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Mechanically isolated stromal vascular fraction improves healing of deep-partial thickness burn in rat model

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ABSTRACT

Background: Despite the advances in treating burn injuries, burn severity remains one of the most challenging cases to be treated. As technologies advance, regenerative therapies using stem cells have been established and found to be effective and friendly as a regenerative tool. Stromal vascular fraction (SVF), extracted from adipose tissue, has a great potential for multipotent mesenchymal stem cells differentiation. Recent research has determined the therapeutic effects of SVF on burn injury. Previous finding has proved the efficacy of using SVF that is isolated enzymatically. This research aimed to examine the efficacy of allogenic mechanically isolated (MI) SVF in treating deep partial-thickness burns in a Wistar rat.

Materials and Methods: After burn induction, 45 rats were divided into three groups of 15 as follows: group one (control, treated with phosphate-buffered saline), group two (treated with silver sulfadiazine (SSD), and group three (injected with SVF). SVF was harvested from the inguinal fat pad of six rats and mechanically processed. All injections were administered intradermally at the four edges of the burn to cover the entire wound bed. Morphological and histopathological analyses were performed for all groups at three different time points (4, 8, and 32 d post-treatment).

Results: Treatment with MI SVF significantly reduced edema formation and dryness of the wound bed on day one compared to the control (P = 0.001). Histopathological results showed that SVF significantly reduced inflammation compared to the control (P = 0.045) on day one and increased neovascularization on day 8 (P = 0.016). Epithelial thickness was significantly greater in the SVF group compared to the SSD group (P = 0.034).

Conclusions: The results of this study indicated the therapeutic potential of MI SVF on deep-partial-thickness burns by increasing neovascularization and epithelial thickness and reducing inflammation.

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INTRODUCTION

Burn could be fatal to injured patients worldwide, with over 73 million injuries globally and around 180 000 deaths annually[1]. For severe burn injury, burn management depends on the burn's size, degree, and the availability of normal skin. Deep-partial thickness burn usually takes long period of time for healing and requires systemic and local treatment. It could end with delayed healing, abnormal healing or abnormal scar formation, depending on the presence of complications in patients. Treatment should be able to enhance healing, reduce fibrosis and improve skin's thickening. Therefore, new and improved methods are required to improve treatment options.

Over the last decade, biological therapies have been used as alternatives to pharmacological and surgical treatments. Research has shifted to using mesenchymal stem cells for treating different types of injuries[2,3]. Mesenchymal stem cells isolated from bone marrow (BMSC) and from adipose tissue (ADSC) have proven their efficacy in treating burn injuries[4-8], by improving skin angiogenesis, collagen deposition, and re-epithelization and reducing inflammation and scarring [9,10].

KEYWORDS:
Stromal Vascular Fraction, burn healing, mechanical, adipose derived stromal stem cells

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However, using both BMSC and ADSC for treating burn injury requires time for their isolation, culturing, and expansion, which could increase the risk of contamination, causing difficulties in clinical applications. Hence, research has shifted toward using a diverse cell population isolated from adipose tissues, called the stromal vascular fraction (SVF).

Since the establishment of stromal cells and connective tissue cells in adipose tissues, the regenerative capability of these cells was documented[11]. SVF contains stromal, pericytic, endothelial, and hematopoietic lineages. SVF contains two types of stromal cells; adipose-derived stromal/stem cells, which are cultured cells, and cells that cannot be cultured. Stromal cells in tissues have reparative, regenerative, and immunological effects on injured tissues[12]. SVF cells can be verified using cell surface identification markers such as $CD45^-$, $CD31^-$, $CD34^+$, $CD73^+$, $CD90^+$, and $CD105^+$ [11].

After liposuction, SVF can be harvested via enzymatic or mechanical processes. Enzymatic isolation (EI) depends on the enzymatic digestion of fat by collagenase, dispase, and trypsin. In this technique, the enzyme destroys the extracellular matrix, then stromal cells are collected by centrifugation. This isolation technique has been used in both research and clinical settings. However, this technique requires an enzyme purification step, which destroys the microenvironment called the cell niche that surrounds cells and aids in cell communication, proliferation, and differentiation. Recently, a mechanical, non-enzymatic, isolation (MI) method, has been developed to reduce the timing of the procedure, avoid contamination of cells and culturing, lower the cost, reduce the regulatory burden, and avoid ethical issues [13]. Compared to EI, MI protects both stromal cells and the extracellular matrix. Tonnrad *et al*. (2013) described a MI method using Luer-Lock syringes. It depends on the mechanical emulsification of lipoaspirate by shifting fat many times to destroy adipose cells, followed by filtration to obtain a liquid suspension [14]. Recently, different tools and devices have been developed to optimize MI to provide minimal manipulation of cells and clinical accessibility. Previous research showed that cell viability and cellular composition did not differ between using non-enzymatic and enzymatic procedures, with slight differences in cell counts[15,16]. Therefore, the treatment in this study used the MI of SVF by microlization. This technique of microfragmentation uses sharpened-edge microblades with a honeycomb design. Previous tests on this gadget showed a high ratio of cell count and viability (around 90%) and the presence of mesenchymal cell markers (CD90 and DC105) [17]. Therefore, this technique offers a cost-effective option with desired cell count and viability.

Previous research has investigated the effect of EI SVF on burn injury when used alone or in combination with other treatments. SVF has improved burn wound healing by reducing inflammation and increasing neovascularization, re-epithelialization, collagen deposition, and tissue regeneration [17-20]. This study aimed to examine the therapeutic potential of allogenic treatment with non-enzymatic or MI SVF in treating deep-partial thickness burn injury and investigate the morphological and histopathological effects post-treatment at three different time points, at 4, 8, and 32 d, in Wistar rats.

METHODS

Experimental animals

This study was approved by the Research Ethics Committee, Faculty of Pharmacy, King Abdulaziz University (Ref: PH-1443-21). Fifty-one male Wistar rats, weighing 265 ± 31 g, were included in this study. All rats were kept at (25 ± 1°C) with a 12-h light vs. dark cycle. They were fed with standard laboratory chow with free access to water. In this study, forty-five rats were assigned into three groups randomly, and six rats were used as donors for adipose tissue extraction. Treatment groups were assigned as follows: control rats (n=15) were treated with saline solution (Group 1, C). In the second treatment group, rats (n=15) were treated with silver sulfadiazine cream (Flamazine®) (Group 2, SSD). Rats from the third treatment group (n=15) were treated with MI SVF (1×10^6 cells/rat) intradermally (Group 3, SVF). The experiment was run for 32 days. The day of burn induction was considered as day (D0) of the experiment and day (1) was the treatment day (D1). Five rats of each group were euthanized on days 4, 8, and 32 (D4, D8 and D32). Day 4 (D4) represents the inflammation phase, day 8 (D8) represents the proliferation phase and day 32 (D32) represents the remodeling phase of burn healing. The cervical dislocation was used to euthanize all rats at the end of the study period.

Deep-partial thickness burn induction

In each rat, the dorsal skin was shaved and disinfected. The burn was induced to the dorsum area by using flat, round-bottom iron stamps 1.8 cm in diameter weighing 22 g for 6 seconds without applying pressure (the stamp was allowed to rest on its weight 22g). All burn induction was done by the same person to ensure the consistency of all wounds. The induction of deep-partial thickness was evaluated by histopathological analysis. The reliability of this method has been validated previously [21], [22]. Ringer lactate solution was used to resuscitate animals immediately to prevent spinal shock intraperitoneally (2 ml/100 g body weight) [21]. Every rat was kept individually in a separate cage bedded with a cotton pad and changed daily for 5 days to minimize wound infection.

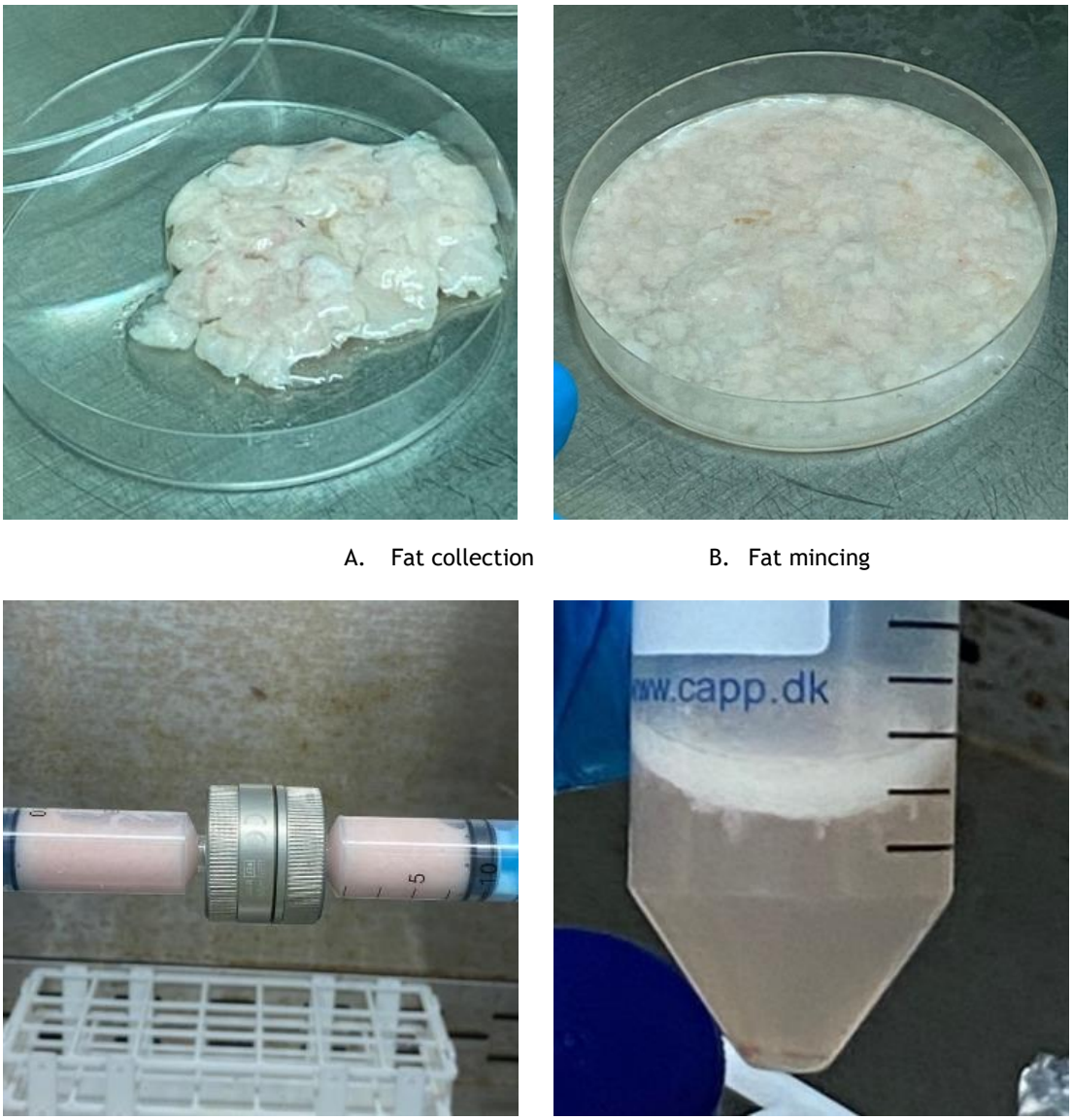
Pain and distress measurement

Post-burn, 2 mg/ml paracetamol was added to rats drinking water for three days[22]. Pain and distress change was examined based on four physical signs (the body weight, grooming, hair coat, animal's appetite, and animal activity and posture). The measurements were observed and recorded daily by the same researcher.

SVF mechanical isolation and preparation

From six rats, the inguinal area was disinfected, and the inguinal adipose tissue was collected as previously described[17]. The fat tissue was washed with normal saline solution and finely minced using two sterilized scalpels. The minced fat was transferred to mycrolizer (BSLrest Inc., Turkey). This gadget provides a closed filtration system by using female-to-female luer-lock adaptors for connecting two syringes together. The fat was shifted manually for 10 times between two syringes to achieve emulsification of fat [23]. The emulsified fat was centrifuged at 400x for 5 minutes to separate the SVF. The fat layer and supernatant were discarded. SVF pellet was washed with saline and centrifuged twice. Then, cells were filtered over the sterile nylon cloth (100 µm) and the effluent was collected in a sterile tube and centrifuged at 400× g for 5 min (Figure 1)[17,18]. After counting cells using Trypan blue, SVF solution was prepared with normal saline resulting in cell volume equivalent to 1×10^6 cells/ml. On the same day of MI of SVF, total cells were injected intradermally at the four edges

of the burn to cover all the wound bed[18,23].



A. Fat collection B. Fat mincing

C. Mechanical isolation of SVF D. SVF pellet

Figure 1: SVF isolation process.

Wound contraction

All photos of rats were taken from the same distance (24 cm) by the same researcher every two days for evaluating the wound contraction. The following equation was used to measure the wound contraction percentage change:

$$CL = \frac{\text{Original wound area} - \text{specific day wound area}}{\text{Original wound area}} \times 100$$

CL= contraction level, Original wound area= (size of the wound on day 1), specific day wound area= the area of wound measured on that day [24]. Wound images were scanned using an image-J analysis program (ImageJ; National Institute of Health, Bethesda, MD, USA) to calculate the wound space. Wound contraction was measured on day 7, 15, 21, 29, and 32 to evaluate wound contraction for deep partial-thickness burns.

Macroscopic analysis

The macroscopic change was examined based on four criteria: skin color, edema, wound bed dryness, and hair growth (Table 1) [24,25]. Changes were examined and recorded daily by the same researcher.

Table 1: Macroscopical wound changes grading system in burn wound healing

Criteria	Score	Parameter
Color (Redness)	0	Absence of color
	1	Presence of color
	2	Mild of color
	3	Moderate of color
	4	High color
Edema	0	Absence of edema
	1	Presence of edema
	2	Mild of edema
	3	Moderate of edema
	4	High edema
Wound bed dryness	0	Absence - wound bed is wet
	1	Presence of dry wound bed
	2	Mild dry wound bed
	3	Moderate dry wound bed
	4	High – complete dry
Hair Growth	0	Absence of hair growth
	1	Presence of hair growth
	2	Mild hair growth
	3	Moderate hair growth
	4	High hair growth

Histological analysis

After H&E staining, the entire wound was evaluated based on three parameters: inflammation, neovascularization, and re-epithelization, and the presence of skin appendages, such as hair follicles and sebaceous glands, on days 4, 8, and 32 of the experiment duration. Measurements were conducted using the Philips IntelliSite Pathology Solution (Philips Digital Pathology Solution, Netherlands) software. The measurements of inflammation, neovascularization, and re-epithelization were conducted for all samples from 20 different areas for every tissue specimen using a grid (0.1 mm2) and graded as described in (Table 2)[17,26,27].

Inflammation

The inflammation was assessed by counting the inflammatory cell density and graded at (x40 magnification) from 20 different areas for every tissue specimen. The mean of the total count was estimated for every tissue specimen. All samples areexamined by the same researcher blindly.

Neovascularization

The neovascularization was assessed by counting newly formed vessels for every tissue specimen at (x40 of magnification) from 20 different areas for every tissue specimen. The mean of the total count was calculated for every tissue specimen. All samples were examined by the same researcher blindly.

Table 2: grading of different histopathological criteria used in this study

Criteria	Score	Parameter
Inflammation (x40)	0	Normal (neutrophil count <20/field)
	1	Moderate - many inflammatory cells (neutrophil count 20-300/field)
	2	Sever - exaggerated inflammatory cellularity (neutrophil count >300/field)
Neovascularization (x40)	0	Absence of vascular formation
	1	Presence of vascular formation (capillary count 1-5/field)
	2	Discrete vascular formation (capillary count 6-10/field)
	3	High vascular formation (capillary count >10/field)
Re-epithelization (x5)	1	Discrete – partial re-epithelization with a small epithelial layer (the epithelial tongue occupies, at most 1/3 of the wound gap)
	2	Moderate - partial re-epithelization with a longer new epithelial layer (the epithelial tongue occupies more than 1/3 of the wound gap)
	3	Complete re-epithelization

Re-epithelization

The re-epithelization was assessed by the length of the newly formed epithelial layer by measuring the ratio between the length of the wound gap and the length of the epithelial tongue. The calculation was done for every tissue specimen at (x5 magnification) as described previously[28]. The mean of the total count was calculated for every tissue specimen. All samples were examined by the same researcher blindly.

Measuring epithelial thickness

The thickness of the epithelial layer was assessed on day 32. Measuring the distance between the stratum basale and the stratum corneum of the epithelial layer in each tissue specimen was measured at (x20 magnification) from 20 different areas for every tissue specimen as described previously[19]. The mean of the measurements was estimated for every tissue specimen. All samples were examined by the same researcher blindly.

Skin appendages regeneration

The skin appendage regeneration was assessed by the hair follicles and sebaceous glands presence on day 32. A grid was used (0.1 mm^2) to count 20 different areas for every tissue specimen at (x10 magnification). The mean of the total count was calculated for every tissue specimen.

Statistical analysis

Statistical analysis was performed using SPSS Win 10.0 software and data were presented as mean ± standard deviation. Normality was tested for all data. The mean was compared between groups using one-way ANOVA and non-parametric Mann-Whitney U test and Kruskal-Wallis H test or t-test were used, depending on normality results The significant level was considered as *P* value less than 0.05.

RESULTS

Deep-partial thickness burn induction

Burn injury was induced using two duration of time (6 and 10 seconds) to ensure burn degree before proceeding with the experiment. Histological analysis showed that scalding for 6 seconds induced partial damage up to the muscular layer while scalding for 10 seconds induced full damage to the muscle layer. Therefore, deep-partial thickness burn was created in all experimental rats by exposure to 100 ° C for 6 seconds.

Pain and distress measurement and management

Pain and distress changes including, the body weight, grooming, hair coat, animal appetite, and animal activity and posture, were examined during the experiment at D1, D4, D8 and D32. On D1, body weight of rats from SSD group was reduced significantly compared to the C group (*P* = 0.032). However, there were no significant differences between all treatment groups at D4, D8 and D32 (Figure 2). Other distress markers did not appear to be significantly different between all treatment groups (*P* = 1). No death was recorded for all experimental rats.

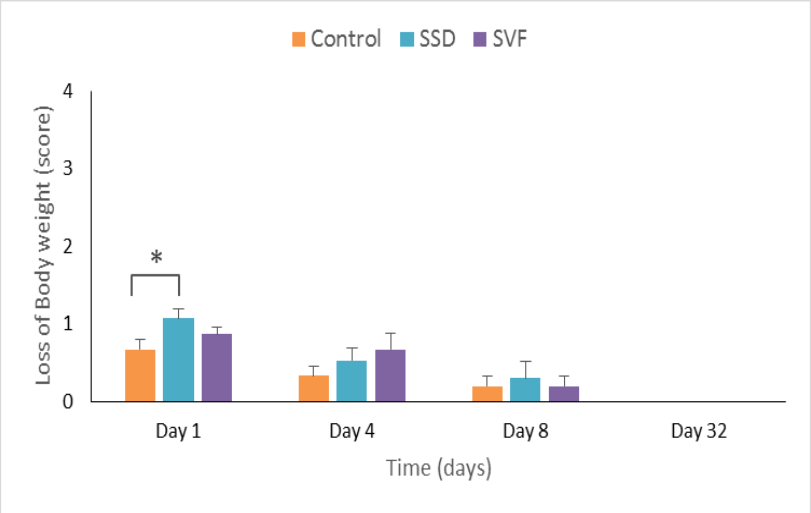


Figure: Meaasurement of body weight on days 1,4, 8, and 32. Control: Normal saline (n=15), SSD: Silver sulfadiazine (n=15), and SVF: Stromal vascular fraction (n=15). Data are expressed as mean ± standard error. * Indicates p<0.05 for comparison between control and SSD groups

SVF preparation

After SVF isolation, a total of 8.12 $X10^6$ cell/ml were isolated and then calculated as described above to be injected in each rat. Cell viability was 98%.

Wound contraction

Wound contraction was measured at D1, D7, D15, D21, D29 and D32. There were no significant differences between all treatment groups (*P*= 1, 0.2, 0.4, 0.2, and 0.4, respectively for all days) (Figure 3).

Macroscopic analysis

Skin color, edema, wound bed dryness, and hair growth were measured for all groups on D1, D4, D8, and D32 to evaluate the treatment's effect on deep partial-thickness burns. At D1, the burn wound area exhibited swelling and edema. Only the SVF group recorded a significant reduction in edema formation on D1 compared to the C and SSD groups (*P*= 0.001 and 0.001, respectively). There were no significant differences between the control and SSD groups. Similarly, SVF group showed a higher rate of dryness on day one compared to C and SSD groups (*P*= 0.001). There were no significant differences between all groups for other criteria. All groups showed hyperemia. All animals recorded no hair growth during the experiment duration (figure 4).

Histological analysis

Inflammation

On day 4, SVF showed a significant reduction in inflammation compared to the C group (*P*= 0.045). However, the reduction was not significantly different compared to the SSD group (*P*= 0.465). There were no significant differences between all groups on day 8 and 32 (*P*=0.5 and 1; respectively) (figure 5)

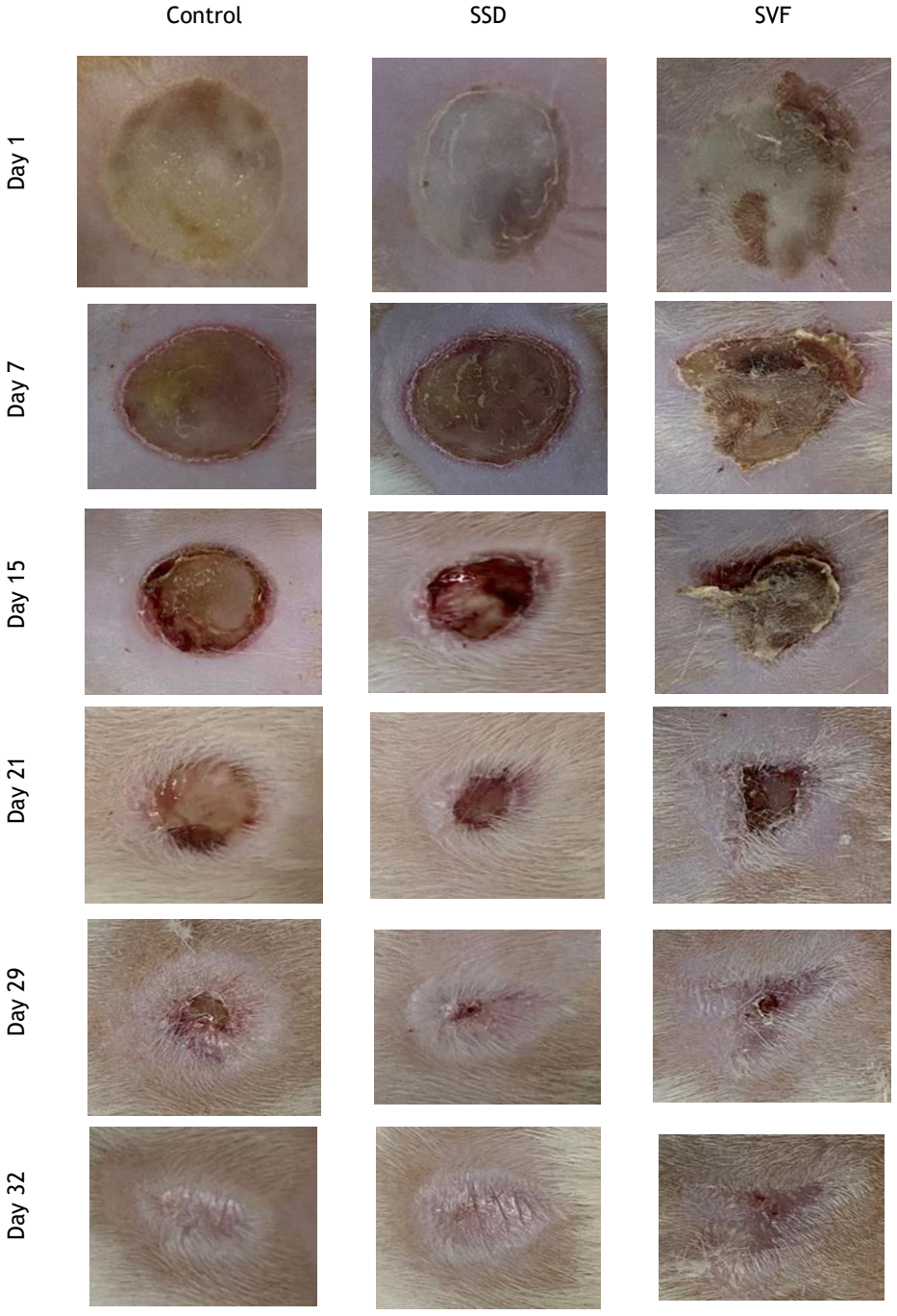
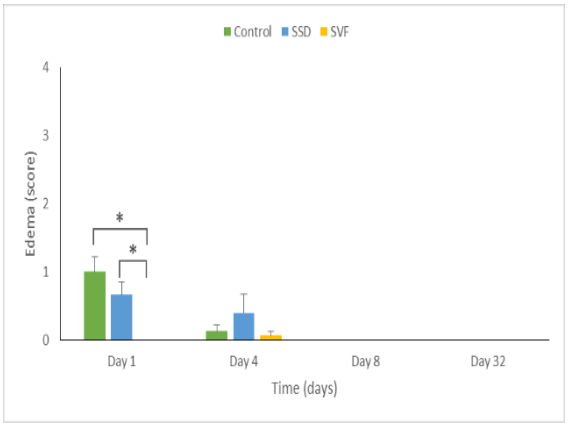


Figure 3: Photographs showing wound contraction rate of rat skin burned wounds on days 1, 7, 15, 21, 29, and 32 post-treatment. Control: Normal saline (n=15), SSD: Silver sulfadiazine (n=15), and SVF: Stromal vascular fraction(n=15)

Neovascularization

Examination of vascular density showed no significant differences between all groups on D4. Rats from SVF group showed significantly higher neovascularization compared to the C group (*P*= 0.016) at D8. However, no significant differences were noted in comparison with the SSD group (*P*= 0.340) (Figure 6). Similarly, at D32, no significant differences were recorded between SVF group compared to the C group (*P*= 0.806), and to the SSD group (*P*= 0.340); and between the SSD group and the control group (*P*= 0.623).



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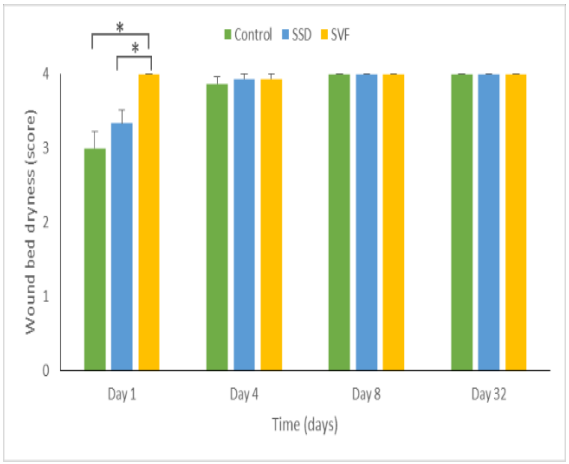

Figure 4: Macroscopic burn wound changes of (a) edema and (B) the wound bed dryness of the deep partial-thickness burn wound on days 1,4, 8, and 32. Control: Normal saline (n=15), SSD: Silver sulfadiazine (n=15), and SVF: Stromal vascular fraction (n=15). Data are expressed as mean ± standard error. * Indicates p<0.05 for comparison between groups.

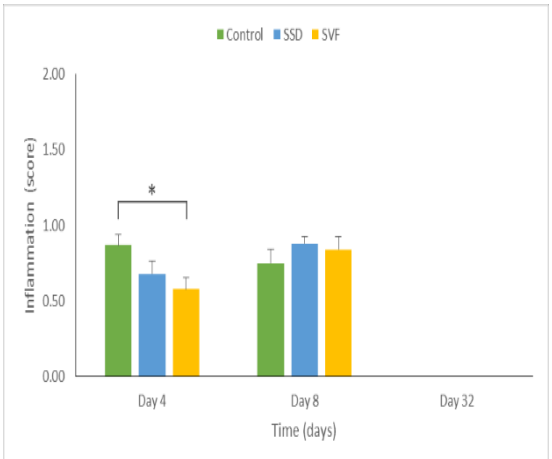

Figure 5: Comparison of inflammation scores between the study groups in deep partial-thickness burn wounds on days 4, 8, and 32. Control: Normal saline (n=14), SSD: Silver sulfadiazine (n=15), and SVF: Stromal vascular fraction (n=15). Data are expressed as mean ± standard error. * Indicates p<0.05 for comparison between control and SVF groups.

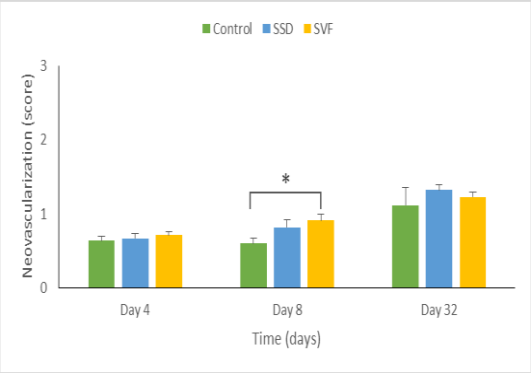

Figure 6: A. Comparison of neovascularization scores between the study groups on days 4, 8, and 32. Control: Normal saline (n=14), SSD: Silver sulfadiazine (n=15), and SVF: Stromal vascular fraction (n=15). Data are expressed as mean ± standard error. * Indicates p<0.05 for comparison between control and SVF groups.

Re-epithelization

The re-epithelization evaluation determined clear signs of recovery in all study groups post-treatment, but no significant differences between all treatment groups were recorded.

Epithelial thickness

The epidermis layer was completely formed as mentioned above on day 32 post-treatment in all groups. The SVF group showed a thicker epidermis layer (87.47 ± 15.575) than the C group (76.13 ± 25.625), but it was not significant (*P*= 0.436). The epidermal thickness of SVF group was significantly thicker than SSD group (58.72 ± 19.832, *P*= 0.034) (Figure 7).

Appendages

The SVF group showed higher presence of skin appendages (1.65 ± 0.799) than the C group (0.79 ± 0.595), but it was not significant (*P*= 0.117). Rats from SSD group showed lesser presence of skin appendages, but it was not significant compared to the SVF group (1.18 ± 1.268, *P*= 0.503). No significant differences were recorded between the C group and the SSD group (*P*= 0.589).

DISCUSSION

The aim of burn treatment is to avoid complications, enhance tissue regeneration, and restore skin functionality. In severe burn injuries, the treatment depends on the burn's severity and size. For severe burn injury, the standard treatment depends on using skin grafting or skin substitutes for full-thickness burn while deep-partial thickness burn usually has delayed healing duration and could lead to scarring. Therefore, there is a need for interventions to reduce fibrosis and enhance burn healing outcomes. Regenerative therapies, using SVF, could provide effective and friendly treatment options.

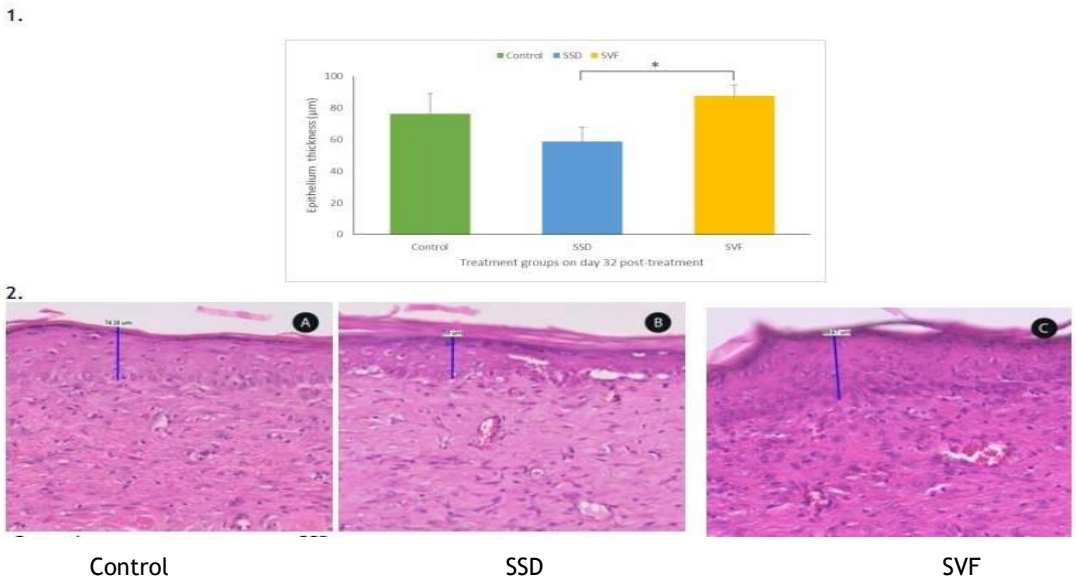

Figure 7: 1. Microscopic evaluation of epithelium thickness (µm) changes in deep partial-thickness burn wound on day 32. Data are expressed as mean ± standard error. * Indicates p<0.05 for comparison between SVF and SSD groups. 2. Histopathological image showed the epithelium thickness on day 32 post-treatment at x20 (H&E, scale bar: 50 µm). (A) Control: Normal saline (n=14), (B) SSD: Silver sulfadiazine (n=15), (C) SVF: Stromal vascular fraction (n=15). Blue line indicates the epithelium thickness (µm).

Burn wound healing requires cell communication by releasing mediators from keratinocytes, macrophages, and fibroblasts. The influx of macrophages into granulation tissue aids in replacing damaged tissues. Thus, cell-based therapies have become promising alternatives to pharmacological and surgical treatments. In the last decade, mesenchymal stem cells derived from various tissues were investigated for their capability to differentiate and modulate the release of various mediators of cell growth and differentiation, such as chemokines, cytokines, and growth factors[29]. Thus, mesenchymal stem/stromal cells can promote burn's healing. Despite advances in the use of ADSC in burn treatment, the isolation method consumes time, is costly, and requires culturing and expansion, which can increase the risk of contamination. Therefore, research has shifted toward using heterogeneous cells (SVF) isolated from adipose tissue for their regenerative and repairing ability. SVF isolation is a fast procedure, affordable, applicable in clinical practice, and costless when compared to ADSC isolation. Therefore, it is appropriate to isolate SVF from the fat of the patient to avoid ethical and allogenic reactions.

EI is the common method for SVF isolation using collagenase or trypsin [30]. This method has proved to be effective, but it is expensive, time-consuming, and destroys the microenvironment[31]. According to the Food and Drug Administration in the United States and the European Medicines Agency in the European Union, adipose tissue needs to be minimally manipulated, and the procedure should be performed on the same day[32]. Enzymatically isolated adipose-derived stem/stromal cells are regarded as more than minimally manipulated and are classified as drugs in the United States[15]. MI was standardized for the minimal manipulation of cells, through preserving cell surface, and glycocalyx arrangement. This method is fast and is applicable in clinical settings. Therefore, this study used MI of SVF by microlyzer as it has not been used previously in burn treatment. However, others have reported that the cell count was lower in MI adipose-derived stem/stromal cells than in EI adipose-derived stem/stromal cells but both isolation methods displayed same CD markers[15]. This study investigated the therapeutic effect of MI SVF macroscopically and microscopically on deep partial-thickness burn in Wistar rat. To our knowledge, this study was the first to investigate the effects of MI technique for SVF on burn healing, without using collagenase.

Previous research has reported the therapeutic effect of SVF in burn injuries using the EI technique. Treatment with enzymatically isolated SVF improves burn wound healing by enhancing re-epithelization, collagen formation, and neovascularization and reduces inflammation and scar formation [8,17,18,28]. Others reported the use of enzyme-free SVF isolation in burn injury[20]. However, the methodology included incubation with collagenase type I. This study demonstrated the efficacy of MI SVF by using microlization and did not involve the usage of collagenase.

In this study, deep-patrial thickness burn was induced by scalding, to have a homogeneous burn. It was done on the skin of the back of the rats at 100 ℃ for 6 s. This method has been reported previously[33,34,21]. The results were confirmed histologically. Burn healing was monitored and evaluated for 32 days post injury.

MI SVF was injected immediately after the extraction. Burn injury conversion is preventable if appropriate treatment is applied within the first 72 h[35]. A total of $1X10^6$ cells per rat were injected intradermally at the four edges of the burn. The effectiveness of this technique has been reported previously [18]. Results from this study showed that injection of allogenic SVF did not increase pain or distress in burned animals. Wound contraction was not significantly different between groups. Similar results have been reported for EI SVF[18,24]. These findings suggest that both isolation methods have the same impact on wound contraction. However, adding platelet-rich plasma (PRP) to SVF increased the contraction rate [24]. No infections or deaths have occurred during the experiment.

In this study, MI SVF reduced edema on day 1 post-treatment and increased wound bed dryness when compared to other groups. Our results agreed with those obtained in studies using EI SVF, showing a reduction in edema formation[24]. Similar results have been reported in rats treated with both SVF and PRP on day 7 post-treatment[24]. The difference in reduction duration between this study's results and previous studies suggested that MI SVF could have higher potency in reducing edema compared to EI SVF. Edema is a sign of inflammation due to increased vascular permeability, an effect of the acute inflammatory response to the injury. Our results showed an early impact on inflammation through the reduction of edema. SVF contains stromal/stem cells that can help reduce the release of tumor necrosis factor-alpha (TNF-α) and interleukin-2 (IL-2) and induce apoptosis in activated macrophages at the transplanted site [36]. Reduction in inflammation resulted in reduced edema formation and thus increases wound bed dryness. Other macroscopic parameters did not differ between the groups in this study. Similarly, treatment with EI SVF did not show differences in wound dryness, hemorrhage, hyperemia, granulation tissue, and fibrosis compared to the control but decreased necrosis[18]. This study showed that using MI SVF is safe and does not cause adverse effects.

After an injury, tissue inflammation increases cells' infiltration to the injury's site. The results from this study showed a reduction in inflammation as early as day 4 post-burn in the SVF treatment group compared to the control, indicating the anti-inflammatory and regulatory effects of SVF. Day 4 represents the inflammation phase of burn healing. Others reported that the anti-inflammatory effect of EI SVF treatment was on days 7 and 10 post-treatment [17,24]. These results showed that MI SVF has an early anti-inflammatory when compared to EI SVF for treating deep-partial thickness burn. After burn injury, an elevation in the levels of proinflammatory cytokines, such as TNF, IL-1β, and IL-6, was observed in the SVF treatment group, but these levels returned to normal between the second and seventh-day post-burn[21]. ADSC reduced inflammation by enhancing anti-inflammatory cytokines, such as IL-10, and lowering proinflammatory cytokines [37]. These data are consistent with our results. Measurement of inflammatory markers is required.

The proliferative phase aids in wound closure and neovascularization. On day 8 post-treatment in this study, an improvement was observed in neovascularization and capillary count in the SVF treatment group compared to the control group. This period represents the proliferative phase of the burn healing process. Wound closure and neovascularization occur in keratinocytes and fibroblasts [38]. Our results are consistent with EI SVF treatment of burn injuries. SVF increased neovascularization at days 7, 10, and 14 through releasing growth factors, such as vascular endothelial growth factor (VEGF) [17,18,24,8]. Stromal cells in the SVF affect endothelial proliferation by releasing VEGF at the injured site[39]. Therefore, both isolation methods positively enhanced neovascularization.

Remodeling is the last phase of burn healing, where re-epithelization and collagen deposition occurs. Re-epithelialization was complete in the epidermis layer for all groups on day 32 post-treatment. Previous research has

reported complete healing between approximately 21–30 days[18, 40, 41]. Completion of re-epithelization depends on the burn's size, depth, and the presence of comorbidities, leading to inflammation. In this study, the epithelial thickness was thicker in the SVF group than in the SSD group. Similar results have been recorded for EI SVF [24]. Previous results showed that EI SVF significantly enhanced Ki67, as an indicator of proliferation, CK17, as an epithelial cell marker, and proliferating cell nuclear antigen (PCNA) index around 21 days post treatment of burn wounds[17,20]. Thus, results from this study suggest that SVF can enhance the proliferation of keratinocytes through cell differentiation or paracrine signaling. Further investigation of proliferation markers is required.

Research has shown that the combined treatment of SVF with other biomaterials enhances healing compared to SVF alone[24,40,42,43]. For instance, applying EI SVF, using both collagenase type I and II, and MI SVF, using collagenase type I, to full-thickness burn significantly improved histopathological scores in rat model[20]. The application of SVF+ PRP increased neovascularization and re-epithelization [24][39]. The effect could be mediated through increasing growth factors such as transforming growth factor–beta (TGF-β) and VEGF levels[39].

EI SVF has been used in the treatment of various injuries and illnesses and has shown promising therapeutic effects. This study showed that MI SVF positively impacts the healing of deep partial-thickness burns by increasing neovascularization and re-epithelization and decreasing early inflammation. These results could be due to different population of cells in the SVF that function through paracrine signaling and their ability to differentiate. Investigating factors underlying the effect of MI SVF is required. Increasing the number of SVF cells and combining treatment with other biomaterials could enhance the therapeutic effect of SVF.

This study used an SVF isolation Microlyzer (BSLrest, Inc., Turky). Currently, other kits and devices with different names are available for mechanical isolation and can be investigated in diverse clinical settings [32]. The availability of these devices could advance the treatment of various diseases for their applicability in clinical setting and to avoid ethical and application issues associated with enzymatically isolated SVF.

CONCLUSIONS

This study showed that MI of the SVF positively impacts deep partial-thickness burns healing. Results from this study are consistent with those for EI SVF. This technique is safe and helps reduce the time for isolation with efficacy in burn healing by enhancing proliferation, neovascularization, and reducing inflammation. Further investigation of signaling pathways and different activated regulatory markers behind these results are required.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

Ethical approval

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