REVIEW ARTICLE

Adipose stromal vascular fraction: a promising treatment for severe burn injury

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Abstract

Thermal skin burn injury affects both adults and children globally. Severe burn injury affects a patient's life psychologically, cosmetically, and socially. The pathophysiology of burn injury is well known. Due to the complexity of burn pathophysiology, the development of specific treatment aiding in tissue regeneration is required. Treatment of burn injury depends on burn severity, size of the burn and availability of donor site. Burn healing requires biochemical and cellular events to ensure better cell response to biochemical signals of the healing process. This led to the consideration of using cell therapy for severe burn injury. Adult mesenchymal stem cells have become a therapeutic option because of their ability for self-renewal and differentiation. Adipose stromal vascular fraction (SVF), isolated from adipose tissues, is a heterogeneous cell population that contains adipose-derived stromal/stem cells (ADSC), stromal, endothelial, hematopoietic and pericytic lineages. SVF isolation has advantages over other types of cells; such as heterogeneity of cells, lower invasive extraction procedure, high yield of cells, and fast and easy isolation. Therefore, SVF has many characteristics that enable them to be a therapeutic option for burn treatment. Studies have been conducted mostly in animal models to investigate their therapeutic potential for burn injury. They can be used alone or in combination with other treatment options. Treatment with both ADSCs and/or SVF enhances burn healing through increasing re-epithelization, angiogenesis and decreasing inflammation and scar formation. Research needs to be conducted for a better understanding of the SVF mechanism in burn healing and to optimize current techniques for enhanced treatment outcomes.

Keywords Stromal vascular fraction · Burn · Treatment · Adipose derived stem cell · Wound

Introduction

Skin is the largest organ in the human body that has many functions, including homeostasis and barrier protection. It consists of three main layers, epidermis, dermis, and subcutaneous tissue, with appendages such as hairs and glands. The epidermis contains keratinocytes, which regenerate every 2–4 weeks, and melanocytes, which regenerate slower than keratinocytes thus leading to pigmentation during burn healing. Epidermis also contains the nerves' endings that arise from the dermis. The dermis contains blood vessels, sensory nerves, sebaceous glands, sweat glands, and hair follicles. It heals by scarring and fibrosis instead of regeneration [80]. Any damage to the skin will inevitably compromise its function, exposing individuals to other health complications.

A wound is characterized by discontinuation of skin tissue resulting in disruption of cellular structure or function by any physical, chemical or biological sources [55]. Burn wounds can be caused by chemical, heat, electrical, radiation, or freezing materials. In the United States, one million burn injuries are recorded annually [9, 80]. More than 100,000 burned patients are hospitalized from severe burn injuries [20]. Out of 0.5 million burns recorded annually, about 3000 people lost their lives and 40,000 require hospitalization, according to the National Burn Repository of the American Burn Association [3]. According to the World Health Organization, burn is the fifth most common factor causing non-fatal injuries. Severe burn injury affects a patient's life psychologically, cosmetically, and socially. Therefore, burn is detrimental to patients and sufficient treatment is essential for a better quality of life.

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Burn treatment depends on the burn size, depth and the viability of donor site. The standard care for severe burn injury is using autologous skin grafts or skin substitutes. However, in burned patients with large total body surface area (TBSA), treatment options are limited and interventions are required.

The objective of this review is to give an overview of the pathophysiology of burn injury, highlight the currently available treatment and provide insights about future possible interventions focusing on using stromal vascular fraction (SVF), methods of its isolation, and the mechanism of action.

Classification of burn

Different classifications of burn can be considered depending on the appearance, duration, or nature of healing. Burn is classified depending on the depth of injury and the appearance into four degrees: first (superficial), second (superficial partial-thickness and deep partial-thickness), third (full thickness) and fourth (extend through other layers up to the subcutaneous fat and down to the bone, fascia, and tendons) [80]. It can also be subdivided according to the affected surface area into local burn injury (< 20% of TBSA) and severe burn injury (> 20–30% of TBSA) [102]. Burn wounds could be acute or chronic depending on the nature of healing.

Thermal burn wound is divided based on the nature of healing by Jackson [48] (Hettiaratchy and Dziewulski [47]) into three zones:

1. *Coagulation zone:* it is the deepest and most damaged zone among the three layers due to the coagulation of constituent proteins. It consists of inevitable tissues that cannot be regenerated due to the ceased circulation in this zone. It needs surgical excision and grafting [80]
2. *Stasis zone:* it is the middle zone that consists of dermal burn, vascular stasis, ischemia, and low tissue perfusion. Often, this zone could become necrotic within 48 h after thermal injury [78]. This zone is challenging since it can be recovered; it has the potential to heal or progress to the full-thickness lesion. Healing can be achieved by increasing tissue perfusion and preventing further damage before it becomes irreversible.
3. *Hyperemia zone:* it is the outermost layer and the least damaged area that is characterized by vasodilation, and inflammatory change without structural damage. Tissues are regenerated unless there is sepsis or prolonged hypoperfusion.

Physiological changes during severe burn injury

Unlike local burn injury, severe burn injury affects the whole body. Severe burn injury consists of two phases: the resuscitation phase and the hyperdynamic hypermetabolic phase. The *Resuscitation phase* (also called hypodynamic or ebb phase) occurs immediately after severe burn injury (24–72 h). It is characterized by increased vascular permeability, edema formation, and inflammatory response syndrome. Thermal burn injury starts with denaturation after long-term exposure to heat (more than 40 °C). This leads to protein misfolding (morphological dysregulation) and loss of plasma membrane integrity [55]. Moreover, released mediators alter vascular permeability and increase the inflammatory response. Increased vascular permeability causes the release of proteins and large molecules from vessels. This leads to the imbalance between oncotic and hydrostatic forces which results in the shift of fluids from intravascular space into the interstitial space resulting in edema formation. Severe burn injury decreases cellular membrane potential due to the disruption of sodium-ATPase pumps. This leads to increased sodium uptake that results in cellular swelling [102]. Therefore, this phase results from hypovolemic and inflammatory mediators' effects to restore and preserve tissue perfusion.

After-burn's shock, the body releases biochemical mediators as a defensive mechanism against injury. Many mediators control vasodilation, such as histamine, kinins (Bradykinin) and xanthine oxidase [102]. The xanthine oxidase leads to the release of free radicals. The balance between the production of free radicals and detoxification is a determinant of clinical response to a burn. Histamine, released by mast cells, causes capillary leakage (arteriolar dilation and venular contraction) and increases hydrostatic pressure [33, 90]. This could lead to loss of circulating plasma volume and edema formation. However, the role of histamine in severe burn injury remains controversial since the administration of histamine receptor antagonists did not show a reduction in edema formation [87]. Reactive oxygen species (ROS), released from neutrophils, infiltrate (approximately after 72 h) at the site of injury. ROS causes the release of lipid peroxidation (LPO), which regulates plasma membrane permeability. Production of ROS and LPO are involved in the biosynthesis of Prostaglandin (PGE2) and prostacyclin (PGI2), hormones, leukotrienes and thromboxane A2 (TXA2) [78]. Both PGE2 and PGI2 increase microvascular permeability and edema formation. Thromboxane A2 (TXA_2), a vasoconstrictor produced by platelets, decreases blood flow and could contribute to the zone of ischemia and thrombus formation [102]. Bradykinin is released at the site of injury and exerts it is effect

locally and systemically. It increases venular dilation, smooth muscle contraction and pain. Therefore, edema formation is a result of interaction between many factors, including histamine, ROS, LPO, PGE2, thromboxane and kinins.

Many agents are activated and released during burn healing and can be targeted as therapeutic agents. In severe burn injury, agents enhancing dendritic cells are released to ensure cell proliferation and accelerate wound healing. Angiogenic agents, such as hypoxia-induced factor 1 and cytokines (ex. VEGF and CXCL12) ensure endothelial progenitor cell proliferation. Wound contraction is ensured by the activation of the transforming growth factors-β (TGF-β) pathway for scar formation. During this phase, many inflammatory mediators are released against injury systemically or locally. This results in systemic inflammatory response syndrome. Macrophage migration inhibitor factor (MIF) can be released from the basal layer of the epidermis in response to thermal injury. It plays a role as an inflammatory cytokine in the innate and adaptive immunity, a neuroendocrine hormone, and a catalytic enzyme [102]. Nuclear factor-κB (NF-κB) is a transcription factor that is released after severe burn injury and regulates several inflammatory mediators, such as tumor necrosis factor (TNF), interleukin-6 (IL-6) and IL-1β, from cardiomyocytes causing cardiac dysfunction [68]. Studies have shown elevated levels of cytokines post-burn, such as IL-6, IL-8, IL-2, IL-13, I-10, and IL-17. It was suggested that pro-inflammatory cytokines that are activated by macrophages, such as IL-1, IL-6, TNF, and PGE-2, are involved in complications of severe burns [49]. Systemically, the release of TNF and IL-6 at the site of the burn is responsible for increasing infectious risk, organ failure, and death [91]. These changes in pro-inflammatory cytokines are part of the immune and endocrine response to burn injury to enhance survival. This phase of activity is followed by an anti-inflammatory response where there is a reduction in immune cells' count to return to normal status after a few weeks [50]. Therefore, mediators involved in the healing process can be enhanced and targeted as a treatment for severe burn injury.

Hyperdynamic hypermetabolic phase (also called flow phase) occurs as a response to heat loss and downregulation of thermoregulation within 3–5 days post-burn. This phase is characterized by a reduction in vascular permeability, exacerbation in the circulation of blood, heart rate thus cardiac output to restore microvascular integrity and peripheral blood flow [102]. It is also characterized by increased metabolic rate, body temperature and oxygen consumption [50]. This phase could last up to 24 months. This phase is driven by regulators, such as catecholamine, glucagon, stress hormones and pro-inflammatory cytokines. The metabolic rate could be increased up to 3 times higher compared to the basal metabolic rate. [102]. The anti-inflammatory effect is characterized by increased T-lymphocytes helper (Th-2) and the release of three main mediators, IL-4, IL-10 and TGF. This phase is characterized by an increased metabolic rate, heart rate and cardiac output and decreased vascular permeability and lean body mass as a result of the overproduction of stress hormones.

Possible treatment

Due to the complexity of burn pathophysiology, the development of a specific treatment that aids in the regeneration of new tissues and limits further complications is required.

Nutrition and rehabilitation

Fluid replacement treatment has been adopted for resuscitation and respiratory care, such as ringer lactate or NaCl solutions. However, fluid replacement does not improve complications associated with burn injury. Significant edema in all tissues leads to complications, such as respiratory failure, and abdominal compartment syndrome, which cannot be prevented only by preventing hypovolemic shock and tissue hypoxia [86]. Several approaches have been used to control hyper-metabolic rates, such as hyperglycemia, to improve patients' outcomes [50].

Infection control

Due to the compromised barrier function of the skin, tissues can become rapidly infected with pathogens. Moreover, hyperinflammation after-burn could cause immunosuppression and increase the infectious risk. Therefore, many topical antimicrobial agents have been developed, including bacitracin, mupirocin, and silver sulfadiazine (SSD) and aqueous solutions (e.g. mafenide acetate aka Sulfamylon and silver nitrate) [95]. The silver-based dressing was found to combat infection and eschar formation and control wound exudate. Unlike other dressing, silver-based dressing has the advantage of needing to be applied less frequently which reduces patients' pain due to the consistent release of silver ions to the wound bed. However, topical antimicrobial agents could have their risks, including delayed healing, incomplete re-epithelization, discolored scar, hypersensitivity and ineffectiveness against some pathogens [95].

Surgical control

Deep-partial and full-thickness burns usually require multiple surgeries and repeated wound care, depending on the injured TBSA. Clinically, burn wound management includes the excision of necrotic tissues until punctate bleeding in the wound bed is visible. Then permanent autologous skin

graft or a temporary skin substitute is applied. This could help in reducing infectious risk and lead to better healing results. However, this procedure could lead to loss of blood and hyperemia. Different techniques could be applied to reduce blood loss, such as thrombin spray, epinephrine soaked gauze and fibrin sealants [110]. To have a successful graft, complete homeostasis is required to prevent hematoma formation [12].

Permanent wound coverage: autograft

Autograft is the first option for physicians, and it is the current standard of care for deep-partial thickness and full-thickness burns with small TBSA. Autologous skin graft requires sufficient availability of donor's skin, usually the epidermis layer with the papillary dermis. However, wounds heal with some degree of contraction [11].

Mesh autograft is usually used when there is a limited source of donor site to cover the wound. This technique depends on using cut slits of skin in a mesh. Applying a high mesh autograft area could delay re-epithelization and thus could increase the risk of infection, contraction, and scarring. The Head, neck and face are among the most important areas. Using meshed autografts in these areas could lead to dramatic cosmetic issues and affect patients' quality of life. Using thick autografts could modulate contraction but increase other complications [94]. Even though using autograft is safe and promising, it is limited by the size of the burn wound, the availability of healthy skin, the time it takes for engrafting, and the healing of the donor site.

Temporary wound coverage: allograft

Autograft is the first choice of treatment when donor skin is available. In case of burn with high TBSA, skin autograft cannot be applied due to the lack of donor site. Temporary wound coverage is the available option in this situation with fresh or cryopreserved allograft. Temporary skin graft was first achieved by James Brown in 1942. It was used until the permanent cover is achieved. Temporary covering provides burn coverage, desiccation, formation of granulation tissues, and protection from microbial contamination.

For temporary burn wound coverage, several options are available. One of which is to use cryopreserved cultured epidermal allograft. This technique has many advantages, such as the ability of repeating the application, availability of frozen tissues at any time, and the possibility of giving early wound closure [105]. The other option is using skin substitute until the availability of donor sites. This includes epidermal substitute by extracting epithelial autologous cells from small biopsy of patient's own skin. These cells are then used to form sheets called cultured epithelial autografts (CEA), such as Epicel, to apply on the burn bed or spray epithelial cells directly on the burn wound after excision such as ReCell [22, 46, 95]. However, it is complex and costly and could not replace all components of the skin [22, 104]. Treatment of burn injury with cultured epidermal grafts consisting of keratinocytes had a risk of reopening and fragility of the wound, and increase wound contraction [103]. Tissue engineered dermo-epidermal skin substitute is a treatment option, but it is sophisticated, costly, and commonly increases the risk of infection and unstable scars. These drawbacks have restricted using them worldwide and directed the research to using cell therapy.

Hypertrophic scar prevention

One of the common complications of severe burn is developing hypertrophic scar, especially when healing process is delayed or un-succeeded. Young age, darker skin color, female gender, and burn severity are among the risk factors of hypertrophic scar formation [95]. Currently, there is no affective therapy for preventing hypertrophic scar formation. Some of the used techniques include messaging, moisturizing, using pressure garments or silicone gel sheets [4]. These techniques are not costly or risky and applicable but are not completely effective.

Burn healing requires biochemical and cellular events. However, many factors could interfere with healing process and delay healing. For instance, persistent inflammatory state could delay healing and lead to complications. Macrophages mediate inflammation through different approaches. When macrophages' functions are dysregulated by development of persistent inflammatory state, they become a type I inflammatory phenotype and impede progress toward burn repair regeneration [32]. Therefore, one of the reasons behind healing's delay is the presence of unresponsive cells that are incapable of responding to normal biochemical signals. These findings led investigators to consider using cell-based therapy as a solution of delayed healing in wound. Since using autograft or allografts have their limitations, stem cells have become a therapeutic option for their ability to self-renew and differentiation to other cell types.

Cell therapy

Burn healing is a process aiming in restoring the normal structure of the skin. Some of the available treatments are promising. However, new therapeutic approaches are needed to improve burn care and wound repair. The alternative of the pharmaceutical treatments could be cell therapy through the application of stem cells. Endogenous stem cells are found in the inter-follicular epidermis and sebaceous glands. Studies have showed rapid healing in superficial burns where hair follicles are intact. However, in severe burn injury, where hair follicles are damaged, burn healing demonstrated

scarring and lacked adnexal structure [63]. Therefore, the presence of stem cells in the injured site is crucial for healing process in severe burn injury.

Stem cells can be derived from a variety of sources, including embryonic (pluripotent) or adult tissues (multipotent), such as bone marrow, adipose tissue, and hair follicles. Due to ethical and logistical issues, research has shifted from employing embryonic stem cells to adult stem cells. Several studies have investigated different types of stem cells in burn healing. For instance, amniotic stem cells enhanced re-epithelization, angiogenesis, wound closure compared to collagen gel in treated mice [92]. Bone marrow-derived mesenchymal stem cells combined with an artificial dermal substitute promoted healing in a full-thickness burn by enhancing vascularization and re-epithelization in mice [64]. Compared to bone marrow, adipose tissue can provide a higher yield of adipose-derived stem cells (ADSC), also called mesenchymal stem cells, which can be acquired readily with a less invasive technique and donor site morbidity. Therefore, ADSCs present a better substitute for bone marrow-derived stem cells in many therapeutic applications.

ADSC in burn healing

ADSCs are among the stem cells that are candidates for burn treatment for many reasons; ADSCs are multipotent and can be easily isolated, have paracrine, tropic, and immune-modulating properties that enable using them in clinical settings. Studies have shown positive outcomes using ADSCs in burn injury [18, 35, 39, 82]. Treatment with ADSCs improved re-epithelization, angiogenesis, collagen deposition, and decreased inflammation and scar formation. ADSCs transplant effectiveness in burn healing depends on the cells' count (optimal dose of 1–3 million cells), timing, and the site of cells infusion. Administration of ADSCs at two-time points (0 and 4 days) post-burn enhanced wound contraction, collagen deposition, and lymphatic vessels and reduced scar formation in the full-thickness burn rat model [38]. Multiple injections of ADSCs improved neovascularization [109]. In other burn models, positive effects were also observed with a single dosage of ADSCs. The majority of ADSCs were administered intradermally or subcutaneously. Intravenous administration of ADSCs, on the other hand, enhanced meshed autologous slit thickness skin transplant for deep- and full-thickness burn injuries [37]. Furthermore, administration of ADSCs could be at the edge or the center of the burn wound. Chang et al. [17] showed that the injection of ADSCs at the four edges of the burn wound improved wound healing when compared to injections at the center of the wound [17]. Optimization of the ADSC delivery method is required for better healing results.

In burn injury, ADSCs can be delivered to the damaged site topically, as a spray, injected at the wound bed, or administered with different dressing materials, such as hydrogel, scaffolds, etc. [21, 76, 79]. One of the treatment applications of ADSCs for skin tissue regeneration is the administration with other dressing materials. For instance, the addition of ADSCs to fat isograft or collagen scaffold improved the maturation of wound bed via boosting certain healing indicators, angiogenesis, and collagen deposition [36, 65]. ADSCs can also be used in combination with other biomaterials, such as platelet-rich plasma (PRP) and hyaluronic acid (HA). Research has been conducted to evaluate the effects of PRP and HA in tissue engineering or tissue repair, favoring wound healing. Clinical application of mixed PRP with fat graft in different dermal diseases (wounds, maxillofacial atrophy, and others) increased re-epithelization significantly when compared to HA and collagen medication [14]. In vitro, PRP increased cell proliferation and synthesis of collagen type I and HA [14]. However, employing the right concentration of PRP cell concentration could affect the outcomes. A systemic review analysis revealed that PRP/media ratio of 1×10^6 Plt/µl provides optimum nutritious supply by avoiding starvation of cells [40]. Therefore, combined administration of PRP or HA with ADSC could enhance the therapeutic effect, in comparison with isolated ADSC administration. The burn treatment therapeutic potential of using ADSCs alone or with platelet-rich plasma (PRP) was previously investigated [17, 18, 37, 38, 70, 71]. In a diabetic burned rat model, combined treatment of PRP and keratinocyte-like cells, isolated from fat, enhanced wound contraction and reduced inflammatory markers [71]. When ADSCs were seeded into PRP gel, they improved skin grafts by enhancing angiogenesis, skin thickness, and collagen arrangement [97]. Recently, it was reported that the treatment with ADSCs seeded in hyaluronic acid hydrogel or gelatin hydrogel provided an effective niche that protects ADSCs and augments their therapeutic potential for burn injury [30, 66]. ADSCs can be combined with other materials and/or used instead of synthetic materials that require a more sophisticated approach.

The other treatment application of ADSC is using ADSC-exosomes. Exosomes are membrane vesicles secreted by cells that operate as a paracrine factor in cell-to-cell communication by regulating inflammatory responses, cell proliferation and differentiation, and neovascularization. ADSC-exosome is composed of different growth factors, such as TGF- β, Growth Differentiation Factor 11 (GDF-11), Insulin-like Growth Factor (IGF) and Wingless 10b (Wnt10b), cytokines, such as IL-6, IL-8, IL-10, TNF, and IL-1 beta, and toll-like receptors (TLR2 and TLR4) [73]. These exosomes direct signaling pathways between and within cells and exert similar effects as their origin cells. Evidence has indicated the secretion of ADSC-exosomes in tissue regeneration and repair. Therefore, exosomes could play a role in burn healing similarly to ADSCs. Recently, research has

shifted toward investigating the impact of ADSC-exosome in wound healing and other diseases. ADSC-exosome promotes wound healing by enhancing proliferation and angiogenesis, mainly when derived from ADSCs overexpressing nuclear factor-E2-related factor2 (Nrf2) [85]. They positively affect wound healing models via Wnt/ß-catenin, PI3K/Akt, and ncRNA H19/miR-19b/SOX9 axis signaling pathways [45, 67, 85, 108]. Consequently, this increases cell proliferation and migration, collagen deposition, and inhibition of inflammation. When ADSC-exosome was co-transplanted on fat graft, it promoted fat graft survival, reduced inflammatory infiltrate, and improved neovascularization [44]. However, the exact underlying mechanism is still unclear, either in the wound or burn healing mechanisms.

A skin substitute is an alternative to a skin graft. Reconstruction of skin substitutes from ADSCs was achieved with promising outcomes [100]. ADSCs were used to regenerate the extracellular matrix endogenously. Then keratinocytes were seeded to achieve the formation of a three-layered skin substitute. With severe burn injuries involving broad TBSA, therapeutic choices are restricted owing to the absence of the donor area. Developing skin substitutes from ADSCs isolated from adipose tissue of the surgically debrided burned skin was achieved, demonstrating vascularized skin structure [16, 74]. Cells differentiated into fibroblast-like cells when seeded into a collagen I-based matrix. They also differentiated into an epithelial layer, a vascularized dermal layer, and a hypodermal layer when seeded into a PEGylated-fibrin-based matrix. Moreover, culturing ADSCs in tropoelastin biometric skin substitute enhanced wound closure and re-epithelization in the animal model [69]. This emphasizes the beneficial role of ADSCs in skin homeostasis and healing and their ability to provide an alternative to wound coverage without the need for exogenous factors. Outcomes from these techniques in severe burn injury are required.

ADSC mechanism of action ADSCs secrete anti-inflammatory cytokines such as interleukin-10 (IL-10) and tumor necrosis factor inducible gene siRNA6 (TSG-6) and pro-inflammatory cytokines, such as IL-1 beta and IL-17 [32]. Therefore, they can modulate the inflammatory response and reduce healing duration, especially the inflammatory phase. They are approved to be safe for clinical use and improve wound healing and other diseases [61]. Fibrogenesis's reduction, angiogenesis, lymphogenesis, skin tissue regeneration instead of scar formation and attenuation of inflammation are crucial characteristics of ADSC in wound healing. However, there are limitations in using ADSC in wound healing that includes cell delivery methods, cell viability, heterogeneity in preparation methods and inconsistent micro-environmental cues [32].

ADSCs have anti-inflammatory and angiogenic effects; which are essential for burn healing. Several experiments in animal models have been undertaken to study the role of ADSCs in thermal burn injuries. ADSCs promoted the proliferation of keratinocytes by mediating keratinocyte growth factor- 1 (KGF-1) and platelet-derived growth factor-BB (PDGF-BB) in mice [2]. ADSC in a conditioned medium lowered apoptosis via the regulation of Bcl-2 and caspase-3 [51]. When ADSCs were compared to bone marrow stem cells and dental pulp stem cells, they showed higher myeloperoxidase activity than other sources [1]. Recently, it was reported that ADSCs reduced scar formation and lymphogenesis and increased collagen III deposition [38]. Others found that ADSC differentiated adipocytes have similar abilities to ADSC and fat grafts [18]. Therefore, ADSCs have therapeutic potential for treating severe burn injuries. However, the long process of isolation, culturing, and expansion of ADSCs increases the risk of contamination, therefore, limiting their usage in clinical applications.

Adipose stromal vascular fraction (SVF)

In the last decade, research has focused on the use of SVF produced from adipose tissues. Since the 1960s, stromal and connective tissue cells were distinguished in adipose tissues. Although adipose tissues were discarded as medical waste in surgical abdominoplasties and lipoaspirates, plastic surgeons and other researchers have documented that this tissue has a regenerative capability [8]. SVF contains a heterogeneous cell population, including adipose stromal/stem cells, endothelial, hematopoietic and pericytic lineages, representing 15–30%, 10–20%, 25–45% and 3–5%, respectively of the total nucleated cells [8]. According to the International Federation of Adipose Therapeutics and Sciences (IFATS) and the International Society for Cellular Therapy (ISCT), stromal cells within the SVF include both non-adherent cells, that cannot be cultured, and cultured stromal/stem cells, called adipose derived-stromal/stem cells [8]. This variation of cells can accelerate healing, modulate immunity, reduce inflammation and communicate locally and systemically through different signaling pathways. They can be phenotypically verified by surface antigen markers, such as $CD45^-$, $CD31^-$, $CD34^+$, $CD73^+$, $CD90^+$, and $CD105^+$ [8]. Even though ADSCs are effective in burn treatment, their isolation from SVF is time-consuming, complicated, and costly. Therefore, it is efficient to use the heterogeneous mix of SVF.

After liposuction, SVF can be isolated directly from the lipoaspirate. To isolate SVF, the standard procedure used initially was the chemical digestion of fat by collagenase, dispase, trypsin or related enzymes. Then, mechanical, non-enzymatic techniques for SVF isolation were developed and applied in clinical practice to avoid contamination of SVF, reduce the duration of the procedure, avoid practical and culturing issues, lower the cost, reduce regulatory burden, and

Fig. 1 illustration of SVF isolation methods from lipoaspirate (mechanical and chemical)
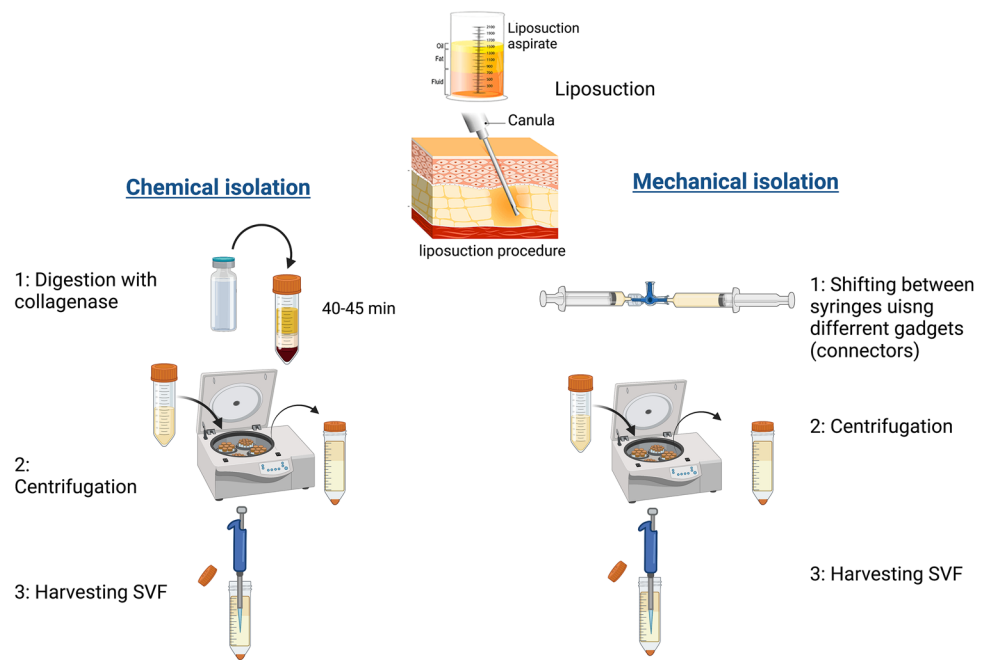
avoid ethical issues (Fig. 1) [99]. For clinical application, many automated devices have been developed to standardize the isolation procedure, increase cell yields, and avoid contamination [57]. The automated isolation of SVF from freshly isolated lipoaspirate is a reliable method as well as the manual isolation, showing similarities in regards to cell yield, viability, and differentiation potential [28]. Developing automated isolation is a promising technique in clinical applications. Due to SVF feasibility and clinical applicability, recent research has shifted to applying SVF for burn or wound injury. Both methods were effective in clinical studies [93]. Research needs to be conducted with standardized isolation methods to compare results.

SVF in burn treatment SVF has shown positive effects in burn healing (Table 1). SVF was used in burn injury alone or combined with other biomaterials. Fat graft burns improved vascularity and decreased fibrosis in burn beds to protect from hypertrophic scar formation [96]. The mechanism behind fat graft treatment includes acceleration of vascular endothelial growth factor (VEGF), stromal cell-derived factor-1 (SDF-1) and decreased fibrotic markers, such as TGF-β and matrix metallopeptidase 9 (MMP-9) [96]. Injection of SVF in the stasis zone of the burn wound preserved cell viability, enhanced re-epithelization and vascularization, and decreased inflammation in the burn model [34]. SVF's effect on vascularization could be due to ADSC's ability to secrete VEGF, FGF2, glutathione, superoxide dismutase, and N-acetylcysteine [34, 77]. Treatment with SVF in deep-partial thickness burned rats showed a reduction in inflammation and increased vascularization and tis-

sue regeneration [5]. Re-epithelization was not measured in this study. Treatment with fat isograft or ADSC or both contributed to scar remodeling by reducing wound depth, area, and apoptotic activity but did not improve vascularization in a mouse model of burn injury [65]. When compared to wound dressing (2% silver sulfadiazine), SVF increased infiltration of the polymorphonuclear cell, fibrin and fibroblast migration, angiogenesis and collagen deposition in the full-thickness burn model [13]. When SVF was compared to human mesenchymal cells in treating a hypertrophic scar, both showed positive results [29]. However, human MSC demonstrated higher expression of anti-fibrotic mediators, such as TGF-β3 and hepatocyte growth factor (HGF), and fibrosis remodeling mediators, such as MMP-2. This suggests that fat not only contribute by biochemical markers but also by improving burn wound outcomes.

Enzymatic isolation, using the collagenase method, is used in the laboratory isolation of SVFs. In clinical practice, mechanical isolation is safer and more reliable. Therefore, Karakol et al. investigated the effect of the isolation method on full-thickness burn using SVF isolated enzymatically and enzyme-free mechanical isolation of skin cell suspension (EMI) as an alternative treatment for burn injury [53]. When SVF treatment was compared with EMI, both applications improved healing histologically. Combined treatment of both applications provided better results than applying each one alone. Both applications can be isolated easily and timelessly, which makes them good alternatives for severe burn injury.

The fate and quality of SVF cells are controlled by the microenvironment that surrounds its niche. Severe burn

Table 1 Research used SVF as a treatment for severe burn injury

Year	Model	Type of burn	Treatment	Outcomes	References
2014	Wistar albino rat	Second-degree	SVF intradermally below the necrotic tissue	+ Vascularity, fibroblastic proliferation, VEGF[†] − Inflammation	[5]
2014	Patients with burns sequelae and scar	–	SVF-enhanced autologous fat grafts + PRP	+ Contour restoring after 1 year	[41]
2016	Wistar rat	Full-thickness burn	SVF	+ Mononuclear infiltration, angiogenesis, fibroblast content and collagen deposition	[13]
2018	Sprague–Dawley rats	–	SVF in stasis zone of burn	+ Angiogenesis, re-epithelization, fibrosis gradient − Inflammation	[34]
2018	Case control	Deep burn	SVF with the MyStem™ device and a scaffold of hyaluronic acid	+ Neoangiogenesis	[42]
2019	Sprague–Dawley rats	Second degree burn	SVF + PRP	+ Wound closure, dermal recovery, vascularization, skin appendages − Inflammation	[54]
2020	Sprague–Dawley rats	Deep dermal burn	SVF + PRP injection and topical	+ TGF-beta[¶] levels in blood	[60]
2020	Nude mice	Irradiation induced lesion	Microfat + SVF	+ Neo-angiogenesis − Wound area	[6]
2021	Sprague–Dawley rats	Full-thickness burn	SVF (enzyme or enzyme-free isolation)	Better histological score compared to control	[53]
2021	Wistar rat	Deep-dermal burn	SVF + PRP	Inhibited local and systematic oxidative response	[52]
2021	Wistar rat	Deep-dermal burn	SVF + PRP (injection or topical application)	Injection increased ↕ VEGF level	[59]

injury response is affected by the quality of SVF or ADSC via increasing the inflammatory response in subcutaneous fat in the un-burned region. To demonstrate the suitable time for SVF or ADSC isolation after burn injury, Prasai et al. [83] demonstrated that SVF and ADSC isolated post-burn injury showed an increase in inflammatory markers and DNA damage (24–48 h) post-burn in an animal model [83]. This suggests that, in severely burned patients, isolation of SVF is recommended after inflammation is resolved.

Regarding using SVF with other biomaterials, researchers investigated the subcutaneous injection of SVF combined with PRP. In the last few years, most studies supported the combined treatment of SVF and platelet-rich plasma (PRP). PRP contains growth factors that are essential for neovascularization. The addition of PRP to SVF was reported to accelerate burn healing by increasing re-epithelization, angiogenesis, fibrosis, and collagen formation. Clinically, treatment with autologous fat graft with SVF or PRP improved graft function and maintenance in burned patients with face scars [41]. In wound injury, the treatment with SVF + PRP enhanced re-epithelization and angiogenesis and decreased inflammation when compared to using SVF or PRP alone [54]. In burn injury, the combined application of SVF and PRP, either by injection or topical administration, was found to accelerate the angiogenic growth factor VEGF serum levels during the healing [60]. VEGF is a growth factor that

mediates vascular permeability and regulation during angiogenesis. The injection route was more effective than the topical application for increasing VEGF release. However, both routes were found to increase the expression of TGF-beta up to 14 days [60]. The combined treatment of both SVF and PRP decreased oxidative stress products, such as malondialdehyde, and nitric oxide, in both blood and tissues post-burn [52]. SVF therapeutic potential can be enhanced with other biomaterials, such as PRP or non-cultured stem cells. Further research is required to assess the effectiveness of using biomaterials with SVF and the optimum methods and dose for severe burn injury.

In wound and burn injury, several studies investigated the role of LASER and LED in the healing process. Both of them promoted healing by reducing inflammation, increasing proliferation, angiogenesis, and forming collagen and granulation tissue [26, 27, 56]. Treatment with both SVF and photobiomodulation enhanced burn healing in a dose-dependent manner [62]. The addition of photobiomodulation (L30) promoted healing by augmentation of the inflammatory response, angiogenesis, fibrosis contraction and collagen concentration. Low-level light using pulsed light-emitting diode light on SVF enhanced cells' vascularization and proliferation capacity [84]. Moreover, clinically, combined treatment of fractional laser and microfat improved scar appearance in burned patients [81]. Therefore, combined

administration of SVF with photomodulation could improve treatment outcomes.

Fat grafting is one of the treatment options for severe burn injury [96]. Fat grafting has been used effectively to treat numerous skin abnormalities since it requires minimal invasive procedures (liposuction). Sometimes the repeated transfer is required. However, grafting could lead to scarring, when incorporated more than 2 mm away from the blood supply, and retention, due to poor vascularization and loss of applied fat. Therefore, extensive research has been conducted to improve fat grafting survival. Cell-enriched fat grafting could include either ADSCs or SVF. ADSCs-enriched fat grafting improved graft retention and fat survival significantly in the animal models [72, 89]. SVF-enriched fat grafting improved face scar appearance in burned patients [41]. SVF-enriched fat graft increased elasticity and resilience and decreased skin contraction [19]. Histological and molecular analysis revealed that SVF increased the thickness and vascularization of the skin by elevating the expression of different growth factors, such as endothelial growth factor (EGF), basic fibroblast growth factor, and hepatocyte growth factor.

Clinically, patients with burns sequelae treated with SVF-enhanced autologous fat graft showed around 63% maintenance contour restoring when compared to patients treated with fat graft alone (39%) [41]. SVF was used to treat post-burn scars showing promising results with improvement in the scar appearance, Vancouver Scar Scale (VSS), and patient satisfaction following treatment [42, 93, 107]. In a case study report of second-degree deep facial burn, applying SVF on the burned face provided good cosmetic outcomes and functional results for the face [106]. Previous investigations were conducted in a smaller group, therefore there is a need to conduct more studies with larger cohort groups.

SVF demonstrated positive effects in the wound and burned injuries treatment either when used alone or combined with other treatment options. However, the exact mechanism behind SVF healing properties for burn injury is not fully understood. Differences in application techniques, including cell counts, purification and delivery methods, lead to a lack of consistency in the documented results. For instance, most of the studies reported that SVF was administered by injection, intradermally or subcutaneously. In contrast, others did not specify the route of administration and the site, such as stasis zone, center, or edges of the burn wound. Therefore, there is a need for establishing the optimal cell count and isolation methods regarding injured surface area to have better healing outcomes.

SVF mechanism of action SVF transplantation can have direct (differentiation into other cell types) or indirect (paracrine) effects. SVF could be the differentiation into endothelial cells, keratinocytes or fibroblasts, etc. The paracrine role is crucial for burn healing through modulating growth factors, inflammatory, angiogenic, and fibrosis mediators. This leads to the activation of different signaling pathways. The differentiation capability of cells within SVF is well-documented [8, 18].

Even though lots of researchers investigated the effect of SVF in wound healing, the exact mechanism behind its healing effect remains unclear. Recent research has investigated the mechanisms behind SVF healing properties. Different signaling pathways are activated in animal models in wounded skin samples treated with SVF [7]. During wound healing, dermal fibroblasts play an important role in dermal thickness by activating extracellular matrix synthesis and growth factors. Fibroblast migration and angiogenesis are crucial for providing blood supply to the wound bed. One of the involved factors in vessel contraction is Leiomodin 1, which plays an important role in vasoconstriction. In wound injury, RNA seq analysis revealed that treatment with SVF decreases the mRNA expression of *Endothelin1* and *Leiomodin 1*, thus leading to vasodilation and improving local blood supply in the wound bed. Moreover, immunohistochemical analysis showed higher vascular endothelial growth factor (VEGF) expression in stromal cells post-burn in rats treated with SVF compared to control [5]. This indicates an increase in blood supply to the wound bed, which indicates SVF's role in angiogenesis.

One of the most activated pathways in wound injury treated with SVF was cytokine-cytokine interaction and chemokines [7]. Gene expression analysis revealed that the cytokine-cytokine receptor interaction" pathway is activated post-SVF treatment and upregulated *Osm*, *Ccl4*, and *Ccr1* gene expressions. The Cytoscape analysis showed an increase in OSM, an IL-6 family protein, which is secreted by macrophages and inhibits the expression of inflammatory cytokines (TNF and IL-1B). Different chemokines are activated after SVF treatment, including CCL2, CCL4, CCL6, CCL7, and CCL9, which attract macrophages to the wound site. Both OSM and chemokines are upregulated by SVF, which chemoattracts macrophages and activates the anti-inflammatory response in the wound site.

Laminins play an important role in re-epithelization and angiogenesis by regulating growth factors that aid in cell adhesion. After treatment with SVF, *Lama3*, *Lamb3*, and *Lamc3* were upregulated in fibroblasts and endothelial cells in the wound site to promote wound healing [7]. Integrins, transmembrane cell surface receptors that regulate cell adhesion and migration, were found to be upregulated after treatment with SVF, such as *a2b, a6, a7, b4*, and *b7* [7]. Treatment with SVF upregulates gene expression of re-epithelization growth factors, such as EGF, stromal cell-derived factor (SDF-1 or CXCL12), neutrophil-activating protein-2 (NAP-2 or CXCL7) [15]. This shows the role of

SVF in regulating the interaction in the extracellular matrix and collagen deposition in wound injury.

It was suggested that both SVF and ADSCs regulate fibroblast and endothelial functions by mediating different signaling pathways that regulate angiogenesis, cell adhesion, collagen deposition, and reducing inflammation in wound injury [7]. It was reported the mechanism of SVF treatment in wounded animal models. However, SVF healing mechanism in the burn was not investigated yet. Further studies are required to fully understand the exact mechanism behind the healing capacity of SVF in burn injury. To our best knowledge, the morphological and histological properties of exosomes isolated from SVF were not studied in burn injury.

Discussion

Thermal burn injury is one of the common health problems globally. A remarkable understanding of the pathophysiology of thermal burn has been achieved so far. Severe burn injury is associated with many complications, including social, functional, and psychological conditions. However, effective treatment of severe burns is still under development. The standard care for severe burn injury is early excision of necrotic tissue and autografting. This treatment minimizes infection hospitalization and reduces hypertrophic scar formation. Standard of care for burn injury is sufficient for superficial, partial- and deep partial-thickness but not for a full-thickness burn with high TBSA. In severe burns with large TBSA, wound closure could delay the healing process, which may increase the risk of infection result in poor aesthetic and functional results. Therefore, interventions are required for best wound closure of severe burn injury.

One of the promising treatments in burn healing is cell therapy. Mesenchymal stem cells from different tissues can be isolated and used for tissue regeneration. Isolation methods, cells' yield, and application techniques are crucial factors in choosing the best candidates for treating severe burn injury. Several studies have investigated the effect of ADSCs in the burn's healing process. ADSCs can improve burn healing by reducing scar formation, cell infiltration, and inflammation, increasing angiogenesis, re-epithelization, granulation, and forming hair follicles and sebaceous glands. Different applications have been developed for using ADSCs in severe burn treatment, either alone or combined with other treatments, such as forming scaffolds with several biomaterials. Promising results were reported from treatment with ADSCs when combined with PRP. However, optimal methodology, including the administration route, timing, dose, and duration, should be optimized for better results. The long time needed for ADSCs culture, which might lead to contamination, highlighted the importance of using uncultured SVFs.

The discovery of SVF from adipose tissues evoked their use for the reconstruction of skin tissues. Many reasons favored using SVF, including cell's heterogeneity, applicability in clinical settings, avoidance of contamination, culturing and ethical issues, reduction of isolation time and cost. SVF is used to amplify the human natural repair and regenerative system by accelerating the number of autologous healing cells into damaged tissues. Autologous stem cells are personalized with a person's DNA and biological profile. SVF cannot be manufactured since it has four different stem cells (mesenchymal, hematopoietic, endothelial, and pericytic progenitors). In contrast, treatment with one type of stem cells, such as ADSC, contains one type of cells and may or may not be autologous. Moreover, stem cells' healing properties could be achieved through their differentiation capability and their paracrine effect. Therefore, SVF has several key advantages that favor using it for severe burn injury.

SVF was first isolated using enzymatic methods since they were proven to be effective. However, enzymatic isolation is a time consuming, expensive, requires purification and destroys cell niche. Therefore, research was directed to use mechanical isolation of SVF. The advantage of mechanical isolation is its reliability, simplicity, minimal tissue manipulation, and protection of extracellular matrix and stromal cells. Tonnard was the first to use the term 'nanofat' for the mechanical isolation of SVF [98]. Many authors then rejected this term since it is not a fat; it consists of stromal cells obtained from adipose tissues. After that, many terms were used for the mechanical isolation, such as fragmented fat, mechanical stromal cell and total-stromal cells [25]. To our best knowledge, there is no standard methodology or protocols for the mechanical isolation of SVFs. The isolation depends on shifting lipoaspirate between Luer-lock or by adenizing [24]. New automated devices have been invented to isolate SVF from lipoaspirate for clinical applications. They showed reliability for cells' viability and differentiation.

SVF's quality could be affected by several factors, such as harvesting site, age, isolation techniques, and health condition. For instance, SVF's isolation from subcutaneous fat in the abdomen demonstrated the best results when compared to other sites of the body since it has richer soluble factors [43]. In females, cell yields obtained from SVF significantly declined with age [31]. Enzymatic harvesting methods give higher cell yields when compared to the mechanical method. However, the enzymatic method is time-consuming (80 min) compared to the mechanical method (15 min) [88]. All these factors should be considered to choose the best method for patients with severe burn injury. All these factors should be considered to choose the best applications for patients with severe burn injury.

The fat injection has been used in severe burn injury and was found to enhance tissue regeneration and improve scar quality [58]. For SVF feasibility, cellular abundance, multilineage differentiation potential, and heterogeneity, SVF has been investigated for burn injury. SVF enhanced burn healing by stimulating angiogenesis, re-epithelization, and reducing inflammation and scarring. The inconsistency in documented results could be due to the differences in therapeutic dose of cells, in the stage of replication and differentiation and in the isolation procedure of SVF. More research is required to optimize the use of SVF in severe burn injury and to understand the underlying mechanisms behind its healing properties. In animal models, a dose of 10×10^6 cells or more can induce a therapeutic effect on burn healing. Route of administration can be intradermally or subcutaneously. Injection could be in the stasis zone, at the edge or the center of the burn injury. The timing of SVF injection for severe burn injury is crucial and was not consistent in most studies. Some suggested that stem cell quality is better after the resolution of the inflammatory phase [10]. However, others injected SVF directly or within 24–48 h post-burn and healing was improved significantly [83].

Research has revealed the capability of SVF to activate different signaling pathways. SVF accelerates vasodilation mediators to improve the blood supply to the injured tissues, cytokine-cytokine interaction to attract macrophages and reduce inflammation, growth factors that improve adhesion, re-epithelization, and angiogenesis. Further investigation is required to understand the exact mechanism of SVF injection post-injury. Moreover, the role of exosomes in burn injury should be investigated.

Both ADSCs and SVF can be used alone or combined with other biomaterials to develop skin substitutes. Most research has investigated ADSCs effect when combined with other treatments, such as PRP, HA, scaffolds and grafts. However, further investigation is needed to better understand the effect of using SVF alone or combined with other treatments in severe burn injury. SVF therapeutic potential can be enhanced when combined with other biomaterials, such as collagen and PRP. Most studies supported the combined treatment of both SVF and PRP for burn injury [52, 60]. Treatment with SVF and PRP or non-cultured stem cells improved graft function and maintenance in burned patients with facial scars. Combined treatment with both SVF and photobiomodulations enhanced burn healing through increased proliferation and neovascularization in a dose-dependent manner.

The standard care of severe burn injury includes the surgical debridement of necrotic tissues with eschar, which involves the removal of subcutaneous tissues and intact adipose tissues. A new approach of using debrided skin from severe burn as a source of adipose-derived stem cells has been investigated by Natesan [75]. The hypodermal adipose region has viable stem cells that can differentiate into multi-lineages. When isolated stem cells from debrided skin were used to create scaffolds, they decreased wound contraction and enhanced re-epithelization [101]. This new approach is promising for patients with high TBSA burn and lower donor sites. This approach could be investigated in different experimental clinical settings. In addition, fat-cellular bio-banking should be considered as a treatment option in the future [40].

Treatment with SVF is safe and appropriate in medical intervention because it's easy to apply and does not cause an allogenic reaction or ethical problems. However, it is difficult to perform it in the emergency setting. Treatment with SVF needs a sterilized surgical room supplied with a centrifuge or automated SVF device which cannot be performed in the clinical setting.

With advances in burn wound treatment, patients' quality of life will be improved. The majority of patients willing to choose autologous stem cells application for burn injury or other diseases [23]. In massive burn, covering TBSA around 81%, treatment with cultured epithelial autografts showed satisfying results specially in young age patients and with low level of infectious complications. Currently, new techniques have promising therapeutic potential for severe burn injury but need further investigation. Without a standardized procedure for using SVF in severe burn injury, its treatment outcome would not be reliable.

Declarations

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