Advances in mesenchymal stem cell therapy on wound healing emphasizing on the alternative cell free techniques

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Abstract

Cell-based treatments have been said to have considerable promise for the healing of wounds especially for those which don't heal or heal with severe scarring. Stem cells have been demonstrated to speed up the healing process by direct integration into regenerated cells and transformation to parenchymal cells. The downside of using stem cells include low probability of MSC survival, engraftment, and the quantity of newly created cells, through cell differentiation or fusion. Stem cells secrete a wide spectrum of physiologically active molecules, including cytokines, growth factors, mRNAs, and active lipids with crucial roles in skin tissue regeneration. MSC secretome and MSC-exosome are products of MSCs discharge, imitating the actions of parental MSCs. They have a great potential to be used as a non-cell-based therapeutic strategy for the management of various wounds that would avoid the adverse effects of stem cell therapy.

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Introduction

Non-healing wounds continue to be a major source of disability and death across the globe and are a significant economic load, despite advancements in our knowledge of the processes involved in acute and chronic wound healing [1]. The healing process for cutaneous wounds typically involves a complex series of stages in which damaged tissues are replaced and supporting structures are re-established through the activities of various cell types with local and systemic stimuli [2]. However, chronic non-healing wounds may develop when these mechanisms go awry and in tandem with an underlying disease state [3]. The goal of the best practice in wound treatment is to encourage healing and avoid problems like scarring. There are still a lot of wound cases that either don't heal or heal with severe scarring, despite the abundance of wound care solutions available. Therefore, it is obvious that alternate wound treatments that encourage healing and minimize scarring are needed. Cell-based treatments have been said to have considerable promise for the healing of wounds. It has been suggested that stem cells can stimulate regenerative restoration as opposed to the repair processes that lead to scar formation since stem cells have been demonstrated to speed up the healing process [4]. The primary method by which mesenchymal stem cells (MSC) exercise their

positive benefits has been believed to be direct integration into regenerated cells and transformation to parenchymal cells [5,6]. The probability of MSC survival, engraftment, and the quantity of newly created cells, through cell differentiation or fusion, have lately been demonstrated to be too low to explain the major effects produced by MSCs [7,8]. Stem cells secrete a wide spectrum of physiologically active molecules, including cytokines, growth factors, mRNAs, and active lipids with crucial roles in skin tissue regeneration, according to a proteomic study of MSC conditioned medium (MSC-CM), which contains MSC secretome (MSC-S) [9]. As a result, the primary mechanism underlying has been proposed to be MSC paracrine signaling [10].

Mesenchymal stromal cells (MSCs) are active participants in all phases of cutaneous wound healing in both animal models and human subjects [11]. Exosomes, one of the main products MSCs discharge, imitate the actions of parental MSCs. They can transport different effector proteins, messenger RNA (mRNA), and microRNAs (miRNAs), which are crucial for modulating the behavior of recipient cells and aiding in the healing process of wounds. Additionally, utilizing exosomes eliminates a number of dangers related to cell transplantation. As a result, MSC-exosome-mediated delivery, a novel kind of cell-free treatment, may be both safer and more effective than whole cell [12]. We present a thorough analysis of the most recent research and findings on the application of MSC-exosome treatment to wound healing and skin regeneration in this review. Additionally, we discuss the MSCs-MExos hypothesis, which must be evaluated and immediately resolved in the study connected to the therapeutic uses of MSC-exosomes. This review may encourage researchers to consider fresh lines of inquiry into MSC-exosome treatment for skin regeneration and repair.

MSC Secretome

Recent research has indicated that MSCs' key therapeutic advantages go beyond just their cell-to-cell interactions [13, 14, 15, 16]. In reaction to their surroundings, MSCs release a wide variety of bioactive substances, generally referred to as the secretome, including proteins, nucleic acids, proteasomes, exosomes, microRNA, and membrane vesicles [17, 18]. The MSC secretome (MSC-S) then affects surrounding cells and controls a variety of biological functions [19]. Paracrine or trophic qualities are currently thought to be the main mechanism by which MSCs exert their therapeutic effects [17, 20]. Despite having similar phenotypic and regenerative properties, MSCs generated from various organs have diverse secretomes that are dependent on their source, which can result in a range of therapeutic prospects [21]. Using in vitro and in vivo models, MSC-S of diverse sources has been utilized to evaluate its impact on skin cell functioning as well as its impact on tissue regeneration.

Potential Mechanism of Action of MSC-S

Before the MSC-S is extensively marketed as a potential new treatment in the clinic, its mode of action must be clarified. Insights into several pathways have been provided by recent developments in cell and molecular biology, and it has been suggested that MSC-S may augment wound healing. When the MSC-S is dissected, a wide variety of proteins that are associated with skin inflammation, hemostasis, and wound healing are seen. These proteins' metabolic routes and mode of action have already been demonstrated [13, 17].

As a result, the MSC-S is a complex combination of bioactive molecules that has been demonstrated to have important therapeutic benefits in the management of inflammatory diseases of the neurological, circulatory, pulmonary, and musculoskeletal systems [22, 23, 24]. Several investigations have shown that the contact between MSCs and immune cells

can be attributable to MSC-secreted cytokines, despite the fact that it is generally accepted that the anti-inflammatory effects of cells depend on direct cell-cell interactions [25]. Interleukin-1 receptor antagonist (IL1-RA), for instance, prevents B cell differentiation [26]. Galectin-1 produced by human MSCs also inhibits the growth of alloreactive CD4+ and CD8+ T lymphocytes [27]. Programmed death ligand 1 (PD-L1), which is secreted by MSCs as well, promotes T cell apoptosis and inhibits T cell activation [28, 29]. Additionally, TGF-1, PGE2, IL-6, nitric oxide, and PGE2 are also released by MSCs and have antagonistic effects on neutrophils, T lymphocytes, macrophages, and NK cells [30, 31]. It has been demonstrated that MSC-S as a whole has immunosuppressive effects by controlling the proliferation and activation of immune cells in vitro. Peripheral blood mononuclear cells treated with MSC-S produced less pro-inflammatory cytokines and more anti-inflammatory cytokines. When BM-MSC-S is injected into the edges of excisional wounds in mice, the inflammation caused by macrophage polymerization is reduced, promoting wound healing. MSC-S has a much more positive impact than the same amount of fibroblast secretome therapy [13].

In vitro stimulation of endothelial cells with MSC-S boosting their growth and recruitment has been postulated as another of the key mechanisms of action for MSC-S in various types of wounds [14, 15]. The secretion of Cyr61 from MSCs is thought to be the mechanism by which MSC-S affects angiogenesis. Vascular development and stability are further aided by the pro-angiogenic proteins released by MSCs, including Ang-1, Ang-2, angiostatin, VEGF, CXCL16, EGF, PDGF, FGF, granulocyte-macrophage colony-stimulating factor (GM-CSF), MCP-1, HGF, MMP-8, and MMP-9 [32]. In preclinical experiments, healing of partial-thickness skin burn in rats was found to be enhanced by BM-MSC-S therapy, which was facilitated by increased angiogenesis [14]. In a different research, topical use of BM-MSC-S to rats with full-thickness burns led to an increased fibroblasts, better angiogenesis, and quicker wound healing [15]. When umbilical cord-derived MSC secretome (UC-MSC-S) was subcutaneously injected into diabetic mice's wounds, the wounds healed quickly and had a high density of capillaries.

In vitro, MSC-S from a variety of sources, including the iliac crest, bone marrow, adipose, Wharton's jelly, and umbilical cords, have been demonstrated to improve the migration patterns and cell proliferation attributes of dermal fibroblasts and epidermal keratinocytes [33]. MSC-S also changes the expression of genes responsible for re-epithelialization and angiogenesis and boosts it. In in vitro models, the secretome from MSCs generated from adipose tissue (ADSC-S) has been demonstrated to shield dermal fibroblasts from oxidative stress-mediated apoptosis, speed up wound healing, and have enhancing effect on fibroblast recruitment. Growth factors (such IGF-1, FGF-2, EGF, KGF, TGF, HGF, VEGF, PDGF, SDF-1, and erythropoietin) and chemokines (like IL-6, IL-8, MCP-1, and RANTES) are thought to promote the positive impact of MSC-S on keratinocytes [33, 34, 35]. The re-epithelialization, collagen deposition, and new tissue growth in wounds treated with MSC-S are all greatly accelerated [13]. Application of BM-MSC-S given in a fibrin carrier to chronic rat wounds boosts re-epithelialization and collagen deposition as well [16]. In a different research, rats with excisional wounds that were topically treated with ADSC secretome showed quicker reepithelialization and wound healing. It has also been shown that the secretome of dental pulp stem cells (DPSC) promotes dermal fibroblast proliferation and migration, increases collagen production, and speeds up wound healing. Through enhanced cell proliferation, MSC-S made from Wharton's jelly (WJ-MSC-S) aids in the healing of excisional wounds in mice. Recently, it has also been demonstrated that WJ-MSC-S helps rats with radiation-induced skin wounds recover faster.

Advantages of MSC-S

Cell-based medicines and products have been there for quite some time now. In truth, there have been recombinant growth factors, cytokines, platelet-rich plasma, and skin replacements for many years. But despite encouraging preclinical findings and fruitful clinical studies, there is still a need for better cell-based treatments, as seen by the spiraling rise in chronic wounds throughout the world. TransCyte, Dermagraft, Apligraf, and OrCel are examples of skin replacements available today that comprise live fibroblasts, keratinocytes, or both [36, 37, 38, 39]. These cell-based skin graft replacements have shown promise in encouraging quicker wound healing (Transcyte), increased re-epithelialization rates (Dermagraft), and superior vascularity, pigmentation, wound height, and scar scores (Apligraf) [40]. The drawbacks of these treatments include their high cost, need for certain storage conditions, possibility for tumorigenicity, infection, and rejection, and difficulty in utilizing them in the general population [41]. Recombinant growth factors were proposed as a treatment for poor healing because it was believed that chronic non-healing wounds lacked certain cytokines and growth factors [42]. Numerous clinical trials were conducted to examine growth factor therapies, such as EGF, KGF, PDGF, and GM-CSF therapies. However, despite these therapies having appeared to be effective in many animal models of wound repair, their translation into human medicine has been hindered by the sizeable quantities of growth factors needed for treatment, the high cost of manufacturing them, and the paucity of clinically significant healing upgrades [43, 44, 45, 46]. Currently, only PDGF has been FDA-approved for treating diabetic foot ulcers, and its use is restricted since it requires frequent dressing changes and may raise the risk of cancer [47, 48]. Given that wounds are diverse and complex systems, it may be necessary to provide numerous growth factors and/or cytokines to promote healing. MSC-S may be a better option than pricey cytokine and growth factor treatments that can only transport one or two proteins to the wounds since it includes a wide variety of proteins at physiological and balanced levels, including growth factors, cytokines, and chemokines.

Delivering live cells to skin wounds poses a special and distinct set of difficulties [49]. It has been demonstrated that injecting cells with a syringe or needle reduces cell viability to just 1-32% and can harm the cell membrane irreparably, often fatally [50, 51]. Additionally, the injection of a significant number of apoptotic or necrotic cells may help to trigger an immune response that might be harmful to the healing process, negating any possible benefits of cell therapy. MSC-S treatment benefits from the simplicity of mass manufacturing, packing, and transportation while avoiding the challenges of live-cell administration in stem cells. These positive elements have increased the possibility of using MSC-S.

Challenges with the MSC-S as a Wound Therapy

Despite the possibility that MSC-S is a possible therapeutic product, it has proven to be exceedingly difficult to determine its biochemical make-up or to gauge the activity and half-life of each of its constituent parts. Exosomes and extracellular vesicles are also present in MSC-S along with proteins [52]. Exosomes may include lipids, long non-coding RNAs, and miRNAs, which control many signaling pathways involved in inflammation [53]. Although it is challenging to identify and characterize every biomolecule that makes up the secretome, doing so will assist us in better comprehending the secreted factor composition and provide light on its modulation, function, and therapeutic application [54]. Clarifying all of the important metabolic and signaling pathways that are facilitating new and creative tissue formation, reduced inflammation, and improved wound closure will require additional research on the MSC-S utilizing high throughput mutational and

chemical testing and next-generation metabolomics-driven approaches.

The efficacy of MSC preparations has reportedly been impacted by donor fitness and age, as well as segregation and growth techniques [55]. Another current issue with the therapeutic application of secretome is the inconsistent secretome harvesting in terms of MSC heterogeneity, donor differences, cell quantity, and time interval. A crucial step in using MSC-S as a therapeutic agent in the clinic is to produce it in accordance with good manufacturing practices (GMP) and pharmaceutical guidelines. Batch-to-batch constancy and MSC-replicable S's efficacy will increase if well-defined good manufacturing practices (GMP) are followed [56].

The hazards associated with employing extrinsic biological molecules are always there, even if there have been fewer reports of these problems in relation to secretome than there have been for cell-based treatments. Before MSC-S are transplanted into specific tissue niches, a thorough investigation is required. For instance, MSC-S comprises extracellular vesicles and exosomes produced from MSCs that have been proven to have a lower immunogenicity than respective parent MSCs [57]. However, MSC-S has been shown to exhibit immunosuppressive qualities, which are thought to be one of its primary modes of action when managing autoimmune illnesses [58]. The use of secretome, unfortunately, may weaken the immune system, increasing the risk of infection, immunosuppression, and tumor progression in treated individuals [59]. To discover the ideal balance between safety and efficacy of any secretome-based therapy, an optimal quantity of secretome must be precisely specified.

In comparison to directly injected live cells, the number of MSCs needed to create an equal amount of secretome for an impact on acute wounds is around 10–25 times greater. Because the biological characteristics and activities of these cells may alter with consecutive cycles, the higher number of cells has an influence on the expenditures of generation and confirmation. However, the impact of this limitation could be reduced with higher output and advancements in cell factories and bioreactors. The unstable nature of proteins and their brief half-lives is another significant issue in secretome treatment. Preconditioning cells to promote the secretome's paracrine synthesis is one of the effective methods for overcoming these disadvantages. To create a proper balance between the stimulatory and suppressive factors generated by these cells, it is crucial to first raise the amount of desirable factors and decrease the production of unfavorable ones.

There are several pre-treatment techniques for MSCs; for instance, it has been observed that transplanted stem cells secrete more growth factors and cytokines when exposed to hypoxia or anoxia [60]. To manage the MSC-S post-transplantation, genetic modification of cells employing transgenes can also change the expression of a certain gene [61]. Small chemicals like inflammatory cytokines and growth factors are one possible method for pre-treating stem cells prior to transplantation [62]. For instance, administering inflammatory cytokines to MSCs enhances their immunosuppressive activity and causes them to secrete more anti-inflammatory chemicals [63]. An additional method to enhance the production of advantageous biomolecules is preconditioning via interactions between cells. In contrast to MSCs in monolayers, Potapova et al. (2007) found that MSCs in 3D spheroids are able to release larger quantities of paracrine molecules such IL-11, VEGF, FGF-2, and angiogenin [64]. This customization of the MSC-S may result in a wide variety of off-the-shelf items made expressly for the management of particular problems or wound types.

MSC-exosomes

The characteristic features of resource cells are displayed by MSC-derived exosomes (MSC-

exosomes), which can encourage cell self-repair and cell growth, reinstate cellular homeostasis, and quicken wound healing in injured regions [65]. Recent research has revealed that MSCs are very capable of producing exosomes [66]. According to the majority of researchers, MSC-exosomes are the primary paracrine component of MSCs that exerts biological functions that are almost identical to those of complete MSCs. MSC-exosomes are superior to MSCs in the following ways: First, since they directly merge with target cells, MSC-exosomes have powerful physiologic effects. Second, MSC-exosomes may be kept and delivered at temperatures below 70 °C for a long time because their active components are shielded by the exosome's tough plasma membrane. Third, it is simple to adjust the dosage, method, concentration, and timing of usage. Last but not least, cell transplantation treatment poses no danger of immunological rejection or cancer [67].

Potential Mechanism of Action of MSC-exosomes

Three overlapping stages may be used to sum up the usual skin tissue repair: the inflammatory phase, the proliferation phase (tissue growth and reepithelization), and the remodelling phase [68, 69, 70]. The involvement of MSC-exosomes in the aforementioned three phases is now the focus of all research on MSC-exosomes in wound repair and skin regeneration.

Excessive cytokine production during the inflammatory stage can result in tissue damage, and MSC-exosomes control inflammatory factors that are crucial for skin regeneration [71]. By down-regulating proinflammatory factors like inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, as well as cytokines and chemokines like tumor necrosis factor (TNF)-, interleukin (IL)-1, and monocyte chemoattractant protein (MCP)-1, exosomes derived from various types of MSCs can reduce the immune response brought on. Additionally, MSCs-exosomes can promote the production of IL-10, an anti-inflammatory cytokine, in several disease models. IL-10 is thought to be crucial in the modulation of skin wound inflammation and tissue repair [72, 73, 74]. Numerous studies have shown that certain miRNAs are used by MSC-exosomes to achieve their immunomodulatory activities. They discovered that hUC-dMSCs had the greatest concentration of three miRNAs (miRNA-21, miRNA-146a, and miRNA-181c) that are especially involved in the control of immunological and inflammatory response by evaluating miRNA expression patterns [75, 76]. Additionally, further investigations showed that via down-regulating the Toll-like receptor 4 (TLR4) signaling pathway, hUC-dMSCs-exosomes harboring miRNA-181c reduced the burn-induced highly strong inflammation [73]. The post-transcriptional repression of chemokines and cytokines (such as TNF- and MCP-1) by anti-inflammatory miRNAs (miRNA-124a, miRNA-125b) found within exosomes has been linked to the maintenance of inflammatory cell infiltrates in healing process [77, 78]. Overall, the unique molecular pathways through which MSC-exosomes reduce inflammation during tissue repair and healing needs to be studied deeply.

MSC-exosomes aid in neoangiogenesis, collagen deposition, granulation tissue development, re-epithelialization, and wound contraction during the proliferation phase [79]. Several angiogenesis-related RNAs and proteins, including miRNAs that may activate several signaling pathways in endothelial cells, are more abundant in MSC-exosomes. Numerous trophic factors can be expressed by the same exosomes [82]. Li et al. also demonstrated that grafted human umbilical cord blood vascular endothelial cells-derived exosomes (EPC-exosomes) could increase the appearance of angiogenesis-related factors, such as vascular endothelial growth factor (VEGF) - A, fibroblast growth factor (FGF)- 1, vascular endothelial growth factor receptor (VEGFR)-2, E-selectin, IL- 8, angiopoietin-1, Chemokine (C-X-C motif) ligand 16, and endothelial nitric oxide synthase (eNOS), in vascular endothelial cells. In addition,

endothelial cells treated with EPC-exosomes showed a significant drop in matrix metalloproteinase (MMP)-9 mRNA levels [83]. According to the study's findings, MMP-9 expression levels were substantially greater and were linked to subpar wound healing [84]. Therefore, MMP-9 suppression by EPC-exosomes may contribute to some of their pro angiogenesis actions. It is still important to disclose their further methods for how angiogenesis affects wound healing.

Skin re-epithelialization and cell proliferation are essential for skin renewal. Skin fibroblasts contribute to wound contraction, extracellular matrix formation, tissue remodeling, and other important skin tissue healing and regeneration processes [85]. MSC-exosomes have the ability to be internalized, allowing them to deliver their contents—such as proteins and RNAs—into the recipient cells to control their migratory and proliferative behavior. It has been established that MSC-exosomes control fibroblast proliferation and movement by altering the expression of genes associated with growth factors, which contributes to the production of collagen, which serves as a structural support for wound healing [86, 87, 88, 89, 90]. Exosomes produced from BMSCs were used by some researchers to treat fibroblasts taken from chronic diabetic ulcer ulcers. The outcomes demonstrated that exosomes might, in a dose-dependent way, encourage fibroblast proliferation and migration. Exosomes generated from human fibrocytes include miRNAs and proteins with a variety of biological functions, and these exosomes sped up wound healing by encouraging epidermal cell proliferation and migration in the diabetic rat model [77].

The study also discovered that skin cells (fibroblasts and keratinocytes) proliferate when exposed to exosomes made from hUC-dMSCs in a dose-dependent fashion. Exosomes were injected around deep II degree burn wound models in in vivo tests using local multiple point injection to assess the impact of exosomes. Exosomes from hUC-dMSCs were found to hasten wound healing, encourage re-epithelialization, and enhance the synthesis of collagen I, cytokeratin-19 (CK19) and proliferating cell nuclear antigen (PCNA) [91, 92]. Additionally, transferring hAEC-exosomes generated from human amniotic epithelial cells to wound sites sped up the healing process [93].

Along with contributing to the cellular impacts listed above, MSC-exosomes have been demonstrated to control ECM re-synthesis throughout the remodelling phase. Exosomes made from human induced pluripotent stem cells (hiPSCs) that had matured into dermal MSCs (dMSCs) are said to be able to induce the production of type I collagen and elastin as well as increase the expression levels of type I collagen, type III collagen, and elastin mRNAs [87, 94]. Our results demonstrate that MSC-exosomes can encourage ECM renewal and speed up wound healing. Exosomes derived from ADSCs (also known as ADSC-exosomes) have the ability to control collagen synthesis at various stages of wound healing, speed up wound healing by increasing the production of type I and type III collagen in the early stages, and impede collagen synthesis in the late stages to prevent the formation of scars [95]. Exosomes from hUC-dMSCs did decrease the development of scars in a mouse model with skin defects by preventing the differentiation of fibroblasts into myofibroblasts [96]. Moreover, hAEC-exosomes were found to have positive benefits in a rat model of scarless wound healing, according to the research. By promoting MMP-1 expression, high concentrations of hAEC-exosomes somewhat decreased ECM deposition [93]. Moreover, according to Wang et al hypothesis , ADSCs-exosomes can prevent the production of type III/type I collagen by directly interacting with fibroblasts [97]. Exosomes were drawn to the skin wounds in a study using traceable ADSCs-exosomes to mend mice's skin flaws in order to carry out their activities and speed up wound healing. According to histological study, exosomes can stimulate collagen production

during the early stages of wound healing and suppress it during the late stages to prevent the development of scar tissue [86]. All of these studies show that MSC-exosomes are essential for the remodelling of the ECM, which may also be the process minimising scar formation.

Advantages of MSC-exosomes

MSC-exosomes, a cell-free alternative therapy, have several benefits, including being simple to manufacture, store, and transport, simple to dose, and simple to deliver at a convenient time. Moreover, they appear to be very therapeutically effective and to pose little threat of immunological rejection or cancer. As a result, MSC-exosomes have a remarkable potential for cutaneous regeneration and might successfully replace therapies based on complete MSCs.

Challenges with the MSC-exosomes as a Wound Therapy

More research is needed to figure out the precise contents and activities of MSC-exosomes to completely understand the molecular processes by which they induce cutaneous regeneration. The majority of the processes mentioned thus far have been examined in rats, however human physiology cannot always be extrapolated from animal physiology. Hence, more clinical studies utilising exosomes of human origin are required to conclusively establish the therapeutic effects of MSCs for skin regeneration in a broad patient population. Yet, there are still significant scientific problems that must be overcome first. The morphological and biological properties of MSCs produced from various tissues exhibit a high degree of consistency, despite the fact that they do not all have the same differentiation potency [98, 99]. MSCs are suitable to create exosomes, hence this may not be viewed as a challenge. Nevertheless, the biological consequences of MSC transplantation vary depending on the illness or the stage of wound healing. In actuality, the microenvironment of the transplanted site has a significant impact on the destiny and paracrine actions of MSCs [100, 101]. In some studies involving tumours, researchers discovered that MSCs can down-regulate the expression of VEGF and impede the angiogenesis, continuing to support the growth of tumours [103, 104, 105]. For example, studies have shown that transplantation of MSCs into organs and tissues can up-regulate the expression of VEGF and accelerate the regeneration and healing of injured tissues [102]. Our research also supports the idea that MSCs derived from various sources can hasten the establishment of granulation tissue and speed up the process of wound re-epithelialization by up-regulating the expression of transforming growth factor (TGF) - 1, collagen I, and angiogenesis factors in fibroblasts and wound tissues. However, in in vivo and in vitro studies using MSCs after wound re-epithelialization, the paracrine effect altered the biological functions (e.g., down-regulating the expression of fibrosis-related factors like TGF-1, -smooth muscle actin (-SMA), and collagen I, and up-regulating the expression of anti-fibrosis factors like TGF-3 and Decorin to prevent the formation of the scar).

Recent studies have also demonstrated that interferon (IFN) stimulation of hUC-dMSCs in a model inflammatory milieu may greatly boost the release of MSC-exosomes. IFN- may strengthen hUC-dMSCs' ability to regulate the immune system by functioning as a license factor and boosting the fraction of CD4+, CD25+, and Foxp3+ T cells in all Regulatory T cells (Tregs) [106, 107]. hUC-dMSCs that have been pretreated with lipopolysaccharide (LPS) emit more proteins than untreated cells do, and the exosomes produced by these cells are abundant in the miRNA let-7b, which can cause macrophages to adopt the anti-inflammatory M2 phenotype. Also, the study demonstrated that the components of MSCs' paracrine factors in vitro culture were dramatically altered by a 1-2% hypoxic environment, medium containing TNF, and a three-dimensional culture method [108]. By altering the cell culture conditions, or more specifically, the microenvironment in

which the cells exist, it is possible to control the release and constituents of MSC-exosomes.

Nevertheless, the MSC-exosomes that have been isolated in vitro from the usual culture media constitute the basis for all of the current investigations on the processes behind MSC-exosome activity. The crucial scientific claim that the paracrine action of MSCs is intimately correlated with their microenvironment is definitely refuted by this. To put it another way, MSC-exosomes released in various microenvironments have various components that have various biological consequences. Almost all scientists that examine MSCs-EVs or MSC-exosomes have disregarded this crucial scientific problem.

Table 1: Advantages and challenges of using MSC secretome and MSC exosomes for wound healing

	MSC Secretome	**MSC-exosomes**
Advantages	quicker wound healing increased re-epithelialization rates superior vascularity, pigmentation, wound height, and scar scores simplicity of mass manufacturing, packing, and transportation	simple to manufacture, store, and transport, simple to dose simple to deliver at a convenient time therapeutically effective pose little threat of immunological rejection or cancer
Challenges	high cost need for certain storage conditions possibility for tumorigenicity, infection, and rejection difficulty in utilizing them in the general population difficult to determine biochemical make-up may contain lipids, long non-coding RNAs, and miRNAs, which control many signaling pathways involved in inflammation Efficacy is impacted by donor fitness and age, as well as segregation and growth techniques inconsistent secretome harvesting in terms of MSC heterogeneity, donor differences, cell quantity, and time interval hazards associated with employing extrinsic biological molecules may weaken the immune system, increasing the risk of infection, immunosuppression, and tumor progression in treated individuals unstable nature of proteins and their brief half-lives	therapeutic effects in human subjects is not fully established Efficacy depends on the microenvironment

Conclusion

The potential future use of the secretome and exosomes instead of MSCs, would provide a non-cell-based therapeutic strategy for the management of various wounds that would avoid the adverse effects of cell based therapy.

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