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Research Article

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Evaluation of the Hemostatic Activity of Multicomponent Polymer Sponge Implants in An In Vitro Experiment

V.A. Lipatov, S.V. Lazarenko, D.A. Severinov*✉*, A.A. Denisov, O.V. Padalkina

Laboratory of Experimental Surgery and Oncology, Research Institute of Experimental Medicine
Kursk State Medical University, Institute of Continuing Education
K. Marx Str. 3, Kursk, Russian Federation 305041

✉ **Contacts:** Dmitry A. Severinov, Candidate of Medical Sciences, Associate Professor, Department of Pediatric Surgery and Pediatrics, Institute of Continuing Education, Kursk State Medical University. Email: dmitriy.severinov.93@mail.ru

BACKGROUND The development of new samples of local hemostatic agents is an intensively developing area of the modern industrial biotechnology due to a high need of clinical bases for such products. In addition to constant search for optimal substances used as a basis (collagen, cellulose and its derivatives, gelatin, etc.) for local hemostatic agents, methods for their comprehensive testing are also being developed.

AIM OF THE STUDY To evaluate the hemostatic activity of multicomponent polymer sponge implants using the coagulometer method developed by the authors in an in vitro experiment.

MATERIAL AND METHODS As research materials, new samples of multicomponent polymer sponge implants developed within the Laboratory of Experimental Surgery and Oncology of the Kursk State Medical University were used. The samples were based on marine collagen made from deep-sea squid in different ratios by weight with carboxymethylcellulose sodium salt (15/85, 25/75, 50/50). The hemostatic activity of these products was evaluated in the in vitro experiment using our method based on coagulometric measurement of blood clotting time of volunteer donors. Statistical processing of the data was carried out using methods of descriptive and variation statistics (Me [25; 75]). The validity of the difference was determined using the Mann–Whitney test ($p \leq 0,05$).

RESULTS The blood clotting time in experimental group No. 2 turned out to be 2.12 s less than in the control group (the tested samples of hemostatic agents were not added). Statistically significant differences were found when comparing the values of coagulation time in experimental groups No. 3 (2.98 s less) and No. 4 (2.37 s less) with the values of the control group (No. 1). A decrease in the blood clotting time indirectly proves the effectiveness of the products used, due to the formation of the blood clot in a shorter period of time. This suggests that when the hemostatic agents are used in bleeding conditions, the products will reduce the time and volume of bleeding due to their hemostatic activity.

CONCLUSION Within the framework of the completed study, it was established that our method for assessing the hemostatic activity of local hemostatic agents, based on the evaluation of blood clotting time using electrocoagulometry, after grinding the tested samples, is easy to perform, accessible to most laboratories, and allows researchers to investigate the effectiveness of various forms of multicomponent polymer sponge implants.

The development of multicomponent polymer sponge implants based on carboxymethylcellulose sodium salt and collagen seems to be a promising direction, since these products accelerate blood clotting time in the in vitro experiment.

Keywords: hemostasis, hemostatic sponges, polymer, in vitro experiment, bleeding, collagen

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Affiliations

Vyacheslav A. Lipatov	Doctor of Medical Sciences, Full Professor, Professor, Department of Operative Surgery and Topographic Anatomy; Head, Laboratory of Experimental Surgery and Oncology, Research Institute of Experimental Medicine, Kursk State Medical University; https//orcid.org/0000-0001-6121-7412, drli@yandex.ru; 25%, research concept and design, text writing and editing, approval of the final version of the article, responsibility for the integrity of all parts of the article
Sergey V. Lazarenko	Candidate of Medical Sciences, Associate Professor, Department of Urology, Kursk State Medical University; https//orcid.org/0000-0002-7200-4508, dok.lazarenko@yandex.ru; 25%, experimental part, statistical data processing, editing, approval of the final version of the article

Dmitry A. Severinov	Candidate of Medical Sciences, Associate Professor, Department of Pediatric Surgery and Pediatrics, Institute of Continuing Education, Kursk State Medical University; https//orcid.org/0000-0003-4460-1353, dmitriy.severinov.93@mail.ru; 20%, experimental part, text editing, approval of the final version of the article
Artem A. Denisov	Assistant, Department of Operative Surgery and Topographic Anatomy, Kursk State Medical University; https//orcid.org/0000-0001-5034-8580, d.artyom21@gmail.com; 15%, experimental part, text writing and editing, analysis of literature data
Olga V. Padalkina	6th year student, Pediatric faculty, Kursk State Medical University; https//orcid.org/0009-0004-1959-2306, olga_padalkina2000@mail.ru; 15%, experimental part, text writing, analysis of literature data

LHAs — local hemostatic agents
MPSIs — multicomponent polymer sponge implants
Na-CMC — collagen/carboxymethylcellulose sodium salt

INTRODUCTION

Currently, despite the significant achievements of modern medical science, mortality from injuries to the parenchymal organs of the abdominal cavity (liver, spleen) remain high [1, 2]. The number of such patients is growing progressively due to a significant number of man-made disasters and road traffic accidents [3, 4]. Damage to these organs is characterized by the rapid development of massive intra-abdominal bleeding due to the peculiarities of the anatomical structure (fragility of the parenchyma, abundant blood supply, proximity of bone structures, etc.) [5, 6]. As a rule, such patients require emergency surgical care, which consists of stopping the bleeding and further stabilizing the condition [7].

In clinical practice, a wide arsenal of methods for intraoperative bleeding control is available to the surgeon. One of the modern methods of hemostasis is the use of local hemostatic agents (LHAs) (sponges, films, adhesive compositions, gels, etc.) [8, 9]. The range of applications for such products is quite diverse - puncture and incised wounds of the liver, superficial injuries. LHAs can be used both as the main method of hemostasis and as an adjuvant in combination with other methods of stopping bleeding [10].

Among the LHAs used in clinical practice, certain groups can be distinguished based on polymers of organic (for example, collagen) and inorganic origin (synthetic products based on gelatin, cellulose, etc.) [11, 12]. LHAs from each group have different advantages (time and reliability of bleeding control, biodegradation) and disadvantages (risk of infection, need for repeated interventions) [13]. Today, new compositions are being actively developed that will have the positive properties inherent to their components.

The aim of the study was to evaluate the hemostatic activity of multicomponent polymer sponge implants using the coagulometric method developed by the authors in an in vitro experiment.

MATERIAL AND METHODS

As part of this research, the hemostatic activity of new samples of multicomponent polymer sponge implants (MPSIs) (groups Nos. 2–4), the description of which is presented below (Table 1), was assessed. These samples were manufactured on the basis of the Kursk State Medical University of the Ministry of Health of the Russian Federation (“Combined hemostatic sponge”, RF patent application No. 2023123284 dated 09/07/2023). The studied MPSI samples were crushed to a powder state before the

Table 1

Characteristics of tested hemostatic materials

	Group number		
	2	3	4
Manufacturer of raw materials	AS RS LLC (Kaliningrad, Russia)		
Small batch production base	Laboratory of Experimental Surgery and Oncology, Research Institute of Experimental Medicine, Kursk State Medical University		
Composition	3% deep sea squid collagen suspension, 1% sodium carboxymethylcellulose gel, Ratio: collagen/carboxymethylcellulose sodium salt, equal to % wt. 15/85	3% deep sea squid collagen suspension, 1% sodium carboxymethylcellulose gel, Ratio: collagen/carboxymethylcellulose sodium salt, equal to % wt. 25/75	3% deep sea squid collagen suspension, 1% sodium carboxymethylcellulose gel, Ratio: collagen/carboxymethylcellulose sodium salt, equal to % wt. 50/50

start of the experiment to ensure complete contact of MPSI particles with blood. We preliminarily weighed 0.006 g (the ratio of the mass of the blood collection tube and the test sample was 1:650) of the studied MPSIs of our own production. This ratio of blood and the test sample was obtained experimentally (this mass of MPSIs does not affect the aggregate state of blood in the test tube). Also, the results of assessing the blood clotting time of volunteer donors without adding samples of hemostatic agents were used as a control group (group No. 1).

All the studies were carried out under the supervision of the regional ethics committee at the Kursk State Medical University in compliance with current international ethical standards (protocol No. 3 of November 16, 2020). After obtaining voluntary consent, in the morning, on an empty stomach, 4.2 ml of blood was taken from the cubital vein of 10 healthy volunteer donors (young men 20–23 years old) with no history of pathology of the hemostatic system. Vacutainers containing sodium citrate were used. Using a micropipette, the donor's blood was transferred into a measuring glass beaker, which was then placed on the platform of a magnetic stirrer, into which the indicated mass of crushed MPSIs was gradually poured over 30 s and stirred for 3 minutes at 1000 rpm.

A steel ball was placed into the cuvette of a semi-automatic coagulometer, 100 ml of thromboplastin-calcium mixture (standardized reagent) was added using a micropipette for recalcification stabilized by blood citrate, after which 100 µl of citrated blood mixed with the test MPSI sample was added there. The timer was started manually to count the start time of the research ("Method for studying the effect of hemostatic agents on the formation of the blood clot" RF patent application No. 2023123665 dated 09/13/2023). The clotting time of blood in the cuvettes was assessed. Four experiments were performed with the blood of each donor according to the number of the experimental groups. In each experimental group, 10 coagulometer tests were performed (according to the number of donors).

Statistical processing of the obtained data was carried out using methods of descriptive and variation statistics - calculation of the median and 25 and 75 percentiles (Me [25; 75]). The trial version of the Statistica 10 (manufactured by Dell Software Company, Round Rock, Texas, United States of America) was used as the software environment. Due to the small sample ($n<30$) in the experimental groups and the non-normal distribution of the sample according to Kolmogorov–Smirnov, the

nonparametric Mann–Whitney U test was used to determine the statistical significance of differences in means. The critical level of statistical significance when testing statistical hypotheses in this study was taken to be $p<0.05$, which is an acceptable value for biomedical research.

RESULTS

According to the results of the study (Table 2), the highest value of blood clotting time was observed in the second experimental group (ratio: collagen/carboxymethylcellulose sodium salt (Na-CMC), 15/85), the smallest - in the third experimental group (ratio: collagen / Na-CMC, 25/75). The blood clotting time in experimental group No. 2 was 2.12 s less than in the control group. Statistically significant differences were found when comparing the clotting time values in experimental groups No. 3 and No. 4 with the values of the control group (No. 1). Thus, in experimental group No. 3 they were 2.98 s less ($p = 0.004$) than in the control group, and in experimental group No. 4 - 2.37 s less ($p = 0.011$).

Table 2

Coagulation time in the experimental groups, Me (25; 75)

Indicator	Group No. 1	Group No. 2	Group No. 3	Group No. 4
Me	15.675	13.45	13.05	13.425
0.25- quantile	14.96	12.06	10.74	12.33
0.75- quantile	16.29	15.5	14.78	14.45
p	–	p_1=0,07	p_2=0,004*	p_3=0,011*

Notes: * — statistically significant values ($p\leq0.05$); p_1 — statistical significance of the differences between the values of group No. 1 and group No. 2; p_2 — statistical significance of the differences between the values of group No. 1 and group No. 3; p_3 — statistical significance of the differences between the values of group No. 1 and group No. 4

When performing the intergroup comparison (groups Nos. 2–4) in clotting time, no statistically significant differences were revealed.

DISCUSSION

Based on the results of our own research, we can note the effectiveness of the developed method for assessing the hemostatic activity of MPSIs. The advantage of this method is greater coverage of various solid forms of local hemostatic agents (sponges, films, powders), which can be crushed for testing. The experiment proved the positive effect of different variants of MPSIs (the ratio of Na-CMC and collagen was varied) on blood clotting time, and the optimal ratio can be considered 25/75, since it was in this group that the lowest clotting time was observed, in contrast to the control group.

The use of hemostatic agents based on Na-CMC and collagen in in vitro and in vivo experiments is described in the works of both domestic and foreign authors [14]. The use of these products has certain advantages and disadvantages. Na-CMC is highly biologically inert and quickly degrades; sponge hemostatic agents based on it are highly porous and quickly stop bleeding due to the capillary properties of the sample [15]. Collagen hemostatic agents have been known for a long time and are widely used in practice. In addition to the obvious advantage, such as high efficiency (quick and reliable stop of bleeding, since collagen potentiates blood clotting processes), collagen hemostatics have a number of disadvantages: a long period of degradation, the individual reaction of the body to protein of animal origin [4, 7]. However, the use of combined products that have the advantages of these substances, in our opinion, is a promising direction in the development of new MPSI samples. This will reduce the negative consequences of using local hemostatic agents and increase their hemostatic activity. The use of dry marine collagen isolated from deep-sea squid, which is less allergenic compared to proteins of animal origin, will help avoid adverse reactions.

CONCLUSION

Within the framework of the completed study, it was established that our method for assessing the hemostatic activity of local hemostatic agents, based on the evaluation of blood clotting time using electrocoagulometry, after grinding the tested samples, is easy to perform, accessible to most laboratories, and allows researchers to investigate the effectiveness of various forms of multicomponent polymer sponge implants.

The development of multicomponent polymer sponge implants based on carboxymethylcellulose sodium salt and collagen seems to be a promising direction, since these products accelerate blood clotting time in the in vitro experiment. However, the multicomponent polymer sponge implants we obtained require further comprehensive experimental testing.

FINDINGS

1. Estimation of blood clotting time in an in vitro experiment is an effective test for studying the hemostatic activity of surgical hemostatic materials, which is confirmed by its changes when powdered agents are added to the blood.

2. The comparative analysis of the results obtained shows that a crushed to a powder state hemostatic sponge based on sodium carboxymethylcellulose with the addition of 25% collagen by weight of sodium carboxymethylcellulose reduces the blood clotting time by 2.98 s compared to the control group.

3. The blood clotting time in experimental group No. 2 is 2.12 s less than in the control group (tested samples of hemostatic agents were not added). Statistically significant differences were found when comparing the values of coagulation time in experimental groups No. 3 (2.98 s less, p_2 = 0.004*) and No. 4 (2.37 s, p_3 = 0.011) with the values of the control group (No. 1). The reduced blood clotting time indirectly proves the effectiveness of the products used, due to the formation of the blood clot in a shorter period of time.

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