D.V. Kuliesh [1], N.A. Galatenko [1], R.A. Rozhnova [1], L.F. Narazhayko [1], S.O. Prymushko [1], D.V. Tarnavskyi [2]

[1] Institute of Macromolecular Chemistry of the National Academy of Sciences of Ukraine
[2] National University of Life and Environmental Sciences of Ukraine
Kyiv, Ukraine

Надійшла: 19.10.2024
Прийнята: 25.11.2024

DOI: https://doi.org/10.26641/1997-9665.2024.4.52-60

UDC 678.664, 57.084.1, 57.085.1

STUDY OF CYTOTOXICITY AND ANALYSIS OF CELLULAR REACTIONS TO THE IMPLANTATION OF POLYURETHANEUREAS WITH IFOSFAMIDE

Kuliesh D.V. ✉, Galatenko N.A., Rozhnova R.A., Narazhayko L.F., Prymushko S.O., Tarnavskyi D.V. Study of cytotoxicity and analysis of cellular reactions to the implantation of polyurethaneureas with ifosfamide.
Institute of Macromolecular Chemistry of the National Academy of Sciences of Ukraine, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine.

ABSTRACT. Background. Society's need for modern implants and tissue engineering products is steadily growing due to the increase in the number of surgical interventions caused by various diseases, injuries and age-related changes. An increasing number of materials based on synthetic polymers, intended for implantation in the tissues of a living organism, are becoming widely used in medical practice. **The aim** of this work was to study the cytotoxicity and analysis of cellular reactions to the implantation of polyurethaneureas with immobilized ifosfamide, synthesized using 4,4'-diaminodiphenylmethane and 3,6-dioxyoctane-1,8-diamine as macrochain extenders by the method of tissue culture of fibroblasts and by means of the implantation test. **Results.** Using the method of tissue culture of fibroblast cells, it was shown that the dynamics and nature of the growth of cellular elements during cultivation in experimental vials with composite materials based on polyurethaneureas, including ifosfamide, did not differ significantly from control cultures, which allows us to conclude that the extracts from the studied cells do not have a cytotoxic effect materials on cultured cells. It was established that the implantation of test samples of polyurethaneureas in the body of experimental animals led to the development of cellular reactions typical for aseptic inflammation, without signs of acute inflammatory and other reactive processes. Histological studies have shown that the studied samples are biocompatible with the tissues of experimental animals. Implantation of polymer samples with ifosfamide led to the development of intense cellular reactions in the area where the implants were placed. The content of ifosfamide in the polymer matrix probably affected the proliferation of cellular elements in implantion site, as a result of which regenerative processes were inhibited in the early stages of the study. **Conclusion.** Based on the results of the research, it was shown that the developed composite materials based on polyurethaneureas with ifosfamide do not have a pronounced cytotoxic effect, are biocompatible and promising materials for use in medical practice during antitumor therapy.
Key words: polyurethaneureas, ifosfamide, fibroblast tissue culture, implantation test, biocompatibility.

Citation:
Kuliesh DV, Galatenko NA, Rozhnova RA, Narazhayko LF, Prymushko SO, Tarnavskyi DV. Study of cytotoxicity and analysis of cellular reactions to the implantation of polyurethaneureas with ifosfamide. Morphologia. 2024;18(4):52-60.
DOI: https://doi.org/10.26641/1997-9665.2024.4.52-60

Kuliesh D.V. 0000-0002-0484-7853; Galatenko N.A. 0000-0002-5961-5750;
Rozhnova R.A. 0000-0003-3284-3435; Prymushko S.O. 0000-0002-3623-1068;
Narazhayko L.F. 0000-0001-7031-9998; Tarnavskyi D.V. 0000-0002-3300-9269
✉ d_kulesh@ukr.net

Background

The need of society for modern implants and tissue engineering products is steadily increasing due to the growing number of surgical interventions caused by various diseases, injuries, and age-related changes. The development, design and modification of implantation materials and products based on metals and al-

loys, bioceramics and bioglasses, natural and synthetic polymers lead to an exponential growth of functional replacements for damaged tissues [1-4].

An increasing number of materials based on synthetic polymers intended for implantation into living tissues are being widely used in medical practice [5-7]. The growing interest in polymer implantation materials is due to their unique mechanical and physicochemical properties, non-toxicity, and biocompatibility. Thanks to this, polymer materials can be "adapted" for various purposes in practical medicine, and the ability to introduce modifications into the structure of synthesized polymers allows the creation of new biocompatible composite materials with the necessary set of specified properties and characteristics. An undeniable advantage of polymer materials for creating modern implants is that polymers can be functionalized with various medicinal drugs and biologically active substances, which significantly expands their application range and substantially increases their bioavailability and, accordingly, their effectiveness [8]. Such implants can become powerful therapeutic agents for diseases that are not amenable to effective treatment, and their biological activity, providing continuous therapy, can exert a targeted effect on the source of the pathological process at the application site for a defined period, sparing the tissues and organs of the body from many side effects of such substances.

One of the main problems in the application of polymer materials in medical practice as implants is their interaction with the surrounding tissues of the body. Studying the cellular reactions at the implant-tissue interface is an extremely important issue for understanding the degree of biocompatibility and effectiveness of implanted materials, as well as ways to minimize the possible negative impact of implanted materials throughout their functional lifespan. It is known that the immune system, which is formed by a complex network of cells, induces an inflammatory response to tissue damage during the implantation procedure, the presence of a foreign body in living tissue or bacterial infection. As a result, varying degrees of cellular reactions are observed, leading to subsequent fibrosis and isolation of the implants, which can impair the diffusion of the drug into the surrounding tissues [9-11], sometimes even to the complete loss of their functionality. Therefore, the search for new and modification of existing polymer implantation materials aimed at minimizing the cellular response of tissues while simultaneously increasing the effectiveness of the use of implanted materials/products without losing their functionality is a relevant task.

One of the types of polymer materials used in medicine as implants are polyurethanes containing biologically active substances and medicinal drugs in their composition [12], which, under the influence of the internal environment of the body, are released into the surrounding tissues, exerting a therapeutic effect at the implantation site and can influence the the growth and development of cellular elements in the connective tissue surrounding the implant and the biocompatibility of the implantation material as a whole. Therefore, studying the effect of prolonged forms of biologically active substances and medicinal drugs in the composition of polymer implants on the growth of cellular elements under *in vitro* conditions is a necessary stage of research, which will allow predicting the effectiveness of the developed materials and assessing their biocompatibility for further implementation in medical practice.

There are known works aimed at developing polymer implantation materials with immobilized antitumor agents, in particular ifosfamide, as means of adjuvant therapy in the treatment of pathological neoplasms [13-15].

Ifosfamide (IFO) is a cytostatic medicinal drug from the group of oxazaphosphorines, which is used for the comprehensive treatment of malignant neoplasms (lung cancer, ovarian cancer, breast cancer, cervical cancer, soft tissue sarcomas). In the liver, IFO is activated to the active metabolite ifosfamide mustard, whose antitumor effect is due to alkylation of nucleophilic centers, disruption of DNA replication and RNA transcription, leading to impaired nucleic acid function [16].

Considering that the prolonged form of IFO in the composition of polymer implants can influence the growth and development of cellular elements under *in vivo* conditions, studies of its cytotoxic effect using the express method of toxicological evaluation of fibroblast tissue culture with subsequent assessment of its biocompatibility upon implantation in experimental animals are relevant.

The aim of this work was to study the cytotoxicity and analyze the cellular reactions to the implantation of polyurethaneureas with immobilized ifosfamide, synthesized using 4,4'-diaminodiphenylmethane (DADPh) and 3,6-dioxyoctane-1,8-diamine (DA2) as chain extenders, using fibroblast tissue culture methods and histological techniques.

Materials and Methods

The objects of the study were polyurethaneureas (PUUs) synthesized based on a diisocyanate prepolymer (DFP) using 4,4'-diaminodiphenylmethane (DADPh) and 3,6-dioxyoctane-1,8-diamine (DA2) as chain extenders in a molar ratio of DADPh to DA2 of 0.3:0.7, and PUUs with immobilized IFO in the amount of 1 wt%; abbreviations: DFP-30DADPh-70DA2, DFP-30DADPh-70DA2+IFO [17, 18].

Holoxan® – (RS)-N-Bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide (Baxter, Germany) – active substance Ifosfamide $C_7H_{15}Cl_2N_2O_2P$ (Molecular weight = 261.09; melting point – (39-41)°C; log P (octanol-water) = 0.86. White crystalline powder, well soluble in water (3780 mg/L at 25 °C); purity 99.999%).

Fibroblast Tissue Culture Method. When study-

ing the cytotoxicity of samples of polymer compositions with IFO, the fibroblast tissue culture method was used, which allows assessing the biocompatibility of polymer materials intended for medical use under *in vitro* conditions [19, 20]. The method is based on culturing under standard conditions the subcutaneous adipose tissue of Wistar rats, which under cultivation conditions gives the growth of fibroblastic elements.

Fibroblast tissue culture was obtained by explanting pieces of subcutaneous adipose tissue from Wistar rats, which were placed in Carrel flasks with a nutrient mixture consisting of solid and liquid phases. Solid phase: rooster blood plasma, chicken embryo extract (obtained under laboratory experimental conditions). Liquid phase: medium 199 (LLC "BIOTESTLAB", Ukraine), bovine serum (LLC "Veterinary Medicine", Ukraine). Source of fibroblastic element growth – cultured tissue (subcutaneous tissue of Wistar rats).

Preparation of Fibroblast Tissue Culture for Explantation. Experimental groups: control (cultured tissues) and experimental (on the 3rd day of cultivation, samples of compositions were added at a rate of 100 mg of product per 1 ml of liquid phase). Each group had 3 explantations.

Explantation Conditions. The animals were euthanized using ether anesthesia. Under sterile conditions, fragments of subcutaneous tissue measuring 1 × 1 cm were isolated from the posterior dorsal part of the torso. The pieces of tissue were placed in physiological solution with the addition of streptomycin (pharmaceutical grade) at a rate of 25 ml of the drug per 10 cm³ of solution. After that, they were rinsed three times with portions of physiological solution without antibiotic. The initial pieces of tissue were divided into separate explants with a width of no more than 1.0-1.5 mm. The explants were transferred into identically sized Carrel flasks with a nutrient mixture consisting of rooster plasma and medium 199. On the bottom of each flask, 4-5 explants were evenly placed. An embryonic extract prepared from 10-day-old chicken embryos was added. After the formation of the solid phase, a mixture of bovine serum and medium 199 was added to the flasks. The ratio of ingredients in the nutrient medium was: medium 199 – 50%, chicken embryo extract – 10%, rooster blood plasma – 20%, bovine serum – 20% (for flasks with a height of 1 cm, diameter 3.5 cm). The culture was incubated at a temperature of 37°C, the liquid phase was replaced every 3 days. On the 3rd day, the migration of cellular elements was observed. Under conditions of cellular element migration, the test sample was introduced into the experimental samples at a rate of 100 mg per 1 ml of liquid medium. On days 3, 7, 10, and 14, observations were made of the growth of the compact, reticular zone and the zone of migrating fibroblastic elements, using a Carl Zeiss Primo Star microscope at ×160 magnification. Microphotography was performed using a Canon PowerShot A640 camera with a Soligor Adapter Tube for Canon A610/A620 52 mm Tele. The criterion for distinguishing these zones was the nature of the arrangement of the growing fibroblastic elements. A qualitative analysis of the growth zones was performed with a description of the nature of the growth of cellular elements. The compact zone included areas of dense arrangement of growing cells; the reticular zone included areas of arrangement of cellular cords that anastomose and branch. At the tips of isolated cellular cords growing into the solid phase of the nutrient medium to the location of isolated cells, the zone of migrating elements was determined.

Implantation Test and Histological Studies. For the purpose of histological study of cellular reactions to the implantation of composite materials with IFO based on PUUs and evaluation of their biocompatibility, implantation of experimental samples was carried out in the bodies of 24 Wistar rats weighing 240 ± 20 g. All manipulations with experimental animals were carried out in compliance with the principles set forth in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [21] and in accordance with the Law of Ukraine "On the Protection of Animals from Cruelty" No. 3447-IV of 21.02.2006. Healthy, young sexually mature animals were used. Model operations were performed under general anesthesia of laboratory animals in aseptic conditions.

Procedure of Implantation. After general anesthesia, the hair on the back of the animals was shaved in the interscapular area. The surgical field was treated with 70% ethanol solution, after which a longitudinal skin incision was made, and subcutaneous "pockets" were formed on both sides of the spine in the subcutaneous adipose tissue, where experimental samples measuring 10 × 10 mm, thickness 2 mm, were placed. Such an implantation area is characterized by good blood supply, high cell density of various differentiation stages, as well as low mobility of this zone and inaccessibility for the animal itself, which minimizes the risk of its interference in the experimental process. After the surgical procedure, the wound was sutured with sterile suture material. The animals were euthanized on the 7th, 14th, 30th, and 90th day after the operation by humane euthanasia. The experimental material (polymer sample with surrounding connective tissue) was fixed in 10% formalin solution and embedded in paraffin after histological processing according to standard methods [22]. Sections 10-15 μm thick were stained with hematoxylin and eosin. Analysis of cellular reactions and evaluation of the biocompatibility of composite materials were carried out by studying histological specimens using light microscopy with a Carl Zeiss Primo Star microscope; microphotography was performed using a Canon PowerShot A640 camera with a Soligor Adapter Tube for Canon A610/A620 52 mm Tele.

Results and Discussion

Study of Biocompatibility of Composite Materials with IFO Using the Fibroblast Tissue Culture Method In Vitro. To study the features of the dynamics of growth and development of fibroblastic elements, the tissue culture method was applied. This method allows for quick and accurate assessment of the cytotoxic effects of test materials and their components on connective tissue cells – rat fibroblasts, which play an important role in the interaction between a polymer implant and connective tissue. It is known that fibroblasts are components of the stroma of parenchymal organs and actively participate in morphogenesis and differentiation of specialized cells, allowing extrapolation of data obtained in fibroblast cultures to *in vivo* studies [19, 20]. The growth and development of cellular elements from the subcutaneous tissue of Wistar rats were studied on days 3, 7, 10, and 14.

Migration of fibroblastic elements in the study of the DFP-30DADPh-70DA2 sample without IFO, as in the control, began on the 3rd day of cultivation. The primary zone was formed by individual cells with a spindle-shaped form and strands oriented mostly perpendicular to the surface of the explant; irregular polygonal cells were also observed (Fig. 1a). For samples with the addition of the drug IFO, migration of fibroblastic elements in Carrel flasks also began on the 3rd day of cultivation, but a characteristic feature was the presence of a greater number of polygonal and round-shaped cells (Fig. 1b).

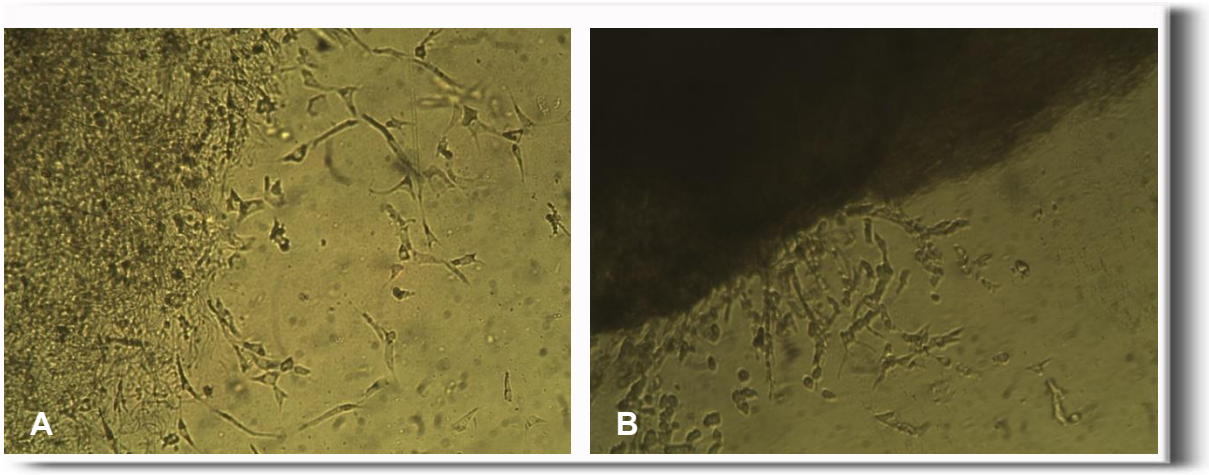

Fig. 1. Growth of fibroblast culture on the 3rd day of cultivation. a) Migration of fibroblast-like cells. DFP-30DADPh-70DA2 without IFO. ×160. b) Migration of polygonal fibroblast-like cells. DFP-30DADPh-70DA2+IFO. ×160.

On the 7th day of cultivation, both in the control and in the experimental samples (DFP-30DADPh-70DA2 and DFP-30DADPh-70DA2+IFO), three growth zones formed around the explants: a compact zone consisting of spindle-shaped and polygonal cells; a reticular zone where bundles and strands of cells formed; and a zone of individual migrating elements (Fig. 2). Notably, in the study of the DFP-30DADPh-70DA2+IFO sample, the compact zone was smaller in area than in the control and consisted of polygonal and oval cells closely adjacent to each other, which may indicate the influence of the prolonged biological action of IFO.

On the 10th day after explantation, the areas of cell growth zones increased in all Carrel flasks. In the study of the DFP-30DADPh-70DA2 polymer without IFO, growth zones similar to those in control flasks were observed, characterized by an expansion of the growth zone of individual migrating cells with diverse cell forms. Tissue-like growth was observed only in the control. In the study of the DFP-30DADPh-70DA2+IFO sample, the pattern of growth zone formation was similar to the polymer sample without IFO and the control, but the areas of growth zones were smaller. Disorientation of cells in the reticular zone and the presence of a large number of polygonal cellular elements with blunt processes were noted. In flasks with the DFP-30DADPh-70DA2+IFO sample, signs of degenerative changes in fibroblastic and fibroblast-like cells in the compact and reticular zones were detected (Fig. 3). Vacuolization and granular degeneration of the cytoplasm were observed.

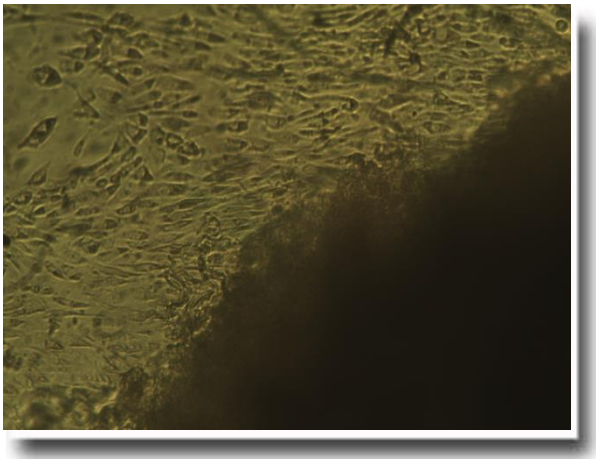

Fig. 2. Compact and reticular growth zones of cellular elements on the 7th day of cultivation for the DFP-30DADPh-70DA2+IFO sample. ×160.

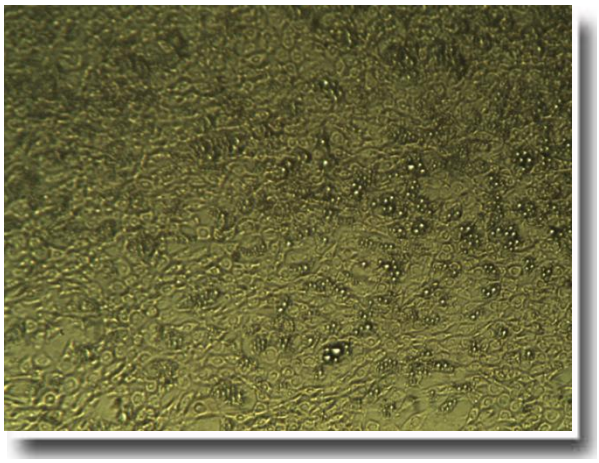

Fig. 3. Degenerative changes of fibroblasts in the compact zone on the 10th day of cultivation for the DFP-30DADPh-70DA2+IFO sample. ×160.

On the 14th day of cultivation, the cell population entered a phase of pronounced degeneration, with rounding of cell bodies and complete loss of intercellular contacts, both in control flasks and in all experimental flasks.

Thus, observations of tissue cultures showed that the dynamics and nature of cellular element growth in experimental flasks did not significantly differ from control cultures. This allows us to conclude that there is no cytotoxic effect of the experimental samples on the cultured cells.

Study of Biocompatibility of PUUs with IFO Using the Histological Method In Vivo. To histologically study cellular reactions and assess the biocompatibility of the obtained composite materials with IFO, model operations for implanting PUUs samples with IFO into the bodies of experimental animals – Wistar rats were conducted. During the experiment, the behavioral reactions of the animals, their external condition, and the postoperative field were monitored. Daily visual assessment of the epithelial reaction at the surgical site showed that the wound healed within 3-5 days after surgery without signs of inflammatory reaction. Morphological examination revealed virtually no degenerative changes, tumors, or tissue necrosis in either the short-term or distant postoperative periods. Throughout the experiment, the implanted materials were palpable through the skin of the animals. Implantation of the test samples did not cause aggression or changes in the behavior of the experimental animals.

The main focus in histological studies was on signs of inflammatory phenomena development in the implantation zone of the polymer samples at the "implant – tissue" interface. Macroscopically, connective tissue was detected around the implanted samples at all study periods, which separated the implanted samples from the surrounding tissues and did not differ in color and structure from tissues distant from the implantation site.

On the 7th day after surgery around the polymer samples DFP+30DADPh+70DA2, the formation of a thick connective tissue capsule was observed, separating the implanted samples from the surrounding connective tissue (Fig. 4a). The capsule exhibited a pronounced round-cell reaction, mainly consisting of leukocytes (polymorphonuclear neutrophils) and monocytic-macrophage cells with pronounced phagocytic activity. In some areas, young forms of fibroblastic elements without clear signs of maturity were observed.

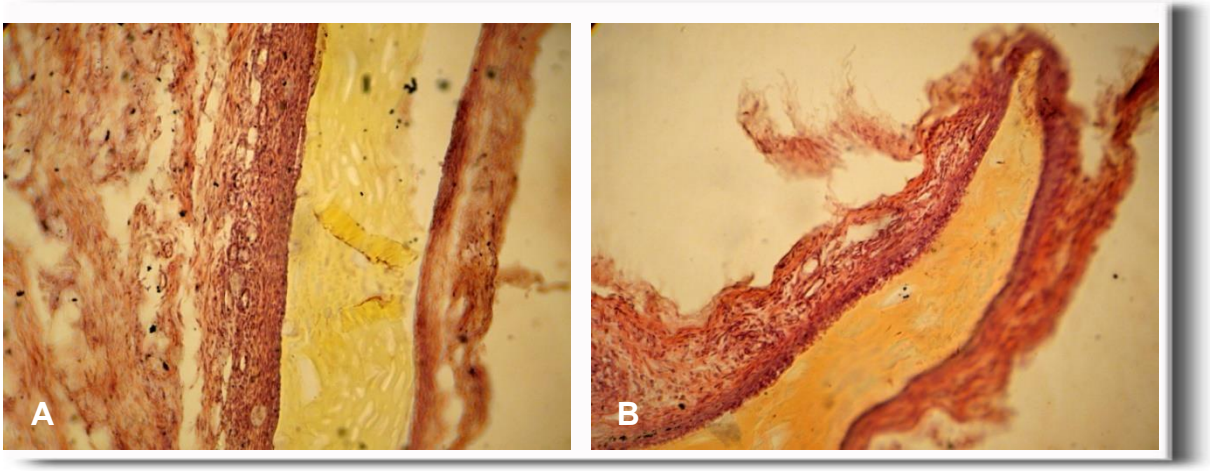

Fig. 4. Formation of connective tissue capsules around implanted samples: DFP-30DADPh-70DA2 (a), DFP-30DADPh-70DA2+IFO (b) on the 7th day after surgery. Hematoxylin and eosin staining. ×200.

Occasionally, a small number of poorly differentiated cells and cellular debris were present. A moderate number of blood vessels were observed, with microcirculatory processes occurring without disturbances. On the 7th day after surgery around the polymer samples DFP-30DADPh-70DA2+IFO, separation of the implanted polymer sample from the surrounding tissues by a connective tissue capsule was observed, differing in cellular composition throughout its length. In some areas, a formed and immature capsule was observed, whose cellular composition

was mainly represented by round-cell elements. In other areas, spindle-shaped fibroblasts located within collagen fiber bundles were observed. Single local infiltrations with round-cell elements were noted. Macrophages were present in significant quantities in certain areas of the capsule, indicating activation of phagocytic processes (Fig. 4b). Blood vessels were present in small numbers, without signs of microcirculatory disturbances.

On the 14th day after surgery around the polymer samples DFP-30DADPh-70DA2, a decrease in the thickness of the connective tissue capsule was observed compared to the previous study period, due to maturation of the capsule and the initiation of active proliferative processes and intercellular substance formation in the implant area. The cellular composition consisted of fibroblasts located within collagen fiber bundles (Fig. 5a). In some areas, the capsule had a low degree of maturity and was represented by round-cell elements – polymorphonuclear leukocytes and an intense monocytic-macrophage reaction, indicating active phagocytosis. A small number of blood vessels without microcirculatory disturbances were characteristic at this study period. On the 14th day after surgery around the polymer samples DFP-30DADPh-70DA2+IFO, a decrease in the thickness of the connective tissue capsule was observed compared to the previous period (Fig. 5b). The capsule had a fairly high degree of maturity throughout its length and was well-formed. The outer layer of the capsule consisted of fibroblasts located within mature collagen fiber bundles. The inner layer sometimes contained young forms of fibroblasts and poorly differentiated cellular elements. In some areas, the capsule had a low degree of maturity and was represented by round-cell elements – polymorphonuclear leukocytes and an intense monocytic-macrophage reaction, indicating active phagocytosis. A large number of blood vessels of various calibers without microcirculatory disturbances were observed at this period.

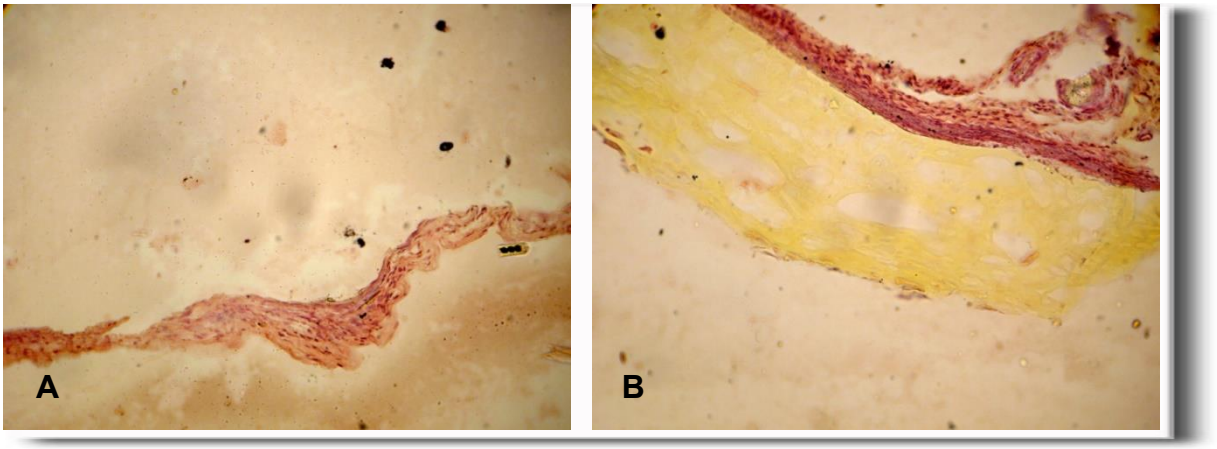

Fig. 5. Formation of connective tissue capsules around implanted samples: DFP-30DADPh-70DA2 (a), DFP-30DADPh-70DA2+IFO (b) on the 14th day after surgery. Hematoxylin and eosin staining. ×200.

On the 30th day after surgery around the DFP-30DADPh-70DA2 sample, a mature connective tissue capsule was observed, consisting mainly of collagen fiber bundles and spindle-shaped fibroblasts between them, which actively synthesized collagen and formed intercellular substance (Fig. 6a). In some areas of the capsule, accumulations of round-cell elements – polymorphonuclear leukocytes and macrophages were observed. A small number of blood vessels without disturbances in microcirculatory processes were noted at this period. On the 30th day after surgery around the DFP-30DADPh-70DA2+IFO sample, a connective tissue capsule characterized by a high degree of maturity was observed. It consisted of densely arranged collagen fiber bundles and spindle-shaped fibroblasts between them, oriented along the implanted material. In some areas of the capsule, slight round-cell infiltration was observed, mainly represented by macrophage reaction and mild leukocyte infiltration. A characteristic feature of the polymer sample implantation at this period was the formation of adipose tissue in the surrounding connective tissue. The number of blood vessels was small, and microcirculatory processes in them were undisturbed (Fig. 6b).

On the 90th day after surgery around the implanted DFP-30DADPh-70DA2 sample, a fairly thin connective tissue capsule was observed, which had a high degree of maturity throughout its length. The capsule consisted of bundles of wavy collagen fibers with spindle-shaped fibroblasts between them. In some areas of the capsule, slight foci of macrophage infiltration were characteristic. At this study period, single blood vessels with normal microcirculation were observed. On the 90th day after surgery around the implanted DFP-30DADPh-70DA2+IFO sample, a thin and mature connective tissue capsule was observed, consisting of bundles of wavy collagen fibers and spindle-shaped fibroblasts between them. It should be noted that the density of the capsule increased due to active synthesis of collagen fibers and other components of the extracellular matrix by fibroblasts. Blood vessels were present in small quantities

with normal microcirculation.

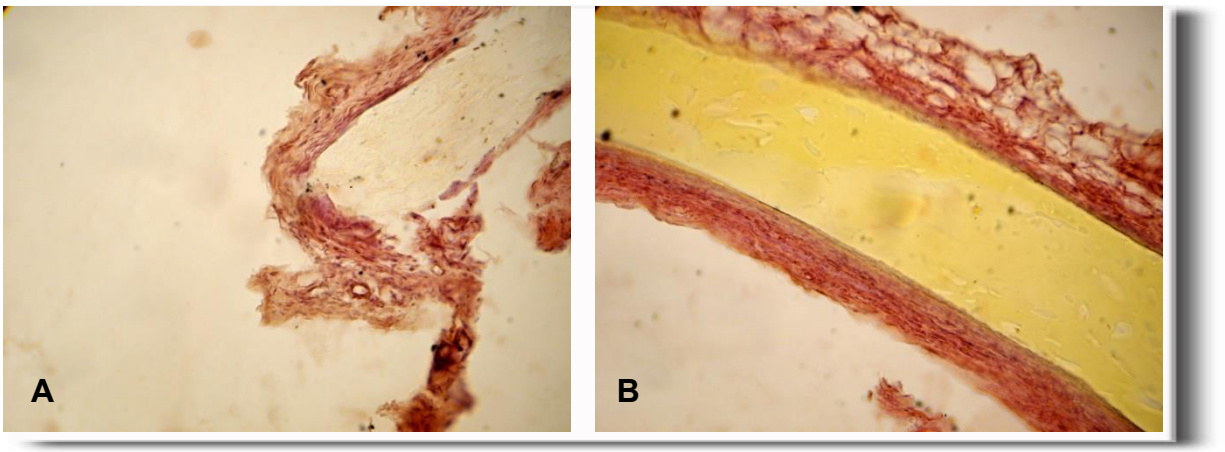

Fig. 6. Formation of connective tissue capsules around implanted samples: DFP-30DADPh-70DA2 (a), DFP-30DADPh-70DA2+IFO (b) on the 30th day after surgery. Hematoxylin and eosin staining. ×200.

Thus, based on the results of histological studies, it was established that implantation of composite materials with IFO based on multiblock PUUs (DFP-30DADPh-70DA2, DFP-30DADPh-70DA2+IFO) into the bodies of experimental animals led to the development of similar cellular reactions at all study periods. Separation of the implanted samples from the surrounding tissues by connective tissue capsules occurred already at early study periods, which did not differ significantly from each other in thickness, cellular composition or degree of maturity. Cellular reactions in the capsules themselves and beyond were typical for the classical development of aseptic inflammation. The dynamics of inflammatory reactions and the nature of their development were largely due to the complex of biological processes in the focus of inflammation, leading to the formation of formed connective tissue capsules already at early study periods, with a tendency for full maturation by the 30th day after implantation. Around the control implanted samples, normalization of cellular reactions occurred only on the 30th day after surgery, whereas with the implantation of the composite material with IFO, pronounced cellular reactions were observed even 30 days after implantation, which was most likely associated not only with the body's reaction to the presence of a foreign material but also with the biological activity of IFO itself and its cytostatic action. Full-fledged formation and maturation of the connective tissue capsule around the implanted DFP-30DADPh-70DA2+IFO sample was also observed 30 days after surgery. At the same time, at almost all study periods, the capsules around the DFP-30DADPh-70DA2+IFO sample exhibited an intense reaction of leukocytes (polymorphonuclear neutrophils) and monocytic-macrophage cells with pronounced phagocytic activity, associated with the process of phagocytosis to implement the body's protective-compensatory mechanisms.

Conclusions

1. The biocompatibility of composite materials based on PUUs with extenders 3,6-dioxyoctane-1,8-diamine (DA2), including with IFO, was studied using the fibroblast cell culture method. It was shown that the dynamics and nature of cellular element growth in the experimental flasks did not significantly differ from control cultures, allowing the conclusion that there is no cytotoxic effect of extracts from the studied materials on the cultured cells.

2. It was shown that implantation of the experimental PUUs samples (DFP-30DADPh-70DA2, DFP-30DADPh-70DA2+IFO) into the bodies of experimental animals led to the development of cellular reactions typical for aseptic inflammation, without signs of acute inflammatory and other reactive processes. Histological studies showed that the studied samples are biocompatible with the tissues of experimental animals.

3. It was established that implantation of polymer samples DFP-30DADPh-70DA2+IFO led to the development of intense cellular reactions in the implant placement zone, primarily reactions of round-cell elements – polymorphonuclear leukocytes and mononuclear cells (macrophages). The content of IFO in the polymer matrix likely influenced the proliferation of cellular elements in the implant placement zone, resulting in inhibition of regenerative processes at early study periods.

Prospects for Further Development

Conducting further medical and biological studies aimed at determining the possibility of using the developed composite materials in medical practice during antitumor therapy.

Conflict of Interest Information

No potential or actual conflicts of interest exist or are anticipated at the time of publication.

References

1. Farag MM. Recent trends on biomaterials for tissue regeneration applications: review. Journal of Materials Science. 2023;58:527-558. https://doi.org/10.1007/s10853-022-08102-x

2. Kovylin RS, Aleynik DY, Fedushkin IL. Modern Porous Polymer Implants: Synthesis, Properties, and Application. Polymer Science, Series C. 2021;63:29-46. https://doi.org/10.1134/S1811238221010033

3. Al-Shalawi FD, Mohamed Ariff AH, Jung D-W, Mohd Ariffin MKA, Seng Kim CL, Brabazon D, Al-Osaimi MO. Biomaterials as Implants in the Orthopedic Field for Regenerative Medicine: Metal versus Synthetic Polymers. Polymers. 2023;15:2601. https://doi.org/10.3390/polym15122601

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

5. Ulery BD, Nair LS, Laurencin CT. Biomedical Applications of Biodegradable Polymers. J Polym Sci B Polym Phys. 2011;49(12):832-864. https://doi.org/10.1002/polb.22259

6. Ornaghi HL Jr, Monticeli FM, Agnol LD. A Review on Polymers for Biomedical Applications on Hard and Soft Tissues and Prosthetic Limbs. Polymers. 2023;15(19):4034. https://doi.org/10.3390/polym15194034

7. Cui M, Chai Z, Lu Y, Zhu J, Chen J. Developments of polyurethane in biomedical applications: A review. Resources Chemicals and Materials. 2023;2(4):262-276. https://doi.org/10.1016/j.recm.2023.07.004

8. Stewart SA, Domínguez-Robles J, Donnelly RF, Larrañeta E. Implantable Polymeric Drug Delivery Devices: Classification, Manufacture, Materials, and Clinical Applications. Polymers. 2018;10(12):1379. https://doi.org/10.3390/polym10121379

9. Teo AJT, Mishra A, Park I, Kim Y-J, Park W-T, Yoon Y-J. Polymeric Biomaterials for Medical Implants & Devices. ACS Biomaterials Science & Engineering. 2016;2(4):454-472. https://doi.org/10.1021/acsbiomaterials.5b00429

10. Avula MN, Rao AN, McGill LD, Grainger DW, Solzbacher F. Foreign body response to subcutaneous biomaterial implants in a mast cell-deficient Kitw-sh murine model. Acta Biomaterialia. 2014;10(5):1856-1863. https://doi.org/10.1016/j.actbio.2013.12.056

11. Anderson JM. Biological responses to materials. Annual Review of Materials Research. 2001;31(1):81-110.

12. Galatenko NA, Rozhnova RA. [Biologically Active Polymeric Materials for Medicine]. Kyiv: Naukova Dumka; 2013. 210 p. Russian.

13. Wang Y, Sun L, Mei Z, Zhang F, He M, Fletcher C, Wang F, Yang J, Bi D, Jiang Y, Liu P. 3D printed biodegradable implants as an individualized drug delivery system for local chemotherapy of osteosarcoma. Materials & Design. 2020;186:108336. https://doi.org/10.1016/j.matdes.2019.108336

14. Gladyr I, Kozlova G, Narazhayko L, Galatenko N. [Development and research of polymeric composite materials of medical purpose with ifosfamide on the basis of polyurethanes with isocyanurate fragments]. Polymer Journal. 2020;42(2):125-135. Ukrainian. https://doi.org/10.15407/polymerj.42.02.125

15. Prymushko SO, Galatenko NA, Rozhnova RA, Kozlova GA, Gladyr II, Danko NO. Synthesis and research of polyurethane urea with 2-(2-aminoethoxy)ethan-1-amine and 3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propane-1-amine as macrochain extenders, and compositions with ifosfamide based on them. Issues of Chemistry and Chemical Technology. 2024;(1):70-80. http://dx.doi.org/10.32434/0321-4095-2024-152-1-70-80

16. Zhang Y, Kawedia J, Myers A, McIntyre C, Anderson P, Kramer M. Physical and chemical stability of high-dose ifosfamide and mesna for prolonged 14-day continuous infusion. Journal of Oncology Pharmacy Practice. 2013;20(1):51-57. https://doi.org/10.1177/1078155213478284

17. Prymushko SO, Galatenko NA, Rozhnova RA, Kozlova GA, Gladyr II. Synthesis and investigation of new polyurethaneureas containing 3,6-dioxyoctane-1,8-diamine as a macrochain extender. Polymer Journal. 2022;44(3):231. https://doi.org/10.15407/polymerj.44.03.231

18. Prymushko SO, Kozlova GA, Gladyr II, Galatenko NA. Development of medical composite materials with ifosfamide based on polyurethaneureas containing 3,6-dioxyoctane-1,8-diamine in their structure. In: [XXIII International Conference of Students, Postgraduates, and Young Scientists "Modern Problems of Chemistry"; 2022 May 18-20; Kyiv, Ukraine]. 2022. p. 114.

19. Burenko HV, Galatenko NA, Kabak KS. [Morfologicheskie i biokhimicheskie aspekti biodestruktsii polimerov]. Kyiv: Naukova Dumka; 1986. 221 p. Russian.

20. Lebedev EV, Konstantinov YuB, Galatenko NA, Yatsenko VP, Rozhnova RA, Maksymenko VB, authors: [Toksikologo-gigiєnichni ta doklinichni doslidzhennya polimernih materialiv i virobiv na їh osnovi medichnogo priznachennya: metod. vkazivki]. Kyiv: Naukova dumka; 2009. 99 p. Ukrainian.

21. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Strasbourg: Council of Europe; 1986. 53 p.

22. Bagrij MM, Dibrova VA, Popadynets OG, Hryshchuk MI, authors: [Metodiki morfologichnikh doslidzhen: monografiya]. Vinniczya: Nova Kniga; 2016. 328 p. Ukrainian.

Кулєш Д.В., Галатенко Н.А., Рожнова Р.А., Наражайко Л.Ф., Примушко С.О., Тарнавський Д.В. Вивчення цитотоксичності та аналіз клітиних реакцій на імплантацію поліуретансечовин з іфосфамідом.

РЕФЕРАТ. Актуальність. Потреба суспільства в сучасних імплантатах і виробах тканинної інженерії неухильно зростає у зв'язку зі збільшенням кількості оперативних втручань, викликаних різноманітними захворюваннями, травмами та віковими змінами. Широкого застосування в медичній практиці набуває все більша кількість матеріалів на основі синтетичних полімерів, що призначені для імплантації в тканини живого організму. **Метою** даної роботи було вивчення цитотоксичності та аналіз клітинних реакцій на імплантацію поліуретансечовин з іммобілізованим іфосфамідом, синтезованих з використанням як подовжувачів макроланцюга 4,4'-діамінодифенилметану та 3,6-діоксиоктан-1,8-діаміну методом тканинної культури фібробластів та за допомогою імплантаційного тесту. **Результати.** Методом тканинної культури клітин фібробластів показано, що динаміка і характер росту клітинних елементів при культивуванні в дослідних флаконах з композиційними матеріалами на основі поліуретансечовин, в тому числі з іфосфамідом, суттєво не відрізнялися від контрольних культур, що дозволяє зробити висновок про відсутність цитоксичного впливу екстрактів з досліджуваних матеріалів на клітини, що культивувались. Встановлено, що імплантація дослідних зразків поліуретансечовин в організм експериментальних тварин приводила до розвитку клітинних реакцій типових для асептичного запалення, без ознак гострих запальних та інших реактивних процесів. Проведені гістологічні дослідження показали, що досліджені зразки є біосумісними з тканинами експериментальних тварин. Імплантація полімерних зразків з іфосфамідом призводила до розвитку інтенсивних клітинних реакцій в зоні розміщення імплантатів. Вміст іфосфаміду в полімерній матриці, ймовірно, впливав на проліферацію клітинних елементів в зоні розміщення імплантату, в результаті чого відбувалося інгібування регенераторних процесів на ранніх термінах дослідження. **Висновок.** За результатами проведених досліджень показано, що розроблені композиційні матеріали на основі поліуретансечовин з іфосфамідом не мають вираженої цитотоксичної дії, є біосумісними та перспективними матеріалами для використання в медичній практиці при протипухлинній терапії.

Ключові слова: поліуретансечовини, іфосфамід, культура тканин фібробластів, імплантаційний тест, біосумісність.