Effect of human breast milk-derived exosomes on the migration and inflammatory response of lipopolysaccharide-exposed dental pulp stem cells

Ehsaneh Azaryan
Birjand University of Medical Sciences
Samira Karbasi
Birjand University of Medical Sciences
Mansoore Saharkhiz
Birjand University of Medical Sciences
Mohammad Yahya Hanafi-Bojd
Birjand University of Medical Sciences
Asghar Zarban
Birjand University of Medical Sciences
Fariba Emadian Razavi
Birjand University of Medical Sciences
mohsen naseri (naseri_m2003@yahoo.com)
Birjand University of Medical Sciences https://orcid.org/0000-0003-3100-6344

Research Article

Keywords: Dental pulp stem cells, Exosomes, Inflammation, Lipopolysaccharide

Posted Date: August 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1889959/v1

Abstract

Aim: The purpose of this study was to investigate the effects of human breast milk-derived exosomes (HM-Exos)on the viability,migration, and inflammatory responses oflipopolysaccharide (LPS)exposed human dental pulp stem cells (HDPSCs) in vitro condition.

Methods: HM-Exoswere isolated, and dynamic light scattering (DLS), scanning electron microscopy (SEM), and transmission electron microscopy(TEM) were used to analyze their physiological properties (size and shape).To construct an in vitro inflammation model, HDPSCs were exposed to LPS.The MTT test and migration assay were used to investigate the effect of HM-Exos on cell proliferation and migration, and the quantitative polymerase chain reaction was used to assess the expression of inflammatory genes in HDPSCs.

Results: DLS measurement revealed that HM-Exos were 116.83.6 nm in diameter. The SEM and TEM images revealed spherical shapes with diameters of 97.2±34.6 nm. According to the results of the cell viability assay, the maximal nontoxic concentration of HM-Exos (400 μg/ml) was chosen for the subsequent investigations. The migration assay results showed that HM-Exos improved the potential of HDPSCs to migrate under LPS-stimulated inflammatory processes. The RT-PCR results indicated that HM-Exos significantly reduced the expression of inflammatory cytokines such as TNF-α, IL-1β and IL-6 in HDPSCs after LPS stimulation.

Conclusions: HM-Exos reduced gene expression of inflammatory cytokinesin inflamed HDPSCs and may be a viable candidate for use as a pulp capping substance in vital pulp therapy.

Highlights

- HM-Exos was successfully isolated using the exosome extraction kit.
- The size and shape of HM-Exos were analyzed using DLS, SEM, and TEM.
- HM-Exos improved the migration potential of LPS-exposed HDPSCs.
- HM-Exos reduced the expression of inflammatory cytokines such as TNF-α, IL-1β and IL-6 in LPS-exposed HDPSCs.

1. Introduction

The dental pulp is a connective tissue that is located in the center of the tooth and contains cells, nerves, and blood vessels. Tooth decay is the most prevalent chronic disease, characterized by the loss of calcified tissue as a result of sugar consumption and the impact of microbes. As caries advances and the infection affects the tooth pulp, immunological and defensive reactions occur in the pulp, resulting in pulp inflammation, or pulpitis. Pulpitis pathogenesis contains a range of substances and bacterial products that enter the dentin and root canal. Lipopolysaccharide (LPS) is a potent stimulator of pulpitis

and has been found in inflamed pulp tissue, causing the release of inflammatory cytokines including TNF-α, IL-1β and IL-6, which suppress odontogenic differentiation of dental pulp cells and the stimulation of necrosis in the pulp.

Because the vitality of the dental pulp is critical to the survival and health of the tooth, in treating inflamed pulp tissue, it is very importantto regulate the inflammation of its healthy sections. The purpose of vital pulp therapy (VPT) is to preserve pulp tissue that has been injured by decay, trauma, or tooth restoration but not totally destroyed.This is done by eliminating caries and employing biocompatible materials to establish resistance to bacterial re-entry.These treatments include direct pulp capping, indirect pulp capping, and pulpotomy, which are utilized based on the degree of pulp inflammation.The most regularly utilized pulp caps, have limited anti-inflammatory capabilities. On the other hand, sodium hypochlorite and normal saline are the two most frequently utilized washing solutions in pulpotomies. However, normal saline has no anti-inflammatory qualities, while sodium hypochlorite in high concentrations damages the pulp tissue. Anti-inflammatory drugs can therefore be used to treat pulpotomy and reduce pulp inflammation[1–6].

Exosomes are extracellular vesicles released by various cell types. They emanatefrom particular cell compartments known as multivesicular bodies (MVBs) and have a diameter of 30-100 nm. Exosomes contain various types of components obtained from parent cells, such as proteins, miRNAs, RNA, and DNA, which conduct a significant role in cell-to-cell interaction. They are involved in regulating important biological processes such as the immune response and inflammation, collagen production, tissue regeneration, blood coagulation, and angiogenesis [7, 8]. Exosomes are generally involved in providing a diversity of antigens to induce an immune andinflammatory responsesthrough the translocation of prostaglandins[9].Exosomes are being employed more often as a component in wound healing and scar-free procedures.Because they are naturally generated, they have the ability to regulate inflammatory responses and encourage cell migration and proliferation[8].

Breast milk includes a wide range of components, including immune competent cells, milk fat globules (MFG), and soluble proteins such as IgA, cytokines, and antimicrobial peptides [10].Exosomes are found in abundance in breast milk [11].Milk exosomes are membranous nanovesicles that are 30-100 nm in size and play an important role in intercellular communication, primarily through their mRNAs, microRNAs, and proteins [12]. Breast milk exosomes have different roles, including: anti-cancer properties, regulation of immune responses, and control of inflammatory responses [13].

Mesenchymal stem cells (MSCs) are multipotent stem cells found in a variety of adult tissues, including bone marrow, adipose tissue, and tooth pulp. These cells are distinguished by their ability to self-renew and multi-differentiate [14, 15].MSC cells have a high paracrine influence on the body. Considering the role of exosomes in cellular communication, it is suggested that these molecules play a role in the paracrine actions of MSCs [16].DPSCs, unlike other well-known MSCs, are the major component of dental pulp tissue and are employed in numerous in vitro dental pulp investigations. Because of their tremendous potential in tissue repair/regeneration and immune response regulation, they have been

indicated as a prospective choice for cell-based therapeutic applications. Therefore, it has been presentedas a promising therapy for inflammatory and damaging disorders such as pulpitis[4, 17–19].

Although the extraction and analysis of exosomes from human blood has been widely published, there have been few studies of exosomes from breast milk. The goals of this study were to prepare an effective extraction protocol for exosomes-drived human breast milk (HM-Exos) and investigate the impact of HM-Exos on proliferation, migration, and inflammatory response of LPS-induced dental pulp stem cells.

2. Materials And Methods

2.1 Human breast milk exosome isolation

350 breastfeeding mothers aged 20 to 35 were recruited from four healthcare centers in southern Khorasan, Birjand, Iran for this cross-sectional study. A cluster random sampling method was used to select participants. Prior to recruitment, subjects provided written informed consent. All participants had infants aged 1 to 6 months, with no history of chronic diseases or medication use in the previous six months. Birjand University of Medical Sciences Ethics Committee (ethical number: IR.BUMS.REC.1399.281) approved the study. At the start of the day, each mother was asked to provide two samples of breast milk in 20 ml volumes expressed from primary breastfeeding.Human breast milk is centrifuged after collection to remove fat and debris. The upper fat layer is removed by centrifuging milk at 2000 g for 10 min at 4 °C, then at 12000 g for 30 min at 4 °C to remove deposited cells. Exosomes are extracted from the remaining milk by the EXOCIB kit (Tehran, Iran), as directed by the manufacturer. A BCA protein assay kit was used to determine the total protein content of exosomes, and the protein concentration of HM-Exos equaled 63 mg/ml.

2.2 Exosome characterization

DLS was used to evaluate the size of Exos, and to further analyze the size and morphology of Exos, SEM and TEM were employed.

2.3 Cell isolation and culture

The HDPSCs were isolated using a technique reported in our prior publication[20].In brief, healthy third molars (wisdom tooth) of patients aged 20 to 25 were collected after obtaining the consent of the patientsat the Dental Center of Imam Reza Hospital in Birjand (Iran), in accordance with the guidelines of the Birjand University of Medical Sciences ethics committee (ethical number: IR.BUMS.REC.1399.090).The pulp tissues were removed from the teeth and then soaked inPBS containing 100U/mL type Icollagenaseenzyme (Bio-Idea, Tehran, Iran) for 1 hour at 37°C.The extracted cells from the toothpulp were cultured in the Dulbecco's modified Eagle's medium mixtureF-12 (DMEM/F12) complemented by 10% fetal bovine serum and 1%antibiotic (penicillin-streptomycin) (all from Gibco, USA) at 37°C in 5% CO_2 and a humidified atmosphere.Cell passage numbers from 3 to 5 were used for the following experiment.

2.4 LPS induction ofHDPSCs

Escherichia coli LPS powder (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water and added to the culture mediumto trigger inflammation of HDPSCs. These LPS-induced HDPSCs were called inflammatory human dental pulp stem cells (iHDPSCs). After 24 h of culture of HDPSCs, the medium was changed to a complete medium supplemented with 2 g/ml of LPS powder for 24 h in order to induce inflammation. The supernatants and cells were taken at each experimental time point for the following experiment.

2.5 Cell viability assay

To cell viability test, HDPSCs were planted in a 96-well plate (SPL) at a density of 1.5 10^4cells per well. After 24 h of LPSinduction, the medium was changed to complete culture medium supplemented with different doses of HM-Exo for 24 h at 37 °C. Then 20 µl of MTT solution (2 mg/ml in PBS) was added to each well, and the plate underwent incubation for 4 h at 37°C incubator in the dark. Afterward, the supernatant was eliminated, 100µl DMSO was added to each well, and the optical absorption of the samples was measured at 570 and 630 nm using a spectrophotometer (Biotek Epoch, winooski, VT).

2.6 Cell migration assay

A scratch test was used to evaluate the migration potential of HDPSCs.HDPSCs were planted into six-well plates. After 24 h of LPS induction, the medium was changed to complete culture medium and a straight scratch was formed with a 200 µl tip. Microscopy was used to observe cell migration at 0, and 24 h after treatment with 400µg of HM-Exos. The lesion's boundary areas were evaluated and photographed using an inverted microscope (ZEISS Axiovert 200, Zeiss, Germany). Cellular migration was evaluated by measuring the ratio between the reduced open space after 24 h and the open space at 0 h.

2.7 Quantitative real-time polymerase chain reaction (qRT-PCR)

The mRNA expression levels of TNF-α, IL-1β and, IL-6 were evaluated using the quantitative real-time polymerase chain reactionand the GAPDH gene was used as an endogenous control. Isolation of mRNA and cDNA synthesis were performed using the Pars Tous kit (Tehran, Iran) according to the manufacturer’s instructions, and real-time was done using the SYBR Green assay. The expression levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method.The sequences of primers used are showed in Table 1.

2.8 Statistical analysis

The data were presented as mean standard deviation (n=3). The statistical analysis was carried out using GraphPad prism software. A one-way analysis of variance (ANOVA) with Tukey's post hoc multiple comparison test was employed to assess statistical significance for noticing significantdifferences between study groups, with $p< 0.05$ as the statistical significance value.

3. Result

Exosome isolation and characterization

The size and shape of HM-Exos were described in figures 1, 2, and 3. According to DLS findings, HM-Exos were 116.8±3.6nm in diameter(Fig.1). The SEM and TEM observations of Exos revealed that they were spherical in form and had a size of 97.2±34.6 nm (Fig.2 and 3).

3.1 Effects of LPS powder and HM-Exoson iHDPSCs viability

The effect of LPS powder (2 μg/ml) and different doses of HM-Exos on the cell viability of iHDPSCs is shown in Fig.4.After 24 hours, the viability of cells treatedwith LPS was not significantly different compared tothe control group ($p > 0.05$).The viability of HM-Exos-treated iHDPSCs increased significantly at doses of 10 and 25 ($p<0.01$) and 50 μg/ml ($p<0.05$). In comparison to the control group, no statistically significant difference in cell toxicity or proliferation was seen at 5 and 100–400 μg/ml doses ($p>0.05$). Furthermore, at 800 μg/ml, cell viability was reduced ($p<0.01$). Based on the findings of the cell viability assay, the maximal nontoxic concentration (400 μg/ml) of HM-Exos was chosen for following investigations.

3.2 The effects of LPS powder and HM-Exos on iHDPSCs migration

To investigate the effects of HM-Exos on the migration capacities of iHDPSCs, the scratch test was done (Fig.5). The results indicated that the concentrations of 400 μg/ml of HM-Exosimprovedthemigratory abilities of iHDPSCs compared to HDPSC and iHDPSC groups ($P < 0.01$).

3.3 The effects of LPS powder and HM-Exos on inflammatory cytokine expression

To investigate the inhibitory impact of HM-Exos on mRNA expression of inflammatory cytokines in iHDPSCs, qRT-PCR was used (Fig.6). LPS significantlyincreased mRNA expression of IL-6, IL1-β and TNFα ($p<0.01$, $p<0.05$, $p<0.001$ respectively) compare to the HDPSC group. HM-Exos significantly reduced LPS-induced expression of IL6, IL1-β ($P<0.01$) and TNFα ($P<0.001$) compared to the iHDPSC group.

4. Discussion

HM-Exos has been demonstrated to have a variety of functions, including anti-cancer characteristics, immune response modulation, and inflammation response regulation[13]**.** However, the role of HM-Exos in pulpitis therapy remained unclear. We isolated vesicles from human breast milk using the exosome extraction kit. Using electron microscopy and DLS, we discovered that the vesicles had an exo-like form and size. For the first time, we have demonstrated that in an in vitro LPS model of pulpal inflammation, HM-Exos had an anti-inflammatory and cell migration effect on HDPSCs.

DPSCs are a type of pluripotent stem cell found in the dental pulp's cell-rich zone. They play an important role in angiogenesis, homeostasis, and tooth pulp health by responding to injury and bacterial

invasion.According to studies,DPSCshave a high potential for healing at the inflamed area[21].LPS is a prominent component of the bacteria's outer membrane and can promote the expression of inflammatory cytokines as well as cellular death in DPSCs [4].In this investigation, we found that LPS might induce an inflammatory condition in DPSCs by increasing the expression ofTNF-α, IL-1β and, IL-6. In addition, we indicated that the level of inflammatory cytokines was enhanced in response to LPS exposure and reduced after treatment with HM-Exos.

Pulpitis is a common inflammation of tooth pulp tissue, and oral microbes are implicated in this opportunistic infection. According to research, various parameters associated to host reaction play an important role in pulpitis. Among these components are immune system inflammatory mediators such as cytokines and chemokines, which contribute to pulpal defense mechanisms[22]. In this regard, the results of a reversible pulpitis model showed that IL-1β, IL- 6, and TNF-α gene expressions were elevated in LPS-exposed inflamed pulp tissues [23].Recent studies on the cellular and molecular basis, inflammatory processes, pulp repair, and the emergence of new drug strategies such as drug delivery systems and tissue engineering have opened up numerous ways for the progress of infectious and inflammatory pulp therapeutic strategies [24]. Jung et al.,revealed that simvastatin inhibits the expression of inflammatory cytokines, cell adhesion molecules, and NF-kβ transcription factors induced by LPS in human dental pulp cells [25]. Li et al.,investigated that epigallocatechin gallate (EGCG) significantly reducedexpression of inflammatory cytokines like TNF-α, IL-1β and IL-6 in dental pulp stem cells after LPS stimulation, and also reduced inflammation of inflamed rat pulp tissue[2].Chen et al.,demonstrated that 4Methylumbelliferone decreased inflammatory cytokines in inflamed DPSCs and promoted DPSCs migration and odontogenic differentiation[26].According to several studies, exosomes have the potential to regulate immunity in inflammatory diseases. For example, exosomes from IL-10-treated dendritic cells were found to be effective in both suppressing the onset of murine collagen-stimulated arthritis and reducing the symptoms of established arthritis[27]. According to one study, Exos derived from MSCs can reduce osteoarthritic symptoms in an inflammatory model of osteoarthritis [28].Another study discovered that bone marrow mesenchymal stem cell-derived boosted M2macrophages, decreased inflammatory cytokines, and induced the secretion of anti-inflammatory cytokines [29].Ahnet al.,found that bovine milk exosomes inhibited the expression level of inflammatory cytokines such as IL-6 and TNF-αin LPS-induced RAW264.7 cells [8].As a result of the findings in this investigation, we propose that suitable in vivo studies be conducted in the future to demonstrate the efficacy of HM-Exo in pulpitis therapy.

5. Conclusion

In conclusion, the results showed that HM-Exos therapy could not only reduce inflammatory cytokine release but also increase HDPSC migration and proliferation. This study discovered that HM-Exos may be a viable candidate for use as a pulp capping substance in vital pulp therapy.

Abbreviations

MSC; Mesenchymal stem cell

HDPSC; Human dental pulp stem cell

HM-Exo; Human breast milk exosome

LPS; Lipopolysaccharide

IL-6; Interleukin-6

TNF-α; Tumor necrosis factor-alpha

IL-1β; Interleukin-1 beta

iHDPSC; Inflammatory human dental pulp stem cells

Declarations

Acknowledgments

We appreciate the staff working in the Central Research Lab of Birjand University of Medical Sciences for their assistance and support. This study was fully supported by a research grant given by the Vice-Chancellor for Research, Birjand University of Medical Sciences, Birjand, Iran.

Authors' contributions

The study was planned byEA, SK, MS, MH,FE, MN and, AZ. EA and SK were executor of plan and carried out data analysis and interpretationand wrote the original draft. The results analysis and interpretation, writing and revising the manuscriptwere conducted by MS,MH, AZ and FE. MN was responsible in the project's administration, supervision, finance,reviewing, and resource acquisition.All authors reviewed the manuscript.

Availability of Data Statement

All data of the study are available from the correspondingauthor upon reasonable request.

Author Disclosure Statement

The authors declare they have no financial conflicts ofinterest.

Funding Information

This study was funded by Birjand University of MedicalSciences, under grant No. 5785

Ethics Approval and Consent to Participate

For DPSCs extraction, third molars (wisdom tooth)were collected after obtaining the consent of the patientsat the Dental Center of Imam Reza Hospital in Birjand (Iran),in accordance with the guidelines of

the Birjand University of Medical Sciences Ethics Committee (ethical number: IR.BUMS.REC.1399.090).

For HM-Exo extraction, 350 breastfeeding mothers aged 20 to 35 were recruited from four healthcare centers in southern Khorasan, Birjand, Iran for this cross-sectional study. Prior to recruitment, subjects provided written informed consent. All participants had infants aged 1 to 6 months with no history of chronic diseases or medication use in the previous six months. Birjand University of Medical SciencesEthics Committee(ethical number: IR.BUMS.REC.1399.281) approved the study.

Consent for publication

Not applicable

References

1. Yu MK, Lee JC, Kim JH et al (2009) Anti-inflammatory Effect of Peroxisome Proliferator Activated Receptor Gamma on Human Dental Pulp Cells. J Endod 35:524–528. https://doi.org/10.1016/j.joen.2008.12.012
2. Li Y, Zhao Y, Han J et al (2021) Effects of epigallocatechin gallate (EGCG) on the biological properties of human dental pulp stem cells and inflammatory pulp tissue. Arch Oral Biol 123. https://doi.org/10.1016/j.archoralbio.2020.105034
3. Nakanishi T, Mukai K, Yumoto H et al (2010) Anti-inflammatory effect of catechin on cultured human dental pulp cells affected by bacteria-derived factors. Eur J Oral Sci 118:145–150. https://doi.org/10.1111/j.1600-0722.2010.00714.x
4. Wang D, Zhu NX, Qin M, Wang YY (2019) Betamethasone suppresses the inflammatory response in LPS-stimulated dental pulp cells through inhibition of NF-κB. Arch Oral Biol 98:156–163. https://doi.org/10.1016/j.archoralbio.2018.11.022
5. Lee JC, Yu MK, Lee R et al (2008) Terrein Reduces Pulpal Inflammation in Human Dental Pulp Cells. J Endod 34:433–437. https://doi.org/10.1016/j.joen.2008.01.015
6. Brodzikowska A, Ciechanowska M, Kopka M, Stachura A (2022) Role of Lipopolysaccharide, Derived from Various Bacterial Species, in Pulpitis — A Systematic Review. 1–30
7. Golchin A, Hosseinzadeh S, Ardeshirylajimi A (2018) The exosomes released from different cell types and their effects in wound healing. J Cell Biochem 119:5043–5052. https://doi.org/10.1002/jcb.26706
8. Ahn G, Kim YH, Ahn JY (2021) Multifaceted effects of milk-exosomes (Mi-Exo) as a modulator of scar-free wound healing. Nanoscale Adv 3:528–537. https://doi.org/10.1039/d0na00665c
9. Aharon A, Brenner B (2009) Microparticles, thrombosis and cancer. Best Pract Res Clin Haematol 22:61–69. https://doi.org/10.1016/j.beha.2008.11.002
10. Admyre C, Johansson SM, Rahman K et al (2022) Exosomes with Immune Modulatory Features Are Present in Human Breast Milk. https://doi.org/10.4049/jimmunol.179.3.1969

11. Miyake H, Lee C, Chusilp S et al (2020) Human breast milk exosomes attenuate intestinal damage. Pediatr Surg Int 36:155–163. https://doi.org/10.1007/s00383-019-04599-7
12. El-kattawy AM, Algezawy O, Alfaifi MY et al (2021) Therapeutic potential of camel milk exosomes against HepaRG cells with potent apoptotic, anti-inflammatory, and anti-angiogenesis effects for colostrum exosomes. Biomed Pharmacother 143:112220. https://doi.org/10.1016/j.biopha.2021.112220
13. de la Torre Gomez C, Goreham RV, Bech Serra JJ et al (2018) "Exosomics"-A review of biophysics, biology and biochemistry of exosomes with a focus on human breast milk. Front Genet 9:1–11. https://doi.org/10.3389/fgene.2018.00092
14. Ayadilord M (2021) Immunomodulatory effects of phytosomal curcumin on related-micro RNAs, CD200 expression and inflammatory pathways in dental pulp stem cells. 1–10. https://doi.org/10.1002/cbf.3659
15. Saharkhiz M, Razavi FE, Riahi SM et al (2021) An in Vitro Study of the Effects of Crocin on the Modulation of DSPP, VEGF-A, HLA-G5, STAT3 and CD200 Expression in Human Dental Pulp Stem Cells. Cell Reprogram 23:239–249. https://doi.org/10.1089/cell.2021.0032
16. Ren G, Zhang L, Zhao X et al (2008) Mesenchymal Stem Cell-Mediated Immunosuppression Occurs via Concerted Action of Chemokines and Nitric Oxide. Cell Stem Cell 2:141–150. https://doi.org/10.1016/j.stem.2007.11.014
17. E K, E A (2013) Dental Stem Cells and their. Applications in Dental Tissue Engineering
18. Potdar PD (2015) Human dental pulp stem cells: Applications in future regenerative medicine. World J Stem Cells 7:839. https://doi.org/10.4252/wjsc.v7.i5.839
19. Mansoore Saharkhiz M, Ayadilord FE, Razavi MN (2021) Effects of phytosomal curcumin treatment on modulation of immunomodulatory and pulp regeneration genes in dental pulp mesenchymal stem cells. Odontology 1–9. https://doi.org/https://doi.org/10.1007/s10266-021-00659-4
20. Saharkhiz M, Ayadilord M, Emadian Razavi F, Naseri M (2021) Effects of phytosomal curcumin treatment on modulation of immunomodulatory and pulp regeneration genes in dental pulp mesenchymal stem cells. Odontology. https://doi.org/10.1007/s10266-021-00659-4
21. Sehgal apoorva (2018) Jhanwar bharat., Gilhotra.K U Immune responses of human dental pulp stem cells in lipopolysaccharide induced microenvironment. Arch Anesthesiol Crit Care 4:527–534
22. Khorasani MMY, Hassanshahi G, Brodzikowska A (2020) Cytokine Role (s) of cytokines in pulpitis: Latest evidence and therapeutic approaches. Cytokine 126:154896. https://doi.org/10.1016/j.cyto.2019.154896
23. Renard E, Gaudin A, Bienvenu G et al (2016) Immune Cells and Molecular Networks in Experimentally Induced Pulpitis. J Dent Res 95:196–205. https://doi.org/10.1177/0022034515612086
24. Komabayashi T, Zhu Q (2010) Innovative endodontic therapy for anti-inflammatory direct pulp capping of permanent teeth with a mature apex. Oral Surgery. Oral Med Oral Pathol Oral Radiol Endodontology 109:e75–e81. https://doi.org/10.1016/j.tripleo.2009.12.034

25. Jung JY, Woo SM, Kim WJ et al (2017) Simvastatin inhibits the expression of inflammatory cytokines and cell adhesion molecules induced by LPS in human dental pulp cells. Int Endod J 50:377–386. https://doi.org/10.1111/iej.12635
26. Chen W, Guan Y, Xu F, Jiang B (2021) 4-Methylumbelliferone promotes the migration and odontogenetic differentiation of human dental pulp stem cells exposed to lipopolysaccharide in vitro. Cell Biol Int 45:1415–1422. https://doi.org/10.1002/cbin.11579
27. Kim S-H, Lechman ER, Bianco N et al (2005) Exosomes Derived from IL-10-Treated Dendritic Cells Can Suppress Inflammation and Collagen-Induced Arthritis. J Immunol 174:6440–6448. https://doi.org/10.4049/jimmunol.174.10.6440
28. Zhu Y, Wang Y, Zhao B et al (2017) Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis. Stem Cell Res Ther 8:1–11. https://doi.org/10.1186/s13287-017-0510-9
29. Zhang J, Rong Y, Luo C, Cui W (2020) Bone marrow mesenchymal stem cell-derived exosomes prevent osteoarthritis by regulating synovial macrophage polarization. 12:25138–25152

Table

Table 1. Sequences of primers used for real-time PCR

Name	Forward	Reverse
TNF alpha	AGGCGGTGCTTGTTCCTCAG	GGCTACAGGCTTGTCACTCG
IL-1β	TCCAGGGACAGGATATGGAG	TCTTTCAACACGCAGGACAG
IL6	AGACTTGCCTGGTGAAAATCA	GCTCTGGCTTGTTCCTCACT
GAPDH	CGAACCTCTCTGCTCCTCCTGTTCG	CATGGTGTCTGAGCGATGTGG

Figures

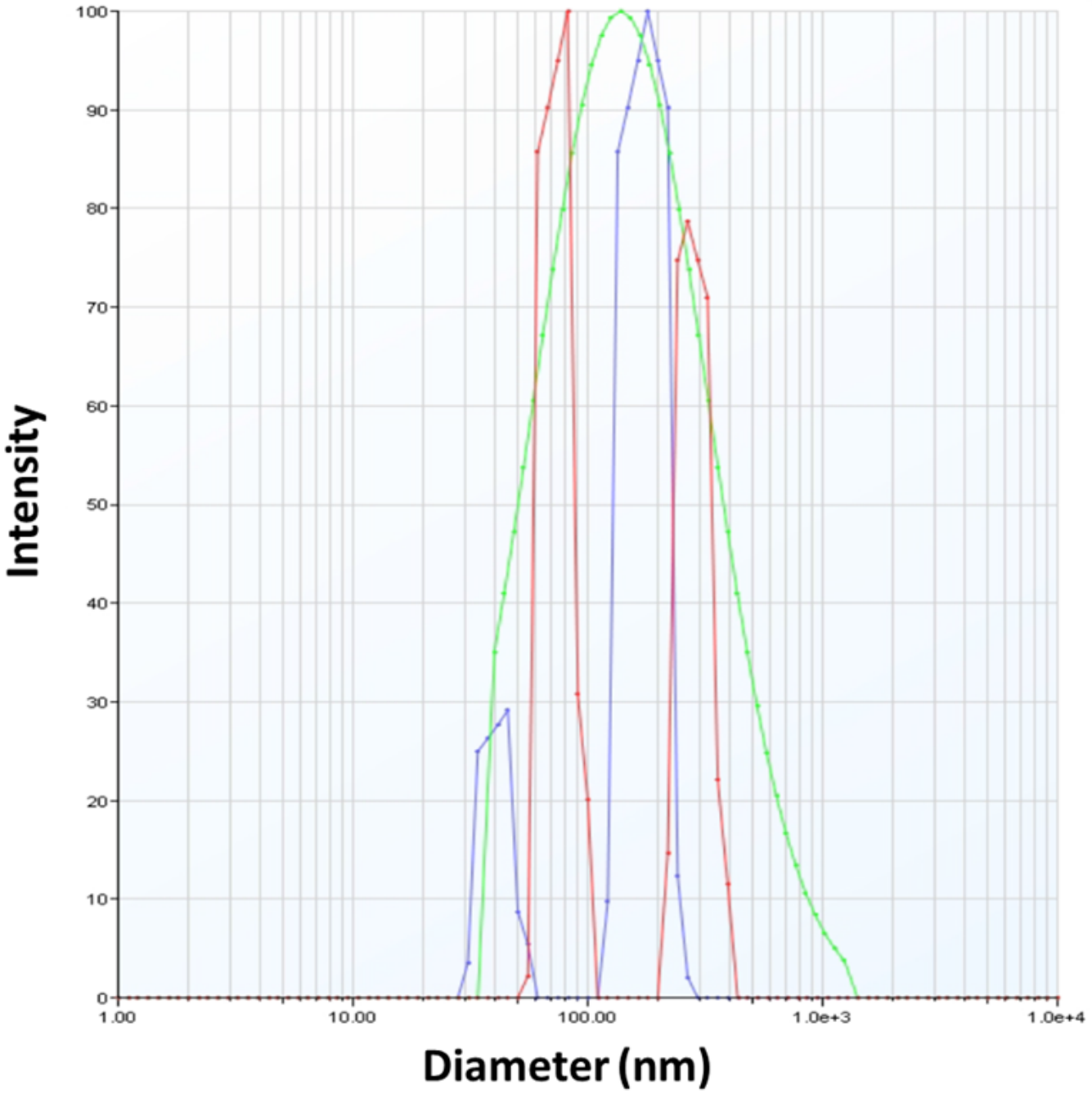

Figure 1

DLS Analysis of HM-Exos Size

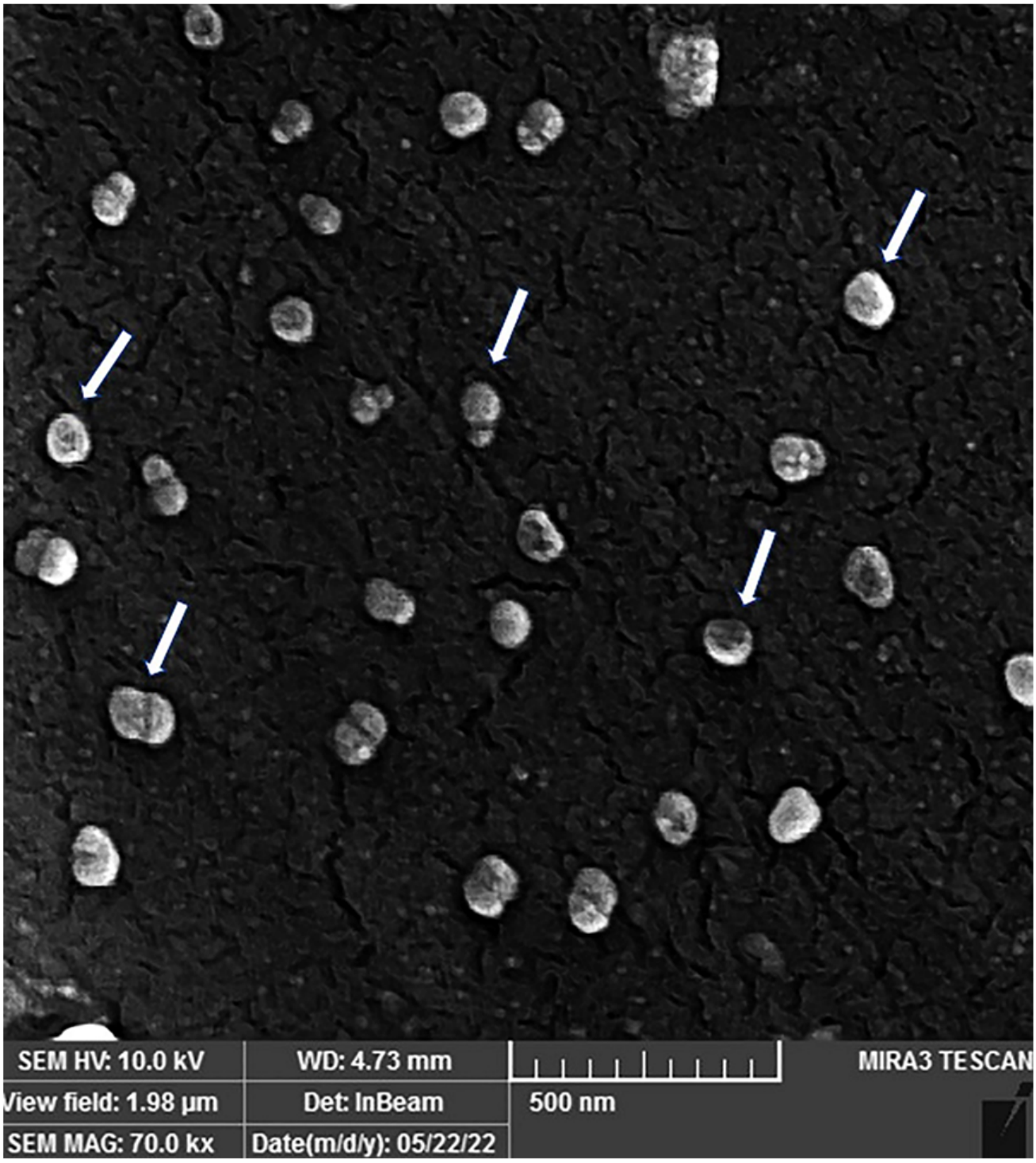

Figure 2

SEM image of HM-Exos

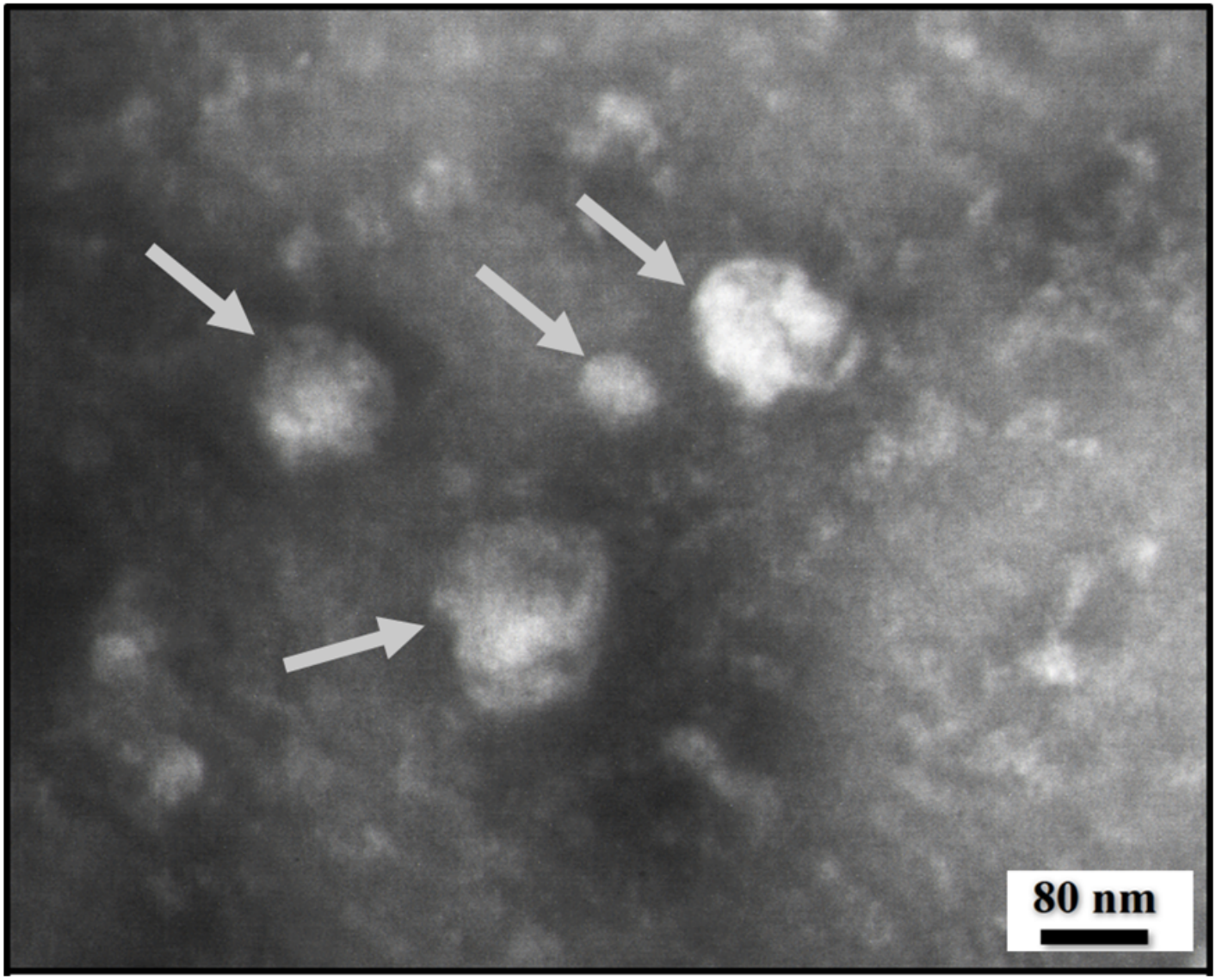

Figure 3

TEM image of HM-Exos

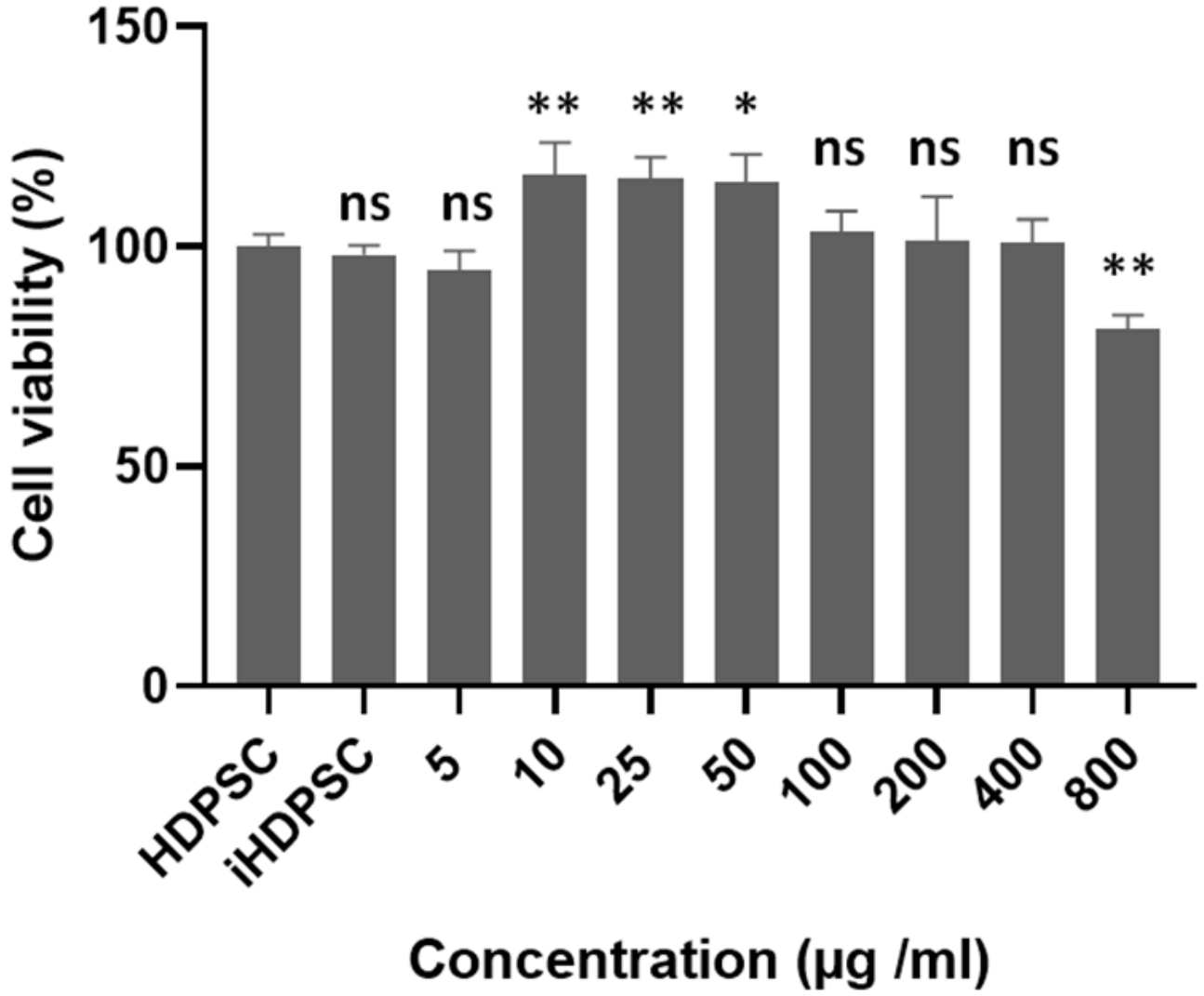

Figure 4

Viability assay for HDPSCs incubated with HM-Exos on 24h. Significant differences compared to control were indicated: *p < 0.05; **p < 0.01; ***p < 0.001.

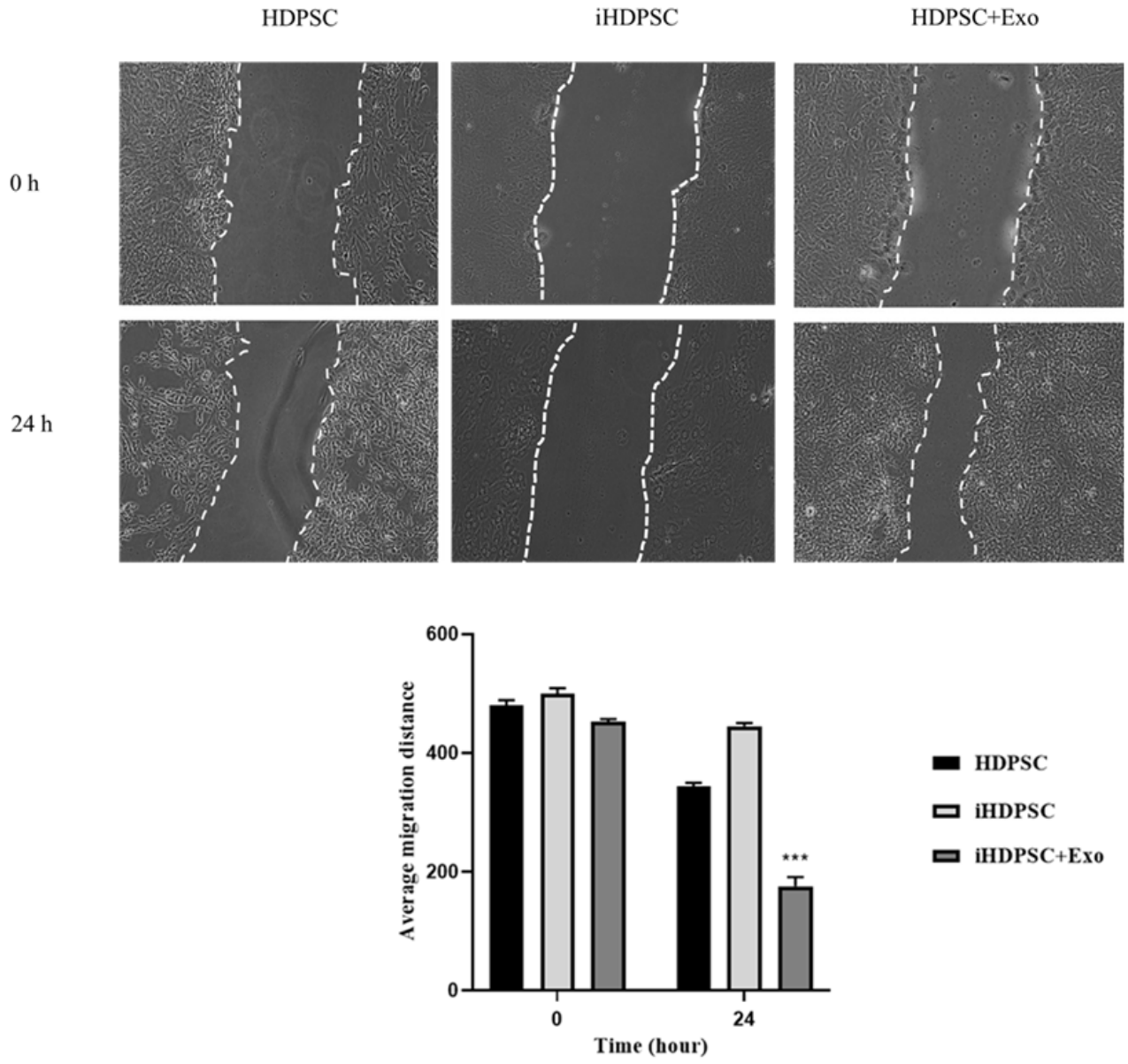

Figure 5

The effects of HM-Exos on the migration of HDPSCs

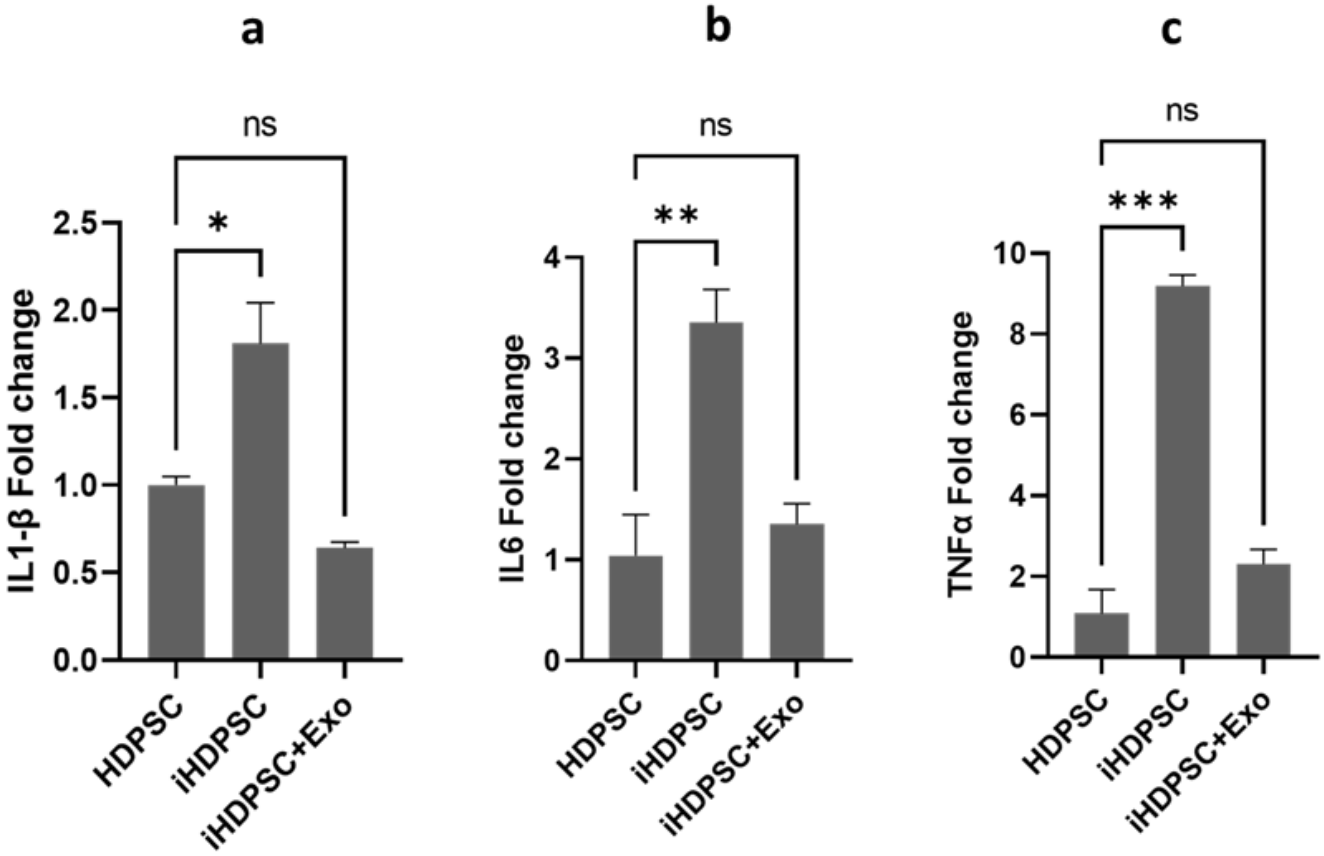

Figure 6

Comparison of the genes expression levels: (a) IL1-β, (b) IL-6, and (c) TNFα. *p<0.05, **p<0.01, ***p<0.001.