J Mater Sci: Mater Med (2008) 19:291–299
DOI 10.1007/s10856-006-0110-1

Surface tailoring of poly(ethylene terephthalate) via ligand-tethered comb-like PEG to enhance endothelialization

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Received: 23 June 2006 / Accepted: 11 December 2006 / Published online: 28 June 2007

Abstract The comb-like PEG (CPEG) end-tethered with L-lysine was explored to surface modification of PET to enhance endothelialization. The hydroxyl end groups of CPEG were oxygenated into aldehyde groups. The CPEG-CHO was grafted onto the aminolysized PET. The L-lysine was then end-tethered onto surface via the residual aldehyde groups. The surface modification was confirmed by ATR-FTIR, contact angle and XPS measurements. The endothelial cell adhesion, proliferation and viability results indicated that the PET-CPEG resisted cell adhesion and growth, where as PET-CPEG-lysine promoted cell adhesion and growth. The MTT assay and total cell protein tests indicated that the endothelial cells on PET-CPEG-lysine had high viability. Cell spread uniformly and covered completely on the PET-CPEG-lysine. The CPEG end tethered with L-lysine could regulate cell adhesion and growth and enhance surface endotheliazation.

Introduction

The endothelialization approach is believed to be an ideal method since it can solve the problems of incomplete cover of endothelial cells on the vascular graft surfaces and the subsequent myointimal hyperplasia [1]. Endothelialization not only improves the long term patency of synthetic vascular grafts but also controls of immunologic barriers to allograft application. The endothelialization depends on the adhesion of endothelial cells, retention of those endothelial cells under shear, and proliferation and migration of endothelial cells on the biomaterial surfaces [2].

The poly(ethylene glycol) with weak immunogenicity, antithrombus formation have been regarded as the most effective structures to reduce the non-specific adsorption of protein [3]. Furthermore, the hydroxyl end groups of PEG provide the possibility of end-tethered ligand to guide cell behavior. There are many reports about enhancing endothelization utilizing end-tethered linear PEG [2, 4]. Generally, it is believed that adsorption resistance of PEG generally increases with increasing grafting density and chain length. The multi-arms PEG can provide a much higher surface concentration than linear PEG in the same grafting density, thus multi-arms polymers may offer enhanced resistance to protein adsorption [5]. In addition, multi-arm PEG has more hydroxyl end groups than linear PEG. So, multi-arm PEG has more sites to be functionalized by ligands so as to obtain specific cell-substrate interactions. But up to now there are not still any reports about enhance endothelization with ligand-tethered multi-arm PEG, such comb-like PEG. So it is worth exploring surface endothelization with ligand-tethered comb-like PEG.

So here we reported a facile method of surface modification via the comb-like PEG (CPEG) end-tethered with L-lysine. The hydroxyl groups of the CPEG were firstly transformed into aldehyde groups. Then it was grafted onto aminolysized Poly(ethylene terephthalate) (PET). The residual aldehyde groups were tailored by lysine to mediate

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the cell adhesion. The endothelial cell, ECV304, was chosen to study the cell behavior on modified PET.

Experiment

Material and characterization

Poly(ethylene terephthalate) (PET) films (Melinexs[@], DuPont) with a thickness of 250 μm were supplied by Dupont Teijin Films[TM]. It was eluted by hexane for 24 h before used. Linear poly(ethylene glycol) (LPEG) (Mn = 4000 and 10,000) was purchased from Shanghai chemical reagent factory. The hydroxyl-capped comb-like poly[poly(ethylene glycol) methacrylate] (CPEG) (Fig. 1) was prepared via atom transfer radical polymerization(ATRP), which described in detail previously [6, 7]. The numerical average molecular weight and polydispersity of CPEG were Mn = 13,500, Mw/Mn = 1.21 determined by gel Permeation chromatography (GPC). Lysine was purchased from Shanghai biochemical reagent factory. Dimethyl sulfone (Shanghai chemical reagent Ltd. Co.) was distilled under vacuum. Poly(ethylenimine) (PEI) (Mn = 25,000) was purchased from Aldrich. Sodium cyanoborohydride ($NaCNBH_3$) was purchased from Fluka. Platelet-rich plasma (PRP) was purchased from Blood Center of Zhejiang Province. Phosphate buffered saline (PBS, pH = 7.4) was prepared in our laboratory, containing 8 mg/mL NaCl, 2.9 mg/mL $Na_2HPO_2 \cdot 2H_2O$, 0.2 mg/mL KH_2PO_4 and 0.2 mg/mL KCl.

X-ray photoelectron spectra (XPS) were recorded with a Perkin-Elmer PHI-550 with Mg K_α excitation (15 kV, 400 W). Spectra were obtained at the take-off angles, 35° (between the plane of the surface and the entrance lens of the detector optics). Individual XPS atomic composition is determined from peak areas and Schofield section factors of individual elements; all data lie within ±5% of the reported values (averages). Surface topological morphology was measured by atomic force microscopy (AFM) (SPA 400, Seiko Instrument Inc.). Contact angle measurement was done in DSA10-MK2(Kruss, Germany) under static mode. The probe fluid used was double distilled deionized water.

Synthesis of aldehyde-PEG

Hydroxyl groups in CPEG and LPEG were oxidized using the Moffatt procedure essentially as described by Zalipsky [8]. Acetic anhydride (0.3 mL, 3 mmol) was added to dry DMSO (1 mL). In a separating stopper flask, a certain linear PEG or comb-like PEG(0.2 mmol hydroxyl groups) and DMSO (1 mL) were added to the acetic anhydride/DMSO solution. After the solution was stirred at ambient temperature for 24 h, further portions of acetic anhydride (0.3 mL, 3 mmol) and triethylamine (0.5 mL) were added, and the solution was stirred at room temperature for a further 24 h. Addition of ethyl ether (5 mL) and ethyl acetate (5 mL) and cooling the flask in the refrigerator resulted in the precipitation of the product. The product was collected by filtration and dried under vacuum.

PET grafted by PEG and conjugation of lysine to PEG

The aminolysis of PET by PEI aqueous solution(1 mg/mL, Mn = 25,000) was carried out at room temperature for 24 h as described previously [9]. The films were washed by water for three times and blew under N_2 stream. The grafting of PEG-CHO was performed in PBS. In brief, the PET-PEI films were immersed in closed vials containing sodium phosphate buffer solution with 1 mg/mL PEG-CHO concentration and 0.6 M K_2SO_4. The vials were kept in a thermostat bath at 60 °C for 24 h. An amount of lysine (5 mg/mL), 20 mM $NaCNBH_3$, in 0.1 M sodium phosphate buffer was added into the above solution. The reaction was kept for 24 h at ambient temperature. Finally, the

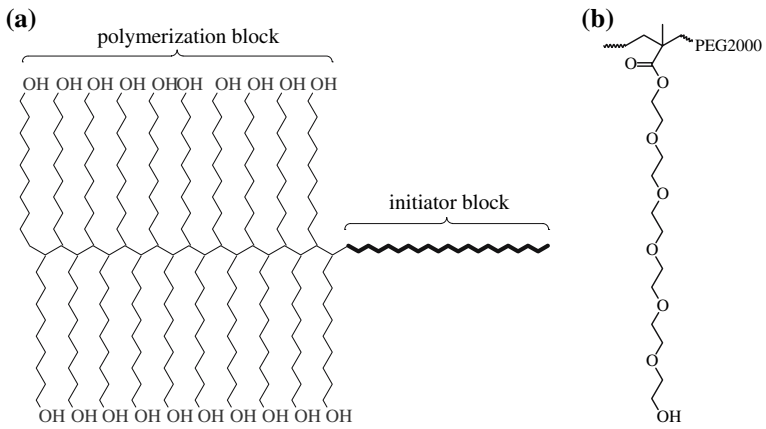

Fig. 1 The scheme of the comb-like PEG (CPEG), (**a**) structural scheme and (**b**) molecular scheme

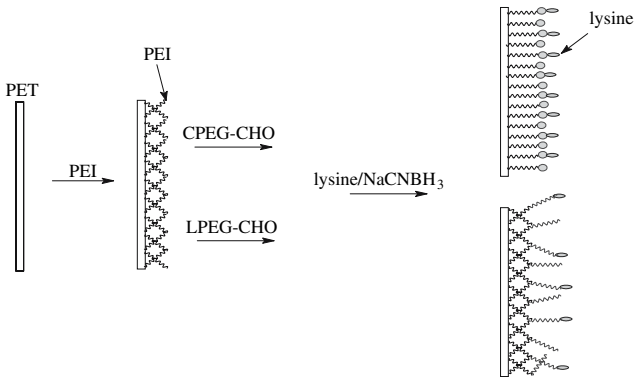

Fig. 2 The scheme of the total modification process of PET

films were rinsed by double deionized water for 3 times under the ultrasonic and dried for 24 h under vacuum. The total reaction scheme was shown in Fig. 2.

Platelet adhesion

The films were washed with PBS and incubated at 37 °C for 1 h with platelet-rich human plasma (PRHP). Samples were rinsed with PBS for three times, treated with 2.5% glutaraldehyde in PBS, and kept at 5 °C overnight. The sample was rinsed with saline and dehydrated by systematic immersion in a series of ethanol–water solutions: 60, 70, 80, 90, and 100% ethanol (v/v). After critical-point drying with carbon dioxide, the samples were coated with gold prior to being observed in an electron probe microanalyzer (Sirion-100, FEI) operated at an accelerating voltage of 20 kV.

Cell culture

The cells investigated in this study were human umbilical vein endothelial cell line ECV304, which were purchased from the China Center for Type Culture Collection (CCTCC, China). Ten million units of penicillin G sodium and 10 million μg of streptomycin sulfate (double antibiotic) were added into 500 mL of sterilized PBS to create dPBS. The cell culture medium was formulated by mixing the sterilized RPMI 1640 with fetal bovine serum that had been inactivated at 56 °C for 30 min. The content of the fetal bovine serum was 10%.

Cell adhesion and proliferation

The polymeric films were sterilized by immersion in 75% ethanol for 2 h, followed by equilibrated in PBS for 24 h. Then, the polymeric films were transferred to cover the bottom of the cell culture wells. The same batch of cells was used for the comparable culture with the cell density of 30,000 per cm^3. Besides all the polymeric samples, ECV304 were also seeded on the blank tissue culture polystyrene (TCPS) bottoms as the control. ECV304 were humidified and incubated in 5% CO_2 at 37 °C. After 24 h of incubation, the medium was discarded. The samples were washed three times with dPBS, by which the poorly adhered and/or suspending cells were removed. Then 100 μL 0.25% trypsin solution was added into each well. After a 15 min digestion, 100 μL of culture medium was added and the cells were re-suspended. The cells were counted with haemacytometer. Cell adhesion was expressed as a proportion of the number of cells adhered to TCPS in the same culture media. For cell proliferation studies, the sample preparation was the same to the adhesion assay except that the cells were incubated for 48 h. The cell proliferation was expressed by comparing with the number of original seeding cells. All data were gotten from three parallel samples from three separate experiments, and the error values are standard deviations. The difference not only between TCPS and other surfaces but also between PET-CPEG and PET-CPEG-lysine was evaluated by the paired *t*-test. The level of statistical significance is defined as $p < 0.05$.

Cell viability

Cell activity was determined by MTT assay [10] which is based on the mitochondrial conversion of the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H- tetrazolium bromide (MTT). Briefly, after the ECV304 were incubated on different samples for 72 h, 20 μL MTT solution (5 mg/mL in PBS) was added to each sample, and incubated at 37 °C for at least 4 h to form MTT formazan. The medium and MTT were replaced by DMSO solution; the samples were incubated at 37 °C for additional 5 min, to dissolve the MTT formazan, also gently shaken for 10 min to ensure the dissolution of formazan. The absorbance values were measured by using microplate reader (BIO-RAD, model 550) at wavelength 490 nm, blanked with DMSO solution. Cell viability was expressed as a proportion of the absorbance value of TCPS in the same culture media. All results represent mean ± SD of five replicates from three separate experiments. The difference not only between TCPS and other surfaces but also between PET-CPEG and PET-CPEG-lysine was evaluated by the paired *t*-test. The level of statistical significance is defined as $p < 0.05$.

Total cell protein

After 3 days when cells were seeded onto films in each experimental group, total cell protein content was assessed. Total cell protein content to a certain extent reflected the cellular activity, i.e. split and proliferation ability of cells.

Here measurement of total cell protein content was based on bicinchoninic acid (BCA) for the colorimetric detection and quantization [11]. The diluted albumin (BSA) standards and the BCA working reagent (WR) were prepared according to the instruction [12]. Collected the cells and crushed them with 1% Triton X-100 under ultrasonic. Then 25μl cell solution was added into 96-well tissue culture plate. Every sample was with three separated replicates. Added 200 μL of the WR to each well and mixed plate thoroughly on a plate shaker for 30 s. After covered plate and incubated at 37 °C for 30 min cool plate to RT and measure the absorbance at 570 nm on a microplate reader.

Cell morphology

Cell monolayer on different PET was stained by fluorescein diacetate (FDA, Sigma) for the morphological observation with confocal laser scanning microscopy (CLSM) (CLSM, Bio-Rad, Radiance 2100; Zeiss AXIOVERT 200) [13]. FDA is an indicator of membrane integrity and cytoplasmic esterase activity [14]. Enzymatic hydrolysis of the fluorogenic ester substrate of FDA results in the intracellular accumulation of the green fluorescent product fluorescein in cells with intact plasma membranes. Stock solutions were prepared by dissolving 5.0 mg/mL FDA in acetone. The working solution was freshly prepared by adding 5.0 μL of FDA stock solution to 5.0 mL of PBS. A 20 μL FDA working solution was added in each well, and the solution was incubated for 5 min. The substrates were then washed twice with PBS and placed on a glass slide for CLSM examination. The laser of 488 nm wavelength was used to excite the fluorescein. Total cell area and cell perimeter were determined using the Image-Pro Plus (Media Cybetnetics). Because spreading included changes in cell shape, the shape factor [(area/perimeter^2) × 4π]] was also calculated [15]. The coverage ratio was calculated as (cell area)/(background area).

Result and discussion

Synthesis of the PEG-CHO

Aldehyde groups had higher reactivity for amino groups so they were efficient for conjugation via reductive amination reaction. The PEG including LPEG and CPEG was oxidized by the mixture of DMSO/acetic anhydride which was described in Fig. 3.

The ^{1}HNMR spectrum of CPEG and CPEG-CHO in $CDCl_3$ showed in Fig. 4. The characteristic hydrogen peaks of CPEG were shown as peak a (–CH_3), peak b(–C–CH_2–C–) and peak c(–O–CH_2–C–) By these characteristic peaks, the $M_{n,NMR}$ of CPEG was determined as 10960. Figure 4(b) showed the spectrum of PEG–CH_2–CHO transformed from CPEG. The aldehyde conversion ratio was determined according to the ratio of the characteristic peaks. The aldehyde conversion ratio of LPEG4K and LPEG10K was 48% and 44%.

Surface characterization of the modified PET

The ester bonds of PET were aminolysized by of PEI in water. The surfaces were characterized by ATR-FTIR. The spectrum (Fig. 5) showed the characteristic peaks of amide I band (λ = 1640 cm^{-1}), amide II band (λ = 1538 cm^{-1}) and ν_{N-H} (λ = 3239 cm^{-1}), respectively.

The different stages of modification were monitored by XPS. N_{1s} and C_{1s} spectrum of the modified PET was

$$(CH_3)_2S{=}O + (CH_3CO)_2O + \text{PEG-}CH_2\text{-OH} \longrightarrow \text{PEG-CHO} + 2CH_3COOH + (CH_3)_2S$$

Fig. 3 The reaction equation of transforming hydroxyl groups into aldehyde groups in PEG

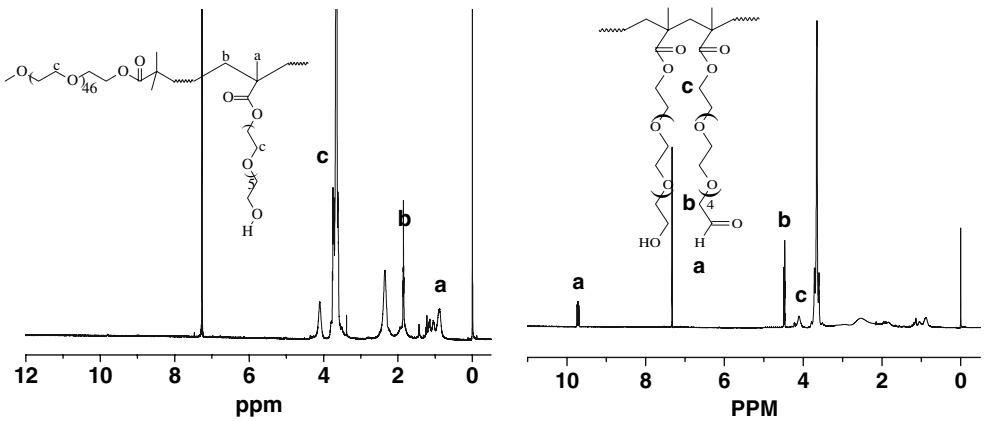

Fig. 4 ^{1}HNMR spectrum of (**a**) CPEG and (**b**) CPEG–CH_2–CHO in $CDCl_3$

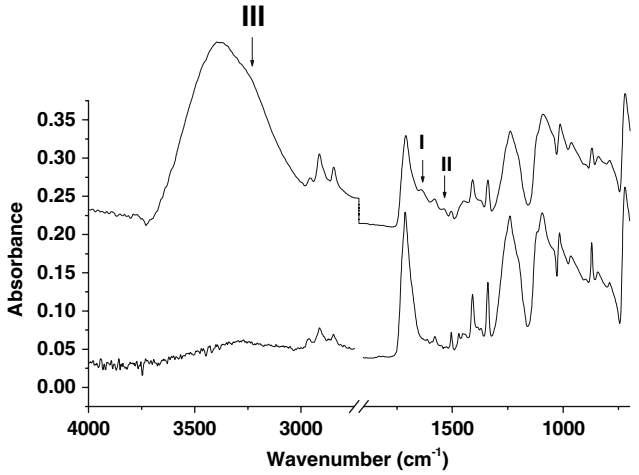

Fig. 5 ATR-FTIR spectrum of PET and PET-PEI. Peak I: Amide I; Peak II: Amide II; Peak III: ν_{N-H}

investigated. The N_{1s} spectra of the PET and PET-PEI were showed in Fig. 5(a). The N content of the PET and PET-PEI was 0 and 6.30% respectively, which indicated that the PEI was grafted onto PET by aminolysis. The content of –C–O– of the PET-PEI, PET-PEI-LPEG10K and PET-PEI-CPEG were 27.18, 35.66 and 50.21%, respectively. The nitrogen content changed from 6.30% in PET-PEI to 1.34% in PET-LPEG and 0.54% in PET-CPEG. The increase of the –C–O-content of and decease of nitrogen content on the surface indicated that both LPEG and CPEG were covalently bound onto PET-PEI surface. The grafting density CPEG was higher than LPEG if we considered the higher content of C–O in PET-PEI-CPEG. The higher grafting density might be explained by more aldehyde groups in CPEG-CHO. The nitrogen content increased from 1.34% and 0.54% to 2.34% and 3.69% for PET-LPEG10K-lysine and PET-CPEG-lysine, which indicated that lysine had been conjugated onto the surface and more lysine was tethered onto CPEG modified surface (Figs. 6, 7, Table 1).

The method of static contact angle was employed to characterize the change of surface wettibility in Fig. 5. The contact angle of the unmodified PET surface was 71.90°. The contact angle of PET-PEI dropped to 64.00° because of the hydrophilicity of PEI chains. When PEG grafted onto the surface the contact angles were 52.06°, 36.90° and 26.98° for PET-CPEG, PET-LPEG4K and PET-LPEG10K, respectively.

Change of surface composition usually led to different surface morphology and subsequently led to change of surface roughness. Here the roughness of surface was studied by atomic force microscopy (AFM) under tapping mode. The typical 3-D AFM pictures were showed in Fig. 8. Degree of surface roughness was denoted by root mean square (RMS). The RMS of PET, PET-PEI, PET-LPEG10k and PET-CPEG was 0.70, 1.62, 0.35 and 0.2 nm, respectively. Grafting PEI led the PET to become rougher. But the surfaces became smoother via LPEG and CPEG grafting.

The above results proved that PEI was grafted onto PET by aminolysis and LPEG10K, CPEG was also

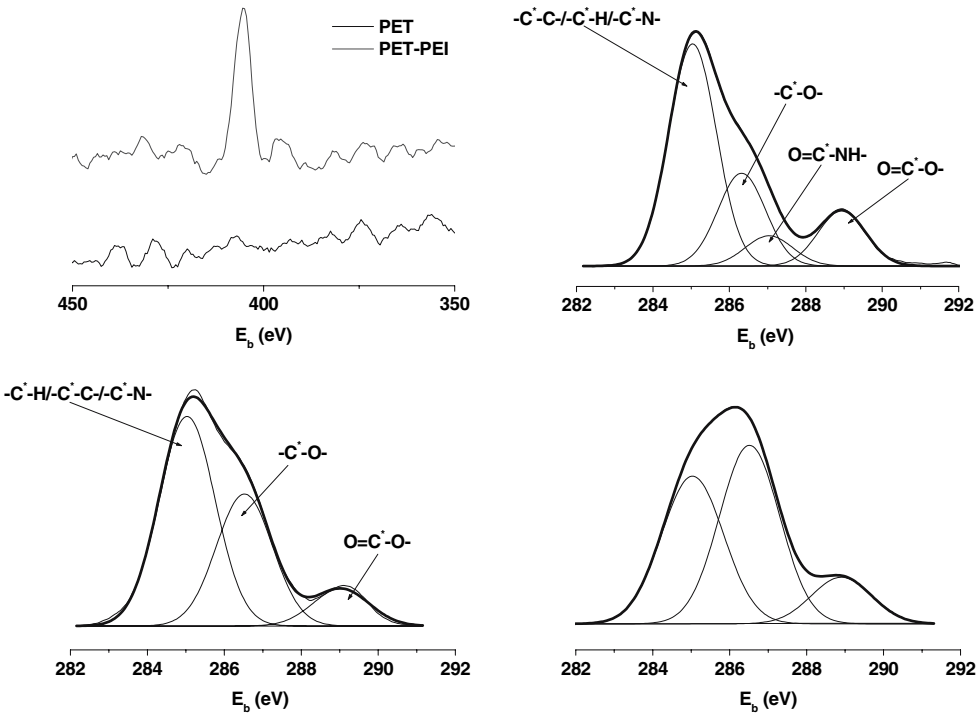

Fig. 6 XPS spectra of different modified PET surface. (**a**) The spectra of PET and aminolysised PET by PEI; (**b**) the C_{1s} spectra of PET-PEI; (**c**) the C_{1s} spectra of PET-LPEG10K-lysine; (**d**) the C_{1s} spectra of PET-CPEG-lysine

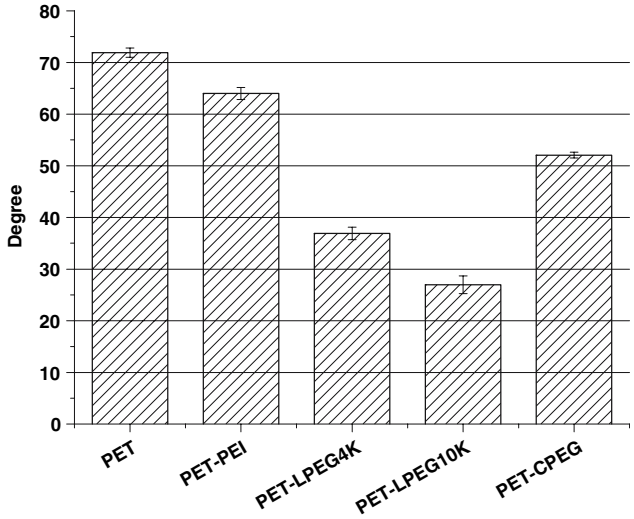

Fig. 7 The contact angle of different modified PET fims

Table 1 XPS surface analysis summary about different modified PET surfaces

Sample	C (%)	N (%)	O (%)	–C–O–/C (%)
PET	64.05	0	35.95	20.83
PET-PEI	44.02	6.30	49.68	27.18
PET-PEI-LPEG10K	45.87	1.23	52.90	35.66
PET-PEI-LPEG10K-lysine	43.60	2.34	54.06	34.84
PET-PEI-CPEG	66.89	0.54	32.57	50.21
PET-PEI-CPEG-lysine	58.01	3.69	38.30	49.44

grafted onto PET-PEI by the reaction between amino groups and aldehyde groups. The L-lysine was then end-tethered onto surface via the residual aldehyde groups. PET-LPEG10k-lysine and PET-CPEG-lysine was hydrophilic and smooth. PET-CPEG-lysine presented a higher PEG content and higher lysine content than that in PET-LPEG10k-lysine.

Platelet adhesion

Hemocompatibility of modified PET films were assessed by the degree and nature of blood platelet adhesion resulting from exposure to platelet-rich plasma for 30 min. The specimens incubated in platelet-rich plasma were viewed by scanning electron microscopy (SEM). Figure 9 showed the typical SEM photographs of adhered platelets on PET, PET-LPEG10k and PET-CPEG. The PET-CPEG showed few platelets adhered and the platelet remained rounded. Although in PET-LPEG10k only a few platelets adhered these platelets aggregated and exhibited their pseudopods which indicated that the platelets had been activated. The platelet density was 32.97×10^3, 13.22×10^3 and $7.51 \times 10^3/mm^2$ for PET, PET-LPEG10k and PET-CPEG, respectively. The result was consistent with XPS result that the higher PEG content on the PET-CPEG led to lower adsorption of platelet adhesion. And the CPEG improved the hemocompatibility of PET films more efficiently than LPEG10K.

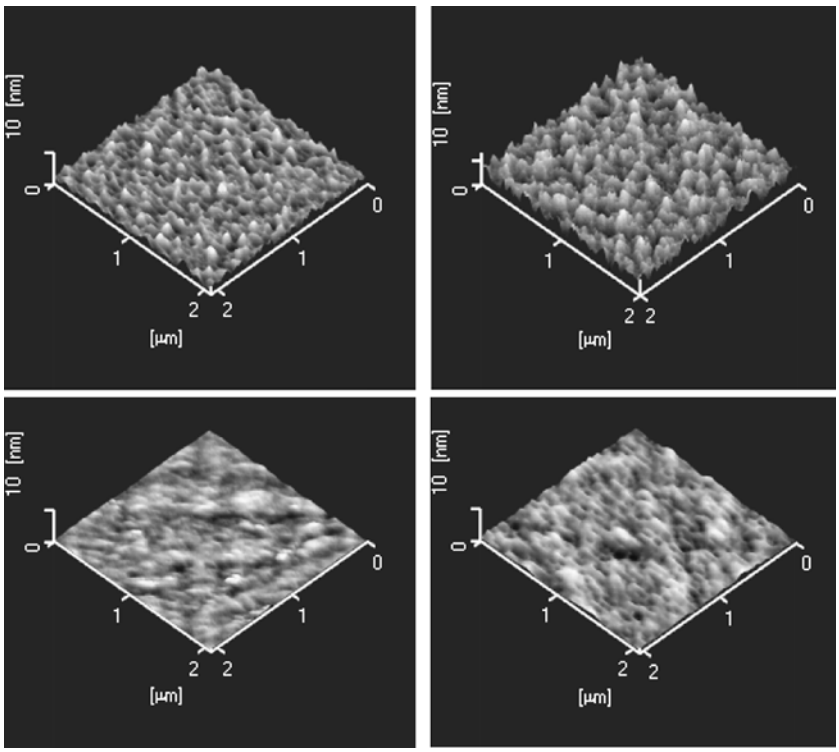

Fig. 8 AFM topology micrographs of different modified PET surface. (**a**) PET; (**b**) PET-PEI; (**c**) PET-CPEG; (**d**) PET-LPEG10K

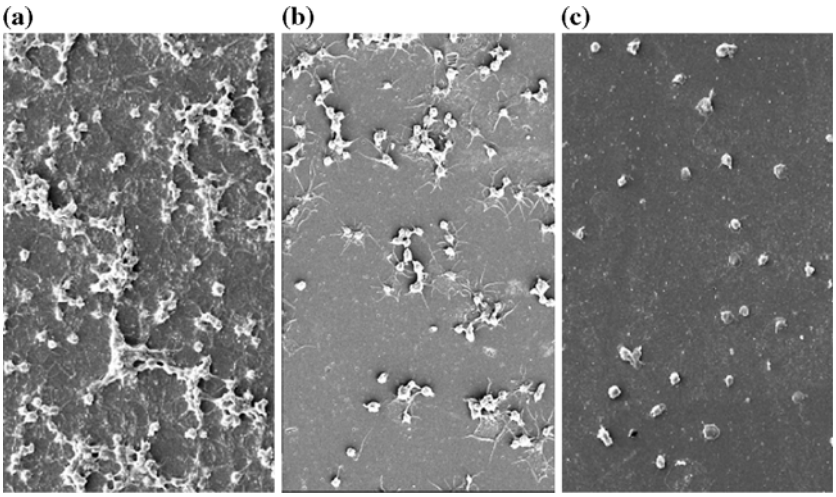

Fig. 9 SEM pictures of adhering platelets on different films, (**a**) PET; (**b**) PET-LPEG10k; (**c**) PET-CPEG. (×2000)

Cell behavior

Cell adhesion and proliferation

Cell behavior such as endothelial cell adhesion and growth appeared to be an important requisite of vascular prosthetic materials, possibly influencing thrombosis, pseudointimal hyperplasia, and accelerated atherosclerosis at the site of blood–material interaction [16].

Results of cell adhesion behavior were showed in Fig. 10. The values for cells adhesion were reported as a proportion of the number of cells that attached to TCPS in the same culture media. Over 24 h the adhesion ratio of PET, PET-LPEG4K, PET-LPEG10K, PET-CPEG, PET-LPEG4k-lysine, PET-LPEG10k-lysine and PET-CPEG-lysine was 66, 54, 60, 48, 71, 73 and 80%, respectively. The adhesion ratio on PET-CPEG was lower than PET-LPEG4k and PET-LPEG10k. The PET-CPEG surface is more resistant to the cell adhesion due to the higher PEG density of PET-CPEG. However, the cell adhesion on PET-CPEG-lysine was improved evidently, which was higher than that on PET-LPEG4k-lysine and PET-LPEG10k-lysine. The higher adhesion ratios maybe were due to higher lysine content.

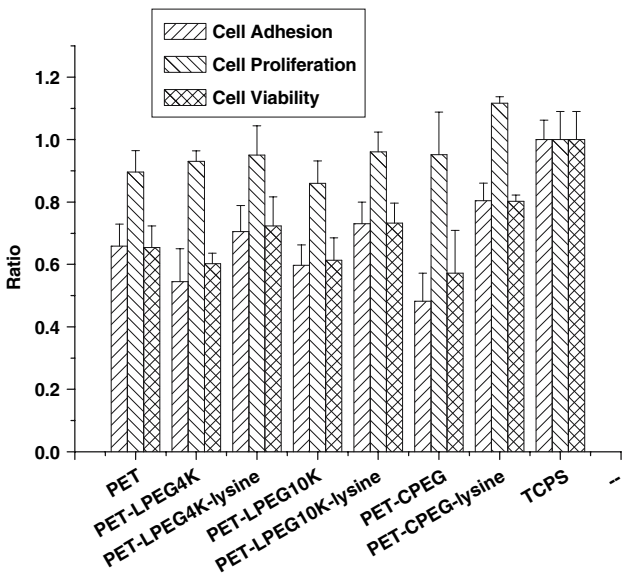

Fig. 10 Results of cell adhesion, proliferation and viability on films. Results represent mean ± SD of triplicates from three separate experiments ($p < 0.05$)

The proliferation ratio of PET, PET-LPEG4K, PET-LPEG10K, PET-CPEG, PET-LPEG4k-lysine, PET-LPEG10k-lysine and PET-CPEG-lysine was 90, 93, 95, 86, 96, 95 and 111.7%, respectively. Cell proliferation ratio was lowest on PET-CPEG in all samples. The higher PEG content in PET-CPEG was not favorable for cell adhesion and subsequently effected cell growth. But cell proliferation on PET-CPEG-lysine was improved evidently. Lysine-tethered comb-like PEG could mediate the cell adhesion and proliferation behavior. The specific cell-substrate interaction could be expected if other selective biomolecules for endothelial cell were conjugated onto PET-CPEG.

Cell viability

Cell viability characterization was achieved by MTT assay and total cell protein assay. The results of MTT assay were shown in Fig. 10. The cell viability of PET, PET-LPEG4K, PET-LPEG10K, PET-CPEG, PET-LPEG4k-lysine, PET-LPEG10k-lysine and PET-CPEG-lysine was 65, 60, 61, 57, 72, 73 and 80%, respectively. The cell viability was the highest on PET-CPEG-lysine and the lowest on PET-CPEG. The absorbance of total cell protein was proportional to its amount. In Fig. 11 the absorbance of total cell protein was 0.245, 0.466, 0.431, 0.386, 0.645, 0.564 and 0.687 for PET, PET-LPEG4K, PET-LPEG10K, PET-

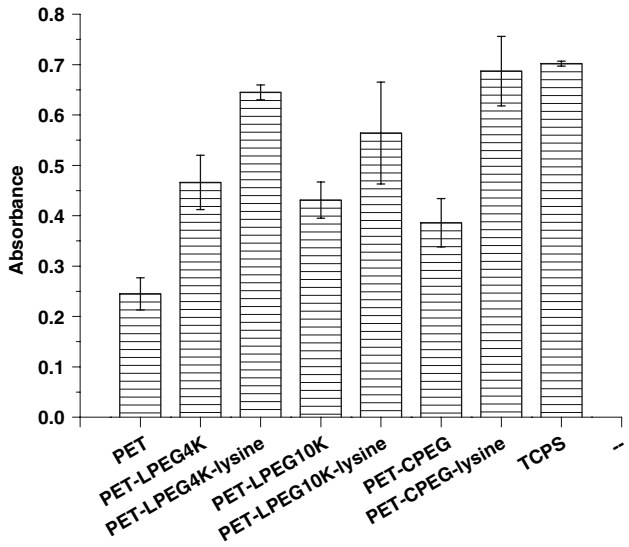

Fig. 11 The cell protein of ECV304 to different modified PET surface conjugated by lysine. Results represent mean ± SD of five replicates from three separate experiments ($p < 0.05$)

CPEG, PET-LPEG4k-lysine, PET-LPEG10k-lysine and PET-CPEG-lysine, respectively. This meant that the content of total cell protein was highest on PET-CPEG-lysine and the lowest on PET-CPEG, which indicated again that the cell viability was the highest on PET-CPEG-lysine and the lowest on PET-CPEG.

Cell morphology

Because cells are inherently sensitive to local chemistry the spreading and morphology would change with the chemistry of surface, especially evidently when the functional groups existed [17]. After initial cell adhesion, cell spreading was generally believed to be required to maintain stable adhesion in vitro. The spreading cells can form a monolayer covering the foreign material surfaces to prevent direct contact between the blood and the foreign material; therefore, preventing immuno-reactions and thrombosis [18]. Using confocal laser scanning microscopy (CLSM), the morphology of cells was determined and morphometric image analysis performed to determine the effect of culture substrate on cell spreading as measured by cell coverage ratio and shape factor [15].

The CLSM images for cells adhering to PET-LPEG4K, PET-LPEG10K, PET-CPEG, PET-LPEG4K, PET-LPEG10K-lysine and PET-CPEG-lysine over 72 h were showed in Fig. 12. The different cell morphology was found. It showed cells on the PET-CPEG-lysine spread uniformly and nearly completely covered. But on PET-CPEG cells showed more regular compact morphology. The result of cell coverage ratio and shape factor was showed in Fig. 13. The cell coverage ratio for PET-LPEG4K, PET-LPEG10K, PET-CPEG, PET-LPEG4K, PET-LPEG10K-lysine and PET-CPEG-lysine was 0.437, 0.406, 0.236, 0.519, 0.503 and 0.755. The cell shape factor

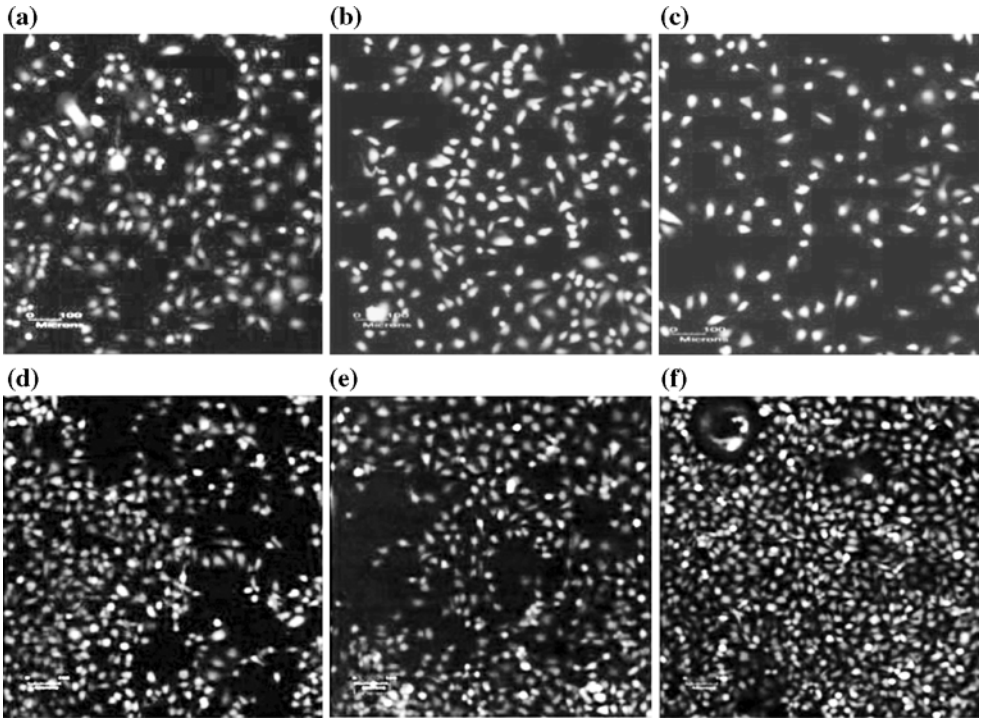

Fig. 12 CLSM images of Cell morphology on different PET surfaces. (**a**) PET-LPEG4K; (**b**) PET-LPEG10K; (**c**) PET-CPEG; (**d**) PET-LPEG4K; (**e**) PET- LPEG10K-lysine; (**f**) PET-CPEG-lysine

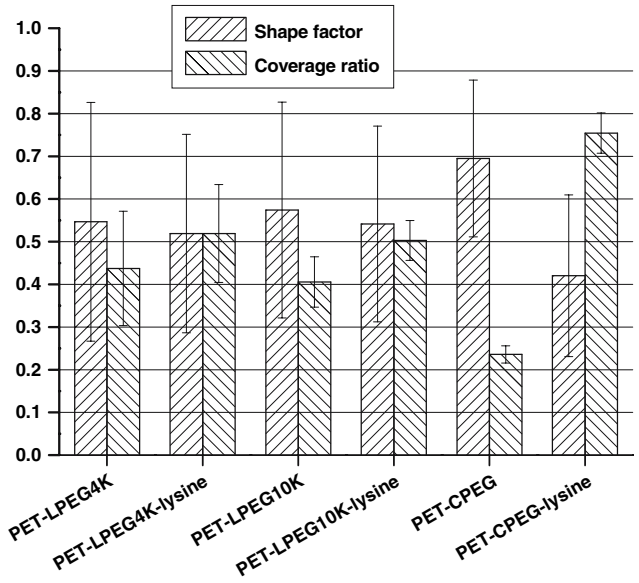

Fig. 13 Effect of substrate on cellular morphology as analyzed by image analysis of cell coverage ratio and cell shape factor

for PET-LPEG4K, PET-LPEG10K, PET-CPEG, PET-LPEG4K, PET-LPEG10K-lysine and PET-CPEG-lysine was 0.547, 0.574, 0.695, 0.519, 0.541 and 0.420. From the above results, the PET-CPEG-lysine was most favorable for cell adhesion and spreading and PET-CPEG was most unfavorable. It was believed that higher PEG density in PET-CPEG led to lower cells adhesion and spreading. Higher lysine content in PET-CPEG-lysine induce cells to adhere and spread.

Conclusion

The hydroxyl groups of LPEG and CPEG were oxided into aldehyde groups by Moffatt procedure. The reactive comb-like PEG with aldehyde groups was then covalent bond to aminolysized PET surfaces, which were further tethered with lysine to enhance surface endotheliazation. Results of ^{1}HNMR, ATR-FTIR, XPS and AFM confirmed the above steps. The cell behavior on PET-CPEG-lysine was evidently different from the other modified PET. PET-CPEG could more resist the cell adhesion because of higher PEG content and also had better hemocompatibility. However, the end-tethered lysine could promote cell adhesion, spread and proliferation. The different cell behavior was perhaps due to higher lysine content in the PET-CPEG-lysine. Both the cell viability and cell protein indicated that cells on PET-PEI-CPEG-lysine surface had higher viability. The ligand-thethered comb-like PEG could serve as an ideal surface modifier to enhance surface endothelialization via the specific interaction of bioactive ligands with cells.

Acknowledgements This research was financially supported by Major State Basic Research Foundation of China (grant No. 2005CB623902), Natural Science Foundation of Chain (NSFC-20174035), Program for New Century Excellent Talents in University (NCET-05-0527) and National High Technology Research and Development Program of China (2006AA03Z329).

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