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STUDY OF BIOLOGICAL ACTIVITY OF BIOMATERIAL BASED ON STRUCTURED FIBROIN

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Abstract

The article presents information on the significance, hemostatic and biological activity of fibroin-based drugs. The study examined samples of hemostatic biomaterial obtained on the basis of carboxymethylinulin and fibroin, depending on the amount of polymer components of carboxymethylinulin and fibroin. It was found that the carboxymethylinulin/fibroin sample at a mass ratio of 1:0.5 exhibits high hemostatic activity and complete resorption occurs within 21 days.

Keywords: *carboxymethylinulin, fibroin, biomaterial, biological activity, hemostatic, resorption*

Introduction

In recent years, the attention of researchers has been drawn to the creation of medical products based on silk fibroin, the determination of their physical and chemical properties and biological activity. This is due to the convenience of developing a biomaterial based on it and the possibility of obtaining various forms of the drug. Research is mainly aimed at creating wound healing and hemostatic agents. The reason for this is that the hemostatic stage is the first stage of wound treatment, and rapid hemostasis prevents major blood loss and potential death. The manifestation of the hemostatic activity of fibroin occurs through two main mechanisms:

– firstly, in the bleeding zone, as a result of its swelling, fibroins surround the surface layer and hemostasis is observed with the formation of a septum. For example, a sponge was obtained by freeze-drying from a composition of silk fibroin and polyethylene glycol (PEG, 1500 Da). In an experiment conducted on a rabbit liver injury model, it was found that in the process of interaction with blood, the sponge turns into a gel and is mechanically blocked in the areas of bleeding (Wei W., Liu J., Peng Z., et al., 2020).

– secondly, silk fibroin directly affects the blood clotting process by activating clotting factors or platelets, thereby stimulating platelet adhesion and aggregation, facilitating the

interaction between platelets and fibrinogen (Chouhan D., Mandal B. B., 2020).

Studies to determine the effect of fibroin on coagulation time were conducted in comparison with platelet-free plasma and platelet-rich plasma. Based on the results of in-depth analysis, it was noted that fibroin activates the platelet-mediated coagulation cascade (Kundu B., Schlimp C. J., Nurnberger S., Redl H., Kundu S. C., 2014). In addition, the ability of fibroin to quickly bind to fibrinogen and platelets was revealed, which stimulates the coagulation cascade and facilitates the hemostasis process (Dahlke H., Dociu N., Thurau K., 1980).

Kukun shaklidagi biomaterial yopishqoq xususiyatga ega bo'lsa va suyuqlik ta'sirida bo'kuvchanligi yuqori bo'lsa, o'ziga suv molekulalarini singdirishi orqali hajmining oshishiga olib keladi. Natijada qon oqayotga sohada to'siq hosil qilib, qon ivish omillarini to'playdi. Ushbu xususiyat ayniqsa tolasimon tuzilishli gemostatik kukunlarda yaqqol kuzatiladi (Babiuc R. D., Purcarea M., Sadagurschi R., Negreanu L., 2013; Wang X. X., Liu Q., Sui J. X., Ramakrishna S., Yu M., Zhou Y., Jiang X. Y., Long Y. Z. 2019; Barkun A. N., Moosavi S., Martel M., 2013).

If the biomaterial in the form of a powder has viscous properties and has a high swelling under the influence of liquid, it increases its volume by absorbing water molecules. As a result, this creates an obstacle in the area of blood flow and accumulates factors of blood coagulation. This feature is particularly pronounced in the fibrous structure of hemostatic powders (Babiuc R. D., Purcarea M., Sadagurschi R., Negreanu L., 2013; Wang X. X., Liu Q., Sui J. X., Ramakrishna S., Yu M., Zhou Y., Jiang X. Y., Long Y. Z., 2019; Barkun A. N., Moosavi S., Martel M., 2013).

In our study, we set the task of determining the degree of swelling of biomaterial samples in the form of powder obtained from polymer compositions of fiproin and carboxymethylinulin (CMI), as well as studying the activity in stopping blood and the dependence of the absorption time in the body on the mass ratio of the polymer components.

The object and methods of research. In the study, biomaterial samples in the form of a powder obtained on the basis of CMI/fibroin compositions in mass ratios of 1:0.25; 1:0.5; 1:0.75 and 1:1 were used.

Determining the degree of swelling. The swelling degree of the samples was determined using the following formula:

$$A = (m - m_0)100/m_0$$

Here: A – swelling degree, %;

m – the mass of the powder soaked in water, g.

m_0 – the mass of the original dry powder, g.

Microscopic analysis. Microscopic examination was performed using the LEICA ICC50 optical microscope (Germany). For this purpose, a small amount of test powders (at least 5 mg) was placed on a microscope bottle. The microscope was then mounted on a stand, adjusted the focus of the microscope until a clear image was obtained, and photographed using a digital camera.

Determining hemostatic activity. The studies were conducted on non-pedigreed white rats weighing 200–210 grams. The rats were anesthetized by introducing etaminal sodium into the abdominal cavity at a dose of 50 mg/kg. The abdominal cavity was opened using a surgical instrument (scalpel). The abdominal cavity is incised longitudinally along the white line of the abdomen and opened with a wide incision using special instruments. The intestines are pushed out and bounded with a special napkin or paper. The anterior surface of the liver was also removed. The liver protrusion was resected with a knife using a special device – a limiter (plastic material with a round hole in the center). A segment cut in a vertical projection looks like a circle or ellipsis, its dimensions are constant. The formation of an evenly bleeding wound with soft edges and uniform curvature has a total area of about 1–1.5 cm^2 and a depth of about 0.3 cm. The aforementioned experiment was conducted simultaneously to conduct a comparative assessment of the tested powder and the control (using gauze). The time of hemostasis was determined using a stopwatch.

Determining biosolvability. The biosolvability of biomaterial samples in powder form was studied in white non-pedigreed rats weighing 180±10 g. In the experiments, 24 rats were divided into 4 groups. In the experiments, a «pocket» was formed in the lumbar region of the rats and placed in an amount of 0.2 g (with an accuracy of 0.001 g) of the test-

ed powder samples. After the operation was completed, the skin was closed, 1–2 sutures were sutured and cleaned in a 5% alcohol iodine solution. To determine the biosolvability of the samples under the skin, the amount of powder was taken from the skin and monitored for 7, 14, 21, and 28 days.

Results and their discussion

When obtaining all types of hemostatic agents, it is important to study the degree of swelling under the influence of liquid. Especially for hemostatic agents in powder form. The high fluid retention capacity of the biomaterial not only increases its hemostatic properties, but also ensures faster absorption by the body. Taking this into account, in our experiments: 1:0.25; 1:0.5; We determined the swelling degree in water of powdered biomaterial samples obtained based on compositions of CMI/fibroin in a mass ratio of 1:0.75 and 1:1 (Fig. 1). The experiments were conducted in room conditions and until the samples swelled in water and became a thick gel.

Figure 1. *The degree of swelling of biomaterial samples in powder form*

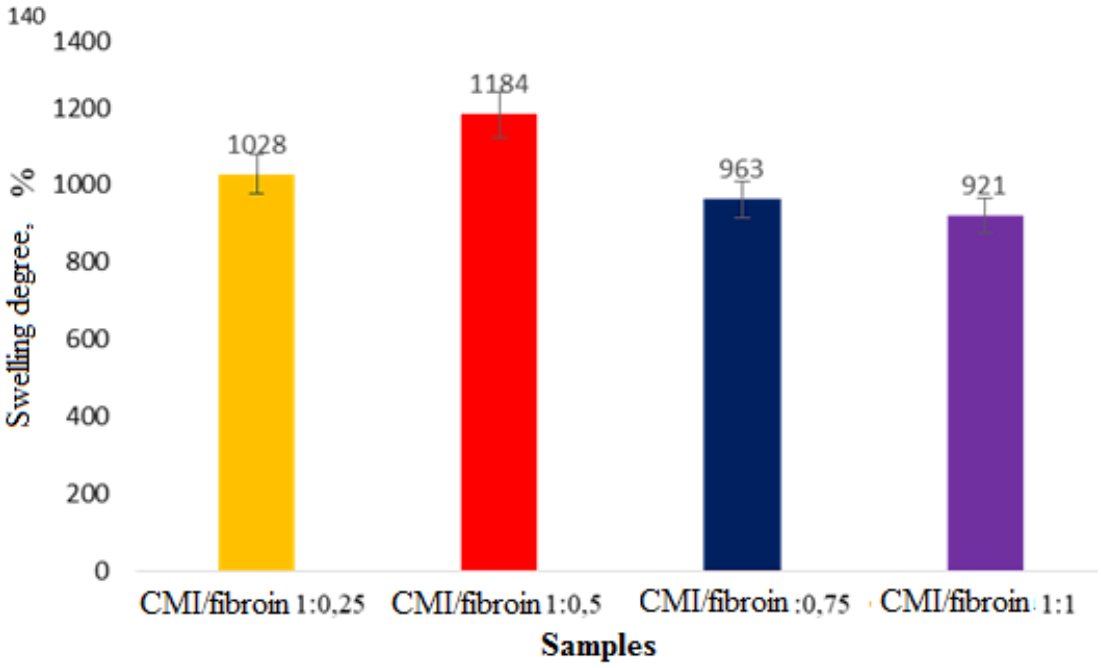


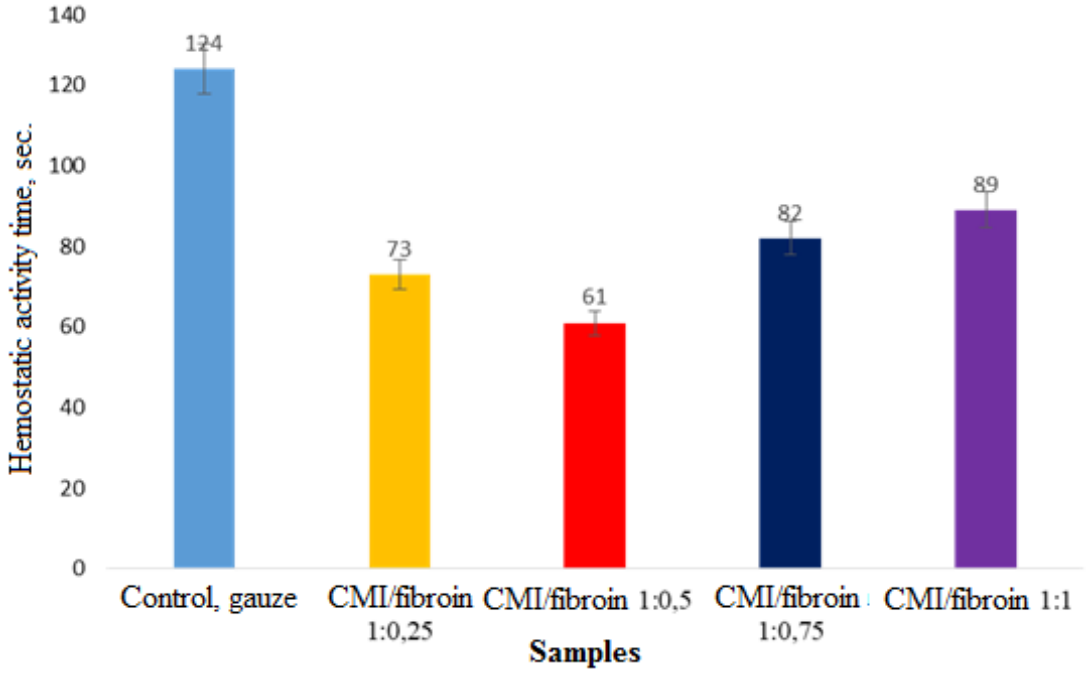
Figure 2. Biomaterial in a mass ratio of CMI/fibroin 1:0.5:
a) powder form; b) magnified x100 times

According to the results of the study of the swelling degree of powdered biomaterials based on compositions of CMI/fibroin in different mass ratios, at mass ratios of 1:0.75 and 1:1, the swelling of biomaterials based on compositions of CMI/fibroin was relatively low (963% and 921%), and at a mass ratio of CMI/fibroin of 1:0.25, the swelling was 1028%. The best indicator was found in a sample with a CMI/fibroin ratio of 1:0.5

(1184%). The main reason for this is that the powdered biomaterial in this mass ratio not only retains the same particle size, but also retains a fibrous structure (Fig. 2).

In our subsequent experiments, the hemostatic activity of biomaterials based on CMI/fibroin in various mass ratios was studied for bleeding in the liver of the parenchymal organ of rats (Fig. 3).

Figure 3. *Hemostatic activity of biomaterial samples*



As a result, compared to the control (control, gauze), the KIM/fibroin was 1:0.25; Experimental samples with mass ratios of 1:0.75 and 1:1 were respectively 1.7; 1.51 and 1.39 times faster. The highest hemostatic activity was observed in the experimental sample with a CMI/fibroin ratio of 1:0.5 mass, which is 2.03 times faster than in the control group.

The absorption rate of powdered biomaterial samples in the body was also determined by implantation under the skin of experimental animals. The results of the experiments are presented in Table 1.

Table 1. *Determining the absorption time of biomaterials implanted under the skin into the body (M±c; n=6)*

Samples	Duration of absorption, a day				
	Initial mass, g.	Mass on the 7 th day, g (%)	Mass on the 14 th day, g (%)	Mass on the 21 st day, g (%)	Mass on the 28 th day, g (%)
CMI/ fibroin 1:0.25	0.2±0.001	0.124± ±0.001(62%)	0.053±0.001(26.5%)	100% absorbed	–
CMI/fibroin 1:0.5	0.2±0.001	0.127± ±0.001(63.5%)	0.058±0.001(29%)	100% absorbed	–
CMI/ fibroin 1:0.75	0.2±0.001	0.143± ±0.001(71.5%)	0.096±0.001(48%)	0.048±0.001(24%)	100% absorbed
CMI/fibroin 1:1	0.2±0.001	0.158± ±0.031(79%)	0.104±0.031(52%)	0.054±0.031(27%)	100% absorbed

As can be seen from the results presented in Table 1, all samples were completely absorbed in the body. The total absorption of powder samples based on CMI/fibroin in mass ratios of 1:0.25 and 1:0.5 in the body was 21 days. Samples based on CMI/fibroin in a mass ratio of 1:0.75 and 1:1 absorbed 76 and 73% respectively on day 21, with a full absorption time of 28 days in the body. Based on the obtained results, it can be concluded that with a low amount of fibroin, the rate of biomaterial absorption in the body is high, and in these samples, fibroin is relatively well structured using CMI.

Conclusion

It has been determined that biomaterials with a CMI/fibroin ratio of 1:0.75 and 1:1 exhibit relatively low swelling (963% and 921%), a biomaterial with a 1:0.25 mass ratio of 1028%, and a sample with a 1:0.5 mass ratio of 1184% exhibit high swelling. A study of the hemostatic activity of biomaterials revealed that a BMI/fibroin sample with a mass ratio of 1:0.5 stops twice as quickly and exhibits high hemostatic activity and is completely absorbed by the body after 21 days compared to the control.

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