

RESEARCH ARTICLE

The Effect of Educated Platelets on the Healing Process of Burn Wounds in Rats

Ilgen Orkun , Kurt Sefa , Yilmaz Osman , Celiloglu Murat 
Dokuz Eylul University, School of Medicine, Department of Obstetrics and Gynecology, Izmir, Turkey

ABSTRACT

Burns are serious life-threatening health problems. This study was performed to determine the effect of educated platelets on burn wound healing process. 28 female wistar albino 200-220 gr rats were randomly divided into four groups. Group A1 rats(n:7) were the first-line burnt group from which blood samples are extracted to develop platelet-rich plasma (PRP) with the educated platelets that have a response to burn injury. Group B1 rats(n:7) were the unburnt group with ordinary platelets. Group A2 rats(n:8) were the second-line burnt group which was given PRP with educated platelets. Group B2 rats(n:6), as control group, were the second-line burnt group which was given PRP with ordinary platelets. Photos of rats dorsum were taken by digital camera on the first day and 21st day of the study. Wound healing was determined by scar surface area. In the study group (Group A2) mean wound area was 53 ± 37 mm², in the control group (Group B2) mean wound area was 114 ± 55 mm² at the last day of the experiment. The sizes of the wounded areas were significantly lower in the study group compared with the control group (p: 0.039). Educated platelets seem to facilitate the recovery period of burn wound healing in rats.

KEYWORDS: Educated Platelet, Burn Wound Healing, Platelet Rich Plasma.

Correspondence: Dr Ilgen Orkun. Mithatpaşa Cad. Dokuz Eylül University Hospital Polyclinics Building 2nd Floor, Department of Obstetrics and Gynecology İnciraltı / Balçova / Izmir, Turkey. Email mdorkunilgen@gmail.com

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INTRODUCTION

Platelets are small, nucleated, discoid cells with a diameter of 2-4 microns. They are produced from megakaryocytes which are precursor cells found in the bone marrow or intravascular regions of the lungs. Their prominent functions are blood coagulation and wound healing. However, platelets have received considerable attention for their complexity and versatility, and are known to be involved in many physiological and pathophysiological processes, such as innate and adaptive immunity atherosclerosis, angiogenesis, and tumor metastasis (1,2). Recently, there is a new concept in tumor evolution called the tumor educated platelets. Tumor educated platelets are the cells that enhance tumor cell proliferation and metastasis by several pathophysiological pathways (3,4). One of these is altered RNA profiles in TEPs that conduct different protein synthesis.

The same mechanism might play a role in burn victims. Burns are among the most dangerous life-threatening health problems all over the world affecting people of every age. Physical scars and chronic disabilities are common in this area. Depth and diameter of burn are the main issues that impact morbidity and mortality. Scar tissue healing is the main problem to cope up with (5). There are various topical agents in the treatment of burn wound. There are some studies and case reports using platelet rich plasma (PRP) in burn scar healing. According to these

studies, PRP enhances wound healing (6). In the light of this, we investigate if there is a relationship between acceleration of burn wound healing and educated platelets which are produced as a response to burn trauma.

MATERIALS AND METHODS

Animals:

A total of 28 mature (12-week-old) female wistar albino rats weighing 200-220 g were used in this study. The animals were fed ad libitum and housed in pairs in steel cages in a temperature-controlled environment ($22 \pm 2^\circ\text{C}$) with 12-h light/dark cycles. The experimental procedures were approved by the Committee for Animal Research of Dokuz Eylül University, Izmir, Turkey. All animal studies strictly conformed to the experimental guidelines of the Committee for Animal Care (Dokuz Eylül University) (Ethical approval no: 17/2019).

Rats were randomly divided into four groups which were called A1, A2, B1 and B2. Group A1 rats(n:7) were the first line burnt group from which blood samples are extracted for PRP with the educated platelets that are response to the burn injury. Group B1 rats(n:7) were the unburnt group from which blood samples are extracted for PRP with ordinary platelets. Group A2 rats (n:8) is the second line burnt group which were given PRP with educated platelets to their blood circulation. Group B2 rats (n:6) were the

second line burnt group which were given PRP with ordinary platelets to their blood circulation served as a control group (table 1).

Table 1: Distribution of rats by groups and experimental design

Unburnt group (group B1) (n:7)	Burnt group (group A1) (n:7)
↓ 15 days later	↓ 15 days later
Second-line burnt group which was given ordinary platelets (group B2) (n: 6)	Second-line burnt group which was given educated platelets (group A2) (n: 8)
↓ 21 days later	↓ 21 days later
Burn follow-up and improvement assessment	Burn follow-up and improvement assessment

For the burning procedure, rats were anesthetized by the intraperitoneal injection of a combination of 50 mg/kg ketamine hydrochloride and 7 mg/kg xylazine hydrochloride (Alfazyne; Alfasan International BV, Woerden, Holland). The dorsal skin around the operation area was shaved and disinfected. The dorsal skin was chosen since it was difficult for the rat to reach by itself. Hot metallic device was used to form burn wounds (diameter: 5x2 cm²) (figure 1). Under sterile conditions, a 5x2x2 cm³ metal was kept in boiling water for 5 minutes and contacted to the rats' shaved dorsum for 10 seconds with its own weight to induce burn injury (7). In the follow-up period all the rats were kept in same conditions. Fentanyl citrate (0.002 µg/kg) was given twice a day for the purpose of postoperative analgesia. Group A1 rats were burnt under these circumstances and the same procedures, except performing burn, were implemented to the group B1 rats (such as anesthesia and shaving). At the 14th day of experiment group A2 and group B2 rats were burnt and group A1 and group A2 rats were sacrificed to take enough blood samples to prepare PRP. PRP was prepared right after the first hour of taken samples and given to the group B1 and group B2 as soon as possible. Photos of rats' dorsum were taken by a digital camera on the first day and 21st day (7). Wound healing was determined by the scar surface area. The surface area of the wound was calculated by the image-j program (ImageJ bundled with 64-bit Java 1.8.0_112) (8). All the rats were sacrificed after administration of high dose anesthetics

(100 mg/kg intraperitoneal ketamine) on the 35th day of the experiment.



Figure 1: Hot metallic device

PRP Preparation:

Blood samples were extracted from vena cava caudalis to the tubes that were buffered by sodium-citrate with the ratio of 1:10 (for example; 9 mL of blood were mixed with 1 mL of 0.1 mol/L sodium citrate). First, the tube was centrifuged for 7 minutes at 1700 rpm. After centrifugation, the plasma layer and buffy coat were collected and located into another tube for the second centrifugation which would last for 5 minutes at 3200 rpm. Then, the bottom half of plasma, which is called PRP, was collected. Platelet number in PRP was calculated by using an automated cell counter (CDA-1000; Sysmex) (9).

Statistical Analysis:

The data were analyzed using SPSS software (version 11.5, Chicago, IL, USA) by non-parametric test of Mann Whitney. Test of normality was analyzed by the Shapiro-Wilk test. P values of less than 0.05 were labeled as significant.

RESULTS

Normal distribution among groups was determined in the Shapiro-Wilk test. All the rats from group A2 and group B2 had the same wound area measured as 1000 mm² on the 14th day of the experiment. In the study group mean wound area was detected 53±37 mm²; By contrast, in the control group mean wound area was detected 114±55 mm² at the last day (35th day) of the experiment (table 2). The wound area sizes were significantly lower in the study group than the control group (p: 0.039) (figure 2,3).

Table 2: Statistical analysis of the results according to the burn areas of the groups

Group	N	Mean (mm ²)	Std. Deviation (mm ²)	Median (mm ²)	Minimum (mm ²)	Maximum (mm ²)
Group B1	8	53.3000	37.45401	58.7500	8.00	105.60
Group B2	6	114.1167	55.1999	137.7500	20.40	166.90

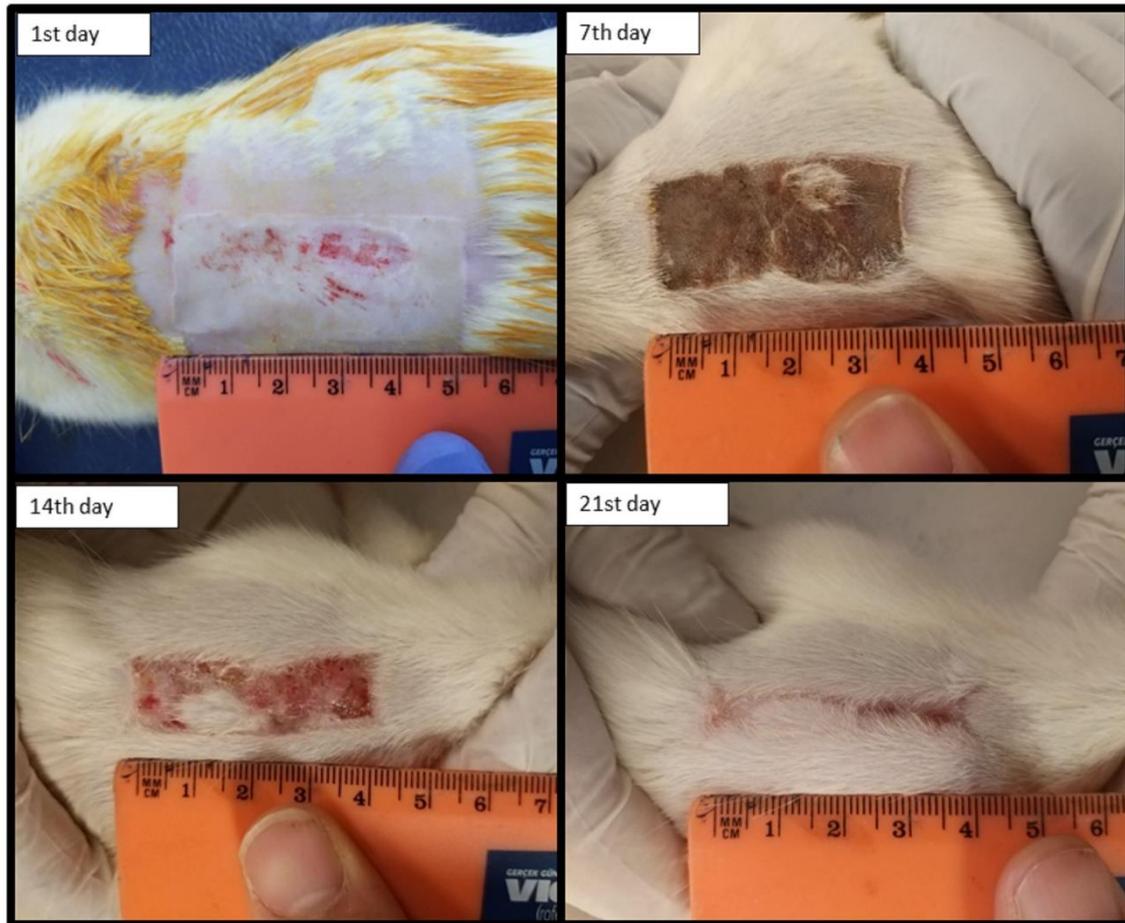


Figure 2: Photos of wound healing process on day 0,7,14 and 21 in group A2 respectively.

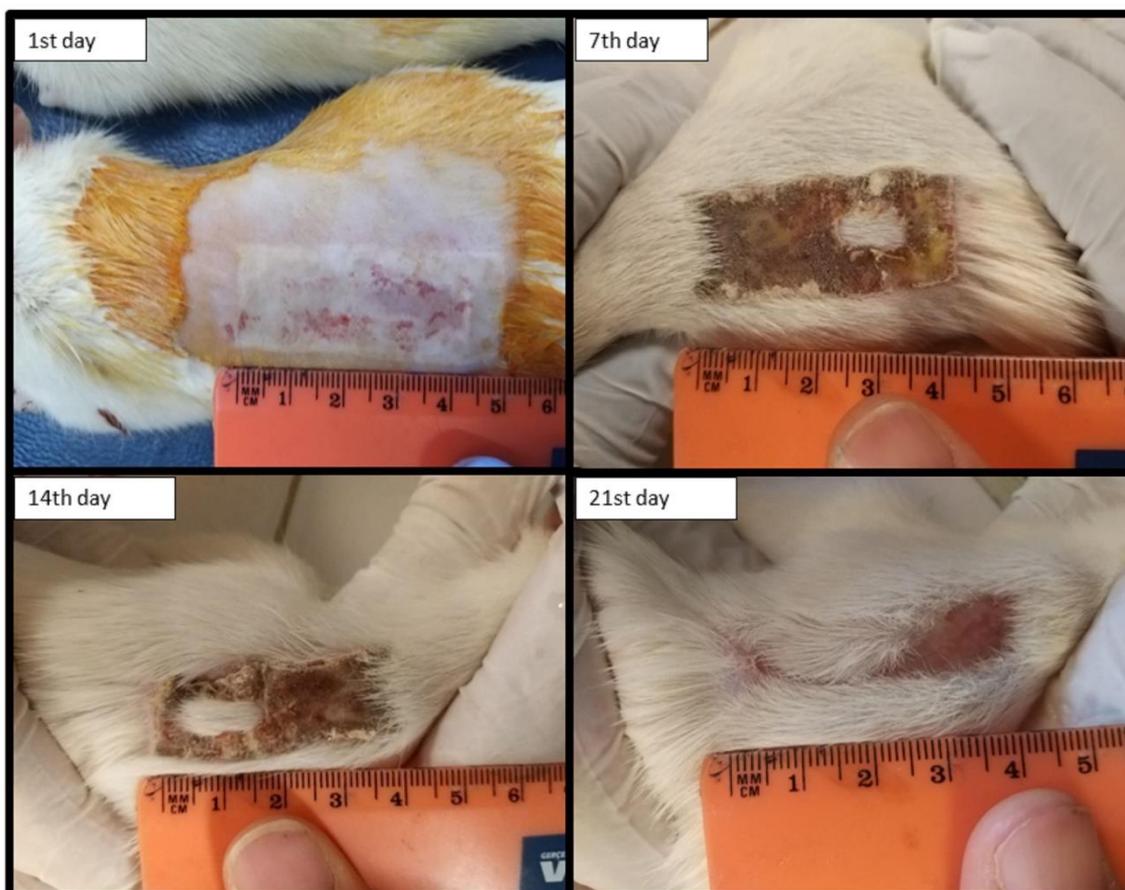


Figure 3: Photo of wound healing process on day 0,7,14 and 21 in group B2 respectively.

DISCUSSION

Wound healing occurs as a cellular response to tissue damage and involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages and platelets. Right after skin injury, platelets trigger the coagulation cascade and release important growth factors and cytokines for initiation and progression of wound healing. The biological roles of platelets include the spread of cancer metastasis, liver regeneration, inflammatory arthritis, and strengthening of immunity to pathogens. Many of these occur by secreting large amounts of cytokine and growth factor granules. Platelet granules being rich in growth factor have led to the use of PRP in wound healing by some researchers. Platelets also have many other functions such as hemostasis, wound healing, angiogenesis, interaction with immune cells and the development of tumor metastases. Educated platelets, particularly the ones observed in cancer patients, have proved to be different from inflammatory and non-cancerous patients. Furthermore, the mRNA biomarker signatures they contain are becoming increasingly significant in terms of diagnosis.

Recent studies have shown that rather than random transfer to platelets during thrombopoiesis, megakaryocytes specifically transfer mRNA. As a result, platelets are released to blood circulation with thousands of mRNAs (10,11).

Several independent studies using serial analysis of gene expression, microarray profiling, or next generation RNA seq have shown that platelets have a different repertoire of mRNAs. These studies identified genes that are important in megakaryocyte and platelet biology, and provided a new notion of the diversity and breadth of megakaryocyte transcriptome. Platelets are able to express thousands of mRNAs which are most likely megakaryocyte-derived (10).

Platelets are known as endocytosis proteins, and recent studies in other cell types have shown that genetic exchange between cells and exosome-mediated transfer of mRNA from cell to cell are possible. It has also been shown that platelets can transfer functional RNA to monocytes, and platelets might play a role as mRNA transfer receptors from other cells (12).

Platelet mRNAs have a different repertoire than other cell mRNAs which also contain DNA. Platelets convert most of these mRNAs into proteins and also transfer them to other cells, allowing them to be used as a template. In addition, platelets communicate with the local and remote environment via horizontal transfer of platelet-derived microparticles, chemokines, cytokines and through direct physical interaction (eg, binding to glycoprotein IIb / IIIa and P-selectin) (12).

This taught memory created in platelets can be used in many situations such as tissue healing, cardiovascular diseases and cancer. Tumor-related biomolecules can be transferred to platelets to educate them, in addition, thermal damage related biomolecules can lead to platelet education (13–15).

Platelet counts exhibit fluctuant patterns after burn. For instance on the first days of post burn it is possible to observe a decrement. However after that platelet counts

tend to increase and on the post-burn 15th-day maximal number is achieved. After second-degree burn, the study demonstrated that EPLT numbers reach the maximum level at the 14th-15th days. For this reason, we took the blood sample and extracted PRP for our trial. On the 24th day of the burn, the values returned to normal (16,17). Also platelets are functionally activated in burn patients. Size of the burnt area is one of the most crucial factors that is related to mortality and morbidity after thermal injuries. Studies have shown that platelet count and activation increase in burn patients (16,17). In vivo studies confirmed that bone marrow cells made an increment on wound healing (18,19). In the literature, few case reports and animal studies indicated that local application of PRP is beneficial in burn wound healing (20,21).

The initial act in our study was to give platelets that we considered having acquired memory to the second group of rats after deriving them from the first group of rats 14 days following the second degree-burn. Wound healing was dramatically faster in the group receiving the platelets, which we thought to be educated, at 1-week intervals. When the healing / non-healing surface areas of both groups were compared, the difference was statistically significant. We think that this result is caused by educated platelets that have been involved in the healing of previous thermal damage and this have improved memory.

CONCLUSION

We were influenced by the dynamic response of platelets to external stimuli, central tasks at all stages of wound healing, intercellular communication and memory formation. We observed that platelets, which we think were educated in response to thermal damage and healing process previously, have positive effects on repair and healing process when used systemically for new thermal damages of the same level. We hope that our conclusion will be supported by the findings of other researchers. Further studies are needed in this issue.

ACKNOWLEDGEMENTS

None

DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest.

STATEMENT OF ETHICS

The experimental procedures were approved by the Committee for Animal Research of Dokuz Eylul University, Izmir, Turkey. All animal studies strictly conformed to the experimental guidelines of the Committee for Animal Care (Dokuz Eylul University).

AUTHOR CONTRIBUTIONS

All authors made equal contributions in all parts of the study. O.I., S.K., O.Y., and M.C. designed and work on the study and participated in data collection, manuscript preparation, and revision. All authors read and approved the final manuscript.

FUNDING

None

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