Biological and Biomechanical Performance of Biomaterials,
edited by P. Christel, A. Meunier and A.J.C. Lee
Elsevier Science Publishers B.V., Amsterdam, 1986 — Printed in The Netherlands

NEW FIBRIN CONTAINING THERMOPLASTIC ELASTOMERS FOR CARDIOVASCULAR APPLICATIONS

P. GIUSTI[1], G. SOLDANI[2], M. PALLA[2], M. PACI[1]

[1]Dipartimento di Ingegneria Chimica - Via Diotisalvi 2, 56100 PISA

[2]Centro Studi Processi Ionici di Polimerizzazione e Proprietà Polimeri - C.N.R., c/o Facoltà di Ingegneria, Via Diotisalvi 2, 56100 PISA - ITALY

ABSTRACT

New biocompatible and hemocompatible materials for applications in the cardiocirculatory system have been obtained by blending segmented polyurethane (SP) and human fibrinogen (H-FBNG) and by subsequent transformation of it into cross-linked human fibrin (H-FBN) by action of thrombin, factor XIII and calcium ions. The fairly low fibrinogen release (15%) from the blend and the DSC analysis seem to prove the presence of strong interaction operative on a molecular level between SP and H-FBNG. The transformation of the SP-(H-FBNG) blend into SP-(H-FBN) blend is confirmed by DSC analysis that shows the disappearance of a characteristic transition at 90°C present in H-FBNG and not in H-FBN. Preliminary results of "in vitro" and "in vivo" experiments of hemocompatibility of these materials are described.

INTRODUCTION

Polymers both for temporary and long-term application in the cardiocirculatory system, should display high hemocompatibility. In spite of the great number of studies in this field, a fully non-thrombogenic material has not so far been synthesized. In particular as far as the construction of prosthetic grafts is concerned, the most suitable materials appear to be those capable of allowing tissue ingrowth and neo-intima formation, by virtue of their surface characteristics.

We have already studied the synthesis and the characterization of new thermoplastic elastomers, mainly segmented polyurethane (SP), with mechanical properties easily modifiable over a wide range by varying the ratio of the hard to soft segments (ref.1).

Althought their hemocompatibility is fairly good as evaluated from "in vitro" tests, long term "in vivo" experiments of small diameter porous vascular grafts manufactured with these materials were not completely satisfactory.

Therefore we supposed that the failure could be related to an insufficient hemocompatibility that could cause an inadequate tissue ingrowth.

In order to improve the performance of the grafts, we studied the properties of biomaterials obtained by blending SP produced in our laboratories with fibrinogen, which is cross-linked into fibrin by thrombin, factor XIII and calcium chloride while the prosthesis is manufactured (ref.2) utilizing the final steps of the natural coagulation pathway (ref.3).

MATERIALS AND METHODS

The synthetic polymers used in these investigations were thermoplastic elastomers, segmented polyurethanes and polyurethaneureas, synthesized in our laboratories as described in previous publications (ref.1).

The human fibrinogen has been supplied by Biagini Farmaceutici Spa, as a lyophilized product of approximately 98% clottability. The thrombin, of bovine origin, has been supplied as a lyophilized product by Merz+Dade AG (CH). The concentrate of factor XIII (Fibrogammin), extracted from human placenta, has been supplied as lyophilized product by Istituto Bëring Spa.

The SP-(H-FBNG) blends were obtained by mixing a SP solution (2.5% w/v in THF-1,4 Dioxane) with H-FBNG in the ratio 2:1, stirring 1 hour at 37°C and drying under vacuum at 37°C for 24 hours.

The SP-(H-FBN) blends were obtained by incubation of the SP-(H-FBNG) blend in distilled water containing 500 NIH units x ml^{-1} of thrombin, 10 U.I. x ml^{-1} of factor XIII and 50 µmol x ml^{-1} of calcium chloride, for 3 hours at 37°C. The blends were then vacuum dried at 37°C for 24 hours.

U.V. analysis was carried out by the use of a Perkin-Elmer Recording Spectrophotometer Mod. 323. The optical determination of H-FBNG was made assuming $E^{1\%}_{1cm}$ (280nm)= 15.06 (ref.4).

DSC was made using a Perkin-Elmer DSC 4 Scanning Calorimeter. Samples were dried under vacuum for 5 hrs at 37°C and then quickly transferred to DSC pans. Scanning was made at a heating rate of 20°C x min^{-1} on samples in dried form. All traces were normalized to 1 mg of substance.

Prothrombin Time (PT) and Partial Thromboplastin Time (PTT) have been carried out according to U.S.-NIH publication (ref.5), by the use of an Elvi 820 Coagulometer.

RESULTS AND DISCUSSION

The aim of this work being to crosslink fibrinogen blended with SP through the action of thrombin, factor XIII and calcium chloride in an aqueous solution and considering the solubility of FBNG in water, the experiments described below sought to prove that the interaction of FBNG-SP significantly reduced the amount of FBNG released into the aqueous solution.

In order to evaluate the fibrinogen release from the SP/(H-FBNG) blend 2:1 ratio in distilled water the concentration of fibrinogen as a function of time was determined by U.V. spectroscopy. As shown in Fig. 1 this release reaches a value of about 15% after 90 min, then remain constant.

This relative low value seems to be due to the presence of strong interactions between SP and H-FBNG at the molecular level (ref.6).

The blend, after crosslinking, owing to the presence of the natural polymer, shows a marked hydrophilicity much higher than that pure SP and increasing with the increase of fibrin in the blend.

In order to prove the crosslinking of fibrinogen in the blend, DSC analysis was carried out, since the electrophoresis analysis in polyacrylammide gel with sodium dodecylsulphate presented some difficulties in separation of the natural from the synthetic material.

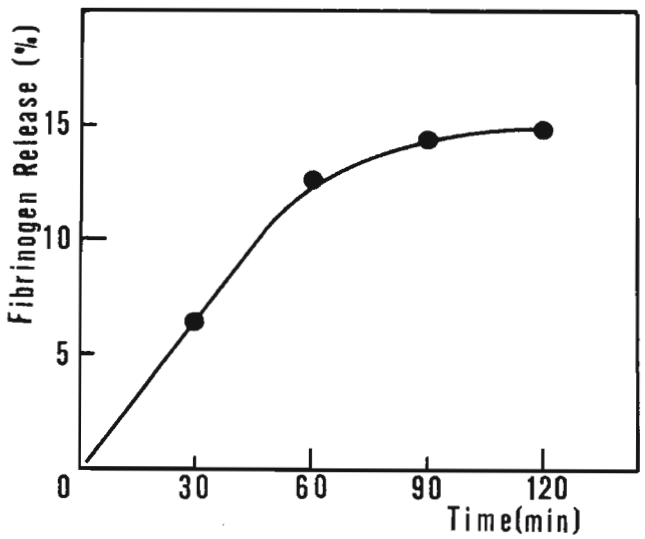

Fig. 1. Fibrinogen release vs time.

As shown in Fig. 2 the fibrinogen trace a is characterized by a broad endotherm centered at 90°C and extended over a range of about 100 deg., plus a smaller one at 150°C. The endoterm at 90°C corresponds to that found by Donovan and Mihalyi (ref.7) for fibrinogen solution and associated to motions of the central portion of the fibrinogen molecule.

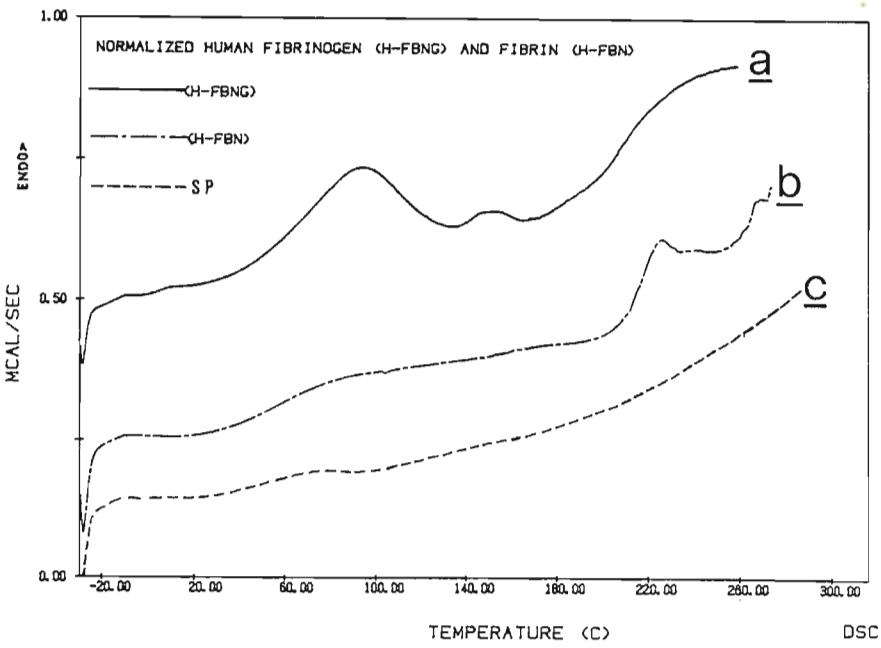

Fig. 2. DSC Thermograms of H-FBNG a, H-FBN b, SP c.

The specific heat change observed above 180°C is probably associated with thermal degradation. The trace of the fibrin sample b displays no endotherm at temperatures up to 180°C, but only a specific heat change at ca. 60°C. This is expected since the structure of the fibrinogen molecule responsible for the endotherm at 90°C is not present in the fibrin molecule arrangement.

It is known that the thermal degradation of fibrin starts at ca. 170°C: therefore, the endotherm observed at ca. 220°C in the fibrin trace can be tentatively associated with some kind of chemical transformation.

The SP trace c shows no singularities over the whole temperature range investigated (-20 + +280°C).

The DSC trace of the SP-(H-FBNG) blend is compared in Fig. 3 with that of the same blend after fibrinogen cross-linking. The first trace appears

qualitatively similar to that of fibrinogen. It is noteworthy, however, that the enthalpy change associated with the 90°C endotherm is now much higher than expected on the basis of the fibrinogen amount in the blend. This indicates that some kind of interaction between H-FBNG and SP must be operative on a molecular level. The second trace is very similar to that of the fibrin, thus confirming that cross-linking led to an almost complete transformation of the SP-(H-FBNG) blend into a SP-(H-FBN) blend. The thermal stability of the fibrin appears much higher in the blend than in the natural condition, as demonstrated by the absence of strong thermal effects above 180°C, and this can be rationalized on the basis of molecular interactions between SP and H-FBN, probably similar to those already mentioned in the case of fibrinogen.

Results of blood-material interaction show that the SP-(H-FBN) blends, during 2 hrs of interaction with normal control plasma, do not alter the parameters of the coagulation evaluated as PT and PTT.

A graft with a 4 mm internal diameter was placed as an interposition graft in the femoral artery of a dog, while a second one with a 2 mm internal diameter were placed in the suprarenal abdominal aorta of a rat.

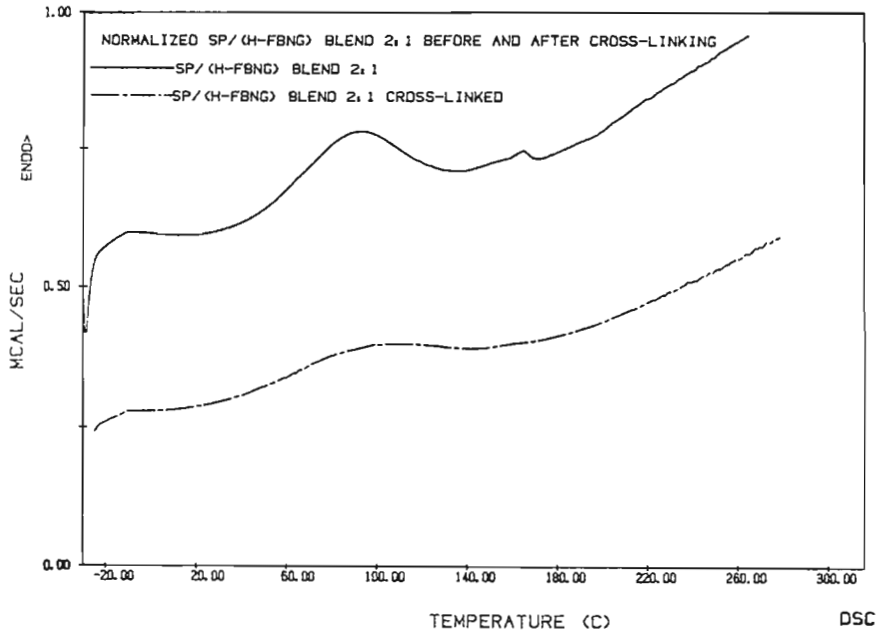

Fig. 3. DSC Thermograms of SP-(H-FBNG)blend 2:1 before and after crosslinking.

These preliminary experiments, still in progress, look promising.

We expect fibrin to increase the blood compatibility of the prosthesis and during its biodegradation to favour tissue ingrowth and neo-intima formation as it has been already shown in the use of fibrin glue for sealing vascular prostheses of high porosity (ref. 8-9).

Acknowledgment: This work was partially supported by the Italian CNR Progetto Finalizzato "Chimica Fine e Secondaria".

REFERENCES

1 P. Giusti, R. Pietrabissa, G. Soldani, P. Miccoli, P. Iacconi. New thermoplastic elastomers for small diameter compliant grafts. Progress in Artificial Organs (1983) 852-857.

2 P. Giusti, G. Soldani, M. Palla. Materiali biocompatibili ed emocompatibili per realizzare endoprotesi, organi e tessuti artificiali e metodo per preparare detti materiali. Italian Patent No. 9429A/85.

3 L. Lorand. Fibrinoligase: The fibrin-stabilizing factor system of blood plasma. Annals New York Academy of Science, 202 (1972) 6-30.

4 E. Mihalyi. Physicochemical Studies of Bovine Fibrinogen. IV. Ultraviolet Absorption and Its Relation to the Structure of the Molecule. Biochemistry, 7 (1968) 208-223.

5 Guidelines for Blood-Material Interactions. U.S. NIH Publication No.80-2185 (1980) 49-50.

6 S.I. Stupp, J.W. Rauffman, S.H. Carr. Interactions between Segmented Polyurethane Surfaces and the Plasma Protein Fibrinogen. J. Biomed. Mater. Res., 11 (1977) 237-250.

7 J.W. Donovan, E. Mihalyi. Conformation of Fibrinogen: Calorimetric Evidence for a Three-Nodular Structure. Proc. Nat. Acad. Sci., USA, 71 (1974) 4125-4128.

8 A. Haverich, G. Welterbusch, H.G. Borst. The use of fibrin glue for sealing vascular prostheses of high porosity. Thorac. Cardiovasc. Surgeon 29 (1981) 252-254.

9 G. Köverek, E.R. de Vivie, K.D. Helberg. Clinical experience with fibrin glue in cardiac surgery. Thorac. Cardiovasc. Surgeon 29 (1981) 287-289.