve damage in rats.^[17]

5.4 Immunomodulation and Anti-Inflammatory Effects

Inflammation control is essential for pulp healing. Exosomes regulate macrophage polarization, facilitating conversion from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype.^[4] They also influence T cell regulation and immune suppression. Through these mechanisms, exosomes create a microenvironment



conducive to tissue regeneration rather than chronic inflammation.^[12]

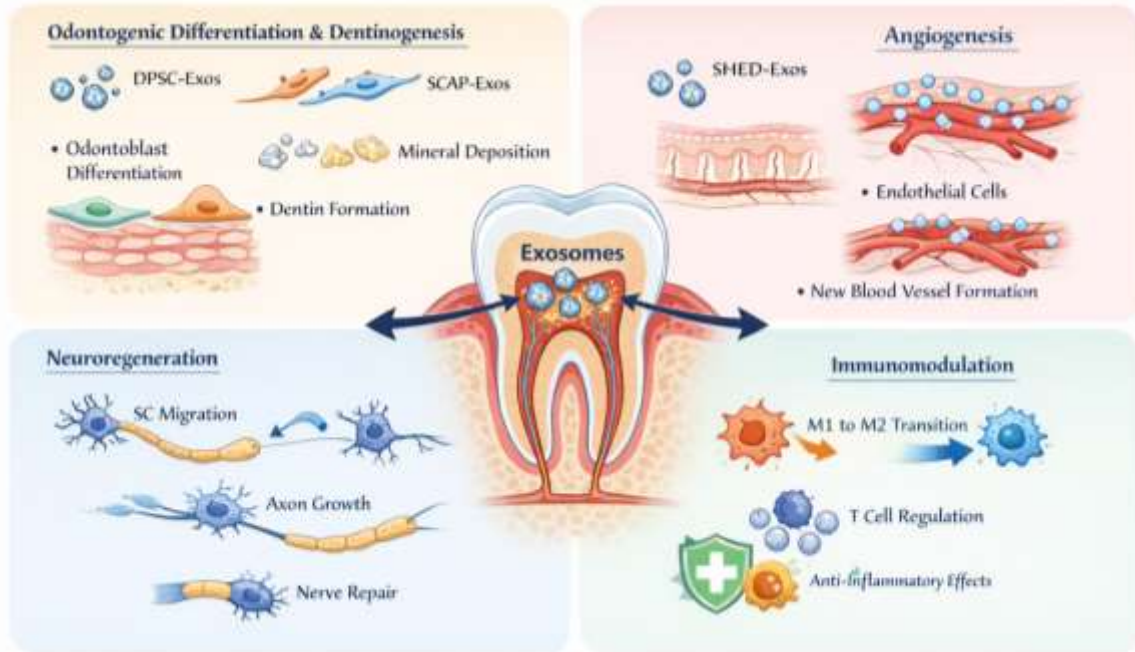


Figure 3- Schematic illustration of biological functions of exosomes

VI. Factors Influencing Exosome-Mediated Regeneration

6.1 Parent Cell Type

The biological activity of exosomes is closely related to their parent cell type. Exosomes derived from odontogenic stem cells exhibit superior regenerative potential compared with those from non-dental sources.^[5]

6.2 Culture Conditions

Exosomes derived under odontogenic or inflammatory stimulation conditions demonstrate enhanced regenerative efficacy, indicating that the microenvironment influences exosomal cargo and function.^[4,5]

6.3 Delivery Systems and Scaffolds

Scaffold-based delivery systems improve the stability and localized retention of exosomes, thereby enhancing their regenerative effects. Biomaterials such as collagen and fibrin gels have been used successfully in experimental models.^[5]

VII. Clinical Implications in Regenerative Endodontics

Exosome-based therapy represents a shift from cell transplantation to cell-free regenerative strategies in endodontics. Stem cell derived exosomes preserve many of the regenerative properties of their parent cells while reducing risks linked to direct stem cell use, including

immunogenicity, safety concerns, storage limitations, and ethical issues. As acellular vesicles, they avoid complications such as uncontrolled proliferation, tumorigenesis, and unpredictable differentiation that may occur with mesenchymal stem cell therapies.^[4]

From a practical perspective, exosomes can be isolated, quantified, and stored more predictably than viable cells, which may improve their translational suitability for clinical use. Experimental studies indicate that exosomes enhance mesenchymal cell proliferation, migration, and odontogenic differentiation in vitro, and promote pulp–dentin like tissue formation in animal models.^[4,5]

However, current evidence is limited to laboratory and animal research. Thus, despite strong preclinical support, their clinical application remains investigational.^[4,11]

VIII. Challenges and Future Perspectives

Although exosome-based regenerative therapy demonstrates substantial promise, several scientific and translational challenges must be addressed before routine clinical implementation.

One of the primary limitations is the absence of standardized isolation protocols. Current methods including differential ultracentrifugation, ultrafiltration, immunoaffinity approaches, and precipitation techniques vary significantly across studies. Even within ultracentrifugation protocols,



centrifugal forces, durations, and purification steps differ considerably, leading to inconsistency in exosome yield and composition. The lack of methodological uniformity complicates comparison between studies and may influence biological performance.^[18]

In addition to procedural variability, differences in exosome yield, purity, and cargo composition remain critical concerns. The heterogeneity of isolated vesicles and potential contamination with other extracellular particles can affect reproducibility and therapeutic outcomes.^[19]

Another major limitation is the scarcity of clinical data. As emphasized in current literature, no human clinical trials have validated the safety or efficacy of exosomes in regenerative endodontics. Consequently, translation into clinical protocols requires rigorous preclinical standardization followed by well-designed clinical investigations.^[4] Future research directions should prioritize:

- Establishing standardized, reproducible isolation and characterization protocols.
- Optimizing delivery systems capable of controlled and sustained exosome release within the root canal environment.
- Investigating scaffold-based strategies to enhance local retention and biological effectiveness.
- Conducting controlled clinical trials to evaluate safety, dosage, and long-term outcome.

Addressing these issues is essential to ensure predictable regenerative outcomes and regulatory approval.^[11,12,20]

IX. Conclusion

Exosomes are increasingly recognized as promising biologic agents in regenerative endodontics. Sourced from dental stem cells, they contribute to pulp-dentin complex repair by regulating differentiation, vascularization, neural responses, and immune balance. Importantly, they provide many advantages of stem cell therapy while avoiding key risks associated with cellular transplantation.^[4,12]

Despite encouraging results from in vitro and animal studies, the lack of standardized protocols and human clinical trials continues to limit their routine clinical application.

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