

The application of human Wharton's jelly mesenchymal stem cells in wound healing: A narrative review

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Abstract

Management and treatment of chronic wounds remain a significant problem in clinical practice. Stem cell therapies are an important and promising approach for regenerative medicine because of their self-renewal and differentiation potential. Mesenchymal stem cells (MSCs), a major cellular source for regeneration, are present in almost all tissues. The use of embryonic stem cells is morally controversial because of the need to nurture and destroy embryonic cells. Therefore, adult umbilical cord tissues are of particular importance as an alternative source of perinatal tissues. Wharton Jelly is a gelatinous connective tissue in the umbilical cord containing MSCs that can differentiate into osteogenic, adipose, chondrogenic, and other lineages. These cells do not express the MHC-II molecule and show immunomodulatory properties that make them viable for allogeneic and xenogeneic transplants in cell therapy. Therefore, the umbilical cord, especially the part named Wharton's jelly, is an important and promising source of mesenchymal stem cells.

Keywords: Stem cells, Wharton's Jelly, Cell therapy, Wound healing, Regenerative medicine

1. Introduction

Wounds are physical injuries caused by breakage of skin [1, 2]. Immediate and appropriate wound healing is essential for the re-establishment of functional tissues and the maintenance of structure following injury [3]. This complex and dynamic phenomenon involves cell-matrix interactions that heal wounds in three different overlapping phases, including the inflammatory, the proliferation, and the regenerative phase [4]. The naturally slow healing of wounds, rising costs, and inconsistency in healing are

the most critical problems of this treatment. These problems have led to the discovery of more advanced therapies such as tissue engineering, gene therapy, platelet-rich plasma (PRP), the use of growth factors (GF) and stem cells (SC) [5]. Numerous studies have been performed using stem cells in different fields of diseases with promising results [6]. Cell therapy involves the replacement of stem cells or tissue made from stem cells for various disorders and injuries [7]. Stem cells are undifferentiated multifunctional cells that can differentiate into various types of cells. These

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cells are of embryonic or adult origin, depending on the type of tissue they are derived from [8]. Mesenchymal stem cells are a group of pluripotent, fibroblast-shaped mature stem cells that have the ability to self-renew, modulate the immune system, and differentiate into several cell lines [9, 10]. Although adult bone marrow is the most common source of mesenchymal stem cell extraction for clinical use, these cells can now be obtained from various tissues, including skin, adipose tissue, peripheral blood, umbilical cord (blood/Wharton jelly), endometrium, and tooth pulp [10-14]. In conditions of tissue damage, proinflammatory factors are usually produced by both innate and compatible immune responses. Studies have shown that in pathological conditions such as tissue damage, mesenchymal stem cells are inherently mobilized to the site of injury and are activated in an inflammatory environment in close interaction with the immune system [15]. These cells then facilitate wound healing by secreting specific cytokines and growth factors, increasing angiogenesis, inhibiting inflammation, increasing fibroblast migration, and collagen production [16]. In fact, the interactions between mesenchymal stem cells (MSCs) and inflammatory cells determine the outcome of tissue repair processes [15]. Today, mesenchymal stem cells isolated from extra-embryonic tissues such as umbilical cord Wharton's Jelly have been considered as a suitable cellular source [14, 17]. The reason for using these cells is easy and unlimited access, low cost, non-invasive in tissue isolation, the ability to differentiate into different cells, non-tumorigenic and abundant sources of these cells [10, 18]. Wharton's jelly is a mucous connective tissue surrounding umbilical arteries and veins covered by an amniotic epithelium. Umbilical cords are considered hospital waste, so their clinical application in research and cell therapy has no ethical concerns [19, 20]. Since the amount of Wharton jelly cells is limited and the amount of extracellular matrix (ECM) compounds is very high, it seems that its cells produce high amounts of collagen and glycosaminoglycans under the induction of existing growth factors [21]. Previous studies show that WJ-MSCs can be used for various diseases such as cancer, neurological disorders, kidney failure, and liver, lung, and orthopedic injuries. Recent advances show that WJ-MSCs reinforced with microparticles and scaffolds can be used more effectively for various clinical applications [22-28].

The advantage of WJ-derived mesenchymal stem cells over bone marrow-derived mesenchymal stem cells (BM-MSCs) and adipose tissue is that they do not express MHC-II. Moreover, these cells have stronger immunomodulatory properties due to the release of large amounts of anti-inflammatory molecules such as TGF β , IL-10, IDO, TSG-6 and PGE2 [29-31]. Although, to date, there have been no reports of the use of human WJ-MSCs in human skin lesions, paracrine effects of WJ-MSC appear to improve wound healing, at least in mice [32].

2. Wound healing

As one of the largest organs of the human body, the skin has several vital roles, including a protective barrier against fluid loss, electrolyte imbalance, and microbial infections [33, 34]. Skin lesions are defined as any mechanical, thermal, or chemical damage to the skin that interferes with its function or fails to maintain its integrity [35, 36]. Wound healing is a complex, multi-step process that is often divided into three stages:

1. The inflammatory phase: In this phase, following the tissue injury, the process of hemostasis is initiated, and the resulting fibrin clots provide an extracellular matrix for the migration of the white blood cells (neutrophils and macrophages) and platelets. These elements play a pivotal role in wound healing. Although platelets affect wound healing by secreting various growth factors such as platelet-derived growth factor (PDGF), they are not essential for wound healing without bleeding. The next steps are the result of neutrophils' function against infections, foreign bodies, and pus. Then, the transformation of monocytes into macrophages indicates the end of this phase and the beginning of the proliferation stage [34, 37].

2. The proliferative phase: This phase is divided into several stages, including neoangiogenesis, fibroblast migration, epithelialization, granulation tissue formation and, contraction [34, 37, 38]. In short, at this stage, the number of macrophages decreases, and granulation tissue begins to form. Migration of other cells, such as fibroblasts and keratinocytes, also begins. These cells secrete various growth factors and help the growth of granulation tissue. As more granulation tissue grows, more collagen is synthesized as a scaffold. The combination of these interactions

causes the wound margin to close and eventually the wound to close.

3. Extracellular matrix regeneration stage: In this stage, which is the longest stage, collagen is regenerated. All these stages overlap to some extent [18, 33, 34, 38, 39].

recovery has stopped at one of these stages, and all evidence suggests that recovery has not been completed within four weeks [37]. Many factors can trigger this delay in healing and increase the risk of turning an acute wound into a chronic wound. These factors can be divided into systemic and local reasons

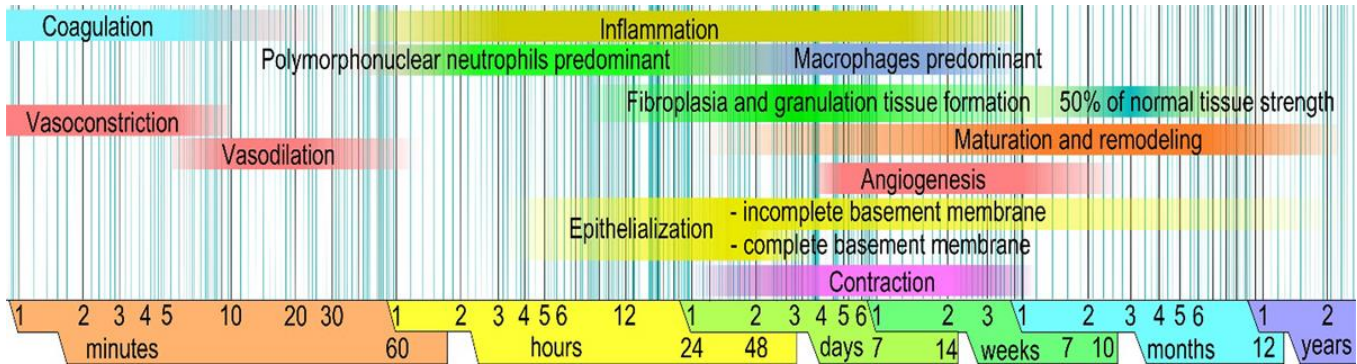


Figure 1. Approximate times of the different phases of wound healing on a logarithmic scale, with faded intervals marking substantial variation, depending mainly on wound size and healing conditions, but the image does not include major impairments that cause chronic wounds.

Table 1. Risk factors of delay in wound healing

Systemic	Local	Miscellaneous
Advanced age	Microbial infections	Excess inflammatory mediators
Obesity	Exudates	Some medications (eg. Chemotherapeutic drugs, Corticosteroids, NSAIDs)
Renal Diseases	Ischemia and necrosis	Inappropriate keratinocytes proliferation
Malnutrition/Poor nutrition	Hypoxia	
Trauma		
Diabetes Mellitus		
Vasculitis		
Hypothermia		

Figure 1 shows phases of wound healing. Limits vary within faded intervals, mainly by wound size and healing conditions, but the image does not include major impairments that cause chronic wounds [40]. Wounds can be classified as acute wounds or chronic wounds based on the healing time. A wound is described as acute when the three phases mentioned above progress as is expected and the healing process reaches a healthy conclusion within four weeks, whereas a chronic wound is one in which the healing process is not complete. It has been claimed that

and include the items listed in Table 1 [34, 41-44]. There are also factors that can facilitate healing such as a more profuse blood flow [45] [45], vitamin C [46], and more [34, 43, 47]. Unfortunately, chronic wounds remain a challenge because there is no sign of complete healing of these wounds [38]. Current treatments include both modern and traditional dressings [42, 48]. One interesting approach has been the use of hydrogel dressings and the conditioned media (stem cell secretome) of stem cells which showed promising results in both reducing scar

formation, and the healing rate in studies [41, 49, 50]. Some strategies to deal with chronic wounds challenge are antibiotics to prevent and treat infections, growth factors such as epidermal growth factors (EGF) or PDGF, or fibroblast growth factors. These factors affect the proliferative phase of wound healing [41, 51, 52]. Also, there are reports of immune-modulating molecules such as IL-4 in wound healing [41, 52, 53]. Despite all of these, stem cells offer a very bright horizon for future research and therapeutic applications [5, 6, 41, 51, 54-57]. Some studies have claimed that MSCs secrete vascular endothelial growth factor (VEGF), which can help regenerate the skin. These findings can potentially change the management of chronic wounds and our view of wound management in general [41, 58]. Stem cells are notable due to their vast potential in solving problems of current medicine, including wound healing [45, 59-62], diseases of the nervous system [63-67], diabetes mellitus [68, 69], Crohn's disease [70], chronic myeloid leukemia [71, 72], heart failure [73, 74], liver cirrhosis [75, 76] and others.

3. Stem cells and their classification

Stem cells are undifferentiated cells with differing degrees of potential for differentiation, varying from unipotent (capable of differentiating into a single lineage) to totipotent (capable of differentiating into all cell types). These cells can "self-renew", which is defined as the generation of daughter cells utterly identical to the original cell. This ability can help preserve the limited pool of stem cells available at birth [77-83]. Stem cells are classified into two main categories based on the source of origin and potential for differentiation.

3.1 Classification of stem cells based on their origin

Embryonic: These cells are derived from the fetus's internal cell mass and can form all three layers of the embryo [77]. Adult: These stem cells are derived from different adult cells with a differentiation capacity of at least one lineage. Recent studies show that some of them have can generate up to three lineages [81, 82, 84, 85].

3.2 Classification of stem cells based on the potential to differentiate

Unipotent: These are stem cells with the potential of differentiating into a single cell type that can produce a lineage, such as muscle stem cells that differentiate into adult muscle cells [77, 79, 86-89]. Oligopotent: These stem cells can generate two or more lineages but are limited to a specific tissue. A good example is the hematopoietic stem cells because these cells can differentiate into both myeloid and lymphoid lineages [90-92]. Multipotent: This type of stem cell is found in most tissues and can differentiate into cells from a single layer. MSCs are the most recognized in this class of stem cells. They can be taken from various tissues such as bone marrow, adipose tissue, Wharton's Jelly, umbilical cord, and peripheral blood [93-96], and differentiated into all mesoderm-derived tissues such as adipose, bone, cartilage, and muscle [96-99]. There have also been reports of "transdifferentiation", the differentiation of cells of one layer (MSCs) into the tissue of another layer (such as ectoderm-derived neuronal tissue) [90, 100]. Pluripotent: These cells have the potential to differentiate into cells from all three germ layers, ectoderm, endoderm, and mesoderm. Embryonic Stem Cells (ESCs) are examples of these stem cells that were first extracted from the inner cell mass of the blastocyst [90, 101]. Totipotent: Totipotent or omnipotent stem cells are the most undifferentiated cells and are only seen in early development. Fertilized oocytes and the daughter cells of the 1st and 2nd generation are examples of these cells that can differentiate into embryonic and extraembryonic tissues [90, 102].

4. Stem cells in wound healing

One issue to consider is that, despite all the evidence, long-term aspects of stem cell therapy and research are unknown, and its potentially harmful effects can be far more devastating than they seem. However, with more and long-term research, these concerns will be reduced [103, 104]. While the exact mechanisms of action of stem cells in wound healing have not yet been discovered, some aspects have been investigated. Various studies have shown that stem cells are involved in eliminating necrosis, neoangiogenesis, vascularization, reduction of scar formation, accelerated epithelialization and wound contraction [105-107]. These effects suggest that stem cell activity is beneficial for wound healing and reducing local inflammation. Most of these cases can

be attributed to signaling, which plays a crucial role in the stem cell effect during wound healing [32, 108, 109]. The studies have shown that transplanted mesenchymal stem cells release various growth factors and cytokines, which trigger the following two processes:

1. Promotes the migration and activity of fibroblasts and keratinocytes, which cause angiogenesis and healing of skin wounds and modulate the migration of leukocytes to the site of injury [110].

2. Release of immunosuppressive and anti-inflammatory agents, which reduce leukocyte proliferation and inflammation. Excessive inflammatory mediators are one of the predisposing factors for chronic skin wounds [47, 106]. Thus, mesenchymal stem cells enhance and improve the complex process of wound healing at all stages [111].

5. Mesenchymal stem cells (MSCs)

MSCs are adult stem cells that originate in the embryonic mesoderm layer. These stem cells are derived from a wide range of different tissues such as bone marrow, adipose tissue, nerve tissue, cord blood, and Wharton jelly [18, 32, 38, 77, 112-115]. These cells can self-renew, and despite years of research, a single specific marker has not yet been identified to identify and differentiate them. The best effort to achieve a uniform definition has been made by the Mesenchymal Stem Cell Committee and the International Cell Therapy Society, which defined mesenchymal stem cells as follows:

1. Plastic adhesive cells when stored in standard culture conditions.

2. Cells that should express (> 95%) CD105, CD73, and CD90 and lack the expression (<2%) of surface molecules CD45, CD34, CD14, or CD11b, CD79 α or CD19, and HLA-DR.

3. Cells that should be differentiated into osteoblasts, adipocytes, and chondroblasts *in vitro*.

These are only a minimal set of standard criteria set up to facilitate data exchange amongst researchers and academia [116]. MSCs remain at the site of the skin wound, even after the wound has been closed. These cells play a pivotal role in almost all of the processes of inflammation, fibrosis, tissue repair, angiogenesis, wound contraction, scar development, and granulation tissue formation [117-120]. The studies have shown that mesenchymal stem cells have

immunomodulatory effects such as inhibition of proliferation and reduced function in various immune cells, including natural killer (NK) cells, dendritic cells (DC), and lymphocytes [121-123]. Mesenchymal stem cells reduce the secretion of inflammatory cytokines [124] and secrete various anti-inflammatory cytokines such as TGF β , IDO, PGE $_2$, nitric oxide, IL-6, semaphorin-3A, and the Gal-1 and Gal-9 of galactins [125-131]. Some studies have used the MSCs conditioned mediums. These studies showed significant contributions to tissue regeneration and wound healing [5, 119, 132-135]. Interestingly, all mesenchymal stem cells appear to have some similarities, regardless of the tissue from which they are isolated. These similarities include nuclear cell markers, growth factors, and cytokines. In addition, there are differences, so when designing MSC-based treatments, differences such as their degree of differentiation and proliferation should be considered [136-138].

5.1 Bone marrow-derived stem cells (BMCs)

These stem cells are pluripotent and include mesenchymal stromal cells, hematopoietic stem cells, and even epithelial progenitor cells [139-141]. BMCs secrete various growth factors, cytokines, and exosomes [142, 143]. Many studies on different diseases such as myocardial infarctions (MI) [144, 145], chronic kidney disease (CKD) [146, 147], spinal cord injuries [140], and uveitis [148] have been undertaken. These stem cells have angiogenesis-inducing effects and a positive effect on microvasculature [139].

5.2 Adipose-derived stem cells (ASCs)

Stem cells derived from adipose tissue are yet another source of multipotent adult stem cells with numerous advantages compared to other sources of adult mesenchymal stem cells, given the massive pool of available adipose tissue and the minimally invasive extraction methods used [149]. Zuk et al. were the first to introduce this new source of mesenchymal stem cells around the turn of the century [150]. Some of the characteristics of these ASCs that have led to the interest taken in them are their anti-apoptotic, anti-inflammatory, proangiogenic, immunomodulatory, and anti-scarring effects [151]. Various studies have evaluated the therapeutic effects of ASCs on soft tissue regeneration, myocardial infarctions, ischemic

injuries, immune disorders such as diabetes mellitus, systemic lupus erythematosus, and many others [152].

5.3 Olfactory-Ecto mesenchymal stem cells (OE-MSCs)

These are a relatively novel population of stem cells present in the olfactory lamina propria [153]. As a population of stem cells, these cells boast a high proliferation rate, the potential to differentiate into multiple various lineages, self-renewal, as well as impressive immunomodulatory effects [154, 155]. One study found that the immunoregulation was mainly through T cell response [156].

5.4 Neural stem cells (NSCs)

NSCs are multipotent stem cells that have been the main target of interest in neural and spinal cord diseases [157, 158]. One major hurdle in their use, despite their beneficial secretions and differentiation into neurons, astrocytes, and oligodendrocytes, is the source from which they are derived, as well as the therapeutic approach that needs to be used be taken [159, 160].

5.5 Wharton's jelly-mesenchymal stem cells

Wharton jelly-mesenchymal stem cells (WJ-MSCs) are mesenchymal stem cells isolated from the umbilical cord, especially connective tissue called Wharton's jelly. What truly makes WJ-MSCs valuable is their immune-privileged status, high differentiation potential, easy isolation and collection, and minor moral issue. These stem cells have many features in common with embryonic stem cells, both phenotypically and genetically, although there are differences. Some common features are high ex vivo expansion capacity and a shorter cell cycle [161]. Wharton's Jelly was first described in 1656 by Thomas Wharton as a mucosal connective tissue that separates the umbilical vessels and amniotic epithelium. Then in 1991, McElreavey et al. first isolated the WJ-MSC from the umbilical cord [162, 163]. Many studies have been performed using stem cells in various fields, including oncology, pulmonology, nephrology, neurology, and orthopedics [22-25, 164]. Due to the immunosuppressive and modulatory effect of mesenchymal stem cells, WJ-MSCs are very suitable candidates for use in allogeneic transplants for cell therapy. Some immunomodulatory properties of WJ-MSCs have been demonstrated in a study by Zhang et

al. [165]. They observed that WJ-MSC grafting in burn wounds significantly showed wound healing and reduction of inflammatory markers. These results meant that the WJ-MSC transplant helped repair the skin by suppressing the secondary inflammatory response. There are reports of several methods of administering WJ-MSCs during stem cell therapy. These include local injections [165-167], topical administration [168-170], and systemic injections [171, 172]. Various methods have been used in studies in which topical administration has been investigated. In one study, Pourfath et al. sprayed WJ-MSC at the wound site [168], while in two other studies by Gholipour et al. [169, 170], mesenchymal stem cells were administered through seeding in tissue-engineered scaffoldings. Although topical application of WJ-MSCs to skin wounds may seem to be the least invasive and available method of application of these cells, the merits of other prescriptions such as systemic injections are so high that they cannot be ignored. One of these competencies is that in local injection, over-cell dosing at the target site of skin ulcers is better controlled. On the other hand, the WJ-MSCs in the scaffold can help improve paracrine signaling and cell survival by keeping them out of the harsh environment after injection [173]. Problems with systemic use are primarily immune responses that do not concern the immune-privileged status of WJ-MSCs, and secondly, whether injected MSCs are present at the site of injury and participate in the healing process [173].

6. Therapeutic effects of WJ-MSCs in skin wound healing

Many studies have already been done on the clinical use of WJ-MSCs in wounds treatment. These studies [109, 174, 175] have been researching the healing properties of these stem cells on the rat, ovine, and sheep animal models. Due to ethical issues related to the unknown long-term effects of stem cells, most research regarding the use of WJ-MSCs in wound healing in animal studies. However, as of September 12, 2021, there are 50 clinical trials registered that are attempting to assess the effects of WJ-MSCs on a wide range of diseases, including erectile dysfunction, osteoarthritis, diabetes mellitus type 1, systemic sclerosis (scleroderma), myocardial infarctions, and no-option critical limb ischemia (NO-CLI). In addition to the aforementioned clinical trials listed, there are also two clinical trials, in which the use of Wharton's

jelly and umbilical cord are more directly assessed in the field of wound healing. The first one is Clinical trial NEOX® CORD 1K vs. Standard of Care in Non-healing Diabetic Foot Ulcers (CONDUCT 1) (NCT02166294), which the researchers on sponsor's demand terminated. The second one is Therapeutic Potential of Stem Cell Conditioned Medium on Chronic Ulcer Wounds (NCT04134676) which despite being completed, has yet to post any results. Furthermore, Hashemi et al. conducted a randomized clinical trial in which [176], they used an acellular amniotic membrane seeded with WJ-MSCs to cover the wounds. They concluded that the use of these stem cells with the scaffolding had significantly decreased the wound size, and wound healing time.

7. Clinical applications of WJ-derived stem cells in wounds treatment

Wharton jelly-derived MSCs have been widely studied as an unlimited, accessible, and promising source for skin wound healing. In 2014, Arno et al. showed that administration of human WJ-MSCs repaired skin wounds in a mouse model by increasing epithelialization, angiogenesis, as well as fibroblast proliferation and migration [32]. In a study, Zhang et al. examined the effect of subcutaneous injection of WJ-MSCs on an animal burn model. According to the results of this study, subcutaneous injection of WJ-MSCs suppresses secondary inflammation by reducing inflammatory cytokines and thus accelerates the skin repair process in burn models of mice [27]. Recently, the results of a study showed that because of the potential for epidermal differentiation and lack of HLA antigen expression, WJ-MSCs are more suitable sources for bioengineered human skin replacement compared to mesenchymal stem cells derived from other tissues such as bone marrow. They are fat and tooth pulp [177]. According to several studies on the mouse skin wound model, the use of WJ-MSCs could accelerate the formation of the epithelial layer, increase wound contraction, neovascularization and increase collagen production [178-180]. It has also been shown that the use of extracellular vesicles and medium conditioning derived from WJ-MSCs at the wound site enhances the proliferation and migration of fibroblasts to the site of injury, epithelialization, angiogenesis, regeneration of sebaceous glands and hair follicles [32, 181-183]. Application of WJ-MSCs combined with biocompatible scaffolds was also

associated with reduced scar formation, wound healing time, and wound size [9, 176].

8. Concerns, dilemmas, ethical issues of the use of Wharton jelly stem cells in wound healing

Ethical issues with stem cells remain a challenge, although the use of cells such as the WJ-MSC can significantly reduce this. The primary concern is that stem cell therapies, besides the limited number of human studies, are ethical [184]. One major point against the use of these cells, especially embryonic stem cells, has always been that to supply enough stem cells, many embryos would have to be raised and killed. While some argue that considering the benefits of stem cell research and therapy, it can be justified in part, but public opinion is still not interested in serious action. On the other hand, in recent years, public opinion on the therapeutic uses of stem cells seems to have greatly improved. This improvement of public opinion is largely achieved through general education about the extraordinary untapped benefits and potential of stem cells in therapeutic fields [184, 185]. Furthermore, a specific study focusing on burn wounds showed that people fully accept stem cell therapy, especially autologous stem cells [186]. Fortunately, Clover et al. also showed that the general acceptance rate of allogeneic stem cells therapy is high and does not differ significantly for other diseases such as diabetes or Parkinson's [186]. Another concern that has diminished is immune system rejection and tumorigenesis that have been addressed in many articles [187-189]. In general, as research expands, our understanding of stem cells and the mechanism by which they affect living cells will improve, and more evidence will be discovered that stem cell therapy is safe [103, 104].

9. Conclusion and future perspectives

In summary, according to what was discussed in our study, the future of the use of stem cells, especially MSCs, in wound healing is auspicious. As science and technology advance, more innovative ways to meet the current challenges of stem cell therapy are being discovered. We believe that given the unique effects of WJ-MSCs on immunomodulation and its moral health, human studies and clinical trials should be conducted to provide further evidence of the safety and

efficacy of these cells as a cornerstone of the future of reconstructive medicine.

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Authors' contributions

All authors contributed equally in data collection and drafting of the manuscript. Also, all authors approved the final version of the manuscript.

Conflict of interests

The authors disclose no competing/conflicting interests.

Ethical declarations

Not applicable.

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