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Surface properties and *in vitro* biocompability of a track membrane based on polyethylene terephthalate after exposure to low-temperature atmospheric plasma and ionizing γ -radionuclide ^{60}Co

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ABSTRACT

Aim. This research studies the effect of a low-temperature atmospheric plasma and the subsequent γ-ray sterilization on topography and properties of track membranes (TM) based on polyethylene terephthalate (PET).

Materials and methods. TM were obtained by irradiating a PET film with a $^{40}Ar^{+8}$ ion beam and then by chemical etching in an aqueous solution of 1.5N NaOH. Modification of the membrane surface was carried out by exposure to an atmospheric low-temperature plasma. The gamma radiation of the radionuclide ^{60}Co with the dosages of 1kGy (SI) and 10 kGy (SI) was used to sterilize the membranes. In vitro studies of the TM biocompatibility were performed by using a culture of prenatal stromal cells isolated from a lung of an 11-week human embryo and maintained ex vivo.

Results. It has been established that the treatment of the membranes with the low-temperature atmospheric plasma leads to an increase in the roughness and hydrophilization of the TM surface. The change in the physical-chemical state of the TM surface as a result of the exposure of cold plasma and subsequent sterilization had practically no effect on the morphofunctional state of the culture of human prenatal stromal cells. In vitro tests on the TM cellular-molecular biocompability with a short-term culture of in vitro fibroblast-like cells have made it possible to indicate their relative bioinerticity with respect to human stromal cells. The conclusion is made about the relative bioinerticity of TM and the proposed regimes for their sterilization with respect to the culture of human stromal cells, the prospects for further research in applying the material to the areas of surgical practice (cardiology, ophthalmology).

Key words: track membranes, polyethylene terephthalate, human stromal cells, biocompatibility, atmospheric low-temperature plasma, sterilization, morphofunctional reaction.

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INTRODUCTION

Polyethylene terephthalate (PET), due to its structure and high biological compatibility, has the potential for use in ophthalmology [1, 2, 3], and is also widely used in cardiosurgery as a material for vascular prosthetics [4]. Nevertheless, existing medical products from PET are far from perfect. Modern technologies make it possible to obtain nanoporous PET films (so-called track etched membranes, TM) [5], which can be considered as a prototype of the basilar membranes of capillaries. However, the amount of the surface energy of the TM based on PET is fairly low (~32 MJ/m^2) [6], resulting in the fact that surface properties of the material, such as hydrophilicity, do not always meet the requirements, which is critical for the usage of a membrane as corneal implant [7].

The exposure to low-temperature plasma is one of the most promising and innovative methods of modifying the surface of polymer materials. The plasma is the source of free radicals in a hydroxy groups, along with oxygen atoms and ions, oxygenated molecules, as well as charged particles and photons. The advantage of the exposure to plasma is the low depth of penetration of its particles into the material and the change of properties only in the surface layer of the material, without any significant heat input [8, 9, 10]. The surface interaction of a number of organic materials, polypropylene, polyethylene terephthalate [11] as well as unsaturated types of rubber [12] with nitrogen or nitrogen-bearing plasma forms nitrogen-bearing groups in the surface layer of a polymer as imide and urethane groups, thus increasing the surface biocompability as well as increasing wettability, which is able to maintain itself for a long time (up to 120 days).

It is necessary to distinguish the ionizing radiation sterilization among the existing methods of sterilizing for medical implants, widely used for the polymers, particularly for polyethylene terephthalate [13]. Still, the exposure of the high-energy radiation creates a risk of decomposing the material, with emission of toxic ethylene glycol monomers, as well as changing material surface and bulk properties, also creating a synergistic effect of the plasma modification and the γ-sterilization on the TM properties [14].

According to the GOST standards group ISO 10993-2011, the in vitro studies of biocompatibility by the way of direct and indirect contact of the material with the cellular culture of modified potential biomedical products is one of the first stages in establishing prospects and risks of its usage [15].

The aim of the research is to study the morphofunctional reaction of the human prenatal stromal cells culture to the changes of the surface properties of a track membrane based on polyethylene terephthalate after the exposure to the low-temperature atmospheric plasma and ionizing γ-radionuclide ^{60}Co.

MATERIALS AND METHODS OF RESEARCH

The track membranes made of polyethylene terephthalate were obtained by irradiating the polymeric film with the $^{40}Ar^{+8}$ ion beam with the radiant energy of 41 MeV, which was followed-up by the chemical etching in an aqueous solution of NaOH with the concentration of 1.5 N in the range of temperature 72–82 °C.

The modification of the track membranes surface was conducted using the atmospheric low-temperature plasma experimental device, based on the charge barrier (Tomsk Polytechnic University) [16]. The time of the plasma exposure on each surface of the membranes comprised 30 seconds.

The gamma radiation of the radionuclide ^{60}Co with the dosages of 1kGy (Si) and 10 kGy (Si) was used to sterilize the membranes. In the course of experiments, the γ-ray irradiation was conducted both before and after the plasma treatment, enabling to assess the influence of the successive exposures on the changes of the main properties in the membranes under study.

Pictures of the membranes surfaces, obtained by using the Hitachi S3400N Type II (Japan) microscope, were used to establish the size and the surface denstiy of the pores in the track membranes.

The surface topography was studied with the multipurpose correlator of optical, spectral and topographical surface objects properties - "Centaur HR" (Russia). The surface roughness was estimated using the Gwyddion software.

The wetting angles for purified water ($\theta_W{}^0$) and glycerol ($\theta_g{}^0$) were calculated using the sessile drop technique with the room temperature of 25±2 °C, the "KRbSS EasyDrop DSA 20"(German) device and the special software, with the measurement accuracy of ± 0.1°. The contact angle was calculated on days 1, 3, 7, 14, 21 after the exposure of plasma and also after the γ-sterilization of the modified samples within the same timeframe.

The full surface energy (σ_s) was viewed within the Owens/Wendt model [17], as superposition for dispersive (σ^d_s) and polar (σ^p_s) parts, calculated using the Owens, Wendt, Rabel and Kaelble method (OWRK).

$$\frac{\sigma_l \cdot (\cos\theta + 1)}{2\sqrt{\sigma_l^d}} = \frac{\sqrt{\sigma_l^p}}{\sqrt{\sigma_l^d}} \cdot \sqrt{\sigma_s^p} + \sqrt{\sigma_s^d} \qquad (1)$$

Polarity of the membranes, defined as a part of proportion of the polar componet in the overall surface energy, was calculated using the following formula [18]:

$$p = \sigma^{p}_{s} / \sigma_{s}. \quad (2)$$

The samples exposed to plasma were stored in the open air and the samples exposed to γ– irradiation were stored in the special bags for sterilization.

The infrared spectrum for the original membranes, modified membranes as well as modified membranes after the sterilization was determined using the infrared-Fourier spectrometer Nicolet 5700 (USA).

Cell culture. In vitro studies of the track through membranes were conducted using the previously mentioned methods [19, 20]. The experiments used the prenatal stromal cells culture, originally isolated from the light 11-week human embryo maintained ex vivo (line FL-42, Stem cell bank, Tomsk). After defrosting, the samples represented a population of cells round in shape and size, with the limited shelf life, preserving stable karyotype during transits and oncogenicaly safe. The cells stick to plastic, take the fibroblast-like morphology and are able to differentiate into fibroblasts and osteoblasts [21]. After defrosting, cells viability equaled 94%, and was defined, according to the ISO 10993-5 standard, by the test using 0.4% trypan blue.

Track membranes (TM) with the modified surface (samples with the linear size of 10Ч10 mm^2 and with the length of up to 10 μm) were put in 24 well culture plates (OrangeScientific, Belgium). Each group had 3 matrices. A control group comprised the cell culture on the plastic surface of the culture plates without the addition of the samples. TM occupied 60% of the surface area of the wells in the culture plates. Non-cellular culture medium or cell suspension with the concentration of 3×10^4 viable karyocytes in 1 ml of the full culture medium were added in the wells of the 24 well plates (OrangeScientific, Belgium). Culture medium composition: 280 mg/L of L-glutamate, 50 mg/L of gentamicin sulfate, 20% of cow embryo blood serum, 80% of the DMEM/F12 culture (1:1).

The membranes were removed with tweezers after 72 hours (3 days) of cultivation under the temperature of 37°C and 100% humidity in the open air. Supernatants (conditioned media) were obtained by gathering the supernatant part of the cell cultures and their subsequent 10-minutes centrifugation with 500 g. The alkaline phosphatase (AP) activity, the concentraions of calcium (total and free), potassium and inorganic phosphate in the interstitial fluid were determined by the standard method of colorimetry, using the biochemisty analyzer Konelab 60i (USA), following the common biochemistry technique and the protocols of the specialized sets by Thermo Fisher Scientific Inc. (USA).

The plates were dried out in the air under the room temperature for 24 hours. Later, the cells that stuck to the plastic around the TM had been fixed in the formaldehyde fumes for 30 seconds to get the immunocytochemical staining for vimentin. Immunocytochemical operations with the cells on the TM were impossible, due to the insufficient optical transparency of the studied material, which did not allow indicating slight changes of the cytoskeleton.

The staining for vimentin was conducted in the following way. The cell culture had been fixed and permeabilized in the cold (-20^{0}C) methanol for 1 minute. The immunocytochemical analysis was conducted after that. Mouse monoclonal antibodies were added to human vimentin (clone V9, Novocastra™, United Kingdom). Working dilution of the primary antibodies was 1:500. The visualization was performed on the basis of the immunoperoxidase method protocol, recommended by the producer of the primary antibodies, using Novolink™ Polymer Detection Systems set (United Kingdom). 3,3'-Diaminobenzidine (DAB) was used to register the immune response. The response to vimentin was considered positive, if the specific brown color would be discovered in the cell culture cytoplasm.

Statistical analysis. The results of the experiment were processed with the software STATISTICA 10.0. The following statistical parameters were calculated: values of mean (M), standard deviation (SD,σ) and the standard error of the mean (m) for the physical parameters, median (Me), 25% quartile (Q_1) and 75% quartile (Q_3) for the biological parameters. A parametric Student's t-test (Pt) and a nonparametric Mann-Whitney test (U-test, P_U) were used to assess the statistical significance. The differences were considered statistically significant if p was < 0.05.

RESULTS AND DISCUSSIONS

Figure 1 shows an electron micrograph of the TM surface element.

As it is seen from figure 1, the pores on the TM surface are distributed rather evenly. The calculations based on the data collected by the electron microscope showed the average size of the pores – 0.5 μm and the surface density - 5×10^8 pores/cm^2. Figure 1c also shows the appearence of the defects of irregular shape (fig. 1c, indicated with an arrow), as a result of the γ–irradiation. Such structure changes were observed when the surface was studied using atomic-force microscopy (fig. 2).

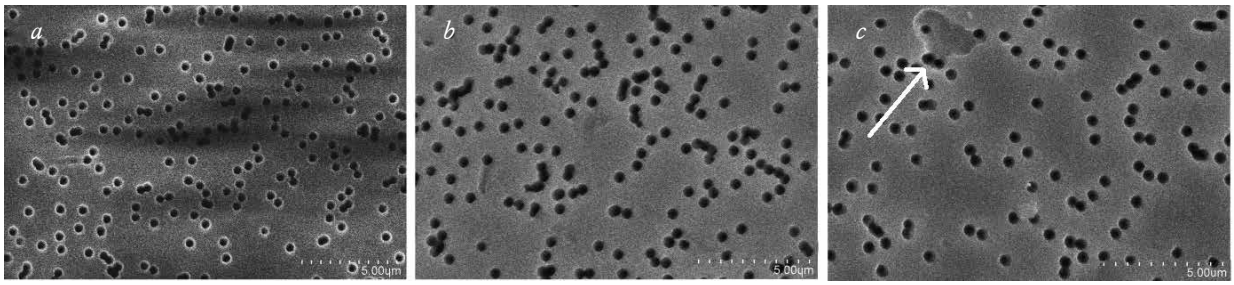


Fig. 1. Electron microscopic image of the surface of the original TM (*a*), TM after plasma modification (*b*) and after sterilization with a dose of 1 kGy (*c*)

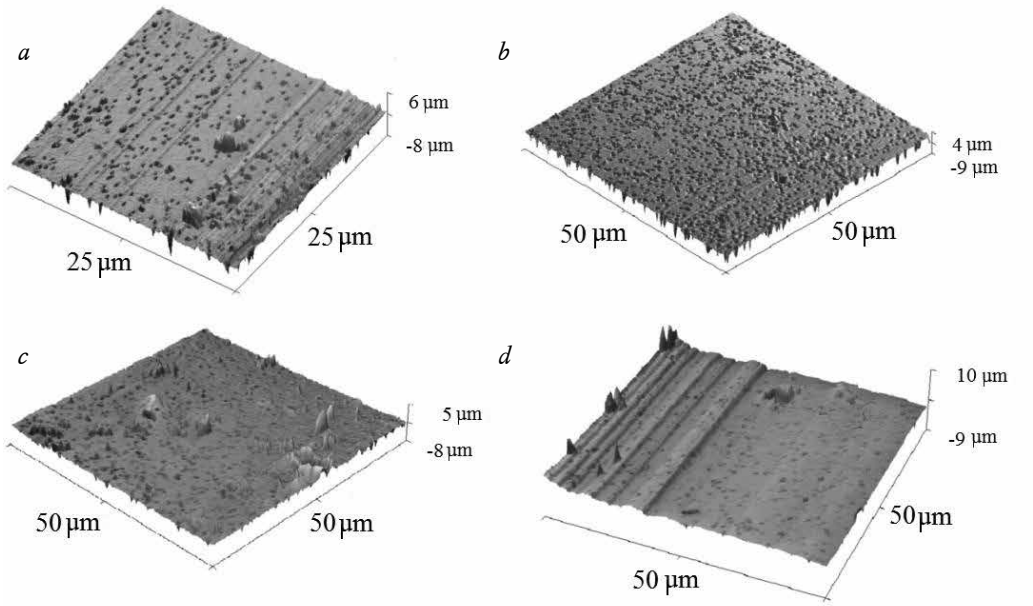


Fig. 2. Surface topography of original TM (*a*), after plasma modification (*b*), after sterilization (1 kGy) of source (*c*) and plasma-modified TM (*d*)

Таблица 1
Table 1

Относительная площадь (%) и глубина дефектов ТМ после γ-стерилизации модифицированных и не модифицированных плазмой образцов, *M ± m*

Relative area (%) and depth of defects of TM after γ-sterilization of modified and not modified by plasma samples, *M ± m*

Доза стерилизации, кГр Sterilization dose, kGy	Без плазменной обработки Without plasma treatment		Обработка поверхности плазмой Plasma treatment	
	Относительная площадь дефектов, % Relative area of defects, %	Глубина дефекта, мкм Depth of defects, μm	Относительная площадь дефектов, % Relative area of defects, %	Глубина дефекта, мкм Depth of defects, μm
1	7,9 ± 0,9	4,25 ± 1,1	8,1 ± 0,4 $p > 0,47$	4,06 ± 2,8 $p > 0,51$
10	6,1 ± 1,3	3,87 ± 2,3	8,1 ± 0,9 $p > 0,32$	4,11 ± 0,7 $p > 0,18$

П р и м е ч а н и е: *p* – уровень статистической значимости различий по сравнению с необработанными плазмой стерилизованными образцами.

N o t e: *p* – the level of statistical significance of differences compared to untreated plasma sterilized samples.

Таблица 2
Table 2

Средние значения параметров поверхности ТМ: шероховатость R_a, поверхностная энергия σ_s, дисперсионная σ^d_s и поляризационная σ^p_s составляющие поверхностной энергии, угол смачивания: θ_W^0 (вода), θ_g^0 (глицерин)

Average values of TM surface parameters: roughness R_a, surface energy σ_s, dispersion σ^d_s and polarization σ^p_s components of surface energy, wetting angle: θ_W^0 (water), θ_g^0 (glycerin)

Образец Sample	R_a*	σ_s	σ^d_s	σ^p_s	Полярность Polarity	θ_W^{0}*	θ_g^{0}*
Пленка ПЭТФ PET film	0,002	36,76	29,15	7,61	0,2	61,1	76,5
ТМ исходная TM naive	0,031	29,95	5,97	23,98	0,8	72,8	74,8
ТМ + 1 кГр TM + 1 kGy	0,028	43,73	0,30	43,43	0,99	68,7	77,2
ТМ + 10 кГр γ TM + 10 kGy γ	0,03	37	0,9	36,34	0,98	72,3	80,9
ТМ + pl 30	0,103	131,53	7,33	124,21	0,94	33,0	73,3
ТМ + pl 30 + 1 кГр TM + pl 30 + 1 kGy	0,055	110,3	3,1	107,2	0,97	36,0	70,5
ТМ + pl 30 + 10 кГр TM + pl 30 +10 kGy	0,055	120,1	7,08	113,02	0,94	39,1	76,9

Примечание: pl – обработка плазмой; параметр шероховатости dim R_a = мкм; поверхностная энергия dim σ = мДж/м²; контактный угол dim θ = градус (θ°).* средние величины трех измерений.
Note: pl – plasma treatment; roughness parameter dim R_a = мкм; surface energy dim σ = mJ/m²; contact angle dim θ = degree (θ°).* the data given are average values.

The relative area of the defected zones, defined as a relation of the defected elements of the area to the overall area, was estimated graphically (table 1).

According to the data in table 1, there are no statistically significant differences between the samples irradiated with the doses of 1 kGy and 10 kGy ($p > 0.05$).

The information on the roughness of the surface is shown in table 2. According to this data, the exposure of the original TM to the γ -rays does not influence the R_a value significantly, unlike the plasma treatment, which leads to a significant (more than by 4 times) increasing in the parameter of roughness for the original samples. γ-sterilization of the plasma modified TM reduces the membranes R_a value by 0.05 μm after the plasma treatment.

The measurement results of the wetting angle show that the original TM has a low-grade

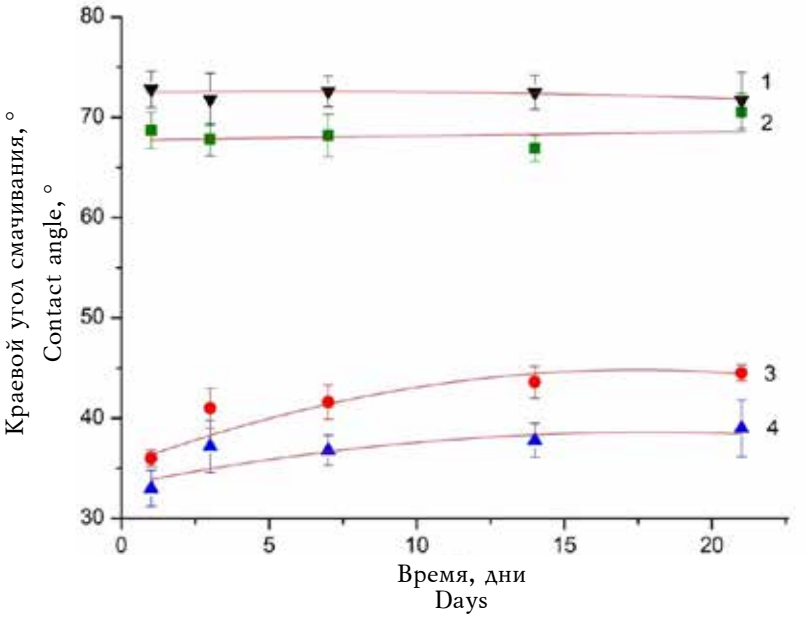


Fig. 3. Dependence of the value of contact angle of wetting θ_W^0 on storage time: *1* – virgin TM; *2* – after γ-sterilization in a dose of 1 kGy; *3* – after plasma treatment and γ-sterilization in a dose of 1 kGy; *4* – after plasma treatment

hydrophilia, with the mean value of θ = 72.0°. The exposure of the low-temperature plasma on the TM surface lead to a rapid increase in hydrophilicity and reduction of the contact angle θ by 40°– 43°. The θ measurement dynamics from the storage time showed the growth of the contact angle during the first 3 days by (5-7) ϵ and the relative stability for the rest of the time (fig. 3).

Sterilization of the original TM by γ-radiation of the ^{60}Co radionuclides did not influence the wettability significantly (table 2), however, it contributed into the θ increase of the modified samples by (3-5)ϵ. The θ measurement dynamics of the TM subsequently modified by plasma and sterilized by γ-irradiation showed the growth of the contact angle by 10°–12° in the first 3 days of storage (fig. 3).

Table 2 shows that PET belongs to the class of the low polarity polymers with the film polarity equal to 0.22 [22]. The track membrane forming process, that includes the ion irradiation and the chemical etching, increased the value of the polar σ^p_s part of the full energy significantly, which defines the membrane surface as highly polar (polarity p = 0.8). The TM exposure to the plasma lead to a significant increase of the surface energy, mostly because of σ^p_s and was equal σ_s 131.5 MJ/m^2 (table 2).

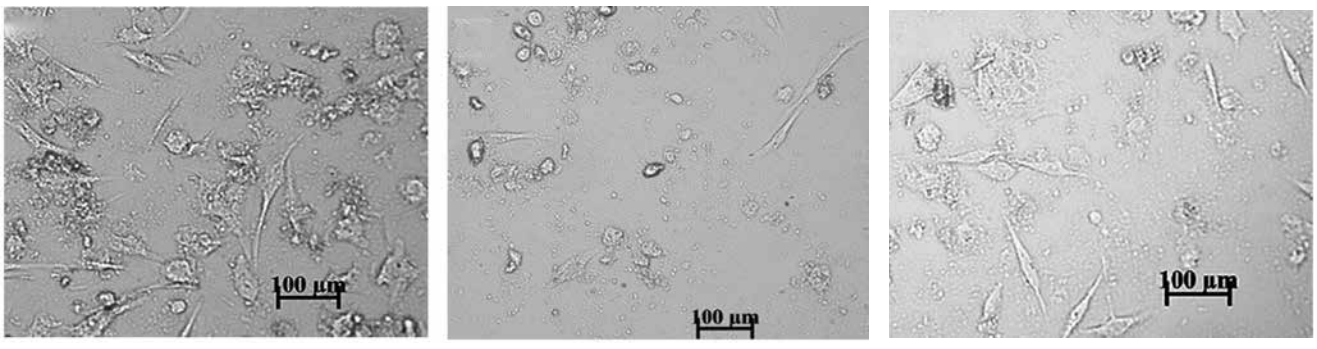
Infrared spectroscopy shows that the exposure to plasma has lead to some decrease in the TM absorption lines 1712 cm^{-1}, 1241 cm^{-1} and 1093 cm^{-1} , that are connected with the non-polar (C=C, C=O) functional groups in the surface layer of the membranes, which corresponds with the results of the works [23, 24, 25], signifying the reduction of the non-polar (hydrophobic) functional groups C=C and C=O after the low-temperature plasma treatment.

Presumably, the exposure to plasma contributes into the destruction of the TM surface polymer chains, which are in the amorphous phase. Such exposure breaks the C-O and C-C bonds and creates carboxyl groups. This determines the increase in roughness and hydrophilicity of the surface.

A slight decrease in the intensity of the absorption line 1716 cm^{-1} was noted in the infrared-absorption spectres, after the sterilization of the TM samples modified by plasma by ^{60}Co γ-irradiation. Such changes illustrate the decrease in the number of the polar functional groups, which resulted in a mild reduction of the surface wettability (sterilization of the modified samples incresead the wettabiltity contact angle by 3°–5°).

Track membranes biocompatibility in vitro. The physical and chemical properties changes of the through track membranes surface as a result of exposure to cold plasma conditioned in vitro studies of the stromal cells reaction on the tested materials. A problem for polymer medical devices is an irritation of the connective tissue cells, which often forms a thick caplsule around the implants [26]. An excessive encapsulation might lead to a failure of their ophthalmological supplements.

The results showed low demonstration of vimentin (an intracellular marker for fibroblasts [27] in the occasional prenatal stromal cells around the TM) in the treatment groups, i. e. similar to the control group (fig. 4). This indicates that possible products of the track membranes destruction after high energy exposure do not influence differentiation and growth of the stem cells of the mesenchymal stromal cells (MSC) pool into fibroblasts and the risk of developing excessive fibrovascular proliferative reaction is minimal.



a *b* *c*

Fig. 4. Status of a 3-day culture of fibroblast-like prenatal stromal cells isolated from the human lungs under conditions of co-cultivation with the test materials. Coloring on vimentin (sections of cells of brown color). *a* – control of cell growth; *b* – TM after sterilization of ^{60}Co; *c* – TM after treatment with cold plasma followed by sterilization

Таблица 3
Table 3

Минеральный и биохимический состав супернатантов 3-суточной культуры пренатальных стромальных клеток легкого человека (контроль роста) при прямом контакте с модельными мембранами после различной обработки их поверхности, *Me* (Q_1–Q_3)

Mineral and biochemical composition of supernatants of a 3-day culture of prenatal stromal cells of the human lung (growth control) with direct contact with model membranes after different treatment of their surface, *Me* (Q_1–Q_3)

№ групп № group	Исследуемая группа Study group	pH	Кальций ионизированный, мМ Ionized calcium, mM	Кальций общий, мМ Calcium total, mM	Фосфат-ионы, мМ Phosphate-ions, mM	Калий ионизированный, мМ Ionized otassium, mM	ЩФ, Ед/л Alkaline phospha-tase, U/l
Контрольная культура без ТМ Control culture without TM							
1	Полная культуральная среда (ПКС), $n = 4$ Full culture medium (FCM), $n = 4$	9,00 (8,89–9,12)	1,15 (0,96–1,17)	1,64 (1,25–1,68)	0,78 (0,73–0,80)	5,85 (5,7–5,95)	22 (19,5–24,5)
2	Контроль роста фибробластоподобных клеток на пластике, $n = 4$ Control of the growth of fibroblast-like cells on plastics, $n = 4$	8,86 (8,78–9,01)	1,21 (0,95–1,24)	1,58 (1,11–1,61)	0,73 (0,67–0,77)	5,70 (5,25–5,95)	44,5* (41,5–47) $p_1 <$ 0,00012
ТМ после стерилизации ^{60}Co в дозе 10 кГр TM after sterilization ^{60}Co in a dose 10 кGry							
3	ТМ в ПКС без клеток, $n = 3$ TM in FCM without cells, $n = 3$	8,88 (8,85–8,88)	1,18	1,33 (1,27–1,36)	0,69	6,1* $p_3 < 0,05$	18 (15–22)
4	ТМ в контакте с клетками, $n = 3$ TM in contact with cells, $n = 3$	9,03* (9,03–9,04) $p_3 < 0,05$	1,17	1,62 (1,59–1,64)	0,77 (0,76–0,77)	6,0* $p_3 < 0,05$	45* (45–47) $p_3 < 0,05$
ТМ после обработки АНП с последующей стерилизацией ^{60}Co в дозе 10 кГр TM after cold plasma treatment followed by sterilization Co^{60} in a dose 10 кGry							
5	ТМ в ПКС без клеток, $n = 3$ TM in FCM without cells, $n = 3$	9,15* (9,13–9,15) $p_3 < 0,05$	1,18 (1,17–1,18)	1,27 (1,25–1,27)	0,65* (0,65–0,67) $p_1 < 0,02$ $p_3 < 0,05$	6,1* $p_1 < 0,05$	17* (16–18) $p_1 < 0,04$
6	ТМ в контакте с клетками, $n = 3$ TM in contact with cells, $n = 3$	9,09* (9,09–9,10) $p_4 < 0,05$	1,13* (1,12–1,13) $p_4 < 0,05$ $p_5 < 0,05$	1,53 (1,52–1,82)	0,76 (0,73–0,77)	6,0* $p_5 < 0,05$	42* (42–44) $p_4 < 0,05$ $p_5 < 0,05$

Примечание: n – количество исследованных образцов (лунок) в планшете.
* статистически значимые различия согласно U-критерию Манна – Уитни.
Note: n – number of samples (wells) in the plate.
* statistically significant differences according to the U test Mann – Whitney.

Sorption of ions (primarily calcium) on the surface of the implants is an adverse event for the soft tissues [19] and may damage their optical performance. Changes in the levels of calcium, inorganic phosphorus and the AP activity in the biological environments in vitro are viewed as markers for osteogenic potential of the fibroblast-like cells [19]. These minerals are AP substrate for forming and storing the calcium phosphates in the osteogenic cells of membranes or artificial surfaces [19].

Table 3 indicates that the control culture of prenatal stromal cells (group 2) does not show any visible metabolic changes in 3-day FCM cultivation without osteogenic additives. Comparing with the control culture, membranes sterilization with Co^{60}

gamma-rays barely leads to any changes in the sorption properties in the culture medium filled with biologically active ions. Before gamma-ray sterilization and after the preliminary treatment of the material by cold plasma, the reduction (up by 6% in comparison with the group 3, with $p < 0.05$) in phosphate groups concentraions in the supernatants (group 5, table 3) was noted, along with some increase of the pH environment. It is possible that high deposits of the inorganic phosphorus anions on the the membranes after the combined sterilization reflect the changes of the surface properties of the membranes (charge, the increase of hydrophilicity). In other words, cold plasma treatment, followed by Co^{60} sterilization in the dosage of 10 kGy, gives the TM a certain bioactivity (the ability to change the ion composition of the environment), which may be the manifestation of the change in TM surface state or the occurence of the TM destruction products, important to cells.

Prenatal stromal cells are able to actively change the ion composition of a culture medium [28]. At the same time, the increase of concentraion in the potassium interstitial fluid, which is mostly located intracellularly, is a sign of cytotoxicity, conditioned by the damage of the membrane channels and the pumps [29].

However, when contacting both the TM modified with only γ-sterilization and combined with cold plasma, the metabolic performance of the cell culture did not differ statistically significant from the same in the control group of the cells on the plastic surface of the culture plates (table 3).

This fact allows excluding any major TM desctruction after sterilization. Apparently, the change in the physico-chemical state of the TM surface still remains within the relative bioinerticity after modification, because it barely influences morphofunctional state of the human prenatal stromal cells culture.

CONCLUSION

The series of the conducted studies allow us to draw the following conclusions.

1. TM surface exposure to low-temperature atmospheric plasma leads to the increase in roughness R_a by more than 4 times. Subsequent γ-sterilization of the plasma modified TM decreases the R_a value of the membranes after plasma treatment without the sterilization by 0.05 μm.

2. Plasma treatment of the membranes surface increases hydrophilicity and decreases the contact angle θ value by 40° – 43°. Sterilization does not contribute to any significant changes in surface wettability of the original and plasma modified TM.

3. The increase in wettability and roughness of the TM surface as a result of plasma treatment is apparently caused by the destruction of the amorphous phased polymeric chains and the formation of the C-O and C-C carboxyl groups in the places where the chains break.

4. Low morphofunctional reaction of the culture of the human prenatal stromal cells on the contact with the modified TM demonstrate preserving of their relative bioinerticity and lack of the significant destruction when exposed to the ionizing γ-irradiation of the ^{60}Co radionuclide in a doses of 1-10 kGy, including the combination with low-temperature atmospheric plasma.

Thus, the surface properties and biocompatibility of PET-based TM after exposure to the ANP and ionizing γ-radiation of the ^{60}Co radionuclide testify to the prospect of further study of them in the application to cardiac surgery, ophthalmology and, possibly, other sections of medicine.

CONFLICT OF INTEREST

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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REFERENCES

1. Filippova E.O., Krivosheina O.I., Zapuskalov I.V. Intrastromal implantation of track polymeric membranes in the treatment of endothelial-epithelial dystrophy of the cornea. *Medical Bulletin of Bashkortostan.* 2015; 10 (2): 137–139. (in Russ.)
2. Filippova E.O., Sokhoreva V.V., Pichugin V.F. Investigation of the possibility of using nuclear track membranes for ophthalmology. *Membranes and Membrane Technologies.* 2014; 4 (4): 267 – 271. (in Russ.)
3. Filippova E.O., Pichugin V.F., Sokhoreva V.V. Potential use of nuclear track membranes in ophthalmology. *Petroleum Chemistry.* 2014; 54 (8): 669 – 672.
4. Lam M.T., Wu. J.C. Biomaterial applications in cardiovascular tissue repair and regeneration. *Expert Rev Cardiovasc Ther.* 2012; 10(8): 1039 – 1049.
5. Apel P.Yu. Tracks of very heavy ions in polymers. *Nucl. Instrum. Meth. in Phys. Res.* 1997; B130: 55 – 63.
6. Mironyuk A.V., Pridatko A.V., Sivolapov P.V., Svidersky V.A. Features of evaluation of wetting polymeric surfaces. Technologies of organic and inorganic substances. *Eastern*

European Journal of Advanced Technology. 2014; 1/6: 23 – 26. (in Russ.)
7. Filippova E.O., Sohoreva VV, Shilova OG Investigation of the possibility of using polymeric track membranes in barrier keratoplasty. *News of Higher Educational Institutions. Physics*. 2013; 56 (11/3): C. 303 – 305. (in Russ.)
8. Dmitriev Sergue N., Lyubov I. Kravets, Vladimir V. Sleptsov, Vera M. Elinson Water permeability of poly(ethylene) terephthalate track membranes modified in plasma. *Desalination*. 2002; 146: 279 – 286.
9. Fortova V.E. Encyclopedia of low-temperature plasma. Introductory volume IV. M.: Science. 2000: 386. (in Russ.)
10. Provotorova D.A. Modification of unsaturated rubbers in low-temperature plasmas in order to improve their adhesion properties. *Adhesives. Sealants. Technologies*. 2013; 9: 7 – 9. (in Russ.)
11. Golovyatinsky S.A. Modification of polymer surfaces by pulsed plasma of atmospheric pressure. *Bulletin of Kharkov University*. 2004; 62: 80 – 86. (in Russ.)
12. Akishev Yu.S. Experimental and theoretical studies of the effect of a non-equilibrium low-temperature plasma of atmospheric pressure on the surface of polymer films.*5th International Symposium on Theoretical and Applied Plasma Chemistry: symposium materials. Ivanovo*.2008: 360 – 363. (in Russ.)
13. GOST ISO 11137 – 2000 Sterilization of medical products. Requirements for validation and ongoing monitoring. Radiation sterilization. (in Russ.)
14. Filippova E.O. Influence of Low-Temperature Plasma and γ-Radiation on the Surface Properties of PET Track Membranes. *Inorganic Materials: Applied Researc*. 2016; 7 (5): 484 – 492.
15. GOST ISO 10993-5-2011 Medical products. Evaluation of biological effects of medical devices. Part 5. Studies on cytotoxicity: invitromethods. 2014. (in Russ.)
16. Filippova E.O., Kalanda N.S.,Pichugin V.F. Investigation of the process of sterilization of track membranes from polyethylene terephthalate using low-temperature atmospheric plasma. *Medical equipment*. 2017; 2: 26 – 29. (in Russ.)
17. Carre A. Polar interactions at liquid/polymer inter faces. *Adhesion Sci. Technol*. 2007; 21(10): 961 – 981.
18. Kuznetsov V.D. Surface energy of solids. M.: State Publishing House of Technical and Theoretical Literature. 1954: 220. (in Russ.)
19. Khlusov I.A., KhlusovaM.Yu., ZaitsevK. . Pilot *in vitro* study of the parameters of artificiall niche for osteogenic differentiation of human stromal stem cell pool. *Bull Exp Biol Med*. 2011; 150 (4): 535 – 542.
20. Bolbasov E.N., Anissimov Y.G., Pustovoytov A.V. Ferroelectric polymer scaffolds based on a copolymer of tetrafluoroethylene with vinylidene fluoride: Fabrication and properties. *Materials Science and Engineering*. 2014; 40: 32– 41.
21. Khlusov I.A., Shevtsova N.M., Khlusova M.Y. Detection in vitro and quantitative estimation of artificial microterritories which promote osteogenic differentiation and maturation of stromal stem cells. *Methods Mol Biol*. 2013; 1035: 103– 119.
22. Guzhova A.A. Influence of the parameters of the electret on the surface and electret properties of polyethylene terephthalate. *News of the Russian State Pedagogical University. AI Herzen. 2013; 157: 55 – 60. (in Russ.)*
23. Dowling D.P. Atmospheric Pressure Plasma Treatment of Amorphous Polyethylene Terephthalate for Enhanced Heatsealing Properties. *International Journal of Adhesion and Adhesives*. 2013; 35: 1 – 8.
24. Kwang-Hyuk Ch. Effect of Ar Ion Beam Pre-Treatment of Poly (ethylene terephthalate) Substrate on the Mechanical and Electrical Stability of Flexible InSnO Films Grown by Roll-to-Roll Sputtering System. *Japanese Journal of Applied Physics*. 2013; 52: 45 – 49.
25. Navaneetha K. Adhesive properties of polypropylene (PP) and polyethylene-terephthalate (PET) film surfaces treated by DC glow discharge plasma. *Vacuum 83*. 2009: 332 – 339.
26. Ratner B.D., Hoffman A.S., Schoen F.J. Biomaterials Science: an introduction to materials in medicine. Third ed. Elsevier Academic Press: Oxford, UK; Waltham, MA. 2013: 1520.
27. Eriksson J.E., Dechat T., Grin B., Helfand B. Introducing intermediate filaments: from discovery to disease. *The Journal of clinical investigation*.2009; 119 (7): 1763–1771.
28. Khlusov IA, KhlusovaM.Yu., Shevtsova N.M. Morphofunctional state of stem cell culture on 2D matrix, imitating "silent" osteogenic and hematopoietic microterritories. *Bulletin of Siberian Medicine*. 2012; 6: 96 – 105. (in Russ.)
29. Lyakhov N.Z. Biocomposites based on calcium phosphate coatings, nanostructured and ultrafine-grained bioinert metals, their biocompatibility and biodegradation.T: Tomsk State University Publishing House. 2014: 596. (in Russ.)

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РЕЗЮМЕ

Цель. Исследование воздействия атмосферной низкотемпературной плазмы (АНП) и последующей стерилизации γ-лучами на топографию и свойства трековых мембран (ТМ) на основе полиэтилентерефталата (ПЭТФ).

Материалы и методы. ТМ были получены путем облучения пленки ПЭТФ потоком ионов $^{40}Ar^{+8}$ и последующего химического травления в 1,5N водном растворе NaOH. Для модификации поверхности на ТМ воздействовали АНП в течении 30 с. Стерилизация мембран проводилась с использованием γ-излучения радионуклида ^{60}Co в дозах 1 и 10 кГр (Si). Биосовместимость ТМ *in vitro* исследовали с использованием культуры пренатальных стромальных клеток (ПСКч), выделенной из легкого 11-недельного эмбриона человека и поддерживаемой *ex vivo*.

Результаты. Установлено, что обработка ТМ с помощью АНП приводит к возрастанию шероховатости и гидрофильности их поверхности ТМ. Изменение физико-химического состояния поверхности ТМ в результате воздействия холодной плазмы и последующей стерилизации практически не влияло на морфофункциональное состояние культуры ПСКч. Сделано заключение об относительной биоинертности ТМ и предложенных режимов их γ-стерилизации в отношении культуры стромальных клеток человека, перспективности дальнейших исследований в приложении материала к направлениям хирургической практики (кардиология, офтальмология).

Ключевые слова: стерилизация, стромальные клетки человека, морфофункциональная реакция.

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