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Bioactive Composite Material Based on Polyurethane with Isoniazid

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Abstract: An urgent task is the development of new implantation materials with a prolonged medicinal effect for the treatment of the extrapulmonary form of tuberculosis. The purpose of this investigation was to obtain an implantation material based on mesh polyurethane with the anti-tuberculosis drug isoniazid, to study the drug release of isoniazid from the polymer, to study the biodegradation of such materials, as well as to analyze cellular reactions to their implantation in the body of experimental animals. According to the results of the study of the dynamics of the release of isoniazid from the structure of mesh polyurethane, it was shown that a little more than 44% of isoniazid was released from the polymer matrix during the 389 days of the study. At the same time, in the first 3 days, a shock dose of the anti-tuberculosis drug was released – more than 18%, which can be important for reducing the pathological process at the location of the implant. When studying the biodegradation of the obtained materials, it was established that there was a redistribution of supramolecular structures, in which the type of these structures remained unchanged, but the size of the globular particles changed. In the model environments, the organization of large, possibly hydrophobic, destruction fragments into large globular formations probably took place, which was reflected in the increase of some physical and mechanical indicators. During implantation, the polymer material was subjected to cellular resorption due to the activity of phagocytic fragments, as well as mechanical loads, which contributed to the removal of destruction products from the mass of the polymer and led to a decrease in the main physical and mechanical parameters. According to the results of the implantation test by histological methods, it was established that around the samples of polyurethane with isoniazid a rather pronounced and long-lasting (up to 1 month) reaction of the type of aseptic inflammation was observed. At the same time, the cellular composition of the connective tissue capsule indicated a fairly good tolerability of the polymer material with prolonged release of isoniazid. It is shown that the obtained bioactive composite material based on polyurethane with isoniazid can be a promising implant material for the treatment of bone and joint tuberculosis.

Keywords: Composite Material, Polyurethane, Isoniazid, Drug Release, Biodegradation, Implantation

1. Introduction

The use of biodegradable polymers as platforms for depositing various medicinal products is a relevant and rapidly developing direction. Prolonged drug systems are able to increase the bioavailability of drugs, ensuring their directed transport to the field of the pathological process [1-3]. The development of prolonged drug delivery systems covers almost all areas of medicine [4-8]. Special attention of such prolonged systems is focused on the selection of materials used as a base, which are subject to a number of requirements, including biocompatibility and harmlessness, a set of physical and mechanical properties, ability to biodegrade, bioavailability of drugs, etc. [9, 10].

It is known that porous polyurethanes with a highly branched surface are capable of intensive biodegradation processes with subsequent replacement by tissue regenerate [11, 12]. Polyurethanes have been successfully used for a long time in the creation of biologically active implants for endoprosthesis of bone defects in medicine [13].

Currently, the development of new materials for the

treatment of the extrapulmonary form of tuberculosis is an urgent issue. It is known that the basis for the treatment of bone and joint tuberculosis is surgical removal of the affected bones and filling of the formed defects with transplants or implants, followed by systemic treatment with anti-tuberculosis drugs. The high resistance factor of tuberculosis mycobacteria can significantly reduce the effectiveness of treatment and does not always allow to achieve therapeutically sufficient concentrations of drugs in the focus of bone tissue damage.

To solve this problem, the ideal form of treatment of bone tissue defects in tuberculosis can be the replacement of the affected defect with a polymer implant with a prolonged medicinal effect.

Considering the above, the purpose of this work was to obtain an implantation material based on mesh polyurethane with the anti-tuberculosis drug isoniazid, to study the drug release of isoniazid from the polymer, to study the biodegradation of such materials, as well as to analyze the reactions of cells to their implantation in the body of experimental animals.

2. Experimental

2.1. Materials

The product for obtaining polyurethane compositions was oligoether urethane diisocyanate obtained by the interaction of polyoxypropylene glycol with toluene diisocyanate. In order to give the polymer material biological activity, the antituberculosis drug isoniazid was immobilized. Polyurethane samples without isoniazid were used as control.

2.2. Drug Release of Isoniazid

Drug release of isoniazid from polymer samples into the model environment (saline solution) was studied by the spectrophotometric method. For this, control samples and polyurethane samples with 4 wt. % of isoniazid was placed in beakers with polished corks, 20 ml of distilled water were added and incubated in a thermostat at a temperature of 37±1°C. Periodically, the obtained solutions were drained and the optical density was recorded. A solution of an extract from polyurethane samples without isoniazid was used as a control.

A series of aqueous isoniazid solutions with concentrations of: 0.005; 0.01; 0.015; 0.02; 0.025 mg/ml was prepared to verify the implementation of Beer's law and to construct a calibration graph of the dependence of the optical density of the solutions on their concentration. The calibration graph was a straight line in the entire range of studied concentrations.

The amount of isoniazid that was released into the saline solution from the studied compositions was determined by the formulas 1 and 2:

$$Isoniazid\ (mg) = C \cdot V \cdot n \qquad (1)$$

$$Isoniazid\ (\%) = \frac{isoniazid\ (mg)}{m} \cdot 100\ \% \qquad (2)$$

where:

C – the concentration of isoniazid in the solution, determined from the calibration graph, mg/ml;

V – the volume of the saline solution in which washing was carried out, ml;

n – the number of dilutions of the solution during the research;

m – mass of isoniazid introduced into the polymer, mg.

2.3. Methods to Determine the Biodegradation

The biodegradation of the obtained polymer materials was evaluated by the change in the main physical and mechanical parameters (tensile strength, relative elongation) after their stay in model environments and in the body of experimental animals. The research was carried out with the help of a modernized bursting machine 2166 R-5. The error of measuring the strength limit (σ) did not exceed ± 0.253 MPa, and the relative elongation (ε) ± 0.13%. Polymer samples in the form of strips measuring 5x50x0.4 mm were placed in sterile test tubes, filled with 30 ml of model medium, sealed and kept in a thermostat at a temperature of (37 ± 1) °C for 30, 90, and 180 days, periodically replacing the solutions of model mediums with freshly prepared. The following were chosen as model environments:

1) biological environment 199, which chemically simulates blood plasma;
2) 0.01% buffer solution of the proteolytic enzyme – chymotrypsin in a 0.1 M solution of Na_2HPO_4 and KH_2PO_4 at pH 8.0;
3) Fenton's reagent – a solution of highly diluted hydrogen peroxide (H_2O_2) and ferrous salt ($FeSO_4$ x $7H_2O$), which simulates the conditions of the inflammatory process when the material comes into contact with blood.

In parallel, the implantation of polymer strips measuring 5x50x0.4 mm into the body of laboratory rats was carried out. After 30, 90 and 180 days, the animals were removed from the experiment, polymer samples were taken, washed with distilled water and dried at room temperature.

2.4. Animal Studies

2.4.1. Implantation Test

The study of the morphological features of the tissues surrounding the implanted samples was carried out on 18 white male laboratory rats (weight 220-260 g), divided into 2 groups: 1) animals implanted with polymer samples without isoniazid, 2) animals implanted with polymeric samples with immobilized isoniazid. All manipulations with experimental animals were carried out under anesthesia, as well as in compliance with the principles set forth in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes [14]. Model operations on laboratory animals were performed in aseptic conditions. After treatment of the operative field, porous polymer samples measuring 10x5x5 mm were placed subcutally in the area of the interscapular space without additional fixation, in order to exclude the influence of the suture material on the wound process. Such an area is characterized by low mobility and

inaccessibility for the animal itself, which minimizes the risk of its intervention in the experimental process. Animals were removed from the experiment 7, 14, 30 and 90 days after the operation by overdose with chloroform.

2.4.2. Histological Evaluation

The test material (a polymer sample with surrounding connective tissue) was fixed in a 10% formalin solution and embedded in paraffin after histological processing according to the standard method [15]. Paraffin sections made with a microtome, 10-15 μm thick, were stained with hematoxylin and eosin. Assessment of biocompatibility of implanted composite materials was carried out by analyzing histological preparations using light microscopy.

3. Results and Discussion

3.1. Study of Drug Release of Isoniazid from Polyurethane Compositions in Vitro

It was of great interest to evaluate the release process of isoniazid deposited in polymer samples into the model medium using the spectrophotometric method. The UV spectrum of isoniazid has an intense absorption maximum at a wavelength of $\lambda = 262 \pm 0.1$ nm. The UV absorption spectra of the studied isoniazid solutions before and after the exposure of the polymer samples under the specified conditions were identical to the spectrum of isoniazid itself, i.e., the medicinal substance did not undergo changes under the experimental conditions.

It was shown that the polymer sample with isoniazid was characterized by gradual and prolonged release of isoniazid from mesh polyurethane over a long period of time. The total amount of released isoniazid was slightly more than 44%. At the same time, in the first 3 days, a shock dose of the antituberculosis drug was released - more than 18% (Figure 1), which can be important for reducing the pathological process at the place of placement of the implant (subject to positive results of the study on the anti-tuberculosis activity of the obtained polymer compositions).

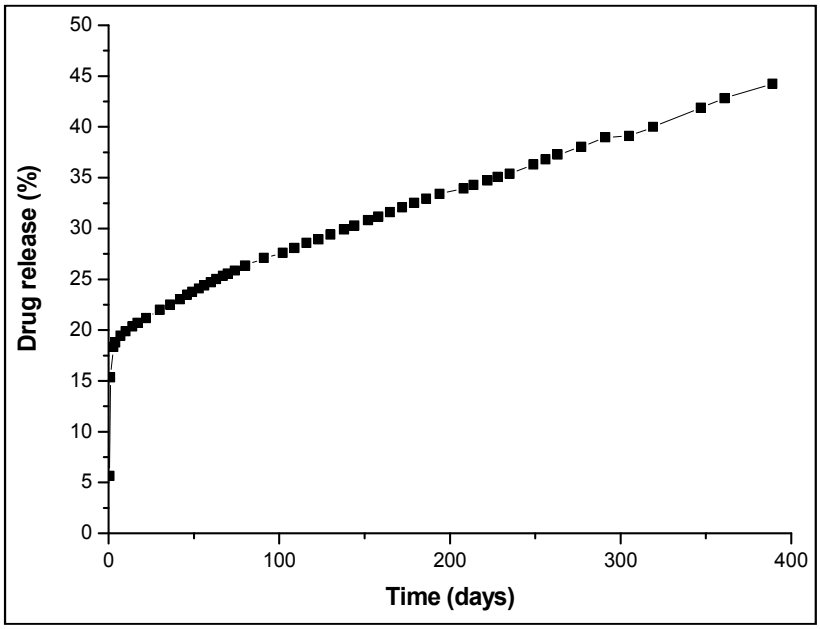


Figure 1. *Drug release of isoniazid from polymer samples.*

3.2. Study of Biodegradation of Polymer Samples

The introduction of isoniazid into the structure of polyurethane led to a decrease in the main physical and mechanical parameters. When polyurethane samples were incubated in model media, biological medium 199, which chemically simulates blood plasma, and Fenton's reagent, which simulates the conditions of the inflammatory process when the material comes into contact with blood, had the strongest hydrolytic effect. When polymer samples were incubated in these media for 180 days, a gradual decrease in the relative elongation index was observed. For polyurethane samples with isoniazid, an increase in breaking strength indicators was observed, as well as a decrease in relative elongation for samples that were in medium 199 and Fenton's reagent. The results of physical and mechanical studies of polymer samples after being in model environments and after their implantation in the body of experimental animals for different periods are presented in the table 1. Incubation of polyurethane samples with isoniazid in a chymotrypsin buffer solution, on the contrary, led to an increase in the breaking strength index and the stability of the relative elongation index. That is, chymotrypsin had some inhibitory effect on the destruction of the polymer, which is in good agreement with previously obtained data [16].

Table 1. *Physico-mechanical properties of polymer samples after their incubation in model environments.*

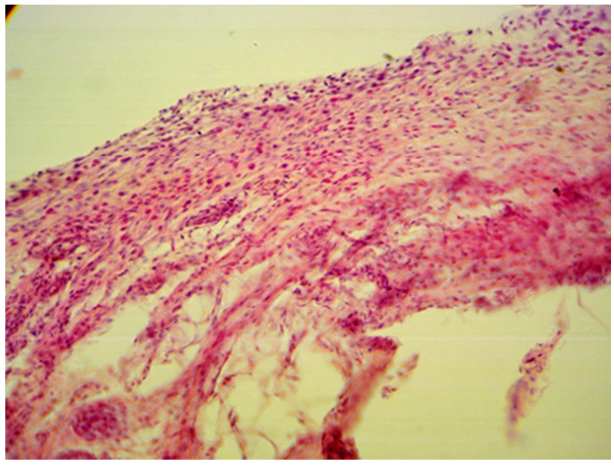
Samples	Control, before incubation		Periods of incubation, days					
			30		90		180	
	σ, МПа	ε, %	σ, МПа	ε, %	σ, МПа	ε, %	σ, МПа	ε, %
Polymer samples without isoniazid								
Biological medium 199	10,08	1796	10,64	1287	12,83	1172	10,85	1088
Chymotrypsin	10,08	1796	11,54	1366	12,3	1554	10,88	1698
Fenton's reagent	10,08	1796	11,47	1369	13,34	1135	10,96	1294
Polymer samples with isoniazid								
Biological medium 199	5,23	771	7,41	787	6,07	612	5,53	641
Chymotrypsin	5,23	771	4,49	616	7,56	896	5,67	763
Fenton's reagent	5,23	771	6,18	692	6,17	533	5,83	470

The study of biodegradation of the obtained materials after implantation in the body of experimental animals revealed that the relative elongation index decreased in almost all samples, and the breaking strength index slightly increased, which can be explained by the redistribution of supramolecular structures in polyurethane samples. Thus, in the process of biodegradation, a redistribution of supramolecular structures occurred in the polymer material, in which the type of these structures remained the same, but the size of the globular particles changed. In the model environments, the organization of large, possibly hydrophobic, destruction fragments into large globular formations probably took place, which was reflected in the increase of some physical and mechanical indicators. During implantation, the polymer material was subjected to cellular resorption due to the activity of phagocytic fragments, as well as mechanical loads, which contributed to the removal of destruction products from the mass of the polymer and led to a decrease in the main physical and mechanical parameters.

3.3. Implantation Test

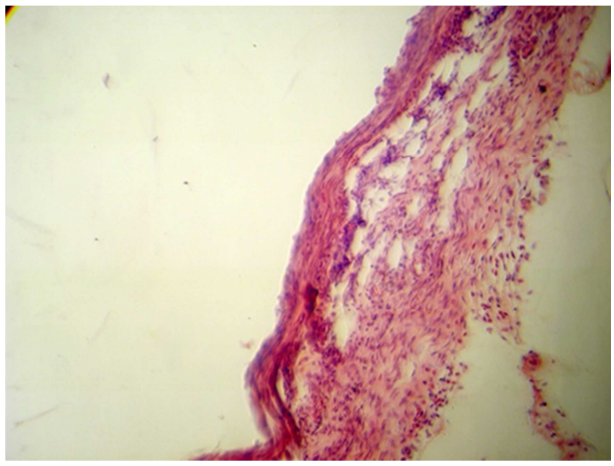
When examining polymer samples by histological methods on the 7th day after surgery, a polyurethane sample without isoniazid was surrounded by immature granulation tissue with a disorderly arrangement of thin immature collagen fibers and few differentiated fibroblasts. Neutrophil, lymphocytic and macrophage infiltration was observed. Blood vessels were mainly represented by arteries and arterioles of various caliber, and were present in large numbers along the entire length of the connective tissue capsule. Mast cells were localized near blood vessels. The connective tissue capsule around the samples of polyurethane with isoniazid was thick, characterized by a different degree of maturity along its entire length. In some areas, immature collagen fibers and poorly differentiated fibroblasts were observed, in others, mature collagen fibers with spindle-shaped fibroblasts oriented along the polymer sample. Marked macrophage, lymphocytic and weak neutrophilic infiltration was noted (Figure 2). Many newly formed blood vessels of various diameters without stasis and thrombosis were observed.

At 14 days postoperatively, the connective tissue capsule around the implanted polyurethane without isoniazid appeared thicker, more mature than at the previous term, with little neutrophilic and marked macrophage infiltration. Mature fibroblasts appeared, actively synthesizing collagen. Blood vessels of various calibers were represented in large numbers. A formed connective tissue capsule was observed around the polyurethane with isoniazid, which, as in the previous term of the study, had a different degree of maturity along its entire length. Although infiltrations should be noted, and the macrophage reaction was pronounced (Figure 3). Blood vessels without stasis and thrombosis were represented in small numbers.



Hematoxylin-eosin staining. ×200

Figure 2. *Round cell infiltration around porous polyurethane with isoniazid on the 7th day of the experiment.*



Hematoxylin-eosin staining. ×200

Figure 3. *Formation of a connective tissue capsule around polyurethane with isoniazid on the 14th day of the experiment.*

30 days after surgery, the connective tissue capsule around the implanted polyurethane without isoniazid was mature and multi-layered. Collagen fibers were stretched along the polymer sample and contained spindle-shaped fibroblasts and fibrocytes. Foci of round cell infiltration were observed in some areas of the capsule. A large number of blood vessels were present in the connective tissue capsule, as in the previous periods of the study. A fairly thin connective tissue capsule was observed around the polyurethane with isoniazid, which was characterized by a different degree of maturity along its entire length. Macrophage reaction was pronounced in separate areas of the capsule. Single blood vessels without elements of stasis and thrombosis were observed.

Thus, by histological methods, it was established that around the polyurethane samples with isoniazid there was a rather pronounced, prolonged (up to 1 month) reaction of the type of aseptic inflammation. At the same time, the cellular composition of the connective tissue capsule indicated a fairly good tolerability of the polymer material with prolonged release of isoniazid. It should be noted that a more pronounced macrophage reaction was observed around the polyurethane samples with isoniazid than around the control samples. Macrophages (cells of mesenchymal origin, capable of actively capturing and digesting particles foreign to the body) were present in large numbers both in the connective tissue capsule itself and in the connective tissue surrounding the polymeric material.

4. Conclusion

As a result of the work, was established that the polymer sample with isoniazid was characterized by prolonged release of isoniazid from mesh polyurethane over a long period of time. The total amount of released isoniazid was slightly more than 44%. At the same time, in the first 3 days, a shock dose of the antituberculosis drug was released – more than 18%, which can be important for reducing the pathological process at the place of placement of the implant. The influence of model environments and the internal environment of the body of experimental animals on the process of biodegradation of bioactive polymers as potential implantation materials was investigated. Immobilization of isoniazid on polyurethane led to the stability of the structure of polymer samples during long-term stay in the body. The process of biodegradation consisted in the reduction of the main physical and mechanical indicators, while no macroscopically visible destruction of the structure of polymer samples was observed. It was established that isoniazid was released from mesh polyurethane for a long time, stimulating active regenerative processes. A gradual growth of the connective tissue deep into the implanted samples was observed with simultaneous reliable fixation of the studied material. It is shown that the bioactive composite material based on polyurethane with isoniazid can be a promising implant material for the treatment of bone and joint tuberculosis and can find wide application in medical practice.

References

[1] Dutta R. C. Drug carriers in pharmaceutical design: Promises and progress. Curr. Pharm. Des. 2007, 13, 761-769. doi: 10.2174/138161207780249119.

[2] Galatenko N. A., Kuliesh D. V., Gritsenko V. P., Narazhayko L. F. Influence of the prolonged form of lysocyme in the composition of polyurethane implant on cells and tissues in vitro and in vivo. Morphologia, 2021, 15 (2), 16-24. doi: https://doi.org/10.26641/1997-9665.2021.2.16-24.

[3] Galatenko N. A., Kulyesh D. V., Maletskyi A. P., Karpenko O. S. Soft-tissue response to synthetic polymer implants made of cross-linked polyurethane and containing a biologically active substance, albucid or dacarbazine, in animals. Journal of Ophthalmology, 2018, № 6, 52-58. http://doi.org/10.31288/oftalmolzh201865258.

[4] Abdelkader H., Fathalla Z., Seyfoddin A., Farahani M., Thrimawithana T., Allahham A., Alani A., Al-Kinani A., Alany R. Polymeric long-acting drug delivery systems (LADDS) for treatment of chronic diseases: Inserts, patches, wafers, and implants, Advanced Drug Delivery Reviews, Volume 177, 2021, 113957, ISSN 0169-409X. https://doi.org/10.1016/j.addr.2021.113957

[5] Domingues Goncalves A, Balestri W, Reinwald Y. Biomedical Implants for Regenerative Therapies. Biomaterials, 2020, Nov 11. http://dx.doi.org/10.5772/intechopen.91295

[6] Kim J. O., Kabanov A. V., Bronich T. K. Polymer micelles with cross-linked polyanion core for delivery of a cationic drug doxorubicin // Journal of Controlled Release, Volume 138, Issue 3, 2009, 197-204. https://doi.org/10.1016/j.jconrel.2009.04.019.

[7] Goforth, R., Salem, A., Zhu, X., Miles, S., & Zhang Immune stimulatory antigen loaded particles combined with depletion of regulatory T-cells induce potent tumor specific immunity in a mouse model of melanoma. // Cancer Immunology. Immunotherapy, 2009, 58 (4), 517-530.

[8] Vasir J. K., Labhasetwar V. Biodegradable nanoparticles for cytosolic delivery of therapeutics. Adv Drug Deliv Rev. 2007 Aug 10; 59 (8): 718-28. doi: 10.1016/j.addr.2007.06.003.

[9] Stewart S. A., Domínguez-Robles J., Donnelly R. F., Larrañeta E. Implantable Polymeric Drug Delivery Devices: Classification, Manufacture, Materials, and Clinical Applications. Polymers. 2018; 10 (12), 1379. https://doi.org/10.3390/polym10121379.

[10] Galatenko N. A., Rozhnova R. A. Biologically active polymeric materials for medicine, Kyiv, Naukova Dumka, 2013, 211 p.

[11] W. Kenneth Ward, Emily P Slobodzian, Kenneth L Tiekotter, Michael D Wood, The effect of microgeometry, implant thickness and polyurethane chemistry on the foreign body response to subcutaneous implants, Biomaterials, Volume 23, Issue 21, 2002, 4185-4192. https://doi.org/10.1016/S0142-9612(02)00160-6.

[12] Galatenko N. A., R. A. Rozhnova, Kuliesh D. V., Denisenko V. D., Maletskyy A. P., Bigun N. M. Rat tissue responses to dacarbazine-containing implants made of cross-linked polyurethane of different densities. Journal of Ophthalmology, 2022, 4 (507), 40-48. http://doi.org/10.31288/oftalmolzh202244048.

[13] Galatenko N. A., Malanchuk V. O., Rozhnova R. A.. Astapenko O. O., Rudenchyk T. V. Biologically active polyurethane compositions for endoprosthesis of bone tissue, Kyiv, Naukova dumka, 2020, 230 p. ISBN 978-966-00-1784-9.

[14] European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Council of Europe, Strasbourg, 1986, 53 p.

[15] Bagrij M. M., Dibrova V. A., Popadinecz O. G., Grishhuk M. I. [Metodiki morfologichnikh doslidzhen: monografiya, Vinniczya, Nova Kniga, 2016, 328 p.

[16] Lipatova T. E., Phakadze G. A. Polymers in endoprosthesis, Kyiv, Naukova dumka, 1983, 160 p.