

Early postoperative changes in hematological, erythrocyte aggregation and blood coagulation parameters after unilateral implantation of polytetrafluoroethylene vascular graft in the femoral artery of beagle dogs¹

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ABSTRACT

PURPOSE: The failure of small-caliber vascular grafts still means a serious problem. Concerning the early postoperative complications we aimed to investigate the hemostaseological and hemorheological aspects of this issue in a canine model.

METHODS: In the Control group only anesthesia was induced. In the Grafted group under general anesthesia a 3.5-cm segment was resected unilaterally from the femoral artery and replaced with a PTFE graft (diameter: 3 mm). On the 1st-3rd-5th-7th and 14th postoperative days the skin temperature of both hind limbs was measured, and blood sampling occurred for hematological, hemostaseological and hemorheological tests.

RESULTS: The skin temperature of the operated versus intact limbs did not differ. In the Grafted group leukocyte count was elevated by the 1st postoperative day, while platelet count increased over the entire follow-up period. Fibrinogen concentration rose on the 1st-5th days, activated partial thromboplastin time increased on the 3rd-7th days. Erythrocyte aggregation was enhanced significantly on the 1st-5th days. In specimens taken on the 14th day, histologically we found matured thrombus narrowing the graft lumen.

CONCLUSIONS: Small-caliber PTFE graft implantation into the femoral artery caused significant changes in several hemostaseological and hemorheological parameters. However, better clarifying the factors leading to early thrombosis of these grafts needs further studies.

Key words: Vascular Grafting. Graft Occlusion, Vascular. Erythrocyte Aggregation. Blood Coagulation. Models, Animal. Dogs.

Introduction

Open surgical procedures, such as bypass operations still have an important role in today's vascular surgery¹⁻⁵. For the bypass implants, the patient's superficial vein or artificial vascular graft is used, what can be made of polyethylene terephthalate (PTE, Dacron®) or polytetrafluoroethylene (PTFE). However, bypass surgeries made with the patients' own veins are statistically proved to have twice as more patency rates than the artificial grafts⁶⁻⁸.

During the last 10-15 years a reduction in the number of infrainguinal bypass operations can be observed^{4,5}. The reasons are not well known but risk factor reduction, modification, early referral and the improvement of the endovascular techniques (even for TASC C,D lesions) could be a reason for that. However, by surgeons' opinion the open surgery remains the first choice for TASC D lesions^{4,6}. The greater saphenous vein (GSV) is the gold standard for infrainguinal bypasses at any level. If the GSV is of poor quality or has been removed (for example CABG or varicectomy was performed), the use of the contralateral GSV has to be considered, rather than arm veins, which have lower patency rates. In case of the surgeries above the knee the implantation of an artificial graft is chosen since with progression of the underlying disease it might be the necessary to do surgery below the knee, where veins are preferred for the bypass^{4,6}.

In absence of vein a prosthetic graft should be used. In this case a vein cuff recommended at the distal anastomosis. The Joint Vascular Research Group RCT of Miller vein cuff versus non-cuff for femoro-distal PTFE grafts demonstrated significantly higher patency rates for prosthetic graft with vein segment at P III level. The number of prosthetic grafts, used for intermittent claudication/critical limb ischemia has fallen. Poor patency rate and the concerns about graft infections are the main reasons for that^{1-5,9-13}.

The first couple of postoperative days are always critical. The problem of early thrombosis in case of small-diameter artificial vascular conduits still means a serious question in vascular surgery⁹. The wall of the artificial graft is more rigid, the arterial three-phased blood flow pattern cannot be observed. After the implantation of an artificial vascular graft, we may see several early and late complications. Early: suture insufficiency, hemorrhage, graft infection, wound infection, vascular and nerve injuries, early obstruction of the graft. Late: pseudoaneurysm formation due to suture insufficiency, obstruction of the graft, stenosis caused by neointima formation

or occlusion, graft infection⁹⁻¹³. The blood flow characteristics change at the anastomoses, the cells may suffer mechanical injury - here the formation of deposits usually leads to another operation^{10,12-14}. Although it is not completely clarified that from which point the flow properties of the altered vascular geometry can lead to thrombotic complications later.

The aim our study was to investigate the effect of the presence of unilaterally implanted PTFE graft into the femoral artery in a canine model, focusing on the early postoperative changes in general haematological parameters, red blood cell aggregation and general blood coagulation parameters.

Methods

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 20/2011. UD CAR), in accordance with the relevant Hungarian Animal Protection Act (Law XVIII/1998) and EU directives.

All the surgical interventions were performed under general anesthesia (10 mg/kg ketamin + 0.1 mg/kg xylazin, i.m.)

In the *Grafted group* (n=5): the left femoral artery was gently exposed and atraumatically clamped proximally and distally. A 3.5 cm long segment was excised and replaced with a polytetrafluoroethylene (PTFE) graft (diameter = 3 mm, Atrium Co.) of the same length (3.52 ± 0.48 cm) using end-to-end anastomoses (continuously suture line, 6/0 polypropylene). The time for the necessary clamping of the vessel was 25 ± 3.1 minutes. In the Control group (n=4) only anesthesia was induced and for a 2-hour-period animals were laid on the operative table under the same circumstances as in the Grafted group.

Animals received 1000 IU sodium-heparin intravenously at the beginning of the operation. Postoperatively, on the 1st and 3rd days 500 IU Clexan was given subcutaneously. Intramuscularly 50 µg/kg sodium-metamizole (Algopyrin 1 g/2 ml ampule) was administered for analgesia just after the operation and on the 1st postoperative day.

Via puncturing the cephalic vein, blood samples were collected before the operation, on the 1st, 3rd, 5th, 7th and on the 14th postoperative days using Vacutainer® system.

Laboratory tests

For testing hematological parameters we used a Sysmex F-800 microcell counter (TOA Medical Electronics Co. Ltd.,

Japan). In this study red blood cell count (RBC [$\times 10^6/\mu\text{l}$]), white blood cell count (WBC [$\times 10^3/\mu\text{l}$]), monocyte-granulocyte ratio and platelet count (Plt [$\times 10^3/\mu\text{l}$]) were analyzed (anticoagulant: 1.5 mg/ml $\text{K}_3\text{-EDTA}$).

Blood coagulation time parameters, such as prothrombin time (PT [s]), activated partial thromboplastin time (APTT [s]), as well as fibrinogen concentration (Fbg [g/dl]) were determined by a Sysmex CA-500 automated coagulometer (TOA Medical Electronics Co. Ltd., Japan) (anticoagulant: 0.129 M sodium-citrate).

Red blood cell aggregation has been tested by two methods: the light-transmittance based Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) and the laser diffraction based LoRRca ektacytometer (Mechatronics BV, The Netherlands) (anticoagulant: 1.5 mg/ml $\text{K}_3\text{-EDTA}$).

Histological investigation

On the 14th postoperative day under general anesthesia the grafts with intact vessel parts over the anastomoses and the contralateral, intact femoral arteries were excised. The specimens were fixed in 10% formalin before the regular dehydration and embedding protocol, and microtomed into 5 μm sections. Standard hematoxylin-eosin (H&E) staining, as well as immunohistochemistry for CD31 was carried out on the specimens.

Statistical analysis

Data are presented as means \pm standard deviation (S.D.). Although the case number was low, for inter-group comparison student t-test or Mann-Whitney RS test were used, and one-way ANOVA tests (Dunn's or Bonferroni method) were carried out for intra-group comparisons, depending on the data distribution, with a level of significance of $p < 0.05$.

Results

General postoperative observations

All experimental animals survived the operations and there was no death during the 2-week postoperative follow-up period. No surgical complication -neither early, nor late- was detected. The motion of the animals were normal during the 2-week follow-up period, there was no sign for hind limb circulatory problem. The skin temperature values of the non-operated and operated legs were identical (Figure 1).

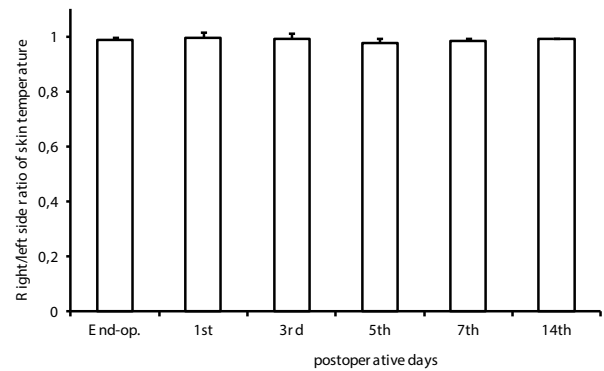


FIGURE 1 – Relative values of skin temperature: right side (non-operated limb) values to left side (operated limb) values measured at the end of the operation (End-op.) and during the postoperative follow-up period. Means \pm S.D.

Hematological parameters

Table 1 shows the blood cell count parameters. The red blood cell count slightly decreased over the 2-week follow-up period in the Control group (versus base values: $p=0.002$ on the 1st, $p=0.018$ on the 7th and $p=0.003$ on the 14th postoperative day). The Grafted group showed the same tendency (versus base values: $p=0.021$ on the 3rd, $p=0.033$ on the 5th, $p < 0.001$ on the 7th, and $p=0.038$ on the 14th day), and expressed moderately lower values compared to the Control group ($p=0.046$ on the 3rd, and $p=0.049$ on the 7th day). By the 7th day a definitive decrease in the red blood cell count was observed, being significant compared to the base values.

TABLE 1 – Changes of red blood cell count (RBC), white blood cell count (WBC) and platelet count (Plt) in the Control and Grafted groups.

Variable	Group	Base	Postoperative days				
			1 st	3 rd	5 th	7 th	14 th
RBC [$\times 10^6/\mu\text{l}$]	Control	7.48 ± 0.67	6.68 ± 0.21 *	7.24 ± 1.23	6.85 ± 0.68	6.52 ± 0.76 *	6.28 ± 0.64 *
	Grafted	6.9 ± 0.45	6.49 ± 0.54 *#	6.31 ± 0.62 *#	6.28 ± 0.72 *	5.85 ± 0.57 *#	6.38 ± 0.58 *
WBC [$\times 10^3/\mu\text{l}$]	Control	10.82 ± 1.49	14.67 ± 2.08 *	12.91 ± 1.87	12.21 ± 2.37	13.48 ± 1.34 *	11.2 ± 0.99
	Grafted	11.41 ± 1.27	22.49 ± 5.34 *#	15.46 ± 4.04 *	14.68 ± 3.1 *	13.49 ± 2.13 *	71.2 ± 2.8
Plt [$\times 10^3/\mu\text{l}$]	Control	229.5 ± 39.7	229 ± 58.7	255 ± 70.5	300.1 ± 95.1	333.1 ± 117.2	332.7 ± 100.2
	Grafted	284.9 ± 94.3	331.2 ± 152.7	400.7 ± 145.5 #	391 ± 82.1 *#	433.2 ± 77.8 *	477.9 ± 74.4 *#

Means \pm S.D., * $p < 0.05$ vs. base; # $p < 0.05$ vs. Control

White blood cell count (total leukocyte count) increased by the 1st postoperative day ($p < 0.001$ in both groups), in a larger magnitude in the Grafted group ($p = 0.002$ vs. Control). The cell count normalized in the Control group, but in the grafted it remained elevated until the end of the first postoperative week (compared to base: $p = 0.019$ on the 3rd day, $p = 0.005$ on the 5th day and $p = 0.016$ on the 7th day.) The monocyte-granulocyte ratio remained between 60-70%, except for the 5th and 7th day, when the values were $81.73 \pm 3.78\%$ and $74.3 \pm 3.98\%$, respectively.

Platelet count of the Grafted group continuously increased over the experimental period. The rise was significant from the 3rd postoperative day compared to the base values (5th day: $p = 0.015$; 7th day: $p = 0.001$; 14th day: $p < 0.001$) and versus the Control group, too (3rd day: $p = 0.03$; 5th day: $p = 0.046$; 14th day: $p = 0.003$).

Blood coagulation parameters

Changes of selected blood coagulation parameters are shown in Table 2. Prothrombin time did not show important changes, however, activated partial thromboplastin time rose twice in the Grafted group: on the 3rd day ($p = 0.048$ vs. base) and on the 7th day ($p = 0.012$ vs. base). Fibrinogen concentration rose by the 1st day and gradually decreased by the end of the follow-up period. Although it remained in physiological manner, there were significant differences (on the 1st day: $p < 0.001$ vs. base and vs. Control; on the 3rd day: $p = 0.023$ vs. base and $p = 0.002$ vs. Control; on the 5th day:

TABLE 2 – Changes of prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen concentration (Fbg) in the Control and Grafted groups.

Variable	Group	Base	Postoperative days				
			1 st	3 rd	5 th	7 th	14 th
PT [s]	Control	8.96 ± 0.15	8.3 ± 0.7	8.2 ± 0.88	7.37 ± 0.38	8.56 ± 1.49	7.33 ± 0.12 *
	Grafted	8.0 ± 1.18	7.37 ± 0.93 #	7.74 ± 1.17	7.04 ± 0.73	7.81 ± 1.23	7.55 ± 0.47
APTT [s]	Control	10.51 ± 7.67	15.68 ± 12.38	13.18 ± 9.75	9.77 ± 5.92	16.08 ± 9.61	17.11 ± 4.51
	Grafted	9.18 ± 6.93	16.24 ± 9.82	22.12 ± 6.44 *	16.72 ± 3.52	25.15 ± 10.89 *	11.43 ± 5.1
Fbg [g/dl]	Control	1.86 ± 0.08	2.18 ± 0.15	2.2 ± 0.85	1.97 ± 0.21	2.07 ± 0.25	2.07 ± 0.28
	Grafted	2.1 ± 0.44	3.3 ± 0.37 *#	2.87 ± 0.24 *#	2.58 ± 0.32 *#	2.47 ± 0.25	2.03 ± 0.21

Means ± S.D., * $p < 0.05$ vs. base; # $p < 0.05$ vs. Control

$p = 0.043$ vs. base and $p = 0.002$ vs. Control).

Changes in erythrocyte aggregation

Table 3 presents the parameters tested by the LoRRca device. The aggregation index (AI [arbitrary unit]) represent the magnitude of aggregation over the tested 120-second period, the amplitude shows the heights of the syllectogram curve compared to the initial values (Amp [au]), while t1/2 [s] shows the time point when the aggregation process reaches the half of the total aggregation index values. AI values were moderately higher in the Grafted group on the 1st, 3rd and 5th postoperative day, together with increased Amp values (at the 7th day it was significant: $p = 0.008$ vs. Control), while the t1/2 values alluded to a faster aggregation.

TABLE 3 – Changes of red blood cell aggregation parameters aggregation index (AI), amplitude (Amp) and aggregation half-time (t1/2) tested by the LoRRca in blood samples of Control and Grafted groups.

Variable	Group	Base	Postoperative days				
			1 st	3 rd	5 th	7 th	14 th
AI [au]	Control	51.35 ± 2.45	48.81 ± 9.68	50.11 ± 4.02	45.2 ± 11.34	49.49 ± 3.27	50.61 ± 5.49
	Grafted	46.27 ± 12.09	55.13 ± 12.17	57.63 ± 5.57	52.21 ± 4.04	49.51 ± 8.12	48.38 ± 5.64
Amp [au]	Control	23.82 ± 2.62	20.13 ± 5.08	24.75 ± 2.61	23.71 ± 4.05	23.94 ± 1.57	23.28 ± 2.99
	Grafted	28.71 ± 2.09	24.8 ± 5.17	29.65 ± 2.34	27.88 ± 2.07	30.42 ± 3.16 #	25.08 ± 5.01
t1/2 [s]	Control	3.8 ± 0.4	4.46 ± 2.07	4.0 ± 0.71	3.92 ± 0.64	4.07 ± 0.64	3.97 ± 0.91
	Grafted	4.11 ± 2.15	3.55 ± 2.05	2.92 ± 0.63	3.67 ± 0.62	4.26 ± 1.38	4.35 ± 1.03

Means ± S.D., # $p < 0.05$ vs. Control

Erythrocyte aggregation index *M 5 s* and *M 10 s* values (tested with Myrenne aggregometer) represent the magnitude of the aggregation at the 5th and 10th second of the process measured at stasis. In the Grafted group these values showed significant increase during the 1st postoperative week, peaking on the 1st and 3rd postoperative days (Figure 2).

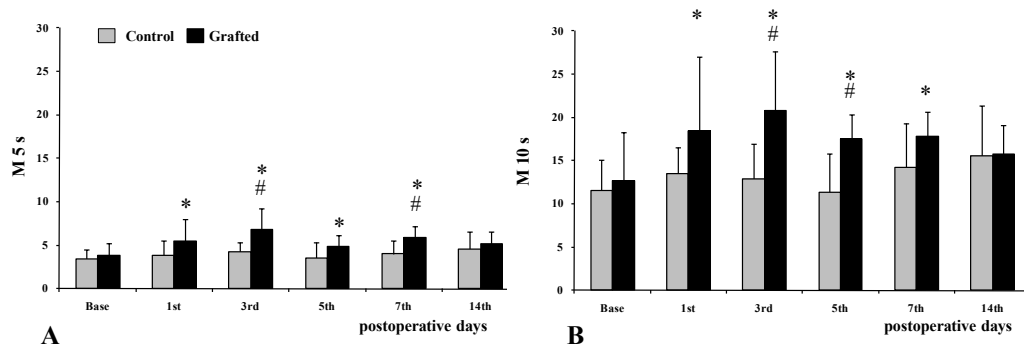


FIGURE 2 – Alterations of red blood cell aggregation index *M 5 s* (A) and *M 10 s* (B) values in the Control and Grafted groups. Means \pm S.D., * $p < 0.05$ vs. base; # $p < 0.05$ vs. Control

The *M 5 s* values (at 5th second of the aggregation process) increased on the 1st ($p = 0.011$ vs. base, $p = 0.03$ vs. Control), 3rd ($p < 0.001$ vs. base and vs. Control) 5th ($p = 0.021$ vs. base) and 7th day ($p < 0.001$ vs. base and vs. Control). The values of *M 10 s* (at 10th second of the aggregation process) resulted in larger values and more prominent differences between the experimental groups (on the 1st day: $p = 0.029$ vs. base; on the 3rd day: $p < 0.001$ vs. base and vs. Control; on the 5th day: $p = 0.006$ vs. base and $p < 0.001$ vs. Control; and on the 7th day: $p = 0.006$ vs. base).

Histological investigations

On the 14th day during the biopsy taking, the diameter of the control-side femoral artery was 3.56 ± 0.13 mm, the graft's one was 3.62 ± 0.17 , while the artery segment just above the graft was 3.5 ± 0.41 mm, but below, it was 2.75 ± 0.28 mm in diameter ($p < 0.001$ vs. graft, $p = 0.024$ vs. above graft and $p = 0.016$ vs. control-side artery).

Histologically we found matured thrombus at the anastomoses narrowing the lumen. Imbedded capillary network lined with endothelium was observed in the fibrin web and connective tissue filled with various sized and shaped red blood cells. It seemed to be fixed to the inner side of the arterial intimal layer. At site of the proximal anastomoses the grafts were observed in the scared thickening of the adventitia. The fixture of thrombus inside the grafts was not obvious, here we could see "free" inner layer. Freshly formed thrombotic layers were seen towards the distal segment. At the site of the distal anastomoses we observed scar tissue with foreign body giant cells and mixed inflammatory cells around the surgical suture material in the adventitia, which was present as continuity along a short section of the graft (Figure 3).

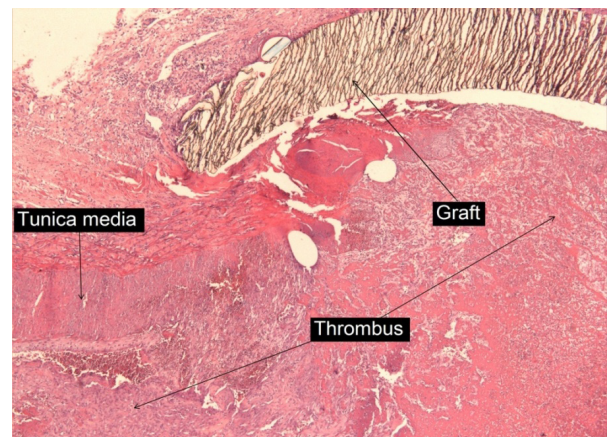


FIGURE 3 – Representative histological photograph of the grafted vessel section, showing matured thrombus at the anastomoses narrowing the lumen (H&E staining, original magnification: x500).

The widening of the internal elastic lamina was observed, but the endothelium lining was not always present. The observed thrombi seem to be the continuity of this widening. In the reticulate wall of the thrombi red blood cells and inflammatory cells were apparent. The endothelialization did not occur during the 2-week period, which was confirmed by the CD31 immunohistochemical examination. However, pseudointima formations made up from thrombotic elements are visible in certain segments. The arterial sections from the control side show regular, intact histological structure.

Discussion

Small-caliber vascular conduits are still an important tool in the surgical management of peripheral vascular diseases. However, the problem of early failure is a serious clinical problem⁹⁻¹¹. Since not only the problem of small-caliber vascular conduits must be considered, but the question of biomechanical tissue remodeling is also a key factor with all the shear stress-

stretching-, fluid biomechanical and mechanical micro-environmental relations^{15,16}. However, vascular tissue engineering provides a wide aspect for finding the possibly best solution for vessel substitutions¹⁵⁻¹⁷.

The healing of arterial graft is a complex and long process, depending on numberless factors, probably including inter-species differences, as well. Canine models may provide important information for vascular surgery research¹⁸⁻²⁵. Kuzuya *et al.*²² investigated the healing process of ePTFE vascular access grafts (internal diameter: 5 mm, length: 15 cm) with various intermodal distances. They followed the process for 12 weeks. According to their findings, in 2 weeks the inflammatory phase almost ended and fibroblast proliferation was almost complete. Kasza *et al.*²¹ found that after peripheral arterial stent implantation the restitution of vascular wall was completed by the end of the first postoperative month. Hess *et al.*²⁶ demonstrated that approximately six months is needed for a 5-cm polyurethane prosthesis for complete endothelialisation. It has also been shown that in a 6-9-cm PTFE graft only in about 60% endothelialisation occurs by the 12th postoperative month²⁷.

Clowes *et al.*²⁷ in their baboon experiment used 4-mm PTFE grafts with 6-8 cm in length implanted into the common iliac artery. They found that by the end of the 1st postoperative week the luminal surface of the graft was covered by thrombus and some patches of endothelial cells. During the 2nd week the thrombus was replaced and by the 4th-12th week the grafts luminal surface was covered with cells resembling endothelium²⁷.

Hess *et al.*²⁸ used endothelial-cell-seeded or non-seeded 3 mm wide, 5 cm long PTFE grafts in beagles, implanted into the femoral artery. The graft size and location were very similar to our experiment. Without anti-platelet therapy (acetylsalicylic acid or dipyridamole) in the 1st postoperative week 7 of 8 seeded prostheses were patent, while only 1 of 8 non-seeded one was patent.

In this model we focused on the changes over the first two postoperative weeks providing a relatively frequent investigation protocol during the 1st postoperative week. We found that the majority of the blood coagulation and red blood cell aggregation changes happened during the 1st week. Previously we studied the effect of hind limb ischemia-reperfusion on hematological and blood coagulation parameters, and we also saw the critical importance of 1st postoperative week²⁹. Inflammatory processes, acute phase reactions can be associated with alterations with hemorheological parameters, too^{30,31}. We saw that fibrinogen concentration closed very quickly and the 2-peak elongation of coagulation time

parameters with a continuously increasing platelet count might suggest that a thrombotic event could happen in the period of 3rd - 7th postoperative day.

Failure of the graft can be due to complex reasons that include the hemodynamic effect of the small-caliber tube, the surface properties, activation of hemostatic cascades and mechanical damage of blood cells, among others⁹⁻¹⁴. Along with the injury of the intima, the inflammatory events induced by the sutures of the anastomoses may contribute to the development of thrombus. Here it may come into play that the vessels incidentally bend and refract above the graft after surgery while positioning the lower limb back into natural position and during the normal everyday movement of the animal. There were no sign of circulatory problems on the operated side, no change in the movement and behavior of the animal, and the skin temperatures were normal; it showed virtually similar values with the contralateral non-operated side. The anastomoses originated from the gluteal region might compensate the circulation of the limb.

Besides the general hematological and blood coagulation time parameters' changes, we observed an early increase in erythrocyte aggregation values, too. Red blood cell aggregation is determined by cellular (cell morphology, deformability, membrane mechanical properties, composition of the surface glycocalyx) and plasmatic factors (e.g. fibrinogen concentration)^{30,32,33}. Free radicals deliberating during ischemia-reperfusion and inflammatory processes, mechanical cell damage, changes in red blood cell deformability, alteration in fibrinogen concentration, as well as micro-environmental conditions (pH, osmolarity), all may result in altered red blood cell aggregation^{30,31}. Furthermore, the mechanical properties of the cell membrane also play an important role. In a separate paper we have analyzed the mechanical stability of the red blood cells using various stress conditions in a complex evaluation process³⁴. An increase in erythrocyte aggregation may have local and even remote effect, too, since this parameter plays an important role in the rheology of the microcirculation. Increased red blood cell aggregation may cause numerous *in vivo* effects resulting in increased flow resistance, so contributing to disturbances of the tissue perfusion and modifying local hemodynamic profile³⁵.

Conclusions

The PTFE graft implantation for the replacement of the resected femoral arterial segment caused changes in the coagulation parameters and hemorheological properties, which lowered then equaled to the Control group by the end of the 2-week follow-up

period. Better clarifying the factors leading to early thrombosis of the small-caliber grafts is a very important issue. Further studies are needed for revealing the optimal conditions on geometry, length, position, hemodynamic and hemorheological factors, moving relations or even impregnated grafts that may decrease the chance for thrombus formation.

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