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RESEARCH ARTICLE

Mesenchymal stem cell derived exosome promotes the viability of ameloblast-like cells

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ABSTRACT

Objective(s): To assess the effects of the exosome produced by mesenchymal stem cells (MSCs) on ameloblast-like cells' capacity to proliferate.

Methods: The exosomes were isolated from the human BM-MSCs and characterized by TEM images and western blotting. Then ALC cells were exposed with the increasing concentration of the exosome within 12, 24, 48 and 72 hours of treatment. Then, the cell viability was assessed by MTT assay. Also, the expression levels of the cyclin A, cyclin B, PI3K/AKT and FOXO3 were measured by real-time PCR upon cell exposure with 40 ng/ml exosome within 24-72 hours of treatment.

Results: BM-MSCs-exosome could to promote the viability of ALC cells, in particular, at higher concentrations. Also, therapy resulted in an increased level of cyclin A/B, PI3K, AKT and FOXO3 in treated cell, more evidently within 72 hours of treatment.

Conclusions: We showed that MSCs-derived exosome as natural nanoparticles could improve the viability of ameloblast-like cells by promoting cell cycle arrest and activating P3K/AKT pathway.

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INTRODUCTION

Despite the challenges in dental tissue regeneration that still need to be overcome, there is strong evidence to support the idea that recent developments in cell-based technology have a great chance of overturning the status quo and enabling dental tissue regeneration [1, 2]. With the help of stem cell technology, it may be possible to improve periodontal defects, encourage the regeneration of the maxillary and mandibular bones, and even repair teeth after carious damage and perhaps even grow back missing teeth [3]. The rapid application of regenerative dentistry into general dental practice will be made possible in the near future as a result of advancements in bioengineering research. This will result in effective treatments and significantly raise patient quality of life.

A key technique for repairing relatively large tissue or organ defects is cell-based tissue

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engineering and regeneration [4]. Exogenous cells that have been obtained from various sources, including autologous and allogenic sources, are also delivered. Due to their rapid in vivo proliferation as well as their differential capacity, stem/progenitor cells are therefore superior to differentiated cells [5, 6]. The earliest cells found in all multicellular organisms, stem cells (SCs), have the capacity for self-renewal and the capacity to differentiate into mature cell types. According to the literature, SCs have a remarkable capacity for regeneration and can be used to replace or restore damaged cells, enabling the treatment of a variety of conditions. The distinct class of adult stem cells known as mesenchymal stem cells (MSCs) can be obtained from bone marrow (BM), as well as the liver, umbilical cord, placenta, adipose tissue (AT), synovial membrane, amniotic fluid (AF), and even teeth [7-9]. They can essentially be divided into a variety of tissues, including adipose, cartilage, and bone. Additionally, MSCs secrete a wide range of biomolecules, which finally facilitate angiogenesis, bone formation, immunomodulation, and ultimately tissue regeneration [10, 11]. In fact, MSCs play a significant role in dentistry because they can help with the regeneration of important tissues like bone, cementum, periodontal ligament fibers, and dental pulp [12, 13]. Due to their immunomodulatory abilities, MSCs may also reduce oral cavity inflammation. Enamel is the hardest substance in the human body, and ameloblasts are cells that secrete the enamel proteins enamelin and amelogenin [14, 15]. Ionic and organic compositions of enamel are under the control of ameloblasts. The range of progressive phenotypes and activities that ameloblasts, a class of epithelial cells, display during the stages of enamel formation, represent a truly unique life cycle [16]. Enamel, a unique type of mineralized and acellular tissue derived from the epithelium in vertebrates, is produced by ameloblasts. A dense network of hydroxyapatite crystals arranged in a prismatic structure makes up this surface-level hypermineralized barrier. Enamel has almost no organic matrix in its mature state.

MATERIALS AND METHODS

Cell culture

Royesh Stem Cell Biotechnology (Tehran, Iran) provided the human bone marrow (BM)-MSCs. Then, they were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Germany) with 10% fetal bovine serum (FBS) (Sigma-Aldrich, Germany) and 1% pen/strep. Cells were then maintained at 37°C in a humidified atmosphere containing 5% CO2. Additionally, 10% heat-inactivated fetal bovine serum (FBS) and 1% pen/strep were added to the DMEM medium used to grow the ALC cells.

Exosome isolation

Using the MagCaptureTM Exosome Isolation Kit PS (FUJIFILM Wako), BM-MSCs-derived exosomes were isolated from the serum-free conditioned media (CM). For the purpose of eliminating other varieties of the vesicles, the serum-free CM was centrifuged at 10,000 g for 40 min. Using 0.2 mm filter membranes, the supernatants were subsequently filtered and ultimately restored.

Western blotting

The BM-MSCs cells and their exosomes were first lysed in RIPA buffer (BioLegend, USA) and then moved to PVDF (polyvinylidene difluoride) membranes for analysis of the expression of CD9, CD81, and CD81. The protein levels of CD63, CD81, and CD9 expression were measured using specific primary and secondary antibodies (Abcam, UK).

MTT

Following the directions on the MTT kit (Abcam, UK), the effects of the exosomes at varying concentrations of 5-80 ng/ml on the ALC cell line were quantified. 4×10^4 cells were initially seeded in 96-well plates and incubated for 24 hours. We then added the exosome at a concentration of 5-80 ng/ml, and the ALC cells were incubated for 24-96 hours. After adding 10 µL of the 5 mg MTT/ml medium, the wells' OD was measured at 570 nm wavelengths after 4 hours of incubation.

Transmission electron microscopy (TEM)

Exosomes that had been isolated were examined under a TEM to gauge their morphology. A pellet of exosomes was suspended in PBS and then applied to carbon-coated grids for electron microscopy for negative staining.

RNA isolation and cDNA synthesis

Total RNA was extracted from ALC cells using the RNX Plus solution kit (Sinaclon, Iran). ALC cells were first lysed, and crops were then kept

at -70°C and thawed when RNA extraction was necessary. The isolated RNA was used to create complementary DNA (cDNA) using a high-capacity kit (Bioneer, USA).

Real-Time PCR

Total RNA was extracted from ALC cells using the RNX Plus solution kit (Sinaclon, Iran). ALC cells were first lysed, and crops were then kept at -70°C and thawed when RNA extraction was necessary. The isolated RNA was used to create complementary DNA (cDNA) using a high-capacity kit (Bioneer, USA).

Statistical analysis

Results from three separate experiments were achieved. Data are presented as Mean± SEM. Statistics were determined using the Student T-test. Graph Pad Prism software was used to conduct all analyses.

RESULTS AND DISCUSSION

MSCs-exosomes promotes proliferation of ALC cells

Exosomes were isolated and their identity and morphology was approved suing TEM images and western blotting (Figure 1 A, B). The ALC cells were treated with 5-80ng/ml concentrations of MSCs-exosome. Based on MTT assay, 10-80ng/ml concentrations of MSCs-exosome were able to enhance to proliferation of ALC cells within 12, 24, 48 and 72 hours of exposure (Figure 2) ($P<0.05$). Although MSCs-exosome 5ng/ml was not able to promote cell viability within 12 hours of treatment, it enhanced cell proliferation within 24-72 hours of exposure. The supportive effect also was time- and dose-dependent (Figure 2) ($P<0.05$). Accordingly, the most evident effect was found within 72 hours of exposure with MSCs-exosome 80 ng/ml.

MSCs-exosomes up-regulate cyclin A and cyclin B expression in ALC cells

The cyclin A and cyclin B expression in ALC cells were assessed upon treatment with 40 ng/ml concentrations of MSCs-exosome within 24, 48 and 72 hours of treatment (Figure 3A, B) ($P<0.05$). Based on Real-Time PCR results, 40 ng/ml concentrations of MSCs-exosome did not affect cyclin A and cyclin B expression in ALC cells within

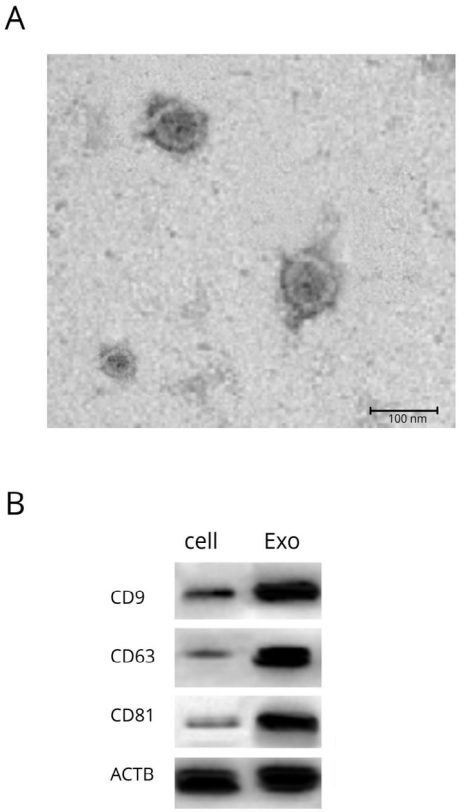

Fig. 1. Exosome characterizing by TEM image (A) and western blotting (B).

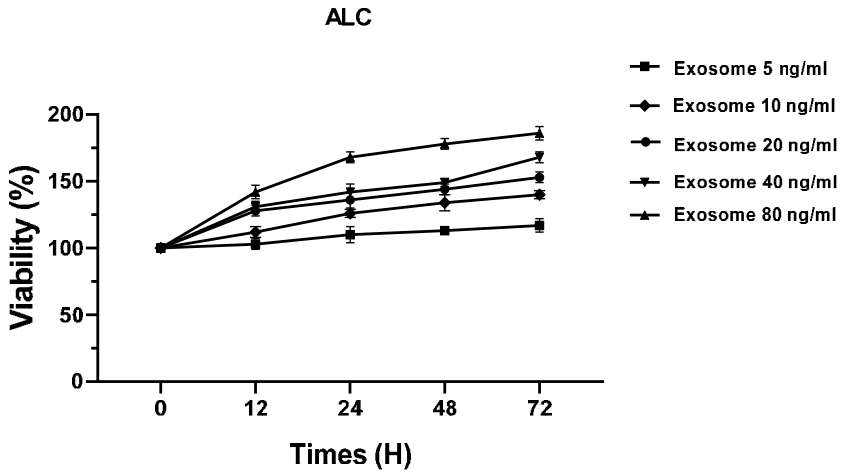

Fig. 2. MTT assay results based on the proliferation of ALC cells upon treatment with various concentrations of MSCs-exosome.

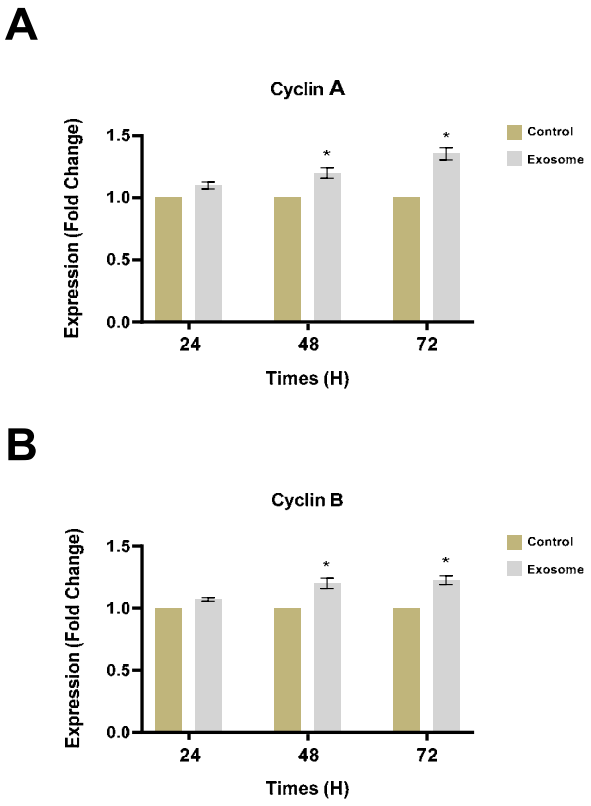

Fig. 3. Real Time PCR results based on the cyclin A (A) and cyclin B (B) expression in ALC cells upon treatment with 40 ng/ml concentration of MSCs-exosome.

24 hours of treatment compared to control group (Figure 3A, B) ($P<0.05$). Besides, an increased levels of the cyclin A and cyclin B expression were found within 48 and 72 hours of ALC cell treatment with 40 ng/ml concentrations of MSCs-exosome compared to control group (Figure 3A, B) ($P<0.05$). The most significant increment was also detected within 72 hours of treatment.

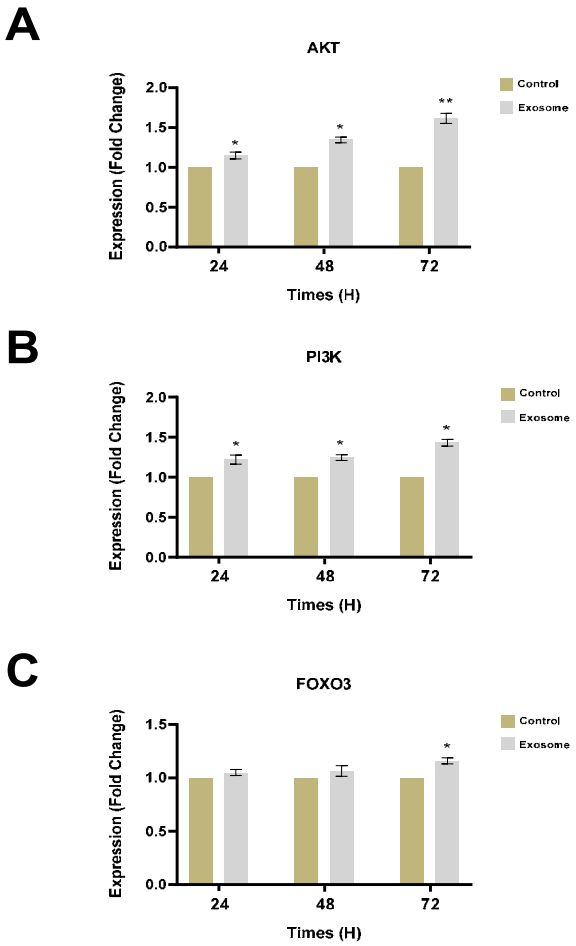

Fig. 4. Real Time PCR results based on the AKT (A), PI3K (B) and FOXO3 (C) expression in ALC cells upon treatment with 40 ng/ml concentration of MSCs-exosome.

Exosomes play a role in various cell-to-cell interactions related to a variety of physiological and pathological functions, according to mounting evidence [17]. Exosomes hold a prominent potential as a treatment for pathological conditions like dental tissue diseases due to their ability to efficiently transport their cargo, such as lipids, RNAs, and proteins, to target recipient cells or tissues [18]. Numerous studies have demonstrated that MSC-derived exosomes can promote human cell proliferation both in vitro and in vivo, which is consistent with these findings. Exosomes made from dental pulp have a strong ability to induce ontogenesis and osteogenesis [19].

MSCs-exosomes up-regulate PI3K/AKT expression in ALC cells

The PI3K and AKT expression in ALC cells were assessed upon treatment with 40 ng/ml concentrations of MSCs-exosome within 24, 48 and 72 hours of treatment (Figure 4A, B) ($P<0.05$). Concerning the Real-Time PCR results, 40 ng/ml concentrations of MSCs-exosome increased both AKT and PI3K expression in ALC cells within 24-72 hours of treatment compared to control group (Figure 4A, B) ($P<0.05$). The most significant increment was also detected within 72 hours of treatment. Also, the enhancement in AKT was more evident compared to PI3K at the same period.

Other studies have demonstrated that MSCs-derived exosomes trigger target cell proliferation by inducing Akt and ERK axis [20, 21]. MSC-exosomes could induce the proliferation, migration, and tube-forming potential of endothelial cells in vitro by up-regulating the PI3K/Akt signaling pathway in target cells [22].

Table 1. Primer pairs used for Real-time PCR

Gene		Primer Sequence (5′-3′)
PI3K	F	GAAGCACCTGAATAGGCAAGTCG
	R	GAGCATCCATGAAATCTGGTCGC
AKT	F	TGGACTACCTGCACTCGGAGAA
	R	GTGCCGCAAAAGGTCTTCATGG
FOXO3	F	CATTCTGTCTACGAGGTGCGTTG
	R	CTCTTGCCTGGATGAGTTCCCT
Cyclin A	F	ATCCAAAGCAGCGGTTAGTCCCA
	R	ATTTCCTCAGGAAAGAATTTTCAGA
Cyclin B	F	TGGGCCTTATGCCCCTTAAATGTC
	R	AAGAGTTTGGGCCAATGTCCTCA
GAPDH	F	GAGTCAACGGATTTGGTCGT
	R	TTGATTTTGGAGGGATCTCG

MSCs-exosomes up-regulate FOXO3 expression in ALC cells

The FOXO3 expression in ALC cells were evaluated post-treatment with 40 ng/ml concentrations of MSCs-exosome within 24, 48 and 72 hours of treatment (Figure 4C) ($P<0.05$). Real-Time PCR results showed that 40 ng/ml concentration of MSCs-exosome also enhanced FOXO3 expression, this enhancement was not significant within 24 and 48 hours of treatment (Figure 4C) ($P<0.05$). However, the 40 ng/ml concentration of MSCs-exosome was able to improve FOXO3 expression significantly within 72 hours of treatment (Figure 4C) ($P<0.05$).

CONCLUSION

Our analysis have shown that MSCs-derived exosome could improve ameloblast like cells proliferation by increasing cyclin A/B expression and promoting PI3K/AKT signaling pathways along with up-regulation of FOXO3 expression. These results open new avenue for treatment of dental tissue diseases resulting from ameloblast cells dysfunction or loss.

CONFLICT OF INTEREST

There is no conflict of interest.

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