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# Combination of platelet-rich plasma and stromal vascular fraction on the level of transforming growth factor-β in rat subjects experiencing deep dermal burn injury

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## ARTICLE INFO

## ABSTRACT

*Introduction:* The combination of stromal vascular fraction (SVF) and platelet-rich plasma (PRP) is effective at increasing the concentration of growth factors including transforming growth factor-β (TGF-β). The increase in this growth factor is expected to accelerate the healing of burns. This study aims to determine the effect of giving a combination of SVFs and PRP on TGF-β levels in the healing process of deep dermal burns.
*Methods:* This was an experimental study in 64 rats using a post-test control group design consisting of 1 group of SVFs and PRP combination injection treatment group, 1 group given a topical combination of SVFs and PRP, 1 group given Vaseline, and 1 control group.
*Results:* There was a significant difference in TGF-β levels between the deep dermal burns group that was given a combination of SVFs and PRP injection and topical, the Vaseline group, and the control group with p-value <0.05.
*Conclusion:* The combination of SVFs and PRP increases the level of TGF-β in the healing process of deep dermal burns.

## 1. Introduction

Burns are defined as damage to the skin and underlying tissue caused by heat, chemicals, or electricity [1–4]. Stromal vascular fraction (SVF) is a component of lipoaspirates obtained from fat tissue liposuction. Lipoaspirates contain a large number of stem cells called adipose-derived stem cells (ASCs) [5–7]. SVFs are processed in such a

way that they contain a consistent, reproducible heterogeneous cell composition. After the production and recording process, SVFs derived from adipose can differentiate into different tissue types, support neo-vascularization, replace cells, and repair injured tissue [6,8,9].

Platelet-rich plasma (PRP) is a concentrated platelet in small volume plasma, which contains at least six major growth factors, including platelet-derived growth factor (PDGF), basic fibroblast growth factor

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(bFGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and transforming growth factor-b (TGF-b), which are released after platelet activation [5, 7,9–14].

TGF-β plays a physiological role by binding to TGF-β receptors (TGF-βR) and regulating developmental pathways, controlling the pattern of mesoderm changes in the early embryonic period through the development of several organ systems [15–17]. TGF-β in the initial wound-healing process is released by activated monocytes to stimulate monocytes to express several other peptides such as TGF-α, IL-1, and PDGF. Apart from being released by monocytes, TGF-β is also released by platelets and fibroblasts in the wound [15–18].

This study aims to determine the effect of stem cell combination (SVFs and PRP) on TGF-β levels in the healing process of deep dermal burns in a rats model.

## 2. Methods

Sixty-four male Sprague-Dawley rats aged 10 weeks, range of weight 150–250 g, were used in this animal experimental study. These experimental animals were fed BR-1 without additional feeding for approximately 1 week for the adaptation period. Feeding took place daily, with aquadest provided as a drink. The cage was standard shaped, cleaned regularly, and given lighting (12 h light/dark photoperiod). Temperature was kept at 24 ± 2 °C. This study was conducted in the animal laboratory Faculty of Medicine, Hasanuddin University, and was approved by the Ethics Commission registration number: 1029/UN4.6.4.5.31/PP36/2019. The work was also carried out in line with the ARRIVE guidelines for reporting animal research [19,20].

Rats were randomly assigned to four groups: 3 treatment groups (sacrificed 1, 4, 7, 10, and 14 days post treatment) and 1 control group (sacrificed day 0). Group A was a control group of TGF levels in healthy rats without treatment. Group B was a control group of rats that were modelled deep dermal burns and Vaseline wound care. Group C was a treatment group given topical PRP + SVFs. Group D was a treatment group of mice given PRP + SVFs injection. PRP from venous blood and SVF from lipoaspirate from the donor mouse were harvested and processed according to Juntendo University School of Medicine, Japan established protocols [21].

### 2.1. PRP preparation

The rats were shaved on their backs, then ether was used as an inhalation anesthetic. The donor group underwent a thoracotomy until the heart was visible. Apex identification was then performed by aspirating blood from the apex using a 3 cc 25G needle. The blood drawn was transferred to a tube containing 3.8% sodium citrate. Blood was centrifuged for 10 min at 2400 rpm (450 g) for the first centrifugation. The supernatant plasma with a buffy coat was collected and centrifuged at 3600 rpm (850 g) for 15 min. The top layer of plasma was discarded until 5 mL of plasma remained at the bottom. The remaining plasma was collected as PRP, frozen at −20 °C, and thawed before use [22].

### 2.2. SVF preparation

SVF preparation was derived from adipose tissue from the inguinal portion of rat donors.The fat taken was washed with phosphate-buffered saline (PBS) (Gibco-BRL, Grand Island, NY, USA), chopped into small pieces until smooth, then digested with 0.15% collagenase (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and centrifuged for 30 min at 37 in a 50 mL centrifuge tube. Dulbecco's Modified Eagle Medium (DMEM, Gibco-BRL) control media with 10% FBS (Gibco-BRL) and 1% antibiotic-antimycotic (Gibco-BRL) was added to neutralize collagenase activation, then centrifuged 1500 rpm (260 g) for 5 min. Cell pellets were resuspended with phosphate-buffered saline with 2% foetal bovine serum. The number of SVF cells was counted using the FACSCalibur

cytometer (Becton Dickinson). 50,000 SVF cells were diluted with distilled water 0.5, transferred to an Eppendorf tube for the final SVF product, and stored at 4 °C before use [21,22].

### 2.3. PRP and SVF combination preparation

In the combination group of PRP and SVFs, 50,000 cells were added with 0.5 cc of PRP, so that the final product of PRP + SVFs was the same for both the injection treatment group and the topical group [22].

### 2.4. Vaseline

Vaseline, also known as white petrolatum, acts as an additive to make a product [23]. Vaseline is often used for hair oils, body lotions, ointments, and topical creams. Vaseline was used in this study because it has the ability to become a wound moisturizer (moist) on a wound site [24].

### 2.5. Deep dermal burn model

Deep dermal burn models were prepared following the method described by Guo et al. [25] with modifications. After an adaptation period of 1 week, rats were anesthetized by inhalation using ether in a special box until the state of consciousness decreased. The affected area was disinfected with Povidone-iodine 1%. After that, aluminium metal with a diameter of 10 mm was heated in hot water at 100 °C for 5 min. The hot metal was affixed for 3 s to the back of the rat. The pressure applied to the skin corresponded to a mass of 51 gr of aluminium metal to induce burns. This method obtained deep dermal burns which were confirmed by the results of histopathological examination (Fig. 1) [26]. Immediately after the procedure, analgesia with metamizole 50 mg/kg and amoxicillin 15 mg/kg antibiotics were given orally [25,26].

### 2.6. Wound analysis

Skin tissue was subjected to histopathological analysis to assess deep dermal burns.

### 2.7. Combination stem cell and vaseline implantation

There was one negative control group and three treatment groups. Group A was the control of TGF levels in healthy rats without treatment. In Group B, wound care was performed using standard moist (Vaseline) which was applied to the wound surface. In Group C, a topical combination of PRP + SVFs was administered in gel form and placed on the wound surface, each with a total volume of 0.5 cc of PRP +50,000 SVFs cells per experimental animal. In Group D, subcutaneous injections of PRP and SVFs were administered to the four edges of the wound at 12, 3, 6, 9, and the center of the wound 0.1 cc each with a total volume of 0.5
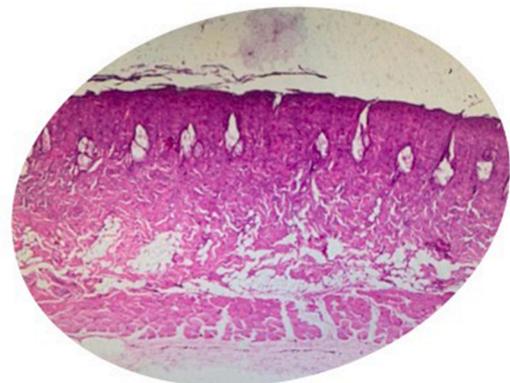


**Fig. 1.** Histopatological deep dermal burn model in our study.

cc of PRP +50,000 SVFs cells per experimental animal. The rats were then fed sufficiently and given oral analgesics and antibiotics.

### 2.8. TGF beta test

TGF beta examination was carried out 6 times, on day 0 (24 h) before the burn treatment, then blood was taken 1, 4, 7, 10, and 14 days after treatment. All blood samples were examined by TGF beta Sandwich ELISA with Catalogue No. MBS175833 which was purchased from MyBioSource, Inc [33,34].

### 2.9. Statistical analysis

All data are presented as mean $\pm$ SD. Data were analysed using the Levene and independent t-tests using SPSS software for Windows 21 (IBM SPSS Statistics for Windows, Version 21.0. IBM Corp., Armonk, NY). A p-value less than 0.05 was considered statistically significant.

## 3. Results

In this study, as many as 64 rats were divided into 4 treatment groups: Group A (negative control), Group B (topical Vaseline and burn wound), Group C (Topical PRP and SVFs and burn wound), and Group D (PRP Injection and SVF and burn wound).

As shown in Table 1, the highest TGF-$\beta$ levels were found in Group B (topical Vaseline and burns) which increased slowly starting the first day (0.267) and reached its peak on the 7th day (0.309), then slowly decreased until the 7th and 14th days (0.290). In Group C (topical PRP and SVFs and burns), levels of TGF-$\beta$ started to increase slowly from the first day (0.314) and continued to increase until the 14th day (0.415). TGF-$\beta$ levels in Group D (PRP injection and SVF and burns) began to increase on the first day of injection (0.333), and continued to increase until the 14th day (0.402) (Fig. 2).

### 3.1. Comparison of Group A (negative control) and Group B (topical vaseline)

As shown in Table 2, the TGF-$\beta$ level after giving Vaseline on the first to the 14th day had a p-value <0.05. Therefore, there was a significant difference from the TGF-$\beta$ level in the control group. These results show that $H_0$ is rejected and $H_1$ is accepted. There was a significant effect on the TGF-$\beta$ levels of deep dermal burns after giving Vaseline on days 1, -4, -7, -10, and $-14$.

### 3.2. Comparison of Group B (vaseline) and Group C (Topical SVFs and PRP)

As shown in Table 3, the ratio of TGF-$\beta$ levels after administering topical stem cells and Vaseline on the first to the 14th days has a value of $p < 0.05$, which represents a significant difference in TGF-$\beta$ levels between the two groups. These results show that $H_0$ is rejected and $H_1$ is accepted; that is, topical stem cell administration performed better than Vaseline for changes in TGF-$\beta$ levels in deep dermal burns on days 1, -4,

-7, -10, and - 14.

### 3.3. Comparison of Group B (vaseline) and Group D (injection of SVFs and PRP)

As shown in Table 4, the ratio of TGF-$\beta$ levels after giving stem cell injection and Vaseline on the first day to the 14th day has a p value < 0.05, which represents a significant difference in TGF-$\beta$ levels between the two groups. Based on these results, $H_0$ is rejected and $H_1$ is accepted; that is, stem cell injection is better than Vaseline for changes in TGF-$\beta$ levels in deep dermal burns on days 1, -4, -7, -10, and $-14$.

### 3.4. Comparison of Group C (Topical SVFs and PRP) and Group D (SVFs and PRP injection)

As shown in Table 5 and Fig. 3, the ratio of TGF-$\beta$ levels after giving stem cell and topical stem cell injections on the first day to the 14th day has a value of $p > 0.05$. Therefore, the difference in TGF-$\beta$ levels is not significant between the two groups. Based on these results, $H_1$ is rejected and $H_0$ is accepted; that is, the administration of stem cell injections is no better than giving topical stem cells to changes in levels of TGF-$\beta$ deep dermal burns on days 1, -4, -7, -10, and $-14$.

## 4. Discussion

The TGF-$\beta$ level of the Vaseline group had a significant difference compared to the control group; levels peaked on the 7th day of Vaseline administration. A study comparing a group given Vaseline to a control group with fibroblast parameters found that the 7th, 14th, and 21st days of Vaseline administration were better than the control group [27]. Zhang YS et al. found that TGF-$\beta$ levels using Vaseline compared to a control group were higher on the 7th and 14th days; the TGF-$\beta$ level in the control group was higher than the Vaseline $\beta$1 group [28]. Another study carried out for 35 days comparing pelnac, Vaseline, and control groups to TGF-$\beta$ found that use of Vaseline was better than the pelnac and control groups with significant results ($p < 0.001$) [29].

This study found that the administration of PRP + SVFs topically or by injection was significantly better at increasing the expression of TGF-$\beta$ up to the 14th day than the administration of Vaseline or controls ($p < 0.05$). PRP contains many growth factors (such as TGF-$\beta$, PDGF, and VEGF) that mediate angiogenesis. These growth factors stimulate cell proliferation, migration, and capillary tube formation via multiple signalling pathways, such as extracellular regulated kinase (ERK) and calcineurin (CaN)/nuclear factor from the activated T-cell signalling pathway (NFAT), whereas SVFs contain many cell types such as ADSCs, pre-adipocytes, fibroblasts, endothelial cells, macrophages, and lymphocytes [22]. ADSCs have been reported to be composed of many cytokines and growth factors that can influence parameters such as angiogenesis, fibroblasts, mononuclear infiltrates, and collagen production. Expression of potent angiogenic agents such as vascular endothelial growth factor (VEGF), fibroblast migration, and stimulators of activity such as transforming growth factor (TGF) and macrophage chemotaxis are expressed by ADSC [22,31]. Collectively, these processes can accelerate the proliferative phase and build more favorable conditions for wound repair as observed in our results.

In our study, the results showed that there was no significant difference between the administration of PRP + SVFs by injection or topically. The administration of PRP + SVFs by injection was not better at increasing the expression of TGF-$\beta$ than by topical administration of PRP + SVFs. In a previous study involving 24 patients with chronic lower limb ulcers, wounds were injected with PRP every 2 weeks, and successful wound closure and reepithelialisation were obtained in 20 wounds. In addition, the use of autologous PRP gel in treating 59 patients with acute soft tissue wounds of the extremities showed that the administration of PRP gel was valuable and effective in the management of acute trauma wounds [5].

**Table 1**
The average change in TGF-$\beta$ levels in each treatment group.

| Treatment Group (n = 16) | Average level of TGF-$\beta$ on termination day (Mean $\pm$ SD) (pg/mL) | | | | |
|---|---|---|---|---|---|
| | 1 | 4 | 7 | 10 | 14 |
| A | $0.239 \pm 0.021$ | | | | |
| B | $0.276 \pm 0.009$ | $0.299 \pm 0.008$ | $0.309 \pm 0.008$ | $0.304 \pm 0.002$ | $0.290 \pm 0.006$ |
| C | $0.314 \pm 0.015$ | $0.355 \pm 0.019$ | $0.378 \pm 0.011$ | $0.409 \pm 0.009$ | $0.415 \pm 0.018$ |
| D | $0.333 \pm 0.028$ | $0.342 \pm 0.013$ | $0.397 \pm 0.010$ | $0.411 \pm 0.014$ | $0.420 \pm 0.012$ |
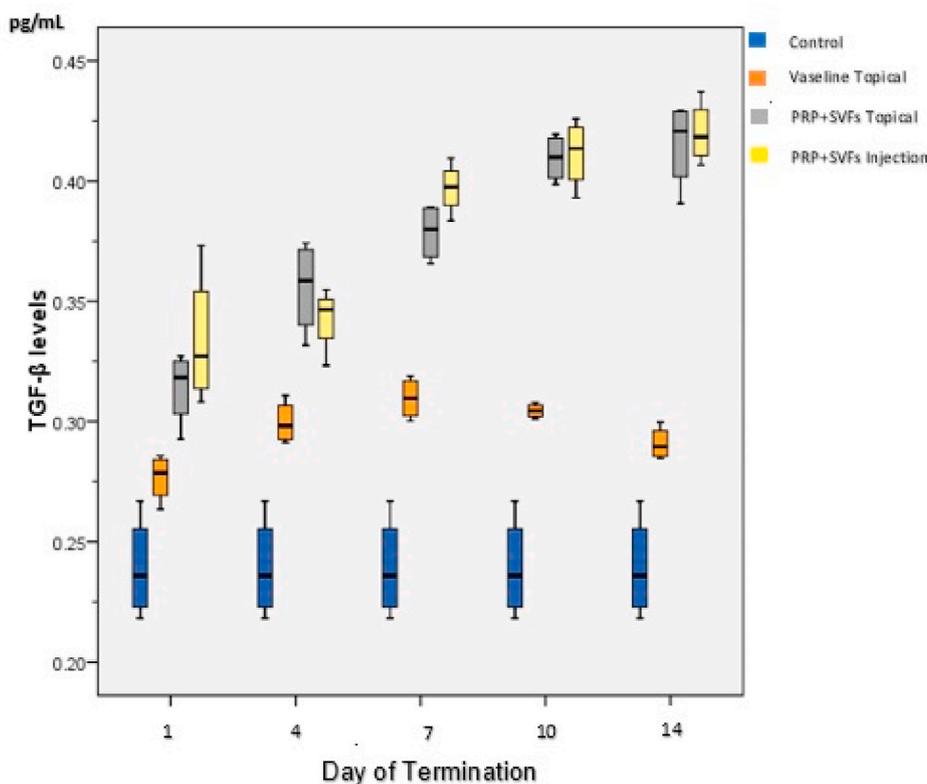
**Fig. 2.** Average changes in TGF-β levels in each treatment group.

**Table 2**
Comparison of TGF-β levels between Groups A and B.

| Day of Termination | Group A (mean ± SD) (pg/mL) | Control B (mean ± SD) (pg/mL) | *p value | **p value |
|---|---|---|---|---|
| 1 |  | 0.276 ± 0.009 | 0.168 | 0.019 |
| 4 |  | 0.299 ± 0.008 | 0.139 | 0.002 |
| 7 | 0.239 ± 0.021 | 0.309 ± 0.008 | 0.134 | 0.001 |
| 10 |  | 0.304 ± 0.002 | 0.035 | 0.008 |
| 14 |  | 0.290 ± 0.006 | 0.085 | 0.004 |

*Levene test **Independent *t*-test.

**Table 3**
Comparison of TGF-β levels between Groups B and C.

| Day of Termination | Group B (mean ± SD) (pg/mL) | Group C (mean ± SD) (pg/mL) | *p value | **p value |
|---|---|---|---|---|
| 1 | 0.276 ± 0.009 | 0.314 ± 0.015 | 0.507 | 0.006 |
| 4 | 0.299 ± 0.008 | 0.355 ± 0.019 | 0.080 | 0.002 |
| 7 | 0.309 ± 0.008 | 0.378 ± 0.011 | 0.134 | <0.001 |
| 10 | 0.304 ± 0.002 | 0.409 ± 0.009 | 0.005 | <0.001 |
| 14 | 0.290 ± 0.006 | 0.415 ± 0.018 | 0.133 | <0.001 |

*Levene test ** Independent *t*-test.

**Table 4**
Comparison of TGF-β levels between Groups B and D.

| Day of Termination | Group B (mean ± SD) (pg/mL) | Group D (mean ± SD) (pg/mL) | *p value | **p value |
|---|---|---|---|---|
| 1 | 0.276 ± 0.009 | 0.333 ± 0.028 | 0.178 | 0.009 |
| 4 | 0.299 ± 0.008 | 0.342 ± 0.013 | 0.539 | 0.002 |
| 7 | 0.309 ± 0.008 | 0.397 ± 0.010 | 0.984 | <0.001 |
| 10 | 0.304 ± 0.002 | 0.411 ± 0.014 | 0.049 | <0.001 |
| 14 | 0.290 ± 0.006 | 0.420 ± 0.012 | 0.305 | <0.001 |

*Levene test ** Independent *t*-test.

**Table 5**
Comparison of TGF-β levels between Groups C and D.

| Day of Termination | Group C (mean ± SD) (pg/mL) | Group D (mean ± SD) (pg/mL) | *p value | *p value |
|---|---|---|---|---|
| 1 | 0.314 ± 0.015 | 0.333 ± 0.028 | 0.349 | 0.266 |
| 4 | 0.355 ± 0.019 | 0.342 ± 0.013 | 0.309 | 0.316 |
| 7 | 0.378 ± 0.011 | 0.397 ± 0.010 | 0.437 | 0.061 |
| 10 | 0.409 ± 0.009 | 0.411 ± 0.014 | 0.507 | 0.825 |
| 14 | 0.415 ± 0.018 | 0.420 ± 0.012 | 0.499 | 0.679 |

*Levene test ** Independent *t*-test.

Wound healing of the skin involves the regulation of inflammation, cell proliferation, remodelling, extracellular matrix deposition, angiogenesis, and epithelialization. PRP combined with adipose-derived stem cells—in this case SVFs—can improve the healing of the skin layer in mice by reducing infiltration of the inflammatory process, increasing collagen deposition, promoting angiogenesis and neurogenesis [29]. The combination of adipose-derived stem cells and PRP is effective in increasing growth factor concentrations (IGF-1, TGF- β 1, HGF, and VEGF) [22].

The most important growth factors in PRP are TGF-β and PDGF. Both affect each stage of wound healing by stimulating cell growth. TGF-β regulates cell differentiation, proliferation, chemotaxis, and synthesis of several extracellular matrix proteins. The level of TGF-β was four times higher in PRP than in PPP (platelet-poor plasma). Furthermore, TGF-β further enhances superior basal cell proliferation and epidermal regeneration. Collagen, fibronectin, and glycosaminoglycan synthesis from fibroblasts are stimulated by TGF-β. TGF-β triggers collagen synthesis and accelerates collagen maturation in the initial period of wound
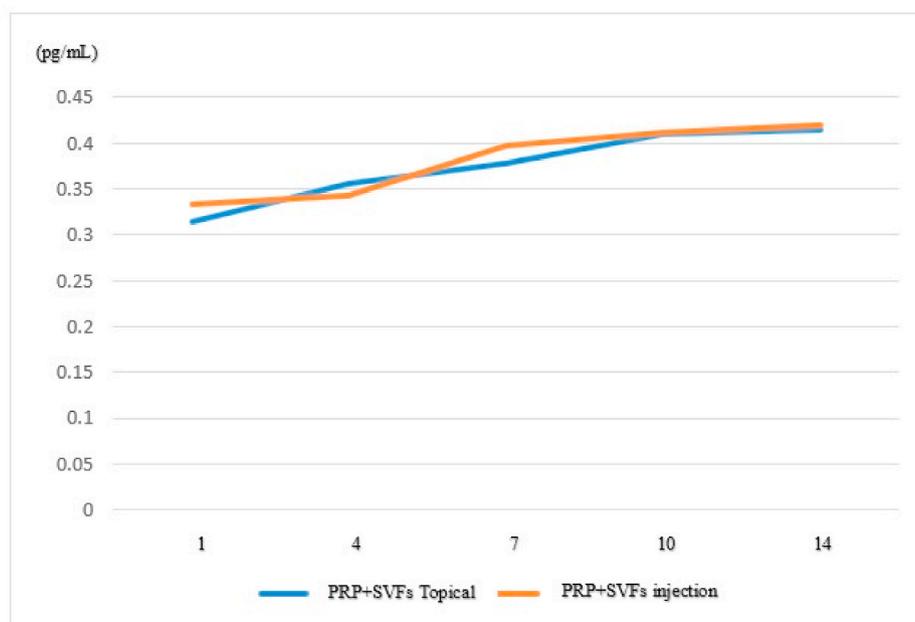
**Fig. 3.** Comparison of TGF-β levels between Groups C and D.

healing [32]. Previous studies have described a role for TGF-β in the formation of hypertrophic scars. TGF-β may be a predictive factor in the formation of burn scars with hypertrophic scars. The study explained that burns that heal well and in the absence of hypertrophic scarring show the formation of TGF-β in plasma for 2 weeks after the burn. However, burns with hypertrophic scarring did not show an initial increase in TGF-β levels [17].

Many studies, including ours, support the superiority of the combination of SVF and PRP compared to Vaseline or control groups for wound healing and tissue regeneration. Given the limitations of conventional approaches for wound healing, including skin grafts, our findings have important practical implications and address a clinical issue. Furthermore, our results may be of general interest to researchers interested in additive and synergistic effects of therapeutic strategies involving stem cells.

Monitoring TGF-β levels in this study was only carried out for 14 days (proliferation phase). It is better to monitor TGF-β levels until the remodelling phase, so that researchers can determine whether the appropriate therapy has been given in burn cases. This study only recorded and evaluated TGF-β levels in the four treatment groups and did not assess other outcomes in rats, such as clinical appearance, wound area, and so on, which supports the assessment of the effectiveness of a therapy. We recommend that further research be carried out by including these variables.

However, further preclinical studies were conducted on the effect of administering a combination of SVFs and PRP on growth factor levels, especially TGF-β in the healing process of deep dermal burns by assessing the quantity of burn healing through histopathology.

## 5. Conclusion

From this study, it can be concluded that the administration of the combination of PRP + SVFs by injection and topically in rats induced by deep dermal burns can increase TGF-β levels significantly compared to the group with Vaseline administration.

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## Registration of research studies

None.

## Ethical approval

All procedure for Animal experiment has been approved by Ethics Commission Faculty of Medicine, Hasanuddin University Number: 1029/UN4.6.4.5.31/PP36/2019.

## Consent

This manuscript does not involve human participants, human data, or human tissue.

## Author contribution

SRL, FJ, FRC, MF, ASP, and BS wrote the manuscript and participated in the study design. SRL, FJ, FRC, WS, ASB, MNM, and AAI drafted and revised the manuscript. SRL, FJ, FRC, MF, ASP, and BS performed deep dermal burn models, stem cell preparation and implantation. SRL, FRC, MF, ASP, and BS performed bioinformatics analyses and revised the manuscript. All authors read and approved the final manuscript.

## Guarantor

Sachraswaty R. Laidding.

## Declaration of competing interest

The authors declare that they have no conflict of interests.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://do

i.org/10.1016/j.amsu.2020.11.088.

## Provenance and peer review

Not commissioned, externally peer-reviewed.

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