

Inducing Corneal Keratocyte Differentiation in Human Wharton's Jelly-derived Mesenchymal Stem Cells via Keratocyte-derived Exosomes

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ABSTRACT

One of the primary and most important components of the corneal stroma is keratocyte cells. These cells are of great interest in cell therapy for corneal diseases such as keratoconus and corneal ectasia due to their unique characteristics, including quiescence and difficulty in cultivation. Therefore, differentiation of mesenchymal stem cells (MSCs) into keratocytes has long been considered as an alternative approach. The present study was designed to explore potential of directing human Wharton's jelly-derived mesenchymal stem cells (hWJMSCs) toward corneal keratocyte-like cells using keratocyte-derived exosomes. First, keratocyte cells were isolated and cultured from discarded corneal tissue. Their culture medium was collected, and keratocyte-derived exosomes were extracted. In the next step, the optimal dose of extracted exosomes was determined using an MTT assay. Differentiation induction in hWJMSCs was then using the optimal dose of keratocyte exosomes (KExo), and the differentiation outcomes were evaluated by two techniques: real-time PCR and immunocytochemistry (ICC) at two time points (7 and 14 days). Exosomes were isolated from the conditioned medium of keratocyte cells, and SEM imaging confirmed their presence. PKH26 staining verified the successful uptake of exosomes by MSCs. Expression of the keratocan, lumican, ALDH3A1, and CD34 genes showed a significant increase ($p < 0.05$) after differentiation induction at both 7 and 14 days compared to the control group. Furthermore, keratocan protein expression significantly increased in cells treated with keratocyte-derived exosomes. Since the conditioned medium of keratocytes is rich in keratocyte growth factors, including exosomes, isolation and application of these exosomes effectively induce differentiation of hWJMSCs into keratocyte-like cells. Therefore, the present method offers as a suitable and cost-effective approach to generate keratocytes needed for corneal tissue engineering.

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Introduction

The cornea, the outermost structure of the eye, is a highly transparent and organized tissue that plays a vital role in refracting light toward the retina and protecting the eye from external factors. When the corneal tissue is damaged, keratocytes—the primary cells of this tissue—are

activated and migrate to the injury site to regenerate the tissue. However, a fibrotic process may also occur following corneal tissue damage, which can lead to scar formation (Ghiasi *et al.*, 2023; A. Zhang, *et al.*, 2022).

Keratocytes are cells with unique characteristics: they are mitotically inactive and morphologically flat and dendritic in shape, forming an

interconnected cellular network between and parallel to the collagen lamellae within the corneal structure. The main function of these cells is to maintain homeostasis and corneal transparency through the production of collagen and proteoglycans (Fini, 1999; Ku, *et al.*, 2008). Studies have shown that the loss of keratocytes contributes to the development of keratoconus, with the severity of the disease correlating with the changes in keratocytes (Swarbrick, 2004). Keratocyte-based cell therapy represents an innovative approach for treating corneal stromal diseases (Alio del Barrio and Alio, 2018). However, because keratocytes are mitotically inactive, culturing these cells faces several challenges, including their low abundance. Additionally, keratocyte culture requires numerous complex growth factors, many of which are costly and not readily available (Chen *et al.*, 2009; He and Bazan, 2008).

Exosomes are spherical biological vesicles composed of a lipid bilayer, with a diameter of about 40 to 100 nm. They contain important biomolecules, including nucleic acids, proteins, lipids, cytokines, transcription factor receptors, and *etc.* One of the primary functions of exosomes is to mediate intercellular communication and exchange of substances during physiological and pathological processes (Ghiasi and Dayani, 2025; Y. Zhang *et al.*, 2020). Extensive research in the field of regenerative medicine and cell biology has demonstrated that exosomes not only act as highly sensitive and specific diagnostic biomarkers for the early detection and monitoring of the progression of diseases such as cancer, neurological disorders and metabolic conditions, but also, due to their inherent ability to directly transfer active biomolecules to target cells, they are considered an exceptional and promising tool for inducing cellular differentiation across various tissues (Huda *et al.*, 2021; Roszkowski, 2024). In addition, with significant advances in the engineering and targeted modification of exosomes have positioned these natural nanocarriers are now at the forefront of cutting-edge therapeutic strategies, including smart drug delivery, immune response modulation and tissue repair (Ghiasi, *et al.*, 2024; Khorrami-Nejad *et al.*, 2025). Therefore, exosomes—with their pivotal roles in diagnosis, differentiation and treatment, are one of the most powerful and versatile tools of

the present era in precision and personalized medicine.

Mesenchymal stem cells (MSCs) are multipotent cells that can be isolated from various tissues throughout the body. These cells have the ability to differentiate into a variety of cell lineages, including neural, bone, and keratocyte cells (Fallah Tafti, *et al.*, 2023; Mojtahedi *et al.*, 2025; Tabari, *et al.*, 2025). Their relative ease of availability, coupled with their high differentiation potential and inherent anti-inflammatory properties, has made them a very powerful tool in the field of regenerative medicine. The human umbilical cord matrix is a rich source of Wharton's jelly mesenchymal stem cells (WJMSCs). These cells exhibit low immunogenicity, lack tumorigenic potential, and demonstrate a high proliferation capacity. In addition, hWJMSCs can reduce the inflammatory response by producing anti-inflammatory cytokines and chemokines and possess anti-apoptotic and anti-fibrotic properties (Kwiecien *et al.*, 2025). Given the limitations in the availability and cultivation of keratocytes, MSCs are considered as one of the main candidates for ocular cell therapy. Extensive and reputable research on the use of stem cells in different layers of the cornea indicates the special attention of researchers around the world to the significant therapeutic potential of these cells (Ghiasi *et al.*, 2021). In a study by Alio del Barrio *et al.* (first human pilot trial: NCT05279157), direct injection of MSCs into the stromal tissue was shown to be effective in improving corneal function (Alio del Barrio *et al.*, 2017).

The aim of this study is to investigate the hypothesis that exosomes derived from human corneal keratocytes, which contain a unique and rich set of signaling molecules (*e.g.*, microRNAs, extracellular matrix proteins, and growth factors), can deliver an ideal molecular template to hWJMSCs, thereby reprogramming and directing their differentiation into keratocyte-like cells.

Materials and Methods

hWJMSCs culture

hWJMSCs were purchased from Stem Cell Technology Research Center (Iran, Tehran) in 25 cm² flask. the cells were cultured in a basal culture medium containing Dulbecco's Modified Eagle

Medium (DMEM) with high glucose, 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin (all materials from Gibco) was used. Cells were passaged after reaching 80% confluence.

Isolation and culture of keratocyte cells

Based on optimized protocols from previous studies (Fallah Tafti *et al.*, 2023; Ghiasi *et al.*, 2023), keratocyte cells in this study were discarded from corneal tissue, isolated, and subsequently cultured. Human keratocytes were isolated from the remaining corneas after the DSEAK procedure, using the following method. After washing with PBS, the tissue was sequentially digested with dispase (1 hour/h at 37°C) and collagenase I (12 h.). After centrifugation, the cells were cultured in a keratocyte-specific culture medium containing DMEM/F12, 1% amniotic stromal extract (Lifecell, Iran), ascorbic acid 2-phosphate (Sigma), Insulin-Transferrin-Selenium (ITS) supplement (Gibco), and non-essential amino acids (Gibco). The culture medium was replaced every 48 h.

Extraction of exosomes from keratocyte conditioned medium

The exosome was extracted from activated keratocyte via protocols in the previous study. (Sanie-Jahromi *et al.*, 2025) First, keratocytes were cultured in a 25 cm² flask. Upon reaching 80% confluence, the culture medium was removed, and the cells were washed and replenished with serum-free culture medium. The cells were then incubated for 24 h to allow for exosome secretion. Following this incubation, the conditioned medium was collected, and exosomes were isolated using the ExoCIB exosome extraction kit (ExoCIB kit, Iran) according to the manufacturer's instructions.

MTT cell viability test

To evaluate and determine the optimal dose of exosomes extracted from keratocytes, the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was employed. The evaluation was performed according to the standard protocol provided by the manufacturer (BIO-IDEA, Iran) and the evaluation steps were in accordance with previous study (Shirkoohi, *et*

al., 2024). Optimal dose assessment was conducted using MTT at concentrations of 40 µg/ml, 4 µg/ml, 0.4 µg/ml, 0.04 µg/ml, and 0.004 µg/ml. Absorbance was measured at 570 nm using an ELISA microplate reader (Bio-Rad, USA). The MTT assay to assess cell proliferation was performed in three independent biological replicates.

PKH26 staining for exosome uptake assay

PKH26 staining was performed to investigate the internalization of exosomes into hWJMSCs, following the manufacturer's protocol. Briefly, 15 µL of exosomes were added to 200 µL of diluent solution. Separately, 1 µL of PKH26 dye was diluted in 200 µL of the same diluent. The two solutions were immediately mixed by vigorous pipetting and incubated at room temperature in complete darkness for 5 minutes (min). To bind the excess dye, 400 µL of exosome-depleted FBS was added to the labeling mixture and incubated at room temperature for 1 min. Subsequently, 40 µL of polyethylene glycol 8000 (PEG8000, Merck, Germany) was added to the 800 µL mixture to achieve a homogeneous solution. The final mixture was incubated at 4°C for 24 h. Following incubation, the solution was centrifuged at 15,000 ×g for 30 min at 4°C. The supernatant was carefully removed, and the pelleted, labeled exosomes were resuspended in 50 µL of PBS. For the uptake assay, 15 µL of the labeled exosome suspension was added to hWJMSCs seeded at a density of 10,000 cells per well and co-cultured for 24 h. After the incubation, the cells were fixed with 4% paraformaldehyde, stained with DAPI (4',6-diamidino-2-phenylindole) for nuclei visualization, and washed three times with PBS. Fluorescent imaging was performed using a fluorescence microscope. The nuclei of the cells appeared blue with DAPI staining, while the internalized exosomes were visualized in red.

Differentiation induction

To assess the potential for differentiation induction, hWJMSCs were subjected to a differentiation protocol and evaluated at two key time points: day 7 and day 14 after induction began. Initially, the cells were cultured and allowed to adhere to the bottom of the culture plate for 24 h. After this period, keratocyte-

derived exosomes were added to the culture medium at a concentration of 0.004 µg/ml. In order to maintain a stable induction stimulus, exosomal treatment was extended every 48 h (every other day) until the end of days 7 and 14.

Evaluation of keratocyte gene expression

Total RNA was extracted from cultured cells subjected to differentiation using TRIzol reagent (Invitrogen, USA). RNA purity and concentration were determined by Nanodrop spectrophotometry (Thermo Scientific, USA). Subsequently, cDNA was synthesized from 1 µg of total RNA using a Reverse Transcription System kit (BioFACT™,

South Korea), following the manufacturer's protocol. Gene expression analysis was performed by qRT-PCR using Maxima SYBR Green qPCR Master Mix (Thermo Scientific, USA) and gene-specific primers (Table 1). The reactions were carried out with the following thermal cycling program: initial denaturation at 95°C for 30 seconds (s), followed by 40 cycles of denaturation at 95°C for 30s, primer annealing at 59°C for 30 s, and extension at 72°C for 30s. Target genes expression were normalized against β -actin (Housekeeping gene) and calibrated to control group (or TCPS/ tissue culture polystyrene).

Table 1. Table 1. PCR program and sequences of primers was used in qRT-PCR.

Gene	Primer sequence (3'→5')	PCR program	Product size (bp)
<i>Keratocan</i>	Forward: 3'- ATCTGCAGCACCTTCACCTT Reverse: 3'- CATTGGAATTGGTGGTTTGA	Initial denaturation at 95°C for 30s; 40 cycles (Denaturation at 95°C for 30s; primer annealing at 59°C for 30 s; and extension at 72°C for 30s), 1 cycle (Final extension at 72°C for 30s)	167
<i>Lumican</i>	Forward: 3'- CCTGGTTGAGCTGGATCTGT Reverse: 3'- TAGGATAATGGCCCCAGGA		194
<i>ALDH3A1</i>	Forward: 3'- CATTGGCACCTGGAACCTACC Reverse: 3'- GGCTTGAGGACCACTGAGTT		191
<i>CD34</i>	Forward: 3'- CTTGGGCATCACTGGCTATT Reverse: 3'- TCCACCGTTTTCCGTGTAAT		292
β -actin	Forward: 3'- GGTCATCACTATTGGCAACG Reverse: 3'- ACGGATGTCAACGTCACACT		133

Study of keratocyte protein expression

In order to evaluate the induction of keratocyte differentiation in hWJMSCs, keratocan protein was examined as a marker. The standard protocol was performed according to previous studies (Ghiasi *et al.*, 2023). In summary, cells cultured in plates were fixed with 4% paraformaldehyde for 20 min, and then washed with PBS. Next, cells were permeabilized with 0.4% Triton X-100 (Sigma, USA) for 30 min. Subsequently, 10% goat serum (Sigma, USA) was added to the samples for 45 min to block the secondary antibody reaction. Then, the diluted primary antibody (1:100) was added to the cells and the plate was incubated at 4°C for 24 h. Then, the cells were washed with PBS and the secondary antibody was added to the sample at a dilution of 1:150 and incubated for 1 h and 30 min in the dark at room temperature. DAPI (4',6-diamidino-2-phenylindole) dye was used to stain the cell nucleus. Finally, fluorescence imaging was performed by microscope (Nikon, Japan).

Statistical analysis

Data analysis was performed using Microsoft Excel and GraphPad Prism software. Standard deviation (SD), analysis of variance (ANOVA), and t-test were used to compare the results. For comparisons involving multiple groups against a single TCPS group, one-way ANOVA followed by Tukey's HSD post-hoc test was performed. In this study, a p-value < 0.05 was considered as a significant level, indicating a significant difference between the groups. All mentioned experiments were performed with at least three biological replicates.

Results

Cell morphological changes

The hWJMSCs exhibit a dendritic-like morphology after 14 days of differentiation induction into keratocyte-like cells (Fig. 1).

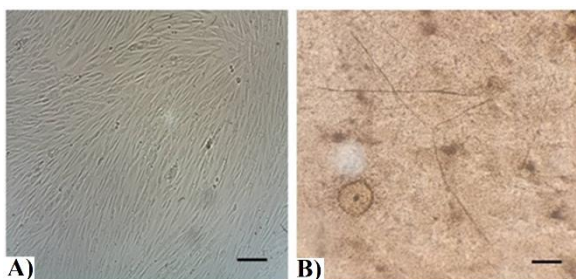


Fig. 1. Light microscope images of hWJMSCs at different growth times: A) Day 1 (before induction of differentiation); B) Day 14 of differentiation into keratocyte-like cells ($\times 400$ magnification).

Examination of isolated exosomes by electron microscopy

To evaluate the morphological characteristics and ensure the success of the isolation process, exosome samples extracted from keratocytes were imaged by scanning electron microscopy (SEM) (Fig. 2). The images clearly revealed the presence of a uniform population of spherical and cup-shaped vesicles that were morphologically consistent with the known characteristics of exosomes. A careful analysis of these images showed that the diameter of the vast majority of these particles was within the standard range, which well confirms that the isolation method used was successful in isolating a pure and high-quality population of exosomes.

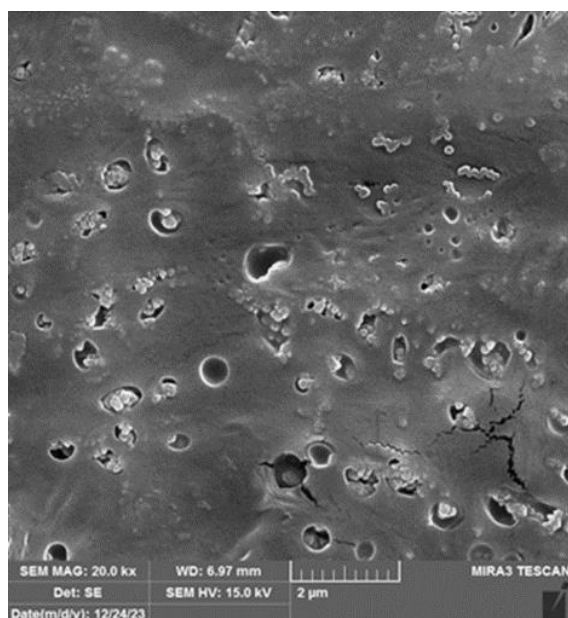


Fig. 2. Examination of exosomes extracted from keratocytic cells using SEM imaging (Scale bars: 2 μ m).

Cytotoxic effects of exosomes

In order to evaluate the effective concentration of exosomes extracted from keratocytes, an MTT assay was performed on stem cells. hWJMSCs were treated with exosomes at concentrations of 40, 4, 0.4, 0.04, and 0.004 μ g/ml. Compared to the TCPS group, treatment with exosomes at 40 and 4 μ g/ml resulted in decreased MTT activity, however, these decreases were not statistically significant (ns: not significant). Lower concentrations did not exhibit any inhibitory effect on cellular metabolic activity or viability. A concentration of 0.004 μ g/ml was selected as the lowest non-toxic dose for subsequent differentiation experiments. Although a slight increase in MTT activity was observed at this concentration compared to the TCPS group, this difference was not statistically significant. Therefore, this finding was not interpreted as a definitive increase in cell proliferation. Because the MTT assay was performed over a short period, it primarily reflects cellular metabolic activity and viability rather than sustained proliferation or keratocyte differentiation. The effects of exosomes on keratocyte differentiation were subsequently evaluated on days 7 and 14 using keratocyte-specific markers (Fig. 3).

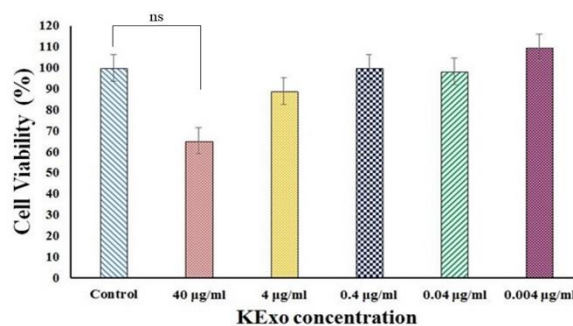


Fig. 3. Investigation of the effect of different concentrations extracted from keratocytic cells on hWJMSCs (Significance level set at $p < 0.05$).

PKH26 staining for exosome uptake assay

PKH26 dye was used to assess the entry of exosomes extracted from keratocytes into hWJMSCs. As shown in figure 4, the exosomes (red) entered the stem cells and were located adjacent to the nuclei of the cells, which were stained with DAPI.

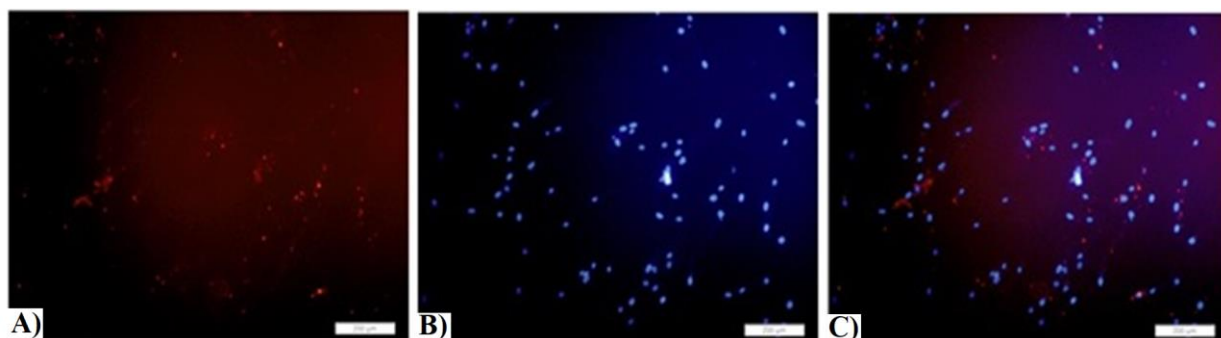


Fig. 4. Fluorescence microscopy images of hWJMSCs incubated with PKH26-labeled exosomes: A) PKH26 dye (red); B) DAPI staining (blue); C) Merged image showing perinuclear accumulation of exosomes (Scale bar: 200 μ m).

Evaluation of gene expression using Real-time PCR technique

After 7 and 14 days of differentiation induction in hWJMSCs using keratocyte-derived exosomes at a concentration of 0.004 μ g/ml, the expression levels of keratocyte indicator markers—namely *lumican*, *keratocan*, *ALDH3A1*, and *CD34* genes—were evaluated. RNA was first extracted from the cells, followed by cDNA synthesis. Gene expression levels of keratocyte indicator genes were evaluated by RT-qPCR method. As shown in figure 5A, the expression levels of genes on day 7 showed a statistically significant increase in all genes compared to the TCPS group ($*p < 0.05$).

Specifically, *keratocan* and *lumican* showed approximately 1.5-fold and 1.7-fold increases in expression, respectively, compared to the TCPS group. Among these, *lumican* and *CD34* genes showed a more significant increase in expression ($***p < 0.001$). Similarly, as shown in figure 5B, the expression levels of all genes on day 14 showed a statistically significant increase compared to the TCPS group ($*p < 0.05$). At this time point, *keratocan* expression increased approximately 2-fold, and *lumican* increased approximately 2.4-fold relative to TCPS. Consistent with day 7 results, the *lumican* and *CD34* genes showed a more significant increases expression ($***p < 0.001$).

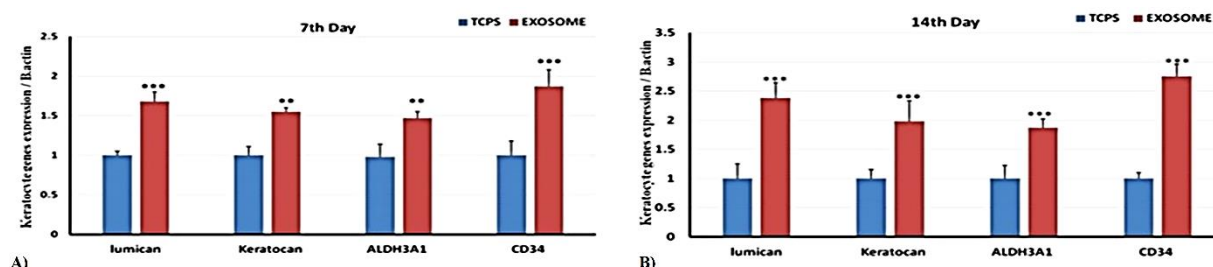


Fig. 5. Expression of keratocytic marker genes (*lumican*, *keratocan*, *ALDH3A1*, and *CD34*) at different induction time of hWJMSCs using 0.004 μ g/ml, KExo): A) Day 7; B) Day 14. TCPS= Tissue culture polystyrene or control group.

Examination of keratocyte indicator protein expression

hWJMSCs were exposed to keratocyte-extracted exosomes at a concentration of 0.004 μ g/ml for two periods: 7 and 14 days. The expression of Keratocan protein was examined as one of the differentiation indicator markers (Fig. 6A and 6B). As shown, an increase in Keratocan protein expression was observed compared to the TCPS

group, indicating successful induction of differentiation in hWJMSCs into keratocyte-like cells.

Discussion

Corneal injuries lead to the loss of keratocytes, and the replacement of these cells as the most important components of the corneal stroma is a major clinical challenge. Cell therapy using mesenchymal stem cells (MSCs) is promising due

to their immunomodulatory ability and differentiation potential. However, efficiently and specifically targeting of these cells to differentiate into a keratocyte fate *in situ* remains a major challenge. The importance of achieving a suitable

protocol for successful differentiation induction of stem cells is of paramount importance to maximize their therapeutic potential in regenerative medicine.

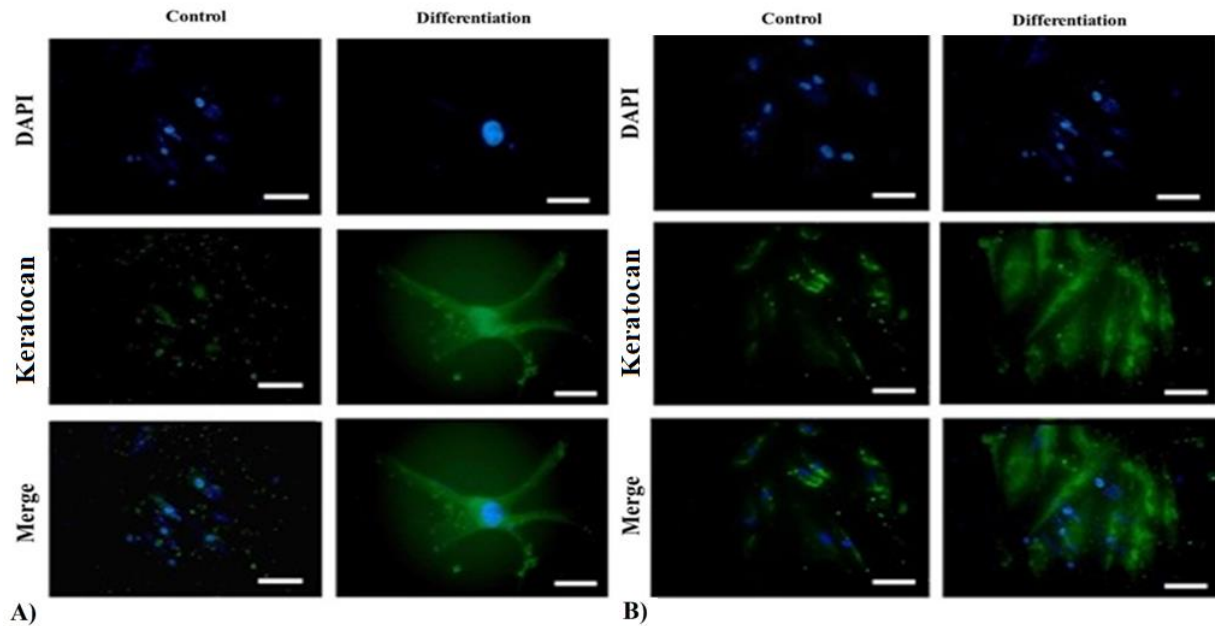


Fig. 6. Immunocytochemical image of keratocan protein expression in hWJMSCs treated with keratocyte-derived exosomes: A) on day 7; B) on day 14 of differentiation induction (Scale bar: 200 μ m).

In the last decade, due to the increasing pace of research in the field of regenerative medicine, extensive studies have been conducted to achieve standardized, suitable, and validated protocols for more successful differentiation induction of stem cells (Ghiasi, *et al.*, 2026; Hemati, *et al.*, 2023; Masoumi, *et al.*, 2023). In the first step, successful culture of human keratocyte was achieved *in vitro*. Subsequently, exosomes were successfully isolated from the conditioned medium of these cells (Fig. 2). Further evaluations confirmed the efficient uptake of exosomes by WJ-MSCs and their high efficacy in promoting differentiation (Fig. 4). Finally, the results showed that the optimal dose (Fig. 3) of these exosomes was able to effectively direct hWJ-MSCs towards a keratocyte-like phenotype (Fig. 5, and 6). The findings of the present study demonstrate that exosomes derived from keratocyte conditioned medium (KCM) are potent inducers of hWJMSCs differentiation into keratocyte-like cells, exhibiting significantly higher efficiency

compared to the use of crude conditioned medium. This observation is consistent with yet refines the conclusions of a recent 2023 study, which reported that exposure to 40% crude KCM could direct human adipose mesenchymal stem cells (hADSCs) differentiation over a 21-day period (Ghiasi *et al.*, 2023). The critical advancement offered by our approach lies in the purification of exosomes. KCM contains a heterogeneous mixture of secreted factors, including metabolites, waste products, and non-specific proteins, which may dilute the inductive signals or even exert counterproductive effects. By employing purified exosomes nanovesicles, known to be rich in specific miRNAs, and proteins responsible for cell-fate determination we provided a more concentrated and defined inductive cue. This likely explains the superior differentiation efficiency observed in our model. The present findings confirm the emerging role of exosomes in stem cell differentiation. Previous studies have shown that exosomes can enhance

cell proliferation and biochemical activities through various signaling pathways, such as MAPK and Wnt (Shi, *et al.*, 2022). Our study also provides convincing and valid evidence supporting the critical role of exosomes as mediators in inducing differentiation of hWJ-MSCs and generating keratocytic-like cells. Recently, the role of exosomes on keratocyte proliferation has been considered. In a study conducted in 2024 showed that amniotic membrane extract and Wharton's jelly mesenchymal stem cells derived-exosomes (WJ-MSC-Exos) increased the proliferation of corneal keratocytes (Erkoc-Biradli *et al.*, 2024). In the present study, the potential of exosomes to induce MSC differentiation and promote the production of keratocytic-like cells was also confirmed. As shown in figure 5, the addition of keratocyte-derived exosomes at an effective concentration (0.004 $\mu\text{g/ml}$) induced an increase in the expression of key genes regulating keratocyte differentiation in hWJ-MSCs. This observation is consistent with previous studies that have shown a significant increase in the expression of keratocyte -specific markers, at both the gene and protein levels, during the differentiation of hADSCs towards the keratocyte-like lineage (Fallah Tafti *et al.*, 2023; Ghiasi *et al.*, 2023). Exosomes directly modulate host cell signaling pathways by delivering specific cargoes—including microRNAs, signaling proteins, and transcription factors. The simultaneous upregulation of keratocan and lumican (key corneal proteoglycans) and ALDH3A1 (an oxidative stress-protective enzyme) indicates the activation of a coherent differentiation program, driving stem cells toward a keratocyte-like fate (Estey, *et al.*, 2007; Kao and Liu, 2002). Furthermore, confirmation of the increased keratocan expression at the protein level (Fig. 6) is critically important, as it demonstrates that exosomal-induced gene induction is not only at the transcriptional level but also results in a sustained functional phenotype. Keratocan plays a fundamental role in maintaining corneal stromal transparency and homeostasis. Therefore, in a successful differentiation process, the simultaneous increase in both gene and protein expression of this key marker (along with other important factors) is essential, making the use of induced keratocyte-like cells with these

characteristics a reliable approach. Compared with traditional methods of differentiation induction, such as the use of growth factors or co-culture, conventional differentiation methods are often expensive, time-consuming, and associated with standardization problems (Engel, *et al.*, 2016; Fowler, *et al.*, 2020; Kovina, *et al.*, 2021). The use of exosomes as a novel strategy in the present study not only reduces these challenges but also addresses issues of immunogenicity and transplant rejection, making it an attractive, innovative, and safe approach to inducing differentiation.

Although the findings of this study are very promising, there are limitations in the present study that need to be addressed in future researches. First, the precise molecular mechanisms by which KExo induces differentiation is not yet fully understood; therefore, precise identification of the contents of exosomes— such as specific microRNAs or proteins— is an effective and necessary step in achieving a precise protocol for inducing differentiation. Also, the lack of functional assessments *in vitro*, including the ability of differentiated cells to produce corneal collagen and display dendritic morphology, is another limitation of this study. In addition, functional assessment of keratocyte-like cells *in vivo* models to confirm their ability to repair the corneal stroma and maintain its transparency will be a crucial step in future studies.

Optimized protocols with respect to cell sources, culture conditions, stable cell performance and high differentiation efficiency are crucial to achieve reliable clinical outcomes. In this study, we present a novel differentiation induction protocol.

Conclusion

In conclusion, this study demonstrates that keratocyte-derived exosomes are a powerful and specific tool for directing hWJ-MSCs towards a keratocyte-like phenotype. The proposed protocol, by simultaneously inducing expression of a functionally relevant genes and protein, offers a standardized and safe alternative to conventional differentiation induction methods. This alternative is particularly advantageous because exosomes are inherently low in immunogenicity, do not have the risk of

uncontrolled proliferation, and address concerns related to injection toxicity, oncological complications, or ethical issues of using living cells (Sadeghi, et al., 2023; Tan et al., 2024). Based on the findings of the present study, it is suggested that future research investigate the therapeutic potential of keratocyte-derived exosomes as an eye drop in animal models as a preclinical evaluation for the treatment of keratoconus.

Ethical Statement

No animal experimentation or plant-based materials were utilized in this study; consequently, ethical clearance regarding these protocols was not applicable.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interests

The authors declare no conflict of interest.

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