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INFLUENCE OF PROTEIN-PEPTIDE BIOREGULATOR ISOLATED FROM BOVINE SCLERA AND INCORPORATED INTO AN ALBUMIN-BASED CRYOGEL ON THE SCLERA IN A MODEL CULTIVATION OF A POSTERIOR EYE SEGMENT

O.V. Yamskova[1], M.S. Krasnov[1], E.V. Sidorsky[1, 2], V.I. Lozinsky[1]

[1] Nesmeyanov Institute of Organoelement Compounds, Moscow, Russian Federation
[2] Institute for Biomedical Problems, Moscow, Russian Federation

Delivering bioactive substances to certain spots in the human and animal body is a crucial task. To address this problem, we have developed a delayed-release bioactive substance carrier – an albumin-based cryogel obtained by cryostructuring. It was tested on an organotypic culture model of the posterior eye segment of a newt. **Objective:** to study the effectiveness of porous albumin-based cryogel obtained by cryostructuring and loaded with a bioregulator isolated from bovine sclera in different quantities in maintaining eye tissue integrity and preserving Iberian ribbed newt fibroblasts on an organotypic culture model. **Materials and methods.** Albumin sponges were obtained after being denatured at temperatures –15 °C, –17.5 °C, and –20 °C, with albumin levels 40 mg/mL, 50 mg/mL, and 60 mg/mL in a thermostatic cooler. Their modulus of elasticity was measured. Eye tissues were isolated from adult sexually mature Iberian ribbed newts of both sexes. The posterior segment of each eye was placed on a sponge sample of albumin cryogel in penicillin vials, sealed and placed in a thermostat. At the end of cultivation, the samples were fixed, washed, dehydrated, and embedded in paraffin. Paraffin sections were made, followed by staining. A Leica microscope (Germany) with an Olympus DP70 camera (Japan) was used to view histological sections. Fibroblast count in the histological sections was estimated using the ImageJ program. **Results.** Cryogel with initial albumin solution levels of 50 mg/mL obtained at –20 °C with 4.50 kPa elastic modulus, was chosen for the organ culture experiment. Histological studies showed that eye tissue integrity was maintained in the experiment when albumin-based scaffold was loaded with the bioregulator at doses of 2.46×10^{-5}, 2.46×10^{-7}, 2.46×10^{-9}, 2.46×10^{-13}, 2.46×10^{-15} μg. Moreover, the statistically significant difference for fibroblast count per unit area in the sclera partially correlates with the qualitative state of the posterior eye tissue itself. Groups where bioregulator isolated from the sclera had a dose of 2.46×10^{-7}, 2.46×10^{-9} and 2.46×10^{-15} μg, showed the best result as compared with the control group. **Conclusion.** Albumin-based scaffold as a carrier with a bioregulator adsorbed on it (doses of 2.46×10^{-5}, 2.46×10^{-7}, 2.46×10^{-9}, 2.46×10^{-13}, 2.46×10^{-15} μg) is effective in maintaining eye tissue integrity and preserving Iberian ribbed newt fibroblasts. Albumin cryogen is an effective carrier for delayed release of bioactive substances.

Keywords: targeted drug-delivery systems, albumin cryogel, elastic modulus, organ culturing, bioregulator, protective properties.

INTRODUCTION

Targeted delivery of bioactive compounds (BAC) to certain organs and tissues of the human or animal body is currently a crucial task [1–3]. Such delivery systems most often consist of two main components: the BAC itself and a carrier that provides transport and release of the active ingredient in the target area [4]. Such carriers are used in various forms, such as nanoparticles and nanocontainers, liposomes, polymeric micelles, gels, etc. [5]. In recent years, various biodegradable sponges, especially those based on naturally occurring polymers such as proteins and polysaccharides, have become increasingly popular among BAC carriers [6–12]. An important advantage of such carriers is their good biocompatibility, low immunogenicity, and nontoxicity both of the sponges themselves and of the products of their resorption or degradation during hydrolysis in the recipient's body. In particular, sponges based on denatured serum albumin proved to be effective carriers of both low-molecular-weight and high-molecular-weight BAC [13–15]. These biopolymer materials were obtained by the so-called cryostructurization method [16–18], which allows formation of macroporous and supermacroporous (spongy) polymer matrices upon shallow freezing of solutions of the corresponding precursors, when polycrystals of the frozen solvent act as pore formers [16, 19, 20]. The

Corresponding author: Mikhail Krasnov. Address: 28, Vavilova str., Moscow, 119991, Russian Federation.
Phone: (499) 135-64-92. E-mail: embrmsk@mail.ru

advantages of exactly such albumin sponges include the possibility, by simple techniques, to vary their size, shape, porosity and elasticity, as well as the BAC dose with which the carrier is loaded to achieve the required therapeutic treatment result.

For example, it has been demonstrated that one of the promising biomedical applications of these cryo-structured albumin sponges is their use as 3D carriers for tissue-specific protein-peptide bioregulators that activate repair processes [15, 21–23]. In the present work, we used a bioregulator isolated from bovine sclera, since the indicated peptide-protein complex added to the culture medium has, as previously shown, a protective effect on the state of posterior eye tissues [24]. The peptide-protein complex isolated from the sclera consists of peptides with molecular masses of 1054–5080 Da, as well as from bovine serum albumin with a molecular mass of 66385 Da. It is the complex of peptides and the indicated protein that has bioactivity since its individual components showed no pronounced effects on maintenance of posterior eye cells and tissues during cultivation [24]. At the same time, special attention has now been paid to the influence of the amount (dose) of such a bioregulator incorporated into the albumin sponge on the protective activity exhibited by this BAC delivery system, which was the aim of this study.

MATERIALS AND METHODS

Preparation of cryo-structured albumin carriers

Albumin sponges were synthesized according to a modified known technique [25]. Bovine serum albumin (99%) (DIA-M, Moscow, RF) was dissolved in a calculated volume of water, then urea (o.p.h.) (REACHIM, Moscow, RF) was added. The solution was placed in an ice bath and the necessary volume of aqueous L-cysteine solution (ultra grade) (Fluka, Switzerland) was added. The concentrations of the components in the reaction solutions prepared in this manner were as follows: albumin, 40 mg/mL or 50 mg/mL or 60 mg/mL; urea, 1.5 mol/L; and cysteine, 0.01 mol/L. These solutions were added in 1.5 mL portions to glass vials (internal diameter, 22 mm) or in 3 mL portions into the cells of a plastic 24-well plate. The solutions were frozen for 24 hours in a Proline 1840 programmable ultra cryostat chamber (Lauda, Germany): samples in vials at –20 °C, samples in a plate at either –15 °C, or –17.5 °C, or –20 °C, and then thawed at room temperature. The resulting spongy albumin cryogels were thoroughly washed with sterilized water to remove soluble substances and stored in a closed container at 4–6 °C until further use.

Determination of the elastic modulus of albumin sponges

The elastic moduli of water-swollen albumin sponges were measured on cylindrical specimens, 16 mm in diameter and 20 mm in height. Such measurements were performed according to the technique previously used for soft sponge cryogels [26]. The swollen albumin sponge was placed in a glass beaker filled with water to compensate for capillary forces during extrusion of liquid from communicating macropores during uniaxial compression of the cylindrical sample. To minimize the Archimedes' buoyant force, a 10-mm diameter, 2-mm thick disc punch connected to a TA-Plus automatic texture analyzer holder (Lloyd Instruments, UK) was used. Compression was performed at a rate of 0.3 mm/min to a 50% strain, and the compression modulus of elasticity (E) was determined using the instrument software. The sample for determining the modulus of elasticity included 6–8 samples per point. The obtained values were averaged in Excel 2010. Quantitative data were also analyzed using the SPSS 26.0 software package (IBM, USA). Elastic modulus values having a distribution other than normal were presented as median (Me) and 25% (Q1) and 75% (Q3) percentiles. Statistical significance of differences between the two independent groups was assessed using the Mann–Whitney U test. When comparing three or more independent groups, nonparametric Kruskal–Wallis one-way analysis of variance with Dunn's correction for posterior analysis was used. Differences were considered significant at significance level $p < 0.05$.

Incorporation of protein-peptide bioregulator into albumin sponge

The bioregulator was isolated from bovine sclera tissue according to the technique described earlier [27]. Working solutions with target concentrations from 3×10^{-4} mg/mL to 3×10^{-20} mg/mL were prepared using the obtained solution with a bioregulator concentration of 0.3 mg/mL by successive tenfold dilutions. To incorporate the bioregulator into the albumin carrier, a water-swollen sponge formed in a glass vial was placed on a glass filter and free liquid was removed for 3 minutes under the vacuum of a water-jet pump. After that, the material "squeezed out" in this way was placed into an aqueous solution with a known concentration of the bioregulator, where the sponge swelled rapidly. The sample was incubated for 4 hours at 4–6 °C, then frozen at –20 °C and freeze-dried using a FreeZone[1] freeze dryer system (Labconco, USA).

The mass (dose in micrograms) of the bioregulator included in the sponge carrier was calculated according to the concentration of the bioregulator and the volume of liquid absorbed by the sponge during swelling.

Comparison samples (2 groups) were sponges that were incubated in water without additives.

Investigation of the activity of the bioregulator-loaded sponge albumin carrier on an organotypic culture model of the posterior eye segment

The studies were performed on eye preparations of adult sexually mature Iberian ribbed newts, both sexes, taken from the aquarium belonging to the Koltzov Institute of Developmental Biology (Russia). At least 17 animals (34 eyes) were used in each experiment. The newts were anesthetized in a 2% solution of urethane (ethyl carbamate) in amphibian physiological solution (0.65% NaCl). After anesthesia, the heads of the animals were rinsed with 70% ethanol and the eyes were enucleated under standard laboratory light. The isolated eyes were placed in sterile 35-mm Petri dishes with amphibian nutrient medium (199–70% medium, 30% distilled water) [28]. The ocular tissue culture medium contained 350 mL of 199 medium, 150 mL of double distilled water, 0.15 mL of 1.0 M HEPES buffer, and 1 mL of 1% gentamicin. Before being added to vials, the medium was cold sterilized by passing it through cellulose acetate (CA) membrane filters (Nalgene, USA) with a pore size of 0.2 μm. Eye tissues were isolated under a binocular lens in the following sequence: the eyes were freed from the skin, then cut along the circumference, proximal to the limbus. The retinal growth area together with iris, cornea, and lens were discarded. The posterior part of each eye, which included the retina, pigment epithelium, vascular membrane, and sclera, was used for subsequent culturing.

When culturing the posterior part of the eye, the experimental specimens were divided into the following 11 groups:

1. Control, at the bottom of a glass vial in the absence of an albumin carrier.
2. Control, on a bioregulator-free albumin carrier.
3. On an albumin sponge, loaded with 2.46×10^{-1} μg of the bioregulator.
4. On an albumin sponge, loaded with 2.46×10^{-3} μg of the bioregulator.
5. On an albumin sponge, loaded with 2.46×10^{-5} μg of the bioregulator.
6. On an albumin sponge, loaded with 2.46×10^{-7} μg of the bioregulator.
7. On an albumin sponge, loaded with 2.46×10^{-9} μg of the bioregulator.
8. On an albumin sponge, loaded with 2.46×10^{-11} μg of the bioregulator.
9. On an albumin sponge, loaded with 2.46×10^{-13} μg of the bioregulator.
10. On an albumin sponge, loaded with 2.46×10^{-15} μg of the bioregulator.
11. On an albumin sponge, loaded with 2.46×10^{-17} μg of the bioregulator.

In the experimental groups (3–11), the posterior eye segment of the newt was placed on a bioregulator-loaded sponge sample in the appropriate concentration, located in a penicillin vial, washed with sterile culture medium, and then filled with serum-free medium (medium 199 without serum added). All vials were covered with sterile caps, then with ParafilmM film (USA), and placed in a thermostat. Cultivation was performed stationary in the dark at 20–22 °C for 72 hours without changing the culture medium.

The study of explant condition after cultivation was carried out on a series of paraffin sections. Eye tissues were fixed in Bouin's solution, washed three times with 70% ethanol after fixation for 12 hours, then dehydrated and embedded in paraffin. 7 μm-thick paraffin sections were made using an ERM 4000 microtome (Hestion, Australia), which were stained with hematoxylin and eosin after deparaffinization and hydration, and placed under a coverslip with adhesive fluid added. A Leica microscope (Germany) with an Olympus DP70 camera (Japan) was used to view histological sections. The number of fibroblasts on histological sections was estimated using the ImageJ program, estimating the number of sclera fibroblasts per 1 mm^2 of tissue. At least 50 sections were examined for each experimental point. The results were processed using Mann–Whitney U test and Student's t test.

RESULTS AND DISCUSSION

Obtaining spongy albumin carriers and their physical/mechanical properties

The physical and mechanical characteristics of BAC carriers are important in terms of the performance properties of delivery systems because they largely determine the operational capabilities of the respective drugs, especially under *in vivo* conditions. Since the cryo-structured albumin sponges obtained in this work were further tested as carriers of the peptide-protein bioregulator in model experiments on cultivation of the posterior eye segment, when the sponge was in a hydrated state, it was important to evaluate the mechanical characteristics of the carrier under the applied conditions, i.e., in aqueous medium. At the same time, we still varied albumin levels in the initial solutions used to obtain the sponge media, as well as the cryogenic treatment temperature of these solutions, which is known [16, 19, 20] to affect the porous morphology of polymeric cryogels.

The compression moduli of elasticity of the obtained sponge materials were measured using an automatic

texture analyzer (see the experimental part) using a disc punch (Fig. 1). The results of these measurements are summarized in Table.

The results obtained clearly indicate that increasing the protein levels in the initial solutions led to the formation, at least in the range of negative temperatures –15 °C to –20 °C, of cryo-structured albumin sponges with higher compression modulus of elasticity (with confidence $p < 0.001$ for samples synthesized at –15 °C; $p = 0.014$ for cryogels formed at –17.5 °C; and $p = 0.005$ for samples obtained at –20 °C). At the same time, the averaged *E* values [(A) in Table] and the corresponding moduli of elasticity found during statistical processing of the experimental data [(B) in Table] almost did not differ in absolute value. This emphasizes that the obtained information was reliable.

With the same protein concentration in the initial gel-forming system, more elastic spongy samples were obtained in the case of –15 °C cryogenic treatment temperature. However, considering also the literature data [25] on the efficiency of cryotropic gelation of albumin depending on the conditions of freezing of its initial solutions at the same negative temperatures of –15.0 °C, –17.5 °C and –20 °C, as well as the information obtained in preliminary experiments to evaluate the convenience of working with the obtained sponge carriers during organ cultivation of biological model, for further use in this study we chose albumin sponges synthesized by freezing at –20 °C of reaction solutions with a protein concentration of 50 mg/mL were chosen for further use in this study.

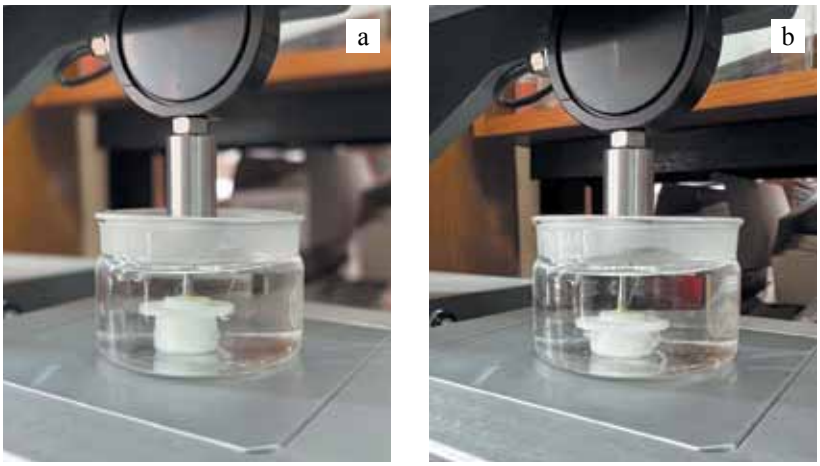

Fig. 1. Photographs of a sample of water-swollen cryo-structured albumin sponge before the onset of the effect of compression load (a) and during deformation under the applied load (b)

Table

Compression modulus of elasticity (*E*) of water-swollen albumin-based cryogels formed from initial solutions with different albumin content by freezing at three negative temperatures

Albumin levels in stock solution (mg/mL)	*E* (kPa) of albumin sponges formed by freezing at the following temperatures:					
	–15.0 °C		–17.5 °C		–20.0 °C	
(A) *E* values averaged using Excel 2010						
40	4.16 ± 0.46		4.07 ± 1.07		2.98 ± 0.45	
50	5.24 ± 0.22		5.03 ± 0.45		4.50 ± 0.76	
60	7.31 ± 0.82		5.71 ± 0.93		4.91 ± 1.08	
(B) *E* values found by statistical processing of the experimental values using the SPSS 26.0 software package						
	Me [Q1–Q3]	p	Me [Q1–Q3]	p	Me [Q1–Q3]	p
40	3.93 [3.74–4.77]	0.001* $p_{40–50} = 0.088$*	4.25 [3.43–4.89]	0.014* $p_{40–50} = 0.105$	2.97 [2.57–3.18]	0.005* $p_{40–50} = 0.006$*
50	5.20 [5.08–5.43]	0.001* $p_{40–60} = 0.001$*	4.96 [4.78–5.22]	0.014* $p_{40–60} = 0.004$*	4.94 [3.64–5.05]	0.005* $p_{40–60} = 0.004$*
60	7.42 [6.06–8.44]	0.001* $p_{50–60} = 0.031$*	5.58 [4.98–6.17]	0.014* $p_{50–60} = 0.194$	4.86 [3.49–5.78]	0.005* $p_{50–60} = 0.092$

* differences are statistically significant ($p < 0.05$).

Results of organ cultivation of the posterior part of a newt's eye using bioregulator-loaded sponge albumin carriers

When counting the number of cells (fibroblasts) in the sclera after culturing the posterior eye segment of Iberian ribbed newt on a bioregulator-free albumin sponge and albumin sponge loaded with bioregulator in different doses (see the experimental part), the results were obtained, shown in the form of diagrams in Fig. 2.

When the posterior part of the eye was cultured on albumin sponges in experimental groups 5, 6, 7, 9, and 10, when the dose of the bioregulator in the carrier was, respectively, 2.46×10^{-5}, 2.46×10^{-7}, 2.46×10^{-9}, 2.46×10^{-13} and 2.46×10^{-15} μg, fibroblast count in the sclera turned out to be significantly higher than in group 1, where the posterior part of the eye was cultured without the albumin sponge, and the posterior part of the eye of the newt was placed directly on the bottom of the cultivation glass vial. In addition, in experimental groups 6, 7, and 10, where the dose of bioregulator included in the sponge carrier was 2.46×10^{-7}, 2.46×10^{-9} and 2.46×10^{-15} μg, respectively, a significantly higher fibroblast count was detected compared to group 2 (cultivation of the posterior eye segment on a bioregulator-free albumin sponge). The graph shows that when sclera bioregulator was added at a dose of 2.46×10^{-11} μg, no significant differences in the fibroblast count per unit area of the sclera were observed relative to the control. It turns out that the dose dependence of the action of the bioregulator included in the cryogel is polymodal in nature.

Fibroblast count per unit area in the sclera partially correlated with the qualitative state of the tissues of the posterior part of the eye themselves. Specifically, their best condition compared to the control group 2 was also observed in groups 6, 7 and 10 (Fig. 3, a, b, g, j), where the dose of the bioregulator isolated from the sclera was, respectively, 2.46×10^{-7}, 2.46×10^{-9} and 2.46×10^{-15} μg.

Below is a description of the histological state of the posterior eye tissues in different experimental groups.

In the control ***group 1*** (Fig. 3, a), when culturing the posterior segment of a newt's eye on the bottom of a glass vial without an albumin carrier, retinal detachment (1) from the pigment epithelial layer (2), as well as detachment of the pigment epithelial layer from the choroid occurred. In the pigment epithelial layer itself, the pigment is shifted to the apical side, which indicates the instability of cells of this layer and their dedifferentiation. Signs of neuronal degradation and damage were observed in the retina. Scleral membrane showed signs of the beginning of tissue degradation, manifested by collagen fiber stratification with formation of cavities (3) between them and by small fibroblast (4) count per unit area (Fig. 2).

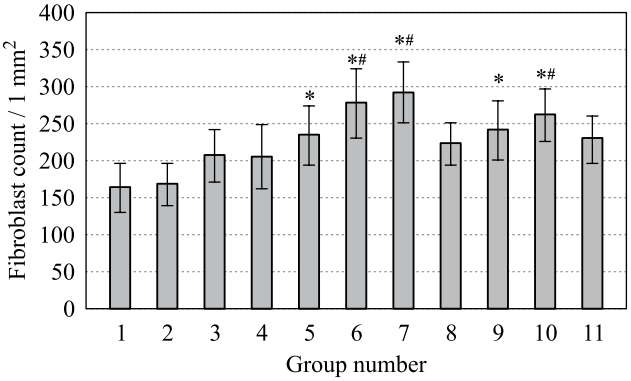

Fig. 2. Fibroblast count per unit sclera area in different experimental groups after cultivation of the posterior part of the eye. *, significant differences $p < 0.05$ from control group 1 (without albumin carrier); #, significant differences $p < 0.05$ from control group 2 (albumin sponge without bioregulator included)

In control ***group 2*** (Fig. 3, b), when culturing the posterior part of a newt's eye on an albumin sponge without adding any factors, a slightly better picture was observed than in control group 1. Retinal detachment (1) from the pigment epithelial layer (2) was partial, photoreceptor cell outgrowths and other retinal neurons were less damaged than in control group 1, but there was retinal neuronal death. The pigment in the pigment epithelial layer also tended to shift to the apical side, but this was not as pronounced as in control 1. The choroid remained dense. There were also elements of tissue degradation in the sclera, manifested by collagen fiber stratification and formation of small cavities (3) and by small fibroblast (4) count per unit area (Fig. 2).

In ***group 3*** (bioregulator in the carrier, 2.46×10^{-1} μg) retinal detachment (1) from the pigment epithelium (2) was also observed. The pigment in the pigment epithelial layer was shifted to the apical side, the choroid was dense without any signs of damage (Fig. 3, c). The sclera showed elements of tissue degradation expressed as cavities (3) between collagen fibers and insignificant difference from control groups in terms of fibroblast (4) count (Fig. 2).

In ***group 4*** (2.46×10^{-3} μg bioregulator in the carrier) (Fig. 3, d), retinal detachment (1) from the pigment epithelium (2) was also detected in places. The pigment in the pigment epithelium layer was shifted to the apical side, as in the previous group. The choroid was in good condition, dense, with no signs of degradation. There was death of retinal neurons. The sclera showed elements of tissue degradation expressed in the stratification of collagen fibers and formation of large cavities (3) and by small fibroblast (4) count per unit area, not significantly different from the control groups (Fig. 2).

In ***group 5*** (2.46×10^{-5} μg bioregulator in the carrier) (Fig. 3, b, e), there was pronounced death of retinal neu-

rons (1). The pigment in the pigment epithelial layer (2) was shifted to the apical side, as in the previous group. The choroid was in good condition, dense, and without signs of degradation. The sclera also showed elements of tissue degradation, manifested by collagen fiber stratification and formation of large cavities (3). In addition, fibroblast (4) count per unit area was not significantly different from the control group 2 (Fig. 2).

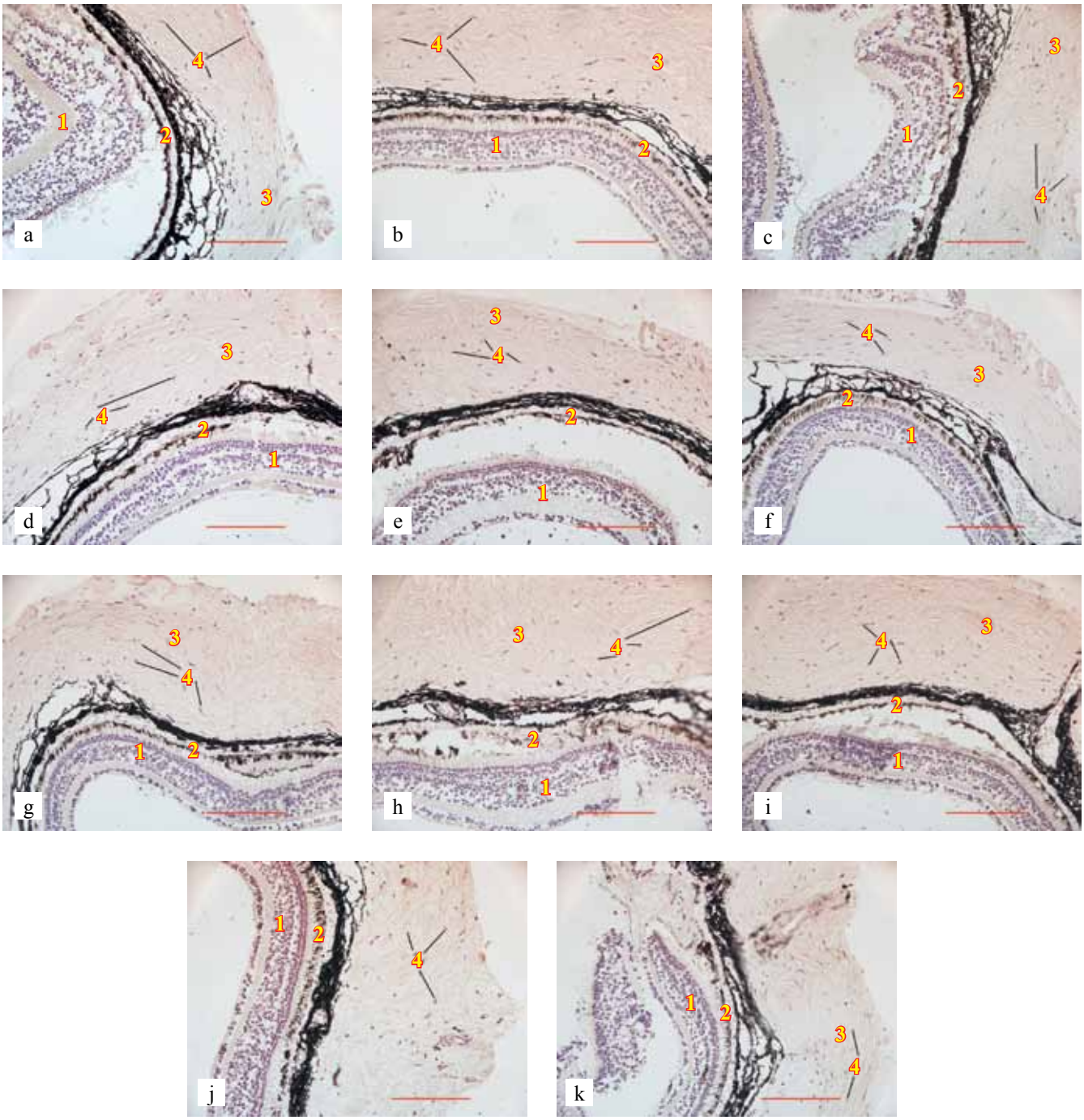

Fig. 3. Histological images of tissues of the posterior segment of the eye of an Iberian ribbed newt, after 3 days of cultivation: (a) without scaffold; (b) on a bioregulator-free albumin-based cryogel scaffold; (c) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-1} μg; (d) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-3} μg; (e) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-5} μg; (f) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-7} μg; (g) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-9} μg; (h) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-11} μg; (i) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-13} μg; (j) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-15} μg; (k) on an albumin-based cryogel scaffold with a sclera-derived bioregulator included in it at a dose of 2.46×10^{-17} μg. H&E stain. Metric scale = 200 μm (red line)

The histological sections of the preparations in ***group 6*** (2.46×10^{-7} μg bioregulator in the carrier) (Fig. 3, a, b, f) showed a markedly better picture than in the other groups. In particular, there was less retinal detachment (1) (partial detachment only from the edges), pigment was more evenly distributed in the pigment epithelial cells (2), indicating a stable differentiated state of these cells; there was almost no neuronal death. The sclera showed less signs of degradation, manifested by a smaller number and size of cavities (3) between collagen fibers, and a higher fibroblast (4) count per unit area of the sclera, significantly different from both control groups 1 and 2 about 1.6-fold (Fig. 2).

Sections in ***group 7*** samples (2.46×10^{-9} μg bioregulator in the carrier) (Fig. 3, a, b, g) showed partial retinal detachment (1) from the pigment epithelium (2) from the edges; the pigment was slightly shifted to the apical side; cavities (3) in the sclera were slightly larger than in group 6 preparations but smaller than in other groups; death of retinal neurons was insignificant. Fibroblast (4) count in the sclera differed significantly from both control groups by 1.7-fold (Fig. 2).

In ***group 8*** (2.46×10^{-11} μg bioregulator in the carrier) (Fig. 3, h), partial retinal detachment (1) from the edges was observed, while pigment displacement in the pigment epithelial cells (2) was practically not observed, but there were significant cavities (3) in the sclera. Fibroblast (4) count in the sclera did not differ significantly from both control groups (Fig. 2).

Histological sections of preparations in ***group 9*** (2.46×10^{-13} μg bioregulator in the carrier) (Fig. 3, b, i) showed retinal detachment (1) from the pigment epithelium (2), pigment shift to the apical side, and minor cavities (3) in the sclera. Fibroblast (4) count in the sclera differed significantly only from control group 1, and did not differ from control group (Fig. 2).

Sections of specimens from ***group 10*** (2.46×10^{-15} μg bioregulator in the carrier) (Fig. 3, a, b, j) had a better picture of the state of the posterior eye tissues compared with specimens from all other groups. The sclera was dense without tears, and there were practically no cavities. Pigment was slightly isolated from the pigment epithelial cells (2), and no displacement to the apical side was detected, indicating that these cells are in a stable differentiated state. Retinal detachment (1) from the pigment epithelium was observed only from the edges. Neuronal death was not severe, the choroid was compact. Fibroblast (4) count in the sclera differed significantly from both control groups about 1.5-fold (Fig. 2).

In ***group 11*** (2.46×10^{-17} μg bioregulator in the carrier) (Fig. 3, a, b, k), there was death of retinal neurons (1) and a slight detachment of the retina from the pigment epithelium (2) in the marginal areas. There was practically no pigment shift to the apical side. This indicates that these cells were in a stable differentiated state. Minor ruptures were found in the sclera (3). Fibroblast (4) count in the sclera did not differ significantly from both control groups (Fig. 2).

The detected differences in tissue state and fibroblast preservation during cultivation suggest that the sclera bioregulator only in certain amounts included in the albumin cryogel has a pronounced protective effect the state of the posterior eye tissues. The most prominent protective effect was observed when the posterior eye tissues of a newt was cultured on an albumin scaffold with the bioregulator included at doses 2.46×10^{-7} μg, 2.46×10^{-9} and 2.46×10^{-15} μg. It has been previously shown that there was a protective effect on the state of the posterior eye tissues when this bioregulator was included in a cryogel carrier at a dose of 2.46×10^{-7} μg [22]. The bioregulator, introduced into a cryo-structured albumin sponge, showed the most pronounced protective effect on the state of tissues and cells of the posterior part of the eye, also at doses 2.46×10^{-9} and 2.46×10^{-15} μg, and had a weak effect at other doses (Fig. 2). This fact revealed in this work also testified to the non-linear dose dependence of the action of the bioregulator. In this case, we did not observe the classical dependence – the higher the dose, the greater the effect. In other words, the activity of this bioregulator is characterized by a complex polymodal dose dependence. These results are consistent with the data obtained on the polymodal action of the sclera bioregulator in aqueous solution, which were obtained earlier [24].

This result also correlates with previously obtained information about a similar polymodal dependence for protein-peptide bioregulators isolated from other eye tissues, such as pigment epithelium [29], when the most pronounced biological effect on the posterior eye condition was observed at bioregulator concentrations of 10^{-9} and 10^{-17} mg/mL in culture medium. In terms of its content in albumin sponge, this corresponds to doses of 2.46×10^{-7} and 2.46×10^{-15} μg. Thus, the level of the protective activity of the bioregulator is determined by the concentration of this agent in the solution, which was used when the bioregulator was incorporated into the sponge albumin carrier.

CONCLUSION

A protein-peptide bioregulator isolated from bovine sclera was incorporated into a spongy cryo-structured carrier that was synthesized on the basis of serum albumin solutions with the addition of denaturant (urea) and thiol-reducing agent (cysteine) in doses ranging from 2.46×10^{-1} to 2.46×10^{-17} μg. The functionality of the BAC delivery systems obtained in this way was tested in experiments on organotypic culture model of the posterior eye segment of Iberian ribbed newts in order to find

out the effect of different doses of this bioregulator on the state of the sclera and on maintenance of the integrity of eye tissue (retina and pigment epithelium, choroid) and fibroblast preservation. According to histology data, it was shown that the most pronounced protective effect on the state of tissues of the posterior eye segment during cultivation occurred on the albumin scaffold with bioregulator doses of 2.46×10^{-7} μg, 2.46×10^{-9} and 2.46×10^{-15} μg. The results obtained indicate that cryostructured sponge albumin cryogel is an effective carrier for the release of bioactive compounds.

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