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INVESTIGATION OF BIODEGRADATION AND PROPERTIES OF POLYURETHANE FOAM COMPOSITE MATERIALS WITH LYSOZYME IN VITRO

The study of biodegradation ability of polyurethane foams composite materials with lysozyme under the influence of saline solution for 2 weeks, 1, 3 and 6 months by IR spectroscopy, DSC, TGA was conducted. According to the results of IR spectroscopy under the influence of model medium there are processes of biodegradation, which are confirmed by a decrease in the intensity of the absorption band νC=O. Along with biodegradation there is a redistribution of hydrogen bonds of NH and CO groups of polymer matrix. According to DSC after incubation in saline solution there is an increase in Tg and ΔCp at the glass-transition (for polyurethane foams and composites with lysozyme in the amount of 5 wt. %), an increase in Tg and decrease in ΔCp (for composites with lysozyme in the amount of 1 and 3 wt. %), which indicates the redistribution of hydrogen bonds under the influence of saline solution and due to lysozyme release. It was found that after incubation in saline solution there is an increase in T0 and Tmax for both polyurethane foams and composite materials with lysozyme by the method of TGA. Thus, composites with lysozyme in vitro are heat-resistant materials. According to the study results of the dynamics of lysozyme release composites are capable to the prolonged release of enzyme for 5 days, the amount of which varies depending on the lysozyme content (43.85-61.97 % of the total amount of the introduced drug) and is sufficient for the manifestation of antimicrobial activity. The tissue culture method has established the biocompatibility of investigated materials. For polyurethane foam composite materials with lysozyme more active growth of fibroblastic elements than in the control and polyurethane foam and slowing down the process of cell degeneration was observed. The obtained results indicate that polyurethane foam composite materials with lysozyme are promising materials that due to the presence of the enzyme will have antimicrobial action and can be used in medical practice as polymer composites for the treatment of wounds and burns.

Keywords: *polyurethane foam, composite material, lysozyme, saline solution, biodegradation.*

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Introduction

Polyurethane foams (PUF) are one of the most widely used materials in medicine due to their biocompatibility, which conditioned by the similarity in chemical structure of the urethane group to the amide group of protein molecule. In addition, they have a porous structure and a developed surface that is capable of stimulating tissue regeneration processes at the implantation site [1] and they are capable to the prolonged release of drugs [2].

Considering the aforesaid, PUF composite materials filled with lysozyme based on diisocyanate prepolymer as polymer composites for the treatment of wounds and burns were obtained. These composite materials due to the presence of enzyme will have hydrolytic and antimicrobial action [3].

The ability to biodegradation in the conditions imitating the environment of the organism is one of the most important characteristics of polymers for medical use. This process is accompanied by changes in the structure of the polymer which cause changes of properties and it can influence on the amount of released drug to the internal environment of an organism.

To date, the biodegradation mechanisms of polyurethanes have been established. Biodegradation of polyurethanes *in vivo* occurs by two complementary mechanisms. It is hydrolytic and macrophagal. Hydrolytic biodegradation is manifested in the hydrolysis of ester, urethane and later ether bonds. Hydrolytic biodegradation *in vivo* is identical to hydrolytic biodegradation *in vitro*. Macrophage-mediated biodegradation occurs due to the phagocytic activity of macrophages and foreign body giant cells [4-6].

Experimental determination of the biodegradation timing of polymer in the body (using labeled preparations) is a time-consuming procedure. Estimation of effective rates of polymer weight loss is also difficult due to the lack of sufficiently clear methods for separation of the implant from connective tissues and experimental errors. Therefore, predictions of the polymer biodegradability in vivo are based on the results of in vitro studies.

It is known that the biodegradation processes are influenced by such factors as the chemical composition of polymer, the hydrophilicity of polymer matrix, the presence of drug substance and the drug type etc. [7, 8]. So, the introduction of drugs into the composition of polymer materials can affect the rate of biodegradation processes [9]. For example, the introduction of tiamulin fumarate into the composition of composite materials based on polyurethaneurea accelerates the biodegradation of the polymer base [10]. At the same time, due to the presence of amizon in the block-copolyurethane the polymer materials remain stable [11]. Hydrophilic drugs accelerate the polymer degradation by facilitating the water penetration in the system. In contrast, hydrophobic drugs slow down the polymer degradation by hindering the water diffusion into the matrix [8].

Also important characteristic of polymers for medical use is the release dynamics of the drug and its quantity. The drug release from polymer composites is influenced by the chemical nature of polymer and drug, their physical-chemical properties etc. [12]. Such factor as water solubility of drug determines the mechanism and kinetics of its release from a polymer matrix. Well water soluble drugs are released due to diffusion that promotes swelling of matrix and degradation of polymer, while poorly water-soluble drugs are released mainly due to degradation of polymer matrix [13, 14]. The ability of polymer materials to prolonged release of drugs to ensure their uniform release into the body in acceptable quantity is important to ensure local therapeutic effect.

Considering the aforesaid, there is a need to study the ability to biodegradation and the ability to release of lysozyme from the polyurethane foam matrix in *vitro*. Results of these researches will allow predicting behaviour of polymer materials *in vivo* at their further application in medical practice.

Therefore, the purpose of the work is to study the biodegradation of PUF composite materials with lysozyme *in vitro*, the ability to prolonged release of lysozyme and biocompatibility.

Experimental

Materials

PUF and PUF composite materials filled by lysozyme in the amount of 1, 3 and 5 wt. % synthesized according to the method [3] were objects of researches.

Sodium chloride solution for infusion (saline solution) (Novofarm-Biosynthesis, Ukraine)

containing NaCl (9 mg/ml) and water for injection was used as a model medium to study biodegradation.

Distilled water and drug lysozyme (molecular weight = 14000) (Merck, Germany) were used to study the dynamics of lysozyme release.

The method of incubation in saline solution

Samples were placed in sterile tubes, poured 25 ml of model biological medium and kept in a thermostat at a temperature of (37 ± 1)°C for 2 weeks, 1, 3 and 6 months. Solutions of model mediums were changed daily. After defined incubation terms in the model medium, the samples were taken out, washed with distilled water and dried to a constant masse at room temperature.

Study Methods

The structure was investigated on a Tensor-37 FTIR spectrometer in the range of 650–4000 cm^{-1} by the MATR method with the aid of a diamond crystal trapezoidal prism (a number of reflections of N = 1, an incidence angle of φ = 39°).

Thermophysical properties (glass-transition temperature (T_g), changes of the heat capacity at the glass-transition temperature (ΔC_p)) have been studied by the DSC method. The study has been carried out within the interval of temperature from -90 to +200 °C (TA Instrument Q2000) at a heating rate 20 °C/min under nitrogen atmosphere. Two heating procedures have been carried out to exclude the influence of the thermal and mechanical prehistory of the material.

Thermogravimetric characteristics (onset temperature of thermal decomposition (T_0), temperature of maximum decomposition rate (T_{max}), weight loss at T_0) were studied by TGA. The study has been carried out within the interval of temperature from +20 to +700 °C (TA Instrument Q50) at a heating rate 20°C/min under an air atmosphere.

The study of lysozyme release from the polymer samples was performed by spectrophotometric method. Absorption spectra of the studied solutions were obtained on the spectrophotometer "SF-46" in cuvettes with a layer thickness of 1 cm relative to the comparison solutions at a wavelength λ = (281 ± 0.5) nm.

Preparation of solutions. Control and experimental samples (average weight 2 g) were placed in weighing bottles with the ground-in stopper, added 20 ml of distilled water. The weighing bottles were kept in a thermostat at a temperature 36.6 °C for 30 minutes, 6 hours, 1, 2, 3, 4 and 5 days. After defined terms the solutions were poured out and investigated their absorption spectra. The experimental solutions received from the samples containing lysozyme in their composition. Solutions of comparative (control solutions) obtained from control samples (from samples which do not contain lysozyme).

The concentration of released lysozyme was found on the calibration graph. For creation of the calibration graph of dependence of optical density of solutions on lysozyme concentration, a series of lysozyme solutions with different percentage concentration: 0.0050; 0.0125; 0.0250; 0.0350; 0.0500; 0.0600 and 0.0700 were prepared. Their absorption spectra were investigated.

The amount of released lysozyme from polymer samples into the solution (*L*, %) was calculated using Equation (1) and (2):

$$L = \frac{m}{m_0} \cdot 100\% \ ,$$

$$m = \frac{C \cdot V}{1000} \ ,$$

where *m* is the amount of released lysozyme for certain period of time, g; m_0 is the masse of lysozyme introduced into the polymer, g; *C* is the concentration of lysozyme in the investigated solution, found according to the calibration graph, mol/L; *V* is the volume of the solution in which performed washing-out, ml.

By tissue culture method was conducted the study of biocompatibility on the culture of tissue of subcutaneous fatty tissue of white laboratory rats. The culture of tissues was received by explantation of pieces of subcutaneous fatty tissue. It was placed in Carrel flacon with a nutritious mixture consisting of the biological medium 199 and chicken plasma. In Carrel flacon the samples of studied polymer materials in the size of 0.5 x 0.5 cm were introduced. Then an embryonic extract was added and a plasma clot (a solid phase) was obtained. After the formation of the solid phase (10-15 min.) the biological medium 199 and the serum of cattle (a liquid phase) was introduced. Cultivations were carried out at a temperature of 37 °C. Flacons with explants of subcutaneous fatty tissue without the addition of polymer samples

were used as control. The cultures were investigated under a microscope in a native state.

Results and Discussion

To study the ability to biodegradation, PUF and PUF composite materials with lysozyme were incubated in the model medium (saline solution) for 2 weeks, 1, 3 and 6 months. The ability to biodegradation of studied polymer materials was evaluated by changes in their structure, thermophysical and thermogravimetric properties before and after incubation in saline solution.

The structure of PUF and PUF composite materials with lysozyme of various concentrations (1, 3 and 5 wt. %) was investigated by IR spectroscopic tests before (control) and after incubation in saline solution. IR spectra were measured in the range of 4000–600 cm^{-1}. Only fragments of spectra that have changes are presented.

The absorption band of valence vibrations of NH groups remained unchanged during 3 months of incubation in saline solution within the frequency interval of spectra of PUF 2800–3800 cm^{-1}. There is an increase in the intensity of the absorption band $\nu_{NH\text{-}free}$ with an approximate maximum at 3571 cm^{-1} after 6 months. It is connected with the increase in the number of hydrogen-free NH groups on the surface layer of the samples (as IR spectra are removed from the surface of polymer materials) (Fig. 1, curve *5*).

Under the influence of saline solution there is a decrease in the intensity of the absorption band $\nu_{C=O}$ of urethane group (1718 cm^{-1}) and the expansion of its maximum in the spectrum range of 1800–1500 cm^{-1}. It indicates the processes of biodegradation and the formation C=O groups with different hydrogen bond strength, respectively (Fig. 1, curves *2–5*).

Under the influence of saline solution for PUF composite materials with lysozyme, changes in the spectral range of 2800–3800 cm^{-1} starting from 2 weeks of incubation are observed. On the IR spectra of PUF containing enzyme in amount of 1 and 3 wt. % there is an increase of intensity of the absorption band $\nu_{NH\text{-}bond}$ with an approximate maximum at 3296 cm^{-1} and the absorption band $\nu_{NH\text{-}free}$ with an approximate maximum at 3571 cm^{-1}. It indicates an increase in the number both free and hydrogen-bonded NH groups in the surface layer molecules of the samples. In the spectral range of 1800-1500 cm^{-1} under the influence of saline solution there is a decrease in the intensity of the absorption band $\nu_{C=O}$ (1718 cm^{-1}) and the expansion of its maximum (Fig. 2, Fig. 3, curve *2–5*).

On the IR spectra of PUF containing lysozyme in the amount of 5 wt. % there is a decrease in the intensity of the absorption band $\nu_{NH\text{-}bond}$ (3296 cm^{-1}) and the absorption band $\nu_{NH\text{-}free}$ (3571 cm^{-1}), which indicates a decrease in the number of free and

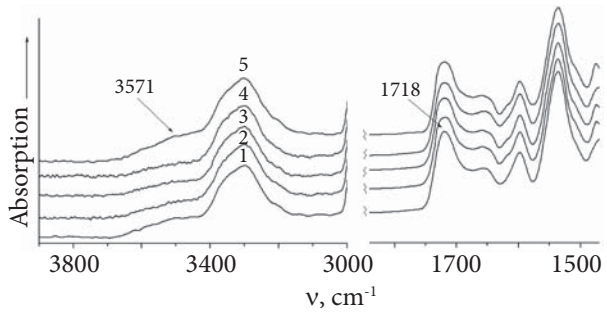

Fig. 1. Fragments of IR spectra of PUF before (*1*) and after incubation in saline solution for 2 weeks (*2*), 1 month (*3*), 3 months (*4*), 6 months (*5*)

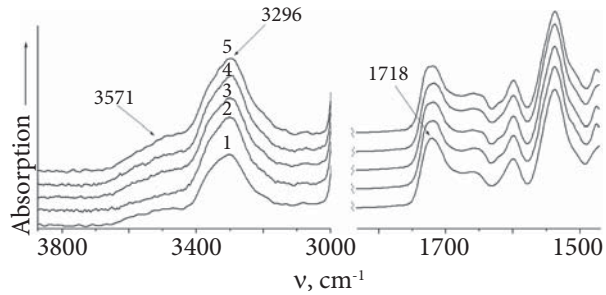

Fig. 2. Fragments of IR spectra of PUF+lysozyme (1 wt. %) before (*1*) and after incubation in saline solution for 2 weeks (*2*), 1 month (*3*), 3 months (*4*), 6 months (*5*)

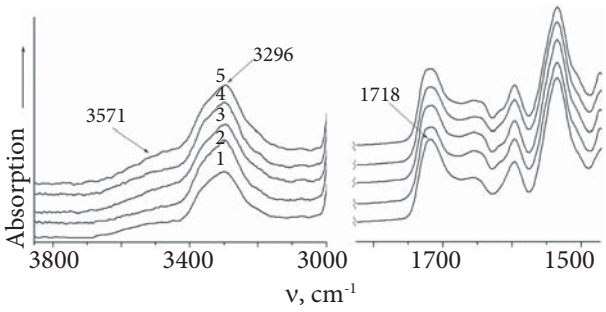

Fig. 3. Fragments of IR spectra of PUF+lysozyme (3 wt. %) before (*1*) and after incubation in saline solution for 2 weeks (*2*), 1 month (*3*), 3 months (*4*), 6 months (*5*)

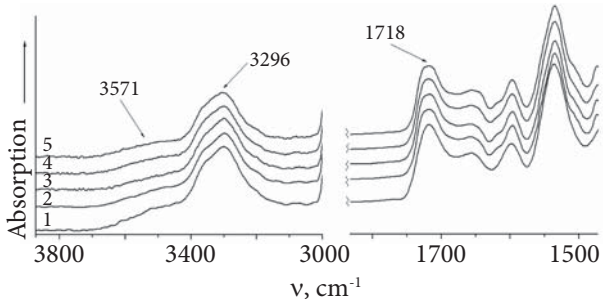

Fig. 4. Fragments of IR spectra of PUF+lysozyme (5 wt. %) before (*1*) and after incubation in saline solution for 2 weeks (*2*), 1 month (*3*), 3 months (*4*), 6 months (*5*)

hydrogen-bound NH-groups in the surface layer molecules of the samples. Also under the influence of saline solution there is a decrease in the intensity of the absorption band $\nu_{C=O}$ (1718 cm^{-1}) and the expansion of its maximum, as well as for all samples (Fig. 4, curve *2–5*).

Thus, according to the results of IR spectroscopic studies of PUF and PUF composites with lysozyme under the influence in saline solution there are processes of biodegradation, which are confirmed by a decrease in the intensity of the absorption band $\nu_{C=O}$. Along with biodegradation there is a redistribution of hydrogen bonds of NH and CO groups of the surface layer of the samples. The dependence of the above changes on the presence and concentration of lysozyme in the composition of composites was established. Thus, for PUF there is an increase in the number of $\nu_{NH\text{-}free}$ of the surface layer only after 6 months, which is probably due to prolonged exposure to saline solution. Whereas for PUF composite materials with lysozyme starting from 2 weeks of incubation there is an increase (for PUF containing 1 and 3 wt. % lysozyme) and a decrease (for PUF containing 5 wt. % lysozyme) in the amount of both $\nu_{NH\text{-}bond}$ and $\nu_{NH\text{-}free}$. It may be connected with the redistribution of hydrogen bonds of surface layer due to lysozyme release from the polymer matrix. The difference in IR spectra of composites with a maximum lysozyme content (5 wt. %) may be connected with release of more enzymes. As a result, the redistribution of hydrogen bonds is different (which is consistent with changes in ΔC_p).

Table 1. **Thermogravimetric characteristics of composites after incubation in saline solution**

Samples	Periods of incubation	T_0, °C	T_{max}, °C	Weight loss at T_0, %
PUF	control	196,39	300,89	0,62
	2 weeks	210,53	333,01	0,86
	1 month	209,12	328,99	0,61
	3 months	214,91	328,66	1,15
	6 months	207,24	329,30	0,50
PUF+lysozyme (1 wt. %)	control	191,13	299,76	0,70
	2 weeks	217,77	335,23	1,10
	1 month	220,49	326,14	1,12
	3 months	213,93	330,88	0,52
	6 months	201,67	329,81	0,57
PUF+lysozyme (3 wt. %)	control	195,73	302,24	0,78
	2 weeks	207,89	331,66	0,60
	1 month	209,29	333,45	0,85
	3 months	221,16	332,36	0,75
	6 months	208,91	332,90	0,58
PUF+lysozyme (5 wt. %)	control	179,95	310,72	1,08
	2 weeks	211,52	333,26	0,95
	1 month	206,46	333,75	1,01
	3 months	211,01	342,31	0,91
	6 months	206,09	333,20	0,86

The change in thermophysical and thermogravimetric properties of PUF and PUF composite materials with lysozyme of various concentrations (1, 3 and 5 wt. %) was evaluated before (control) and after incubation for 2 weeks, 1, 3 and 6 months in saline solution.

According to TGA, onset temperature of thermal decomposition (T_0) of studied PUF before incubation is 196.39 °C, while after 6 months of incubation in saline solution it is in the range from 207.24 °C to 214.91 °C. T_0 of PUF composites with lysozyme before incubation is in the range of 179.95–195.73 °C, while after 6 months of incubation it is in the range of 201.67–221.16 °C (Table 1). Therefore, under the influence of saline solution there is an increase of T_0 for both PUF and PUF composite materials with lysozyme.

T_0 accompanied by a slight weight loss for all samples. For PUF before incubation the weight loss is 0.62 %, after 6 months of incubation in saline solution it is 0.50–1.15 %. For PUF composites with lysozyme the weight loss before incubation is 0.70–1.08 %, after 6 months it is 0.52-1.12 % and depends on the period of incubation in the model medium. So, after incubation of PUF with lysozyme for up to 1 month an increase in weight loss is observed. In the following terms there is a decrease in weight loss. After 6 months of incubation the percentage of weight loss of samples with lysozyme became less than it was before incubation.

The temperature of maximum decomposition rate (T_{max}) after incubation in saline solution for both PUF and PUF composite materials with lysozyme also increases. For PUF T_{max} before incubation is 300.89 °C, after incubation it is 329.30-333.01 °C. For PUF composites with lysozyme T_{max} before incubation is 299.76–310.72 °C, after incubation it is in the range from 326.14 to 342.31 °C (Table 1).

According to DSC, the T_g of the 2nd heating procedure for PUF before incubation in saline solution is minus 49.20 °C, while after 6 months of

Table 2. **Thermophysical properties of composites after incubation in saline solution**

Samples	Periods of incubation	T_g, °C		ΔC_p, J/(g·°C)	
		1st heating procedure	2nd heating procedure	1st heating procedure	2nd heating procedure
PUF	control	-47,10	-49,20	0,2005	0,2135
	2 weeks	-47,57	-49,23	0,2666	0,2787
	1 month	-46,30	-47,63	0,2480	0,2711
	3 months	-47,18	-47,53	0,2561	0,2494
	6 months	-45,58	-46,68	0,2875	0,3096
PUF+lysozyme (1 wt. %)	control	-50,44	-49,48	0,2502	0,2669
	2 weeks	-47,09	-48,48	0,2533	0,2721
	1 month	-48,31	-49,28	0,2652	0,2790
	3 months	-47,67	-48,27	0,2425	0,2802
	6 months	-46,92	-46,73	0,2422	0,2444
PUF+lysozyme (3 wt. %)	control	-48,39	-49,23	0,2807	0,2894
	2 weeks	-47,81	-48,56	0,2466	0,2681
	1 month	-48,21	-48,79	0,2482	0,2683
	3 months	-46,81	-47,81	0,2521	0,2650
	6 months	-45,70	-47,80	0,2266	0,2558
PUF+lysozyme (5 wt. %)	control	-48,30	-49,86	0,2104	0,2385
	2 weeks	-47,85	-48,90	0,2300	0,2525
	1 month	-46,79	-48,02	0,2315	0,2546
	3 months	-48,33	-49,23	0,2531	0,2664
	6 months	-47,91	-48,11	0,2451	0,2534

Table 3. **Spectrophotometric study of lysozyme solutions**

Solution concentrations lysozyme (*C*), %	Wavelength (λ), nm	Optical density ($D_{avg.}$)
0,0050	281,2	0,1230
0,0125	281,0	0,2981
0,0250	281,2	0,5953
0,0350	281,4	0,8322
0,0500	281,5	1,1850
0,0600	280,8	1,4210
0,0700	280,7	1,6580

incubation it is in the range from minus 46.68 °C to minus 49.23 °C. T_g for PUF composite materials with lysozyme before incubation is in the range from minus 49.23 °C to minus 49.86 °C, after 6 months of incubation it is in the range from minus 46.73 °C to minus 49.28 °C (Table 2). Thus, under the influence of saline solution there is an increase in T_g of PUF and PUF composites with lysozyme.

The value of ΔC_p at the second heating procedure for PUF before incubation is 0.2135, while after 6 months of incubation is in the range of 0.2494–0.3096. The value of ΔC_p for PUF composites with lysozyme before incubation is 0.2385–0.2894, after 6 months of incubation it is 0.2444–0.2802 (Table 2). Therefore the value of ΔC_p for PPU after incubation in the model medium increased. The presence of lysozyme affects the value of ΔC_p. After 6 months of incubation in saline solution for PUF composite materials with lysozyme filled in the amount of 1 and 3 wt. % there is a decrease of ΔC_p, whereas for PUF composites containing 5 wt. % of enzyme there is an increase compared to control. Therefore, after incubation there is an increase in ΔC_p for unfilled samples and a decrease in ΔC_p for PUF containing lysozyme. The resulting changes indicate the redistribution of hydrogen bonds under the influence of saline solution and due to the release of lysozyme. The difference is the increase of ΔC_p for PUF filled with lysozyme in the amount of 5 wt. %, which can be due to an increase in the segmental mobility of the macrochain as a result of enzyme release in large amount (compared to 1 and 3 wt. %) and as a consequence the redistribution of hydrogen bonds different from other filled samples (which is consistent with data infrared spectroscopy).

The value of ΔC_p for composite materials with lysozyme depends on the period of their incubation in saline solution. Up to 3 months there is an increase in ΔC_p, further period there is a decrease.

Therefore, according to DSC the presence of lysozyme affects the thermophysical properties of materials *in vitro*. After incubation in saline solution there is an increase in T_g and ΔC_p at the glass-transition (for PUF and PUF with lysozyme in the amount of 5 wt. %), an increase in T_g and decrease in ΔC_p (for PUF of composites with lysozyme in the amount of 1 and 3 wt. %), which indicates the redistribution of hydrogen bonds under the influence of saline solution and due to the release of lysozyme. According to TGA after incubation in saline solution there is an increase in T_0 and T_{max} for both PUF and PUF composite materials with lysozyme. Thus, PUF composites with lysozyme *in vitro* are heat-resistant materials.

Obtained results of IR spectroscopic and thermophysical tests allowed concluding that the release of lysozyme from the polymer matrix is probable. However, quantitative proofs are necessary for polymers of medical appointment. Therefore, the study of its release was carried out by spectrophotometric method.

PUF composite materials with lysozyme of various concentrations (1, 3 and 5 wt. %) were objects of researches of the dynamics of lysozyme release from polymer matrix.

The value of optical density of lysozyme solutions (prepared to create a calibration graph) at the maximum of the band at wavelength λ = (281±0.5) nm are presented in Table. 3.

The calibration graph of dependence of the optical density on concentration of lysozyme solutions is a straight line passing through the origin of coordinates. Thus, the possibility of quantitative analysis of lysozyme release by this method is confirmed.

Table 4. **The study results of the dynamics of lysozyme release**

Period of study	Optical density of solution, ($D_{avg.}$)	Volume / dilution of extract, V, ml / times	Concentration of lysozyme, C, %	The amount of released lysozyme		
				from the moment of the previous definition, m, g	from the beginning of the study	
					m, g	%
PUF+lysozyme (1 wt. %)						
30 minutes	1,2827	10 / –	0,0543	0,00543	0,00543	27,15
6 hours	0,3638	10 / –	0,0154	0,00154	0,00697	34,85
1 day	0,1795	10 / –	0,0076	0,00076	0,00773	38,65
2 days	0,1039	10 / –	0,0044	0,00044	0,00817	40,85
3 days	0,0685	10 / –	0,0028	0,00028	0,00845	42,25
4 days	0,0472	10 / –	0,0022	0,00022	0,00867	43,35
5 days	0,0260	10 / –	0,0010	0,00010	0,00877	43,85
PUF+lysozyme (3 wt. %)						
30 minutes	4,2309	10 / 3	0,0597	0,01791	0,01791	29,85
6 hours	1,2686	10 / –	0,0537	0,00537	0,02328	38,80
1 day	0,7276	10 / –	0,0308	0,00308	0,02636	43,93
2 days	0,3945	10 / –	0,0167	0,00167	0,02803	46,72
3 days	0,2315	10 / –	0,0098	0,00098	0,02901	48,35
4 days	0,1441	10 / –	0,0061	0,00061	0,02962	49,37
5 days	0,0780	10 / –	0,0033	0,00033	0,02995	49,92
PUF+lysozyme (5 wt. %)						
30 minutes	9,3973	10 / 6	0,0663	0,03978	0,03978	39,78
6 hours	1,9985	10 / 2	0,0423	0,00846	0,04824	48,24
1 day	1,3300	10 / –	0,0563	0,00563	0,05387	53,87
2 days	0,8433	10 / –	0,0357	0,00357	0,05744	57,44
3 days	0,4229	10 / –	0,0179	0,00179	0,05923	59,23
4 days	0,3803	10 / –	0,0161	0,00161	0,06084	60,84
5 days	0,2669	10 / –	0,0113	0,00113	0,06197	61,97

The results of measurements and calculations of lysozyme release from polymer samples are presented in Table. 4.

According to table 4, the release of lysozyme from the PUF matrix occurs in the range from 43.85 to 61.97 % from the total amount of introduced drug in 5 days. For samples of composite materials filled with lysozyme in the amount of 5 wt. % there is a maximum enzyme release, which is 61.97 % in 5 days.

Graphically the results of the dynamics of release are presented in Figure 5.

It is known that for the treatment of purulent wounds, burns and frostbite wipes soaked in 0.05 % lysozyme solution are applied [15]. The obtained results (Table 4) indicate that the concentration of

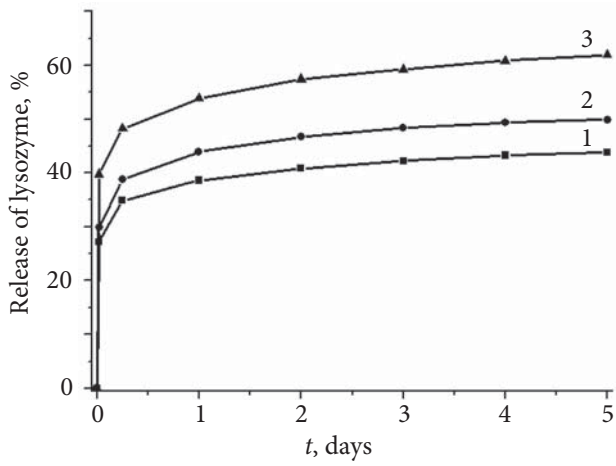

Fig. 5. The dynamics of lysozyme release from composite materials filled with enzyme in the amount of 1 wt. % (*1*), 3 wt. % (*2*), and 5 wt. % (*3*)

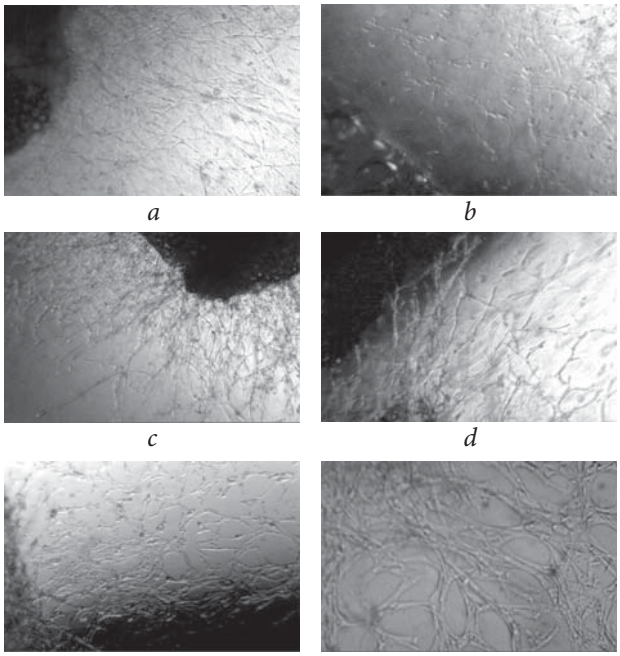

Fig. 6. The Growth of fibroblasts culture on the 7th day of cultivation in a flacon with control (*a*), PUF (*b*), PUF+lysozyme (1 wt. %) (*c*), PUF+lysozyme (3 wt. %) (*d*), PUF+lysozyme (5 wt. %) (*e*, *f*)

released lysozyme is sufficient for the exhibit of antimicrobial activity.

Data on intramuscular use of lysozyme in the amount of 0.15 g are also known [15]. Thus, the amount of released enzyme (Table 4) is not excessive, so it will not have a toxic effect. In addition, the absence of toxic effects of the drug was proved by the results of tissue culture studies, which indicate the biocompatibility of PUF composites with lysozyme.

Therefore, according to the study results of the dynamics of lysozyme release it was found that PUF composite materials are capable to the prolonged

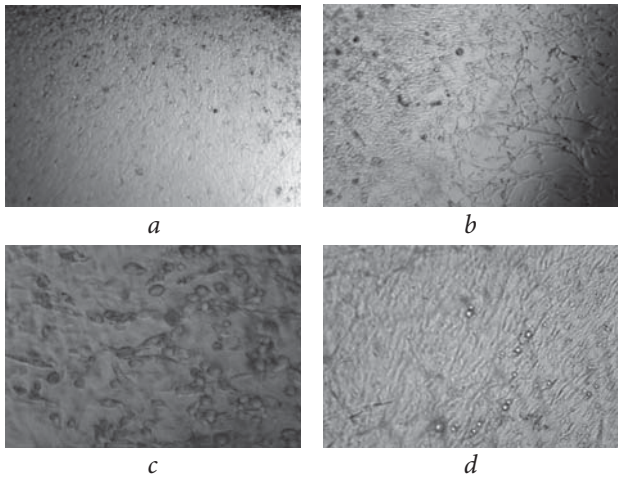

Fig. 7. The beginning of degenerative changes of fibroblasts on the 10th day of cultivation in flacon with control (*a*), PUF (*b*), PUF+lysozyme (1 wt. %) (*c*), PUF+lysozyme (5 wt. %) (*d*)

release of lysozyme. It is possible to obtain a coating for medicine with different amounts of released drug depending on requirements by varying the lysozyme content in the PUF.

To study the biocompatibility of polymer materials and the influence of prolonged form of lysozyme on the growth and development of the culture of fibroblastic elements, researches of PUF and PUF composite materials with various concentrations of lysozyme (1, 3 and 5 wt. %) were conducted by tissue culture method. Flacons with explants of subcutaneous fatty tissue without the addition of polymer samples were used as control. The study of growth and development of cellular elements of the subcutaneous fatty tissue of white rats after 3, 7, 10 and 14 days of cultivation were carried out.

According to the obtained results, the migration of fibroblastic elements in flacons with PUF and

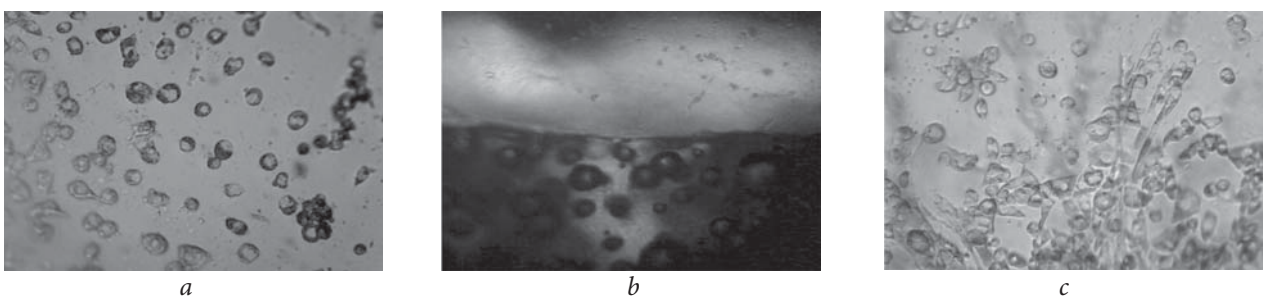

Fig. 8. The degenerative changes of fibroblasts culture on the 14th day of cultivation in flacon with control (*a*), PUF (*b*), PUF+lysozyme (3 wt. %) (*c*)

PUF composite materials with lysozyme began on the 3rd day of cultivation, as well as in control flacons. The primary zone was formed by single spindle-shaped cells, and thread-like structures oriented perpendicular to the surface of the explants in most cases. There were also single cells of irregular polygonal form.

On the 7th day of cultivation there was the formation of three growth zones around the explants, as well as the control. It is a compact zone consisting of cells of spindle-shaped and polygonal form, a mesh-like zone consisting of bunches and thread-like structures of cells located in a grid, and zones of single migratory elements (Fig. 6).

It should be noted that the growth of fibroblastic elements around the PUF composites with lysozyme is much better than around the sample without lysozyme. There is a compaction of the zone of migratory fibroblasts due to the formation of new spindle-shaped cells and there are cells that migrate into the polymer pores (Fig. 6 *c, d, e*).

In flacon with PUF without lysozyme (Fig. 6 *b*) the migrating cells are located at a distance from the sample; the shape of the cells varies from spindle-shaped to polygonal.

On the 10th day of cultivation tissue-like growth around explants was observed. In Carrel flacons with composite materials with lysozyme the areas of growth zones of cell were significantly wider compared with control and PUF without lysozyme. The growth zone of single migrating cells expanded and was characterized by a large variety of cell forms. In the compact zone there were signs of degenerative changes of individual (Fig. 7).

In flacon with PUF without lysozyme (Fig. 7 b) on the 10th day of cultivation an increase of cellular elements in most polygonal forms is observed. But unlike control and polymer samples with lysozyme degenerative changes are detected in compact and mesh-like zones.

On the 14th day of cultivation the cell population entered the phase of degeneration, which manifests itself in considerable vacuolization of the cytoplasm and its granular rebirth in the cells in both control and experimental flacons (Fig. 8).

In flacons with samples of PUF with lysozyme (Fig. 8 *c*) on the 14th day the amount of degenerate cells continues to increase. But despite the degeneration of culture new spindle-like and polygonal cells are present in zone of migratory fibroblasts.

Thus, studies of tissue culture showed that the dynamics and character of growth of cellular elements in the experimental flacons did not differ significantly from control cultures. But for composite materials with lysozyme more active growth of fibroblastic elements than in the control was observed. And on the 14th day of cultivation the process of cell degeneration slowed down.

It allows us to conclude that there is no histotoxic effect of the investigated materials on cultured cells. Therefore, the samples can be characterized as biocompatible.

Conclusions

Thus, studies of the ability to biodegradation of PUF and PUF composite materials with lysozyme under the influence of a model biological medium for 2 weeks, 1, 3 and 6 months *in vitro* were carried out. After incubation of PUF and PUF composites with lysozyme in saline solution there are processes of biodegradation, which are confirmed by a decrease in the intensity of the absorption band $\nu_{C=O}$. Along with biodegradation there is a redistribution of hydrogen bonds of NH and CO groups of the surface layer of the samples. A change of ΔC_p at the glass-transition indicates the redistribution of hydrogen bonds under the influence of saline solution and due to the release of lysozyme. Also under the influence of model environment there is an increase in heat resistance of the studied materials. It is established that PUF composite materials are capable to the prolonged release of lysozyme for 5 days. According to the test results the studied materials are biocompatible. For composite materials with lysozyme more active growth of fibroblastic elements than in the control was observed. And on the 14th day of cultivation the process of cell degeneration slowed down.

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ДОСЛІДЖЕННЯ БІОДЕГРАДАЦІЇ ТА ВЛАСТИВОСТЕЙ ПІНОПОЛІУРЕТАНОВИХ КОМПОЗИЦІЙНИХ МАТЕРІАЛІВ З ЛІЗОЦИМОМ ЗА УМОВ *IN VITRO*

Досліджено здатність до біодеградації пінополіуретанових композиційних матеріалів з лізоцимом під впливом фізіологічного розчину протягом 2 тижнів, 1, 3 і 6 місяців інкубації методом ІЧ-спектроскопії, ДСК, ТГА. За результатами ІЧ-спектроскопії під впливом модельного середовища відбуваються процеси біодеградації, про що свідчить зниження інтенсивності смуги поглинання ($\nu_{C=O}$) з одночасним перерозподілом водневих зв'язків NH- і CO-груп полімерної матриці. За даними ДСК після інкубації у фізіологічному розчині спостерігали підвищення T_c і ΔC_p при склуванні (для пінополіуретанів та композитів з лізоцимом у кількості 5 % мас.), підвищення T_c та зниження ΔC_p (для композитів з лізоцимом у кількості 1 і 3 % мас.), що свідчить про перерозподіл водневих зв'язків під впливом фізіологічного розчину та внаслідок вивільнення лізоциму. Методом ТГА встановлено, що після інкубації у фізіологічному розчині спостерігається підвищення $T_{\text{поч. розкл.}}$ та $T_{\text{макс. шв. розкл.}}$ як для пінополіуретанів так і для композиційних матеріалів з лізоцимом. Отже, композити з лізоцимом за умов *in vitro* залишаються термостійкими матеріалами. За результатами досліджень динаміки вивільнення лізоциму, композити здатні до пролонгованого вивільнення ферменту протягом 5 діб, кількість якого варіює залежно від вмісту лізоциму (43,85–61,97 % від загальної кількості введеного препарату) та є достатньою для прояву антимікробної активності. Методом культури тканин встановлено, що досліджувані матеріали є біосумісними. Для пінополіуретанових композиційних матеріалів з лізоцимом спостерігали більш активний ріст фібробластичних елементів, ніж у контролі й ППУ та сповільнення процесу дегенерації клітин. Отримані результати свідчать, що пінополіуретанові композиційні матеріали з лізоцимом є перспективними матеріалами, які завдяки наявності ферменту будуть мати антимікробну дію та можуть бути використані в медичній практиці як полімерні композити для лікування ран та опіків.

Ключові слова: пінополіуретан, композиційний матеріал, лізоцим, фізіологічний розчин, біодеградація.