Surface modification of polyurethane for promotion of cell adhesion and growth 1: Surface photo-grafting with N,N-dimethylaminoethyl methacrylate and cytocompatibility of the modified surface

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Functional polyurethane (PU) surface was prepared by photo-grafting N,N-dimethylaminoethyl methacrylate (DMAEM) onto the membrane surface. Grafting copolymerization was conducted by the combined use of the photo-oxidation and irradiation grafting. PU membrane was photo-oxidized to introduce the hydroperoxide groups onto the surface, then the membrane previously immersed in monomer solution was irradiated by UV light. The X-ray photoelectron spectroscopy and water contact angle characterized the grafted copolymers and verified the occurrence of graft copolymerization. The results showed that UV irradiation could realize the graft copolymerization effectively. The grafted membrane showed minimal surface morphology. Human umbilical vein endothelium (HUVE) cells were seeded on the grafted surfaces. The performance of the surface in cell attachment correlated with the content of oxygen and nitrogen. Cells were spread more extensive and grown faster on the surface with lower degree of grafting.

1. Introduction

Polyurethane (PU) has been widely used as biomaterial due to its excellent physical property and blood compatibility. Nonthrombogenicity and resistance to biodegradation of PU had led to their use in both commercial and experimental blood-contacting applications, such as in catheters, chambers for artificial heart and pacemaker wire lead insulation [1, 2]. However, the insufficient nonthrombogenicity of conventional PU restrict their use in long-term *in vivo* applications such as vascular graft prostheses. It has been known that certain surface features of polymer can influence the interactions between cells and material. Many studies have shown that cellular adhesion tend to correlate with surface hydrophilicity [3] and surface charge [4] of the polymer. This has lead to the development of different physical and/or chemical methods to modify the surface characteristics of polymers in favor of the cellular behavior [5, 6]. Some authors reported that the nitrogen-containing groups effected the cytocompatibility because they could adjust the surface hydrophilicity and interact with peptide of cells [4, 7, 8]. However, the effect of pure amino groups has not been described because the surface they tested were complicated and there were more than one kind of functional group on the surface that could effect the cytocompatibility, which was resulted from the modification methods they used [4, 7, 8].

In this paper, PU was modified by graft copolymerization with N,N-dimethylaminoethyl methacrylate to introduce the amino groups onto the surface. Graft copolymerization was conducted by the combined use of the photo-oxidation and irradiation grafting. To realize the UV irradiation polymerization, the photosensitive groups on the PU surface was critical. As hydroperoxide group was UV sensitive [9], this paper we attempted to introduce the hydroperoxide groups onto the PU surface by photo-oxidizing the PU membrane under UV irradiation. To modify only the polymer surface we used the UV irradiation polymerization because the penetration ability of UV light was low, moreover the hydroperoxide group was UV sensitive [9] and UV light treated surface was cleaner than that treated by other methods [10]. When irradiating under UV light, the hydroperoxide groups decomposed into macromolecular oxygen radicals, which initiated the graft copolymerization. We have found that the graft copolymerization conducted in monomer solution directly yielded surface morphology even when the degree of grafting was low, and it was difficult to determine which factors between the surface morphology and hydrophilicity effect cytocompatibility largely [11]. To minimize the surface

morphology, we immersed the membrane in monomer solution and then UV irradiation the membrane, which obtained the minimal surface morphology. Human umbilical vein endothelium (HUVE) cells were seeded on the grafted surfaces to investigate the cytocompatibility.

2. Materials and methods

2.1. Preparation of materials

The substrate PU employed was poly(ester-urethane) purchased from BFGoodrich Co. A PU membrane with thickness 0.3 mm was obtained by spreading a DMF solution of base PU onto a glass plate and evaporated the solvent. The membrane was purified by extraction with ethanol for 24 h, followed by vacuum drying at 50 °C overnight.

N,N-dimethylaminoethyl methacrylate (DMAEM) was prepared by reacting of N,N-dimethylethanolamine with methacryol chloride. FTIR and ^{1}HNMR characterized the monomer obtained.

2.2. Photo-oxidization and graft polymerization

Photo-oxidization was conducted by placing PU membrane into hydrogen peroxide solution (30%) and UV irradiated 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 free excess of hydrogen peroxide and dried.

The membrane was immersed in aqueous monomer solution with concentration of 1, 5, 10 wt % for 1 h, then placed into Pyrex glass tube closed with a rubber stopper and replaced with nitrogen. Graft copolymerization was carried out under UV irradiation at a distance of 12.5 cm for 1 h at 35 °C. The grafted membrane was extracted with hot water (70 °C) for at least 48 h to remove the homopolymer [12] and then dried.

2.3. Analysis of modified surface

The content of hydroperoxide groups on the membrane surface was determined by the iodometry method [13].

The static water contact angle was conducted by sessile drop method. Ten measurements on different parts of film were taken and averaged. The water used for the measurement was purified by deionization after double distillation.

XPS spectra were conducted on a ESCA LAB Mark II spectrometer employing A*l*K$_\alpha$ excitation radiation. The charging shift was referred to the C_{1s} line emitted from the saturated hydrocarbon. The roughness of gold-coated PU and grafted PU membranes were examined by scanning electron microscopy (HITACHI S-570 microscopy).

2.4. Cell culture

HUVE cells were isolated form human umbilical vein using 0.1% collagenase (Sigma Co.) in phosphate buffered saline (PBS) by a method adapted from Jaffe *et al*. [14] and Schwartz [15]. The method has been described in detail elsewhere [16]. The isolated cells were incubated in PRMI 1640 medium (Gibcobrl Co.) supplemented with 20% fetal bovine serum (Sijiqin Biotech. Co., China) at 37 °C in a humidified atmosphere of 5% CO_2.

2.5. Evaluation of Cell Attachment

PU and PU-g-PDMAEM membranes were sterilized in 75% ethanol and swollen in PBS were placed into 96-well TCPS plates (NUNCLON™, Cat. No. 167008). Cells were then seeded in culture media to give a final density of 3×10^4 cells per well and incubated for 12 h at 37° in an atmosphere of 5% CO_2 in air. Following incubation, the wells were washed twice with PBS to remove non-attached cells. The remaining cells were removed by digestion with solution of 0.25% trypsin. The number of cells attached to the polymer surfaces was determined by hemocytometric countings [17]. Cell attachment was expressed as a proportion of the number of cells that attached to TCPS in the same culture media.

2.6. Evaluation of Cell Growth

For cell growth studies, the sample preparation was the same as for the attachment assay. Cells were seeded at a density of 3×10^4 cells per well. The culture media was replenished every sencond day. The randomly selected wells were washed twice with PBS and digested with solution of 0.25% trypsin per day up to 4 days. The number of cells grown on the polymer surfaces was determined by hemocytometric countings.

3. Results and discussion

The effect of photo-oxidization on the chemical composition was investigated with ESCA. All data were reproducible within 0.2 eV. In O1s spectra of starting and photo-oxidized membranes, there appeared two peaks at 532.9 and 534.3 eV which assigned to the oxygen in C–O bond and C═O bond respectively (Fig. 1(a)) [18, 19]. The photo-oxidized membrane also showed these two peaks (Fig. 1b). Comparing the relative intensity of two peaks, it was found that the peak at 534.3 eV was increased after photo-oxidization, that was, the content of oxygen with the binding energy at 534.3 eV increased. According to Ikada *et al*. [10], it could be considered that the peroxide groups were formed on the surface. After photo-oxidization, the O1s/C1s ratio of the PU surface increased (Fig. 2(a)). It could consider that the peroxide groups were introduced onto the surface [10, 20]. To determine the content of hydroperoxide groups on membrane surface, which could initiate the graft copolymerization, we used the iodometry method [13]. As can be seen from Fig. 2(b), the content of hydroperoxide groups increased with irradiation time and reached maximum at about 8 h, thereafter decreased. It indicated that PU membrane could be effectively oxidized to yield hydroperoxide groups under UV irradiation.

We have found that the graft copolymerization conducted in monomer solution yielded surface morphology even when the degree of grafting was low [11].

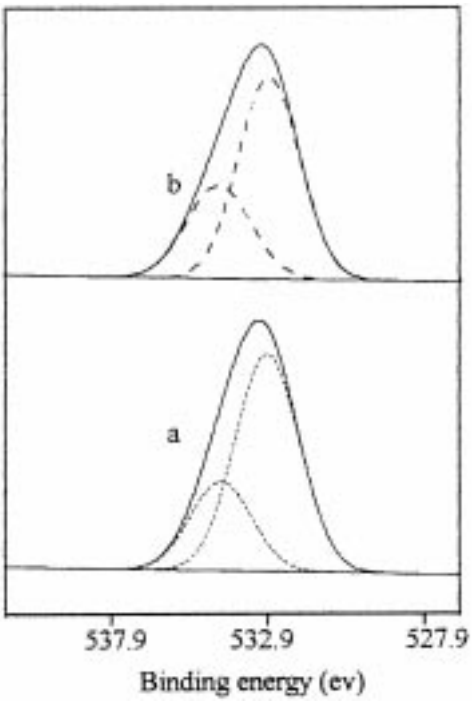

Figure 1 O1s spectra of PU and photo-oxidized PU.

The membrane surfaces thus obtained showed poor cytocompatibility. The reasons lied in two ways, i.e. the length of grafted chains was long and the hydrophilicity was high which did not favor for cell adhesion; the morphology of the grafted membrane effect the cell adhesion. In order to control the length of the grafted chains and subsequently the morphology of the grafted membrane, another method called ''pre-absorbing'' was used. The photo-oxidized membrane was immersed firstly in monomer solution with concentration of 1, 5 and 10 wt % for one hour and then irradiated under UV light. The occurrence of graft copolymerization could be verified by the surface analysis results.

Surface chemical structure of the membranes was analyzed by XPS spectra. By comparison the difference of membranes before and after grafting, it could confirm the occurrence of graft copolymerization. Fig. 3 showed

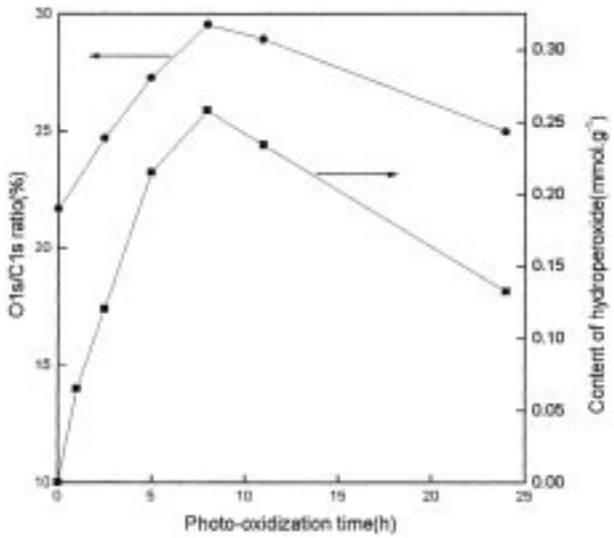

Figure 2 Effect of photo-oxidization time on the O1s/C1s ratio (emission angle 60°) and content of hydroperoxide on the photo-oxidized membrane surface.

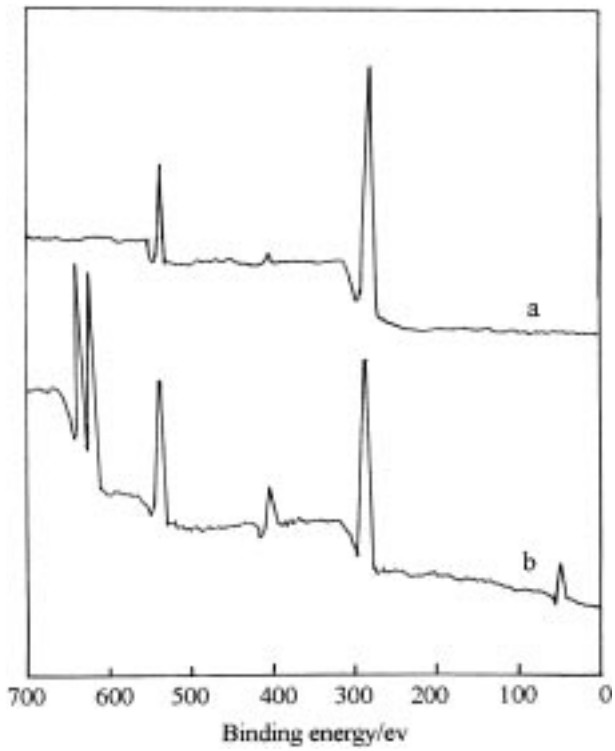

Figure 3 XPS spectra of PU (a) and PU-g-PDMAEM (b) treated with iodomethane.

the XPS spectra of iodomethane treated control and grafted membranes. As the tert-amine could be quaternized by iodomethane while the amide groups could not, it was reliable to characterize the membrane by this method. It was seen from Fig. 3 that the grafted membrane showed the characteristic peaks of iodine at 631.0, 619.5 and 51.0 eV, which attributed to I3d3, I3d5 and I4d, respectively [21]. However, the control membrane did not show these three peaks. This indicated that there were amino groups on the grafted surface. As comparing the C1s spectra of PU and PU-g-PDMAEM, it was found that there appeared an extra peak at 286.0 eV (Fig. 4), which designated to C–N bonds coming from PDMAEM grafted [22]. Correspondingly, the intensity of carbon in ester group at 289.1 eV increased and the intensity of carbon in urethane groups decreased. From above results, it could be concluded that graft copolymerization was occurred.

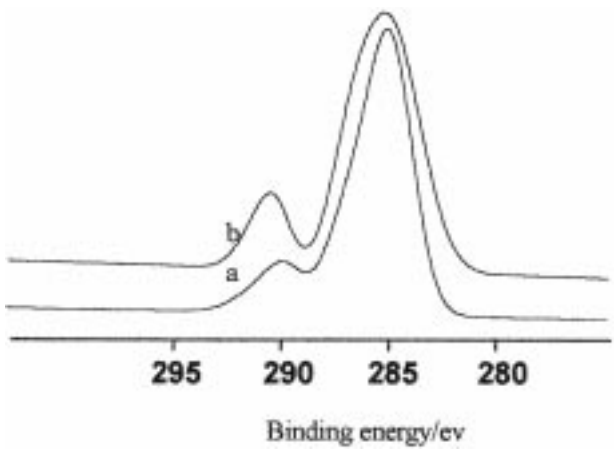

Figure 4 C1s spectra for PU (a) and PU-g-PDMAEM (b).

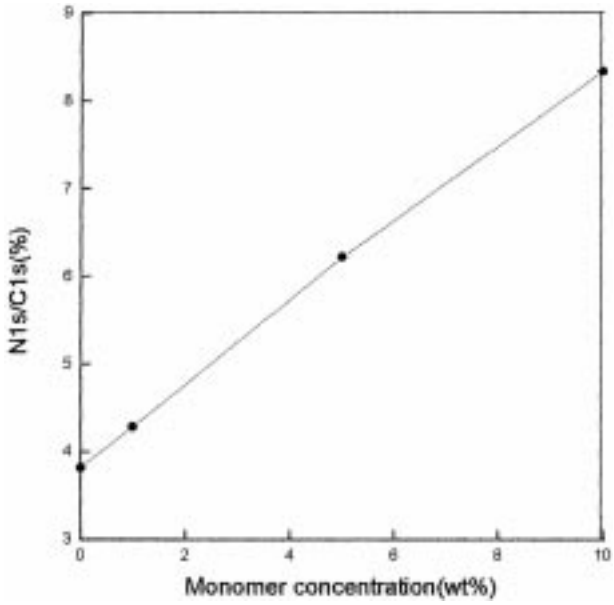

Figure 5 Effect of monomer concentration on the N1s/C1s ratio.

The surface composition of membranes was characterized by N1s/C1s ratio. The results were shown in Fig. 5. After grafted with DMAEM, the N1s/C1s ratio increased, and increased with the concentration of the monomer solution. As the increase of the concentration of monomer solution, the amount of DMAEM molecules absorbed onto the membrane surface increased and the amount of DMAEM grafted increased which resulted in the increase of nitrogen content.

Graft polymerization of DMAEM on the PU surface altered the PU surface from much hydrophobic to hydrophilic. Fig. 6 showed that the static water contact angle decreased after grafted, which also confirmed the occurrence of the graft copolymerization. As the nitrogen content increased, the contact angle decreased and the surface hydrophilicity increased further.

Surface morphology of the control and grafted membranes were showed in Fig. 7. The control membrane (Fig. 7(a)) showed minimal surface morphology and had some flaws on the surface, presumably derived from membrane processing. When the grafting was processed by pre-absorbing method, the grafted membranes showed minimal morphology, there were no obvious differences between the control membrane and grafted membrane (Fig. 7(b–d)). In our previous paper, we have found that the graft copolymerization conducted in solution yielded surface morphology even when the degree of grafting was low [11]. The differences in morphology between the membranes grafted with the same monomer by two methods implied that the length of grafted chains were different. When the grafting was proceeded in monomer solution, the monomer diffused onto the membrane surface continuously and the grafted chains propagated and lengthened because the rate of propagating reaction was larger than that of initiating reaction once the initiating reaction occurred. In current experiment, the diffusion ability of monomer absorbed onto the membrane surface in pre-absorbing grafting was lessened and the chain propagating reaction was restricted which produced the chain with almost the same length. Moreover, the graft chain was short because only the monomer near the active sites could be grafted.

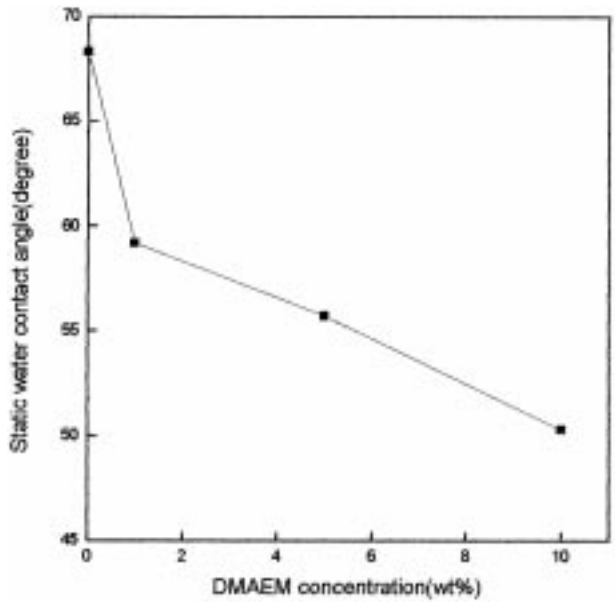

Figure 6 Effect of N1s/C1s ratio on the static water contact angle.

Attachment characteristics of Human Umbilical Vein Endothelial (HUVE) cells to the grafted surface were studied, and the results were shown in Fig. 8. The values for attachment were reported as a proportion of the number of cells that attached to TCPS in the same culture media. The comparison of PU and DMAEM grafted membranes showed that cell attachment is better on the grafted membranes. It suggested that cell attachment was promoted by the presence of amino groups. As the tert-amine groups could be quaternized by the hydrogen ions in the culture media, which resulted in the surface was positively-charged, the increase of cell attachment indicated that a strong electrostatic interaction between the negatively charged cell membrane and the positively-charged surface occurred. The adhesion of cells onto the surfaces may be ascribed to interactions between cell surface and proteins adsorbed onto the polymeric surface, because cell culture studies were carried out in the presence of fetal calf serum [23]. Fig. 8 also showed that as the N1s/C1s ratio was higher than 8.4%, the cell attachment was decreased. The reason was that as the nitrogen content on the surface was increased, the content of positive charge on the surface was increased, which resulted in the increase of the surface hydrophilicity, and the highly hydrophilic surface did not favor for cell adhesion. So the amino groups on the surface effected the cell adhesion in two parts, i.e. in suitable range, it could make the surface positively-charged and improve the surface hydrophilicity, which result in the increase of cell attachment; when the content of amino was much higher, the effect of hydrophilicity of the surface on cell attachment was higher than that of positive charge, which resulted in the decrease of cell attachment.

Fig. 9 showed the growth curves of HUVE cells on various surfaces over a period of 4 days. It was found that cells grew faster on the grafted membrane than that on the PU, and the cell growth rate was relative to the N1s/C1s ratio. The highest cell density was obtained on the

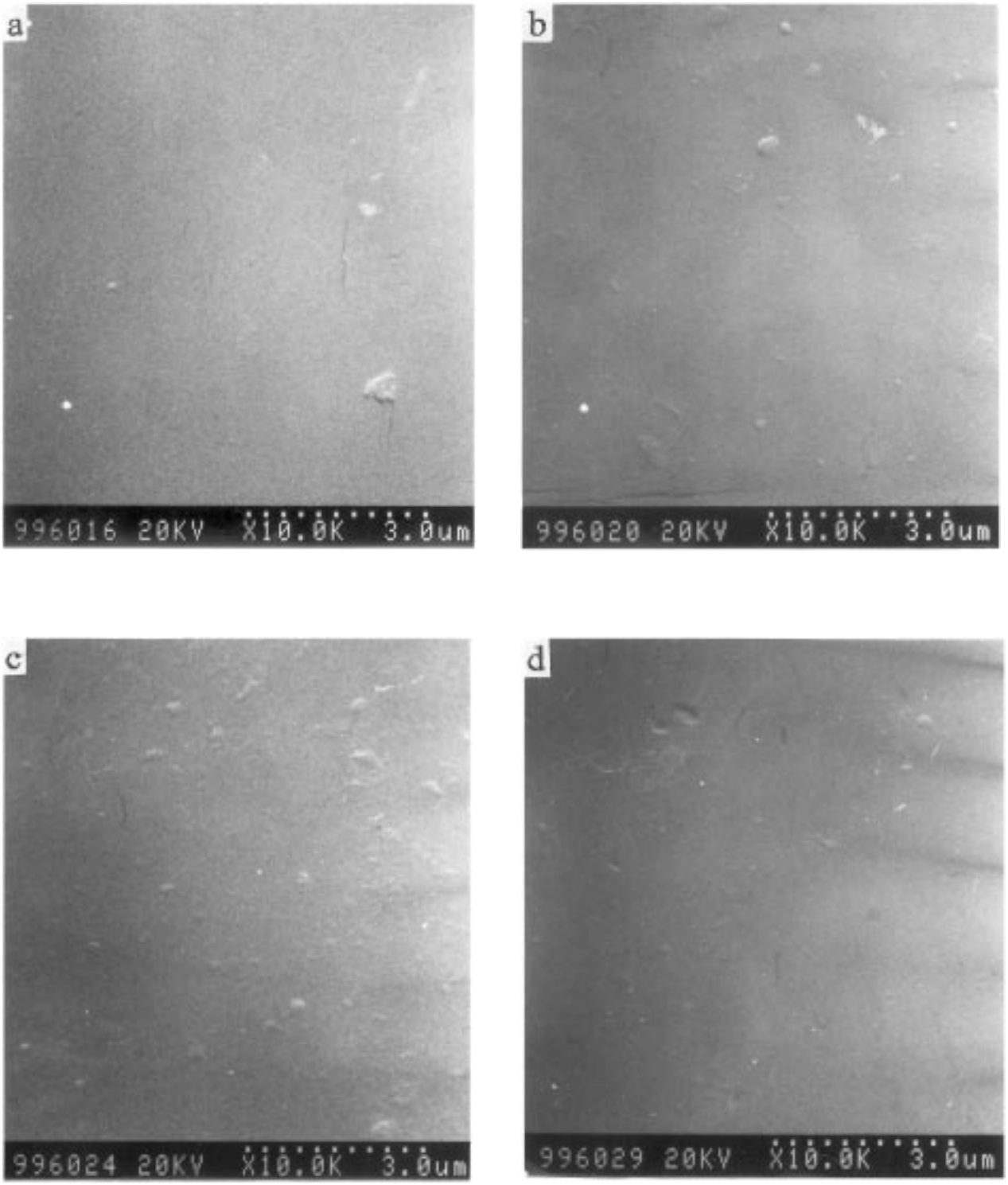

Figure 7 SEM photos of PU (a), PU-g-PDMAEM with the N1s/C1s ratio 4.4 (b), 6.2 (c) and 8.4% (d) respectively.

moderate N1s/C1s ratio surface. It implied that the cells were preferred to attach and grow on surface with moderate content of charge.

From the results of this study, it could be concluded that the surface modification by combining use of photo-oxidization of UV irradiation grafting of DMAEM onto PU substrata provided a good support for colonization by HUVE cells. The surface modification by this method appeared to be a promising strategy for the efficient endothelialization of vascular grafts.

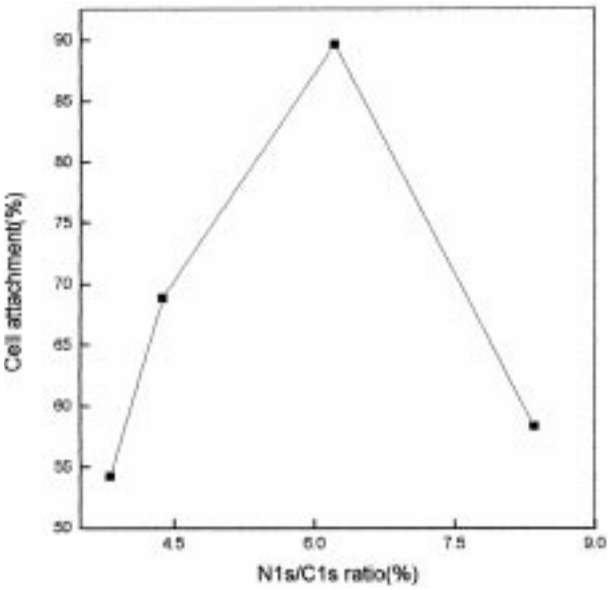

Figure 8 Effect of N1s/C1s ratio on the HUVE cell attachment (relative to TCPS).

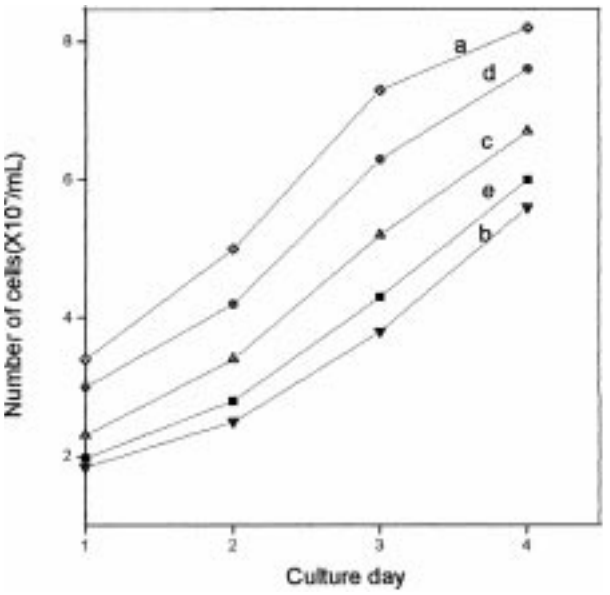

Figure 9 Relative HUVE cell growth rate seeded on TCPS (a), PU (b), PU-g-PDMAEM with the N1s/C1s ratio 4.4 (c), 6.2 (d) and 8.4% (e) respectively.

Acknowledgments

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