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Cellular Therapy for Wounds: Applications of Mesenchymal Stem Cells in Wound Healing

Moyassar B. H. Al-Shaibani, Xiao-nong Wang,

Penny E. Lovat and Anne M. Dickinson

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Abstract

Despite progress in wound treatment including gene therapy, biological dresses and engineered skin equivalents, present treatment options for chronic wounds are restricted and not always effective. For example, inability to get consistent product from the introduced gene, biological covers may give rise to hypoxic conditions and engineered skin models are limited by their construction from substances which are hard to be degraded, and do not always result in complete replication into normal uninjured skin. A growing body of evidence suggests mesenchymal stem cells (MSCs), and their secreted growth factors and microvesicles, may potentiate the wound-healing process and as such their addition to novel wound-healing treatments may improve the efficacy of current therapeutic strategies. Recent studies report the ability of bone marrow-derived MSCs (BM-MSCs) to migrate and differentiate into skin cells *in vivo*.

Therefore, this chapter aims to review the important concepts of wound healing at the cellular and kinetic level and the potential therapeutic strategies of MSCs through which to improve their treatment. Focus to this chapter will begin with a description of wound types, phases of healing process, followed by an outline of the role of MSC secretions in each phase, leading to potential novel treatment strategies and the requirements for a successful healing process.

Keywords: cellular therapy, conditioned medium, growth factors, mesenchymal stem cells, skin regeneration

1. Introduction

In the United Kingdom, around 200,000 patients experience a chronic wound of varying type, ranging between ulcerations, scars, trauma and burns. Unfortunately, patient morbidity and in some cases mortality may result from such injuries for which chronic ulceration is a major factor [1–3]. One of these impacts is the reduced contribution to society by the individuals suffering from these chronic wounds including their inability to work [3]. In addition, healthcare treatment and hospitalisation for chronic wounds are costly [2] involving lengthy treatment and nursing care. In 2005 and 2006, the care of patients with a chronic wound costs the UK NHS approximately £2.3bn–£3.1bn each year, with £6.08 million in England lone being attributed to nursing care [4]. In the United States, approximately 6.5 million patients suffered from chronic wounds with expenses for wound care management exceeding US $25 billion in 2009 [5]. Furthermore, infection is inevitable, which not only negatively affect wound healing but can also be life threatening, requiring more hospitalisation and increased healthcare expenditure [6] and repetitive treatment [7]. Consequently, both the society and the health sector are negatively affected by the burden of chronic wounds. Moreover, despite great progress in wound treatment including the implementation of growth factors and biological engineering of skin equivalents, present treatment options for burns and non-healing chronic wounds are restricted and not always effective [8]. Engineered skin to aid the development of novel wound care strategies is limited by their construction from substances that are hard to be degraded, and do not always result in complete replication into normal uninjured skin. Furthermore, complete renewal of this model requires the alteration of immune responses to reduce fibrotic reactions in order to diminish scar production [9]. Gene therapy may also be limited by insufficient selection of target cells, the identification of the factors which may affect the introduction of genes or the inability to produce stable prolonged specific gene product which is the main problem with systemic gene therapy [10]. Additionally, biofilms give rise to hypoxic conditions. Therefore, there is an urgent need for new therapies for wounds with delayed healing [8]. Specific extracellular matrix (ECM) proteins equivalent to the skin, specific growth factors, mesenchymal stem cells (MSCs), fibroblasts or viable epithelial cells may, however, aid the wound-healing process, and their addition to potential wound-healing treatments may improve the efficacy of current therapeutic strategies [11]. The availability of MSCs in normal human skin [12], and their vital function in wound healing suggests that the exogenous application of such cells may represent a promising solution for the treatment of non-healing wounds [13].

Mesenchymal stem cells (MSCs) are generally defined as self-renewable, multi-potent pro-genitor adult stem cells present in peripheral blood. *In vivo*, they have the ability to differentiate widely into many mesenchymal lineages such as cartilage, bone, muscle and adipose tissues [14]. Furthermore, MSCs have the ability to migrate from the bone marrow to an injured site and differentiate into functional skin cells [15]. *In vitro* they can be defined as fibroblast-like cells capable of self-renewal with the ability to adhere to plastic and subsequently differentiate into adipose, bone, cartilage tissue [16] as well as a multi-layered epidermis-like structure [17]. Paracrine factors secreted by MSCs are considered the principle factors with therapeutic potential for tissue wound healing [18] including growth factors, cytokines and chemokines

which promote angiogenesis and wound repair [19–22]. Moreover, MSCs produce soluble factors that regulate cellular responses, angiogenesis formation and tissue remodelling [23] and play a vital role in each of the five phases of wound-healing process including haemostasis, inflammation, proliferation, contraction and remodelling [11]. In addition, MSCs exert antimicrobial activity via the secretion of the antimicrobial peptide LL-37 thereby preventing wound infection [24]. Furthermore, Tamama and Kerpedjieva [25] report that conditioned medium derived from the cell culture of MSCs (MSC-CM) contains all the effector molecules secreted by MSCs which could be effectively utilised in tissue regeneration and wound healing. Collectively these data thus suggest that MSC-CM may represent a novel therapeutic strategy for wound therapy, but the mechanisms mediating these events and exactly how MSCs contribute in skin regeneration remain undefined.

2. Skin wounds and healing process

Generally, wounds are classified on the basis of location, depth and tissue loss into three categories: superficial wounds where damage affects the epidermis only; partial thickness wounds when both the epidermis and dermis are involved; and full thickness wounds which involve the dermis, subcutaneous fats and sometimes, bones. However, depending on normal healing trajectory, there are two principal categories of skin wounds: acute and chronic wounds [26, 27].

2.1. Acute wounds

Acute wounds arise either as a result of surgical incision or following traumatic accidents including abrasions, superficial burns and partial thickness injuries with significant loss of tissues. Irrespective to their causes, the healing process of acute wounds is complex and utilises different types of cells and cytokines [26].

2.2. Chronic skin wounds

Wounds are defined as chronic when they fail to heal during one or all of the phases of the healing process causing an injury that cannot be repaired within the expected time period of normal wound repairs [11]. Chronic wounds mainly accompany disorders such as pressure ulcers, diabetes, burns, vascular insufficiency and vasculitis [5]. The chronic state of non-healing wounds is exacerbated by many factors including tissue hypoxia, microbial infection, necrosis, exudates and an elevated ratio of inflammatory cytokines during the different healing stages [28]. Neutrophils also contribute by releasing excessive amounts of collagenase which leads to break down the ECM [29] and enzyme elastase destroying important healing factors such as PDGF and transforming growth factor-beta (TGF-β). Chronic wounds do not respond to therapeutic methods unless the prolonged inflammation is targeted [11]. Consequently, human skin with its limited abilities will fail to heal itself in cases of wounds penetrating the epidermis [30] due to the deficiency in growth factors and cytokines which are depleted during the healing process [31, 32]

2.3. Phases of wound-healing process

Each wound undergoes a series of successive events for repairing and healing. These processes take from several minutes such as coagulation, several days such as inflammation to several months or years such as remodelling and can be divided into three, four or five overlapping phases and stages. Monaco and Lawrence [26] state the wound-healing process consists of five distinct phases: (a) haemostasis, (b) inflammation, (c) cellular migration and proliferation, (d) protein synthesis and wound contraction and (e) remodelling, while Gosain and DiPietro [33] and Zhou et al. [34] describe the healing process as consisting of four highly integrated and overlapping phases: (a) haemostasis, (b) inflammation, (c) proliferation and (d) tissue remodelling or resolution [7]. A normal wound-healing mechanism is a dynamic and complex process involving a series of coordinated events, including (a) bleeding and coagulation, (b) acute inflammation, (c) cell migration, (d) proliferation, (e) differentiation, (f) angiogenesis, re-epithelialisation and (g) synthesis and remodelling of ECM. Conversely, Maxson et al. [11] report that the healing process is a complex event occurring in three overlapping phases: (a) inflammatory, (b) proliferative and (c) remodelling. These phases and their biophysiological functions must occur in the proper sequence, at a specific time, and continue for a specific duration and intensity [35]. There are many factors that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair [28]. All in all, a successful healing process cannot be accomplished without any one of these processes; haemostasis, inflammation, angiogenesis, proliferation, contraction, re-epithelialisation and remodelling [36]. To better understand the healing process, we will discuss the five phases and how they overlap.

2.3.1. Haemostasis phase (coagulation)

During blood circulation in an intact blood vessel, endothelial cells of the blood vessel secrete coagulation and aggregation inhibitors, that is they release heparin-like molecules to prevent blood coagulation and thrombomodulin to prevent platelet aggregation. Prostacyclin and nitric oxide are also involved in this process [37]. In contrast, the endothelial cells of broken blood vessels replace the secretions of clot inhibitors with a blood glycoprotein called von Willebrand factor (vWF) which initiates haemostasis [37, 38].

Haemostasis is the first phase of wound healing and consists of three successive steps: vasoconstriction, blockage the wound by platelet aggregation and blood coagulation. When skin is injured, a blood extravasation begins to fill the injured site. Immediately after the skin injury and bleeding, the blood vessel contracts and reduces the blood flow to the wounded site thereby keeping the blood within the damaged vessel and causing bleeding to stop [38, 39]. Not only do vessel contractions stop haemorrhage, but also blood changing from a liquid phase to a gel phase forming a blood clot (coagulation) and platelet aggregation generates a haemostatic buffer (plasma) which is rich in fibrin, thereby stopping the haemorrhage and restoring a barrier protecting the wound from infection by invading microorganisms. This process constitutes a matrix what encourages cell migration [40, 41]. In this phase, the role of platelets is not only restricted to blocking the damaged area and in clot formation, but also in the formation of a transient extracellular matrix by secreting adhesion molecules such as

fibronectin and thrombospondin, as well as growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor-alpha (TGF-α) and beta (TGF-β), and vascular endothelial growth factor (VEGF) [42]. This matrix serves as a reservoir for growth factors and cytokines critical to subsequent healing phases [41]. Collectively, the matrix, activated cascade coagulation and parenchymatous cells make the injured vessel a chemotactic environment to attract inflammatory cells at the wound site and initiate the start of inflammatory phase [43].

2.3.2. Inflammation phase

An inflammatory reaction begins soon after the haemorrhage stops at the site of injury. This reaction promotes mobility of various cells toward the injured tissue giving rise to a multitude of complicated and successive series of reactions ending with rebuilding of a tissue-like structure [44]. The main advantages of this phase are isolating the injured tissues from the surrounding contaminated environment, cleaning out cell debris and damaged tissues and the initiation of the healing process [45]. The main reactivity observed in this phase is an increased migration of inflammatory cells from intravascular tissue towards the extracellular wound site due to increased vascular permeability. This permeability increases due to vasodilation when both fibrin and thrombin are activated by the coagulation cascade. Meanwhile, clot formation and their stimuli are dissipated and plasminogen converted to plasmin [46]. Three main cell types are involved in the inflammatory phase: neutrophils, macrophages and lymphocytes whose activity is initiated within hours of injury [44, 45]. Neutrophils seem to be the most dominant cell type during the first 48 hours, cleaning the wound site from bacteria, cell debris and damaged tissue by releasing free radicals; however, they are not essential for the healing process [40, 41, 47]. Approximately 48 hours following the injury, stimuli for neutrophils no longer persist and neutrophil numbers cease when macrophages (monocyte-derived macrophage) then penetrate the wound site via the blood and become the dominant cellular component of the inflammatory phase by phagocytosing cell debris and bacteria including expended neutrophils. Macrophages also secrete collagenases and elastases to break down the damaged tissues [48]. In contrast to neutrophils, the role of macrophages is not restricted to cleaning of the tissues as they also play a crucial role in the healing process by secreting prostaglandins, which act as vasodilators increasing microvessel permeability and attracting other inflammatory cells into the wounded site [41, 49, 50]. In addition, macrophages secrete fibroblast growth factor (FGF), PDGF, TGF-α and VEGF which are important for proliferation and migration of fibroblasts as well as cytokines, which attract endothelial cells to the injury site promoting their proliferation and the development of a new tissue [48, 49, 51]. Within three days of the inflammatory phase, T lymphocytes home to the injury site by the activity of interlukin-1 and secret lymphokines such heparin-binding epidermal growth factor (HB-EGF) and basic fibroblast growth factor (bFGF), promoting fibroblast proliferation [52].

2.3.3. Proliferation phase (epithelialisation)

The proliferation phase (epithelial proliferation phase) represents the main phase responsible for actual wound closure. In the case of skin wounds, endothelial non-inflammatory cells such

as keratinocytes and fibroblasts start to proliferate and migrate towards the edges of the wound-producing collagen for the development of new tissues [53–55]. Within a few hours (between 6 and 24 hours) of injury, TGF-β and EGF act as mitogenic and chemotactic stimulators attracting keratinocytes which migrate towards the wound and start epithelialisation [54]. Fibroblasts are activated and start to differentiate into myofibroblasts which participate in reducing the wound size by contracting and secreting extracellular matrix (ECM) proteins giving rise to healing of the connective tissue [56, 57]. Meanwhile, angiogenesis progresses, coordinating the transfer of nutrients and oxygen from newly formed capillaries to the wound site enhancing metabolic activity [58]. Epithelisation, fibroplasia and angiogenesis collectively comprise granulation tissue which covers the damaged tissues within four days of injury [55].

2.3.4. Contraction phase

Wound contraction could be defined as mobility of wound margins towards the wound core to facilitate closure; this phase begins when fibroblasts stop proliferating and undergo apoptosis within 5–15 days post-injury which occurs concurrently with collagen synthesis [59, 60]. The rate of movement of wound edges depends on tissue laxity and wound shape; for instance, the looser tissues tend to contract more rapidly than the compact tissues and squared wounds contract more quickly than rounded wounds. The contraction rate also depends on the availability of myofibroblast and their proliferation and connection to the surrounding extracellular matrix [61].

2.3.5. Remodelling phase (resolution)

Remodelling or resolution is the last phase of the wound-healing process. The biological processes observed in this step involve gradual resolution of the inflammatory phase, collagen deposition, complete coverage of the injured site by the new tissues and formation of scar tissue [62]. Successful remodelling requires stable collagen content; therefore, the important step in this phase is controlling collagen remodelling [34]. Although collagen synthesis is continuing during this phase, its level is restricted due to the activity of collagenases and metalloproteinases which aid in removing the excess collagen [63, 64]. For optimal remodelling, collagen levels need to be balanced by the activity of metalloproteinases inhibitors secreted by tissue arresting the collagenolytic enzymes and balancing the production of new collagen with that of the removed old collagen [64]. The outcome of this process is that collagen type III is replaced by collagen type I, hence replacing both hyaluronic acid and glycosaminoglycans by proteoglycans and the disappearance of fibronectin as well as resorbing water from scar tissues. These events start approximately 3 weeks after the injury and may last indefinitely as collagen fibres stack closer to each other decreasing scar thickness and increasing wound bursting strength 'resistance to rupture' [65].

As described above, the main issues in the wound-healing process are how cells are attracted to the site of injury site and how to enhance their proliferation and differentiate at the wounded region. These cells include inflammatory cells (neutrophils, macrophages and lymphocytes) and epithelial cells (fibroblasts and keratinocytes). All these activities are mainly regulated by growth factors and cytokines. In many cases, these cells fail to migrate, proliferate and

differentiate due to deficiency in growth factors and cytokines; consequently, the healing process will be impaired and chronic wounds will arise [54]. Therefore, in order to improve wound healing, there is a need for an alternative source of healing cytokines and growth factors to enrich the injury site. MSC-CM acts as a rich source of 36 growth factors, cytokines and chemokines which collected from MSC *in vitro* under good manufacturing practice could be used as therapy for wounds in the future [66]. The main events and phases of the wound-healing process are summarised in **Table 1**.

Phase	Haemostasis	Inflammation	Proliferation migration	Contraction	Remodelling
Starts post-injury	Immediately	First hours	Day 4	Day 5	Day 20
Duration	(minutes–hour)	(3 days to 14 days)	(21 days)	(10 days to 20 days)	(Months to 2 years)
Events	Haemorrhage	Phagocytosis	Endothelial cells migration	Fibroblast apoptosis	Collagen control remodelling
	Vasoconstriction	Growth factors secretion	Epithelialisation	Wound edges pull	Replacing collagen type III by type I
	Platelet aggregation	Cytokines secretion	Fibroblast differentiate into myofibroblasts	Wound closure	Disappearance of fibronectin
	Blood coagulation	Synthesis of preliminary ECM	ECM production	Scar maturation	
		Immunomodulation by T lymphocytes	Angiogenesis	Collagen fibre cross-linking	
		Migration of inflammatory cells	Collagen production		
		Activation of coagulation cascade	Granulation		
		Fibroblast migration			
		Fibroplasia			
Healing progress	(a) Wound initiated	(b) Healing not initiated	(c) Progressive healing	(d) Healed	(e) Healing complete

This table shows the main phases and events of the wound-healing process which are divided into five overlapping phases. For example, the phase (a) indicates that the wound is not healed and there is a possibility to reach a chronic state if the coagulation phase failed. The phase (b) indicates that the wound is still not healed, but it is progressing towards healing; however, if the inflammation is not terminated, a chronic condition has a chance to be initiated. The phase (c) indicates that the active healing process has been initiated. The phase (d) represents further development of the healing process with less chance of progression to a chronic condition. The phase (e) represents the complete healing and remodelling.

Table 1. The main phases and events of the wound-healing process.

3. Utilisation of mesenchymal stem cells in wound healing

3.1. Definition of mesenchymal stem cells

MSCs can be defined as a heterogeneous population of cells which are non-hematopoietic stem cells with the potential capacity to differentiate into various somatic lineages and tissues of both mesenchymal and non-mesenchymal origin [67]. Song et al. [68] have defined MSCs as a type of stem cell population capable of self-renewing and differentiating into different cell types with pluripotent potential. On the other hand, MSCs have also been termed marrow stromal cells, or fibroblastoid colony-forming unit (FCFU) [69], mesenchymal stem cells, multipotent mesenchymal stromal cells or stromal progenitor cells [23]. MSCs can be isolated from different tissues; however, they share the major criteria defining MSCs with minor differences related to their differentiation capacity and cell surface expression profile [67, 70]. These differences have challenged the definition of MSCs. In 2006, the International Society for Cellular Therapy (ISCT) attempted to demystify the nomenclature of MSCs suggesting that the term mesenchymal stem cells should only be referred to the cells which are characterised by the specific criteria, while the nomenclature of multipotent mesenchymal stromal cells should be used to describe the fibroblast-like plastic-adherent population irrespective of their source of origin [16, 23, 71]. Three minimal criteria have been agreed to become consensus characteristics shared by human MSCs [16, 23, 72, 73]. These criteria are:

1. The isolated MSCs should possess plastic adherence ability.

2. More than 95 % of the isolated MSCs must express CD73 (SH3), CD90 and CD105 (HS2), and more than 98 % of the isolated MSCs do not express CD14, D19, CD34, CD45, CD11b, CD79a and HLA-DR surface molecules.

3. The isolated MSCs have the capacity to differentiate into osteoblastic, chondrogenic and adipogenic lineages under *in vitro* standard differentiation conditions.

3.2. Clinical applications of mesenchymal stem cells

MSCs have been considered as safe irrespective to therapeutics since there is no critical inverse or side effect of MSCs has been detected on disease conditions [74, 75]. The characteristics of MSCs make them good candidates for regenerative medicine and tissue engineering [8, 25]. The most popular application of MSCs in regenerative medicine is in wound healing and skin regeneration [76]. However, MSCs have other clinical applications including ameliorating tissue damage in nearly all the major organs in the body such as skin regeneration, cardiac therapy, hepatic cirrhosis [23, 77, 78], brain, lung and kidney repair [23, 77]. In addition, MSCs can be used for pancreatic regeneration, neurological defects, limb ischemia, graft-versus-host disease (GvHD), rheumatoid arthritis, osteoarthritis (OA) and other bone and cartilage disorders [78]. The availability of MSCs in normal human skin suggests that these cells potentiate vital functions in wound healing and could be a promising solution for the treatment of chronic wounds [8, 25].

3.3. Mesenchymal stem cells in skin regeneration

Wounding in mammals evokes two types of biological responses: tissue regeneration and wound repair. Recently, skin regeneration especially cutaneous regeneration via MSCs leads to accelerated wound closure, re-epithelialisation and angiogenesis [7]. BM-MSCs transplanted into the injury site expressing keratinocyte-specific protein (KSP) form glandular structures [79, 80]. One of these successful studies is the induction of BM-MSCs to acquire phenotypic characteristics of sweat gland cells (SGCs) *in vitro* followed by re-transplantation of these cells into fresh wounds in five patients and resulted in recovery of functional sweat gland participating in perspiration function during 2 to 12 months follow-up [81]. Another study focusing on chronic diabetic foot ulcers showed that injection of a biografts consisting of a combination of MSCs and autologous skin fibroblasts resulted in increase of both dermal thickness and vascularity and decreased wound size [82, 83]. Another study has showed that MSCs acquire phenotypic characteristics of epidermal cells or vascular endothelial cells after *in vitro* culture in media supplemented with EGF or VEGF, respectively [82]. MSCs also undergo trans-differentiation into keratinocytes enabling them to interact with the original epidermal cells suggesting that MSCs can participate directly in tissue regeneration of both dermal and epidermal cells [30]. These characteristics, collectively, reveal the plasticity of MSCs and suggesting them promising therapeutic for the regeneration of skin and consequently wound healing [81].

3.4. Modes of action of mesenchymal stem cells in wound healing

The wound-healing process requires interaction between cells, extracellular matrix proteins (EMP) and biomolecules such as growth factors, cytokines and chemokines in which MSCs are a pivotal player in the coordination of the repair processes [11, 84]. Differentiation and
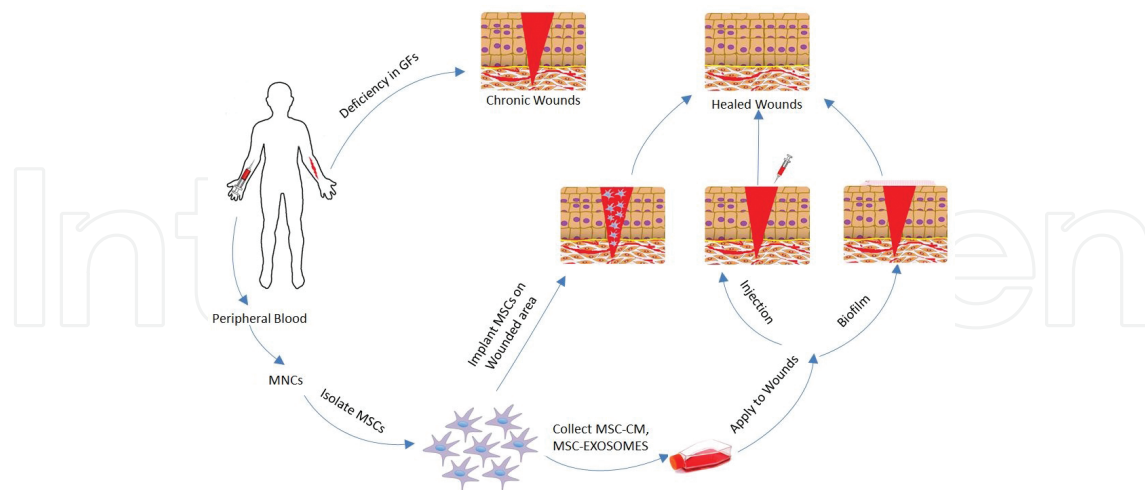

Figure 1. Potential applications of MSCs in wound healing. MSC therapy contributes to skin wound healing via two mechanisms: (1) Differentiation into skin-like cells, thereby compensating for the loss cells due to damaged tissue. (2) Promote proliferation and migration of skin cells into the injury site by secreting soluble factors and macrovesicles. MSC secretions represented by MSC-CM and MSC-EXOSOME can be either injected onto the wounded skin area or applied on skin wound using biofilm dressings.

paracrine signalling have both been implicated as mechanisms by which MSCs recruit other host cells in all steps of healing process to improve tissue repair [23]. To better understand the role of MSCs in wound healing, we have divided their participation in repair into two major mechanisms: (1) cell-mediated repair and (2) secretory-mediated repair (**Figure 1**).

3.4.1. Cell-mediated repair

In vitro studies have shown that MSCs possess phenotypic properties resembling native dermal fibroblasts or myoblasts [85]. Furthermore, BM-MSCs may accelerate wound closure by differentiating into epidermal keratinocytes and other skin cells [15, 23, 79, 86, 87]. Recent studies have shown that MSCs undergo trans-differentiation into keratinocyte, epidermal cells and microvascular endothelial cells when cultured under defined culture conditions [30] and express keratinocyte-specific protein (KSP) [79, 88, 89]. MSCs therefore could be utilised for wound healing by transplanting aggregated MSCs into the injured tissue to increase collagen deposition and improve epithelisation [80]. They can also differentiate into other skin cells such as endothelial cells, keratin 14-positive cells and pericytes, when localised to blood vessels and dermis [86], sebaceous glands and hair follicles [23].

3.4.2. Secretory-mediated repair–role of mesenchymal stem cell-conditioned media (MSC-CM)

Paracrine signalling of BM-MSCs is the major mechanism by which these cells contribute to wound repair, in which their secretory products impact on inflammation, fibrotic proliferation and angiogenesis [18]. Many studies have reported that MSC-CM is the supernatant from MSC *in vitro culture* significantly promotes wound healing by affecting the pivotal steps of the repair process. The components of MSC-CM have accelerated epithelialisation and via chemotaxis recruit endothelial cells and macrophages to the injured site *in vivo* [21]. The MSC-CM recruits both epidermal keratinocytes and dermal fibroblasts to the wound site *in vitro* [21, 23]. As well as to its activity as chemoattractant, MSC paracrine secretions serve as regulators for cell migration in response to wounding leading to faster wound closure by regulating dermal fibroblast migration [90]. MSC secretory mitogenic molecules stimulate the proliferation of keratinocytes, dermal fibroblasts and endothelial cells [91]. Conditioned medium derived from the cell culture of MSCs (MSC-CM) contains all the effector biomolecules which could be effectively utilised in tissue regeneration and wound healing by promoting migration, proliferation and differentiation of human skin cells such as fibroblast and keratinocytes. Collectively, these data suggest MSC-CM may represent a novel therapeutic strategy for wound therapy [25].

3.5. Biologically active substances secreted by mesenchymal stem cells

The potential of MSCs in regenerative medicine and wound healing has been reflected by their secretion of biomolecules including growth factors, cytokines and chemokines [18, 21, 25]. Some 36 biomolecules have been reported to be released by human MSCs (h-MSCs) which act in concert to promote the wound-healing process [66].

3.5.1. Growth factors

Human MSCs secrete a wide range of growth factors that play a significant role in the wound-healing process. These are angiopoietins (ANGPT), connective tissue growth factors (CTGFs), epidermal growth factor (EGF), fibroblast growth factors (FGFs), insulin-like growth factors (IGF), keratinocyte growth factor (KGF), nerve growth factor (NGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF) and scatter factors which are a family of growth factors also known as plasminogen-related growth factors (PRGFs), which include two members: hepatocyte growth factor (HGF) also referred to as plasminogen-related growth factor-1 (PRGF-1) and macrophage-stimulating protein (MSP) which is also known as scatter factor-2 (SF-2) or hepatocyte growth factor-like protein (HGFL) (**Table 2**).

Growth factors	Function(s)	References
ANGPT	ANGPT-1 is responsible for the stabilisation of blood vessels and promotes wound closure	[21, 22]
	ANGPT-2 causes vessel destabilisation and remodelling	[40]
CTGFs	Stimulation of chemotaxis, proliferation of fibroblasts and the induction of extracellular matrix proteins including fibronectin and collagen type I	[40]
	Promote endothelial angiogenesis, survival, migration, proliferation and adhesion	[92]
EGF	Re-epithelialisation of skin wounds and	[40]
	promotion of wound closure	[22]
FGFs	Exert a cytoprotective function in would repair, supporting cell survival under stress conditions	[93]
	Promotes mitogenic activity for keratinocytes and fibroblasts at the wound site. FGF1 and FGF2 stimulate angiogenesis. Basic fibroblast growth factor (bFGF) enhances the proliferation of endothelial cells and smooth muscle cells	[94]
IGF	In association with HB-EGF, IGF enhances the proliferation of keratinocyte *in vitro*. Mitogenesis and survival of many cells are stimulated by IGF-I and IGF-II, promotes wound closure	[40]
KGF	Promotes wound closure in two ways:	[21, 22]
	(1) It serves as a transporter for alveolar epithelial fluid	[94]
	(2) It plays a role in tissue remodelling	[11]
NGF	Involved in fibroblast migration, increasing expression of actin by smooth muscle and collagen gel contraction by these cells	[95]
	Performs two functions in wound healing: (1) it stimulates proliferation of keratinocytes and inhibits apoptosis *in vitro*, (2) it supports the proliferation of human dermal	[40]

Growth factors	Function(s)	References
	microvascular endothelial cells and their adherence molecule expression	
PDGF	Stimulates DNA synthesis, attracts fibroblasts to wound sites, enhances their production of collagenase, collagen and glycosaminoglycan	[96]
	The first chemotactic growth factor participating in migration of fibroblasts, monocytes and neutrophils into the skin wound, subsequently stimulating the production of extracellular matrix and the induction of a myofibroblast phenotype	[97]
HGF or PRGF-1	It inhibits fibrosis and promotes re-epithelialisation	[98]
	Enhances keratinocytes to migrate, proliferate and produce matrix metalloproteinase and stimulates new blood vessel formation	[99]
MSP	Accelerates cell migration and proliferation with regulation of proliferation and differentiation of keratinocytes and macrophages. plays an integral role in inflammation, proliferation and the remodelling phases of the healing process	[40, 100]
TGF	Enhances proliferation of epithelial cells, expression of antimicrobial peptides and release of chemotactic cytokines	[40]
	TGF-β1 activates keratinocytes and macrophages, while suppressing T lymphocytes	[94]
	TGF-β3 stimulates remodelling	[11]
	Activins which are members of TGF-β family act as enhancers for granulation tissue fibroblasts and the induction of extracellular matrix deposition	[40]
	Activin B supports wound repair and regeneration of hair follicles, promotes wound closure	[21, 22]
VEGF	Regulates angiogenesis	[20, 40]
	VEGF-α promotes wound closure	[21, 22]
	Promotes proliferation of endothelial cells	[11]

MSCs secrete a wide spectrum of growth factors. These biological substances participate in wound healing from early stages starting with haemostasis and coagulation and ending with remodelling. These growth factors promote angiogenesis, accelerate proliferation and also migration of endothelial cells. In addition, they are involved in the contraction phase, ending at the last stages of remodelling, leading to wound healing in the absence of scar formation.

Table 2. The main growth factors secreted by MSCs and their roles and functions in the wound-healing process.

3.5.2. Cytokines

Cytokines are small proteins secreted by many cell types which affect the activity of other cells including immune cells; they include interleukins, lymphokines and other signalling biomolecules including interferons and tumour necrosis factor [40]. Here, they are categorised into groups depending on their role in the wound-healing process (**Table 3**).

Cytokines	Function(s)	References
Pro-inflammatory cytokines		
IL-1α	They influence the inflammatory phase	[101–103]
IL-1β	They promote wound healing by controlling the proliferation	[40]
IL-6	of fibroblast and keratinocyte and regulate the synthesis and breakdown of extracellular	
TNF-β	matrix proteins. They also control fibroblast chemotaxis and regulate the immune response	
Anti-inflammatory cytokines		
PGE2	They play a primary role in limitation and termination of inflammatory responses	[11, 40, 102–106]
IL-1		
IL-4		
LL-37	It acts as antimicrobial peptide and reduce inflammation	[24]
Proliferative cytokines		
IL-6	Plays an axial role in wound healing by regulating cellular responses	[8, 23]
	Promotes epithelial cell migration	[25]
	Promotes angiogenesis formation	[8, 25, 107]
	Regulates leukocyte recruitment and infiltration to the inflammatory sites and regulates collagen deposition	[107]
	Possess both pro-inflammatory and anti-inflammatory activities under different conditions of the wound-healing process	[25, 105]
IL-10	Regulates differentiation and/or growth of keratinocytes, endothelial and various immune cells	[108]
	Regulates the infiltration of macrophage-derived neutrophils into the wound site, promotes the expression of pro-inflammatory cytokines and reduces matrix deposition and thereby inhibiting scar formation	[40]
GM-CSF	Enhances wound healing either indirectly via stimulating secondary cytokines such as TGF-β1	[109]
	Directly via its mitogenic activity for keratinocytes, and the stimulation of endothelial cell proliferation and migration	[40]
	Regulating angiogenesis formation, cellular responses and tissue remodelling	[8]

MSCs secrete a wide range of cytokines. These secretions initiate and terminate the inflammatory phase and accelerate proliferation and also migration of endothelial cells. In addition, they are involved in the contraction phase, ending at the last stages of remodelling, leading to wound healing in the absence of scar formation.

Table 3. The main cytokines secreted by MSCs and their roles and functions in the wound-healing process.

3.5.3. Chemokines

Chemokines are a subtype of small cytokines responsible for stimulating chemotaxis and extravasation of leukocytes; hence, referred to as so, they are called chemotactic cytokines [40]. Human MSCs release several chemokines that participate in wound healing such as IL-8 and

its receptor (CXCL8), macrophage chemoattractant protein-1 (MCP-1) and its receptor (CCL2), macrophage inflammatory protein-1-alpha and macrophage inflammatory protein-1-beta (MIP-1α and MIP-1β) and stromal-derived factor 1 (SDF-1) (**Table 4**).

Chemokines	Function(s)	References
IL-8	Increases keratinocyte proliferation and stimulate re-epithelialisation in human skin grafts, both in *vitro* and in *vivo*. IL-8 and its receptor (CXCL8) act as a chemoattractant for neutrophils	[110]
	Enhances the migration of epithelial cells *in vitro*	[25, 111]
MCP-1	MCP-1 and its receptor (CCL2) are primarily involved in macrophage infiltration	[66, 105]
	Inflammation regulatory chemokines in the wound-healing process	[40]
MIP-1	MIP-1α and MIP-1β promote wound closure	[21, 22]
	MIP-1α and MIP-1β increase macrophage trafficking	[105]
SDF-1	Plays a role in regulating skin homeostasis and tissue remodelling	[40]
	Promotes wound closure	[21, 22]
	Induces cell migration	[74]

MSCs secrete some chemokines. These biological substances participate in wound healing from early stages starting with haemostasis and coagulation and ending with remodelling. They also participate in the inflammatory phase and accelerate proliferation and also migration of endothelial cells. In addition, they are involved in the contraction phase, ending at the last stages of remodelling, leading to wound healing in the absence of scar formation.

Table 4. The main chemokines secreted by MSCs and their roles and functions in the wound-healing process.

3.5.4. Mesenchymal stem cell exosomes (MSC-EXOSOME)

Exosomes are tiny vesicles (30–100 nm in diameter) present in blood and urine and perhaps all other biological fluids that may also be collected from *in vitro* cell culture [112, 113]. Originating from endosomal sections and released from the plasma membrane into the extracellular environment these vesicles participate in coagulation, intracellular communication and signalling and cytoplasmic cleaning [112–115]. It has been reported that MSC-EXOSOME repair renal injury indicating that MSC-EXOSOME as a potential mechanism that could be harnessed for wound healing [116, 117]. With respect to wound healing, MSC-EXOSOME plays an important role in collagen synthesis, the acceleration of cell migration and proliferation and in the formation of new and mature blood vessel [113]. Exosome healing action could be attributed to its ability to transfer RNA, miRNA and proteins such as Wint-4 into the injured tissues which participate in skin repair by promoting re-epithelialisation and cell proliferation as well as through the activation of β-catenin which plays a pivotal role in skin development and wound healing [116]. Additionally, MSC-EXOSOME has been shown to accelerate wound healing through mediating pathway signalling of some genes such as Akt, ERK and STAT3 as well as by enhancing the expression of important growth factors, Such as;

HGF, IGF-1, NGF and SDF-1 which collectively accelerate migration and proliferation of fibroblasts in normal and diabetic wounds [118]. Moreover, MSC-EXOSOME reduces levels of pro-apoptotic Bax thereby inhibiting apoptosis of skin cells such keratinocytes and fibroblasts [117, 119]. Collectively, these data suggest the MSC-EXOSOMES thus play a significant role in wound healing.

The application of MSC-CM or MSC-EXOSOME onto chronic wounds either by direct injection or by designing biological dressings enriched with MSC-CM collected from autologous MSCs may therefore provide a valuable therapeutic strategy.

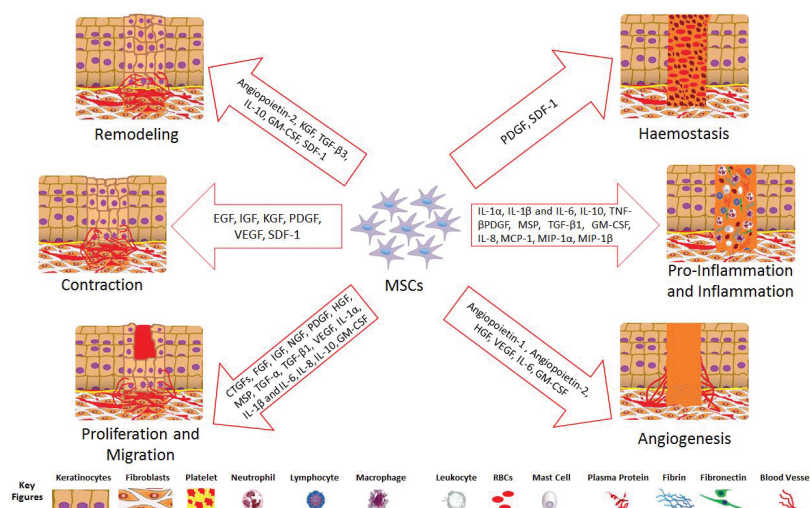The participation of MSC secretions in wound healing is summarised in **Figure 2**.

Figure 2. Participation of MSC secretions in wound-healing phases and events. MSCs secrete a wide spectrum of growth factors, cytokines and chemokines. These biological substances participate in wound healing from early stages starting with haemostasis and coagulation and ending with remodelling. These secretions initiate and terminate the inflammatory phase and promote angiogenesis, accelerate proliferation and also migration of endothelial cells. In addition, they are involved in the contraction phase, ending at the last stages of remodelling, leading to wound healing in the absence of scar formation.

3.6. Benefits of the use of mesenchymal stem cells in treating wounds

As we previously mentioned, MSCs have been considered safe irrespective their use in different clinical indications, since no critical adverse or side effect of MSCs has been shown when used therapeutically in humans [74, 75, 120]. Interestingly, patients with conditions such as sepsis [106], acute limb ischemia, acute GvHD [78, 120], critical myocardial infarction, Crohn's disease, acute tubular necrosis and multiple sclerosis can obtain benefit from the use of MSC therapies with no reported contraindications [120].

3.6.1. Immunomodulatory features of MSCs

In 2000, Liechty et al. [121] were the first to recognise that MSCs possess unique immunologic features allowing them to persist in a xenogeneic environment and modulate the immune

response. They have the potential to reduce inflammation and enhance repair wounds [122]. The exact mechanism by which MSCs modulate the immune system is not fully understood. The potential mechanism includes cell-to-cell direct contact and by the secretion of immune suppressive factors, interacting with other immune cells such as T lymphocytes, B lymphocytes, dendritic cells (DC) and natural killer cells (NKCs) [123]. In 2013, Patel et al. [74] reported that MSCs suppress both the activation and proliferation of lymphocytes in response to allogeneic antigens as well as enhancing the development of CD8+ regulatory T cells (Treg) in the suppression of an allogeneic lymphocyte response. Additional immune suppressive activities of MSC include inhibition of differentiation of peripheral blood monocyte progenitor cells and CD34+ haematopoietic progenitor cells (HPCs) into antigen-presenting cells (APCs) [124]. MSCs also inhibit the proliferation of NK cells mediated by IL-2 or IL-15 [125]. MSCs have been shown to exert other immunomodulatory activities including altering the proliferation and activation of B cells, IgG production, antibody secretion, chemoattractant behaviour, and reducing the expression of CD40, and CD86 and major histocompatibility complex class II (MHC-II) [126].

The ability of MSCs to modulate T cell and their proliferation, participation and activity [127] and suppress the proliferation of B cells [128] and NK cells [125] is well documented. By attenuating the function of these cells, MSCs reduce the pro-fibrotic process [129]. Importantly, by the secretion of Prostaglandin E2 (PGE2) [102, 103] and 1L-10 [11, 40, 106], MSCs regulate macrophage and lymphocyte function [30]. For instance, PGE2 attenuates mitogenesis and proliferation of T cells in the wound [124] acting as co-parameter in regulating the transition from T_{h1} cells into T_{h2} cells [130]. On the other hand, IL-10 prevents the deposition of excessive collagen and inhibits the invasion of neutrophils into the wound and their release of reactive oxygen species (ROS) factors, which collectively participate in the prevention of scar tissue formation [30].

3.6.2. Migration and engraftment capacity

Various studies have reported the capability of MSCs to selectively migrate to and engraft into the wound site and exert local functional effects on inflammatory reactions regardless of tissue type [30, 73]. In this context, murine studies have shown that MSCs can home to the lung, adopting phenotypic characteristics of epithelium-like cells and reducing inflammation in response to injury [131]. Another study in mdx mice, a strain of mice arising from a spontaneous mutation (mdx) in inbred C57BL mice showed that MSCs may migrate to muscle tissues [132]. MSC migration has been shown to be regulated by a multitude of signals [133] ranging from growth factors such as PDGF and IGF-1, cytokine such as SDF-1, chemokine such as CCL5 and chemokine receptors, including CCR2, CCR3 and CCR4 [73, 134].

3.6.3. Wound closure acceleration

MSCs play a role not only in wound healing but also in accelerating the healing process by increasing the strength of wound and by reducing scaring [23]. The effects of MSCs during wound healing include acceleration of epithelialisation, an increase in angiogenesis and the formation of granulation tissue [11]. These activities are attributed to the ability of MSCs to

produce biologically active substances capable of accelerating the regeneration process [135] including IL-8 and CXCL1, responsible for stimulating the migration of epithelial cells and accelerating wound closure [111].

3.6.4. Antimicrobial activity

Conditioned medium of hBM-MSCs is capable of inhibiting bacterial growth directly indicating the ability of MSCs to produce and release substantial amounts of antibacterial substances known as human cathelicidin peptide-18 (hCAP-18) or LL-37 peptide which are characterised by their ability to retard *in vitro* growth of *Pseudomonas aeruginosa* and *Escherichia coli* [24], thus avoiding wound contamination and infections complications which exacerbate the healing process [28].

3.6.5. Prevent chronic condition

Besides having immune modulatory activities, the effective biomolecules secreted by MSCs can prevent wounds from reaching a chronic state by their angiogenic and antiapoptotic characteristics [105]. For instance, transplantation of hMSCs intramyocardially has the ability to improve cardiac function via enhancing myogenesis and angiogenesis in the ischemic myocardium [136]. Also, MSCs enable a wound to progress to healing beyond the inflammation stage and not regress into a chronic state [11].

3.6.6. Attenuation of scar formation

The tissues in the scar have many disadvantages including their undesirable visual appearance and lack of structures that are present in the native skin such as hair follicles, sebaceous glands and sensory nerve receptors [30]. In addition, scar tissue weakens the skin making it more susceptible for re-injury [137]. MSCs have been shown to overcome these disadvantages via attenuating scar formation [138].

3.6.7. Neutralising the reactive oxygen species (ROS)

Although IL-10 participates in preventing the invasion of neutrophils into the site of tissue injury and the enhancement of collagen deposition, the penetrations of some populations result in the release of ROS, which are oxygen molecules have an unpaired electron making them extremely reactive such as superoxide, hydrogen peroxide and alkyl peroxides [139]. Many tissues are susceptible to attack by ROS contributing to dangerous diseases including heart disease and cancer. Also, prolonged persistence of ROS induces fibrogenesis and the accumulation of fibrotic tissues [30]. To counter act such effects, MSCs significantly upregulate the expression of nitric oxide synthase [140] which alters the ROS balance preventing the formation of fibrotic tissues [141].

3.6.8. Producing antifibrotic factors

MSCs release growth factors and cytokines characterised by their antifibrotic activities such as HGF and IL-10 [142]. HGF has been shown to downregulate the expression of collagen type

I and type III by fibroblasts therefore attenuating fibrosis and scar formation [143]. Moreover, HGF impacts on the keratinocyte behaviour by promoting their migration, proliferation and expression of VEGF-A, thereby generating a well-granulated tissue with a high degree of vascularisation and re-epithelialisation [30].

3.6.9. Enhancing dermal fibroblast function

In response to the wounding process, fibroblasts present at the injury site produce additional quantities of ECM to restore the integrity of the skin leading to scarred tissue [144]. Also, many endothelial cells undergo epithelial-to-mesenchymal transition (EMT) under the effect of TGF-β1 and become wound-healing myofibroblasts [138]. Both of these actions affect the function of dermal fibroblasts. Therefore, MSCs present in the wound site enhance dermal fibroblast function by producing HGF and PGE2 which both play a role in inhibiting EMT [145] and secrete biomolecules promoting the function of dermal fibroblast in wound healing [90]. MSCs therefore enable the cells present in the wound site to release ECM similar to those produced by neighbouring dermal cells [30].

3.6.10. Promoting angiogenesis and vascular stability

It has been well documented that BM-MSCs play a major role in angiogenesis and microvascularisation via promoting proliferation, migration and differentiation of microvascular endothelial cells by producing basic FGF and VEGF-A [146].

3.7. Requirements for the healing process

3.7.1. Infection fighting

Open wounds are at risk of infections by bacteria and other microorganisms causing serious conditions such tetanus and gangrene and giving rise to chronic wounds, bone necrosis, long-term disabilities and death. Unfortunately, in some wound types, the necrotic tissues exudate secretions which act as a medium for bacterial growth inside the wound and protect the bacteria from the host's immune defence [147]. The use of disinfectant is useless, because they damage the injured tissues and arrest wound contraction. Also, they are easily suppressed by the inorganic substances present in the wound including blood components and other tissue secretions [148]. Therefore, appropriate care is required to protect open wounds and reduce the possibility of bacterial infection. Included in such cares are the treatments of wounds with topical antibiotics to kill the invading bacteria and moisturise the wounded area [149, 150], accelerate the healing process [151] and modulate inflammation [152]. Failure of antibiotic treatment leads to non-healing wounds in which bacteria thrive on dead tissue giving rise to uncontrolled infection leading to additional complicated treatments including draining and removal of dead tissues from the injured site or even amputation in the case of diabetic ulcers [147, 150].

3.7.2. Prevention of ischemia and hypoxia

Ischemia is defined as the failure of blood to reach the target tissue and characterised by insufficient nutrient and oxygen supply resulting in turn in hypoxia and the insufficient availability or very low oxygen concentration at the injury site [153]. Therefore, the prevention of such circumstances is critical to the acceleration of wound-healing process [154]. Cold environment causes continuous constriction of blood vessels thereby reducing blood flow into the injury site and causing ischemia. Therefore, keeping tissue warm will dilate blood vessels facilitating blood flow and decrease the probability of ischemia initiation; however, hyperthermia is not recommended as this increases the likelihood of post-surgical infection [147]. In some cases of diabetic ulcers and venous ulcers, surgery is required to treat ischemia by revascularisation the veins and arteries to correct their function [155]. Another approaches to combat ischemia include pressure-assisted treatment or negative pressure wound therapy (NPWT), involving the creation of a vacuum to drain and remove wound exudate and their bacterial component, reducing tissue swelling, enhancing cell proliferation at the wounded site and the production of extracellular matrix thereby improving the healing process [156, 157]. Hypoxemia could arise due to vascular disease that arrests oxygen transfer, high demand for oxygen by tissue metabolism at the injured site and the formation of reactive oxygen species (ROS) [153]. The best therapy for hypoxemia is increasing the oxygenation of injured tissue by hyperbaric oxygen therapy (HBOT) to compensate oxygen limitations [155]. Also, higher oxygen content results in bacterial death, the acceleration of growth factor production, the enhancement of fibroblast growth and the promotion of angiogenesis [155, 157]. Another method to treat hypoxemia is the use of antioxidants to reduce the presence of oxidant substances [32].

MSCs could potentially be used to treat ischemia and hypoxia because they release angiogenic and mitogenic factors such as VEGF, IGF-1 and HGF which are well known to induce angiogenesis and myogenesis [158]. Intravenous (IV) administration of BM-MSCs increased the activity of matrix metalloproteinase-2 and decreased the activity of matrix metalloproteinase-9 resulting in improved cardiac function in a rat model of diabetic cardiomyopathy [159]. Wang et al. [160] showed that MSCs secrete hypoxia-regulated haem oxygenase-1, frizzled-related protein-2, hypoxic Akt-regulated stem cell factor, heat-shock protein-20, adrenomedullin (AM) and SDF which collectively contribute to regeneration, neovascularisation and remodelling. MSCs were also be used to treat diabetic limb ischemia in the ischemic hind limb of type II diabetic mice due to the secretion of proangiogenic factors including hypoxia-inducible factor and VEGF, responsible for vasculogenesis, blood flow regulation [161] and improvement of arterial perfusion in type 1 diabetic patients with gangrene [162]. A pilot study carried on patients of critical limb ischemia, who did not respond to other therapies, showed that multiple intramuscular injections of MSCs induced formation of vascular networks across the closed arteries, resulting in successful improvement of limb ischemia in terms of pain reduction and claudication [163]. In another study using a pig model of stenotic kidney blood flow, administration of MSCs improved the architecture of microvessels in term of size and density [164].

3.7.3. Regulation of growth factors and hormones

As previously described, growth mediatory factors play pivotal roles in wound healing. Therefore, more quantities of these growth factors are required to progress the healing process, in particular in case of chronic wounds, so they should be continuously upregulated [31, 32]. Several methodologies have been proposed to maintain efficient concentrations of growth factors at the injury site. Direct application of such biomolecules, however, requires large quantities and repetitive application. Spreading autologous platelets over the injury site which later secrete growth factors such as EGF, IGF-1, IGF-2, TGF-β and VEGF has been proposed [165] as has the utilisation of keratinocytes and fibroblasts on a collagen matrix to enhance further secretion of growth factors when applied on the wound [32, 166]. Another potential way to protect efficient concentrations of growth factors at the wound site is through the prevention of their breakdown by analytical enzymes and thereby preventing the formation of proteases such as elastase [31, 32]. Oestrogen and prostaglandins have also been showed to play a role in the healing process; maintenance of their concentration at efficient levels may thus prevent excess neutrophils from reaching the injury site and produce more elastase [41, 49, 50, 167].

3.8. Limitations of using MSCs for wound healing

MSCs can be considered as a promising tool for treating non-healing wounds; however, some aspects of MSC biology need to be intensively studied before use in clinical application. One of these is the problem of finding a source of MSC isolation with no invasive procedure for autologous treatment, for example isolation of MSCs from peripheral blood instead of bone marrow. Other questions include the following: What is the ideal number and timing of MSC administration/implantation? How long can MSCs survive at the injury site after implantation? Are multiple administrations or implantations required for successful healing? When do the implanted MSCs start releasing their soluble secretions after implantation/administration? And finally, are the secretions of MSCs controllable? Answers to these questions are important and essential for the therapeutic use of MSCs and a safer and a more effective treatment for non-healing wounds.

4. Conclusion

Mesenchymal stem cells (MSCs) and their secretions are promising therapeutics for use in accelerating wound healing. Ease of availability, isolation and *in vitro* expansion make MSCs the best candidates for wound-healing therapies in comparison with other stem cells including embryonic stem cells (ESCs). Two main strategies could be used in the application of MSCs to the treatment of non-healing wounds. MSCs show the ability to differentiate into different cells of the epidermis. Also, MSC secretions collected from their *in vitro* cultures (MSC-CM) and their small vesicles (MSC-EXOSOME) are important for promoting proliferation and migration of skin cells, such as keratinocytes and fibroblasts, into the injury site. MSC-CM contains a wide range of at least 36 known growth factors, and cytokines work in synergy to accelerate

wound healing. These characteristics make MSCs unique when compared to other cells, such as keratinocytes and fibroblasts, since keratinocytes are unable to migrate by themselves without growth factors and cytokines and fibroblasts cannot differentiate into skin-like cells. Therefore, MSCs are bi-functional combining wound healing and skin regeneration. MSC-CM and MSC-EXOSOME if developed into a medicinal product could potentially be applied directly onto the wounded area either by injection or by enriching biological dressings with these growth factors and exosomes and aid in combating the problems of non-healing wounds.

Author details

Moyassar B. H. Al-Shaibani[1,2*], Xiao-nong Wang[1], Penny E. Lovat[1] and Anne M. Dickinson[1]

*Address all correspondence to: m.b.h.al-shaibani@newcastle.ac.uk

1 Faculty of Medical Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

2 Department of Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

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