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Containers and Various Packaging Devices Made of Polymeric Material for Storage of Allogeneic Grafts, the Surface of Which is Treated by Applying Carbon-Containing Nanofilms to their Polymeric Surface

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Abbreviations

PET - polyethylene terephthalate

PTFE - polytetrafluoroethylene

PVDF - polyvinylidene fluoride

NSPS - nanostructured polymer surface

NMPS - nanomodified polymer surface

CCF - carbon-containing film

IR spectroscopy - spectroscopy in the infrared spectrum

ESHA - electron spectroscopy for chemical analysis

AFM - atomic force microscope

HF - high frequency

cfu/ml - colony forming unit

Staphylococcus aureus ATCC 29213- museum strain of a gram-positive microorganism

Pseudomonas aeruginosa ATCC 27853 - museum strain of a gram-negative microorganism

nm- nanometer

μ - microne

r- horizontal dimension

R - air 1.5 - universal gas constant

L-perimeter

V - volume (of surface features, of surface roughness)

Rq - standard deviation

S- square

DF - fractal dimension.

θ -contact angle (of the wetting)

γ- the value of the total surface energy

γP- the polar component of γ

γd -dispersion component γ

EnC - endothelial cell

ECD -corneal endothelial cell density

Introduction

Proper preservation of viable tissues and organs for successful further transplantation is an urgent problem of modern transplantology.

To solve this problem, various packaging materials and systems used in biology, medicine and made from synthetic polymeric materials must have a certain set of properties, including: biocompatibility, aseptic properties, antibacterial activity, if necessary, in our further successful research and developments is the achievement of providing the phenomenon of bioepitaxy for living tissue. These properties and qualities of the treated surface can be achieved through nanostructuring (NSPS) and nanomodification (NMPS) of the surface of the treated polymer material.

Developing this problem on the basis of the Tissue Bank of the Russian Centre for Eye and Plastic Surgery (Ufa), we turned to the achievements of modern nanotechnologies applicable in medicine. The research was carried out as part of a joint project with the departments of Advanced Technologies of MATI and Radio Electronics of Moscow State University, where various technologies were developed.

Obtaining NSPS and NMPP for obtaining treated polymeric surfaces with different physicochemical properties. Previous results allowed the initial development of containers for aseptic storage of contact and intraocular lenses. Preliminary studies have shown that aseptic storage containers can maintain the viability of donor tissues, including the donor cornea.

Such features of modernity as urbanization, the emergence of many new megacities, increased danger and the likelihood of man-made disasters require the modernization of the healthcare system. The work of modern multidisciplinary tissue banks that provide the necessary amount of high-quality donor material, especially for cases of mass emergency operations, is one of the main conditions for the development of transplantology and plastic surgery.

Today the advances in physics, chemistry, biology, medicine, technology, computerization and mathematical modelling dictate new requirements for the quality of transplanted material, too which partly explains the existence of many ways to preserve donor material. Recently, we have been witnessing advances in the field of nanotechnologies, which have emerged and are developing at the intersection of sciences. The use of nanotechnologies in medical clinical practice, in addition to knowledge in the field of physiology of cell membranes, molecular biology, regenerative medicine, requires fundamental knowledge, for example, in materials science, chemical synthesis.

Preservation of proper viable tissues and organs for further successful transplantation is an urgent problem of modern transplantology. Methods used by modern tissue and organ banks: low-temperature cryopreservation, silicone drying, storage on nutrient media, etc. (Filatov V.P., Eroshevsky T.I., Kovalenko P.P., Makkari B., Kaufman H., Volkov V.V., Travkin A. G., Dronov M.M.), do not always allow to achieve long-term preservation and bio plasticity of the allogeneic transplants. Conservation of donor tissues and organs, which ensures the reduction of metabolic, enzymatic autolytic processes and the preservation of isolated tissues and organs in the state structural integrity and viability are quite a challenge, and the search for the best method for preserving grafts continues.

The trend in the use of disposable products made from available polymeric material in modern life, medicine, biology, dictated by the achievements of scientific and technological progress and sanitary and hygienic requirements, today is due to the widespread use in medicine of various disposable products, that is, products made of polymers. However, paradoxically, the widespread use of various polymers, including for hygienic purposes, has given rise to another, no less dangerous problem - the frequency of infections caused by microbes originating from biofilms, which, almost as a rule, can form on the surface of any polymers. Thus, over time, it became necessary to define the class of diseases "caused by biofilm microbes." To ensure the biocompatibility of various products made of polymeric materials with viable tissue, as well as to prevent the formation of a biofilm on the polymer material surface, in the late 90s, various methods of modification of the surface of polymers began to be used in medicine, in particular by means of NMPS by the surface treating carbon-containing coatings: diamond-like, nanotubular, fullerene-containing, carbyne-containing. The various polymeric packaging materials and devices used in biology, medicine and made from synthetic polymeric materials must have a certain set of healthy safe properties of their polymeric surface, including: biocompatibility, asepsis. Preliminary studies have shown that the proposed aseptic containers are also capable of maintaining the viability of living tissue.

Thus, we set ourselves a new goal - to improve the method of prolonged term preservation of the quality of allogeneic grafts by creating containers and film packaging materials, the surface of which is nanomodified by applying carbon-containing coatings, in order to use processed polymer containers during storage and safe transportation of various grafts

Materials and Methods

Methods for physicochemical investigation of the characteristics of aseptic biocompatible carbon-containing coatings on the surface of polymeric materials (IR spectroscopy, X-ray photoelectron spectroscopy, electron spectroscopy for chemical analysis (ESCA); study of electrostatic properties, nanomodified surface topology were applying.

On the way to improving the method of long-term preservation of allogeneic transplants by creating containers and packaging devices with a nanostructured carbon-containing coating of the polymer surface of a special container for storage and safe transportation, for example, a donor cornea, the following tasks are being solving:

1. Study of the main physical and chemical characteristics of an aseptic carbon-containing coating of the polymer surface in order to develop a special container and packaging material for the preservation of various transplants and items.
2. Study of antibacterial and adhesive properties of carbon-containing nanomodified coatings and testing them for toxicity.
3. Development of special containers and packaging devices with a nanomodified carbon-containing polymer surface coating.
4. Carrying out a comparative morphological analysis of native grafts on the example of a donor cornea with different methods of their preservation.
5. Optimization of system parameters: donor cornea - nanomodified carbon-containing polymer coating - nutrient medium.

The processes of formation of NSPP (nanostructured polymer surface) of polymeric materials: PET, PTFE and PVDF have been studied, as their modified surface characteristics and the ability to variated them. The choice of these polymers was due to both the wide use of these materials and the possibility of providing a wide range of surface properties during its formation, for example, PET has a high-energy polar surface, and PTFE has low dielectric constant and dielectric losses, a low-energy non-polar surface and a wide range of operating temperatures. NSPPs based on PET, PET, PVDF were formed by treating the initial surface with ionic flows of active and inert gases and their mixtures (CF4, Ar, O2). Modification of the formed NSPs was carried out in two ways: by deposition of carbon films 5–120 nm thick from directed ion-plasma flows of hydrocarbon vapors and by magnetron deposition of highly porous films. In the first case, it is possible to control the properties of the surface by controlling the phase and cluster composition of the film, the number of layers, and the thickness of

the layers with the ratio of the real surface to the geometric one within 8–10; and in the second case, this ratio increases by 100 times. The effect of pretreatment of the polymer surface with a beam of nitrogen and oxygen ions on the composition of the surface and its physicochemical properties has been studied. To determine the change in the chemical structure of the surface, the chemical compounds of a carbon-based nanoscale coating formed by ion-plasma methods of processing the surface of the processed material of the packaging film material and special containers were studied, these are: IR spectroscopy, X-ray photoelectron spectroscopy, electron spectroscopy for chemical analysis (ESCA).

The surface charge was measured by the dynamic capacitor method.

The absolute value of the electrostatic potential was measured with an S-95 kilovoltmeter. For topological studies of the NSPP structure, a FemtoScan atomic force microscope with a maximum scanning field of 10 × 10 (μm) was used. For each of the samples, images of the surface were obtained at different its points and at different magnifications of the researched surface. The main parameters to be determined were: characteristic horizontal size - r, perimeter - L, volume - V signs of surface topography, surface roughness (standard deviation - Rq), surface area - S and fractal dimension - DF. The goniometric method for measuring the contact angles (θ) was also used to study the surface characteristics of the samples. The working fluids were water (bidistillate) and glycerol (chemically pure). The contact angle values were obtained as the average of 5 experiments over 10 measurements. Based on the data obtained, the values of the total surface energy (γ), polar (γP), and dispersion components (γd) were calculated. For calculations, we used the values of the adhesion work obtained on the basis of the experimental values of the contact angles of wetting and the Dupre-Young formula for two liquids.

Methods for studying the antimicrobial activity of carbon-containing films and their toxicology. Study of the antimicrobial activity of carbon-containing films. Methods for studying the adhesive properties of carbon-containing coatings.

The study of the antimicrobial activity of diamond-like and carbon-containing films was carried out according to the standard method by applying the test samples to the surface of meat-peptone agar contaminated with microorganisms in Petri dishes. Silicon wafers coated with diamond-like and carbon-containing films were used as substrates for culturing mouse embryonic fibroblasts. The deposited carbon films had different characteristics on surface charge level, contact angle, topography,

and surface chemical composition. The relationship between the data of studies of the charge properties of the surface and the data of studies of the degree of adhesiveness of connective tissue cells (mouse embryonic fibroblasts) was studied. In the course of the studies, the antibacterial activity of samples nanomodified with carbon-containing coatings was evaluated according to the standard in vitro method.

Gram-positive (Staphylococcus aureus ATCC 29213) and gram-negative (Pseudomonas aeruginosa ATCC 27853) microorganisms of museum strains were used. 3–4 colonies of an 18–20-hour culture of the studied microorganisms was suspended in 3 ml of saline. The resulting suspension was adjusted to a 0.5 McFarland turbidity standard (1.5 x 108 cfu/ml). The base suspension was then diluted in saline solution serum dilutions until the concentration was 15 cfu/ml.

NSS polymers foils were cut into fragments of size 15x15 μm. The fragment of substrate was put into a tube with liquid nutrition medium for control of the sterility of the coatings. Muller-Hinton bouillon was used as a nutrition media. From every tube with different concentration 25 μl of suspension was extracted using a pipette and were deposited on separate fragments of the polymer substrate with NSS for control of the growth of the culture.

Such infected fragments were incubated in wet media with a thermostat at 37ºC for 2 hours. After incubation, all fragments were put into tubes with 4 ml of nutrition media again incubated in the thermostat at 37ºC for 48 hours. Antibacterial activity was estimated by the appearance of visible growth in tubes with nutrition media after 48 hours.

The effectiveness of the antibacterial activity of the samples was conditionally determined by "three degrees".

Sanitary-chemical tests were carried out for the content of reducing impurities, determined by the consumption of 0.02 N $Na_2S_2O_3$ solution; on the content of organic impurities, determined in terms of optical density in the wavelength range of 220 nm, as well as the content of metals such as Cd, Pb, Sn, Cu, Cr, Ba.

Adhesiveness "in vitro" in relation to cultured cells, mouse embryonic fibroblasts, was studied as follows: the substrates were treated with 96% ethanol solution, then washed with distilled water and, after drying, placed in culture dishes. Substrate dishes were UV sterilized for 1 hour. Cells were suspended in culture medium 199 supplemented with 10% fetal calf serum and antibiotics. Cells were cultured for three days at 37°C in air with 5% CO_2 containing.

At the end of cultivation, the cells were fixed with a 2% solution of glutaraldehyde, washed in water, and dried; some preparations were stained with 1% methylene blue solution. The preparations were studied using light and stereomicroscopy.

The "in vivo" experiment was carried out as follows: fluoroplastic plates (0.6 x 2 cm2) covered with carbon films were implanted subcutaneously under sterile conditions in mice of the CBA x C57Bl line (one plate in both sides of the experimental mouse body). Four months after the implantation, the plates with the capsules formed around them were removed and fixed in 10% neutral formalin. From plates with capsules, histological preparations were prepared according to the standard method; serial sections were stained with hematoxylin-eosin.

The preparations were examined using light microscopy

Morphological studies of the donor corneal material in the experiment, demonstrating the dependence on the method of storage of the donor cornea, the type of preservative medium, the duration and temperature of storage of the donor cornea.

For morphological studies, corneoscleral discs were excised and stored in organ culture in accordance with the standards of tissue banks.

Study of the endothelium

After a certain period of storage of the donor cornea, the density of endothelial cells (EnC) and their viability was determined in a zone with an area of 1.54 mm2 using a phase-contrast light microscope. Corneal endothelium was stained with 45% trypan blue solution (Sigma, UK). Staining lasted 1 minute, after which the number of necrotic cells was counted and the nature of their distribution. After washing in sterile 0.9% (w/v) NaCl, a hypotonic solution of 1.8% sucrose was used to visualize cell boundaries. The number of cells in the central zone of the corneal endothelium was determined per square millimetre using the graduation of the ocular grid on the microscope, taking into account the degree-degree of the fold.

The quality of the EnC was assessed according to the degree from 1 to 5, according to an increasing criterion, according to such criteria as: the shape of the cell, its boundaries and dimensions. Cell shape was assessed on a 4-point scale: from 1 - hexagonal to 4 - polymorphism; cell boundaries: from 1 - clear boundaries without violating their integrity to 4 - partial loss of cell boundaries and their complete

absence; cell sizes: from 1 - flat cells without signs of edema to 4 - swollen EC; apical surface of EnC, with pinocytic vesicles: 1 - 0% vesicles-holes, 2 - 10-20% vesicles, 3 - 40-50% vesicles, 4 - 70-100% vesicles.

Summing up these changes, we have identified the 4 main groups for assessing the morphological state of EnC: 1 – unchanged EnC up to 4 - destructured EnC. 20 cadaveric eyes were studied, which were stored in different modes of conservation: in a humid chamber, in Eagle's medium, in special containers with various media (Optisol, Dexol, donor blood plasma). After this study, corneoscleral discs immediately placed in 10% neutral buffered formalin solution and stored at 4°C.

To study tissues in an electron microscope, which makes it possible to reveal the ultrastructure of cells and their components, methods of fixation (usually using osmic acid and glutaraldehyde) and embedding media (usually epoxy resins) were used. A special ultramicrotome with a glass or diamond knife makes it possible to obtain sections with a thickness of less than 1 micron. The study of the antimicrobial activity of diamond-like and carbon-containing films was carried out according to the standard method described above by applying the test samples to the microorganism-contaminated surface of meat-peptone agar in Petri dishes. For culturing mouse embryonic fibroblasts, silicon wafers coated with diamond-like and carbon-containing films were used as substrates. The deposited carbon films had different characteristics of surface charge, contact angle, topography, and surface composition. The relationship between the data of studies of the charge properties of the surface and the data of studies of the degree of adhesiveness of connective tissue cells (mouse embryonic fibroblasts) was studied. In the course of the studies, the antibacterial activity of samples nanomodified with carbon-containing coatings was evaluated according to the standard “in vitro” method.

Pieces of corneal tissue of cadaveric eyes 1–2 mm in size served as preparations for electron microscopy; To do this, the samples were sequentially treated with 2.5% glutaraldehyde (30 min), 2.5% solution of osmic acid in phosphate buffer according to Caulfield (2 hours at pH=7.4). Then they were washed with phosphate buffer and placed in uracil acetate with 70% alcohol (24 hours). Then, in alcohols of increasing concentration and in acetone, the preparation was dehydrated and filled with a fixed Epon-araldite-treatment method; the original method of corneal tissue processing, proposed by L.V. Ilatovskaya, was also used (1980). To determine the tissue area required for the study, as well as for orientation in the layers of the preparation, sections were made, which were stained (methylene blue, hematoxylin blue, toluidine blue). Ultrathin sections were made on a microtome on the desired tissue section selected under a light microscope, which were contrasted (for example: lead according to Reynolds).

For morphological investigations, corneoscleral discs were excised and stored in organ culture in accordance with the standards of tissue banks. The disks were excised after treating the entire eyeball:

the eyeball was washed four times in sterile 0.9% [w/v] NaCl, then immersed in a 5% solution of polyvinylpyrrolidone-iodine, after which the eyeball was treated with 2% sodium thiosulfate solution (for neutralization) and again placed in a solution of 0.9% [w/v] NaCl.

A special container for storing and transporting a viable cornea is made of PVDF material by pressing, such a special container has the shape of a corneoscleral contact lens (like a cradle for a donor cornea), 17 mm. in diameter, 0.3 mm thick, with a modified NMPP. The peripheral edge along the entire circumference of such a cradle-lens is flattened along the entire circumference, perpendicular to the main surface of the lens, forming protrusions of the torsion edge of the lens-container: inner and outer. The donor cornea is placed in this special cradle with a 3-4 mm scleral rim. The inner torsion edge with the inner surface of the lens forms a puzzle, where the edge of the donor cornea is neatly placed around the entire circumference inside the cradle-container; The outer ledge of the torsion ledge of the container serves to fix the container by sewing the cradle container through this outer ledge and hanging it with Mersilk 5/0 thread (Ethicon, AAH, Bristol, UK). In such a fixed state, donor corneas in a special container were placed in a 120 ml glass vial with 100 ml of preservative medium, Eagle (MEM), Optisol, Dexol (McCarey-Kaufman medium) and donor plasma were used as preservative media. Sterile closed vials are placed in cabinets for storage at a temperature of 27-28°C. Donor corneas were stored in a standard incubator at 27°C, the storage periods varied up to 20 days, without changing the conservation medium. (Musina A.D., 2000; 2005).

The study of the antimicrobial activity of diamond-like and carbon-containing films was carried out according to the standard method by applying the test samples to the surface of meat-peptone agar contaminated with microorganisms in Petri dishes. Silicon wafers coated with diamond-like and carbon-containing films were used as substrates for culturing mouse embryonic fibroblasts. The deposited carbon films had different characteristics of surface charge, contact angle, topography, and surface composition. The relationship between the data of studies of the charge properties of the surface and the data of studies of the degree of adhesiveness of connective tissue cells (mouse embryonic fibroblasts) was studied. In the course of the studies, the antibacterial activity of samples nanomodified with carbon-containing coatings was evaluated according to the standard in vitro method.

To research of the tissues in an electron microscope, which makes it possible to reveal the ultrastructure of cells and their components, methods of fixation (usually using osmic acid and glutaraldehyde) and embedding media (usually epoxy resins) were used.

A special ultramicrotome with a glass or diamond knife makes it possible to obtain sections with a thickness of less than 1 micron(μm), and permanent preparations are mounted not on glass slides, but on copper grids.

Pieces of corneal tissue of cadaveric eyes 1–2 mm in size served as preparations for electron microscopy; To do this, the samples were successively treated with 2.5% glutaraldehyde (30 min), 2.5% osmic acid solution in phosphate buffer by Caulfield (2 hours at pH=7.4). Then they were washed with phosphate buffer and placed in uracil acetate with 70% alcohol (24 hours). Then, in alcohols of increasing concentration and in acetone, the preparation was dehydrated and fixed using Epon-Araldite-Processing method; at the same time, the original method of filling the corneal tissue, proposed by Ilatovskaya L.V., was used. (1980). To determine the tissue area required for the study, as well as for orientation in the preparation layers, sections were made, which were stained (methylene blue, haematoxylin blue, toluidine blue). On the desired area of the fabric selected under a light microscope, ultrathin sections were made on a microtome, which were contrasted (for example: lead according to Reynolds).

Morphological studies were carried out at x200 and x500 magnifications, in groups of donor material under study, differing: the method for storing of the viable donor cornea; type of preservative medium; duration and temperature of storage of the donor cornea.

Also had been compared such methods of storage of the donor cornea as: the method of storage in a humid chamber according to Filatov, on a nutrient medium Eagle, and the storage in a suggested special container, in use of various preservative media, as Optisol, Dexol (McCarey-Kaufman medium), donor plasma, which were selected.

Results and discussion

The research of the charge level on NSPS, NMPS and the possibility of controlling its magnitude and direction suggest that this vector of research is promising not only in the creation of plastic containers for aseptic storage of contact and intraocular lenses and in the manufacture of special containers for maintaining a viable donor cornea, various alloplants, but also to create modified functional surfaces of artificial devices in medicine and biology of the future.

The control of the applying chemical composition and physicochemical properties, as atomic structure, charge level on the polymer surface, its relief are important and primary tasks in the formation of NSPSs, NMPSs and depend on the goals set by researchers and technologists.

Study of antibacterial activity NSPS was our first study on how to overcome biofilm formation on a polymer surface and achieve biocompatibility with surrounding tissues. Biocompatible NSPSs, NMPSs is provided by means of the charge on the treated surface and certain chemical composition, atomic structure, which can provide the effect of polarization of cell membranes and can influence on the shape and function of living tissue cells when they come into contact with the NSP-surface interaction (for example: avascular structure of the corneal tissue in contact with NSPS, NMPS (Alvina D. Musina, 2002).

Thus, IR spectroscopy indicates that the nanosized carbon-based coating synthesized from an ion beam has an inhomogeneous structure, including linear chains (CH2) n and six-membered rings (CH2)6 containing ketone groups in their composition, as well as amorphous carbon α-C chains. The results of studying the composition of carbon-based films during the treatment of polymers with a flow of argon ions showed the absence of ketone groups, which gave great prospects for obtaining biocompatible materials. In the case of treatment in an air mixture and the deposition of carbon-containing films (CCF) on the surface of the polymer, the antibacterial properties of the surface treated in this way were obtained.

Electrostatic studies of carbon-containing films have shown that during pre-treatment of the polymer surface, the thickness of the deposited coating, the energy of the contained particles significantly affect the charge change on the polymer surface. Films of PTFE, PET, and PVDF acquire an excess positive charge of the treated surface upon deposition of a carbon layer, up to several μC/m2. Pretreatment of the PET, PTFE, and PVDF film surface in Ar + O mixtures of plasma surface treatment contributes to a more efficient charge of the sample surface compared to treatment in carbon tetrafluoride (CF4) plasma.

The study of the electrical properties of the surface of polymers after treatment and subsequent deposition of carbon films made it possible to reveal some features of the formation and growth of films on the surface of polymers with different surface structures. The possibility of influencing the electrical properties of polymeric surfaces with the help of NSPS and NMPS is also shown.

Topographic studies using atomic force microscopy (AFM) of a nanostructured surface showed that after pretreatment of PET, PTFE, and PVDF samples and after application to their surface, the surface

microroughness is smoothed at the first moment of time, i.e., the roughness decreases. Further, with an increase in the film thickness, the height of the irregularities begins to increase, and the diameter of the conglomerates remains practically unchanged, however, when a certain coating thickness is reached, the size of the characteristic features of the relief increases, preliminarily surface treatment of the CF4 substrate leads to the development of the surface relief. After a sufficiently long treatment, the surface after coating has a greater roughness than without pretreatment. Increasing the pre-treatment time increases the surface roughness obtained after coating. At the same time, the surface roughness increases by almost 100 times compared to the film deposited without pretreatment. All these changes in the surface structure cause a change in the fractal dimension. Later, when conducting studies of the aseptic properties of NPPS and its biocompatibility, it was found that the highest bactericidal activity is manifested in samples with the most developed surface relief (the ratio of real area to geometric ~100). Correlation of charge study results and careful data analysis allow state that the interaction of nanoparticles with microorganisms proceeds, apparently, by two mechanisms, and one of them is associated with electrostatic interaction, when the real surface can be increased by 5-8 times, and the charge reaches a few mmillicoulomb per Square Millimeter (μC/m2); the intensity of the second mechanism of interaction with microorganisms is related to the degree of dispersity of NPPSs.

Thus, the physicochemical properties of NSP made it possible to determine the optimal parameters for the characteristics of carbon-containing films for obtaining special containers and carbon-containing films applied to various polymeric devices in the form of aseptic biocompatible carbon coatings. The thickness of the films deposited on the surface varies from 5 to 120 nm and depends both on the substrate material (PET, PTFE, PVDF) and on the specified properties of the finished product.

The deposition of a C6H12 film reduces the charge on the surface of the films, and with an increase in the content of N (nitrogen) in the gas mixture, it increases NSPS charge. Surface pretreatment, for example with CF4 leads to the development of the relief, and pretreatment of the surface with beams of O2 and N ions leads to the destruction of carbonyl bonds with the formation of a hydrophobic surface.

That is, the technological mode of the process of deposition of carbon-containing films on a polymer surface is determined by the specified properties aseptic biocompatible surface of a special container or for surface treatment of other polymer storage devices.

The development of special containers and various packaging devices made of polymeric materials treated by applying CCF deposition to the polymer surface for to ensure aseptic storage of various items, preventing the formation of biofilms in the first place

When developing special containers for aseptic storage and transportation of native tissues of the donor cornea, in addition to antibacterial properties, some barrier properties were identified that were previously discovered in the course of experimental and clinical studies, other properties of the nanomodified polymer surface obtained after applying carbon-containing coatings, for example: modeling electrostatic properties of the polymer surface, which form the interface activity with the underlying biological tissue. This made it possible to start developing a method for preserving viable donor tissue to create conditions for prolonged-term storage of high-quality donor material, as close as possible to physiological conditions.

The surface of a special container can be modified in two ways: by deposition of carbon films 5–120 nm thick from directed ion-plasma flows of hydrocarbon vapors (V.M. Elinson, V.V. Sleptsov, A.N. Lamin, V.V. Shake, L.N. Kostichenko, A.D. Musina, 1999; V.V. Sleptsov, V.M. Elinson, N.V. Simakina, A.N. Lamin, I.V. Tsygankov, A.A. Kivaev, A.D. Musina, 1996) and magnetron deposition of highly porous films (V.T. Grinchenko, V.V. Sleptsov, S.A. Fedorov, 1988). In the first case, it is possible to control the properties of the surface by controlling the phase and cluster composition of the CC film, the number of layers and layer thickness at the ratio of the real surface to the geometric interval 8-10; and in the second case, this ratio increases by 100 times.

Results of the study of the antimicrobial activity of carbon-containing films and their toxicology.

The results of the study of adhesive properties of carbon-containing coatings.

Experimental results of studies of the antibacterial activity of nanostructured carbon-containing films are presented in Table1.

№	Polymer / deposition conditions	Indicator m/o	Innoculation dose (cfu/ml)				
			105	104	103	102	10
1	PTFE: 1) treated 2)α-C:H (50 nm) 3)Al (aluminum foil film)	Ps. aerug.	+	+	+	±	±
		S. aureus	+	+	±	-	-
2	PTFE: 1. treated CF4 2. α-C:H (50 nm) 2) AL aluminum foil film)	Ps. aerug.	+	+	+	+	+
		S. aureus	+	+	+	+	+
3	PTFE: 1) treated CF4	Ps. aerug.	+	+	+	+	+

	2)α-C:H (50 nm)	S. aureus	+	+	+	+	+
4	PTFE smooth surface film: α-C:H (50 nm)	Ps. aerug.	+	+	+	+	+
		S. aureus	+	+	+	+	+
5	PTFE: 1) treated CF4 2)α-C:H (10 nm)	Ps. aerug.	+	+	+	+	+
		S. aureus	+	+	+	+	+
6	PTFE smooth surface film 1) α-C:H (10 nm)	Ps. aerug.	+	+	+	+	+
		S. aureus	+	+	±	-	-
7	PTFE: 1) treated CF4	Ps. aerug.	+	+	+	+	+
		S. aureus	+	-	-	-	-
8	PET with a developed relief surface+ Al) 1)"R" /air.1/ 2)"R" /α-C:H 10 nm	Ps. aerug.	+	+	+	+	+
		S. aureus	+	+	+	+	+
9	PET with a developed relief surface+ Al) 1)"R" /air.1/ 2)"R" /α-C:H 50 nм	Ps. aerug.	+	+	+	+	+
		S. aureus	+	+	-	-	-
10	PET with a developed relief surface+ Al/Al2O3) 1)"R"/air .1/ 2)"R" /α-C:H 50 nm	Ps. aerug.	+	+	+	+	+
		S. aureus	-	-	-	-	-
11	PET relief surface+ Al/Al2O3) 1)"R" /air.3/ 2)"R" /α-C:H 50 nm	Ps. aerug.	+	+	+	-	-
		S. aureus	+	-	-	-	-
12	PET relief surface+ Al/Al2O3) 1)"R" /air.3/ 2)"R" /α-C:H 100 nm	Ps. aerug.	+	+	-	-	-
		S. aureus	+	-	-	-	-
13	PET relief surface+ Al/Al2O3) 1)"R" /air.1,5/ 2)"R" /α-C:H (30+20) nm	Ps. aerug.	+	+	±	±	-
14	PET 1)"R" / CF4 2)"R" /α-C:H 30 nm	Ps. aerug.	+	+	+	-	-
15	PET 1)"R" /air.1,5/ 2)"R" /α-C:H 40 nм	Ps. aerug.	+	-	-	-	-
16	PET: "R" /α-C:H 40 nm	Ps. aerug.	+	+	+	+	-
17	PET 1)"R" /air.1,5/ 2)"R" /α-C:H (20+ nm)	Ps. aerug.	+	+	+	+	+

Results of studying the antimicrobial activity of carbon-containing films and their toxicology.

Table 1. Results of experimental studies of antimicrobial activity of nanostructured carbon-containing cells.

Results of the study of the adhesive properties of carbon-containing coatings.

Analysis of the data obtained, presented in table. 1. In all the experiments, as well as the control samples for growth of cultures, control of sterility of samples was also provided. Experimental results of antimicrobial activity study are presented in Table (1). In tubes with polymer samples without CF coatings growth of microorganisms in all the dilutions was observed, which demonstrated high turbidity of the media. The absence of a CF antimicrobial effect in relation to Pseudomonas aeruginosa in some samples may be explained by the fact that this microorganism is more resistant to the influence of different aggressive factors compared with Staphylococcus aureus. Of greater interest are results demonstrating the CF coatings antimicrobial activity to Pseudomonas aeruginosa in samples 11-16. Sample 15 has suppressed the growth even at concentration of microorganisms of 104 cfu/ml. Samples 14 and 15 which have suppressed the growth of both types of microorganisms are of most interest.

Analysis of Table (1) has shown that maximum CF antibacterial activity was observed for samples with the maximum relief (ratio of real surface to geometrical one is about ~ 100). A comparison between the results from the study of surface charge and careful analysis of Table (1) data show that the interaction between NSPS and microorganisms may depend upon through mechanisms of two types, one of which is associated with electrostatic interaction, and the intensity of the second mechanism is related to the degree of dispersion of NSPS.

The main factors affecting the antibacterial activity of surfaces with carbon coatings are: the composition and structure of the initial treated surface (polymer), the conditions and method of film formation on the surface, the physicochemical features of the applied film, and the surface treatment mode.

Results of the study of the adhesive properties of carbon-containing coatings presented in the Table 2. Table 2 shows adhesion results of mouse embryonic fibroblasts on smooth PET and PET-based film.

The deposition of films was carried out by various methods of deposition of carbon-containing coatings and in various modes of surface treatment.

№	Polymer Substrat	Application method	Deposition conditions	Average number of cells per S=0.6 mm2	Number of weakly scattered cells, %	(φ)/V, ± 25%
1	Cover glass (control)	-	-	300,0	5	-
2	PTEF TM	2	C_6H_{12}	11,0	51,6	1200
3	PTEF 0,1 mm	2	C_6H_{12}, dC=0,5 μm	13,2	27,2	1300
4	PTEF 0,1 mm	2	C_6H_{12}	15,2	75,9	1500
5	PTEF 0,1 mm	1	C_6H_{12}+N2 3 1	34,6	13,6	30
6	PTEF 0,1 mm without coatings (control)	-	-	46,5	14,6	10
7	PTEF 0,1 mm	1	C_6H_{12}	82,7	76,7	5
8	PTEF 0,1 mm	1	C_6H_{12}+N2 1 1	92,4	29,6	0
9	PTEF 0,1 mm	1	C_6H_{12}	189,4	35,3	5
10	PTEF 0,1 mm	2	C_6H_{12}, d>3 μm	196,7	12,2	-100

Table 2. Adhesion of connective tissue cells (mouse fibroblasts) to carbon films deposited on PET by various methods.

The average number of cells and the percentage of weakly scattered cells, which included cells with a strongly reduced cytoplasm, are also given. The total number of attached cells (both deformed and slightly deformed or not deformed) characterizes the adhesive properties of CCF on the NMPS surface of the polymer material, its ability to relatively firmly bind living cells from the surrounding biological environment to the surface of the polymer material being processed. The percentage of cells that have reached a sufficiently pronounced "spreading", depending on the level of deformation, characterizes

the combination of properties of the treated surface of the polymer material, such as: the ability to stick the living cells, the ability to bind certain blood serum proteins, that are determined by the nature of the microrelief of the treated surface, and the absence of cytotoxicity, preventing cell fusion and their subsequent death. The results also show that the smaller φ (the value of electrostatic potential), the higher the adhesion for cells, while the percentage of weakly flattened cells does not change monotonously and proportionally to the magnitude of the charge. The main factors in interaction with the surrounding living tissue are the structure and composition of the CCF of the treated surface.

When studying the adhesive properties of the CCF, the results of the experiment "in vivo" with the implantation of plates with NMPS showed that 4 months after implantation, a connective tissue capsule is formed around the plates with NMPS, which is a standard reaction to the biocompatible implant;

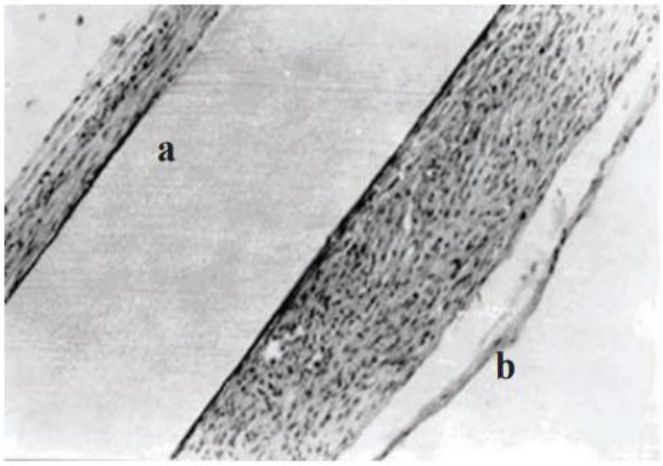

Fig.1. Capsule formed around a carbon film-coated PTFE plate 4 months after mouse subcutaneous implantation. Thin (a) and multi-layered (b) sections of the capsule. Magnification X60

The thickness of the capsule around the same plate varied considerably: in some places the capsule was thin (no more than 2–4 rows of cells), in other places it looked multi-layered (Fig.1). Thin sections of the capsule young poorly differentiated fibroblasts, sometimes in direct contact with the surface of the plate; thin collagen fibres were determined between the cells.

In the thick sections of the capsule, three layers could be distinguished: inner (adjacent directly to the plate), morphologically similar to the thin sections of the capsule described above; the middle layer, consisting of mature fibroblasts and collagen fibres, oriented parallel to the surface of the plate; the outer layer, consisting of fibroblasts of varying degrees of maturity and loosely arranged collagen

fibres. No leukocytes were observed in any of the cases infiltration, indicating the absence of an inflammatory response.

The described morphological picture of the structure of the capsule was similar for all implanted plates.

Experimental studies carried out on PET, PTFE, PVDF with NSPP formed by ion-plasma technology showed that the degree of surface dispersion and the method of its modification determines the presence and effectiveness of surface characteristics, in particular, biocompatibility, antibacterial activity, lack of toxicity, developed surface with good adhesive properties for cells.

When studying a nanostructured surface (NSPS), such phenomena were discovered as: tracking, correction, design and control over biological systems at the molecular level. All natural materials and systems are built from nano-objects. It is in the range of nano sizes, at the molecular level, that nature determines the main characteristics of substances, phenomena and processes. Features of the properties of substances and materials in the nanometre range are determined not only by a decrease in the size of structural elements, but also by the manifestation of quantum mechanical effects, the wave nature of transfer processes, and the dominant role of interfaces. By controlling the size and shape of nanostructures, it is possible to impart completely new functional characteristics to the materials, which differ significantly from the characteristics of bulk materials.

In addition, in a number of special cases, NSPSs must have special physicochemical or chemical properties, for example, biodegradability, or special strength or flexibility, or have a porous structure for the formation of intercellular substance (matrix). On the basis of appropriately modified NSPSs, biocatalysts for various biotechnological processes can also be implemented.

The obtained results of experimental studies open up the new technological approaches to the creation of biologically active systems.

As a result of morphological studies of the donor material of the cornea, it was found that after storage of the donor cornea for 1-2 days, no significant morphological changes were revealed for any of the observed donor corneas (DC), regardless of the method of its storage, more precisely: the type of preservative medium, the duration and temperature of storage of the donor cornea, with the obligatory observance of the prescribed mode of storage of the DC. Pathological morphological changes appeared on the 4th day of storage of the donor cornea in a humid chamber, on the 5th-6th day of storage in Eagle's medium. Donor corneas were stored in special containers in Optisol and Dexol preserving media at a temperature of 25–27°C to maintain the proper quality of the donor cornea for up to 17 days. Encouraging results were obtained when the donor cornea was stored in a special container in

the donor's blood plasma. The donor cornea retained its structure: the preservation of the apical and, more importantly, of the DC"s lateral borders up to 20 days. As is known, the state of the lateral boundaries of the DC cell membranes is very important for the implementation of the mechanism of the transport through the DC (J. Fischbarg, 1997). Under the conditions of storage of the donor cornea in own blood plasma, had not be observed the appearance of a precipitate in a preservative medium, which occurred during storage in Optisol, Dexol, after 15 days of storage of the donor cornea in 10% of cases. The density and number of endothelial cells (EnC) were determined and compared before and after conservation, on the 5, 10, 15, 20 days of storage 20 donor corneas, divided into 5 groups according to the method of storage. Research data are presented in table 3.

Culture Medium	Before conservation ECD	In 5 days ECD	In 10 days ECD	In 15 days ECD	In 20 days ECD
Humid chamber	2260	1490			
Eagle’s	2190	1740	1320		
Optisol	2360	2200	1930	1880	1740
Dexol	2280	2150	1950	1860	1770
Donor’s blood plasma	2330	2240	2200	1970	1930

Table 3. Results of determining the number of ECs from donor corneas preserved with various storage

It is known that the greatest loss of the number of EnC of the donor cornea (DC) occurs during the first 5-10 days of preservation of the donor material. Structural (morphological) changes in DC were established during storage in a humid chamber and in Eagle's medium after 5 and 10 days of storage, respectively, which led to the exclusion of these DRs from further studies. Donor corneas stored in special containers with preservatives retained the structure and density of EnC, i.e., remained viable for a relatively longer period of conservation. The electrostatic properties of NSPP, NSPP can either promote adhesion on the surface of chemical composites, protein structures, cells, cell structures, or NSPP, NSPP can remain unchanged for some of the above compounds. The loss of EnC by the donor cornea can be prevented by placing DC in a special container, which, in addition to the function of a

dressing, functions as a biologically active system, since the NSPP and NMPS of the special container help to maintain of the potential of the cell membranes of corneal tissue cells.

Polarization of cell membranes is ensured by constant vectorial transfusion of humoral fluids, and the direction of the motion vector is determined by the cell concentration gradient coefficient EnC.

In 1974 Temirov N.E. proposed the use of separated media to preserve DC, which is based on the principle of artificial modeling of the conditions for the existence of the cornea in a living eyeball. For the first time, a dynamic model was proposed for recreating the conditions of an isolated cornea, simulating the physiological conditions of the interaction of preserving fluids with a donor cornea in a device specially designed for this purpose, consisting of 2 isolated chambers, which, apparently, was the first (empirical) attempt to artificially recreate the vector the movement of fluid through the cornea, through the structures of the donor cornea, in order to maintain, thus, its viability.

In the presented work, the system which has been developed and proposed: donor tissue - nanomodified, biocompatible, aseptic surface - conservating medium. The research results showed that the storage of the donor cornea in the proposed special container seems to be a more perfect dynamic and original in its simplicity model for the storage of viable donor material.

Conclusion

It is difficult to imagine modern life without the use of a huge mass of products made of polymer materials, which is especially common in our everyday life, as well as in medicine and biology, primarily as a material for disponible items, as a guarantee of safety and hygienic safety for health. However, in reality, everything turned out to be exactly the opposite! A polymer is a material used in laboratories to grow microorganisms; a biofilm is inevitably formed on the surface of the polymer material, which causes severe diseases by infection with microorganisms from the biofilm, which are very difficult to treat, even with antibiotics and sulfonamides. that is why the separate class of diseases caused by microorganisms from biofilm has separately isolated nosologically! The solution to this dangerous and large-scale problem is possible either by abandoning cheap polymers or by creating more expensive polymers, which is difficult to imagine economically and technologically. Any way this problem can also be successfully solved by improving the properties of the polymer surface of materials, by surface treatment, for example, by Nano structuring, Nano modification of the polymer surface, by means of applying carbon-containing coatings that provide the creation of specified physicochemical properties of the polymer surface, with certain specified properties of the polymer

surface. This would allow the successful use of polymeric materials in the future. Our tasks, research methods and achievement of our goals were aimed at prolonging the quality and bioplastic properties of biomaterials during their preservation, as well as the quality of preserved donor materials and their safe transportation. This is achieved by imparting certain specified properties to the surfaces of polymer products by treating the surface of polymer products using modern nanotechnology methods. The optimal parameters of aseptic biocompatible carbon-containing coatings are substantiated when creating special containers and packaging devices treated with carbon-containing films deposited on their polymer surface for the safe storage of donor materials and other items of medical and biological use that require aseptic storage. The developed coatings with NSPP, NMPP can be used to treat the surface of special containers for donor cornea preservation and various polymer packaging devices for the preservation of other donor materials and products requiring aseptic storage by applying carbon-containing coatings with antibacterial properties that are biocompatible to the biological tissues. In a physiological buffer solution used as a storage medium, nanofilms also create a weak cationic environment, which eliminates the presence of any contamination during preservation, This is also contributes to the polarization of cell structures, restoring the potentials of cell membranes, thereby maintaining the shape and structure of the tissue, for example, of a donor cell. The adhesive properties of these coatings are determined by the electrostatic properties of NPPS, NMPS and can both promote adhesion on the treated surface: chemical compounds, protein structures, cells, cellular structures, and the nanofilm can behave as a repulsive surface. The use of special plastic containers and packaging devices with an aseptic, biocompatible surface makes it possible to create aseptic storage conditions, increase the shelf life of the donor material and, thus, have stocks of the donor material. The use of high-quality donor material that has retained its viability for transplantation ensures a successful outcome of the operation and reduces the risk of postoperative complications. The creation of a biologically active system by forming an interface between the nanostructured surface and the underlying biological tissue can be a solution to the problem of prolonged storage of viable donor tissues in high-quality condition. So, on the basis of these results and conclusions, a method for the preservation of viable donor corneal tissue by creating conditions for prolongated-term storage that are as close to physiological as possible was developed. Patent RU No. 2690153, 06/28/2019).

In our investigation, the donor cornea was also used as a model for allogeneic graft preservation. Since the cornea is a unique biological tissue that develops ontogenetically from 3 different germ layers, this can be considered as a hint at the preservation of somatic cells of other organs and body systems. That is, such a principle of applying the proposed method of preservation could be studied in more depth in order to develop methods for storing other donor tissues and organs.

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