Study on Influence of Gelatin-Alginate Matrixes on the Coagulation System and Morphotic Blood Elements

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Summary: The paper presents evaluation hemolytic and hemostatic activity of gelatin-alginate matrixes in contact with human blood *in vitro* studies. The percentage of hemolysis and free hemoglobin concentration were normal. The blood recalcyfication time (CT) was shortened and activated partial thromboplastin time (APTT) was prolonged. That sugested that coagulation processes were activated. Discrabed changes were depended an component composition of sponge.

Keywords: biocompatibility; biodegradable; coagulation system; hemolytic action; sponge

Introduction

Assessment ralations between biomaterias and blood may allow to charakterize determinate property of that materials. Interactions of biomaterials with blood should not cause damages of blood cells, alter protein activity.[1–3] The porous gelatin-alginate matrixes may be useful for medicine in future. Polymers like gelatine and sodium alginate are biocompatibilites, biodegradablilites and bioresorbables. Glycerol is a subtances which accurs naturally in human organism.[4–6]

The aim of the study was to evaluate the influence of gelatin-alginate matrixes on morphotic blood elements and blood proteins *in vitro*.

Material

Four kinds of gelatin-alginate matrices in a form of a sponge prepared at Department of Drug Form Technology Medical University of Wrocław were used in the study. In order to obtain a form of sponge a liofilization of foam originated from foaming of mixture of sterile solution of gelatin (20%), natrium alginate (2% or 4%) and glycerol (3% or 5%) selected in an appropriate ratio was performed (Table 1). Human blood preserved in citrate was used in the study and citrate plasma. Hemocompatibility of gelatin-alginate matrixes with the use of citrated human bood. Studies were made on whole citrated human blood on static *in vitro* model.[7–12]

Methods

Hemolytic Activity Studies

Hemolytic action of gelatin-alginate matrixes was assessed by measure of the hemolysis percentage, free plasma hemoglobin concentration and evaluation of morphotic blood elements.[13–15]

Erythrocyte Preparation

Fresh human blood $Rh^{+}0$ (sodium citrate solution, 0.11 mol/l, ratio 10:1) was centrifugated ($1050 \times g$,10 min). Erythrocyte sediment was rinsed three times with 0.15 mol/l of sodium chloride solution, stirred and centrifugated ($550 \times g$,10 min)

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Table 1.
Content of the gelatin-alginate matrixes.

Matrix	Gelatin	Natrium alginate	Glycerol
A3	8 cz 20%	2 cz 2%	3%
A5	8 cz 20%	2 cz 2%	5%
B3	8 cz 20%	2 cz 4%	3%
B5	8 cz 20%	2 cz 4%	5%

each time. The concentrated erythrocytes were in studies the hemolysis percent.[7–9]

Hemolysis Determination

The sponges incubated in sodium in citrate solution (0,020 g/5 ml) in 37 °C for 24 hours.

Isotonic extract from the sponge, isotonic water for injection (negative control) and water for injection (positive control) and 20 ul erythrocytes concentrated were incubated at 37 °C for 4 hours. Next, the erythrocyte suspension was examined microscopic alls. After centrifugation ($550 \times g$, 10 min) absorbance of the liquid over the erythrocyte sediment was measured at 540 nm by a spectrofotometer Marcel s 330 by Marcel, Poland. The percent hemolysis was calculated.[15]

Plasma Free Hemoglobin Determination

The sponges in citrate blood (0,0070 g/1,3 ml) incubated in a thermostat for 24 hours at 37 °C. Simultaneously with the tested sample a only blood was prepared as the control sample. Determination of plasma hemoglobin Drabkin,s metod. After centrifugation ($550 \times g$, 10 min) absorbance of the liquid over the erythrocyte sediment was measured at 540 nm and 680 nm by analyzed spectrophotometrically.[10–12]

Blood Cell Morphology

Blood smear was used in order to evaluate morphotic blood elements. A drop of citrate blood was put on a basic glass and smear was made which was dried in room temperature. Smears of control blood and after contact with evaluated sponges were stained with Maya, Grunwalda and Giemsy methods. The smears were observed under oil in a light microscope and erythrocytes and leukocytes and thrombocytes were evaluated.[10–12]

Hemostatic Activity Studies

Recalcification Time Blood Determination

The samples sponge (0,0024 ± g/0,5 ml) were incubated in a thermostat in temperature 37 °C for 15, 30 and 60 minutes. Next all the samples were delicately stirred of blood was taken from each tested and control sample. After incubation in temperature 37 °C through 120 minutes, 0.5 ml of 25 mmol/l of calcium chloride was added, delicately stirred and coagulation time was measured.[11–12]

Plasma Coagulation System Determination

Plasma coagulation system studies were done on average-platelet-rich plasma, which was obtained after centrifuging (1500 g, 10 min) control citrate blood and after contact with the tested sponges.

The samples sponge in plasma (0.0054 g/1,4 ml) and only plasma (control) incubation at 37 °C over 15, 30, 60 and 120 minutes. Activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrinogen concentration (FB) were determined in the plasma. The studies at 37 °C were performed using coagulation Coag Crom 3003 by Bio-Ksel, Poland.[7–12]

Results

The mean value of hemolysis percetagle, no matter kind of matrix, did not exceed normal values. Free hemoglobin concentration in

Table 2.
Hemolysis percent and free plasma hemoglobin concentration of blood by gelatin-alginate matrixes.

Matrix	H[a)]	HGB[b)]
	%	g/dl
A3	0,83 ± 0,12	32,09 ± 2,53
A5	0,78 ± 0,12	27,18 ± 2,53
B3	0,88 ± 0,10	31,03 ± 3,14
B5	0,81 ± 0,16	31,56 ± 3,26
Control	–	23,16 ± 2,27

[a)] H, hemolysis;
[b)] HGB, free plasma hemoglobin.

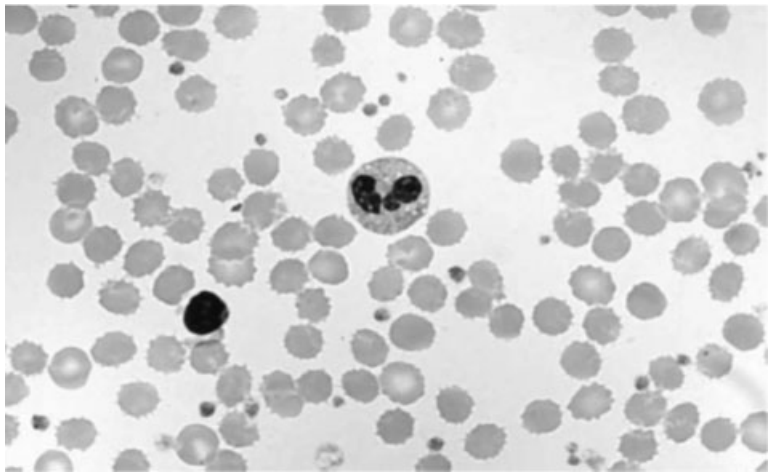

Figure 1.
Citrate blood – control after incubation for 24 hours in 37 °C. Acantocytes form of erytrocytes. Normal leukocytes (lymphocytes and neutrocytes) and thrombocytes blood. Stain. MMG. Magn. 1400×.

plasma obtained from blood after contact with matrix was in the range of values observed in the control group (Table 2). Leukocytes were normal in blood smear from somples with sponges and no changes were observed in comparsion with control samples. Erythrocytes were in the form of accantocytes. The cells were aglutinated together in the blood after contact with gelatin. Rapid sedimentation and rouleaux formation of erytrocyteswas observed to the contact with sponges The thrombocytes were interlinked, creating bigger or smaller aggregates (Figure 1 and 2).

The reference value parameters blood coagulation for human plasma give in Table 3 and after contact citrate plasma with sponge in Table 4 and Figure 3–6.

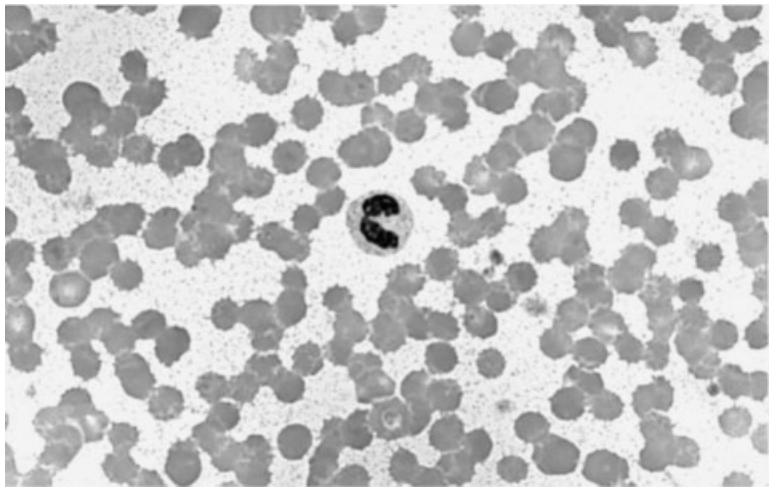

Figure 2.
Citrate blood after incubation whit gelatin-alginate matrix for 24 hours in 37 °C. Acantocytes and rouleaux. Normal leukocytes (neutrocytes) and thrombocytes blood. Stain. MMG. Magn. 1400×.

Table 3.
Values parameter normal human plasma.

Matrix	Values parameters of normal human plasma	Values parameters of experimental human plasma
APTT (s)	29,2–36,5–43,8	33,25 ± 0,98
PT (s)	10,6–13,2–15,8	14,65 ± 0,35
TT (s)	10,3–12,5–15,5	11,60 ± 0,18
FB (g/l)	0,3–6,0	3,14 ± 0,06

APTT, activated partial thromboplastin time; PT, prothrombin time; TT, trombin time; FB, fibrynogen.

Table 4.
Recalcification time (CT) blood and activated partial thromboplastin time (APTT) in plasma after contact with sponge.

Matrix	Shortened CT in comparsion with control				Prolonged APTT in comparsion with control			
	15 s	30 s	60 s	120 s	15 s	30 s	60 s	120 s
	%				%			
A3	55	54	49	–	23	19	21	23
A5	51	52	48	–	13	15	16	17
B3	56	53	56	–	13	20	18	19
B5	31	38	60	–	15	16	15	18

Recalcification time (CT) was significantly shortened in blood samples with sponges-about 55% ($p < 0{,}001$) in relation to control value (Table 4).

After contact with a sponge the plasma APTT was prolonged and significantly ($p < 0{,}01$, $p < 0{,}001$) longer in comparison to controls (Figure 3, Table 4). Prothrombin time (PT), thrombin time (TT) and fibrinogen concentration (FB) in all measures were comparable and did not differ from controls (Figure 3–6).

Discussion

During the study gelatin-alginate matrixes were in solid form in study environment only for a short period of time (15 minutes), hence later the study was performed on

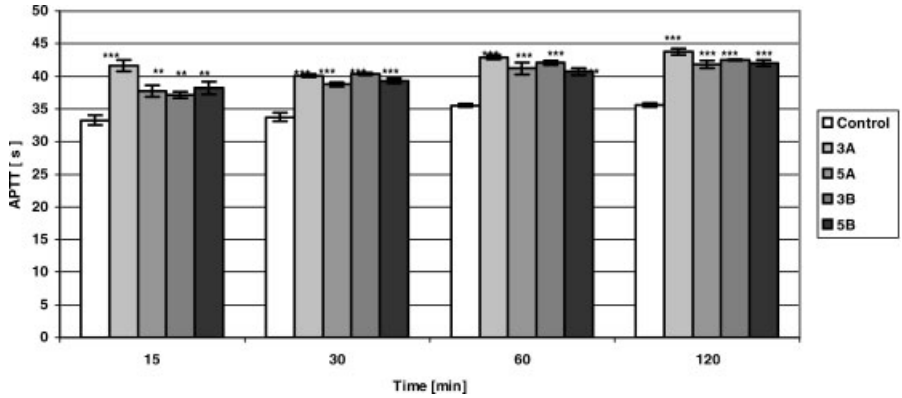

Figure 3.
Changes activated partial thromboplastin time (APTT) in the plasma control and after contact with gelatin-alginate matrixes in time function $^{**}p < 0{,}01$, $^{***}p < 0{,}001$-differences of statistic inportance in relation to the control.

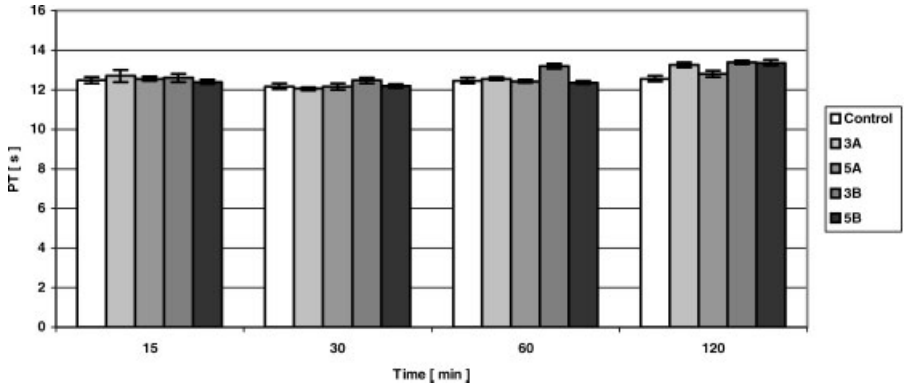

Figure 4.
Changes prothrombin time (PT) in the plasma control and after contact with gelatin-alginate matrixes in time function.

samples in liquid state. Hemolytic action studies mean value of hemolysis rate, no matter kind of matrix, did not exceed normal values 3%.[15] Free plasma hemoglobin concentration did exceed the value of 40 mg/dl what is regarded as the highest accepted value of this parameter and was in range of values measured in control group.[11–12] The erytrocytes were aglutinated together, rouleaux, because of the presens in the blood of the sol sponge. The change thrombocytes in thei shape shows their activation.[11–12]

Coagulation activation dependent on contact factors (endogenous system) was evaluated by a test activated partial thromboplastin (APTT). Coagulation activation dependent on tissue thromboplastin (exogenous system) was evaluated by a test of prothrombin (PT). Those two systems were joined by the test of trombine time (TT), the measurement of the conversion of fibrinogen into fibrin. Additionally the fibrinogen concentration (FB) was evaluated.[7–12] The analysis of the changes in the value of the above mentioned parameters

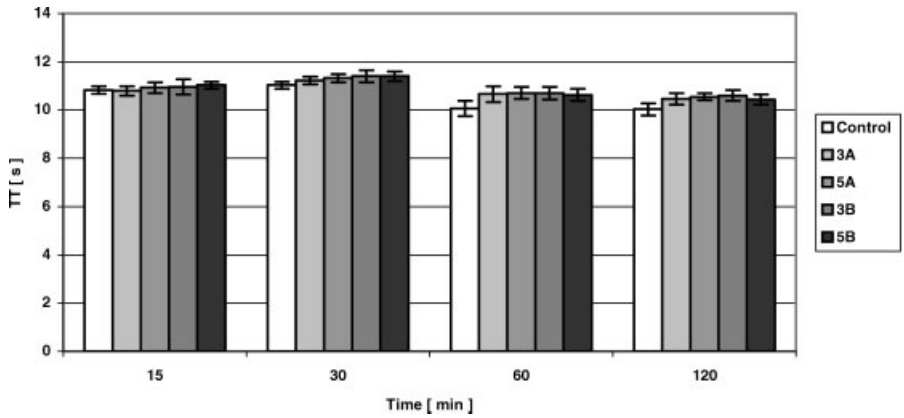

Figure 5.
Changes prothrombin time (TT) in the plasma control and after contact with gelatin-alginate matrixes in time function.

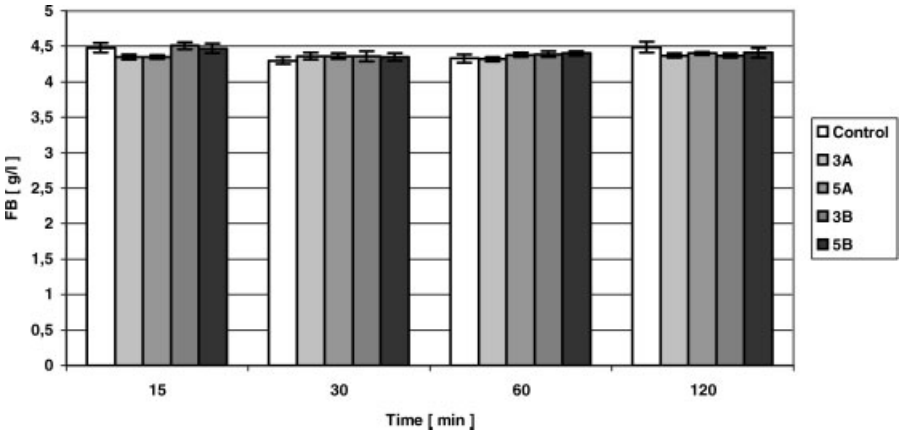

Figure 6.
Fibrinogen concentration (FB) in the plasma control and after contact with gelatin-alginate matrixes in time function.

allowed us to evaluated the activation of blood coagulation system. The sponges under our evaluation significantly shortened the recalcification time and prolonged APTT, but did not change the values of PT, TT and FB. The changes observed in the coagulation system are the result of the temporary contact of the tested sponges with blood and plasma.The result values are not higher then the reference values (Table 3).

This is important for the diagnostic and is the proof that during gelatin-alginate matrixes contact with blood and clot formation the use of blood coagulation components will not be higher. The matrixes may be use like a hemostatic materials.[8]

Conclusion

The obtained results indicate that gelatin-alginate matrixes in a form of sponges do not have hemolytic features and they activate the coagulation system.The shortest recalcination time and the smallest changes in plasma coagulation system were observed in sponges with high natruim alginate concentration.

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