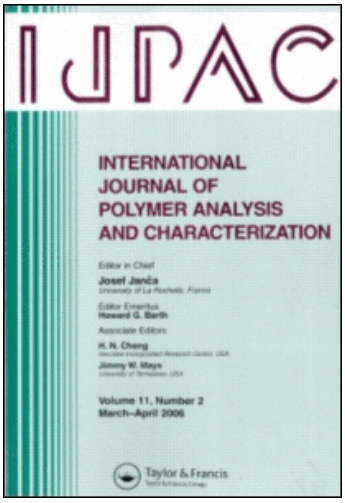


International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Blood-Compatible Polymers and Their Characterization: A Simple Sensitive Assay for Hemocompatibility Evaluation

L. Lelli[a]; G. D. Guerra[a]; L. Lazzeri[b]; N. Barbani[b]; A. Bonaretti[b]; P. Giusti[b]
[a] Centro Studi Processi Ionici di Polimerizzazione e Proprietà Fisiche e Tecniche di Sistemi di Macromolecole, Pisa, Italy [b] Dipartimento di Ingegneria Chimica, University of Pisa, Pisa, Italy

To cite this Article Lelli, L. , Guerra, G. D. , Lazzeri, L. , Barbani, N. , Bonaretti, A. and Giusti, P.(1995) 'Blood-Compatible Polymers and Their Characterization: A Simple Sensitive Assay for Hemocompatibility Evaluation', International Journal of Polymer Analysis and Characterization, 1: 2, 131 — 136
To link to this Article: DOI: 10.1080/10236669508233868
URL: http://dx.doi.org/10.1080/10236669508233868

Int. J. Polymer Analysis & Characterization
1995, Vol. 1, pp. 131–136
Reprints available directly from the publisher
Photocopying permitted by license only

Blood-Compatible Polymers and Their Characterization: A Simple Sensitive Assay for Hemocompatibility Evaluation

L. LELLI and G. D. GUERRA

Centro Studi Processi Ionici di Polimerizzazione e Proprietà Fisiche e Tecniche di Sistemi di Macromolecole, C.N.R., Via Diotisalvi 2, I-56126 Pisa, Italy

and

L. LAZZERI, N. BARBANI, A. BONARETTI and P. GIUSTI

Dipartimento di Ingegneria Chimica, University of Pisa, Via Diotisalvi 2, I-56126 Pisa, Italy

(Received September 22, 1993)

The contact activation of plasma prekallikrein to kallikrein has been used as a blood compatibility test for two series of materials. Samples of diluted human plasma were held in contact with the materials to be tested, the activated plasma was then reacted with the chromogenic substrate H-D-Pro-Phe-Arg-*p*NA, which releases *p*-nitroaniline under the proteolytic action of kallikrein. The two series of materials tested are: an ethylene-vinyl alcohol copolymer (EVAL) and three graft copolymers (EVAL-SMA) obtained by reacting EVAL with a styrene-maleic anhydride alternating copolymer; four fluorinated polyurethanes (FPU). Each series of materials was compared with borosilicate glass, the high-activation reference; silicone, the low-activation reference; Cardiothane® 51, a blend of poly(ether-urethane) and poly(dimethylsiloxane) used for cardiovascular applications. Both series of materials show a low thrombogenicity; in particular, the FPUs are less activating than Cardiothane 51. To check the correlation between the prekallikrein activation and the hydrophilicity of these materials, their contact angles with water and plasma were measured. The results of the prekallikrein activation test suggest that EVAL-SMA copolymers and FPUs could find a use as blood-contacting biomaterials.

KEY WORDS Blood compatibility, kallikrein, EVAL-SMA graft copolymers, fluorinated polyurethanes, biomaterials

INTRODUCTION

Synthetic polymers have found a large use as biomaterials because they are available with a wide variety of compositions, properties, and forms, and because they can be fabricated readily into complex shapes and structures.

The methods for the physicochemical and mechanical characterization of biomaterials are not very different from those for polymeric materials employed in other fields. Polymers selected for the fabrication of blood-contacting devices (see Table I) require also characterization for blood compatibility to establish their safety.

TABLE 1

Polymeric materials used for blood contacting devices

Devices	Materials
Vascular prostheses	Expanded polytetrafluoroethylene (GoreTex®) Woven or knitted polyethyleneterephthalate (Dacron®)
Hemodialysis membranes	Cellulose acetate (Cuprophan®) Ethylene-vinyl alcohol copolymers (EVAL)
Intravenous catheters	Polytetrafluoroethylene (Teflon®) Polyethyleneterephthalate (PET) Polyurethanes (PU)

The contact between blood and foreign materials starts the coagulation cascade (Figure 1), leading to thrombus formation and embolization. It is therefore necessary that the biomaterials used for blood-contacting devices induce such a process as little as possible.

Four proteins that circulate freely in the blood are involved in contact activation: factor XII (F.XII), factor XI (F.XI), prekallikrein (PKK) and high-molecular-weight kininogen (HMWK). Absorbed F.XII is activated to F.XIIa which, together with HMWK, converts F.XI to F.XIa and PKK to kallikrein (KK) which, in turn, is the most effective activator of F.XII by a feedback mechanism. Blood compatibility of a material can be therefore pointed out by testing the formation of KK, by means of its proteolytic reaction on the chromogenic substrate H-D-Pro-Phe-Arg-*p*NA. Such a reaction releases *p*-nitroaniline, spectrophotometrically detectable by its absorption at 405 nm (A_{405}). The "kallikrein-like activity" (KLA) of the activated plasma can be easily evaluated from the "initial velocity" of the KK-substrate reaction [1, 2], carried out in a spectrophotometric cell at 37°C.

The KLA evaluation was carried out in our laboratories on some materials potentially useful as biomaterials; the KLA values so obtained were compared with those measured on the following materials: borosilicate glass (high-activation reference); silicone (low-activation reference); Cardiothane® 51, a blend of poly(ether-urethane) and poly(dimethylsiloxane) commonly used for cardiovascular applications.

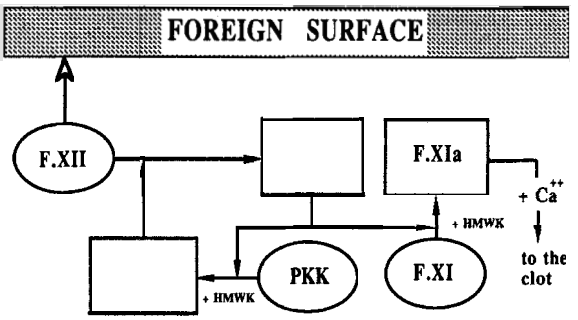
EXPERIMENTAL

Materials

Silicone (*PDMS, Kontron Cardiovascular Inc.*). A 5% aqueous emulsion was obtained and repeatedly cast on the inner surfaces of the borosilicate glass tubes.

Cardiothane 51 (9 : 1 *PEU/PDMS blend, Kontron Cardiovascular Inc.*) was dissolved (5% wt) in 1 : 2 THF/1,4-dioxane and repeatedly cast on the inner surfaces of the borosilicate glass tubes.

EVAL (*Clarene L6® Solvay, 29 mol% ethylene*) was dissolved (5% wt) in dimethylsulfoxide and repeatedly cast on the inner surfaces of the borosilicate glass tubes.



EVAL-SMA copolymers were synthesised from Clarene L6 and styrene-maleic anhydride copolymer (Aldrich) by a procedure already described [2] and used as the sodium salt. Their structure most likely consists of SMA chains grafted onto the vinyl alcohol domains of EVAL through ester links, as inferred from IR, DSC, and NMR data [3]. The three copolymers, EVAL-SMA 1, EVAL-SMA 2, and EVAL-SMA 3, with different grafting degrees, were dissolved (5% wt) in dimethylsulfoxide and repeatedly cast on the inner surfaces of the borosilicate glass tubes.

Fluorinated polyurethanes (FPUs, patented by Ausimont, Milan, Italy), differing for both fluorine content and macro-glycol nature, were dissolved (5% wt) in 1:9 DMF/THF and repeatedly cast on the inner surfaces of the borosilicate glass tubes.

The chromogenic substrate H-D-Pro-Phe-Arg-*p*NA (S-L2302, Kabi Diagnostica) was used as supplied.

Hemocompatibility and Hydrophilicity Tests

To evaluate the contact PKK activation, a pool of citrated plasma from nine healthy donors was dispensed in samples of 0.5 mL, and then frozen at −20°C. Before carrying out the activation test, the plasma was thawed at 37°C and diluted 1:10 with a 0.05 M TRIS-HCl buffer (pH 7.8). Volumes of 0.52 mL of diluted plasma were placed in 0.50-cm inner-radius borosilicate glass tubes, both uncoated and internally coated with the materials to be tested. The plasma was incubated at 37°C under a constant stirring speed (1,100 rpm) for 3 min; 0.2 mL of activated plasma was added to 0.2 mL of 2 mM solution of the chromogenic substrate and to 0.6 mL of the same buffer in a spectrophotometric cell thermostatted at 37°C. The releasing of *p*-nitroaniline was followed at 405 nm (molar absorptivity 9950) by means of a Shimadzu 2100 UV-visible spectrophotometer; the initial rates were obtained by "differential plots", [4] calculated by a computer program. Kallikrein-like activity values were obtained from the initial rates by the "initial velocity" method, using the kinetic constants ($K_M = 1.84 \times 10^{-4}$ mol/L, $k_c = 4.04 \times 10^{-7}$ mol/U · min) evaluated from the reaction between S-2302 and purified kallikrein [2]. Two different pools of plasma, later named as "Pool I" and "Pool II", were used to carry out the PKK tests on EVAL-SMA copolymers and FPUs.

The contact angles with both distilled water and human plasma drops were measured by a Ramé-Hart normal contact angle goniometer.

RESULTS AND DISCUSSION

Figure 2 shows the plasma PKK activation induced by EVAL and by the three graft copolymers, EVAL-SMA 1, EVAL-SMA 2 and EVAL-SMA 3, with different grafting degrees.

The results show that EVAL induces a KLA of the same order than those induced by Cardiothane 51 and by silicone; concerning the three graft copolymers, only EVAL-SMA 1 shows an activation of the same order as unmodified EVAL, while the other two materials give markedly higher KLA values. Since EVAL is a biomaterial used in the fabrication of hemodialysis membranes (see Table I), and EVAL-SMA copolymers can be easily processed into water-permeable hollow fibres with good mechanical properties, [3] a possibility of their use in blood dialysis devices could be proposed. Although from the results in Figure 2 their hemocompatibility seems generally less than that of EVAL, the presence of ionic groups in SMA chains might improve their perm-selectivity toward substances in blood.

Figure 3 shows the plasma PKK activation induced by four FPUs, differing for both fluorine content and macro-glycol nature. The PKK activation appears very low, even lower than that induced by the silicone and Cardiothane 51, as if the presence of fluorine in the polyurethane structure had a positive influence toward the hemocompatibility. Moreover, FPU 52 and FPU 60, which contain poly(ε-caprolactone) glycol as soft segment, show a PKK activation lower than FPU 58 and FPU 42, which contain poly(tetramethylene ether) glycol. Such a positive effect of the presence of a poly(ε-caprolactone) segment is in good agreement with the results of the PKK activation induced by some tri-block poly(ε-caprolactone)-poly(oxyethylene)-poly(ε-caprolactone) copolymers, which generally show a good hemocompatibility [5].

As regards the lowering of PKK activation due to the presence of fluorine in the polyurethane chains, it is known that fluorinated polyurethanes are generally more thromboresistant than the nonfluorinated ones [6]. Since the hydrophilicity degree

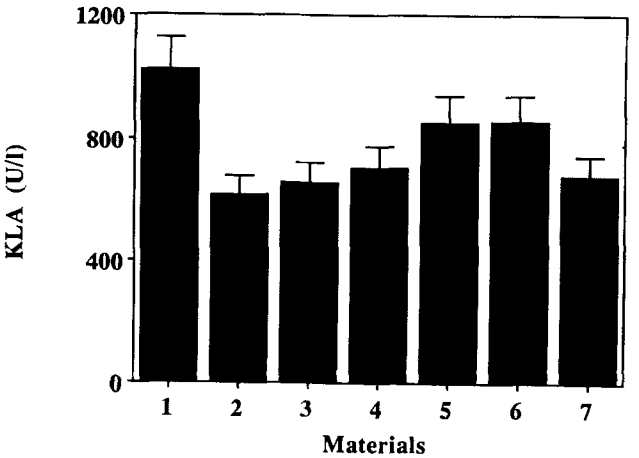


FIGURE 2 Plasma PKK activation by reference materials, EVAL and EVAL-SMA copolymers. Columns: 1, glass; 2, silicone; 3, Cardiothane 51; 4, EVAL-SMA 1; 5, EVAL-SMA 2; 6, EVAL-SMA 3; 7, EVAL (Clarene L6). Plasma contact time: 3 min. Plasma samples from Pool I.

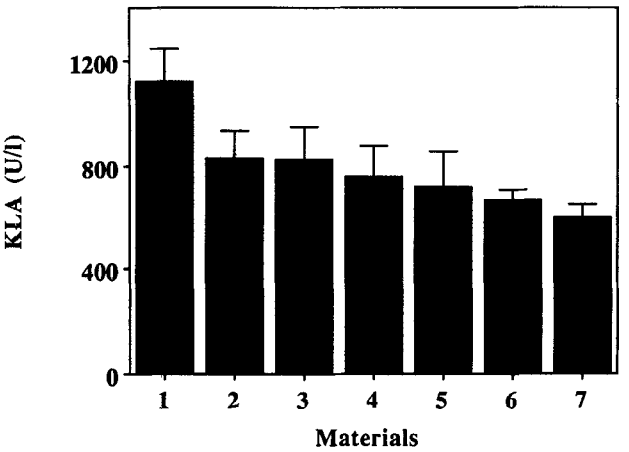


FIGURE 3 Plasma PKK activation by reference materials and fluorinated polyurethanes. Columns: 1, glass; 2, silicone; 3, Cardiothane 51; 4, FPU 58; 5, FPU 42; 6, FPU 52; 7, FPU 60. Plasma contact time: 3 min. Plasma samples from Pool II.

of the surface, which is lowered by the presence of fluorine, [7] is correlated to blood compatibility, [6, 8] the hydrophilicity degree of the materials has been evaluated by measuring the contact angles with both distilled water and human plasma drops. The results obtained (expressed as mean ± standard error) are reported in Table II.

In all cases, the contact angles on FPUs have values that are greater than typical values for polyurethanes [8, 9]. In addition, they are quite similar to contact angles on Cardiothane 51, in which the hydrophilicity is lowered by the presence of PDMS. Probably, the presence of hydrophobic fluorine atoms in the polyurethane chain enhances the blood compatibility more than the blending with a hydrophobic macromolecule.

The low thrombogenicity of these FPUs, together with the elastomeric properties of the polyurethanes, suggests that such materials, processed by a spraying-phase inversion process, [10] could be used for the construction of small diameter vascular prostheses.

CONCLUSIONS

The low thrombogenicity of the EVAL-SMA graft copolymers and of the fluorinated polyurethanes, as shown by the PKK contact activation test, suggests a

TABLE II

Contact angle measurements

Material	Surface-water-air	Surface-plasma-air
Cardiothane 51	101.6 ± 0.5	90.0 ± 0.2
FPU 42	103.7 ± 0.6	94.8 ± 0.6
FPU 52	103.6 ± 0.4	96.5 ± 0.5
FPU 58	102.5 ± 0.7	96.7 ± 0.7
FPU 60	103.3 ± 0.4	95.0 ± 0.5

possibility of their use as biomaterials. Obviously, a single test is not sufficientto state the hemo- and biocompatibility of such materials, so that further investigation must be carried out before suggesting them for specific applications. Such studies will be reported in subsequent papers.

Acknowledgments

This work has been supported partially by Italian C.N.R., P. F. Chimica Fine II. The authors thank Ausimont S.p.A., Milan, Italy, for supplying the fluorinated polyurethanes.

References

1. U. Christensen, *Thromb. Haemostasis*, **43**, 169 (1980).
2. G. D. Guerra, N. Barbani, L. Lazzeri, L. Lelli, M. Palla, and C. Rizzo, *J. Biomat. Sci. Polym. Ed.* **4**, 643 (1993).
3. S. Schifano, *Produzione di fibre cave e tubi capillari e loro applicazioni.* Tesi di Laurea in Ingegneria Chimica, Università di Pisa, Pisa (1989).
4. P. Giusti, F. Andruzzi, P. Cerrai, and G. L. Possanzini, *Makromol. Chem.*, **136**, 97 (1970).
5. P. Cerrai, G. D. Guerra, L. Lelli, M. Tricoli, R. Sbarbati Del Guerra, M. G. Cascone, and P. Giusti, *J. Mater. Sci.: Mater. Med.*, **5**, 33 (1994).
6. T. Takakura, M. Kato, and M. Yamabe, *Makromol. Chem.*, **191**, 625 (1990).
7. P. G. Edelman, D. G. Castner, and B. D. Ratner, *Polym. Prepr.* **31**, 314 (1990).
8. A. Takahara, A. Z. Okkema, H. Wabers, and S. L. Cooper, *J. Biomed. Mater. Res.*, **25**, 1095 (1991).
9. P. D. Nair, M. Mohanty, K. Rathinam, M. Jayabalan, and V. N. Krishnamurthy, *Biomaterials*, **13**, 537 (1992).
10. G. Soldani, M. Steiner, P. M. Galletti, L. Lelli, M. Palla, and P. Giusti, *Clin. Mater.* **8**, 81 (1991).