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Effect of platelet-rich plasma microinjections on the healing and histostructure of extended fasciocutaneous flaps based on axial blood supply

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Purpose To reveal a possible effect of platelet-rich plasma (PRP) on healing and histostructure of extended fasciocutaneous flaps with axial blood supply **Materials and methods** A flap based on the superficial inferior epigastric artery (SIEA) and extended to the cranial side according to a template with an area of 18 cm^2 was produced in 24 rats. After one-and-half hour femoral artery clipping for temporary ischemia, it was re-perfused and re-planted at the site of SIEA origination. After the operation, six microinjections of platelet-rich plasma (0.1 ml) were performed subdermally that were equally distributed on the flap. Comparison group had injections of saline in the similar number and zones. Control group had flaps without injections. Reference norm sites were skin contralateral areas. Twelve days after the operation, the animals are euthanized. Methods of the study were computer planimetry, histomorphometry, and immunohistochemistry. **Results** When compared with the control and placebo groups, a more than ten-fold reduction in the area of epidermal defects that were associated with delayed wound healing and necrotic complications was achieved. Polymorphic cell infiltration of the dermis decreased 1.7 times under the conditions of PRP microinjections when compared with the control group. Numerical density of capillaries in the subpapillary plexus doubled, and the number of dermal papillae increased 1.5 times. **Discussion** Enhancement of capillarization of the superficial skin structures proceeded with inhibition of inflammatory reactions and was probably caused by direct action of growth factors of platelets on endothelial cells. **Conclusio**n PRP microinjections are able to effectively prevent partial necrosis of the extended fasciocutaneous flap based on axial blood supply, accelerate epithelization of the wound along the flap perimeter, and maintain the normal plasticity of the epidermis. There was no significant effect of the drug on the state of deep flap structures (subcutaneous fat and skin appendages).

Keywords: vascularized fasciocutaneous flap, platelet-rich plasma, angiogenesis

INTRODUCTION

Flaps based on axial blood supply have been widely used in reconstructive surgery, traumatology and orthopedics [1]. When they are transplanted or transferred, complications such as partial necrosis occur in 7–20 % and in 20–30 % of cases, respectively [2]. The main causes of necrosis are damage to the vascular pedicle, inadequate vascularization of remote to the pedicle areas, microthrombosis of blood supply vessels, and activation of proinflammatory mediators. To develop the methods for preventive treatment of these complications, hyperbaric oxygenation, ischemic preconditioning, pharmacological support, and the use of biomolecular regulators such as growth factors have been investigated [3, 4]. Preparations of platelet-rich plasma (PRP) contain a number of growth factors and cytokines, which play an important role in wound healing and engraftment. Among them is platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), type I insulin-like growth factor (IGF-I), platelet-derived endothelial growth factor (PDEGF), platelet-derived angiogenesis factor (PDAF), and vascular endothelial growth factor (VEGF) [5]. The possibility to prepare PRP from autologous blood ensures the safety and economy of the method. Reviews on the use of PRP in plastic surgery reveal its positive effect on wound healing, survival of fat and bone grafts. In addition to regeneration stimulation, a decrease in edema, ecchymosis, and pain was noted [6]. Improved survival of skin flaps based on a random blood supply under the influence of PRP injections, mediated by an increased expression of matrix RNAs of proangiogenic genes, was shown in several publications [7]. It is not known how PRP influences the survival of fasciocutanous flaps with axial blood supply, including in cases where their area exceeds the territory of the blood supply artery.

Our **aim** was to reveal a possible effect of platelet-rich plasma on healing and histostructure of extended fasciocutaneous flaps based on axial blood supply.

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MATERIAL AND METHODS

The experiments were carried out on 24 male Wistar rats in the age of 8 to12 months and having weight from 380 to 560 g. The requirements of the order of the Ministry of Health of the Russian Federation No. 267 of 19.06.2003 were followed. Under general anesthesia and aseptic conditions of the operating room, a template that measured 3 × 6 cm was used to elevate and reposition the fasciocutaneous flap with the axial type of blood supply based on a. epigastrica inferior superficialis (SIEA). The incisions were made with a scalpel; the medial one was 0.5 cm lateral to the white line of the abdomen; the cranial one was along the lower edge of the rib arc; the caudal incision was parallel to the cranial one and distanced by 6 cm, and the lateral one was parallel to the medial incision and 3 cm away from it. The feeding pedicle (SIEA, accompanying veins and nerves) was isolated with surrounding tissues. The cranial portion of the flap was related to the a. thoracica lateralis angiosom, and the flap was not perfused with it (**Fig. 1 a, b**). Simulation of a 1.5-hour ischemia relevant to the clinical transplantation and transposition of flaps was performed by clipping the feeding artery for 90 minutes. The rats were randomly assigned to 3 groups: a negative control group without any therapeutic effects on the process (n = 10); a comparison (placebo) group that had six 0.1-ml microinjections of saline solution, evenly distributed (**Fig. 1 c**) over the the flap surface (n = 8), and an experimental group had a similar number and sites of PRP microinjections (n = 6).

To obtain autologous PRP, rats of the experimental group were injected 2 ml of saline subcutaneously into the withers area half an hour before the operation. Next, 2 ml of blood was taken from the tail veins into vacutainers with 3.2 % sodium citrate. The blood was centrifuged at 1500 r/m for 15 minutes at room temperature. The supernatant plasma layers in the amount of 0.6 ml were taken from the vacutainer with the needle into an insulin syringe for injections.

Twelve days after the operation, the rats were euthanized. The degree of flap contraction (% of the template area) was determined with computer-aided planimetry in scaled full-color digital images of the cutaneous surface of the flaps. The state of the flaps was assessed visually and stereologically (results of calculating the portion (%) of the epidermal defects in the flap using dot-counting planimetry).

Standard fragments of flaps in the experimental and control group animals were excised for histological examination. A reference norm (positive control) was samples of the contralateral skin areas. Paraffin sections that were 5–7 microns thick were stained by Van Gieson and hematoxylin-eosin. A part of them was studied on adhesive glasses coated with poly-L-lysine; the expression of ki-67 (proliferation marker) and vWF (endotheliocyte marker) was determined by the protocol of the manufacturing company using the Novolink Polymer imaging systems (Novocastra, UK). The preparations were studied with a stereomicroscope AxioScope.A1 supplied with a digital camera AxioCam (Carl Zeiss MicroImaging GmbH, Germany). Histomorphometry was performed using the computer program VideoTesT Master Morphology 4.0. Statistical processing used the software program Attestat 9.3.1 (developed be I.P. Gaidyshev) with application of Mann-Whitney criteria and interval randomization criterion.

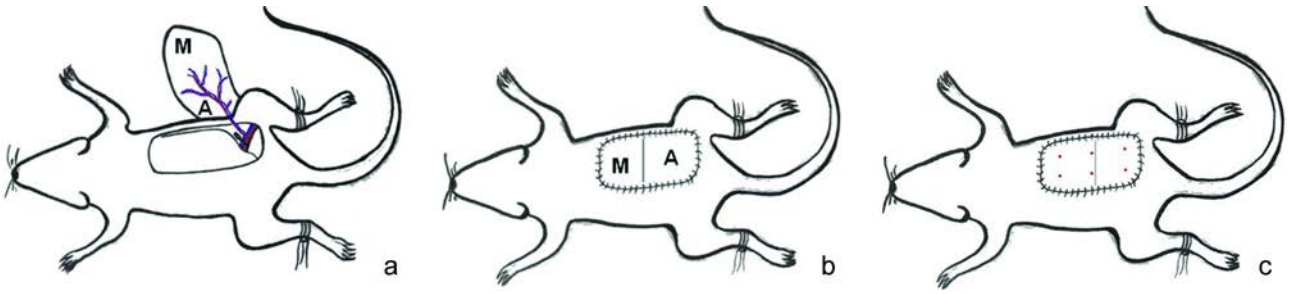

Fig. 1 Schemes of the experiment: **a** clipping of the artery that feeds the extended flap with axial blood supply, **b** angiosomal (A) and interangiosomal (M) parts of the flap; **c** topography of subdermal PRP microinjections in the experimental group and saline injection in the comparison group

RESULTS

The number of platelets in the PRP samples ranged from 900,000 to 1,174,000 / μl (on average, their concentration exceeded twice the amount in the native peripheral blood, which varied from 475,000 to 615,000 / μl).

In the first week after the operation, the experimental group differed from the control and placebo groups. Its animals had a less pronounced postoperative swelling and inflammation, a more rapid recovery of the orientation reflex and motor activity.

By day 12 after the operation , all the animals in the

experimental group showed slight manifestations of incomplete wound healing (**Table 1**) but necrotic complications were observed in none of them, which significantly distinguished them from the groups without pharmacological effects ($p < 0.05$ by Barnard test).

Epidermal defects in the flap averaged 13.72 ± 9.17 % in the control group, 11.33 ± 3.68 % in the placebo group, and 0.95 ± 0.25 % in the PRP group. The difference between control and placebo group for this parameter did not reach the level of statistical significance. The experimental group was significantly different from the control and placebo groups ($p < 0.05$).

Examples of the outcomes of the replantation of the extended SIEA murine flap on day 12 after an hour and a half of ischemia-reperfusion in the comparison group and in the experimental group are shown in Figures 2 and 3. Visual intergroup differences of wound healing in the rats revealed that in rats with delayed healing, non-healed spaces between the stitches prevailed (**Fig. 2 c**) in the control and in the comparison groups while almost a complete healing of the interstitching spaces was seen in the experimental group, with only ligature canals unhealed (**Fig. 3 b**).

The micro-preparations of animals of the experimental group were visually distinguished from the control by signs of active epithelialization of the wound along the perimeter of the flap (**Fig. 4 a**), good preservation of the papillae and capillaries of the dermis, moderate post-ischemic changes in the hypodermis (**Fig. 4 b**). Variability in the size of hypodermal adipocytes, areas of its polymorphic cell infiltration and fibrotic substitution were more pronounced in the control group. The state of the epidermis varied in different parts of the flap (**Fig. 4 c**), both in the experimental and control groups, which was determined by the state of the dermis.

Table 1

Outcomes of extended flap replantation at 12 days post-surgery

Criterion/group	Complete survival and wound healing	Defect of healing and survival		
		Incomplete wound healing	+ rim necrosis (epidermolysis)	+ necrosis of interangiosomal zone
PRP group (n = 6)	0	6	0	0
Control group (n = 10)	1	3	5	1
Placebo group (n = 8)	0	3	5	2

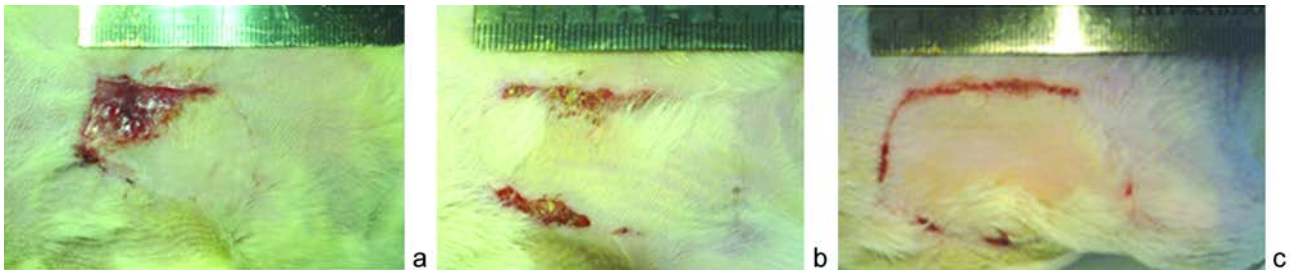

Fig. 2 Variants of the outcomes of flap replantation in the comparison group (saline injections): **a** necrosis of the interangiosomal zone; **b** severe marginal necrosis; **c** incomplete wound healing of spaces between the stitches

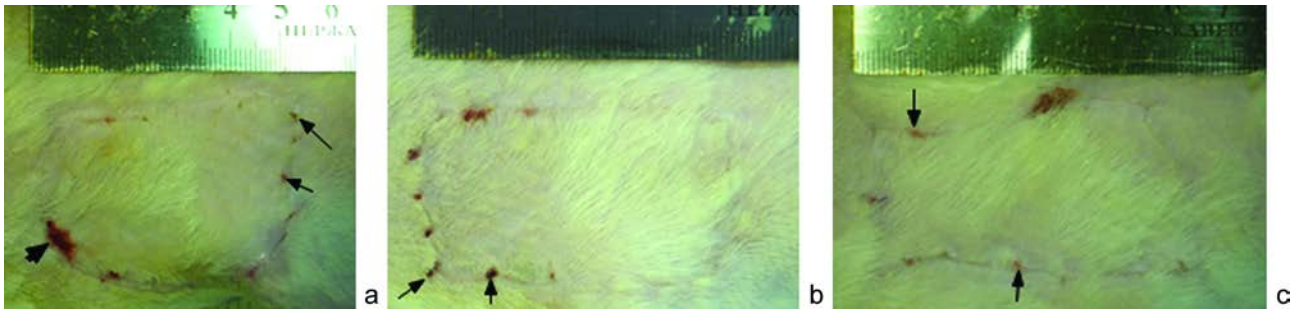

Fig. 3 Variants of the outcomes of flap replantation in the experimental (PRP injection) group: **a** site of incomplete wound healing in the suture space (thick arrow); **a, b, c** incomplete wound healing in the ligature canals (thin arrows)

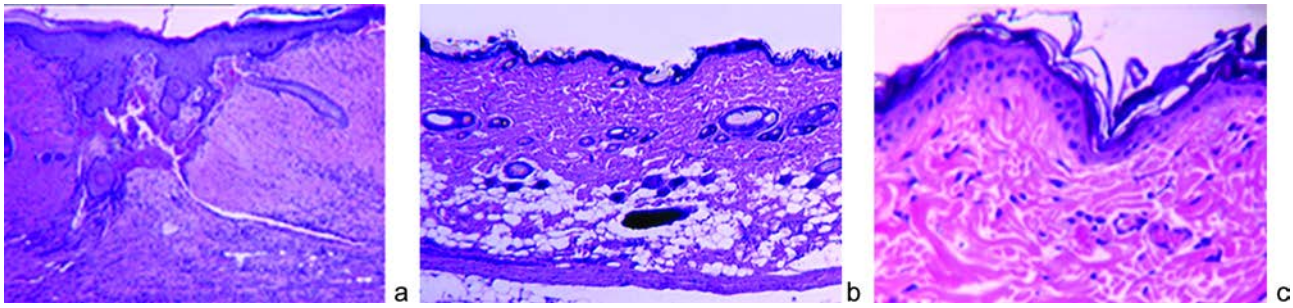

Fig. 4 Fragments of paraffin sections of survived flaps in the experimental group: **a** wound epithelization along the flap perimeter**; b** mild postischemic changes in hypodermis**; c** changes in the expression of the epidermis layers in various sites of the flap slice

The preservation of the papillary vascular plexus and, accordingly, the expressiveness of the papillae of the dermis predetermined the normoplastic state of the epidermis, which was characterized by the presence of five to six cell layers and the localization of mitosis figures and ki-67-positive cells in the basal layer. In the sites of atrophy of the dermal papillae, the epidermis became thinned due to a decrease in the number of cell layers and cell sizes. In the basal layer, there was not only a decrease in the number of proliferating cells but also pycnotic nuclei appeared. In the areas of fibrotic post-necrotic replacement of the dermis, the epidermis thickened (hyperplasia) due to an increase in the number of spiny, and sometimes granular cells (post-proliferation acanthosis and hypergranulosis). The histograms of frequency distribution of epidermis thickness measurements (**Fig. 5**) revealed that the control group was characterized by a large representation of the hypotrophic epidermis (first class, 40 % versus 21 % in the experimental group). The normotrophic epidermis was more frequently seen in the experimental group (second class, 64 % in the experimental group and only 41 % in the control one). The total portion of hypertrophic epidermis in the experimental group was 15 % versus 19 % in the control one. Moreover, in the control group, 8 % of the measurements fall on classes where the thickness of the epidermis was two to three times higher than normal.

When compared with the controls, the experimental group showed that the average thickness of the epidermis was increased by 8.7 %, the number of dermal papillae in 1 mm of the slice length was greater by 56.5 %; the numeral density of the capillaries of the dermis was bigger by 94.9 %, the hypodermis was thicker by 38. 5 %, while the cellularity of the dermis was reduced by 40.3 % (**Table 2**).

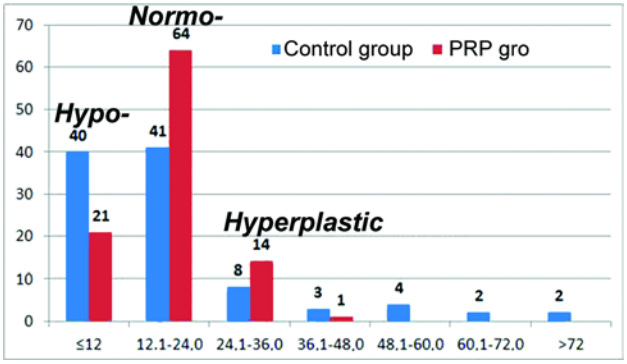

Fig. 5 Frequency distribution of epidermal thickness measurements in the control and experimental group (PRP): abscissa – size ranges (μm), ordinate – frequency of ranges (%)

Table 2

Histomorphometric characteristics of flap skin

Group	Parameter (M ± m)		
	Negative control group	PRP group	Reference norm group
Thickness (mcm)			
Epidermis	16.12 ± 0.61	17.53 ± 0.24*!	22.14 ± 1.90
Dermis	543.43 ± 7.38	533.36 ± 6.58	527.08 ± 19.22
Hypodermis	200.69 ± 3.40	278.01 ± 4.63*!	351.03 ± 42.82
Number			
Dermal papillae (in 1 mm of slice length)	5.89 ± 0.37	9.22 ± 0.64*!	12.82 ± 0.40
Numeric density (in mm^2)			
Dermal cells	2112.25 ± 354.05	1261.11 ± 66.81*	1285.57 ± 110.93
Skin appendages	16.69±1.95	18.80 ± 2.67	37.54 ± 5.70
Dermal capillaries	34.87±5.13	67.95 ± 7.03*!	32.53 ± 2.69
Hypodermal capillaries	111.33±9.18	108.73 ± 7.23	113.85 ± 9.24

Note: ***** differences between the experimental and control groups; **!** differences between the experimental and the reference norm are reliable by the Wilcoxon test for independent samples ($p < 0.05$).

DISCUSSION

As the analysis of the available literature shows, most studies of the PRP effects that were performed on animals used a two-stage centrifugation protocol for its preparation. The platelet content in the preparation was 2.46–6.9 times higher than in the blood [6]. In clinical trials, a single-stage centrifugation protocol is frequently used in a conventional laboratory centrifuge [6, 8]. It excludes expenditures for acquiring specialized equipment and supplies but at the same time allows for an effective platelet concentration in the PRP of about 1 million per μl. A lower concentration provides suboptimal effects, and a greater concentration may lead to inhibition of regenerative processes [9].

Our study used a one-stage protocol of centrifugation. The concentration of platelets in the PRP was twice as high as their concentration in blood. Compared with untreated control and placebo groups, more than a tenfold decrease in the area of epidermal defects associated with delayed wound healing and necrotic complications was achieved. The histological study revealed that polymorphic cell infiltration of the dermis under the conditions of PRP microinjections decreased by 1.7 times when compared with the controls. However, the numerical density of the capillaries of the subpapillary plexus doubled, and the number of papillae of the dermis increased by 1.5 times. It indicates an angiogenic effect, unrelated with inflammation. A similar decrease in inflammatory cell infiltration and an angiogenic effect were observed in the flap based on a random blood supply in the conditions of using a rich versus poor platelet-rich plasma [7].

CONCLUSIONS

Microinjections of platelet-rich plasma are able to effectively prevent partial necrosis of the extended fasciocutaneous flap based on axial blood supply, accelerate epithelization of the wound along the flap perimeter, and maintain the normal plasticity of the epidermis. There was no significant effect of the preparation on the state of deep flap structures (subcutaneous fat and skin appendages).

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