The Dynamic Duo for Acid Burn Healing: Non-Cultured Autologous Skin Cells and Adipose-Derived Mesenchymal Stem Cells

Muhammad Rauf Ahmed[1]*,Fatima Jaweria[1], Iffat[1], Aizazullah Shah[1] Abdullah Ahmad[1] ,Madiha Islam[2], Nabila Kauser[1], Tanveer A Q[1], Mohsin Shahzad[1], and Tariq Iqbal[1]

[1]*Department of Molecular Biology and Biochemistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan*

[2] *Tehsil Headquarter Muree, Hospital, Pakistan*

*Corresponding author

Abstract

This study is aimed to investigate the regenerative potential of a mixture of non-cultured skin cells (fibroblasts, keratinocytes, Langerhans cells, and melanocytes) combined with ADMSCs (adipose-derived mesenchymal stem cells) in rat models with acid burns. The non-cultured skin cells were obtained through enzymatic digestion and then transplanted (1 million cells) into the dermal layer of rat burn models (n=10), both with and without the addition of ADMSCs. Acid-inflicted burn wounds measuring approximately 2 cm^2 were created on the rats' backs. Following transplantation, the healing capacity of the non-cultured skin cells with ADMSCs was evaluated through macroscopic analysis (photographs taken at different time intervals to assess wound closure percentage), histological examination (using H&E staining), and analysis of healing-related molecular markers. The results indicated that the combination of non-cultured cells with ADMSCs led to earlier and more effective recovery compared to the group treated with non-cultured cells alone. This combination therapy demonstrated faster wound closure, accelerated re-epithelization, formation of granulation tissue, increased collagen deposition, enhanced angiogenesis, and a significant reduction in the number of inflammatory cells. Additionally, the group receiving the combined therapy showed a noteworthy decrease in the expression of pro-inflammatory markers

(IL-1 beta, IL-6, TNF alpha), an improvement in the oxidative marker (SOD1), and elevated levels of angiogenesis factors such as VEGF and HGF. Furthermore, markers associated with collagen production (collagen type 1 alpha 1, fibroblast growth factor-2, transforming growth factor beta 1) displayed marked improvement. Based on these findings, we propose that the combination of non-cultured skin cells with ADMSCs represents a superior treatment modality for achieving earlier healing and regeneration of the skin in partial thickness burn wounds.

Introduction

A burn wound refers to an injury that affects the skin and its underlying layers, caused by various factors such as electricity, chemicals, radiation, steam, and fire. The severity of a burn depends on the duration of exposure, temperature, and concentration of the substances involved (1). Globally, approximately 11 million individuals experience burn injuries each year, which translates to an average of 30 thousand people per day, with up to 180,000 fatalities annually (2). In Pakistan, the mortality rate associated with burns stands at around 7.1%. Unfortunately, many individuals endure additional complications due to inadequate rehabilitation. A recent study conducted at Karachi Civil Hospital revealed that over 89% of cases were accidental, while 1.5% were categorized as suicidal (3).

Chemical burns are among the most significant types of burns, with alkalis and acids being common causative agents. When chemicals are absorbed or inhaled, they can cause injuries to the skin, eyes, and even systemic side effects (4). Partial thickness burns involve damage to the entire epidermis and variable depths of the dermis. Unlike full thickness burns, partial thickness burns can undergo re-epithelialization without requiring surgical intervention. It is crucial for the re-epithelialization process to commence within 24 hours of the injury to minimize the risk of scar

formation and achieve optimal cosmesis (5). Severe burn injuries are characterized by the loss of skin architecture, function, and progenitor cell populations responsible for regenerating and restoring the structures and functions of the skin. Several studies have highlighted these hallmarks of severe burn injuries(1, 6, 7).

Previous studies have highlighted the significant positive effects of adipose-derived mesenchymal stem cells (ADMSCs) in skin regeneration, both as standalone treatments and in combination with other modalities. These studies have demonstrated that ADMSCs exhibit higher expression of healing markers in the skin. The application of ADMSCs in burn rat models has yielded promising results in the repair of damaged skin tissue. It has been observed that ADMSCs can effectively reduce skin wear and tear and prevent the development of skin contracture. Furthermore, ADMSCs have the potential to address age-related issues associated with the differentiation of skin-specific cells such as fibroblasts and keratinocytes. An important advantage of MSCs is their ability to remain at the injection site, ensuring prolonged activity. Additionally, the effectiveness of this approach can be further enhanced through transplantation with an appropriate scaffold. While ADMSCs have demonstrated promising potential for regeneration, it is believed that their performance could be optimized in the presence of skin cells.

Autologous skin grafting is a commonly employed procedure for the treatment of severe burns. However, it can be challenging to treat patients with limited donor sites available for harvesting skin grafts. This limitation may result in significant delays in wound healing, increased susceptibility to infections, scarring, or even fatalities. To address the shortage of autografts, various alternatives such as allogeneic skin, xenografts, and synthetic skin substitutes have been extensively utilized in burn wound care. In the context of skin regeneration, non-cultured

autologous skin cells, including fibroblasts, keratinocytes, melanocytes, and Langerhans cells, are combined with different treatment modalities. These cells can be directly injected onto the wound or sprayed along with hydrogel-like fibrin to ensure their adherence to the wound surface (8). To increase cell numbers before application, culturing of these autologous cells can also be performed. The first reported use of a combination of autologous cultured keratinocytes with fibrin for the treatment of full-thickness wounds in pigs was documented by Grant et al. in 2002 (9, 10)

In recent years, there has been a growing utilization of cell-based therapies as viable alternatives to traditional skin grafting techniques in the treatment of burn wounds, aiming to promote wound healing and restore skin structure and function (7, 11). This article intends to assess the effectiveness of autologous skin cells, both as standalone treatments and in combination with ADMSCs, specifically focusing on their impact on partial thickness burn wounds

Materials and methods

Experimental design:

The study involved a total of 40 male Sprague Dawley (SD) rats, aged between 3 and 4 months, with an average weight of approximately 300-400g. These rats were housed in a controlled environment (12) throughout the duration of the experiment. The rats were randomly divided into four equal groups, with each group consisting of 10 rats. Group I served as the control group and comprised of rat burn models who did not receive any treatment. Group II consisted of rat burn models treated with non-cultured skin cells. Group III included rat burn models treated with ADMSCs. Lastly, group IV consisted of rat burn models that received a combination therapy of ADMSCs and non-cultured autologous skin cells.

Isolation and preparation of ADMSCs

As mentioned in our previous report (13), fat tissue weighing between 2 to 4 g was extracted from the abdominal cavity of SD rats aged 3-4 months. ADMSCs were isolated from the fat tissue through enzymatic digestion. The cells were then cultured in DMEM-low glucose supplemented with 15% fetal bovine serum (FBS) (Sigma, MO, USA), 100U/ml penicillin, and 100 mg/ml streptomycin (MP Biomedicals, CA, USA). For this study, the ADMSCs were used in the second passage of culturing.

Preparation of non-cultured skin cells:

Following the removal of hair, a biopsy was performed on the skin, specifically obtaining a small split thickness sample measuring approximately one square centimeter. This sample contained both the epidermis and a portion of the dermis. Subsequently, the skin sample was immersed in a solution containing trypsin (Gibco, USA) at a temperature of 37 degrees Celsius for a duration of 10 minutes. This enzymatic treatment facilitated the separation of the two layers of skin. After removing the skin sample from the solution, the inner side of both skin layers was gently scraped using a scalpel.

To create a cellular suspension, the cells were aspirated and passed through a filter. The total number of cells transplanted per rat in the dermal part of the burn wound was one million, as determined using the Countess TM Automated Cell Counter (Thermofisher scientific). The suspension contained all subtypes of cells, including keratinocytes, papillary dermal fibroblasts, Langerhans cells, and melanocytes. These cell subtypes were characterized by a histopathologist (14).

Rat model of acid burns:

For the acid burn injury model, SD rats were selected. To induce general anesthesia, ketamine at a dosage of 88 mg/kg of body weight and xylazine at a dosage of 14 mg/kg of body weight were injected into the peritoneal cavity. This ensured that the rats were adequately anesthetized during the procedure.

To prepare the rats for the acid burn, the backside of the rats was shaved using an electric hair clipper (Dingling hair clipper, RF-608). The positioning of the rats' bodies was adjusted to achieve an optimal dorsal surface area for the application of the acid.

Filter sheets were then cut into circular shapes with a diameter of 2 cm. These filter sheets were soaked in a strong solution of 12.01 N hydrochloric acid (HCl) obtained from Merck, USA, for a duration of 2 minutes. Each rat received two adjacent wounds, as two pieces of the soaked filter sheets were applied to the upper dorsal area of each rat for a period of 10 minutes (15)

Transplantation of skin cells & ADMSCs:

For the *in vivo* study, a total of 40 SD rats were utilized and were randomly divided into four study groups, with each group consisting of 10 rats, as previously mentioned. All groups were assessed at specific time points, including days 4, 7, 10, 14, and 18. On the first day of the experiment, the rats in the second group were treated by administering 200 μL of ADMSCs to each wound site, while the rats in the third group received autologous skin cells suspended in 200 μL of PBS. These treatments were administered according to the protocols outlined in the previous studies (16, 17). In contrast, the fourth group of rats received injections of one million ADMSCs and one million autologous skin cells suspension into the dermis, adjacent to the wound site.

Wound assessment:

To evaluate wound healing progress, the percentage of wound closure was analyzed using the same methodology as previously reported in our article. Photographs of the wounds were captured on days 4, 7, 10, 14, and 18 during the treatment period to document the healing process. Subsequently, upon complete healing, the rats were euthanized, and skin biopsies were obtained from the site of healing, following the same procedures as described (18).

Histological evaluation:

Skin samples measuring 0.5 cm^2, obtained from the healed wound area, were fixed in 10% formalin (Sigma, USA). Following fixation, the skin samples underwent a dehydration process, after which they were embedded in wax. Fine sections measuring 5 μm in thickness were then obtained from the embedded samples using an HM-340E microtome (Microm Inc., USA).

To visualize the skin architecture, the sections were stained with hematoxylin and eosin (H&E) using standard protocols. The stained sections were examined and analyzed under an Olympus BX-61 microscope (19).

***In vivo* gene expression analysis:**

Skin samples obtained from all groups were minced, and Trizol reagent (Sigma Aldrich, USA) was utilized to isolate total RNA from the samples. A reverse transcriptase system (Invitrogen, USA) was employed to synthesize complementary DNA (cDNA), with 1.5 μg of RNA serving as the template for the PCR reaction. For quantification, SYBR Green PCR Super Mix (Fermentas, USA) was used on the Bio-Rad System iQ5. The genes of interest that were quantified in this study

include SOD1, VEGF, HGF, HIF-1α, TGF-β1, Col1-α1, and FGF2, as previously described in the studies (18, 19).

Statistical analysis:

Statistical analysis was conducted using GraphPad Prism 8.0 software. The results were expressed as the mean value along with the standard deviation. To compare groups at a specific time point, a t-test was employed. On the other hand, a two-way analysis of variance (ANOVA) was utilized to compare groups across multiple time points. A p-value less than 0.05 ($P < 0.05$) was considered to be statistically significant, as indicated in the previous study (17).

Ethical approval

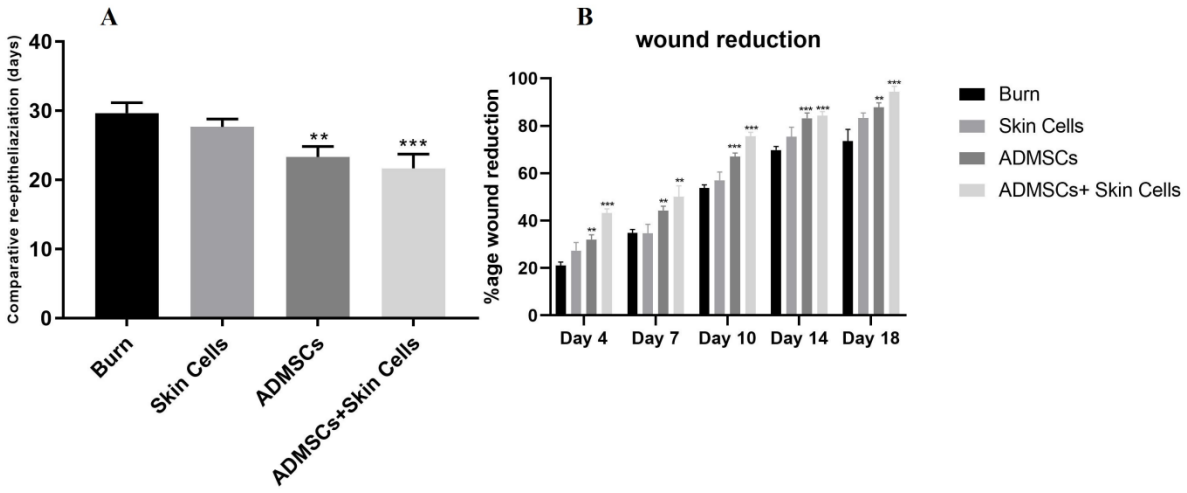
The research study received ethical approval from the Ethical Review Board of Shaheed Zulfiqar Ali Bhutto Medical University. The approval was granted via a letter with the reference number F.1-1/2015/ERB/SZAMBU/826.

Consent to participate and consent to publish

No human subjects were involved in this study. No anesthesia or euthanasia is part of the study.

Results:

It was observed that wounds started close at day 4th of treatment in a group of rats treated by a combination of ADMSCs and autologous non-culture skin cells(Fibroblasts, keratinocytes, langerhans, melanocytes cells), which shows faster healing when compared with autologous non-culture skin cells treated and ADMSCs treated group separately (Fig 1).



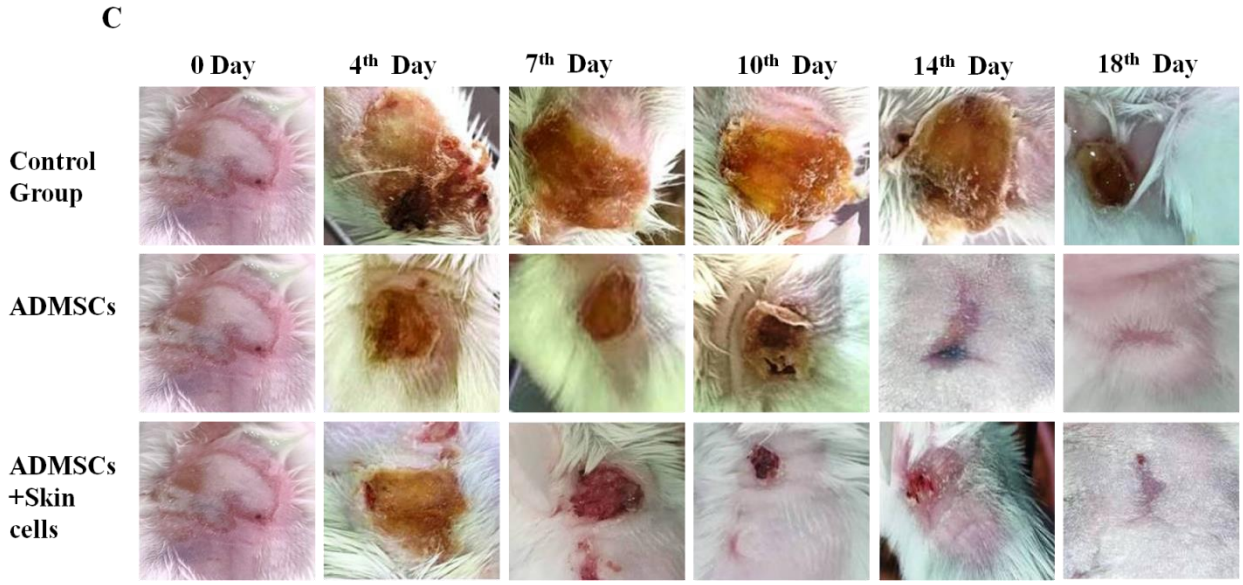


Figure 1: Re-epithelialization of the burn wound in experimental groups. (A) Comparative re-epithelization shows that autologous non-cultured skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and the combined treatment of ADMSCs with autologous non-cultured skin cells shows significant improvement whereas skin cells alone treatment was not significant when compared with the burn control. Data are presented as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001 (versus the Burn non-treatment group). (B) Wound reduction was noted for the first 14 days and then till the complete wound healing. Data shows the combined treatment of ADMSCs + skin cells completely heal the wound after the 18 days of treatment. Data are presented as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001 (versus the Burn non-treatment group). (C) Macroscopic analysis of the wounds treated with ADMSCs and Combined therapy of ADMSCs+ Skin cells comparative to control group. Representative photographs of wounds from postoperative days 0, 4, 7, 10, 14 and 18 for each of the following groups: Saline injected, ADMSCs injected, ADMSCs and Skin cells.

All experimental wounds were observed by taking photos at different time intervals day, 4^{th} day, 7^{th} day, 10^{th} day, 14^{th} day, till the day when the wounds of the combined group (autologous non-culture skin cell treated + ADMSCs) were healed completely at day 18 of treatment. The average number of days for re-epithelization reduced after the treatment compared to the control (burn). Days for re-epithelization were less in the skin cells with ADMSCs combined group than in the separate skin cells and ADMSCs group compared with burn control. The percentage wound reduction at day 4 was 43.2 ± 1.78 $p < 0.001$ in skin cells with ADMSCs combined, 32.1 ± 2.03 $p = 0.004$ in ADMSCs, 27.4 ± 3.36, $p = 0.080$, in skin cells vs 21.58 ± 1.59 burn control. As compared to days 7, 10 and 14 (50.1 ± 4.74 $p = 0.007$, 75.6 ± 1.6 $p < 0.001$ and 84.4 ± 1.65 $p < 0.001$ in the combined group, 44.2 ± 1.92 $p = 0.004$, 67.3 ± 1.52 < 0.001 and 82.6 ± 1.7 $p < 0.001$ in ADMSCs, 34.6 ± 3.84, $p = 0.99$, 57.6 ± 2.3, $p = 0.026$ and 74.4 ± 2.07, $p = 0.014$, in skin cells vs 34.8 ± 1.48, 53.8 ± 1.3, 69.87 ± 1. 47 in burn control, respectively). It was observed that the burn wounds of the control group rats healed slowly and showed 75.3% wound reduction at day 18. On the other side, ADMSCs and the mixture of skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) treated groups showed comparatively better healing, but the wound could not heal completely on day 18. However, combined treatment (autologous non-culture skin

cells + ADMSCs) showed significant improvement in healing. Wounds were completely closed on day 18 of treatment. The percentage of wound reduction at day 18 was 94.4 ± 2.18 $p < 0.001$ in the combined group, 87.8 ± 1.9 $p = 0.002$ in ADMSCs, 83.4 ± 2.10, $p = 0.014$, in skin cells vs 75.2 ± 2.68 burn control.

The combination therapy of non-cultured skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs exhibited suppression of wound inflammation and enhancement of collagen deposition and granulation.

Images of H&E staining showed fast and early improvement in skin architecture of a group of rats treated with combination therapy than other groups by decreasing the inflammatory cells because inflammatory cells in excess are a key factor that impedes the healing process. Histological scoring revealed that inflammatory cells were three times less (1.2 ± 0.44 $p < 0.001$ vs 3.6 ± 0.49) in the group treated with a combination of autologous non-culture skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs combined it positively improves the collagen deposition and granulation tissue formation than ADMSCs and skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) alone when compared to control group (3.6 ± 0.55 $p < 0.001$ and 3.4 ± 0.56 $p < 0.001$, 3.4 ± 0.45 $p < 0.001$ and 2.8 ± 0.46 $p < 0.001$, 2.2 ± 0.39 $p = 0.071$ and 1.4 ± 0.55 $p = 0.863$ vs 1.44 ± 0.51 and 1.2 ± 0.44 respectively) (fig.2).

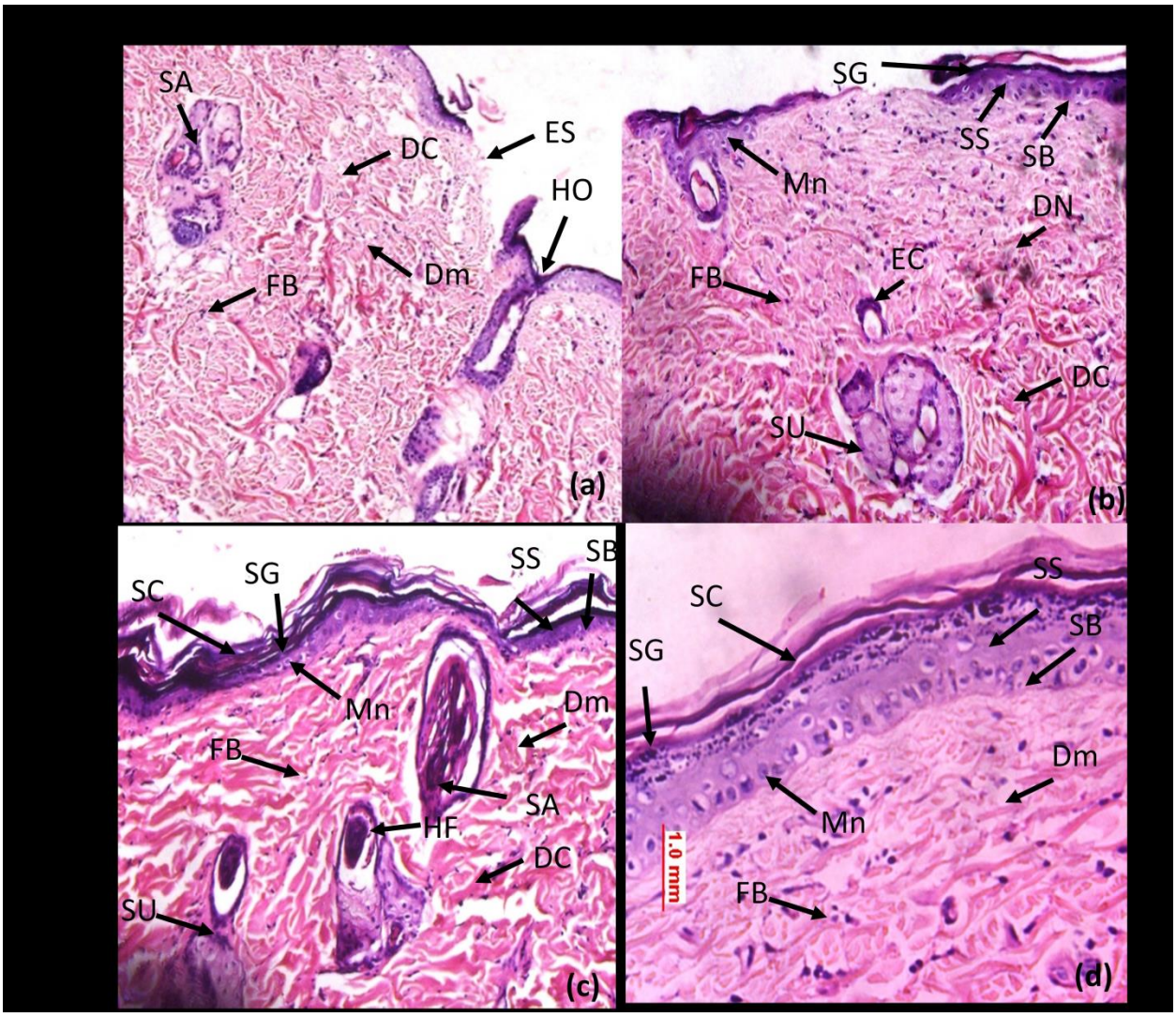


Figure 2: H and E staining of burn tissues with treatment at 18th day (a) burn as control (b) skin cells spray (c) ADMSCs (d) ADMSCs + cell spray. Arrows indicate stratum corneum (SC), stratum spinosum (SS), stratum basale (SB), stratum granulosum (SG), dermis (Dm), dermis extensive collagen (DC) fibroblast (FB) showing collagen as purple dots, melanocytes (Mn) and nucleus having little blob of spidery cytoplasm around it, sebaceus unit (SU), hair follicle (HF), skin adnexal structure (SA), eccrine glands (EC), dermal necrosis (DN), Opening of hair shaft (OH) and epidermal sloughed off (ES).

All the molecular markers showed a significant difference between the groups with combined therapy vs. control group (without treatment). Inflammatory markers like IL-1β and TNF-α were decreased in combined therapy than ADMSCs and skin cells (Fibroblasts, keratinocytes,

langerhans, melanocytes cells) alone when compared with burn control (0.312 ± 0.008 p< 0.001 and 0.244 ± 0.020 p < 0.001, 0.51±0.015 p < 0.001 and 0.43 ± 0.016 p < 0.001, 0.825 ± 0.07 p = 0.012 and 0.78 ± 0.101 p = 0.019). The other markers i.e., collagen deposition, SOD1, VEGF, HGF, HIF-1α, TGF-β1, Col1-α1 and FGF2 significantly increased in skin cells + ADMSCs combined (3.6 ± 0.55 p < 0.001 vs 1.44 ± 0.51, 2.8 ± 0.53 p < 0.001, 3.87 ± 0.087 p < 0.001, 4.04 ± 0.301 p < 0.001, 4.654 ± 0.213 p < 0.001, 6.2 ± 0.142 p < 0.001, 6.2 ± 0.142 p < 0.001, 6.034 ± 0.109 p < 0.001, 6.422 ± 0.62 p < 0.001) than ADMSCs (2.8 ± 0.45 p < 0.001, 2.7 ± 0.42 p < 0.001, 3.57 ± 0.055 p < 0.001, 3.23 ± 0.319 p < 0.001, 3.506 ± 0.46 p < 0.001, 5.72 ± 0.81 p < 0.001, 5.75 ± 0.16 p < 0.001 and 6.14 ± 0.151 p < 0.001) and skin cells treated groups (1.4 ± 0.39 p = 0.863, 1.2 ± 0.46 p = 0.755, 1.69 ± 0.281 p = 0.012, 2.45 ± 0.35 p = 0.002, 2.0 ± 0.37 p = 0.007, 2.62 ± 0.71 p = 0.016, 2.3 ± 0.68 p= 0.030 and2.86 ± 0.44 p = 0.002) when compared with the control group (without treatment) respectively (fig.3, fig.4).

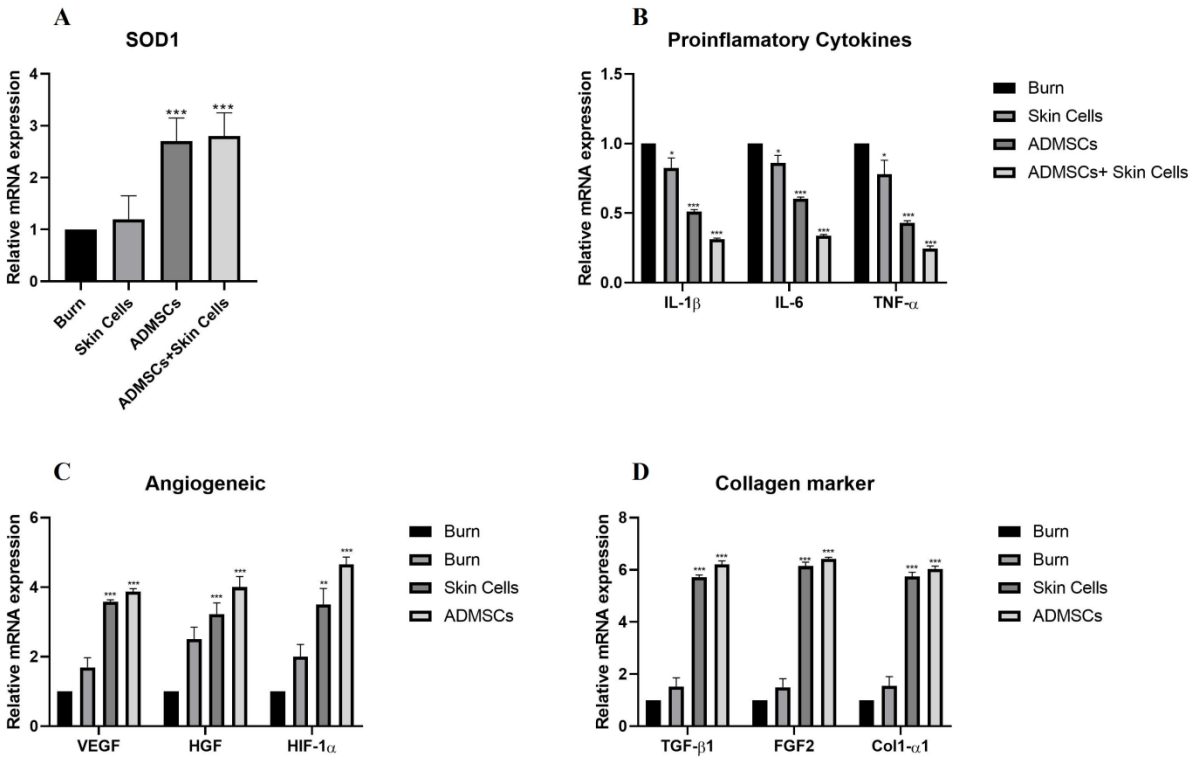


Figure 3: Molecular analysis for the wound healing through Real-Time PCR for gene expression at 18th day (a) oxidative analysis shows that SOD1has increased in ADMSCs + Skin cells combined treatment (b) pro-inflammatory markers IL-1β, IL6 and TNF-α shows maximum decrease in expression in combined therapy of ADMSCs +Skin cells (c) Pro-angiogenic markers VEGF, HGF and HIF-1α shows increase in expression in combined therapy (d) the collagen markers of TGF-β1, FGF2 and Col1-α1 also showed significantly increases expression in combined therapy when compared to burn control. Data are presented as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001 (versus the Burn non-treatment group).

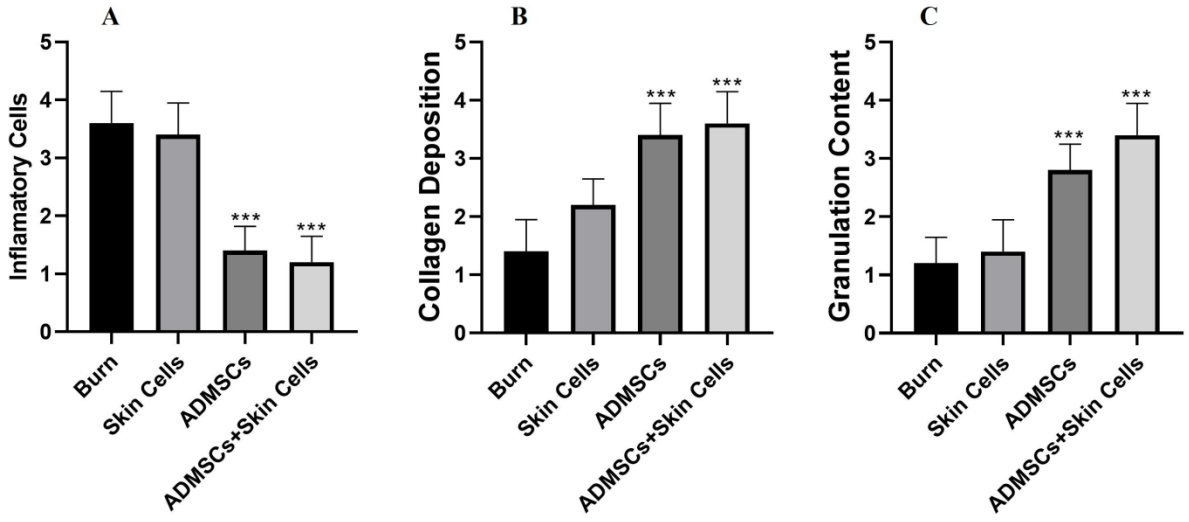


Figure 4: Histological evaluation of wound-healed skin after the 18th day. (a) Number of inflammatory cells decreased in ADMSCs and skin cell combined group. (b) Collagen deposition increased significantly in ADMSCs and skin cell combined group (c) the granulation content was also increases in ADMSCs and skin cell combined group. Data are presented as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001 (versus the Burn non-treatment group).

Discussion:

The main factors investigated in this study are non-cultured autologous skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs. The objective of the study was to assess the impact of a combined therapy involving non-cultured skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs on the rate of healing in acid-burn wounds. To evaluate the outcomes, various methods were employed, including morphological observation, histological assessment using H&E staining, and gene expression analysis of different markers associated with healing. The findings of our study indicated that the combined therapy, consisting of both skin cells and ADMSCs, outperformed the use of ADMSCs or autologous non-cultured skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) alone in terms of

wound healing, re-epithelization, inhibition of the inflammatory response, promotion of angiogenesis, collagen deposition, and granulation.

Previous studies have demonstrated the beneficial effects of cell-based therapies in wound repair, primarily by enhancing the biological functions of skin cells (10, 20). Interestingly, even a small number of transplanted cells can exert a significant positive impact on damaged tissue (21). Building upon these findings, it is hypothesized that a minority of viable cells can effectively contribute to the regeneration of various tissues. This hypothesis holds promise for facilitating the clinical introduction of cell-based therapies without the need for cell culture (22, 23).

In our study, we strictly avoided the cell culture step and instead isolated and transplanted purely differentiated cells directly onto the burn wounds. This mixed population of cells, including keratinocytes, melanocytes, Langerhans cells, and fibroblasts, notably promoted faster re-epithelization and enhanced wound healing, all without any complications. Our study findings demonstrate that the combination of ADMSCs and non-cultured skin cells confers resistance to the adverse effects of acid burns, further supporting the potential of this therapeutic approach.

Inflammation and the infiltration of inflammatory cells play a pivotal role in the process of wound repair and healing (24). Previous studies have highlighted the importance of maintaining a balanced ratio of pro-inflammatory and anti-inflammatory cytokines for optimal wound healing (25).

In alignment with these observations, our study yielded similar findings. At 18^{th} day, in burn rats who did not receive any treatment, we observed a substantial presence of inflammatory cells, which correlated with a delayed healing process. Conversely, in the groups of rats treated with a

mixture of skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs, as well as those treated with ADMSCs alone, we noted a reduced number of inflammatory cells, indicating an enhanced healing process.

In addition to conducting histological analysis, at 18^{th} day, we observed elevated expression levels of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, in the untreated burn group. Notably, in both the combined therapy group of skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) + ADMSCs and the group treated with ADMSCs alone, we observed a significant reduction in inflammatory cell infiltration compared to the group treated with skin cells alone. Furthermore, in the skin tissue healed by the combined treatment of mixture of skin cells + ADMSCs, we observed a decrease in the expression of pro-inflammatory cytokines compared to both the skin cells treated group and the untreated group. These findings align with previous studies that have reported the intrinsic ability of cell-based therapy to reduce inflammation (21, 26, 27). However, it is important to note that the decrease in inflammatory cell infiltration during the healing phase could also be attributed to the proteins and growth factors secreted by platelets, as reported in several studies.

Delayed healing wounds are often characterized by a prolonged inflammatory phase and an oxidative micro-environment (28). The various cells involved in inflammation within the wound bed produce reactive radicals, leading to an oxidative imbalance that negatively impacts the healing process (29, 30). Superoxide dismutase, an antioxidant enzyme encoded by the SOD1 gene, plays a crucial role in protecting cells from oxidative damage by scavenging reactive radicals (31, 32).

In our study, we observed no significant change in the expression of Sod1 in the untreated burn group compared to the group treated with skin cells alone at 18th day. However, we did find higher expression levels of Sod1 in the group that received the combined treatment of mixture of skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) + ADMSCs. This finding suggests that the use of this treatment modality may result in the release of antioxidant enzymes that can neutralize reactive species and contribute to the overall healing process.

Poor angiogenesis is a significant factor that can delay wound repair and healing (33). Cell-based treatments have been shown to induce angiogenesis through the release of paracrine secretions upon transplantation (34). Consistent with findings from previous studies (35, 36), we observed significant expressions of pro-angiogenic growth factors such as HGF, VEGF, and HIF-1α in the group treated with a combination of mixture of skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) + ADMSCs at the molecular level. In contrast, the expression of these growth factors was poor in the group treated with skin cells alone and in the group of burn rats that did not receive any treatment. These findings provide further support to our previous findings that cell-based transplantation improves micro circulation and enhances the blood supply in wounds inflicted by acid burns.

When combined with platelet-rich plasma (PRP), ADMSCs have the ability to induce the production of multiple growth factors, including VEGF (37), EGF(38), TGF-β (7, 39), FGF(40, 41), IGF1 (42) through autocrine or paracrine mechanisms. Transforming Growth Factor-β1 (TGF-β1) plays a crucial role in the wound healing process by converting fibroblasts into myofibroblasts, which in turn leads to collagen production (43, 44). Therefore, in our study, we evaluated the expression of TGF-β1, COL1α1, and FGF-2 genes.

As expected, we found elevated expression levels of these genes in the healed skin of rat models treated with the combination of skin cells and ADMSCs, as well as in the group treated with ADMSCs alone. However, the expression of these genes was not significantly improved in the group treated with skin cells alone. Moreover, in the burn rats group without treatment, there was a decreased expression of these genes. These findings further support the notion that the combined therapy of skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs, or ADMSCs alone, can effectively enhance the expression of crucial genes involved in the wound healing process, while the use of skin cells alone or no treatment leads to sub optimal gene expression levels.

Histological assessments using various methods, such as macroscopic observation and H&E staining, revealed distinct histological features in the healed skin of wounds treated with different methods. In particular, the combination of mixture of skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs, as well as the treatment with skin cells alone, demonstrated significantly improved formation of granulation tissue, notable fibroblast proliferation, and increased collagen deposition at 18^{th} day. These findings are consistent with a previous study that reported enhanced expressions of FGF-2, COL1α1, and TGF-β1 in healed skin (45).

Conclusion

Based on our study, the results demonstrate the effectiveness of combining non-cultured skin cells (Fibroblasts, keratinocytes, langerhans, melanocytes cells) with ADMSCs in the treatment of acid burn wounds. The absence of cell culture allowed for the utilization of highly pure and differentiated cells, leading to accelerated re-epithelization, granulation tissue formation, and

improved collagen deposition and angiogenesis. While further investigation is required to understand the underlying molecular mechanisms, our findings provide compelling evidence supporting the use of ADMSCs in conjunction with skin cells as an adjunctive therapy to enhance wound repair and healing. Consequently, the combination of autologous non-cultured skin cells(Fibroblasts, keratinocytes, langerhans, melanocytes cells) and ADMSCs shows promise as a viable option for the clinical management of acid burn wounds.

Key Points

- Autologous non-cultured skin cells accelerate the rate of wound healing.
- When used in conjunction with ADMSCs, autologous non-cultured skin cells enhance work efficiency more effectively than ADMSCs or skin cells used alone.
- The expression of pro-inflammatory cytokines such as IL-1 beta, IL-6, and TNF-alpha decreases, while the levels of growth factors such as VEGF, HGF, HGF1 alpha, and collagen markers improve significantly in the combined therapy approach.

References

1. Hüsnü G, Soylu S, İNAN DŞ, ÇOŞKUN H, YILDIRIR A. Comparison of the effect of stem cell, platelete rich plasma and ovarium folicular fluid on burn stasis zone (Experimental study). Cumhuriyet Medical Journal. 2021;43(1):62-71.

2. Abdul Kareem N, Aijaz A, Jeschke MG. Stem cell therapy for burns: story so far. Biologics: Targets and Therapy. 2021:379-97.

3. Ali MB, Ali MB. Psychological and physiological complications of post-burn patients in Pakistan: A narrative review. Sultan Qaboos University Medical Journal. 2022;22(1):8.

4. Abbasi H, Dehghani A, Mohammadi AA, Ghadimi T, Keshavarzi A. The Epidemiology of Chemical Burns Among the Patients Referred to Burn Centers in Shiraz, Southern Iran, 2008–2018. Bulletin of Emergency & Trauma. 2021;9(4):195.

5. Bairagi A, Griffin B, Banani T, McPhail SM, Kimble R, Tyack Z. A systematic review and meta-analysis of randomized trials evaluating the efficacy of autologous skin cell suspensions for re-epithelialization of acute partial thickness burn injuries and split-thickness skin graft donor sites. Burns. 2021;47(6):1225-40.

6. Darwish A, Hassan KM, Saad DA, Al-Ahmady M. Evaluation of the Effect of Cell Spray Technique in the Treatment of Partial-thickness Skin Loss. Minia Journal of Medical Research. 2020;31(2):12-20.

7. Li Z, Maitz P. Cell therapy for severe burn wound healing. Burns & trauma. 2018;6.

8. Mirshekar M, Afkhami H, Razavi S, Jazi FM, Darban-Sarokhalil D, Ohadi E, et al. Potential antibacterial activity and healing effect of topical administration of bone marrow and adipose mesenchymal stem cells encapsulated in collagen-fibrin hydrogel scaffold on full-thickness burn wound infection caused by Pseudomonas aeruginosa. Burns. 2023.

9. Domaszewska-Szostek AP, Krzyżanowska MO, Czarnecka AM, Siemionow M. Local treatment of burns with cell-based therapies tested in clinical studies. Journal of clinical medicine. 2021;10(3):396.

10. Wood FM, Stoner ML. Cell suspension preparation technique and device. Google Patents; 2018.

11. Mahjoor M, Afkhami H, Najafi M, Nasr A, Khorrami S. The role of microRNA-30c in targeting interleukin 6, as an inflammatory cytokine, in the mesenchymal stem cell: a therapeutic approach in colorectal cancer. Journal of Cancer Research and Clinical Oncology. 2023;149(7):3149-60.

12. Azam M, Ghufran H, Butt H, Mehmood A, Ashfaq R, Ilyas AM, et al. Curcumin preconditioning enhances the efficacy of adipose-derived mesenchymal stem cells to accelerate healing of burn wounds. Burns & Trauma. 2021;9:tkab021.

13. Ahmad MR, Badar W, Ullah Khan MA, Mahmood A, Latif N, Iqbal T, et al. Combination of preconditioned adipose-derived mesenchymal stem cells and platelet-rich plasma improves the repair of osteoarthritis in rat. Regenerative Medicine. 2020;15(11):2285-95.

14. Cooper-Jones B, Visintini S. A noncultured autologous skin cell spray graft for the treatment of burns. 2019.

15. Lauder J. Measuring Response to Pain Mitigation for Ovariectomy in Bos taurus Yearling Beef Heifers: University of Saskatchewan; 2018.

16. Pleguezuelos-Beltrán P, Gálvez-Martín P, Nieto-García D, Marchal JA, López-Ruiz E. Advances in spray products for skin regeneration. Bioactive materials. 2022.

17. Xu P, Wu Y, Zhou L, Yang Z, Zhang X, Hu X, et al. Platelet-rich plasma accelerates skin wound healing by promoting re-epithelialization. Burns & trauma. 2020;8.

18. Azam M, Ghufran H, Butt H, Mehmood A, Ashfaq R, Ilyas AM, et al. Curcumin preconditioning enhances the efficacy of adipose-derived mesenchymal stem cells to accelerate healing of burn wounds. Burns & Trauma. 2021;9.

19. Mahmood R, Mehmood A, Choudhery MS, Awan SJ, Khan SN, Riazuddin S. Human neonatal stem cell-derived skin substitute improves healing of severe burn wounds in a rat model. Cell biology international. 2019;43(2):147-57.

20. Otero-Viñas M, Falanga V. Mesenchymal stem cells in chronic wounds: the spectrum from basic to advanced therapy. Advances in Wound Care. 2016;5(4):149-63.

21. Coalson E, Bishop E, Liu W, Feng Y, Spezia M, Liu B, et al. Stem cell therapy for chronic skin wounds in the era of personalized medicine: From bench to bedside. Genes & diseases. 2019;6(4):342-58.

22. Ruzicka J, Urdzikova LM, Kloudova A, Amin AG, Vallova J, Kubinova S, et al. Anti-inflammatory compound curcumin and mesenchymal stem cells in the treatment of spinal cord injury in rats. Acta Neurobiol Exp. 2018;78(4):358-74.

23. Wang Y-L, Liu X-S, Wang S-S, Xue P, Zeng Z-L, Yang X-P, et al. Curcumin-activated mesenchymal stem cells derived from human umbilical cord and their effects on MPTP-mouse model of Parkinson's disease: a new biological therapy for Parkinson's disease. Stem Cells International. 2020;2020.

24. Delavary BM, van der Veer WM, van Egmond M, Niessen FB, Beelen RH. Macrophages in skin injury and repair. Immunobiology. 2011;216(7):753-62.

25. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. International journal of molecular sciences. 2016;17(12):2085.

26. Pedrazza L, Cubillos-Rojas M, de Mesquita FC, Luft C, Cunha AA, Rosa JL, et al. Mesenchymal stem cells decrease lung inflammation during sepsis, acting through inhibition of the MAPK pathway. Stem Cell Research & Therapy. 2017;8(1):1-14.

27. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nature Reviews Nephrology. 2018;14(8):493-507.

28. Schäfer M, Werner S. Oxidative stress in normal and impaired wound repair. Pharmacological research. 2008;58(2):165-71.

29. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxidants & redox signaling. 2014;20(7):1126-67.

30. Park N-Y, Lim Y. Short term supplementation of dietary antioxidants selectively regulates the inflammatory responses during early cutaneous wound healing in diabetic mice. Nutrition & Metabolism. 2011;8(1):1-9.

31. Gol S, Pena RN, Rothschild MF, Tor M, Estany J. A polymorphism in the fatty acid desaturase-2 gene is associated with the arachidonic acid metabolism in pigs. Scientific reports. 2018;8(1):1-9.

32. Lin Z-F, Xu H-B, Wang J-Y, Lin Q, Ruan Z, Liu F-B, et al. SIRT5 desuccinylates and activates SOD1 to eliminate ROS. Biochemical and biophysical research communications. 2013;441(1):191-5.

33. Zhang X, Wei X, Liu L, Marti GP, Ghanamah MS, Arshad MJ, et al. Association of increasing burn severity in mice with delayed mobilization of circulating angiogenic cells. Archives of Surgery. 2010;145(3):259-66.

34. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011;146(6):873-87.

35. Yu C, Xu Z-X, Hao Y-H, Gao Y-B, Yao B-W, Zhang J, et al. A novel microcurrent dressing for wound healing in a rat skin defect model. Military Medical Research. 2019;6(1):1-9.

36. Zachary I. VEGF signalling: integration and multi-tasking in endothelial cell biology. Biochemical Society Transactions. 2003;31(6):1171-7.

37. Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. Journal of Surgical Research. 2009;153(2):347-58.

38. Brown GL, Nanney LB, Griffen J, Cramer AB, Yancey JM, Curtsinger III LJ, et al. Enhancement of wound healing by topical treatment with epidermal growth factor. New England Journal of Medicine. 1989;321(2):76-9.

39. Chong DL, Trinder S, Labelle M, Rodriguez-Justo M, Hughes S, Holmes AM, et al. Platelet-derived transforming growth factor-β1 promotes keratinocyte proliferation in cutaneous wound healing. Journal of Tissue Engineering and Regenerative Medicine. 2020;14(4):645-9.

40. Choi SM, Lee K-M, Kim HJ, Park IK, Kang HJ, Shin H-C, et al. Effects of structurally stabilized EGF and bFGF on wound healing in type I and type II diabetic mice. Acta biomaterialia. 2018;66:325-34.

41. Xie J, Bian H, Qi S, Xu Y, Tang J, Li T, et al. Effects of basic fibroblast growth factor on the expression of extracellular matrix and matrix metalloproteinase-1 in wound healing. Clinical and Experimental Dermatology: Continuing professional development (CPD). 2008;33(2):176-82.

42. Le M, Naridze R, Morrison J, Biggs LC, Rhea L, Schutte BC, et al. Transforming growth factor Beta 3 is required for excisional wound repair in vivo. PloS one. 2012;7(10):e48040.

43. Liarte S, Bernabé-García Á, Nicolás FJ. Role of TGF-β in skin chronic wounds: a keratinocyte perspective. Cells. 2020;9(2):306.

44. Lichtman MK, Otero-Vinas M, Falanga V. Transforming growth factor beta (TGF-β) isoforms in wound healing and fibrosis. Wound Repair and Regeneration. 2016;24(2):215-22.

45. Sarkar S, Bhattacharya S, Alam MJ, Yadav R, Banerjee SK. Hypoxia aggravates non-alcoholic fatty liver disease in presence of high fat choline deficient diet: a pilot study. Life Sciences. 2020;260:118404.