Technical Note

Contact activation during incubation of five different polyurethanes or glass in plasma

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During blood–material interaction, the enzymes factor XII fragment (factor XIIf) and kallikrein are generated (contact activation). In this study, the enzymatic activities of factor XIIf and kallikrein were examined with an assay based on the conversion of tripeptide-*p*-nitroanilide substrate. With the use of aprotinin to inhibit kallikrein, the proteolytic activities of factor XIIf and kallikrein could be separately determined. In this *in vitro* study, two commercially available polyurethanes, Pellethane and Biomer®; three custom synthesized polyurethanes; a biomerlike 2000 M_W polytetramethyleneoxide containing polyurethane (PU-2000); an octadecyl extended (ODCE) biomer-like 2000 M_W polytetramethyleneoxide containing polyurethane (PU-2000–ODCE); a hard-segment polyurethane (HS-PU); and glass (reference material) were incubated in 25% diluted plasma. In both series of experiments, glass caused the highest amidolytic activities by factor XIIf and kallikrein compared with any of the polyurethanes. In contrast, within the polyurethane group of materials, lower amidolytic activities by factor XIIf and kallikrein were measured on the custom-made polyurethanes than on the commercially available polyurethanes, although the differences among the polyurethanes were small. In addition, the influence of different ratios of material surface to the plasma incubation volume was studied. An increased ratio of surface area over plasma volume resulted in reduced contact activation, suggesting that plasma components are the limiting factor.

INTRODUCTION

Exposure of polymeric materials to blood causes protein adsorption at the blood polymer interface.[1] Among the proteins which adsorb at the polymer surface are the contact factors.[2] These proteins, which are part of the intrinsic coagulation pathway, may then become activated as a result of the alteration in their structure induced by the polymeric surface. The contact factors include factor XII, prekallikrein, and high molecular weight kininogen, which cooperate during the initial interactions of blood with the polymer surface.[3] The activation of factor XII and prekallikrein are considered to have a minor role in hemostasis. Both proenzymes become considerably activated during interaction with negatively charged surfaces such as collagen or glass.[4] The activation of these factors by other surfaces such as polymeric materials has not been studied as much, partly because of the nonspecificity and insensitivity of hematologic assays (e.g., activated partial thromboplastin time, prothrombin time, kallikrein inhibiting capacity, or factor Xa). Thus, a more specific assay was developed to determine the contact activation on polymer surfaces.[5] The purpose of this study was to determine whether different polyurethanes incubated for 2 h with 25% diluted plasma cause significant differences in contact activation (the initial step of the intrinsic coagulation pathway in blood). In addition, the effect of the ratio of the material surface area to plasma incubation volume was studied.

MATERIALS AND METHODS

Polymer preparation

Two commercially available polyurethanes, Biomer®[6] (Ethicon, Somerville, NJ) and Pellethane[6]

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(Pellethane, Upjohn, Torrance, CA), and three custom-synthesized polyurethanes, a biomer-like 2000 M_W polytetramethyleneoxide (PTMO) containing polyurethane[6] (PU-2000), an octadecyl extended (ODCE) biomer-like 2000 M_W PTMO containing polyurethane[7] (PU-2000–ODCE), and a hard-segmented polyurethane[8] (HS-PU), were included in this study. Purification of the two commercially available polymers and the preparation of the three custom-made polymers are described elsewhere.[9] Polyethylene terephthalate (Lux Thermanox; Nunc, Naperville, IL) coverslips were coated with polyurethanes as described previously.[10] Briefly, uniform, thin films were coated onto coverslips with a photoresist spinner (Model EC101; Headway Research, Garland, TX). All coverslips including glass coverslips obtained from VWR Scientific (San Francisco, CA) were cleaned in an ultrasonic cleaner prior to use. The chemical analysis of the used material surfaces was performed by use of electron spectroscopy (ESCA). These material surfaces characterizations have been described and discussed by Chinn et al.[9] Methods of ESCA and data analysis have been described and discussed elsewhere.[11,12]

Plasma incubation technique

Plasma was isolated from citrated blood (final concentration of sodium citrate 0.32%) of a healthy volunteer, frozen, and stored at −80°C, and thawed just before use. In a first series of experiments (Experiment 1), 0.250 mL of 25% aliquots of plasma was added to each well of the 24-well plates (290-8130-01F; Evergreen Scientific, Los Angeles, CA) in which each well contained one coverslip of each material. During the incubation of plasma with the coverslips, the contact system became activated. Plasma was diluted to a final concentration of 25% with citrate phosphate-buffered saline (CBPS; sodium citrate: 10 mM, sodium phosphate: 10 mM, sodium chloride: 120 mM, pH 7.4). Each well contained one polyurethane coated or a glass coverslip. Incubation was done for 2 h at 20°C. In a second series of experiments (Experiment 2), a three-times larger coverslip area of each material was incubated with a two-times larger plasma volume at the same temperature. After both series of experiments, the incubated plasma samples were collected and assayed immediately to determine factor XIIf and kallikrein amidolytic activity.

Contact activation measured after material–plasma incubation

Immediately after the incubation of 25% diluted plasma with the polyurethane-coated coverslips and glass coverslips for 2 h, plasma aliquots were mixed with a specific tripeptide-*p*-nitroanilide (Z-Lys-Phe-Arg-pNA · 2HCl; Nova Biochem, Läufelfingen, Switzerland) substrate for factor XIIf and kallikrein. Evidence for substrate specificity for factor XIIf and kallikrein has been described in detail elsewhere.[13,14] The substrate was dissolved in phosphate-buffered saline (PBS; sodium phosphate: 10 mM, sodium chloride: 120 mM, pH 7.4) and stored in aliquots of 1 mg/mL at −80°C. To measure factor XIIf and kallikrein activity, two dilutions of plasma were prepared. Plasma from each sample was diluted 10 (2.5%) and five (5%) times with saline. From the 10-times diluted sample, 100 μL of the plasma dilution, and from the five-times diluted samples, 50 μL of the plasma dilution were stored in 96-well plates (290-185-01F; Evergreen Scientific). In the wells containing 50 μL plasma (5.0%), 50 μL of 800 KIU/mL aprotinin (Bayer, Leverkusen, Germany) was added to inhibit substrate cleavage by kallikrein. At this step in the assay, all comparative plasma samples were diluted 40 times (2.5%) and the plasma volume was 100 μl. Thereafter, the diluted plasma samples were mixed 1:1 with saline-diluted substrate. The final plasma dilutions were 1.25% and the final substrate concentration was 0.125 mg/mL. The change in optical density by release of the yellow-colored pNA was recorded at 405 nm at 5-min time intervals, up to 25 min using a plate recorder (V_{max} kinetic 96-well plate reader; Molecular Devices Corporation, Palo Alto, CA). Blanks obtained in plasma samples incubated in polystyrene wells with no coverslip were subtracted from the measured values from wells which contained test material. With the use of this assay, factor XIIf and kallikrein amidolytic activities could be separately calculated. The substrate conversions were expressed as nanomoles pNA related activity of factor XIIf or kallikrein per minute per square centimeter of material.

Statistical analysis

The one-factorial analysis of variance test was used for statistical analysis to discriminate between the activity with and without aprotinin obtained after incubation with the materials. Results were expressed as mean ± standard error of the mean, and $P < .05$ was regarded as significant.

RESULTS

In all experiments significantly higher activities of factor XIIf and kallikrein were generated by glass as compared to the polyurethanes tested. On Biomer®

TABLE I
Activation of the Contact System Using Polyethylene Terephthalate Coverslips Coated with Five Different Polyurethanes and Coverslips of Glass

Material	Reference	Experiment 1 (nmol pNA/min cm^2)		Experiment 2 (nmol pNA/min cm^2)	
		Factor XIIf	Kallikrein	Factor XIIf	Kallikrein
Biomer®	6	0.56 ± 0.03*	0.98 ± 0.07*	0.25 ± 0.06*	0.38 ± 0.11*
Pellethane	6	0.41 ± 0.18*	0.96 ± 0.04*	0.61 ± 0.25*	0.45 ± 0.11*
PU-2000	6	0.14 ± 0.01	0.55 ± 0.06	0.08 ± 0.02	0.23 ± 0.09
PU-2000–ODCE	7	0.24 ± 0.01	0.55 ± 0.02	0.08 ± 0.01	0.19 ± 0.09
HS-PU	8	0.20 ± 0.04	0.63 ± 0.13	0.11 ± 0.01	0.14 ± 0.12
Glass		3.80 ± 0.16†	2.12 ± 0.07†	2.67 ± 0.67†	1.47 ± 0.21†

Statistically significant ($P < .05$) increased amidolytic activities were measured on the two commercially available polyurethanes (Biomer® and Pellethane) in comparison with the custom-made polyurethanes (*), and statistically significant ($P < .05$) increased amidolytic activities were measured using glass compared to all polyurethanes (†). HS-PU, hard-segmented polyurethane; PU-2000, biomer-like 2000 M_w polytetramethyleneoxide chain-extended polyurethane; PU-2000–ODCE, octadecyl extended (ODCE) biomer-like 2000 M_w polytetramethyleneoxide chain-extended polyurethane.

and Pellethane, statistically significant more factor XIIf and kallikrein was induced than on the three custom-synthesized polyurethanes. In Experiment 1, with the ratio of coverslip area to plasma incubation volume (0.56 cm^2: 0.25 mL), higher amidolytic activities were measured using all materials than in Experiment 2, with a higher ratio (1.50 cm^2: 0.5 mL). In both experiments, within the polyurethane group, lower amidolytic activities by factor XIIf and kallikrein were measured using the custom-made polyurethanes than with the commercially available polyurethanes. No differences could be determined between either the factor XIIf or kallikrein activities induced by the different custom-made polyurethanes. However, the differences in factor XIIf and kallikrein activities measured using the polyurethanes were small compared to the difference of factor XIIf and kallikrein activities measured on glass (Table I).

DISCUSSION

The interaction of blood with materials used in medical devices has an important role in the activation of the coagulation cascade. Activation of the contact system results in the activation of the intrinsic coagulation, kinin, and fibrinolytic systems.[15,16] Factor XII, high molecular weight kininogen, and prekallikrein are the proteins involved in the contact system activation process.[17] During the interaction of blood with materials, the proenzyme factor XII releases the factor XIIf, while prekallikrein converts to kallikrein. Kallikrein acts as a positive feedback activator during the interaction of factor XII with the material surface.[18] With the use of the previously described method, factor XIIf and kallikrein activities generated on different polyurethanes and glass under static conditions were measured.[9] Briefly, the chromogenic assay to determine the protease activities was based on the addition of a substrate to which human factor XIIf is known to have the highest affinity compared to the bovine factors IXa, Xa, XIa, thrombin, and trypsin, and the human activated protein C.[13] However, human kallikrein was observed to possess considerable ability to cleave this substrate for factor XIIf.[19] With the use of aprotinin, a specific inhibitor of kallikrein which does not affect factor XIIf,[14] it is possible to measure both the factor XIIf activity and kallikrein activity in plasma. From all polyurethanes tested in this study, the custom-made polyurethanes were identified as weaker activators of the contact system than the commercially available ones. However, the differences in the ability of polyurethanes to activate the contact system were relatively small compared to glass. In addition, by comparing the results of Experiment 2 with those of Experiment 1, it turned out that a higher level of contact activation could be obtained with the ratio of coverslip area of 0.56 cm^2 to plasma incubation volume of 0.25 mL (Experiment 1) than with the ratio of coverslip area of 1.50 cm^2 to plasma incubation volume of 0.50 mL (Experiment 2). This suggests that the contact activation process is limited by the amount of available plasma components from the contact system.

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