Investigation of the Hemostatic Action of Low-Dimensional Electropositive Structures

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Abstract. The paper studies the hemostatic action of the fibrous material containing agglomerates of low-dimensional electropositive structures of aluminum oxyhydroxide. The time of parenchymatous bleeding is found to decrease from 292.5 s at spontaneous hemostasis to 177.8 s with the attached fibrous material and to 215.7 s with gauze. The mixed mechanism of the hemostatic action is possible, i.e. along with the hygroscopic mechanism, plateletes aggregate due to destruction of a diffuse part of the double electrical layer in contact with low-dimensional electropositive structures of aluminum oxyhydroxide.

Keywords: low-dimensional electropositive structures, aluminum oxyhydroxide, fibrous material, hemostatic action

INTRODUCTION

Despite the abundance of commercially available hemostatic and dressing materials, the problem of rapid control of bleeding remains relevant. According to the reported data, blood loss in case of disasters, accidents, or emergency causes a considerable percentage of medical complications and heavy mortality.

The prospect for developing new hemostatic agents can lie in the preparation of electropositive materials. As known, blood cells of mammals bear negative electric charges at the outer surface of their membranes. The cell surface demonstrates a double layer of electric charges, which consists of negative charges firmly connected to membranes and a balancing diffuse layer of positive charges. Cell motion causes the electrokinetic potential to form at the interface of adsorption and diffusion layers, which plays an important role in stabilization of cells and prevention of their aggregation. At changed ionic conditions the diffuse layer is compressed and the barrier to cell aggregation is reduced. Consequently, the contact of blood cells with the electropositive material should result in blood clotting and stopping the bleeding. This effect is already used in hemostatic agents based on biological materials, such as oxidized cellulose and chitosan [1, 2].

The present paper considers possibilities of the fibrous material containing low-dimensional electropositive structures of aluminum oxyhydroxide for stopping the bleeding.

EXPERIMENTAL PROCEDURE

The material for studying the hemostatic action is prepared in the following way [3]. A sample of the polymer fibrous matrix of cellulose acetate with the fiber diameter 1.5–3 μm is placed into a water suspension (1 wt.%) of nanoparticles of alluminum nitide Al/AlN, which are obtained by electrical explosion of aluminum wire in nitrogen.

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The size of Al/AlN particles is 80–100 nm, specific surface area 15 m^2/g, and the content of the AlN phase 70 wt.%. The reaction mixture is heated to 60°C during 20 min for water oxidation of Al/AlN nanoparticles. Reaction products, namely, agglomerates of low-dimensional structures of aluminum oxyhydroxide up to 3 µm in size, are fixed at the surface of polymer fibers. The formed fibrous material is removed from the reaction medium and dried at 120°C for 4 hours.

The TEM images were obtained on finely powdered samples using a JEM-2100 transmission electron microscope (JEOL, Japan). The SEM analysis was performed with a LEO EVO 50 (Zeiss, Germany) electron microscope on samples covered with a thin silver layer. The zeta-potential of agglomerates is determined with the ZetaSizer Nano ZS analyser (Malvern Instruments, UK).

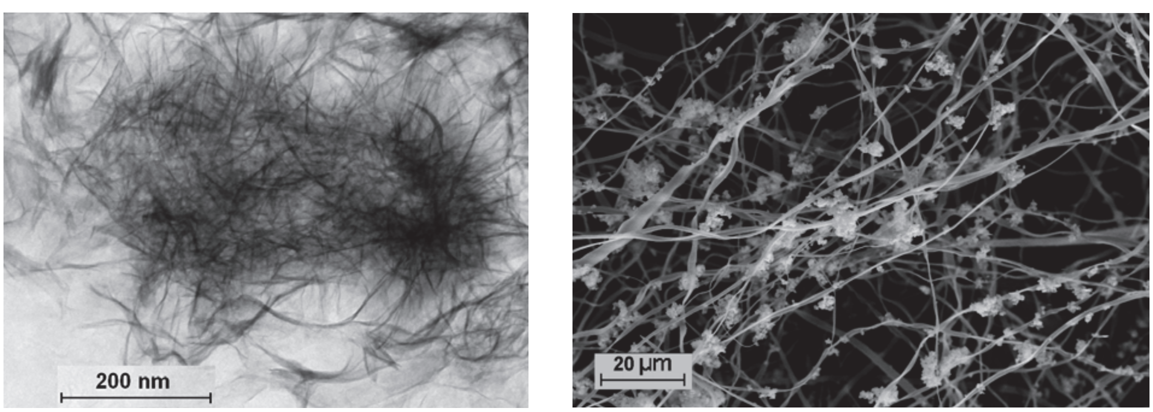
The effect of the fibrous material on time of parenchymatous bleeding was studied on adult white outbred male rats 200–220 g in weight [4]. The rats were kept in accordance with the rules of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986. The animals were divided into groups as follows: group I had 3 rats while groups II and III contained 7 rats in each. Rats under ether anesthesia were fixed at the back and laparotomized after shaving hair on the abdomen. Two tissue pieces 1×1 cm and about 1 mm in thickness were knifed from the diaphragmatic surface of the liver of anasthesized rats and the time to total spontaneous hemostasis is recorded (group I). The liver of group II and III rats was similarly injured and dressed with the fibrous material (group II) or a gauze piece of the same size (group III), which were pressed slightly to fix. The time to total hemostasis was registered. At the end of the experimental period, the rats were sacrificed with ether overdose.

The effect of the fibrous material on rheological properties of the human blood are investigated with the analyzer of blood rheological properties ARP-01 (Mednord, Russia). The method [5] is based on the registration of changes in the aggregate state of blood due to clotting by measuring the resistance of the investigated medium to resonant vibrations of the needle attached to the tuning fork and dipped into a blood cuvette. The measurements are performed with initial venous blood (10 male donors aged from 20 to 40 years) and blood in contact with the fibrous material.

DISCUSSION OF THE RESULTS

Characteristics of the Fibrous Material

The fibrous material presents polymer fibers with fixed agglomerates of low-dimensional structures of aluminum oxyhydroxide. The scanning and transmission electron microscopy of the fibrous material shows that the reaction of Al/AlN with water results in the formation of nano-demensional folded structures of aluminum oxyhydroxide (Fig. 1(a)) 5–10 nm in thickness and 300 nm in size, which are joint into spherical porous agglomerates 0.5–3 µm in size [6].



(a) (b)

FIGURE 1. Transmission electron image of agglomerates of low-dimensional structures of aluminum oxyhydroxide (a) and scanning electron image of the fibrous material (b)

TABLE 1. Time of paranchymatous bleeding in rats

Characteristic	Group I, Spontaneous Hemostasis	Group II, Fibrous Material	Group III, Gauze
Bleeding time, s	292.5 ± 9.4	177.8 ± 7.9	215.7 ± 13.0

Electropositive agglomerates of low-dimensional structures are fixed on fibers and uniformly distributed in the bulk of the polymer matrix (Fig. 1(b)).

The effectiveness of electropositive low-dimensional structures for blood corpuscles can be governed by their electrical properties, in particular, by surface density of charge. Distribution of δ charge at the outer surface of the charged body depends largely on the surface shape, namely, the lower is the radius curvature k, the higher is the surface charge density, $\delta \sim k$, where $k \sim 1/r$ (r is the curvature radius). Consequently, charged low-dimensional structures several nanometers in thickness produce a highly inhomogeneous electrical field affecting negatively charged blood cells.

The electric field strength formed by agglomerates of low-dimensional structures can be estimated. The zeta potential of low-dimensional structures of aluminum oxyhydroxide is about 60 mV. Assuming the face thickness to average 5 nm, the electric field strength E can be estimated at the face edge:

$$E = \frac{\varphi}{R},$$

where φ is the agglomerate potential and R is the face thickness.

The electric field strength at the face edge E = 12 mV/nm or 1.2×10^7 V/m. The electric field strength at the surface of a nonporous spherical particle of aluminum oxyhydroxide 3 μm in size would comprise 20 mV/μm or 2×10^4 V/m, which is approximately 3 orders of magnitude lower than that at the face edge.

Study of the Hemostatic Action of the Fibrous Material

The surface density of the fibrous material comprises 70–80 g/m^2 and the absorbent capacity is about 13 g/g. The fibrous material adheres well to a wound due to its structure, which allows applying it to wound surfaces of any configuration. Immobilization of agglomerates of low-dimensional structures on polymer fibers prevents them from washing away by blood during the wound process.

The average time of parenchymatous bleeding in rats to total hemostasis is 292.5 seconds (Table 1). In using the gauze the average bleeding time is reduced to 215.7 s and in using the fiber material up to 177.8 s. The significance level to determine the difference in the average hemostasis time without application of any material, with the fiber material, and with gauze is $p = 0.001$.

The study of the effect of the fibrous material on rheological properties of human blood has shown a pronounced tendency to an increase in platelet aggregation by approximately 50%, which points to intensification of the aggregation process. Other parameters of the thromboelastogram change inconsiderably. The significance level to determine the difference in parameters of the initial thromboelastogram and that obtained in the blood contact with the fibrous material is $p = 0.064$.

The experimental results on the degree of platelet aggregation suggest that particles of electropositive aluminum oxyhydroxide interact with negatively charged membranes of blood cells and cause adhesion and aggregation of platelets due to the destruction of a diffuse part of the double electrical layer.

CONCLUSION

The performed investigation has revealed that the electropositive fibrous material has a hemostatic action at its local application. Probably the hemostatic action of the fibrous material is related to both a hygroscopic mechanism and mechanism of platelet aggregation due to the destruction of a diffuse part of the double electrical layer in contact with low-dimensional structures of aluminum oxyhydroxide being electropositive in aqueous media. However, in order to estimate the effectiveness of the fibrous material and to presicely reveal the mechanism of its hemostatic action it should be compared with the available hemostatics in the same experimental model.

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