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Research Article

Preservation Conditions of Donor Cornea Ensure its Proper Quality, As Optical Characteristics and Biocompatibility

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Abstract

The proposed method for preserving grafts relates to ophthalmology and transplantology and can also be used for the safe preservation and transportation of various viable grafts using, as an example, the donor cornea (DC) preservation method. According to embryogenesis, the cornea develops from 3 germ layers: ectodermal, mesodermal and endodermal. This means that the basic principles of the DC preservation and transportation method can be used as a universal method for the preservation and transportation of some other viable donor tissues and materials by modifying the proposed DC preservation method. Consequently, to the method described above, a special container (SC) is used from a polymeric material, the shape of which repeats the shape of the cornea and this SC is manufactured on the same contact lens production line. The graft preservation buffer may contain silver nanoclusters.

So, DC is placed in a special container in the form of a corneoscleral lens which is perforated over the entire SC's surface; then the SC, containing DC, is placed in a cylindrical vial with a buffer solution for further storage and transportation. For safe aseptic prolongated storage and secure transportation of various viable grafts, they are placed in a container in a shape corresponding to the shape of the storing graft; the polymer surface of such containers is also treated by means of carbon-containing nanofilms deposition to its polymer surface. Carbon-containing nanofilms can be diamond-like, carbyne-containing, fullerene- containing, 0.01-1 μm thick, with an integral surface charge. The use of the proposed method is aimed at maintaining the proper quality of the donor material during their storage, because of prevent the deformation of donor materials and tissues, as well as eliminate the risk of biofilm formation or the occurrence of various contaminants on the surface of the nanomodified polymeric containers when in contact with biological media.

Most of the current works devoted to and aimed at improving the quality of the organ culture of the donor cornea (DC) indicate the need, first of all, to modify the nutrient medium by changing the quantitative and qualitative combination of its components. However, exposure to various components of the nutrient medium can lead to an overload of the recipient's immune system and early graft rejection.

Introduction

One of the first methods for preserving the DC was the method of V.P. Filatov, who in 1935 proposed a method for conservation the eyeball in a "wet chamber". In this case, the entire cadaveric eye is stored in a closed sterile glass jar filled with saturated moist air at an average temperature of 4 °C. If the time between the death of the donor and the enucleation of the eye is from 4 to 6 hours, then the donor eye preserved by this method can be stored and used for penetrating keratoplasty (PKP) for 2 days, with a longer period - for anterior lamellar keratoplasty (ALKP). It has been observed that the shorter the storage period, the better the clinical results of postoperative graft engraftment. The main disadvantage of this method is the post-mortem contact of the endothelial layer with altered depletion of moisture in the anterior chamber of the eyeball.

Research Approach

The main idea and meaning of preserving the donor cornea using the Filatov method, as it seemed logical, was primarily to maintain the right shape of the cornea and preserve the structures of the eyeball before excision of the corneal-scleral segment from the entire eyeball and its immediate transplantation into the patient's eye.

In the modern activity of eye banks, the main methods for preserving the donor cornea, corneoscleral flaps are the medium-term method of hypothermia and the prolonged method of normothermic conservation, which ensure adequate preservation of the corneal tissue and its endothelial layer, throughout the successful completion of therapeutic terminal and layered corneal transplantation, which also allows for systematic delivery cadaveric corneoscleral flaps to banks of medical institutions for planned and emergency ophthalmic reconstructive interventions. At the same time, discarding DCs cannot be avoided, which, according to ophthalmic banks, can reach up to 50% as unusable during the DC preservation.

Materials and Methods

Based on the study of these issues, we have researched, developed and proposed a Method for the preservation and safe transportation of DC, as the first method of aseptic storage proposed in ophthalmologic medical practice "Method for the preservation and safe transportation of allogeneic implants, donor tissues", on the example of the DC, in a special polymer container (SC), the inner polymer surface of which is processed by the method of Nano structuring and Nano modification of the polymeric surface. 2017; EN 2690153 C2)

The proposed method is focused on solving the problems associated with long-term storage and safe transportation of high-quality viable DC, which eliminates, first of all, any deformation of the corneal tissue. The implementation of the method is aimed at reducing the number of discarded DC and reducing the number of various postoperative ophthalmic complications, such as graft disease. This can be achieved by improving the method of preserving and transporting the DC in SC, the shape of which is most convenient and corresponds to the shape of the native cornea, since it completely repeats in shape and contour when placed in a container in the form of a corneoscleral lens.

The aseptic DC storage method implies that the DCis placed into a SC like polymer contact lens shape (diameter 17,0 mm, radius 8,8 mm, curve radius 8,8 mm, sagittal height 5,8-6,2 mm). The suggested DC into SC in the shape of a contact lens manufactured from a polymer on the equipment for polymer soft corneoscleral lenses production, that has a NSS (nanostructured surface) and is perforated. In addition, the shape of the container has to have an empirically aspheric surface with the angle on the whole surface not less than 164 degrees what can provide the state of corneal cells close to physiological condition. The cradle packing form of the SC prevents any deformation of the DC placed in this shape of the SC. These facts are not considered in the papers on the topic before.

Results

The essence of the invention is illustrated in the following graphic materials.

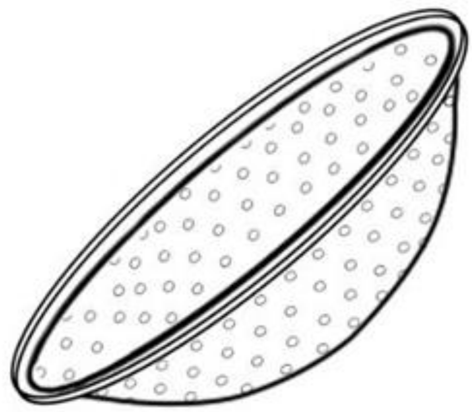

Fig. 1 Container made of polymer, in form corresponding to the corneoscleral contact lens, in the form of an aspherical segment, with surface angle, at least of 164 degrees and not less, across the empirically aspherical shape of the surface of the container; the container is perforated and has the following dimensions: diameter 18 mm, thickness 0.3 mm, radius of the central curvature of the container 8.8, sagittal height 5.8-6.2 mm, the torsion edge of the container is flattened around the entire circumference and has a tape shape 4 mm wide (2 mm outside and inward to form a groove with the inner surface of the container where the edge of the donor cornea fits.

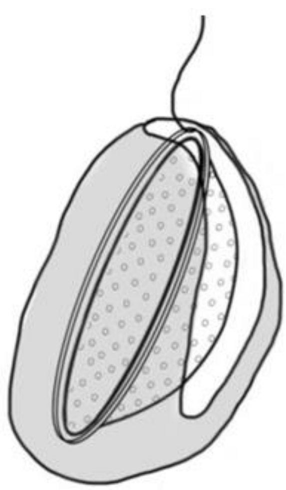

Fig. 2 The outer protrusion of the torsion edge of the container is stitched with a 5/0 Mersilk thread (Ethicon, AAH, Bristol, UK) for fixation.

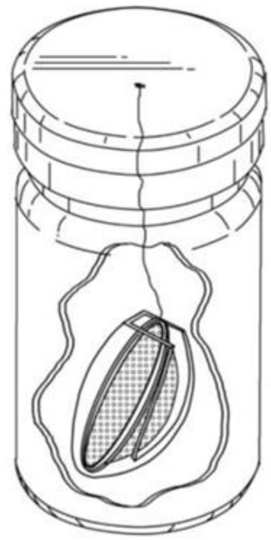

Fig.3 A container made of polymeric material, perforated, which surface is nano-modified, where a donor cornea neatly packed in it and soon is placed in a 120 ml cylindrical plastic bottle with 100 ml of preservative medium, which can contain the silver nanoclusters for prolonged of high quality viable material storage.

The proposed container has a flattened toric edge, the inner protrusion of which forms a recess along the entire circumference of the special container with the inner surface of the SC. The edge of the excised DC is carefully placed in this recess, then the entire DC is carefully placed in the SC, which, as already described above, has the shape of an exactly corneoscleral lens. The container is then stitch through the outer edge with Mersilk 5/0 thread (Ethicon, AAH, Bristol, UK). Next, such a container, with a DC placed in it, is placed in a cylindrical container filled with the storage medium.

The special container surface can be treated with carbon-, carbyne-, diamond- like, fullerene-, nanotube-containing films, as well as their combination, mono-, multilayer, mono-, hetero -phase. The methods variation can give the possibility to obtain certain specified polymer surface characteristics, which guarantee aseptic features, biocompatibility. Certain chemical composition of the treated polymer surface, its charge provide security for living tissues in contact with such treated polymer NSS. Obtaining antimicrobial Nano composite polymer materials by forming NSS is done by means of preliminary treatment of the polymeric surface with ions of chemically active and/or inert gasses.

The composition of the gasses is altered to perform the ionic treatment or other modes of this operation to gain the relief with the desired root-mean-square roughness (Rq), and further application of the Nano sized film obtained on the base of carbon by means of ion-stimulated deposition from the gaseous phase of vapors of carbon-containing compounds which include sp2- and sp3-hybridized states of carbon. The nanosized carbon-containing film is manufactured in the form of a multilayered structure. The tension of average ionic energy is altered periodically or the gasses forming vapors of carbon- containing compounds at constant ionic energy are interchanged to regulate the number of sp3- and sp2-hibridized carbon states in the molecular content of Nano layers. At that the ratio of sp3-/sp2-hibridized carbon states in Nano layers is sustained within the interval from 0,7 to 1,8 resulting in antimicrobial Nano composite polymer material. The surface features play a great role in the bio system as most reactions occur on the surface and interface. The size of nano structurization is similar to that of most biomolecules and structures of NSS intended to create any properties of surfaces, from chemical inertness to a CF treated surface with proper characteristics: surface charge, surface oxide hydration degrees, surface relief, and types of conductivity. As a result, these can assist with the adhesion or impart the repelling of a chemical compound, protein, cells from interface through the creation of active functional groups of composites by means of NSS, improving the polymer surface biocompatibility, which is directly proportional to the magnitude of the surface charge potential, and through elimination of a certain composite off the surface.

The development of perfect surfaces production can provoke specific cellular responses and direct new tissue regeneration. The level of this area of technique characterizes the way to obtain Nano composite biologically active polymer materials described in the article by V.M. Elinson, V.V. Sleptsov, A.N. Lyamin, V.V. Potryasay, L.N. Kostyuchenko, A.D. Musina "Barrier properties of carbon films applied to a polymer base in conditions of aggressive environment". Engineering journal; №11 (32); 61-64;1999 and in the Works of V International scientific-technical conference «High technologies in Russia’s industry». M.: MSTU n.a. N.E. Bauman, pp 419-426, 1999, which include nanostructured surface formation in the base layer made of a biocompatible polymer material by means of treating the base surface with ions of chemically active and/or inert gasses with the further modification of the formed developed surface by means of applying the carbon-based film.

Discussion

The features of the deposition of carbon-, carbyne-containing films, fluorocarbon nanofilms on the polymer surface have been studied. At present day, the modification, for example, of a polymer surface in order to obtain the certain surface with desired properties is possible only through the application of nanotechnology.

The industry of polymer material production is a large-scale and rapidly expanding area. As new technological processes of polymer production are being developed and introduced, polymer waste is growing even faster.

At the same time, these wastes can be avoided if in the process of polymer products and films manufacturing, additionally, in processing of the product at its output is supplementing by applying a polymer material surface modification. Modern, advanced technologies are also aimed at the development of technologies forwarded at the producing the quality of materials that do not harm the environment. Nanotechnology can be viewed as such an advanced technology. Nano modification- treating as additional stage in technology process stage as an opportunity to polymer material protection and improving.

The wide use of polymers has resulted in a variety of diseases associated with, for example, microorganisms of the biofilm formed on the polymer surface when it contacts with organic environment and then complications are inevitable. The process of biofilm formation goes due to the loose friable porous structure of polymer materials, which could be as a nutrient medium for microorganisms. There exists a class of diseases called "diseases caused by the biofilm pathogens." It is a separate class of severe diseases, which sometimes are not amenable to conventional treatment. Considering the fact that the microorganisms have the ability to synthesize proteins with inorganic compounds, it enhances the harmfulness of biofilm agents, for example, increases the danger of new, stronger and more resistant strains. The aforementioned reasons either require using more expensive materials than polymers or the application the modification of the polymer material surface. But the production of polymeric products from cheap and affordable raw materials, films from PET, PTFE, is already in full bloom and stopping it does not seem realistic today.

And it is difficult to imagine how to reduce or abandon the ubiquitous and large-scale production of polymer products. But it seems possible at the final stage of the conveyor technology process of polymer products obtaining to add the Polymer Surface Treating by means of application of the nanotechnology, which is aimed at the Nano modification of the physical-chemical surface characteristics: the relief, chemical element composition, atomic structure, charge on the surface, all of this can assist the adhesion features or impart the repelling of a certain element, biochemical compounds, proteins, cells from the interface. The creation of active functional groups of composite NSS gives a new quality of material with the certain NSS features. In recent years, significant advances in the field of nanotechnology have emerged and developed at the junction of R&D.

Nanotechnologies allow using carbon as one of the smallest chemical elements to create unlimited number of nanocomposite materials. Carbon as no other element has a unique range of quite opposite properties: it is a dielectric and a metal, a semiconductor and a semimetal, a super hard and super soft heat insulator, the best heat conductor, the model of transparency and an absolutely black; above that it is super tough, has a specific surface, and is various when in different chemical compositions. The following materials have unique carbon properties: graphite’s, graphene’s, fullerenes, carbyne, diamond, nanotubes. The creation of active functional groups of composite NSS gives a new quality of the created material with certain preset NSS features.

Living organisms for more than 98% consist of four chemical elements: oxygen (O), carbon (C), hydrogen (H) and nitrogen (N). Carbon is the second common element present in living tissues and cells. This chemical element with one of the smallest sizes allows obtaining an unlimited number of chemical compounds or gas mixtures for surface treatment to get a wide range of surface characteristics with any programmed desired properties. The use of carbon- or carbyne-containing films treated with surface nanomodification (SNM) by preliminary surface Nano structuring (SNS) - the technology for protecting the surface of polymeric materials is a solution to the problem of biofilm formation, which also ensures the biocompatibility of the treated surface with living tissues when is in contact.

As it is known, the surface properties of the material are determined by its topography, surface charge, and atomic structure.

The materials obtained by the described method are biocompatible, possess bacteriostatic and hypoallergenic properties, high chemical resistance to the human biosphere and could be recommended for use in medical pharmaceutical, hygiene.

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And it is difficult to imagine how to reduce or abandon the ubiquitous and large-scale production of polymer products. But it seems possible at the final stage of the conveyor technology process of polymer products obtaining to add the Polymer Surface Treating by means of nanotechnology application, which is aimed at the modification of the physical-chemical surface characteristics: the relief, chemical element composition, atomic structure, charge on the surface, which can assist the adhesion features or impart the repelling of a certain element, biochemical compounds, proteins, cells from the interface. The creation of active functional groups of composite NSS (nanostructured surface) gives a new quality of material with the certain NSS features. In recent years, significant advances in the field of nanotechnology have emerged and developed at the junction of R&D.

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The materials obtained by the described method are biocompatible, possess bacteriostatic and hypoallergenic properties, high chemical resistance to the human biosphere and could be recommended for use in medical pharmaceutical, hygiene.

Thus, the use of processing the polymer surface of a container for storage of DC by applying carbon-containing films that determine the physicochemical properties of the treated surface, subsequently in contact with the donor cornea, seems to be more appropriate and expedient in the procedure for DC storage. Besides such maintenance allow one to carry out the test of individual biocompatibility before DC transplantation. This will also help preserve one of the most important optical properties of the transplanted DC— its transparency, structure, and biocompatibility.

Basic provisions of the work

1. The DC is placed into a special polymer container the inner surface of which is NSS and NSM; then the container is arranged within a cylindrical flacon filled with supporting storage medium.

2. The DC is preliminarily placed in a special container with a thoroughly perforated surface in the form of a corneoscleral lens, which also performs the bandage function of the "cradle" to avoid any deformation of the DC.

3. One of the most important advantages of the proposed method is the nanomodification of the polymer surface of a special container in contact with DC by depositing Nano films containing carbon, carbyne, diamond-like, fullerenes, nanotubes, as well as their combinations, mono-, multilayer, mono-, heterophases.

4. The method differs as the films applied to the container surface are 00,1-1mkm.

5. The NSS surface has an integrated charge; the nanosized film is made in the form of a multilayered structure, periodically changing the tension of average energy of ions or interchanging the gases forming vapors of carbon-containing compounds with constant ionic energy thus regulating the molecular content of the film nanolayers sp3- и sp2-hibridized carbon states. At that the ratio of sp3-/sp2 hibridized carbon states is sustained as not less than 0,5 mA/cm2.

Conclusion

The polymer surface treatment method can be implemented and used for conservation, storage and transportation of DC in a special polymer container or simply in the production of a nanomodified polymer sheet suitable for wrapping, for example, various transplant materials, for further successful implantation and grafting. It can be used in the Tissue, Blood and Eye Banks.

The cradle form of the special container is produced from a polymer on the same equipment as is used for manufacturing of soft contact lenses Its surface is treated with carbon-, carbyne-, diamond-like, fullerene, nanotube-containing films as well as their combinations in mono-, multilayer, mono-, heterophase. It is meant to be a special container for DC storage having a fitting shape for native cornea thus protecting DC from any deformation. In addition, the shape of the container has to have an empirically aspheric surface with the angle of the overall surface not less than 164 degrees, what can provide the state of corneal cells close to physiological condition. After being placed into a special container to protect DC from any deformation, contamination and similar physiological conditions, DC is placed in a cylindrical flacon filled with storage medium. The possibility of control in wide ranges of NSS characteristics of the polymers used for the manufacture of containers and packaging material for viable tissue and implants storage, applying carbon films ion-plasma methods has established. It is shown that the biocompatibility of carbon films is directly proportional to the magnitude of the electrostatic potential, and the antibacterial activity is determined by the structure and surface relief.

The proposed method for the preservation, storage and transportation using of DC preservation method, as an example, makes it possible to reduce the number of discarded donor corneas and other grafts that have become unusable due to improper storage, as well as to reduce the number of possible postoperative complications.