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# EVALUATION OF AN IRRITATING AND ALLERGIZING ACTION OF A BIOPELLICLE FOR THE TREATMENT OF PENETRATING EYE INJURIES

[1]ZebinisoNiyazova,[2]Lyubov Khegay, [2]Alisher Rakhmanov
[1]*Tashkent pediatric medical institute*
[2]*Tashkent medical academy Republic of Uzbekistan*
*zebooriginal@gmail.com*

***Abstract: The article presents the results of a study of acute and chronic toxicity, irritating and allergenic effects of biocoating for the treatment of penetrating eye injuries. It has been established that a bioactive, biodegradable pellicle does not have a toxic effect, is safe and, according to the classification of toxicity, belongs to the 5th group of substances "practically non-toxic substance" and can be recommended for use in the manufacture of medicines intended for the treatment of eye injuries.***
***Keywords: preclinical studies, medical device, bioactive pellicle, wound coating, toxicity, irritant effect, allergenic property, ophthalmology.***

**Introduction** One of the main tasks identified in the Program for the Development of the Pharmaceutical Industry of Uzbekistan is the creation of import-substituting medical products based on modern technologies. As part of the implementation of the State scientific and technical program of the Ministry of innovative development of the Republic of Uzbekistan. according to the applied grant OR-201709292910 "Development of semipermeable and biodegradable wound coatings for the treatment of skin and soft tissues damage from various origins" at the Tashkent Medical Academy together with scientists from the Institute of Chemistry and Polymer Physics of the Academy of Sciences of the Republic of Uzbekistan. A coating technology has been developed for the treatment of penetrating eye injuries. Full-scale biocoating studies included toxicological studies on biomedical safety assessments.
**The purpose of the study:**to study acute, chronic toxicity, irritating and allergenic effects of biocoating for the treatment of penetrating eye injuries.

**Materials and research methods.** A bioactive coating based on Na-CMC (Na carboxymethyl cellulose), oxidized cellulose, glycerin, methylene blue, which has photosensitizing and bactericidal activity, was synthesized by scientists of Uzbekistan [1]. Preclinical studies of the pellicle were carried out on the basis of the Interuniversity Research Laboratory of the Tashkent Medical Academy (IURL TMA) on the basis of the agreement on scientific and technical cooperation No. 01 dated September 03, 2018 and as part of an applied grant of the Ministry of Innovative Development of the Republic of Uzbekistan 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". Received conclusions of the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan (Protocol N. 5 dated December 05, 2015). The experiments were carried out taking into account the requirements of the European Convention [2].The study of acute and chronic toxicity, irritating and allergenic effects was carried out in accordance with GOV ISO 10993-5-2011 and other methodological documents [3-6].

The general toxic effect in acute experiments was studied on 36 male rats weighing 150-170 g. Animals were divided into 1-5 experimental groups, group 6 served as a control. In each group there were 6 animals. Toxicity testing for 30-day intragastric inoculation was carried out on 24 white rats weighing 150-180 g. Animals were divided into 1-3 experimental and 4 (control) groups of 6 animals in each group. An aqueous solution of the pellicle was introduced into the stomach of animals through a special probe using a syringe. The injected volume did not exceed 5 ml. Before the introduction of the solution and after it, the animals did not receive food for 2-3 hours.

A study of the local irritant effect was carried out on 12 white rats (6 animals in the control and experimental groups). The day before the experiment, the animal's hair was carefully cut in symmetrical sections of the sides measuring 2x2 cm, the left side served as a control, and the right side as an experienced side. Three complementary methods were applied: two-drop and application tests, lowering 2/3 of the tail into a test tube with a pellicle solution.

The allergic effect was studied in 12 rabbits of 6 animals in the control and experimental groups. The study was conducted using a conjunctival test by subconjunctival injection of the test pellicle solution in a volume of 1 drop using an eye pipette in the transition zone of the mucous membranes of the eyelids and the eyeball of the rabbits right eye. After making the solution for 1 min. pressed the lacrimal-nasal canal at the inner corner of the eyes. The control was the left eye, into which distilled water was injected in an equivalent volume.

Peripheral blood results were studied on a BC-3000 hematology analyzer (Mindray, P.R.China). Biochemical parameters of blood serum were determined on a BA-88A biochemical analyzer (Mindray, P.R. China) using reagents from CYPRESS Diagnostics, Belgium.

After the completion of the experiments, all animals were clogged by decapitation for histological studies of the skin of the back and tails. Micropreparations were stained with hematoxylin-eosin [7–9].

Statistical processing of the research results was carried out on a Pentium-IV personal computer using the Statplus.9.0 office applications with calculation of the arithmetic mean of the studying indicator (M), its standard error (m), reliability indicators (P), and Styudent criterion [10].

**Results and discussions.**A bioactive coating for treatment of eye injuries is synthesized based on Na-carboxymethyl cellulose, oxidized powder cellulose, methylene blue and distilled water.

In acute experiments, animals of the 1st group were exposed to a dose of 1000 mg / kg, the 2nd group - 2000, the 3rd group - 3000, the 4th - 4000 and the 5th - 5000 mg / kg. The animals in the control group were injected with distilled water. Observation of the general condition of the animals was carried out daily for 14 days. In the experimental groups during the experiments there were no signs of intoxication. Integral indicators: the general condition of animals, food and water consumption, behavior, coordination of movements, reaction to external stimuli, the condition of the coat and skin, and the color of the mucous membranes did not differ from those in the control group. Due to the absence of animal death, it was not possible to establish an FD50. The results of the studies made it possible to classify biocoating to the 5th class - a practically non-toxic substance.

For 30 days, to study the toxicity of the pellicle, animals of the 1st group were injected per os daily with 5 ml of solutions at a dose of 10 mg / kg, 2 groups - 100 mg / kg, 3 groups - 200 mg / kg. 4 group served as a control. It was found that prolonged exposure to

doses is well tolerated by experimental animals. Indicators of the general condition, behavior, weight gain, hematological parameters of peripheral blood (the number of red blood cells and white blood cells, Hb, color indicator, ESR, hemogram with counting reticulocytes, platelets, basophils, eosinophils, stab leukocytes, segmented white blood cells, lymphocytes) biochemical indicators of the content of total protein, protein fractions, urea, creatinine, bilirubin total, direct, indirect, AST, ALT, cholesterol, LDH, beta-lipoproteins did not reveal statistically significant differences in animals of the experimental groups compared with the control data. Thus, the domestic bioactive coating for the treatment of penetrating eye injuries does not have a toxic effect on the body of experimental animals.

The local irritating effect of the native pellicle for treatment of eye injuries was evaluated according to the results of 3 complementary methods: two-drop and application tests, lowering 2/3 of the tail into a test tube with a pellicle solution. A day before the experiments using two-drop and application methods, the hair of 12 animals was carefully cut in symmetrical sections of the sides measuring 2x2 cm, the left side was control and was treated with distilled water, the right was experimental. To study the local irritant effect, a pellicle solution with a maximum concentration of 200 mg / kg tolerated in a chronic experiment was used.

After pretreatment of the skin with 70% alcohol, an experimental solution was applied to the experimental animals with a pellicle solution, and to the rats of the control group, 2 drops each. The results of observations of rats of the experimental group in the first 20 minutes of the experiment did not reveal signs of an immediate reaction. At the place of applying the drops of the pellicle solution, erythema, edema, papules, and vesicle elements were not detected. Observation for 48 hours showed no signs of a delayed reaction. The results of the droplet test suggest that the bioactive pellicle at a concentration of 200 mg / kg does not have a sensitizing property.

In order to confirm the results of a two-drop test, an application test was applied. Application with a pellicle solution was applied to the shaved skin daily by an open method. When applying for 4 hours, the animals were in a fixed state. A piece of gauze 1 cm in size moistened with a pellicle solution was applied to the skin and fixed with adhesive tape. At the site of application for 24-48 hours, the skin reaction was taken into account daily on the scale of assessment of skin samples. The reaction was observed on the outer surface of the skin and scored on a scale: visible reaction; pale pink erythema throughout the site or on its periphery; bright pink erythema throughout the site or its periphery; red erythema throughout the area; infiltration and swelling of the skin.

The results of the experiments established that during the period of observation of signs of irritation from the skin was not observed, which indicates the absence of a skin-irritating effect. Thus, with a cutaneous application of the test pellicle solution at a dose of 200 mg / kg, an irritating effect on the surrounding tissue in the area of application of the substance was not detected.

The study of skin-resorptive action was carried out in a subacute experiment under conditions of immersion in test tubes with the studied solution of the pellicle 2/3 of the length of the tails of rats, which is 5% of the body surface. The daily exposure of the studied solutions was 4 hours with an experiment duration of 30 days. Every day after a 4-hour exposure, the tails were washed with soap and warm water and dried. The results of studies found that pellicle solutions at a concentration of 200 mg / kg do not cause damage to the integrity of the skin of the tails.

The allergenic effect of pellicle solutions was studied in 12 rabbits. Animals were divided into 2 groups: experimental and control 6 animals in each. The study was conducted using a conjunctival test by subconjunctival injection of the test pellicle solution in a volume of 1 drop using an eye pipette in the transition zone of the mucous membranes of the eyelids and

the eyeball of rabbits in the right eye. After adding the substance for 1 min. pressed the lacrimal-nasal canal at the inner corner of the eyes. The control was the left eye, into which 1 drop of distilled water was injected. Assessment of the allergic effect with subconjunctival administration was carried out visually. The reaction was taken into account after 15 minutes (fast reaction) and after 24-48 hours (hypersensitivity) of the delayed type and was evaluated on a scale (in points):

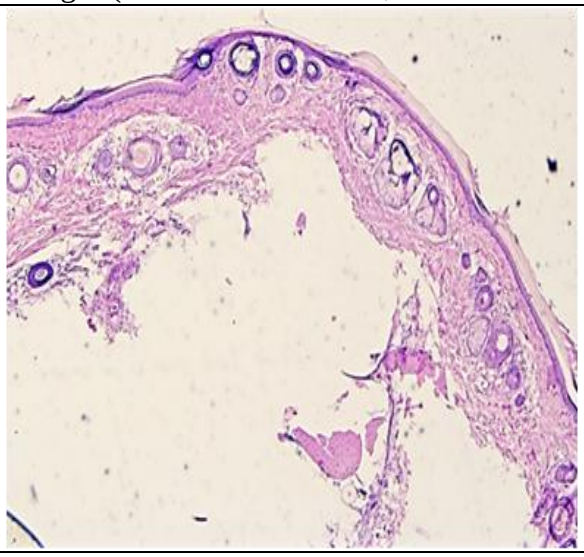
0 - absence of action;
1 - slight redness of the lacrimal duct;
2 - redness of the lacrimal duct and sclera in the direction of the cornea;
3 - redness of the entire conjunctiva and sclera.

During the observation period, signs of an allergic effect from the mucous membrane of the eyes were not observed, which indicates the absence of sensitization to the pellicle solution. The general condition of the animals during the entire observation period was without deviations from the norm. Thus, it was found that the studied pellicle solution in a dose of 200 mg / kg does not irritate the mucous membranes of the eyes.

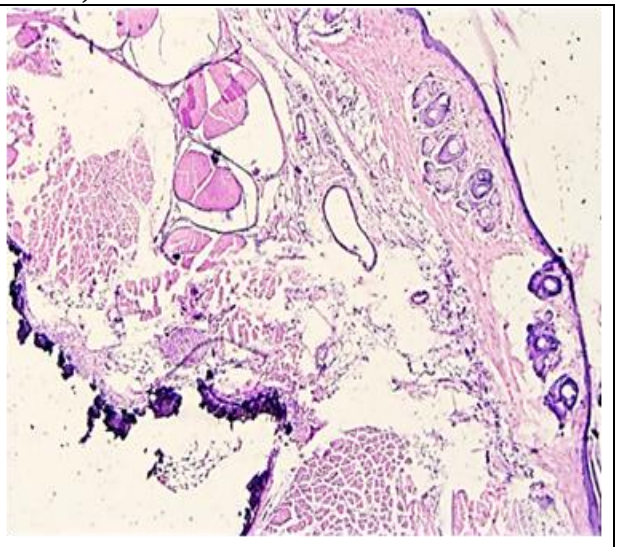
In order to confirm the results of studying the skin-irritating and skin-resorptive action of the native pellicle for the treatment of eye injuries, histological studies of the skin of the back and tails of white rats were performed.

After decapitation of the animals, the skin rags of the back and tails of 2 cm in size and not more than 5-10 mm thick were excised with a scalpel. Cut pieces were fixed in a 10% aqueous formalin solution. The solvent used is tap water, as distilled causes tissue swelling. The period of tissue fixation in formalin did not exceed 48 hours. After posting, the pieces were poured into paraffin. The thickness of paraffin sections ranged from 2 to 3 microns. The method of staining with hematoxylin-eosin was applied.

Histological studies of micropreparations were carried out using a DN-200M colour microscope (China). In a hystomorphological study conducted 30 days after exposure to a pellicle solution at a concentration of 200 mg / kg, the structure of the skin of the tails of white rats, regardless of the time of exposure to the solution, did not reveal pathological changes (in Pic. 1-2 - Ob. 5x5, in Pic. 3- 4 - Ob. 5x10).



***Pic 1.***The tail of white rats of the experimental group after 30 days of exposure to a solution of the pellicle at a concentration of 200 mg / kg Dermis, epidermis, appendages saved. Coloring HE. Ob. 5x5.



***Pic. 2.***The tail of white rats in the control group after 30 days of exposure to distilled water The epidermis, dermis, appendages are not broken. Coloring HE. Ob. 5x5.

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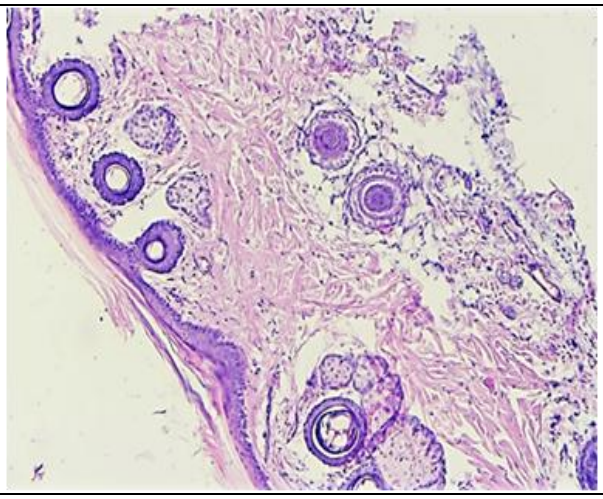
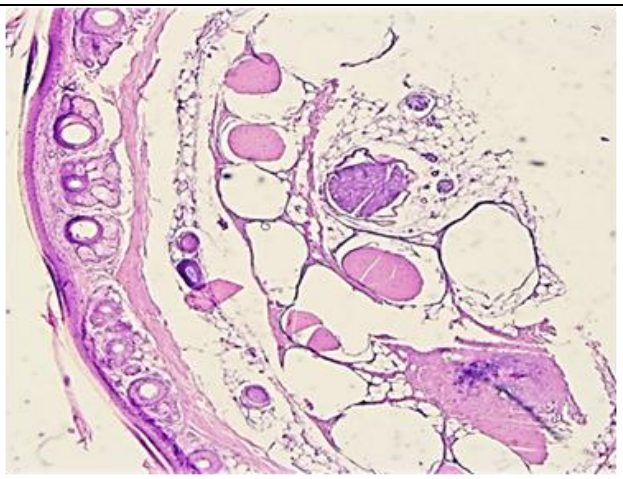
|  |  |
| ***Pic3.***The tail of white rats of the experimental group after 30 days of exposure to a solution of the pellicle at a concentration of 200 mg / kg There are no foci of dystrophy, necrosis, infiltration, or edema. Coloring HE. Ob. 5x10 | ***Pic. 4.***The tail of white rats of the control group after 30 days of exposure to distilled water. The epidermis, dermis, appendages are preserved. Coloring HE. Ob. 5x10. |

Thus, a comparative analysis of the histological picture of the skin of the tails of white rats of the experimental and control groups did not reveal any changes in the cell structure, which indicates the absence of skin-irritating and skin-resorptive action of the native bioactive pellicle for the treatment of penetrating eye injuries.

The results of histological studies of the skin of the backs of white rats of the experimental group after 48 hours of application of a pellicle solution at a concentration of 200 mg / kg are shown in Pics. 5-6. No foci of infiltration, edema, necrosis were detected. The cellular structure of the epidermis, dermis, skin appendages is not broken.

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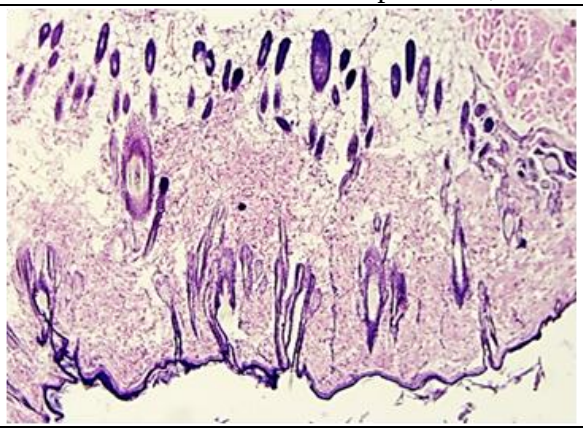
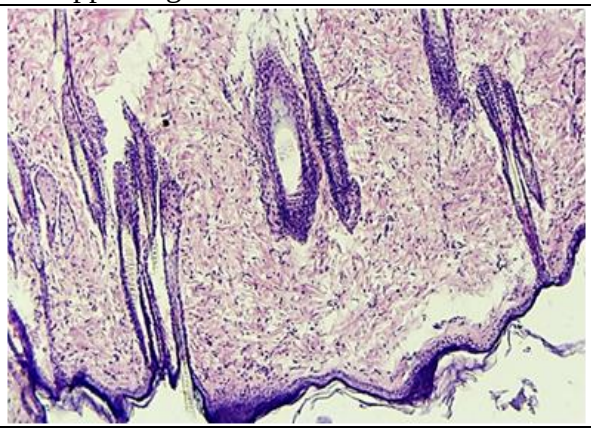
|  |  |
| ***Pic.5.***The skin of the backs of white rats of the experimental group after 48 hours of application of the pellicle solution at a concentration of 200 mg / kg The epidermis, dermis, appendages are not broken. Coloring HE. Ob. 5x5. | ***Pic.6.***The skin of the backs of white rats of the experimental group after 48 hours of application of the pellicle solution at a concentration of 200 mg / kg There are no foci of edema, necrosis, or infiltration.Ob. 5x20. |

In the control group after 48 hours of application of distilled water, no foci of infiltration, edema, necrosis, and disturbances in the cellular structures of the epidermis, dermis, and appendages of the skin of the backs of white rats were also revealed (Pic. 7-8).

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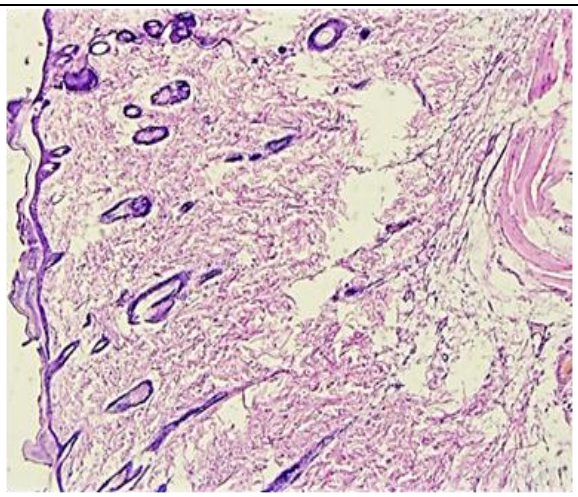
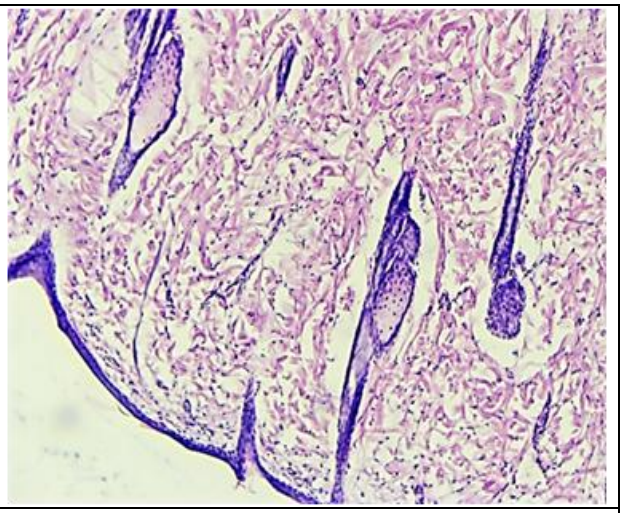
|  |  |
| ***Pic. 7.***The skin of the backs of white rats of the control group after 48 hours of application of distilled water The epidermis, dermis, appendages are not broken. Coloring HE. Ob. 5x5. | ***Pic.8.***The skin of the backs of white rats of the control group after 48 hours of application of distilled water. Coloring HE. Ob. 5x20. |

Thus, a comparative analysis of the histological picture of the skin of the backs of white rats of the experimental group after 48 hours of application did not reveal any changes in the cell structure, which indicates the absence of skin-irritating and skin-resorptive action of the native bioactive pellicle for the treatment of penetrating eye injuries.

**Conclusions.**The results of studies of the native coating for the treatment of penetrating eye injuries in doses of 1000,2000,3000,4000 and 5000 mg / kg made it possible to classify biocoating to class 5 - a practically non-toxic substance. Prolonged intragastric administration of solutions of bioactive coating in doses of 10, 100 and 200 mg / kg does not have a toxic effect on hematological and biochemical parameters of blood. A solution of the pellicle at a dose of 200 mg / kg does not exert a local irritant and sensitizing effect on the skin and irritating effect on the mucous membranes of the eyes of experimental animals. The results of histological studies of the skin of the backs and tails of white rats of the experimental groups did not reveal any changes in the cell structure, which confirms the absence of a skin-irritating and skin-resorptive effect on the native coating for the treatment of penetrating eye injuries.

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