

GRAFTING OF HYDROPHILIC MONOMERS ONTO POLYURETHANE MEMBRANES BY SOLUTION OR PRE-ABSORBING METHODS FOR ACCELERATION OF CELL COMPATIBILITY*

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Abstract Two grafting methods, *i.e.* solution grafting and pre-adsorbing, are introduced to covalently immobilize hydrophilic polymers on segmented polyurethane (SPU) to modify its hydrophilicity and to improve its cell compatibility. Solution grafting results in higher degree of grafting and rougher surface morphology. Cell culture evaluation demonstrates that the modified membranes thus obtained are disadvantageous to the endothelial cell (ECs) growth probably because the hydrophilic groups on the surface are over-crowded. However, pre-absorbing grafting generates lower degree of grafting, which is detected and confirmed by ATR-FTIR spectra and water contact angle. Scanning electron microscopic (SEM) measurement shows that the latter method produces a plane and smooth morphology, which is similar to the SPU control sample controlled. When grafting with lower monomer concentration, ECs could grow on SPU-g-PHEA [poly(2-hydroxyethyl acrylate)], SPU-g-PAAm (polyacrylamide), SPU-g-PDMA [poly(2-(dimethylamino)ethyl methacrylate)] or quaternized SPU-g-PDMA surface with elongated cell shapes. Hence, the cell compatibility of SPU is improved and a useful method to construct a cell compatible layer on the polymer surface has been developed.

Keywords Segmented polyurethane, Cell compatibility, Surface grafting, Endothelial cell

INTRODUCTION

When artificial implants are introduced into the organism for long-term application, they are sure to be finally covered with the organism tissues. In many instances such as for blood vessel, it is favorable to cover the implant in a period as short as possible. This means a cell growth-promoting surface is more advantageous to tissue growth around the implant. Segmented polyurethanes (SPU) have been widely used as implantable biomaterials especially for blood vessels and artificial heart, but ordinary SPU does not facilitate tissue growth quickly around the implant surface. Therefore, improvement of its cell compatibility is required. One important approach is to introduce some functional groups that can accelerate cells adhesion and growth on the material surface^[1, 2], among which amino, carboxyl, hydroxyl and amide groups have proved effective and easy to introduce with a stable bond to the material surface^[3-6].

In this paper, 2-(dimethylamino)ethyl methacrylate (DMA), 2-hydroxyethyl acrylate (HEA) as well as acrylamide (AAM) which contain the amino, hydroxyl and amide groups, respectively, were grafted onto the SPU membranes through photo-oxidation and UV irradiation polymerization. Photo-oxidizing the SPU membrane in hydroperoxide solution will first generate macromolecular hydroperoxide groups ($P-O-O-H$, where P represents the SPU polymer chain)^[7] on its surface. When irradiated under UV light, the hydroperoxide groups will decompose into macromolecular oxygen radicals ($P-O\cdot$) that have the ability to initiate the grafting copolymerization, while hydroxyl radicals $\cdot O-H$ initiate mainly the homopolymerization of the corresponding

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monomers. The latter reaction is undesired and can be retarded by ferrous ions that convert the hydroxyl radicals into hydroxyl ions^[8]. In solution grafting, the SPU membrane was immersed in the monomer solution when grafting reaction was conducted, yielding a higher degree of grafting and rougher surface morphology compared with pre-absorbing grafting. Evaluation of endothelial cell (EC) cultures revealed that these two grafting methods can generate different degrees of cell compatibility.

EXPERIMENTAL

Photo-oxidization and Grafting Copolymerization

SPU (BF Goodrich Co.) membrane of 0.3 mm thickness was prepared by casting a 30% SPU solution in 1,4-dioxane, and then evaporating the solvent at 40°C. The SPU membrane was cut into 1.5 × 3 cm strips, and was placed in 40 mL hydrogen peroxide solution (30%) and irradiated by UV light generated from a high-pressure mercury lamp (250 W) for a given time under stirring at 35°C. The photo-oxidized membrane was rinsed with water to remove the excess hydrogen peroxide and dried. For solution grafting, the required amount of monomer, 15 mL aqueous solution of ammonium iron(II) sulfate hexahydrate and photo-oxidized membrane were placed in a glass tube. For pre-absorbing grafting, the photo-oxidized membrane was immersed in monomer solution of 1%, 5% or 10% concentration for 1 h initially, then was taken out and placed in a glass tube without any solution. The glass tube was closed with a rubber stopper and the air inside then replaced with nitrogen. Grafting copolymerization was carried out under UV irradiation at a distance of 12.5 cm for a given time at 35°C. The grafted membrane was rinsed with hot water for at least 48 h to remove the homopolymers^[9-11], and subsequently dried in vacuum at 50°C for 24 h. The DMA grafted membrane was further quaternized by iodomethane (10% ethanol) at room temperature for 3 h.

Endothelial Cell Culture

Endothelial cells (ECs) were isolated from the human umbilical cord veins of a newborn baby through digestion with 0.1% collagenase (type I, Sigma) for 15 to 20 min at room temperature^[12]. The isolated ECs were routinely cultured in the beds that were pre-laid with SPU-g-PDMA, SPU-g-PHEA or SPU-g-PAAm membranes, as well as in the beds of tissue culture polystyrene (TCPS) (Nunc, Denmark) as control. The endothelial cells were maintained in a humidified atmosphere of 5% CO₂ in air at 37°C, in a serum-containing culture medium which consisted of 20% little calf serum and 80% RPMI1640 (Gibco, containing *L*-glutamine), supplemented with 100 U/mL of penicillin and 100 µg/mL of streptomycin. After incubation for 24 h, the culture medium was changed and then changed every 2 days. The cultured ECs were fixed with 2.5% glutaraldehyde for 30 min, and the cell nuclei then stained with Giemsa for 5 to 10 min.

Characterization

The content of hydroperoxide groups on the membrane surface was determined by iodometry^[13]. The degree of grafting is defined as $(W_2 - W_1)/W_1$, where W_1 and W_2 represent the weight of photo-oxidized membrane and grafted membrane, respectively. The Infra-red spectra were recorded on a Nicolet Magna-IR560 spectrometer by using the ATR technique. An ESCA LAB Mark II spectrometer was employed to analyze the relative element contents on the membrane surfaces using Al K α excitation radiation. The charging shift was referred to the C_{1s} line emitted from the saturated hydrocarbon. The static water contact angle (θ) was measured by the sessile drop method employing a JY-82 apparatus. Scanning electron microscopy images were recorded with a Hitachi S-570 microscope. The morphology of cultured ECs was observed with an inverted microscope (LOIC).

RESULTS AND DISCUSSION

Figure 1 shows that the content of hydroperoxide groups on the SPU membrane surface increases with the irradiation time and reaches its maximum at about 8 h, and then decreases. UV light can also cause the decomposition of hydroperoxide. Therefore, a longer oxidation time is unfavorable.

When the grafting copolymerization was conducted in monomer solution, the ferrous ions (Fe²⁺) added will retard the homopolymerization. Figure 2 shows that the degree of grafting was increased almost linearly with the

content of hydroperoxide groups for all monomers, especially for DMA and AAm, whose degree of grafting is lower than that of HEA in the same hydroperoxide content. The degree of grafting by this method is so high that the amount of grafted polymers could be simply measured gravimetrically.

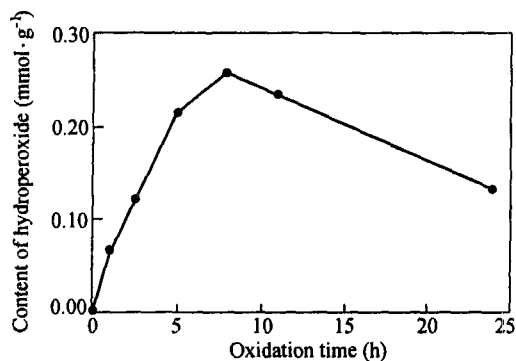


Fig. 1 The content of hydroperoxide as a function of photo-oxidation time

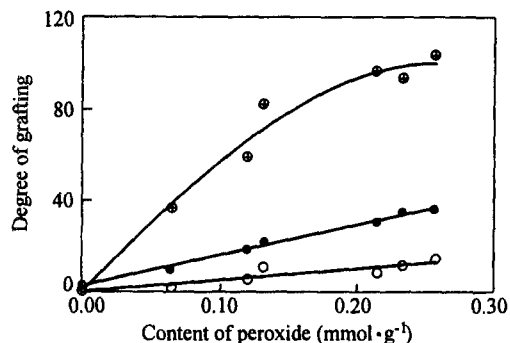


Fig. 2 Degree of grafting on the surface of SPU membranes as a function of peroxide content

● SPU-g-PDMA, ⊕ SPU-g-PHEA, ○ SPU-g-PAAm

When the pre-absorbing grafting method was employed, a smaller amount of polymers will be grafted on the SPU surface. The degree of grafting could not be estimated by weighing, because the grafted SPU membrane was usually lighter than the ungrafted one, probably due to the slight decomposition of SPU induced by the photo-oxidation. Yet ATR-FTIR spectra could easily identify that the amount of grafted polymers on the SPU membrane surface increases with the monomer concentration, as shown in Table 1. Since DMA does not have an absorption peak at 3335 cm^{-1} (N—H stretching vibration of amide groups in SPU), therefore the peak intensity ratio of I_{3335}/I_{2955} (2955 cm^{-1} , C—H stretching vibration) for SPU-g-PDMA copolymer can represent the amount of SPU on the top surface of the grafted membrane. Table 1 shows that this ratio decreases with the increase of monomer concentration. Thus, it can be inferred that more PDMA was immobilized on the SPU membrane surfaces at a higher DMA content. On the other hand, DMA has an absorption at 1724 cm^{-1} (—O—C=O ester group). Therefore, the peak intensity ratio I_{1724}/I_{3335} and I_{1724}/I_{1596} (1596 cm^{-1} , N—H deformation vibration of an amide groups in SPU) increases. The same analysis process proving the occurrence of graft polymerization can also be used for AAm and HEA grafted SPU from the peak intensity ratio in IR spectrum. For example, AAm has an amide group that shows absorption at 3335 cm^{-1} . Hence, the increase of I_{3335}/I_{2955} with the monomer concentration demonstrates that PAAm was immobilized on SPU membrane surfaces.

Table 1. IR spectroscopic results of grafted SPU membranes obtained by pre-absorbing grafting method using different kinds of monomer and monomer concentrations

Peak intensity ratio	Control	DMA (%)			AAm (%)			HEA (%)		
		1	5	10	1	5	10	1	5	10
I_{3335}/I_{2955}	0.724	0.599	0.453	0.249	0.763	0.841	1.196	—	—	—
I_{1724}/I_{3335}	2.353	2.972	3.263	3.691	1.798	1.628	1.479	—	—	—
I_{1724}/I_{1596}	2.148	2.441	2.631	2.878	—	—	—	2.310	2.592	2.786

The static water contact angle shows the same trend as the IR results (Fig. 3), *i.e.* the hydrophilicity of the grafted membranes is improved (but contact angle decreases) with increasing monomer concentration. This result further confirms the occurrence of the graft copolymerization. Figure 3 also shows that SPU-g-PHEA has better hydrophilicity than SPU-g-PAAm, and SPU-g-PDMA is the most hydrophobic one.

The occurrence of grafting is further evidenced by ESCA analysis. It was found that the O_{1s}/C_{1s} ratio of grafted SPU was always larger than that of photo-oxidized SPU membrane, and a higher value was obtained at a higher monomer concentration (for further information, refer to Refs. [10] and [11]). It is worth noting that a

photoelectron emission angle of 60° is necessary in order to obtain an increase of O_{1s}/C_{1s} ratio for grafted SPU membranes prepared by pre-absorbing method. Measuring at an angle of 0° will generate a constant value. Therefore, it can be deduced that graft copolymerization occurs only on the SPU superficial layer for pre-adsorbing grafting.

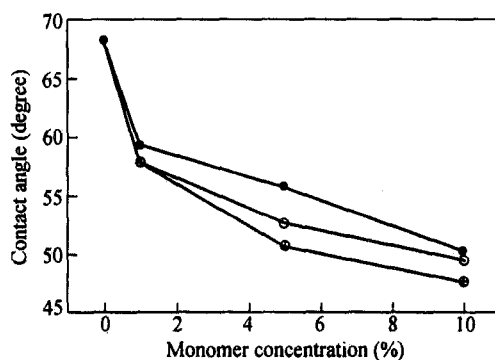


Fig. 3 Static water contact angle of SPU grafted with PDMA (●), PAAm (○) or PHEA (⊕) by pre-absorbing grafting

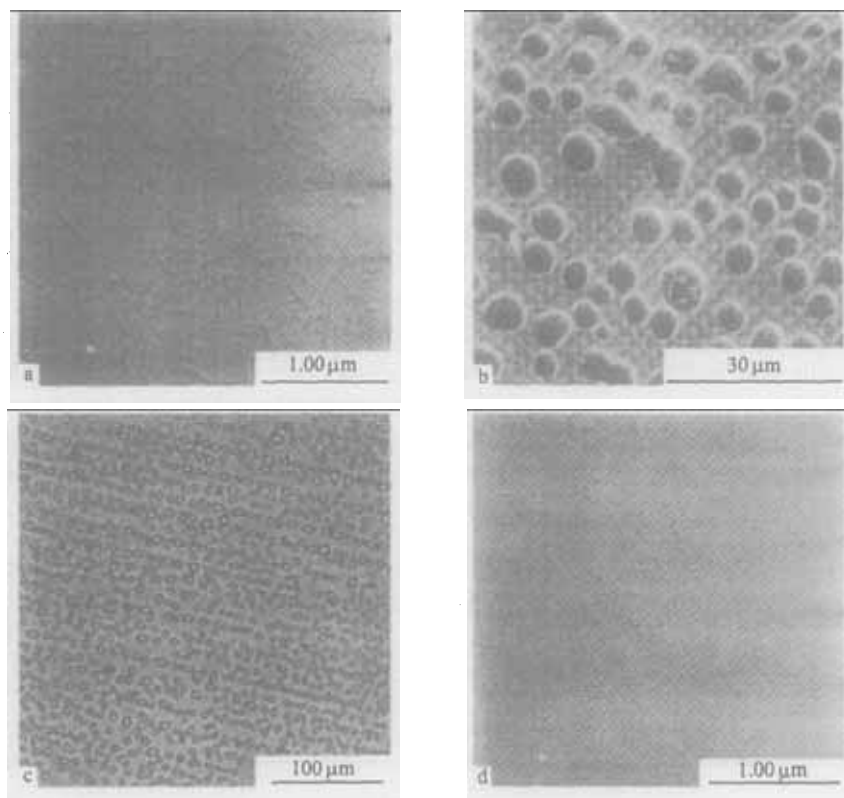


Fig. 4 The morphology of SPU membranes
a) SEM image of the SPU control
b), c) SEM and optical images of SPU-g-PDMA by solution grafting, respectively
d) SEM image of SPU-g-PDMA by pre-absorbing grafting

The SPU-g-PDMA membranes acquired from both methods were further quaternized with iodomethane. Peaks at 631.0 eV, 619.5 eV and 51.0 eV in ESCA spectra, which were attributed to the I3d3, I3d5 and I4d^[14],

respectively, reveal that quaternization has occurred.

Solution grafting and pre-absorbing grafting produce different surface morphology (Fig. 4). SPU-g-PAAm and SPU-g-PHEA membranes prepared by solution grafting show an irregular and much rougher surface structure^[15], while SPU-g-PDMA (Figs. 4b, c) and quaternized SPU-g-PDMA (image is not shown) show a regular distribution of spots having a diameter around several to micrometers on the whole surface. On the other hand, grafted SPU membranes prepared from pre-absorbing grafting show a flat and smooth surface structure (Fig. 4d) which exhibits no difference from the control (Fig. 4a). The difference in morphology between the two grafting methods is mainly caused by the different degree of grafting and different grafting process. When the grafting is carried out in monomer solution, the monomers diffuse onto the membrane surface steadily and then the grafted chains propagate continuously, to yield higher degree of grafting. As a result, a larger amount of hydrophilic polymers grafted on the hydrophobic SPU membrane surface can accumulate to form a separate phase. By contrast the monomers absorbed on the membrane surface in pre-absorbing grafting are almost unable to move or shift, the chain propagation is thus restricted within the surrounding of initiators that produced the chain with almost the same length, and lower degree of grafting. As a result the grafted polymers could only form a very thin layer on the SPU membrane. At a very low monomer concentration, *e.g.* 1%, it perhaps could only cover the SPU membrane partially to form a hydrophilic-hydrophobic micro-phase separated surface structure, which is proved to be favorable for cell growth by cell culture evaluation. The mechanism of spot formation on SPU-g-PDMA surface (Figs. 4b, c) is not clear yet. A possible explanation would be that it might be related with the stiffness of the polymer chain and the repulsion of hydrophilic-hydrophobic interaction. If the hydrophilic polymer chain is soft enough, the repulsion by the hydrophobic SPU substrate may cause the grafted hydrophilic polymer droplets to segregate. The segregated polymer droplets may self accumulate again to form the spots corresponding to the lower hydrophilic-hydrophobic repulsion state.

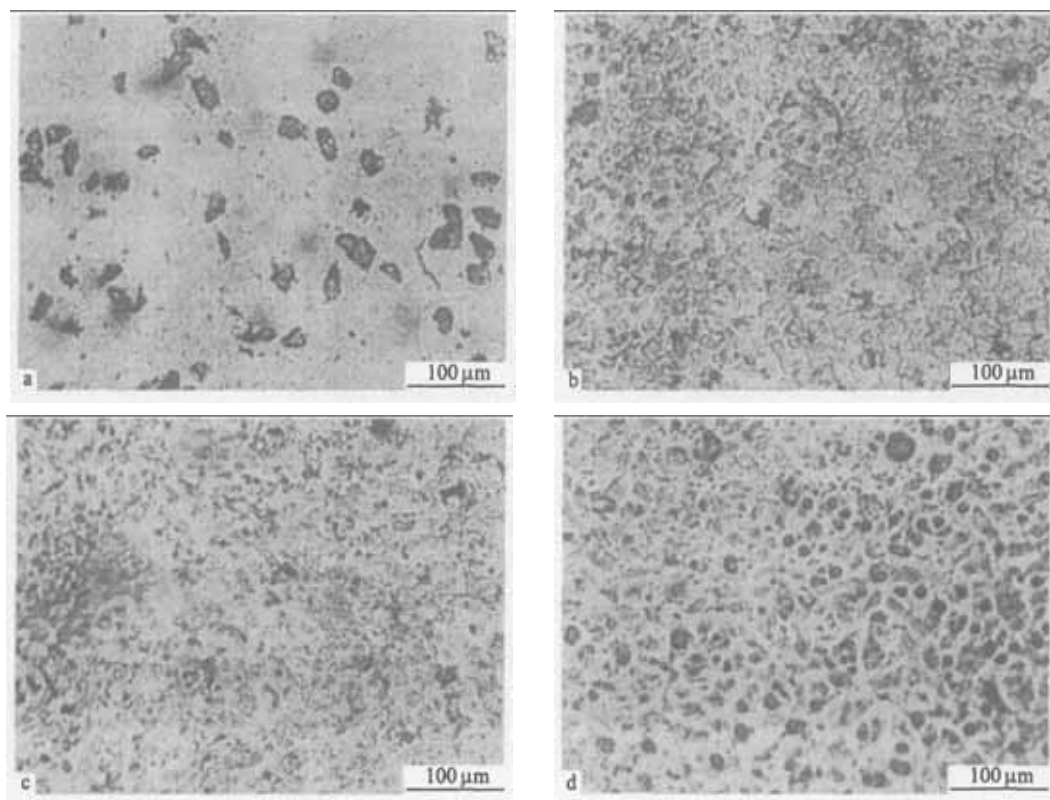


Fig. 5 EC culture results on (a) SPU control, (b) SPU-g-PDMA, (c) quaternized SPU-g-PDMA and (d) SPU-g-PHEA grafted by pre-absorbing grafting method

The endothelial cells (ECs) were isolated and cultured on the modified SPU membranes. The results show that all kinds of grafted SPU membranes obtained from solution grafting, whether they contain hydroxyl, amino or amide groups or are further quaternized, are disadvantageous for EC growth. No ECs were observed on the surface of membranes after they were cultured for 4 days. Repeated experiments generate the same results. The entire surface of these membranes is covered with the grafted hydrophilic polymers. Materials with too hydrophilic surface are disadvantageous for cell adhesion and growth as reported by many researchers. However, ECs could adhere and grow on SPU-g-PDMA and SPU-g-PHEA surfaces obtained from pre-absorbing grafting with 1% monomer concentration (Fig. 5). The elongated cell shapes (Figs. 5b, d) suggest that the cell compatibility of SPU modified either by DMA, HEA or AAm in this way is effectively improved when they are compared with the result of the control (Fig. 5a). ECs could adhere on unmodified SPU but could not undergo cell division and cell proliferation. The cells still kept a ball-like morphology (Fig. 5a). The quaternized surface does not show higher ability to promote cell growth as compared with the original (Fig. 5c). Yet, the surfaces of both SPU-g-PDMA and quaternized SPU-g-PDMA are not as good as tissue culture polystyrene (TCPS, image is not shown) at accelerating EC growth. However, we have succeeded in constructing a cell compatible layer on the SPU surface to promote the cell growth.

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