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Experimental study to investigate the safety of new intraocular bio-degradable implant for drug delivery to the posterior segment of an eye

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Purpose: *to prove the safety of dexamethasone-free and dexamethasone-loaded intraocular implants in the experiment in vivo.*

Materials and methods*. The study included 60 Chinchilla rabbits (120 eyes). In the first series of animals, dexamethasone-free implants were inserted into the anterior chamber of the eye in the rabbits of the 1st group,*

and into the vitreous cavity in the rabbits of the 2nd group. In the second series, dexamethasone-containing implants were inserted into the anterior chamber of the eye in the rabbits of the 3rd group, and into the vitreous cavity in the rabbits of the 4th group. The intraocular structures were evaluated by ophthalmologic examinations: slit lamp biomicroscopy, fundus photographic images, electroretinography (ERG) performed before the implantation and in dynamics. In the same time period, the animals were withdrawn from the experiment, the eyes enucleated, and morphological studies performed.

Results*. Being inserted into the anterior chamber of the eyes in the rabbits of the 1st and 3rd group, the implants settled on a surface of an iris, occupied the position in the anterior chamber bottom. The implants were resorbed within 31–33 days. Throughout the whole study period, no intraocular structure abnormalities were identified by the slit lamp biomicroscopy in the rabbits of the 1st and 3rd groups. Morphological studies of the eyes demonstrated no structural abnormalities in the cornea, iris, or the ciliary body, either. While studying the effects of dexamethasone-free and dexamethasone-containing implants on the posterior structural segments of the eyes in the rabbits of the 2nd and 4th groups immediately after intravitreous implantation, the implants were identified positioned in the anterior third of the vitreous of a rabbit eye. Their gradual resorption took the whole study period. Implants were not identifiable any more in the vitreous body at day 35. No evident abnormalities were seen in the structures of the eye anterior segments, vitreous body or retina throughout the study period. Histological examination showed no abnormalities of the retinal or other intraocular structures.*

Conclusions: *in the experimental study in vivo, the developed implant has proved to be an inert, biocompatible intraocular system for drug delivery and posing no toxic effects on eyeball structures of a rabbit.*

Keywords: implant, drug, biodegradable polymer, dexamethasone.

Today, doctors in clinical practice possess a large arsenal of techniques to treat various eye diseases. The purpose of medicinal drug delivery to damaged tissues is to establish and maintain therapeutic drug concentrations for a sufficient time period. However, existing histohematogenous barriers impede the drug delivery to a diseased tissue in adequate concentration for a required time period thus reducing the treatment efficacy. So, the search for novel posterior segment ocular drug delivery strategies is of a great practical importance.

According to a number of Russian and foreign investigators, the d is preferable while treating the diseases of the retina, choroid, and optic nerve [1-3]. This would reduce the drug dose and minimize its impact on other tissues [4, 5]. Drug administration into the vitreous cavity allows the therapeutic concentrations to be maintained over an extended period when compared to other routes of drug delivery [6-8]. Moreover, the intravitreal drug administration reduces the risk of systemic side effects due to less systemic exposure in terms of a smaller dose, and the substance being eliminated from an eye, thus by-passing the systemic circulation.

One of the most important trends in ophthalmology nowadays is the development of microinvasive systems capable of drug delivery to the pathological foci overpassing the anatomic and physiologic barriers. All existing systems for intravitreal drug delivery can be classified into two groups: non-biodegradable implants and biodegradable implants [9, 10]. Of

note, the main disadvantage of all non-biodegradable systems is the need for their subsequent surgical removal which increases the risk of postoperative complications [11-15]. Biodegradable implants, unlike non-degradable ones, are subjected to a complete absorption in the vitreous cavity over time, requiring no subsequent removal which significantly reduces the risk of postoperative complications [16-20].

According to literature reports, the use of controlled release intraocular drug delivery systems may be associated with adverse events such as a cataract development, persistent uncompensated hypertension, retinal detachment, choroidal detachment, a transient decrease in visual acuity, vitreous hemorrhage, and others. [20-22].

All the above makes promising the research and developments of a home-manufactured implant that would act as a drug carrier; there is an essential need to investigate its properties, the ability of the implant to ensure a controlled drug release to achieve sustained therapeutic levels in the vitreous cavity and to reduce the risk of various complications [4, 6].

In cooperation with the *Eye Microsurgery Research and Experimental Production, LLC*, we have developed a biodegradable intravitreal implant for posterior segment ocular drug delivery that was based on a lactic acid, polyvinylpyrrolidone, and glycosaminoglycans, of a rod-shape, 0.3 mm in diameter, and 4.0 mm length designed for implantation in the vitreous cavity using special gauge 27 devices (Fig.1). The system can be loaded with various medicinal substances, including dexamethasone.

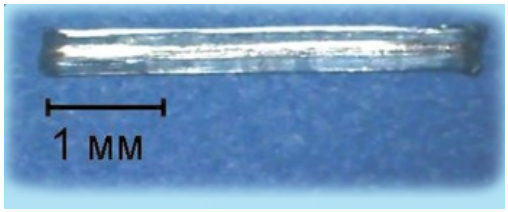

Fig. 1. Layered biodegradable implant designed in the *Eye Microsurgery Research and Experimental Production, LLC, Moscow*

The experimental sample system was loaded with dexamethasone at a dose of 300 micrograms in laboratory conditions in such a way that drug-saturated layers alternated with drug-unsaturated layers to prevent an excessive release of the active substance. Varying the number of cross-linking, we created the implant comprising the following dissolution profile of the layers: 3 days for a drug-saturated layer, 1 day for a drug-unsaturated layer. The dexamethasone release from the designed drug-loaded implant was investigated in vitro by spectrophotometry (Lambda EZ 201 Spectrophotometer, Perkin Elmer Corporation, USA) in the ultraviolet region of the electromagnetic spectrum with a wavelength corresponding to the maximum absorption of dexamethasone (λmax = 242 nm).

The obtained results reflecting the dexamethasone release profile presented as a "time-concentration" (exponential) curve demonstrated a gradual increase in dexamethasone concentration over the first 3 days with the fall in its concentration by the mid of the 4^{th} day that corresponded to the time of drug-unsaturated layer dissolution. The drug concentration resumed its rise at day 5 and was increasing till day 7 that again was followed by the decrease in dexamethasone concentration at day 8 (Fig. 2). In general, the drug release cycle repeated 8 times. By day 31, all the active substance had released, meanwhile, the biopolymer matrix had completely resorbed.

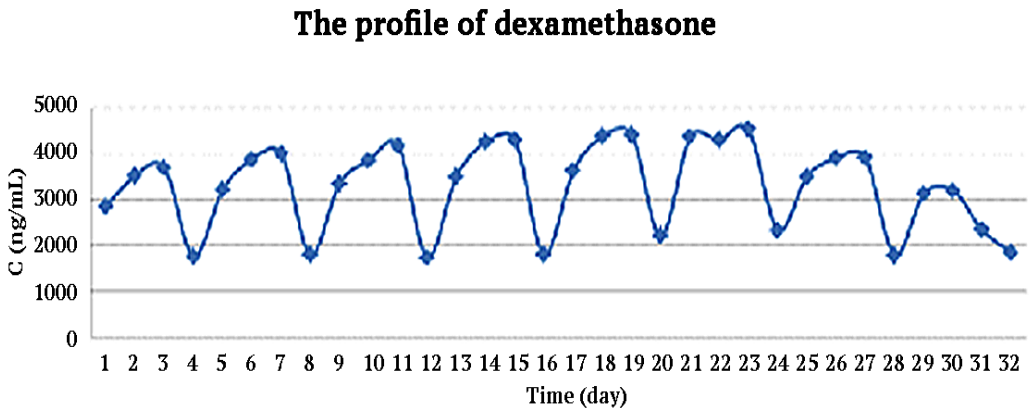

Fig. 2. The profile of dexamethasone release during the entire period of observation

After the completion of in vitro experiments and obtaining the results, we performed a safety study of intraocular biodegradable implant administration on an animal model. The aim was to study clinical and morphological safety of dexamethasone-loaded and dexamethasone-free implants at intraocular administration.

Material and Methods

The study material comprised drug-free implants and drug-loaded (dexamethasone) implants at a dose of 300 micrograms (mcg). The drug-loaded implant was designed in a way where 8 drug-saturated layers interlaced with 7 drug-unsaturated layers so that the top and bottom layers contained the active drug. Dexamethasone was distributed in equal amounts between the drug-saturated layers. The layer dissolution profile in the device was as follows: 3 days for a drug-saturated layer, 1 day for a drug-unsaturated layer.

The study was performed on the base of he Kaluga Branch of Academician S.N.Fedorov Eye Microsurgery Interdisciplinary Science and Technology Complex of the Russian Healthcare Ministry, (Director of the Institution: A.V.Tereshchenko, Dr. Med. Sci.), under the leadership and supervision of Professor Yu.A.Belyy, the Deputy Director of the Eye Microsurgery Complex, and A.A.Temnov, Dr. Med.Sci, the Head of the Laboratory for Cellular and Physico-Chemical Medical Technologies of the N.V.Sklifosovsky Institute for Emergency Medicine.

The study included 60 Chinchilla rabbits (120 eyes) and was conducted as two series of experiments. In the first series, dexamethasone-free implants were inserted into the anterior ocular chamber in 15 rabbits (15 eyes) of the 1st group, and into the vitreous cavity of 15 rabbits (15 eyes) of the 2nd group. Intra-group comparisons were made versus intact contralateral rabbit eyes, and contralateral eyes after intravitreous administration of 0.1 ml balanced saline, for the 1st and the 2nd group, respectively. In the second series, the 3rd and the 4th groups of rabbits were allocated. Dexamethasone-loaded implants were inserted into the anterior chamber of an eye in 15 rabbits (15 eyes) of the 3rd group, and into the vitreous cavity in 15 rabbits (15 eyes) of the 4th group. Comparisons in the 3rd group were made versus intact rabbit eyes. In the 4th group, comparisons were made versus rabbit eyes of the 2nd group from the first series of experiments where drug-free implant was inserted into the vitreous cavity.

All rabbits received instillation of 1-2 drops of 1% tropicamide solution in conjunctival sac of both eyes at 30 minutes before surgery to maintain the maximum of pharmacologically-induced mydriasis. A local anesthesia in all the rabbits comprised instillation of 1% alcaine solution in

the conjunctival sac, and a retrobulbar administration of 2% novocaine, 1.0 ml. The surgery was performed under intravenous anesthesia (with 10% hexenal at a dosage of 10-15 mg/kg of body weight). Prior to surgery, the conjunctival sac was rinsed with an antiseptic solution.

Eyelids were fixed using blepharostat, the eyeball was secured in position using fixation forceps grasping the limbal conjunctiva. The injection into the anterior chamber was performed at the 3-o'clock position. Corneal paracentesis was performed with a 2.0 mm lanceolated knife, and the implant was inserted using a 27 gauge cannula.

To insert the implant into the vitreous cavity, the eyeball coats were punctured with 27 gauge port at 2 mm apart from the limbus towards the equator in upper outer quadrant. A straight 27 gauge cannula was carried deep into the vitreous, parallel to the lens; the implant was inserted into the upper third of the vitreous cavity. Then the 27 gauge port was removed. After the eye surgery, the sklerotomy site was sealed without suturing.

The intraocular structures were evaluated ophthalmologically before the implantation, and afterwards on the 1^{st}, 7^{th}, 14^{th}, 28^{th}, and 35^{th} days post implantation using the slit lamp biomicroscopy (Opton, Germany) of the anterior eye segment, ophthalmoscopy using a binocular ophthalmoscope (Heine, Germany), obtaining photographic images of the eye fundus using a *Ret Cam-120* retinal diagnostic system (USA) and electroretinography (ERG) on Tomey Electrodiagnostic System (Japan). ERG was performed in the animals that received drug-free and drug-loaded implants intravitreally.

After all the above mentioned examinations, the animals were withdrawn from the experiment by means of air embolism in the same period, the eyes were enucleated and morphological studies were performed.

Results

Being inserted into the anterior chamber of an eye in the rabbits of the 1st and 3rd group, the implants settled on a surface of an iris, and on the 1st day they took the position at the anterior chamber bottom, and moved freely in accordance with eyeball movements (Fig. 3). The implant resorption in the anterior chamber took 31–33 days. Ophthalmologic examinations of the eyeballs from the 1st and 3rd experimental groups demonstrated no evident abnormalities of the implant surface throughout the entire study period. The implants were gradually reducing in the length, their edges becoming smooth, as observed during the study period. At day 28, the implants were oval in shape and significantly reduced in size by more than 2/3 of the baseline length (Fig. 4). Implants ceased to be identifiable in the anterior chamber by day 33.

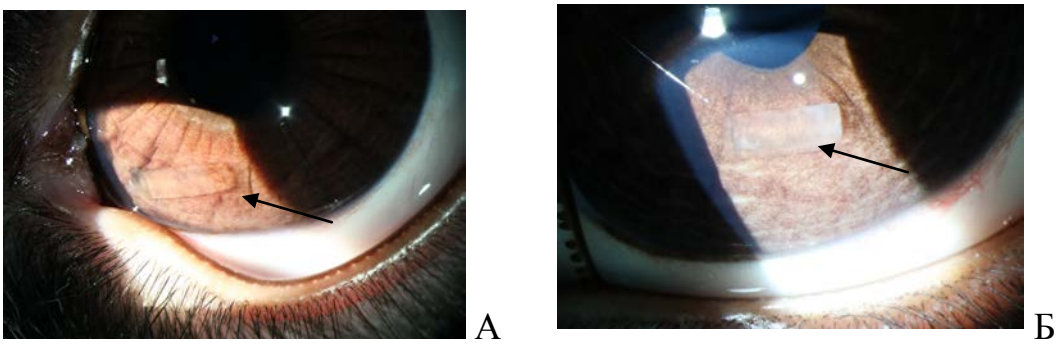
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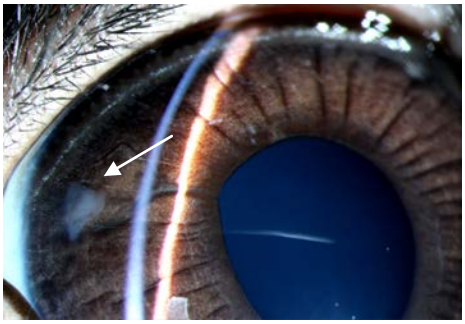
Fig. 3. Drug-free (A) and drug-loaded (B) implants (*arrows*) in the anterior chamber of the rabbit eye on the 1st day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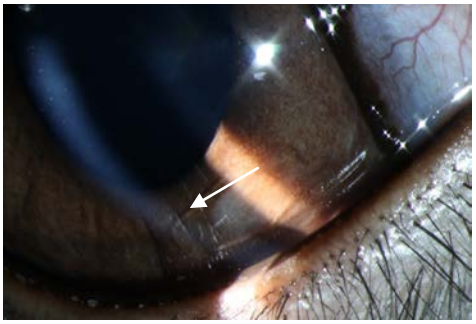
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Fig. 4. Drug-free (A) and drug-loaded (B) implants (*arrows*) in the anterior chamber of the rabbit eye on the 28th day

In one of 15 eyes from the 1st group, an implant edge fixation to the surface of the iris near the pupillary margin was noted at day 7. The implant was located in the lower part of the anterior chamber without following the eyeball movements (Fig. 5). No intraocular inflammation, fibrin deposits, structural abnormalities, or implant discoloration were seen. The follow-up assessment of the implant resorption demonstrated a gradual decrease in the implant size while keeping fixed to the surface of the iris. At day 21, the decision was taken to withdraw the animal from the experiment to perform morphological studies of the anterior segment ocular structures and the implant fixation area.

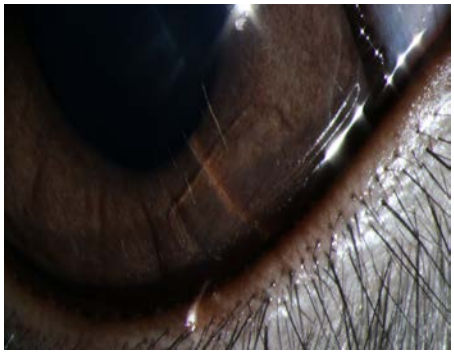
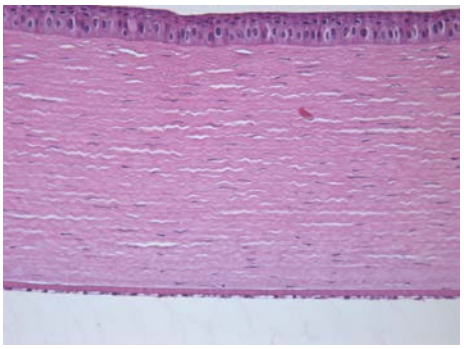


Fig. 5. Drug-free implant fixation to the surface of the iris

Biomicroscopy of all rabbit eyes from the 1st and 3rd study groups and from the 1st and 3rd control groups demonstrated the transparency of the cornea and the anterior chamber humid throughout the whole study period; the anterior chamber was of medium depth, a papillary light reflex maintained normal, the color and structure of the iris were without alterations. During the observation period, the lens remained clear, the deep-lying media and structures the fundus were without evident abnormalities. Neither inflammation, no abnormalities (i.e. conjunctival hyperemia, corneal precipitates, hypopyon, hyphema, cataracts, pupillary shape alteration, or others) occurred in the anterior segment ocular structures in any of the animal eyes from the 1st group during the entire observation period.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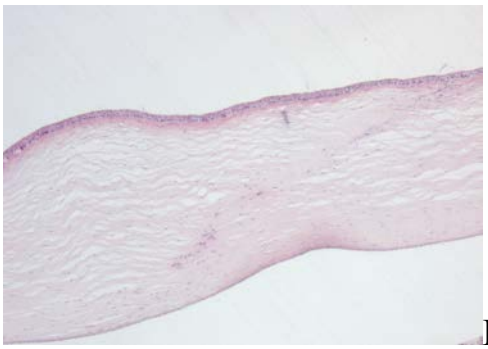
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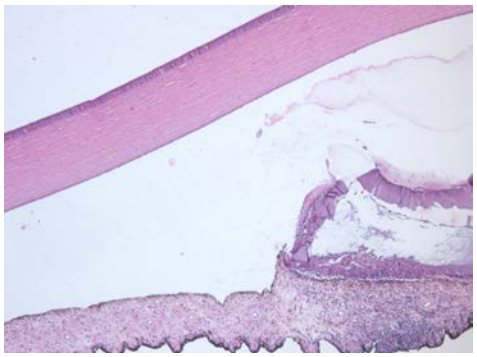
Fig. 6. Cornea at day 7 after the placement of the drug-free (A) and drug-loaded (B) implants in the anterior chamber of the rabbit eye (H & E stain; Magnification: A - x100)

Histological examinations of the eye specimens from the 1st, 3rd study groups and corresponding controls revealed no structural abnormalities in the cornea, iris, or ciliary body (Fig. 6). The histology of the rabbit eyeball with a fixed implant performed on day 21 revealed the implant adhesion to the iris, and the implant incomplete biodegradation. A local reactive

inflammatory infiltration of the iris was seen at the contact site. Meanwhile, no pathological alterations were identified in any other anterior segment structures (Fig. 7).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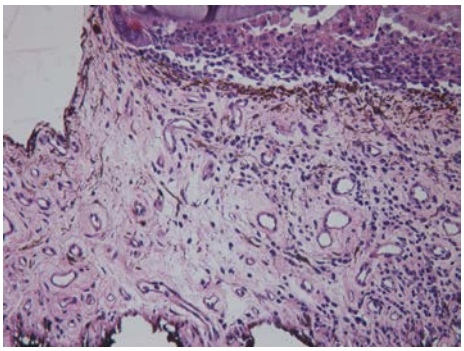
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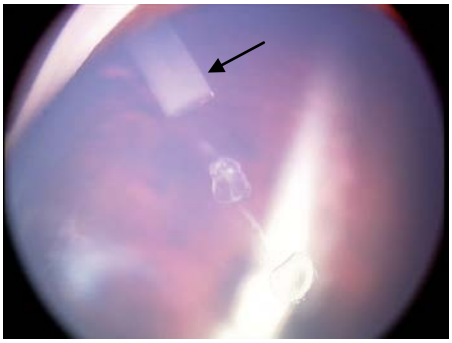
Fig. 7. The implant (*arrow*) located in the anterior chamber of the rabbit eye on the 21st day. Incomplete biodegradation of the implant (A), local reactive inflammatory infiltration of the iris at the contact site (A, B), other anterior chamber structures being intact (H & E stain; Magnification: A – x50; B - x200)

While studying the effects of dexamethasone-free and dexamethasone-loaded implants on the posterior segment ocular structures in the rabbits of the 2nd and 4th groups immediately after intravitreous implantation, the implants were identified as positioned in the anterior third of the vitreous of a rabbit eye (Fig. 8). The implant gradual resorption took place in the vitreous cavity during the whole study period. The implants had a round shape as were visualized in the vitreous cavity of the rabbit eyes from both study groups on the 28th day (Fig. 9). They occupied the position mainly in the middle and lower parts of the vitreous. At day 35, the implants were not identifiable in the vitreous any longer.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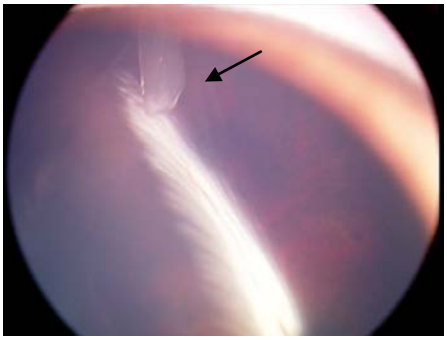
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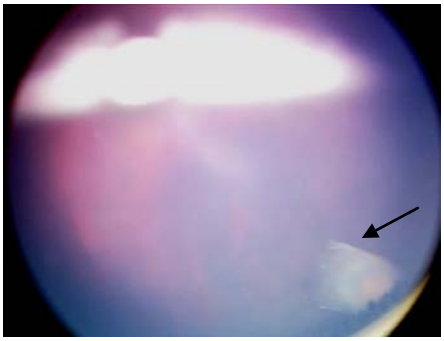
Fig. 8. Drug-free (A) and drug-loaded (B) implants (*arrows*) in the vitreous of the rabbit eye on the 1st day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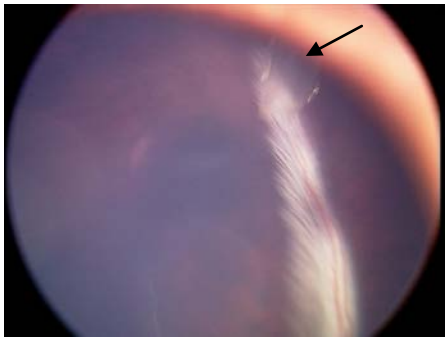
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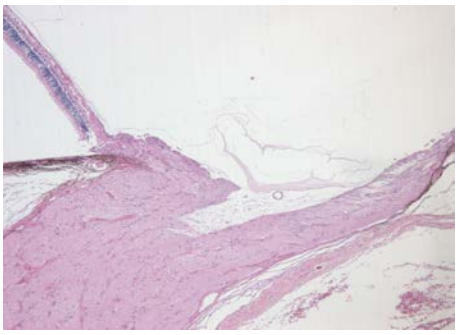
Fig. 9. Drug-free (A) and drug-loaded (B) implants (*arrows*) in the vitreous of the rabbit eye on the 28th day

A local swelling of the conjunctiva, a minor mixed injection of eyeball blood vessels at the site of scleral puncture were seen biomicroscopically in the eyes of the 2nd and 4th study groups and corresponding controls on the first day after the intravitreal implant placement, those were completely resolved on the third day. There were no evident alterations in the anterior segment ocular structures, the retina, and the vitreous throughout the entire study period. ERG findings in the study groups were similar to those in the comparator groups, indicating an eye minor response to the intravitreal placement of dexamethasone-loaded and dexamethasone-free implants. A

month later after the implantation, the bioelectrical activity parameters were consistent with normal values.

Histological studies of the eyes with intravitreally placed implant demonstrated neither structural abnormalities, nor proliferative process in the retina and other intraocular structures (Fig. 10).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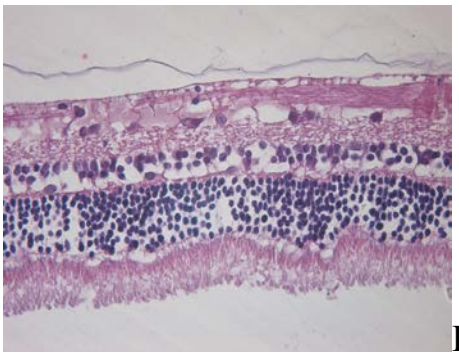
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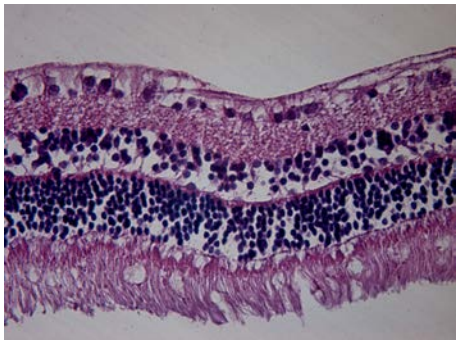
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Fig. 10. Posterior segment structures of the rabbit eye on the 28th day. No abnormalities in the disc of the optic nerve and central retinal vessels (A), no destructive inflammation in the retina after the placement of drug-free implant (B), after the placement of the drug-loaded implant (C).

Conclusions

The originally designed implant was proved experimentally in vivo to be an inert biocompatible intraocular drug delivery system that poses no toxic effect on the ocular structures of a rabbit. The implant may be used as a medicinal drug carrier (reservoir); and the placement of the drug into this

delivery system ensures the therapeutic agent to be intact until its contact and interaction with the affected tissue. This enables to provide a sustained release of the drug in the required dose without exceeding its therapeutic concentrations, as confirmed in the experimental safety study of intraocular implant placement. These results suggest the possibility to offer the designed biodegradable implant to be used for posterior segment ocular drug delivery and to proceed to clinical studies.

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