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Comparative efficacy of the hemostatic implant made of the cellulose derivatives on the model of parenchymatous hemorrhage from liver

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ABSTRACT

Aim. The aim was to study the comparative efficiency of the hemostatic implant made of the cellulose derivatives on a model of the parenchymatous hemorrhage from a liver. Methods. Experimental studies on the biocompatibility's evaluation were conducted in accordance with the Russian national standard ISO 10993-6-2011. Operations have been performed under the general anaesthetizing with the modeling the parenchymatous hemorrhage from the wound of liver. A total of 72 white mature outbred rats of both sexes weighing 196.5±2.8 g were used from which 36 ones made up group of comparison using the application hemostatic material, Sergicel® Fibrillar™. In the basic group of rodents (36) powder Heprocel in equal amounts by weight of 30 mg was applied on a wound. Results. From the results it is possible to come to a conclusion that the hemostatic Heprocel implant causes on the first day morphological reaction of liver as an inflammation and a spread of the connecting tissue, but these processes calm down quickly. An inflammatory reaction was less expressed than the control group. To the 30th day in the basic group after application of Heprocel biodecomposition of hemostatic implant was being marked, there were regenerator processes in the liver's parenchima especially in the zone of lesion that testifies to renewal of liver's tissue, while in a comparison group an active degradation of the application hemostatic material began on the 30th day and an expressed adhesion process in an abdominal cavity took place. Conclusion. Hemostatic powder closely adjoins the liver's tissue, stops bleeding, cases of relapse of bleeding were not marked. Histological researches conducted in the dynamics of the healing showed that the wounds of liver educed that Heprocel did not cause the expressed inflammatory reaction, the zone of lesion did not exceed 150 µm, and the biodecomposition started after 14 days.

Original Article

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Hemostasis, Parenchymatous Hemorrhage, Liver, Cellulose's derivatives, Experimental surgery, Hemostatic implant, Morphology of liver.

INTRODUCTION

Nowadays a lot of preparations and their combinations possessing the hemostatic activity at local application are well-known. All of them, however, have certain limitations. It is possible to attribute materials based on gelatin, cellulose and collagen (Spongostan, USA) and combined hemostatic materials (Tachocomb, Austria) to traditional hemostatic means. For example, preparations based on collagen possess a low hemostatic activity in humid media, badly stop hemorrhage at systemic coagulopathies and thromboblasthemia, have a potential possibility of the infection, and are inactivated at autoclaving that substantially reduces their efficiency and limits a sphere of application [1].

Modern hemostatic material Tachocomb presents itself as a sponge of collagen of the horse tendons, human lyophilized fibrinogen, pig fibrin, and animal's aprotinin. Its basic defect is a presence of components of animal origin which can cause an allergic reaction [2]. Application of preparations on the basis of gelatin is connected with a high probability of the infecting in a zone of the applying the implant [3].

Implants on the basis of polymeric materials find an increasingly wider use in surgery [4]. Hemostatic preparations obtained from the polysaccharides' derivatives and, first of all, from oxidized cellulose (OC) are of a considerable interest [3, 5, 6]. OC accepted internationally after it firstly used in 1945 [7], because of its bactericidal properties, favorable biocompatibility, and overall ease of use [8]. Oxidized cellulose possesses a hemostatic action and is widely used in surgery for treatment of skin wounds, protractedly non-healing chronic ulcers, resection of kidney [9-11]. The principle of the regenerated OC's hemostatic action is in the change of pH media in anoxidosis side (pH 2.5-3.0) that creates favorable conditions for the forming the thrombocyte clot. Also anoxidosis environment in the zone of lesion contributes to the nonspecific anti-microbe activity of OC [5].

OC possesses a good biocompatibility and biodegradation, is not toxic, chemically inert, non-soluble in the water, has a fibred structure and high mechanical durability [12].

A hemostatic implant on the plants basis Ankaferd Blood Stopper (ABS, Turkey) was examined for the hemostasis from the bone tissue. An inflammatory reaction was evaluated on the amount of inflammation cells: 0-25% - weak, 25-50% - moderate, 75-100% - expressed. The factors of necrosis were considered on a qualitative sign: presence or absence. Besides the inflammatory reaction a number of newly formed osteoblasts were estimated. On the scale of Moretton for the implants of bone tissue Ankaferd was determined as an agent which does not cause an active inflammation and but assisting the tissue healing [9]. The tissue reaction caused by a hemostatic implant is one of the reliable factors of the estimating the biocompatibility of a product and conditioned by terms and character of biodegradation [13, 14]. Therefore, the aim of this study was to investigate the comparative efficiency of the hemostatic implant made of the cellulose derivatives on a model of the parenchymatous hemorrhage from a liver.

MATERIAL AND METHODS

Hemostatic Heprocel implant was worked out in SI "Republican Specialized Scientific and Practical Medical Center of Surgery named after Academician V.Vakhidov". Basic components of implants were as follow: sodium salt of carboxymethyl cellulose, oxidized cellulose and nanocellulose (Patent No. IAP 20160273) [1]. Experimental studies on the biocompatibility's evaluation were conducted in accordance with the Russian national standard ISO 10993-6-2011. Operations were performed under the general anaesthetizing with the modeling the parenchymatous hemorrhage from the wound of liver. 72 white mature outbred rats of both sexes weighing 196.5±2.8 g were used from which 36 ones made up a group of comparison with the using the application hemostatic material of Sergicel® Fibrillar™. In the basic group of rodents (n=36) powder Heprocel in equal amounts by weight of 30 mg was applied on a wound.

Under inhalation anesthesia (Halothanum) a supramedian laparotomy was performed. On the surface of liver a flat wound was formed with a diameter up to one cm, and a depth up to 0.1 cm. From the liver's wound an active parenchymatous bleeding was marked. In the comparative group hemostasis was conducted by the applying the hemostatic means Sergicel® Fibrillar™ till the stopping the bleeding completely. In the experience group a hemostatic powder Heprocel was applied on the bleeding surface. An observance over the possible bleeding resumption was conducted during 10 minutes. In the fixed terms animals were taken out of an experiment for the estimating the macroscopic changes, and also for the intake of material for histological researches. Laboratorial animals were withdrawn from an experiment on the 1 -, 7 -, 14 -, 21 - and the 30thday after an operation. Euthanasia was carried out according to the Provisions of ISO 10993-2-2011 under the general anesthesia. An analysis of macroscopic picture of the abdominal cavity was made at the dissection of animals after euthanasia.

For the making the morphological preparations ready the investigated area of liver was excised and fixed in 10% solution of neutral for malin. After expiration of the fixing terms bioptate was inundated by paraffin in a shape of blocks. Series of sections with a thickness of three-four μm were made. Histological preparations were painted by hematoxylin and eosin. For the estimation of histological changes in liver a system of points was employed in accordance with ISO 10993-6-2011 where the parameters of semi-quantitative estimation of the number and distribution of cells characterizing the inflammatory process (polymorphonuclear neutrophils, lymphocytes, plasmatic cells, macrophages, eosinophils 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.

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. 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) and distinctions of the examined selections on the Student's t-criterion. Distinctions in the compared groups were considered reliable at the level of value 95% ($P<0.05$).

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. Study protocol as well as the study itself was approved.

RESULTS AND DISCUSSION

An active parenchymatous bleeding was being marked in a formed wound of liver. In a basic group at the using Heprocel hemostasis started during 34.0±2.5 seconds (Picture 1), in a control group with the using the application hemostatic material Sergicel® Fibrillar™ the time of the stopping the bleeding made up 69.5±5.5 seconds (Picture 2). At the 10-minutes supervision a renewal in bleeding was observed in a basic group in two (5%) cases, and in a control – in eight (22.2%) cases.

It was noted that after application of hemostatic preparation Heprocel the wound surface due to its high hygroscopic it remained dry, while at the using the application hemostatic material Sergicel® Fibrillar™ a seepage of blood between fibers with the continuing bleeding reaching the friable hemostasis had been marked (Picture 2). The results of experimental researches on the achievement of hemostasis were presented in table 1.

The obtained results showed a reliable ($P<0.05$) distinction in the shortening the time of the hemorrhage stopping and the blood loss at the using the hemostatic implant Heprocel with respect to the indexes of Sergicel® Fibrillar™ . In the fixed terms animals were subjected to euthanasia and an intake of material for the conducting the morphological estimation had been carried out. After the first day an expressed inflammatory reaction and adhesive process had not been marked in the abdominal cavity (Picture 3). A vacuolar dystrophy of hepatocytes with expansion of sinusoidal spaces was marked microscopically subcapsularly (Picture 5). The amount of elements of inflammation in visual field made up as follows: polymorphonuclear- three, lymphocytes- four, plasmatic cells- three, macrophages- two. Necrosis zone – 500 μm (Picture 4).

On the seventh day of the experiment pellicle coverage of a pellucid-whitishcolor had been formed in an abdominal cavity on the front surface of liver in the area of lesion. With thata formation of the adhesive process was not found (Picture 5). Fibrosis of the liver's capsule was microscopically marked with the reducingof the inflammatory infiltrate which was expressed in the form of reduction in amount: polymorphonuclear- two, lymphocytes- three, plasmatic cells- three, macrophages- two. Necrosiszone- 200 μm. Neovascularization - three. Signs of debris (Heprocel) (Picture 6).

On the 14th day of the experiment a moderate-mildadhesive process was marked in an abdominal cavity. There was a thin pellucid coverage on the surface of liver (Picture 7). Strengthening of regenerator processes was microscopically marked in a parenchyma, especially in a zone of lesion which was manifested as a restoration of the frameconstruction of liver, disappearance of edema and plethora, reduction in thickness of fibrotic pellicle of liver up to 100 μm. A moderate lymphoid infiltration of the capsule of liver was marked with reduction in visual fieldin an amount: polymorphonuclear- three, lymphocytes- three, plasmatic cells- three, macrophages- two. Necrosiszone- null. Neovascularization- one, fibrosis- two, fatty infiltration - one. Signs of debris (Heprocel) were determined (Picture 8).

On the 30th day of the experiment a calming down of the adhesive process was noted in an abdominal cavity. The surface of liver was smooth and of a soft consistency, without the signs of inflammation (Picture 10). A reduction in thickness of fibrotic pellicle, decrease in amount of elements of inflammatory character (singular amount of lymphocytes) were microscopically marked. Singular elements of debris (Picture 11).

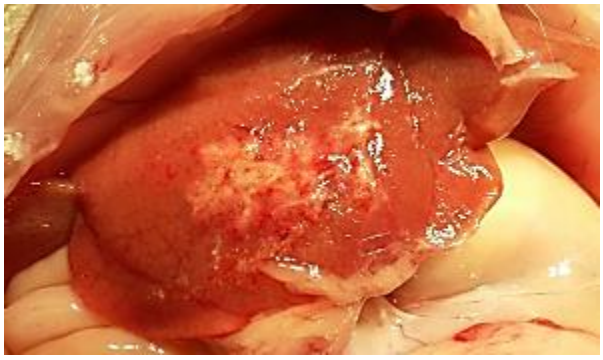
Picture 1. Stopping the bleeding from the wound of liver with the help of hemostatic Heprocel powder

Picture 2. Application of hemostatic material Sergicel® Fibrillar™

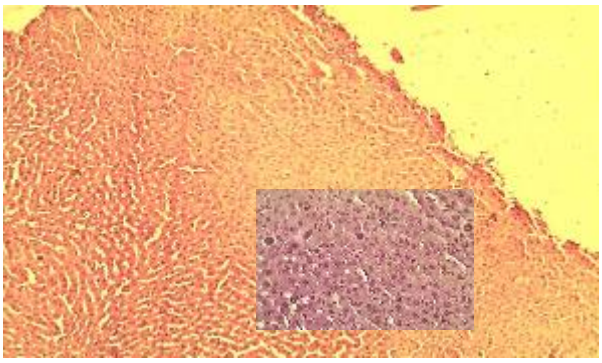
Table 1. Indexes of the bleeding time at the modeling the experimental parenchymatous hemorrhage from the wound of liver in rats

№	Groups	n	Time of the hemorrhage stopping, seconds	P*	ABS	Repeated hemorrhage, %	P-value*
1	Comparative	36	69.5 ± 5.5	-	2	5,6±3.9	-
2	Basic	36	34.0 ± 2.5	P<0.001	8	22.2±7.0	P<0.05

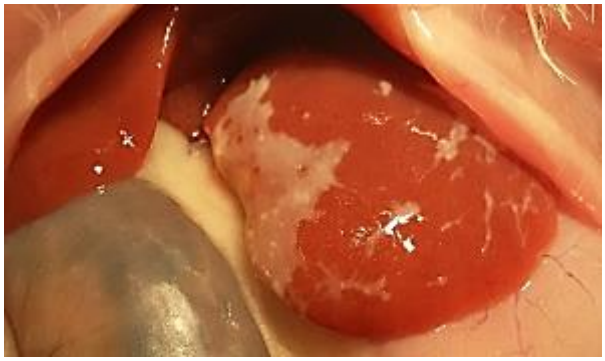
P: an authenticity of the average values' distinctions with respect to the comparative group (accounted with the use of the Student's t-test).



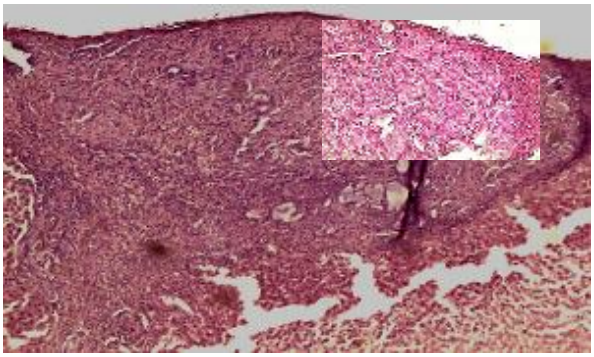
Picture 3. Macropictureon the first day after application of Heprocel.



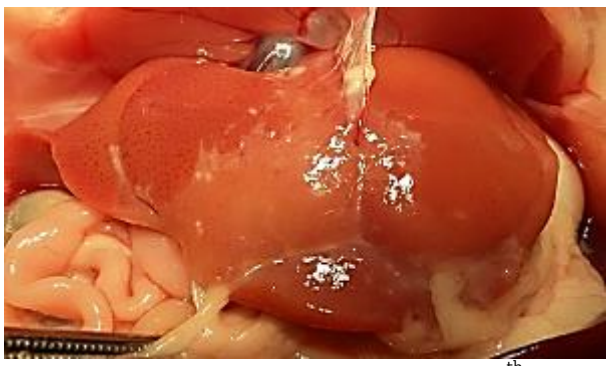
Picture 4. Tissue of liver in an area of Heprocel application on the first day. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



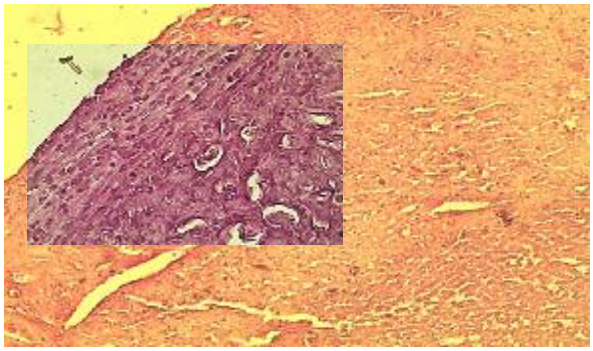
Picture 5. Macropicture on the seventh day after application of Heprocel. Formation of pellicle coverage.



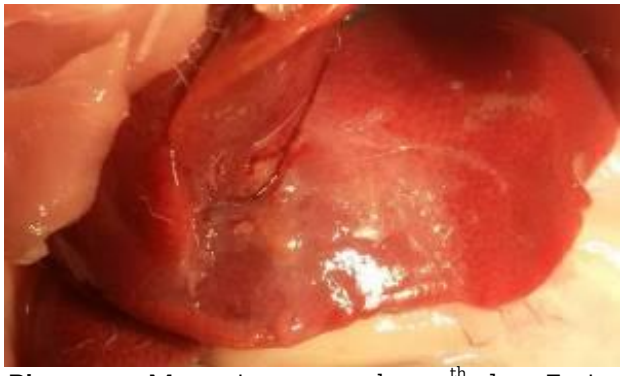
Picture 6. Tissue of liver in an area of Heprocel application on the seventh day. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



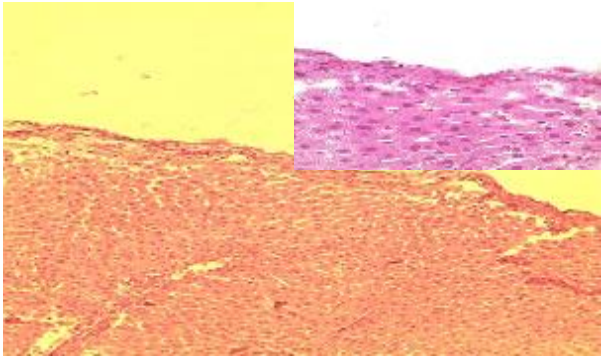
Picture 7. Macropicture on the 14th day. Application of Heprocel, the inning of pellicle coverage.



Picture 8. Tissue of liver in an area of Heprocel application on the 14th day. Absence of necrosis zone. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



Picture 9. Macropicture on the 30th day. Entire degradation of the hemostatic implant Heprocel.



Picture 10. Tissue of on the 30th day. Singular elements of Heprocel. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.

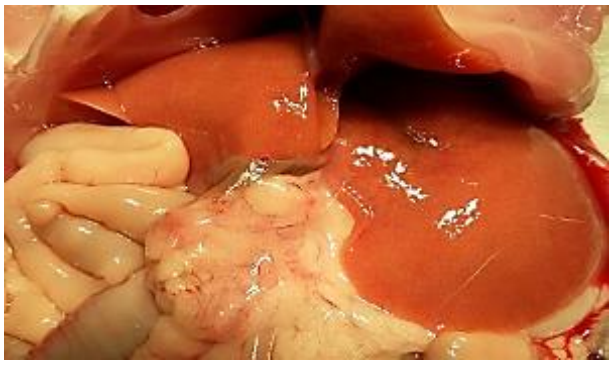
Expressed pathohistological changes in the tissue of liver were not found. The capsule of liver was not incrassated, contained the longitudinally oriented bunches of collagen fibers. Interlobular connecting tissue was developed poorly, and signs of inflammatory infiltration and fibrosis of liver were not detected. Hepatocytes 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 under lobular 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 cholangiolandinter lobular 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.

In a comparison group a day after the applying the hemostatic material Sergicel® Fibrillar™ a macroscopic picture showed a hemostatic material fully saturated with blood in the wound area and a formation of adhesive process with participation of an epiploon (Picture 11). Necrosis of hepatocytes, edema of sinusoids, plethora of vessels were microscopically marked (Picture 12). The quantitative index of inflammatory process showed: polymorphonuclear- one, lymphocytes-one, plasmatic cells–one, Necrosis zone-100 μm. Neovascularization–ne, fibrosis-one. Debris-plasts.

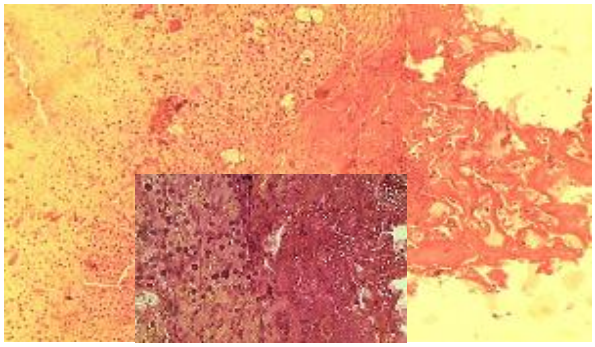
On the seventh day of the experiment on the surface of the liver's wound a preservation of structure of hemostatic material Sergicel® Fibrillar™ was noted by the formation of an adhesive process with the involving of nearby organs (Picture 13). Microscopically fibers of Sergicel® Fibrillar™ were abundantly infiltrated by neutrophil leucocytes, a preservation of hemorrhage, edema of sinusoids, and plethora were marked (Picture 14). The frame structure of liver was destroyed, and the focuses of coagulative necrosis were marked. Quantitative index of the inflammation elements: polymorphonuclear-three, lymphocytes- three, plasmatic cells - three, macrophages – one, Necrosis zone-220 μm. Neovascularization-one, fibrosis–two. Debris-plasts.

On the 14th day on the hepatic wound's surface it was marked a continuation of the preservation of hemostatic material Sergicel® Fibrillar™ fully wrapped up by an epiploon, an expressed inflammatory reaction with development of massive adhesive process took place from the side of abdominal cavity (Picture 15). An excrescence of granulation tissue rich in blood vessels with a negligible quantity of collagen fibers was microscopically marked (Picture 16). There was a strengthening of fatty infiltration. Quantitatively a composition of inflammatory elements made up: polymorphonuclear- three, lymphocytes- three, plasmatic cells - two, macrophages- one, giant cell-one, Neovascularization-two, fibrosis-two, fatty infiltrate-four. Debris-plasts.

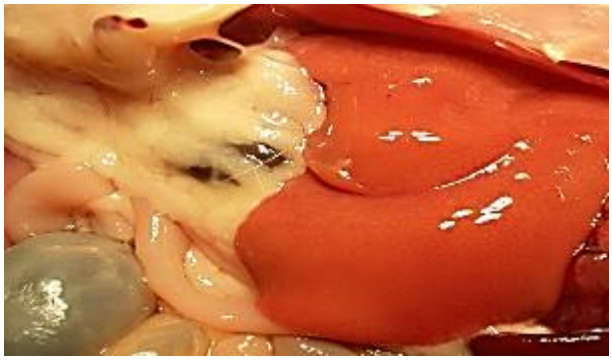
On the 30th day of the experiment on the surface of the liver's wound a reduction in sizes of hemostatic material Sergicel® Fibrillar™ are with the preservation of adhesive process were marked (Picture 17). A quantitative reduction in elements of inflammation was also marked microscopically: polymorphonuclear-two, lymphocytes-two, plasmatic cells-one, macrophages-one. Neovascularization-one, fibrosis-two. Debris-plasts (Picture 18).



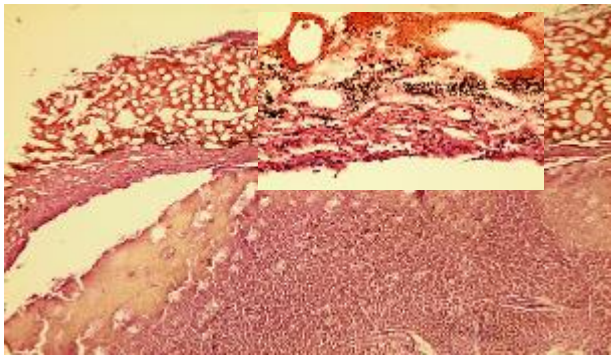
Picture 11. The first day. Hemostatic material Surgicel fibrillar, adhesive process with involving the epiploon and small intestine.



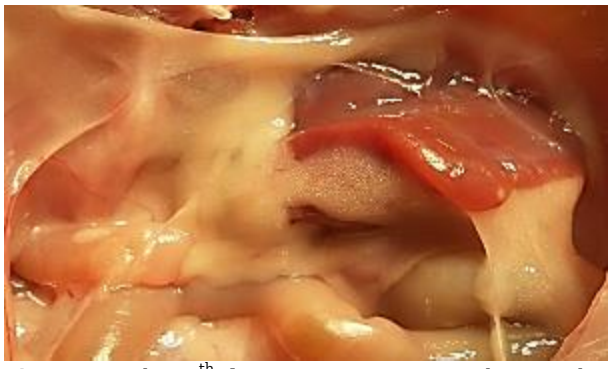
Picture 12. Tissue of liver on the first day. Application of Surgicel fibrillar, saturation with neutrophil leucocytes. Necrosis of hepatocytes, presence of debris. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



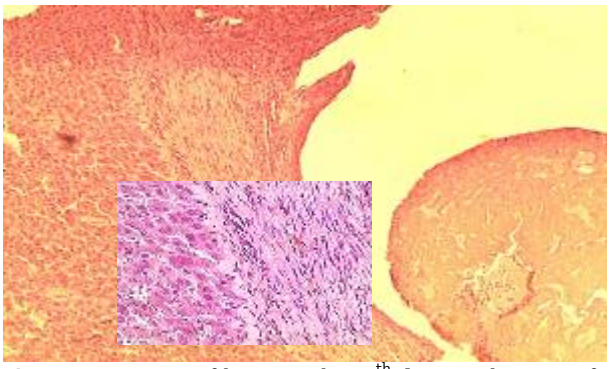
Picture 13. The seventh day. Hemostatic material Sergicel® Fibrillar™, formation not adhesive process with involving the small intestine and stomach.



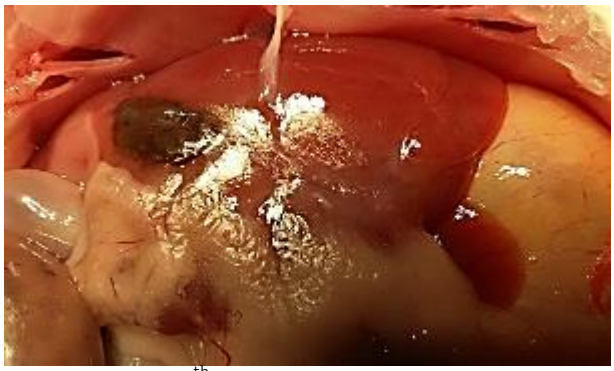
Picture 14. Tissue of liver on the seventh day. Application of Sergicel® Fibrillar™. Preservation of plethora and edema of sinusoids. Presence of debris. Light microscopy. Magnification x200, x40. Coloration Hematoxylin-Eosin.



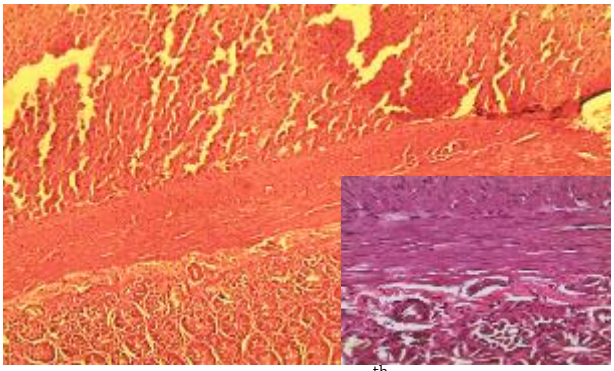
Picture 15. The 14th day. Hemostatic material Sergicel® Fibrillar™, formation of massive adhesive process



Picture 16. Tissue of liver on the 14th day. Application of Sergicel® Fibrillar™, excrescence of granulation tissue, fatty infiltration. Presence of debris. Light microscopy. Magnification x200, x40.Coloration Hematoxylin-Eosin.



Picture 17. The 30th day. Reduction in sizes of hemostatic materialSurgicelfibrillar.



Picture 18. Tissue of liver on the 30th day. Fragmentation of fibers of Surgicel fibrillar. Light microscopy. Magnification x200, x400.Coloration Hematoxylin-Eosin.

At the macroscopic evaluation the structure of the application hemostatic material Sergicel® Fibrillar™ was preserved up to 30 days. Using the application hemostatic material Sergicel® Fibrillar™ promoted the development to inflammatory reaction, on the 14th day after an operation an expressed adhesive process of the abdominal cavity was marked with the involving the nearby organs which began to calm down on 30th day. On the same day an expressed biodegradation of Surgicel fibrillary began, a preparation disintegrated on fragments and was phagocyted bymacrophages, and here a number of inflammatory elements diminished.

As a result of researches it is possible to come to conclusion that a hemostatic implant Heprocel causes on the first day a morphological reaction of liver in a kind of inflammation and excrescence of the connecting tissue, but these processes calm down quickly. An inflammatory reaction is less expressed than in the group of control. To the 30th day in a basic group after application of Heprocel a biodecomposition of hemostatic implant is marked, and regenerator processes in the parenchyma of liver, especially in a zone of lesion, were observed that testifies to the restoration of the liver's tissue, while in a comparative group an active degradation of application hemostatic material began on the 30th day an dan expressed adhesive process took place in an abdominal cavity.

CONCLUSION

Thus, the results of the conducted research of the comparative efficiency showed that the created implant Heprocel comparable with a foreign analogue differed by a greater hemostatic activity, good adhesiveness to the parenchyma of liver. In in vivo researches it was marked that the acceleration of the blood coagulability's time had been by as many as two times faster than in the comparative group. From the data of our researches hemostatic powder closely adjoins the tissue of liver, stops the bleeding, cases of relapse of bleeding were not marked. Histological researches conducted in the dynamics of the healing the wounds of liver revealed that Heprocel did not cause the expressed inflammatory reaction, the zone of lesion did not exceed 150 μm, bio-decomposition started after 14 days.

DECLARATIONS

Acknowledgements

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Authors' contributions

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests.

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