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In Vitro Justification of Using Endobiliary Stents Made of Polyhydroxyalkanoates

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Summary: Treatment of patients with mechanical jaundice (MJ) has been one of the topical problems of current medicine. The fraction of patients having MJ of oncologic character that can undergo radical surgery does not exceed 25–30%. A way out of such situation is to use minimally invasive endobiliary interventions, the main type of which is endoprosthesis replacement of bile duct lumens. The aim of the work was to study the biological properties of the experimental models of polymer stents made of PHA for endobiliary prosthetics and to investigate the biological properties of PHA suture material for forming of biliary-enteric anastomoses (cholecystoduodenostomy). Experiments were performed on 20 adult mongrel dogs, weighing 10–12 kg. The animals were divided to three groups: the negative control group (intact animals); the positive control group (the animals with implanted endobiliary silicon stents); and the experimental group (the animals with the PHA stents). The animals were monitored for 100 days. The clinical blood analysis was made before the operation, on day 7, 30, 60 and 100 days. During autopsy the presence of exudate, commissural process in the free abdominal cavity and subhepatic spatium, the appearance of choledoch where the prosthesis was located, as well as the appearance of cholecystoenterostomy, liver and duodenum. We did not found any signs of inflammation, cicatrical changes were estimated in the free abdominal cavity and subhepatic spatium. All implanted PHA stents were at their initial places of implantation. After the end of the experiment inflammatory reaction and anastomositis were absent. Macroscopic changes of liver and duodenum were also not detected. Liver function did not have any pathological deviations. These positive results give grounds to conclude that application of PHA as endobiliary stents in reconstructive surgery of bile passages and as suture material is a promising technology.

Keywords: bioresorbable materials; endobiliary stents; mechanical jaundice (MJ); PHAs

Introduction

One of most serious problems of using polymers in various spheres of practical medicine is the interaction of implanted polymer devices with organism tissues. The highly topical problem of using polymers in various spheres of medicine, except fundamental issues connected with interaction of new polymers with organism tissues, is of profound importance to practical medicine. Surgery is one of those medical sciences that lacks materials for clinical practice most of all. It is impossible to improve the results of operative interventions in reconstructive surgery of bile ducts without introduction of new biocompatible materials. The number of operations aimed at restoration of bile outflow is increasing corresponding to the increasing number of patients with malignant neoplasms, cholelithiasis, inflammatory diseases of liver and bile ducts.[1–2]

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Bile-excreting system diseases occupy the leading place in the structure of surgical pathologies of abdominal cavity. According to the data of IV International Congress of Gastroenterologists, cholecysto- and choledocholithiasis are the second most frequent diseases after atherosclerosis and cause up to 2.5 mln. elective and emergency operations on bile ducts every year.[1,3] Lately, treatment of patients with mechanical jaundice (MJ) has been one of the topical problems of biliary surgery. Due to the advances of the present-day techinques, it is possible to significantly improve the results of surgical treatment and reduce the number of complications and lethality. What favored such advances was the introduction into clinical practice of draining instrumental interventions aimed at reduction of cholaemia syndrome and the related affection of life-supporting organs and organism systems.[2,4]

Despite the impressive results of reconstructive biliary surgery, there are still many limiting moments that do not allow medics to solve the problem of treatment of patients with mechanical jaundice completely. The number of patients having MJ of oncologic character that can undergo radical surgery does not exceed 25–30%. 38–46% of these patients have the tumorous block localizes in the liver porta and affected the adjacent organs, which makes radical intervention difficult or even impossible. A way out of such situation is to use minimally invasive endobiliary interventions, the main of which is endoprosthetic restoration of bile ducts lumen.[3,5,6] The success of the operation to a large extent depends on the properties of a construction designed to provide adequate bile passage. Despite on vascular stenting using bioactive devices,[7–10] which is actively developing recent years, mini-invasive treatment of mechanical obstacles of bile passage with non-metallic, based on biocompatible polymers, stents is developed on a limited scale. Now, stent occlusion occurs in up to 25% of patients, and stent migration in up to 6%. Recurrence of biliary stricture after stenting occurs in 15–45% of patients after an average time of four to nine years.[11] The most frequently used synthetic materials for making endobiliary stents are silicon, Teflon, polyurethane, polyethylene and Percuflex.[12]

The clinical use of synthetic stents revealed a series of shortcomings such as quick obturation with components of bile within 3–6 months, migration of implants, increased traumaticity of transhepatic intervention. As Moss et al. reported in a review considering twenty-one clinical trials involving 1,454 people[13] in the palliative treatment of obstructing pancreatic carcinoma, where endoscopic stenting with plastic stents appeared to be associated with a reduced risk of complications, but with higher risk of recurrent biliary obstruction, compared with surgery. Neither Teflon, hydrourethane, or hydrophilic coating appeared to improve the patency of plastic stents above polyethylene in the trials reviewed. Only perfluoroalkoxy plastic stents had superior outcome to polyethylene stents in one trial. The single eligible trial comparing types of metal stents reported higher patency with covered stents, but also a higher risk of complications. Investigations revealed a number of limitations including invasion of metal mesh stents by tumor tissues, coupled with recurrence of MJ, impossibility of surgical removal of stent after invasion and occlusion, and necrosis of the mucous membrane of bile ducts.[14–15] The major alternative to biliary stenting is surgical repair of the stricture. The most common method is resection of the narrowed area followed by the creation of biliary-enteric anastomoses (cholecystoenterostomy and cholecistoduodenostomy). Surgical structure repair results in a cure for 85–98% of patients and is associated with a low risk of complications[11]. For such kind of operations requirements for properties of suture materials are also very stringent.

Lately, due to the advances in the sphere of polymer synthesis, the search for the optimal material for construction of endobiliary prostheses and suture materials has become more intensive. Unfortunately, up

to date there is no ideal material for production of stents and prostheses that would comply with all present-day requirements of biliary surgery.

The materials that are now actively being studied are linear polyesters, polyhydroxyalkanoates (PHAs). They are biocompatible and biodegradable polymers of microbial origin, which have many medical applications in perspective. PHA which causes minimal immune reactions after implantation, in particular with a view for prosthesis of tubular structures.[16,17]

There is an advanced experimental base at the Institute of Biophysics of the Siberian Branch of Russian Academy of Sciences (Krasnoyarsk, Russia) for biotechnology of PHAs of high and suitable for medicine purity.[19] Positive results of toxicological and biomedical tests[18] allowed us to carry out investigations of the applicability of these polymers for construction of biocompatible endoprostheses. PHA were used to develop and study two experimental models of vascular endoprostheses (coated metal stent and drug eluting stent loaded with a cytostatic drug). In experiments on animals the PHA coating showed high efficiency in terms of reduction of the vascular wall reaction and prevention of complications caused by metallic stents.[1]

The aim of our work was to study the biological properties of the stent experimental models made of PHA for endobiliary prosthesis and to investigate the biological properties of polymer suture material for biliary-enteric anastomoses.

Materials and Methods

Materials

The tested material was polyhydroxybutyrate (PHB) synthesized by *Wautersia eutropha* B5786 bacteria in the Institute of Biophysics SB RAS.[1] The polymers were extracted from bacterial biomass with methylene chloride and precipitated with ethanol. The PHB sample had the weight-average molecular mass (M_w) 300–320 kDa and crystallinities 72–75%. The procedure of re-dissolution and further precipitation of polymers was repeated several times to prepare specimens that would not contain organic impurities of protein, carbohydrate or lipid nature. All the organic solvents used in the procedure were preliminarily distilled to remove impurities. Polymers prepared and purified using this procedure can be used in medicine, including blood contact applications.[19] The trade mark of the material is Bioplastotan™.[20]

Design of Stents and Fibers

The stents were made by solvent-evaporation method from highly purified polyhydroxybutyrate samples. PHB, dissolved in methylene chloride (6%), was applied to the surface of metal tubules; after evaporation of the solvent the polymer was applied again, which resulted in a family of experimental stent models of various diameters. For this work stents 3.5 mm in diameter, up to 22 mm long, and wall thickness 0.09 mm were selected. The initial mass of the stents was around 0.1139 ± 0.016 g.

As the silicone stents in these experiments fragments of standard silicone tubes of similar dimensions (''Forvard Complect'' company, Russia) were used.

The suture fibers were produced by PHB melt flowing on a single-screw mini-extruder with round nozzle of 1 mm diameter (Brabender, Germany)[18]; the diameter of the fibers after orienting was 0.15 mm; strength - 320 MPa, elasticity coefficient 3.5 GPa.

In Vivo Model

Experiments were performed on 20 adult mongrel dogs, weighing 10–12 kg (Table 1). All works with animals were carried out in accordance with International and Russian regulations on laboratory animal care,[21–23] and by the permission of the Ethical committees of the Krasnoyarsk State Medical Academy and IBP SB RAS.

The animals were divided into three groups: the negative control (untreated animals), the positive control (the animals with silicone stents), and the treatment group (the animals with the PHA-coated

Table 1.
Group distribution of animals.

	Number of animals, n	Group characteristic
Negative control – comparison group	N = 7	Intact animals
1 test group – positive control	N = 5	Implantation of endobiliary stents made of silicon, cholecystoduodenostomy
2 test group – experimental	N = 8	Implantation of endobiliary stents made of PHA, cholecystoduodenostomy
Total	N = 20	

stents). The animals were kept in a vivarium and fed a standard diet. Feeding was stopped 24 h before surgery. In the early postoperative period the animals received antibiotic prophylaxis - Oframax (ceftriaxone) at the dose of 1.0 g two times a day during 3 days, as well as analgesics and spasmolytics.

Surgical Protocol

The animals were operated at room temperature in sterile conditions. The dogs were premedicated with intramuscular administration of Kalipsol (the dose of Kalipsol at the basic narcosis stage was 2 mg/kg of body weight, maintenance dose was 1 mg/kg of body weight). After supramedian laparotomy, the choledoch was verified, and choledochotomy was made. In the 2nd group a PHB stents were implanted to the supraduodenal part of the choledoch and fixed to the choledoch wall by PHB suture material. A silicone stent was implanted to the animals of the 1st group (positive control) and cholecystoduodenostomy was made using the widely spread suture material Vicryl. The final stage of the operation included control of hemostasis and foreign bodies in abdomina, layer-by-layer tight closure of the wound with Vicril sutures and application of aseptic dressing.

Clinical and Laboratory Tests

In the course of the experiment, the clinical blood analyses of animals were made before the operation and on the days 7, 30, 60 and 100 days after the surgery, including determination of content of hemoglobin, globular value, erythrocyte sedimentation rate, blood corpuscles.[24] The complete analyses of blood biochemical values were made using standard techniques[24]; the following parameters were determined: total blood protein, sugar, urea, total bilirubin; hemodiastase activity; liver function tests: alanine aminotransferase (ALT) and aspartate aminotransferase (AST), by Rightman-Frenkel method using the set ALT-, AST-1 ''FL'' (''Olvex Diagnosticum'' company, Russia).

To monitor the state of the non-specific immune system of animals we observed the phagocytic activity of lymphocytes, that was determined using NBT-test (Nitroblue tetrazolium test)[24] and spontaneous and stimulated luminal-dependent chemiluminescence of lymphocytes using luminometer – registering the time of reaching the maximum intensity (i-max) and the area (s-max) of chemiluminescence curve.[25]

The animals were monitored for 100 days. The experiment was finished by administering letal dose of thiopental sodium to the animals intravenously.

During autopsy we estimated the presence of exudate, commissural process in the free abdominal cavity and subhepatic spatium, the appearance of choledoch where the prosthesis was located, as well as the appearance of cholecystoenterostomy, liver and duodenum. Analising choledoch, the following factors were taken into account: choledoch wall thickness, presence of visible inflammatory changes, state of choledoch mucous membrane, reliability of endoprosthesis adhesion to choledoch wall, presence of defects in prosthesis construction as a result of

polymer biodegradation, size of endoprosthesis lumen, presence of sludge and concrement sedimentation inside the stents, thickness of endoprosthesis wall. Choledoch was examined in the place of stent implantation for infiltration, lumen expansion and cicatrical changes.

Histological Investigation

The morphological methods of tissue investigation included macroscopic description and morphometric characteristic of tissue specimens. The segment was prepared by making 5–10 cross-sectional cuts. Five histological specimens were prepared from each fragment. The specimens were embedded in paraffin and 5 μm-thick sections were cut. The sections were stained by Van-Gieson method with hematoxylin-eosine, and with fuchselin and examined under a light microscope ''Lyumam I-1''. A Carl Zeiss Image Analysis System (Germany) was used for viewing and analysis of microscopic images.

Statistical Analysis

The data were presented as means and standard errors of means. Statistical comparisons were performed using Student's *t*-test. *P*-values <0.05 were considered as statistically significant.

Results and Discussion

All the surgeries successful and the animals recovered from narcosis. The sutures were removed on the 14th day, healing of post-operative wound - per prima. The animals were fed with standard diet from the 5th day; there were no clinical or laboratory data indicating a lack of anastomoses or a non-specific inflammatory reaction to the implant. During the whole period of observation the animals were active and had good appetite. The dogs were weighed every week; no body weight loss was detected.

After the autopsy of choledoch lumen, the following factors were taken into account: choledoch wall thickness, presence of visible inflammatory changes, state of choledoch mucous membrane, reliability of endoprosthesis fixation to choledoch wall, presence of defects in prosthesis construction as a result of polymer biodegradation, size of endoprosthesis lumen, presence of diminution as a result of sludge and concrement sedimentation, thickness of endoprosthesis wall.

The autopsy detected no pathologies of abdominal cavity in the animals of the comparison group.

3 animals of the 1st test group (silicone stents) had an insignificant amount of serous exudate in the abdominal cavity (up to 30–40 ml), and a moderate commissural process in the subhepatic spatium. It was verified that 2 animals of this group had stent migration towards Vater's ampulla. The appearance of cholecystoduodenostomy (presense of hyperaemia, infiltration, cicatrical deformation in the area of anastomosis and duodenum) allowed us to conclude that 2 animals had anastomositis. The macroscopic examination of liver showed that it had normal characteristics, 2 animals had moderate hepatomegaly. The autopsy of choledoch lumen of all animals showed that the choledoch wall had thickening, sclerosis and infiltration. The silicon stent was easily extracted from the lumen. Choledoch mucous membrane in the place of contact with the stent was of pale pink color, there were atrophied zones detected, 3 animals had the stent lumen narrowed by 40–50% of the initial one as a result of sludge and sedimentation of bile components. All silicone stents were fragile and had sediments of salts and bile pigments. Cholecystoduodenostomy was obturated in 2 animals of this group. The other animals had a sufficient anastomosis lumen, a moderate inflammatory process was registered in the area of mucous membrane, and Vicryl sutures were visualized.

After the withdrawal of the 2nd group of animals from the experiment, the autopsy did not reveal exudates, commissural changes in the free abdominal cavity and subhepatic spatium. The choledoch in the place of stent implantation had normal appearance; no its expansion, inflammatory

reaction or cicatrical process was visualized. All implanted PHA stents were at their initial places of implantation, no cases of migration were detected. All animals had adequate cholecystoduodenostomy, no inflammatory reaction or data indicating anastomosites were detected. No macroscopic changes were revealed during the examination of liver and duodenum. Choledoch lumen remained in the place of stent implantation in all animals and had a normal size (0.4–0.5 mm), without any deformations, strictures, cicatrical or inflammatory changes in the stent implantation zone. Extracting the stents, we registered their leaky adhesion to the choledoch mucous membrane, a small effort was enough to extract the stents from the choledoch lumen. The stents retained their initial physical properties, were not calcificated, there was no narrowing of stent lumens, their diameters were of 3.5 ± 0.1 mm. Biodegradation did not cause any visible defects, except that the stent wall became a lightly thinner, the average stent wall thickness was 0.05–0.08 mm. No narrowed areas of stent lumens were detected. The macroscopic examination of cholecystoduodenostomies did not reveal any data indicating inflammatory, infiltrative or cicatrical changes; all anastomosis were functioning. The average diameter for anastomosis lumens was 12.3 ± 4.3 mm. No traces of PHA suture material were detected in the place of single-layer continuous anastomosis to 100th day.

The results of the clinical and biochemical blood values of the animals are specified in Table 2 and 3.

The analysis of the composition of peripheral blood in the control and test groups showed that generally these parameters were within the limits of physiological values and norms. The insignificant increase of amount of leukocytes (from 10 to 12×10^9/L) and ESR (up to 10–15 mm/h) were registered on the 7th day after the operation in all groups of operated animals versus the control. By the end of the period of observation, the animals of the 1st test group had a moderately increased ESR (up to 10.1 ± 3.3 mm/h). No shifts in the leucogram of the experimental animals during the whole period of observation were registered (Table 2).

The analysis of the values of urea and total protein in the blood serum of the animals shows that the implanted polymer PHB-based stents did not have negative effect on the nitrogen metabolism indices and kidney function of the animals. There were no significant differences between the comparison group and test groups with regard to the indices characterizing the function of pancreas (blood amylase). Beginning from the 30th day, the animals of the 1st group had a moderately increased total bilirubin level caused by bile flow disorder. On the 100th

Table 2.
Full-scale blood count indices of test group animals

Parameters	Comparison group – negative control –	Group 1 Positive control	Group 2 Experimental
Hemoglobin	142 ± 3.5 g/L	137 ± 1.9 g/L	149 ± 2.8 g/L
Erythrocytes	$5.12 \pm 1.2 \times 10^{12}$/L	$4.89 \pm 1.5 \times 10^{12}$/L	$5.68 \pm 0.9 \times 10^{12}$/L
Globular value	1.0 ± 0.1	0.9 ± 0.4	0.8 ± 0.2
Leucocytes	$7.2 \pm 0.9 \times 10^9$/L	$9.8^a \pm 1.3 \times 10^9$/L	$7.6 \pm 1.6 \times 10^9$/L
ESR	3.2 ± 1.6 mm/h	$10.1^a \pm 3.3$ mm/h	4.7 ± 2.1 mm/h
Leucogram			
Stab, %	2.3 ± 0.1	5.2 ± 0.2	3.1 ± 0.3
Segmental, %	68.4 ± 3.6	64.2 ± 4.1	67.9 ± 3.9
Monocytes, %	2.5 ± 0.3	1.1 ± 0.2	3.2 ± 0.1
Lymphocytes, %	26.8 ± 3.2	29.9 ± 4.3^a	24.8 ± 4.8
Eosinophils, %	2.1 ± 0.1	1.3 ± 0.3	2.2 ± 0.2
Plasmocytes, %	1.1 ± 0.1	1.4 ± 0.1	1.2 ± 0.1

[a] Differences are significant as compared to the control group.

Table 3.
Biochemical blood count indices of animals on the 100th day.

Parameters	Comparison group negative control	Group 1 Positive control	Group 2 Experimental
Amylase	64.6 ± 4.39 g × h/L	89.9 ± 6.7 g × h/L	73.3 ± 5.9 g × h/L
Total protein	71.9 ± 3.2 g/L	59 ± 4.6 g/L	67.6 ± 4.8 g/L
Total bilirubin	8.8 ± 1.8 mmol/L	39.9[a] ± 4.3 mmol/L	9.1 ± 2.0 mmol/L
Sugar	4.2 ± 0.9 mmol/L	4.9 ± 1.9 mmol/L	4.5 ± 1.2 mmol/L
Urea	4.9 ± 1.7 mmol/L	7.1 ± 2.3 mmol/L[a]	5.6 ± 1.8 mmol/L[a]
AST	0.88 ± 0.4 c.u.	1.01 ± 1.9 c.u.	0.96 ± 0.2 c.u.
ALT	0.84 ± 0.7 c.u.	2.15 ± 3.7 c.u.[a]	0.86 ± 0.6 c.u.

[a] Differences are significant as compared to the control group.

day the animals of this group demonstrated an increased ALT level to 0.84 ± 0.7 c.u. which indicates cholestasis progression and compromised liver function (Table 3).

Registration of liver function indices of the animals during the experiment did not reveal any pathological deviations in the experimental group. The total bilirubin (cholestasis main marker) of test and control animals was within the physiological norm (Table 3). The activity of hepatic enzymes, ALT and AST during the experiment did not reveal AST deviations. The slight increased ALT activity registered in the first period of observation in the experimental animals and can have been caused either by operational trauma or by the toxic effect of narcosis drugs.

The results of the study aimed at determination of the degree of influence of implanted polymer stents and PHB fibers on the nonspecific immunity of the animals are specified in Table 4. These nonspecific immunity indices of the test group animals are characterized by a moderately increased activity, which is indicated by an insignificant increase of phagocytosis level and stimulated chemiluminescence. However, no inhibition or decrease of phagocytic activity was detected, which suggests that

Table 4.
The indices of the phagocytic activity and luminol-dependent chemiluminescence of animal blood lymphocytes on the 100th day of observation.

Parameters	Comparison group negative control	Group 1 Positive control	Group 2 Experimental
Phagocytosis	32 ± 3.6	58 ± 5.4[a]	53 ± 4.3[a]
Complete 30 min.	21 ± 2.1	47 ± 3.2[a]	44 ± 5.4[a]
Complete 90 min.	19 ± 2.3	45 ± 3.4[a]	42 ± 3.1[a]
Index of phagocytosis completeness	1.02 ± 0.25	1.08 ± 0.13[a]	1.05 ± 0.25
NBT-test			
Spontaneous	413 ± 13.2	434 ± 16.8	426 ± 14.2
Stimulated	445 ± 11.2	471 ± 12.7	465 ± 12.4
Stimulation index (S_{cn}/S_{cm})	1.06 ± 0.32	1.08 ± 0.23	1.09 ± 0.31

Chemiluminescence	Spont.	Spont.	Stimul.	Stimul.	Stimul.	Stimul.
t-max – time of reaching the peak by the curve (sec)	255 ± 10.2	298 ± 9.6	341 ± 12.4[a]	311 ± 14.3[a]	240 ± 10.5	285 ± 11.7
i-max – number of impulses at the peak	1511 ± 32.8	1487 ± 38.9	3248 ± 56.9[a]	4248 ± 45.7[a]	1457 ± 27.9	1329 ± 34.1
s-max – area of the peak	1.71 ± 0.4 × 10^5	1.92 ± 0.6 × 10^5	3.88[a] ± 0.9 × 10^5	5.08[a] ± 0.6 × 10^5	1.67 ± 0.6 × 10^5	1.8 ± 0.5 × 10^5
Stimulation index (S_{cn}/S_{cm})	1.08 ± 0.24		1.31 ± 0.21		1.07 ± 0.14	

[a] Differences are significant as compared to the control group.

there was no long-term antigenic stress and ''immunological paralysis'' phenomenon.

No pathological changes were detected during morphologic investigation of the specimens of choledoch, gallbladder, duodenum and liver.

All specimens of the 1st test group animals had signs of inflammatory cellular reaction and fibrosis. The choledoch mucous membrane was atrophied, with necrotic patches. The liver had signs of cholestasis, beam and hepatocyte fracture. In the zone of anastomoses, too, there were signs of inflammatory cellular reaction, large number of leucocytes, macrophages, rough scar tissue.

The morphologic investigation of the choledoch area of the 2nd test group animals with PHB stent did not reveal any pathological changes. Common bile duct is lined with a mucous membrane with high cylinder-shaped epithelium, each cell resembles the adjacent one. The lumen has traces of bile. Epithelium has microfibers (Figure 1a). The interfacial cytoplasm of epithelial cells has granules. Epithelium forms numerous folds, it is located on the proper mucous plate with its loose connective tissue, and the whole mucous membrane lies on the layer of smooth muscle tissue, which are interleaved with connective tissue and elastic fibers (Figure 1b and 2a). Behind the muscular layer there is a subserous membrane (Figure 2b) with its loose connective tissue, in which fat cell groups, arteries, veins, lymphatic vessels and nerves are located. The serous membrane of choledoch consists of a thin layer of mesothelium. This morphologic pattern is within the limits of the norm, which is explained by the absence of inflammatory cellular reaction and proliferation of any tissue.

The mucous membrane of gall bladder is lined with high cylinder-shaped epithelium, the latter has microfibers. The interfacial cytoplasm of epithelial cells has granules. Epithelium forms numerous folds, making an impression that the mucous membrane has glands, but this is explained by bladder wall contraction. Epithelium is located on the proper mucous plate with its loose connective tissue, and the whole mucous membrane lies on the layer of smooth muscle tissue, which are interleaved with connective tissue and elastic fibers. Behind the muscular layer there is a subserous membrane with its loose connective tissue, in which fat cell groups, arteries, veins, lymphatic vessels and nerves are located.

The histologic pattern of duodenum revealed fibers in the form of digitules out of mucous membrane proper layer covered with epithelium on the surface of mucous membrane. The fibers are wide and are covered with cylinder-shaped one-row epithelium with single caliciform cells. The fibers of the stroma consists of connective tissue – it is a prominence of mucous membrane proper layer – and is formed by loose and reticular connective tissue,

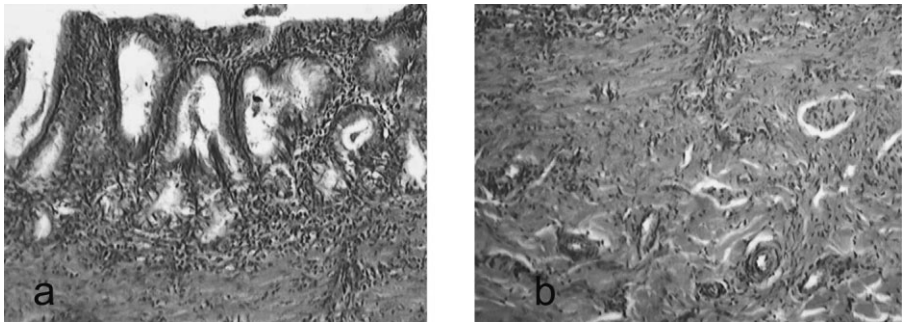

Figure 1.
Mucous (a) and muscular (b) layers of choledoch in the place of stent implantation in animals with implanted PHA stents.

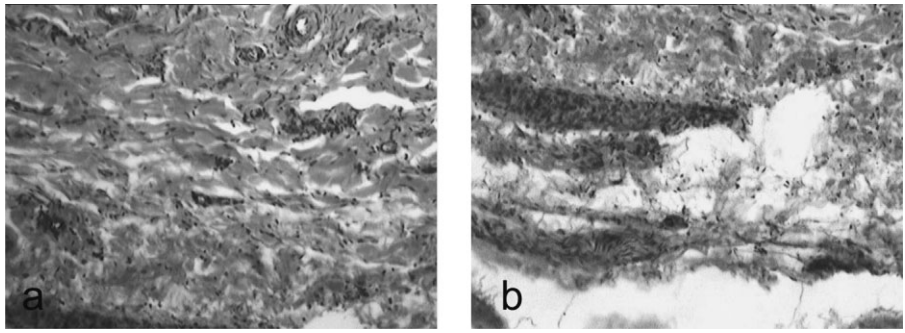

Figure 2.
Subserous layer (a) and serous membrane (b) of choledoch in the place of stent implantation in animals with implanted PHA stents.

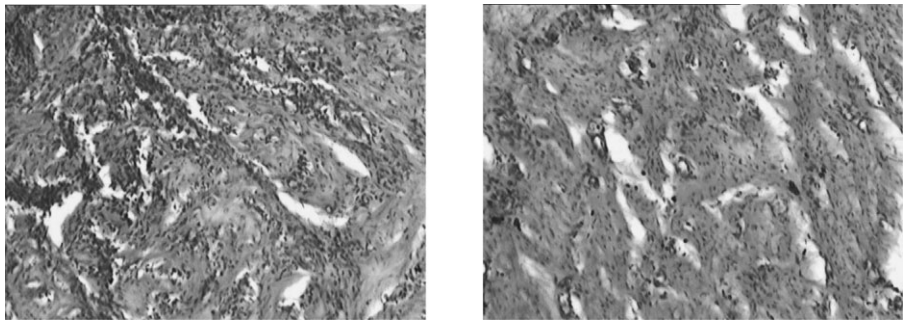

Figure 3.
Granulation tissue and mature fibrous tissue in the cholecystoduodenostomy zone of the animals with implanted PHA stents.

smooth muscle fascicles and blood vessels. Under the base of the fibers there are Lieberkuhn's glands in the form of tubules, lined like tectorial epithelium, which open between the fibers into the intestine lumen (crypth openings). Under the mucous membrane layer there is a muscular layer, consisting of smooth muscle cells. Below is a submucous layer where among loose connective tissue there are Brunner's glands (which are present only in duodenum, like Lieberkuhn's glands). They have flat nuclei and weakly basophilic cytoplasm.

No pathological changes were revealed in the histological specimen of the liver of the 2nd group animals.

The morphologic study of the anastomosis area between the duodenum and gall bladder (cholecystoduodenostomy), too, returned positive results (Figure 3).

At the anastomosis level, closer to the submucous layer, there was localized developing granulation tissue with vessels of capillaceous type, as well as fibroblasts, epithelial, plasmatic cells, lymphocytes, eosinophils and single leucocytes. Such pattern complies with the anastomosis formation period (100 days) and indicates that the regeneration process is nearing completion. The study detected formed vessels, smooth muscular cells, connective tissue, vessel sections, nerve cells and a thin layer of mesothelium. No traces of suture material were detected in the specimen.

Conclusion

The physiological, biochemical and morphological investigations of the experimen-

tal group animals that had the implants of the experimental PHA sample products did not reveal any data indicating that polymer products (stents and monofilaments) cause pathological reactions of the tissues of bile passages. These positive results give grounds to conclude that application of PHA as endobiliary stents in reconstructive surgery of bile passages and as suture material is a promising technology which requires further investigation.

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Correction

In Vitro Justification of Using Endobiliary Stents Made of Polyhydroxyalkanoates

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The author likes to announce the following corrections:

In Vivo Justification of Using Endobiliary Stents Made of Polyhydroxyalkanoates

The author likes to apologize for any inconvenience this mistake may have caused!