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Impact of Modification on the Energy Characteristics of Surfaces and Matrix Properties of the New Effective Polymer Vascular Implants

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Abstract. New tissue-engineered vascular prostheses of small diameter (4mm) based on biodegradable polymer backbone – poly(ε-caprolactone) (PCL) and its composition with poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV/ PCL) were created. The full cycle of surface modification of the backbone with polyvinylpyrrolidone and drugs permitted to increase significantly the atrombogenic and antimicrobial properties of prostheses and provide its effective matrix properties. Both types of the developed constructs are suitable for testing *in vivo*. The energy characteristics of the prosthesis surfaces at the different interfaces were determined. It was established that the value of the energy of the "polymer, saturated with octane/water" interface can be used as a parameter for predicting cell adhesion and proliferation in the case when it is difficult to determine or to distinguish the energy characteristics of the surfaces of tissue-engineered materials at the interface with air.

Introduction

Tissue engineering is a promising area of modern biomedical materials science, which task is to develop and implement scientific principles for creating effective blood vessel prostheses [1]. Current methods of vascular tissue engineering are aimed at creating of functional implants that have a structure similar to that of native vascular tissue organization, and are able to provide patency in the long-term postoperative period (7 days after implantation of prostheses and until the end of implantation). In addition, full migration of autologous cells (i.e., the patient's cells) from the bloodstream and surrounding tissues into the highly porous wall of the prosthesis should be ensured, with the cells subsequent proliferation and differentiation in a certain direction. The basis of such vascular prostheses is an artificial tubular matrix, most often made of biodegradable natural and/or synthetic polymers (polyhydroxybutyrate, polycaprolactone and their derivatives), which have high biocompatibility, implying, first of all, low toxicity and stability of the functional characteristics of materials based on such polymers in biological environments. The matrix is a framework that is inoculated by the autologous cells under i*n vitro* or *in situ* conditions. The factor that ensures proliferation, differentiation of autologous cells and formation of extracellular matrix is their good adhesion on the surface of the vascular prosthesis [2-4].

In addition to biocompatibility, modern materials for vascular prostheses should be characterized by high antibacterial activity and low probability of inducing drug resistance. This requirement is due to the emergence of a global problem of the effectiveness of antibacterial drugs deterioration due to the occurrence of new pharmacologically resistant bacteria. Thus, despite the placement of sterile vascular prostheses in an uninfected field (under sterile conditions), about 20% of them become infected, and their occlusion (violation of patency, including that provoked by infection) can range from 21% to 27% [5]. The adhesion of pathogenic microorganisms to the

implant surfaces, followed by cell growth and colonies formation, leads to the development of a biofilm with high resistance to both antibiotics and defense mechanisms of host [6, 7]. In this case, in clinical practice, the replacement of the prosthesis is usually carried out, which increases the risk for the patient, up to the refusal of implantation.

Another important characteristic of tissue-engineered vascular prostheses is their thrombosis resistance. It is known that thrombosis of implanted prostheses occurs in the first hours and days after implantation when sheep model is used [8], while the formation of newly formed vascular tissue takes several months [9]. Thromboembolism blocks not only the blood flow, but also the further mechanisms of self-assembly of the elements of the newly formed vascular tissue *in situ*. The problem of thrombosis resistance of materials for small-diameter vascular implants is particularly relevant, which is due to the low blood flow rate in them [10, 11]. So, to date, all modern developments in field of the creation of small-diameter vascular prostheses are at an experimental level, and the market of products addressing the needs of cardiovascular surgery still lacks vascular prostheses with a diameter of less than 3 mm.

The choice of material and method to manufacture small-diameter vascular prosthesis matrixes with specified mechanical characteristics that ensure the migration of autologous cells and the self-assembly of elements and the adaptive growth of new vascular tissue *in situ* is a separate scientific issue. However, in addition to this, there is a complex task of their antimicrobial protection and giving atrombogenic properties to their inner surface.

One of the solutions to the problem of antimicrobial protection is the introduction of an antibacterial drug directly into the structure of the prosthesis. This approach solves the problem of local delivery of the drug in combination with its bactericidal effect on microorganisms until they form biofilms [12]. The most attractive compounds with antimicrobial activity, to which microorganisms do not develop resistance, are cationic surfactants – diphilic molecules with one or more hydrophilic positively charged groups and lipophilic fragments [13, 14], which can cause a violation of the transmembrane potential, leakage of cytoplasmic contents and, eventually, cell death [13]. Most of these compounds are highly effective against both gram-positive and gram-negative bacteria (including antibiotic-resistant strains), and have good selectivity relative to mammalian cells. The producibility of cationic surfactants destroying bacterial membranes and at the same time some other bacterial targets significantly reduces the likelihood of resistance to such compounds. The high stability of cationic surfactants, including stability 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 polymer materials with antibacterial properties.

The task of ensuring the thrombosis resistance of vascular matrixes can be solved by modifying their inner surface with drugs with antiplatelet and anticoagulant activity (heparin, hirudin, acetylsalicylic acid) [15, 16]. The undoubted advantage of such modifications is that this approach allows one to directly adjust the surface properties of the material without changing its mechanical characteristics.

An express and informative method for monitoring changes in the surface properties of materials as a result of modification is the wetting method, namely, measurment of the contact angles of liquids θ on the surface of solids under various conditions: advancing (θa - when applying a drop of water to the surface in air environment), receding (θr - when bringing an air bubble to the surface of a liquid immersed in a liquid) and selective wetting (θ_{WO} - when applying a drop of liquid to the surface in a medium of another liquid) (Fig.1).

For low-energy polymer surfaces, the molecular theory of wetting allows one to calculate the specific surface free energy γ_{SV} of the material, as well as its dispersive γ^{d}_{SV} and polar γ^{p}_{SV} components; their value is a measure of uncompensation of dispersion and polar Van der Waals forces at the interface: $\gamma_{SV} = \gamma^{d}_{SV} + \gamma^{p}_{SV}$. In turn, γ^{d}_{SV} and γ^{p}_{SV} determine the value of the surface free energy of the polymer-liquid interface, which is the driving force of the adhesion and adsorption processes that occur when the material comes into contact with the biological medium [17-20].

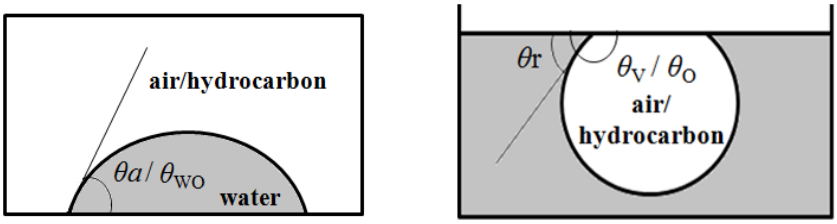

Figure 1. Scheme for measuring advancing θa / selective θ_{WO} (left) and receding θr (right) contact angles; $\theta r + \theta_V = 180°$(air), $\theta r + \theta_O = 180°$(hydrocarbon).

Despite the fact that the problem of the relationship between the degree of hydrophilicity, the energy characteristics of the surface and its interaction with biological objects has been studied for more than 50 years, by now it has not lost its relevance due to the emergence of new polymers and approaches to the production of materials based on them.

The simplest way to assess the properties of a surface is evaluation of its wettability with water under the advancing conditions. In the first approximation, at $0 < \theta a(H_2O) < 90°$, the surface is wetted with water and is considered hydrophilic; such surfaces, especially at low values of $\theta a(H_2O)$, are characterized by high γ^p_{SV}. At $\theta a(H_2O) > 90°$, the surface is considered hydrophobic, and $\gamma^p_{SV} \rightarrow 0$. In addition to the chemical nature of the polymer, the value of γ^d_{SV} is sensitive to the packing density of polymer chains in the surface layer of the material [21]. The optimal value of $\theta a(H_2O) = (50–60)°$ indicated in [2, 22] which corresponds to good cell adhesion to a solid surface, is difficult to consider as universal, since for other systems in which good cell adhesion to the material is realized, lower $\theta a(H_2O)$ values are also obtained [3, 23-25].

The γ^d_{SV} and γ^p_{SV} values are more unambiguous parameters for predicting cell adhesion. It is generally recognized that they correlate with cell adhesion (which is a key factor in the formation of new tissue on the implant surface [2-4]) and have even more significant effect on adhesion than surface roughness [26]. The crucial role belongs to γ^p_{SV}: the greater this value is, the better is cell adhesion. In [23], it is indicated that at $\gamma^p_{SV} \leq 5$ mJ/m^2, the cells do not adhere well to the surface of the material, and at $\gamma^p_{SV} > 15$ mJ/m^2, good adhesion is realized. However, the validity of these semiempirical criterion values is questionable. Unfortunately, some researchers neglect the limitations of the applicability of the molecular theory of wetting to high-energy surfaces (metals, glass, silicon), which leads to erroneous values in the calculations of γ_{SV} and its components within the framework of the molecular theory of wetting, the algorithms of which are embedded in all modern devices for determining the contact angle. Incorrect application of the wetting method results in the values of the specific surface free energy of gold γ_{SV} = 49.0 mJ/m^2 [3] instead of 1400 mJ/m^2[27], specific surface free energy of silicon γ_{SV} = 39.6 mJ/m^2 [22] instead of 1510 - 2130 mJ/m^2 [28]. The orders of magnitude of the errors in the calculations on the glass are illustrated by the data in Table 1. For high-energy surfaces, it is customary to use other methods for determining the specific surface free energy [16] or to take into account the presence of a thin wetting film of the test liquid in front of the drop front [26].

Table 1. Energy characteristics of the high-energy glass surface [17, 29], obtained in various experiments.

γ_{SV} (γ^p_{SV}), mJ/m^2	66.6 (38.0)	35.2 (not determined)	47.5 (7.6)	106-112 (80)
Reference	[3]	[4]	[23]	[29]
Method	Wetting			Ellipsometry, Zeta potential

Despite these inaccuracies, the overall picture remains unchanged: for good cell adhesion, a hydrophilic polar surface is required. In this picture, there should only be other orders of magnitude γ_{SV} and γ^p_{SV} for non-organic surfaces. Poor adhesion of gram-negative bacteria on such surfaces [3] is an additional factor in inhibiting infections after the introduction of a tissue-engineered vascular prosthesis into a living organism.

The hydrophilicity of the surface is a factor that ensures the potential hemocompatibility of the implant material in contact with blood at low blood flow rates [18, 30, 31]. The high hydrophilicity and polarity of the implant surface provides a low "polymer/aqueous medium" interfacial energy, creating a thermodynamic restriction on the adsorption of plasma proteins on the implant surface. This is especially important in the case of fibrinogen, the largest plasma protein, which conformation change upon contact with the implant surface provokes thrombosis [18]. To determine the interfacial energy in the simplest system "polymer/water" ($\gamma_{S(W)W}$), which simulates the behavior of the implant in blood plasma, a special methodological approach was developed, taking into account the possible lability of the polymer surface groups [18]. In the same work, a criterion value of $\gamma_{S(W)W}$ = (1-3) mJ/m^2 was proposed for a hemocompatible material.

The approach considering the possibility of reorientation of the polymer surface groups has been extended to other cases, in particular, to the determination of the "polymer/nonpolar liquid (octane)" γ_{SO} and "polymer saturated with octane/water" $\gamma_{S(O)W}$ interfacial energies. However, the question of whether these values can be used as parameters for predicting the biocompatibility of a polymer material (for example, in relation to cell adhesion) was not discussed by the authors [18].

Due to the above-mentioned biomedical and physicochemical aspects of the problem of creating matrixes of tissue-engineered vascular prostheses and predicting their behavior in a living system, the following tasks were set in this study:

1) creation of a tissue-engineered vascular prosthesis of small diameter in accordance with the principles of temporary protection of its polymer backbone from thrombosis and microbial contamination by modifying the surface and ensuring its effective matrix properties, namely, the formation of new tissue on its basis due to hydrophilicity and highly porous microstructure of the surface;

2) determination of the energy characteristics of the prosthesis surface at the interfaces with air and liquids of different polarities under different conditions of surface modification and search for new parameters for predicting the matrix properties of the prosthesis material.

Experimental Details

Preparation of tissue-engineered vascular prostheses

Within this work two types of polymer tubular frames with a diameter of 4 mm were made by electrospinning: first was made of a 12% solution of poly(ε-caprolactone), (PCL, Sigma-Aldrich), and second was made of a polymeric composition containing a 2% solution of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), (PHBV, Sigma-Aldrich) and a 12% solution of poly(ε-caprolactone), (PCL, Sigma-Aldrich) in 1:2 volume ratio. 1,1,1,3,3,3-hexafluoropropan-2-ol (Sigma-Aldrich) was used as a solvent. Electrospinning was performed using Nanon-01A apparatus (MECC, Japan) with needle voltages of 22 and 20 kV for PCL and PHBV/PCL grafts, respectively; the polymer solution feed rate was 0.5 ml/h, the collector rotation speed was 1000 rpm, the needle movement speed was 60 mm/sec, and the distance from the needle to the roller collector was 15 cm.

Then a hydrogel coating was formed on the inner surface of the polymer frame by the radiation-induced graft polymerization – a carrier of drugs that provide antibacterial and antithrombogenic protection. To do this, the frames were immersed in a 5% solution of polyvinylpyrrolidone (PVP; PanReac, Germany) in ethanol for 30 minutes until the internal channels were completely filled with gel; then the finished products were dried in horizontal position for 24 hours at 23°C. Covalent grafting of PVP to the surface of the prostheses was carried out in an inert atmosphere (Ar) using ionizing radiation in two different modes (with a total absorbed dose of 10 and 15 kGy) on a pulse linear accelerator ILU-10 with a beam energy of 5 MeV at 50 kW (manufacturer: G.I. Budker INP SB RAS, Russia).

The addition of drugs to the hydrogel coating was performed by complexation. For this purpose, the prostheses were kept in a modifying solution for 30 minutes, followed by drying for 24 hours in sterile conditions at 23°C. The modifying solution used is a mixture of 0.25 mg/ml solution of cationic surfactant (TBP) in methanol and 0.2 mg/ml solution of Iloprostum (Ilo) in sterile distilled water in a volume ratio of 1:99.

The drug Iloprostum (ilomedin) is a synthetic analogue of prostacyclin, registered in the territory of the Russian Federation and approved for medical use, which provides antithrombogenic protection of the prosthesis. 1,5-bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octane-1-yl) pentane tetrabromide was used as a cationic surfactant, which exhibits bactericidal activity against gram-negative and gram-positive microorganisms [32] and is used as an active substance in the veterinary drug Trividgekt 5% (Trividgektum 5%; 50.0 mg of aRNase (1.5-6ns-[N,N-1-(4-tetradecyl)diazoniabicyclo[2.2.2]octyl] pentane tetrobromide; Stovek LLC, Russia).

The designations of the examined prostheses and control samples are presented in Table 2.

Table 2. Designations of experimental and control samples of polymer matrixes.

№	Sample Name	Group
1.	PHBV/PCL	Control 1
2.	PCL	Control 2
3.	PHBV/PCL/PVP-10 kGr	Comparison 1
4.	PHBV/PCL/PVP-15 kGr	Comparison 2
5.	PCL/PVP-10 kGr	Comparison 3
6.	PCL/PVP-15 kGr	Comparison 4
7.	PHBV/PCL/PVP/Ilo/TBP -10 kGr	Test 1
8.	PHBV/PCL/PVP/Ilo/TBP -15 kGr	Test 2
9.	PCL/PVP/Ilo/TBP -10 kGr	Test 3
10.	PCL/PVP/Ilo/TBP -15 kGr	Test 4

Determination of energy characteristics of polymer matrix surfaces at the interfaces with air and liquid media

To study the surface properties, the prostheses were cut so that they were in the form of films. The specific surface free energy (γ_{SV}), its dispersive (γ^d_{SV}) and polar (γ^p_{SV}) components were calculated using the experimental values of the contact angles of distilled water (n^d_{20} = 1.333) and ethylene glycol (Sigma-Aldrich) in accordance with the two-liquid Owens-Wendt-Kaelble approach in the framework of the molecular theory of wetting [20]. The contact angles of the test liquids were measured under receding conditions (when an air bubble is brought to the surface immersed in the liquid) to ensure good reproducibility of the results on samples of high porosity. The interfacial energy of the polymer/water interface ($\gamma_{S(W)W}$) and polymer, saturated with octane/water ($\gamma_{S(O)W}$) were determined in accordance with the approach [18] using the contact angles of water and octane measured under receding and selective wetting conditions after 24-hour exposure of polymer matrixes in model systems (θ_V, θ_O and θ_{WO} at the Fig. 1). The volume of drops (bubbles) was 10-20 μl. The contact angles were determined by processing the profile of the video image of drops (bubbles) using the author's program "Promer" written by employees of the Department of Chemistry of M.V. Lomonosov Moscow State University; the program is based on the search for an approximate solution of the Laplace equation describing the profiles of curved interfaces in a gravitational field [17]. Statistical processing of the experimental data was carried out by determining the standard deviation of the measured value for each specific sample (based on the results of at least 8 measurements). The accuracy of the contact angle value determination is ±1º; all measurements were made at 20ºC.

Cell Experiment

For the study, the Ea.hy 926 cell culture was selected, representing a hybridoma line derived from human endothelium and A549/8 cells and demonstrating stable endothelial characteristics. The cells were cultured in DMEM/F12 growth medium (11320033, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with HAT (H0262, Sigma Aldrich, St. Louis, MO, USA), 10% fetal bovine serum (26140079, Thermo Fisher Scientific, Waltham, MA, USA), 1% L-glutamine solution with penicillin and streptomycin, (0378016, Thermo Fisher Scientific, Waltham, MA, USA) and 0.4% amphotericin B (15290018, Thermo Fisher Scientific, Waltham, MA, USA). The passage of the culture was carried out after 70% confluence was reached, the cells were removed from the

surface with 0.025% trypsin-EDTA solution (15400054, Thermo Fisher Scientific, Waltham, MA, USA). The experiments were carried out under sterile conditions, the cells were cultured in a CO_2 incubator at 37°C with a 5% of CO_2 and high humidity.

The matrix inoculation with cells was performed as follows. Fragments of 1.8 cm^2 were cut out of the matrix samples and placed on the bottom of the wells of a 24-well plate (3524, Corning, NY, USA). The samples were inoculated with 200,000 cells per well then cultured in 1 ml of growth medium. Renewal of the growth medium was carried out daily. The samples in two (assessment of cell proliferative activity) and three (assessment of cell viability) replicates were used in the experiment. The control group consisted of cells cultured on a culture plastic under similar conditions. The cultivation period was 4 days.

To assess the viability of cells, they were stained with Hoechst 33342 nuclear dyes (10 μg/ml, 14533, Sigma Aldrich, USA, St. Louis, MO) for 10 minutes, and with ethidium bromide (30 μg/ml, 46067, Sigma Aldrich, Sigma Aldrich, USA, St. Louis, MO) for 1 minute. Cell count on samples and culture plastic was performed using an inverted Axio Observer Z1 microscope (Carl Zeiss, Germany, Oberkochen) with 5 random fields of view (on an area of S=1 mm^2) for each replicate.

The relative degree of dead cells (n) was calculated using the formula: $n = 100\%^* (n_m/n_o)$, where n_m is an absolute number of dead cells, n_o is an absolute number of all adherent cells. The relative degree of living cells was determined by subtracting the fraction of dead cells from 100% of the adherent cells.

Cell proliferative activity was evaluated using the Click-iT™ Plus EdU Cell Proliferation Kit for Imaging (C10637, Thermo Fisher Scientific, Waltham, MA, USA). The cells were incubated with the EdU reagent for 16 hours, then stained in accordance with the manufacturer's instructions. After the procedure, the cells were stained with DAPI nuclear dye (10 μg/ml, D9542, Sigma Aldrich, St. Louis, MO, USA) for 30 minutes. Visualization was performed using a scanning confocal microscope LSM700 (Carl Zeiss, Germany, Oberkochen); 10 randomly selected fields of view with an area of S=1 mm2were analyzed for each sample at x200 magnification; 2 samples for each type of polymer were evaluated. Quantitative analysis of the images was performed via ImageJ program (National Institutes of Health, Bethesda, MD, USA), the total number of cells and the number of proliferating cells were estimated. The results were presented as a percentage of proliferating cells relative to the total number of cells.

The data collected were processed by the software package "Graph Pad Prism 7.04". The normality of distribution of values was determined using the Kolmogorov-Smirnov test. The data is presented as the median, 25th, and 75th percentiles (Me [25%; 75%]). The differences between the groups were evaluated using the Kruskal-Wallis test, adjusted using multiple comparisons Dunn-test. The differences between the values were considered statistically significant at a significance level of $p < 0.05$.

Results and Discussion

The use of PVP as a modifying component of both PCL and PHBV/PCL matrixes leads to improved adhesion and increased viability of endothelial cells. The maximum improvement in adhesion was observed on samples, PCL and PHBV/PCL, modified by PVP at a radiation dose of 10 kGr (Fig. 2). Cell viability also increased: on the surface of PCL/PVP-10 kGr viability increased to 98% (20% higher relative to the PCL matrix), and on PHBV/PVP-10 kGr it increased to 97% (5% higher relative to PHBV/PCL matrix) (Fig. 3).

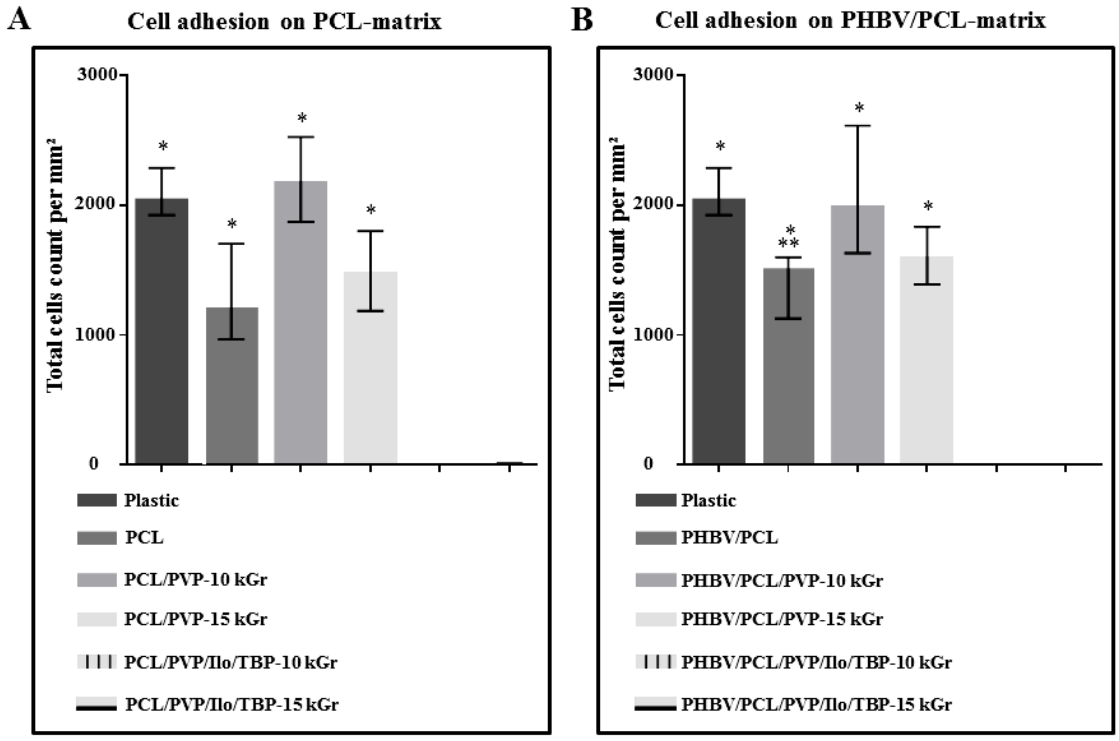

Figure 2. Cell adhesion to the matrix surfaces on PCL (A) and PHBV/PCL (B) backbone; (A): * means $p < 0.05$ compared to PCL/PVP/Ilo/TBP-10 kGr and PCL/PVP/Ilo/TBP-15 kGr; (B): * means $p < 0.05$ compared to PHBV/PCL/PVP/Ilo/TBP-10 kGr and PHBV/PCL/PVP/Ilo/TBP-15 kGr; ** means $p < 0.05$ compared to Plastic.

An increase of the radiation dose to 15 kGr led to a statistically unreliable 30% decrease in the adhesion of endothelial cells to the surface of PCL/PVP-15 kGr and PHBV/PVP-15 kGr matrixes compared to their analogues prepared with the irradiation of 10 kGr (Fig. 2). However, compared to unmodified analogues of PCL and PHBV/PCL, the adhesion of endotheliocytes to the surface of PCL/PVP-15 kGr and PHBV/PVP-15 kGr matrixes increased by 12 %, which also shows a tendency to improve the biocompatibility of the matrixes when the surface modification option is used. Against this background, the cell viability did not change (Fig. 3).

Modification of PCL/PVP and PHBV/PCL/PVP matrixes with target drugs (iloprostum and cationic surfactant) allowed us to obtain a functionally active surface that prevents the attachment of endothelial cells (Fig. 2). It is also important that the insignificant number of cells (on average, 1.26 cells/mm^2) of Ea. hy 926, which was still detected on matrixes with drugs, remained viable in 100% of cases.

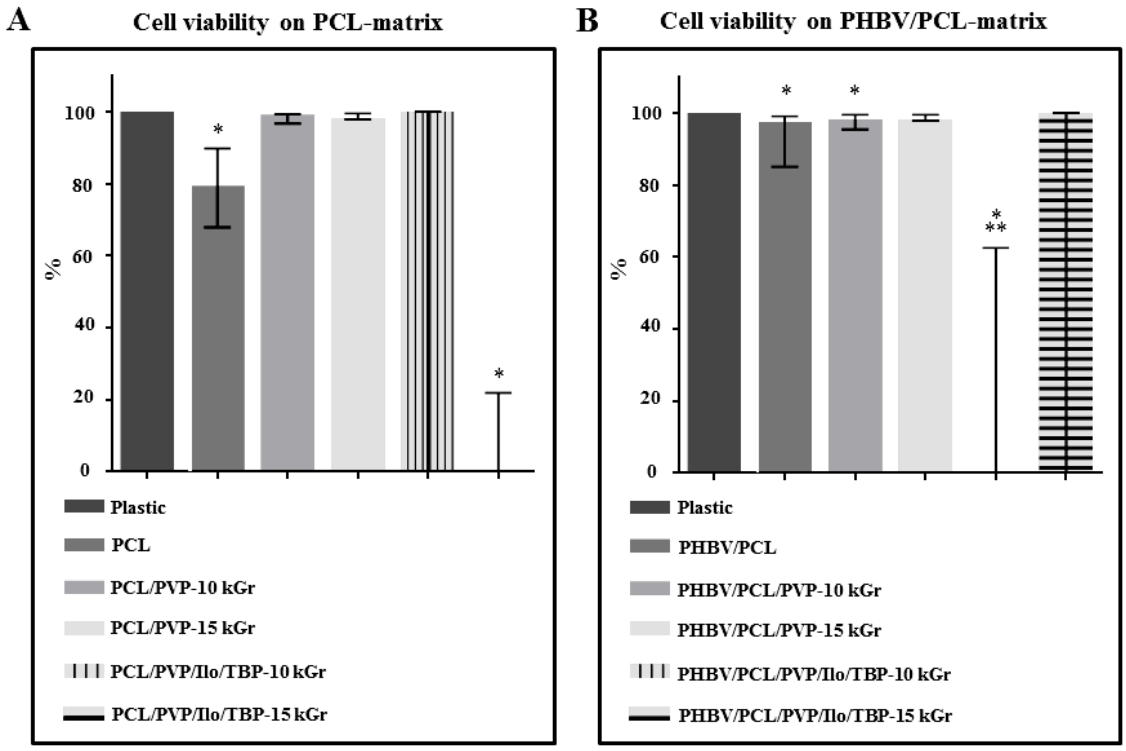

Figure 3. Cell valiability at the matrix surfaces on PCL (A) and PHBV/PCL (B) backbone; (A): * means $p < 0.05$ compared to Plastic; ** means $p < 0.05$ compared to PCL/PVP/Ilo/TBP-15 kGr. (B): * means $p < 0.05$ compared to Plastic.

The highest proliferative activity of cells was observed in control samples of cells cultured on culture plastic (Fig. 4), which is the "gold standard" surface for the cultivation of adhesive cell cultures (Fig. 4). There were no statistically significant differences between the control on plastic and the PCL and PHBV/PCL matrixes that do not contain drugs, which characterizes the high biocompatibility of the polymers used both for the preparation of the matrixes and for their subsequent surface modification. Nevertheless, some trends can be noted.

For unmodified PHBV/PCL matrixes, the percentage of proliferating cells was less than that for PCL (Fig.4). The addition of PVP with an increase in the radiation dose led to an increase in the proliferative activity of cells (Fig. 4) on PHBV/PCL matrixes, which may be associated with a decrease in the density of the cell layer, which is observed with an increase in the radiation dose. In the case of PCL matrixes, an increase in the radiation dose during PVP modification also stimulated the proliferative activity of cells, but its parameters remained below the initial values obtained on PCL matrixes (Fig.4).

The single cells observed on the PHBV/PCL/PVP and PCL/PVP samples modified with drugs were in the vast majority of cases proliferating, which in general gave very high (up to 100%) proliferation rates, having statistically significant difference from those for matrixes without drugs (Fig. 4).

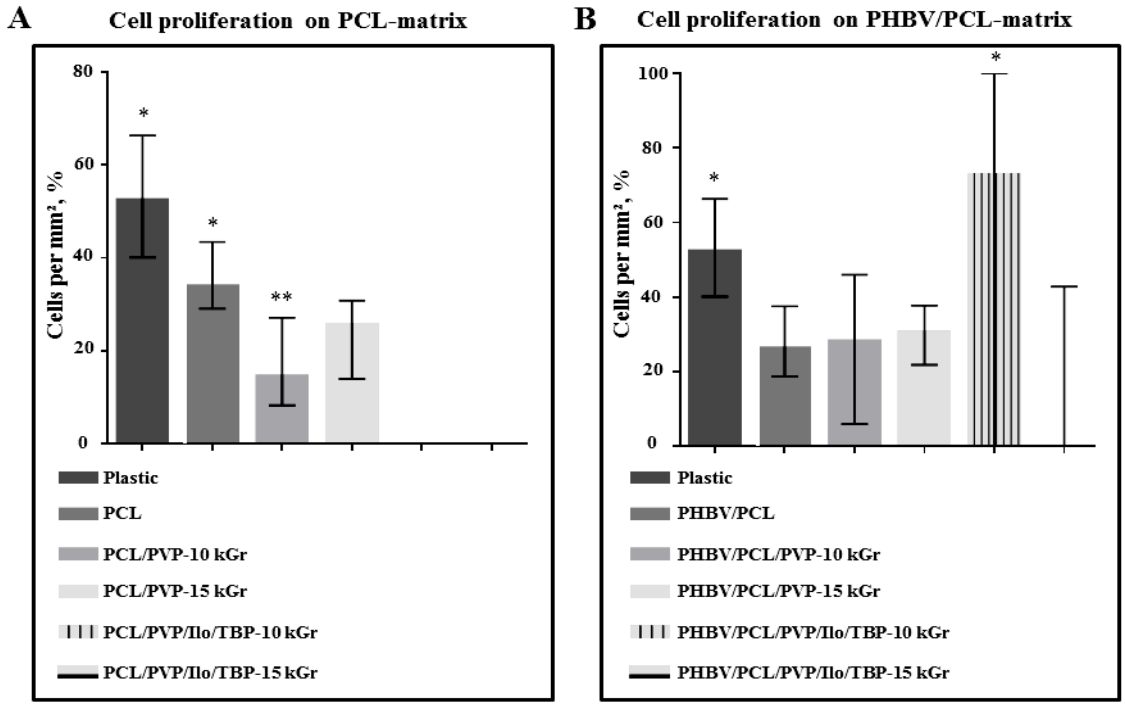

Figure 4. Proliferative activity Ea.hy 926 on PCL (A) and PHBV/PCL (B) matrixes; (A): * means $p < 0.05$ compared to PCL/PVP/Ilo/TBP-10 kGr and PCL/PVP/Ilo/TBP-15 kGr; ** means $p < 0.05$ compared to PCL/PVP-10 kGr; (B): * means $p < 0.05$ compared to PCL/PVP/Ilo/TBP-15 kGr.

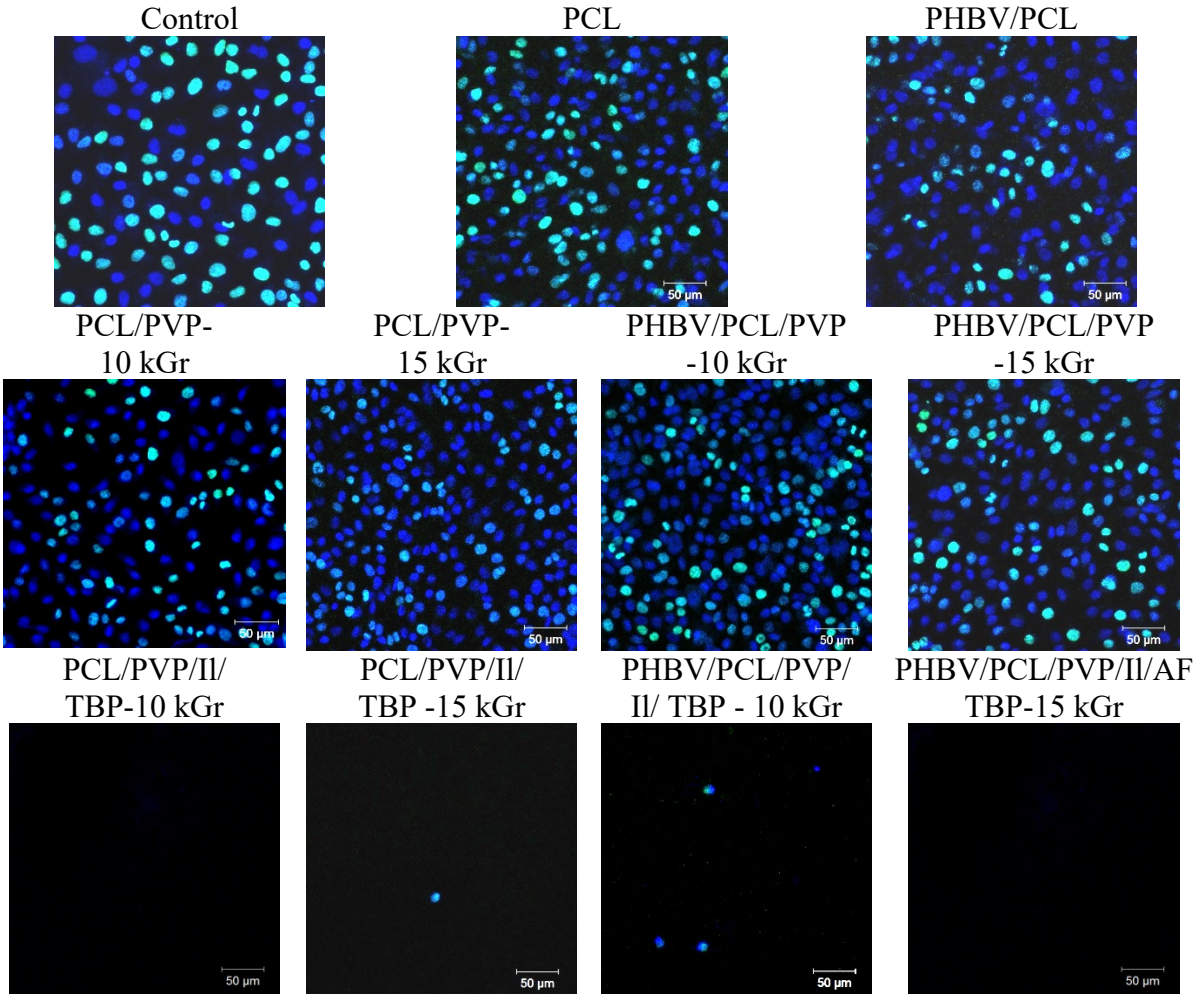

Figure 5. Confocal microscopy of Ea. hy 926 with proliferation staining (green) and DAPI finishing staining (blue). Representative micrographs, x200 magnification.

The surfaces of all the studied materials were hydrophilic, and the water contact angles on them differed insignificantly (Table 3). However, the introduction of PHBV into PCL (samples 1 and 2) and the modification of PCL and PHBV/PCL matrixes by PVP in combination with irradiation up to 15 kGr resulted in a slight hydrophilization of the surface (samples 1 and 4, 2 and 6).

Table 3. Receding contact angles (θr) of water and ethylene glycol (EG) at the matrix surfaces; contact angles in "matrix/liquid" model systems (θ)[18]; energy characteriscics of matrix surfaces at the interface with air (γ_{SV}) and in model system ($\gamma_{S(O)W}$) [18]; X = γ^{p}_{SV}/ γ_{SV}.

No. of sample	θr, deg		γ_{SV}, mJ/m^2			X, %	θ, deg			$\gamma_{S(O)W}$ mJ/m^2
	water	EG	dispersive	polar	total		O	V	WO	
1	30	20	14	50	62	76	154	153	156	47
2	36	25	14	46	60	77	152	152	154	46
3	34	26	14	48	62	77	163	157	138	49
4	31	20	15	49	64	77	166	156	146	50
5	34	28	13	49	62	79	166	164	148	50
6	30	24	13	51	64	80	165	164	151	50
7	37	22	15	44	59	75	152	155	154	46
8	29	20	14	50	64	78	153	142	153	46
9	41	22	16	41	57	72	148	152	147	44
10	30	24	13	51	64	80	152	150	149	46

The values of the specific surface free energy and its polar and dispersion components are similar in most of the studied samples (Table 3). This may be due to minor changes in the cosines of the contact angles, the values of which are used in the calculations, in the region of the experimentally fixed receding contact angles of the test liquids. The obtained result does not allow us to unambiguously link the degree of hydrophilicity of the matrix surface and cell adhesion, although samples 7 and 9 show a lower surface polarity, which correlates with a deterioration in cell adhesion when modifying the PBV/PCL/PVP and PCL/PVP matrixes with drugs at irradiation dose of 10 kGr. An increase in the radiation dose seems to have an effect on the microrelief of the matrixes surface, which leads to an improvement in wettability and overestimated values of the energy characteristics of the surfaces calculated using the effective values of the contact angles.

Thus, the polarity of the surface at the interface with the air in this case cannot serve as a reliable criterion for good cell adhesion, despite the fact that this concept is accepted and used to interpret the results of research in this area [3, 23-25].

The values of $\gamma_{S(W)W}$ for all of the samples studied are also close (tenths to 1 mJ/m^2). This means that the adsorption of plasma proteins on the sample surfaces is not thermodynamically advantageous, and all of the materials are thromboresistant [18]. At the same time, such low values of interfacial energy indicate the possibility of spontaneous dispersion of the material in an aqueous medium, which is provided for in the technology of tissue engineering. In this case, it is necessary to study in detail the kinetic aspects of matrix dispersion and the formation of new tissue on their basis.

With a slight difference in the contact angles of water on the matrix surface and close values of the dispersion and polar components of the specific surface free energy, the values of $\gamma_{S(O)W}$ can be a more reliable indicator of the degree of hydrophilicity of the material and the surface polarity; $\gamma_{S(O)W}$ will have the maximum values for the most hydrophilic and polar surfaces. Indeed, for samples 3 and 5, for which the highest cell adhesion is observed, the highest values of $\gamma_{S(O)W} \geq 49$ mJ/m^2 were obtained (Table 3). Similarly high values of $\gamma_{S(O)W}$ against the backdrop of reduced cell adhesion (samples 4 and 6) may be due to an increase in the roughness of the matrix surface with an increase in the radiation dose, which leads to an improvement in wetting and distortion of the results of the calculation of $\gamma_{S(O)W}$. On the other hand, an increase in the radiation

dose during modification stimulates cell proliferation, and, thus, $\gamma_{S(O)W} \geq 49$ mJ/m^2 can act as a parameter for predicting the formation of new tissue based on a prosthesis as a matrix. To test the effectiveness and universality of such a prediction, it is advisable to conduct a similar study on other polymer and cellular objects.

Conclusion

In accordance with the tasks set in the work, the following conclusions can be drawn.

(1) New tissue-engineered vascular prostheses of small diameter with effective matrix properties have been created. Modification of the surface of the polymer frameworks of prostheses with polyvinylpyrrolidone (capable of swelling and spontaneous dispersion in an aqueous medium) and drugs (suppressing cell adhesion at the biochemical level) is able to prevent thrombosis and microbial contamination of prostheses implanted in the vascular bed in the early postoperative period. Based on the conducted cellular experiment, it is expected that after the complete dispersion of the modifying layer and the release of drugs with athrombogenic and antimicrobial properties, passable vascular prostheses based on PHBV/PCL and PCL will be maximally suitable for the formation of new vascular tissue on their basis.

(2) The PVP was grafted to the surface of the biodegradable prostheses using the radiation-induced graft polymerization method. At the same time, a dose of 15 kGr was used for the sterilization of products. However, ionizing radiation can affect biocompatibility properties. We proved that the use of a total absorbed dose of 10 or 15 kGr do not significantly reduce the adhesive characteristics of the matrixes and the number of viable cells compared to the matrixes that were not exposed to radiation. Therefore, in the future, the choice of the radiation dose of 15 kGr will be preferable, since, without adversely affecting the biocompatibility, it will allow polymerization of polyvinylpyrrolidone with the surface of biodegradable prostheses and sterilization of prostheses to be performed in one step.

(3) The energy characteristics of the prosthesis surfaces at the interfaces with air and liquids of different polarities (water, octane) were determined. It is established that the value of the energy of "polymer saturated with octane/water" interface can be used as a parameter for predicting cell adhesion and proliferation in the case when it is difficult to determine or to distinguish the energy characteristics of the surfaces of tissue-engineered materials at the interface with air.

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