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Function and Therapeutic Potential of Mesenchymal Stem Cells and Their Acellular Derivatives on Non-Healing Chronic Skin Ulcers

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Abstract

Non-healing chronic skin ulcers are considered a major biological, psychological, and financial burden for both patients and health systems. Multidisciplinary endeavors are required to address this refractory disease, in order to find definitive solutions that lead to improved living conditions. Diabetes, venous stasis, arterial insufficiency, pressure and radiation are common risk factors associated with chronic wounds. Unfortunately, the cured state for these wounds has a high relapse rate, which adversely affects the patient's quality of life. Nevertheless, advances on regenerative medicine have allowed the development of cell-based therapies that promote wound healing by increasing cell migration and differentiation. Particularly, mesenchymal stem cells (MSCs) and their acellular derivatives have emerged as an attractive therapeutic agent in various diseases, including chronic skin ulcers, due to their role in immunomodulation and tissue regeneration. In this review discusses the characteristics of MSCs as well as their regenerative properties and their action mechanisms on wound healing. Finally, the perspectives of MSCs and their acellular derivatives in clinical chronic skin ulcer therapy are also explored.

Keywords: Mesenchymal Stem Cells; Acellular derivatives; Regenerative medicine; Chronic skin ulcers

Introduction

The skin is an important organ that effectively protects the body from the outside environment. This organ has developed intrinsic mechanisms that not only defend the organism from a wide range of external threats, such as bacteria, xenobiotic substances and dehydration, but also enable rapid restoration of tissue integrity and organ-specific function. Indeed, when a degloving injury occurs, the body initiates a series of complex events to recover skin protection. A normal cutaneous wound healing process is divided into sequential and overlapping phases that include early and late events. The initial events involve homeostasis, immediate inflammatory response (infiltration of cytokine-releasing leukocytes with antimicrobial functions), as well as cell proliferation and migration to form new epithelium, blood vessels, and extracellular matrix (ECM). In the late stage, the wound contracts as the ECM is remodeled [1].

In order to achieve the most favorable repair, at each wound healing phase, different cell types, specific cytokines, chemokines and growth factors must interact at the target site with their respective receptors, growth factors, and ECM components [2]. These highly regulated cellular, humoral and molecular processes have been described as an orchestral performance that leads to perfect regeneration; however, human adult wounds usually undergo a repair process that leads to scarring, and, in some cases, to non-healing chronic wounds [3].

Non-healing chronic wounds are characterized by a loss of epidermal and dermal tissue, as well as pathologically extensive inflammation. They are more frequently found in ageing patients, or in those suffering from conditions such as obesity, chronic disease, vascular insufficiency, diabetes, and malnutrition. Additionally, chronic wounds are affected by local factors, including hypoxia, ischemia-reperfusion, injury, pressure, bacterial colonization and edema, which play a major role in the disruption of the normal wound healing cascade [4,5]. In wounds for which the repair process has been disrupted, a sustained anatomical and functional progress is not reached within an appropriate time frame (usually three months) and remain intractable despite adequate wound management [5].

Non-healing ulcers are considered a major burden for patients and their families. In fact, the incidence of wounds has been called the "silent epidemic" [6], due to the large impact they have on the life quality of over 40 million people worldwide [5], and the significant economic cost they represent for the health care system.

Patients suffering from non-healing ulcers report pain, loss of function, and infections that often lead to amputations or sepsis [6], in addition to the severe physical, mental and social consequences associated with this condition [7]. Currently available treatments for chronic wounds involve debridement, dressings, and antibiotics. Nevertheless, around 50% of chronic wounds are resistant to these therapies, even when using promising techniques such as chemicals, dressings and skin grafts [8,9]. Therefore, new strategies to stimulate skin regeneration may provide novel therapeutic approaches to reduce non-healing ulcer disease [2].

In this context, multipotent mesenchymal stromal cells, also referred to as mesenchymal stem cells (MSCs), have been explored as an attractive therapeutic agent to treat non-healing ulcers [10].

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MSCs offer outstanding advantages over other stem cell populations: low immunogenicity, anti-inflammatory properties, and their culture and expansion *in vitro* is relatively simple. Moreover, MSC acellular derivatives could also be potentially used as a convenient therapeutic tool. The goal of this review was to highlight the features, function and action mechanism of MSCs in the context of repair and regeneration of wounds that are resistant to healing. Furthermore, relevant pre-clinical and clinical studies illustrating the impact of allogeneic and autologous MSCs obtained from different sources, as well as their derivatives on wound healing are exposed.

Characteristics of MSCs

Tissue sources

Bone marrow-derived MSCs (BM-MSCs) were first described by Alexander Friedenstein et al. [11] as adherent, fibroblast-like, clonogenic cells (colony forming unit-fibroblast, CFU-F), which possess high replicative capacity *in vitro* [11-13], are able to differentiate into several mesenchymal cell lineages (osteoblasts, chondrocytes and adipocytes), and support the hematopoietic stroma [11-15]. These pioneer studies demonstrated that BM contains a cell population distinct from haematopoietic stem cells, with stem cell features.

MSCs are a heterogeneous subset of stromal cells distributed throughout the stroma of almost all tissues/organs *in vivo* [16], giving rise to a variety of sources for their isolation, including adult peripheral blood, adipose tissue, BM, as well as fetal (e.g. umbilical cord blood, Wharton's jelly, amnion, amniotic fluid, and placenta) and embryonic tissues [16,17]. Despite the number of sources, most of the MSCs used for clinical trials are primarily derived from BM, adipose tissue (AD), and umbilical cord blood (UCB) [17], being BM considered the gold standard [17]. Nonetheless, BM-MSC isolation involves a highly invasive aspiration procedure that often causes severe pain and high risk of infection [18]. Furthermore, limited volume of BM is collected at a time, resulting in a low MSC yield, which appears to be detrimental for MSC proliferation and differentiation potential, as indicated by the presence of senescence [19]. In an effort to overcome these obstacles, other MSC sources have been explored. MSCs derived from AD (AD-MSCs) show similar morphology and phenotype as BM-MSCs, and offer the advantage of a less invasive isolation procedure. In fact, AD-MSCs can be easily obtained from biological material generated during liposuction, lipoplasty or lipectomy [18]. Even though these cells are considered an excellent alternative to BM-MSCs in the context of innovative approaches for MSC treatments [19], the literature presents conflicting reports regarding the similarities between AD-MSCs and BM-MSCs. Although they share many biological characteristics, there are some differences in their immunophenotype, differentiation potential, transcriptome, proteome, and immunomodulatory activity [20,21]. These differences should be taken into account when selecting the MSC source to be used in research and for therapeutic purposes [22,23].

To surmount the barriers associated with MSC precedence and isolation procedures, the use of cadaveric MSCs (CMSCs) from BM has recently emerged as a new approach. Mansilla and coworkers were the first research group that reported the use of CMSCs for treating severe thermal burns in a 26-year old male patient [24]. After isolation and expansion of CMSCs, combined treatment (conventional and CMSCs) was administered to the patient, who did not have any immunological rejection and was monitored during 35 days. The authors observed a faster growth of granulation dermal-like tissue and new epidermis compared to the control group (patients treated with conventional methods). After three years of follow-up, no adverse events were detected. This is the first time CMSCs were employed as a means for improving burn closures; nevertheless, additional studies to further demonstrate its safe use are indeed required.

Isolation and expansion

MSC-based therapies demand large cell numbers per treatment (hundreds of millions), which implies extensive expansion *in vitro*, since MSCs are scarce in the body even though they are present in several types of tissues [25]. The age and clinical characteristics of the MSC donors play an essential role in optimizing the cell culture conditions in order to scale-up the process for clinical applications [26]. Depending on the MSC source, different procedures have been used to perform MSC isolation. For instance, the most common method to isolate BM-MSCs is the density gradient procedure or the direct cell plating on a solid surface due to their adhesion capacity [27]. In contrast, AD-MSCs are obtained by enzymatic treatment (collagenase digestion) and centrifugation (density gradient separation) in order to collect the pre-adipocyte stromal vascular fraction and remove the adipocyte fraction [28].

After cell isolation, MSCs are typically expanded in monolayer culture on standard tissue dishes using basal medium that contains 10% fetal bovine serum (FBS) [29]. These cells display a spindle-shaped morphology during culture, retaining their stemness characteristics. Nevertheless, xenogeneic components have to be avoided in cell maintenance, and good manufacturing practice guidelines need to be followed in order to use these cells in cell-based therapy treatments. In this context, human platelet lysate has recently been proposed as a promising FBS substitute [30], and several authors have reported its higher influence on promoting MSC proliferation, relative to FBS [31-34].

Cell seeding density is another essential parameter in MSC *in vitro* expansion, and it depends on the MSC source. For example, BM-MSCs are suggested to be seeded at 4 - 22 $\times 10^3$ BM mononuclear cells/cm^2, yielding up to 9.8 $\times 10^8$ MSCs when they are harvested after one passage [35,36]. In contrast, MSCs derived from UCB (UCB-MSCs) should be seeded at higher densities (around 1 $\times 10^6$/cm^2) because of their low quantity [35,37]. In the case of MSCs obtained from embryonic tissues, it has been suggested to use lower cell densities since they have higher proliferative capacity and life span, as well as higher differentiation potential and biological properties compared with MSCs derived from adult tissues [38].

On the other hand, the inconsistency found in the results of clinical studies reported in literature, may be due to in part to highly variable quality of MSCs, and more specifically, the lack of a robust manufacturing process. The latter does not allow the production of sufficient doses of MSCs with a bath-to-bath consistency. In consequence, recent studies have proposed the creation of a MSC bank by generating a pool of bone marrow mononuclear cells from multiple donors as a novel strategy, which may allow the patients to receive the same standardized MSC therapy in clinical studies [39,40].

Minimal criteria for MSC characterization

The International Society for Cell Therapy lists the minimal criteria to define human MSCs [41]. First, MSCs must be plastic-adherent cells in standard culture conditions. Second, MSCs must be able to differentiate into chondrocytes, osteoblasts, and adipocytes *in vitro*. Third, MSCs must express CD29, CD73, CD90, CD44 and CD105, and lack expression of hematopoietic markers (CD14, CD34, CD45),

endothelial markers (CD31), human leukocyte antigen (HLA) class II, costimulatory molecules (CD80, CD86), and HLA-DR surface molecules [42]. However, these markers may also vary among different MSC sources. For example, UCB-MSCs express CD45, CD14, and CD31 and lack the expression of CD34, CD1a, and CD80, expression profile that is quite different when BM-MSCs are studied [35,43].

MSC delivery, homing and engraftment capacity

Although it has been demonstrated that MSCs play a role in the wound healing process, there is not currently a recommended approach for delivering MSCs as a treatment for chronic wounds. The most common routes of MSC administration are intradermal (into the dermis) and subcutaneous (below the epidermis and dermis) injections into or around the wound site; however, topical MSC application to the wound, immediately covered with a dressing, is also used. In these methods, MSCs are usually suspended in sterile PBS and applied around the edges of the ulcer [44-46]. Indeed, some pre-clinical studies have shown MSC homing and engraftment on non-healing wounds by using these routes. In particular, Pratheesh et al. labeled caprine MSCs with PKH26 (a fluorescent dye that binds to the cell membrane) in order to track the grafted cells and investigate their direct action and migration pattern at the incisional wound site in rabbits [47]. After creating the incisional wounds and intradermally administering the PKH26-labeled cells, the authors found that the MSCs were trapped within both the hair follicles and the injured area close to the wounds. After 14 days, wounds were healed up and the red fluorescent dye was still present, indicating the integration of the labeled cells into the host skin and suggesting a synergic role in the wound healing process [47]. Likewise, Hanson et al. showed the presence of pig MSC DNA after 21 days of being intradermally applied to partial thickness cutaneous wounds in a porcine model [48]. Some other studies have also demonstrated that, after engraftment, MSCs start to migrate to the regenerated tissue [49].

In addition, MSC local administration has been also combined with different methods in order to improve their survival and proliferation at the wound site. Recently, Yu et al. utilized MSC administration in a full-thickness excisional wound rat model along with negative pressure wound therapy (values at continuous −150 mmHg), for improving the viability of the MSCs and induce MSC differentiation into cutaneous tissue-related cell types. The results demonstrated that MSCs combined with negative pressure could significantly promote cutaneous wound healing, characterized by robust and improved vascularization at wound sites [50]. More importantly, the authors found that negative pressure provided a beneficial microenvironment supporting better MSC viability as well as inducing neoangiogenesis and maturation of blood vessels, suggesting that this strategy may serve as an alternative to soft tissue reconstruction for wound healing.

Several clinical studies have evidenced safety and efficacy of MSCs after local injection Table 1 [51-54]. In particular, Conget et al. evaluated the improvement of ulcers in two patients with recessive dystrophic epidermolysis bullosa (RDEB) by intradermally administering allogenic MSCs on intact and chronic ulcerated sites. After one week of the procedure, type VII collagen was detected in the MSC-treated ulcers along the basement membrane zone, as well as a continuous dermal-epidermal junction. Also, re-epithelialization of chronic ulcerated skin was observed only near MSC administration sites. Although the observed clinical benefits lasted for four months in both patients, the intradermal administration of allogeneic MSCs was associated with type VII collagen replenishment at the dermal–epidermal junction, prevention of blistering and improvement of wound healing in unconditioned patients with RDEB [51]. Similarly, Dash et al. conducted a clinical trial with 24 patients with non-healing ulcers of the lower limb that was followed up for twelve months. The participants were randomly distributed into two groups: the implant and the control group. Both groups received standard wound dressings, but the first group also received autologous BM-MSCs. The authors reported that the implant group showed a significant improvement compared to the control group in terms of wound size, pain-free walking distance, and liver and renal function [52].

On the other hand, new MSC delivery methods, such as bioengineered scaffolds, have been developed to enhance cell engrafting capacity, and have become a promising strategy for wound repair [55]. Wang et al. prepared acellular dermal matrixes (ADM) from mice in which BM-MSCs were seeded and used in full-thickness wounds in mice. The mice treated with the ADM presented not only an accelerated wound healing process, but also improved blood vessel formation as well as re-epithelialization and appendage regeneration [55]. Similarly, available artificial dermal matrices, such as Integra®, have been modified to behave as an ECM for MSC culture. Formigli et al. seeded BM-MSCs on Integra® matrices pre-coated with platelet-rich plasma in order to optimize MSC engraftment on the wound area and elucidate the mechanism of MSC action in a full thickness model using rats [56]. The authors demonstrated that the MSC-seeded Integra® matrix accelerated healing, promoted complete re-epithelization, induced hair follicle appearance, and enhanced blood vessel formation.

Another promising strategy for MSC delivery on wound tissues is the use of engineered microspheres as a skin substitute. Indeed, Huang et al. designed epidermal growth factor (EGF) microspheres on which BM-MSCs were seeded and then incorporated into a biomimetic scaffold for the generation of a skin construct [57]. After implanting these MSC-seeded-EGF microspheres into excisional wounds in mice, the healing rate was accelerated by increasing re-epithelialization and decreasing skin contraction. In addition, the data revealed the appearance of repaired sweat glands after 3 weeks of wound healing [57].

Despite the fact that, due to their remarkable intrinsic properties, MSCs are attractive for the treatment of non-healing wounds, there is still a lack of standardized routes and delivery methods to guarantee MSC optimal engraftment. Therefore, controlled studies may be required to investigate the appropriate approach to be used to deliver MSCs and ensure their survival at the wound site.

MSC oxidative stress management

MSCs are characterized by their ability to tolerate *ex vivo* culture and ionizing radiation, two conditions that generate strong oxidative stress (OS) [58,59]. In this context, MSCs prove to be useful in the treatment of pathologies that provoke tissue damage such as acute myocardial infarction [60], cerebral ischemia [61], and diabetes [62]. Specifically, Conget et al. showed that human BM-MSCs are highly resistant to OS-induced death [63]. This low susceptibility to reactive species correlates with the ability of human BM-MSCs to effectively scavenge peroxide and peroxynitrite, being the latter associated with the constitutive expression and activity of superoxide dismutase (SOD1, SOD2), catalase, glutathione peroxidase 1 enzymes and the high level of intracellular total glutathione (GSx) [62,63]. Furthermore, human BM-MSCs expressed constitutively and at a high level methionine sulfoxide reductase A, a crucial enzyme for the repair of oxidized proteins and for the recovery of methionine residues that act as oxidant scavengers [63,64]. Likewise, it has also been reported that human BM-MSCs produce the enzymes required for DNA repair [65].

Author	MSC source	Wound model	Used model	Delivery method	Time of study (days)	Mechanism of action	Therapeutic effect
Rustad KC [45]	Goat(BM)	Full thickness	Rabbit	Intradermally	14	Graftment	Complete healing
Nie C [46]	Pig (BM and AT)	Partial thickness	Pig	Intradermally	21	Graftment	Appearance Re-epithelialization Epidermal maturation
Dash NR [52]	Mouse (BM)	Full thickness	Mouse	Acellular dermal matrix	21	Graftment and migration	Neovascularization Skin appendage regeneration Re-Eithelialization
Yoshikawa T [53]	Rabbit (BM and AT)	Full thickness	Rabbit	Intradermally	21	No reported	Re-epithelialization Collagen deposition Restoration of skin architecture Inflammatory infiltration
Mansilla E [24]	Human (CBM)	Burn	Clinical trial	Sprayed with fibrinogen	35	Graftment and differentiation	Granulation dermal-like tissue
Falanga V [54]	Rat (BM)	Full thickness	Rat	Artificial dermal matrix	28	Paracrine signaling	Re-epithelialization Neoangiogenesis Return of hair follicles Collagen deposition
Wang Q [55]	Human (UC)	Burn	Rat	Tail vein injection	21	Migration	Wound closure Neo-vascularization Ratio of Collagen I/III Inflammatory responsec
Formigli L [56]	Dog (BM)	Full thickness	Canine	Intradermally	35	Paracrine signaling	Wound closure Collagen synthesis Cell proliferation Angiogenesis Cytokine Eroduction
Huang SP [57]	Human (BM)	Full thickness	Rat	Biomatrix	7	Graftment and paracrine signaling	Wound closure Re-epithelialization Neovascularization Granulation tissue formation Immune cell infiltration Giant cell formation
Halliwell B [58]	Mouse (BM)	Burn	Mouse	Transfusion	28	Migration	Re-epithelialization
Chen MF [59]	Mouse (BM)	Full thickness	Mouse	Microspheres	21	Differentiation and paracrine signaling	Re-epithelialization Sweet-glands like structures skin contractions
Le Blanc K [42]	Mouse (AT)	Full thickness	Mouse	Extracellular matrix Eatch	14	Paracrine signaling	Wound healing rate Fibrosis
Mareschi K [43]	Mouse (BM)	Full thickness	Mouse	Hydrogel	28	Engraftment	Skin appendages Angiogenesis
Chen SL [60]	Dog (AT)	Full thickness	Mouse	Intradermally	21	Differentiation and paracrine signaling	Wound closure Neovascularization Regeneration of skin appendages
Kurozumi K [61]	Rat (AT)	Full thickness	Rat	Intradermally	9	No reported	Wound healing Density of fibroblasts
Lam MT [44]	Rat (AT)	Full thickness	Rat	Intradermally	28	Differentiation	Epithelialization Granulation tissue deposition Time for wound closure
Hanson SE [48]	Human (BM)	Ulcerated sites	Clinical trial	Intradermally	7	No reported	Re-epithelialization Replenishment of collagen VII at the dermal-epidermal junction
Ouma GO [49]	Human (BM)	Diabetic foot ulcers	Clinical trial	Intramuscularly	84	No reported	Pain-free walking distance Ulcer size
Lee RH [62]	Mouse (BM)	Full thickness	Mouse	Tail vein injection	14	Migration and differentiation	Wound size Wound repair
Valle-Prieto A [63]	Mouse (BM)	Full thickness	Mouse	Intradermally	28	Differentiation and paracrine signaling	Wound closure Re-epithelialization Cellularity Angiogenesis Skin appendages
Conget P [51]	Human (BM)	Acute wounds	Clinical trial	Fibrin polymer spray	84	Paracrine signaling	Pain relief Resurfacing Wound size

Abbreviations: BM: Bone Marrow, AT: Adipose Tissue, CBM: Cadaveric Bone Marrow, UC: Umbilical Cordon

Table 1: Pre-clinical and clinical studies that evidence the the safety and efficacy of MSCs after local administration.

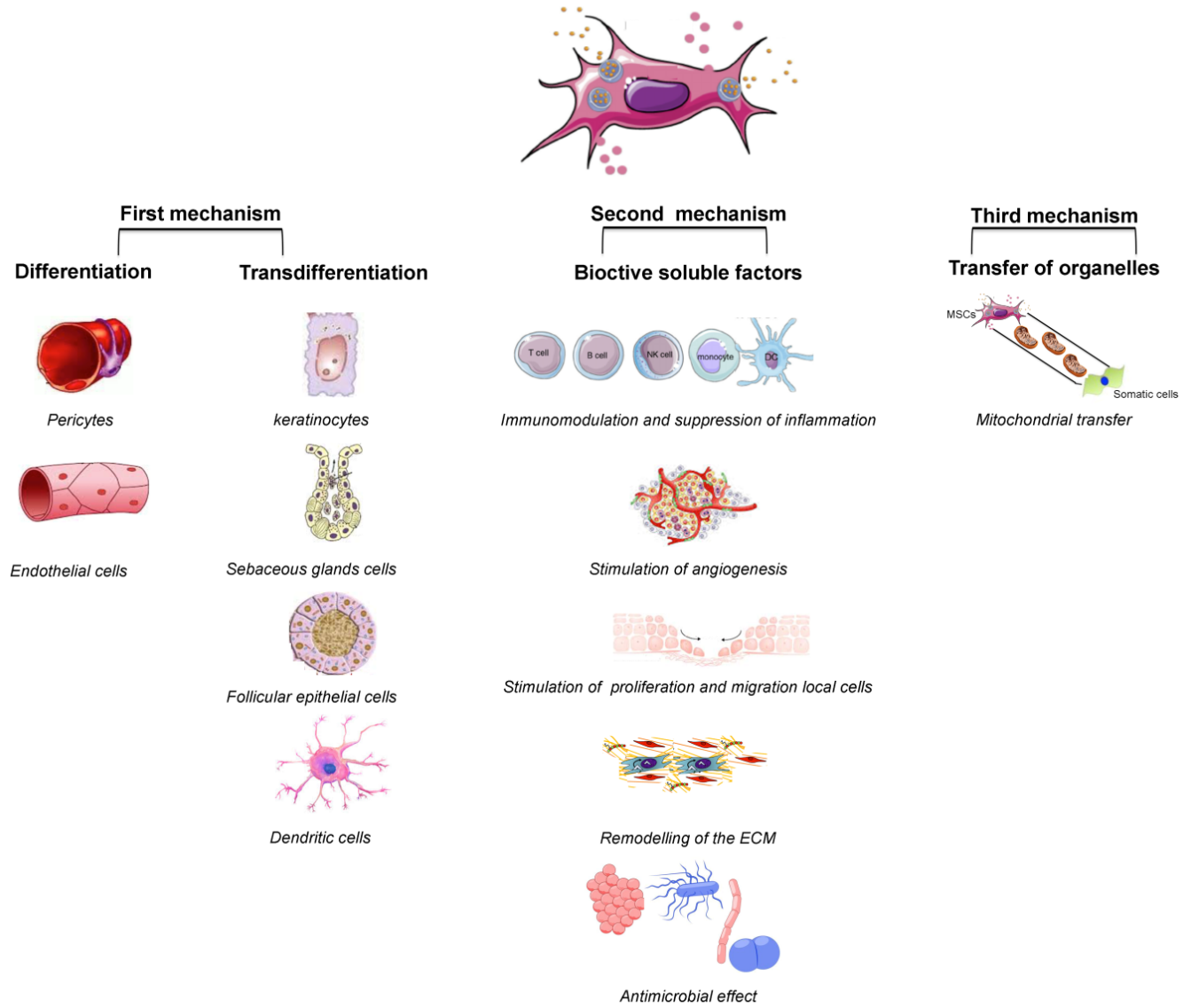

Figure 1: Mechanisms by which MSCs ameliorate skin damage. Three mechanisms might explain the therapeutic effect of MSCs. The first mechanisms includes the MSC differentiation into pericytes and endothelial cells and/or transdifferentiation into dermal and epidermal cells (keratinocytes, sebaceous glands cells, follicular epithelial cells and dendritic cells). The second mechanisms involves the MSC production of bioactive soluble factors which contribute to wound repair and regeneration by: i) immunomodulating and suppressing inflammation, ii) stimulating angiogenesis, proliferation and migration of local keratinocytes and dermal fibroblasts, iii) aiding in the formation and remodeling of the ECM, and iv) providing an antimicrobial effect. The third mechanisms involves the MSC transfer orgallellas (mitochondria transfer).

Cumulatively, human BM-MSCs possess the main enzymatic and non-enzymatic mechanisms for reactive species detoxification as well as proteome and genome oxidative damage repair, which ensure efficient OS management.

Role of metabolism in MSC self-renewal

In BM, MSCs reside under a hypoxic environment [66], with oxygen (O_2) tensions (PO_2) ranging from 10-32 mmHg [67]. The low O_2 levels of the MSC niche promote the activation of hypoxia-inducible factor (HIF) dependent pathways, which regulate the metabolic fate and pluripotency of MSCs [68]. In general, hypoxia triggers adaptive responses to reduced PO_2, enhancing the ability of cells to survive under O_2 deprivation [69]. This effect is mediated by the transcription of HIF-1α controlled genes, including vascular endothelial growth factor (VEGF), which promotes the formation of new blood vessels [70] and erythropoietin, a hormone involved in red cell production. This in turn favors O_2 tissue delivery [69] and the activation of glycolytic gene promoters [71]. The metabolic features of MSCs have been tested *in vitro*, demonstrating that culture of MSCs in normoxia share similar metabolic responses to reduced PO_2 [72], with a concomitant metabolic plasticity of the MSC mitochondria [66]. Essentially, Pattappa, et al. showed that oxidative phosphorylation (OXPHOS) in MSCs cultured in normoxia accounts for at least 30% of total ATP production. OXPHOS dependence *in vitro* has been previously associated with increased reactive oxygen species (ROS) production and premature senescence of expanding MSCs [73], which can affect MSC overall therapeutic

efficacy [72]. Thus, the ability of MSCs to retain their hypoxic signals in culture is an important feature to maintain their stem cell properties *in vitro* [72].

When compared to differentiated progeny, the MSC metabolic profile exhibits higher levels of glycolytic enzymes and lactate production [74], with diminished levels of OXPHOS proteins [75]. This demonstrates that undifferentiated BM-MSCs mainly rely on glycolysis for energy purposes, relative to their derived-differentiated cells (e.g. osteoblasts) [75]. Differentiation of expanding MSCs *in vitro* involves a metabolic switch that favors OXPHOS over glycolysis [75]. This effect on early-differentiated MSC metabolism redirects cell fate by increasing the expression of OXPHOS proteins, oxygen consumption rates, intracellular ATP levels [76], and mitochondrial ROS production [77]. As demonstrated before, hypoxic preconditioning in MSC culture enhanced MSC ability to maintain cell self-renewing properties after transplantation [78]. The effects of maintaining a hypoxic environment during MSC culture involved HIF-1α stabilization, which triggered increased growth factor production, including VEGF and its receptor Flk-1, insulin-like growth factor 1 (IGF-1) and basic fibroblastic growth factor (bFGF), as well as reduced pro-inflammatory molecule release [72,79]. Together, the improved production of these protective molecules enhanced the MSC abilities for tissue regeneration and self-renewal. Similarly, another key factor to sustain MSC renewal potential involves an increased glycolytic metabolism, which has been successfully proven during MSC high-glucose culture *in vitro* [80]. As a result, the role of glucose has been previously recognized as a key approach to enduring cell survival and function after construct transplantation [80].

Role of MSCs in OS-related diseases

The observed therapeutic effects after MSC transplantation into individuals with OS-related diseases might be attributed, among other mechanisms, to their potential to effectively scavenge exogenous ROS and reactive nitrogen species, once homed into the niche of damaged tissues. Indeed, mice with experimental diseases (liver and neurodegenerative diseases) that received MSCs showed a discrete but statistically significant lower ratio of reduced GSx to oxidized GSx [81], as well as a lower increase of disease-induced oxidative markers [82].

In particular, the surroundings of diabetic foot ulcers are characterized by a high-glucose environment, along with an extremely anoxic microenvironment [83]. These two conditions lead to increased production of pro-inflammatory molecules such as tumor necrosis factor alpha (TNF-α), which subsequently enhance local inflammatory responses and thus result in wound healing disorders [84]. In animal studies, the presence of a high glucose microenvironment affects the vascular regeneration of skin ulcers in comparison to low glucose surrounding environments [83-85]. However, one of the key characteristics in successful MSC transplantation for treating diabetic foot ulcers relies on the ability of MSCs to sustain vascularization and angiogenesis. Based on this, there is a need for modulation of cell metabolic responses to the microenvironment surrounding diabetic foot ulcers, in order to control the MSC paracrine effects and cell survival, which might be accomplished by regulating nutrient bioavailability and intrinsic cellular metabolic pathways as well as using pharmacological approaches. A comprehensive understanding of the metabolic features that regulate and control stem cell fate during ulcer regeneration will provide a powerful tool to overcome the challenge of maintaining cell proliferation and differentiation in the hostile environment of chronic ulcers, where excessive inflammation prevents healing.

Clinical Potential of MSCs

Due to their intrinsic properties and regenerative capacity, MSCs are considered to have therapeutic potential, which makes them a favorable candidate for cell-based therapies and tissue engineering applications [86]. MSCs are able to migrate to the exact site of injury, differentiate into various cell lineages, and secrete abundant soluble growth factors and cytokines that are crucial for cell survival, proliferation, as well as host immune response modulation [87]. As a result, MSCs show a remarkable potential for the treatment of a number of diseases, including both immunological and non-immunological disorders. In particular, more than 756 clinical trials involving the use of MSCs are currently in progress (www.clinicaltrials.gov). These include the treatment of different conditions such as: myocardial infarction, osteogenesis imperfecta, hematologic malignancies, graft-versus-host disease, Crohn's disease, spinal cord injury, multiple sclerosis, and diabetes (for the healing of refractory wounds), without any reported serious adverse events [88]. Cumulatively, the results of these early-phase studies indicate that the use of autologous and allogenic MSCs obtained from different sources appears to be safe. Nonetheless, the efficacy of these treatments remains to be demonstrated in late-stage clinical trials [87].

Molecular mechanisms associated with the clinical potential of MSCs

The therapeutic effects of MSCs to repair injured tissues have been largely associated to three mechanisms: i) differentiation or transdifferentiation into functional cells, ii) paracrine signals and iii) transfer of organelles and molecules to cells in the injury sites (Figure 1) [89]. In brief, the mechanisms through which MSCs could potentially enhance tissue repair are described below.

Cell differentiation and/or trans-differentiation: this mechanism includes the migration of MSCs to injury sites after administration in response to chemotactic signals *in vivo* [45]. Once MSCs are located at these sites, they start to engraft, differentiate and/or trans-differentiate to actively participate in tissue regeneration [89,90]. However, recent studies have suggested that MSC differentiation and/or trans-differentiation could be limited due to poor engraftment [91].

Paracrine signals: the production of bioactive soluble factors that modulate immune responses at injury sites has been suggested to contribute into the MSC therapeutic potency by promoting proliferation, migration and gene expression in several cell types [92,93]. These factors include cytokines, growth factors, enzymes, microparticles, miRNA and exosomes that are secreted without a direct cell-to-cell interaction. Also, it has been recently considered that MSCs could transfer their contents such as proteins and peptides, lipids, nucleic acids, and calcium and magnesium ions to local recipient cells at injury sites to stimulate cell survival and potentiate clinical responses [89,92,94-96].

Transfer of organelles: some studies have suggested that other paracrine mechanisms may play a part on cell signaling communication, mediated by cell-to-cell contacts by using tunneling nanotubes (TNTs) or cytonemes [97].

Cell-to-cell communication through highly dynamic TNTs, was described 40 years ago as a result of sea urchin cell studies [98]. At present, different authors have reported that MSCs may modulate cell responses by vesicle trafficking through TNTs. In particular, some authors have demonstrated MSC mitochondria transfer to several different cell types, including epithelial cells, endothelial cells, and

cardiac myocytes [96-100]; as a result, intracellular mitochondrial transfer has been lately proposed as a potential molecular mechanism of MSC-induced therapeutic potential. Spees et al. showed trafficking of MSC mitochondria when these were co-cultured with injured lung epithelial cells (lacked functional mitochondria), which allowed lung cells to restore aerobic respiration and enhance cell growth [96]. Similarly, it was evidenced that MSCs rescued injured endothelial cells *in vitro* using an ischemia-reperfusion model via TNT-mediated mitochondrial transfer [100].

The efficient mitochondrial transfer between MSCs and mitochondrial-deficient cells has been showed to be dependent on TNT formation. Li et al. showed that human-induced pluripotent stem cell-derived MSCs transfer their functional mitochondria to airway epithelial cells that were exposed to cigarette smoke (chronic obstructive pulmonary disease) through the formation of TNTs using a rat model [101]. Similarly, Jiang et al. reported that TNT formation induced corneal protection to corneal epithelial cells via mitochondrial donation through the Rot/NF-κB/TNFαip2 signaling pathway [102]. The effective transfer of mitochondria from MSCs to somatic cells could potentially abrogate associated mitochondrial-dysfunction damage in several pathological diseases. Nonetheless, the potential transfer of this organelle from MSCs to cells located at the wound injury sites still remain to be widely studied.

MSC-based therapy for chronic wound healing

Currently, MSC-based therapy for treating non-healing chronic wounds has shown supportive results. Particularly, a variety of clinical trials have revealed that MSCs are safe and therapeutic for healing chronic wounds [54], limb ischemia [103], diabetic foot ulcers [83] and radiation burns [104]. These studies reported that the administration of MSCs produced a significant recovery that entailed increased perfusion, decreased pain, ulcer size reduction, modulation of the radiation inflammatory processes, and a more appropriate wound repair. Specifically, the effect of MSCs on chronic wound healing is primarily reflected on the repair and replacement of cellular substrates, as well as the increased wound closure rates, tensile strength and angiogenesis. In addition, the use of MSCs allows to decrease scarring, attenuate inflammation, enhance migration of reparative cells and improve histological characteristics, such as superior rete ridge architecture, multilayered structure, major dermal-epidermal junction and the formation of new skin appendage structures (hair follicles and sebaceous glands) [105-109].

MSCs have the unique ability to initiate different wound-healing programs depending on the environmental milieu. Nevertheless, the exact mechanisms by which MSCs ameliorate skin damage are still under debate. In fact, two theories might explain the therapeutic effect of MSCs: MSC differentiation and/or transdifferentiation into dermal and epidermal cells and MSC production of bioactive soluble factors (growth factors, cytokines and specific proteins) Figure 1 [110]. Most studies agree on the fact that, although MSCs can migrate to injury sites in response to chemotactic signals *in vivo* [45], only a small percentage of the engrafted MSCs becomes incorporated and survives within the damaged tissue [111]. Also, several studies have evidenced that the implantation time of MSCs is usually too short to have an effective impact [112]. Indeed, it has been reported that less than 1% of MSCs survive more than one week in the wound site after systemic administration [113,114]. In contrast, other studies indicate that transplanted MSCs do not necessarily have to be in close proximity to the damaged tissue in order to promote wound repair and functional recovery, since the secretion of paracrine factors appears to be the main MSC therapeutic action involved in skin disorder repair [115,116].

First theory: the role of MSC differentiation and transdifferentiation in chronic non-healing wounds: Different pre-clinical and clinical studies have described that MSCs help to restore the normal function of chronic wounds by: i) differentiating into pericytes [45,110] and endothelial cells (ECs) [45,105,106,110], and ii) transdifferentiating into keratinocytes, sebaceous glands cells, follicular epithelial cells and dendritic cells Table 1 [64,105,110,117,118]. Various studies have reported the differentiation of MSCs into EC lineage after their delivery at the ulcer sites. These cells expressed endothelial-type markers, such as Von Willebran Factor (vWF), Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), Vascular Cell Adhesion Molecule (VCAM), and helped to stabilize and promote the formation of new vessel walls. Huang et al. observed that AD-MSCs enhanced wound healing in full-thickness defects in mice by promoting greater invasion of blood vessels, relative to the control. Also, the grafted cells were positive stained for VEGF and vWF after transplantation in mice, suggesting that MSCs might promote angiogenesis by differentiating into ECs [57]. These findings are supported by the ability of MSCs to differentiate into mesoderm cells and transdifferentiate into endoderm functional cells, depending on culture conditions. Indeed, placenta-derived MSCs undergo *in vitro* differentiation into ECs, which is evidenced by expression of specific endothelial cell markers such as vWF, CD31 and VE-cadherin, after being exposed to several inducers during 10 days [106]. On the other hand, Hu et al. suggested that BM-MSCs migrated to the wound site and enhanced epithelialization by transdifferentiation into keratinocytes. They used a chimeric mouse model by inserting fluorescently-labeled male MSCs into a female mouse. The results showed that Y-chromosome positive MSCs were co-localized with pancytokeratin-positive cells, revealing self-transdifferentiation or cell fusion into keratinocytes [119]. Nevertheless, a study conducted by Sasaki et al. demonstrated that transdifferentiation of BM-MSCs into keratinocytes was not a result from spontaneous cell fusion; instead, the fluorescently-labeled male MSCs contained XY chromosomes, indicating that cell fusion was a rare event [110]. In contrast, other authors have reported conflicting data regarding MSC transdifferentiation capacity [120]. Rustad et al. assessed the *in vivo* differentiation of engrafted BM-MSCs after 14 days of wound healing in mice, and showed their capacity to differentiate into pericytes and ECs but not into keratinocytes [45]. Similarly, Formigli et al. showed that BM-MSCs did not transdifferentiate into keratinocytes, but instead promoted the differentiation of neighboring cells [56]. That said, the transdifferentiation process may depend on the wound microenvironment as well as the delivery system used to administer the MSCs, which might indicate their potential role in the wound healing process.

Despite the fact that MSC differentiation and transdifferentiation might play a critical role in wound healing, a number of studies have revealed poor MSC engraftment when they are injected in the wounds [56,121]. In this context, Wu et al. demonstrated by means of a tracing assay that injected BM-MSCs disappeared in the first 24 hours after delivery into dermal fibrotic skin regions in mice. Similar results were reported by Formigli et al., who studied BM-MSC grafting in rats. As a result, several authors have implied that the secretion of paracrine factors is the major MSC therapeutic mechanism involved in skin ulcer repair [79,122-124].

Second theory: MSC production of bioactive soluble factors: MSC acellular derivatives are defined as the set of factors/molecules secreted by MSCs to the extracellular space. These factors include trophic

factors, soluble proteins, chemokines, cytokines, glycosaminoglycans, free nucleic acids, lipids, and extracellular vesicles (apoptotic bodies, microparticles and exosomes) [125].

Several groups have reported successful wound healing of surgical wounds [122,126], diabetic wounds [107,124] and burns [127-129] after the delivery of MSC acellular derivatives [129]. The effective wound healing has been associated with the secretion of trophic factors, such as VEGF, IGF-1, bFGF, platelet-derived growth factor BB (PDGF-BB), angiopoietin 1 (Ang-1), stromal cell-derived factor 1 (SDF-1), EGF, and keratinocyte growth factor (KGF), as well as the secretion of matrix metalloproteinase 9 (MMP9), and cytokines, including tumor necrosis factor beta 1 (TGF-β1), interleukin 6 (IL-6) and IL-8. These molecules contribute to wound repair and regeneration by: i) immunomodulating and suppressing inflammation, ii) stimulating angiogenesis, proliferation and migration of local keratinocytes and dermal fibroblasts, iii) aiding in the formation and remodeling of the ECM [118], and iv) providing an antimicrobial effect [130].

Immunomodulation and suppression of inflammation: MSCs have an immunomodulatory effect by mediating the proliferation, activation and function of immune cells since they typically have a low expression of the major histocompatibility complex (MHC) class I and lack the expression of MHC class II, CD40, CD80, and CD86. This allows MSCs to avoid T cell recognition, and often results in the absence of an immune response [131]. Indeed, pre-clinical studies have shown a suppressive effect on both the innate and adaptive immune response when MSCs are applied [132-134]. MSCs play a role in several phases of the immune response through the production of different soluble factors, especially in the phases of antigen recognition and presentation, T cell activation, proliferation, and differentiation as well as the effector stage of T cells [135]. In particular, MSCs produce factors such as TGF-β1, hepatocyte growth factor (HGF), IGF-1, prostaglandin E2 (PGE_2), nitric oxide (NO), hemeoxigenase-1 and indoleamine-2,3-dioxygenase (IDO) [136,137].

On the other hand, MSCs also inhibit the following: proliferation of monocytes and their differentiation into macrophages [138]; differentiation of monocytes and haematopoietic progenitors into mature dendritic cells [139,140], and the de-differentiation of macrophages into monocytes [138]. In addition, MSCs induce dendritic cells to lose their ability to stimulate allo-responses and acquire a regulatory phenotype due to the production of large amounts of IL-10 [133]. Similarly, MSC-derived PGE_2 alters the cytokine secretion profile of dendritic cells and MSCs alter natural killer (NK) cell phenotype as well as suppress NK proliferation and cytokine secretion [141] through the production of soluble factors such as TGF-β1 and PGE_2.

MSC anti-inflammatory effect is mediated by cytokines such as TGF-β1 [142], IL-10, IL-12p70, IL-17E, IL-27 IL-13 [142,143], IL-1 receptor antagonist (IL1RA), IL-18 binding protein (IL-18BP), ciliary neurotrophic factor (CNTF), neurotrophin 3 (NT-3) factors [142,143], among others. On the other hand, MSC acellular derivatives have also been found to contain pro-inflammatory cytokines, such as IL-1b [142], IL-6 [144,145], IL-8 [146,147] and IL-9 [147], that are in balance with the anti-inflammatory cytokines, and this balance may determine the ultimate response in the tissue. Nevertheless, it is also remarkable that MSC acellular derivatives inhibit pro-inflammatory cytokines (for example, interferon (IFN) and TNFα), while increasing anti-inflammatory IL-10 release [143,148]. Specifically, Legaki et al. reported that MSC acellular derivatives significantly reduced the mRNA expression of IL-6, IL-8, TNFα and macrophage inflammatory proteins 1(MIP-1), and increased the mRNA expression of the IL-10 anti-inflammatory cytokine [149].

A number of these pro-inflammatory factors are involved in the acute inflammation period, a crucial phase in the wound healing process that leads to structural and functional repair of the injured tissue. Particularly, the inflammatory mediators that are released at the wound site and significantly contribute to the wound healing process are TGF-β1, IL-6, and IL-8. In a similar way, IL-6 plays a major role in both the balancing of the pro-inflammatory/anti-inflammatory pathways, and the stress response.

Stimulation of angiogenesis: Because of the fact that MSC acellular derivatives have shown to play a more relevant role in angiogenesis than MSCs, therapeutic approaches are currently developed using only the bioactive factors produced by MSCs [25,150,151].

MSC acellular derivatives can trigger vessel regeneration in ulcers by different mechanisms, mainly through vasculogenesis (the novo blood vessel formation from endothelial precursors or angioblasts), angiogenesis (the sprouting of existing vessels or intussusceptive angiogenesis), and arteriogenesis (the growth of collateral vessels), which have been mostly associated with angiogenic factors that are present in the secretome of MSCs [152,153]. They have been shown to induce proliferation, migration, and tube formation of endothelial colony-forming cells [152].

MSC acellular derivatives induce EC migration and chemiotaxis through factors such as CXCL-12/16 [154], CCN3 [155], and HGF [156]. EC migration initiates vascular reconstruction and allows endothelial tip cells to become invasive and to form both filopodia and lammellipodia, in response to guidance cues. At the same time, stalk cells, which lie behind tip cells, proliferate, extend the vessels and form extracellular matrix, junctions and lumens [157]. During this angiogenic process, the MSC acellular derivatives support the entire neo-vascular niche as well as rise the proliferation, survival and maturation of the cells involved in this process [152]. Some of these essential acellular derivatives are Ang-2 [158], endothelin-1 [159], Upa [160], VEGF [161], PDGF-AA/BB [162], placental growth factor (PIGF) [163] and FGF-7 [164].

Despite the fact that MSC acellular derivatives induce angiogenesis, it is important to highlight that this secretome also contains anti-angiogenic regulators, such as TIMP-1/4 [165], serpin F1 and Thrombospondin-1/2 [166], which may block the migration of ECs. In this context, MSC acellular derivatives may modulate the angiogenesis mechanism in the wound healing process through complex interactions that may occur between both their pro-angiogenic and anti-angiogenic regulators [152].

Stimulation of proliferation and migration of local keratinocytes and dermal fibroblasts: MSC acellular derivatives are being rigorously investigated as a means to accelerate the proliferation, migration and differentiation of keratinocytes and dermal fibroblasts, in order to regulate the complex interactions that occur during wound healing [123,167,168]. Scratch assays revealed that, relative to the control (medium with serum), dermal fibroblasts and keratinocytes enhanced their rate of wound closure when exposed to MSC acellular derivatives by increasing their migration instead of their proliferation rates [167]. However, Seung et al. reported a significant increase in the proliferation rate of both keratinocytes and dermal fibroblasts when exposed to MSC acellular derivatives obtained from AD-MSCs [169]. These discrepancies may have arisen because of differences in the MSC sources and the concentration of the MSC acellular derivatives employed in

the studies. In fact, these derivatives appeared to influence dermal fibroblast migration rate in a dose-dependent manner [170]. Indeed, by increasing MSC concentration (by 40% or more), the migration rate of fibroblasts significantly decreased [170]. These results might suggest that the production of chemoattractant cytokines by MSCs varies depending on their confluency, creating a distinct microenvironment and secreting variable amounts of the attractant molecules.

To gain insight into the role of MSC acellular derivatives on wound healing progression, some researchers have compared the effect of MSC and fibroblast acellular derivatives on keratinocyte function and behavior, since dermal fibroblasts are known to be essential in the skin regeneration process. Specifically, Liwen et al. mimicked the normal wound healing environment by growing BM-MSCs and fibroblasts under hypoxic conditions and collected their acellular derivatives. Proliferation and migration assays performed on keratinocytes and ECs demonstrated that MSC acellular derivatives had a greater mitogenic and chemoattractive effect than fibroblast acellular derivatives. Indeed, MSC acellular derivatives analysis confirmed that MSCs expressed higher levels of KGF-1, PDGF, EGF, IGF-1 compared to dermal fibroblasts [122,171]. In addition, data from *in vivo* studies showed an accelerated wound closure when MSC acellular derivatives were used [122,171]. Similarly, AD-MSCs and fibroblasts have been used as a support for keratinocyte growth in two-dimensional (2D) and three-dimensional (3D) contexts, in order to better understand the paracrine factors secreted by these two cell types that are involved in the improvement of cutaneous wound healing [171]. By growing keratinocytes in MSC acellular derivatives, the number of cells in the transition from G_2 phase to mitosis significantly increased compared to cells grown in fibroblast acellular derivatives, which sustained the cells on G_0/G_1 phases. However, in 3D contexts, AD-MSC acellular derivatives stimulated the abnormal keratinocyte expression of cytokeratins 5, 14 and 19, suggesting the induction of unusual hyperproliferation [169]. That said, future studies would need to incorporate a higher number of 3D-biomimetic culture systems to obtain more physiologically appropriate results.

On the other hand, during normal wound healing, keratinocyte migration is accelerated by EGF and TGF-β [172], while keratinocyte proliferation is induced by EGF, bFGF, keratinocyte growth factor-1 (KGF-1) and IGF-1 [172,173]. Likewise, PDGF, TGF-β, connective tissue growth factor (CTGF) and nerve growth factors act as chemoattractants for dermal fibroblasts, while their proliferation is influenced by the presence of EGF, FGF, PDGF, TGF-β, CTGF and IGF-1 [174]. Collectively, the MSC acellular derivative effect over dermal fibroblasts and keratinocytes merit further investigation as "off-the-shelf" therapeutic options to promote healing of chronic ulcers.

ECM remodeling: ECM plays a number of critical roles in the wound healing process, which include supplying information and signals to the surrounding cells, as well as providing structural support [175]. In this context, MSC acellular derivatives can modulate the ECM healing microenvironment by remodeling the matrix and promoting its biosynthesis, stimulating different biological activities at the tissue or cellular levels [150,175].

In fact, Arango et al. conducted a study to elucidate the role of MSC acellular derivatives on wound healing using different animal models, in particular, a diabetic mouse model. The results revealed that the wounds treated with the derivatives improved the synthesis, deposition and organization of collagen fibers at the dermal matrix, relative to the wounds treated with MSCs only [124,176]. Another recent study showed that the intravenous injection of acellular derivatives promoted cutaneous wound repair when exosomes secreted by human AD-MSCs (AD-Exos) were administered in murine incisional wounds. Wang et al. observed an improved wound healing process *in vivo,* which was mediated by the following mechanisms: i) increase in the ratio of collagen III to collagen I, ii) prevention of fibroblast differentiation into myofibroblasts, and iii) increase in the ratio of TGF-β3 to TGF-β1. In addition, AD-Exos enhanced the matrix metalloproteinase-3 (MMP-3) expression of skin dermal fibroblasts by activating the ERK/MAPK pathway, leading to a high ratio of MMP3 to tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), which was also beneficial for ECM remodeling [177]. That said, MMP production is inhibited by TIMPs, some of which are found in the MSC acellular derivatives, such as TIMP-1 and TIMP-4 [152]. Also, MMPs have been shown to regulate the cell–cell and cell–matrix signaling through the release of cytokines and growth factors sequestered in the ECM, as well as the exhibition of bioactive domains in the components of the ECM. Similarly, MMPs modify cell surface receptors and junctional proteins, regulating signaling processes in the cell in the wound healing microenvironment, which include: migration, proliferation, differentiation, mobility and cell death, thus, playing a pleiotropic role in the wound healing process [178,179]. Consequently, the degradation of the matrix allows to activate the cells in the wound microenvironment, which can initiate an indirect remodeling process.

There are important components of MSC acellular derivatives that produce an anti-fibrotic effect, which significantly allows the attenuation of scar formation during wound healing by ECM remodeling, being the most prominent factors HGF and IL-10 [180]. Fibroblasts respond to HGF by down-regulating their expression of TGF-β1 and collagen type I/III [181]. In addition, HGF not also stimulates the up-regulation of MMP-1/3/13 expression in fibroblasts, promoting ECM turnover, but also increases the keratinocyte migration and proliferation as well as their expression of VEGF-A [182]. Therefore, HGF contributes to the generation of a high-quality and well-vascularized granulation tissue, while enhancing re-epithelialization of the wound [182].

Similarly, IL10 is able to reprogram wound fibroblasts to favor ECM remodeling by up-regulating the expression of MMPs and down-regulating the expression of collagens [183], as well as attenuating the expression of pro-inflammatory cytokines in the wound, such as IL-6 and IL-8 [184]. Furthermore, IL-10 inhibits neutrophil invasion into the wound and prevents oxidative tissue damage [185]. As a result, expression of IL-10 contributes to both a resolution of the inflammatory stage and acceleration of the wound into the proliferation stage [184,186].

Antimicrobial effect: One of the most common complications of chronic skin wounds is the presence of opportunistic pathogens that colonize the skin ulcer, which constitutes one of the main reasons why chronic wounds do not heal in a short time [187]. Up-to-date literature shows conflicting data regarding the influence of MSCs on wound infection. Reported evidence suggests that MSCs may have pro- as well as anti-microbial effects, [188,189] which seem to depend on MSC isolation and expansion conditions, cell source, doses, administration route, timing and wound microenvironment.

Several studies have shown that MSCs may provide an antimicrobial effect. Both un-stimulated and IFN-γ stimulated human MSCs can inhibit the growth of Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*, as well as the growth of Gram-positive pathogens, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, group B *Streptococci* and *Enterococcus faecium* [190,191]. Recent data has also shown that MSCs exerted a strong antimicrobial effect on

preclinical models of polymicrobial sepsis [130,188,192,193], acute respiratory distress syndrome [194,195], cystic fibrosis infection [130,196,197], and endotoxemic rat models (involving intravenous LPS injection) [198]. Indeed, the results suggest that MSCs are responsible for inhibiting and clearing bacterial growth, decreasing subject mortality, as well as reducing systemic inflammation and decreasing inflammatory cytokine levels.

MSC antimicrobial activity has also been proven in a clinical study aimed at treating patients suffering from acute respiratory distress syndrome (NCT01902082). Specifically, one intravenous dose of 1×10^6 cells/kg allogeneic AD-MSCs acted as a safe and feasible therapeutic tool for this infection [199], through the secretion of antimicrobial peptides such as: cathelicidin LL-37 [191,196,197], defensins [200], hepcidin [201], and lipocalin 2 [202], which prevented bacterial growth or killed the pathogens. The secretion of these soluble peptides improved resident phagocyte ability to clear bacteria by the up-regulation of pathways associated with monocyte/macrophage, phagocytosis, NK cell activity and antigen presentation [188]. Likewise, MSC antifungal activity has been associated with an increased amount of TH17 cells in the blood, promoting TH1-type immune responses and restraining the TH2-type ones [29,203]. Cumulatively, MSC acellular derivatives might become an innovative therapeutic tool for preventing and treating infected skin wounds by improving the conditions of the chronic cutaneous wound healing process [130,187,204].

Future Perspective

The fascinating regenerative therapeutic effects of MSCs in a number of life-threatening human diseases have led them to become the most common and effective cell source in cell-based treatments. Nevertheless, some issues still require to be addressed in order to propose optimized therapeutic strategies, for instance: which route is more suited for the administration of MSCs? Which would be the most suitable biomaterials used for optimizing stem cells' transplant effectiveness? How does the local environment affect delivered MSC performance and action? Which is the best alternative culture protocol for the *in vitro* MSC expansion using xeno-free media before transplant? Which is the best source of donor cells for the degenerative disease under investigation?

On the other hand, several investigators have recently explored the possibility of replacing MSCs by their acellular derivatives for therapeutic applications since MSCs exert many of their effects via paracrine signaling. In fact, acellular derivatives could be a more promising therapeutic tool due to both their good manufacturing practice production and their release is less complex compared to living cells, resulting in reduced costs. In addition, the acellular derivatives could circumvent the current limitations associated with poor cell survival upon transplantation as well as provide the possibility to apply one or combined trophic factors as oriented therapies for diseases. Although it is undisputable that MSC therapy contributes to restoration of structural integrity and functionality of damaged tissue, resulting in functional advantage over other conventional strategies, these series of gaps still need to be addressed so that these potential therapeutic tools could have transition from bench to bedside and become more feasible in the near future.

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