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STUDY OF THE EFFECTIVENESS OF DOMESTIC OPHTHALMIC FILM IN EXPERIMENT

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Abstract. To date, there are no film coatings that have antibacterial properties in the practice of ophthalmologists for use in penetrating wounds of the eye. **Objective**: to study the properties of domestic ophthalmic film in the experiment. **Material and methods**. The studies were conducted at the Interuniversity research laboratory of the Tashkent Medical Academy. The specific wound healing activity of film biological coating was studied on the model of primary penetrating wound of the cornea of 12 rabbits: 6 animals, 1 control and 2 experimental groups. **The results** of the study showed that the domestic wound dressing based on Na-CMC is effective in the treatment of stabbing wounds of the cornea, sclera and conjunctiva of the eyes, accelerates the regeneration processes by 14 days and there is a greater increase in body weight compared to the control group in which healing observed after 30 days and there is a smaller increase in body weight. **Conclusion.** The developed coating for the treatment of stabbing wounds of the cornea, sclera and conjunctiva of the eyes does not cause local irritating effect, hypoallergenic, which corresponds to the properties of wound coverings.
Keywords: biological coating, disco-diffusion method, infected eye wound, wound dressings, cornea, sensitivity to antibiotics, histologic-morphological studies.

Introduction.

Traumatic eye injuries and their consequences are still one of the main causes of blindness, poor vision of the injured eye and occupational disability. The prognostic criteria for the development and severity of complications determining the outcome of the injury, the frequency of removal of the eyeball, which varies from 6.5 to 26.3% of cases, largely depend on the size and location of penetrating wounds. Complications of penetrating eye injuries include post-traumatic cataract (65%), endophthalmitis (30%), intraocular infections (10%) [1,2]. The risk of developing complications is directly related to the time from injury to hospitalization. Crucial in preventing the development of severe complications of penetrating eye injuries are diagnostics and qualified primary surgical treatment, which are not always performed at the proper level [3,4]. Over the past decades, in addition to the traditional ophthalmic preparations used for penetrating eye injuries (gels, ointments, aqueous suspensions), ophthalmic films, colloidal systems consisting of nano- and microparticles, nanoemulsions, nanosuspensions have been developed. Compared with traditional subconjunctival injection, film coatings are characterized by a longer exposure, high bioavailability for the membranes of the eye [5,6,15]. The creation and implementation of new non-toxic, biodegradable coatings for the treatment of penetrating eye injuries is a priority for scientists, technologists and ophthalmologists.

The growth of pathogenic microorganisms resistant to antibiotics determines the search for new alternative antibacterial strategies, in particular in the field of ophthalmology [16]. At this stage of the development of science, innovative ideas are aimed at the development of new medical devices with certain properties. The interest of scientists is drawn at creating new treatments that provide ease of use and provide targeted therapeutic effect. Ophthalmic films for treating eye

injuries deserve special attention in this regard, as bacterial aggression is one of the factors in the development of complications and disability.

When developing new technologies, an integral part of experimental research is the assessment of its antimicrobial activity [17]. The annual death rate from infections caused by methicillin-resistant strain of Staphylococcus aureus (MRSA) is equivalent to the total number of deaths from AIDS, tuberculosis and viral hepatitis [18]. In this regard, the search for new effective biocoatings for the treatment of penetrating eye wounds that do not cause resistance in microorganisms is relevant for ophthalmologists.

Biomaterials remain one of the most complex and relevant tasks of modern ophthalmology and implantology. Difficulties consist in low bioavailability due to the peculiarities of the anatomy and physiology of the eye, the relative low permeability of the corneal epithelial membrane, the dynamics of tear fluid, nasolacrimal drainage, etc. [7,8,9,10].

For treatment, an ideal product could be a film coating that was applied for the damaged sclera for barrier function and further adequate therapy. The implant

t should have an adequate coating area, adhesive properties, be biocompatible, with a low frequency of complications, should not cause systemic and side effects, be easy to use, economically affordable [11,12]. Many existing implants inhibit the germination of fibroblasts and cause a chronic response to inflammation of the tissues of the eye. Biodegradable eye films based on water-soluble polymers are used as delivery systems. Film coatings can be used for both systemic and topical applications. Compared with traditional means, ophthalmic films have the following advantages: increased contact time, bioavailability, the ability to prolong the release of the drug, buffering efficiency, reduced systemic side effects [13,14]. Full biodegradation of the films leads to the absence of the need to remove the excess amount of remedy from the application area. Bioactive coatings for the treatment of penetrating wounds of the eye have high demands: the creation of an optimal microenvironment for wound healing, the ability to prevent the penetration of microorganisms, high absorption capacity, sufficient permeability to gases, water vapor and ultraviolet, infrared rays when using photodynamic therapy, elasticity, the absence of irritating, allergenic, pyrogenic and toxic effects.

In this regard, one of the urgent problems facing industry experts is raising the quality of medical care to a new level, developing modern methods of treating penetrating eye injuries.

Thus, an analysis of literature sources showed that to date, no domestic film coatings have been developed for the ophthalmic practice of treating penetrating eye injuries.

Objective: to study the properties of domestic ophthalmic film.

Material and methods. The State Scientific and Technical Program of the Ministry of Innovative Development of the Republic of Uzbekistan is being implemented on the basis of the Tashkent Medical Academy (TMA) under grant ORDER-201709292910 on the following topic: “Development of semipermeable and biodegradable wound coatings for the treatment of skin and soft tissues damage from various origins”. (02.01.2018 to 12.31.2020).

In vitro studies were performed on 12 rabbits (males weighing 2.5-3.5 kg) to select the most effective concentration of methylene blue photosensitizer for inclusion in the biological coating. The film biological coating from local raw materials is a multicomponent, biodegradable implant based on Na-CMC with methylene blue (a dye with photosensitizing and antimicrobial activity) and a plasticizer glycerin. A method similar to the disco-diffusion for determining the sensitivity of antibiotics was used.

Experimental studies were carried out on sexually mature male Chinchilla rabbits weighing 2800-3500 kg. After 2 weeks of quarantine, the animals were carefully examined, taking into account the appearance, physical activity and reaction to reflexes. A solution of sodium etaminal

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was prepared and was injected into the ear vein slowly at a dose of 40 mg / kg. After the stage of anesthesia, the animal was fixed on the operating table for further surgical research. In the experimental groups, retrobulbar anesthesia was performed on animals under local anesthesia with 2 ml of a 2% percent lidocaine solution. After determining the deep sensitivity, we started modeling a penetrating infected wound.

With the help of a cataract Gref knife, at 11 o'clock 2–3 mm from the limb, a penetrating lesion of the cornea 2-3 mm long and 0.7 mm deep was inflicted in the paraoptic zone. The front camera is empty of moisture. 200 µl of a suspension of pathogenic strains (St. aureus, E. colli, Candida spp.) In a ratio of 1: 1: 1 with a concentration of 2 x 107 CFU / ml was introduced into the wound area using a mechanical pipette dispenser.

2-3 nodal sutures (nylon 10-0) were applied to the wound. Further, in the experimental group-2 a film polymer bio-coating BIKO with a diameter of 8 mm with the addition of methylene blue 0.05% was applied over the sutures. In test group 1, sutures were applied similarly without applying a film coating.

Bacteriological examination of the material from the conjunctiva of the eyes of rabbits was taken before and after infection on the 1st, 3rd, 5th, 7th, 14th and 21st days, seeding of bacterial culture in growth media was carried out no later than 0.5-1 hours from the time of collection.

The detachable eye was taken with a sterile cotton swab and, after preliminary microscopy, was sown on solid nutrient media using the Gold's method for subsequent quantification of grown colonies, and then the swab was lowered into sugar broth. In the absence of growth on a solid nutrient medium, the study was continued by seeding from sugar broth onto dense nutrient media.

The following culture media were used to isolate aerobic and facultative anaerobic microorganisms: 5% blood agar, yolk-salt agar, Endo, Saburo. After the appearance of growth, the number of grown colonies was counted and recalculated per 1 ml of sample, expressing the degree of colonization in CFU / swab or CFU / ml. The table shows the used culture media. Crops were cultivated under normal conditions for 18-24 hours, at t-37 ° C.

All isolated facultative anaerobic microorganisms were identified by genus or species on the basis of cultural, tinctorial, morphological and biochemical properties, in accordance with Order 535 "On the Unification of Microbiological Research Methods", according to the identification tests of the 9th edition of Bergey's Guide (1986) and Extended Schemes for the Short "Bergi Identifier of Bacteria" (1997).

The specific wound healing activity of film biocoating was studied on the model of primary penetrating wound of the cornea of 12 rabbits (6 animals in the 1st (true control) and 2nd experimental groups) in the modification of C. Hanna, J.E. O'Brien (1960). All animals underwent anesthesia by the intravenous administration of pentobarbitone; analgesia was achieved by retrobulbar injection of 0.5 ml of a salt solution containing 5 mg of lignocaine. The pupils of the eyes of rabbits are dilated with 1 drop of 1% cyclopentolate and 1 drop of 10% phenylephrine. A needle with a diameter of 5 mm was used to simulate a shallow punctured penetrating wound of the cornea, sclera, and conjunctiva of rabbit eyes. The observation was carried out for 1, 3, 7, 14, and 21 days. Assessed the general condition of the animals and local status. Direct ophthalmoscopy was used. Enucleation of the left eye was performed under ether anesthesia. The animals were removed from the experiment by an overdose of ether. Macro preparations were placed in a 10% formalin solution, poured into paraffin blocks. Sections 4-5 µm thick were stained with hematoxylin-eosin.

Results. According to research, the domestic implant has characteristics comparable to foreign ones and meets the requirements for medical devices in terms of bioinertness, biodegradation, adhesion, strength, bactericidal property, as well as the possibility of sterilization in

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a clinic. Commercial availability of products is due to the fact that the production uses cellulose obtained from cotton fiber.

In in vivo experiments, 12 rabbits were divided into 2 groups. The first truly experimental group consisted of 6 animals, whose eyes were covered with biocoating with a penetrating wound. The 2nd (experimental group) consisted of animals that underwent primary surgical treatment without the use of film biocoating.

Table 1.

Weight dynamics (kg) in animals in the control group

Dynamics of weight (kg) in animals of the 2nd experimental group (without film)							**mass gain,%**
Number of animals	**Initial weight**	**1- day**	**3- day**	**7-e day**	**14-e day**	**21-day**	
1	2,5	2,5	2,48	2,46	2,45	2,49	0,4
2	2,8	2,8	2,78	2,77	2,76	2,79	0,35
3	2,6	2,6	2,58	2,56	2,55	2,57	1,15
4	2,7	2,7	2,76	2,74	2,66	2,65	1,85
5	2,9	2,9	2,88	2,86	2,85	2,87	1
6	2,8	2,8	2,78	2,76	2,75	2,78	0,7

Table 2.

Weight dynamics (kg) in animals in the main group

Dynamics of weight (kg) in animals of the 1st experimental group (with film)							**mass gain,%**
Number of animals	**Initial weight**	**1- day**	**3- day**	**7- day**	**14-day**	**21-day**	
1	2,65	2,6	2,58	2,7	2,79	2,93	10,56
2	2,58	2,5	2,48	2,6	2,69	2,83	9,68
3	2,78	2,7	2,68	2,8	2,87	3,07	10,43
4	2,85	2,8	2,78	2,83	2,9	3,1	8,77
5	2,67	2,6	2,55	2,66	2,75	2,9	8,61
6	2,95	2,9	2,85	2,97	3,06	3,2	8,47

Note: P * - significance of differences in weight gain in rabbits with control group data (P <0.05).

In tables 2 and 3, an assessment of the increase in body weight in both groups was made, from which it is seen that in the experimental group with a film there is a greater increase in body weight compared to the control.

Based on the results of in vitro studies, it was determined that exposure to a film-coated polymer coating led to the formation of a St. aureus. It was found that the most effective

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bacteriostatic effect has a film polymer coating with a methylene blue concentration of 0.05% (Table 2).

Table 3.

The results of a bacteriological study of the growth of microorganisms depending on the concentration of methylene blue in the polymer biocoating

№ пп	Duration of light exposure, min	The number of strains St. aureus	Concentration of methylene blue in a film polymer coating,%			
			0,01%	0,05%	0,1%	0,5%
1	1	6	++++	+	++	++
2	5	6	++++	0	++	+
3	10	6	+++	0	+	+
4	15	6	++	0	+	0
5	Control (without exposure)	6	++++	++++	++++	++++

The biopolymer in the first experimental group was overlaid with a diameter of 8 mm over the sutures. In the true control (2nd) group, sutures were similarly applied without applying a film coating. Histomorphological studies were carried out in dynamics after 1,3,7,14, 21 and 30 days. 1 day after corneal damage in all rabbits, destruction of the anterior multilayer squamous epithelium and anterior border plate, connective tissue discompletion, interstitial edema, and foci of necrosis were revealed.

After 3 days, corneal damage in the form of destruction of the stratified squamous epithelium is noted. Cell degeneration and the appearance of lymphocytic infiltration are noted. In the cornea's own substance, pronounced edema and hypocellularity are noted. In animals of the 2nd experimental group, the revealed violations persisted up to 21 days. In the 1st experimental group, starting from 7 days, regenerative changes in the cornea were revealed. Metaplasia of squamous epithelium into cylindrical and proliferation of the epithelial layer is noted. In its own substance, a large number of lymphocytes and neutrophils, which is characteristic of a locally irritating reaction. On the 14th day, histological preparations showed complete regeneration of the cornea, sometimes with proliferation and hyperplasia of the integumentary multilayer squamous epithelium. In the cornea's own substance, pathological changes or elements of inflammation are not noted. It should be noted that during the test, no secondary infection was noted.

Thus, based on the results of histomorphological studies, it was found that when using film biocoating, complete regeneration of the wound surface occurs on the 14th day, while in the 2nd experimental group, pronounced edema and hypocellularity persist up to 21 days, complete healing in the control is noted through 30 days

Therefore, the domestic multicomposite, biodegradable implant based on Na-CMC with methylene blue corresponds to the properties shown for wound dressings.

Conclusion.

Domestic wound dressing based on Na-CMC is effective in the treatment of stabbing wounds of the cornea, sclera and conjunctiva of the eyes, accelerates the regeneration processes by 14 days and there is a greater increase in body weight compared to the control group in which healing is noted after 30 days and less weight gain. The developed coating for the treatment of

stabbing wounds of the cornea, sclera and conjunctiva of the eyes does not cause local irritating effect, hypoallergenic, which corresponds to the properties of wound coverings. The results of experimental studies confirm the effectiveness of domestic ophthalmic film biocoating.

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