

ХИРУРГИЯ/SURGERY

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MORPHOLOGY OF THE IMPLANTATION ZONE OF SYNTHETIC HERNIOPLASTY PROSTHESES MODIFIED WITH CARBON

Research article

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Abstract

Objective: to improve the baseline biocompatibility properties of synthetic hernioplasty prostheses by surface modification of APP (DLC coating).

Polypropylene meshes (Eafil Light) and perforated porous polytetrafluoroethylene films (Ecoflon) were used as base endoprostheses. APP was applied using a pulsed carbon plasma source with a graphite cathode. Surgical interventions were performed under aseptic conditions; prostheses sized 1.5×1.5 cm were implanted subcutaneously into the anterior abdominal wall of animals outside the incision field without additional fixation.

In the group with polypropylene endoprostheses modified with APP, a uniform granulation tissue zone with visually lower cell density formed around the mesh by day 7. By day 14, a continuous granulation tissue layer formed around the APP-coated prosthesis with fragmentation of the coating into plate-like and needle-like fragments observed. After 180 days, morphological signs showed full encapsulation of the material by a capsule fully integrated structurally with surrounding connective tissue. No acute inflammatory or giant cell reaction to coating fragments was detected. In the group with polytetrafluoroethylene prostheses, no acute inflammatory changes were observed at days 7 and 14. After 180 days, distinctive features included pronounced deposits of adhesive proteins (fibronectin and laminin) and type I collagen; the coating layer remained intact without mechanical damage. No inflammatory reaction was found in adjacent connective tissue.

Polypropylene and polytetrafluoroethylene mesh endoprostheses with APP exhibit greater biocompatibility and better biointegration into surrounding tissues during encapsulation over long-term periods compared to the original materials.

Keywords: carbon, biocompatibility, polypropylene, polytetrafluoroethylene, hernias, prostheses.

**МОРФОЛОГИЯ ЗОНЫ ИМПЛАНТАЦИИ СИНТЕТИЧЕСКИХ ГЕРНИОПРОТЕЗОВ,
МОДИФИЦИРОВАННЫХ УГЛЕРОДОМ**

Научная статья

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Аннотация

Цель исследования: улучшение исходных свойств биосовместимости синтетических герниопротезов путем модификации поверхности АПП. (DLC-покрытием).

В качестве исходных эндопротезов использовали полипропиленовые сетки (Эсфил легкий) и пленки политетрафторэтиленовые перфорированные пористые (Экофлон). АПП наносили с использованием импульсного

источника углеродной плазмы с графитовым катодом. Оперативные вмешательства выполнены в асептических условиях, эндопротезы размерами 1,5×1,5 см помещали подкожно в переднюю брюшную стенку животных вне поля разреза без дополнительной фиксации.

В группе с применением эндопротеза из полипропилена с АПП на 7-е сутки эксперимента вокруг сетчатого протеза определялась равномерная зона грануляционной ткани с визуально меньшей плотностью клеточного состава. На 14-е сутки вокруг протеза с АПП сформировался непрерывный слой грануляционной ткани, было выявлено фрагментирование покрытия в виде пластинчатых и игольчатых фрагментов. Через 180 суток имела место морфологическая картина полной инкапсуляции материала сформированной капсулой, структурно полностью интегрированной в окружающую соединительную ткань. Острой воспалительной и гигантоклеточной реакции на фрагменты покрытия не было выявлено. В группе с эндопротезом из политетрафторэтилена на 7-е и 14-е сутки острые воспалительные изменения отсутствовали. Через 180 суток установлены отличительные особенности в виде выраженных депозитов адгезивных белков (фибронектина и ламина) и коллагена I типа, слой покрытия сохраняется и не подвергается механическому разрушению. В прилежащей соединительной ткани не выявлено воспалительной реакции.

Сетчатые эндопротезы полипропилен и политетрафторэтилен с АПП обладают большей биосовместимостью и лучшей биоинтеграцией в окружающие ткани в процессе инкапсуляции в отдаленные сроки, в сравнении с исходными материалами.

Ключевые слова: углерод, биосовместимость, полипропилен, политетрафторэтилен, грыжи, протезы.

Introduction

Currently, there is a steady increase in the number of reconstructive and restorative surgical interventions using biomaterials in various fields of surgery. At the same time, many authors note unresolved issues regarding the biocompatibility of surgical endoprostheses implanted into the body. This is due to the fact that during the integration of synthetic material with body tissues, a response occurs in the form of aseptic inflammation, resulting in the formation of a connective tissue capsule, which can lead to tumor development, implant calcification, infection, and prosthesis dysfunction.

These complications lead to impaired function of synthetic prostheses, causing deterioration in the patient's health and requiring repeat interventions.

Reducing the risk of complications is currently achieved through two main approaches: modifying the structure of the synthetic implant itself and applying various coatings.

DLC coating (nanoscale diamond-like carbon coating — APP) is an amorphous carbon-based film containing mixed sp₃ and sp₂ carbon-carbon bonds. Currently, this coating is used as a polymer coating for various medical devices (mesh implants, stents, and prostheses for cardiovascular surgeries, endoprostheses in trauma operations). However, it has not gained wide application in surgical practice, as its application requires special expensive equipment and due to the complexity of the technology for applying it to various synthetic coatings.

The method developed at NIU "BelSU" allows applying APP to synthetic materials, which can also be used for closing soft tissue defects (Patent No. 2430986).

Thus, the aim of our study is to investigate the main biocompatibility properties of synthetic hernioplasty endoprostheses whose surfaces are modified with APP.

Research methods and principles

The fabrication of original endoprostheses with carbon coating was carried out at NIU "BelSU." Polypropylene mesh endoprostheses Esfil Light (Lintex, Russia) and perforated porous polytetrafluoroethylene films Ecoflon (Ecoflon, Russia) were used as base implants.

APP was applied to these samples using a pulsed carbon plasma source with a graphite cathode under the following parameters: capacitor capacity — 2300 μF, capacitor charging voltage — 300 V, pulse discharge frequency 1–2 Hz.

Animal studies were conducted at the Laboratory of the Research Institute of "Pharmacology of Living Systems" at NIU "BelSU" (Director: Doctor of Medical Sciences, Professor M.V. Pokrovsky). The work was performed in accordance with the approved research protocol and standard laboratory operating procedures. All stages complied with the Russian National Standard GOST R-53434-2009 "Principles of Good Laboratory Practice," the Order of the Ministry of Health and Social Development of the Russian Federation dated August 23, 2010, No. 708n "On Approval of Laboratory Practice Rules," in compliance with the interstate standard "Guidelines for the Care and Use of Laboratory Animals" (GOST 33216-2014), and ethical principles concerning laboratory animals following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes CETS No. 123, as amended by ETS No. 170.

The study involved 220 male Wistar rats weighing 200 g.

In the experimental study, animals were divided into a group using polypropylene prostheses (n=120) with coating (n=60) and without coating (n=60), and a group using polytetrafluoroethylene prostheses (n=100) with coating (n=50) and without coating (n=50).

Surgical interventions were performed under aseptic conditions. After preparing the surgical field, experimental endoprostheses sized 1.5×1.5 cm were implanted subcutaneously into the anterior abdominal wall outside the incision field without additional fixation. The wound was sutured. Animals were withdrawn from the experiment on days 7, 14, and 180.

Histological specimens were scanned using the "Mirax Desk" system (Carl Zeiss) for scanning, archiving, and image analysis. Specimens were stained with hematoxylin and eosin according to Van Gieson and Mallory methods.

Microscopic images and morphometry were analyzed on scanned micropreparations using the "Pannoramic Viewer 1.14" software. Microphotographs were captured using the same software and with "Fast Stone Capture" image capture software.

Statistical significance between groups was assessed using the Mann–Whitney test. The calculation of the test statistic and p-value was performed with the Statistica 12.5 statistical software package. At $p < 0.05$, the null hypothesis (H_0) of equal means is rejected, and the alternative hypothesis of statistically significant differences between compared groups is accepted.

Main results

Morphology of the implantation zone of polypropylene and polytetrafluoroethylene hernioplasty prostheses without coating. Upon implantation of both materials up to 180 days, no signs of damaging toxic effects on subcutaneous tissues were detected. The observed tissue reactions reflect the typical sequence of changes in the foreign body implantation zone characteristic for each material.

Implantation of uncoated polypropylene mesh was characterized by long-lasting inflammatory changes — in day 14 samples, granulation tissue with signs of incomplete maturity and foci of inflammatory reaction were observed, persisting even after 6 months (Fig. 1).

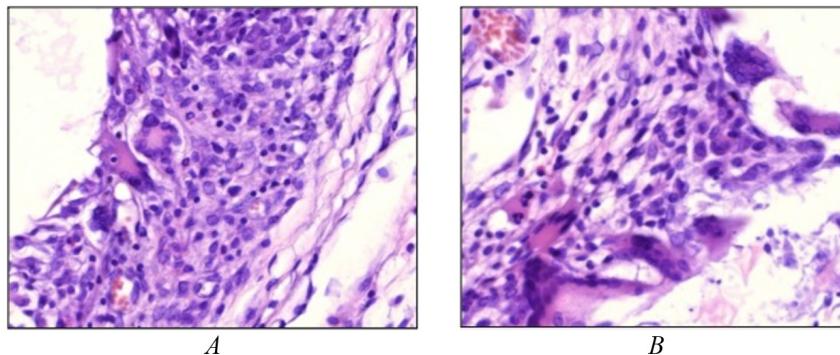


Figure 1 - Implantation area of polypropylene hernioplasty meshes without coating:

A – structure of granulation tissue around the uncoated polypropylene implant on day 14 of the experiment: mixed polymorphic cellular composition with a predominance of leukocytes and histiocytes, presence of multinucleated foreign body giant cells; B – numerous giant multinucleated foreign body cells around the implant on day 14 of the experiment

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Note: stained with hematoxylin and eosin; microphoto. $\times 400$

Tissue interaction with polytetrafluoroethylene did not show similar changes — acute inflammatory changes in histological specimens were absent, showing only typical isolation tissue reactions. At the same time, giant foreign body cell clusters were present on the surface of polytetrafluoroethylene implants, mainly near the edges. Additional Kosa staining revealed the presence of large calcifications in the areas with these cell clusters (Fig. 2).

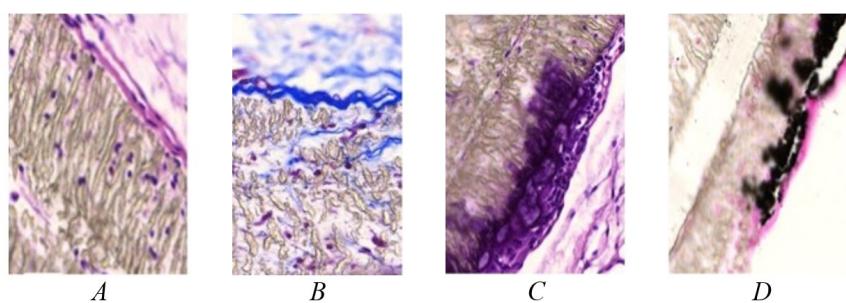


Figure 2 - 180 days after implantation of an uncoated polytetrafluoroethylene plate:

A – the implant structure is moderately loosened; on the outside, single loosened collagen fibers of the connective tissue of the anterior abdominal wall¹; B – leukocyte migration into the pores of the material, ingrowth of single thin collagen fibers²; C – giant foreign body cell layer³; D – area of calcification in the region of foreign body cell accumulation⁴

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Note: 1 – stained with hematoxylin and eosin; microphoto. $\times 100$; 2 – mallory staining; microphoto. $\times 200$; 3 – stained with hematoxylin and eosin; microphoto. $\times 100$; 4 – kossa impregnation; microphoto. $\times 100$

The giant cell macrophage reaction to the foreign body (implant) was also characteristic for uncoated polypropylene mesh, increasing between days 7 and 14. The surrounding layer predominantly contained pro-inflammatory agents (polymorphonuclear leukocytes, lymphocytes, and histiocytic elements) (Fig. 1).

The common response to implantation of both materials was the formation of a characteristic connective tissue capsule after 180 days. On the surface of the polypropylene mesh, formed connective tissue appeared as a heterogeneous capsule 80–100 μm thick, with prevalently concentrically organized dense fibrous, focally hyalinized connective tissue with few fibrocyte-type cells (Fig. 1). However, up to one-third of the encapsulating connective tissue area, especially in regions of implant element intertwining, showed moderate leukocyte infiltration with significant eosinophil content. These areas also contained a moderate number of blood capillaries. Foreign body cells were absent, but epithelioid histiocyte plates were segmentally arranged on the inner capsule surface. There was no full integration of capsule structures into the surrounding connective tissue — a distinct boundary was visible.

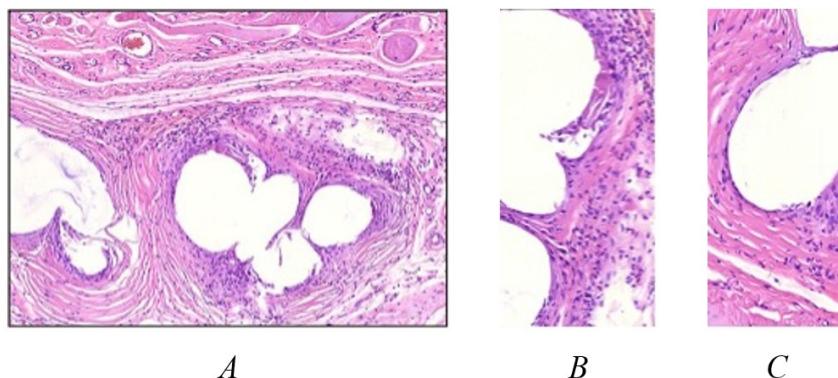


Figure 3 - Structure of the capsule of an uncoated polypropylene implant after 6 months: the connective tissue is heterogeneous, with predominance of areas of dense fibrous structure (C) containing few fibrocyte-like cells; up to one-third of the area consists of regions with pronounced infiltration by polymorphonuclear leukocytes, predominantly eosinophils (B); foreign body cells are absent, but epithelioid histiocyte-like cells are present on the inner surface of the capsule

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Note: stained with hematoxylin and eosin; microphoto. $\times 20$ (A), $\times 400$ (B, C)

The structure of uncoated polytetrafluoroethylene implants at this experimental term was moderately loosening and edematous. The film surface was tightly adjacent to the surrounding connective tissue, which showed no neutrophilic or eosinophilic infiltration; mast cells present showed no signs of degranulation, indicating the absence of an allergenic effect of the material (Fig. 2). A capsule formed directly on the implant surface, represented by bundles of immature collagen fibers, with no inflammatory changes in the surrounding tissues and signs of completed organization with mature collagen fiber bundles forming. Leukocyte migration into the porous implant structure and varying degrees of collagen fiber ingrowth into it were detected in more porous areas. Immunohistochemical analysis showed pronounced deposits of adhesive proteins (fibronectin and laminin) and type I collagen in both implant thickness and surface (Fig. 4).

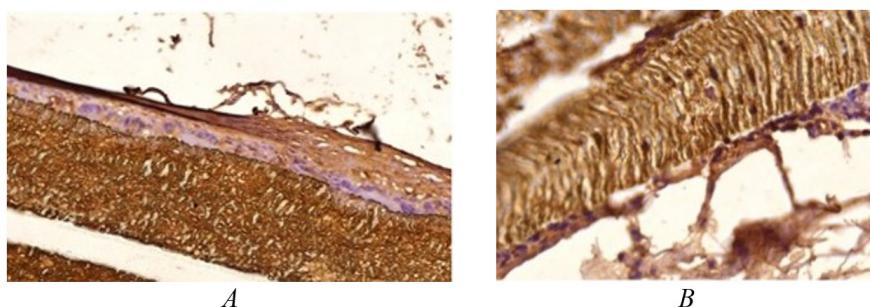


Figure 4 - Deposits of fibronectin (A) and type I collagen (B) in the bulk and on the surface of an uncoated polytetrafluoroethylene implant

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Note: immunohistochemical reactions; microphoto. $\times 100$

Tissue reaction features in the implantation zone of polypropylene and polytetrafluoroethylene hernioplasty prostheses with diamond-like carbon coating surface modification. Modification of polypropylene and polytetrafluoroethylene hernioplasty prostheses with diamond-like carbon coating altered their surface properties and, accordingly, the character of

connective tissue reactions to the foreign body —after 6 months of the experiment, significant differences compared with the originals and substantial surface changes compared to earlier timepoints were detected.

The overall implantation picture of polypropylene with carbon coating showed complete material encapsulation with a capsule fully integrated structurally into the surrounding connective tissue (Fig. 5).

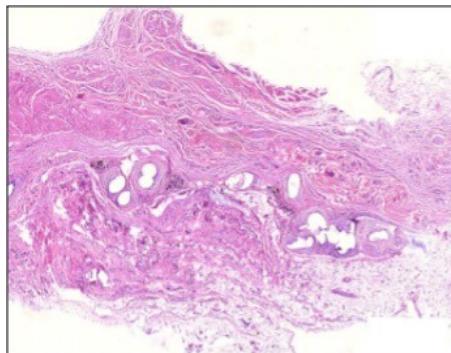


Figure 5 - Overall view of the implantation area of carbon-coated polypropylene mesh after 6 months of the experiment.

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Note: A continuous layer of connective tissue surrounds the implant without clear boundaries from the surrounding tissues. Stained with hematoxylin and eosin. Microphoto. ×20.

The capsule was smaller, 60–70 μm thick, represented by 5–10 compact collagen fiber layers with hyalinized areas evenly interspersed with few fibrocytes. Significant cellular composition differences prevailed: immature fibroblasts dominated, some showing mitotic figures; leukocytes and histiocytic elements were present. Foreign body giant cells were absent. Blood vessels were almost absent. The inner capsule layers had concentric orientation around the mesh implant structures, smoothly transitioning externally into bundles of collagen fibers of subcutaneous connective tissue (Fig. 6 A).

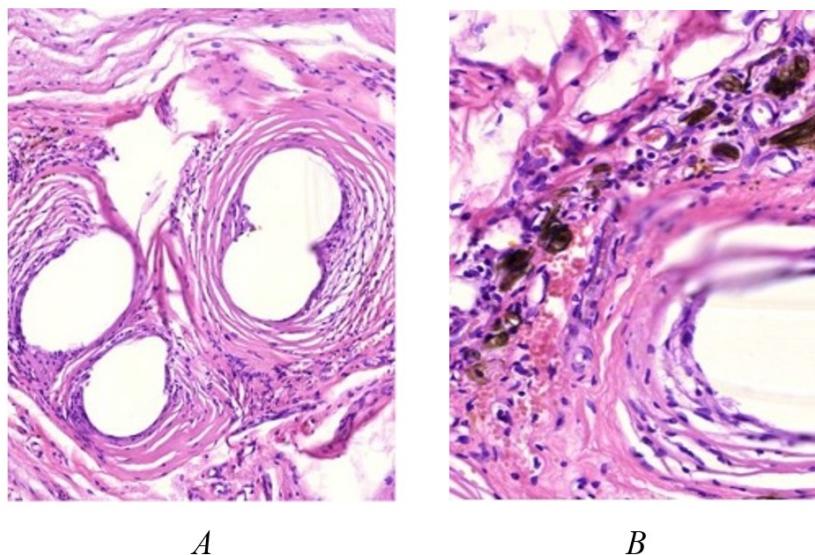


Figure 6 - Structure of the connective tissue capsule around the polypropylene mesh:

A – concentric low-cell-density layers of collagen fibers with a smooth transition into the surrounding connective tissue, absence of inflammatory changes and giant cell reaction to the implant; B – plate-like and needle-like coating particles in close proximity to the implant, surrounded by vascularized loose fibrous connective tissue with a moderate number of lymphohistiocytic elements

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Note: stained with hematoxylin and eosin; microphoto. ×20 (A), ×400 (B)

The gray-brown coating layer appeared fragmented. Most of the coating in the form of plate-like and needle-like structures was partially encapsulated adjacent to mesh elements (Fig. 6 B). The surrounding connective tissue resembled a pseudocapsule due to a somewhat higher cellular density consisting of few macrophages and small lymphocytes and blurred concentric

orientation of collagen fiber bundles. Focal blood capillary concentrations were found here. There was no acute inflammatory or giant cell reaction to the coating fragments.

On the surface of polytetrafluoroethylene, 180 days after implantation, the carbon layer in histological specimens showed no mechanical damage, indicating strong fixation (Fig. 7). No significant inflammatory changes were observed in adjacent connective tissue, and mast cells were intact. No calcification foci were detected. The overall picture suggested reduced implant porosity and permeability for inflammatory elements and intercellular substance components, including growing collagen fibers. Implant encapsulation occurred without connective tissue ingrowth, via capsule formation with a more external location and broad tissue delimitation around the implant.

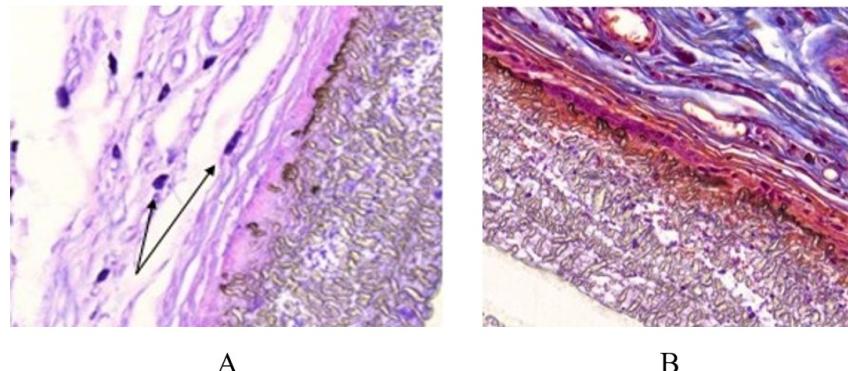


Figure 7 - 180 days after implantation of a polytetrafluoroethylene plate with a carbon coating:

A – tight adhesion of the implant to the connective tissue, the carbon coating layer is preserved as a narrow brown stripe; intact mast cells are indicated by arrows; B – section of the implant surface showing layers of immature collagen fibers of the forming capsule and surrounding abdominal wall tissues with bundles of mature collagen fibers (blue color); connective tissue does not grow into the thickness of the implant; between the capsule fibers and the implant surface, there are few small foreign body cells

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Note: stained with Phenaf dye (A), Mallory staining (B); Microphoto. ×100

A statistical evaluation of differences between study groups was performed and is reflected in Tables 1 and 2.

Table 1 - Study group "Ecoflon," Subgroups with Coating and without Coating

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	Groups	Observation periods			Reliability of differences
		Day 7	Day 14	180th day	
Infiltrate thickness, µm	Control	175±25	-	-	P1*=0.04 P2*>>0.05 P3*>>0.05
	With coating	125±25	-	-	
Polymorphonuclear leukocytes, %	Control	52±8	30±5	-	P1*<<0.01 P2*=0.01 P3*>>0.05
	With coating	-	12±3	-	
Small lymphocyte type cells, %	Control	22±3	17±3	-	P1*<<0.01 P2*=0.08 P3*<<0.01
	With coating	-	12±3	9±1	
Histiocytes, %	Control	22±3	30±2	40±4	P1*=0.02 P2*=0.01 P3*<<0.01
	With coating	37±7	7±1	-	
Foreign body giant multinucleated cells (FBGCs)	Control	3±2	10±1	-	P1*<<0.01 P2*<<0.01 P3*<<0.01
	With coating	-	-	-	
Capsule thickness, µm	Control	-	200±30	90±10	P1*>>0.05 P2*=0.04 P3*=0.02
	With coating	-	147±22	55±5	

P1*- valuep-value for the group indicator on day 7;P2*- valuep-value for the group indicator on day 14;P3*- valuep-value for the group indicator at day 180;

Table 2 - Study group "Eafil-light," Subgroups with Coating and without Coating

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	Groups	Observation periods			Reliability of differences
		Day 7	Day 14	180th day	
Infiltrate thickness, μm	Control	125 \pm 25	-	-	P1*=0.04 P2*>>0.05 P3*>>0.05
	With coating	90 \pm 10	-	-	
Polymorphonuclear leukocytes, %	Control	47 \pm 3	25 \pm 4	-	P1*=0.1 P2*=0.04 P3*>>0.05
	With coating	42 \pm 3	17 \pm 3	-	
Small lymphocyte type cells, %	Control	20 \pm 2	15 \pm 2	-	P1*=0.1 P2*=0.04 P3*<<0.01
	With coating	17 \pm 2	10 \pm 1	7 \pm 1	
Histiocytes, %	Control	26 \pm 2	27 \pm 2	35 \pm 3	P1*=0.08 P2*=0.02 P3*=0.004
	With coating	22 \pm 2	17 \pm 2	10 \pm 2	
Foreign body giant multinucleated cells (FBGCs)	Control	7 \pm 1	9 \pm 1	-	P1*<<0.01 P2*<<0.01 P3*<<0.01
	With coating	-	-	-	
Capsule thickness, μm	Control	-	175 \pm 35	70 \pm 10	P1*>>0.05 P2*=0.05 P3*=0.03
	With coating	-	130 \pm 10	52 \pm 5	

P1*- valuep-value for the group indicator on day 7;P2*- valuep-value for the group indicator on day 14;P3*- valuep-value for the group indicator at day 180.

Discussion

Modern surgical endoprostheses for hernioplasty are predominantly made from non-resorbable synthetic materials, including polypropylene and polytetrafluoroethylene. Despite the achieved reduction in recurrence rates, the persistent risk of specific postoperative complications indicates insufficient effectiveness of current technologies, necessitating further research and development in this area. Current studies focus on developing endoprostheses capable of mimicking native tissue and promoting healing without postoperative complications. Researchers actively investigate advanced biodegradable materials, both synthetic polymers and natural biopolymers, as well as the efficacy of therapeutic agents, surface modification methods, and advanced manufacturing technologies such as 4D printing. Our work is specifically aimed at modifying the surface of materials used for hernia repair by applying a novel diamond-like carbon coating developed at NIU "BelsU" and evaluating its use.

Upon implantation of polypropylene with carbon coating, significant differences were observed as early as day 7, manifested by earlier formation of the fibroblastic component in granulation tissue, as reflected by the relative count of cellular elements in infiltrates. By day 14, fibroblasts with morphological features of functionally active forms and zones of amorphous and fibrous intercellular substance formation were clearly observed. At late time points (6 months), full biointegration of the implant into subcutaneous connective tissue was identified as reactive encapsulation by connective tissue directly connected with surrounding structures. No inflammatory or other reactive changes were noted.

At the same time, disintegration of the implant's surface coating was clearly observed. Its fragments were compactly deposited nearby and surrounded by loose connective tissue. However, no signs of inflammatory reaction or typical foreign body responses were detected, allowing us to classify the coating particles as bioinert. The implant surface at this stage was open, but surrounded by a formed, practically avascular dense fibrous tissue. The avascularity of the formed fibrous capsule determines its areactivity. It can be assumed that the duration of intactness of the fully bioinert coating is sufficient for the formation of the areactive capsule, and subsequent coating disintegration no longer plays a role given the established biointegration of the implant. This is indicated by comparison of the morphological pictures at late stages for implants without coating and with coating.

A somewhat different picture emerged after implantation of polytetrafluoroethylene with carbon coating. The original microporous polytetrafluoroethylene implant absorbed plasma and matrix proteins and was subsequently infiltrated by collagen fibers, which could cause rigid fixation of the implant to the anterior abdominal wall tissues. A negative consequence of this was mechanical impact of implant edges on tissues, manifested as calcification foci with giant cell reaction in these areas. Surface modification of polytetrafluoroethylene plate implants by nanoscale diamond-like carbon coating improved their properties by eliminating microporosity and preventing infiltration of connective tissue components and protein exudation,

including inflammatory reaction components, thereby reducing connective tissue fiber ingrowth and the degree of secondary calcification.

One of the most significant differences in tissue reactions to carbon surface modification of synthetic prostheses was the absence, at all time points, of macrophage giant cell transformation and foreign body cell formation. In current literature, the response of the mononuclear phagocyte system, including giant cell formation, is regarded as a key indicator of implant biocompatibility. However, the dependence of these processes on the physico-chemical surface characteristics of implant materials remains under discussion. Shen M. et al. demonstrated that adsorbed proteins and surface chemical composition strongly influence giant cell formation, monocyte TNF-alpha release, and procoagulant activity in vitro, supporting the idea that these variables may also affect macrophage-mediated foreign body response to biomaterials in vivo.

Surface modification of biomaterials determines their interaction with cells, including adhesion and programmed cell death processes. Notably, implant surface-induced apoptosis of immunocompetent cells (monocytes/macrophages) may create conditions for persistence of infectious agents, supported by clinical data on cardiovascular implants.

Our study established that the most pronounced giant cell reaction occurred with implantation of non-modified materials, accompanied by accumulation of fibronectin and type IV collagen. Use of the specialized coating significantly reduced the intensity of this cellular response.

Several explanations for this difference exist, yet literature data do not provide a definitive conclusion, since calcification signs were observed in tissue zones in direct contact with implant surfaces in giant cell reaction areas.

Critically, the created coating modulates tissue response to the implant, defining two possible scenarios: full biomechanical integration by connective tissue incorporation or formation of a connective tissue capsule as a manifestation of foreign body rejection. Further studies on implant biodegradation kinetics depending on surface modification types should be promising.

Conclusion

The obtained data indicate that synthetic implants with nanoscale diamond-like carbon surface modification, compared to the original materials, possess higher biocompatibility and better biointegration into surrounding connective tissue during late-stage encapsulation.

The developed technology for applying coatings with specified properties to polymer implants is an effective method for controlling implant characteristics depending on the desired application outcome. Based on these results, a clinical study is needed to investigate the interaction of DLC-покрытия with the human anterior abdominal wall.

Конфликт интересов

Не указан.

Рецензия

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Conflict of Interest

None declared.

Review

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