EFFECTS OF PLATELET-RICH PLASMA (PRP) AND STROMAL VASCULAR FRACTION (SVF) ADDITION ON EPIDERMAL GROWTH FACTOR (EGF) SERUM LEVELS IN FULL-THICKNESS BURN HEALING IN RATS

EFFET DE L'INJECTION DE PLASMA RICHE EN PLAQUETTES (PRP) ET DE FRACTION STROMALE VASCULAIRE (FSV) SUR LES TAUX SÉRIQUES DE FACTEUR DE CROISSANCE DE L'ÉPIDERME (FCE) CHEZ DES RATS EN PÉRIODE DE CICATRISATION D'UNE BRÛLURE PROFONDE

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SUMMARY. Platelet-rich plasma (PRP) and stromal vascular fraction (SVF) cells are clinically proven to aid in cellular regeneration and accelerate wound healing. The healing effect can be measured by epidermal growth factor (EGF) levels. This study aims to determine the effect of a combination of PRP and SVF injections on EGF levels during the healing of full-thickness burns in Wistar rats. Forty-eight adult Wistar rats were divided into 4 groups. Group A consisted of healthy rats. Groups B, C and D underwent modified full-thickness dermal burns. Group B was treated with local injections of PRP and SVF, Group C was treated with topical Vaseline, and Group D was treated with local injections of sterile water. EGF levels were subsequently assessed on days 1, 4, 7, 14 and 21 post-burn. EGF levels were generally increased in all groups, with the largest increase observed in the PRP and SVF injection group. A one-way ANOVA showed a significant increase in EGF levels on all days ($p = <0.05$). Based on the results of the linear regression test, local injection of PRP and SVF after full-thickness burns increases EGF levels by 27.3%. Combination PRP and SVF injections can increase EGF levels during the healing process of full-thickness burns. EGF is a critical growth factor in accelerating the healing process of full-thickness burns.

Keywords: platelet-rich plasma, stromal vascular fraction, epidermal growth factor, wound healing, full-thickness burn

RÉSUMÉ. *Le PRP et les cellules de FSV ont prouvé leur intérêt dans la régénération cellulaire, promouvant ainsi la cicatrisation. Cet effet cicatrisant peut être évalué par les taux de FCE. Cette étude a pour but de mesurer les taux sériques de FCE après l'injection, à des rats Wistar en phase de cicatrisation de brûlure profonde, d'un mélange de PRP et de FSV. Quarante- huit rats Wistar adultes ont été répartis en 4 groupes : A- contrôle sain, B- brûlure profonde et injections locales de PRP+FSV, C- brûlure profonde et vaseline topique, D- brûlure profonde et injections locale d'eau stérile. Les taux de FCE ont été mesurés à J1, 4, 7, 14 et 21. Ils étaient augmentés dans tous les groupes, singulièrement le groupe B. En ANOVA unilatérale, cette augmentation était significative sur tous les prélèvements ($p<0,05$). En effectuant un test de régression linéaire, on évalue l'augmentation des taux de FCE sous PRP/FSV à 27,3%. L'injection de PRP+FSV est donc à même d'augmenter les taux de FCE, facteur clé de la cicatrisation, après brûlure profonde.*

Mots-clés : *plasma riche en plaquettes, fraction stromale vasculaire, facteur de croissance de l'épiderme, brûlure profonde, cicatrisation*

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Introduction

Burns are defined as damage to the skin and underlying tissue caused by heat, chemicals or electricity.[1] Every year, 450,000 people in the United States receive medical treatment for burns. An estimated 4,000 people die each year from fires and burns.[2] The goal of wound management is to functionally and aesthetically accelerate wound healing.[3]

Various studies have been carried out to obtain methods that can accelerate the healing process of burns. Stem cell therapy is a leading candidate in this field.[4] Stem cells are primitive cells that have not yet differentiated but can differentiate from only being one type of cell (unipotent) into several types of cells (multipotent) and even into various types of cells (totipotent). This ability can be used to repair tissue damaged by disease or trauma.[5] Hopefully, this method can accelerate healing and will decrease the treatment period for full-thickness burns, resulting in lower treatment costs.[4]

Visceral and subcutaneous adipose tissue has been shown to contain progenitor cells that are capable of differentiating into several different cell types. The stromal vascular fraction (SVF) of cells is obtained by centrifuging lipoaspirate obtained from liposuction of fat tissue, resulting in a heterogeneous population of cells.[6-8] Lipoaspirates contain a large population of adipose-derived stem cells (ASCs). The SVF obtained from adipose tissue is known to contain regulatory T cells, endothelial precursor cells, pre-adipocytes (known to be anti-inflammatory macrophages), adipose tissue-derived stromal cells, hematopoietic stem and progenitor cells, endothelial cells, erythrocytes, fibroblasts, lymphocytes, monocytes/macrophages, and pericytes.[7,9,10] The SVF also contains the enzymes and growth factors superoxide dismutase (SOD), IGF, TGF, FGF, hepatocyte growth factor (HGF), and interleukins (IL). SVF increases cell proliferation and vascularization, enhances inflammation, and increases fibroblast activity. Therefore, it improves the healing process in burns.[8,10,11]

Platelet-rich plasma (PRP) is a small volume of concentrated platelet plasma, which contains at least six major growth factors, including platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and transforming growth factor-β (TGF-β) which are released after platelet activation.[12-15]

PRP stimulates the process of angiogenesis and the proliferation of undifferentiated stem cells. Eppley et al. reported that PRP stimulates endothelial cells near the wound site, increasing proliferation and the formation of new capillaries.[16] An in vitro study conducted by Hu et al. concluded that PRP contains potential donor cells that initiate the process of angiogenesis, recruiting the vascular endothelium to the area to support bone regeneration.[17] PRP can induce undifferentiated stem cell proliferation and cell differentiation for tissue regeneration.[18] Undifferentiated stem cells migrate to sites of PRP growth factor concentration, and growth factors promote the proliferation of these cells into the wound site.[19]

Wound healing is a highly regulated process where coagulation, inflammation, fibroplasia and remodeling overlap to close the wound and regenerate new tissue.[20] Cytokines are messengers that mediate all events from the healing process from the time of injury until the end of tissue repair. Cytokines, from the coagulation process and throughout the inflammatory process (PDGF, TGF-β, EGF, bFGF, and others), are major factors in wound healing.[21]

EGF was one of the first growth factors to be identified based on its biochemical properties, and the name describes EGF's ability to stimulate mitosis and hypertrophy of the epidermis.[22] EGF has potent mitogenic properties when used on epidermal cells, fibroblasts and vascular endothelial cells in vitro, all of which are involved in wound healing. EGF and TGF-β promote the migration of human keratinocytes,[23,24] and EGF is a chemotactic factor for corneal cells[22,25] and vascular endothelial cells that stimulates angiogenesis in animal experiments.[23] In animal wound healing studies, EGF increases the amount of extracellular matrix deposited in the subcutaneous tissue by promoting cellular infiltration of the wound and increasing the synthesis of extracellular matrix components.[22] In clinical trials, topical administration of EGF increases epithelialization and shortens wound healing time in skin grafts, venous ulcers, and diabetic foot ulcers.[26]

Research by Laidding et al. demonstrated that the combination of PRP-SVF accelerated the healing of deep dermal burns compared with PRP alone, SVF alone, or a Vaseline control group.[27] Moreover, topical and injected PRP-SVF therapy significantly increased serum VEGF and TGF-β compared to controls and Vaseline in rat deep dermal burns. This indicates that the injection of stem cells is more effective than topical application in increasing VEGF levels.[28,29] Josh et al. assessed the effect of combination SVF and locally injected PRP to assess serum and tissue malondialdehyde (MDA) and nitric oxide (NO) levels in a deep dermal burn model. The results showed that the combination of SVF and PRP reduced MDA and NO levels in blood and tissues compared to the Vaseline and placebo groups. Injection of these two preparations in combination inhibits local and systemic oxidative stress responses, as illustrated by decreased serum and tissue MDA and NO levels that may play an important role in burn healing.[30]

This study is the continuation of a series of previous studies,[26-29] proving the effect of the combination of PRP and SVFs on EGF levels in Wistar rats with full-thickness burn models.

Materials and methods

This is an experimental study on Wistar rats using a post-test control group design consisting of three treatment groups (sacrificed on day 1, 4, 7, 14 and 21 post-treatment), one control group (sacrificed on day 0), and one donor group for PRP and SVF.

All research procedures were performed at the Animal Laboratory of Faculty Medicine, Indonesian Muslim University, and the Hasanuddin University Medical Research Center (HUM-RC) within six months of obtaining approval from the Animal Research Ethics Committee of the Faculty of Medicine, Hasanuddin University (499/UN4.6.4.5.31 /PP36/2021) and according to the ARRIVE Guidelines (Animal Research: Reporting of In Vivo Experiments) for animal research.

Population and samples

The subjects were 48 (Federer Formula) Wistar rats (Rattus norvegicus). All rats were adult males aged 10 weeks, weighing 150-250 grams, and obtained from the Animal Laboratory at the Indonesian Muslim University. The subjects were divided into three groups. The first group consisted of three healthy rats - the normal control group - used as baseline data (Group A). The other three groups, addressed as experimental groups (Group B, C and D; each consisting of 15 rats), underwent full-thickness dermal burns (sacrificed on days 1, 4, 7, 14 and 21 post-treatment); three rats from each group were sacrificed for each day and blood was taken for ELISA (enzyme-linked immunosorbent assay) examination. After the full-thickness burn, Group B was treated with PRP and SVF injections. Group C was treated with topical Vaseline. Group D was injected intradermally with sterile water in the same way as group B.

Platelet-rich plasma (PRP) preparation

Blood samples were collected from all rat donors through a heart puncture to prepare PRP. The blood was transferred to an ethylenediaminetetraacetic acid tube. Blood was centrifuged for 10 min at 2400 rpm (450×g) for the first centrifugation. Supernatant plasma with buffy coat was collected and centrifuged at 3600 rpm (850×g) for 15 min. The infranatant buffy coat was then suspended to prepare the final PRP product.[30,31]

SVF preparation

SVF was derived from adipose tissue collected from all rat donors. The fat was collected from the left and right inguinal areas. It was washed using phosphate-buffered saline (PBS; Gibco-BRL, Grand Island, NY, USA), minced, and then inserted into a tube. The tissue was digested using 0.15% collagenase (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 37°C for 30 min. Dulbecco's modified eagle media (DMEM; Gibco-BRL) with 10% fetal bovine serum (FBS; Gibco-BRL) and 1% antibiotic-antimycotic (Gibco-BRL) was added to neutralize collagenase activation and centrifuged at 1500 rpm for 5 min. The cell pellet was resuspended with aqua dest. The number of cells present in the SVF was calculated using trypan blue (Gibco-BRL) and a Neubauer counting chamber. A total of 50,000 SVF cells combined with 0.5 ccs of

aqua dest were then transferred to a microtube for the final product.[30]

PRP and SVF preparation

In the PRP and SVF combination group, 50,000 SVF cells were combined with PRP until the volume reached 0.5 ccs, so that the final product was 0.5 cc for each.[30,31]

Vaseline

Vaseline, which is also known as white petrolatum, is usually used as an additive to make a product such as hair oil, body lotion, ointment or cream.[32] Vaseline was chosen for this study as it moisturizes the wound bed.[33]

Modeling full-thickness burns in Wistar rats

In each treatment group, hair was removed in the treatment area using the chemical Jolen (Veet®). Prior to the burn procedure, the rats were anesthetized in a special box using ether inhalation until their state of awareness decreased. The wound area was disinfected with 1% povidone-iodine. Burn wounds were induced according to the modified Guo method using a 10×10 mm piece of hot aluminum heated in 100°C water. Then the hot metal was placed on the rat's back for 15 seconds. The pressure applied to the animal skin corresponds to a mass of 51 grams of aluminum metal to induce burns. In this method, full-thickness burns were confirmed by histopathology (*Fig. 1*). After the burn procedure, the experimental animals were administered an oral analgesic (sodium dipiron/metamizole, 40 mg/kg BW) and antibiotic (Amoxycillin, 200 mg/kg BW) for three consecutive days.[34,35]

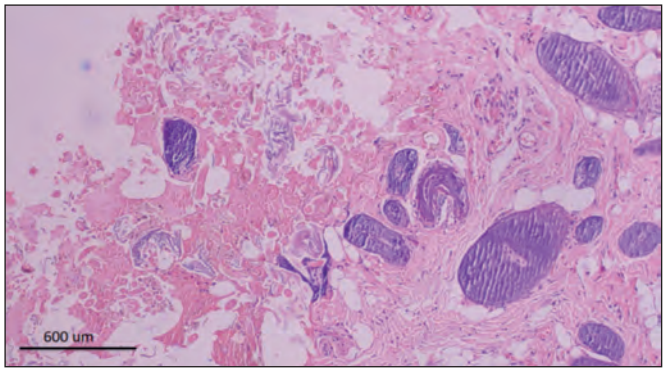

Fig. 1 - Histopathology of full-thickness burn procedure. Histopathological view of the full-thickness burn model with hematoxylin-eosin staining shows the absence of the epidermal and dermal layers of the skin (HE staining, magnification 40x)

Subcutaneous injection and Vaseline procedure

The four edges of the wound and the center of the wound were marked using gentian violet (Efisol®) to facilitate the evaluation of wound healing. The combination PRP-SVF injections were administered to the four edges of the wound at the 12, 3, 6 and 9 o'clock positions and in the center of the wound. Each injection was 0.1 cc for a total volume of 0.5 cc PRP and 50,000 SVF cells per experimental animal. In Group C, topical Vaseline was administered to four edges of the wound at the 12, 3, 6 and 9 o'clock positions for each experimental animal. For Group D, the injection procedure for Group B was repeated using sterile water. All wounds were covered with transparent film and a girdle was placed around the wound. Antibiotics and analgesics were administered as described above. The rats were sacrificed on days 1, 4, 7, 14 and 21 in groups of three animals.

Epidermal growth factor (EGF) ELISA

Blood EGF concentration was assessed using an EGF ELISA Kit (SIGMA-RAB0567-1KT Lot #: 0414I740) following the manufacturer's instructions.

Euthanasia

Prior to euthanasia, each rat was anesthetized by inhaling ether. Rats were then fixed to the surgery table and underwent a thoracotomy. The apex of the heart was identified and punctured using a 25 G needle with a 3 cc syringe. Blood was then aspirated and delivered to the laboratory for testing. Liquid formalin was then injected using the cardiac puncture.

Statistical analysis

Data analysis was carried out using IBM SPSS statistic software version 25 (IBM SPSS Statistics for Windows, Version 25.0. IBM Corp., Armonk, NY). Parametric data were analyzed using a one-way ANOVA and linear regression with a 95% confidence interval.

Results

There were fifteen rats in each group with the inclusion criteria; 3 rats were not given any treatment (Group A), 15 rats underwent full-thickness burn and treatment with a local injection of PRP and

SVF combination (Group B), 15 rats underwent full-thickness burn and treatment with topical Vaseline (Group C), and 15 rats underwent full-thickness burn and were given no local therapy (Group D). All rats observed were healthy and active before sacrifice on days 1, 4, 7, 14 and 21.

Based on *Table I* and *Fig. 2*, the mean EGF value in healthy rats was 1188.00 ± 174.00 pg/mL. On day 1 the mean value of EGF in Group B was 1450.67 ± 137.66 pg/mL, Group C was 778.00 ± 147.49 pg/mL, and Group D was 676.33 ± 129.09 pg/mL. On day 4, Groups B, C and D experienced an overall increase in scores. Group B had a mean of 3022.33 ± 557.24 pg/mL, Group C had a mean of 1010.33 ± 103.08 pg/mL, while Group D had a mean of 939.00 ± 104.24 pg/mL. On day 7, Group B had a mean of 5698.00 ± 611.34 pg/mL, Group C had a mean of 1423.67 ± 321.36 pg/mL, while Group D had a mean of 1078.33 ± 104 99 pg/mL. On day 14, Group B had a mean EGF of 7664.67 ± 885.39 pg/mL, Group C of 4990.33 ± 421.67 pg/mL, and 3042.00 ± 615.92 pg/mL for Group D. On day 21, Groups B, C and D still experienced an increase in overall scores. Group B had a mean of 16411.00 ± 1143.99 pg/mL, Group C had a mean of 7712.00 ± 2284.92 pg/mL, while Group D had a mean of 6023.00 ± 1368.14 pg/mL. Overall, the highest score was obtained from Group B on day 21 (16411.00 ± 1143.99 pg/mL), while the lowest score was from Group D on day 1 (676.33 ± 129.09 pg/mL).

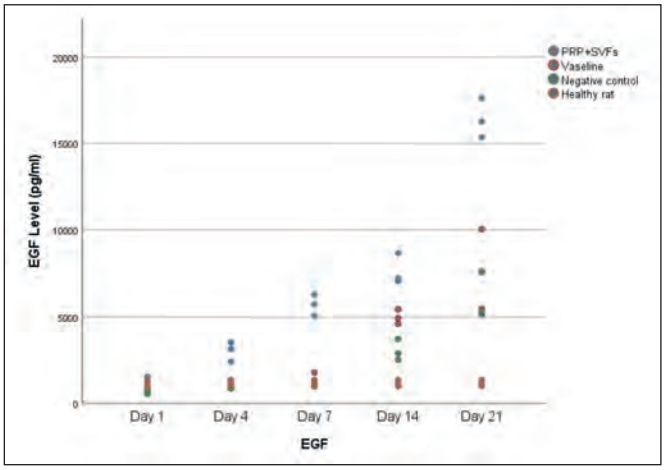

Fig. 2 - Comparison of average EGF levels in each group

Further testing was carried out on the EGF data at each time point. A normality test was performed on each group, and we obtained p >0.05. A Levene homogeneity test was conducted and showed p >0.05, which indicates homogeneous data. A one-way ANOVA test for each time point was conducted, as shown in *Table II*, with p = <0.05 indicating a statistically significant difference between the 4 groups. The post-hoc test determined that there was a significant difference (p = <0.05; *Table III*, *Fig. 3*) in some groups on days 1, 4, 7, 14 and 21.

Table I - EGF concentration based on univariate analysis

Time	Group	N	Mean ± Standard Deviation (pg/mL)	Min	Max
	Group A	3	1188.00 ± 174.00	1014	1362
Day 1	Group B	3	1450.67 ± 137.66	1293	1547
	Group C	3	778.00 ± 147.49	608	872
	Group D	3	676.33 ± 129.09	558	814
Day 4	Group B	3	3022.33 ± 557.24	2416	3512
	Group C	3	1010.33 ± 103.08	943	1129
	Group D	3	939.00 ± 104.24	856	1056
Day 7	Group B	3	5698.00 ± 611.34	5069	6290
	Group C	3	1423.67 ± 321.36	1161	1782
	Group D	3	1078.33 ± 104.99	968	1177
Day 14	Group B	3	7664.67 ± 885.39	7077	8683
	Group C	3	4990.33 ± 421.67	4607	5442
	Group D	3	3042.00 ± 615.92	2517	3720
Day 21	Group B	3	16411.00 ± 1143.99	15350	17623
	Group C	3	7712.00 ± 2284.92	5475	10042
	Group D	3	6023.00 ± 1368.14	5129	7598

Table II - Comparison of EGF levels per day in each group

Day	Group	Mean ± Standard Deviation (pg/mL)	Shapiro-Wilk	Levene test	One Way ANOVA
Day 1	Group B	1450.67 ± 137.66	0.243	0.972	0.001*
	Group C	778.00 ± 147.49	0.117		
	Group D	676.33 ± 129.09	0.752		
	Group A	1188.00 ± 174.00	1.000		
Day 4	Group B	3022.33 ± 557.236	0.652	0.058	0.0001*
	Group C	1010.33 ± 103.08	0.148		
	Group D	939.00 ± 104.24	0.453		
	Group A	1188.00 ± 174.00	1.000		
Day 7	Group B	5698.00 ± 611.34	0.900	0.185	0.0001*
	Group C	1423.67 ± 321.36	0.502		
	Group D	1078.33 ± 104.99	0.816		
	Group A	1188.00 ± 174.00	1.000		
Day 14	Group B	7664.67 ± 885.39	0.170	0.087	0.0001*
	Group C	4990.33 ± 421.67	0.731		
	Group D	3042.00 ± 615.92	0.586		
	Group A	1188.00 ± 174.00	1.000		
Day 21	Group B	16411.00 ± 1143.99	0.781	0.195	0.0001*
	Group C	7712.00 ± 2284.92	0.933		
	Group D	6023.00 ± 1368.14	0.149		
	Group A	1188.00 ± 174.00	1.000		
*95% confidence interval					

Table III - Post Hoc LSD in each group

Time	Group	Group	Mean Difference	P-value
Day 1	Group B	Group C	.0672667*	.001*
		Group D	.0774333*	.000*
		Group A	.0262667	.062
	Group C	Group B	-.0672667*	.001*
		Group D	.0101667	.425
		Group A	-.0410000*	.009*
	Group D	Group B	-.0774333*	.000*
		Group C	-.0101667	.425
		Group A	-.0511667*	.003*
	Group A	Group B	-.0262667	.062
		Group C	.0410000*	.009*
		Group D	.0511667*	.003*
Day 4	Group B	Group C	.2012000*	.000*
		Group D	.2083333*	.000*
		Group A	.1834333*	.000*
	Group C	Group B	-.2012000*	.000*
		Group D	.0071333	.779
		Group A	-.0177667	.490
	Group D	Group B	-.2083333*	.000*
		Group C	-.0071333	.779
		Group A	-.0249000	.341
	Group A	Group B	-.1834333*	.000*
		Group C	.0177667	.490
		Group D	.0249000	.341

Day 7	Group B	Group C	.4274333*	.000*
		Group D	.4619667*	.000*
		Group A	.4510000*	.000*
	Group C	Group B	-.4274333*	.000*
		Group D	.0345333	.274
		Group A	.0235667	.446
	Group D	Group B	-.4619667*	.000*
		Group C	-.0345333	.274
		Group A	-.0109667	.719
	Group A	Group B	-.4510000*	.000*
		Group C	-.0235667	.446
		Group D	.0109667	.719
Day 14	Group B	Group C	.2674333*	.001*
		Group D	.4622667*	.000*
		Group A	.6476667*	.000*
	Group C	Group B	-.2674333*	.001*
		Group D	.1948333*	.004*
		Group A	.3802333*	.000*
	Group D	Group B	-.4622667*	.000*
		Group C	-.1948333*	.004*
		Group A	.1854000*	.005*
	Group A	Group B	-.6476667*	.000*
		Group C	-.3802333*	.000*
		Group D	-.1854000*	.005*
Day 21	Group B	Group C	.8699000*	.000*
		Group D	1.0388000*	.000*
		Group A	1.5223000*	.000*
	Group C	Group B	-.8699000*	.000*
		Group D	.1689000	.192
		Group A	.6524000*	.001*
	Group D	Group B	-1.0388000*	.000*
		Group C	-.1689000	.192
		Group A	.4835000*	.004*
	Group A	Group B	-1.5223000*	.000*
		Group C	-.6524000*	.001*
		Group D	-.4835000*	.004*

*95% confidence interval

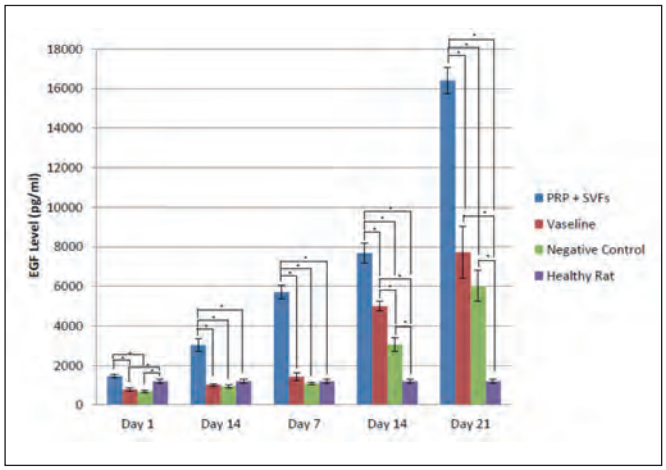

Fig. 3 - EGF levels over time. *p< 0.05

Linear regression was conducted to assess the effect of PRP and SVF combination therapy on full-thickness burns, as shown in *Table IV*. The regression test p-value was 0.001, indicating that PRP and SVF therapy in full-thickness burns had a significant effect on EGF value, resulting in a 27.3% increase in EGF. Each PRP and SVF injection increased EGF value by 5.079.64 pg/mL, consistent with the equation (Y=1769,867 + 5079,467x).

Table IV - Regression test on PRP and SVFs therapy group

Group	P-value	R	R *square*	Equation
PRP+SVF	0.0001	0.522	0.273	Y=1769.867 + 5079.467x

Discussion

The process of healing a wound consists of coagulation, inflammation and remodeling.[28] EGF is a primary mediator of this healing process as it is actively involved in the processes of inflammation and proliferation. A study conducted in diabetic mice, healthy mice and rabbits showed that exogenously administered EGF accelerated the healing process. The effect of EFG is increased when used in combination with other growth factors, such as PDGF, promoting keratinocyte migration and tissue repair.[36]

PRP is a small volume of platelets in autologous plasma, containing 1,000,000 platelets/μl within a 5 mL volume of plasma. PRP is known to contain six kinds of growth factors, including TGF-β, bFG, PDGFa, PDGFb, EGF and VEGF.[7,37,38] Clinical studies have shown that PRP is an effective therapy for the treatment of burns.[39] A meta-analysis conducted by Chen et al. evaluated the efficacy and safety of PRP in the treatment of severe burns. The results show that PRP has strong efficacy in the treatment of severe burns and provides reliable evidence for the clinical use of PRP in the clinical improvement of severe burns.[39] Another result by Cervelli et al. showed that in vitro PRP induced a significant increase in adipose-derived stem cells compared to control cultures. These results demonstrated that PRP accelerates re-epithelialization of chronic skin ulcers and improves fat graft maintenance and function in patients undergoing plastic reconstructive surgery by stimulating ASC proliferation.[40] Mansoub et al. conducted a study on burns in diabetic Wistar mice which were divided into four groups; untreated, injection of keratinocyte-like cells (KLC) only, injection with PRP alone, or treated with a combination injection of PRP and KLC. The levels of EGF, FGF-2, TGF-β1, COL1α2, MCP-1 and VEGF-α were measured on days 3, 7, 10 and 14. They found that there was a significant improvement in the outcomes with the combination of PRP and KLC.[41]

SVF cells act as agents to accelerate the healing process. They are characterized by the processes of fibroplasia, angiogenesis and modulation of inflammatory mediator cells from destructive to constructive phenotypes. They also increase collagen type I and III. PRP is also rich in growth factors that play a role in wound healing by supporting the migration of inflammatory cells to the burn site, inducing cell proliferation and differentiation.[29] Combination PRP and SVF injections can increase the concentration of several growth factors including EGF. In this study, EGF levels were elevated in the combination PRP and SVF injection group throughout the 21-day course of wound healing in comparison to the Vaseline and negative control groups. A significant increase in EGF levels was found at all time points during the 21-day follow-up period, with a 27.3% increase when compared to the administration of Vaseline. In burns, subendothelial thrombin secretion causes the degranulation of platelets.[42] Platelets activated by thrombin release several growth factors that form a hemostatic plug.[43] One of the growth factors released by platelets is EGF.[44,45] EGF facilitates the regeneration of epidermal cells and plays an important role in the healing process by stimulating the proliferation and migration of keratinocytes. EGF also promotes tissue granulation and stimulates fibroblast motility. In vitro mitogenic responses, including increased proliferation, cell migration and synthesis of type I collagen fibers in skin fibroblasts, occur to cells treated with EGF continuously for three to four days (onset of therapeutic effect). This accelerates wound healing, increases the rate of re-epithelialization, and reduces inflammatory infiltration. In contrast, the absence of EGF decreases receptor activity within four hours.[46] In addition, EGF is a key factor in skin inflammation, skin barrier function, and defense against infection. It appears to be significant in the expression and activation of the complement system in the epidermis and human keratinocytes.[47,48] Local injection of a combination of PRP and SVF increases blood serum EGF levels continuously throughout the 21-day follow-up period and accelerates the healing process of full-thickness burns.

In this study, PRP preparation used differential centrifugation with buffy-coat method, and for SVF we used enzymatic digestion. The final result of the preparation was injected subcutaneously in rats. Previous studies also used the same method and got satisfactory results for wound healing. However, all of these studies are still limited to in vitro tests on rat models.[27-30,49-54] PRP is a biological product, prepared using different protocols, sometimes without

even controlling whether platelets were effectively concentrated and purified or whether an early activation occurred, discarding all of the secreted growth factors within the platelet poor plasma (PPP). Another issue is a correlation between the platelet concentration or the PRP volume applied per injured area or volume. Studies have already demonstrated that low platelet concentration is inefficient and that high concentrations have an inhibitory effect on cell growth, but results are still contradictory. Blood processing was optimized in two centrifugation steps known as differential centrifugation. The aim of the first step was to deplete the product of red and white blood cells with minimal loss of platelets, and the aim of the second step was to obtain the highest recovery and the best yield of platelets in the smallest final plasma volume. This procedure recovered more than 50% of the initial platelets with a low amount of other blood cells.[55] Various factors influence the yield of PRP, such as draw of blood; speed, time and temperature of centrifugation and use of anticoagulants.[56] Activation of PRP is not required when it is injected into soft tissues. Activation of PRP primarily refers to two processes - release of growth factors (GFs) from platelets following degranulation and cleavage of fibrinogen to form the matrix. This process turns liquid plasma into a solid clot or a membrane. When PRP is injected into a soft tissue, it does not need to be activated as the natural collagen type I acts as a natural activator.[57] Enzymatic digestion is commonly performed with collagenase, which is subsequently inactivated, and the pool of mononuclear cells is isolated through filtration or centrifugation. This product is also termed "cellular-derived SVF" (cSVF), that obviously lacks intercellular connections and extracellular matrix (ECM). The ECM is an important reservoir of regenerative growth factors while ECM molecules and their degradation products also contribute to the regenerative power of the ECM.[58,59] Research of Sun et al. uses a novel adipose-tissue derived injectable ECM/SVF-gel. Taking advantage of shearing force to selectively break mature adipocytes eliminates most of the lipids and other unwanted components. ECM scaffold in the ECM/SVF-gel accommodates ASCs within their natural niche, thereby providing a beneficial environment for attached ASCs to maintain optimal cell survival and potency. Finally, ECM components regulate proliferation and migration of cells as well as angiogenesis.[60]

There are several limitations to this study. Firstly, this was a short-duration study that has not yet reached the maturation phase, so further research can be carried out until the maturation phase ends. Secondly, the research variables can be expanded to include an assessment based on the route of administration, as well as the measurement of other growth factors as outcome measures. Large-scale clinical studies and an expanded set of variables are needed to assess the efficacy and safety of combined therapy with PRP and SVF in burns and other types of wounds.

Conclusion

EGF is one of the most important growth factors in the burn healing process. This study demonstrates that a single dose of combination therapy with PRP and SVF can increase EGF levels during the healing process of full-thickness burns. These elevated levels can be observed from day one post-burn with a peak EGF concentration occurring on day 21. Therefore, combination therapy with PRP and SVF is a promising treatment for increasing EGF levels during burn healing.

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