ELSEVIER

S0032-3861(96)00538-1

Polymer Vol. 37 No. 22, pp. 4885–4889, 1996

0032-3861/96/$15.00 + 0.00

Studies in polymer surface modification and grafting for biomedical uses: 2. Application to arterial blood filters and oxygenators

C. H. Bamford and K. G. Al-Lamee*
Department of Clinical Engineering, University of Liverpool, Liverpool L69 3BX, UK
(Received 8 November 1995)

The technique for polymer surface modification described in earlier papers has been applied to two components of an extracorporeal blood circulatory system used in open-heart surgery, with the object of improving their haemocompatibility. The devices were the arterial blood filter composed of nylon membranes and the blood oxygenator consisting of polypropylene microporous hollow fibres. Polyacrylamide and, in some cases, a mixture of polyacrylamide and poly(3-aminopropyl methacrylamide) have been covalently attached to the blood-contacting surfaces. The basic groups of the latter polymers provided a means of coupling heparin to the surface. The extent of heparin coupling was determined by radioactivity measurements with the aid of ^{35}S-labelled heparin. The following determinations were made to assess haemocompatibilities: blood plasma clotting times (PTT); platelet adhesion to the nylon filters; concentrations of lymphocytes and neutrophils in whole blood after exposure to the surfaces. It is concluded that the overall process, which has the merits of cheapness and simplicity in application, has a beneficial effect on haemocompatibility. Copyright © 1996 Elsevier Science Ltd.

(Keywords: surface-functionalization; biocompatibility; anticoagulants)

INTRODUCTION

In earlier papers[1,2] we described a simple and inexpensive procedure for functionalizing and grafting to 'difficult' polymer surfaces, e.g. polypropylene, polystyrene and polyacrylonitrile. We anticipated that a process of this type, requiring only relatively mild conditions and aqueous media, would be particularly useful for treating biomedical devices, which often include such polymers but can suffer damage under severe conditions. The procedure has been applied to two components of an extra-corporeal blood circulatory system used in open-heart surgery—the arterial blood filter and the blood oxygenator. These were of a commercial type supplied by Polystan A/S, Copenhagen, Denmark and their blood-contacting surfaces were composed of nylon membranes and polypropylene microporous hollow fibres, respectively. Both polymers are relatively haemocompatible but, as will appear, may cause some damage to the patient's blood. One object of the surface modification treatment is to minimize this.

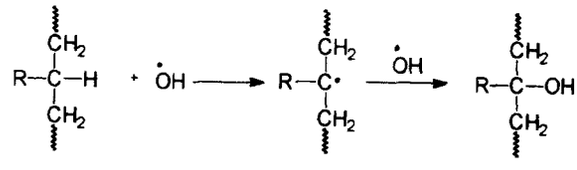
We have grafted polyacrylamide (AcAm) and poly(3-aminopropyl methacrylamide) (APMAcAm) to the blood-contacting surfaces of the filter and the oxygenator. It would be anticipated that these hydrophilic polymers would improve the haemocompatibility. In addition, we have coupled heparin to the surfaces through the amino groups of the basic polymer (APMAcAm). At present, it is customary to heparinize patients undergoing open-heart surgery to minimize the possibility of clot formation, but this procedure is in many ways undesirable. It is generally thought that suitable heparinization of all blood-contacting surfaces might enable this practice to be avoided.

Details of the surface modifications of the filters and oxygenator and the effects of the treatment on the haemocompatibility form the subjects of this paper.

The process described previously[1,2] consists of two stages, hydroxylation and grafting. Hydroxylation is effected by treating the polymer with potassium peroxydisulfate solution at a temperature of about 70°C; the hydroxylated polymer is then submitted to grafting by the familiar ceric ion technique. Under the conditions mentioned hydroxylation is likely to occur by a number of routes, of which the simplest involves hydroxyl radicals formed in the thermal decomposition of the peroxydisulfate[3]:

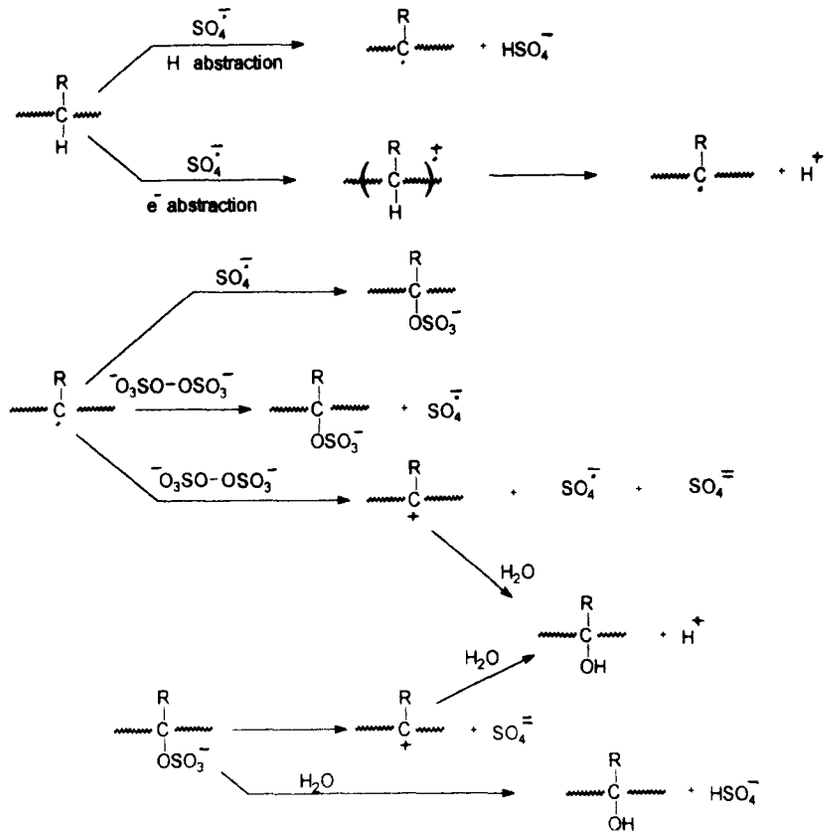
$$\begin{aligned} {}^{-}O_3SO{-}OSO_3^{-} &\rightarrow 2SO_4^{-} \\ SO_4^{-} + H_2O &\rightarrow HSO_4^{-} + {}^{\cdot}OH \\ 2\,{}^{\cdot}OH &\rightarrow H_2O + 1/2O_2 \end{aligned} \quad (1)$$

With a simple vinyl polymer, for example, the reactions involved could be:



(2)

* Present address: Innovative Technologies Ltd, Winsford, Cheshire CW7 3PD, UK



(3a)
(3b)
(3c)
(3d)
(3e)
(3f)
(3g)

Other processes which do not involve hydroxyl radicals may also bring about hydroxylation. Some of these are indicated in reactions (3a–g); they are basically of types discussed by Needles and Whitfield[4] and by Norman and his colleagues[5]; the latter studied them using a rapid-flow system with e.s.r. detection. The nature of the substituent group R influences the relative rates of the reactions, as discussed previously[1]. An important practical point emerges in connection with the generation of oxygen during the peroxydisulfate decomposition (reaction 1). Since oxygen reacts very rapidly[6,7] with hydrocarbon radicals such as $-CH_2-\dot{C}R-CH_2-$ (equation (2)) to form unwanted peroxy radicals, its concentration must be kept as low as possible. This can be achieved satisfactorily by means of a vigorous nitrogen purge, which is found in practice to be very beneficial.

Hydroxylation of polypropylene microporous hollow fibres causes some degradation[2] and this also occurs with nylon membranes to a somewhat larger extent. As shown in *Figure 1*, treatment with the peroxydisulfate solution at 70°C for 1 h brings about a reduction in M_w and M_n by about 50%.

Haworth and Holker[8] have reported the graft polymerization of several vinyl monomers to nylon staple of various types. They used aqueous solutions of Ce^{4+} containing the monomer and a strong mineral acid in concentrations 0.2–2 N. Grafting was markedly dependent on the acid concentration as well as the type of acid. For example, with acrylamide, 0.3 N HNO_3 was used. We have not been able to reproduce these findings; indeed we could obtain only insignificant grafting by Ce^{4+} without a preliminary hydroxylation. In any case these high concentrations of strong mineral acid would be likely to cause considerable damage to the filter membrane.

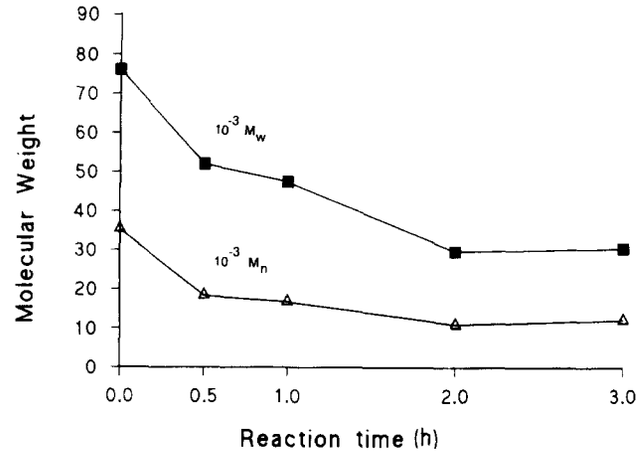


Figure 1 Reduction in the molecular weight of nylon in membranes during hydroxylation

EXPERIMENTAL

Materials

Ceric ammonium nitrate and acrylamide were obtained from BDH and 3-aminopropyl methacrylamide from Kodak. Heparin was supplied by Novo (Denmark) and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) by Sigma and potassium peroxydisulfate by Aldrich. Amersham International supplied heparin labelled with ^{35}S. Aqueous scintillant was obtained from BDH. Molecular weight determinations of nylon were made by RAPRA.

Arterial blood filters

Hydroxylation and grafting. Filters were filled with a 10% (w/v) aqueous solution of potassium

peroxydisulfate made up at 40°C; they were then placed in an oven at 70°C and the solutions circulated by a peristaltic pump for 1 h. Nitrogen gas was injected into the liquid continuously to remove some of the oxygen from the contents of the main containers.

The hydroxylated filters were then grafted with AcAm or a mixture of AcAm and APMAcAm, the feed ratio of the monomers being 1 : 1 or 9 : 1 by weight. The total concentration of monomers was initially 10% w/v; nitric acid and ceric ammonium nitrate concentration were initially 0.04 and 2×10^{-3} M, respectively. Grafting was carried out at 50°C for 1 h. The filters were then washed extensively with 0.05 M NaOH followed by hot water.

Heparinization. Filters grafted with the mixture of AcAm and APMAcAm carried free amino groups and were therefore suitable for heparinization. This was carried out by filling the filters with an aqueous solution of heparin (1% w/v) at pH 4.5 containing 0.1% w/v of EDC as coupling agent. Reaction was continued for 48 h at ambient temperatures. The filters were then washed extensively with 0.05 N NaOH and water.

Estimation of heparin covalently coupled to the surface of the nylon filter. This estimation was carried out with the aid of ^{35}S-labelled heparin. Discs of nylon 2.54 cm in diameter which had been grafted with AcAm and APMAcAm as described above were reacted in the aqueous solution containing EDC for 4 h or 24 h. The discs were then washed thoroughly with phosphate buffer saline (PBS), 0.05 N NaOH and finally with water. For radioactivity counting each disc was dissolved in 1 ml of concentrated hydrochloric acid then 8 ml aqueous scintillant added.

Haemocompatibility tests

Platelet adhesion to nylon filters. This was studied by direct observation of the platelets adhering to the surface by means of scanning electron microscopy (SEM). Discs (1.27 cm in diameter) from a filter were incubated in whole blood (3 ml) under agitation at 37°C for 4 h. The blood was then removed and each disc was washed very carefully with PBS to remove non-adherent platelets. Adhering platelets were then fixed by immersion in glutaraldehyde in PBS for 30 min. After washing the discs in distilled water they were allowed to stand sequentially in the following liquids: 70% methanol, 90% methanol, 100% methanol, then dried in vacuum overnight. Each sample was then mounted on a stent and sputter-coated for viewing with SEM.

Lymphocyte and neutrophil concentrations in blood were measured with standard Coulter Stack equipment.

Blood plasma clotting time. The standard partial thromboplastin time (PTT) test was used in this determination, which was carried out as follows. A disc of the membrane was immersed in 200 μl of platelet-poor plasma at 37°C then 200 μl of platelet substitute were added, followed after 1 min by 200 μl of 25 mM calcium chloride solution. Clotting times were measured from the addition of the latter.

Treatment of blood oxygenator. Hydroxylation and grafting were carried out essentially as described for the blood filters, with minor differences. For hydroxylation, a temperature of 70°C and a reaction time of 1.5 h were used and nitrogen was passed through the equipment following the course taken by oxygen when the apparatus is in use. The nitrogen therefore issued from the micropores in the polypropylene tubes and bubbled through the peroxydisulfate solution. This arrangement proved very satisfactory; effective purging of the oxygen was brought about by the small nitrogen bubbles, and the liquid was kept from contact with the walls of the pores, which were therefore not subsequently grafted. This is an important point, since grafting of the pore walls with a hydrophilic material is liable to cause leakage of the blood into the oxygen stream. Grafting was carried out at 50°C for times of 1–3 h.

RESULTS

Nylon membranes

Heparin coupling. *Table 1* shows the extent of coupling of heparin to grafted nylon membranes. Under these conditions the extent of coupling depends on the base content of the graft, as expected, and also on the reaction time with heparin. The covalent character of the coupling is indicated by the fact that prolonged contact (72 h) with water failed to extract any heparin. These couplings are much higher than those reported in the literature by Lin *et al.*[9], who obtained a maximum heparin coupling of 1.62 $\mu g\,cm^{-2}$.

Haemocompatibility tests

Blood plasma clotting times. Partial thromboplastin times, determined by the technique described earlier, are presented in *Table 2*. The results indicate that the bound heparin is highly active in preventing clotting of the plasma.

Platelet adhesion to nylon filters. A control membrane of untreated nylon, after immersion in blood as described earlier, acquires numerous attached platelets

Table 1 Covalent coupling of heparin to nylon membrane

No.	Feed composition in grafting AcAm : APMAcAm	Reaction time (h)	Heparin coupling ($\mu g\,cm^{-2}$)
1	1 : 1	4	5.20
2	1 : 1	24	13.40
3	9 : 1	4	6.72
4	9 : 1	24	10.61

Table 2 PTT data for nylon membrane, before and after heparinization

Sample	PTT(s)
Blood plasma	280, 300
Untreated membrane	120, fibrin deposition starts 200, complete clotting
No. 2 (*Table 1*)	no fibrin deposited and no clotting in 1 h
No. 4 (*Table 1*)	no clotting in 16 h
Control, unheparinized	160

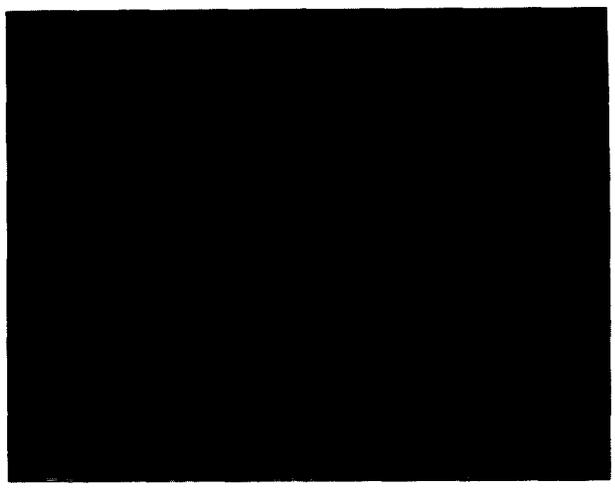


Figure 2 Adhesion of platelets to untreated nylon membrane after exposure to whole blood at 37°C for 4 h

Figure 3 Enlargement of *Figure 2*, indicating activation of platelets

Figure 4 Nylon membrane grafted with poly(acrylamide) after exposure to whole blood at 37°C for 4 h. No platelet adhesion is visible

(*Figure 2*) which are shown by the enlarged diagram (*Figure 3*) to be activated. This means that they have undergone the 'release reaction' in which there is liberation of the contents of platelet granules, including ADP, Ca^{2+} serotonin, fibrinogen, lysosomal enzymes

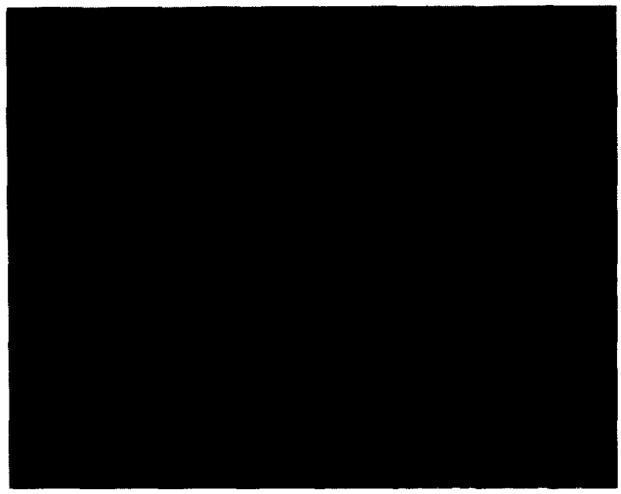


Figure 5 Enlargement of *Figure 4*, confirming absence of adhering platelets

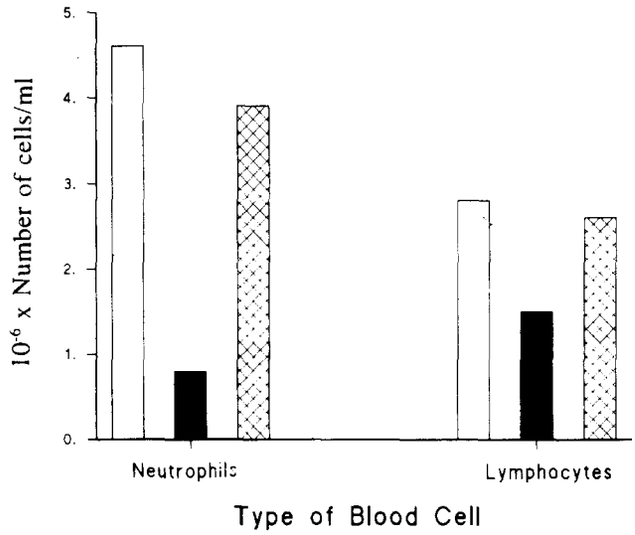


Figure 6 Blood content of neutrophils and lymphocytes after exposure to nylon filter membrane for 4 h at 37°C. Discs of nylon 1.27 cm diameter, 3 ml whole blood: □ whole blood; ■ untreated membrane; ⊠ membrane grafted with polyacrylamide (11% w/w)

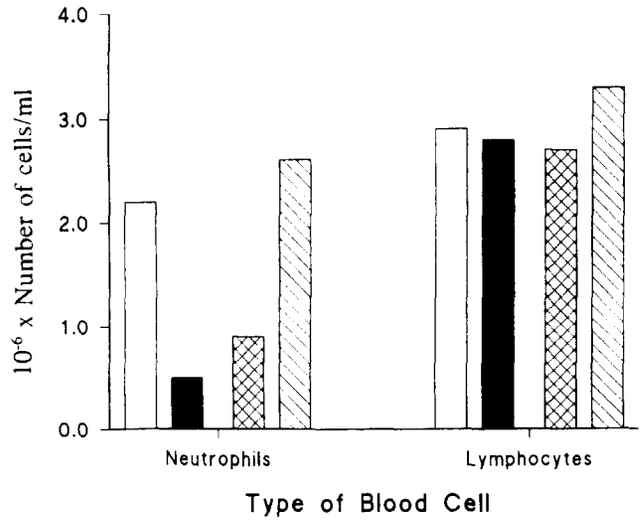


Figure 7 Blood content of neutrophils and lymphocytes after exposure to nylon filter membrane for 4 h at 37°C. Discs of nylon 1.27 cm diameter, 3 ml whole blood: □ whole blood; ■ untreated membrane; ⊠ membrane grafted with AcAm + APMAcAm (9 : 1); ▨ membrane grafted with AcAm + APMAcAm + heparin

and heparin neutralizing factor. These compounds bring about the activation of further platelets, thus initiating an autocatalytic process resulting in the irreversible aggregation of platelets to a haemostatic plug.

Membranes grafted with polyacrylamide examined in the same manner showed no detectable platelet adhesion (*Figures 4* and *5*). Modification of the membrane surface has therefore greatly reduced the number of activated platelets arising from blood in contact with the filter and correspondingly decreased the possibility of blood clot formation.

Depletion of lymphocytes and neutrophils by nylon filters. Lymphocytes and neutrophils are white blood-cells forming part of the body's immune system. Lymphocyte B- and T- ('helper') cells produce highly specific responses to foreign stimulating antigens while neutrophils exert a phagocytic action towards materials such as bacteria, fungi and damaged cells.

Concentration of neutrophils and lymphocytes remaining in whole blood after contact with a nylon membrane for 4 h at 37°C are presented in *Figure 6*; an untreated membrane is shown for comparison to a membrane grafted with polyacrylamide. Clearly, both types of cell are removed by the control, the reduction being greater with the neutrophils. On the other hand, the grafted membranes have little effect.

Figure 7 shows data obtained with membranes grafted with AcAm + APMAcAm (9 : 1) and also heparinized membranes. Neutrophil concentration is clearly markedly reduced by the untreated membrane and also (to a somewhat smaller extent) by the grafted membrane. The heparinized membrane has little effect; however, it is not clear whether this is attributable to the presence of heparin, or to the masking of the base groups in APMAcAm on coupling. The lymphocyte concentrations were relatively unchanged by any of the membranes.

CONCLUSIONS

We believe the work described has shown that the grafting technique developed may be simply applied to the major components of an extracorporeal blood circuit. The results, although somewhat preliminary in character, indicate that the overall process has a beneficial effect on the haemocompatibility of the system. It may readily be extended to other components.

REFERENCES

1 Bamford, C. H. and Al-Lamee, K. G. *Polymer* 1994, **35**, 2844
2 Bamford, C. H. and Al-Lamee, K. G. *Macromol. Rapid Commun.* 1994, **15**, 379
3 Bartlett, P. D. and Cotman, J. D. *J. Am. Chem. Soc.* 1949, **71**, 1419
4 Needles, H. L. and Whitfield, R. E. *J. Org. Chem.* 1964, **29**, 3632
5 Norman, R. O. C., Storey, P. M. and West, P. R. *J. Chem. Soc. B* 1970, 1087
6 Bamford, C. H. and Dewar, M. J. S. *Nature* 1949, **163**, 215
7 Bamford, C. H. and Dewar, M. J. S. *Proc. Roy. Soc., A* 1949, **198**, 252
8 Haworth, S. and Holker, J. R. *J. Soc. Dyers Col.* 1966, **82**, 257
9 Lin, S. C., Jacobs, H. A. and Kim, S. W. *J. Biomed. Mater. Res.* 1991, **25**, 791