

PREVENTION AND TREATMENT OF POTENTIALLY PROBLEMATIC ACUTE WOUNDS TO ACHIEVE FULL RECOVERY BY USING PLATELET-RICH PLASMA: RESEARCH RESULTS

Tsvetan V. Sokolov

*Clinic of Orthopaedics and
Traumatology,
Kanev University Hospital - Ruse*

Corresponding Author:

Tsvetan Sokolov
Clinic of Orthopedics and Traumatology,
Kanev University Hospital - Ruse
2, Nezavisimost Str.
Ruse, 7002
Bulgaria
e-mail: sokolovi2001@abv.bg

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Summary

The study presents the outcomes from platelet-rich plasma (PRP) application in treating acute and potentially problematic skin wounds from 2009 to 2016. Fifty-six hospitalized patients with acute skin wounds were divided into two groups: an experimental group of 30 patients treated with platelet-rich plasma and 26 controls with similar wounds, treated by conventional methods. After platelet-rich plasma treatment of 30 acute and potentially problematic wounds in the Experimental Group patients, 29 (96.7%) of the wounds healed for 16 weeks on average. After applying conventional treatment with surgical wound debridement, of the 26 acute, potentially problematic wounds in the controls, only 2 (7.6%) healed. Since acute and potentially problematic wounds are very likely to become chronic and difficult to heal, we recommend PRP application as a preventive treatment in patients at risk.

Keywords: platelet-rich plasma, acute and potentially problematic skin wounds, chronic wounds

Introduction

The skin is the largest organ in the human body. It plays a significant role in sustaining life by maintaining the water-electrolyte balance, regulating temperature, and acting as a barrier against various harmful external agents, including microorganisms. When this barrier is broken due to wounds, burns, neoplasms, or trauma, these functions can no longer be sustained adequately. This is why it is so important to restore the integrity of the skin as soon as possible. Skin ulcers represent tissue loss from the epidermis and derma, and sometimes they affect adipose tissue and muscle fascia. If spontaneous recovery is not achieved, a tissue lesion turns into a fibrous scar [1,2]. It has been proven that 15% of acute skin wounds become chronic [3].

Problematic skin wounds (PSW) cover a broad range of injuries, including acute, potentially problematic, such as in cases of high energy trauma and elderly patients. A wide variety of intrinsic and extrinsic factors interfere with healing, and PSWs are hard to heal and turn into chronic skin wounds (CSW). Acute skin wounds (ASW) affect skin integrity and sometimes the underlying tissues, and the recovery process follows strictly defined consecutive biology stages. ASWs are particularly difficult to treat because

they are located in bone prominences, underlying tendons, plantar surface of the foot, and around joints. When there are skin defects, the latter can turn into potentially problematic skin wounds [1]. The wound healing process involves a strictly defined set of consecutive events, which starts at the time of injury and lasts for several months. It could be divided into three phases: inflammation, proliferation, and remodeling (differentiation) [4,5,6,7].

The patient's venous blood produces autologous platelet-rich plasma (PRP), which consists of growth factors (GF), cytokines, fibrin scaffold, and chemokines. Molecular and cellular induction of normal wound healing is achieved by adding GF from PRP to the wound, similar to that observed when platelets are activated [1].

The purpose of our research was to evaluate the outcomes of platelet-rich plasma application in treating acute skin wounds.

Materials and Methods

The research took place from 2009 to 2016. There were 56 hospitalized patients with acute skin wounds in total. The experimental group included 30 patients treated with platelet-rich plasma. The control group consisted of 26 patients with similar wounds, treated by conventional methods such as surgical wound debridement. A detailed interview was conducted with patients with clarifying questions on the therapy applied. Informed consent for treatment with PRP was obtained from the 30 patients included in the experimental group. The remainder were put in the control group. According to the Helsinki Declaration's principles, all surveyed

participants in the study signed their informed consent [8].

Wounds were assessed based on scores introduced by Cancelli AM [1]. Total Wound Score (TWS), total anatomic score (TAS) of a wound, and Total Score of Wound data (TSWD) were used to evaluate specific wound parameters.

The data obtained were analyzed using the software SPSS (Statistical Package for the Social Sciences). Non-parametric tests (Kolmogorov-Smirnov, Mann-Whitney, and Wilcoxon) were also used to verify the significance of relations between features. The level of significance for p was assumed to be at least 0.05 [9].

All patients in the experimental group received treatment with PRP derived from PRGF Endoret, based on a treatment algorithm we designed. The first step was surgical treatment and thorough wound debridement. On the second postoperative day, we applied PRP to the wound by infiltrating wound edges and adding PRP to the wound bed. PRP was prepared as follows: blood was drawn in blood collection tubes with sodium citrate. The tubes were then centrifuged for 8 minutes at 1800 r/min. Plasma was drawn with micropipettes, and a 10% CaCl_2 activator was added. The ratio was 1 ml plasma to 50 μl activator. After 5 minutes, the PRP obtained became active. In about 40 minutes, a fibrin clot was formed. Wound edges were infiltrated with the activated plasma in a liquid state, while the fibrin clot was used to fill the wound. Then the wound was dressed with a soft bandage soaked in saline. This procedure was repeated every seven days until the wound had entirely healed.

Table 1 shows systematic data about the

Table 1. Distribution of EG patients based on anatomic location of ASW, infectious agent and wound depth

DEPTH		LOCATION		AGENT	
Anatomic location	Number	Anatomic location	Number	Anatomic location	Number
hypodermis	11	Achilles	2	S. aureus	9
fascia	9	knee	5	E. coli	5
tendon	3	lateral malleolus	6	P.aeruginosa	1
bone	7	ankle	6	K. pneumoniae	1
		shin	7	E. faecalis	1
		heel	2		
		shoulder	1		
		palm	1		

Table 2. Distribution of EG patients based on anatomic location of ASW, infectious agent and wound depth

DEPTH		LOCATION		AGENT	
Anatomic location	Number	Anatomic location	Number	Anatomic location	Number
hypodermis	10	Achilles	2	S. aureus	9
fascia	8	knee	4	E. coli	6
tendon	3	lateral malleolus	5	P. aeruginosa	2
bone	5	ankle	5	K. pneumoniae	2
		shin	6	E. faecalis	2
		heel	2		
		shoulder	1		
		palm	1		

experimental group (EG) patients with acute skin wounds based on anatomic location, infectious agent, and wound depth of ASW. The average area of wounds was 1490mm², and the patients' median age was 54.2 years. Ten patients had controlled type 2 diabetes.

Table 2 shows the control group (CG) patients' systematic data with acute skin wounds based on anatomic location, infectious agent, and wound depth. The average wound area was 1310 mm², and the median age was 55.2 years. There were 8 patients with controlled type 2 diabetes in the CG.

Results

After PRP treatment of 30 acute skin wounds of patients from the experimental group, all 29 wounds (96.7%) healed for 16 weeks on average. No data proving wound recovery was found for one wound (3.33%) in the experimental group.

After conventional treatment with surgical wound debridement of the 26 acute skin wounds in the control group, full recovery occurred only in two patients (7.7%). No recovery was achieved in the remainder of 24 (92.3%) patients.

Figure 1 shows the clinical case of a 40-year-old man with traumatic arthrotomy on the left ankle after an accident with a chainsaw. The patient underwent surgical treatment of the wound and tendraphy of extensor tendons. On the 14th day after the trauma, a wound with a 400 mm² area formed, with 10 mm wound edges. PRP treatment protocol was initiated. The photos show the recovery process dynamics and full recovery on week 16. Figure 2 shows the ASW treatment trend of the same patient by

using different wound assessment scores. The microbiological wound tests isolated S. aureus. No antibiotics were administered.



Figure 1. Clinical course of ASW healing with PRP treatment of a 40-year-old man, successfully treated for 16 weeks

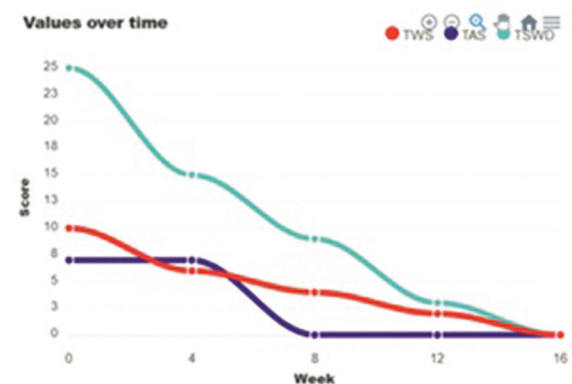


Figure 2. Treatment trend of acute skin wounds of the same patient

Figure 3 shows the clinical case of a 17-year-old woman with wound *dehiscence* after surgery for hemangioma in the *Achilles* area. On the 12th day after surgery, a wound with an area of 300 mm² formed, with 5 mm wound edges. PRP treatment protocol was started. The photos illustrate the dynamics of the recovery process. Full recovery was seen on week 12. Figure 4 shows the ASW treatment trend of the same patient by using different wound assessment scores. The microbiological wound tests isolated *S. Aureus*. No antibiotics were administered.

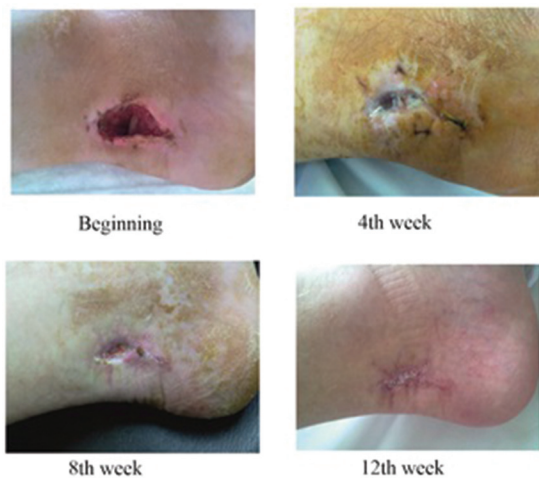


Figure 3. Clinical course of ASW healing with PRP treatment of a 17-year-old woman, successfully treated for 16 weeks

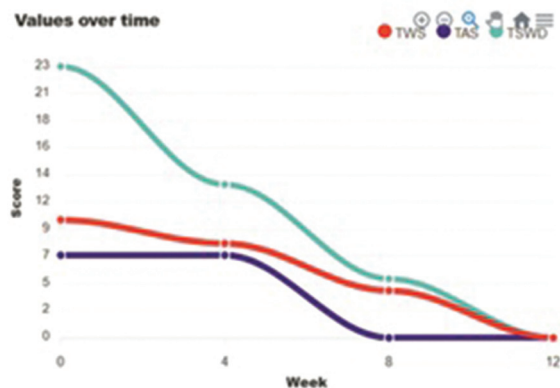


Figure 4. Treatment trend of acute skin wounds of the same patient

Figure 5 shows a 62-year-old woman's clinical case with a bimalleolar fracture, metal osteosynthesis, and a skin defect. On the second day after the surgery, a wound of 560 mm² formed with wound edges of 3mm. PRP treatment protocol was initiated. The photos



Figure 5. Clinical course of ASW healing with PRP treatment of a 62-year-old woman, successfully treated for 16 weeks

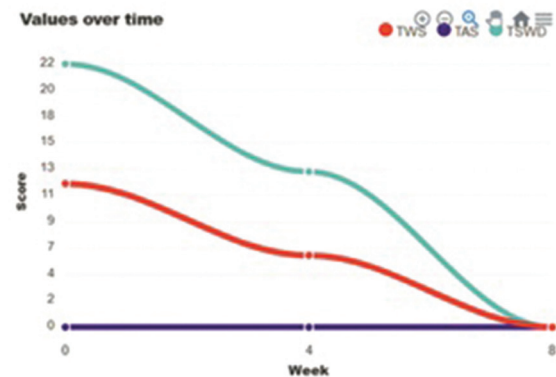


Figure 6. Treatment trend of acute skin wounds of the same patient

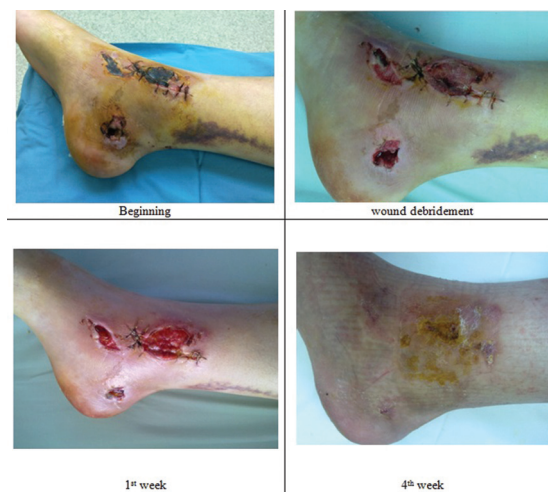


Figure 7. Clinical course of ASW healing with PRP treatment of a 40-year-old man, successfully treated for 4 weeks

show the recovery process dynamics and full recovery on week 8. Figure 6 shows the ASW treatment trend of the same patient by using

wound assessment scores. The microbiological wound tests isolated *S. Aureus*. No antibiotics were administered.

Figure 7 shows a 42-year-old man's clinical case with a bimalleolar fracture, metal osteosynthesis, and a skin defect. On the second day after the surgery, a wound of 600 mm² had formed with 4 mm wound edges. The PRP treatment protocol was applied. The photos illustrate the dynamics of the recovery process. Full recovery was achieved on week 4.

Discussion

The results obtained and analyzed in our study showed that PRP application in acute, potentially problematic skin wounds PSW helps achieve excellent results. Treatment resulted in accelerating healing, shorter hospital stay, and minimizing wound complications. The prophylactic use of PRP on high-risk patients could contribute to full tissue recovery and prevent wounds from turning into chronic wounds that are difficult to heal. We aimed to determine whether the PRP method can be effectively used for acute and potentially problematic skin wounds. The PRP application results were compared with results in the control group, in which surgical wound debridement and a soft bandage dressing were applied. Comparing the other possible alternative methods for the treatment of such wounds will be made in another study.

Many sources prove PRP's positive effect and the benefits of its applications on the healing process of hard-to-heal wounds. Data on the application of PRP for the prevention and treatment of ASW is limited. Meta-analyses of studies on the treatment of ASW with PRP found the following:

Picard et al. report the results from applying PRP to support wound healing. The purpose of this study was to test whether using PRP would support the full recovery of acute wounds, burns, and laser therapy outcomes. Three randomized controlled acute wound studies have statistically demonstrated benefits on healing time, time to return to work, quality of life, and pain. Three prospective controlled studies have found a statistically significant difference in terms of healing time. Authors have concluded that PRP application enhanced ASW healing process [10].

Wang et al. evaluated the PRP efficacy for the treatment of acute wounds. Many studies have been reviewed, including 13 reports on 982 patients. Meta-analyses results have shown that healing time was shorter in the PRP group than the control group with no PRP. PRP may reduce ASW healing time and hospitalization time. It may also minimize wound healing complications and the need for blood transfusion and may relieve posttraumatic pain. Furthermore, PRP has a positive effect on wound infection control [11].

Another PRP application in the PSW treatment process is to prevent and treat wound infection. Numerous sources have proved the positive effect of PRP treatment of wound infections.

El-Sharkawy et al. analyzed GF in PRP and the effects of PRP application on the release of cytokines from monocytes and lipoxin A4. The authors concluded that PRP is a rich source of growth factors and supports significant changes in the monocyte-mediated proinflammatory release of cytokines and chemokines. PRP may inhibit cytokine release, limit inflammation, and promote tissue regeneration because of increased lipoxin A4 [12].

Cieslik-Bielecka et al. reported that PRP in the form of gel inhibits the growth of *S. aureus* and *E.coli*, but no similar effect on wounds with *Kl.pneumoniae*, *E.faecalis*, and *Ps.aeruginosa*. No correlation between leukocytes and platelet concentrations and antimicrobial activity was observed. The activity of antimicrobial peptides of platelets against fungi was less pronounced as compared to the activity against bacteria. Gel PRP induced in vitro growth of *Ps. Aeruginosa* which means that PRP may exacerbate infections with this agent. Authors consider that the combination of gel-like PRP inductive and antimicrobial properties may enhance recovery of infected delayed adhesion or non-adhesion [13].

In their study, Anitua et al. have reported on the antimicrobial potential of the growth factor-rich plasma when used against methicillin-sensitive and methicillin-resistant *S.aureus* and *S.epidermidis*. They also report the effect of incorporated leucocytes in growth factor-rich plasma. Blood samples were obtained from five healthy volunteers and used to prepare each type of plasma rich in growth factors (F1, F3, F3+leu). The Microbiological activity of growth factor-rich plasma against two staphylococcal strains

was tested, based on counting surviving bacterial colonies after incubation for 0.4 and 8 hours with various forms. The authors found out that all types had a bacteriostatic effect, especially in the first 4 hours of administration. All types had an antibacterial effect on the 4th hour on three out of four strains, except for methicillin-sensitive *S.epidermidis*. The researchers concluded that plasma rich in growth factors could be used to fight and prevent postoperative wound infections [14].

Our study also supports these results from PRP application for PSW treatment, reviewing which risk patients are suitable for the treatment method [15].

The use of the respective antimicrobial, antibiotic treatment for an acute and potentially problematic skin wound depends on the derived microbiological research. The laboratory results determine the use of only one or more antibiotics. The duration of antibiotic use depends on the antimicrobial agent and the respective antibiotic, to which the former is susceptible.

Patients from EG and CG fall into age groups in which change in biological status is due to physiological changes. One of the factors contributing to the development of PSW is the patients' advanced age. The skin of elderly patients is easily injured. There is also a lack of adequate stress response regarding gene regulation of stress-related proteins [16,17,18]. There are numerous changes in the cellular and molecular characteristics of mature skin, which inhibit the wound healing process [19,20]. The normal wound healing process in elderly patients is disrupted by delayed cell proliferation, changes in production and composition of the extracellular matrix, and different responses to growth factors. They, alone or in combination, may contribute to a rise in incidence and duration of wounds in elderly patients [16].

The PRP, however, is not a panacea. The modern treatment of acute skin wounds and especially contaminated wounds does not include only wounds debridement but also other therapeutic methods. We switch to alternative therapies such as various skin flaps, different medications, electrical stimulation, mechanical stimulation-vacuum aspiration, cell-based therapy (epithelial stem cells), gene therapy, or hyperbaric oxygenation, in the event of a poor

outcome of PSW treatment with PRP [21,22,23].

This research was conducted on a limited number of patients with acute skin wounds, treated within the research period at the Clinic of Orthopedics and Traumatology, after signing informed consent. Patients with problematic skin wounds might be treated with PRP in other surgical healthcare facilities, which is to be part of future research.

Conclusions

The use of activated platelets from PRP for treating PSW is a preventive and therapeutic method that dramatically supports the tissue-building process. The physical recovery and normal wound healing process are enhanced by delivering necessary growth factors, which play a major role. ASW often turn into hard-to-heal and chronic wounds, and we recommend preventive use of PRP in patients at risk.

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