Synthesis, characterization and preliminary *in vitro* blood compatibility evaluation of poly(methyl methacrylate-*g*-urethane)

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(Received 1 May 1991; revised 11 September 1991; accepted 23 September 1991)

To understand the variation in blood contacting properties of chemically modified polyurethane, methyl methacrylate has been grafted on to polyurethane by the γ-irradiation method. The preliminary *in vitro* blood compatibility studies indicated an improvement in the blood contacting properties of polyurethane by the introduction of poly(methyl methacrylate) chains.

(Keywords: polyurethane; poly(methyl methacrylate-*g*-urethane); platelet adhesion)

INTRODUCTION

The search for an ideal polymeric material for fabricating devices for substituting diseased vital organs has resulted in the routine use of several polymers as biomaterials[1,2]. Among these diversified materials, polyurethanes (PUs) possess an unparalleled position due to their unique properties arising from their specific structural features[3]. In spite of the several desirable characteristics as an ideal biomaterial, PU is inferior in certain aspects[4,5]. One of the easiest ways to reduce the drawbacks of PU, as suggested by several researchers, is by grafting other entities[5–8]. By optimizing the grafting procedure and by choosing appropriate monomers, it is possible to preserve the vital features of the trunk polymer simultaneously modulating the required biological responses. To the best of our knowledge, most of the chemical modifications of PU for improving the blood contacting properties by grafting are restricted to hydrophilic vinyl monomers[8–13]. Often the grafting adversely affects the mechanical properties. This is particularly true in the case of 2-hydroxy ethyl methacrylate grafted PU. As far as we know, hydrophobic monomers have not been grafted on to PU to test for blood compatibility.

Poly(methyl methacrylate) (PMMA) is well known for its biocompatibility and has a long history as a biomaterial in diversified applications[14,15]. This paper addresses the grafting of methyl methacrylate (MMA) on to PU and preliminary *in vitro* blood compatibility evaluation.

EXPERIMENTAL

The PU used consists of methylene bis (*p*-cyclohexyl diisocyanate) (H_{12}MDI), polytetramethylene glycol 990 (PTMEG) and 1,4-butanediol (BD). MMA (Merck) was purified by vacuum distillation. All the solvents used were either of chromatographic grade or spectroscopic grade and used as received. PU with a hard segment content of 46 wt% was synthesized by a two-stage process as reported elsewhere[16]. The relevant parameters of the polymers are summarized in *Table 1*.

Grafting

Grafting of MMA on to PU was achieved by γ-irradiation. Cleaned polymer strips (8×1 cm^2) with a thickness of 0.10–0.12 cm were immersed in the monomer for varied periods of time, taken out, pressed slightly between filter paper strips to remove surface adhered monomer and immediately subjected to γ-irradiation under nitrogen from a ^{60}Co source (Panoramic batch irradiator) to a total dose of 0.5 Mrad. Varied grafting yields were realized by increasing the exposure time of the polymer to the monomer rather than increasing the γ-ray flux.

The materials were extracted extensively with toluene after the grafting procedure to remove ungrafted PMMA chains. The grafting yield was determined from:

$$\frac{(W - W_0)}{W_0} \times 100$$

where W is the final weight of the polymer strip and W_0 is the initial weight.

Equipment

The attenuated total internal reflection (ATR) spectra of the materials were recorded using a Perkin–Elmer model 597 i.r. spectrophotometer together with an ATR accessory (Perkin–Elmer) and a KRS-crystal.

A Waters chromatographic system, consisting of

Table 1 Relevant parameters of the polymer

Weight average molecular weight (M_w)	222 000
Number average molecular weight (M_n)	110 000
Ultimate stress (kg cm^{-2})	528 ± 16
Ultimate strain (%)	486 ± 9
Glass transition temperature of soft segment (°C)	−30

0032-3861/92/132848-04

6000 Å solvent delivery pump, U6K injecter R-401 differential refractometer and 730 data module, was used for estimating the molecular weight parameters of the polymers. A set of three μ-styragel columns of pore size 10^5, 10^4 and 10^3 Å were used in series for analysing the polymers. Dichloromethane was used as a mobile phase at a flow rate of 2 ml min^{-1}. The columns were calibrated using polystyrene standard under the same chromatographic conditions.

A Jeol 35C scanning electron microscope was used to study the surface morphology of the polymeric samples. The samples were coated with a thin layer of gold prior to observation.

An Instron (model 1193) universal testing machine was used for estimating the ultimate stress and strain parameters of the polymer as per ASTM D-882. The cross-head speed was 100 mm min^{-1}.

Blood platelet adhesion

Calf blood was collected in a polyethylene tube containing aqueous trisodium citrate solution as an anticoagulant. The ratio of anticoagulant to blood was 1:10 (v/v). The platelet-rich plasma (PRP) was collected by centrifugation at 1000 rev min^{-1} for 10 min. The platelet count in the PRP was adjusted to 10^5 per microlitre by diluting the PRP with platelet-poor plasma. The polymer strips were immersed in platelet suspension at 37°C for 1 h under static conditions. The platelets in the suspension before and after polymer exposure were counted using a Neubeaur counting chamber on a Nikon 35 A phase contrast microscope. The difference in count expressed as a percentage was taken as the amount of platelet deposition.

RESULTS AND DISCUSSION

Figure 1 shows the ATR-i.r. spectrum of poly(methyl methacrylate-*g*-urethane). The peaks centred at 1145 and 740 cm^{-1} could be assigned to the grafted PMMA chains[17]. The carbonyl peak of the PMMA chains, however, merges with the carbonyl absorption peak of PU (1702 cm^{-1}). The broadening of the peak in the –CO– absorption region is possibly a manifestation of the mixing of these two peaks.

Figure 2 shows the g.p.c. chromatographs of PU and the graft copolymer. The elution profile of the graft (*Figure 2B*) shifts to a lower time-scale suggesting higher molecular weight or in other words the g.p.c. trace confirms the grafting process. The molecular weights obtained from the g.p.c. traces are summarized in *Table 2*.

Figures 3a and *b* are SEM photographs of PU and the grafted sample. The surface of PU appears to be smooth. The distinctively altered morphology resulting from grafting is apparent in *Figure 3b*. The grafted chains, in the form of clusters, nearly cover the entire surface.

Table 2 Molecular weight parameters of polymers as obtained from g.p.c. analysis

Polymer	Grafting (wt%)	M_w	M_n
PU	–	222000	110000
Poly(methyl methacrylate-*g*-urethane)	19	334000	142000
	59	472000	183000

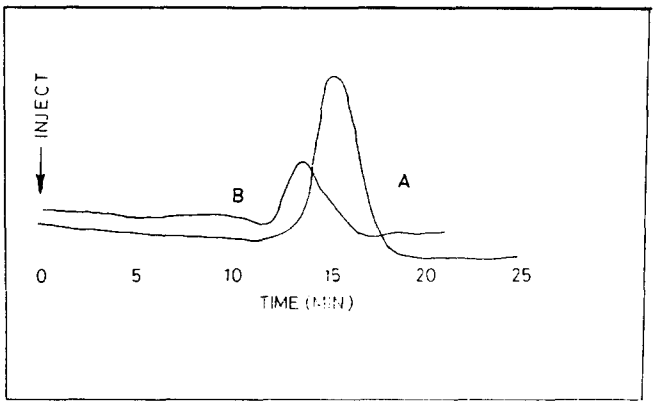

Figure 2 G.p.c. traces of (A) PU and (B) poly(methyl methacrylate-*g*-urethane) (19 wt% grafting)

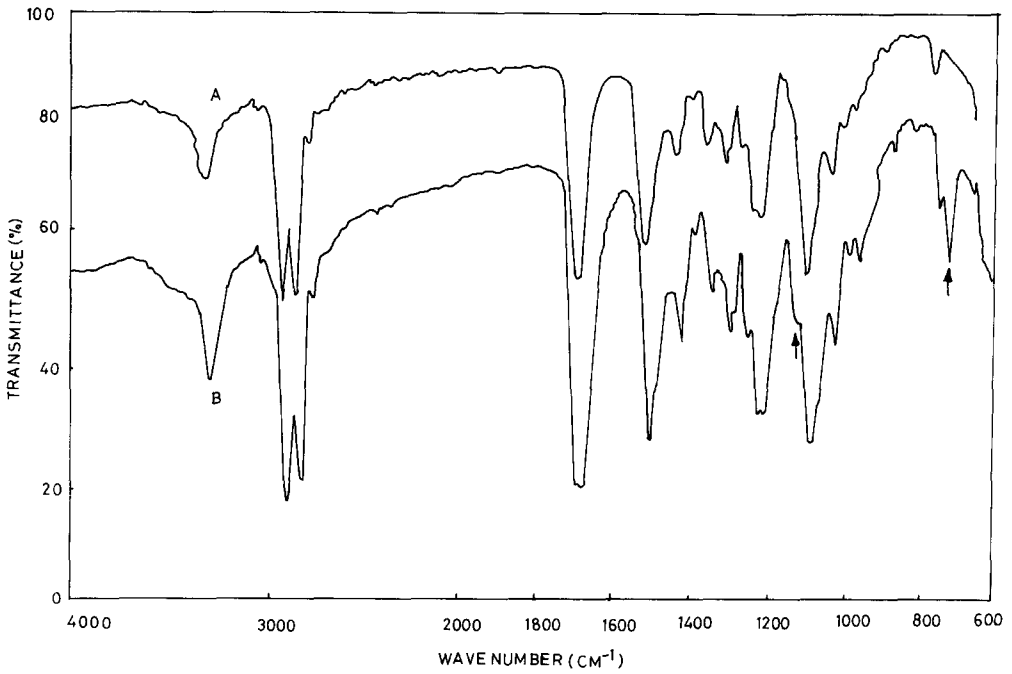

Figure 1 ATR-i.r. spectra of (A) PU and (B) poly(methyl methacrylate-*g*-urethane) (19 wt% grafting)

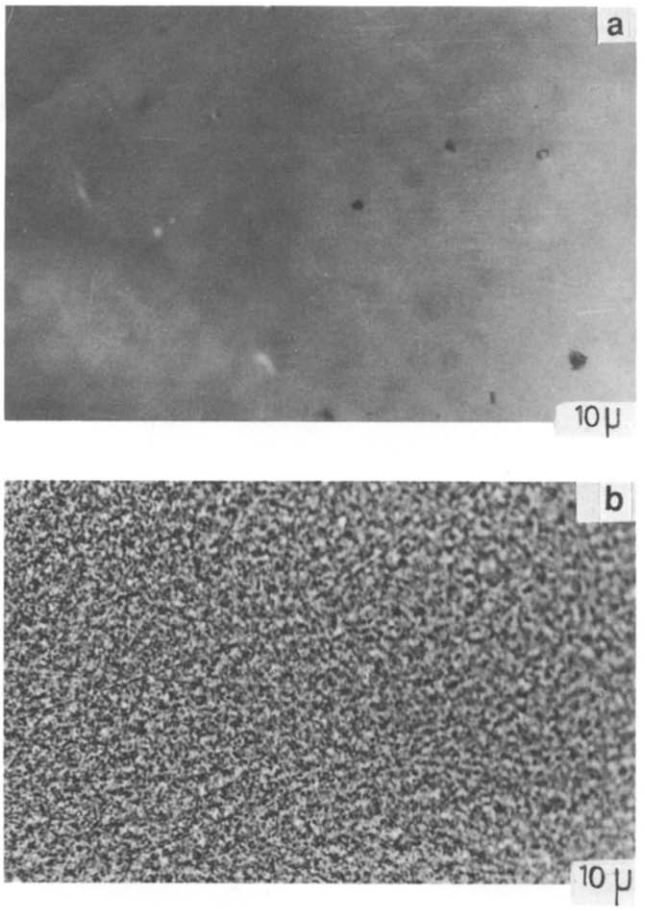

Figure 3 SEM micrographs of (a) PU and (b) poly(methyl methacrylate-*g*-urethane) (5 wt% grafting)

Table 3 Stress–strain parameters of polymers

Polymer	Grafting (wt%)	Ultimate stress ($kg\ cm^{-2}$)	Ultimate strain (%)
PU	–	528 ± 16	486 ± 9
Poly(methyl methacrylate-*g*-urethane)	5	454 ± 11	406 ± 12
	19	398 ± 14	375 ± 15
	59	346 ± 8	310 ± 14

Table 4 Variation in platelet adhesion and protein adsorption with the grafting of MMA

Polymer	Grafting (wt%)	Platelet adhesion (%)	Protein adsorption	
			Albumin ($\mu g\ cm^{-2}$)	Fibrinogen ($\mu g\ cm^{-2}$)
PU	–	74 ± 1.6	1.01 ± 0.13	0.68 ± 0.09
Poly(methyl methacrylate-*g*-urethane)	5	34 ± 2.2	1.98 ± 0.16	0.37 ± 0.06
	19	31 ± 3.4	2.10 ± 0.14	0.34 ± 0.05
	59	33 ± 2.6	1.97 ± 0.11	0.36 ± 0.07

Table 3 summarizes the ultimate stress–strain parameters of the graft polymer. Compared with PU, the grafts register lower values. This could be attributed to the glassy nature of PMMA. The hard domains of the PU are impermeable to the monomers[18] and therefore the grafting is confined to the soft segment. The flexibility of PUs, as is well known, arises from the soft segment content. Apart from the structural features, the ultimate stress of PUs depends heavily on the orientation of the soft segment along the stress and the stress-induced soft segment crystallinity[19]. The grafting of glassy PMMA could affect these factors affecting the ultimate mechanical parameters. The reduction in stress–strain parameters with the increased percentage of grafting presumably indicates the tremendous changes due to grafting in the morphological features of PUs. Surface modification, however, requires only a low percentage of grafting which does not seriously affect the mechanical properties (*Table 3*).

The extent of platelet adhesion and its variation with grafting of PMMA is shown in *Table 4*. The table suggests that the grafting of PMMA results in a reduction in platelet adsorption which is indicative of improved blood compatibility[20]. The platelet adsorption is practically invariant with increased percentage of grafting of PMMA, indicating that bulk modification does not have any specific role in modulating the biological responses.

The first event that occurs after blood contact with a foreign surface is the adsorption of proteins[21–23]. The bulk of blood–material interaction research has focused on the *in vitro* measurement of the adsorption of proteins to a large number of surfaces[24,25]. An understanding of the formation of the protein layer should lead to an understanding of the specific factors involved in blood–material interaction and ultimately to knowledge of clot formation. To visualize the protein adsorption pattern on the modified PU, protein adsorption studies using bovine albumin (Sigma) and fibrinogen (Sigma) have been carried out as detailed elsewhere[26]. The adsorption values obtained for these two proteins are also summarized in *Table 4*. It is interesting to note that PMMA-*g*-PU adsorbs more albumin than fibrinogen. Also thromboresistant surfaces adsorb more albumin while thrombogenic materials adsorb more fibrinogen and γ-globulin[27]. The reduced platelet adhesion on the PMMA–graft could be traced to the increased albumin adsorption on this surface. Although the altered protein adsorption behaviour in terms of the surface features of the graft copolymer is not studied here, it seems that MMA grafting improves the blood contacting properties of PU to an appreciable level.

CONCLUSIONS

MMA was grafted on to PU by the γ-irradiation method. The graft copolymer has been characterized and subjected to preliminary blood compatibility studies. The enrichment of PU surfaces with MMA by grafting seems to improve the blood contacting properties of PU.

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