Topical Adipose Mesenchymal Stem Cell Secretome Cream Improves Wound Healing in Partial-Thickness Burned Mice

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Abstract

Burn injury has a high prevalence worldwide; it is estimated that there were nearly 9 million burn cases in 2019. This injury impacts the physical and mental well-being of the patient, as well as their overall quality of life. This study aims to analyze the therapeutical effect of Mesenchymal Stem Cells Secretome (MSCS) for partial-thickness burn wounds. This research is experimental research with a Posttest-Only Control Group Design. The sample consisted of 24 white mice divided into three groups: the control group, the 1% silver sulfadiazine (SSD) group and the MSCS group. A histopathological examination of the wound was performed by hematoxylin-eosin staining.

The average thickness measurement of the burn surface epidermis in the SSD and MSCS treatment groups showed a thicker epidermis than the control group. The vascular density in the granulation tissue of the SSD (35.63) and MSCS (34.00) treatment groups decreased compared to the control group. The fibroblast density in the granulation tissue of the SSD (66.75) and MSCS (70.25) treatment groups was reduced compared to that of the control group. The inflammatory cell density in the granulation tissue of the SSD (70.75) and MSCS (50.88) treatment groups decreased than the control group. The administration of MSCS cream showed a better histopathological image than the control and was comparable to SSD treatment groups.

Keywords: Mesenchymal stem cell, secretome, burn, wound.

Introduction

Burns are a form of injury resulting from accidental contact with substances at high temperatures such as hot liquids, solids, or gases including cooking stoves, smoke, steam, beverages, machinery, appliances, tools, radiators and items emitting thermal energy. Burn injuries can result in enduring and significant changes, even after the wounds, have healed. These changes have an impact on both the physical and mental well-being of the patient, as well as their overall quality of life. This places a substantial strain not just on the patient's family but also on healthcare systems worldwide. Globally, it is estimated that there were nearly 9 million burn cases in 2019.[30]

When burns reach the dermis, they are called partial-thickness or second-degree burns which can be categorized as either superficial or deep partial-thickness burns. The superficial partial-thickness burn affects the epidermis and approximately 50% of the dermis. The blister will be accompanied by pain, although the hair remains undamaged.

Typically, the slight burn injury will undergo natural healing within a period of one to three weeks without the need for surgical intervention. The profound partial-thickness burn can result in significant damage to the deep dermis.[6]

The common treatment for burns is 1% silver sulfadiazine (SSD). SSD is known to accelerate the wound healing process because it revitalizes and forms tissue granules. However, some studies find that topical silver delays rather than promotes wound healing and continuous use of SSD can have a toxic effect on the skin and inhibit fibroblast regeneration and collagen deposition in the dermis, which causes the membrane to become brittle and stiff.[10]

Advances in biotechnology science raise societal expectations. Pharmaceutical and biotechnology companies nowadays focus on product development using cutting-edge techniques such as stem cell culture and exosome extraction. The secretome is the factors/molecules secreted to the extracellular space. It comprises of soluble proteins, free nucleic acids, lipids and extracellular vesicles.[25]

MSCs secrete a bioactive molecule in a conditioned medium, which is referred to as the secretome. Numerous growth factors, cytokines and a variety of macromolecules and extracellular vesicles, such as microvesicles and exosomes, can induce a variety of biological reactions, particularly in the regulation of the formation of new tissues. The MSC secretome has the potential to regenerate skin by enhancing the production of collagen, elastic fiber and fibroids decreasing the MMP-1.

Furthermore, it can facilitate skin regeneration by increasing the thickness of the regenerated epidermis and restoring the

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lost skin structure. It can also improve the process of angiogenesis by increasing the production of VEGF and HGF, increasing blood vessel density and increasing the migration of fibroblasts and keratinocytes, thereby expediting wound closure.[13] Therefore, MSC-sourced secretome is beneficial for burn recovery. This study compares the effect of using MSC secretome cream with SSD on healing partial-thickness burns *in vivo*.

Material and Methods

This experimental study uses a control group design and a post-test-only approach to compare the histopathological features of partial-thickness burn under SSD administration and MSC secretome. The investigation utilized twenty-four white mice in good health. According to Frederer's formula, a total of 8 mice per group were deemed necessary.

Mice that are eight weeks old, male, weigh 20-30 grams and have a health condition characterized by active movement, clean fur, no defects and clear eyes are included in the study. Additionally, they must not be isolated in the corner of the cage. This research has been authorized by the Research Ethics Commission of the Faculty of Medicine, Andalas University (No.1087/UN.16.2/KEPFK/2022).

The experimental animals were divided into the control group (giving PBS), the MSC secretome cream treatment group and the 1% silver sulfadiazine (SSD) treatment group. MSCs were isolated from human adipose tissue using the explant culture method. The MSCs were subcultured until passage 6 using DMEM + 20% FBS medium. MSC criteria are examined according to ISCT standards.[3] The cream preparation is made with a content of 5% secretome.

The experimental animals were subjected to burn induction after a week of acclimatization. The mice were ketamine-anesthetized intraperitoneally at a dose of 80 mg/kg BW before burn induction. An electric clipper was employed to shave the animal's dorsum and commercial depilatory lotion was employed to defile it. Afterwards, the burn was induced using a 2 cm diameter iron plate that had been heated to 100°C for 3 minutes. Iron plates were affixed to the mice's backs for 10 seconds after the fur was cleaned and aseptic procedures were performed.

The lesion was then dehydrated with graded ethanol and xylene after being immersed in a 10% NBF (normal buffered formalin) solution. After the dehydration process, paraffinization was carried out and a microtome was used to generate 2mm slides. Rehydration was started by placing the microtome slide on a glass surface. Hematoxylin-eosin (HE) was used for further staining.

For statistical analysis, the average value of the repetitions was obtained in each experiment. The results were presented as the mean in a One-way ANOVA analysis, with Bonferroni's multiple comparisons test employed as a post-hoc test for group comparisons. The studies were conducted using the Statistical Package for the Social Sciences (SPSS) V25 software (SPSS Inc., Chicago, IL, USA). A significance level of $P<0.05$ was used to determine statistical significance.

Results and Discussion

Table 1 shows that the average thickness measurement of the burn surface epidermis in the SSD and Secretome groups showed a thicker epidermis than the control group (p = 0,001). MSC secretome treatment groups have the lowest vascular and inflammatory cell density levels among all groups, significantly lower than the control group (p < 0,001). The secretome group has the highest level of fibroblast density, but there is no statistical difference between the groups.

Some factors that affect the effectiveness of secretome therapy include the source of MSCs, secretome concentration and secretome administration method. Tran et al[24] reported that conditioned medium/secretome derived from different MSC sources have different therapeutic potentials. Li et al[14] reported that high-dose secretome on day 13 showed complete reepithelialization and higher levels of vascularization than the administration of low-concentration secretome.[14] Our study proves that secretome derived from adipose tissue administered once daily in the form of a 5% cream preparation has therapeutic potential for burn healing comparable to 1% sulfur sulfadiazine (SSD).

Reepithelialization and the density of inflammatory cells in this study are consistent with other reports that study the administration of MSC secretome as therapy for burns. Aryan et al[1] reported that intra-peritoneal administration of secretome from BW-MSC provides better second-degree burn healing results than topical 1% SSD.

Table 1
Comparison of histopathological assessment between groups

Group	Vascular density	Fibroblast density	Epidermal thickness	Inflamatory cell density
Secretome	34 ± 7,151[a]	70,25 ± 7,996	109,1338 ± 22,446[a]	50,88 ± 35,474[a]
SSD	35,63 ± 8,895[b]	66,75 ± 28,019	101,9425 ± 18,198[b]	70,75 ± 33,251[b]
Control	59,38 ± 10,954[a,b]	58,5 ± 21,863	46,7875 ± 16,065[a,b]	166,5 ± 53,147[a,b]

Comparison between group with superscript a or b = p < 0,001

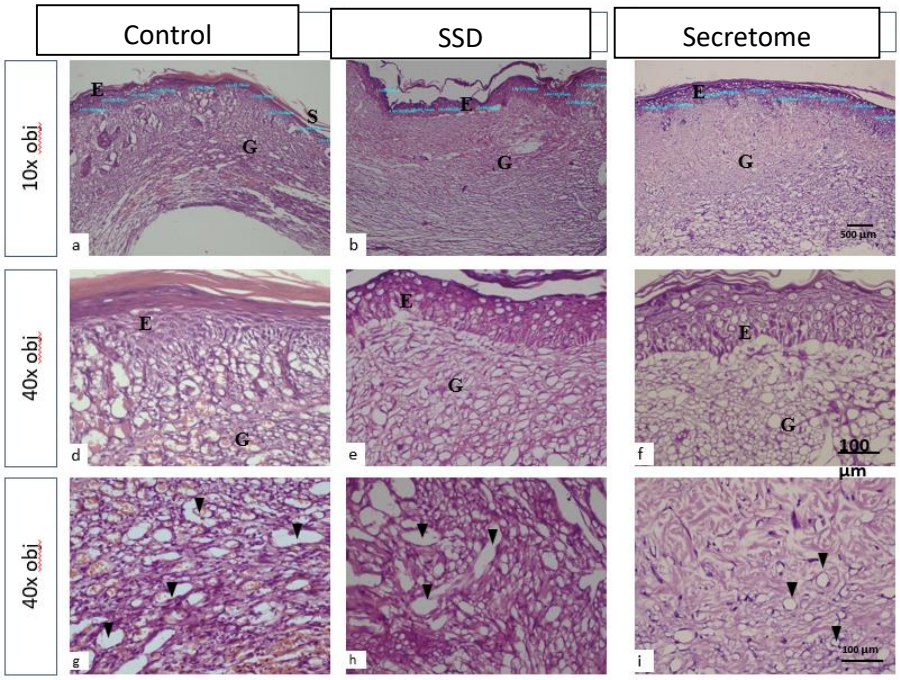

Figure 1: The histology of the experimental animal skin tissue showed the epidermis (E), the burn surface area with scabs (S) and the dermis area with post-burn granulation (G). Granulation tissue with collagen fibers, fibroblast cells, inflammatory cells and blood vessels. The control group (a, d, g) has a thin epidermis, loose granulation tissue with many inflammatory cells, few fibroblasts, thin collagen and many hyperemic dilated blood vessels (▼). Treatment with SSD (b, e, h) showed complete epithelialization, less inflammatory cells and denser collagen. Treatment with secretome (c, f, i) showed better wound repair with complete epithelialization, less inflammatory cells and denser collagen, less vascularity and smaller vessel size, suggesting a healing phase. Hematoxylin-eosin. The top panel objective is 10x and the middle and bottom objectives are 40x.

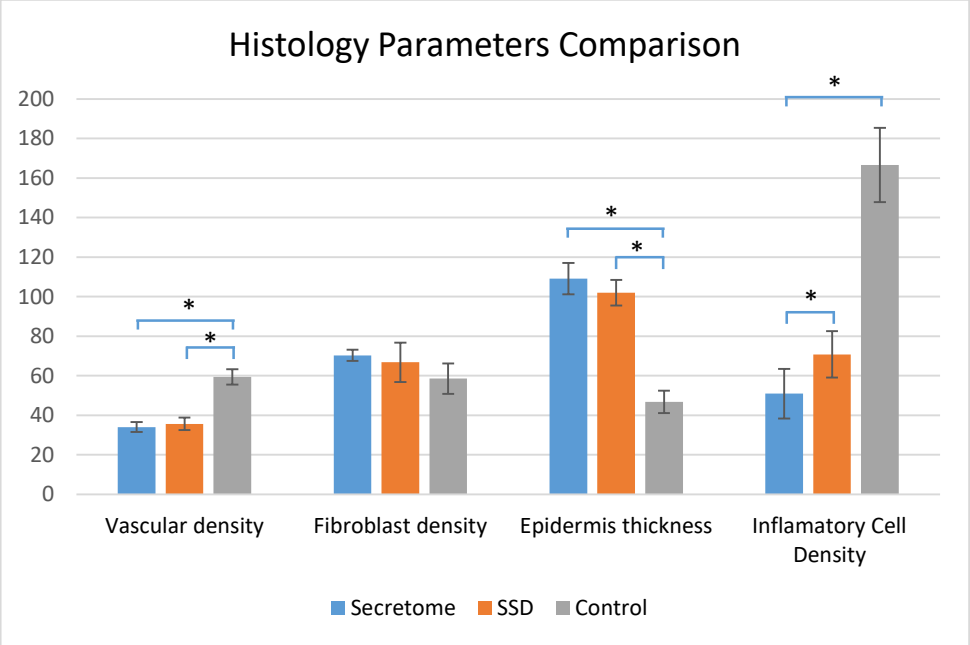

Figure 2: Comparison chart of histology parameters between groups. *p<0,001

The study reported that the number of neutrophils in the wound area on days 7, 15 and 28 was less in the secretome group than in the SSD and control groups, indicating that the anti-inflammatory effect of the secretome was better than that of SSD. Laksmitawati et al[12] compared secretome gel and SSD as burn therapy in rat test animals. The study reported that the treatment group that used secretome gel from MSC adipose tissue, experienced faster reepithelialization and hair growth in the wound area than the group that received SSD.[12] Zhou et al[37] reported that the secretome provides better reepithelization and lower density of inflammatory cells in third-degree burns. The lower

number of inflammatory cells showed not only the secretome cream's anti-inflammatory effect but also a possible therapeutic effect that increased the speed of the wound healing phase from the inflammatory phase to the next.

The vascular density of the secretome group in this study was lower than that of the SSD and control group on day 14. A different result was reported by Aryan et al[1] who stated that the vascular density of the secretome group is significantly higher on day seven and remains higher on days 14 and 28 (not statistically significant). This may be related to the difference in secretome administration routes. Topical administration of secretome may cause the wound healing process to enter the remodeling phase more quickly compared to intra-peritoneal administration.

Following the hemostasis phase, the wound healing process of wounds, including burn wounds, comprises three interrelated and overlapping phases: the inflammatory, proliferation and remodeling phases. The inflammatory phase prevents infection throughout the healing process, breaks down dead tissue and initiates the necessary signals for wound repair.[19] The proliferative phase, which occurs after and alongside the inflammatory reaction, is distinguished by the activation of keratinocytes and fibroblasts by the action of cytokines and growth factors.[27] During this stage, keratinocytes move across the wound to aid in the closure and regeneration of a vascular network, which is a crucial step in wound healing.[18]

The interconnection of stromal, endothelial and immunological cells plays a crucial role in determining the progression of healing including wound closure and reestablishment of blood supply. The last phase of healing, which occurs concurrently with the proliferative phase, involves remodeling the wound.[28] In this study, the lower vascular density in the SSD and secretome groups compared to the control group may indicate that they have entered the remodeling phase. In the remodeling phase, angiogenesis is reduced and blood flow in the wound area is reduced.[7,19] Some characteristics of mature wounds are avascular and acellular.[5]

Fibroblasts play important roles in the proliferation and remodeling phases. The use of secretome as a burn therapy has been reported to increase the number of fibroblasts in the wound area significantly.[1,12] In this study, the number of fibroblasts in the secretome and SSD groups was not significantly different from that of the control group. This may be related to the fact that the remodeling phase had been achieved in the secretome and SSD groups while the control group was still in the proliferative phase. In the remodeling phase, fibroblasts transform into myofibroblasts.[20,23]

The therapeutic effects of the secretome used in this study can come from protein molecules and their vesicular fractions. MSCs from bone marrow, adipose tissue and the umbilical cord are already known to release paracrine factors such as EGF, HGF and bFGF associated with keratinocyte migration and significantly increase wound re-epithelialization.[9,16,17] You and Nam[31] reported that MSCs with EGF overexpression caused increased migration and proliferation of fibroblasts in skin cells compared to MSCs without EGF overexpression. Chen et al[2] reported that using hydrogels containing BMSC might reduce the healing scar of diabetic ulcer wounds in rats and it may be related to the bFGF produced by BMSC. Exosomes isolated from secretome/conditioned media of MSCs have been reported to improve wound healing at various healing phases.

In the inflammatory stage, the MSC exosome affects the polarization of the M2 macrophage which has an anti-inflammatory effect.[8,15,21,22] In the proliferation stage, the administration of MSC exosomes increases fibroblast proliferation and migration, optimizes collagen deposition, improves reepithelialization, reduces heat-stress apoptosis in burns and increases collagen synthesis and angiogenesis.[11,32–34] In the remodeling stage, it suppresses scar formation and improves extracellular matrix reconstruction.[4,26,35,36]

Conclusion

Burn healing has a similar grouping of healing phases to wounds in general which consists of inflammation, proliferative and remodeling phases. Protein molecules and vesicular fractions in the MSC secretome are effective therapeutic agents in burn healing and have a role in every stage of wound healing. The use of adipose-MSC secretome in the form of a 5% cream once a day for partial-thickness burns showed comparable results to sulfur sulfadiazine cream 1% histologically.

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