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ORIGINAL ARTICLE

In vivo hemostatic efficacy of polyurethane foam compared to collagen and gelatin

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Abstract
Objectives Topical hemostatic agents are used in all surgical disciplines. Most of these hemostats are based on animal-derived products like collagen and gelatin. They carry the potential risk of pathogen transmission. A newly developed biodegradable, fully synthetic hemostatic agent based on polyurethane foam (PU) with 55 % polyethylene glycol (PEG) would prevent these potential risks.
Materials and methods The hemostatic efficacy of this new agent was compared to gelatin and collagen in humans who underwent extraction of an upper and lower molar (split-mouth model). After extraction of a molar in the maxilla and mandible, a PU foam and collagen or gelatin were inserted in the extraction socket for 2 min. Hereafter, the agents were removed and stored in ethylenediaminetetraacetic acid to stop coagulation. Then, the concentration of coagulation parameters thrombin–antithrombin III (TAT) complexes, fibrinogen, and thromboxane B_2 (TxB_2) in blood extracts from the agents was measured. The concentrations were also determined in baseline blood samples which were collected from the extraction socket.
Results The concentrations of TAT and TxB_2 were significantly increased, and fibrinogen concentration was significantly reduced compared to baseline wound blood concentrations indicating enhanced hemostasis. No significant differences were seen in the concentrations of these coagulation parameters in the three different hemostatic agents.
Conclusions These results show that PU combined with 55 % PEG is a promising alternative for the animal-derived hemostatic agents.
Clinical relevance The synthetic hemostatic agent could replace the animal-derived products like collagen and gelatin and therewith prevent the potential risk of pathogen transmission.

Keywords Polyurethane · Collagen · Gelatin · Hemostasis · In vivo test

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Introduction

Topical hemostatic agents are used in all surgical disciplines. Most of the mechanical hemostats are based on animal-derived products like collagen and gelatin. Therefore, they carry the potential risk of pathogen transmission which can result in diseases like Creutzfeld–Jacob or bovine spongiform encephalopathy [1].

A newly developed fully synthetic hemostatic agent would avoid these potential risks. Furthermore, the production process of a synthetic material allows greater control over material properties and tissue responses, which gives a

synthetic material another advantage over animal-derived products [2]. The animal-derived hemostatic agents are relatively cheap materials. Therefore, a new synthetic topical hemostatic agent should preferably be made of a material that can be produced at the same low cost.

A blend of modified polyurethane foam (PU) and polyethylene glycol (PEG) was processed to a synthetic, biodegradable material with possible hemostatic properties. The biodegradability and biocompatibility of a comparable PU has been demonstrated in a previous study [3]. In an animal study in which this PU foam was used to close oroantral communications, complete bony regeneration was seen after 1 year with only small PU fragments at a microscopic level [4]. This was a PU with 5 % PEG, whereas our PU with 55 % PEG will be resorbed much faster. The synthetic PU foam can be produced at low cost, but the development of bioresorbable products has become expensive due to laws and regulations. Therewith, it can be expected that the PU will be more expensive than the collagen- and gelatin-based hemostatic agents.

When a biomaterial like PU comes into contact with blood, adsorption of factor XII initiates the contact activation pathway of the coagulation cascade and leads to platelet adhesion and activation [5]. The hemostatic efficacy of this material was improved by increasing the concentration of PEG up to 55 % as shown by in vitro study [6]. Hemostasis, however, is complex and difficult to resemble in vitro [7]. Therefore, an in vivo study was performed in which the polyurethane foam was compared with a commercially available collagen- and gelatin-based hemostatic agent. The purpose of this study was to investigate if the modified PU could achieve similar results as the collagen- and gelatin-based hemostatic agents. The hemostatic efficacy of the materials was analyzed by measurement of blood coagulation parameters. Thrombin–antithrombin III (TAT) complexes, fibrinogen, and thromboxane B_2 (TxB_2) were measured in blood which was derived out of the materials after use in a human wound.

The concentration of TAT complexes is accepted as an index of thrombin generation in vivo and thus a measurement for the amount of coagulation [8]. Fibrinogen is converted into fibrin by thrombin [9]. Reduction of fibrinogen is therefore also an indicator of the amount of coagulation. Thromboxane B_2 is an inactive metabolite of thromboxane A_2 which can be used to quantify platelet aggregation [10].

Materials and methods

Subjects

The study population consisted of 60 patients who were referred to the Department of Oral and Maxillofacial Surgery of the University Hospital Groningen, The Netherlands, for extraction of an upper and lower molar. The molars were extracted because of insufficient space in the dental arch or because they were difficult to clean for the patient. None of the patients had an active pericoronitis or periodontal disease during the time of extraction. The patients were otherwise healthy, and none of them used any medication. The study protocol was approved by the local ethical committee and conducted according to the guidelines for good clinical practice and the Declaration of Helsinki. Written informed consent was obtained from each patient before inclusion.

Materials

In this study, PU foam was compared to a collagen and gelatin hemostatic agent. The used PU was a block copolymer composed of urethane hard segments and co-poly(ether-ester) soft segments. The soft segments consisted of 50 % DL-lactide and 50 % ε-caprolactone. PEG 1000 was used as initiator for the soft segments synthesis, and after that, PEG 20000 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 five 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 55 wt%. Overall porosity was calculated after determining the weight and dimensions of the foams. The foams had a cylindrical shape with a size of 1 cm^3 and were ultimately sterilized using ethylene oxide. The polymers and foams were manufactured by Polyganics BV (Groningen, The Netherlands).

For the collagen hemostat, Hémocollagène (absorbable collagen hemostat; Septodont, Saint-Maur-des-Fossés, France) was used with a size of 1 cm^3. For the gelatin hemostat, Spongostan (absorbable gelatin sponge; Johnson & Johnson, Skipton, UK) was used with a size of 1 cm^3.

Study protocol

We designed an experimental, prospective, randomized, split-mouth protocol in which PU foam, collagen, and gelatin were tested. Randomization was generated with a computer random number generator. PU foam was tested in all patients and compared to a collagen or gelatin sponge in random order. After extraction of the lower molar, 0.2 ml of blood was collected from the extraction socket with a syringe. The blood was stored in a cup with 200 μl of 0.2 M ethylenediaminetetraacetic acid (EDTA) solution to chelate calcium and prevent further activation of coagulation proteins [11].

Hereafter, one of the test materials was inserted in the alveolus during 2 min. After extraction of the upper molar, another test material was inserted in this alveolus for 2 min. Continuous suction was performed around the alveolus to ensure that saliva did not flow in the alveolus. After the 2 min had passed, each material was stored in a cup filled with EDTA solution. The amount of EDTA solution was adjusted for each test material to match the mean amount of blood that was absorbed by each material. In the cups for the PU foams, 250 μl of EDTA solution was inserted, for the collagen sponge 170 μl and for the gelatin sponge 160 μl. Thus, the ratio of blood/EDTA solution was kept at 1:1.

The cups with blood and materials were centrifuged at 13,000 rpm during 1 min in a MSE Micro Centaur. This causes the plasma, cellular release products, and EDTA solution to poor out of the foams. The aliquots of plasma were collected and stored at −80 °C. The samples were thawed just before analysis.

TAT complex immunoassays

The plasma samples were diluted 1 in 1,000 in dilution buffer (0.1 % BSA in PBS pH 7.4). Hereafter, the samples were assayed for TAT complexes using a commercially available immunoassay kit (Enzygnost TAT micro; Behring Diagnostics, Westwood, MA). This assay is a sandwich enzyme-linked immunosorbent assay (ELISA).

Fibrinogen immunoassays

Thirty paired plasma samples were subjected to the detection of fibrinogen. The plasma samples were diluted 1 in 5,000 in dilution buffer. Fibrinogen was measured with an ELISA (Enzyme Research Laboratories Ltd., South Bend, IN, USA).

Thromboxane B_2 immunoassays

TxB_2 generation was measured in plasma samples diluted 1 in 1,000 in dilution buffer using immunoassay (Cayman Chemical, Ann Arbor, MI, USA) as indicated by the manufacturer's protocol.

Statistical analysis

Data were analyzed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as mean and standard deviation (SD). The results for the group of 30 patients who received PU and gelatin were analyzed separately from the group of 30 patients who received PU and collagen. The paired samples were analyzed with a Wilcoxon's signed rank test.

Linear regression analysis was used to determine the relationship between extraction time and baseline values. A two-sided p value <0.05 was considered statistically significant.

Results

Sixty patients (33 males, 27 females) with a mean age (range) of 26 (19–47) were included in the study. To analyze the degree of coagulation in the different samples, we measured the concentration of TAT complexes, fibrinogen, and TxB_2. We found that the use of test materials significantly increased the concentration of TAT complexes and TxB_2 compared with baseline values. The fibrinogen was decreased significantly compared with baseline values. Moreover, we found that baseline values, obtained from wound blood, were significantly different from reported normal blood values. In this wound blood, TAT and TxB_2 were markedly increased and fibrinogen decreased.

The results were separately analyzed for the two groups of 30 patients and are therefore shown in different bar charts. Figure 1 shows the TAT concentration for the different test materials and baseline samples.

The mean TAT concentrations for PU (1.01±0.29 μg/ml) and collagen (0.96±0.30 μg/ml) were higher than the baseline concentration of 0.52±0.20 μg/ml (p<0.001) in this group. Between PU and collagen, there was no significant difference (p=0.13).

The concentrations of TAT complexes in the group with PU and gelatin were 0.97±0.20 and 1.03±0.27 μg/ml, respectively. Therewith, the concentrations were significantly higher than the mean concentration of TAT complexes in the baseline samples which was 0.57±0.19 μg/ml (p<0.001). Between PU and gelatin, there were no significant differences in concentration of TAT complexes (p=0.30).

Figure 2 shows the results for the fibrinogen concentration of the test materials and baseline samples. The mean concentration of fibrinogen in PU (0.16±0.11 mg/ml) and collagen (0.14±0.12 mg/ml) was significantly lower than the mean concentration of fibrinogen in the baseline samples (0.50±0.24 mg/ml) with a p value <0.001 for this group. Between PU and collagen, no significant differences were seen for the concentration of fibrinogen (p=0.88).

In the other group, the fibrinogen concentration was also significantly lower in PU (0.16±0.24 mg/ml) and gelatin (0.16±0.17 mg/ml) than in the baseline blood samples (0.51±0.30 mg/ml) with a p value <0.001. The fibrinogen concentration was not significantly different between PU and gelatin (p=0.49).

To investigate the influence of the test materials on platelet aggregation, the TxB_2 concentration was measured. In Fig. 3, the data for the two groups are presented.

The mean concentration of TxB_2 in the first group was 28.5±24.3 ng/ml for the PU and 20.3±17.5 ng/ml for the

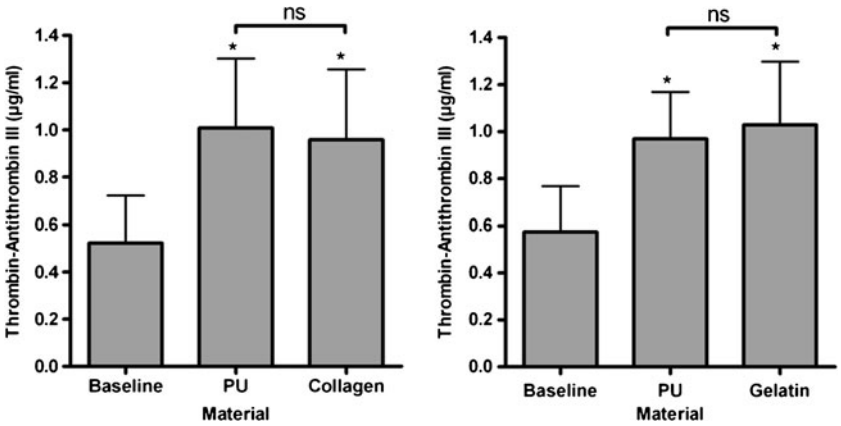

Fig. 1 Mean (±SD) thrombin–antithrombin III concentration for the different test materials and baseline samples. *PU* polyurethane foam, *ns* not significant. The asterisk indicates significance compared to baseline

collagen. This was significantly higher than the mean concentration of 8.5±4.3 ng/ml in the baseline samples ($p<0.001$). Between PU and collagen, the concentration of TxB_2 was not significant ($p=0.16$).

The mean concentration of TxB_2 in the second group was 31.9±20.0 ng/ml for the PU and 28.8±22.5 ng/ml for the gelatin. This was again significantly higher than the mean concentration of 7.7±4.6 ng/ml in the baseline samples ($p<0.001$). No significant differences were seen between the TxB_2 concentrations for PU and gelatin ($p=0.70$).

The extraction time for the molars varied in the different subjects ranging from 2 to 15 min with a mean extraction time of 6.8 min. To investigate if the extraction time correlated with the baseline concentrations of TAT complexes, fibrinogen, and TXB_2, a linear regression analysis was performed. This analysis revealed that the extraction time was not correlated with the baseline concentrations of TAT complexes, fibrinogen, and TxB_2 (Table 1).

Discussion

Our study examined the hemostatic efficacy of modified polyurethane foam by measuring different coagulation parameters in a human model. The coagulation parameters showed no differences between the PU foam and gelatin- or collagen-based materials. The differences between the parameters measured from the test materials and the baseline values however were significant, indicating that all tested materials substantially increased hemostasis supplementary to the activation induced by the wound area.

A lot of research has been done on the hemostatic properties of biomaterials like polyurethane [5, 12, 13]. The polyurethane foam we tested was combined with 55 % PEG to increase the absorbing properties of the foam. The higher absorbability should increase the concentration of endogenous coagulation factors and platelets. The hemostatic effect of cellulose- and polysaccharide-based hemostatic agents is partially based on this mechanism [14, 15].

We used a human in vivo model in which it is possible to compare different hemostatic agents in similar wounds. The use of extraction sockets of upper and lower molars has the advantage that the test materials could be analyzed under similar conditions within the same patient. We used coagulation parameters to determine hemostatic efficacy because time to hemostasis could not be accurately measured in this split-mouth model. This could be considered a drawback as time to hemostasis is the most essential parameter.

The different structures of the test materials made it impossible to ensure complete blinding of the surgeons. We do not expect that this has influenced the results because the time in the wound was exactly 2 min for all test materials and the

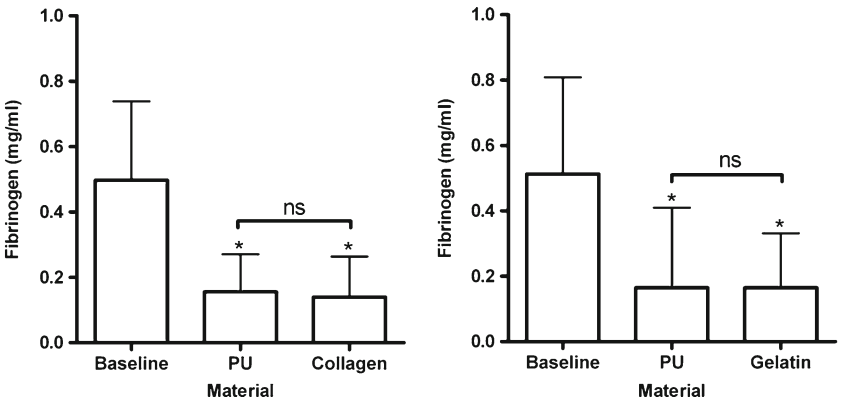

Fig. 2 Mean (±SD) fibrinogen concentrations for the different test materials and baseline samples. *PU* polyurethane foam, *ns* not significant. The asterisk indicates significance compared to baseline

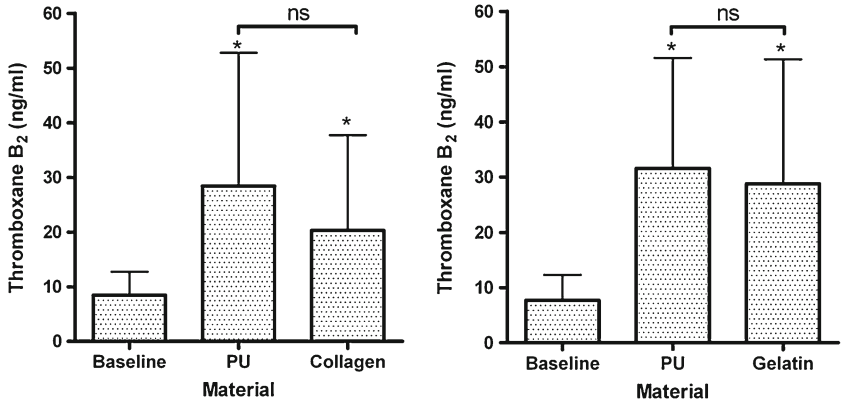

Fig. 3 Mean (±SD) thromboxane B_2 concentrations for the different test materials and baseline samples. *PU* polyurethane foam, *ns* not significant. The asterisk indicates significance compared to baseline

parameter values were measured by ELISA. Because the outcome of the ELISA was determined on numerical-coded samples by computer measurement, the possible bias was restricted to a minimum for both surgeons and researchers.

We compared the PU foam with a collagen and gelatin hemostatic agent. These agents are together with oxidized regenerated cellulose traditionally the most widely used hemostatic agents in surgery [16]. They promote platelet aggregation and coagulation by providing a three-dimensional meshwork for clotting to take place [17]. The oxidized regenerated cellulose was not tested in this study because the structure of the material made it impossible to obtain plasma samples after centrifugation.

There is no consensus on which of these materials is the best hemostatic agent [18, 19], although some studies indicated that collagen is more effective than gelatin and oxidized regenerated cellulose [20, 21]. In this study, no differences were seen in efficacy between the materials. The study design is unsuitable for comparison between collagen and gelatin, but the differences appear to be minimal.

The mean baseline concentrations for the different coagulation parameters show great differences from the mean concentrations that are reported for circulating blood. The mean concentration of TAT complexes in circulating blood is 1.0–4.1 μg/l [22], whereas we found a mean baseline concentration of 0.55 μg/ml which is roughly 500 times higher. The mean baseline concentration of fibrinogen we found in this study is 0.51 mg/ml and therewith is six times lower than the mean concentration in circulating blood of 3.0 mg/ml that is reported in the literature [23]. For TxB_2, the maximal estimate of the circulating concentration is 2.0 pg/ml [24]. The mean concentration of 8.1 ng/ml that we found in the baseline samples is 4,000 times higher. These differences are due to activation of the coagulation cascade in vivo as the baseline blood was collected from a wound [9]. For this study, these differences are of minor importance as every test was performed in a wound situation.

The extraction time could theoretically influence the baseline values as there is more time for the coagulation cascade to be completed. In our results, we did not find a correlation between extraction time and baseline concentrations. This could be due to a constant blood flow from the wound thus preventing a higher baseline value when the extraction time was longer. Another explanation would be that maximum baseline value was already reached after 2 min, which was the shortest extraction time.

The mouth is known as a fibrinolytic environment predominantly due to the saliva [25]. During our tests, the influence of saliva was minimal due to continuous suction around the alveolus and furthermore equal for all tests. Therefore, this factor is not expected to have influenced the test results.

For this study, only healthy subjects were included. The future use of the PU foam will be in patients with an impaired hemostasis. Future studies will have to show if the hemostatic efficacy of PU is strong enough to achieve complete hemostasis in those compromised patients. Further research should therefore also aim on the improvement of the hemostatic efficacy of the PU. This could be done by combining the PU with a procoagulant substance.

Table 1 Regression analysis of the correlation of extraction time with the baseline concentrations of TAT complexes, fibrinogen en TxB2

Coagulation parameter	β value	Standard error	p value
TAT complexes	−0.004	0.003	0.19
Fibrinogen	−0.002	0.003	0.57
TxB2	−0.005	0.004	0.29

TAT thrombin–antithrombin III, *TxB2* thromboxane B2, β regression coefficient

Conclusions

In this study, PU foam was compared with collagen and gelatin for its hemostatic efficacy in a human model. In this model, PU foam showed a similar hemostatic efficacy as the collagen and gelatin hemostatic agents. The synthetic PU is therefore a promising alternative for the animal-derived hemostatic agents used nowadays.

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Conflict of interest This study was financially supported by Polyganics BV and the University Medical Center Groningen, The Netherlands.

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