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EVALUATION OF THE BIOCOMPATIBILITY AND ANTIMICROBIAL PROPERTIES OF BIODEGRADABLE VASCULAR GRAFTS OF VARIOUS POLYMER COMPOSITION WITH ATROMBOGENIC AND ANTIMICROBIAL DRUG COATING

L.V. Antonova[1], E.O. Krivkina[1], V.N. Silnikov[2], O.V. Gruzdeva[1], M.A. Rezvova[1], T.N. Akentieva[1], T.V. Glushkova[1], V.O. Tkachenko[3], V.M. Sakharova[1], L.S. Barbarash[1]

[1] Research Institute for Complex Problems of Cardiovascular Diseases, Kemerovo, Russian Federation
[2] Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation
[3] Budker Institute of Nuclear Physics, Novosibirsk, Russian Federation

Creation of vascular grafts with atrombogenic and antimicrobial coating is a very important area. **Objective:** to evaluate the biocompatibility and antimicrobial properties of biodegradable vascular grafts of various polymer compositions with atrombogenic and antimicrobial drug coating. **Materials and methods.** Modification of the surface of the biodegradable vascular grafts was performed through complexation with polyvinylpyrrolidone, which was polymerized with polymer scaffold surface by means of ionizing radiation at 10 and 15 kGy. Physical and mechanical properties, as well as hemocompatibility were evaluated. Bacteriological studies were carried out using test strains of gram-negative and gram-positive microorganisms: *Klebsiella pneumoniae spp. ozaena* No. 5055, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Proteus mirabillis* ATCC3177, *Pseudomonas aeruginosa*. **Results.** There was no influence of modifying manipulations with ionizing radiation on the physical and mechanical characteristics of biodegradable prostheses. Vascular grafts with atrombogenic and antimicrobial coatings exhibited atrombogenic properties upon contact with blood, reducing platelet aggregation by 5–7 times ($p < 0.05$). Also decrease in adhesion and platelets deformation index was found on the surface of drug-eluting scaffolds (for PCL-based prostheses, the latter decreased by 1.9 times relative to unmodified counterparts ($p < 0.05$), for PHBV/PCL-based prostheses – by 1.3 times relative to unmodified counterparts and 1.5 times relative to scaffolds with polyvinylpyrrolidone ($p < 0.05$). Bacteriological studies revealed a local inhibitory effect in the place where scaffolds with cationic amphiphile were applied on agar. No growth retardation zones were identified. Polymeric composition of the scaffolds and the used dose of ionizing radiation did not lead to a difference in the bacteriostatic properties of the scaffolds with amphiphile. **Conclusion.** A full cycle of surface modification of biodegradable polymer prostheses based on both PCL and PHBV/PCL composition resulted in significant increase in the atrombogenic and antimicrobial properties of prostheses and did not worsen the physical-mechanical and biocompatible properties of the structures being developed.

Keywords: biodegradable polymers, small diameter vascular grafts, atrombogenic drug coating, cationic amphiphile.

INTRODUCTION

Vascular tissue engineering is one of the promising modern areas involved in the development of effective blood vessel prostheses [1]. There are various approaches to tissue engineering of blood vessels, but all of them are aimed at creating a functional vascular implant with a structure similar to the organization of native artery tissues and demonstrating patency in the long-term postoperative period. The basis of such vascular grafts is artificial tubular matrix, most often made of biodegradable natural and/or synthetic polymers with high biocompatibility. The matrix is a scaffold that is populated by autologous cells in vitro or in situ.

Widespread use of vascular grafts in medicine has intensified the need for polymeric materials with antibacterial properties. It is difficult to establish the exact etiology of prosthetic vascular graft infection, since in most cases it is multifactorial [2]. Microbial contamination of the prosthetic vascular graft can occur either exogenously or endogenously (bacteremia) [3, 4]. Despite the placement of a sterile prosthetic vascular graft in an uninfected field, about 20% of them become infected, increasing the percentage of subsequent graft occlusion to 27% [5]. These circumstances require additional antimicrobial and anti-thrombogenic protection of the vascular graft surface.

Corresponding author: Larisa Antonova. Address: 6, Sosnovy Boulevard, Kemerovo, 650002, Russian Federation. Phone: (905) 906-04-51. E-mail: antonova.la@mail.ru

Pathogenic microbial adhesion to the surfaces with subsequent cell growth and colonization leads to biofilm formation with high resistance to antibiotics and host defense mechanisms [6, 7], which in clinical practice leads to the need for prosthesis replacement. This creates inconvenience and increases the risk for the patient. The most effective way to prevent biofilm formation on the surface of the prosthetic material is to prevent the adhesion of bacterial cells at the initial stage of infection of the prosthetic material. This can be achieved by replacing conventional prosthetic materials with antimicrobial biocompatible polymers [8, 9].

In combination with the general problems of decreasing effectiveness of antibacterial drugs due to the emergence of multidrug-resistant bacteria, the development of a prosthetic vascular graft with high antibacterial activity and low probability of inducing drug resistance becomes extremely urgent. A way out of this situation can be the approach of introducing an antibacterial drug directly into the prosthesis structure. On one hand, this approach solves the problem of local drug delivery and, on the other hand, ensures the effect of the drug on microorganisms before they form biofilms.

Over the past decade, hydrogels, three-dimensional polymeric networks obtained from water-soluble or biodegradable natural or synthetic polymers in which the antibacterial agent is bound to the polymer matrix through non-covalent interactions, have been widely used for drug delivery and prolonged release of drugs [10].

The most attractive compounds with antimicrobial activity to which microorganisms do not develop resistance are cationic amphiphiles (CAms) – molecules with one or more positively charged groups and lipophilic fragments [11, 12]. These compounds are synthetic analogues of naturally occurring cationic antimicrobials: they can also disrupt transmembrane potential, cause leakage of cytoplasmic contents and, ultimately, cell death [13]. Most of these compounds are highly effective against both Gram-positive and Gram-negative bacteria (including antibiotic-resistant strains), along with good selectivity in respect of mammalian cells. The possibility of obtaining amphiphiles that destroy bacterial membranes and, at the same time, have other bacterial targets significantly reduces the likelihood of resistance to such compounds. The high stability of these compounds, including in physiological fluids, combined with the low cost of their synthesis, makes these compounds the most promising candidates for the role of low molecular weight modifiers of polymeric materials with antibacterial properties.

The creation of a tissue-engineered vascular graft with a highly porous wall is extremely important for the full migration of cells into the prosthesis wall from the bloodstream and surrounding tissues and subsequent cell proliferation and differentiation in the vascular direction. However, a prosthetic graft with such surface architectonics is certainly capable of provoking thrombus formation. Therefore, additional modification of the surface of tissue-engineered highly porous small-diameter vascular grafts with drugs with antiaggregant and anticoagulant activity can prevent the initiation of thrombosis after implantation of such prostheses into the vascular bed.

The aim of the study was to evaluate the biocompatibility and antimicrobial properties of biodegradable vascular grafts of various polymer compositions with anti-thrombogenic and antimicrobial drug coating.

MATERIALS AND METHODS

Fabrication of biodegradable vascular grafts

Two varieties of 4 mm diameter polymeric tubular scaffolds were made by electrospinning: from 12% poly(ε-caprolactone) solution, (PCL; Sigma-Aldrich, USA) and from a polymer composition of 2% poly(3-hydroxybutyrate-co-3-hydroxyvalerate) solution, (PHBV, Sigma-Aldrich) and 12% poly(ε-caprolactone) solution, (PCL, Sigma-Aldrich) at 1:2 ratio. 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma-Aldrich, USA) was used as a solvent. Electrospinning was performed on a Nanon-01A device (MECC, Japan). The following mode was selected for PCL-based graft fabrication: needle voltage 22 kV, polymer solution feed rate 0.5 mL/h, manifold rotation speed 1000 rpm, needle movement speed 60 mm/s, distance from the needle to the winding manifold 15 cm. For PHBV/PCL-based prosthetic grafts, the following mode was used: needle voltage 20 kV, polymer solution feed rate 0.5 mL/h, manifold rotation speed 1000 rpm, needle movement speed 60 mm/s, distance from needle to winding manifold 15 cm.

Formation of an anti-thrombogenic coating on the surface of biodegradable vascular graft

Additional modification of the surface of PCL- and PHBV/PCL-based grafts to increase thromboresistance was carried out according to our own original technique [14]. Hydrogel coating on the inner surface of the polymer graft was formed using radiation-induced graft polymerization. For this purpose, 4 mm-diameter PCL- and PHBV/PCL-based grafts were immersed for 30 minutes in a 5% solution of polyvinylpyrrolidone (PVP, K 90; PanReac AppliChem, Germany) in ethyl alcohol with complete filling of the graft inner channel. Next, the grafts were removed from the solution and dried horizontally for 24 hours in air at room temperature. For PVP-based grafting to the graft surface, the products were placed in glass tubes that were filled with inert argon gas, sealed with paraphilm, and irradiated with ionizing radiation in two different modes (with a total absorbed dose of 10 and 15 kGy) using a pulsed linear gas pedal ILU-10 with 5 MeV 50 kW beam energy (Budker Institute of Nuclear Physics, Russia). Thus, the vascular grafts were sterilized simultaneously with the

modification; therefore, further manipulations to form the drug coating were carried out under sterile conditions. Non-grafted polymer was washed with injection water for 60 minutes. The grafting quality was evaluated by attenuated total reflectance spectroscopy of the inner surface of the modified grafts on a Bruker Vertex 80v (Germany) instrument with an ATR attachment (Germany) in 4000–500 cm^{-1} spectral range.

To create a drug coating, two substances were chosen – iloprost and cationic amphiphile 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl)pentane tetrabromide without additional reactive groups.

Iloprost (Ilo, Bayer, Germany) is a drug registered in the Russian Federation and approved for medical use. It is a synthetic analog of prostacyclin, inhibits platelet aggregation, adhesion and release reaction; restores impaired microcirculation by induction of vasodilation, inhibition of platelet activation, activation of fibrinolysis, endothelial repair and protection; inhibits adhesion and migration of white blood cells after endothelial damage.

Cationic amphiphiles (A) in general, and 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl)pentane tetrabromide (Nano Tech-C, Russia) in particular, possess ribonuclease activity and, consequently, high antibacterial and antiviral activity [15–17]. An additional argument in favor of choosing this compound is the fact that it has passed all the necessary tests and is used in veterinary medicine as antiviral drug Triviject (Trionis-Vet LLC, Russia) in the form of an injection solution.

Cationic amphiphile, 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl)pentane tetrabromide without additional reactive groups (compound 3, Fig. 1), was synthesized as described earlier [16]. The nuclear magnetic resonance (1H NMR) spectrum of the obtained compound (3) is consistent with the literature data [16].

Addition of iloprost and cationic amphiphile 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl)pentane tetrabromide to the hydrogel coating was performed by complexation. A sterile modifying solution containing cationic amphiphile (A) at 0.25 mg/mL concentration was prepared. For this purpose, 0.025 g of amphiphile 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl)pentane tetrabromide was dissolved in 1 to 2 mL of methanol. The resulting solution was slowly added to a flask with sterile water for injection while stirring and the volume was brought to 98 mL. After that, 2 mL of iloprost (Ilo) was added to the solution at 20 μg per 100 mL (0.2 μg/mL). To attach the drugs to the PVP coating, the vascular grafts were incubated in the modifying solution containing the drugs for 30 minutes. Then, the finished PCL/PVP/Ilo/A and PHBV/PCL/PVP/Ilo/A vascular grafts were air-dried under sterile conditions and placed in sterile storage containers.

PCL and PHBV/PCL samples were unmodified controls; PCL/PVP and PHBV/PCL/PVP samples exposed to 10 kGy and 15 kGy of ionizing radiation were used as comparison groups. All work was carried out inside a sterile box. The list of experimental and control groups is presented in Table 1.

Table 1

Types of polymer samples

Name of samples	
PHBV/PCL	PCL
PHBV/PCL/PVP-10 kGy	PCL/PVP-10 kGy
PHBV/PCL/PVP-15 kGy	PCL/PVP-15 kGy
PHBV/PCL/PVP/Ilo/A-10 kGy	PCL/PVP/Ilo/A-10 kGy
PHBV/PCL/PVP/Ilo/A-15 kGy	PCL/PVP/Ilo/A-15 kGy

Assessment of physical and mechanical properties

The mechanical properties of the biodegradable vascular grafts before and after additional anti-thrombogenic drug coating were evaluated under uniaxial tension on a Z-series universal testing machine (Zwick/Roell) using a transducer with 50 N nominal force. The crosshead movement speed during the test was 50 mm/min. The ultimate tensile strength of the material was estimated as the maximum tensile stress (MPa) prior to failure. The

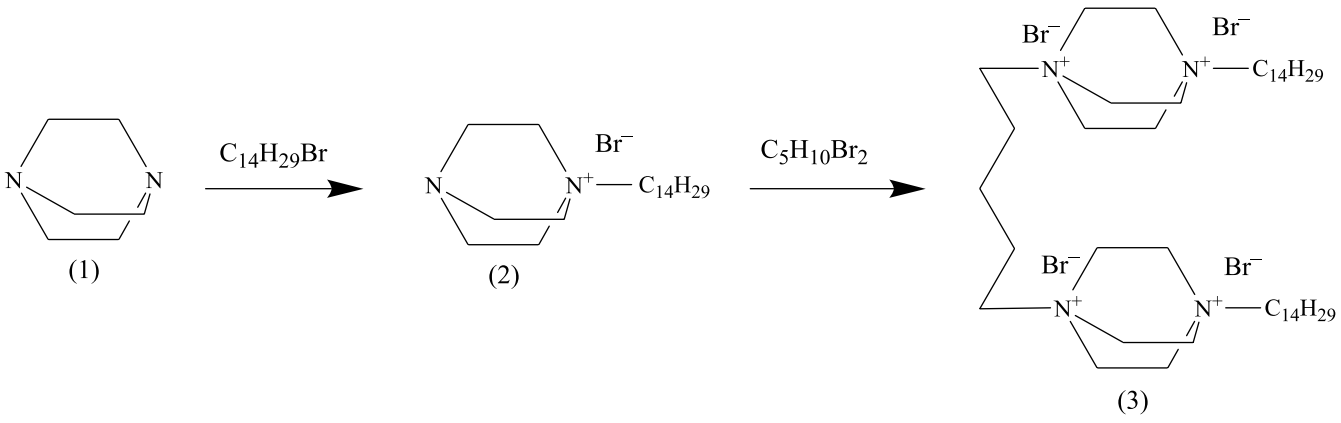

Fig. 1. Synthesis scheme for 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl)pentane tetrabromide

stress-strain properties of the material were evaluated by the relative elongation before failure (%) and Young's modulus (MPa), which was determined in the physiological pressure range (80 to 120 mmHg). Intact sheep carotid artery was used as the control.

Hemocompatibility assessment

Erythrocyte hemolysis

Hemolysis of erythrocytes after contact with the surface of polymeric samples was studied according to the ISO 10993.4 standard. Fresh donor blood was used, to which sodium citrate 3.8% was added in a 1:9 ratio (citrate:blood). 25 cm^2 polymer samples of 5 pieces for each type of material were placed in weighing bottles containing 10 mL of saline. The weighing bottles were incubated in the thermostat at 37 °C for 120 minutes. Saline and distilled water were used as positive and negative controls, respectively. Two hours after incubation, 200 μL of citrate blood was added to each weighing bottle and placed again in the thermostat for 60 min at 37 °C. After incubation, polymer samples were removed from the weighing bottles into appropriate tubes and centrifuged for 10 minutes at 2800 rpm to precipitate erythrocytes. The optical density of the obtained solutions was measured on a GENESYS 6 spectrophotometer (Thermo SCIENTIFIC, USA) at 545 nm.

The degree of hemolysis (H) in % was calculated using the formula [18, 19]:

$$H\ (\%) = \frac{D_t - D_{ne}}{D_{pe} - D_{ne}} \times 100\%,$$

where, D_t is the optical density of the sample incubated with the test material, D_{ne} is the optical density of positive control, and D_{pe} is the optical density of the sample after 100% hemolysis.

The mean optical density when measuring saline with blood (positive control), equal to 0, was taken as complete absence of hemolysis. The mean optical density of the device when measuring distilled water with blood (negative control), which was 0.279, was taken as 100% hemolysis.

Platelet aggregation

The study was performed according to the ISO 10993.4 standard. Fresh donor blood was used, to which sodium citrate 3.8% was added in a 1:9 ratio (citrate:blood). To obtain platelet-rich plasma (PRP), blood was centrifuged for 10 minutes at 1000 rpm. Platelet-poor plasma (PPP) was obtained by repeated centrifugation of PRP for 20 minutes at 4000 rpm. The PPP was used to calibrate the instrument. Intact PRP served as a positive control. Measurements were taken spontaneously without aggregation inducers. Ca^{+2} ion level was restored in citrated blood using $CaCl_2$ solution with 0.025 M molecular mass, after which the measurements were taken. The sample/reagent ratio was 250 μL of PRP + 25 μL of $CaCl_2$. The time of contact of the tested samples with PRP was 3 minutes. Measurement of maximum platelet aggregation was performed on a semi-automatic platelet aggregation analyzer "APAST 4004" (LABiTec, Germany).

Platelet adhesion

Samples of 0.5 cm^2 polymer matrices (n = 2) were incubated for 2 hours at 37 °C in 300 μL of PRP obtained from fresh citrated donor blood when centrifuged for 10 minutes at 1200 rpm. The samples were then washed with phosphate-buffered saline (PBS) (pH – 7.4) to remove non-adherent plasma components. The samples were then fixed in a 2% glutaraldehyde solution for 24 hours, washed with PBS, and dehydrated in a series of alcohols of ascending concentration from 30% to 100% for 15 minutes each, followed by drying at room temperature. The samples were then mounted on special tables using carbon tape and a conductive (Au/Pd) coating was formed on their surface using an EM ACE200 vacuum unit (Leica Mikrosysteme GmbH, Austria). We used the 8 most characteristic fields selected at random for the analysis. The adhesive ability of the material surface was evaluated by the number of platelets per 1 mm^2, by the predominance of platelet type on the surface, and by their deformation index (DI), which was calculated using the formula [18–20]:

DI = (Type I count × 1 + Type II count × 2 + Type III count × 3 + Type IV count × 4 + Type V count × 5) / total platelet count

Types of platelets depending on activation:

I Disc-shaped platelet, no deformity;
II Platelet is enlarged with the rudiments of pseudopodia in the form of protrusions;
III Platelet significantly enlarged, irregularly shaped, with pronounced pseudopodia, platelets tend to accumulate into larger conglomerates;
IV Proliferated platelet, cytoplasm proliferating between the pseudopodia;
V Platelet in the form of a stain with granules, due to proliferation of cytoplasm, the pseudopodia cannot be identified.

Bacteriological examinations

The following microbial strains were used for bacteriological examination: Klebsiella pneumonia spp. ozaena 5055, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Proteus mirabillis ATCC3177, Pseudomonas aeruginosa ATCC27853. These microorganisms were chosen for the experiment because they are the most common in infectious complications in cardiac surgery. Sensitivity of control microbial strains to the polymer matrices under study was measured using a disk-diffusion method to assess the sensitivity of bacteria

to cationic amphiphile (A) introduced into the matrix by complexation with PVP at 0.25 mg/mL concentration. Microbial suspension was prepared by direct suspension of the colonies in sterile isotonic solution to a 0.5 density according to the McFarland turbidity standards. No later than 60 minutes later, the bacterial suspension was applied to agar plates by stroking in three directions over the entire agar surface so that the strokes could adhere tightly to each other. The polymer matrix discs were spread no later than 15 minutes after inoculation of the plates. The plates were incubated in the thermostat at 35–36 °C for 24–48 hours.

Statistical analysis

Data were statistically processed using the Prism 7 software (GraphPad, USA). The nature of distribution in the samples was assessed using the Kolmogorov–Smirnov test. Data were presented as median and percentiles (Me, 25–75%). Kruskal–Wallis H test with FDR correction was used for multiple comparisons; differences were considered significant at significance level $p < 0.05$.

RESULTS

Results of graft polymerization of polyvinylpyrrolidone

In the IR spectra of PVP-modified and unmodified PHBV/PCL matrices, there were bands of C=O (ether carbonyl) stretching vibrations at 1724 cm^{-1}, C–O stretch bands were observed at 1278 and 1054 cm^{-1}, (Fig. 2), [21]. In the spectra of all samples under study, we also observed low intensity bands at 2942 cm^{-1} and 2865 cm^{-1}, corresponding to asymmetric vibrations of the methylene-oxygen group (CH_2–O) and symmetric vibrations of the methylene group (CH_2–), respectively, (Fig. 2) [22]. In contrast to unmodified samples, the spectra of PVP-modified matrices contained a 1654 cm^{-1} band related to the carbonyl amide group of the pyrrolidine ring [23], indicating grafting of the polymer to the surface of the vascular graft (Fig. 2). The absence of changes in the position and intensity of characteristic bands in the infrared spectrum of PHBV/PCL indicates an insignificant effect of modification conditions, in particular, ionizing radiation on the structure of the material. A similar picture was obtained in the study of PCL-based polymer vessels.

Physical and mechanical properties

Assessment of the long-term permeability of the developed prosthetic vascular grafts under is planned to be performed on a sheep model. Therefore, in the process of studying the physical and mechanical properties of the prostheses, the sheep carotid artery was chosen as an additional comparison group.

The procedure of polymerization of intermediate PVP polymer with PCL-based matrix surface using ionizing radiation at 10 and 15 kGy decreased the strength limit by 1.6 times ($p < 0.05$) and slightly increased the Young's modulus of these matrices (Table 2).

Meanwhile, modification of PHBV/PCL matrices by PVP had no effect on their strength properties but resulted in a 1.9-fold increase in Young's modulus ($p < 0.05$). Subsequent modification with amphiphile and iloprost by complexation did not affect the strength index of the PVP-modified PCL matrices, but decreased the stiffness of the PHBV/PCL matrices acquired during polymerization with PVP. Thus, the procedure for forming a drug coating on the surface of PHBV/PCL and PCL vascular grafts involving ionizing radiation did not result in a significant change in the physical and mechanical characteristics of the grafts.

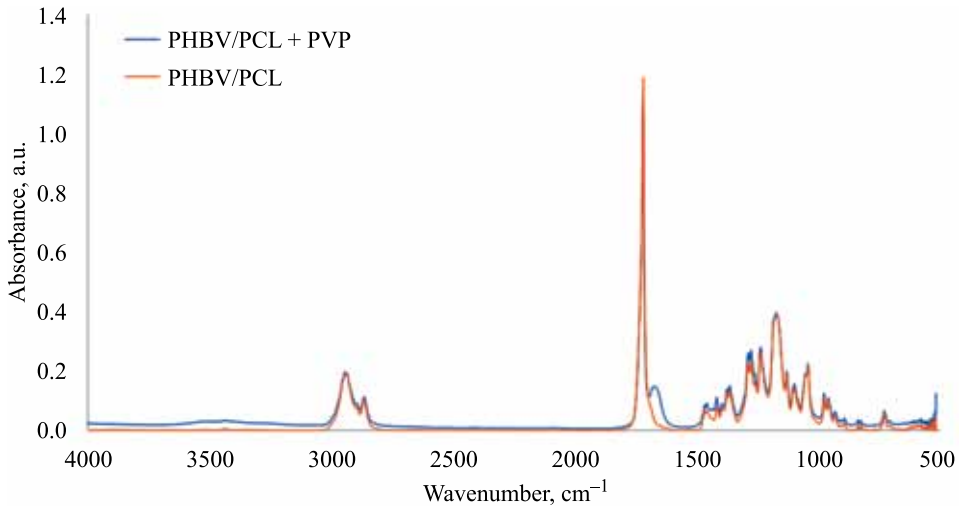

Fig. 2. IR spectra of PVP-modified and unmodified inner surface of a polymer vascular graft using PHBV/PCL as an example

Hemocompatible properties

The study of platelet aggregation activity revealed that there are statistically significant differences between intact PRP and all the matrix groups studied (Table 3).

When comparing the two types of unmodified matrices PHBV/PCL and PCL, no significant differences were found. Polymerization of the matrix surface with PVP under 15 kGy ionizing radiation slightly increased platelet aggregation to the matrix surface (Table 3). However, subsequent complexation of polymerized PVP with iloprost and amphiphile decreased platelet aggregation 6- to 7-fold compared with the results of intact PRP (Table 3; $p < 0.05$). This significant decrease in platelet aggregation appears to be related to the action of iloprost. The presence of amphiphile at a 0.25 mg/mL dose did not result in significant increase in platelet aggregation.

Hemolysis of erythrocytes did not exceed the permissible 2% when erythrocytes came into contact with the surface of all matrix varieties (Table 3). There were no significant differences between the groups either.

Platelet adhesion

After contact of unmodified matrices PHBV/PCL and PCL with intact PRP, platelet count on the surface of PCL matrices was 3 times higher than on the PHBV/PCL surface (Table 4, Fig. 3, Fig. 4). Type II and III platelets predominated on unmodified matrices. However, platelet DI after contact with the PCL surface was 1.4 times higher ($p < 0.05$) than that of platelets contacting the surface of PHBV/PCL matrices (Table 4).

After polymerization of the surface of PCL-based matrixes with PVP at radiation dose of 10 and 15 kGy, no significant changes in the degree of platelet adhesion relative to unmodified PCL matrices were observed (Table 4, Fig. 3). However, the increase in the proportion of type IV platelets using 15 kGy radiation is noteworthy.

Table 2

Mechanical properties of polymer tubular scaffolds before and after modification in comparison with sheep carotid artery

Sample type	n	Tension, MPa	Relative extension, %	Young's modulus, MPa
Sheep carotid artery	14	1.2 (1.06–1.9)	158.5 (126.0–169.5)	0.49 (0.39–0.66)
PHBV/PCL	7	3.99 (3.71–4.23)•	1438.0 (1403.0–1510.0)•	11.52 (10.66–12.21)•
PCL	7	5.84 (5.56–6.13)•	1391.0 (1350.0–1413.0)•	9.33 (9.23–9.55)•
PHBV/PCL/PVP-10 kGy	7	3.74 (3.5–3.83)•	1302.0 (1234.0–1360.0)*•	22.39 (21.59–23.71)*•
PHBV/PCL/PVP-15 kGy	7	3.61 (3.23–4.01)•	1202.0 (1120.0–1298.0)*•	19.8 (18.23–20.9)*•
PCL/PVP-10 kGy	7	3.56 (3.51–3.7)*•	1297.0 (1258.0–1342.0)*•	10.66 (10.37–11.01)*•
PCL/PVP-15 kGy	7	3.75 (3.48–4.01)*•	1183.0 (1157.0–1215.0)*•	12.86 (11.86–14.06)*•
PHBV/PCL/PVP/Ilo/A-10 kGy	7	3.57 (3.28–3.93)•	1258.0 (1200.0–1392.0)*•	15.24 (14.78–15.84)*•
PHBV/PCL/PVP/Ilo/A-15 kGy	7	3.89 (3.88–3.99)•	1364.0 (1343.0–1393.0)*•	14.55 (13.22–15.24)*•
PCL/PVP/Ilo/A-10 kGy	7	3.77 (3.66–3.87)*•	1421.0 (1380.0–1467.0)•▲	9.54 (9.43–9.76)•▲
PCL/PVP/Ilo/A-15 kGy	7	4.02 (3.8–4.18)*•	1454.0 (1433.0–1458.0)*•▲	10.15 (9.82–10.57)*•▲

* – $p < 0.05$ relative to PHBV/PCL or PCL; • – $p < 0.05$ relative to the sheep carotid artery; ▲ – $p < 0.05$ relative to 15 kGy PHBV/PCL-PVP radiation or 15 kGy PCL-PVP radiation.

Table 3

Maximum aggregation of human blood platelets after contact with modified and unmodified polymer matrices

Sample type	Maximum platelet aggregation, % Me (25%–75%)	Degree of red blood cell hemolysis, % Me (25%–75%)
PHBV/PCL	87.23 (83.95–89.84)*	0.504 (0.0–1.01)
PCL	87.23 (83.27–89.35)*	0.504 (0.0–1.01)
PHBV/PCL/PVP-10 kGy	85.8 (84.37–89.03)*	0.704 (0.5–1.01)
PHBV/PCL/PVP-15 kGy	88.53 (86.59–89.37)*	0.2 (0.0–0.5)
PCL/PVP-10 kGy	83.51 (82.68–86.60)*	0.2 (0.0–0.5)
PCL/PVP-15 kGy	90.12 (82.57–90.60)*	0.704 (0.5–1.01)
PHBV/PCL/PVP/Ilo/A-10 kGy	12.1 (11.05–12.78)**	0.2 (0.0–0.5)
PHBV/PCL/PVP/Ilo/A-15 kGy	12.18 (11.15–12.24)**	0.5 (0.0–0.5)
PCL/PVP/Ilo/A-10 kGy	10.86 (9.04–11.39)**	0.504 (0.0–1.01)
PCL/PVP/Ilo/A-15 kGy	10.7 (10.38–17.23)**	0.404 (0.0–1.01)
Intact platelet-rich plasma (PRP)	74.65 (72.45–75.31)	–

* – $p < 0.05$ relative to intact PRP; ** – $p < 0.05$ relative to unmodified PCL and PHBV/PCL matrices.

Grafting of PVP to the surface of PHBV/PCL matrices increased the degree of platelet adhesion almost 2-fold regardless of the radiation dose used (Table 4, Fig. 4) with some increase in type IV platelet count when 15 kGy radiation dose was used. However, platelet DI after polymerization of the matrix surface with PVP did not undergo significant changes.

Analysis of PCL/PVP/Ilo/A-10 kGy matrix surface after contact with intact PRP revealed significant decrease in both adhered platelet count and platelets DI relative to the PCL/PVP-10 kGy matrices – 1.5-fold and 1.7-fold, respectively ($p < 0.05$). At contact with the surface of the PCL/PVP/Ilo/A-15 kGy matrix, the studied parameters decreased 1.3- and 1.5-fold, respectively.

Formation of an anti-thrombogenic and antimicrobial drug coating Ilo/A on the surface of PHBV/PCL/PVP-15 kGy matrices reduced the count of adhered platelets by 1.5 times and the count of type III platelets by 2 times with prevalence of type I and II platelets on the surface of PHBV/PCL/PVP/Ilo/A-10 kGy and PHBV/PCL/PVP/Ilo/A-15 kGy matrices of type I and II platelets, which resulted in a 1.5-fold decrease in platelet DI ($p < 0.05$).

Results of bacteriological examination

Figs. 5 and 6 present the photos obtained when evaluating the bacteriostatic properties of matrices with different polymer composition, containing cationic amphiphile at a 0.25 mg/mL concentration at radiation doses of 10 and 15 kGy. The bacteriostatic properties of unmodified matrices were studied in a comparative aspect. It was found that in all samples containing cationic amphiphile, suppression of the growth of Klebsiella pneumonia spp. ozaena No. 5055, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Proteus mirabillis ATCC3177, Pseudomonas aeruginosa ATCC27853 had local inhibitory effect on the site of matrix application on agar. No growth retardation zones were detected (Figs. 5 and 6). The polymer composition of the matrices and the ionizing radiation dose used did not lead to a difference in the bacteriostatic properties of the amphiphilic matrices.

The unmodified matrices had no bacteriostatic effect: colonies continued to grow at the site where the matrices were applied (Fig. 5, 6).

In order to check the ability of the matrices to induce hemolysis of erythrocytes, Mueller–Hinton blood agar was used (Fig. 7). K. pneumonia does not have hemolytic properties, so this particular microorganism was chosen. As a result, there was no hemolysis both around the matrices and in the area of its location (Fig. 7).

Table 4

Platelet adhesion indicators after contact with polymer matrices PHBV/PCL and PCL depending on the surface modification variant

Sample type	Platelet type, %					Platelet count per 1 mm^2 **Me** (25–75%)	Strain index **Me** (25–75%)
	I	II	III	IV	V		
PHBV/PCL	7.7	30.8	53.8	7.7	0.0	**578.0** (0.0–1349.0)	**1.75** (0.0–2.9)
PCL	4.7	46.5	41.9	4.7	2.3	**1734.0** (866.9–3179.0)*	**2.5** (2.0–2.7)*
PHBV/PCL/PVP-10 kGy	49.0	1.0	48.0	2.0	0.0	**953.0** (1.0–2689.0)*	**1.85** (0.0–2.9)
PHBV/PCL/PVP-15 kGy	3.0	27.3	45.5	21.2	3.0	**1156.0** (0.0–3082.0)*	**1.91** (0.0–2.9)
PCL/PVP-10 kGy	8.1	64.9	16.2	10.8	0.0	**1927.0** (96.3–3082.0)**	**2.2** (0.5–2.5)
PCL/PVP-15 kGy	12.5	25.5	12.5	50.0	0.0	**1728.0** (846.4–3058.0)$^{\sigma}$	**1.9** (0.0–2.8)
PHBV/PCL/PVP/Ilo/A-10 kGy	25.0	50.0	21.6	3.4	0.0	**768.6** (0–1366.0)	**1.3** (0.0–2.6)**
PHBV/PCL/PVP/Ilo/A-15 kGy	12.5	62.5	18.8	6.2	0.0	**770.6** (0–1445.0)$^{\sigma}$	**1.3** (0.0–2.4)$^{\sigma}$
PCL/PVP/Ilo/A-10 kGy	3.7	56.0	31.4	8.9		**1310.0** (0.0–3176.0)$^{\alpha}$	**1.3** (0.0–2.6)$^{\#\,\alpha}$
PCL/PVP/Ilo/A-15 kGy	4.7	6.3	71.8	17.2		**1349.0** (0.0–3275.0)	**1.3** (0.0–2.8)$^{\#\,\beta}$

* – $p < 0.05$ relative to the parameters of the PHBV/PCL scaffold; ** – $p < 0.05$ relative to the parameters of the PHBV/PCL/PVP-10 kGy matrix; $^{\sigma}$ – $p < 0.05$ relative to the parameters of the PHBV/PCL/PVP-15 kGy matrix; $^{\#}$ – $p < 0.05$ relative to the parameters of the PCL scaffold; $^{\alpha}$ – $p < 0.05$ relative to the parameters of the PCL/PVP-10 kGy matrix; $^{\beta}$ – $p < 0.05$ relative to the parameters of the PCL/PVP-15 kGy matrix.

DISCUSSION

Surface modification of PCL- and PHBV/PCL-based biodegradable vascular grafts is done by forming a PVP hydrogel coating on their inner surface, which is capable not only of binding drugs as a result of complexation, but also of temporarily (until its complete resorption) occupying the pore cavity, which significantly reduces the risk of platelet adhesion to the surface of the graft after its implantation into the vascular bed. In addition, the

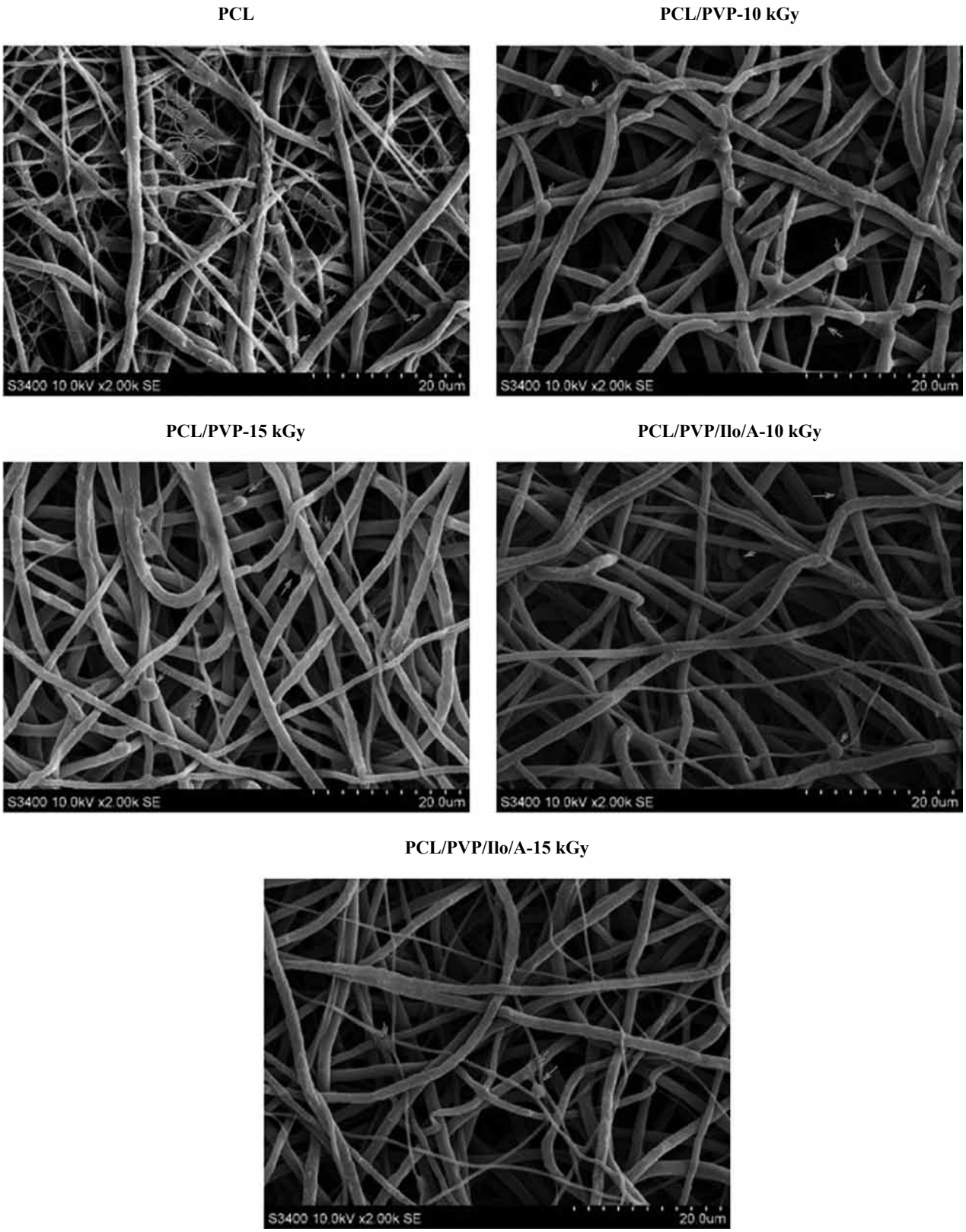

Fig. 3. Surface of PCL matrices after contact with platelet-rich plasma, 2000× magnification. Arrows and circles indicate platelets

known hydrophilicity of PVP contributes to the reduction of the degree of adhesion of protein molecules and blood cells, in particular, platelets, as well as prevention of conformational changes in protein structures. The mobility of macromolecular chains in hydrogels also determines the high rate of desorption of protein molecules, which enhances the anti-thrombogenic potential of PVP [24].

PVP was sewn onto the surface of biodegradable grafts using the radiation-induced graft polymerization method using total absorbed doses of 10 and 15 kGy. At

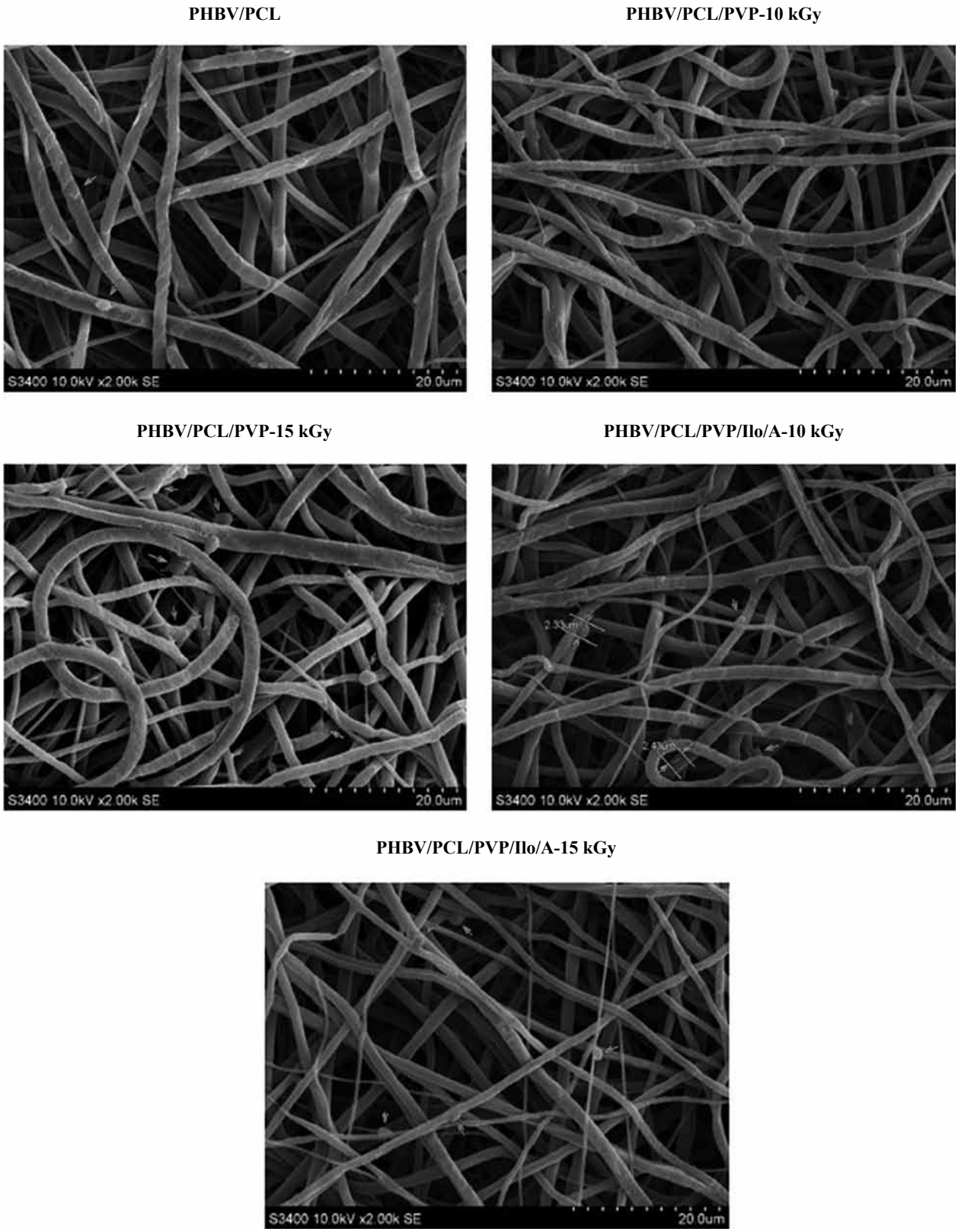

Fig. 4. Surface of PHBV/PCL matrices after contact with platelet-rich plasma, magnification 2000×. Arrows and circles indicate platelets

the same time, the 15 kGy dose was used to sterilize the products. However, ionizing radiation can affect biocompatibility properties. The use of 15 kGy total absorbed dose has been shown not to significantly increase platelet adhesion and deformation, with no significant change in the physical and mechanical properties of the grafts. Nevertheless, the subsequent stages of washing and drug adhesion by complexation neutralized these negative aspects of ionizing radiation with respect to the increase in platelet adhesion and deformation. Consequently, the

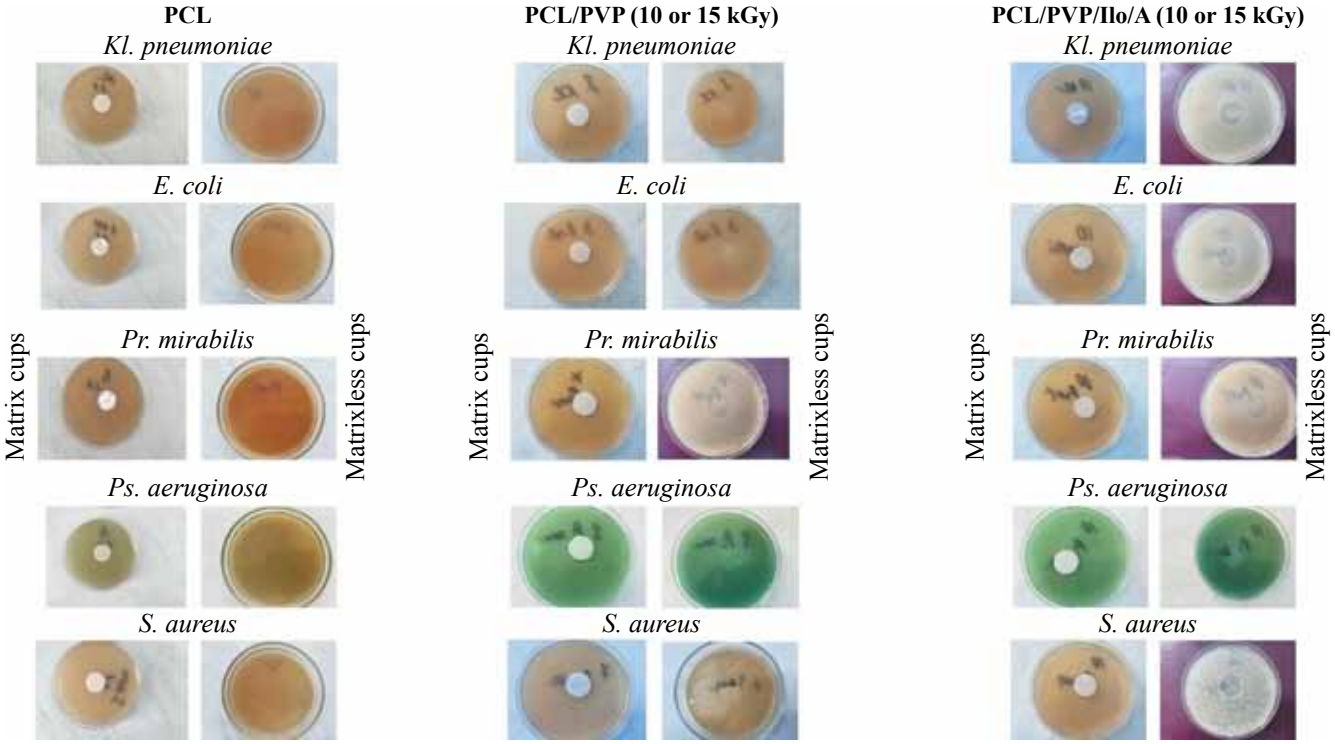

Fig. 5. Bacteriostatic properties of PCL scaffolds before and after modification with cationic amphiphile and iloprost

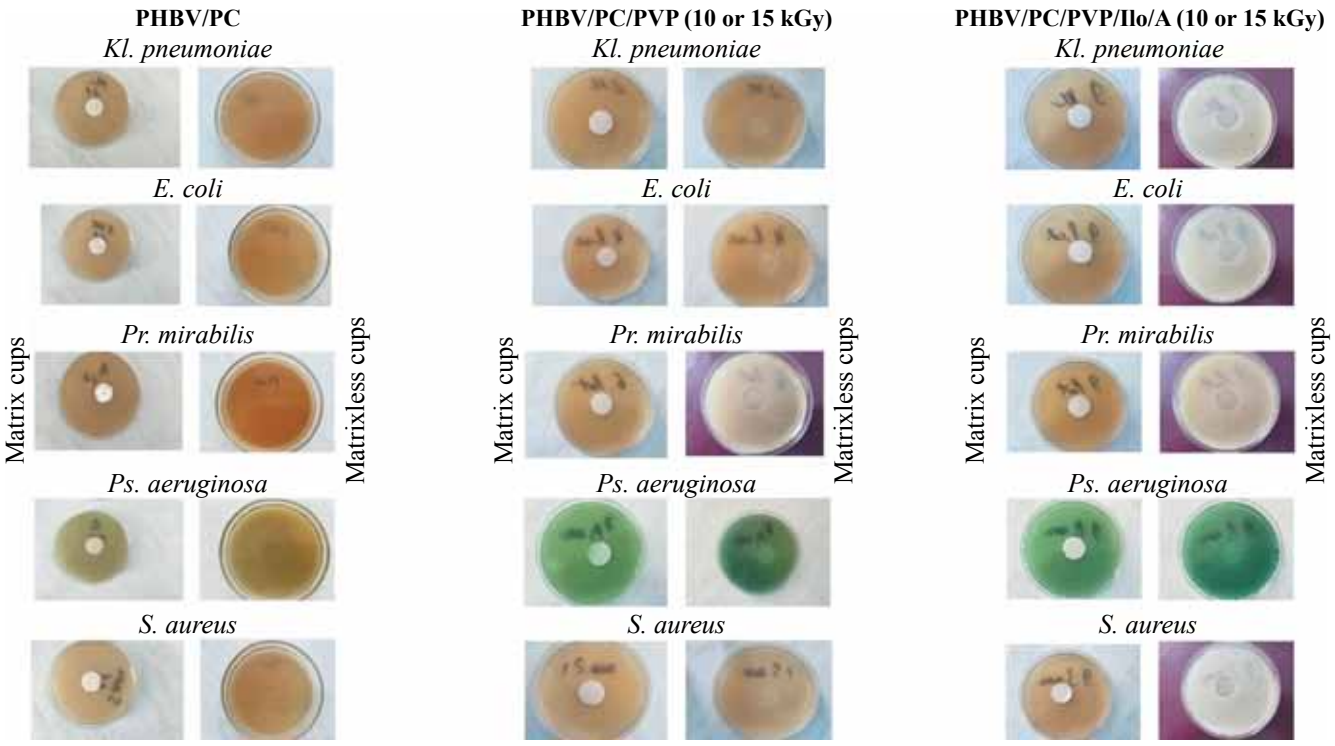

Fig. 6. Bacteriostatic properties of PHBV/PCL scaffolds before and after modification with cationic amphiphile and iloprost

subsequent choice of a 15 kGy radiation dose would be preferable, since, without adversely affecting the biocompatibility of the final product, it allows to simultaneously polymerize polyvinylpyrrolidone with the surface of biodegradable grafts and sterilize the grafts.

Based on PVP ability to form complexes, attachment of drugs to the free reactive groups of the PVP-based hydrogel is done by complexation. This method of drug incorporation, unlike covalent binding, allows for maximum preservation of the biological activity of drugs without creating steric hindrances and without blocking the molecule binding centers with blood clotting factors. The efficacy of iloprost and 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl)pentane tetrabromide after complexation with PVP has been proven both in hemocompatibility tests and in bacteriological examinations. A significant decrease in platelet adhesion to the surface of drug-eluting grafts compared to the unmodified counterparts and matrices with polymerized PVP was revealed. Cationic amphiphilic polymer matrices exhibited convincing bacteriostatic properties at the site where the matrices were applied on the agar without causing hemolysis.

CONCLUSION

A full cycle of surface modification of PCL- and PHV/PCL-based biodegradable polymer grafts resulted in a significant increase in the anti-thrombogenic and antimicrobial properties of prosthetic grafts and did not worsen the stress-strain and biocompatible properties of the developed constructs.

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The authors declare no conflict of interest.

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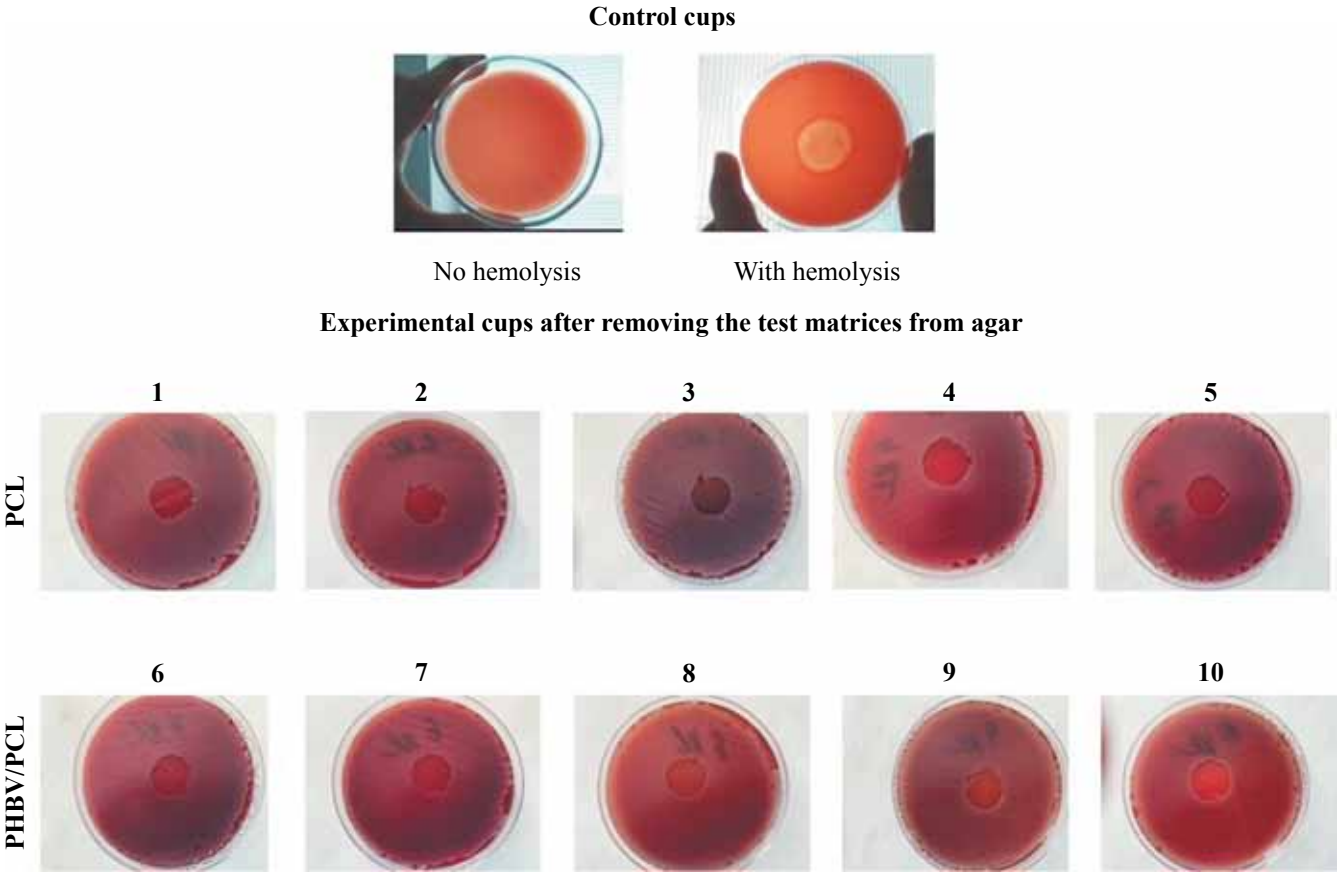

Fig. 7. Delayed growth of K. pneumonia and absence of hemolysis at the location of polymer scaffolds: 1 – PCL; 2 – PCL/PVP-10 kGy; 3 – PCL/PVP-15 kGy; 4 – PCL/PVP/Ilo/A-10 kGy; 5 – PCL/PVP/Ilo/A-15 kGy; 6 – PHBV/PCL; 7 – PHBV/PCL/PVP-10 kGy; 8 – PHBV/PCL/PVP-15 kGy; 9 – PHBV/PCL/PVP/Ilo/A-10 kGy; 10 – PHBV/PCL/PVP/Ilo/A-15 kGy

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