Promoting the cytocompatibility of polyurethane scaffolds via surface photo-grafting polymerization of acrylamide

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Polyurethane (PU) porous scaffolds were modified by grafting polymerization of acrylamide (AAm) initiated under UV light. A pre-adsorbing-monomer method was used beforehand. FTIR-ATR spectroscopy and X-ray photoelectron spectroscopy (XPS) measurements confirmed the presence of grafted PAAm on PU scaffolds. The measurement of water adsorption demonstrated the improvement of hydrophilicity after PU scaffolds were grafted with polyacrylamide (PAAm). The PAAm grafting degree related to the amount of AAm adsorbed, average pore diameter, and the degree of porosity. This study shows that higher degree of porosity and bigger porous areas yielded larger amounts of absorbed AAm and higher grafting degrees. *In vitro* human endothelial cell cultures of PU scaffolds modified with hydrophilic PAAm showed better cytocompatibility than the control matrix.

1. Introduction

Polyurethane (PU) plane and porous scaffolds have been widely used as biomaterials due to its excellent physical property and blood compatibility. Hemocompatibility and resistance to biodegradation has led to application of PU materials in blood-contacting devices, such as in catheters, chambers for artificial heart and pacemaker etc. [1–3]. Porous scaffolds mimic the body's own extracellular matrix on which cells can attach, multiply, migrate and function. These implanted substitutes become encapsulated by surrounding tissues to form an intima after long-term application. A surface that can accelerate this process and promote the formation of an endothelial layer in short time leads to better hemocompatibility. However, the inert property of PU results in a weak cell–material interaction that is unfavorable for endothelium formation. This study focuses on surface modification of PU properties to improve cytocompatibility.

Modifications are made on the material surface because the cell–material interaction is mostly modulated by the superlayer of a material, whilst the bulk properties such as mechanical strength are retained. Several methods such as plasma treatment, ozone or photo-induced grafting and surface oxidation have been employed to introduce hydrophilic compounds onto polymeric scaffold surfaces [4–8]. Photo-induced surface grafting was used in this study because it is the least invasive. Hydrophilic macromolecules polymerized *in situ* from 2-dimethylaminoethyl methacrylate (DMA), 2-hydroxyl ethyl acrylate (HEA) and 2-hydroxylethyl methacrylate (HEMA) etc. have been grafted on PU and poly(L-lactic acid)(PLLA) nonporous membranes under UV irradiation previously [9–13]. The introduction of the above-mentioned functional groups generates more favorable interaction with endothelial cells and chondrocytes, and increases the surface hydrophilicity.

This paper reports the immobilization of hydrophilic polyacrylamide (PAAm) onto PU plane and porous scaffolds through photo-oxidation and UV irradiation polymerization of AAm. The cytocompatibility of these modified PU plane or porous scaffolds was assessed using human endothelial cells *in vitro*. The results showed that the surface modification method, i.e. grafting polymerization of hydrophilic AAm on PU, is effective to improve the cytocomopatibility.

2. Materials and methods

2.1. Preparation of PU plane or porous membrane

PU (Estane 58271, BF Goodrich Co.) plane membrane was prepared by casting a 12% PU/N,N-dimethylformamide (DMF) solution onto a stainless plate. The solvent was evaporated at 50 °C for 24 h, and further dried under vacuum for another 24 h at 30 °C. This yielded a transparent PU membrane with a thickness of approximately 300 μm. The membrane was cut into 1.5 × 3 cm strips for photo-oxidation and grafting polymerization.

Porous PU membrane was fabricated via thermally

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induced phase separation (TIPS) technique described previously [14]. The purified PU was dissolved at 50 °C in 1,4-dioxane in a glass container bearing a stainless steel membrane-forming platform as the cover. A homogeneous solution (0.1 g/ml) was formed and then degassed and inverted upside-down to form a solution layer on the stainless steel platform. The mold was put onto a brass cold plate that had been cooled to the desired temperature by liquid nitrogen. When the membrane was totally solidified the glass mold was removed and the stainless steel platform with the PU membrane was transferred into a desiccator to remove 1,4-dioxane by freeze-drying. The membranes were cut into round pieces with a diameter of 6 mm (slightly smaller than that of the 96-well culture plate (= 6.4 mm)) to form specimens for culture. Membranes were also cut into 15 × 15 mm pieces, for surface modification studies.

2.2. Photo-oxidation and pre-adsorbing grafting polymerization

PU membrane was immersed and stirred in 40 ml hydrogen peroxide solution (30%) and irradiated by UV light generated from a high-pressure mercury lamp (250 W) at 35 °C for 8 h. The photo-oxidized membrane was rinsed with water to remove the excess hydrogen peroxide and dried. Grafting polymerization was employed by the pre-adsorbing-monomer (i.e. AAm) method [13]. The photo-oxidized membrane was immersed in AAm aqueous solution with concentration of 1%, 5%, or 10% for 1 h, 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 was then replaced with nitrogen. Grafting polymerization was carried out under UV irradiation at a distance of 12.5 cm for 1 h at 35 °C. The grafted membrane was rinsed with hot water (70 °C) for at least 48 h to remove homopolymers [9–12], and subsequently dried under reduced pressure at 50 °C.

2.3. Human umbilical vein endothelial cell (HUVEC) culture

The human umbilical vein endothelial cell (HUVECs) were isolated from human umbilical veins with 1.0 mg/ml collagenase (type I, Sigma)/phosphate buffer solution (PBS, pH = 7.4) for 20–25 min at room temperature [15, 16]. The isolated HUVECs were seeded in beds prelaid with control or modified PU membranes as well as in control beds of tissue culture polystyrene (TCPS) (Nunc™, Denmark). The HUVECs were incubated in a culture medium consisting of 20% (v/v) calf serum (Sijiqin Biotech. Co., China) and 80% RPMI1640 (Gibcobrl Co.), supplemented with 100 unit/ml of penicillin and 100 μg/ml of streptomycin. Incubation took place in a humidified air of 5% CO_2 at 37 °C. The culture medium was changed at 24 h, and then changed every 48 h. The cell attachment and proliferation ratios were averaged from 5 parallel measurements at 12 h and 96 h, respectively, by trypsinization of the HUVECs and counting the cell number under a hemocytometer. The cell proliferation ratio was defined as $(N_2 - N_1)/N_1$, where N_1 and N_2 represented the cell number per well at 12 h and 96 h, respectively. The cell activity was measured using methylthiazoletetrazolium (MTT) method [17, 18] and defined as the absorbance percentage to TCPS at a wavelength of 492 nm. The morphology of the cultured HUVECs was observed under an inverted microscope (LOIC) after the cells were fixed with 2.5% glutaraldehyde for 30 min and stained with Giemsa for 10 min.

2.4. Characterizations

The morphology of PU porous scaffolds was observed by a HITACHI S-570 scanning electron microscope (SEM). The pore diameter was measured and averaged from the SEM images. The porous membrane (W_1) was immersed in ethanol for 24 h to measure the porosity. After the ethanol was removed on the surface by filter paper, the weight (W_2) was immediately measured. The porosity was calculated by the following equation, where ρ_1 and ρ_2 represent the density of PU (1.18 g/cm^3) and ethanol, respectively.

$$\text{Porosity} = (W_2 - W_1)\rho_1/[\rho_2 W_1 + \rho_1(W_2 - W_1)] \times 100\% \quad (1)$$

Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectrum was recorded on a Nicolet Magna-IR560 infrared spectrometer. X-ray photoelectron spectroscopy (XPS) spectrum was recorded on an ESCA LAB Mark II spectrometer employing Al k_α excitation radiation. The charging shift was referred to the C_{1s} line emitted from saturated hydrocarbons. Water contact angle was measured at room temperature on a JY-82 apparatus using sessile drop method. The water adsorption ratio (WAR) and the grafting degree (GD) were defined as follows, where W_1 is PU membrane weight, W_1' is PU porous scaffold weight, W_3 is the weight of the PU plane membrane soaked in water for at least 24 h at 37 °C, W_4 is the weight of PAAm grafted membrane, and W_4' is the weight of PAAm grafted porous scaffold.

$$\text{WAR} = (W_3 - W_1)/W_1 \times 100\% \quad (2)$$

For plane membrane,

$$GA = (W_4 - W_1)/(\text{surface area}) \quad (3)$$

For porous scaffold,

$$GA = (W_4' - W_1')/W_1' \times 100\% \quad (4)$$

3. Results and discussion

3.1. Surface modification of PU plane membrane with AAm

Cells differentiate and proliferate *in vitro* if they can adhere on a substratum with proper surface properties like wettability and charge. Previous research has proven that modified PU membranes fabricated from solution grafting, whether they contained hydroxyl, amino or amide groups, were disadvantageous for HUVECs growth. No HUVECs were observed on the membranes

TABLE I Effect of AAm concentration on the adsorbed amount of AAm* and the grafting degree

AAm concentration (wt %)	1	5	10
Adsorbed amount of AAm (10^{-2} mg/cm^2)	4.17	9.51	15.32
Grafting degree (10^{-2} mg/cm^2)	2.75	5.68	8.47

*Adsorbing time 60 min.

after 4 d of culture [10, 13]. The cause of this is attributed to the higher grafting degree that leads to the resistance of cell anchorage. To reduce the surface concentration of hydrophilic groups to a proper degree, an alteration of the grafting strategy is necessary. It is well known that a solid material tends to adsorb those substances that can decrease the surface free energy. According to this mechanism, the PU membrane was immersed into hydrophilic AAm aqueous solution for a period to make it adsorb automatically onto the PU surface. By controlling the AAm concentration, the adsorbed amount of AAm can be modulated, hence the grafting degree can be controlled (Table I). The adsorbed amount of AAm increased with the increase of the AAm concentration from 1% to 10%, while the grafting degree also increased from 2.75 to 8.47×10^{-2} mg/cm^2. More adsorbed AAm on PU membrane produced more grafting sites and higher molecular weight chains of PAAm to be reached. Therefore, a higher grafting degree was obtained. These results were confirmed also by XPS measurement. Both control PU and PU-g-PAAm displayed the absorbance of C_{1s} at 285.0 eV and N_{1s} at 400.3 eV, respectively. But the N_{1s} peak intensity of PU-g-PAAm was stronger than that of control PU (spectra were not shown). The peak area ratio of N_{1s}/C_{1s} was 3.05% for the control PU when the take-off angle was 60°. After the PAAm was grafted, the ratio increased to 3.23% when the grafting degree is 2.75×10^{-2} mg/cm^2. It increased further with increasing grafting degree (Table II). This is reasonable because the theoretical ratio (N_{1s}/C_{1s}) (i.e. the atom ratio of N and C in AAm molecular formula) of PAAm is 1/3. The nitrogen composition increased with more grafted PAAm. Table II also shows the N_{1s}/C_{1s} ratio at different take-off angle as a function of grafting degree. The nitrogen composition increased when the take-off angle decreased. A maximum was reached when the take-off angle tended to 0°, although it was dependent on the grafting degree. This result suggests that the grafted PAAm is mainly located on the top-most layer.

After the PAAm was grafted, the hydrophilicity of the PU surface was improved. Fig. 1 shows that the water contact angle decreased from 68.3 to 48.5° when the PAAm grafting degree increased from 0 to 8.47×10^{-2}mg/cm^2. This again verified the occurrence of PAAm grafting.

3.2. Modification of PU porous scaffold with AAm

Porous PU membranes were prepared via (TIPS) technique. The surface morphology, pore size and porosity of the porous PU scaffolds were influenced by polymer concentration, coarsening time, quenching temperature, temperature of the coldplate and composition of solvents [19]. Table III shows three different PU porous scaffolds generated by three different quenching temperatures (designated as P10, P20 and P30, respectively). The porosity and average pore diameter decreased with decreasing quench temperature. Fig. 2 presents the surface morphology of the porous PU membranes observed under SEM.

The porous PU scaffolds were modified by AAm grafting polymerization with a pre-adsorbing-monomer method. The grafting degree on PU scaffolds was related to the adsorbed amount of AAm. Table III shows that the adsorbed amount of AAm decreased from P10 to P30 under the same resting time, whilst the grafting degree decreased from 0.48% to 0.20%. It is understandable that the scaffolds with larger pore size have greater areas exposure to AAm. Therefore, the higher amounts of AAm will be adsorbed. It is worth noting that the existence of the hydroperoxide groups on the photo-oxidized PU membranes has been previously proven by the iodomethry titration method [10].

The existence of the grafted PAAm on PU scaffold has been verified by ATR-FTIR analysis (Fig. 3). Both

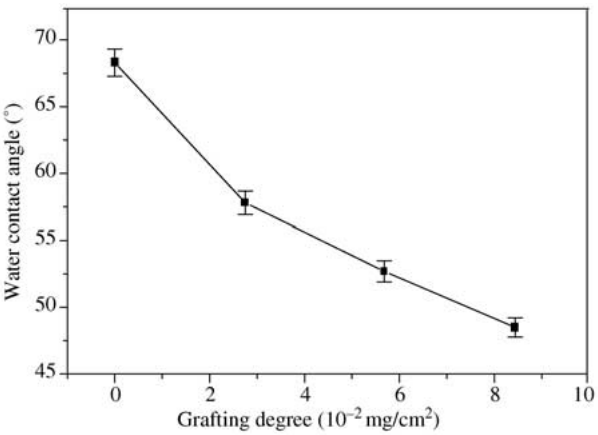

Figure 1 Effect of grafting degree on the static water contact angle.

TABLE II The N_{1s}/C_{1s} ratio at different take-off angles as a function of the grafting degree

Grafting degree (10^{-2}mg/cm^2)		2.75	5.68	8.47
N_{1s}/C_{1s} (%)	0°	4.88	4.94	5.13
	30°	3.82	4.09	4.52
	60°	3.23	3.47	3.65

TABLE III PU scaffold characteristics as a function of quenching temperature and their influence on grafting degree, adsorbed amount of AAm and N_{1s}/C_{1s} ratio

Quenching temperature (°C)	− 10 (P10)	− 20 (P20)	− 30 (P30)
Average pore diameter (μm)	21.0	12.0	7.2
Porosity (%)	79.5	69.7	62.6
Adsorbed amount of AAm (%)	1.08	0.92	0.75
Grafting degree (%)	0.48	0.31	0.20
N_{1s}/C_{1s} ratio (%)*	4.12 (2.37)	4.65 (2.76)	6.75 (3.10)

*The data in parentheses represent the N_{1s}/C_{1s} ratio before grafting. Take-off angle 60°.

spectra (a) and (b) show similar absorbance bands. However, the absorbance of -$CONH_2$- group at 3325 and 1650 cm^{-1}, which attributed to PAAm, is clearly displayed in the spectrum of Fig. 3(c) showing the difference of spectra (a) and (b). The XPS measurement also confirmed the occurrence of the PAAm grafting on PU porous scaffold (Table III). The N_{1s}/C_{1s} ratio of PAAm grafted PU scaffold (P10, P20, P30) increased compared with the control matrix (Table III, the data in parentheses). However, it also increased slightly with the decrease of the grafting degree. This inconsistency between the N_{1s}/C_{1s} ratio and the grafting degree was attributed to the different aspects being revealed. The grafting degree (or the adsorbed amount of AAm) reflects a bulk property of the entire scaffold rather than that of the superficial layer, which is proportional to the inner face area of the scaffold. Because higher porosity corresponds to higher inner face area, larger grafting degree should be obtained. On the other hand, XPS can only detect the elemental composition on the superficial layer of the scaffold. A scaffold with smaller pore size on surface, which generally corresponds to larger occupation of the material, shall have higher percentage of N_{1s}/C_{1s} ratio.

The water adsorption ratio of PU scaffolds either before or after grafted also demonstrated the similar alteration tendency to the pore diameter and porosity. However, after PAAm grafting it slightly increased compared with the control matrix (P10, P20, P30) (Fig. 4).

3.3. The cytocompatibility of the modified PU plane or porous scaffolds

The introduction of the hydrophilic groups onto the PU membrane surfaces after PAAm grafting provides the possibility of cytocompatibility improvement. The endothelial cell culture results showed that the cytocompatibility of the modified PU membrane was related to the ratio of N_{1s}/C_{1s} (Figs. 5 and 6). The cell attachment and proliferation ratio increased with the increase of PAAm amount grafted on PU membrane within the range we studied. The cell activity also increased with the increase of PAAm amount grafted (Fig. 6(a)). The activity per cell reached the maximum value at N_{1s}/C_{1s} ratio of 3.23%, and then decreased slightly. This fluctuation may be caused by impingement among the large numbers of cells on PU-g-PAAm membrane with N_{1s}/C_{1s} ratio of 3.47% or 3.95%, which could limit further growth. The cell morphology was observed under an inverted microscope after stained with Giemsa and fixed with glutaraldehyde. Fig. 7 showed the cobble shape of endothelial cells, and presented an endothelial layer on the modified PU membrane.

The 12 h cell attachment ratio and the 96 h proliferation ratio cultured for 4 d increased with the decrease of the average pore diameter (Figs. 8 and 9). This occurred because the smaller pores facilitated HUVECs to adhere onto the PU substratum. Since HUVECs grow only when they adhere and spread well on the substratum surface, the cell proliferation ratio increased when the average pore diameter decreased. Cell attachment and prolifera-

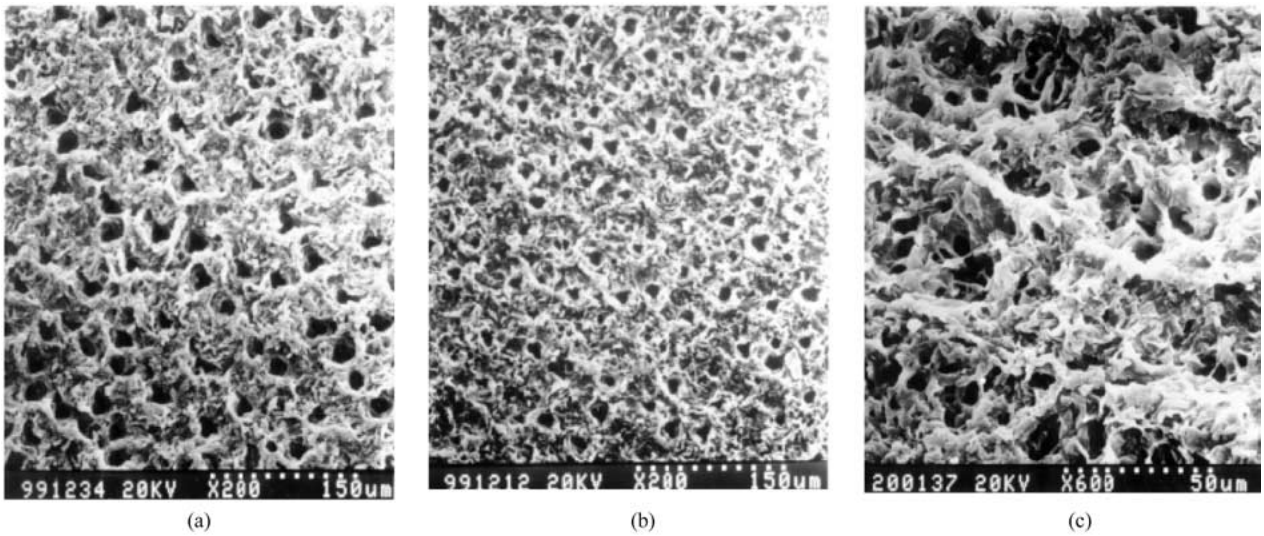

Figure 2 SEM images of porous PU membranes obtained at different quenching temperature. (a) − 10 °C (P10), (b) − 20 °C (P20), (c) − 30 °C (P20). Polymer solution concentration was 0.1 g/ml.

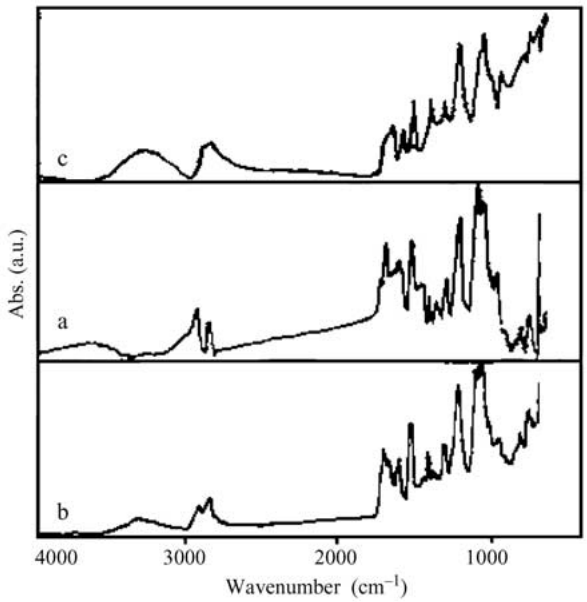

Figure 3 ATR-FTIR spectra of the porous PU (P10) before (a) and after grafted with AAm (P10-g-PAAm), (b), and their subtraction result (c).

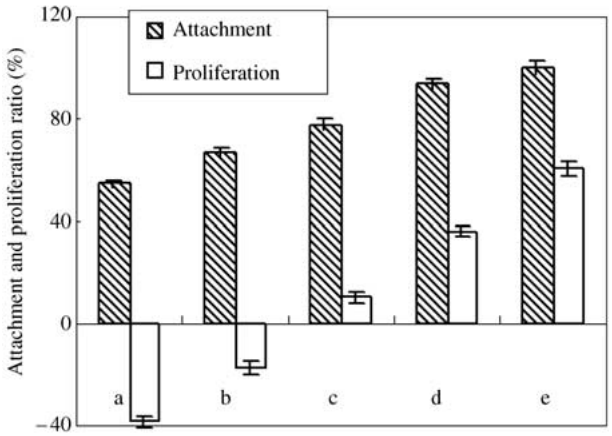

Figure 5 The cell attachment and proliferation ratio on PU and PU-g-PAAm as a function of N_{1s}/C_{1s} ratio (take-off angle 60°). (a) control PU, (b) PU-g-PAAm with N_{1s}/C_{1s} ratio 3.23%, (c) 3.47%, (d) 3.94% and (e) TCPS, respectively. Cell seeding density $1.0 \times 10^5/\text{cm}^2$.

tion increased after grafting PAAm onto the scaffolds. It is interesting that the smaller the pore is, the bigger the increase of attachment and proliferation has, although the PAAm grafting degree decreased with the decrease of pore size. It has been reported elsewhere that the physical pattern besides the chemical structure had an influence on cell growth [20]. Previous experiments have shown that HUVECs exhibit better cytocompatibility on PU surfaces with a nano-scale roughness than on plane or micro-scale pores [21]. In conclusion, porous PU scaffolds with improved cytocompatibility to endothelial cells can be fabricated through grafting of PAAm.

4. Conclusion

PAAm was successfully grafted onto PU plane and porous scaffolds through pre-adsorbing-monomer and grafting polymerization methods. The wettability of both plane and porous PU membrane was improved. The grafting degree was related to the amount of AAm adsorbed, and both were proportional to the porosity. Endothelial cell culture showed that the PU membrane modified with hydrophilic PAAm had better cytocompatibility. For PU porous scaffolds, both the cell attachment and the proliferation were related to the average pore diameter and the porosity. Cytocompatible PU scaffolds that effectively support endothelial cell anchorage and proliferation have been fabricated through AAm pre-adsorbing method followed by photo-grafting polymerization.

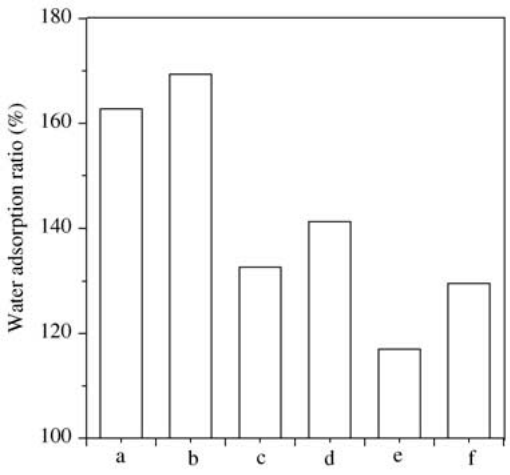

Figure 4 Water adsorption ratio of the porous PU before and after grafted with PAAm. (a) P10, (b) P10-g-PAAm, (c) P20, (d) P20-g-PAAm, (e) P30, and (f) P30-g-PAAm. Porous PU was grafted for 1 h after it was photo-oxidized for 8 h at 35 °C and pre-adsorbed for 1 h in an AAm solution with concentration of 5 wt %.

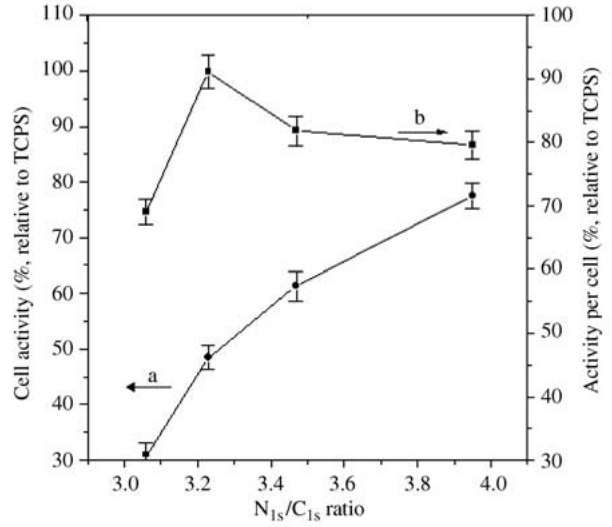

Figure 6 The cell activity (a) and the activity per cell (b) (%, relative to TCPS) as a function of N_{1s}/C_{1s} ratio. Cell seeding density $1.0 \times 10^5/\text{cm}^2$. Cell cultured for 4 d.

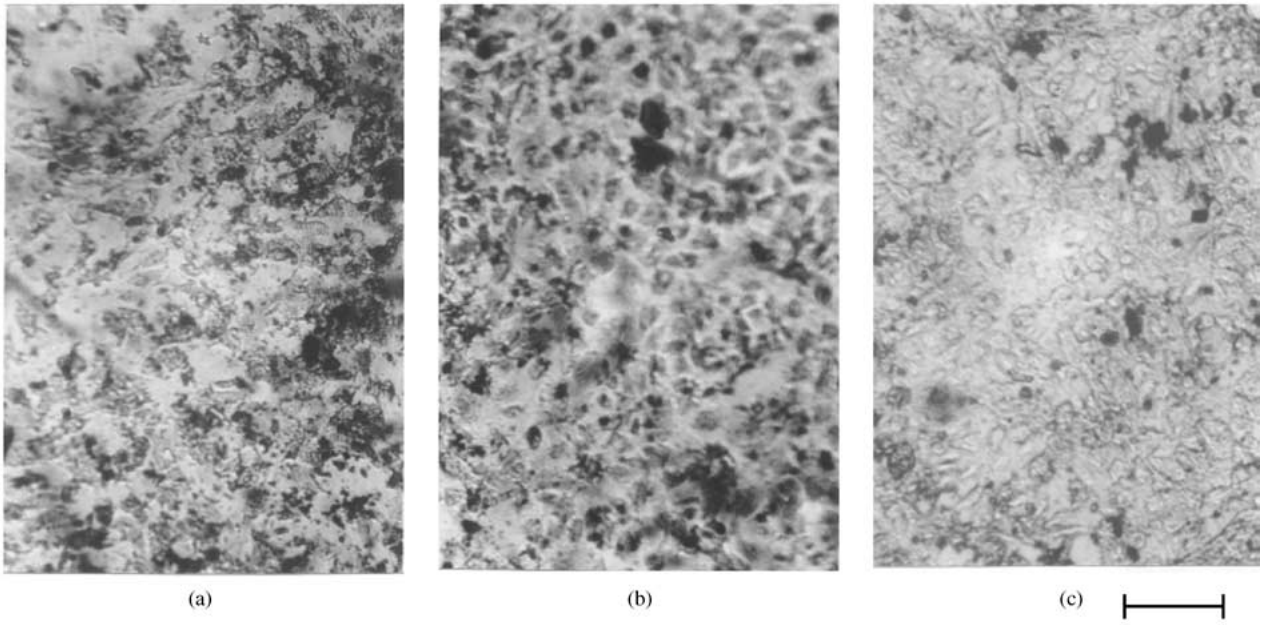

Figure 7 Morphologies of HUVECs seeded on PU-g-PAAm with N_{1s}/C_{1s} ratio 3.23% (a), 3.47% (b) and 3.94% (c), respectively. Cell seeding density $1.0 \times 10^5/cm^2$. Cell cultured for 4 d. Scale bar 200 μm.

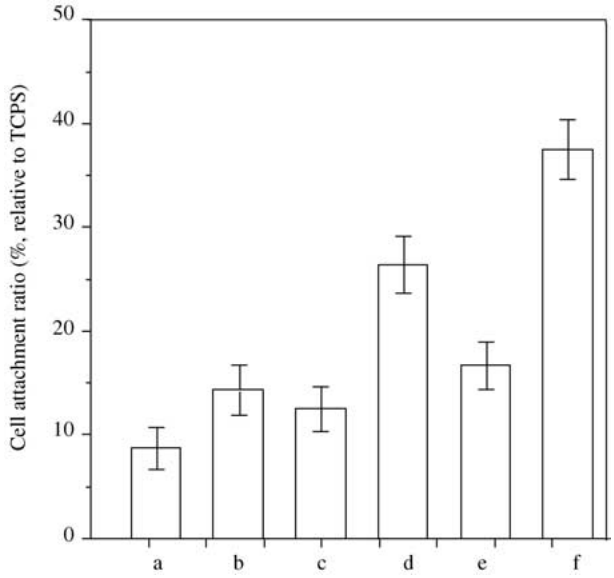

Figure 8 HUVECs attachment ratio after cultured for 12 h on (a) P10, (b) P10-g-PAAm, (c) P20, (d) P20-g-PAAm, (e) P30, (f) P30-g-PAAm. Porous PU was grafted for 1 h after it was photo-oxidized for 8 h at 35 °C and pre-adsorbed for 1 h in AAm solution with a concentration of 5 wt %. Cell seeding density $2.0 \times 10^5/ml$.

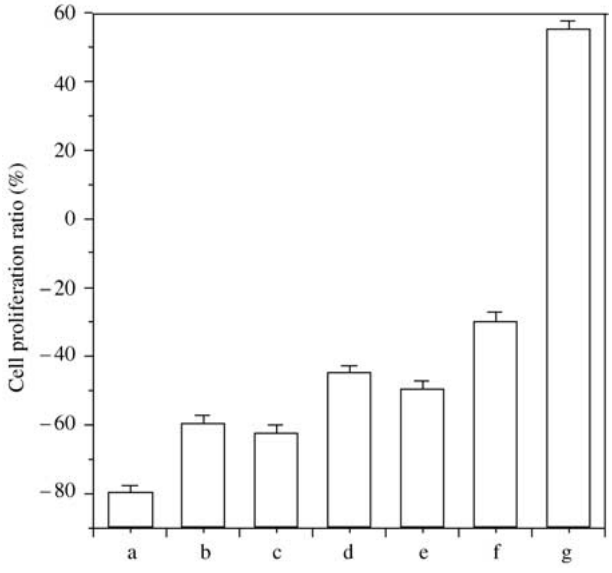

Figure 9 HUVECs proliferation ratio after cultured for 4 d on (a) P10, (b) P10-g-PAAm, (c) P20, (d) P20-g-PAAm, (e) P30, (f) P30-g-PAAm, and (g) TCPS. Porous PU was grafted for 1 h after it was photo-oxidized for 8 h at 35 °C and pre-adsorbed for 1 h in AAm solution with a concentration of 5 wt %. Cell seeding density $2.0 \times 10^5/ml$.

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