PERSPECTIVE ARTICLE

Role of adipose-derived stem cells in wound healing

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ABSTRACT

Impaired wound healing remains a challenge to date and causes debilitating effects with tremendous suffering. Recent advances in tissue engineering approaches in the area of cell therapy have provided promising treatment options to meet the challenges of impaired skin wound healing such as diabetic foot ulcers. Over the last few years, stem cell therapy has emerged as a novel therapeutic approach for various diseases including wound repair and tissue regeneration. Several different types of stem cells have been studied in both preclinical and clinical settings such as bone marrow-derived stem cells, adipose-derived stem cells (ASCs), circulating angiogenic cells (e.g., endothelial progenitor cells), human dermal fibroblasts, and keratinocytes for wound healing. Adipose tissue is an abundant source of mesenchymal stem cells, which have shown an improved outcome in wound healing studies. ASCs are pluripotent stem cells with the ability to differentiate into different lineages and to secrete paracrine factors initiating tissue regeneration process. The abundant supply of fat tissue, ease of isolation, extensive proliferative capacities ex vivo, and their ability to secrete pro-angiogenic growth factors make them an ideal cell type to use in therapies for the treatment of nonhealing wounds. In this review, we look at the pathogenesis of chronic wounds, role of stem cells in wound healing, and more specifically look at the role of ASCs, their mechanism of action and their safety profile in wound repair and tissue regeneration.

Skin is a soft tissue that forms about 8% of the total body mass and covers the entire surface area. It is a self-repairing, self-renewing organ in the body that forms an important barrier from the outer environment to the inner environment.[1] Therefore, damage to the skin leads to debilitating effects forming wounds. A wound is an impairment of the anatomical structure and function of the skin.[2] Cutaneous wounds vary in etiology and treatment depends on the wound type. Main types of chronic wounds include one induced by surgical procedures and failed to heal, pressure ulcer, venous and diabetic ulcers, and burns. Nonhealing wounds usually fail to follow the normal healing cascade leading to arresting in chronic inflammatory phase.[3] The quality of life for patients with chronic wounds is significantly affected with pain as a primary complaint.[4] Chronic wounds affect up to 1% of the population at any given

ACW	Acute wound fluid	HGF	Hepatocyte growth factor
ASC	Adipose-derived stem cells	HIF-1α	Hypoxia induced factor-1 alpha
bFGF	Basic fibroblast growth factor	ICAM	Intracellular adhesion molecule
BM-MNC	Bone marrow mononuclear cells	IGF	Insulin-like growth factor
BMP	Bone morphogenetic protein	IL	Interleukin
BMSC	Bone marrow–derived stem cells	iPSC	Induce pluripotent stem cells
CD	Cluster differentiation	KGF	Keratinocyte growth factor
CM	Conditioned medium	LPA	Lipoaspirate
CWF	Chronic wound fluid	MMP	Matrix metalloproteinase
EC	Endothelial cells	MSC	Mesenchymal stem cells
ECM	Extracellular matrix	PDGF	Platelet-derived growth factor
EPC	Endothelial progenitor cells	PLA	Processed lipoaspirate
FDA	Food and Drug Administration	SDF-1	Stromal cell derived factor-1
FGF	Fibroblast growth factor	SVF	Stromal vascular fraction
GFP	Green fluorescent protein	TGF-β	Transforming growth factor-beta
GMP	Good manufacturing practice	UVB	Ultraviolet B
GvHD	Graft-vs-host disease	VCAM-1	Vascular cell adhesion molecule-1
HEK	Human epidermal keratinocytes	VEGF	Vascular endothelial growth factor

time.[5,6] To put this into perspective, it is estimated that in excess of $20 billion are spent annually for the treatment of chronic wounds in the US,[7,8] with 500,000–1.5 million people suffering from venous leg ulcers.[9] Approximately, 70,000 suffer from serious burns and 15–20% of patients with diabetes go on to develop nonhealing diabetic ulcers of which 3–5% progress to partial or full amputations of the lower limbs. The average cost of treatment for diabetic ulcers is in the range of $30,000 to $50,000.[10,11]

In the past two decades, great progress in understanding the pathophysiology of wound healing and the developments of new therapeutic methods has been made[12]; unfortunately, the healing process of many chronic wounds remain unsatisfactory.

In recent years, stem cell therapies have emerged as therapeutic alternatives for regeneration and repair of damaged organs and tissues in various diseases.[13–17] Mesenchymal stem cells (MSCs) have been the main focus of research in recent years,[18–21] although embryonic stem cells[22,23] and induce pluripotent stem cells (iPSCs) are also being tested.[24,25] However, these cells come with various limitations: genetic manipulation of iPSCs, ethical consideration, and control of cellular differentiation of embryonic stem cells make them less attractive for translational approaches.

Adult stem cells on the other hand do not pose any ethical issues and are also available in abundant supply. To date, lot of research has focused on bone marrow-derived stem cells (BMSCs). However, the procurement of these cells is a painful procedure and usually leads to low yield and may lead to donor site morbidity. On the other hand, stem cells derived from adipose tissue are easy to obtain and provide a much higher yield. When comparing these cells to BMSCs, they show similar morphology, differentiation potential, and cell surface markers. Transcriptomic and proteomics analysis also shows similar profile for adipose-derived stem cells (ASCs) and BMSCs.[26–33] Due to their ease of collection and similar properties, ASCs are another attractive source and worthy of attention for clinical translation.

PATHOPHYSIOLOGY OF THE CHRONIC WOUNDS

Wound healing is a normal process in response to soft tissue injury, which involves a highly organized cascade of events. These phases include hemostasis, inflammation, proliferation, and remodeling.[34] For a normal healing process, all of the above stages must function in a proper sequence and on specific times.[35,36] Therefore, interference with any of the four stages during the wound healing can lead to impairment of the healing process. In most chronic wounds, the tissue regeneration is arrested in the inflammatory phase that leads to pathologic inflammation and failure of the endogenous repair response to progress to the advanced stages of wound healing. The continuous inflammatory phase leads to abnormal production of inflammatory cytokines.[37] Therefore, increased presence of neutrophils can be used as a marker for chronic wounds.[38] The increase in inflammatory cells leads to degradation of extracellular matrix (ECM) due to increase in secretion of matrix metalloproteinases (MMPs) and loss of important wound healing growth factors (transforming growth factor-beta [TGF-β], platelet-derived growth factor [PDGF], and hepatocyte growth factor [HGF]).

Table 1. Factors affecting chronic wounds

Local factors	Systemic factors
Infection	Aging
Ischemia	Chronic diseases (diabetes mellitus)
Cancer	Alcoholism, smoking
Radiation	Drugs (glucocorticoids)
Trauma	Nutritional deficiencies
Local toxins	Uremia
Arterial/venous insufficiency	Neuropathies

Due to a highly inflamed environment, these wounds fail to respond to conventional or even advanced therapies. Chronic wounds usually give rise to ulcers that are mainly associated with vascular insufficiency, neuropathies, burns, diabetes mellitus, venous diseases, and ischemia.[35,36,39] The main factors that are involved in chronic wounds are stated in Table 1.

Wounds in the dermal layer or partially extended wounds in the dermal layer are usually capable of regeneration, unfortunately deep dermal injuries are not healed adequately by the body. Often deep skin wounds do not resolve completely and usually heal with contractures, scar formation and without completely regenerating the dermis.[40] In the case of deep ulcers or full-thickness burns, there are no remaining sources of cells for regeneration except from the wound edges. Complete healing and reepithelialization therefore takes a long time and is complicated by scarring.[41] Chronic wounds have been a major burden on healthcare sectors, as diabetic ulcer, pressure ulcers, and bed sores do not heal properly with current standard treatments and this applies especially to some wounds that tend to heal with scar formation and low mechanical stability.

CONVENTIONAL AND CURRENT TREATMENTS

Autologous skin grafts are a common way to treat full-thickness wounds, where harvested skin from another site on the body is transplanted to the injured area. Although this strategy provides a complete epidermal layer, it lacks the dermal component leading to poor contour and limited availability of the graft.[41]

Autologous keratinocytes produced in vitro have been used to treat serious burns for a long time.[42,43] Therefore, it is possible to obtain a large amount of epidermis by combining silastic epidermis with bovine hide collagen and chondroitin 6-sulfate from shark cartilage to form a temporary silastic epidermis. Recently, the focus has shifted to regenerate the dermal component of the skin as well to overcome the lack of dermis in skin grafts.[44,45] Different strategies are being used to generate dermal component, one of which is to grow the cells in vitro. However, allowing the regeneration of dermis endogenously by providing the relevant cues may be a more sought after strategy. Implantation of biodegradable scaffolds with or without cells can achieve this by degrading slowly and allowing the infiltration of host cells.[46–48]

Commercially available skin substitute such as Integra™ (Johnson & Johnson, New Brunswick, NJ) can regenerate dermal components and is a prime example of the strategy described above. Integra a bilayered scaffold, composed of dermal component derived from bovine collagen and chondroitin-6-sulphate forms the dermal component, degrades slowly. Subsequently, the host's fibroblasts infiltrate and form the autologous connective tissue. After which the top layer is removed and replaced by split-thickness skin grafts or cultured epithelial cells derived from the patients.[11,42] Cell free skin substitutes have also been used for the treatment of deep burn wounds.[49,50] The cell-free dermis is derived from cadavers and when applied to the wounds, become incorporated into the wound site by revascularization and cellular infiltration. The cell free scaffold then is replaced by regenerated tissue rather than scar formation. The immunogenic reaction and risk of rejection is significantly reduced by using this approach as the scaffold has no cellular components.[51] Although a great progress has been made in treating of deep skin wounds, these scaffolds still have limitations associated with allografts and xenografts as well as developmental costs. Another major concern is the possibility of disease transmission as the sources of these scaffolds are of animal origin.[42,52]

Despite the therapeutic efficiency of tissue-engineered skin substitutes, the products face several challenges and difficulties that limit their clinical application. Some disadvantages associated with the clinical use of dermal substitutes include slow vascularization, poor integration, and rejection. Furthermore, the dermal substitute Integra is applied in a two-step grafting procedure that can be uneconomical.[53] Other disadvantages include poor handling properties, short shelf life, high manufacturing and distribution costs, and restriction to wounds of relatively low severity. Therefore, the interest in the use of stem cells for potential wound healing applications is increasing.

STEM CELLS IN WOUND HEALING

Stem cells are defined as cells that can maintain prolonged self-renewal capacity and can differentiate into other tissue types by asymmetric replication. Therefore, stem cells that can differentiate and replenish at the same time could be useful to make skin tissue. Stem cells can be derived from different sources such as embryonic stem cells, induced pluripotent stem cells, BMSCs, ASCs, and other tissue specific stem cells.

In recent years, stem cells have been extensively researched for their wound healing potential in both preclinical and clinical settings, mainly in critical limb ischemia and diabetic wounds.[54–59] Patients in advanced stages of disease such as diabetic ulcers or deep chronic wounds who have no option left apart from limb amputation may benefit from cell therapy. BMSCs and peripheral-derived mononuclear cells have been administered to patients with chronic wounds with varying degree of results.[60]

Topical delivery of bone marrow-derived MSCs on a collagen sponge scaffold showed significant improvement in wound healing.[61] This therapy led to enhanced wound healing in 18 of 20 patients. In separate studies, autologous biograft and MSCs have shown improved healing in hard to heal diabetic wounds.[62] Therefore, cell therapy could be the way forward for treatment of hard to heal chronic wounds. However, the appropriate cell type for the treatment of chronic wounds still needs to be identified.

ASCs

Adipose tissue is a favorable source of stem cells as it can be extracted in large amounts with minor donor site morbidity and ASCs isolated from this tissue can be cryo-preserved for up to 6 months guaranteeing that these cells are accessible for their therapeutic use in the future.[63] Typical isolation procedures for ASCs involve digestion of the lipoaspirated tissue with collagenase and subsequent centrifugation; a high density of stromal-vascular fraction is produced. Subculturing is then performed to detach the ASCs from the primary adipocytes.[64] Platelet-rich plasma has been shown to improve the proliferation of human ASCs, and initial studies promote its utilization for cell-based soft-tissue engineering and healing of wounds.[65]

Liposuction along with surgical excision of excess fat tissue has facilitated the extraction of adipose tissue for many years. An adaptation of this isolation procedure was carried out in 2001 when this tissue was further processed to attain a population of fibroblast-like cells known as processed lipoaspirate (PLA). These cells could be expanded by in vitro culturing methods for prolonged periods of time with substantial population doublings and moderate levels of senescence.[66] Human PLA consists of preadipocytes that, like MSCs, have the ability to differentiate in vitro into mesenchymal lineages. These multipotent stem cells referred to as ASCs have shown to possess powerful developmental flexibility, as they are competent in differentiating into multiple lineages and have the capacity to self-renew. The centrifuged pellet that is obtained from these lipoaspirates contains a relatively high concentration of ASCs. It has been shown that survival, growth, and differential capacity of these adipose precursor cells is dependent on the site of isolation. The superficial abdominal depot is less susceptible to apoptosis compared with other subcutaneous depots, and differentiation potential is more significant in all depots of young individuals compared to the elderly where differentiation is only substantial in the arm and thigh subcutaneous depots.[67]

Stem cells are described as cells that have the ability to undergo self-renewal, be proliferative, and differentiate into multiple lineages.[68] Recent developments in methods to extract and grow stem cells allowed researchers to study them more vigorously. These new methods of extracting cells have allowed use of autologous adult stem cells directly from the patient without any ethical concerns or immunological barriers. ASCs express similar surface markers to bone marrow MSCs such as CD10, CD13, CD29, CD44, CD54, CD71, CD90, CD105, CD106, CD117, and STRO-1. They are negative for the hematopoietic lineage markers CD45, CD14, CD16, CD56, CD61, CD62E, CD104, and CD106 and for the endothelial cell (EC) markers CD31, CD144, and von Willebrand factor.[27,66]

ASCs have the potential to promote angiogenesis, secretion of growth factors, and differentiate into multiple lineages upon appropriate stimulation. Therefore, ASCs can promote human dermal fibroblast proliferation by directly contacting cells and paracrine activation in reepithelialization phase of wound healing.[69]

A great progress in understanding the pathophysiology of wound healing in the past couple of decades has been made.

The developments of new therapeutic methods and market approval of novel skin substitutes such as Apligraf (Organogenesis, Canton, MA), Dermagraft (Organogenesis), OrCel, and Integra to name a few, have helped in the management of wounds; however, the healing process of many chronic wounds remains unsatisfactory. The synthetic skin substitutes lack the structure to resemble the native architecture and lack basement membrane leading to problems in proper healing.[70]

Among the many factors contributing to impaired wound healing, the reduction of cytokines released by local inflammatory cells and the decreased neovascularization are the main cellular responses.[71] To address these major obstacles, BMSCs have been studied along with ASCs, which have shown to modulate the immune response and provide the building block for the regeneration of the wound. They have also shown to exert their function by paracrine effects.[59,69,72–78] ASCs have surface markers and gene profiling similar to BMSCs and their soluble factors are not significantly different. Given their convenient isolation compared with BMSCs and extensive proliferative capacities ex vivo, ASCs have generated interest as the most favored cell type for wound repair and regeneration.

Comparing with BMSCs, ASCs are abundantly present in fat tissue and can yield 500-fold higher cell numbers from the same amount of tissue as bone marrow. ASCs are clinically more attractive due to the ease of extraction and high recovery yield.[33,79] Furthermore, lipoaspirate can be directly used clinically without culturing in vitro.[80]

Direct use of lipoaspirate could be safer and more efficacious as it avoids in vitro manipulation of ASCs that could alter their biological functions. Another advantage of using lipoaspirate is that regulatory issues such as good manufacturing practice production could be circumvented.[81]

Adipose tissue is mainly composed of a heterogeneous population of different cells along with adipocytes. Cells isolated from fat tissue are referred to as stromal vascular fraction (SVF).[82] Adipocytes can be further purified by centrifugation and plastic adherence methods in culture. Generally, SVF contain 30–40% of ASCs along with endothelial, smooth muscle, and different blood cells.[83,84]

To date most clinical applications using adipose tissue have utilized freshly isolated SVF rather than purified ASCs,[85–87] because SVF consists of various components such as pericytes, ECs, and macrophages, and it is thought that this composition may offer unique benefits in wound healing applications. The components of SVF may work synergistically to enhance the ASCs regenerative potential.[80] However, some studies challenge the idea of SVF as a better source for treatment than purified ASCs. Bai et al. recently showed that the cultured ASCs and SVF had similar efficacy and none of them were superior to each other when tested in mouse models of myocardial infraction. In another, study Garcia-Olmo et al. showed that ASCs were superior to SVF in the treatment of Crohn's disease.[88,89] These studies indicate that SVF and ASCs can both promote regeneration. However, there are not many studies available in the context of wound healing that compare both treatment modalities. For an in-depth analysis of the differences and the use of SVF and ASCs, interested readers are suggested to study the excellent reviews by Bunnel et al. and Guilk et al. where the use of SVF and ASCs for preclinical and clinical use and the regulatory hurdles involved are discussed in quite detail.[90,91]

ASCs IN WOUND HEALING

ASCs have a fibroblastic morphology, which consists of a large endoplasmic reticulum and large nucleus.[92] In comparison with stem cells obtained from the bone marrow, stem cell yields derived from adipose tissue are generally 40-fold higher.[93] Skin wounds treated with ASCs have shown enhanced healing via epithelial migration, angiogenesis with better healing rate and less scar formation. In a study by Yuan et al., human epidermal keratinocytes (HEK) were co-cultured with ASCs that resulted in increased HEK migration when compared with controls.[94] In a different study, media obtained from ASCs was examined to study the secretion profile of ASCs. It was found that the cells secreted TGF-β, vascular endothelial growth factor (VEGF), keratinocyte growth factor (KGF), fibroblast growth factor 2 (FGF2), PDGF, HGF, fibronectin, and collagen I[95], and these factors have previously been shown to stimulate wound healing process in normal and chronic wounds. These studies suggest that ASCs can affect other cell types specifically in skin tissue via paracrine method.[96–102] In another study, a preclinical model of chronic wound (diabetic mice wound model) was used to assess the efficacy of ASCs. Here, autologous ASCs in an atelocollagen matrix were implanted in a full-thickness skin wound. Histological examination showed significantly increased healing time, higher epithelialization rate, increased granulation tissue formulation, and capillary formation when compared with control groups. This study showed that ASCs secreted high levels of growth factors; however, this could be associated with the use of biomaterials (atelocollagen matrix with silicone membrane)[103] ASCs, when combined with different scaffolds, have shown improved wound healing. Altman et al. showed that seeded ASCs on silk suture can close full-thickness skin wounds in mice.[104] It was noted that wound closure was significantly higher compared with non-ASC-coated suture groups.[105] However, ASCs did not significantly improve wound healing in porcine skin wounds but showed better cosmetic results when compared with control treatments.

These findings suggest that different factors released from ASCs can have cosmetic affects as well as physiological improvements. ASCs have also been tested for delaying the formation of photo-induced wrinkles both in vitro and in vivo conditions. In another study, human dermal fibroblasts co-cultured with ASCs were exposed to UVB. UVB exposure decreased the rate of proliferation for fibroblasts; however, it did not affect the fibroblasts co-cultured with ASCs. The in vitro findings translated well in a preclinical study where mice injected with ASCs subcutaneously were exposed to ultraviolet B light for 8 weeks. ASCs delayed wrinkle formation in ASC-injected mice compared with control treatment thus suggesting a possible paracrine effect of ASCs.[106] Overall, in many studies, ASCs have shown potential therapeutic effects for skin wound healing.

The summary of recent studies using ASCs has been listed below (Table 2). Although ASCs have shown to be effective in treatment of acute and chronic wounds in preclinical settings exact mechanism of their functions is still under investigation. It has been postulated that ASCs can initiate or enhance tissue regeneration by two different mechanisms, either by differentiating into skin cells or by secretion of paracrine factors, which can initiate the healing process via recruiting endogenous stem cells and ECs or down-regulating the inflammatory response (Figure 1).

Table 2. Summary of preclinical published data of efficacy of ASCs

Authors	Use of adipose-derived stem cells
Kim et al.[69]	ASCs in collagen gel
Nambu et al.[107]	ASCs on atelocollagen scaffold
Altman et al.[104]	ASCs on silk fibroin scaffold
Kim et al.[106]	Direct injection of ASCs
Park et al.[95]	Direct injection of ASCs
Lu et al.[108]	Direct injection of ASCs
Nambu et al.[103]	Noncultured ASCs in atelocollagen matrix
Blanton et al.[105]	ASCs in platelet-rich plasma
Amos et al.[109]	Direct injection of ASCs into the wounds
Ebrahimian et al.[110]	Direct or IV injection of ASCs into the wound
Uysal et al.[111]	Injection of ASCs cells into the flap
Rocco et al.[112]	Topical administration of genetically modified SDF-1 expressing ASCs
Nie et al.[113]	Direct injection of ASCs around the wound site
Lee et al.[114]	ASCs in collagen gels
Huang et al.[115]	ASCs on acellular dermal matrix
Song et al.[116]	Conditioned medium of genetically modified ASCs

WOUND HEALING BY PARACRINE EFFECTS OF ASCs

ASCs secrete many different growth factors such as insulin-like growth factor (IGF), hepatocyte growth factor (HGF), transforming growth factor-beta 1 (TGF-β1), and VEGF. In recent studies, these growth factors have shown to be efficacious in preclinical wound healing models of animals[34,39] and becaplermin, a topical gel containing PDGF-BB, has been used for diabetic foot ulcers in clinical settings for a decade.[117]

In another study, Tsang et al. tested the efficacy of recombinant human epidermal growth factor (hEGF) on diabetic foot ulcers in a double-blind randomized controlled study.[118] In this study, they showed a significant improvement in healing time of diabetic ulcers in a group treated with 0.04% hEGF cream (95%) when compared with control (42.1%). It is hard to draw conclusions from such studies as these treatments were carried out in combination with standard care treatment. However, these studies showed limited efficacy and were not translated into clinical studies. This could be due to the fact that only single growth factors have been delivered and that during wound healing synergistic effects of multiple growth factors may be required in an in vivo environment.

Wu et al. showed that injection of bone marrow stem cells significantly enhanced the wound closure and strength in diabetic mouse model for wound healing.[119] In a similar study, conditioned medium (CM) alone from BMSCs also mediated enhanced wound healing response. Kim et al. used condition medium alone from ASCs instead of BMSCs. This study showed increased collagen synthesis leading to improved wound healing outcome.[95] Although this study did not look at the mechanism involved in the wound healing process, the fact that ASC-CM showed similar collagen production with high levels of growth factors indicate a paracrine mechanism.

Galiano et al. also showed enhanced wound healing in a genetically diabetic mice model with topical application of

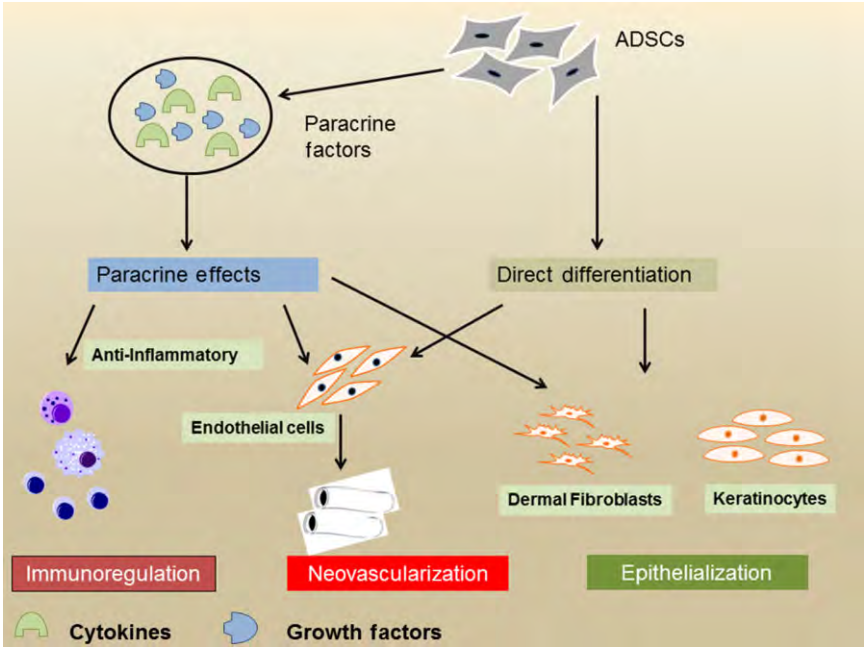

Figure 1. Possible mechanism of skin repair by adipose-derived stem cells (ASCs). In response to injury, ASCs may migrate and differentiate into skin cells to repopulate the injured skin or activate the dermal fibroblasts and keratinocytes by secretion of growth factors for accelerated wound healing.

VEGF.[99] The healing observed was due to the formation of new vasculature in the wound bed and recruitment of BMSCs. ASCs secrete factors that can stimulate proliferation of fibroblasts, keratinocytes, and ECs in vitro. Further analysis revealed increased collagen production and altered gene expression with CM obtained from ASCs.[69,120] Overall, these findings suggest that ASCs release wound healing factors and can stimulate recruitment, migration, and proliferation of endogenous cells in the wound environment. Consequently, ASCs can stimulate angiogenesis, epithelialization, and wound remodeling through paracrine secretion during wound repair.

WOUND HEALING VIA DIFFERENTIATION OF ASCs

Several studies have highlighted the therapeutic efficacy of ASCs for wound healing. Administration of implanted ASCs in rat, pigs, and mice wounds have indicated enhanced healing via epithelialization and angiogenesis.[103,105,107] Topical administration of ASCs on ulcers in diabetic mice was effective for enhanced wound closure. Neovascularization and epithelialization were the main reasons behind this process. Tamarat et al. in particular showed enhanced healing in physiological and pathological wounds of mice by differentiation of green fluorescent protein-positive ASCs into ECs as well as keratinocytes.[76]

Transdifferentiation of ASCs into skin cells has not been extensively investigated; however, Trottier et al. recently replaced skin substitutes that commonly use dermal fibroblasts with ASCs.[121] This cell replacement strategy generated a more intact trilayered skin alternative that consisted of the epidermis, dermis, and hypodermis, which is the deepest layer of the skin. Little progress has been made on the effect of ASCs on fibroblasts, which have a pivotal function in skin biology. Kim et al. investigated the likely function of ASCs in skin wound healing.[69] Co-culture experiments showed that proliferation of fibroblasts was boosted either by direct cell–cell contact of ASCs and human dermal fibroblasts.[69] This CM augmented the release of type 1 collagen in the human dermal fibroblasts by controlling the mRNA levels of ECM proteins. As a consequence, there were both up- and down-regulation of certain factors with the expression of collagen type I and III and fibronectin being increased 1,000-fold compared with MMP-1, which was down-regulated. ASC-CM also provoked migration of human dermal fibroblasts in vitro.

Further confirmation that ASCs are associated with wound healing was clarified in vivo animal models where the delivered cells greatly decreased the size of the wound as well as advancing the reepithelialization from the edge inwards. Factors that aid in this process have been identified in the CM and are namely PDGF, IGF, and KGF.[69,76,113,122]

Additional studies have shown that ASCs seeded onto a biomaterial such as a silk fibroin-chitosan scaffold transformed into fibrovascular, endothelial, and epithelial elements of repaired tissue and improved wound healing.[104] The magnitude of microvessels was considerably higher at the site of the wound, and ASCs that homed to the site of injury and adhered were positive for fibroblastic markers, heat shock protein 47, smooth muscle actin, and von Willebrand factor. These studies indicate that ASCs not only secrete pro-healing factors but also take part in the healing process and act on the local environmental cues.

Although it has been shown that ASCs can differentiate into resident cell types when delivered into the wounds, it is thought that the primary mechanism involved is paracrine secretion that then leads to differentiation of stem cells into ECs, fibroblasts, or keratinocytes. Therefore, it is conceivable that strategies aiming at the enhancement of the paracrine secretion of ASCs by modulation the in vitro environment would result in successful wound healing. Hypoxia is one of these factors in the wound environment that can lead to enhanced secretion of growth factors and will be analyzed in the next section.

HYPOXIA AND ASCs FOR WOUND HEALING

Similar to the culture criteria required in BMSCs proliferation, ASCs proliferation is instigated by the culture medium used plastic surface quality and oxygen levels.[120] Hypoxia or lack of oxygen has shown to improve cellular functions of ASCs via increased proliferation and up-regulation of certain genes, mainly angiogenic and anti-apoptotic factors (Figure 2). Many studies have shown that ASCs proliferate better under hypoxic conditions compared with normoxic conditions.[123124] An oxygen strain of between 1% and 5% during ASC culture favors adequate proliferation, whereas the cells are still retaining their morphology and differential capacity. Transplanted ASCs also have the potential to combine and attach to ECM proteins and this can be manipulated in vitro to enhance the migratory properties of these cells. Amos et al. demonstrated that culturing ASCs under low oxygen levels improved their capability to attach to vascular cell adhesion molecule-1 and endothelial intercellular adhesion molecule-1.[125] ASCs have exhibited homing to factors that are up-regulated as a result of an ischemic tissue environment, such as the chemokine stromal cell-derived factor-1; hypoxic culture conditions increase the expression of this chemokine's receptor and therefore enhance homing.[126]

Hypoxic environments also have sustained levels of hypoxia-inducible factor-1α, which has been shown to

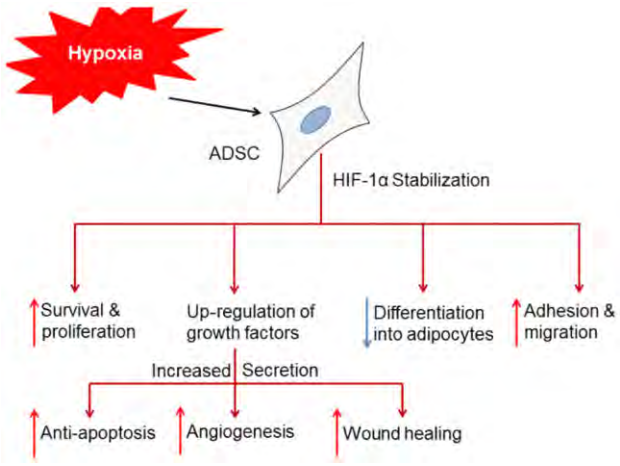

Figure 2. Adipose-derived stem cells response to hypoxia and wound healing potential.

promote expression of certain target genes associated with angiogenesis, cell proliferation, and energy metabolism.[127] Chung et al. supported this finding and identified that levels of VEGF, basic fibroblast growth factor (bFGF), and IGF were substantially elevated (by >1.5 fold).[64] Adjusting stem cell characteristics by inducing a hypoxic environment that mimics the in vivo wound environment greatly strengthens the regenerative potential of these therapeutic cells. Hypoxic conditioning strengthens ASCs function in neoangiogenesis as ASCs stimulate, through a paracrine effect in hypoxic culture medium, a fivefold rise in the levels of VEGF. This growth factor was then implicated in encouraging angiogenesis and anti-apoptosis.[78] In a matrigel implant model, it was shown that ASCs are capable of provoking formation and maintaining vessel-like structures and that hypoxia-induced ASCs had an improved production of capillary-like structures.[127]

The great potential of ASCs in the tissue engineering area has been mentioned previously. However, these cells do pose limitations such as the risk of inducing cancer,[128] large-scale industrial-scale handling and commercialization. These obstacles, however, can be circumvented by using the paracrine factors that are secreted by ASCs. These factors are durable, relatively void of safety concerns and therefore ideal for commercialization. Extracting CM for skin regeneration provides a readily available source of growth factors released by the cells themselves.[64] This secretion can be further improved by ascertaining the ideal oxygen tension to increase the amount of regenerative proteins in the CM.

CONSIDERATION OF WOUND TYPES FOR ASCs THERAPY

During acute wound healing, various stages such as infection control, inflammatory response, angiogenesis, epithelialization, and tissue remodeling occur in a well-orchestrated time-dependant sequence.[3] However, during chronic wounds, these mechanisms are disturbed, thus leading to persistence of inflammation and long-term injury.[129,130]

The factors involved in healing can be classified into systemic and local factors. Lack of oxygen in chronic wounds leads to decreased activity of fibroblasts and increased inflammatory cells along with increase in apoptosis of ECs. The resident cells in chronic wounds show marked changes in phenotype, becoming senescent and less responsive to growth factors.[131] The presence of infections can lead to increase in inflammatory cytokines and proteases and subsequent degradation of growth factors and local ECM.[129]

In earlier studies, Schultz et al. showed that acute wound fluids (AWFs) are significantly different from chronic wound fluids (CWFs) in their composition. They demonstrated that MMP activity is significantly elevated in CWF (30-fold) when compared with AWF. They also noted a higher degradation rate of growth factor EGF (28%) in chronic wounds compared with the acute wounds (0.6%).[132,133] In another study, Hipler et al. showed similar results of an increase in MMP activity in chronic wounds when compared with acute wounds. In addition to this, a significant increase in the production of pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and IL-8 was reported in CWF compared with AWF.[134] Therefore, before deciding on a therapeutic modality, it is imperative to understand the wound pathology and the type of wounds.

Different types of wounds can have different healing outcomes when using ASCs as a therapy. This is particularly true, as in a recent study, Thamm et al. analyzed the impact of AWF and CWF on ASCs. The study results indicated that wound environment can regulate ASC function when in contact. Here, ASCs were treated with AWF obtained from human surgical wounds and CWF obtained from chronic sacral decubitus. The ability of ASCs to proliferate and migrate was significantly hampered when treated with CWF that could lead to impaired regenerative potential of ASCs. However, the expression of growth factors (VEGF, bFGF, and MMP9) was strongly induced in CWF-treated cells as opposed to AWF-treated ASCs.[135] Therefore, the use of ASC for wound healing should be considered carefully, as well as delivery methods and dosing regimen as the use of stem cells may differ depending on the wound type.

ASCs IN CLINICAL TRIALS

As described above, interest in the use of ASCs as therapeutic modalities is ever increasing due to the ease of access of fat tissue, abundant supply, and lack of ethical concerns. Because of the recent efficacy shown by ASCs in preclinical models of chronic wounds, it is no surprise that ASCs have rapidly moved into translational phase, and many clinical trials are being carried out (Table 3).[136]

The first clinical application of ASCs used SVF in a case report to treat defect of the calvaria after injury.[137] In this case study, fibrin glue was used along with SVF. Three months post treatment, new bone formation was detected and resulted in near complete healing of calvaria defect. Unfortunately, in another trial, undesirable events were noted and a possible risk of cancer formation lead to questions regarding the safety of stem cells.[86] The efficacy of SVF in the cardiovascular field for acute myocardial infarction is also being tested in another clinical trial. In a clinical environment, expansion of cells is a major hurdle, and growing cells in a lab can lead to delayed treatment unless allogeneic or frozen cells are used. However, from previous experiences in the field with BMSCs where similar protocols were used with little or no clinical impact.[138] There may be some optimism for the use of SVF in the treatment of critical hind limb ischemia as the injection of bone marrow mononuclear cells was relatively successful even after 2 years.[139,140]

In some clinical trials, ASCs are utilized after being purified from SVF to obtain a pure population of ASCs. ASCs have been used in a trial for the treatment of depressed scars; however, the results have not been published in any peer-reviewed journals after the trials have been concluded. In another report that was successfully completed and documented with 36 months follow-up, it was shown that ASCs along with BMP-2 and tricalcium phosphate scaffold lead to successful healing of osteogenic defect.[141] Although encouraging, this is only a single report and conclusions for long-term safety of these cells cannot be drawn until successful phase I/II clinical trials are completed.

Other ASC-related clinical trials focus on auto-immune inflammatory diseases such as Crohn's disease and fistula complications that result from tissue degeneration following an uncontrolled inflammatory process and graft-vs-host disease (GvHD). Garcia-Olmo et al. have shown successful healing with expanded ASCs (rather than the freshly prepared cells) in treating Crohn's disease.[89] In trials that focused on treating fistula, ASCs have shown to be very efficient in controlling inflammation and improving the healing process.[62–64]

Table 3. A list of some clinical trials, currently recruiting or completed using adipose-derived stem cells

Clinical trials	Status	Ref.
Allogenic Adipose-Derived Stem Cells in Crohn's Fistula	Recruiting	NCT01440699
Autologous Cultured Adipose-Derived Stem Cells (ANTG-ASC) on Complex and Crohn's Fistula	Recruiting	NCT01314092
Development of Bone Grafts Using Adipose-Derived Stem Cells and Different Scaffolds	Recruiting	NCT01218945
Adipose-Derived Stem Cells to Treat Complex Perianal Fistulas	Completed	NCT01020825
Safety and Effects of Autologous Adipose-Derived Stromal Cells Delivered in Patients with Renal Failure	Recruiting	NCT01453816
Human Adipose-Derived Mesenchymal Stem Cells for Critical Limb Ischemia in Diabetic Patients	Recruiting	NCT01257776
Autologous Adipose-Derived Stromal Cells in Patients after Stroke	Recruiting	NCT01453829
Autologous-Cultured Adipocytes in Patient with Depressed Scar	Completed	NCT00992147

The list was compiled by searching for "Adipose derived stem cells" in the web-site clinicaltrials.gov and PubMed.

Two separate clinical trials have focused on GvHD using ASCs as similar trials using BM-MSCs have been done for GvHD.[142] It is not surprising that the use of ASCs for GvHD may show positive results as immunomodulation properties of BMSCs and ASCs are very similar.[66,67]

Multiple sclerosis is another disease that is under investigation as it can also be included in the field of the modulation of inflammation. This is due to the fact that in a preclinical rodent model of MS administration of ASCs via IV injection has shown to home to lymph nodes and brain and dampens down the inflammatory response. So far only two trials are underway for the safety and efficacy of ASCs in critical limb ischemia.[143] However, further trials are either underway or starting in the context of fistula and allogeneic use of ASCs. Therefore, the results of currently ongoing clinical trials, if encouraging, can open up the field for ASC related regenerative medicine.

CONCLUSION

ASCs and their potential for wound healing has been shown in several studies. To date, ASCs have been mainly tested in in vitro or in in vivo settings. However, recently a few clinical trials involving ASCs are either ongoing or recruiting patients. In preclinical studies, ASCs and their secretory factors have shown enhanced wound healing in animal models of chronic wounds. Until now, the mechanisms of their action have not been fully understood and therefore, further research in this particular area is needed. Clinical application of ASCs in skin wound healing is in its early stages and can be hazardous considering the risk of inducing cancer. Stem cells and more specifically ASCs are known to secrete growth factors, cytokines, and chemoattractants that can enhance angiogenesis and increase blood supply thus creating/providing support for tumor cells.[78,144,145] Stem cells can home to tumor sites in animal models when delivered systemically and provide trophic support.[146,147]

Given their secretion of angiogenic and anti-inflammatory molecules, it is postulated that certain stem cell populations may stimulate tumor growth.[148,149] A recent report from the FDA on becaplermin, a gel consisting of 0.01% PDGF-BB, was indicated for increased formation of malignancies. The follow-up study from two randomized controlled trials indicated increased risk of tumor formation at distant sites in patients treated with three or more tubes of PDGF-BB.

With the use of stem cells, it has been hypothesized that the immunomodulatory effects of ASCs may provide favorable conditions for tumor growths that may be hard to detect.[150] However, to date, no cases have been reported from patients treated with lipoinjections or ASCs directly.[151] Recent clinical trials using ASCs in other pathologies have not indicated risk of tumor formation that provides a positive outlook for ASCs. However, long-term follow-up studies are necessary to rule out any negative side effects. Identification of growth factors, proteins, and healing pathways involved in chronic wound healing can lead to safer and quicker translation to clinical setting. Delivery of stem cells or growth factors via biomaterials has shown promise and could be the way forward. ASCs have an important role in the future therapeutics for chronic wound healing.

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