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Investigation of The Efficacy of Mesenchymal Stem Cell-Derived Conditioned Medium on Wound Healing Through Scratch Test and Teer Analysis

Tolga AKKOÇ [1] *, Burak AKSU [2]

[1] TUBİTAK Marmara Research Center, Life Sciences Vice Presidency, Kocaeli, Türkiye.
[2] Department of Medical Microbiology, School of Medicine, Marmara University, İstanbul, Türkiye.
* Corresponding Author. E-mail: akkoc.tolga79@gmail.com (T.A.); Tel. +90-530-601 90 01.

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ABSTRACT:
The aim is to investigate how Umbilical Cord-Derived Mesenchymal Stem Cells conditioned medium (UC-MSC-CM) can promote wound healing and epithelial barrier function.
UC-MSCs were differentiated into osteocytes, adipocytes, and chondrocytes to determine their multipotency. Flow cytometric analyses confirmed positive surface markers (CD90, CD73, CD105) and the absence of negative markers (CD34, CD11b, CD19, HLA_DR). We prepared conditioned medium (CM) from these cells. UC-MSC CM was tested in in vitro wound healing experiments using scratch assays and transepithelial electrical resistance measurements at 6th, 12th, and 24th hours to assess its regenerative effects.
UC-MSCs were found to be multipotent in the characterization and differentiation analysis, demonstrating their potential for use in regenerative medicine. In our wound model design, UC-MSC-CM significantly accelerated the healing process 75% and 92.7% at 12 and 24 hours respectively compared to the control group. According to TEER analyzes epithelial barrier resistance showed 200 Ω.cm2 with UC-MSC-CM healing process at the 12th and 24th hours while the control group showed 145 Ω.cm2 and 185 Ω.cm2 at the same time points, respectively.
The study demonstrates the potential of UC-MSCs in repairing epithelial barriers and wound injuries. The UC-MSC-CM accelerates wound closure as the healing process advances and enhances barrier integrity and permeability. These findings highlight UC-MSC-CM as a cell-free therapy for regenerative medicine and wound healing. Further research should focus on understanding the underlying mechanisms and optimizing its therapeutic applications to revolutionize wound management and treatment.
KEYWORDS: Umbilical cord derived mesenchymal stem cell; conditions medium; scratch assay; wound healing; TEER analysis.

1. INTRODUCTION

A wound's healing is a highly complex and dynamic physiological process aimed at re-establishing the tissue's integrity after it has been damaged. The process involves a series of coordinated cellular and molecular events, including inflammation, cell proliferation, extracellular matrix deposition, and tissue remodeling [1,2]. Although the human body can regenerate itself, several wounds pose significant challenges to the natural healing process, including chronic non-healing ulcers and extensive tissue damage. Therefore, regenerative medicine has focused on innovative therapeutic approaches to accelerate and enhance wound healing [1].

The multipotent nature of mesenchymal stem cells (MSCs), which are capable of dividing into fibroblasts, endothelial cells, and immune-regulatory cells, has made them a promising candidate for wound healing [3]. MSC-derived exosomes are also likely to benefit the development of cell-free therapeutic tools since recent advances have revealed their role in intercellular communication [6,10].

In comparison to other MSC sources, the umbilical cord has gained attention for its abundance and ethical accessibility. The multipotent proliferative properties, immunomodulating properties, and low immunogenicity of umbilical cord MSCs (UC-MSCs) make them viable therapeutic options [3]. UC-MSCs have also demonstrated therapeutic effects through their secreted exosomes despite the fact that their cell-based functions play only a small part in their therapeutic abilities [4,6].

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In addition to acting as main mediators of cell-to-cell communication, exosomes are nanosized extracellular vesicles secreted by various types of cells, including MSCs [7]. MicroRNAs, which can affect the behavior and functions of recipient cells [7], can be carried by them and encapsulate a cargo of proteins, lipids, nucleic acids, and microRNAs [8, 9]. In addition to overcoming the challenges associated with direct cell transplantation, exosomes have an unusual feature that makes them suitable for harnessing the therapeutic potential of MSCs. Exosomes derived from UC-MSCs have gained significant attention in wound healing due to their ability to regulate multiple stages of healing [9,10]. Studies have shown that exosomes derived from UC-MSCs can promote cell migration, proliferation, and angiogenesis in various wound models while reducing inflammation and promoting tissue regeneration. Based on these findings, using UC-MSC exosomes may represent a novel, cell-free therapeutic approach for accelerated wound healing and dealing with complications related to impaired healing [9,10].

There could be significant implications for the development of regenerative medicines and tissue repair therapies by understanding the properties and potential therapeutic applications of UC-MSCs. As a result, wound healing treatments could be revolutionized through exosome-derived UC-MSC-based therapeutics. Developing innovative strategies to harness the regenerative power of UC-MSC exosomes and improve patient outcomes in wound management will require understanding how exosomes interact with target cells within wound microenvironments [9,10]. This study investigates UC-MSCs, induces their differentiation into specific lineages, and investigates the therapeutic effects of conditioned medium derived from these cells on wound healing and epithelial barrier function.

2. RESULTS

2.1. Characterization And Differentiation of Umbilical Cord (UM) Derived Mesenchymal Stem Cells (UC-MSC)

UC-MSC cell pellets were cultivated in sterile T75 flasks using c-DMEM medium at a density of 4x105 cells/flask. The culture medium of UC-MSCs was refreshed 3 times within a week. One week later, we examined the morphological characteristics of the UC-MSCs under an inverted microscope at a magnifying power of 10x (Figure 1).

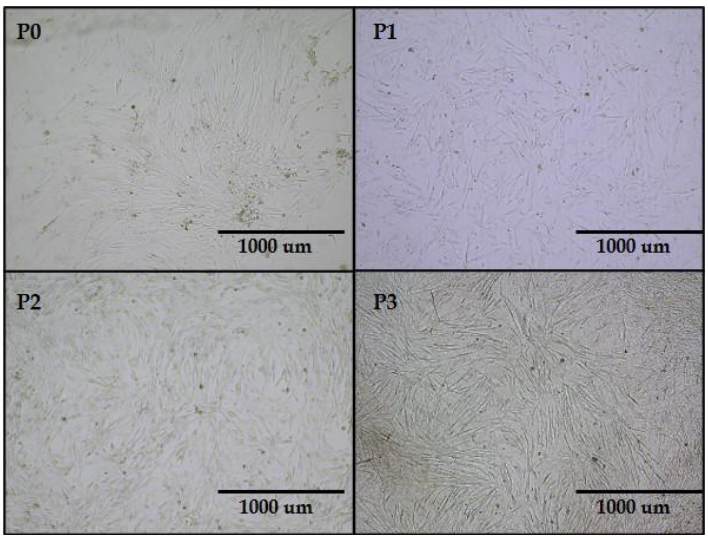

Figure 1. UC-MSCs microscopical morphology (10X). Passage 0 (P0) shows UC-MSCs showing fibroblast-like morphology, passage 1 (P1) shows UC-MSCs showing fibroblast-like morphology, passage 2 (P2) shows UC-MSCs showing fibroblast-like morphology, and passage 3 (P3) shows UC-MSCs showing fibroblast-like morphology.

The MSCs were analyzed by flow cytometry, demonstrating CD29, CD90, CD146, CD73, and CD106 positive expressions and CD34, CD45, CD14, CD28, and CD25 negative expressions (Figure 2).

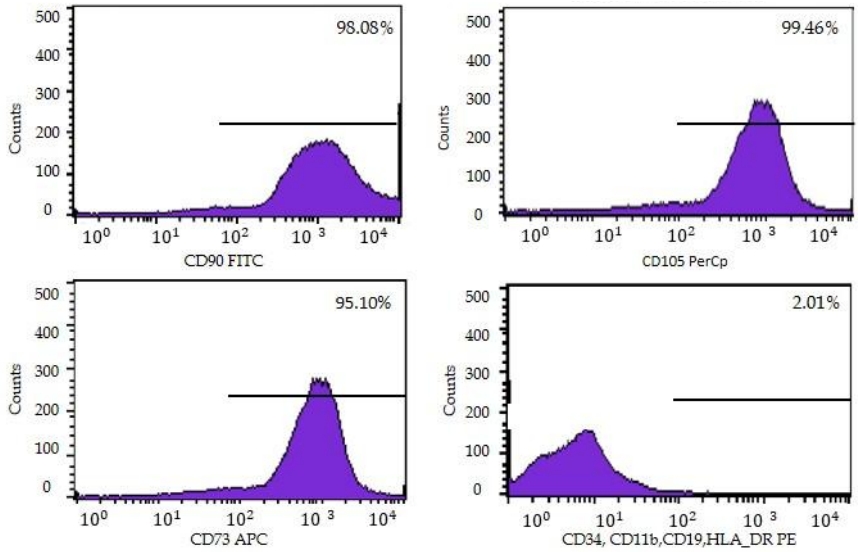

Figure 2. Flow cytometry analysis of positive surface markers CD105, CD90, CD73 and negative surface markers CD11b, CD19, HLA_DR, CD34 for UC-MSCs at the third passage.

The differentiation experiments were conducted on the UC-MSCs, which resulted in their successful differentiation into osteocytes, adipocytes, and chondrocytes. The cells were cultured in an osteogenic induction medium for twenty-eight days during the osteogenic differentiation process. UC-MSCs formed calcium nodule structures upon being stained with Alizarin red. Afterward, the cells were cultivated in an adipogenic induction medium and stained with Oil Red O to reveal intracellular lipid droplets indicating adipogenic differentiation. Cultured UC-MSCs achieved chondrogenic differentiation for fourteen days in a chondrogenic induction medium. Following Alcian Blue staining, these cells displayed intracellular proteoglycans, indicating chondrocyte differentiation (Figure 3).

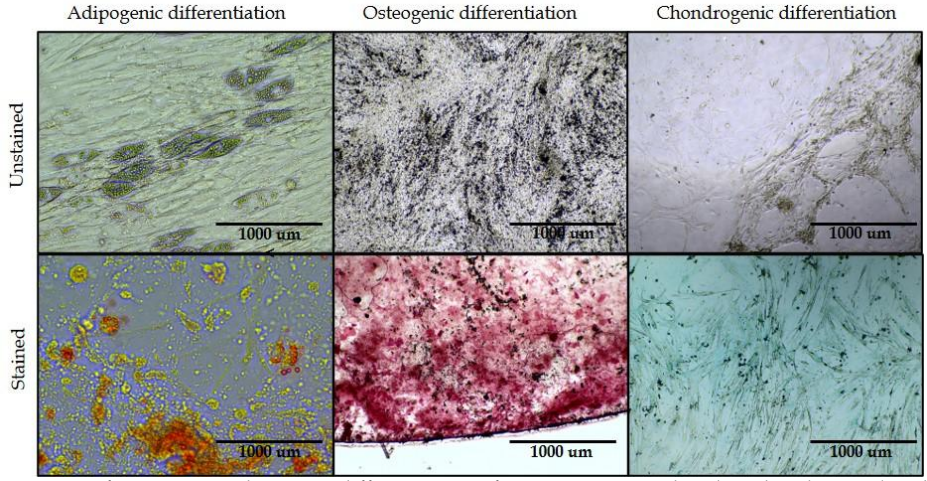

Figure 3. Differentiation of UC-MSCs. Adipogenic differentiation of UC-MSCs. stained with and without Oil Red O; Osteogenic differentiation of UC-MSCs. stained with and without Alizarin Red; Chondrogenic differentiation of UC-MSCs stained with and without Alcian Blue, 1000 µm.

2.2. Wound Healing and Scratch Assay

To analyze the wound closure area calculations and the impact of the different groups on the wound model, we utilized the FUJI ImageJ software (Figure 4A).

In the context of our experimental wound model design, we investigated the potential of UC-MSC-CM (Umbilical Cord Mesenchymal Stem Cell-Conditioned Media) to expedite the healing process. Through meticulous evaluation, we observed remarkable outcomes, revealing a substantial acceleration in wound healing upon UC-MSC-CM treatment. Notably, at the 12-hour mark, the healing process exhibited an enhancement of 75% compared to the control group (60%). Even more impressively, at the 24-hour time point, the healing process exhibited a remarkable enhancement of 92.7% when compared to the control group (76%)

(P<0.05) (Figure 4B). These findings indicate the robust effectiveness of UC-MSC-CM in significantly promoting and expediting the wound healing process. The considerable improvements observed in this study warrant further exploration and hold promising implications for the development of therapeutic strategies targeting wound healing and tissue regeneration. Nevertheless, a comprehensive understanding of the underlying mechanisms involved in the observed beneficial effects of UC-MSC-CM treatment requires further investigation to unlock its full therapeutic potential.

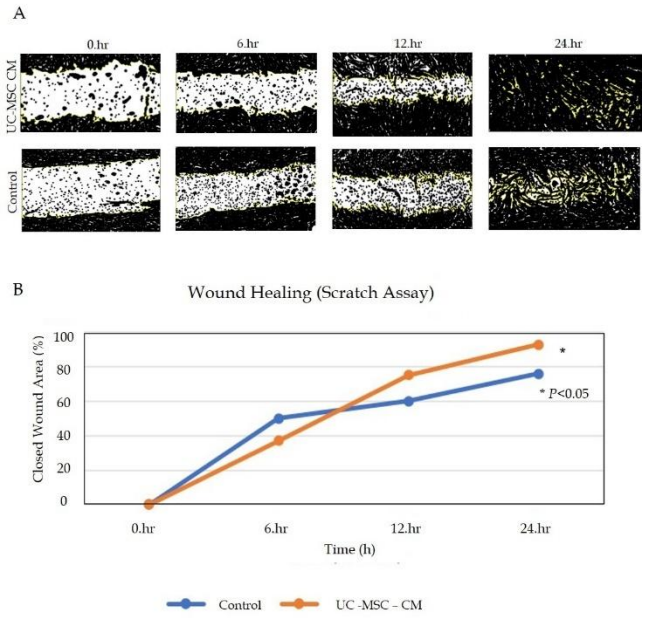

Figure 4. A) An analysis of wound closure percentages over time using the scratch assay for the control group and UC-MSC-CM (Conditioned Medium) group. **B)** A comparison of the effects of control and UC-MSC-CM (Conditioned Medium) on a wound model that was created with images from the Image J program was conducted over time. Sequential comparisons of 0 hours, 6 hours, 12 hours, and 24 hours are carried out.

2.3. TEER Analysis

Studies have previously found that TEER values of healthy epithelia under normal conditions range from 150 to 400 ohms.cm2 (Srinivasan, Balaji, et al.).

UC-derived mesenchymal stem cells secrete cytokines, present in the conditioned medium (UC-MSC-CM), that were evaluated against a damaged epithelial barrier in this study. In order to evaluate the permeability of the damaged epithelial barrier and the tight junction status, the TEER system is commonly used.

According to the results, the epithelial damage in the control group recovered faster than that in group B within six hours. The UC-MSC-CM helped to repair the barrier during the early stages, accelerating the healing process. It is however noteworthy that group B demonstrated a faster return to normal values around the 12-hour when compared to group A (P<0.05). In conclusion, UC-MSC-CM significantly strengthens tight junctions and accelerates the repair of the barrier (Figure 5).

In this study, we employed Transwell Epithelial Electric Resistance (TEER) analyzes to assess the efficacy of UC-MSC-CM (Umbilical Cord Mesenchymal Stem Cell-Conditioned Media) in promoting the healing process of the epithelial barrier. The measurements of epithelial barrier resistance were recorded at 12 and 24-hour time points for both the experimental group treated with UC-MSC-CM and the control group. Remarkably, the UC-MSC-CM treatment demonstrated a significant enhancement in barrier resistance, displaying values of 200 Ω.cm² at both 12 and 24 hours (P<0.05). In contrast, the control group exhibited lower barrier resistances, with values of 145 Ω.cm² and 185 Ω.cm² at 12 and 24 hours, respectively. These findings suggest that the UC-MSC-CM treatment exhibits a beneficial effect on the healing process of the epithelial barrier, potentially indicating its therapeutic potential for various epithelial-related conditions. Further investigation and understanding of the underlying mechanisms are warranted to elucidate the precise role of UC-MSC-CM in promoting epithelial barrier integrity and function.

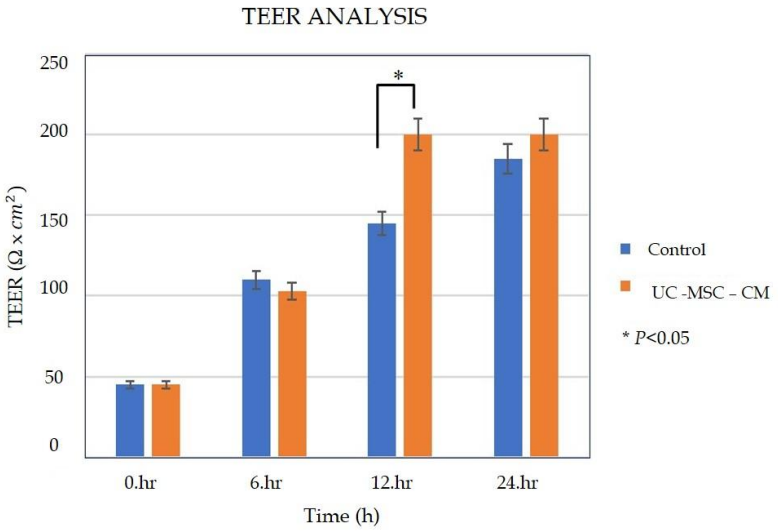

Figure 5. An analysis of TEER data comparing the healing of damaged epithelial barriers using UC-MSC-CM (Conditioned Medium) with a control group

3. DISCUSSION

The present study aimed to investigate the efficacy of mesenchymal stem cell-derived conditioned medium (UC-MSC-CM) on wound healing through in vitro scratch assay and TEER analysis. The process of wound healing involves coordinated cellular and molecular events, which are being enhanced and accelerated by regenerative medicine [13].

The investigation effectively accomplished the characterization and differentiation of umbilical cord-derived mesenchymal stem cells (UC-MSCs) into osteocytes, adipocytes, and chondrocytes, thereby confirming their multipotent attributes. The successful differentiation of UC-MSCs into these distinct lineages further substantiates their therapeutic promise.

As per existing literature, mesenchymal stem cells (MSCs) represent adult multipotent progenitor cells with the capacity for self-renewal and differentiation into diverse specialized cell lineages. Since a singular biomarker for identifying human MSCs (hMSCs) remains elusive, the International Society for Cellular Therapy proposed a set of markers and cell characteristics in 2006 for their recognition (10.1080/14653240600855905). In our investigation, the exhaustive characterization analysis confirmed the presence of positive surface markers (CD73, CD90, CD105) and the absence of negative markers (CD11b, CD19, CD34, HLA-DR) in umbilical cord-derived mesenchymal stem cells (UC-MSCs). Additionally, we observed successful osteogenic, chondrogenic, and adipogenic differentiation in these cells, further solidifying their mesenchymal stem cell phenotype. This rigorous characterization holds crucial significance in guaranteeing the purity and quality of MSCs employed for potential therapeutic applications.

As indicated in the investigations carried out by Bian et al. and Huang et al., a substantial body of research has consistently demonstrated that mesenchymal stem cells (MSCs) exhibit remarkable promise as potential agents for wound healing, owing to their multipotent characteristics [14,15].

Current consensus suggests that the therapeutic effects of MSCs are predominantly mediated through paracrine signaling and the activation of host cells, rather than direct engraftment and cell replacement. In this context, emerging evidence highlights the significance of stem cell-derived extracellular vesicles (EVs), which serve as carriers transferring regulatory miRNAs, cytokines, growth factors, and signaling lipids. Pioneering studies demonstrated that fractions enriched from stem cell supernatants using centrifugation or size exclusion chromatography contained vesicular material and retained MSC activity. This growing body of research underscores the potential of EVs as key mediators of MSC-based therapeutic mechanisms, opening new avenues for advancing regenerative medicine approaches. (10.1681/ASN.2008070798), 10.1016/j.scr.2009.12.003)

Stem cell secretomes are groups of molecules secreted by stromal cells. A variety of proteins, microRNAs, growth factors, antioxidants, proteasomes, and exosomes are found within these secretomes. The cell culture medium in which secretomes are released and accumulated is called the conditioned medium [16]. Because of their enclosed secretomes, conditioned mediums contain a wealth of paracrine factors. Known for their therapeutic potential, conditioned mediums are supported by paracrine factors such as vascular

endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), and insulin-like growth factor-2 [17].

Sukmana et al. demonstrated the significant potential of exosomes derived from adipose-derived stem cells (AD-MSCs) in promoting wound healing through their modulation of immune responses, promotion of angiogenesis, and regulation of cell proliferation and collagen remodeling, offering promise for clinical applications. (10.1111/cpr.12993) . Several clinical and preclinical studies have shown a therapeutic effect of mesenchymal stem cells (MSCs) on wound healing. There has, however, been relatively limited research on the therapeutic potential of mesenchymal stem cells' secretomes in various types of wounds. In a study conducted by Hui Ma et al., the therapeutic impact of secretomes derived from mesenchymal stem cells on wound healing was examined at a molecular level using a rat skin excision model. The findings of this investigation demonstrated that secretomes derived from MSCs contributed to an accelerated wound-healing process [18].

The scratch assay analysis in our study demonstrated that Group B had a slower wound closure rate at the 6-hour time point compared to Group A, control. However, wound closure rates in Group B significantly increased at the 12- and 24-hour time points ($P<0.05$). However, compared to previous findings, Group B showed a deceleration of wound closure within the first few hours. In spite of that, Group B exhibited a faster wound healing process than the control group, consistent with existing literature, substantiating its potential to support early wound healing. These outcomes are consistent with prior research indicating that UC-MSCs conditioned medium promotes cell migration and proliferation as well as alleviates inflammation, all of which play crucial roles in wound healing.

Skin, gastrointestinal system, and airways are the main interfaces controlling and attempting to balance communication between the host and the environment. In addition to providing protection against environmental factors, the epithelial barrier provides physical, chemical, and immunologic defenses [19]. An epidermal barrier hypothesis proposes a mechanism for the development of allergic, autoimmune, and neurodegenerative diseases characterized by inflammation and tissue damage in affected organs or distant organs distant from the skin and mucosal surfaces; explains the relationship between hygiene and biodiversity, epithelial barrier defects, and microbial dysbiosis, demonstrates ways to prevent allergy and autoimmune disease, and suggests future research directions [20]. A comprehensive literature review reveals that a variety of factors, including genetic and epigenetic influences, are responsible for damaging epithelial barrier integrity and disrupting homeostasis. There have been several studies in this area, with a particular focus on corneal, colonic, and pulmonary epithelia [21-23]. However, mesenchymal stem cells and their secretomes have the potential to aid in the regeneration of epithelial barriers by fostering cellular and tissue regeneration within regenerative therapies. There are currently limited studies that examine how acellular treatments, commonly referred to as conditioned medium, affect the epithelial barrier. As far as the therapeutic effect of a conditioned medium on disruption of the skin epithelial barrier is concerned, no relevant studies have been identified in the literature. However, in our study, we conducted TEER analysis to understand how UC-MSC-CM influences epithelial barrier function. A UC-MSC-CM treatment significantly accelerated the healing process by restoring the epithelial barrier in Group B at the 12th hour in TEER analysis ($P<0.05$). Results showed that UC-MSC-CM strengthened tight junctions, which are critical to maintaining barrier integrity and permeability. The UC-MSC-CM therapy offers a promising approach for treating epithelial barrier-disrupting conditions by enhancing barrier function.

In summary, these results demonstrate that UC-MSC-CM is a promising therapeutic candidate for wound healing. The delayed initial wound closure rate followed by an accelerated closure rate suggests that UC-MSC-CM may have a sequential and staged impact on different aspects of wound healing. Additionally, the TEER analysis highlights UC-MSC-CM's beneficial effect on epithelial barrier repair and reinforcement. A cell-free approach to wound healing using UC-MSC exosomes is supported by these results, as well as the importance of further exploring the mechanisms through which UC-MSC-CM works.

4. CONCLUSION

To conclude, this study provides valuable insights into UC-MSCs regenerative potential and the conditioned medium derived from them. It provides a comprehensive understanding of the therapeutic potential of UC-MSCs by characterizing, demonstrating, and assessing their differentiation capacity, as well as their impact on wound healing and epithelial barrier function. According to the findings, UC-MSC-CM could be a viable and innovative therapeutic strategy for promoting wound healing and addressing complications associated with impaired healing. UC-MSC-CM clearly shows promise for wound management

and regenerative medicine; however, more research is necessary to delve deeper into the mechanisms involved.

5. MATERIALS AND METHODS

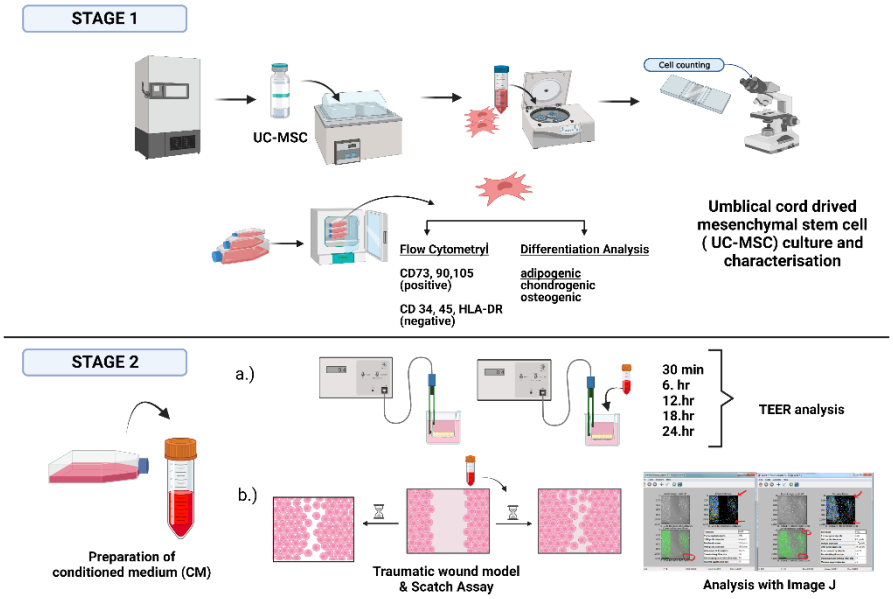

Figure 7. Graphical abstract of present study

5.1. Propagation of Umbilical Cord (UM) Derived Mesenchymal Stem Cells (UC-MSC)

A commercially purchased umbilical cord mesenchymal stem cell suspension was thawed in a water bath at 37 °C and centrifuged with DPBS (wash solution) containing 2% FBS. Cells were cultured in T75 sterile cell culture flasks in complete DMEM (containing 10% FBS, 1% penicillin/streptomycin, C-DMEM) at 37 °C 5% CO_2. Cell cultures that have reached confluence were sub-passaged. The passage is summarized as follows. Cells were inoculated into a T75 culture plate in C-DMEM (10% FBS, 1% penicillin/streptomycin, 1% Amphotericin B) medium and cultured at 37 °C. When the cells that grow and adhere to the base reach a spread rate of 70-80%, trypsinization was performed by incubating them with 0.25% trypsin-EDTA (Biowest, cat no: L0940) for 4 minutes at 37°C, after checking under an inverted microscope. At the end of 4 minutes, enzyme activity was stopped with c-DMEM, and cells were collected. The collected cells were transferred to a sterile 15 ml Falcon tube. They were centrifuged at 1500 RPM for 5 minutes, the supernatant on the surface was discarded, the remaining cell pellets were suspended in 1 ml of c-DMEM solution, and the cell number was calculated. Similarly, the 3rd passage was conducted with 1 T75 culture dish for every $5x10^5$ cells. About $1x10^6$ UC-MSCs from P2-P3 were used for differentiation analyses, while the remaining cells were reserved for characterization.

5.2. UC-MSC Characterization Analysis

UC-MSCs were subjected to flow cytometry analysis to determine their phenotypic characteristics after the third passage. Stem cells were removed from culture dishes by trypsinization. A cell count was made after washing 2 times in PBS containing Ca^{2+} and Mg^{2+}. Cells were homogenized in PBS (DPBS) without Ca^{2+} and Mg^{2+} at a density of $1x10^6$ cells/ml. After the homogenized cells were transferred to polystyrene tubes, special fluorescent isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC) conjugated monoclonal antibodies; CD73 APC, CD90 FITC, CD105 PerCP positive surface markers; CD34 PE, CD11b PE, CD19 PE, HLA-DR PE was used as negative surface markers and incubated in the dark at room temperature for 30 minutes by adding 10 µl of isotype controls (Mouse IgG1) (BD Biosciences, San Diego, CA, USA). After incubation, 400 µl of DPBS was added and centrifuged at 1500 rpm for 5 minutes. The supernatant was

removed and 500 µl of cell washing solution was counted and resuspended. The prepared cell suspension was read on a FACSCalibur flow cytometer (BD San Jose, CA, USA).

5.3. Adipogenic, Osteogenic, and Chondrogenic Differentiation of UC-MSC

First, cells with 80% or more surface area were removed from the T75 flask. Afterward, DBPS was used to wash off the DPBS from the flask bottom. A T75 flask containing Trypsin EDTA (0.25%) and 37°C was used to keep the cells for 4 minutes. At the end of the incubation period, trypsin was inactivated with K-aMEM, and the cells in the T75 flask were pipetted together with K-aMEM and collected into a 15 ml Falcon. After centrifugation at 1500 rpm / 5 minutes, the supernatant was discarded.

K-aMEM was added to cell pellets and pipetted. After centrifugation is repeated and the supernatant is discarded, cell counts were made with Trypan blue on a Thoma slide. 50,000 cells were seeded on the collagen coverslip in each well of the 6-well plate. It was incubated at 37°C in a 5% CO_2 incubator until 80-90% confluency. The medium was drawn from the confluent cells and discarded.

Cells are separated in 3 different culture wells and evaluated for differentiation as follows:

2 ml of osteogenic differentiation medium was added to a well and incubated in a 5% CO_2 incubator at 37°C. The medium was changed every 3 days and the incubation was continued for 21-28 days. At the end of 21-28 days, osteoblast cells were observed by applying the Alizarin Red staining protocol.

2 ml of chondrogenic differentiation medium was added to one well. It was incubated in a 5% CO_2 incubator at 37°C. The medium was changed every 3 days and the incubation was continued for 21-28 days. At the end of 21-28 days, chondrocyte cells were observed by Alcian Blue staining protocol.

2 ml of adipogenic differentiation medium was added to one well. It was incubated in a 5% CO_2 incubator at 37°C. The medium was changed every 3 days and the incubation was continued for 21-28 days. At the end of 21-28 days, the formation of adipocyte cells and oil drops were observed by applying the Oil Red staining protocol.

5.4. Preparation of Conditioned Medium From UC-MSC

Cells were kept at 37°C in a 5% CO_2 incubator. We changed the culture medium every 2-3 days and allowed the cells to reach a satisfactory confluence before conducting further experiments. A third passage of UC-MSC cultures was used to collect conditioned media. To avoid disturbing the cell monolayer, the medium was aspirated carefully. Sterile Falcon tubes were used to transfer the medium, and a 0.22-micron sterile filter was used to remove any cellular debris or contaminants. To preserve the biological activity of the secreted factors, the conditioned medium was stored at +4°C. The therapeutic efficacy was maintained by avoiding freezing.

5.5. Wound Modeling

The cells were incubated in a DMEM medium containing 10% fetal bovine serum (FBS) for 48 hours at 37°C under 5% CO_2. When the culture reached a confluent state, a sterile pipette tip was used to scratch the confluent cell layer with a sterile tip once the culture had become confluent.

5.6. Experimental Groups and Scratch Assay

Experimental groups were as follows; Group A, the control group, receives no intervention; and Group B, the UC-MSC - CM group (1 day), cultured with a conditioned medium, Following the determination of experimental groups, no intervention was made in the control group. An inverted microscope was used to observe only the physical wound and the self-healing process. Group B cultured the wound model with a conditioned medium and examined the wound healing after one day under an inverted microscope after creating the wound model. Data analysis was performed after the observation period based on the images or videos collected for analysis. Each group's wound closure was quantified. Statistical comparisons were made between the control group and the conditioned medium-treated groups to assess the impact of the conditioned medium on the wound-healing process.

5.7. TEER Analysis

In order to examine the therapeutic effect of UC-MSC-derived conditioned medium in case of epithelial barrier disruption, fibroblast cells were seeded into the transwell designed according to the TEER measurement. With a 200 ul sterile pipette tip, mechanical damage was applied to the epithelial barrier as soon as the fibroblast cells reached 100% confluence in the insert. The epithelial surface was treated with a conditioned medium derived from UC-MSCs. As a result of this procedure, regular TEER measurements were

performed and the results were compared to the control group, the group that was not interfered with. Based on the formula below, the measured TEER values were calculated and finalized (Srinivasan, Balaji, et al.).

$$R_{tissue}\ (\Omega) = R_{total}\ (\Omega) - R_{blank}\ (\Omega)$$
$$TEER_{reported} = R_{tissue}\ (\Omega) \times M_{area}\ (cm_2)$$

5.8. Analysis with FUJI ImageJ

The TEER analysis assesses the integrity and tightness of a barrier based on a confluent monolayer of cells grown on permeable support. In order to measure resistance, electrodes were attached to the apical and basolateral sides of the cell layer, and electrodes were then connected to the electrodes for stabilization. To calculate the TEER value, subtract the blank resistance (without cells in the transwell) from the measured resistance. Cell migration and wound closure are also studied using scratch assays. In this experiment, a confluent monolayer of cells is scratched, with images taken at time 0 and after incubation. The wound closure percentage is measured using image analysis software, providing insight into how cells migrate under different conditions.

5.9. Statistical Analysis

One-way ANOVA was used for statistical analysis. Data were represented as mean ± standard error of mean (SEM). $P<0.05$ was accepted for statistical significance. Statistical analysis was performed using SPSS software ver. 13.0 (SPSS Inc., Chicago, IL, USA).

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Author contributions: Concept – T.A., B.A.; Design – T.A., B.A.; Supervision – T.A.; Resources – T.A., B.A.; Materials – B.A.; Data Collection and/or Processing – T.A..; Analysis and/or Interpretation – T.A., B.A.; Literature Search – T.A.; Writing – T.A., B.A.; Critical Reviews – T.A., B.A.

Conflict of interest statement: The authors declared no conflict of interest

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