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Biodegradable stent coatings on the basis of PLGA polymers of different molecular mass, sustaining a steady release of the thrombolityc enzyme streptokinase

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ABSTRACT

The work describes synthesis of novel biodegradable polymer membranes based on poly(lactic-*co*-glycolic acid) (PLGA), as well as testing of their physicochemical properties. The membranes synthesized have been shown to possess the necessary mechanical properties and to be capable of sustained and directed release of large biomacromolecules, in particular, the molecules of streptokinase (m.w. 47 kDa). Streptokinase is a pharmaceutical drug with a high thrombolytic activity. The technology developed allows one to synthesize membranes with a relative elongation of 25 to 165% and a tensile strength of 20 to 55 MPa. The membranes are biodegradable; the rate of the polymer degradation in an isotonic solution is 0.5–1.0% per day. The streptokinase-infused membranes were demonstrated to release the protein in a controlled manner, with ~90% of the enzyme molecules retaining their activity. The rate of streptokinase release from the membranes was in the range of 0.01–0.07 mg/cm^2 per day. The polymer films did not exert any short-term toxic effects on the cells cultivated de novo on the film surface. The mitotic index of cells growing on the surface of polymer films was ~1.5%. The implantation of the synthesized polymers in animals – in the form of film samples and as a component of the coating of nitinol stents – had no complications during the postoperative period. The histological examination of the implant-surrounding tissues did not reveal any abnormalities. When the polymers were implanted as separate film samples, only traces of PGLA were detected in the tissues two months after the surgery. The implantation of stents coated with the streptokinase-infused polymers resulted in the formation of a mature and thick connective-tissue capsule. Thus, novel biodegradable PLGA-based polymer membranes have been synthesized and tested in this work – membranes which possess the necessary mechanical properties and are capable of a sustained and directional release of high-molecular biological compounds.

1. Introduction

Designing systems for the controlled drug delivery which are based on biodegradable polymers is one of the most promising and rapidly developing areas of chemical technology [1–5]. Such polymers are used to manufacture nano- and micro-particles, membranes and coatings. Nano- and micro-particles are usually used as carriers [6]. Membranes are used to make isolating materials and bandages [7]. Coatings are applied to implants – e.g., prostheses, pins and stents, medical devices used to expand narrowed hollow organs [8]. Local release of drugs from biodegradable polymers can solve a number of potential postoperative complications at the site of implantation. In the case of stents, this is about prevention of such complications as restenosis (recurrent narrowing), inflammation, thrombosis, etc. [9]. The postoperative complications often require extra surgical intervention (e.g., stent replacement), which poses additional risks for the patient. Accordingly, the problems actively studied today are (1) the search for materials that can make a basis for biodegradable coatings and (2) the search for pharmaceuticals that retain their chemical structure upon incorporation into polymer coatings and can diffuse from them in a controlled manner,

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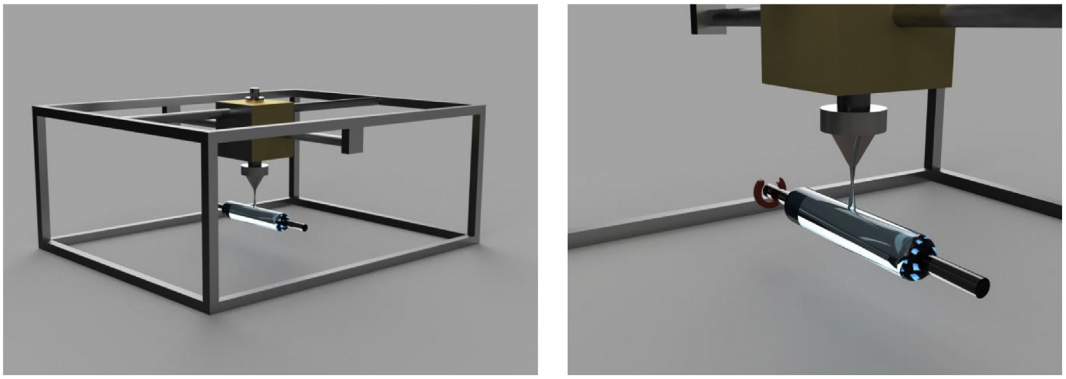

Fig. 1. A scheme of the device for the application of polymer to the surface of stents. (A) A general view of the device. (B) A general view of the head and wire with polymer. The arrow indicates the direction of wire movement.

depending on the external conditions [10]. The main advantage of controlled drug delivery systems is their ability to maintain the required level of a preparation in tissues and biological fluids for a long time – long enough to complete the therapy [11]. The effectiveness of controlled drug delivery systems is largely determined by the properties of the materials that the systems are made of. [12]. In addition to the key ability to release pharmaceutical preparations in a controlled manner, such materials should be biocompatible, biodegradable and atraumatic – and should also have a number of necessary physicochemical and mechanical properties [13]. A promising material for controlled drug delivery systems is considered to be poly(lactic-*co*-glycolic acid) (PLGA) [14]. It is usually synthesized from lactic and glycolic acids or obtained by enzymatic fermentation of carbohydrates. In both cases, renewable raw materials of biological origin are used to produce PLGA [15]. The physicochemical properties of PLGA are determined by the molar ratio and the sequential arrangement of lactic and glycolic acids in the polymer chain. PLGA copolymers can be obtained with different molecular weights and structure, making it possible to vary the degree of interaction between macromolecules [16]. Hence, the physicochemical properties of PLGA can vary quite a lot depending on the composition of the polymer. PLGA polymers are known to be biodegradable and biocompatible. [17]. They are already used in medicine to manufacture surgical sutures and subcutaneous implants [18], as well as to treat the surface of dental implants [19]. PLGA polymers are also used in orthopedy and cosmetology [20]. It is already possible to make PLGA-based systems for the controlled delivery of low molecular weight pharmaceuticals [21]. PLGA-based matrices can also be used to incapsulate enzymes [22]. The possibility of using PLGA-based systems for the controlled delivery of high molecular weight compounds – systems which would have all the necessary physicochemical properties – is yet to be confirmed though. In this work, we offer a number of technological solutions for the synthesis of PLGA-based polymer membranes and coatings with necessary mechanical properties. Our objective was to create biodegradable PLGA-based coatings for stents, which would be capable of prolonged release of the antithrombotic agent streptokinase. Upon entering the bloodstream, streptokinase interacts with plasminogen (profibrinolysin) and forms an “activator complex”, which catalyzes the conversion of blood plasminogen and blood clots into plasmin (fibrinolysin) [23]. Plasmin, a proteolytic enzyme, cleavages fibrin filaments of blood clots and catalyzes degradation of fibrinogen and other plasma proteins, including V (accelerin) and VII (convertin) coagulation factors [24]. Thus, streptokinase dissolves blood clots, acting both on their surface and from the inside [25]. It is most effective for dissolving fresh clots of fibrin (before retraction). The enzyme restores patency of thrombosed blood vessels and is inactivated by the antibodies circulating in the blood [26]. The novelty of this work lies in the synthesis and testing of a range of biodegradable PLGA-based polymer membranes, possessing the necessary mechanical properties and capable of a directional and sustained release of biomacromolecules with high molecular weight.

2. Methods

2.1. Synthesis of PLGA-based membranes

To synthesize PLGA-based membranes, copolymers of PLGA (50:50) (Creative Biolabs, USA) of various molecular weights (45, 90, and 180 kDa) were used. Since it is not quite correct to apply the concept of molecular weight to a polymer product, hereafter we shall use the term molar mass (kg/mol). Preliminary experiments showed that solutions with the concentration of PLGA in the range of 2.8–3.2% are most suitable for the formation of membranes. The solutions of 3% PLGA (Creative Biolabs, USA) in chloroform (Irea 2000, Russia) were prepared by stirring for 1 h at 57°C, until homogeneity. To incorporate streptokinase into the membrane, a colloidal solution of streptokinase in phosphate-buffered saline (15 mM, pH = 7.4) was added to the homogeneous PLGA solution. The resulting mixture was poured into 12-mL plastic molds (85 mm in diameter). The PLGA-based membranes were prepared by the method of casting of the polymer solution followed by a two-step solvent evaporation [27]. The first evaporation step was carried out for 3 h at a pressure of 50 Torr; the second step, for 5 h at 3 Torr. After solidification, the membranes were deposited for 1 day at the atmospheric pressure and temperature of 40 °C.

To apply polymers to the stent wire, we constructed a device consisting of three parts: (1) head for the application of polymer to the wire (ultrasound extruder); (2) multi-axis manipulator for head movement; and (3) system for pulling and rotating the wire (Fig. 1).

2.2. Examination of the mechanical properties of polymer films

The tensile strength of PLGA polymer films was tested on a universal testing machine INSTRON 3382 at the loading speed of 10 mm/min. Samples of PLGA polymer films (100 μm thick) were made in the shape of a double spade. [28]. The morphology of film surface was examined with a scanning electron microscope (SEM) TESCAN VEGA II SBU (TESCAN, Czech Republic).

2.3. Fibrinolytic assay for streptokinase activity

The activity of streptokinase was assessed by its ability to dissolve a clot made with bovine reagents, according to the Christensen method [29] According to the assay protocol, the solution of streptokinase was serially diluted with buffer followed by the addition of fibrinogen and thrombin. When streptokinase was used as an activating agent for plasminogen, 1000 units of the enzyme were sampled into assay tubes prior to the addition of thrombin. A fibrinolytic unit was defined as the

least amount of enzyme needed to lyse a clot within 30 min under the conditions of the test.

2.4. Release of streptokinase from the polymer membranes and dissolution of the polymer

The optical spectra of polylactide and streptokinase aqueous solutions differ substantially. This fact was used as a basis for the spectroscopic monitoring of changes in the streptokinase and polylactide concentrations in aqueous systems. The changes were comparatively monitored in the systems of "enzyme + polymer + water" versus "polymer + water" and "polymer + water" versus "water" [30]. Absorbance of the solutions was measured using a spectroscopic system Ocean Optics USB 2000. Streptokinase absorbance was measured at 280 nm; it varied in the range of 0.1–2.5, with the mass absorption coefficient of the enzyme being equal to 0.19 L/g. In addition to being measured spectroscopically, the dissolution of polylactide was also assessed by weighing the dry residue after evaporation of samples. The measurements of streptokinase release were accompanied by the measurements of the enzyme activity.

2.5. Cell culture assays

The studies of the polymer biocompatibility were performed using standard in vitro test systems. A culture of human neuroblastoma cells (SH-SY5Y) was used as a standard experimental model. SH-SY5Y cells were cultivated in a DMEM medium (Biolot, Russia) supplemented with 10% fetal calf serum (Gibco, USA) and 30 μg/mL gentamicin in a CO_2 incubator (Binder, Germany) at 37 °C and 5% CO_2 [31]. Samples of polylactide films (20 × 20 mm) were placed in 35-mm Petri dishes (1 sample per dish), followed by inoculation with SH-SY5Y cells (104 cells/cm^2; 3 mL per dish). After cultivation for 3 days, the cells growing on the surface of the polymer samples were stained with the fluorescent dyes Hoechst 33342 (Sigma, USA; 2 μg/mL;) and propidium iodide (Sigma, USA; 2 μg/mL). Hoechst 33342 stains both alive and dead cells. Propidium iodide practically does not stain alive cells within the time frame of the staining procedure (about 10 min); it only penetrates into the cells whose plasma membrane is damaged, which is a characteristic feature of dead cells. After staining the polymer samples, the number of alive and dead cells on their surface was counted using an imaging system Leica DMI6000 (Leica, Germany) [32]. At least 500 cells in total was counted per each sample.

The number of cells undergoing mitosis was counted under a fluorescence microscope using the technique of vital staining with Hoechst 33342 (Sigma, USA). Mitotic cells were identified by the distribution of chromatin characteristic of prophase (P), metaphase (M), anaphase (A) and telophase (T). At least 500 cells in total was counted per each sample. The mitotic index (MI) was calculated using the formula MI = (P + M + A + T)/N · 100%, where (P + M + A + T) is the number of cells at different mitotic phases and N is the total number of analyzed cells [33].

2.6. Experimental animals

In the experiments, male Wistar rats (180–200 g) were used. The animals were kept under standard conditions (temperature, 22 ± 2 °C; humidity, 30–70%; 12-h light period; feed and water ad libitum). All animal manipulations were approved by the Bioethics Committee of the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences. At least 5 animals were used to study each material sample.

2.7. Implantation model

For the assessment of biocompatibility of polylactide films, a rat model of subcutaneous biomaterial implantation was used [34]. To sterilize the film surface and prevent the primary acute reaction of the recipient organism, the polymer samples were conditioned for 4 h at 37 °C in a nutrient medium containing 10% cattle serum, a mixture of antibiotics and an antimycotic. Samples were labeled with colored silk threads and implanted subcutaneously by blunt dissection through a cut in the lower part of the back using a trocar. The samples were implanted along the back line of the animal (3 samples per rat; spaced by 3 cm from each other). The surgery was carried out under Zoletil/xylazine anesthesia (6/12 mg per 1 kg of body weight). The operated animals were sacrificed 60 days after the implantation, and the explanted material was subjected to macro- and microscopic examination [35].

2.8. Diffusion mechanisms

To describe medium absorption and streptokinase release behaviours from PLGA, diffusion mechanisms were analyzed by using the Ritger-Peppas equation $M_t/M_\infty = k \times t^n$ [36]. Where M_t and M_∞ are the amounts of the released streptokinase at a time t and at equilibrium, respectively, n is the diffusion exponent which determines the type of mechanism and k, the constant of the streptokinase-polymer system that depends on the structural and geometric characteristics of the polymer. According to the diffusion exponent values, the diffusion mechanism can be classified for a thin polymer film as quasi-Fickian ($n < 0.5$), Fickian ($n = 0.5$), non-Fickian or anomalous ($0.5 < n < 1$), case II ($n = 1$) or super-case II ($n > 1$) transports [37,38].

3. Results

It was shown that both the concentration of streptokinase in the polymer and the molar mass of PLGA significantly affected the relative elongation of polymer films (Fig. 2). With the molar mass of PLGA becoming higher, the ability of polymer films to elongate increased. The incorporation of streptokinase into the film, on the contrary, lowered the ability of the polymer to elongate. In general, the relative elongation of a polymer film linearly depended on the concentration of streptokinase – in all the examined film samples. When the concentration of streptokinase was increased by 1%, the relative elongation decreased by 10–15%.

Fig. 3 presents data on the effect of streptokinase on the tensile strength of PLGA polymer films of various molar masses. An increase in the molar mass of PLGA polymer was shown to lead to the growth of the

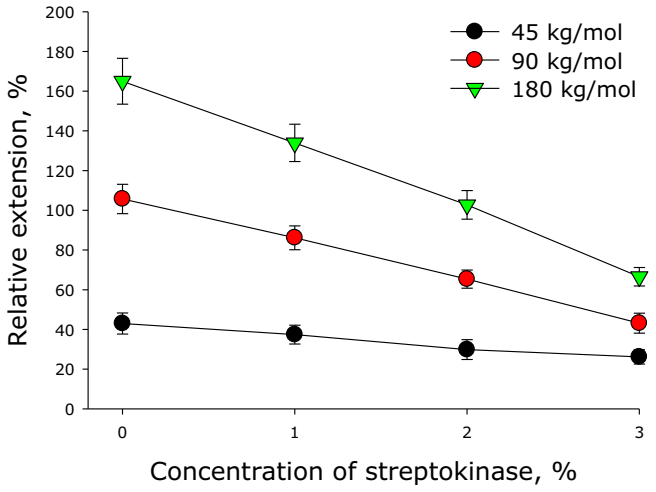

Fig. 2. The effect of streptokinase on the elongation of PGLA polymer films of different molar masses. Mean values and their standard errors for three independent experiments are presented.

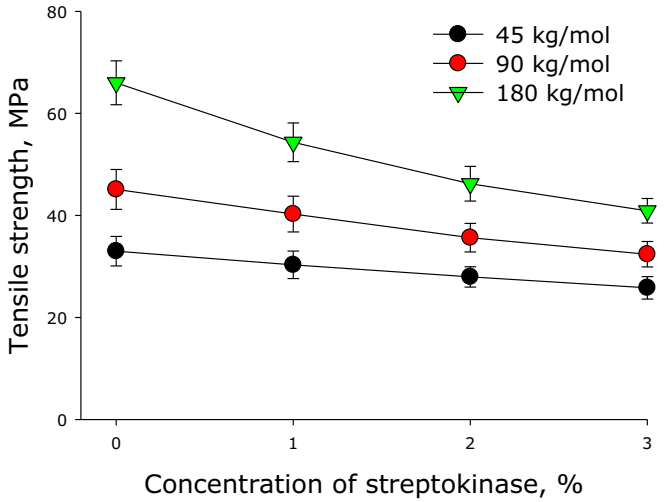

Fig. 3. The effect of streptokinase on the tensile strength of PGLA polymer films of various molar masses. Mean values and their standard errors for three independent experiments are presented.

tensile strength of polymer films. The incorporation of streptokinase into the polymer, on the contrary, decreased the tensile strength of polymer films. In general, the tensile strength of polymer films linearly depended on the concentration of streptokinase. When the concentration of streptokinase was increased by 1%, the tensile strength of polymers decreased by 10–15%.

Fig. 4 shows data on the effect of streptokinase on the yield strength of PLGA polymer films of various molar masses. The higher was the molar mass of PGLA polymers, the higher was the yield point of the polymer films. The introduction of streptokinase into the polymer led to a decrease in the tensile strength of polymer films.

Table 1 shows data on the effect of streptokinase on Young's modulus of PLGA polymer films of various molar masses. The higher was the molar mass of PGLA polymers, the lower was the Young's modulus of the polymer films. The introduction of streptokinase into the polymer led to a decrease in the Young's modulus of polymer films.

Fig. 5 presents micrographs of the surface of PLGA films containing streptokinase at different concentrations. The images showed that all the samples had smooth, flawless surfaces, validating the technology used to obtain polymer films. In rare cases, when the concentration of

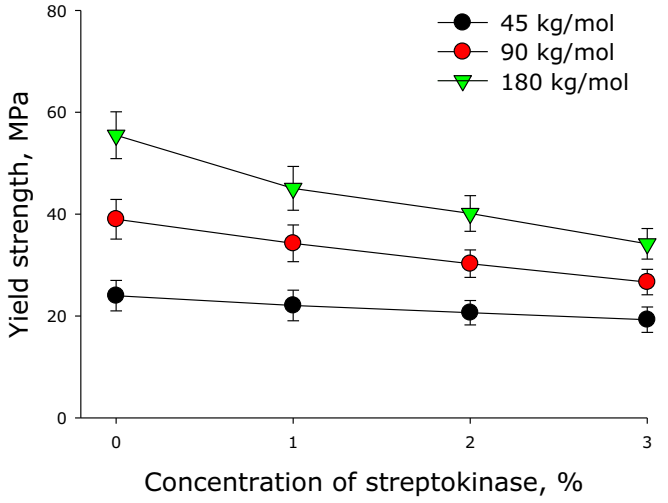

Fig. 4. The effect of streptokinase on the yield strength of PGLA polymer films of various molar masses. Mean values and their standard errors for three independent experiments are presented.

Table 1
The effect of streptokinase on the Young's modulus of PGLA polymer films of various molar masses.

Sample		Young's modulus, GPa
PGLA molar masses, kg/mol	Concentration of streptokinase, %	
45	1	1,44
45	2	1,34
45	3	1,02
90	1	1,01
90	2	0,92
90	3	0,86
180	1	0,81
180	2	0,75
180	3	0,54

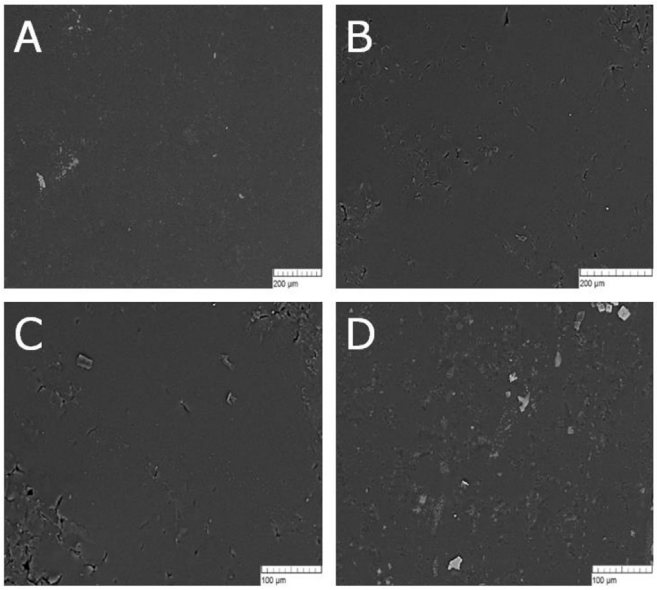

Fig. 5. Micrographs of the surface of PLGA films without streptokinase (A) and with streptokinase at concentrations of 1% (B), 2% (C) and 3% (D). The microphotographs were taken with a scanning electron microscope at a magnification of ×300. The films were obtained from PLGA with a molar mass of 45 kg/mol.

streptokinase was 2–3%, sample batches contained protein aggregates and dried drops of saline solution on the film surface. The streptokinase aggregates mainly appeared when the molar mass of PLGA used to obtain films was 45 kg/mol. When the molar mass of PLGA was higher, no such problems were observed.

Fig. 6 shows the rate of polymer film degradation depending on the molar mass of PLGA. As it turned out, the lower was the molar mass of PLGA, the slower was the degradation of PLGA-based polymer films. The polymer degradation was found to occur in two phases. The first phase, which took about a month from the moment the films were placed in physiological saline, was characterized by a rather high rate of polymer degradation and was followed by the second phase, whose rate was lower. For the films made of 45 kg/mol PLGA, the rate of polymer degradation was approximately 0.6% of polymer mass per day during the first phase and thrice as lower during the second phase (0.2% of polymer mass per day). For the films made of 90 kg/mol PLGA, the rates of polymer degradation were approximately 1.0 and 0.3% of polymer mass per day during the first and second phase respectively. For the films made of 180 kg/mol PLGA, the respective values were 1.3 and 0.3% of polymer mass per day. The experiments, in which degradation of PLGA films was measured in phosphate buffers of

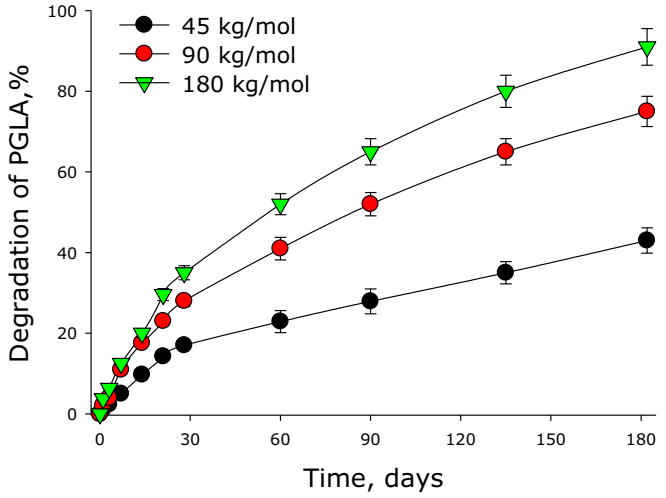

Fig. 6. The rate of degradation of PGLA-based polymer films depending on the molar mass of PLGA. Mean values and their standard errors for three independent experiments are presented.

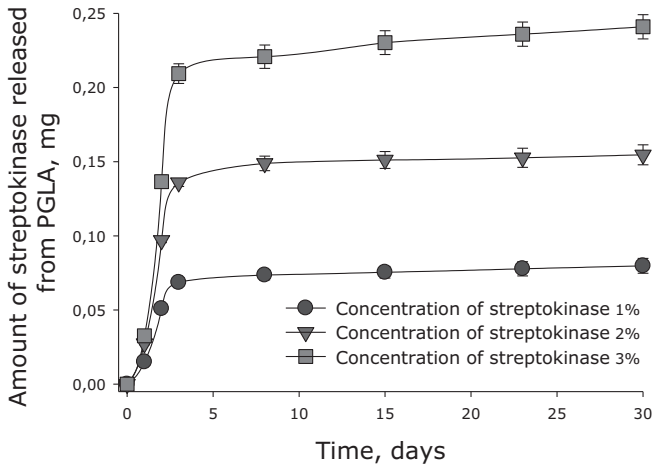

Fig. 7. Dynamics of streptokinase release from polymer membranes made of 45 kg/mol PLGA. Mean values and their standard errors for three independent experiments are presented.

Table 2
Main information provided by power model law fitting in the burst interval (Peppas-Korsmeyer).

Author statement sample		n	k	R^2
PGLA molar masses, kg/mol	Concentration of streptokinase, %			
45	1	1.422	0.201	0.966
45	2	1.499	0.188	0.971
45	3	1.734	0.144	0.977
90	1	1.112	0.191	0.997
90	2	1.33	0.201	0.986
90	3	1.474	0.167	0.997
180	1	1.208	0.202	0.989
180	2	1.187	0.214	0.999
180	3	1.131	0.258	0.971

varying capacity and pH (7.0–8.0), showed that these parameters had no particular effect on the rate of polymer degradation; the values did not differ from those obtained in the physiological saline. The incorporation of streptokinase into the polymer films increased the rate of

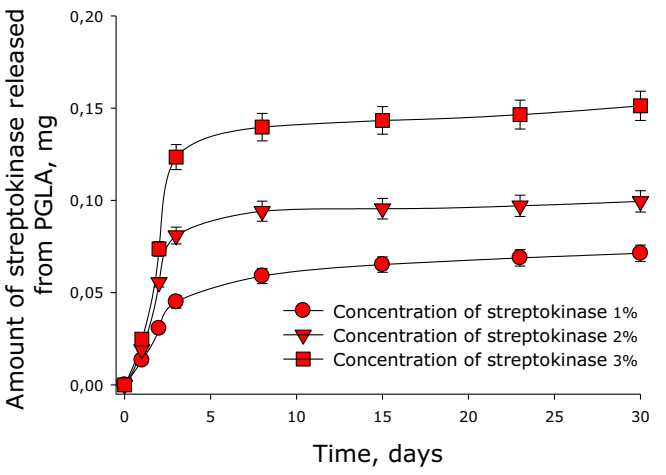

Fig. 8. Dynamics of streptokinase release from polymer membranes made of 90 kg/mol PLGA. Mean values and their standard errors for three independent experiments are presented.

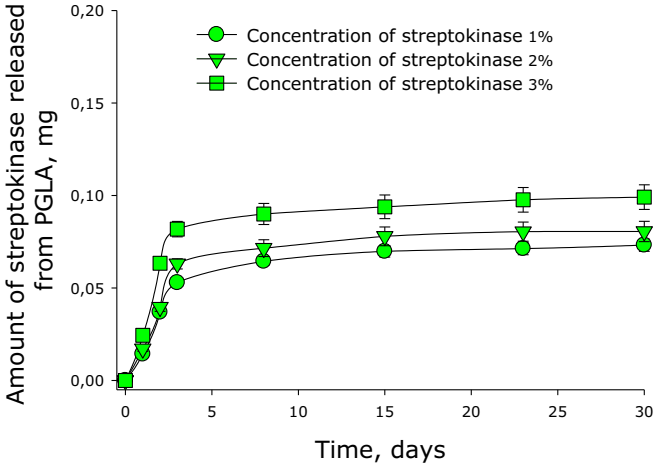

Fig. 9. Dynamics of streptokinase release from polymer membranes made of 180 kg/mol PLGA. Mean values and their standard errors for three independent experiments are presented.

their degradation by 10–20%. In general, however, the degradation of streptokinase-containing films showed the same tendencies that were revealed for the enzyme-free polymer films.

The experiments showed that in solutions, streptokinase gradually diffused out of polymer membranes made of 45 kg/mol PLGA (Fig. 7). The rate of streptokinase release depended on the initial concentration of the enzyme in the polymer membrane. The higher was the initial concentration, the faster was the diffusion of streptokinase out of the membrane. The initial rates of streptokinase release from the membranes containing 1, 2 and 3% of the enzyme were approximately 0.023, 0.045 and 0.070 mg/day respectively. In other words, the initial rate of streptokinase release from the polymer membrane was proportional to its initial amount. It should be noted that after 3 days, the rate of streptokinase release from the membrane decreased significantly, and the remaining streptokinase molecules (~75% of the initial amount) were slowly released into the medium in parallel with the dissolution of polymer membranes. The rate of streptokinase release from the membranes decreased to 0.001 mg/day. The molecules of streptokinase extracted from the solution retained more than 90% of their enzymatic activity, which indicated a high degree of preservation of the native enzyme folding.

It was also shown that streptokinase was gradually released from

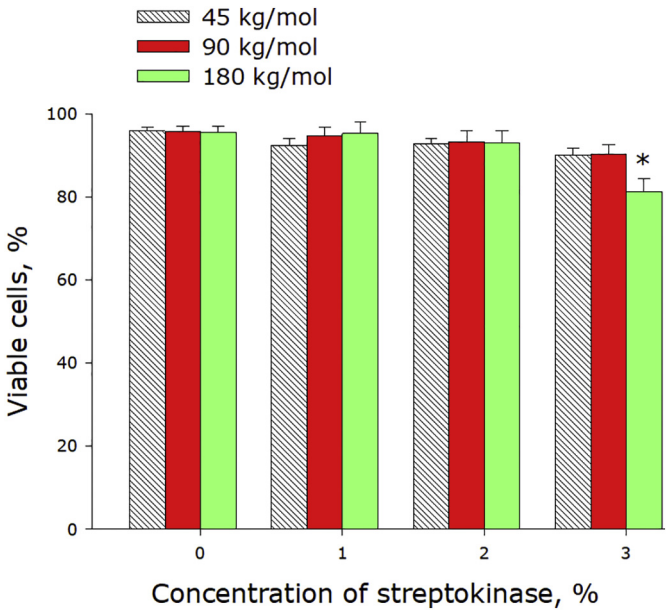

Fig. 10. The effect of streptokinase-containing PLGA-based polymer films on the viability of cell cultures. The averaged data of three independent experiments are presented.

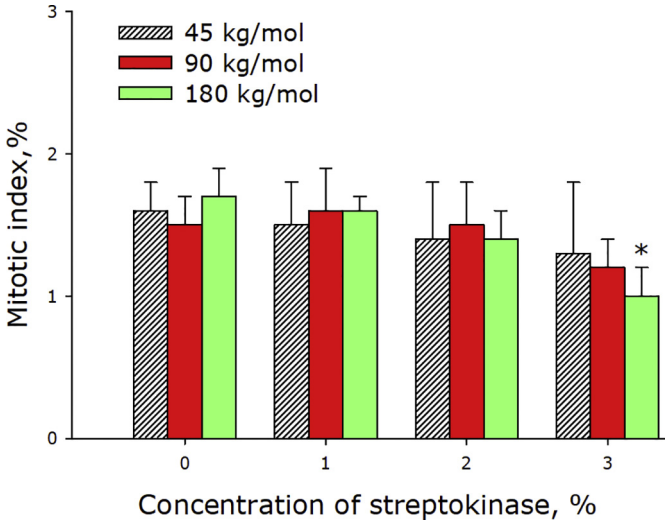

Fig. 11. The effect of streptokinase-containing PLGA-based polymer films on the mitotic index of cell cultures. The averaged data of three independent experiments are presented.

polymer membranes made of 45 kg/mol PLGA (Fig. 7). The rate of streptokinase release depended on the initial concentration of the enzyme in the polymer membrane. The higher was the initial concentration, the faster was the diffusion of streptokinase out of the membrane. The initial rates of streptokinase release from the membranes containing 1, 2 and 3% of the enzyme were approximately 0.015, 0.027 and 0.041 mg/day respectively. That is, the initial rate of streptokinase release from the polymer membrane was proportional to its initial amount. It should be noted that after 3 days, the rate of streptokinase release from the membrane decreased significantly (to a level of 0.001 mg/day). The remaining streptokinase molecules (~75% of the initial amount) slowly diffused into the medium in parallel with the dissolution of polymer membranes.

Streptokinase was also demonstrated to diffuse out of polymer membranes made of 180 kg/mol PLGA (Fig. 9). The picture of the dynamics of streptokinase release was similar to that observed in the

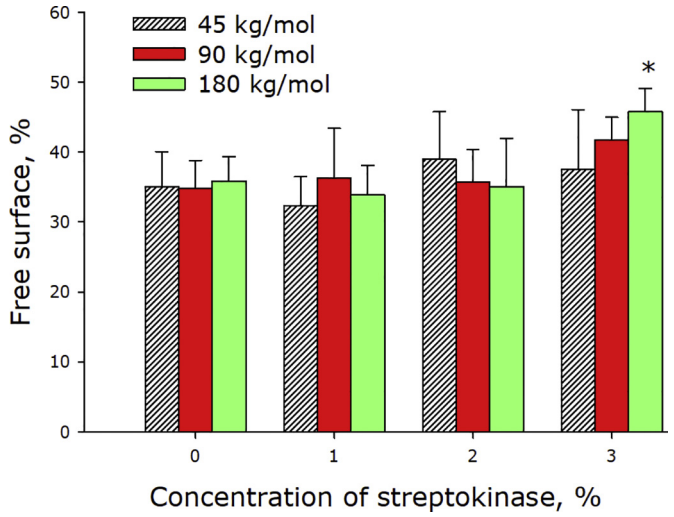

Fig. 12. Population of the surface of streptokinase-containing PLGA-based polymer films by cells. The averaged data of three independent experiments are presented.

experiments with other polymer membranes. The initial rates of streptokinase release from the membranes containing 1, 2 and 3% of the enzyme were approximately 0.016, 0.020 and 0.026 mg/day respectively. After 3 days, the rate of streptokinase release from the membrane decreased significantly and was about 0.001 mg/day.

To find out the possible mechanism of diffusion of streptokinase from the polymer, the Peppas-Korsmeyer model was used (Table 2). As can be observed, the determination coefficients, in general, were higher than 96%, which indicates a good reliability of the transport models, except for the diffusion of streptokinase from the PGLA in water. Type of transport for PGLA in water was super-case II, characterized by $n > 1$. This mechanism is an extreme case of case II diffusion ($n = 1$), where the diffusion of the medium is faster than the relaxation of the polymeric chains when it penetrates the polymer.

Fig. 10 shows the effect of PLGA-based polymer films of different molar masses on the viability of cell cultures. The tests showed that the number of non-viable cells on the surface of streptokinase-free polymer coatings did not exceed 10% of their total amount (for comparison, the number of non-viable cells on the surface of culture glass was 5.3 ± 1.3%). The incorporation of streptokinase into the films did not increase the percentage of non-viable cells on their surface. The only case when statistically significant differences were registered was the case with the films made of 180 kg/mol PLGA containing 3% streptokinase. Thus, all but one film samples tested did not have a short-term toxic effect on the cells growing de novo on their surface.

To evaluate the effect of polymers on the rate of cell growth, we calculated the mitotic index of cell cultures in the logarithmic phase of their growth (3 days after inoculation) (Fig. 11). The cells undergoing division (mitosis) were detected using fluorescence microscopy and identified, on the basis of characteristic chromatin distribution, as going through prophase (P), metaphase (M), anaphase (A), and telophase (T). For quantitative analysis, at least 500 cells on the surface of each sample were counted. The mitotic index of cells growing on the surface of streptokinase-free polymer films was ~1.5%. The presence of 1–2% streptokinase in the polymer film did not lead to significant changes in the mitotic index. When the film was made of 180 kg/mol PLGA and the concentration of streptokinase in the film was 3%, the mitotic index decreased to a level of 1.0%.

Fig. 12 shows how cells populated accessible surfaces of streptokinase-containing PLGA-based polymer films. In the case of streptokinase-free films, approximately 35% of their surface remained uninhabited by the third day of cell cultivation. When streptokinase was incorporated in the films, the situation did not change. The only exception were films

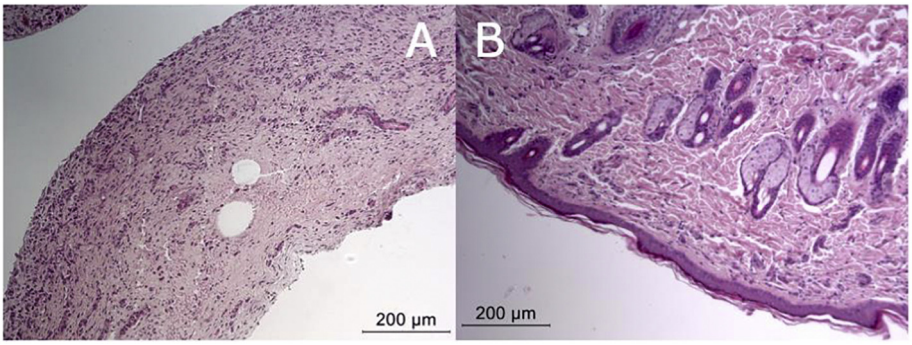

Fig. 13. Sections of tissues surrounding the samples of streptokinase-containing (1–2%) PLGA-based polymer films obtained after 8 weeks of subcutaneous implantation of the films in rats.

made of 180 kg/mol PLGA with 3% streptokinase; in that case, ~45% of their surface remained uninhabited.

The biocompatibility of streptokinase-containing PLGA-based polymer films was assessed in the in vivo experiments using the method of subcutaneous implantation. All the test animals felt satisfactory during the postoperative period and were withdrawn from the experiment 60 days after the operation. After that, the sites of implant placement were examined microscopically. The examination revealed no traces of streptokinase-containing PLGA in the tissues (Fig. 13A). In some cases, there were channels left by the threads used to label the implants and flag the postoperative topology (Fig. 13B). When PLGA polymers were used as coatings for Nitinol stents, the material was explanted and studied macroscopically. Only traces of polymers were found of the stent surface, and mature connective-tissue capsules were formed on the periphery of the samples. The capsules were characterized by a predominance of extracellular over cellular components, a regular arrangement of fibers and the absence of vessels. Those characteristics indicated a partial involution of capsules around the implanted samples. The structure and thickness of capsules were standard for non-resorptive samples of this type, indicating both their bioinertness and biocompatibility.

4. Discussion

In this work, we have established basic mechanical characteristics for all the polymer coatings synthesized. In particular, the elongation of PLGA-based polymer films of various molar masses has been examined in respect to the concentration of streptokinase in the films (Fig. 2). In accordance with the theory of rubber elasticity, we consider a polymer chain in a cross-linked network as an entropy spring. When the chain stretches, entropy decreases with a large margin, since fewer conformations are available [39]. It can be assumed that the presence of protein would affect the conformation of chains – i.e., the chains would be already somewhat stretched in undeformed material, so their ability to further stretch would be lower. Other mechanical properties of PLGA polymers have also been investigated (Figs. 3, 4). An increase in the protein concentration in the polymer has been shown to decrease its tensile and yield strength. The dependence of the polymer yield strength on the concentration of streptokinase can be extrapolated by a linear equation $y = y_0 + ax$, where $y_0 = 24$ and $a = -3$ for 45 kg/mol PLGA; $y_0 = 38$ and $a = -5$ for 90 kg/mol PLGA; and $y_0 = 54$ and $a = -7$ for 180 kg/mol PLGA. Correspondingly, these three straight lines should intersect when the concentration of streptokinase in the polymer is around 8%. This is a point beyond which the yield strength of PLGA polymer films will depend not as much on the molar mass of PLGA as on the concentration of the enzyme. Such a behavior of the polymer can be explained on the basis of the assumption that there is a mechanical weakness at the protein-PLGA interface. When the molecular weight of PLGA polymers increases, their tensile strength grows as well. This can be explained by an increased number of chain entanglements in high-molecular polymers. It should be noted that the PLGA polymers synthesized in this work have a much higher yield strength compared to other PLGA polymers [40,41] and Young's modulus comparable to other polymers [42].

The polymer films studied in the paper have been shown to undergo degradation over time in aqueous solutions – even in the absence of biological objects (Fig. 5). The lower is the molar weight of PLGA used to make a film, the slower is its degradation. This is probably due to the degree of crystallinity of the polymer. In general, similar patterns were observed earlier [43].

The degradation of the polymer occurs in two phases. The first, rapid phase of degradation seems to be primarily associated with the swelling and depolymerization of bonds between the initial polymer particles. The second phase is probably related to the destruction of the polymer base. Similar processes are observed during thermal degradation of PLGA [44]. Often, the rate of exit of compounds from thin coatings and the rate of degradation of the coatings themselves are affected by pH [45]. In our case, a change in pH in the range of 7–8 units did not lead to a significant change in the rate of degradation.

The kinetics of streptokinase release from PLGA-based membranes depends on the concentration of the enzyme in the membrane and the molar mass of PLGA (Fig. 6-8). It is noteworthy that about 75% of the enzyme molecules remain encapsulated in the membrane. 25% of streptokinase molecules release from the membrane into the surrounding medium relatively quickly. By their physicochemical and mechanical characteristics, the biodegradable polymer membranes synthesized in this work are suitable to make coatings of stents and prostheses which would be capable of sustained and controlled release of drugs into the surrounding tissues. Over the past 20 years, there have been many attempts to immobilize drugs in the membrane [46–48], yet all the drugs listed in the references are low-molecular substances. In the present work, we have managed to achieve controlled release of a substance with the molecular weight of 47 kDa.

The biocompatibility of polymer films made of PLGA of various molar masses has been assessed on in vitro models (Fig. 9-11). In general, there is no doubt that PLGA polymers are highly biocompatible. The results obtained on cell cultures have been confirmed in the experiments on animal models (Fig. 12). On the other hand, toxicity of high doses of streptokinase (above 5000 units/g) has been known for more than 70 years [49]. In our experiments, toxic effects: a decrease in the mitotic index and increase in the number of non-viable cells – were observed in the case of 180 kg/mol PLGA polymer containing 3% streptokinase. The toxicity of streptokinase is mainly related to its main function. High doses of streptokinase can cause bradycardia, arrhythmia, ventricular fibrillation and, less often, fever and severe hypotension. Allergic and immune reactions may also occur. In our experiments, none of those adverse effects were observed in the experimental animals. Furthermore, no traces of streptokinase-containing PLGA were found in the tissues 60 days after the surgery. When PLGA polymers were used as components of stent coatings, only traces

of polymers were detected in the implant-surrounding tissues. The implantation experiments have, therefore, shown that all the examined materials are not toxic to the recipient's organism and appear to be biocompatible.

Thus, novel biodegradable PLGA-based polymer membranes have been synthesized and tested in this work – membranes which possess the necessary mechanical properties and are capable of a sustained and directional release of high-molecular biological compounds.

Declaration of Competing Interest

None.

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