REVIEW ARTICLE

In vitro study of the mesenchymal stem cells-conditional media role in skin wound healing process: A systematic review

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Abstract
Mesenchymal stromal cell (MSC)-conditioned medium (CM) offers a potential opportunity in the skin wound healing treatment. In this systematic review, an overview of the knowledge on this topic has been provided. A multistep search of the PubMed, Scopus and Science Direct database has been performed to identify papers on MSCs-conditional media used in skin wound healing. Eligibility checks were performed based upon predefined selection criteria. Of the 485 articles initially identified, consequently, only 96 articles apparently related to MSC-conditional media were initially assessed for eligibility. Finally, the 32 articles, strictly regarding the in vitro use of MSCs-conditional media in skin wounds, were analysed. The information analysed highlights the efficacy of MSCs-conditional media on skin wound healing in vitro models. The outcome of this review may be used to guide pre-clinical and clinical studies on the role of MSCs-conditional media in skin wound healing.

KEYWORDS
conditional media, mesenchymal stem cells, skin, wound healing

Key Messages
- MSC-conditioned media includes a variety of cytokines, chemokines, and growth factors
- MSC-conditioned media can participate in skin regeneration and wound repair through stimulation of cutaneous stem cell migration, proliferation and differentiation into new keratinocytes, inducing angiogenesis and inhibiting inflammation
- MSC-CM is a promising treatment for skin conditions

1 INTRODUCTION

Cutaneous wound healing is a complex process comprised of a network of processes, which are collectively responsible for restoring skin architecture after injury. Wound healing is a sequential process involving cell migration, angiogenesis, inflammation, granulation tissue formation, extracellular matrix remodelling and...
re-epithelialization. Effective skin wound healing still represents a major concern for global healthcare as presently available skin substitutes and alternative therapeutics lead to unsatisfactory outcomes and results are variables with wound types resulting from traumatic injuries, burns and diabetes, where delayed healing and scarring is a reality. In this regard, investigators are looking for the cost-effective and safe methods to wound management.

Conventional wound care methods, with the risk of atrophic pigmentary abnormalities and scars, include skin flap transplantation, skin grafting, biological stents and laser therapy. Moreover, biological scaffolds are slow and costly, and they are not suitable for treating large scale trauma. Other treatments include gene therapy and local application of specific growth factors. Nonetheless, local growth factors are easily degraded in body fluids, whereas dosage cannot be easily controlled at the wound site. Henceforth, there is an urgent and crucial need for alternative efficient and safe approaches for promoting wound healing.

Though different researches have focused on facilitating the wound healing process, at present definitive therapies are not available. In recent years' progression in stem cell therapy have given the promise to expand the wound healing and the majority of studies have concentrated on the importance of applying mesenchymal stem cells (MSCs) in wound regeneration. In spite of the advantages of cell therapies, some limitations like genetic variation and immunological rejection still exist.

Stem cells have been shown to improve wound healing in a paracrine way via regulation of macrophages, B cells, T cells and others to decrease inflammation, secreting VEGF to increase angiogenesis, enhancing proliferation and differentiation of fibroblasts and keratinocyte-forming cells, production of anti-fibrosis cytokines and transforming into keratinocytes and microvascular endothelial cells.

More recent studies have indicated that the role of stem cells in tissue regeneration and wound healing have been mostly associated with their secretome and paracrine impacts rather than their differentiation capability. The molecules secreted via stromal cells are referred to as the stromal cell secretome and include microRNA, proteins, growth factors, antioxidants, exosomes and proteasomes. The stromal cell culture media that comprise the secretome are recognised as the conditioned media (CM), and they are considered to be an abundant resource of paracrine factors. The paracrine factors secreted in vitro include hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), stromal cell-derived factor 1 (SDF-1) and IGF-2. The administration of these factors to the site of an injured organ enhances its oxygen supply and metabolic activity and remodels the extracellular matrix.

MSCs-CM provide a possible opportunity in the skin disease treatment, and there is enhanced evidence justifying its application for treating cutaneous conditions like hair growth, wound healing, skin rejuvenation, or inflammatory skin disease. The purpose of this review is to assess the use of MSC-CM for skin diseases treatment in the in vitro models.

2 | MATERIALS AND METHODS

2.1 | Search strategy and literature screening

The research was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. A multistep search of the PubMed, Scopus, web of science and science direct was conducted to identify studies published before September 17, 2021, on skin wound healing treatment with MSCs derived exosomes and conditioned media, searching in English language.

“(MSC OR Mesenchymal Stem Cell OR Mesenchymal Stromal Cell OR Multipotent Stem Cell OR Multipotent Stromal Cell OR Stem Cell) AND (Conditioned Medium OR Conditioned Culture Media) AND (skin wound healing or cutaneous wound healing).”

2.2 | Exclusion criteria

We applied the following exclusion criteria: (a) conference abstracts, book chapters, books and unpublished results; (b) non-English papers; (c) systematic reviews, reviews, meta-analysis and letters; (d) in vivo study; (e) CM in combination with another factors; (f) comparative study.

2.3 | Inclusion criteria

Experimental studies (in vitro) up to September 2021 in the English language which assessed the wound healing effect of CM (in any cell line and/or model) were included.

2.4 | Data extraction, synthesis and analysis

Finally, 32 articles were included in this study as indicated in a flowchart of the literature search and selection
process (Figure 1). Data extraction and quality assessment were performed, with the following variables being recorded from each study: cell source, wound healing outcome, CM preparation technique and methods (Table 1).

2.5 | Skin wound healing process

Wound healing begins immediately in wound and includes both resident and migratory cell populations, with the action of soluble factors and extracellular matrix. The mechanisms underlying the Skin wound Healing process include: (a) secretion of inflammatory and growth factors; (b) cell–extracellular matrix and cell–cell interactions that is because of cell differentiation, migration and proliferation; (c) epithelialization, angiogenesis and fibroplasia; (d) wound contraction; and (e) remodelling.58-63

In this systematic review, the impact of CM isolated from different source (include amnion, adipose tissue, bone marrow, umbilical cord blood, Wharton's jelly, moreover, murine, human and rat tissues) on divers wound Healing mechanisms were assessed. Furthermore, CM was collected from MSCs between passages 3 and 8 at 60%–90% confluence.

2.6 | Inflammation

The inflammation following tissue injury plays an important role both in normal and pathological healing. Quickly after injury, the innate immune system is activated. This response starts with the degranulation of platelets as well as the resident mast cells injury-induced degranulation. In response to injury, local immune cells, such as resident macrophages, are activated via pro-inflammatory mediators released.64 The hypoxic environment of the wound also increase inflammation, as hypoxia stimulates numerous cell types, like macrophages, to produce mediators critical to inflammation.65 In response to these many signals, leukocyte recruitment rise. The most abundant white cell in the circulation are neutrophils that infiltrate the wound rapidly and are the dominant leukocyte in the earliest stages. Along with the neutrophils recruitment, circulating monocytes enter the wound and differentiate into mature tissue macrophages.64 Mast cell numbers in the wound also increase, with most of the infiltrating mast cells originating in the adjacent tissue.66 T lymphocytes appear in the wound bed, in the late inflammatory phase of wound repair, and might impact the resolution and remodelling of the wound.67-69 As inflammation resolves and the number of leukocytes reduces, the wound undergoes a lengthy period of resolution and remodelling.70,71

One study demonstrated that expression of the inflammatory gene of TNF, IFN, IL-1 and IL-6 after MSC-CM culture for 48 hours were significantly down-regulated in the HUVECs while the key angiogenesis genes included EGF, VEGF, bFGF and KDR were up-regulated.33 In another study it has been indicated that HUMSCM might reduce the inflammation at the beginning of wound healing and enhance cell migration and angiogenesis.39
| Author | MSC source | Preparation of MSC-CM | Method | Outcome |
|--------|------------|------------------------|--------|---------|
| Yuan Li et al | Human amniotic mesenchymal stem cells (hAMSC) | Cells between passages 3 and 7 were used. For the collection of hAMSC-CM, once the cells reached 80% confluence, the medium was changed to high-glucose Dulbecco’s modified Eagle’s medium containing 100 U/mL penicillin/streptomycin. CM was collected after 48 h and centrifuged at 1500 rpm for 5 min to ensure complete removal of cellular debris. CM was then concentrated 10-fold by using an Amicon Ultra 3 K device. | - In vitro skin cells proliferation - apoptosis analysis - In vitro scratch-wound closure assay | Stress-induced apoptosis | HaCaT and DFL cells proliferation ↑ Skin cell migration ↑ Wound closure ↑ PI3K/AKT/mTOR and GSK3β/β-catenin pathway ↑ |
| Saheli et al | Human bone marrow mesenchymal stem cells (hBM-MSCs) | The passage 4 MSCs cultured in a TP75 culture flask reached about 80% confluence, it was incubated for 48 h in 12 mL of serum-free culture medium. Then, the supernatant was harvested and concentrated to 20- or 50-fold via lyophilised drying and the CMs were cryopreserved at -80 °C for further use. | - Scratch assay - MTT assay - Gene expression analysis | Fibroblast migration ↑ Cellular viability and proliferation of the HG-HDFs ↑ HG-HDFs bFGF gene expression ↑ |
| Ahangar et al | Human multipotent adult progenitor cell | 2 × 10^3 cells per cm^2 were seeded into T75 flasks. Upon reaching 70% confluency, MAPC medium was removed, flasks were washed twice with phosphate-buffered saline (PBS) and 10 mL FBS-free DMEM medium was added to the flask. Following 24 h incubation under hypoxia conditions, the conditioned medium was collected from each flask. The conditioned media of MAPC cells in 20 flasks were combined, centrifuged at 350 g for 10 min and sterilised using 0.22 μm filters and kept in −80 °C freezer until use. | - Scratch wound closure assay - Collagen I and III staining of fibroblasts - Matrigel tube formation assay | Proliferation and migration of fibroblasts, Keratinocytes and endothelial cells ↑ Tube formation of endothelial cells ↑ Halftime life of formed tubes ↑ Collagens I and III deposition of fibroblasts ↑ |
| WALTER et al | Human bone marrow-derived MSC | For CM collection, pre-confluent MSC from passage 4-7 were washed with PBS and incubated with DMEM supplemented with 1% non-essential amino acids for 24 h. After that, CM was collected and centrifuged at 2000 g for 25 min, and the supernatant stored at −80 °C. | - Scratch test - Chemotaxis assay - Real-time PCR | Dermal fibroblast and keratinocyte Migration ↑ Formation of extracellular matrix ↑ |
| Rong et al | Antler stem cell | ASCs, hU-MSCs and FPCs at the third passage were plated in six-well plates at a density of 1 × 10^5 cells per well. After 48 h, when cells reached approximately 80% confluence, the normal medium (containing 10% FBS) was replaced with serum-free DMEM after three times of washing with PBS. At 48 h after incubation, the supernatants were harvested as CMs for use in experiments. The supernatants were collected, pooled, centrifuged at 1000 g, and filtered using 0.22-μm filters. All batches of each type of the collected CMs were pooled together, lyophilized, stored at −80 ° C and dissolved in DMEM after thawing for the use in in vitro studies. | Cell proliferation assay Cell growth on the CM-coated plates Immunofluorescence (IF) staining Cell cycle analysis | Proliferation of the HUVEC and NIH-3T3 cells ↑ |
| Sriramulu et al | Human umbilical cord-mesenchymal stem cells | Human UC-MSCs were cultured in T-25 flasks with 20% FBS supplemented DMEM medium maintained at 37 °C and 5% CO₂. Cells begin to release certain soluble factors into the medium. After 72 h, the medium was collected and centrifuged to remove the cell debris. Then, the medium was filtered by using 0.22 μm syringe filter and it was stored at −20 °C until use. | - Cell proliferation assay - Colony forming unit (CFU) assay - Real-time PCR | HaCaT cells proliferation ↑ HaCaT cells expression Col1A1 ↑ |

(Continues)
| Author          | MSC source                                           | Preparation of MSC-CM                                                                 | Method                                      | Outcome                                                                 |
|-----------------|------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------|-------------------------------------------------------------------------|
| Sun et al       | Wharton’s jelly-derived mesenchymal stem cells (WJ-MSCs) | Passage 3 cells were cultured to 60% confluence in normal culture media then replaced with serum-free medium (SFM) composed of an UltraCulture SFM with 2% supplements. After 48 h, the conditioned medium was harvested as MSC-CM. After that, supernatants were collected, pooled and centrifuged at 1000 g and filtered with a 0.22-μm filter. Finally, the CM was lyophilized, stored at −80°C and dissolved in SFM when being used. | -MSC-CM Coating Plate                      | The cell proliferation | inflammatory genes of IFN, TNF, IL-1 and IL-6 expression of the HUVECs |
| He et al        | human amniotic mesenchymal stem cells                | When hAEcs and hAMSCs reached 90% confluence in passage 2, 2.5 × 10^6 cells were seeded into 75 cm^2 cell culture flasks in serum-free EpiLife medium for 24 h. The culture medium was collected from each sample and used as CM. | -MTS cell proliferation assay               | Keratinocyte migration | Proliferation | Differentiation |
| Liang et al     | Induced pluripotent stem cell-derived mesenchymal stem cells | Cells at passage 4 through passage 8 were seeded into 10 cm culture dishes and allowed to reach 60% to 70% confluence. The medium was then changed to α-minimum essential medium (5 mL) and the cells were cultured for another 24 h. Next, the conditioned medium was collected, centrifuged to remove the debris, filtered and stored at −80°C. | -Transwell assay                           | The HUVECs cytoprotective | HUVECs angiogenic potential | Energy metabolism and angiogenesis via activating ERK signalling pathway | H_2O_2-induced mitochondrial fragmentation and apoptosis of HUVECs |
| Collawn et al   | Adipose-Derived Stromal Cells                        | The ADSC-CM was collected from 90% confluent P0 or P1 cells after 3 days in DMEM without FBS. The CM was centrifuged at 1000 rpm for 5 min. The supernatant was recovered without filtration and was stored at −20°C. | -3-D Skin Cultures of Primary Human Keratinocytes | Healing in 3-D Skin Cultures |
| BUSSCHE et al   | Microencapsulated mesenchymal stromal cells         | CM was collected from MSCs after 2 days of culture, when cells were 70% confluent. To this end, 6 × 10^5 MSCs were seeded in a T75 flask in 8 mL of expansion medium. After 48 h, medium was collected, centrifuged twice for 7 min at 300 g to remove any cellular debris and used for further experiments. | -In vitro scratch assays                   | Migration of dermal fibroblasts | Alterations in the expression of genes involved in wound healing |
| Li et al        | BM-MSCs                                              | Rat MSCs were plated at 5000 cells per cm^2 and were then incubated in proliferation medium overnight. The attached cells were washed 3 times with PBS and the medium was replaced with serum-free DMEM/F12 to generate CM. The medium was collected after 48 h of culture. The collected CMs were centrifuged at 1000 rpm for 5 min and filtered through a 0.20-μm syringe filter. The CM was then concentrated to 20-fold of the original concentration by Vivaspin 20 (cutoff of 3 kDa). The concentrated conditioned media were frozen and stored at −80°C for future use. | -Real-Time PCR                             | Proliferation and Migration of Keratinocytes | Level of ROS in Keratinocytes | The Erk Signalling Pathway |
| Author                  | MSC source                                      | Preparation of MSC-CM                                                                                                                                                                                                 | Method                                      | Outcome                                      |
|-------------------------|-------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|----------------------------------------------|
| Dwi Liliek Kusindarta   | Human umbilical mesenchymal stem cells           | When umbilical MSCs reach passage 4 in 60% confluence, cells were harvesting with trypsinization and following by centrifugation for 300 x g, 10 min. Aspirate the supernatant, pellet was wash with phosphate-buffered saline (PBS) for 3 times, 10^7 cells/ml was resuspended with complete medium (DMEM), then cells would promote to become embryoid bodies and cultured on the disc. After embryoid bodies become confluence, cells were washed with PBS and complete medium without foetal bovine serum were added, after being incubated for 48 h at 37 °C, 5% CO2. HU-MSCM was harvesting by centrifugation 300 x g for 2 min. | -Tube formation                             | Inflammation |
|                         |                                                 |                                                                                                                                                                                                                      |                                             | Cell migration and angiogenesis ↑           |
| Ribeiro et al           | BMSCs derived from SCD patients                 | BMSCs were expanded to subconfluence in standard culture medium at 37 °C under 5% CO2 and humidity (standard conditions). To generate normoxic (nor) or hypoxic (hypo) conditions, confluent cells in 6-well plates (average of 20 000 cells/cm²) were washed twice and cultured for 48 h with 3 mL/well of serum-free EBM-2 medium. Briefly, the cell-cultured supernatant was aspirated, pooled, centrifuged at 2000 g for 20 min at 4 °C and stored at −70 °C. | HUVEC proliferation assay                  | Proliferation and survival of endothelial cells ↑ | Skin wound healing ↑ |
| ZOMER et al             | Human mesenchymal stromal cells from dermis and adipose tissue | DSC or ASC monolayers at 90% of confluence were washed three times in PBS and maintained for 48 h in DMEM without BFS. Cell supernatants were collected, centrifuged (5 min, 300 g) and filtered through a 0.22-μm filter to remove cell debris. The conditioned medium was then concentrated 10 times using Ultra-15 Centrifugal Filter Ultracel 3 K by centrifugation (45 min, 5000 g) according to the manufacturer’s instructions. Conditioned medium was stored at −80 °C. | --Wound healing assay                      |                                             |                                             |                                             |
|                         |                                                 |                                                                                                                                                                                                                      | -Tubule formation assay                      |                                             |                                             |                                             |
| Jeon et al              | Human UCB-MSCs                                   | Cultured MSCs were harvested and seeded on 100 mm culture dishes (4105 cells). After 3 days, the media were replaced with fresh media containing 2 or 5% BFS. After another 3 days, media with a different PBS concentration (2% and 5%) were collected and used as CM (2% and 5% CM) for the following experiments. | Cell viability assay, Cell migration assay, Determination of the MMP-1 level, Collagen and elastin assay, RT-PCR, SOD and GPx | Proliferation and migration of CCD-986sk cells ↑ |
|                         |                                                 |                                                                                                                                                                                                                      |                                             | Regulation of collagen, elastin, fibronectin, MMP-1 production, production of SOD and GPx ↑ |                                             |                                             |
| Robertet et al          | Human skin-derived multipotent stromal cells    | The SD-MSC cultures (90% confluency) were washed with PBS and cultured for 10 days in DMEM without BFS. After the media was collected, the samples were filtered through a 0.22-μm filter mesh and concentrated using centrifugal filter units with a 10-kDa cutoff. The concentrated CM samples were stored at −80 °C until further use. | In vitro tube formation assay               | Angiogenesis ↑                              |                                             |                                             |
| Xin et al               | Dermis-derived stem/progenitor cells (DSPCs)    | Third passage hADSCs and hFDSPCs were washed with PBS thrice and starved in DMEM for 48 h. Cell supernatants were collected, centrifuged at 3000 g for 10 min and filtered through a 0.22-μm filter to remove cell debris. The conditioned medium was then concentrated with a cut-off value of 10 kDa and centrifuged at 3000 g for 1 h, eventually condensed into 25 mL. The concentrated CM was frozen and stored at −80 °C until use. | -Cell viability assay, -Scratch assay, -RT-qPCR, -Western blot analysis, -Tube formation assay | Proliferation of HaCaT cells and human fibroblasts ↑ |
|                         |                                                 |                                                                                                                                                                                                                      |                                             | Migration of HaCaT and human fibroblasts ↑ |
|                         |                                                 |                                                                                                                                                                                                                      |                                             | Tube formation of HUVECs ↑, ECM production of human fibroblasts by activating TGF-β/Smad pathways ↑ |                                             |                                             |

(Continues)
### TABLE 1 (Continued)

| Author          | MSC source                                              | Preparation of MSC-CM                                                                 | Method                                      | Outcome                                                                                           |
|-----------------|---------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------|---------------------------------------------------------------------------------------------------|
| Junget al       | Adipose derived stem cells                              | ADSCs (4 × 105 cells) of passage 3–5 were seeded on a 100 mm dish and cultured with a serum free medium for 72 h and the medium was then collected. The collected ADSC-CM was sterilised with 0.22 μm syringe after centrifugation at 300 × g for 5 min. | -ELISA assay                                | Collagen deposition ↑ Hyaluronic acid synthesis of HDFs through HAS-1 and HAS-2 expression ↑ |
| Lee et al       | ASCs                                                    | Eighty percent confluent ASCs at passage 4 in 100 mm-diameter culture dishes were fed with 5 mL of serum-free DMEM and incubated at 37 °C in an atmosphere of 5% CO₂/95% air for 48 h. At the end of the incubation period, the media were collected and centrifuged at 300 × g for 10 min to avoid contamination of cell fragments and the supernatant was used as ASC-CM. | -Viability - In vitro wound healing - Fibroblast-populated collagen lattice contraction assays - RT-PCR | The proliferation of HaCaT cells and fibroblasts ↑ Wound healing of HaCaT cells ↑ Contraction of the fibroblast-populated collagen lattice ↑ The type I procollagen α1 chain gene in fibroblasts ↑ |
| Zhao et al      | HAESCs                                                  | CM was collected as described. Briefly, the HAESCs were seeded into T75 culture flasks (Corning Costar, USA). On reaching approximately 60%–70% confluence, the cells were washed with PBS. The medium was then replaced by serum-free medium for further culture until 100% confluence. Culture medium from each sample was collected and used as CM in this study. | - Scratch wound assay - Transwell coculture assay - Cell cycle analysis - Western blot analysis - Real-time reverse transcription plus polymerase chain reaction | Wound healing ↑ Migration and proliferation of keratinocytes via ERK, JNK and AKT signalling pathways ↑ |
| LEE et al       | Human embryonic stem cell (hESC)-derived endothelial precursor cells (EPC) | CM was generated as follows: 80% confluent passage 5–8 hESC-EPC and CB-EPC in 150-mm tissue culture dishes were fed with 15 mL/dish serum-free EGM-2 medium and incubated for 48 hours. For in vitro and in vivo experiments, the CM was further concentrated (50) by using a TFF membrane filter system (Millipore) unit with a 10-kDa cut-off (Millipore) following the manufacturer's instructions. The multiplex cytokine array was performed using the Milliplex and Luminex systems (Millipore) with concentrated CM. | - Cell proliferation - Migration analysis - Enzyme-linked immunosorbent assay | Proliferation of dermal fibroblasts and epidermal keratinocytes ↑ Migration of fibroblasts and epidermal keratinocytes ↑ The extracellular matrix synthesis of fibroblasts' normal angiogenesis and wound healing related genes ↑ |
| Fong et al      | Human Umbilical Cord Wharton's Jelly Stem Cells          | hWJSCs and CCDs at passages 3-4 (P3-P4) were grown to 80% confluence in KOSR medium and the medium separated after 72 h as hWJSC conditioned medium (hWJSC-CM) and CCD conditioned medium (CCD-CM) respectively. The conditioned media were filter-sterilised using a 0.22 μm Millipore syringe filter (Millipore, Billerica, MA) and the pH and osmolality of the media standardised before use in experiments. The mean ± SEM pH and osmolality of the hWJSC-CM and CCD-CM were 7.75 ± 0.26 and 332.67 ± 1.20 and 7.88 ± 0.18 and 332.33 ± 0.88 respectively. Both hWJSC-CM and CCD-CM were diluted 1:1 v/v in KOSR medium and used as 50% hWJSC-CM and 50% CCD-CM for all experiments. | Scratch wound assay, Sircol (collagen) and Fastin (elastin) assays, Quantitative real time polymerase chain reaction (qRT-PCR) | hWJSCs enhance healing of excisional and diabetic wounds via differentiation into keratinocytes and release of important molecules |
| Yoon et al      | human amniotic fluid-derived mesenchymal stem cells (AF-MSCs) | AF-MSCs were plated at a concentration of 5 × 10^4, 1 × 10^5, 2.5 × 10^5, or 5 × 10^5 cells/100-mm plate and incubated in proliferation medium overnight. The attached cells were washed 3 times with PBS and the medium was replaced with serum-free DMEM/F12. The conditioned medium was prepared by incubating the cells for 3 days. The medium was then collected, centrifuged at 1000 rpm for 5 min and filtered through a 0.20-μm syringe filter. | - proliferation assay - Incorporation of BrdU - wound-healing assay | Proliferation of dermal fibroblasts ↑ Wound healing by dermal fibroblasts via the TGF-β/SMAD2 pathway ↑ Levels of p-SMAD2 ↑ Migration of dermal fibroblasts ↑ |
| Author          | MSC source                                      | Preparation of MSC-CM                                                                                                                                                                                                 | Method                                                                 | Outcome                                                                                                                                                                                                                                                                 |
|-----------------|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Huo et al       | Bone marrow-derived mesenchymal stem cells     | For the preparation of BMSC-CM, confluent BMSC were washed with phosphate-buffered saline (PBS), transferred to basic Epilife medium (Invitrogen) and incubated for 48 hours. The culture supernatants were collected and then concentrated by ultrafiltration with a 3 kDa molecular weight cut-off concentrating columns according to the manufacturer's protocol. | -Cell viability assay  
- Single cell migration assay  
- Scratch wound assay  
- Scratch wound assay  
- Western blot analysis | Migration of human epidermal keratinocytes through β2-AR signalling ↑  
EMT-like changes in human epidermal keratinocytes through β2-AR signalling ↑  
Migration of human epidermal keratinocytes through β2-AR signalling ↑  
Expression of genes involved in re-epithelialization (transforming growth factor-β2), neovascularization (hypoxia-inducible factor-1α) and fibroproliferation (plasminogen activator inhibitor-1) ↑  
Normal skin fibroblast proliferation ↑  
Normal skin fibroblast migration ↑                                                                 |
| ARNO et al      | Human Wharton’s jelly mesenchymal stem cells   | —                                                                                                                                                                                                                      | - Real-time quantitative polymerase chain analysis  
- Proliferation assay  
- Scratch wound assay  
- Immunofluorescence | Expression of genes involved in re-epithelialization (transforming growth factor-β2), neovascularization (hypoxia-inducible factor-1α) and fibroproliferation (plasminogen activator inhibitor-1) ↑  
Normal skin fibroblast proliferation ↑  
Normal skin fibroblast migration ↑                                                                 |
| ZHAO et al, 2013 | ASCs                                            | ASCs were cultured until reaching 80% confluence. The culture medium was then replaced with serum-free DMEM/F-12 and ASCs were cultured for another 48 hours. The ASCs-conditioned medium (ASC-CM) was collected, centrifuged at 1000 rpm for 5 min and filtered through 0.22 μm syringe filter. ASC-CM was stored at −20°C. | - Migration Assays  
- Proliferation Assays | Fibroblasts proliferation ↑  
Fibroblasts migration ↑                                                                 |
| HU et al        | ASCs                                            | ASCs were cultured in DMEM/F-12 containing 10% fetal bovine serum until the cells reached 80% confluence. The culture medium was then replaced by serum-free DMEM/F-12 and incubated for an additional 48 h. The conditioned medium was collected, centrifuged at 165 g for 5 min and filtered through a 0.22-μm syringe filter. The ASC-CM was stored at −20°C. | - Migration assays | Migration of vascular endothelial cells, fibroblasts and keratinocytes ↑                                                                 |
| KIM et al       | ADSC                                            | ADSCs (4 × 10^5 cells) were plated on 100 mm dish overnight with control medium, and were cultured in DMEM/F12 serum-free medium. CM was collected after 72 h of culture, centrifuged at 300 × g for 5 min, and filtered through 0.22 μm syringe filter. Collected CM was applied to HDFs at varying dilution folds (0, 10, 50 and 100%) in DMEM/F12 with FBS concentration adjusted to 2%. | - migration assay  
- RT-PCR  
- Western blot  
- Proliferation assay | Secretion of type I collagen ↑  
mRNA levels of extracellular matrix (ECM) proteins ↑  
Collagen type I, III and fibronectin EXPERSION ↑  
MMP-1 ↑  
Migration of HDFs ↑                                                                 |
| Walter et al    | Human MSC were derived from iliac crest biopsies | Conditioned medium was generated from MSC cultures in serum free DMEM/F12 medium supplemented with insulin, transferrin, selenium and antibiotics when cultures were approximately 70% confluent, then removed after 72 h and all cellular debris were removed by filtration (0.2 μm filter). The number of MSC present in these MSC cultures was 698 500 (±19 020) (mean ± SEM) per flask. | - Scratch wound assays  
- Microscopy  
- Cell proliferation assays | L929 fibroblasts and HaCaT keratinocytes wound closure ↑                                                                 |
2.7 Migration

The healing of human skin wounds is a complex and highly coordinated biological process. Of these processes, fibroplasia, re-epithelialization and neo-angiogenesis are three critical processes for wound healing. These processes need cell migration. Re-epithelialization is the lateral migration of basal keratinocytes in the epidermis across the wound bed and, when successful, closes the wound. The wound bed is initially an amorphous mass of clotted serum within the rent in the skin. DFs from around the wound must migrate into the wound bed and synthesise and secrete new extracellular matrix (ECM) molecules, particularly type I and type III collagen, to begin building a new dermis. This process has been called fibroplasia.

Similarly, the neodermis must become vascularized if the wound is going to heal and transform into functional human skin. Like peri-wound fibroblasts, peri-wound human dermal microvascular endothelial cells (HDMECs) should migrate into the wound bed and establish new blood vessels, a process called neo-vascularization or angiogenesis. For these three core wound healing processes to be accomplished, there needs to be the keratinocytes migration (re-epithelialization), the DFs migration (fibroplasia) and the HDMECs migration (angiogenesis/neo-vascularization).72-74

hAMSC-CM promoted heat-injured skin cell migration and wound closure.27 hBM-MSCs-CM effectively stimulated migration of fibroblast in a diabetic-like microenvironment.28 MAPC-CM enhances the migration of keratinocytes, fibroblasts and endothelial cells.29 We observed enhanced migration when keratinocytes were cocultured in media conditioned via hAMSCs (hAMSCs-CM) and hAECs (hAECs-CM).34 CM from microencapsulated mesenchymal stromal cells stimulates dermal fibroblasts migration in scratch assay.37 MSC-CM increases the keratinocytes migration in diabetes-like microenvironments.38 In vitro models elucidate HU-MSCM at the beginning of wound healing, increase cell migration.39 The BMSCs secrete some SCD patients increases the endothelial cells migration.40 HFDSPC-CM increased cell migration of human fibroblasts and HaCaT in vitro.44 HAECs enhance wound healing via facilitating the keratinocytes migration through JNK, ERK and AKT signalling pathways.47 hESC-EPC CM considerably enhance the epidermal keratinocytes and dermal fibroblasts migration.48 BMSC-CM increased the human epidermal keratinocytes migration in vitro via β2-AR signalling.51 WJ-MSC-CM increased proliferation and migration of normal skin fibroblast.52 ASC-CM induces migration of fibroblasts.53 Adipose stem cell-conditioned medium promoted the migration of fibroblasts, vascular endothelial cells and keratinocytes.54 UC-MSC-CM increases dermal fibroblast migration.56
2.8 | Angiogenesis

Neovascularization represents a vital component in uncompromised wound healing due to its fundamental impact from the very beginning after skin injury until the end of the wound remodelling. The (micro)vasculature contributes to the initial haemostasis, reduces blood loss and establishes a provisional wound matrix. Blood clot-derived growth factors and cytokines drive the recruitment of pivotal cells that are critical for the healing process. This provisional wound microenvironment depicts the starting point for new vessel regeneration and formation thus ensuring the wound nutritive perfusion and the immune cells delivery that remove the cell debris.75-77

MAPC-CM promotes tube formation of endothelial cells and enhances the halftime life of formed tubes.29 Wharton’s jelly-derived mesenchymal stem cells (WJ-MSCs) the key genes involved with angiogenesis of VEGF, EGF, bFGF and KDR expressions were up-regulated.34 iMSC-CdM increased angiogenesis by activation of ERK signalling pathway.35 Human umbilical mesenchymal stem cells increase angiogenesis and cell migration.39 SD-MSC-CM stimulates angiogenesis in vitro.40 It has been shown that hESC-EPC secreted distinctively different chemokines and cytokines, including fibroblast growth factor (bFGF), epidermal growth factor (EGF), fractalkine, interleukin (IL)-6, granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-8, vascular endothelial growth factor (VEGF) and platelet-derived growth factor-A (PDGF-AA) which are well known to be critical in wound healing and normal angiogenesis.41 The commonest model was tube formation assay as angiogenesis model was also used.

2.9 | Proliferation

The proliferative phase of healing is characterised via widespread activation of fibroblasts, keratinocytes, endothelial cells and macrophages to orchestrate wound closure, angiogenesis and matrix deposition. As early as 12 hours post-injury, keratinocytes are activated via modifications in electrical gradients and mechanical tension, and exposure to hydrogen peroxide, cytokines, pathogens and growth factors.78

In the process of skin wound healing, the integration of skin cell migration, differentiation, apoptosis and proliferation plays an important role in skin tissue repair. The wound after healing possess the characteristic of regenerative epithelialization, which is linked to two basic functions of keratinocytes, namely migration and proliferation.79

Most studies assessed the potential of MSC-CM for targeting cell proliferation (Table 1). These studies assessed the MSC-CM effect on HaCAT, HG-HDFs, DFL, fibroblasts, endothelial cells and keratinocytes. hAMSCs and hAMSC-CM increased proliferation of DFL and HaCAT cells in vitro.27 Human bone marrow mesenchymal stem cells (hBM-MSCs)-CM significantly stimulated cellular proliferation and viability of the HG-HDFs.28 MAPC-CM enhances the migration and proliferation of keratinocytes, fibroblasts and endothelial cells.29 Bone marrow mesenchymal stem cell CM accelerated cell proliferation in human HS-derived fibroblasts.30 ASC-CM considerably stimulated the HUVEC and NIH-3T3 cells proliferation in vitro.31 Wharton’s jelly-derived mesenchymal stem cells (WJ-MSCs) efficiently increased the HUVECs proliferation.32

We observed enhanced migration and decreased differentiation and proliferation when keratinocytes were cocultured in media conditioned via hAMSCs (hAMSCs-CM) and hAECs (hAECs-CM) and.34 The secreteme of BMSCs from SCD patients increases the survival and proliferation of endothelial cells.40 hFDSPC-CM enhanced cell proliferation of HaCaT cells and human fibroblasts in vitro.44 The HaCaT cells and fibroblasts proliferation was enhanced via ASC-CM in the viability assay.46 HAESCs promote wound healing via easing the proliferation and migration of keratinocytes.47 hESC-EPC CM considerably enhanced the migration and proliferation of epidermal keratinocytes and dermal fibroblasts.48 AF-MSC-conditioned media (AF-MSC-CM) considerably increased dermal fibroblasts proliferation.50 Wharton’s jelly mesenchymal stem cells-CM increased the normal skin fibroblast proliferation.52

ASC-CM increased the proliferation of fibroblasts.53 CM obtained from UCB-MSCs considerably increased the cell survival capability of fibroblast cells.54 UC-MSC-CM enhances the proliferation of dermal fibroblast proliferation.56

2.10 | Differentiation

Fibroblasts are the key cell type responsible for replacing the provisional fibrin-rich matrix with a more substantial granulation tissue. Resident and mesenchymally derived fibroblasts respond to a milieu of signalling molecules from platelets, macrophages and endothelial cells, such as PDGF and transforming growth factor (TGF-β). These signals direct fibroblasts to either differentiate into myofibroblasts which drive wound contraction or become pro-fibrotic, laying down ECM proteins.80

We detected decreased differentiation when keratinocytes were cocultured in media conditioned via hAMSCs (hAMSCs-CM) and hAECs (hAECs-CM).34 hWJMSCs increase
healing of diabetic and excisional wounds by differentiation into keratinocytes. UC-MSC-CM suppresses the dermal fibroblast differentiation in vitro. 

3 | EMT

It has been established that epithelial cells own a range of inherent plasticity such as the capability to become mesenchymal cells. The EMT process is known to produce migratory mesenchymal cell types, like mesoderm and neural crest, throughout embryogenesis. EMT is also widely studied in cancer, as it is believed to play an important role in cancer metastasis, invasion and chemoresistance. Initially EMT is now considered a transition implying a transient and reversible process. The EMT reverse process is termed mesenchymal-to-epithelial transition (MET).

During the EMT process, epithelial cells undergo cytoskeleton re-arrangement, lose their apical-basal polarity and cell–cell junctions, alter their interaction with the ECM and acquire mesenchymal features such as increased invasiveness and motility. To ease such cellular alterations, EMT cells change their gene expression program, including downregulation of the epithelial junctional components expression and upregulation of the genes expression involved in enhancing cytoskeletal modifications and adhesion to mesenchymal cells. The notion that partial EMT occurs in adult epidermal wound healing to ease the epidermal cells migration throughout re-epithelialization was proposed in 2005, and has been extensively accepted. In one study it has been indicated that BMSC-CM induced EMT-like modifications in human epidermal keratinocytes in vitro via β2-AR signalling.

3.1 | ECM components production

Skin repair is the result of interactive and dynamic processes that include blood elements, soluble factors, cells and extracellular matrix (ECM) components.

Both fibroblasts and keratinocytes synthesise key ECM components that are required for re-forming the basement membrane (BM) beneath the epidermal basal layer. There is wound contraction because of the fibroblasts pulling on the ECM; this eases remodelling of human wound, and the associated traction forces contribute to scarring. The orchestration of these procedures relies upon the temporal and spatial expression and the activation of a variety of proteins, like cytokines, growth factors, ECM components and matrix metalloproteinases (MMPs). HFDSPC-CM enhances extracellular matrix (ECM) production of human fibroblasts via activation of TGF-β/Smad pathways in vitro. ADSC-CM regulated the expression of ECM proteins in HDFs via fibronectin and collagen type I and III up-regulation, and MMP-1 down-regulation.

4 | DISCUSSION

Desirable healing of skin wounds entails a complex cascade of molecular and cellular events such as angiogenesis, cellular proliferation and migration, extracellular matrix (ECM) deposition and production and finally tissue remodelling. Impairment in some of these processes might result in wound chronicity. These complicated processes can be accelerated via using mesenchymal stem cells because of paracrine mechanisms and their anti-inflammation, differentiation and regenerative potentials. Besides the cells themselves, conditioned culture media provide abilities for regenerating and repairing damaged tissues because of the secretion of growth factors and their bioactive substances, like cytokines.

Nevertheless, there was significant variability among studies in the cell source, CM preparation, in vitro model used to evaluate efficacy, ways of assessment and outcome assessment. This systematic review provides a valuable summary regarding the application of MSC-CM for skin conditions and highlights the processes involved in wound healing that are influenced via the MSC-CM. MSC-CMs, in most studies, were capable to enhance the proliferation of fibroblasts, keratinocytes and endothelial cells. Moreover, MSC-CMs can enhance cell migration and angiogenesis. In another study, the positive impact of MSC-CMs on EMT process, inflammation and ECM components production was indicated.

Many studies have indicated that MSC-conditioned media includes a variety of cytokines, chemokines and growth factors that participate in an intricate integration of cell signalling which coordinating tissue and cell behaviours. These signalling molecules can participate in skin regeneration and wound repair through stimulation of cutaneous stem cell migration, proliferation and differentiation into new keratinocytes, inducing angiogenesis and inhibiting inflammation. The MSC-CM properties vary depending upon the cell source. More than 10 MSCs sources were used for treating skin conditions. MSCs were mostly isolated from human bone marrow, human adipose tissue and human umbilical cord blood. Another significant aspect is the timing of the CM collection from the cells and concentration steps conducted for obtaining CM. MSC-CMs were mostly collected in 24–48 hours after MSC culture. Most of the studies used cells from passages 3 to 8 at 60%–90% confluence. The secretome of cells might also differ depending upon the age of
the cells.21 Taken together, MSC-CM is a promising treatment for skin conditions. In vitro studies indicated critical rates of wound closure after treatment of MSC-CM. Further studies are required to corroborate effectiveness and safety and to standardise CM manufacturing.

**DATA AVAILABILITY STATEMENT**

No data are available.

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