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Management of Leg Ulcers Using Combined PRP Therapy on a Nanofiber Carrier: Results of a Pilot Study

Léčení bércového vředu s použitím kombinované terapie PRP na nanovláknovém nosiči: výsledky pilotní studie

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ABSTRACT

PURPOSE OF THE STUDY
Population aging is connected with an increased incidence of chronic diseases. A common related problem is chronic skin ulcers, which, while not life-threatening, can significantly decrease the quality of the patient's life. The present study aims to evaluate new materials and methods to improve and accelerate the treatment of leg ulcers.

MATERIAL AND METHODS
Twenty-five patients with chronic ulcers treated using autologous growth factors applied on a nanofiber carrier were included in the cohort. The control group consisted of 15 patients treated using standard moist wound therapy. The surface area of the ulcer was measured on the 0th, 14th, 28th, 56th, 84th, 112th, 140th, 140th, and 168th day of treatment. Ulcer depth was measured on the 0th, 5th, 28th, 84th, and 168th day of treatment. Results were statistically processed and evaluated.

RESULTS
During the study, the defect area decreased in both the control and experimental group. Statistically significantly better results were observed in the experimental group relative to the progress of ulcer depth. The experimental group also had more healed ulcers.

DISCUSSION
Moistness is necessary for chronic wounds to heal; it is needed to ensure optimal cell growth, angiogenesis, and fibrinolysis. Wounds can be treated using non-active dressings with high absorption qualities; however, these do not guarantee optimal conditions for healing, or wounds can be treated with an interactive dressing that interacts with the wound surface. The third option for treatment is the use of bioactive materials that adhere to the wound and participate directly in the individual stages of healing.

CONCLUSIONS
The study found that autologous growth factors had statistically significant effects on the treatment of chronic ulcers. The authors believe that this method can accelerate the healing of primary post-injury or secondary postoperative wounds of lower leg soft tissues.

Key words: trophic ulcer, autologous growth factors, microangiopathy, polyneuropathy, diabetes mellitus.

INTRODUCTION

Population aging is one of the causes of the increased number of chronic diseases (8), which includes an increased number of patients with chronic ulcers of soft tissues, mainly in the lower legs, and often resulting from microangiopathy or peripheral neuropathy (10). Treatment of soft-tissue ulcers on a trophic basis is complicated and represents, at present, serious medical as well as social and economic problems. We carried out a pilot study focused on monitoring the healing efficiency of platelet-rich plasma (PRP) placed on a nanofiber carrier to treat chronic soft tissue ulcers of the lower legs. PRP is thought to promote healing. This study presents the results of our study.

MATERIAL AND METHODS

Ulcer dressings for both the experimental and control group were changed at three or four-day intervals.

Preparation of dressing material for the experimental group

Preparation of the PRP concentrate involved the collection of 60 ml of the patient's venous blood. PRP was separated immediately after sample collection. Separation

used gravitational centrifugation in a RegenLab 80 centrifuge at −2 °C (RegenLab SA, Le Mont-sur-Lausanne, Switzerland), 3200 rpm, for 15 minutes in a special closed system. Afterward, individual components were separated, i.e., platelet-rich plasma (PRP), platelet-poor plasma (PPP), and red blood cells (RBC). The final required quantity of 6 ml of PRP (10% of the original sample) was collected using a special single-use syringe Artrex ACP Double Syringe (Artrex, Inc., Naples, Florida, USA).

Another step involved applying the collected PRP onto a VUP fabric mesh (VUP Medical, a.s., Brno, Czech Republic) coated with polymer nanofibers made from polycaprolactone (PLC). The surface area of collected PRP applied to the carrier always corresponded to the surface area of the defect. PRP was applied to the fabric mesh as drops dispensed from a pipette. The ratio between PRP and nanofiber carrier was 1 ml of PRP onto 1 cm^2 of the carrier.

The collected liquid lyophilisate suspension of the patient's PRP contained autologous growth factors (especially the platelet-derived growth factor – PDGF, fibroblast growth factor – FGF, and transforming growth factor-beta – TGF). Ensuring standardized application of the PRP onto the carrier and verifying all the above-mentioned autologous growth factors in the concentrate was a part of a grant from Technologická agentura České republiky (TA02011402), describing these methods in detail (13).

Dressing material for the control group

Control group ulcers received standard "moist" treatment with antimicrobial foam dressings from Mepilex Ag (Mölnlyke Health Care AB, Göteborg, Sweden).

Formation of the experimental and control group

The study inclusion criteria were

(1) at least five months of previous conventional treatment of a venous, arterial, diabetic, or post-traumatic ulcer,

(2) an ulcer surface area less than 40 cm^2, and the absence of a circular character,

(3) older than 18 years,

(4) diabetics needed to long-term glycemia under 10 mmol/l, and glycated hemoglobin under 45 mmol/mol,

(5) no immunosuppressive therapy,

(6) basic blood clotting factors (INR, aPTT) within normal limits, and

(7) signed informed consent.

Randomized selection created an experimental group of 25 patients and a control group of 15 patients.

The exclusion criterion was a failure to meet any of the seven inclusion conditions.

Clinical monitoring

The surface area of ulcers was measured using an mm ruler on the 0^{th}, 14^{th}, 28^{th}, 56^{th}, 84^{th}, 112^{th}, 140^{th}, and 168^{th} day of treatment. The depth of the ulcers was measured using a calibrated depth probe on the 0^{th}, 5^{th}, 28^{th}, 84^{th}, and 168^{th} day of treatment. The values obtained were recorded in MS Excel tables (Microsoft Corporation, Redmond, Washington, USA)

Statistical evaluation

The results were processed using standard statistical methods. The Wilcoxon two-sample test was used to compare the distribution of parameters in both groups. Frequency and difference in frequency of categorical variables in individual groups were evaluated using the Chi-squared test and Fisher's exact test. Time-based progress of surface area and depth was tested using the nonparametric Wilcoxon test for paired samples, Friedman's ANOVA test, and the parametric "repeated" ANOVA test. The dependency of the parameters with respect to a non-gaussian distribution of variables was evaluated using the Spearman correlation coefficient. All calculations were done using SAS software (SAS Institute, Inc., Cary, North Carolina, USA) and STATISTICA 9.0 software (StatSoft, Inc., Tulsa, Oklahoma, USA); p-values were set at 0.05.

RESULTS

The experimental group included 25 patients, 14 women and 11 men, with an average age of 62.2 years (28–79). The control group included 15 patients, nine women and six men, with an average age of 59.7 years (38–82).

The change in the surface area and depth of ulcers in both groups of patients are presented in Tables 1 and 2. The surface area and depth of ulcers improved in both groups of patients during the study. It is important to note that at time zero, no statistical difference was

Table 1. Surface area of ulcers in patients in the experimental and control group

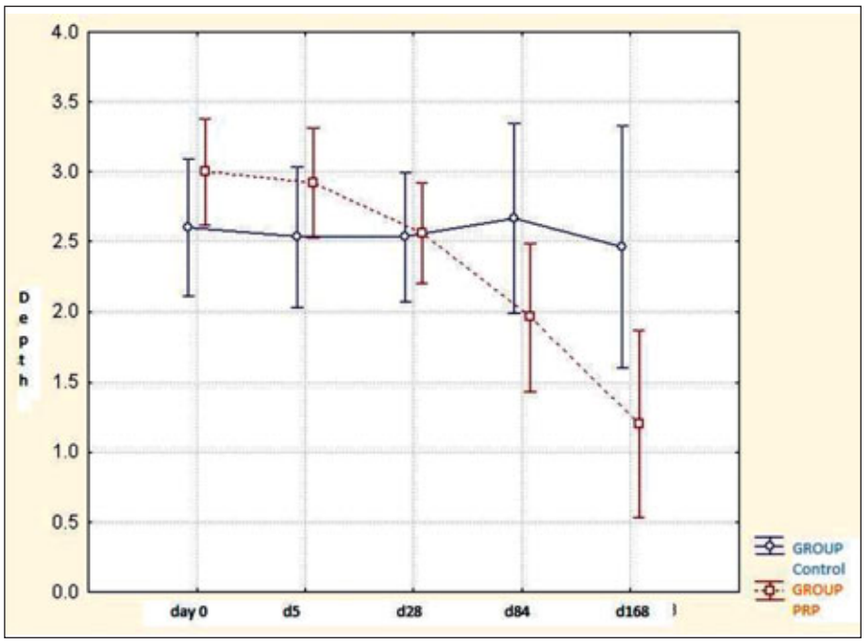
Day	Experimental group		Control group	
	Minimal (cm^2)	Maximal (cm^2)	Minimal (cm^2)	Maximal (cm^2)
Zero	3.0	30.7	1.1	18.7
14^{th}	2.6	31.2	0.9	18.7
28^{th}	2.2	31.2	0.9	18.5
56^{th}	1.8	30.6	0.0	17.4
84^{th}	0.0	30.6	0.0	17.2
112^{th}	0.0	28.9	0.0	17.1
140^{th}	0.0	27.9	0.0	16.5
168^{th}	0.0	27.2	0.0	16.2

Table 2. Depth of ulcers in patients in the experimental and control group

Day	Experimental group		Control group	
	Minimal (mm)	Maximal (mm)	Minimal (mm)	Maximal (mm)
Zero	1	5	1	4
5^{th}	1	5	1	4
28^{th}	1	4	1	5
84^{th}	0	4	0	6
168^{th}	0	4	0	7

recorded between groups relative to surface area (p = 0.3916) or depth (p = 0.2309), although the maximal surface area of ulcers in the control group was markedly smaller than in the experimental group (see Table 1). At the end of monitoring, there were relatively more healed ulcers in the experimental group (8 of 25) than in the control group (2 of 15); however, the difference was not statistically significant (p = 0.2686).

When comparing ulcer surface area progress between the 0th and 168th day, a statistically significant decrease in surface area was observed in both groups of patients (1) treated with growth factors (p = 0.0017) and (2) treated with the traditional "moist" method (p = 0.0189). This result means that both methods were effective over the course of the study (see Table 1).



Graph 1. Graph of the depth of ulcers in patients in the experimental and control group.

When comparing the progress of ulcer depth between the 0th and 168th day, a statistical difference between groups was found (p = 0.0196); patients treated with growth-factor responded better (Fig. 1) than patients treated with the "moist" method (Fig. 2). Moreover, some defects in the control group were seen to deepen over the course of treatment, which was not seen in the experimental group (see Table 2, Graph 1).

DISCUSSION

Treatment of chronic ulcers requires keeping the wounds moist. A moist environment promotes cell growth, angiogenesis, and fibrinolysis. If the wound dries out, extensive amounts of necrotic tissue can start to develop, which worsens the healing process and results in poor functional and cosmetic outcomes (4, 15).

Adequate wound hygiene is also critically important for good healing. Dressings used to cover wounds must act as a barrier against bacterial colonization of the wound and thus prevent infection (9, 11).

In chronic wound management, three methods are distinguished: non-active, interactive, and (bio)active (1).

For non-active or conventional treatment, highly absorbent dressings are used. They are made from cotton, synthetic fibers, or multiple layers of these materials. To keep the wound environment moist, dressings must

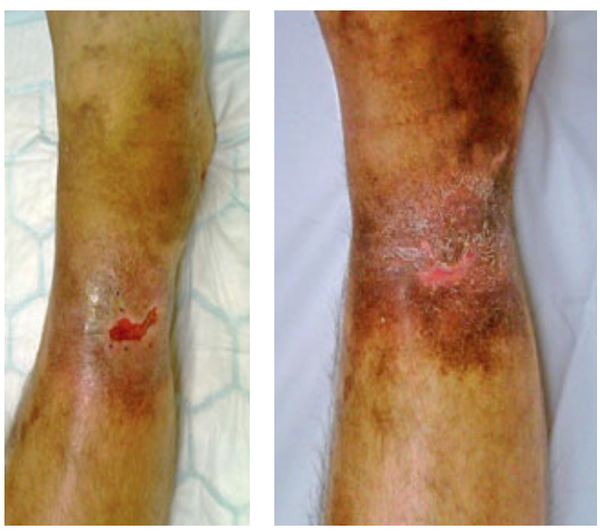


Fig. 1. An example of an ulcer successfully treated with platelet-rich plasma (PRP).

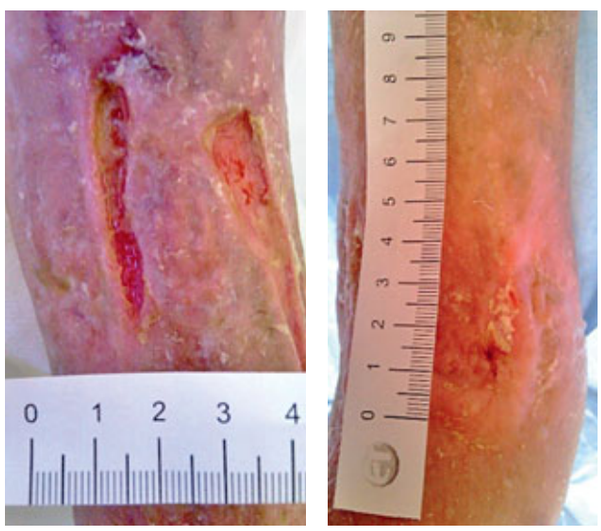


Fig. 2. An example of an ulcer successfully treated with the classic "moist" method.

be soaked in physiological solution and then covered with a waterproofing material. A key advantage of these dressings is their high absorptive quality and low price, but the disadvantage is the risk of them drying out the wound and adhesion of the dressing to the wound. When these dressings are changed, the newly formed granulation tissue is repeatedly traumatized, making the dressing change painful for patients.

Interactive treatment of moist therapy uses dressings made from special materials that interact with the wound. These types of dressings must meet several requirements:

– maintenance of a moist environment and suitable pH (pH ≈ 5.5),
– absorption of toxic components from bacterial degradation,
– protection against secondary infection,
– non-traumatizing and painless dressing changes.

These dressings promote optimal conditions for wound healing and are used in different stages of healing. Dressing changes are less painful, and dressings can be left in place for several days (2, 4, 15).

Currently, there are about 300 different types of dressings on the market, many of which were analyzed as 42 controlled randomized studies presented in the Cochrane Review. None of the studies provided evidence that better healing was achieved using hydrocolloids, alginates, foam dressings, or hydrogels. Similar results were achieved using dressings that simply maintained a moist environment (1, 5).

New trends in chronic wound healing are based on **bioactive dressings** that directly adhere to the wound and directly participate in the healing processes. They often remain in the wound, or they form a durable wound cover after healing. These active dressings should be reserved for use in specialist clinics and specific indications (3 ,7, 12).

The method described in this study offers hope to patients with skin primarily ulcers resulting from injury, or secondarily, in postoperative wounds in the region of heavy post-traumatic contusions of soft tissues accompanying fractures, because regeneration of soft tissues using the described method before definite cover with dermoepidermal skin substitutes can be faster and safer (6, 14).

In our clinical study, we examined a new wound cover that promotes healing with autologous growth factors. The development of the wound cover used in this study was carried out under a grant from the Technological Agency of the Czech Republic (TA02011402) in 2012–2013, and the primary recipient was PrimeCell Therapeutics, a.s. An interval of seven years between the completion of the grant and publication of outcomes was a requirement of the grant holder and concerned the protection of the "carrier model" and the method of placing growth factors onto the carrier.

CONCLUSIONS

The results of our study showed statistically significantly better healing of trophic leg ulcers treated with active autologous growth factors on a nanofiber carrier compared to the more traditional "moist" method of treatment. Although we believe that this method can be used widely, possible treatment of other soft-tissue defects post-injury or post-surgery using autologous growth factors must be demonstrated with targeted prospective studies.

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