JOURNAL OF PLASTIC SURGERY AND HAND SURGERY
2023, VOL. 57, NOS. 1–6, 78–94
https://doi.org/10.1080/2000656X.2021.1993234

ARTICLE

Efficacy of stromal vascular fraction and enzyme-free mechanical isolation therapy in experimental full thickness burn wounds

Percin Karakol[a], Mehmet Bozkurt[a], Caner Gelbal[a] and Mehmet Ibrahim Tuglu[b]

[a]Department of Plastic, Reconstructive and Aesthetic Surgery, Health Science University Bağcilar Education and Training Hospital, Istanbul, Turkey; [b]Faculty of Medicine, Department of Histology, Celal Bayar University, Manisa, Turkey

ABSTRACT

Background: Autologous cell suspensions obtained by a stromal vascular fraction (SVF) and enzyme-free mechanical isolation (EMI) are an alternative in the treatment of burn wounds. In this study, we aimed to investigate the effect of autologous cell suspensions obtained by SVF and EMI on full-thickness skin burn wounds.

Methods: A total of 45 male Sprague–Dawley rats were divided into three groups, SVF group, EMI group, and SVF + EMI group. The groups were also classified as the first, second, and third week of the burn to reveal the effect of the treatment on the burn in the early, middle, and late stages. For treatment, 0.2 ml SVF or 0.2 ml EMI was injected subcutaneously into the burn lesions of the subjects. Histopathological examination was performed on the burn wounds taken at the end of the experiment, and Ki67, CD44, CD73, CD90, and CK17 expressions were evaluated.

Results: Histological examination revealed that there was no improvement in the control samples, but the skin was multicellular, vascularization was present. Histologic scores in all groups was significantly better than control, and SVF + EMI was the best group in terms of recovery ($p < 0.05$). Ki67, CK17, CD44, CD73, and CD90 expressions were significantly higher in the treatment groups compared to the control ($p < 0.05$).

Conclusion: We found in our study that both applications significantly increased the healing of the burn wound. Moreover, SVF + EMI application provided more improvement than SVF or EMI alone.

ARTICLE HISTORY
Received 23 February 2021
Revised 10 August 2021
Accepted 7 October 2021

KEYWORDS
Burn wound; stromal vascular fraction; enzyme-free mechanical isolation; rat; immunohistochemistry

Introduction

Burn injuries are traumas that can happen anytime and anywhere and can affect anyone. Burn injuries can be caused by heat, cold, friction, radiation, electricity, and chemicals. However, most burn injuries are caused by heat [1]. The World Health Organization (WHO) estimates that around 265,000 people worldwide die each year from burns [2]. Burn injuries cause lifelong physical and psychological injuries that affect the quality of life [3,4]. Burns is classically divided into three degrees: first-degree, affecting the superficial layer of the epidermis; second-degree, affecting the dermis; and third-degree, affecting the full-thickness skin and can even reach muscle and bone. Severe burns can affect almost all organs and cause serious morbidity and mortality. Therefore, treating serious burns is important, but the ideal treatment for severe burns has not been developed [5].

Treatment of burn wounds varies according to the affected body area, the size of the lesion, patient age, and individual health status. Routine treatment of burn wounds includes the elimination of necrosis, split-thickness autologous skin grafts (STSG), and prevention of infections [6]. Surgical debridement followed by occlusive dressing is usually routinely used in full-thickness burn injuries in most burn centers [7]. However, it is necessary to develop new interventions to increase functional recovery and morphological reconstruction and to reduce hypertrophic scar formation [8].

As a result of the demonstration that stem cells can prevent further tissue loss with their capacity for regeneration and differentiation, stem cell therapies have become a new option in medical treatment [9–11]. The stromal vascular fraction (SVF) contains a rich source of adipose-derived stem cells (ADSC) that have the potential to differentiate into adipocytes, myocytes, chondrocytes, osteoblasts, and neuron-like cells [12–14]. SVF, the source of ADSCs, has been shown to have antioxidant, anti-inflammatory, antiapoptotic, and immune-modulatory effects [9,11,15]. Foubert et al. [16] reported that ADSC regulated inflammation and improved wound epithelialization and angiogenesis in burn wounds. Atalay et al. [17] reported that SVF improves deep partial-thickness burn wound healing. Cardoso et al. [18] found that SVF was helpful in the treatment of full-thickness burns in rats.

Non-cultured stem cells (NCSC), called ReCell, obtained by enzyme-free mechanical isolation (EMI) method were put into clinical practice for wound healing in 2005. ReCell is an autologous cell suspension containing keratinocytes, fibroblasts, melanocytes, and Langerhans cells [19]. This cell suspension can be applied to the burn wound at a ratio of 1:80 without any delay in place in the operating room, as it does not require culture and can be prepared quickly [20]. ADCS created by ReCell has been used in various areas such as burns, graft donor sites, hypopigmented wounds, chronic wounds, vitiligo, and large congenital melanotic nevus [21–27].

CONTACT Percin Karakol ppercin@gmail.com Department of Plastic, Reconstructive and Aesthetic Surgery, Health Science University Bağcılar Education and Training Hospital, Istanbul 34744, Turkey

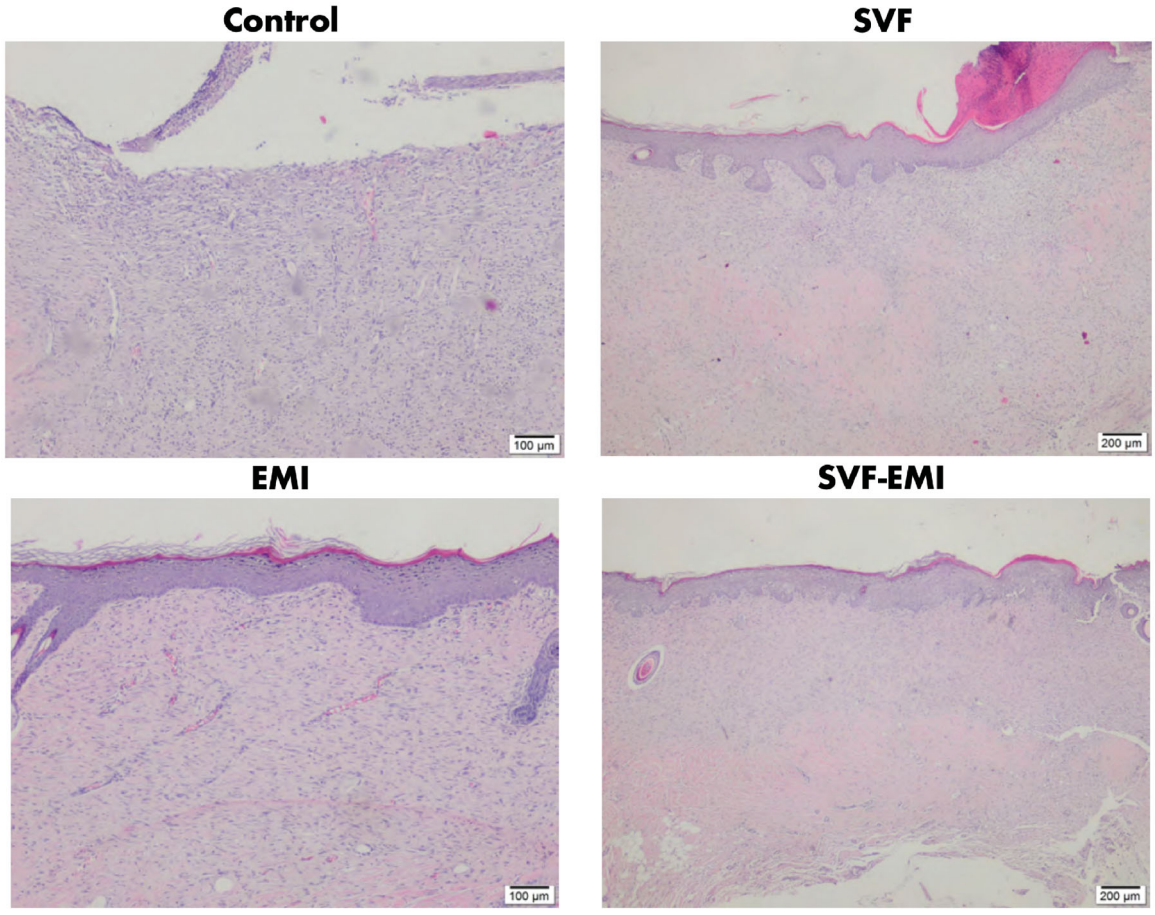

Figure 1. Light microscopic view of the burn wound at the end of the first week. Tissue sections were stained with H&E.

In our study, the aim was to investigate the effects of SVF and EMI applications on the healing of the burn wound in the early period (first week of the burn), early late period (second week of the burn), and late period (third week of the burn) in the rat thermal burn model. For this purpose, the expression profiles of the cell proliferation marker Ki-67, keratinocyte differentiation marker cytokeratin 17 (CK17), and stem cell markers CD44, CD73, and CD90 were investigated immunohistochemically including histological healing.

Materials and methods

All animal experiments adhered to the guidelines detailed in the NIH Guide for the Care and Use of Laboratory Animals. The institutional animal study review committee approved this study. Male Sprague–Dawley rats weighing 250 g were housed in an animal facility with a 12-h light and dark cycle for 1 week to acclimate prior to the initiation of the experiment. All animals received water and food *ad libitum* and were monitored for the entire study period. A total of 45 subjects were divided into three groups with 15 subjects each, SVF group, EMI group, and SVF + EMI group. The groups were also classified as the first week, second week, and third week of the burn to reveal the effect of the treatment on the burn in the early, middle and late stages.

Stromal vascular fraction (SVF) preparation

Fat pads were removed by dissection from the inguinal areas of each rat, and hematopoietic cells were removed by washing with saline. The washed adipose tissue was mechanically cut into small pieces with fine-tipped tissue scissors, and then 0.1% collagenase type I (C0130, Sigma Aldrich^{TM}, St. Louis, MO) and type II (C6885, Sigma Aldrich^{TM}, St. Louis, MO) were added and kept at 37 °C for 30 min. After removing the accumulated triglyceride-rich oil layer, the sample was centrifuged at 3000 rpm for 5 min. The supernatant consisting of mature adipocytes was removed. The bottom precipitate was used as SVF [17].

Enzyme-free mechanical isolation (EMI)

In order to prepare the autologous suspension to be applied, hairy tissue samples were taken with a 3 mm punch biopsy pen. The material was conducted to laminar flux in transport solution (Sterile Phosphate Buffer Solution at 2% (PBS) and 1% antibiotics: 105 units/ml penicillin and 105 µg/ml streptomycin) washed in PBS to remove debris and red blood cells and minced with fine tissue scissors. The fragmented tissues were incubated with type I collagenase (1.5 mg/ml) and kept in a water bath at 37 °C for 50 min. After enzymatic digestion, the tissue was filtered using a 70 µm filter and collagenase was removed by dilution with phosphate buffer solution (PBS). The cell suspension was centrifuged at 363 *g* (1800 rpm)/7 min and the supernatant, composed primarily of mature adipocytes, was removed. The pellet was formed by cells that were resuspended in PBS. The counting of viable cells was performed using the Tripan Blue stain exclusion method. Injections were prepared by adding PBS and an SVF volume equivalent to 5×10^5 cells/ml in 1 ml syringes. Four injections of 0.2 ml were made in each wound edge and dripping of 0.2 ml covering the wound bed [21–27].

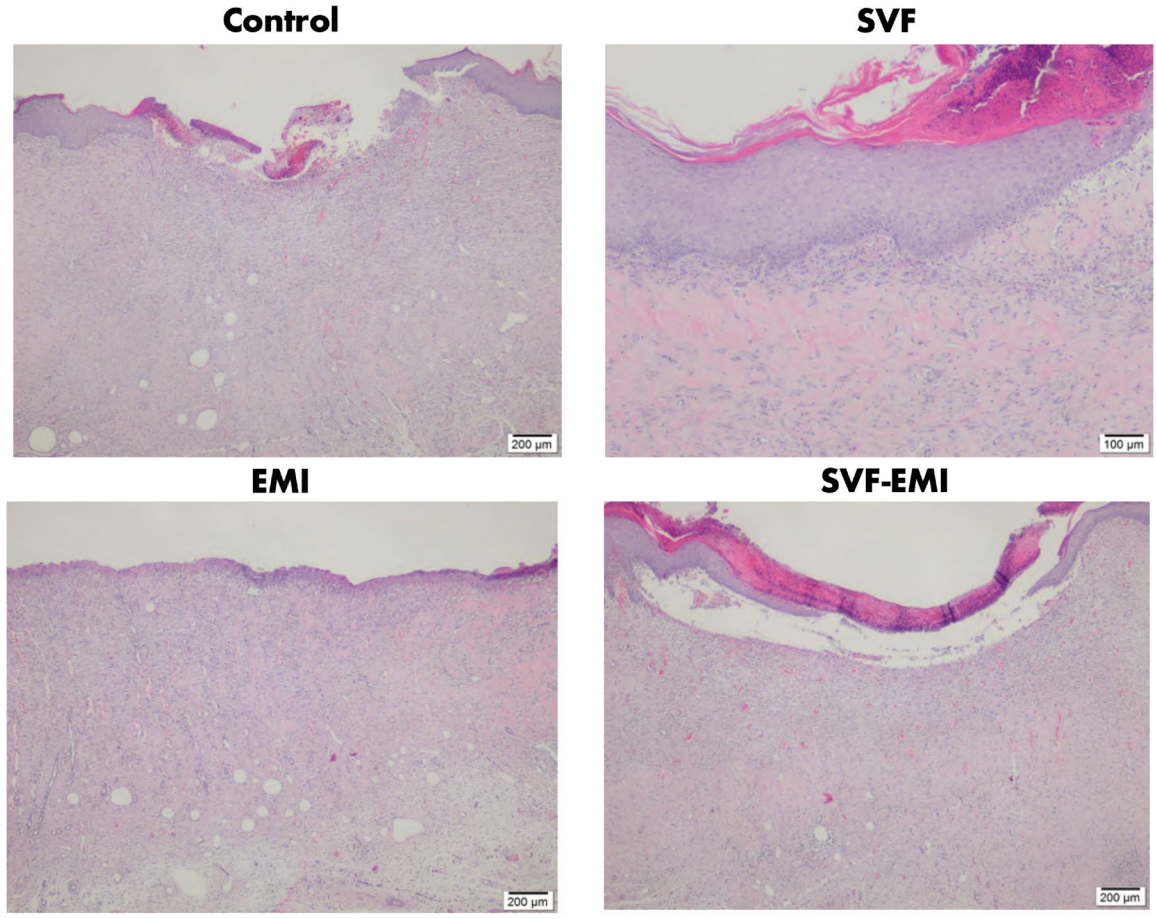

Figure 2. Light microscopic view of the burn wound at the end of the second week. Tissue sections were stained with H&E.

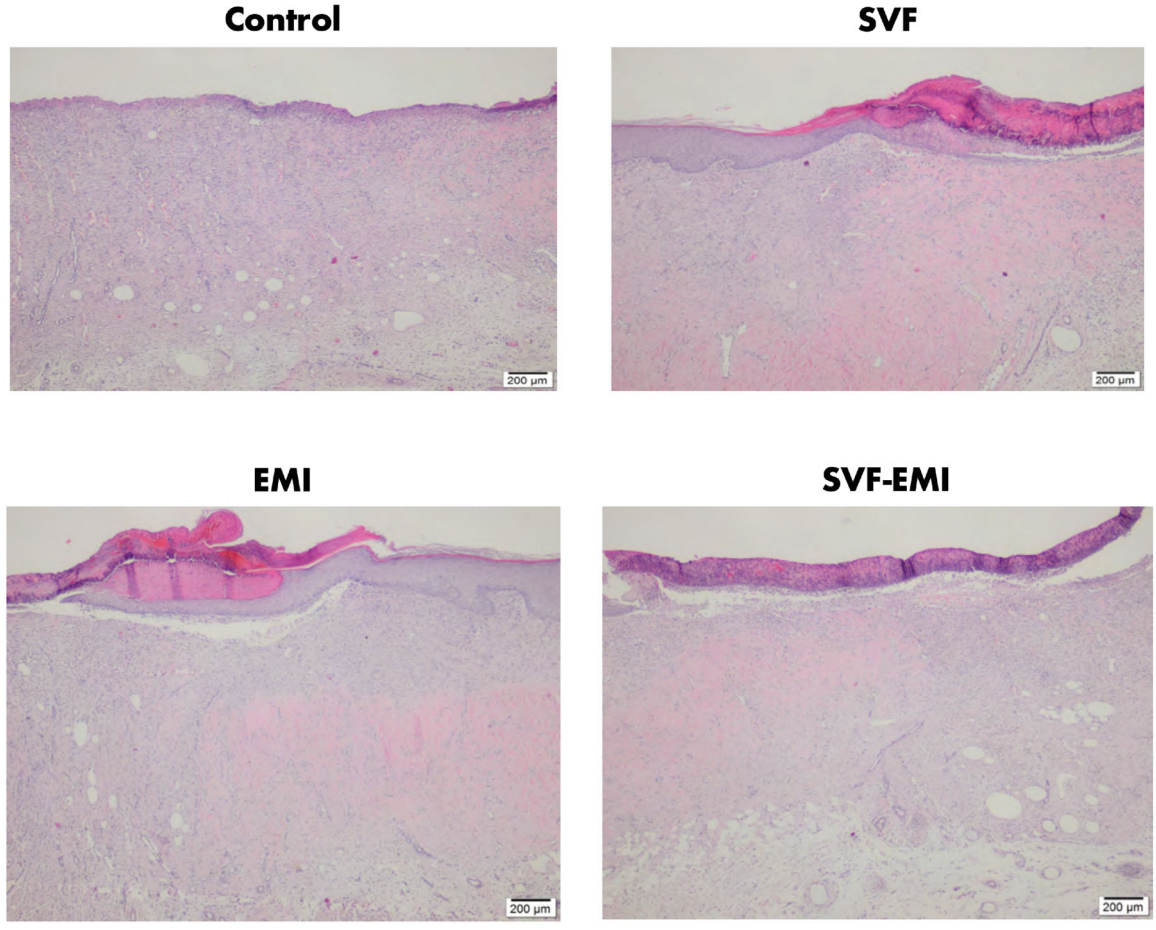

Figure 3. Light microscopic view of the burn wound at the end of the third week. Tissue sections were stained with H&E.

Table 1. Histoscore and H score of Ki67, CD73, CD90, CD44 and CK17 in all groups.

	First week	Second week	Third week
Histoscore			
Control	2.85 ± 0.10	2.15 ± 0.07	1.60 ± 0.12
SVF	3.05 ± 0.11	2.75 ± 0.08**	2.45 ± 0.13***
EMI	3.00 ± 0.07	2.55 ± 0.13*	2.25 ± 0.11**
SVF + EMI	3.85 ± 0.07**	3.10 ± 0.10***,a	3.10 ± 0.14***, a, b
Ki67			
Control	137.10 ± 7.60	90.40 ± 5.91	67.40 ± 6.82
SVF	207.90 ± 10.84***	167.70 ± 11.42***	140.10 ± 13.06***
EMI	187.10 ± 12.56**	148.30 ± 10.39***	125.70 ± 9.69***
SVF + EMI	237.20 ± 9.74***, a	198.90 ± 8.72***, a	180.80 ± 7.55***, a, b
CD73			
Control	213.80 ± 2.35	76.80 ± 5.24	67.40 ± 5.13
SVF	220.80 ± 3.13**	151.00 ± 9.27***	145.50 ± 10.52***
EMI	235.40 ± 3.42***	137.10 ± 8.27***	176.50 ± 6.59***
SVF + EMI	253.90 ± 4.77***, a	176.30 ± 5.37***, a	212.50 ± 5.67***, a, b
CD90			
Control	139.00 ± 2.12	135.30 ± 2.27	135.30 ± 2.27
SVF	159.40 ± 4.49**, a	157.30 ± 4.57**	157.30 ± 4.57**
EMI	187.60 ± 3.92***	170.80 ± 5.86***	170.80 ± 5.86***
SVF + EMI	176.10 ± 4.07***, b	166.00 ± 4.91***	166.00 ± 4.91***
CD44			
Control	195.30 ± 3.71	195.80 ± 5.00	231.60 ± 3.25
SVF	205.50 ± 3.82	202.10 ± 4.23	240.50 ± 3.46
EMI	237.30 ± 5.21***, b	242.60 ± 5.99***, b	264.50 ± 2.70***, b
SVF + EMI	229.10 ± 4.55***, b	231.60 ± 5.05***, b	270.50 ± 4.47***, b
CK17			
Control	135.40 ± 15.72	90.40 ± 5.91	67.40 ± 6.82
SVF	168.50 ± 7.01	167.70 ± 11.42***	140.10 ± 13.06***
EMI	176.20 ± 5.12*	148.30 ± 10.39***	125.70 ± 9.69***
SVF + EMI	214.70 ± 3.89***, a,b	198.90 ± 8.72***, a	180.80 ± 7.55***, a,b

EMI, Enzyme-free Mechanical Isolation; SVF, Stromal Vascular Fraction.
*$p < 0.05$ compared to control; **$p < 0.01$ compared to control; ***$p < 0.001$ compared to control.
[a]$p < 0.01$ compared to EMI; [b]$p < 0.05$ compared to SVF.
Values are expressed as mean ± SEM. Statistical analysis was used: one-way ANOVA.

The kit provided a sterile, single-use, tissue collection double bag with an inner filter bag of 120 μm mesh. A volume of lipoaspirate (25–30 ml) was transferred into the inner bag by the upper port. Placing the bag vertically, the Klein solution containing part of blood cells was recovered in the lower part of the processing bag and removed while the adipose tissue remained in the inner filter bag. An equal volume of PBS solution equal to Klein solution removed was introduced into the processing bag through the upper port, and the fat was processed according to the instruction for use. Briefly, fat tissue was massaged for 5 min and disaggregated by using a small plastic rod and enforced to pass through the filter bag by manual massaging. The disaggregated tissue was collected with a syringe using the lower valve port of the outer bag and centrifuged at 400 G for 10 min at room temperature, followed by resuspension in 1 ml of Dulbecco Minimum Essential Medium (DMEM) complete culture medium Cells 2021 of 14 (Sigma-Aldrich, Milan, Italy) with 10% of Fetal Bovine Serum (Sigma-Aldrich, Milan, Italy)), 0.5% of amphotericin B (GIBCO Life Technology, Monza, Italy), and 1% of a mixture of penicillin/streptomycin 1:1 (GIBCO Life Technology, Monza, Italy) to count the number of cells inside. The product obtained by this method was named 'SVF' [21–27].

Experimental burn model

Surgical procedures were performed on subjects under general anesthesia. For anesthesia, an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar® 50 mg/ml vial, Pfizer) and 0.65 mg/kg xylazine hydrochloride (Rompun® vial, Bayer) was administered. Following anesthesia, the backs of the subjects were first shaved with an electric shaver, then with a razor blade, and disinfected with Savlon (10%) solution. To create the burn, an electric heater with an automatic thermostat with a copper plate with a diameter of 15 mm was used. The areas to be burned were marked as 15 mm in diameter on the dorsal skin of the rats on the scapula, on the right and left sides of the midline, with approximately 20 mm of intact skin between them. A deep dermal burn was created by applying a temperature probe with a temperature of 120 °C to the marked areas for 16 s. With the dermal burn created, the basal layer, dermal cytokines, and stem cells, which could be used as a source for wound healing, were eliminated.

Treatment protocol

Leftside burn-in subjects were used for study groups, and right-side burns were used for control purposes. The rats in all groups were divided into three subgroups among themselves, with five treated at the end of the first week, another five at the end of the second week, and the remaining five at the end of the third week. Subdermal injection of 0.2 ml SVF or 0.2 ml EMI was injected into the left-sided burn lesions of the subjects for treatment. In the SVF + EMI group, a total of 0.4 ml was injected, including 0.2 ml SVF and 0.2 ml EMI. 0.2 ml of NaCl was applied to right-sided control lesions in SVF and EMI groups, and 0.4 ml 0.9% NaCl in SVF + EMI group. Four weeks after the burn model was created, the subjects were sacrificed, and tissue samples were taken and examined.

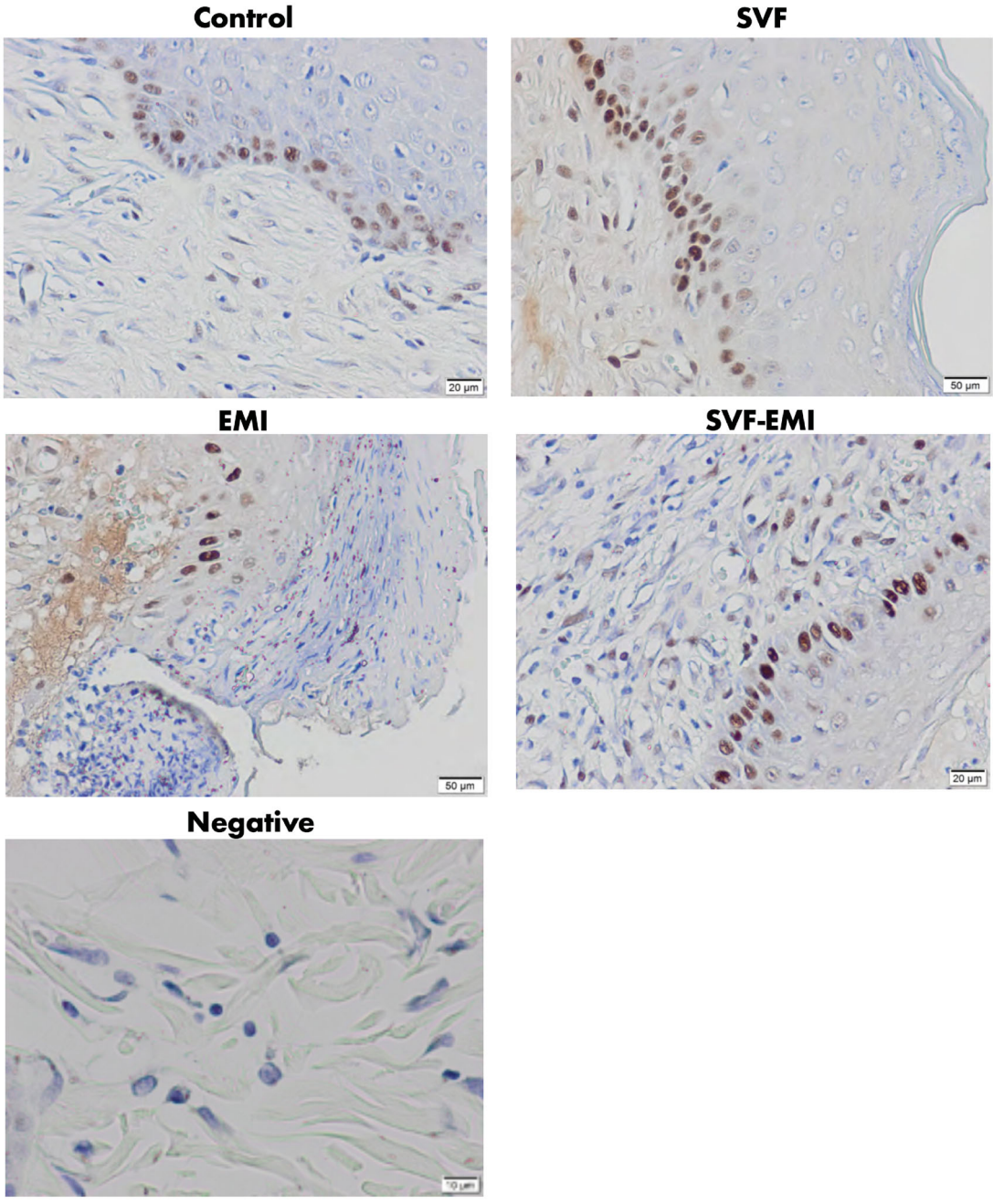

Figure 4. Immunohistochemical staining for Ki67 of the burn wound at the end of the first week.

Histopathological evaluation

At the end of the experiment, after euthanasia of rats with 10 mg/kg xylazine and 75 mg/kg Ketamine under general anesthesia, samples containing injured and intact skin areas were removed and fixed in 10% formalin for 24–72 h. Tissues were then embedded in paraffin blocks following the routine tissue follow-up procedure. Approximately 5 µm thick sections were cut from the paraffin blocks with a microtome. The sections were stained with Hematoxylin-Eosin stain and Masson's Trichrome for histopathological examination. Re-epithelization, granulation tissue, and collagen deposition in the range of at least +1 and maximum +4 were evaluated by two histologists who were blinded to the study groups [28].

Indirect immunohistochemistry staining

After the sections were kept in an oven at 60 °C for one night for immunohistochemical staining, they were made transparent with xylene. Then they were kept in distilled water for 5 min by rehydrating with alcohol series in a degree decreasing from 95% to 60%. The sections restricted with Dakopene were treated in 0.5% trypsin solution at room temperature for 15 min. Then, 3% H_2O_2 was applied for 5 min in order to inhibit endogenous tissue peroxidase. The sections were washed with phosphate-buffered saline (PBS) three times for 5 min and were treated with blocking solution for 1 h for blocking. After removing the blocking solution from the tissue, the sections were incubated overnight with the primary antibodies Ki67, CD44, CD73, CD90, or CK17. The sections

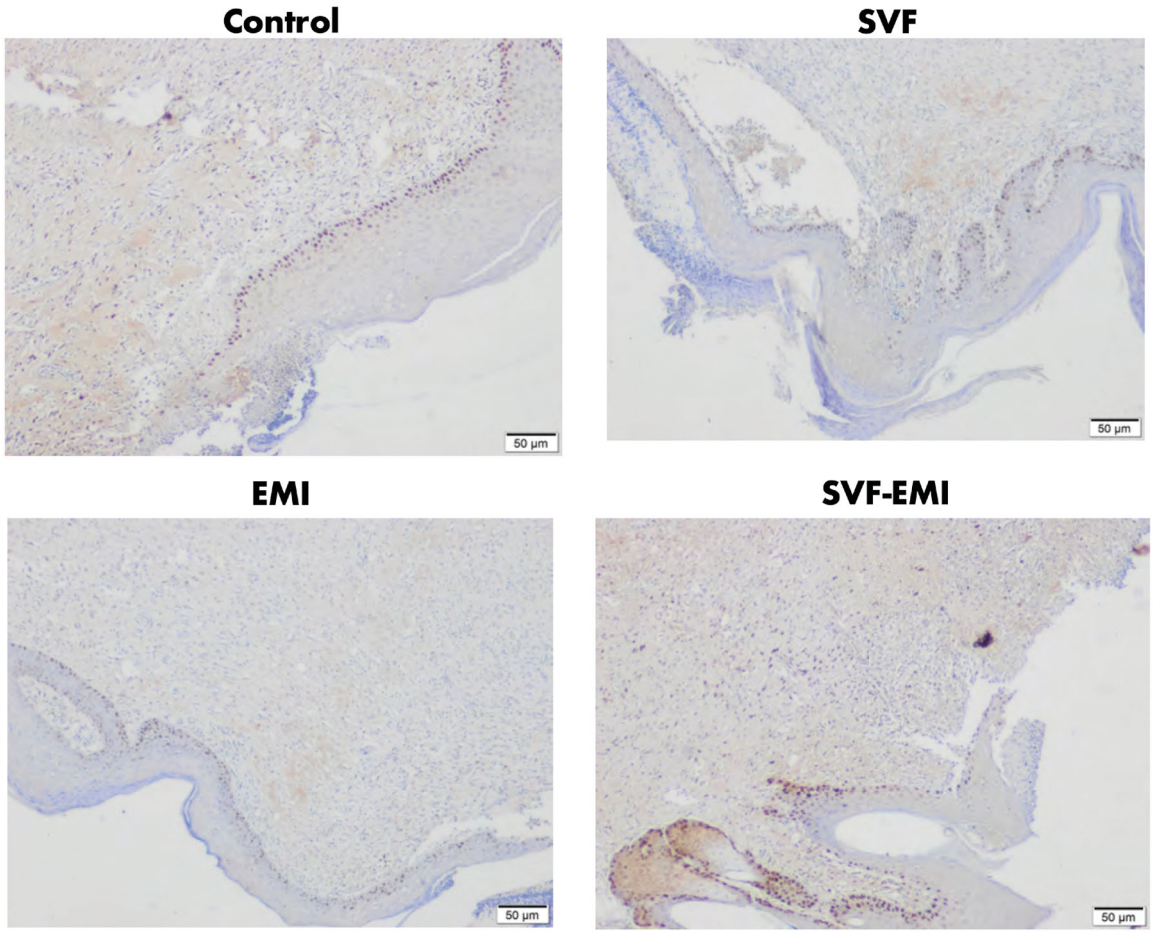

Figure 5. Immunohistochemical staining for Ki67 of the burn wound at the end of the second week.

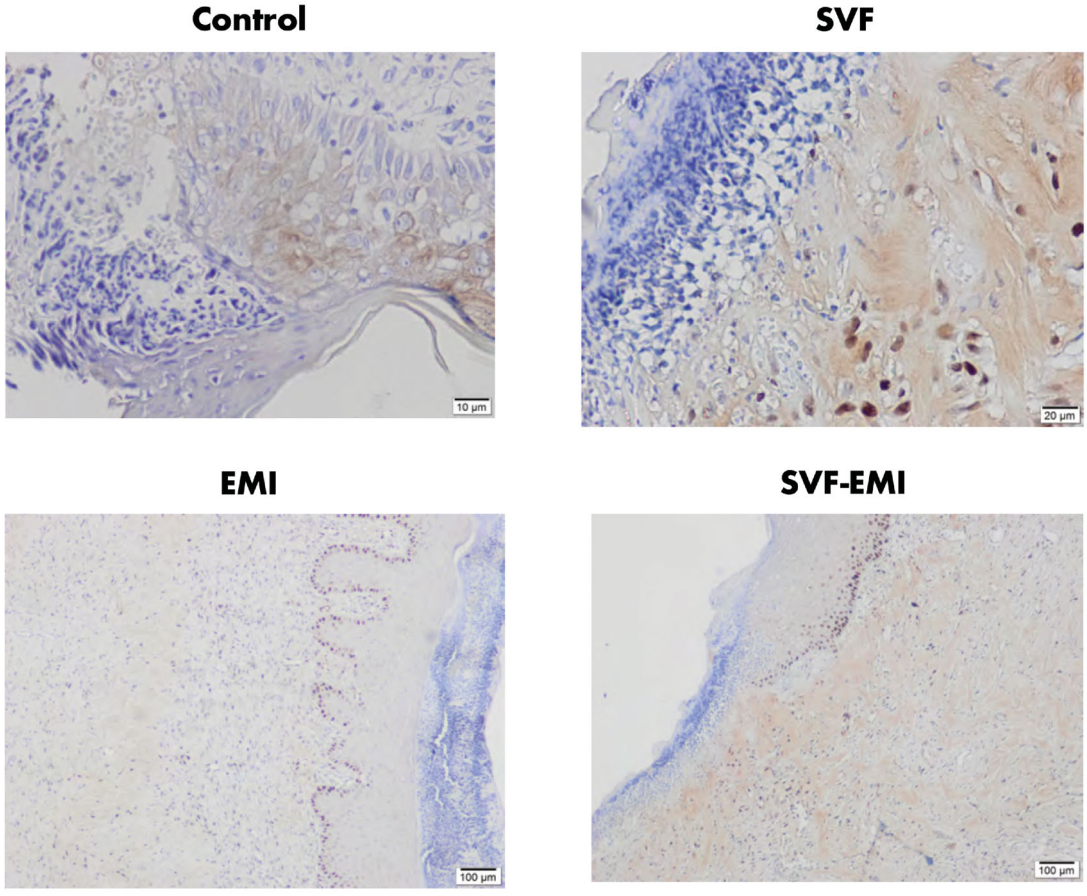

Figure 6. Immunohistochemical staining for Ki67 of the burn wound at the end of the third week.

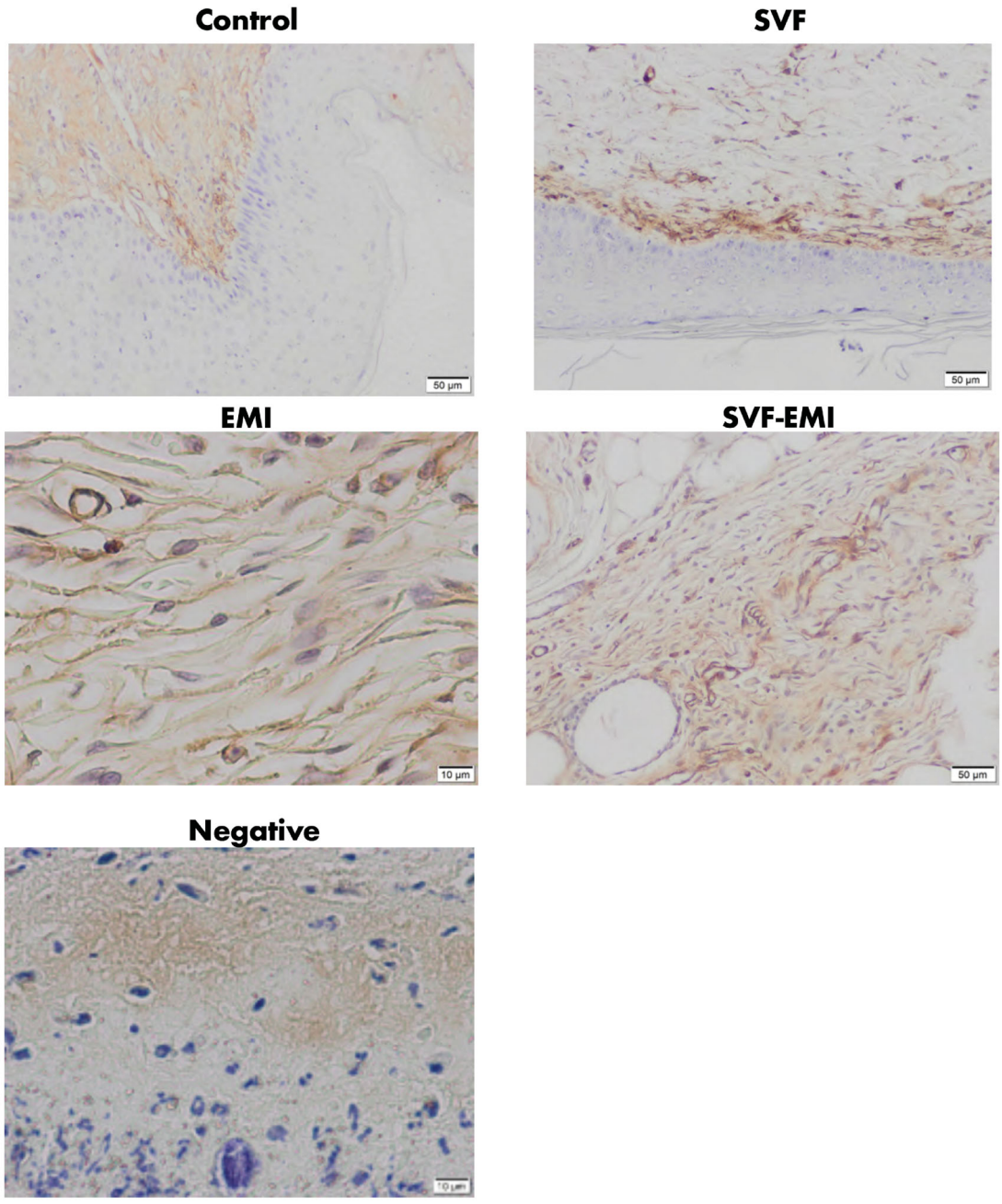

Figure 7. Immunohistochemical staining for CD90 of the burn wound at the end of the first week.

were washed three times with PBS the next day and were incubated with anti-mouse biotin-streptavidin hydrogen peroxidase secondary antibody for 30 min. The sections were washed three times with PBS for 5 min and stained with DAB for 5 min in order to determine the visibility of the immunohistochemical reaction. Contrast stained sections with Mayer's hematoxylin were washed several times with deionized water. For the final step, serially increasing concentrations of alcohol were used to remove water, passed from xylene, and at the end, sections were covered with a coverslip.

Statistical analysis

Immunohistochemical staining results were evaluated by examining the H-score [29–31]. The H-score was calculated with the formula (1 + staining intensity/3) × staining ratio. The staining ratio was graded semi-quantitatively, 0 = staining in less than 1% of cells; 1+ = staining in 1–10% of cells; 2+ = staining in 11–50% of cells; 3+ = staining in 51–80% of cells; 4+ = staining in more than 80% of cells. Staining intensity was determined as 0 = no staining; 1 = pale; 2 = moderate; 3= heavy. The difference between the findings and the groups was determined using the one-way ANOVA test, and $p < 0.05$ was considered statistically significant.

Results

In histological examination, that the control samples did not improve in the first-week group, but improvement started in the treatment groups. The skin was multicellular, vascularization was present, and the wound was closed (Figure 1). The control

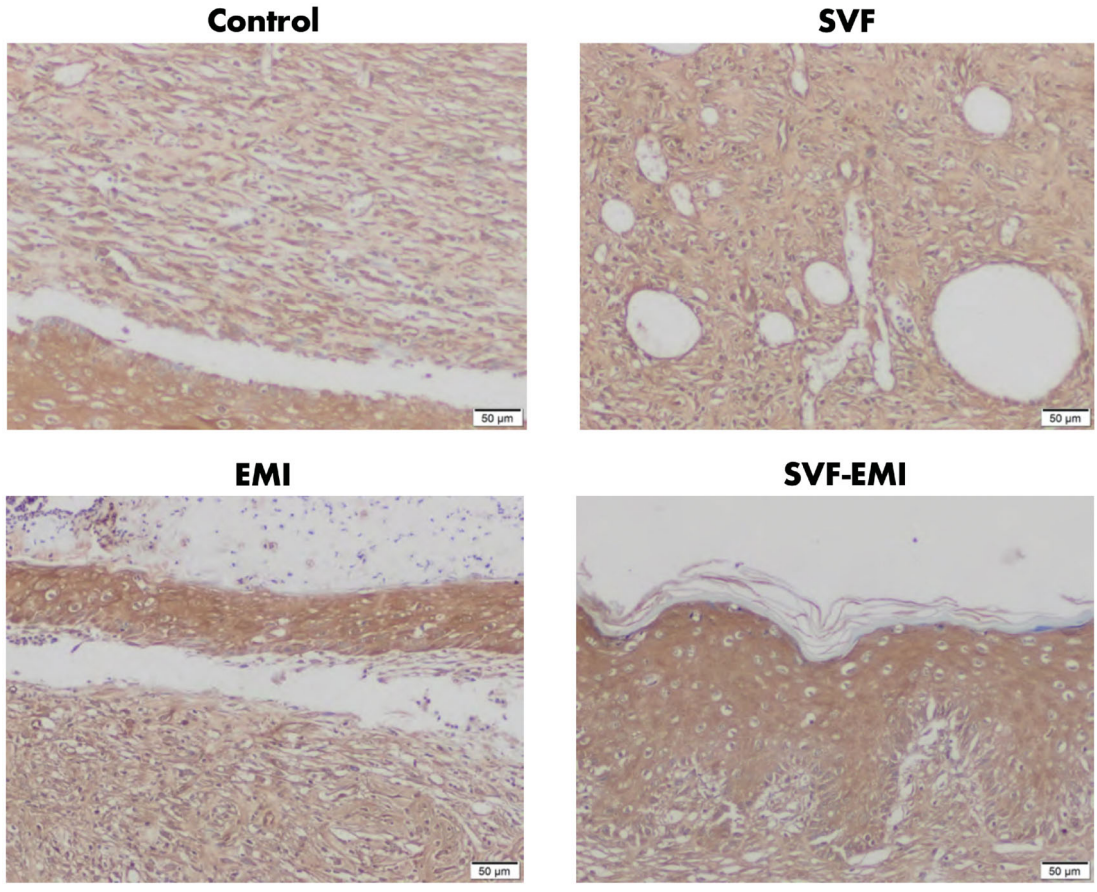

Figure 8. Immunohistochemical staining for CD90 of the burn wound at the end of the second week.

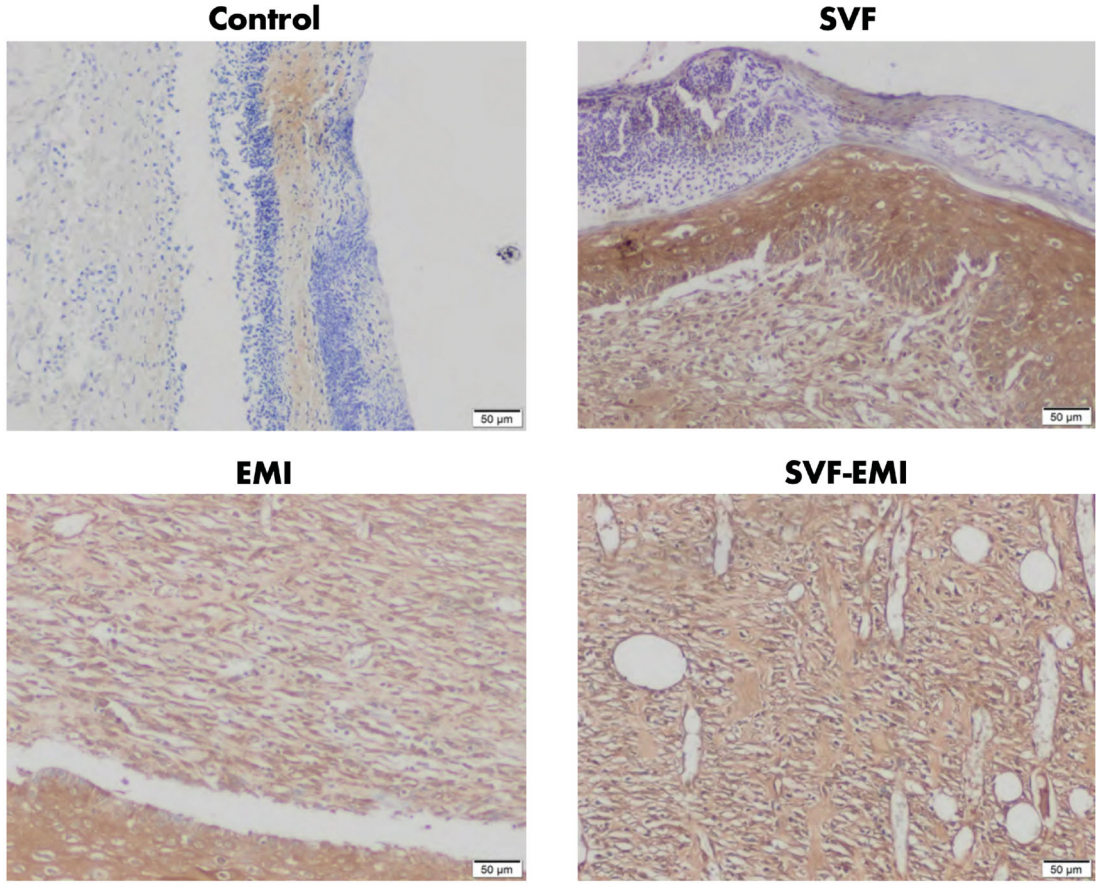

Figure 9. Immunohistochemical staining for CD90 of the burn wound at the end of the third week.

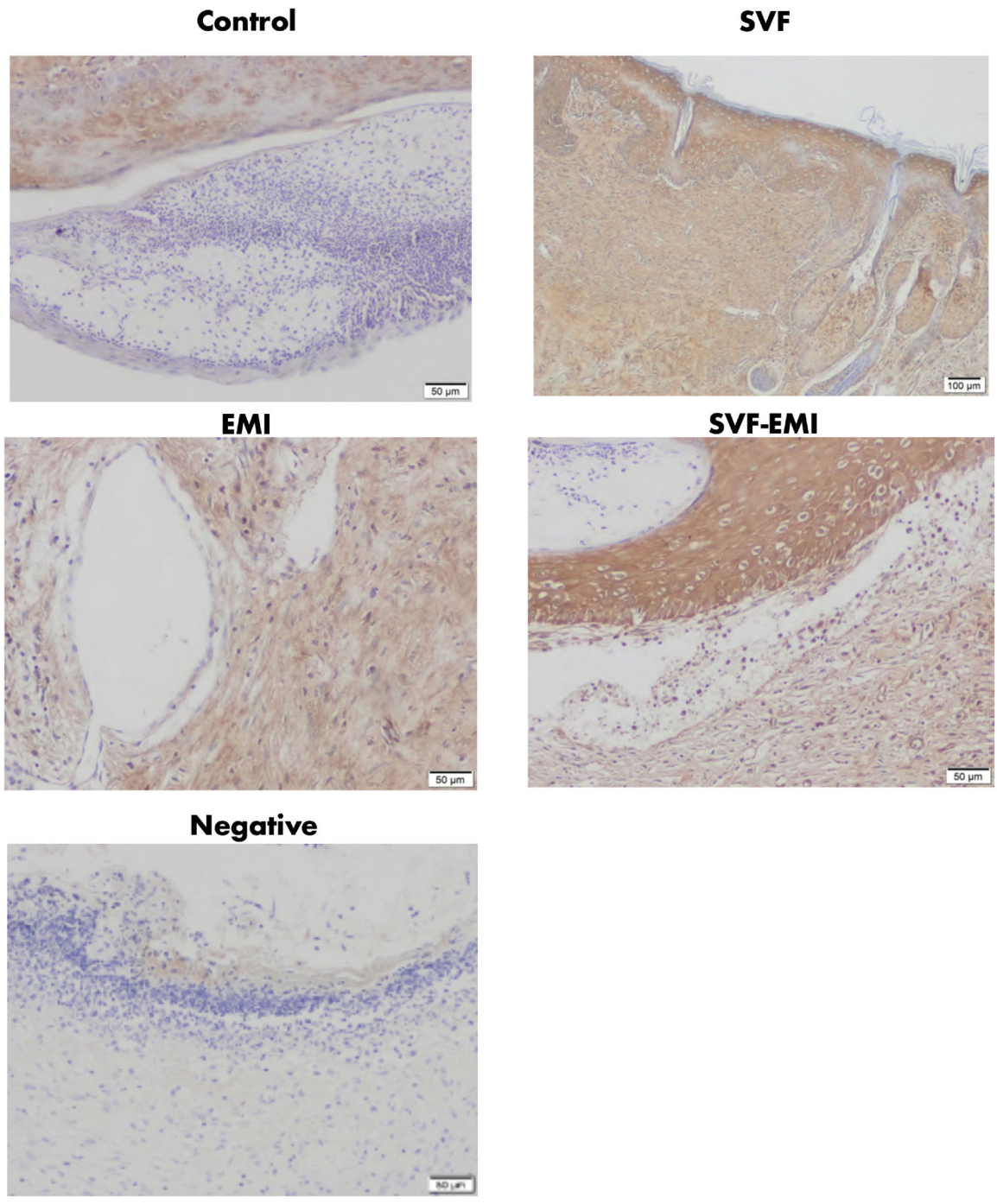

Figure 10. Immunohistochemical staining for CD73 of the burn wound at the end of the first week.

samples did not improve in the second week (Figure 2) and the third week (Figure 3) groups but improved in the treatment groups.

When the groups and weeks were examined in terms of cell proliferation, cell migration, re-epithelialization, granulation tissue, vessel formation, collagen accumulation, and fibril structure parameters during the healing process, a significant improvement ($p < 0.05$) was achieved in all groups compared to the control. The best group in terms of recovery was SVF + EMI, followed by SVF and EMI groups. Results of the first-week application were numerically better than the other weeks (Table 1).

Positive cells were evident, especially in the basal layer of the epidermis, in Ki67 immunohistochemistry stains, which were examined as a marker of proliferation in cells in the skin (Figures 4–6). Ki67 H-score was significantly higher in all experimental groups than in controls ($p < 0.01$). Among the groups, the best group in terms of recovery was SVF + EMI, followed by SVF and EMI. Results of the first-week application were numerically better than the other weeks (Table 1).

Immunohistochemical staining for CD90 (Figures 7–9), CD73 (Figures 10–12), and CD44 (Figures 13–15) were performed as ADSC markers in SVF and EMI applied for treatment. CD73 and CD90 staining were significantly higher than in controls in all groups and weeks. CD44 staining was significantly increased in the EMI and SVF + EMI groups compared to the control group (Table 1).

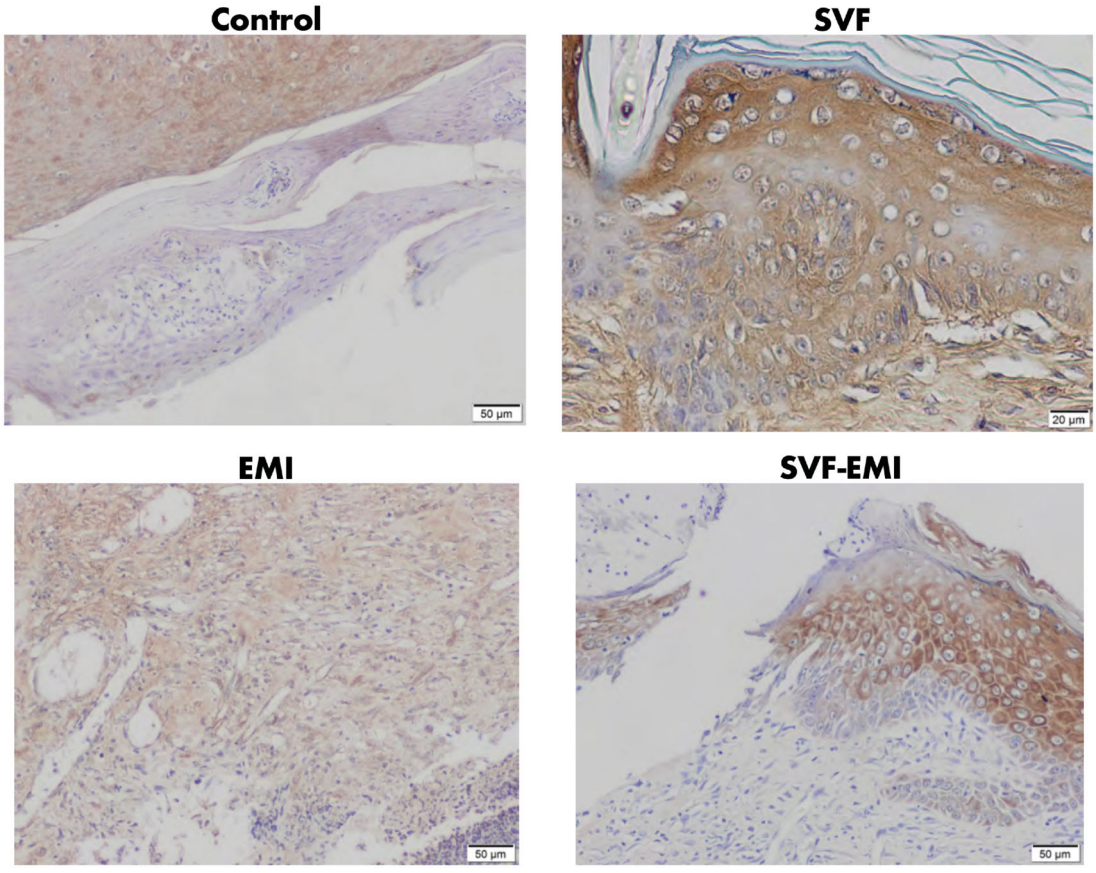

Figure 11. Immunohistochemical staining for CD73 of the burn wound at the end of the second week.

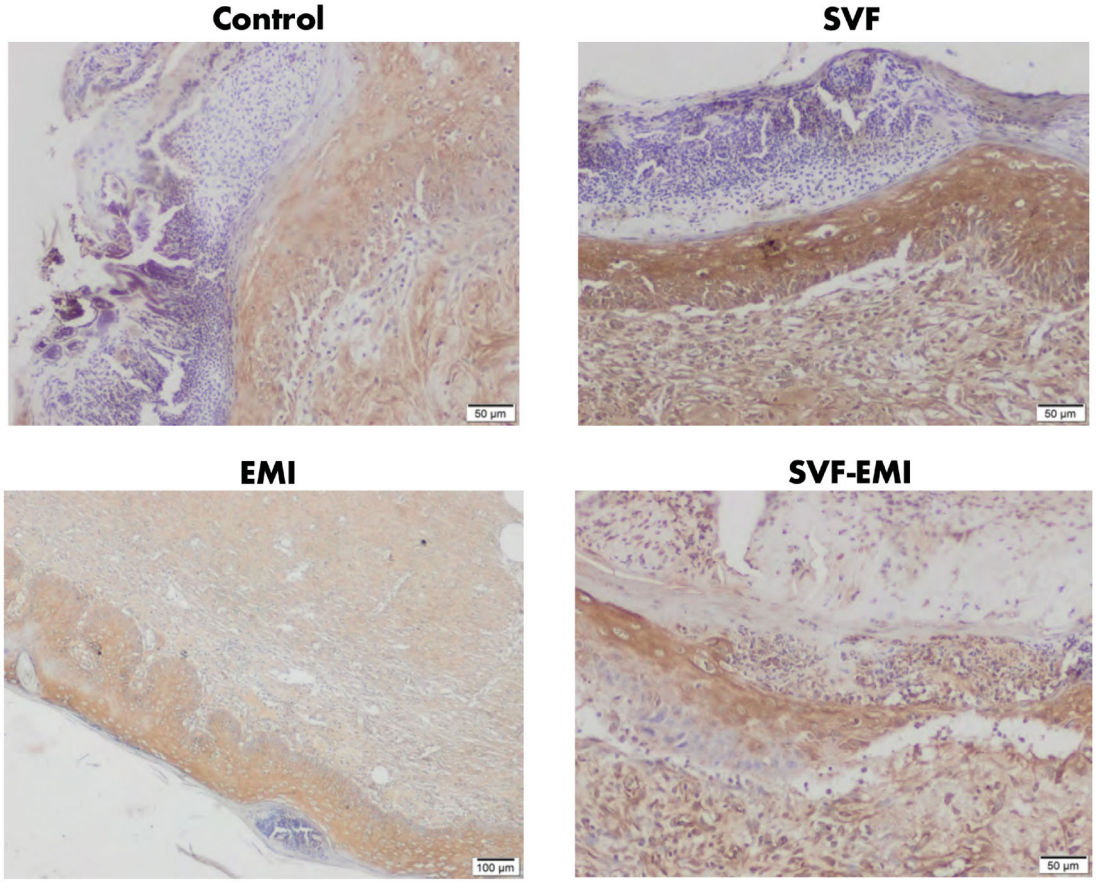

Figure 12. Immunohistochemical staining for CD73 of the burn wound at the end of the third week.

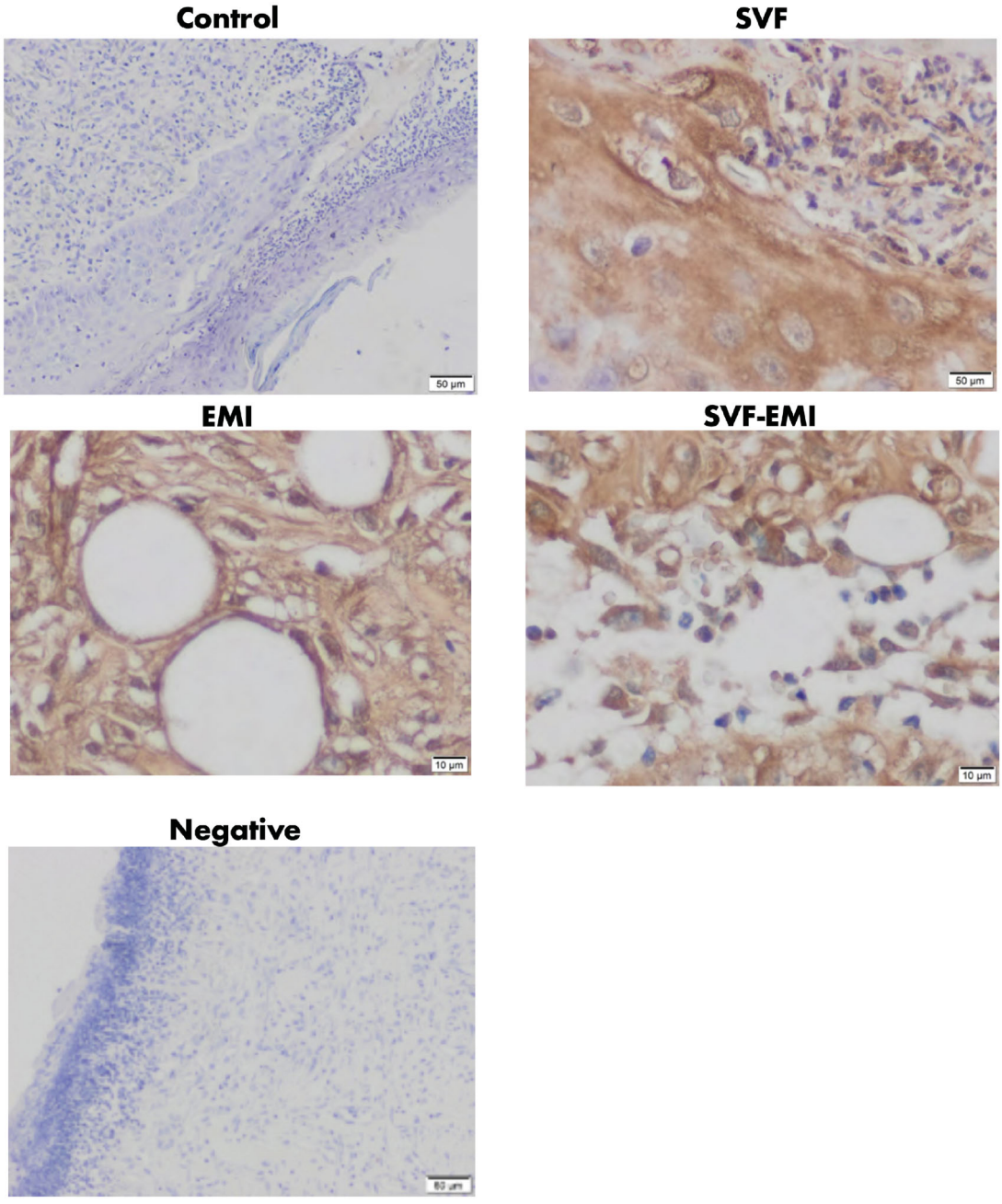

Figure 13. Immunohistochemical staining for CD44 of the burn wound at the end of the first week.

Positive cells were evident in the epithelial layer in CK17 immunohistochemistry staining, which was examined as a cytokeratin marker in cells in the skin (Figures 16–18). The CK17 H-score was significantly higher in all groups in the second and the third-week applications, and in the EMI and SVF + EMI groups in the first week compared to the control group (Table 1). Among the groups, the best group in terms of improvement was SVF + EMI.

A representative macroscopic wound healing process is imaged in Figure 19.

Discussion

Care and treatment of burn patients are carried out by an interprofessional team consisting of burn specialists, plastic surgeons, intensive care, wound care specialists, physiotherapists, dieticians, and nurses. The main goal in burn treatment is to prevent complications and restore functionality. However, treatment results depend on the degree and size of the burn. Recovery is slow with most second and third-degree burns, and patients may have to stay in hospital for a long time [32,33]. Deeper injuries may require surgical treatment, including excision and STSG [34,35]. Early STSG is avoided in the treatment of large and deep partial-thickness wounds due to the risk of delay in wound closure. This can lead to infection and poor aesthetic and functional results [36]. Therefore, early autologous cell spray grafting may be an interesting therapeutic option for the treatment of large, deep partial-thickness wounds [37].

Cutaneous wound healing due to injury and burn can generally be summarized as inflammation, epithelization, and tissue remodeling. During normal wound healing, the functional state and biomechanics of the skin should be sufficient to allow its

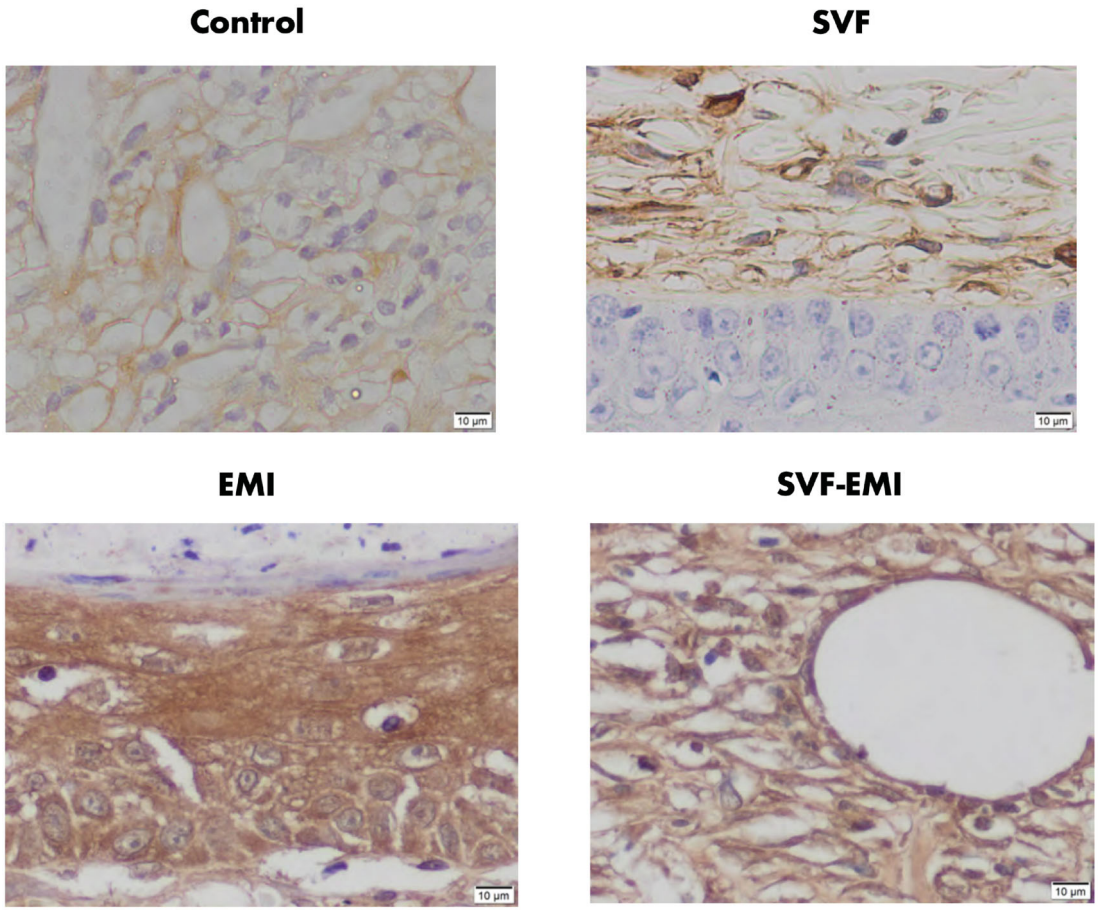

Figure 14. Immunohistochemical staining for CD44 of the burn wound at the end of the second week.

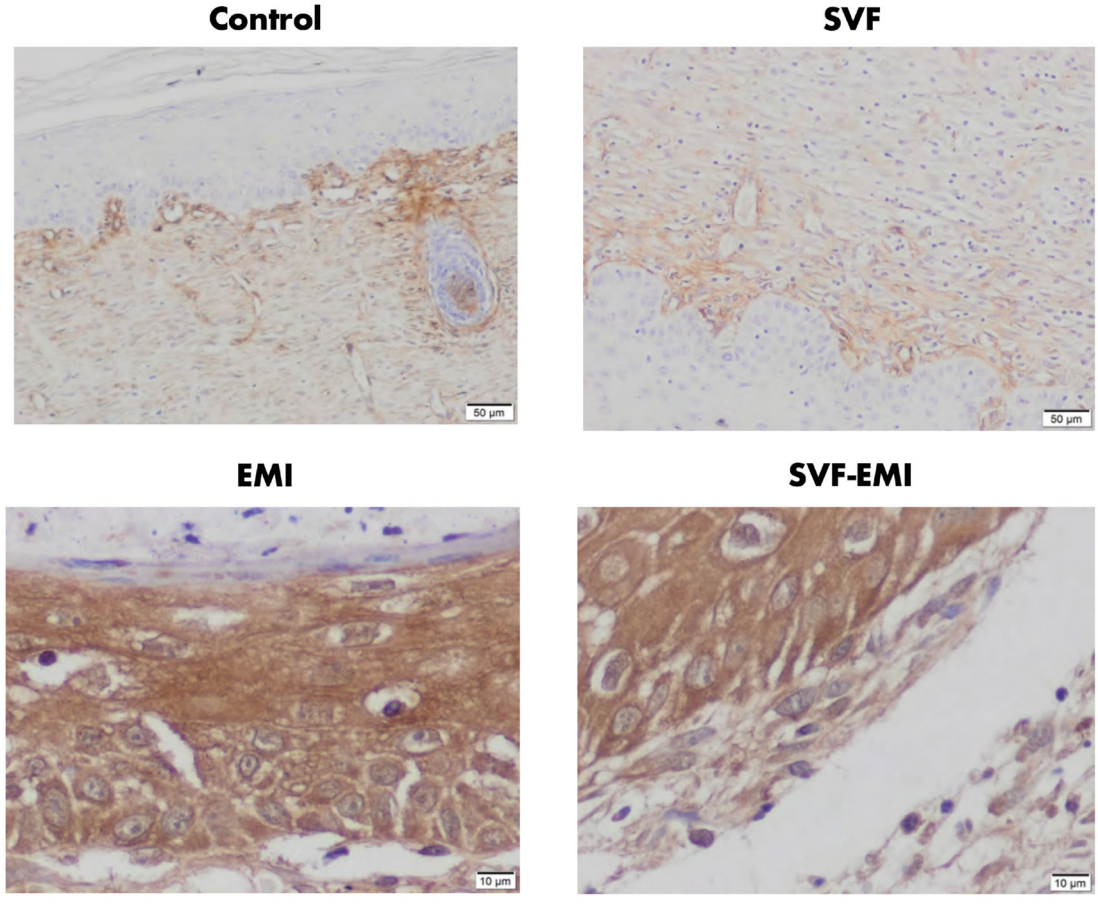

Figure 15. Immunohistochemical staining for CD44 of the burn wound at the end of the third week.

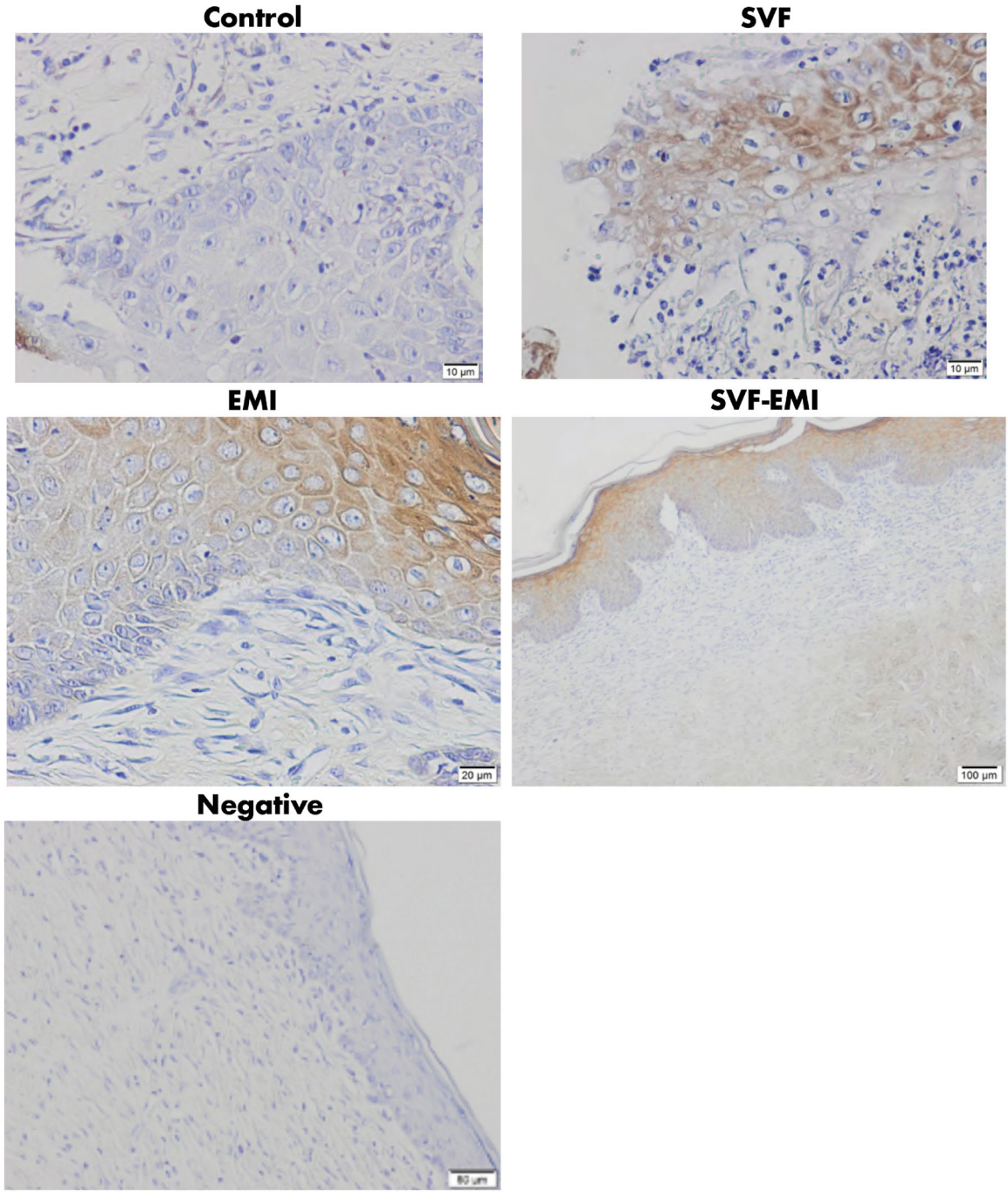

Figure 16. Immunohistochemical staining for CK17 of the burn wound at the end of the first week.

restoration. However, this is not the case in burn-like injuries, and the wound closes without epithelization with excessive accumulation of collagen. Therefore, it is important to know the state of the mechanisms governing the cellular functions and extracellular protein reorganization to be able to accomplish the studies for the treatment of wound healing. CD44 is the cell surface adhesion receptor expressed in all cells in the dermis. It is also known to have a role in the migration of leukocytes, T cell extravasation, and hyaluronic acid metabolism in the inflammatory and fibrotic process. CD44-null mice have been reported to have a severe scarring potential. This data shows that CD44 plays a very important role in epithelization [38,39].

Numerous successes have been achieved with mesenchymal stem cells (MSC) used in regenerative therapy since the 1970s [40]. MSCs are more advantageous than other stem cells in that they differentiate into a variety of cell lineages including muscle, bone, cartilage, and fat, and secrete biologically active paracrine factors [41]. In addition, MSCs, which can be easily isolated from a variety of adult tissues such as adipose tissue, bone marrow, skin, and periodontal ligament, possess immunomodulatory properties [42,43]. ADSCs have been widely studied after being characterized by Zuk et al. in 2001 [43]. ADSCs with multi-potential differentiation capacities, surface markers, and adhesion to plastic can be easily obtained from adipose tissue [44]. Although the efficacy of purified ADSCs has a greater potential for differentiation than SVF consisting of heterogeneous cell mixes, ADSC isolation from SVF requires longer time, more equipment, and higher cost [12]. For this reason, it would be more appropriate to use SVF obtained

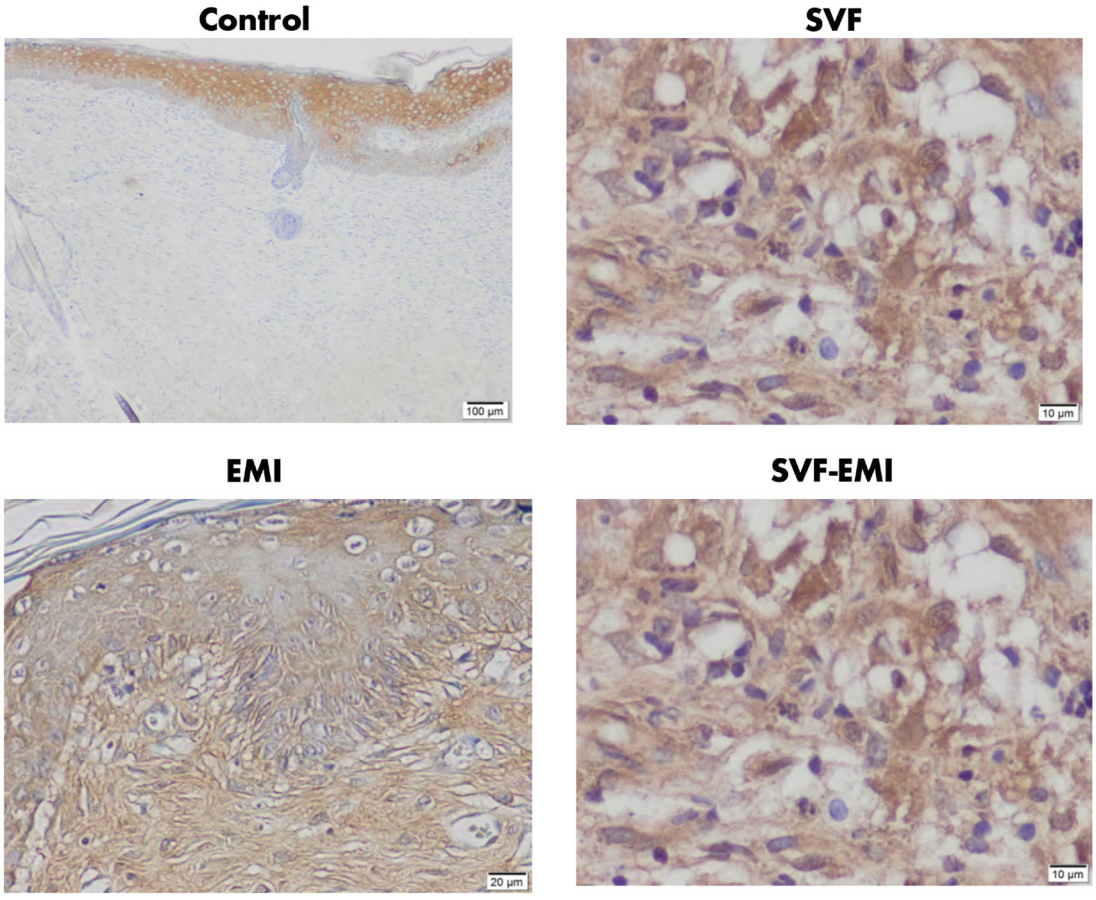

Figure 17. Immunohistochemical staining for CK17 of the burn wound at the end of the second week.

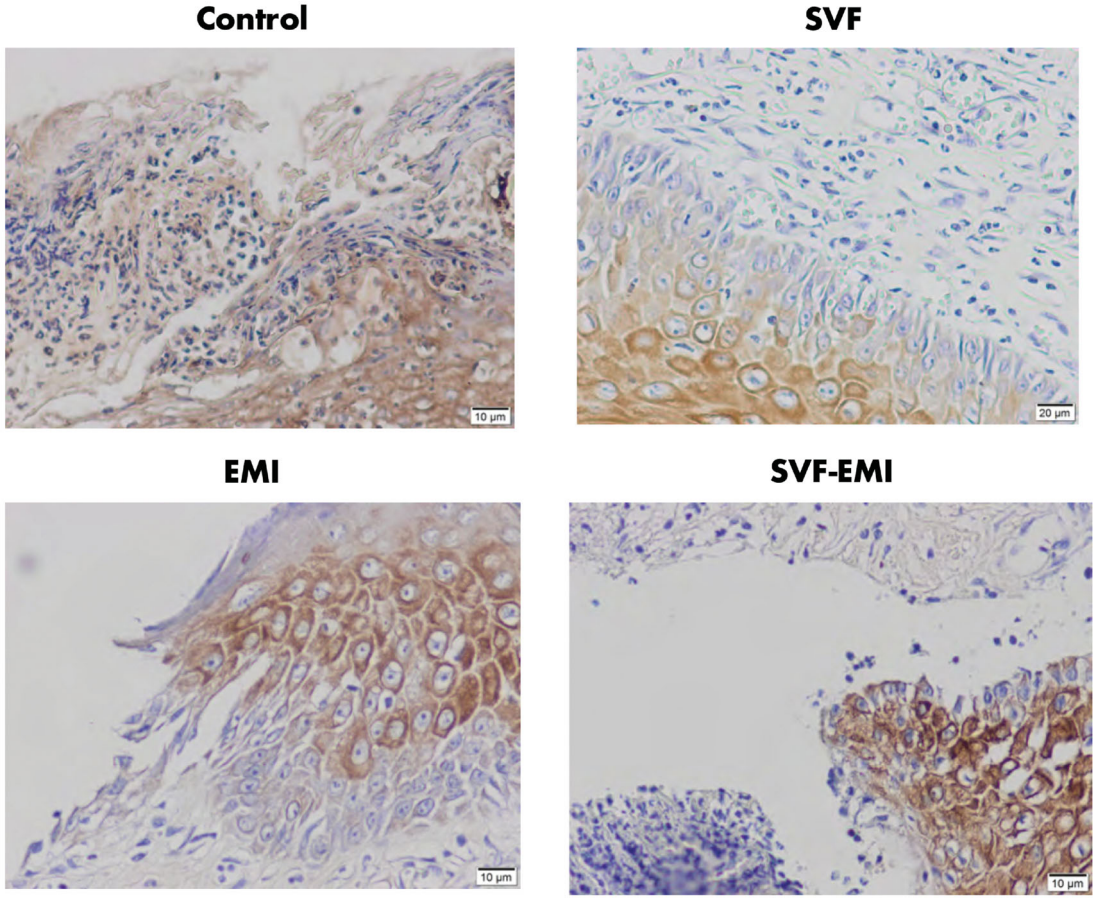

Figure 18. Immunohistochemical staining for CK17 of the burn wound at the end of the third week.

from the patient's own adipose tissue, which does not cause an ethical problem and allogeneic reaction, in cases requiring immediate medical intervention, such as in burn wounds. The application of SVF obtained by lipoaspiration is both an easy method and does not cause significant increases in morbidity [17]. Indeed, in a study conducted in 2012, 174 cases of ADSC application published in clinical settings were analyzed and found to have no adverse effects [45]. In addition, there are clinical studies that acknowledge the safety and efficacy of ADSCs for wound healing [46–48].

Studies have shown that ADSCs accelerate wound healing in an ischemic wound model [11] and a full-thickness excisional wound model [10]. Ebrahimian et al. [49] determined that SVF therapy healed radiation-induced wounds. Another study showed that SVF improved both the microscopic and macroscopic aspects of the wound healing process in experimentally induced full-thickness burn wounds in rats [18]. In animal experimental models to monitor the wound healing process, ADSCs increase angiogenesis and epithelialization and lead to the survival of grafted cells [50].

Re-epithelialization is known to depend on the ability of keratinocytes to co-clone [51]. Additionally, it is suggested that melanocytes and fibroblasts contribute to the repigmentation process [52]. With ReCell® technology, one of the recently developed NCSC and EMI methods, an autologous, heterogeneous skin cell suspension containing approximately 65% keratinocytes, 30% fibroblasts, and 3–5% melanocytes was obtained [53]. By this method, a small donor population of basal layer cells is sufficient for the autologous cell suspension and can be administered immediately. The cell suspension obtained by this method provides an alternative treatment option in burn injuries, as it can be applied without any delay, especially when the affected area is very large or there is not enough donor skin [19]. The easy and fast preparation of the autologous cell suspension with ReCell and the ability of the obtained cell suspension to cover a wound 80 times larger than the donor site has been interesting [54]. ReCell has been used in studies on cutaneous injuries and skin lesions, including vitiligo and congenital melanocytic nevi, and donor sites [25,27,55]. De Angelis et al. [56] reported that 70% of patients achieved re-epithelialization between 40 and 60 days postoperatively of skin cells obtained with the ReCell system to treat chronic ulcers. NCSCs derived from skin tissues of burn patients without the use of biochemical reagents consists of keratinocytes, melanocytes, and fibroblasts. In the rat model of burn healing, NCSCs significantly improved wound healing compared to PBS alone [20]. In another study, ADCS obtained with ReCell were shown to be a safe and effective alternative to STSG for the treatment of deep partial-thickness burns. In addition, increased healing, less scarring, and reduced pain were noted in the donor areas where ReCell was taken [57]. In our study, it was observed that SVF and EMI applied to a full-thickness burn wound provided a significant improvement in all groups compared to the control group in terms of their respective histopathological scores. There was no difference between the groups. Our results are similar to the results of the previous study in which SVF and EMI were applied. Hu et al. [54] showed that the combination of STSG and the autologous skin cell suspension is more effective and safer than STSG alone. Karina et al. [58] reported on wound healing that the efficacy of SVF + PRP (platelet-rich plasma) in terms of both macroscopic and microscopic findings was greater than SVF or PRP alone. In our study, the SVF + EMI group showed more improvement than the SVF alone and EMI alone groups.

The presence of proliferating cells at the injury site is considered an indicator of wound healing. Bromodeoxyuridine and PCNA have been used to detect proliferating cells in the burn wound [59,60]. We used Ki67 as an indicator of proliferation in our study. Positive cells were observed in Ki67 immunohistochemistry staining in skin cells, especially in the basal layer of the epidermis. Ki67 H-score was significantly higher in all experimental groups than in the control group. SVF + EMI was determined to be the best among the groups in terms of recovery. There was no difference between the SVF and EMI groups. Moreover, CK17 expression, which is an epithelial cell marker in the evaluation of recovery, was significantly higher than in the control group in the second and the third-week applications in the SVF group and in all application weeks in the EMI and SVF + EMI groups. Among the groups, the best group in terms of improvement was SVF + EMI. The increased expression of both CK17 and Ki67 in the treatment groups was due to the applied SVF and EMI.

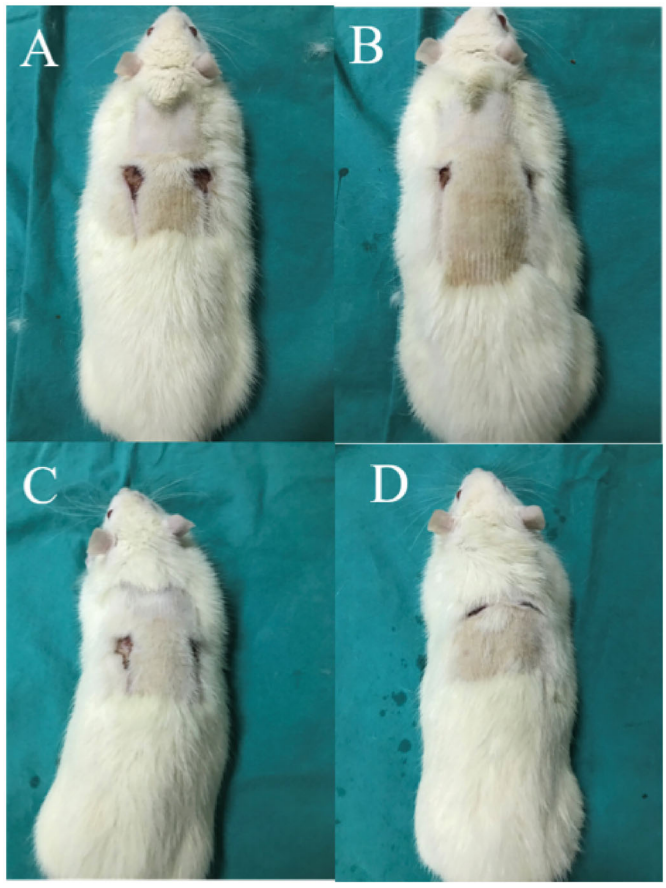

Figure 19. The wound induced is shown to heal gradually. (A) Wound onset, (B) SVF and EMI application (C) After application process, and (D) Final wound healing as images.

MSCs are generally characterized as positive for surface markers CD44, CD73, CD90, and CD105 and negative for surface markers HLADR, CD45, CD14, and CD34 [61]. Bourin et al. [44] provided guidelines for the characterization of stromal elements of SVF using markers CD13, CD29, CD44, CD73, and CD90 (>40% expression). Prasi et al. [62] found that there was no significant difference in their studies comparing both mRNA and protein levels of CD11b, CD34, CD44, CD105, CD29, CD73, CD90, or CD36 in burn wound with control. In our study, we determined that CD73 and CD90 expression increased significantly in all groups and weeks, and CD44 expression in EMI and SVF + EMI groups compared to the control. The fact that Prasi et al. showed that there was no difference in stem cell markers between control and burn wound tissue in their study suggests that the difference between the control and treatment groups in our study is due to the treatment applied, SVF and EMI.

In conclusion, we determined in our study that both SVF and EMI application significantly increased the healing of the burn wound. Moreover, SVF + EMI application provided more improvement than SVF or EMI alone. Since both SVF and EMI can be obtained easily and in a short time, it shows that they can be treatment alternatives, especially in deep and large burns.

Authors' contributions

MIT and PK analysed and interpreted the patient data regarding the immunohistological staining. MB, CG and PK were the major contributors in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank Enes Akyuz, PhD. for his assistance during the revision phase. There are no conflicts of interest in connection with this paper and the study did not use any sources of financial assistance.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Scientific Research Projects Coordination Unit of the University of Health Sciences in Istanbul. Project number BAP-2019/04-madde-2.

ORCID

Percin Karakol http://orcid.org/0000-0003-0068-2139
Mehmet Bozkurt http://orcid.org/0000-0002-4480-513X
Caner Gelbal http://orcid.org/0000-0002-8372-7509
Mehmet Ibrahim Tuglu http://orcid.org/0000-0002-0569-8415

References

[1] Jeschke MG, van Baar ME, Choudhry MA, et al. Burn injury. Nat Rev Dis Primers. 2020;6(1):11.
[2] World Health Organization. Burns fact sheet worldwide [Internet]. Geneva (Switzerland): WHO; 2016 [cited 2017]. Available from: http://www.who.int/mediacentre/factsheets/fs365/en/
[3] Mason SA, Nathens AB, Byrne JP, et al. Increased rate of long-term mortality among burn survivors: a population-based matched cohort study. Ann Surg. 2019;269(6):1192–1199.
[4] Stone J, Gawaziuk JP, Khan S, et al. Outcomes in adult survivors of childhood burn injuries as compared with matched controls. J Burn Care Res. 2016;37(2):e166–e173.
[5] Peck MD. Epidemiology of burns throughout the world. Part I: distribution and risk factors. Burns. 2011;37(7):1087–1100.
[6] Sheridan RL, Greenhalgh D. Special problems in burns. Surg Clin North Am. 2014;94(4):781–791.
[7] Wolf SE, Tompkins RG, Herndon DN. On the horizon: research priorities in burns for the next decade. Surg Clin North Am. 2014;94(4):917–930.
[8] You HJ, Han SK. Cell therapy for wound healing. J Korean Med Sci. 2014;29(3):311–319.
[9] Ichim TE, Harman RJ, Min WP, et al. Autologous stromal vascular fraction cells: a tool for facilitating tolerance in rheumatic disease. Cell Immunol. 2010;264(1):7–17.
[10] Kim WS, Park BS, Kim HK, et al. Evidence supporting antioxidant action of adipose-derived stem cells: protection of human dermal fibroblasts from oxidative stress. J Dermatol Sci. 2008;49(2):133–142.
[11] Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation. 2004;109(10):1292–1298.
[12] Faustini M, Bucco M, Chlapanidas T, et al. Nonexpanded mesenchymal stem cells for regenerative medicine: yield in stromal vascular fraction from adipose tissues. Tissue Eng Part C Methods. 2010;16(6):1515–1521.
[13] Gentile P, Orlandi A, Scioli MG, et al. Concise review: adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical implications for tissue engineering therapies in regenerative surgery. Stem Cells Transl Med. 2012;1(3):230–236.
[14] Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002;13(12):4279–4295.
[15] Gonzalez MA, Gonzalez-Rey E, Rico L, et al. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum. 2009;60(4):1006–1019.
[16] Foubert P, Gonzalez AD, Teodosescu S, et al. Adipose-derived regenerative cell therapy for burn wound healing: a comparison of two delivery Methods. Adv Wound Care. 2016;5(7):288–298.
[17] Atalay S, Coruh A, Deniz K. Stromal vascular fraction improves deep partial thickness burn wound healing. Burns. 2014;40(7):1375–1383.
[18] Cardoso AL, Bachion MM, Morais JM, et al. Adipose tissue stromal vascular fraction in the treatment of full thickness burns in rats. Acta Cir Bras. 2016;31(9):578–585.
[19] Gravante G, Di Fede MC, Araco A, et al. A randomized trial comparing ReCell system of epidermal cells delivery versus classic skin grafts for the treatment of deep partial thickness burns. Burns. 2007;33(8):966–972.
[20] Yoon C, Lee J, Jeong H, et al. Rapid preparation of a non-cultured skin cell suspension that promotes wound healing. Cell Tissue Bank. 2017;18(2):131–141.
[21] Busch KH, Bender R, Walezko N, et al. Combination of medical needling and non-cultured autologous skin cell transplantation (ReNovaCell) for repigmentation of hypopigmented burn scars. Burns. 2016;42(7):1556–1566.
[22] Cervelli V, De Angelis B, Balzani A, et al. Treatment of stable vitiligo by ReCell system. Acta Dermatovenerol Croat. 2009;17(4):273–278.
[23] Chant H, Woodrow T, Manley J. Autologous skin cells: a new technique for skin regeneration in diabetic and vascular ulcers. J Wound Care. 2013;22(10):S11–S15.
[24] Hu Z, Guo D, Liu P, et al. Randomized clinical trial of autologous skin cell suspension for accelerating re-epithelialization of split-thickness donor sites. Br J Surg. 2017;104(7):836–842.
[25] Mulekar SV, Ghwish B, Al Issa A, et al. Treatment of vitiligo lesions by ReCell vs. conventional melanocyte-keratinocyte transplantation: a pilot study. Br J Dermatol. 2008;158(1):45–49.
[26] Busato A, De Francesco F, Biswas R, et al. Simple and rapid Non-Enzymatic procedure allows the isolation of structurally preserved connective tissue Micro-Fragments enriched with SVF. Cells. 2020;10(1):36.

[27] Sood R, Roggy DE, Zieger MJ, et al. A comparative study of spray keratinocytes and autologous meshed split-thickness skin graft in the treatment of acute burn injuries. Wounds. 2015;27(2):31–40.
[28] Meyerholz DK, Piester TL, Sokolich JC, et al. Morphological parameters for assessment of burn severity in an acute burn injury rat model. Int J Exp Pathol. 2009;90(1):26–33.
[29] Mohajeri G, Masoudpour H, Heidarpour M, et al. The effect of dressing with fresh kiwifruit on burn wound healing. Surgery. 2010;148(5):963–968.
[30] Wu XB, Luo XQ, Gu SY, et al. The effects of polygonum cuspidatum extract on wound healing in rats. J Ethnopharmacol. 2012;141(3):934–937.
[31] Guo HF, Mohd Ali RA, Hamid R, et al. A new histological score grade for deep partial-thickness burn wound healing process. Int J Burns Trauma. 2020;10(5):218–224.
[32] Mason SA, Nathens AB, Byrne JP, et al. Association between burn injury and mental illness among burn survivors: a population-based, self-matched, longitudinal cohort study. J Am Coll Surg. 2017;225(4):516–524.
[33] Passaretti D, Billmire DA. Management of pediatric burns. J Craniofac Surg. 2003;14(5):713–718.
[34] Jackson D, Topley E, Cason JS, et al. Primary excision and grafting of large burns. Ann Surg. 1960;152:167–189.
[35] Johnson RM, Richard R. Partial-thickness burns: identification and management. Adv Skin Wound Care. 2003;16(4):178–187.
[36] Balakrishnan C, Hashim M, Gao D. The effect of partial-thickness facial burns on social functioning. J Burn Care Rehabil. 1999;20(3):224–225.
[37] Gerlach JC, Johnen C, McCoy E, et al. Autologous skin cell spray-transplantation for a deep dermal burn patient in an ambulant treatment room setting. Burns. 2011;37(4):e19–e23.
[38] Govindaraju P, Todd L, Shetye S, et al. CD44-dependent inflammation, fibrogenesis, and collagenolysis regulates extracellular matrix remodeling and tensile strength during cutaneous wound healing. Matrix Biol. 2019;75–76:314–330.
[39] Mansilla E, Spretz R, Larsen G, et al. Outstanding survival and regeneration process by the use of intelligent acellular dermal matrices and mesenchymal stem cells in a burn pig model. Transplant Proc. 2010;42(10):4275–4278.
[40] Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970;3(4):393–403.
[41] Chamberlain G, Fox J, Ashton B, et al. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739–2749.
[42] Wexler SA, Donaldson C, Denning-Kendall P, et al. Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not. Br J Haematol. 2003;121(2):368–374.
[43] Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7(2):211–228.
[44] Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;15(6):641–648.
[45] Gir P, Oni G, Brown SA, et al. Human adipose stem cells: current clinical applications. Plast Reconstr Surg. 2012;129(6):1277–1290.
[46] Jeong JH. Adipose stem cells and skin repair. Curr Stem Cell Res Ther. 2010;5(2):137–140.
[47] Jo DI, Yang HJ, Kim SH, et al. Coverage of skin defects without skin grafts using adipose-derived stem cells. Aesthetic Plast Surg. 2013;37(5):1041–1051.
[48] Sung HM, Suh IS, Lee HB, et al. Case reports of adipose-derived stem cell therapy for nasal skin necrosis after filler injection. Arch Plast Surg. 2012;39(1):51–54.
[49] Ebrahimian TG, Pouzoulet F, Squiban C, et al. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. Arterioscler Thromb Vasc Biol. 2009;29(4):503–510.
[50] Zografou A, Papadopoulos O, Tsigris C, et al. Autologous transplantation of adipose-derived stem cells enhances skin graft survival and wound healing in diabetic rats. Ann Plast Surg. 2013;71(2):225–232.
[51] Barrandon Y, Green H. Three clonal types of keratinocyte with different capacities for multiplication. Proc Natl Acad Sci USA. 1987;84(8):2302–2306.
[52] Yamaguchi Y, Morita A, Maeda A, et al. Regulation of skin pigmentation and thickness by dickkopf 1 (DKK1). J Investig Dermatol Symp Proc. 2009;14(1):73–75.
[53] Wood FM, Giles N, Stevenson A, et al. Characterisation of the cell suspension harvested from the dermal epidermal junction using a ReCell® kit. Burns. 2012;38(1):44–51.
[54] Hu ZC, Chen D, Guo D, et al. Randomized clinical trial of autologous skin cell suspension combined with skin grafting for chronic wounds. Br J Surg. 2015;102(2):e117–e123.
[55] Campanella SD, Rapley P, Ramelet AS. A randomised controlled pilot study comparing Mepitel® and SurfaSoft® on paediatric donor sites treated with Recell®. Burns. 2011;37(8):1334–1342.
[56] De Angelis B, Migner A, Lucarini L, et al. The use of a non cultured autologous cell suspension to repair chronic ulcers. Int Wound J. 2015;12(1):32–39.
[57] Holmes JHt, Molnar JA, Shupp JW, et al. Demonstration of the safety and effectiveness of the RECELL® system combined with split-thickness meshed autografts for the reduction of donor skin to treat mixed-depth burn injuries. Burns. 2019;45(4):772–782.
[58] Karina , Samudra MF, Rosadi I, et al. Combination of the stromal vascular fraction and platelet-rich plasma accelerates the wound healing process: pre-clinical study in a Sprague-Dawley rat model. Stem Cell Investig. 2019;6:18.
[59] Alemdaroglu C, Degim Z, Celebi N, et al. An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor. Burns. 2006;32(3):319–327.
[60] Romana-Souza B, Nascimento AP, Monte-Alto-Costa A. Low-dose propranolol improves cutaneous wound healing of burn-injured rats. Plast Reconstr Surg. 2008;122(6):1690–1699.
[61] Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. Cell Transplant. 2011;20(1):5–14.
[62] Prasai A, El Ayadi A, Mifflin RC, et al. Characterization of adipose-derived stem cells following burn injury. Stem Cell Rev Rep. 2017;13(6):781–792.