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New hemostatic preparation made of the cellulose derivatives

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ABSTRACT

Aim. The aim of this study was to investigate the indexes the indexes of biocompatible pellicle hemostatic coverage *in vitro* and *in vivo* conditions. Methods. Samples of pellicle hemostatic coverage on the basis of the cellulose derivatives were used in researches. Breaking strength, estimation of the implant's structure and adhesion power were evaluated according to the Ts 05957837-28:2014 instructions and documents of the National certification system of the Republic of Uzbekistan and with the using the apparatus "Zwick" (Germany) and atomic-power microscope of Agilent technologies (USA). Hemostatic activity of the coverage on the basis of the cellulose derivatives was estimated by the Lee and White test for the blood coagulation time. For *in vivo* research, 30 mature rats were required. Operations were performed under inhalation anesthesia, and the wound of liver was formed. Both macroscopic and microscopic studies had been undertaken. Morphological changes were studied in terms of 3 and 12 hours and then on the 1^{st}, 3^{d}, 7^{th}, 14^{th} and 30^{th} day after an operation. Results. An adhesion power of the pellicle coverage on the basis of the cellulose derivatives was 7.3±0.2 N/cm^2, breaking strength was 390±4.8 kGf/cm^2. In presence of polymer, a coagulation time on Lee and White test was shortened by as many as 2.1 times in relation to control that made up 2.4±0.6 min. In *in vivo* conditions hemostasis started during 3-5 sec. A weak inflammatory reaction of tissue was histologically determined. Further observations over dogs showed that an hour after an operation, an implant had been preserved on the surface of liver as a white pellicle and had not been separated from the wound surface. Bleeding signs were not marked. An abdominal cavity remained intact. Conclusion. Rapid enough biodegradation of polymer along with the unexpressed inflammatory reaction allows preventing the infecting related to the presence of foreign body. The rapid forming of fibrotic tissue in a zone of lesion makes it possible to obtain a durable hemostasis. A poorly expressed reaction was also marked from the side of peritoneum and surrounding organs. Recommendations. The oxidized regenerated cellulose can result in an intensive inflammation of the surrounding tissues because of the low level of pH that had not happened in the present research due to the selected correlation of ingredients of hemostatic pellicle.

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Hemostasis, Morphology, Carboxymethylcellulose, Oxidized Cellulose, Hemostatic Substance.

INTRODUCTION

Cellulose is a natural polymer of vegetable origin, not water-soluble. It possesses a good biocompatibility, does not cause an allergic reaction, and in this connection is widely used in medicine and pharmaceutical industry. Oxidized regenerated cellulose (ORC) – is the water-soluble cellulose's derivative which is synthesized by the influence of oxidizing agents. ORC in the organism of a human biodegrades without the forming the toxic substances and does not cause the expressed inflammatory reaction of tissue [1]. Additionally ORC possesses an antibacterial effect initiating the forming the unfavorable pH environment. Bio-absorption of ORC proceeds during two weeks [2]. ORC is successfully used in plastic surgery because it does not cause an edema or deformation of tissue [3]. Mechanisms of the favorable healing and fibrogenesis are explained by the ORC's absorption of free radicals, ions of metals, stabilizing of growing factors and others [4].

Carboxymethylcellulose (CMC) is a nontoxic and biocompatible polymer owing to what it finds a wide use in pharmaceutical, cosmetic, and food industry [5-7]. Ca-CMC unlike Na-CMC is not dissolved to the end in water, swells and forms a gel substance [8]. The mechanism of hemostatic action of Ca-CMC is in a point that at

a contact with blood the ions of calcium accelerate the coagulation process however the surplus amount of calcium can provoke a burn and necrosis of tissue [9].

The aim of this study was to investigate the indexes the indexes of biocompatible pellicle hemostatic coverage *in vitro* and *in vivo* conditions.

MATERIAL AND METHODS

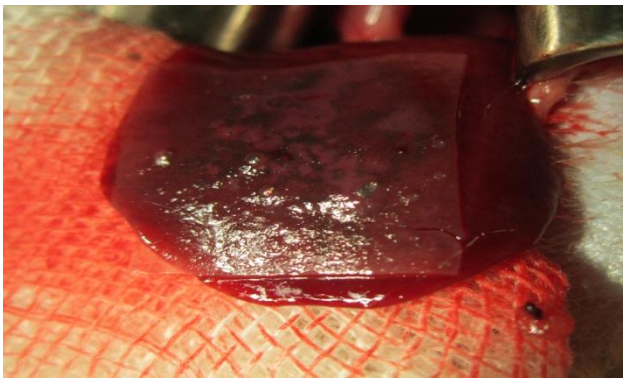
The authors together with the scientific research center of chemistry and physics of polymers under the Academy of sciences of the Republic of Uzbekistan, worked out a new composite polymer material (CPM) possessing hemostatic property for the surgery of liver (30 white mature outbred male rats weighing 198±2.7 gr were used). (Patent No.IAP 20160273 "Bio-absorbable surgical hemostatic substance", registration from 13.06.2016, Nazirov F.G., Rashidova S.Sh., Sadykov R.A., Sarymsakov A.A., Alimov M.M., Ismailov B.A., Li Yu.B.). It presents itself as a semi-transparent flexible pellicle moderately water-soluble coverage (Picture 1). The temperature of melting is 220°C. It is stable at pH 5-7. Rapidly hydrolyzing in an alkaline environment and more steady in an acidic environment.

At the light microscopy of implant fibers of the oxidized cellulose were located evenly on the entire surface of pellicle. Methodologies of the evaluating the breaking strength, estimation of the implant's structure and the determining the adhesion power were conducted according to the Ts 05957837-28:2014 instructions and documents of the national certification system of the Republic of Uzbekistan and using the apparatus "Zwick" (Germany) and atomic-power microscope of Agilent Technologies (USA).

At the studying the blood coagulation time (BCT) in presence of hemostatic coverage on the basis of the cellulose derivatives on Lee-White test a polymer in the kind of a pellicle with the size one cm^2 was placed in a test tube with one ml of venous blood. The test tube was placed in water bath at t - 37° and incubated within two minutes, and then after every 30 sec the test tube was inclined under the angle of 45°. The time of the coagulum appearance was fixed. Control samples were investigated by analogy without adding the polymer to the whole blood.

Experimental studies of the new composition hemostatic coverage were undertaken in accordance with the Russian national standard ISO 10993-6-2011. Guideline: 30 white mature outbred male rats weighing 198±2.7 gr were used. Operations were performed under inhalation anesthesia (Isofluranum) with the modeling the wound of liver with an active parenchymatous bleeding. A hemostatic effect was achieved by the application of pellicle coverage (Picture 2). Morphological changes were studied in terms of 3 and 12 hours and then on the 1^{st}, 3^{d}, 7^{th}, 14^{th} and 30^{th} day after an operation. Macroscopic and microscopic studies were undertaken.

Picture 1. Sample of the Composite polymer material



Picture 2. Stopping the bleeding with pellicle coverage (Composite polymer material)

Methodology of operation

Under inhalation anesthesia a supramedian laparotomy was performed with 3-4 cm lengthwise. A left hepatic lobe was withdrawn in a wound. On the surface of liver a flat wound with a diameter up to one cm and a depth up to 0.3 cm had been modeled. From the hepatic wound an active parenchymatous bleeding was marked. The pellicle coverage was applied on the bleeding surface. The control over the possible renewal of bleeding was kept by the supervision during ten minutes. A postoperative wound was sutured by interrupted sutures. Animals were withdrawn from anesthesia, and subsequently a dynamic supervision over the post-operational state was conducted.

In the fixed terms animals were withdrawn from the experiment for the estimating the macroscopic changes, and also for the intake of material for histological researches. Euthanasia was carried out according to the Provisions of ISO 10993-2 under the general anesthesia. During the experiment a macroscopic estimation of changes in an abdominal cavity was analyzed at the dissection of animals after euthanasia. For the getting ready the morphological preparations the tissue of liver was excised and fixed in 10% solution of neutral formalin. After expiration of the fixing terms bioptate was inundated by paraffin. Paraffin blocks had been manufactured. Series of sections with a thickness of three-four μm were made. Histological preparations were painted by hematoxylin-eosin.

For the estimating the histological changes in liver, a system of points was employed in accordance with Russian national standard ISO 10993-6-2011 where the parameters of semi-quantitative estimation of the number and distribution of cells characterizing the inflammatory process such as polymorphonuclear neutrophils, lymphocytes, plasmatic cells, macrophages, eosinophiles and multinuclear cells were taken into account. At the microscopy, the dynamics of the inflammatory reaction's development, features of the liver's parenchyma regeneration as well as the degree of the investigated implant's destruction, were estimated.

Ethical approval

The review board and ethics committee of RSCS named after acad. V.Vakhidov approved the study protocol and informed consents were taken from all the participants. Experimental studies had been undertaken with the observance of the rules accepted by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS N 123), Strasbourg, 18.03.1986.

Statistical analysis

The obtained results were subjected to the statistical processing with the using the standard package of Microsoft Excel 2010software by the method of variation statistics with the estimation of indexes' values (M±m)

RESULTS AND DISCUSSION

It was set by researches that adhesion required for medical aims had to provide the dense adherence of pellicle to the wound surface that conditioned an instantaneous hemostatic effect in the final outcome. An adhesion power of pellicle coverage made up 7.3±0.2 N/cm^2. Such a high adhesion of implant was related to its flexibility and moderate hydrophilism that strengthens this effect in the aggregate.

At the estimating the breaking strength an attention was paid to the ability of implant to hold the edges of the wound surface that provides the effect of the strengthening the tissue. It was set from the data of comparative researches that the worked out implant had a limit of the breaking strength within the limits of 390±4.8 kGf/cm^2 that fully satisfied the requirements to the implants of a similar origin.

In the polymer's presence BCT on Lee-White test was shortened by as many as 2.1 times in regards of the control that made up 2.4±0.6 min. The obtained results testified to the strengthening the process of the donor blood's coagulation in vitro in the presence of implant due to the activating the factors of an external and internal way of the coagulation's hemostasis.

The results of *in vivo* researches, showed that hemostasis had started after the applying the polymeric coverage within a short time. A complete hemostasis was marked during 3-5 sec. After the supervision during 10 minutes the resumption of bleeding was not marked.

Subsequently, an activation of factors of the strengthening the hemopexis led to the forming the fibrinous pellicle on the surface of the hepatic wound. Further observations on animals showed that in an hour after an operation an implant had been preserved on the surface of liver as a white pellicle and had not been isolated from a wound surface. Bleeding signs were not marked. An abdominal cavity remained intact. In subsequent terms a substitution of pellicle coverage for fibrin without a considerable inflammatory reaction had been marked. It was paid attention to the absence of the expressed accretions in the place of the hemostatic coverage application.

The result of dissection in 3 hours after an implantation showed that there was not free liquid in the abdominal cavity, a renewal of bleeding had not been marked, fibrin pellicle was in the area of the wound. At the microscopic research it was marked an inflammatory process which was characterized in visual field by a presence of polymorphonuclear neutrophils – 18.1±0.6, lymphocytes – 14.3±0.8, plasmatic cells – 4.4±0.4, macrophages – 2.5±0.2. The pellicle fitted closely to the hepatic tissue covering the zones with signs of the

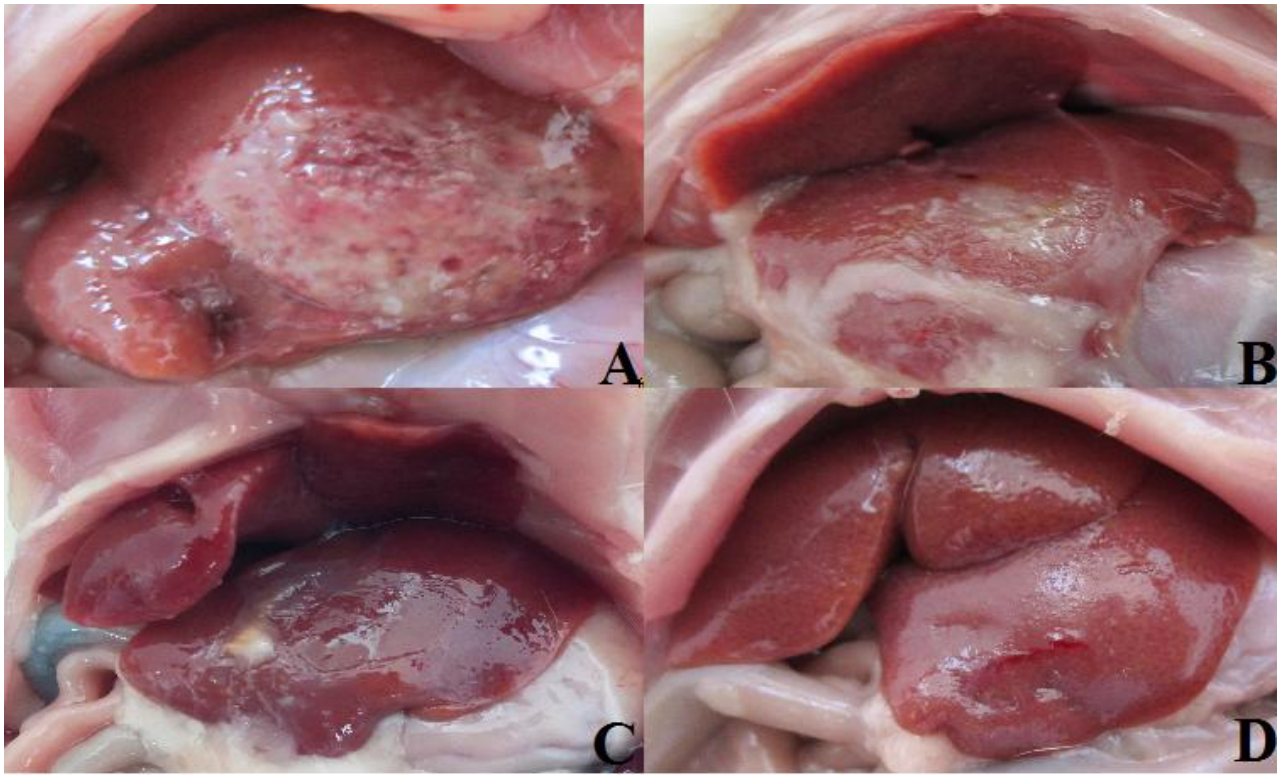
stopped bleeding. The light microscopy of the wound surface of liver showed the presence of a considerable number of hemolyzed erythrocytes testifying to their appearance in the moment of trauma, and also a lot of micro-thrombus in vessels pointing to the hemostatic effect of implant.

After 12 hours, the presence of free liquid in the abdominal cavity was not revealed, a friable adhesion process with the involving the anterior abdominal wall and a dense fibrin pellicle on the wound surface were marked. The necrotic tissue with thickness 80 μm was marked microscopically. Around the hemostatic coverage as a reaction on a foreign body an inflammatory process was marked with an increase in amount of polymorphonuclear neutrophils up to 27.3±0.9, lymphocytes – 18.8±0.9, plasmatic cells – 5.8±0.3, macrophages - 10±0.3 in visual field.

At the macroscopic research on the 1st day in an abdominal cavity a friable adhesion process with the involving the epiploon and an expressed coverage of wound surface with fibrin were noted. Microscopically subcapsularly a vacuolar dystrophy of hepatocytes with the expansion of sinusoid spaces was marked. The amount of elements of inflammation in visual field made up: polymorphonuclear neutrophils – 29.8±0.7, lymphocytes – 13.2±0.7, plasmatic cells – 2.9±0.4, macrophages –7.7±0.3. A vacuolar-granular dystrophy of hepatocytes and a necrotic layer of tissue with thickness up to 120 μm were marked.

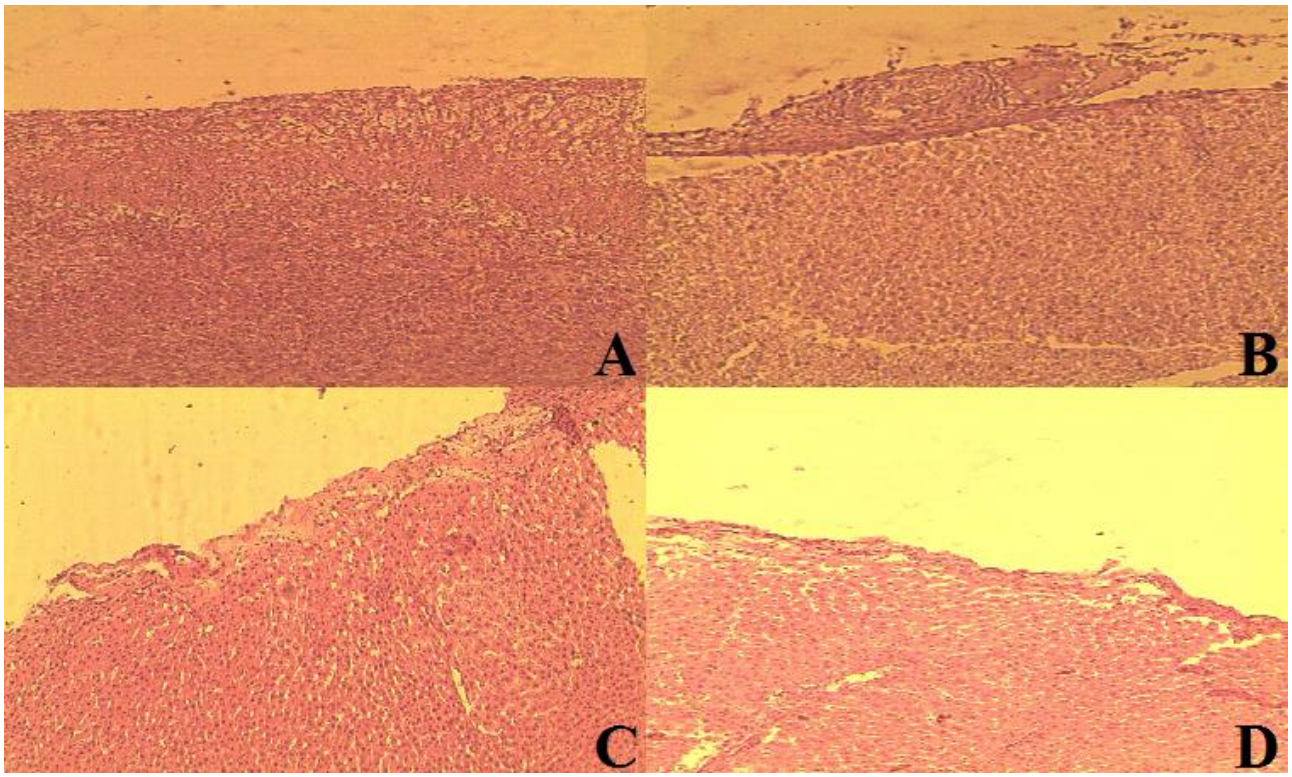
On the 3^{d} day in an abdominal cavity a moderate adhesion process with the epiploon's areas was registered. The loops of small intestine were not engaged in a process. An insignificant decrease in fibrin pellicle was marked (Picture 3A). At the microscopy an abatement of inflammatory process was marked. The vacuolar dystrophy of hepatocytes was visualized in a subcapsular zone (Picture 4A). The thickness of the necrotic layer made up 93±4.6 μm.

On the 7th day of the experiment in an abdominal cavity an adhesion process was preserved with the surface of liver, epiploon and xiphoid process. A decrease in fibrin pellicle was marked (Picture 3B). Microscopically the fibrosis of the hepatic capsule was marked with an abatement of inflammatory infiltrate which was expressed as a reduction in the amount of polymorphonuclear neutrophils up to 4.7±0.6, lymphocytes – 7.7±0.4, plasmatic cells – 1.5±0.3. The thickness of the necrotic layer made up 68±5.3 μm (Picture 4A).



Picture 3. Macroscopy. The 3^{d} day after application of composite polymer material, formation of fibrin pellicle (**A**). The 7th day after application of composite polymer material decrease in fibrin pellicle with the forming the moderate adhesion process (**B**). The 14th day after application of composite polymer material, calming down of the adhesion process with the forming the pellicle coverage (**C**). The 30th day after application of composite polymer material, regeneration of the hepatic wound surface (**D**).

On the 14th day the expressed adhesion process in the abdominal cavity was not marked. A thin transparent fibrin pellicle was marked on the surface of liver (Picture 3C). Microscopically a moderate lymphoid infiltration of the hepatic capsule was marked with decrease in visual field in amount of polymorphonuclear neutrophils – 1.3±0.4, lymphocytes – 5.9±0.4, plasmatic cells – 1.2±0.2. The necrotic layer was 19.8±2.3 μm. The fragments of polymer were determined that testified to its resolution (Picture 4C). On the 30th day a moderate adhesion process was marked in the abdominal cavity. The wound surface of liver was of soft consistency, smooth and without a cicatricial change and signs of inflammation (Picture 3D). Microscopically a reduction in thickness of fibrotic pellicle up to 9.5±1.0 μm was marked. A decrease in the amount of elements of inflammatory character (a rare quantity of lymphocytes) was noted. Complications and lethal outcomes during the operation and after it had not been observed (Picture 4D).



Picture 4. Morphology. Light microscopy. Magnification x200. Coloration Hematoxylin-Eosin. The 3d day after application of composite polymer material, vacuolar dystrophy of hepatocytes (**A**). The 7th day after application of composite polymer material, reduction in inflammatory process (**B**). The 14th day after application of composite polymer material, calming down of inflammatory process, fragments of implant (**C**). The 30th day after application of composite polymer material, single elements of inflammation, regenerative process (**D**).

Microscopically the expressed patho-histologic changes were not discovered in the hepatic tissue. A capsule of liver was not incrassate and it contained longitudinally oriented fascicles of collagen fibers. Interlobular connecting tissue was poorly developed, the signs of inflammatory infiltration and fibrosis of liver were not found. Hepatocytes were of polygonal form with a centrally located nucleus, frequently a nucleolus was determined. Quite often binuclear hepatocytes occurred. Sinusoid capillaries were of ordinary sizes. Singular erythrocytes and leucocytes were determined in a lumen. In the wall of sinusoid hemocapillaries and Disse spaces singular Kupffer cells having an intact structure were revealed at a large magnification. A moderate dilation and blood filling of sinusoid hemocapillaries, central and underlobular veins were noted in some cases. An endothelial lining was without destructive changes, in some places swelled endotheliocytes with hyperchromic nucleus were marked. The structure of cholangiol and interlobular biliary ducts was without pathological changes. And all this pointed to the fact that the studied preparation did not influence the microscopic structures of liver negatively.

From the presented data it is possible to conclude that hemostatic coverage caused a morphological reaction of liver as an inflammation and excrescence of the connecting tissue on the 1st day. But these processes calmed down quickly. A complete resolution of implant was being observed on the 7-14th days. To the 30th day after the implantation regenerator processes in the hepatic parenchyma, especially, in the zone of lesion were

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