# In vitro analysis of polyurethane foam as a topical hemostatic agent

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**Abstract** Topical hemostatic agents can be used to treat problematic bleedings in patients who undergo surgery. Widely used are the collagen- and gelatin-based hemostats. This study aimed to develop a fully synthetic, biodegradable hemostatic agent to avoid exposure to animal antigens. In this in vitro study the suitability of different newly developed polyurethane-based foams as a hemostatic agent has been evaluated and compared to commonly used agents. An experimental in vitro test model was used in which human blood flowed through the test material. Different modified polyurethane foams were compared to collagen and gelatin. The best coagulation was achieved with collagen. The results of the polyurethane foam improved significantly by increasing the amount of polyethylene glycol. Therefore, the increase of the PEG concentration seems a promising approach. Additional in vivo studies will have to be implemented to assess the application of polyurethane foam as a topical hemostatic agent.

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## 1 Introduction

Topical hemostatic agents can be used to treat problematic bleedings in patients who undergo surgery. The benefits of local hemostats include improving blood conservation by reducing blood loss, shortening the time of hemostasis, avoiding the adverse effects of systemic hemostatic drugs and saving transfusion blood. Adverse effects can be foreign body reaction, infection, and granuloma formation [1]. Different types of hemostatic agents are available, each with their own characteristics. The following characteristics should be considered in search of an ideal hemostatic material: ease of application and removal, bioresorption potential, suturability, antigenicity, and tissue reactivity effects [2]. The possibility to adjust absorbability and flexibility of the material would be a further advantage [3]. Some of the most widely used topical hemostatic agents nowadays are collagen and gelatin which are animal-derived products. Procoagulant substances like thrombin or fibrin are frequently added to these agents to increase their hemostatic efficacy [1].

However, the use of products from animal or human origin has the potential risk of blood-borne pathogens and immunization. Examples are Bovine Spongiform Encephalopathy (BSE) and Creutzfeldt-Jakob disease. Bovine collagen intended for collagen-based medical devices should therefore not come from a country with known BSE cases [1]. The use of bovine-derived components in hemostats has resulted in a number of reports of immunization to bovine thrombin. The resulting antibodies can cross-react with blood clotting factor V and lead to coagulopathy [4].

A fully synthetic, biodegradable material with hemostatic properties would prevent these potential risks. Polyurethane (PU) with uniform hard segments composed

of butanediol and 1,4-butanediisocyanate and soft segments of DL-lactide, ε-caprolactone and polyethylene glycol (PEG) is a synthetic biodegradable material with possible hemostatic properties. Coagulation is initiated upon exposure to a PU surface while the PEG makes the material more hydrophilic which increases the attraction of platelets [5]. In a previous in vivo study the good biocompatibility of a comparable PU without PEG moieties has been demonstrated [6].

It is not known whether PU foam has hemostatic properties or can be modified to a hemostatic agent. We questioned in this in vitro study if PU foam can achieve similar hemostatic properties as commercially available products. Therefore, PU foams were compared to an absorbable collagen hemostat (Novacol) and an absorbable gelatin sponge (Spongostan). Different modifications were applied to the PU foam to test whether they might increase its hemostatic efficacy. The PU foam was enriched with rFVIIa (recombinant factor VIIa), phospholipids, ADP (adenosine diphosphate) and thrombin. Factor VII is one of the central proteins in the coagulation cascade. When factor VIIa forms a complex with tissue factor (TF) the extrinsic pathway of the coagulation cascade is initiated [7]. Phospholipids greatly enhance the binding of factor VIIa to TF. ADP interacts with platelets, leading to further platelet activation [8]. Thrombin is a coagulation protein that has many effects in the coagulation cascade including platelet activation and the conversion of fibrinogen into fibrin [9]. Furthermore, some of the PU foams were treated with 'plasma glow discharge' which creates a more hydrophilic foam with enhanced platelet binding and activation [10].

An experimental in vitro test model was used in which fresh human whole blood flowed through the test materials. This test model was based on the Thrombostat 4000® which is used to measure in vitro bleeding time and volume [11].

## 2 Materials and methods

### 2.1 Materials

In this study tests have been performed with PU, Novacol (absorbable collagen hemostat; Bioprof BV, Moerkapelle, the Netherlands) and Spongostan (absorbable gelatin sponge; Johnson & Johnson, Skipton, UK).

The used PU is a block-copolymer composed of urethane hard segments and co-polyether-ester soft segments. This was done to achieve rapid degradation. The soft segments consisted of 50% DL-lactide and 50% ε-caprolactone. In a first PU foam formulation, PEG 1000 was used as initiator for the soft segments synthesis, and in a

second one PEG 20.000 was added in a ratio of 3–1 to prepare a blend. The urethane segments were synthesized with 1,4-butanediisocyanate (BDI) and 1,4-butanediol (BDO). They had a uniform length of 5 urethane moieties, which resulted in a PU with BDI–BDO–BDI–BDO–BDI urethane segments in the polymer. The PU was then dissolved in 1,4-dioxane. After dissolving, the solution was poured into a mold and cooled down to −18°C. The solution was freeze-dried at 3 mbar to remove the 1,4-dioxane crystals, resulting in an highly porous foam with a porosity of 97% and an overall PEG-content of 40 wt%. Overall porosity was calculated after determining the weight and dimensions of the foams.

The second PU foam formulation created a much more hydrophilic foam due to the increased PEG concentration. This foam had a porosity of 97% and an overall PEG-content of 55 wt%.

The foams were processed to round slices with a diameter of 4 mm and a thickness of 2 mm. The foams were ultimately sterilized using ethylene oxide (EtO). The polymers and foams were manufactured by Polyganics BV (Groningen, The Netherlands). The collagen and gelatin materials were, like the PU foams, processed to round slices with a diameter of 4 mm and a thickness of 2 mm.

After the sterilisation process 145 foams were modified to potentially increase their hemostatic efficacy. This was done by adding 10 μl of a procoagulant substance or surface treatment. In Table 1 an overview is presented of the added procoagulant substances and surface treatment.

### 2.1.1 Procoagulant substances

Prior to adding the substances, the PU foams were treated for 15 min with 5 μl of a 15% glutaraldehyde solution to increase the attachment of the substances. Treatment with glutaraldehyde is a procedure which can be employed to stabilize the binding of enzymes [12]. The excess of glutaraldehyde was washed away with copious amounts of phosphate buffer solution (pH 7.4) and ultimately with

**Table 1** Overview of the added substances to the PU foams

| PEG concentration of PU foam (wt%) | Added substance or treatment | Number of treated foams |
|---|---|---|
| 40 | Recombinant FVIIa (0.1 mg/ml) | 30 |
| 40 | Phospholipid solution (100 mg/ml) | 20 |
| 40 | Adenosine diphosphate (ADP) (0.085 mg/ml) | 20 |
| 40 | Bovine thrombin (100 NIH U/ml) | 10 |
| 55 | Recombinant FVIIa (0.1 mg/ml) | 25 |
| 40 | Saline (0.9% NaCl) | 10 |
| 40 | Plasma glow discharge | 30 |

ultra-pure water. The substances were immediately applied to the foams 1 h before testing using a volumetric pipette.

### 2.1.2 Surface treatment

Glow discharge treatment was performed at 50 W during 5 min at both sides. Vacuum was achieved by a dual trap vacuum pump and was always lower than 1800 Pa prior to argon (99.996%, Hoekloos Nederland B·V., The Netherlands) inlet. The argon pressure during plasma treatment was 500 Pa. After glow discharge, the reactor chamber was ventilated with ambient air. The surface characterisation of the treated PU foams was analysed by X-ray photoemission spectroscopy. This showed an increase of oxygen and nitrogen which resulted in a lower contact angle and thus higher hydrophilicity.

## 2.2 Methods

### 2.2.1 Blood preparation

The study was approved by the ethics committee of the University Medical Center Groningen, the Netherlands. The blood that was used for this study was obtained from 10 healthy adult volunteers. Exclusion criteria were a known disease or use of drugs that could have influence on blood coagulation. Each volunteer donated 30 ml of blood which was tested for normal trombocyte count. To prevent the blood from instant clotting, heparin was added to a concentration of 1.5 IU of heparin per ml blood. The blood was then divided over 30 microcentrifuge tubes, one ml per tube and kept at room temperature. This way 30 tests could be performed from each blood donation, the tests were finished within 2 h after the blood was obtained. Each session the materials were tested in a different order to level the influence of time after the blood donation. A series of tests was performed without material as a control group. Recombinant tissue factor (rTF) in a concentration of 5 pM was added to each ml of blood to resemble the wound situation in vivo. This was necessary because no detectible concentrations of active TF are present in the blood of healthy donors [13]. While in a wound situation, extravascular TF is exposed to the blood and binds plasma factor VIIa [7].

### 2.2.2 Test device

An experimental in vitro test model was used which was based on the Thrombostat 4000® (Von der Goltz, Seeon, Germany). This device was used because of the possibility to insert different test materials in an existing model for measuring hemostasis in vitro. In this model blood flowed through the fixated test materials with a constant pressure
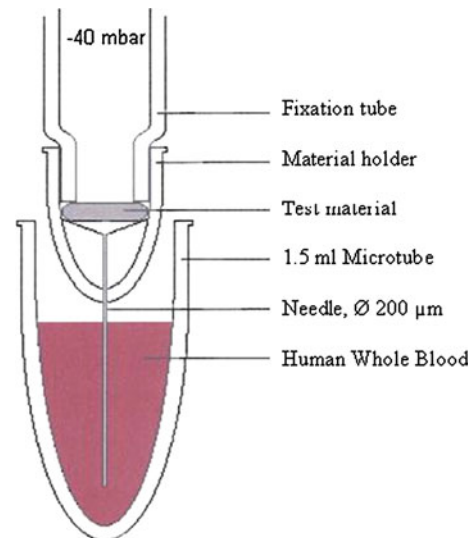


**Fig. 1** In vitro test model. Suction of human whole blood was performed with a constant pressure of −40 mbar. Ø, diameter

of −40 mbar. Suction of the blood was performed through a needle with a diameter of 200 μm to create shear stress (Fig. 1). The shear stress was necessary to mimic capillary bleeding which is an important platelet activator in vivo. The thrombostat calculated the amount of blood flow through the test material over a period of 90 s. As a derivative for the extent of coagulation the blood flow deceleration was determined. This was calculated out of the blood volume that had passed the test material after 30, 60 and 90 s. The volume after 30 s was used to obtain the velocity of blood flow at 30 s. This was done by dividing the volume after 30 s by 30 and defined as initial velocity ($V_i$) in μl/s. The final velocity ($V_f$) in μl/s was calculated by dividing the volume that had flowed between 60 and 90 s by 30. The deceleration (d) in μl/s$^2$ could be calculated by dividing the difference between initial and final velocity by the time interval in seconds (t) using the formula $d = (V_f - V_i)/t$.

### 2.2.3 Thrombin-antithrombin complexes (TAT)

The extent of thrombin generation in vitro was determined by measuring thrombin-antithrombin complexes (TAT) in two of the blood samples. The measurement was performed 30 min after venous blood collection. In two of the blood samples that had flowed through the PU foam (55% PEG) with and without rFVIIa the amount of TAT was also determined. After the experiment 500 μl of blood was collected and added to 50 μl EDTA-solution (0.1 mol/ml) to prevent further generation of thrombin. The blood sample was then centrifuged, and 200 μl of the supernatants were collected and stored at −20°C until further analysis. Enzyme-linked immunosorbent assay (Cedarlane

Ltd., Burlington, Ont., Canada) was employed for measurement of the TAT complexes.

### 2.2.4 Statistical analysis

Statistical analysis was performed using SPSS 16.0. Group differences were calculated by the Mann–Whitney-$U$-test. $P$ values less than 0.05 were considered statistically significant.

## 3 Results

The mean decelerations and their standard deviations were calculated per material and are presented in order of efficacy in Table 2.

For a clear overview the mean decelerations are also presented in a boxplot (Fig. 2).

The best results were achieved with collagen which performed significantly better than the other materials ($P < 0.001$). The second best results were achieved with gelatin which performed significantly better than the control group without material and than the PU foams with 40% PEG, with or without rFVIIa ($P < 0.001$). The PU foams with 40% PEG showed no significant difference compared to the control group without material. The PU foams with 55% PEG showed a significantly higher deceleration than the PU foams with 40% PEG ($P < 0.001$). Again the addition of rFVIIa to the foams gave no improvement of the results. No significant difference was observed between the PU foams with 55% PEG and gelatin.

The results of the modified PU foams (40% PEG) are also presented in a boxplot (Fig. 3).
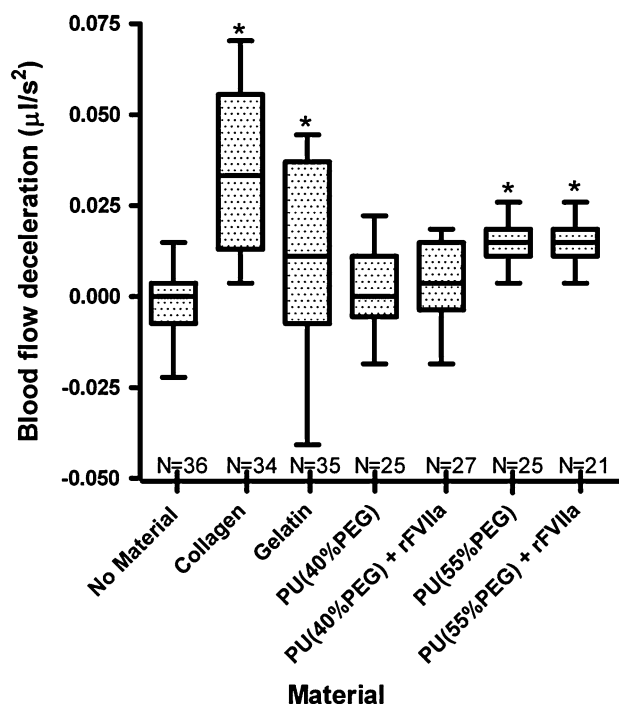


**Fig. 2** Schematic representation of decelerations for the different test materials. The number of tested samples (*n*) is noted. *PU* polyurethane; *PEG* polyethylene glycol; *rFVIIa* recombinant factor VIIa. For each group, the *line in the middle* of the *box* represents the median. The *lower* and the *upper edges* of the *box* are the 1st and 3rd quartile, respectively. The fences are drawn to the nearest value not exceeding 1.5 (interquartile range). *$P < 0.001$ compared with control group (No Material)

In the group with the modified PU foams, collagen again performed significantly better than the other materials ($P < 0.001$). The PU foams which were treated with 'plasma glow discharge' showed a significantly higher deceleration than the tests without material ($P < 0.02$). The PU foams which were enriched with phospholipids, adenosine diphosphate, saline and thrombin showed no significantly different results from the tests without material.

The blood took an average time of 12.1 s (SD: 0.83) to reach the material after suction was started. When the blood took more than 14 s to reach the material the results from these tests were excluded. This was the case in 20 out of the 305 tests.

### 3.1 Thrombin-antithrombin complexes (TAT)

Three duplo measurements were performed to assess the amount of TAT complexes before and after flow through the PU foam (55% PEG) with and without rFVIIa. Results of this measurement are presented in Table 3.

The plasma concentration of TAT complexes in vitro 30 min after venous blood collection is above the normal range (1.0–4.1 μg/l) in vivo [14]. Approximately the same concentration of TAT was found in the blood samples that

**Table 2** Mean deceleration and standard deviation of the different materials

| Material | Mean deceleration ($\mu l/s^2$) | Standard deviation |
|---|---|---|
| Collagen | 0.036 | 0.022 |
| Gelatin | 0.021 | 0.016 |
| PU (55% PEG) | 0.015 | 0.007 |
| PU (55% PEG) + rFVIIa | 0.015 | 0.006 |
| PU + glow discharge | 0.012 | 0.011 |
| PU + thrombin | 0.009 | 0.011 |
| PU + saline | 0.008 | 0.005 |
| PU (40% PEG) + rFVIIa | 0.004 | 0.011 |
| PU (40% PEG) | 0.002 | 0.010 |
| No material | −0.001 | 0.009 |
| PU + ADP | −0.002 | 0.015 |
| PU + PL | −0.003 | 0.015 |

*PU* polyurethane; *PEG* polyethylene glycol; *rFVIIa* recombinant factor VIIa; *ADP* adenosine diphosphate; *PL* phospholipids
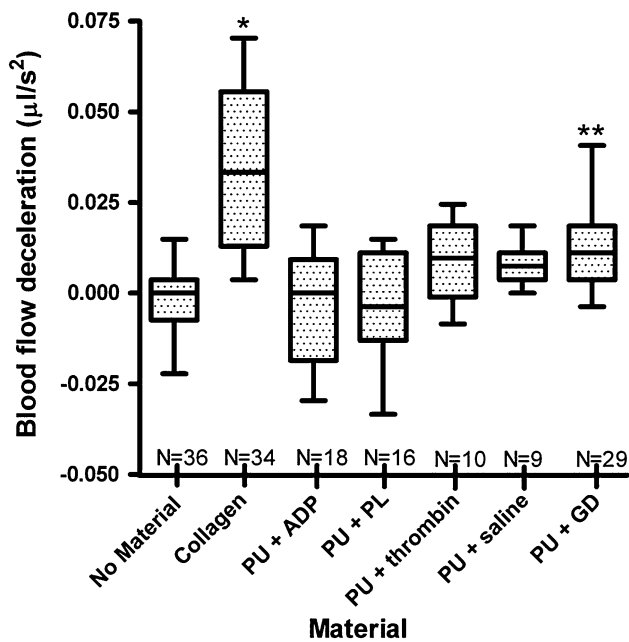
**Fig. 3** Schematic representation of decelerations for the different modified PU foams (40% PEG). The number of tested samples (*n*) is noted. The results of collagen have been added to this boxplot as a reference. *PU* polyurethane; *PL* phospholipids; *ADP* adenosine diphosphate; *GD* glow discharge. For each group, the *line in the middle* of the *box* represents the median. The *lower* and the *upper edges* of the *box* are the 1st and 3rd quartile, respectively. The fences are drawn to the nearest value not exceeding 1.5 (interquartile range). *$P < 0.001$ compared with control group (No Material). **$P < 0.05$ compared with control group (No Material)

**Table 3** Thrombin-antithrombin complexes in the different blood samples

| Blood sample | TAT (mg/L) | Standard deviation |
|---|---|---|
| Before testing | 1.38 | 0.06 |
| After PU | 1.36 | 0.07 |
| After PU + rFVIIa | 2.40 | 0.12 |

*PU* polyurethane; *rFVIIa* recombinant factor VIIa

had flowed through the PU foam (55% PEG) without rFVIIa. The blood samples that had flowed through the PU foam (55% PEG) with rFVIIa showed a higher concentration of TAT complexes.

## 4 Discussion

An experimental in vitro test model was used to compare the synthetic PU foam to commercially available hemostatic materials. Additional rTF was added to the blood to more closely resemble the wound situation in vivo. Different modifications have been applied to the PU foam to increase its hemostatic efficacy. The addition of rFVIIa to the PU foam did not influence the results. The effect of

intravenous rFVIIa has been proven to reduce blood loss in severe blunt trauma patients in a multicenter randomized controlled trial [15]. In a study in rats the local use of a microporous polysaccharide hemosphere (MPH) in combination with freeze-dried rFVIIa significantly improved hemostasis [16]. The failure of rFVIIa to improve the results of PU foam in this study could indicate that the extrinsic pathway of the coagulation cascade was not adequately activated in the current test model. The extrinsic pathway is initiated by the combination of TF and FVIIa and clotting time with normal protein concentrations is approximately 8.6 s [17]. In the used test model the blood flowed through the material during 90 s. Under the influence of heparin which inhibits thrombin and factor Xa this time period might have been too short [18]. Another explanation could be that the applied rFVIIa was flushed out of the PU foam by the blood and therefore did not contribute to the coagulation. The same could be true for the other applied substances which gave no improved blood coagulation. Another explanation is that the complexity of the coagulation cascade might not be adequately resembled in vitro. This process was further complicated by the necessary addition of heparin which inhibits the coagulation cascade [18].

The PU foams which have been enriched with phospholipids, adenosine diphosphate and thrombin gave no improved results. With the addition of thrombin to a local hemostatic agent good results have been achieved in in vivo studies [19, 20].

The good results of collagen in this study could be explained by its action mechanism which is based predominantly on platelet aggregation. Collagen-based hemostats attract platelets when they get in contact with blood. The platelets adhere to collagen fibrils and degranulate, thereby triggering platelet aggregation [21].

The improved results of PU foam after modification of the PEG concentration could indicate that this enhanced the attraction of platelets. In a study by Lee et al. [22] the platelet adhesion to a PU surface increased slightly when short PEG chains were added whereas long PEG chains prevented platelet adhesion. The absorbable properties of PEG might contribute to its hemostatic effect by concentration of the endogenous coagulation factors and platelets. The effect of cellulose- and polysaccharide-based hemostats is based partly on this mechanism [17, 23].

To analyse the influence of adding fluids to the foam some PU foams have been enriched with saline. As expected these foams gave no improved results compared to the PU foam without modifications.

The treatment of PU foams with glow discharge led to significantly better results. This should be contributed to the electrostatic charge at the membrane surface which creates a more hydrophilic material. By increasing the

hydrophilicity of polyethylene Spijker et al. [10] found more platelet adhesion and activation of the clotting system. In 20 out of the 305 tests the blood took more than 14 s to reach the material and therefore the results from these tests were excluded. The delay in blood flow could be due to obstruction of the 200 μm needle.

An advantage of our in vitro model was the easy way to test the efficacy of a large number of modifications in multifold experiments with human blood. A few drawbacks of the in vitro model were also demonstrated. Generation of thrombin after collection of blood can lead to activation of platelets and subsequent loss of response to agonists [24]. The addition of heparin to the blood does not completely block the generation of thrombin in vitro [25]. This is supported by the results of the TAT generation test. The TAT concentration in blood before contact with any hemostatic agent showed a 1000-fold increase compared to normal blood. The lowering blood temperature might also have contributed to a diminished platelet function [26].

In this in vitro coagulation study the best results were achieved with collagen. Increase of the PEG concentration improved the results of PU foam significantly and seems a promising approach. There were no significant differences between this modified PU foam and gelatin. The results of collagen, however, could not be matched. Additional in vivo studies will have to be implemented to assess the application of polyurethane foam as a local hemostatic agent.

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