

Exosomes Derived from Dental Pulp Stem Cells in Tissue Repair and Regeneration

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Abstract. Dental pulp stem cells (DPSCs) are multipotent stem cells with strong differentiation capacity and immunomodulatory properties, making them a promising source for regenerative therapies. Traditional cell-centric approaches such as stem cell transplantation face limitations including high cost, ethical concerns and technical complexity, which has shifted attention toward cell-free strategies. Exosomes, nanosized extracellular vesicles enriched in proteins, RNAs and bioactive molecules, offer advantages such as low immunogenicity, stability and efficient intercellular communication. Exosomes derived from DPSCs (DPSC-Exo) have demonstrated notable regenerative potential. They promote angiogenesis and dentin-pulp complex formation, thereby facilitating pulp regeneration. They also enhance osteogenic differentiation and contribute to bone defect repair, while exerting neuroprotective effects and supporting neural repair through secreting neurotrophic factors and regulating inflammatory responses. Despite these advances, major challenges remain regarding large-scale isolation, precise delivery and long-term safety evaluation. This review aims to summarize current knowledge of DPSC-Exo in pulp regeneration, bone formation and neural repair, highlight their clinical prospects and outline key directions for further studies.

Keywords: Dental pulp stem cells, exosomes, osteogenesis, pulp regeneration

1. Introduction

DPSCs, a population of adult stem cells obtained from dental pulp tissue, exhibit a strong potential to differentiate into diverse lineages. They can differentiate into osteoblasts, chondrocytes, and neurons, and also exert immunomodulatory functions [1]. With the rapid advances in stem cell biology, DPSC-based tissue engineering has been increasingly explored for tissue repair and organ function restoration. Evidence has demonstrated that DPSCs contribute to the treatment of cardiac, cerebral, hepatic, and neurological disorders, underscoring their value as seed cells in regenerative medicine with substantial translational potential [2]. Despite these advances, conventional dental pulp treatment in clinical practice still relies mainly on root canal therapy, which suffers from limitations such as insufficient biocompatibility of filling materials. Moreover, although cell-based therapies have shown promise in preclinical studies, their clinical application remains constrained by high costs, ethical concerns, and technical challenges. To overcome these limitations, research has recently shifted toward cell-free strategies, particularly the therapeutic use of exosomes. Compared

with direct stem cell transplantation, exosome-based therapy offers distinct advantages including efficient cargo loading, enhanced intercellular communication, reduced immunogenicity, and lower cost [3].

Exosomes, also referred to as small extracellular vesicles (sEVs), are a subclass of extracellular vesicles characterized by a lipid bilayer membrane and a diameter of 30-150 nm [4]. They are widely distributed in biological fluids and serve as essential mediators of intracellular and intercellular communication by transporting a diverse cargo of biomolecules, including lipids, proteins, and nucleic acids. Surface markers including CD9, CD63, and CD81 are widely employed to identify and purify exosomes [5]. Several isolation techniques have been developed, among which ultracentrifugation, density-gradient centrifugation, and precipitation kits are most widely applied. Ultracentrifugation, although time consuming, requires minimal sample preprocessing and remains the most cost-effective method. Following isolation, exosomes are routinely identified and analyzed by transmission electron microscopy, nanoparticle tracking analysis, and Western blotting of marker proteins, thereby ensuring the reliability of experimental results [6]. These approaches provide a technical foundation for clarifying the biological functions of exosomes and their clinical potential.

Exosomes derived from dental pulp stem cells (DPSC-Exo) have gained increasing interest due to their promising application in pulp regeneration, bone-like tissue formation, and neuroprotection. DPSC-Exo have been shown to stimulate angiogenesis, thereby facilitating the formation of the dentin–pulp complex and promoting odontogenic differentiation of DPSCs [7]. In a rat mandibular defect model, mesenchymal stem cells derived from the bone marrow of the jaw were able to internalize DPSC-Exo, leading to the elevated expression of osteogenic markers such as osteocalcin (OCN) and alkaline phosphatase (ALP), which significantly promoted new bone formation and mineralization [8]. Furthermore, DPSC-Exo display low cytotoxicity and neuroprotective capacity by delivering neurotrophic factors such as NGF and BDNF, thereby reducing neuronal apoptosis and stimulating axonal regeneration [9]. Collectively, these results demonstrate the extensive application prospects of DPSC-Exo in diverse tissue repair settings. The objective of this article is to provide a comprehensive overview of the molecular mechanisms, delivery strategies, and clinical applications of DPSC-Exo in pulp regeneration, bone regeneration, and neuroprotection. In addition, it discusses the major challenges of large-scale production and safety evaluation, offering insights to guide future translational research and the integration of exosome-based therapies into regenerative medicine and dentistry.

2. Applications of DPSC-Exo in pulp tissue regeneration

Angiogenesis is a central event in pulp–dentin complex repair, providing the vascular supply necessary for nutrient and oxygen delivery. Immunostaining for CD31 in human umbilical vein endothelial cells (HUVECs) has demonstrated that treatment with DPSC-Exo leads to significantly greater neovascularization and capillary formation in vivo, accompanied by elevated CD31 expression. Mechanistic analyses further indicate that DPSC-Exo promote angiogenesis through activation of the Cdc42/p38 MAPK signaling pathway [10]. In addition, DPSC-Exo enhance the proliferation, motility, and tube formation capacity of HUVECs, while upregulating angiogenic factors including vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF-2), and matrix metalloproteinase 9 (MMP-9) [11]. These findings highlight the pivotal role of DPSC-Exo in vascular formation, which supports pulp regeneration and lays a foundation for engineering functional pulp–dentin complexes.

The induction of odontoblastic differentiation is another prerequisite for complete pulp–dentin regeneration. Studies comparing undifferentiated and odontogenic differentiation states of DPSC-

Exo (UN-Exo, OD-Exo) revealed that DPSC-Exo engage with extracellular matrix components, notably type I collagen and are taken by recipient cells both in a manner that both dose-dependent and saturable. This uptake activates the p38 MAPK pathway, upregulates BMP2 and BMP9, and drives odontoblastic differentiation [12]. Transcriptomic sequencing identified 28 differentially expressed microRNAs in OD-Exo, with miR-27a-5p elevated by nearly eleven-fold. Functional analyses demonstrated that OD-Exo enhance odontogenesis by downregulating LTBP1 and activating the TGF β 1 pathway, as evidenced by the upregulation of TGF β 1, TGFR1, Smad4, and p-Smad2/3 [13]. These data suggest that OD-Exo possess enhanced odontogenic potential compared with exosomes from undifferentiated states.

Despite these advances, the rapid clearance of exosomes *in vivo* necessitates the use of biomaterial scaffolds to prolong retention and control release. Hydrogels are increasingly regarded as the most promising scaffolding systems for tissue repair [14]. A thermosensitive hydroxypropyl chitin/chitin whisker (HPCH/CW) hydrogel loaded with DPSC-Exo was shown to enhance angiogenic activity and promote the formation of pulp-like tissue when injected into irregular root canal spaces [15]. However, research in this area remains limited, and further studies are required to establish the therapeutic mechanisms and clinical feasibility of such delivery systems.

3. Applications of DPSC-Exo in bone-like tissue regeneration

Exosomes from dental pulp stem cells also play a significant role in osteogenesis. Exosomes derived from stem cells of human exfoliated deciduous teeth (SHED-Exo) activate the Wnt/ β -catenin and BMP/Smad signaling pathways, thereby enhancing the osteogenic differentiation of periodontal ligament stem cells [16]. High-throughput sequencing revealed dynamic changes in the microRNA profiles of DPSC-Exo under osteogenic conditions. Osteogenesis-related miRNAs including miR-29c-5p, miR-10b-5p, miR-378a-5p, and miR-9-3p were upregulated. In contrast, inhibitory miRNAs such as miR-31-3p, miR-221-3p, miR-183-5p, and miR-503-5p were downregulated. These regulatory miRNAs are predicted to interact with osteogenesis-related targets including connexins, Hmox1, Stab2, and RUNX2, ultimately driving the expression of osteogenic proteins [17].

At the cellular level, mesenchymal stem cells derived from jaw bone marrow (JB-MSCs) efficiently internalize DPSC-Exo, resulting in robust upregulation of key osteogenic markers, such as OCN, ALP, and RUNX2, and a pronounced enhancement of osteogenic differentiation. *In vivo* studies further confirmed these findings: administration of DPSC-Exo in rat mandibular defect models led to significant bone regeneration, reduced defect area, and increased bone surface density, indicating potent osteoinductive effects [8]. These results elucidate the underlying mechanisms of the osteogenic activity of DPSC-Exo and support their application in maxillofacial defect repair and broader bone regeneration therapies.

Integration of DPSC-Exo with biomaterials has shown further promise in bone repair. A controlled-release system embedding DPSC-Exo into PLGA-PEG-PLGA microspheres and incorporating them into a nanofiber PLA scaffold facilitated endogenous cell recruitment, differentiation, and accelerated bone regeneration *in vivo* [18]. A subsequent dual-release hydrogel combining VEGF-loaded and DPSC-Exo-loaded microspheres enabled staged release, with VEGF promoting rapid angiogenesis and DPSC-Exo sustaining osteogenic differentiation. This synergistic approach significantly accelerated bone defect healing [19], offering an advanced strategy for clinical bone tissue engineering.

4. Applications of DPSC-Exo in neuroprotection and regeneration

Given their neural crest origin, dental pulp stem cells exhibit superior neuroregenerative potential compared with mesenchymal stem cells obtained from alternative sources [20]. Exosomes derived from SHEDs cultured in three-dimensional laminin-coated alginate hydrogels were shown to protect dopaminergic neurons from 6-hydroxydopamine-induced oxidative stress, suppress apoptosis, and hold therapeutic potential for Parkinson's disease [21].

Axonal regeneration is critical for neural repair and is influenced by remyelination. Schwann cells (SCs), are responsible for supporting axon ensheathment and myelin formation as the primary glial cells of the peripheral nervous system [22]. DPSC-Exo enhance SC proliferation, motility, and neurotrophic factor secretion, thereby promoting axonal growth and remyelination following peripheral nerve injury [23]. In rat sciatic nerve crush models, DPSC-Exo administration facilitated axonal and myelin regeneration through modulation of the miR-122-5p/p53 pathway, which inhibited SC autophagy triggered by nerve injury [24].

DPSC-Exo also demonstrate strong immunomodulatory effects, surpassing exosomes from other MSC sources. They promote CD4⁺ T cell apoptosis, suppress differentiation into helper T cells, enhance regulatory T cell polarization, and shift cytokine profiles toward an anti-inflammatory state [25]. In models of cerebral ischemia-reperfusion injury, repeated intravenous administration of DPSC-Exo for seven days alleviated brain edema and infarct size while improving neurological outcomes. These benefits were associated with suppression of the TLR4/MyD88/NF- κ B axis and downregulation of proinflammatory cytokines including IL-6, IL-1 β , and TNF- α [26]. Collectively, these findings suggest that DPSC-Exo exert neuroprotective effects by rebalancing immune responses and optimizing the inflammatory microenvironment after injury, highlighting their translational potential in neurological disorders.

5. Challenges and future perspectives

Although numerous preclinical studies have demonstrated the therapeutic effects of DPSC-Exo in pulpitis, periodontitis, bone defects, ischemic stroke, and neurodegenerative disorders, several barriers remain before clinical translation can be achieved. The scalable manufacturing of DPSC-Exo with consistent quality and composition remains a major challenge. Ultracentrifugation, the most common isolation method, is cost-effective but time consuming, susceptible to contamination, and unsuitable for clinical-grade manufacturing [6]. Future work should focus on developing scalable and standardized isolation techniques.

Another obstacle lies in the targeted delivery of DPSC-Exo. Their rapid clearance in vivo limits therapeutic efficacy, and current understanding of the mechanisms governing exosome biodistribution and uptake remains incomplete. Incorporating exosomes into biomaterial scaffolds offers one potential solution by prolonging retention, enabling sustained release, and increasing local concentrations at target sites. Finally, ensuring clinical safety is essential. Exosomes contain diverse proteins, lipids, and genetic material, necessitating comprehensive multi-omics analyses to assess the risk of transferring harmful components such as oncogenes or toxic molecules. Moreover, optimal dosing regimens, delivery routes, and treatment frequencies must be established to maximize efficacy while minimizing risk [5].

6. Conclusion

Cell-based therapies face hurdles including immunogenicity, technical complexity, and high cost. In contrast, exosome-based therapies represent a promising cell-free alternative with lower immunogenicity, higher intercellular communication efficiency, and improved scalability. This review highlights the multifaceted roles of DPSC-Exo in tissue regeneration, including their ability to promote angiogenesis and pulp–dentin regeneration, facilitate bone repair in combination with biomaterials, and exert neuroprotective effects through immunomodulation.

Despite these advances, clinical application remains limited by insufficient in vivo evidence, lack of standardized large-scale production protocols, and incomplete understanding of underlying mechanisms. Future studies should focus on clarifying the molecular pathways through which DPSC-Exo mediate regenerative effects, optimizing biomaterial-based delivery systems, and establishing safety and efficacy through rigorous clinical trials. Collectively, these efforts will be essential to fully realize the clinical promise of DPSC-Exo in regenerative medicine and dentistry.

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