Case Report

The Effect of Exosomes Derived from Human Umbilical Cord Mesenchymal Stromal/ Stem Cells on the Regeneration of Human Pulpectomized Tooth: A Case Report

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What's Known

- The dental pulp is vital for tooth health. Conventional treatments, such as root canals, compromise tooth vitality. The purpose of regenerative endodontic procedures (REPs) is to regenerate pulp using scaffolds, growth factors, and stem cells. Human umbilical cord mesenchymal stem cells (hUCMSCs) are promising due to their availability and low immunogenicity.

What's New

- This study investigated the use of hUCMSC-derived exosomes for dental pulp regeneration, which could fill gaps in the present regenerative approaches. It investigated exosome-based therapy as an alternative to traditional cell-based methods, combining hUCMSC-derived exosomes with chitosan for pulpectomized teeth treatment.

Abstract

The dental pulp is essential for tooth health and sensory function. However, conventional treatments for infected or necrotic pulp, such as root canal therapy, frequently result in tooth loss and increased fracture risk. Regenerative endodontic procedures (REPs) aim to regenerate pulp tissue while preserving tooth vitality. Exosomes from human umbilical cord mesenchymal stromal/stem cells (hUCMSCs) demonstrated potential for tissue regeneration. In this case report, the potential of hUCMSC-derived exosomes for regenerating the dental pulp of a pulpectomized tooth was investigated. The patient, a 40-year-old man, was presented with irreversible pulpitis in the mandibular second premolar. The patient underwent a pulpectomy, followed by the application of a chitosan and hUCMSC-derived exosome mixture into the root canal. This project was conceived and executed as a joint project in Bushehr (Iran), Shiraz (Iran), and Aktobe (Kazakhstan) from 2022 to 2024. Exosomes were isolated and characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Clinical evaluations, including visual inspection, palpation, pulp vitality tests, radiography, and cone beam computed tomography (CBCT) imaging, were conducted over 24 weeks post-treatment. The SEM and TEM images confirmed the effective isolation and characterization of exosomes. Clinical follow-ups showed no signs of infection, swelling, or tenderness around the treated tooth. Radiographic assessments indicated periapical radiolucency and periodontal ligament widening, suggesting active healing. Despite these radiographic changes, the absence of clinical symptoms indicated successful tissue regeneration and integration. This case report demonstrated the potential of hUCMSC-derived exosomes for dental pulp regeneration, with promising results in maintaining tooth vitality and promoting healing.

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Keywords ● Exosomes ● Regenerative endodontics ● Dental pulp ● Regeneration ● Mesenchymal stem cells

Introduction

The dental pulp is the innermost part of the tooth, comprising connective tissue, blood vessels, and nerves, which are essential

for maintaining the tooth healthy and functional. When the dental pulp becomes infected or necrotic due to caries, trauma, or other pathologies, conventional treatment options include root canal therapy (RCT) or pulpectomy. These procedures aim to remove damaged pulp tissue, preventing further infection and restoring tooth function. However, removing pulp tissue also eliminates the tooth's vitality, which may compromise its structural integrity and increase the risk of fracture over time.[1]

A novel treatment approach is regenerative endodontic procedures (REPs). The field of REPs aims to overcome these limitations by promoting pulp tissue regeneration, thereby preserving the vitality of the teeth and increasing their longevity. Traditional approaches to pulp regeneration included scaffolds, growth factors, and stem cells. Among these, mesenchymal stromal/stem cells (MSCs) have shown significant promise due to their multipotency, ability to differentiate into various cell types, and immunomodulatory properties.[2]

Human umbilical cord mesenchymal stromal/stem cells (hUCMSCs) are particularly attractive for regenerative applications. Unlike MSCs derived from bone marrow or adipose tissue, hUCMSCs are harvested from the umbilical cord, which is a medical waste product, making them readily available and ethically non-controversial. Additionally, hUCMSCs have a robust proliferative capacity and low immunogenicity, which makes them ideal for therapeutic utilization.[3]

Recent research has focused on the paracrine effects of MSCs, which are mediated through the secretion of extracellular vesicles, specifically exosomes. Exosomes are nano-sized vesicles (30-150 nm in diameter) that facilitate intercellular communication by delivering bioactive molecules, such as proteins, lipids, and various RNAs. These vesicles can modulate the behavior of recipient cells, promoting processes such as cell proliferation, differentiation, and angiogenesis, which are critical for tissue regeneration.[4]

This case report aimed to investigate the effect of exosomes derived from hUCMSCs on the regeneration of human pulpectomized teeth. This innovative approach was investigated to provide preliminary evidence supporting the feasibility and potential efficacy of exosome therapy in dental pulp regeneration. The findings of this study could pave the way for future research and clinical applications, ultimately contributing to the development of more effective treatments for endodontic diseases.

Case Presentation

This project was conceived and executed as a joint project in Bushehr (Iran), Shiraz (Iran), and Aktobe (Kazakhstan) from 2022 to 2024. This case report described the treatment of a 40-year-old man, who was diagnosed with irreversible pulpitis in a mandibular second premolar. The study was conducted in accordance with the Local Bioethical Committee of NJSC "West Kazakhstan Marat Ospanov Medical University" (registration No. 1,1/01). Once written informed consent was obtained, the patient, who met the inclusion criteria, received treatment. The inclusion criteria were as follows: participants aged 20–55 years with a clinical diagnosis of irreversible pulpitis in a single root canal, no tooth fractures, and periapical radiographs showing no radiolucency. The exclusion criteria included infections, severe diseases, pregnancy, mental illness, and allergies that would interfere with cone beam computed tomography (CBCT) imaging.

Blood and urine tests confirmed the absence of infections or disqualifying conditions. The hUCMSCs were obtained from a commercial cell bank and cultured under optimal conditions. After achieving confluence, the cells were prepared for exosome isolation using a commercial Exovista kit (PerciaVista R&D Co., Iran). The exosomes were characterized with scanning electron microscopy (SEM, TESCAN MIRA3, TESCAN Co., Czech Republic) and transmission electron microscopy (TEM, Zeiss EM10C transmission electron microscope, Zeiss Co., Germany), which revealed sizes ranging from 20 to 180 nm, with a mean diameter of 101.06 nm (figure 1). This characterization confirmed their suitability for use in dental pulp regeneration.

The patient had a pulpectomy under local anesthetic during the first treatment session, followed by conventional root canal preparation (figure 2). The canal was irrigated and filled with temporary material. Then, the temporary infill was removed, and the canal was thoroughly cleaned during the second session, which was two weeks later. Afterward, a mixture of chitosan powder and hUCMSC-derived exosomes was placed into the root canal and sealed with glass ionomer cement.

The patient underwent follow-up assessments at 1, 2, 4, 12, 16, and 24 weeks post-treatment. Clinical examinations, such as percussion, palpation, and pulp vitality tests, revealed normal responses throughout the follow-up period, with no signs of infection or swelling. Radiographic assessments revealed initial signs of periapical radiolucency and periodontal ligament widening, which healed gradually over time. CBCT imaging indicated that the exosome-chitosan mixture was well integrated into the tooth structure, with no missing canals or adverse reactions (figure 3). The patient reported no pain, indicating that the tissue had successfully regenerated and healed.

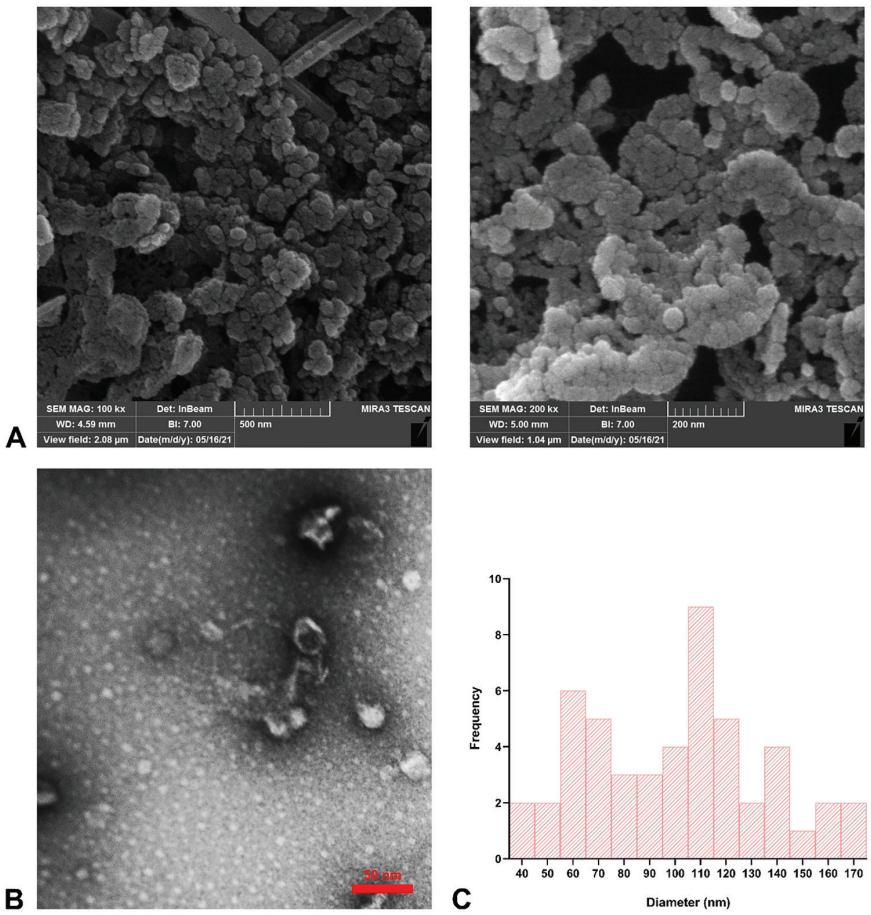

Figure 1: Isolated exosomes from human umbilical cord mesenchymal stromal/stem cells (hUCMSCs) were confirmed using different imaging techniques. Panel A presents scanning electron microscopy (SEM) images, demonstrating the size and morphology of the exosomes. Panel B displays transmission electron microscopy (TEM) images, further verifying their lipid bilayer structure. Panel C provides an analysis of exosome diameter frequencies, illustrating the size distribution of the exosomes, with a mean diameter of 101.06 nm, and sizes ranging from 20 to 180 nm. These findings confirmed the successful isolation and characterization of hUCMSC-derived exosomes.

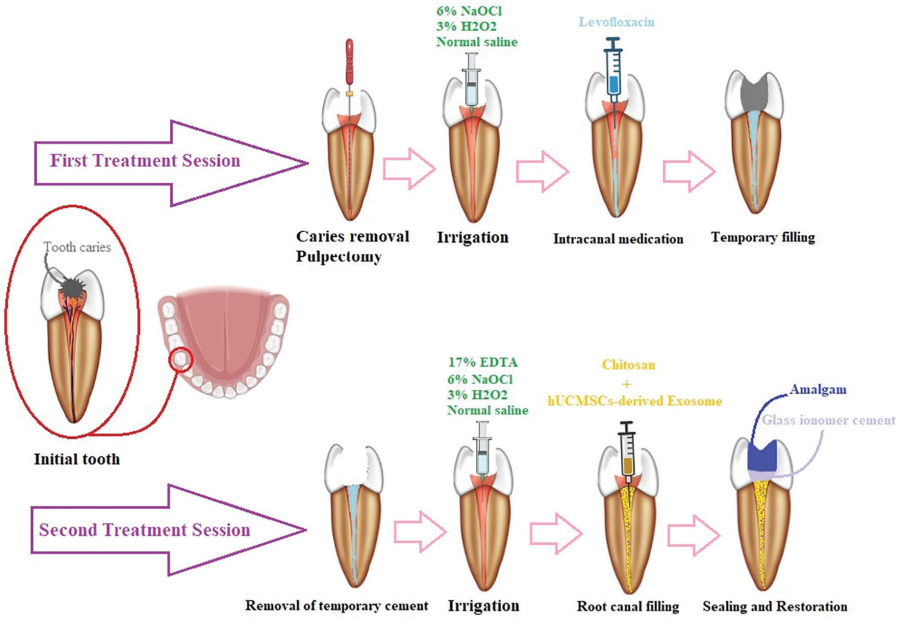

Figure 2: To clarify the process, the treatment procedure is illustrated schematically. It illustrates the step-by-step process of root canal preparation, the injection of human umbilical cord mesenchymal stromal/stem cells (hUCMSCs)-derived exosomes mixed with chitosan into the root canal, and the final sealing of the cavity with glass ionomer cement. The diagram outlines the use of dental tools, exosome injection, and restoration procedures, providing a clear visual overview of the entire regenerative endodontic therapy process.

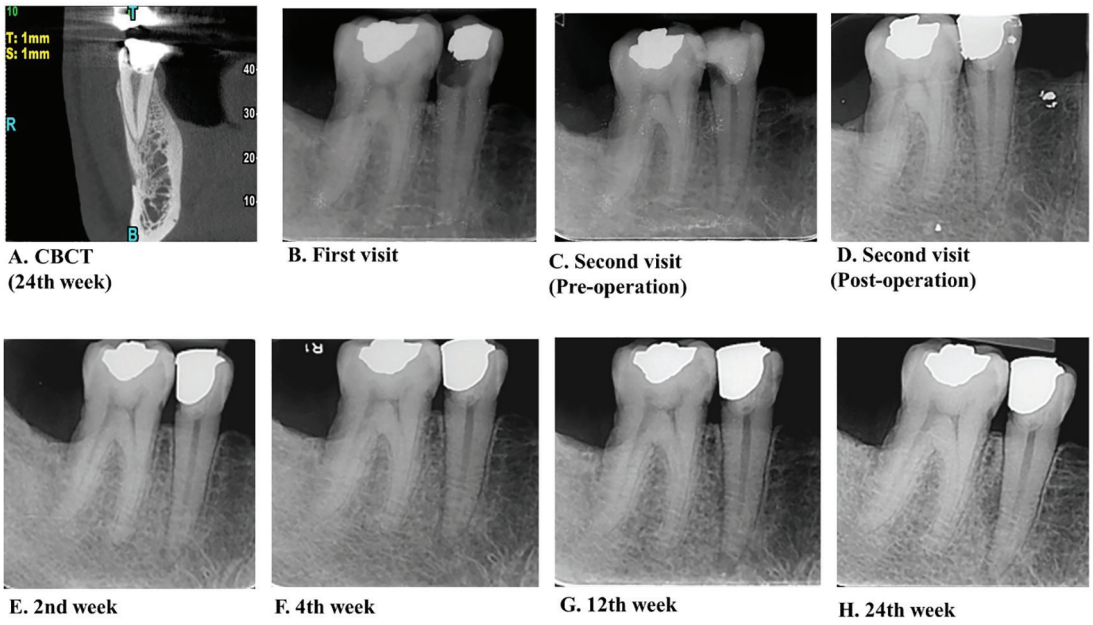

Figure 3: Mandibular second premolar was imaged with cone beam computed tomography (CBCT) and periapical radiographs. The CBCT imaging shows the target tooth at the 24th week after transplantation of chitosan and human umbilical cord mesenchymal stromal/stem cells (hUCMSCs)-derived exosomes in pulpectomized teeth (A). The periapical imaging analysis shows the changes and evolution of periapical tissues at the first visit (B), pre-operation just before chitosan and hUCMSCs-derived exosomes transplantation (C), post-operation just after chitosan and hUCMSCs-derived exosomes transplantation and completing tooth restoration (D), and 2 weeks (E), 4 weeks (F), 12 weeks (G), and 24 weeks (H) following the transplantation of chitosan and hUCMSCs-derived exosomes in pulpectomized teeth.

Discussion

This study investigated the therapeutic potential of exosomes derived from human umbilical cord mesenchymal stromal cells (hUCMSCs) for dental pulp regeneration. While previous research primarily focused on cell-based therapies using dental pulp stromal/stem cells (DPSCs) combined with granulocyte colony-stimulating factor (G-CSF),[1] this study introduced exosome-based therapy as an innovative approach in regenerative endodontics. In contrast to earlier studies, which employed hUCMSCs directly,[5,6] this research used hUCMSCs-derived exosomes in combination with chitosan to treat pulpectomized teeth. The treatment resulted in successful pulp regeneration with no adverse reactions, as confirmed by clinical assessments and radiographic evaluations. Despite the presence of radiolucency and periodontal ligament (PDL) widening, which indicated an active healing process, no signs of inflammation or infection were found, implying effective tissue regeneration and integration.

Several studies supported the potential of exosomes in dental applications. Zhang and others showed that dental pulp-derived exosomes facilitated neovascularization and collagen deposition.[7] Zhuang and colleagues demonstrated that exosomes from stem cells promoted dentin-pulp complex regeneration.[2] Swanson and others confirmed that exosomes induce dentinogenesis when combined with a biodegradable polymer.[8] Exosomes have also been demonstrated to prevent apoptosis, promote cell proliferation, and enhance angiogenesis, making them promising candidates for regenerative therapies.

Despite these encouraging findings, the limitations of the present study must be acknowledged. Firstly, it was based on a single case report, underscoring the need for further research with larger sample sizes and longer follow-up periods. The exact mechanisms through which exosomes promote tissue regeneration remain unclear, necessitating further investigation of the molecular pathways involved. Thus, it is recommended that future studies concentrate on improving treatment protocols to maximize clinical outcomes and gain a better understanding of the long-term effects of exosome-based therapies for pulp vitality.

Conclusion

This study highlighted the potential of hUCMSCs-derived exosomes as a promising alternative to cell-based therapies for dental pulp regeneration. However, more extensive research is required to confirm their efficacy.

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Authors' Contribution

N. J: Study design and reviewing the manuscript; M.SH: Data gathering, drafting, and reviewing the manuscript; A.A: Data gathering, drafting, and reviewing the manuscript; M.Gh: Data gathering, drafting, and reviewing the manuscript; M.AK: Data gathering, drafting, and reviewing the manuscript; N.MM: Data interpretation and reviewing the manuscript; A.AK: Data interpretation and reviewing the manuscript; Sh.Z: Data interpretation and reviewing the manuscript; Z.Kh: Data interpretation and reviewing the manuscript; N.T: Data interpretation and reviewing the manuscript; A.T: Study design, data interpretation and reviewing the manuscript. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

The authors declared no potential conflict of interest as some of the authors are affiliated with PerciaVista R&D Co., Iran, the manufacturer of the Exovista kit used in this study. This affiliation could be perceived as a potential source of bias; however, the authors affirmed that all efforts were made to ensure objectivity and integrity in the conduct of the research, analysis of data, and reporting of results.

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