Feasibility Evaluation of Chitosan Coatings on Polyethylene Tubing for Biliary Stent Applications

Chih-Hsiu Lin,[1] Jui-Che Lin,[1] Chiung-Yu Chen,[2] Chu-Yuan Cheng,[1] Xi-Zhang Lin,[2] Jiunn-Jong Wu[3]

[1]*Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan 701*
[2]*Division of Gastroenterology and Department of Medicine, National Cheng Kung University, Tainan, Taiwan 701*
[3]*Department of Medical Technology, National Cheng Kung University, Tainan, Taiwan 701*

Received 15 September 2004; accepted 21 December 2004
DOI 10.1002/app.21844
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Endoscopic palliation for the obstructive jaundice caused by biliary or pancreatic malignant tumors is a commonly used medical treatment. However, plastic biliary stents are occluded by sludge deposition caused by bacterial adhesion in 3–5 months. Chitosan, which has both good biocompatibility and antimicrobial capability, was used to modify the inner surface of polyethylene (PE) tubing in this study. Chitosan was deposited onto the inner surface of oxidized PE tubing with the methanol precipitation technique. Attenuated total reflection/Fourier transform infrared and electron spectroscopy for chemical analysis indicated that the chitosan coating was feasible. Contact-angle measurements revealed that the surface hydrophilicity of the PE tubing increased with the chitosan coating. Morphological analysis with scanning electron microscopy showed that the PE surface became rougher and exhibited micropores after the chitosan coating. The adhesion of living *Escherichia coli* to chitosan-coated PE stents, characterized by the spreading plate method and scanning electron microscopy analysis, was more significant than that to unmodified stents after a 24-h phosphate-buffered saline or bile perfusion test. This finding may be attributed to the rougher and slightly positively charged surface of chitosan-coated PE tubing and to the —CH_3 hydrophobic functional groups in the chitosan structure. Because of its good biocompatibility, chitosan coated on the surface of PE can still be used for biliary stent applications with further chemical modification, such as sulfonation and quaternization, to increase its antimicrobial ability. J Appl Polym Sci 97: 893–902, 2005

Key words: adhesion; biopolymers; coatings; polyethylene (PE); surfaces

INTRODUCTION

The endoscopic insertion of plastic and/or metallic biliary stents is one of the medical treatments that can relieve the obstructive jaundice caused by biliary or pancreatic malignant tumors.[1,2] It is a useful method for patients, such as the elderly, who cannot tolerate surgery.[3] For many years, the plastic stent has been a profitable option.[4] However, inserted plastic stents are restricted by the occlusion of the stents: replacing them in 3–5 months is necessary.[5] Although the precise mechanism of the blockage is still unknown, it is generally believed that protein adsorption, adherence of bacteria, formation of biofilms, and precipitation of biliary sludge (composed of bacteria, proteins, calcium bilirubinate, etc.[1,6,7]) are the main factors leading to stent occlusion (Fig. 1). A prospective method of preventing the blockage is to reduce the bacterial adhesion and biofilm formation with a useful stent material or by the modification of the surfaces of existing plastic stents.[1,8–11] Moreover, live *Escherichia coli*, being capable of producing β-glucuronidase in bile, can deconjugate bilirubin glucuronide effectively, and this leads to the precipitation of calcium bilirubinate.[6,11] Lately, self-expanding metal stents have been proved to be effective in prolonging the lifetime of stents because of their larger diameters and better mechanical tension, but they are obstructed by tumor ingrowth or overgrowth. Therefore, metal stents are limited by the difficulties of removal and high costs.[4,12,13] Therefore, plastic stents continue to be the focus of advanced research.[14]

Chitosan, a natural polycationic polymer, is obtained by the N-deacetylation of chitin, the second most abundant natural polysaccharide, which is a component of the skeletal structures of crustaceans and the cell walls of fungi. Chitosan is composed of β-(1→4)-linked 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose units and has good biocompatibility, antimicrobial ability, and biodegradability.[15–17] Many reports have discussed chitosan's antimicrobial ability against *E. coli* under

Correspondence to: J.-C. Lin (jclin@mail.ncku.edu.tw).
Contract grant sponsor: National Science Council of Taiwan; contract grant number: NSC 92–2815-C-006–068-E.

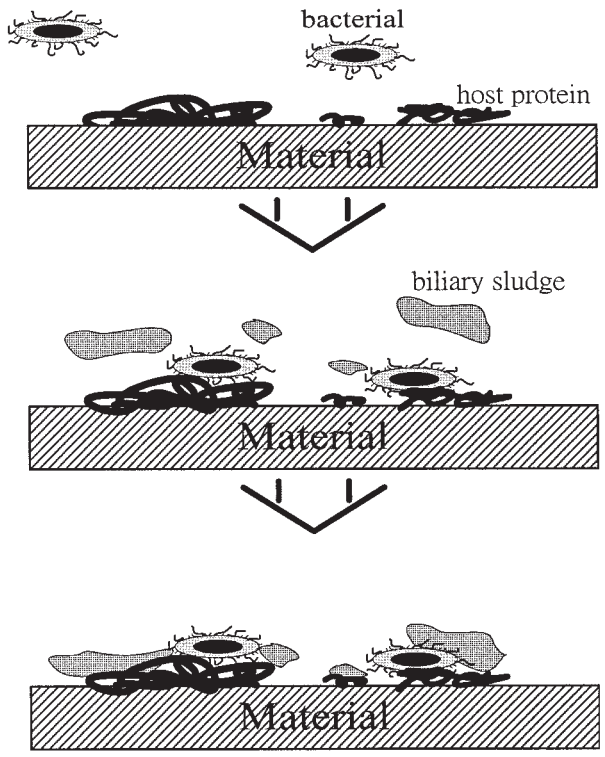

Figure 1 Schematic drawing for the possible mechanism leading to the occlusion of the biliary stent.[6]

different conditions. Studies have shown that chitosan can inhibit the growth of *E. coli* with the shaking cultivation testing method in an acid environment.[18–23] Chitosan has a better antibacterial effect at pH 6.5[18] or pH 3–4.5.[19,23] Moreover, with the agar plate method, other experiments have indicated that chitosan can suppress the growth of *E. coli* effectively.[15,23–25] However, these reported antimicrobial activities of chitosan have been tested in either a static or shaking acid aqueous environment. Only a few studies have measured the antibacterial effect of chitosan against *E. coli* at pH > 7, the condition under which acid solvents, which may be toxic for cells, can be avoided.[26,27]

In this study, because of the possible antimicrobial capability of chitosan in the flow system at pH > 7, chitosan was coated onto the inner surfaces of polyethylene (PE) tubing in an attempt to decrease the bacterial adhesion and, in turn, prolong the patency rate of biliary stents. The surface properties of chitosan-coated PE tubing were examined with attenuated total reflection/Fourier transform infrared (ATR–FTIR), electron spectroscopy for chemical analysis (ESCA), a contact-angle technique, and scanning electron microscopy (SEM). Additionally, a closed phosphate-buffered saline (PBS) or bile perfusion system containing *E. coli* as a simulated infected bile was used to test the antibacterial capability of chitosan-coated PE stents *in vitro*.

EXPERIMENTAL

Chitosan coating of PE tubing

The 12-cm PE tubes (i.d. = 2.92 mm, o.d. = 3.73 mm; PE 330, Intramedic, Becton Dickinson, San Jose, CA) were first ultrasonicatively cleaned with a detergent and then with distilled water for 2 h. After the tubes were dried in a vacuum oven for 24 h, the inner surfaces were oxidized with Chromerge (Aldrich, St. Louis, MO) at 50°C for 6 h oxidized-PE (OxPE) before the chitosan coating. These oxidized-PE (OxPE) tubes were washed with 98% H_2SO_4, 50% H_2SO_4, 3% H_2SO_4, and deionized water sequentially and were dried at 50°C overnight.

Chitosan (weight-average molecular weight ~ 630,000, deacetylation degree ~ 84%; Koyo Chem-

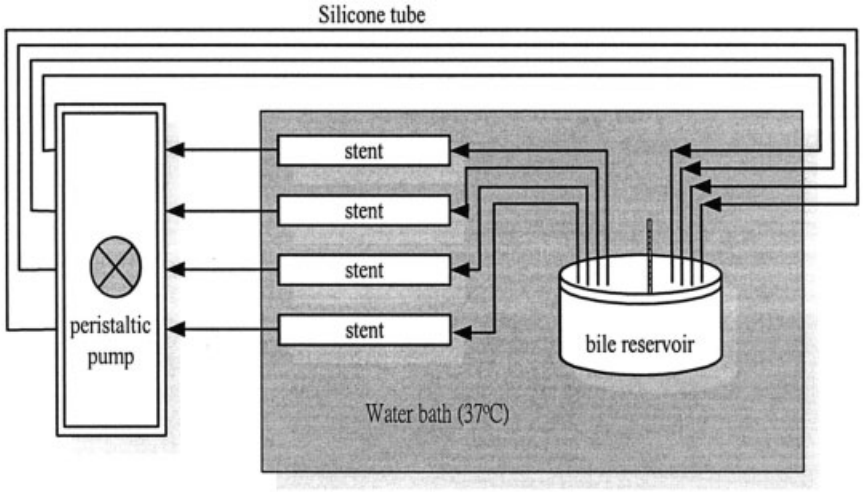

Figure 2 *In vitro E. coli* stent perfusion model.[6]

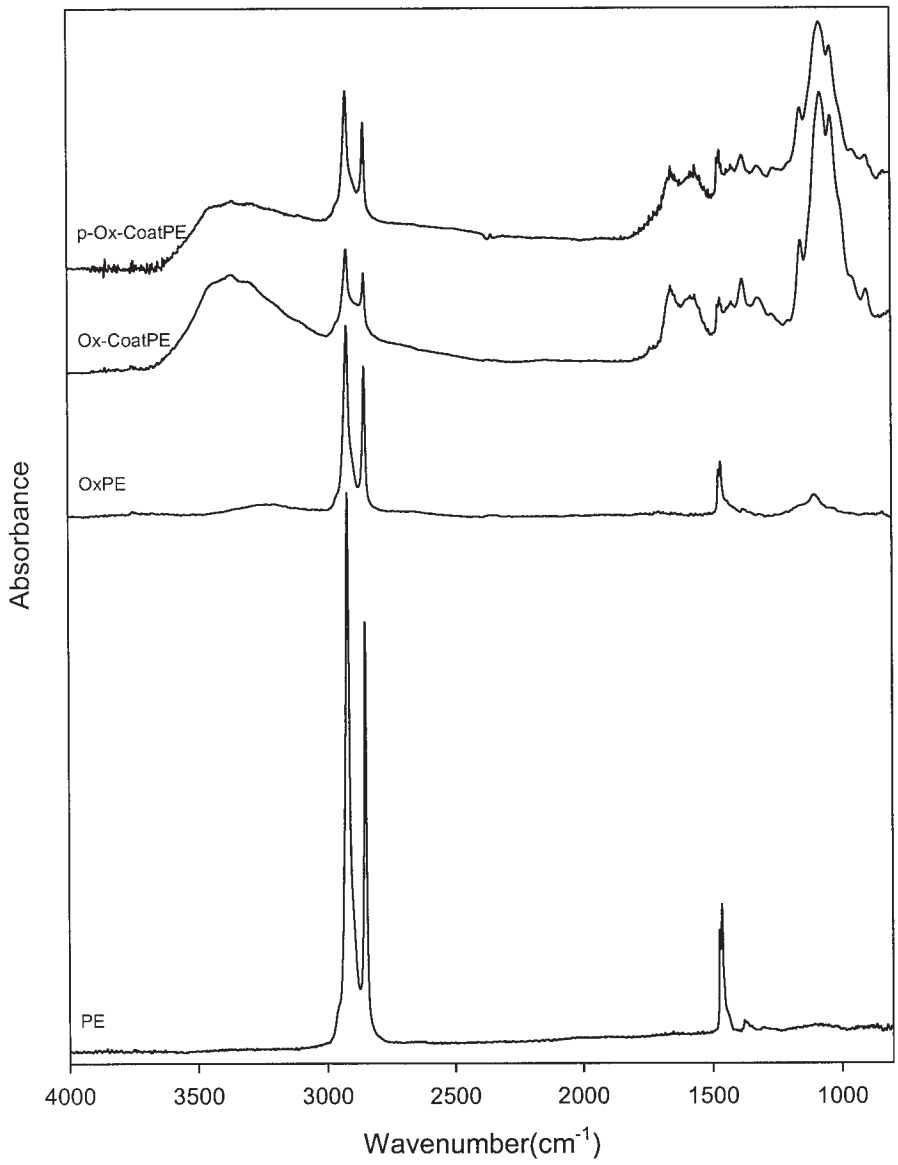

Figure 3 ATR–FTIR spectra of all tested tubing.

ical, Tokyo, Japan) was extracted by acetone and then by alcohol for 24 h in a Soxhlet extraction apparatus to remove the impurities. A chitosan solution (2 wt % in 1 vol % acetic acid) was injected into the OxPE tubes and capped for a 1-h incubation at room temperature. Then, the chitosan solution was replaced with methanol for further incubation of 30 min. After the removal of methanol, these tubes were dried at 40°C for 24 h and then neutralized with 5 wt % NaOH for 10 min. Finally, these tubes were dried in a vacuum oven at 40°C overnight. These chitosan-coated OxPE tubes are called Ox-CoatPE tubes in the following discussion.

Analysis of the tested tubing surfaces

The surface chemistry was analyzed with ATR–FTIR and ESCA. The ATR–FTIR spectra were collected after 256 scans at a resolution of 4 cm^{-1} with a Bio-Rad FTS-40A (Bio-Rad, Cambridge, MA). The ESCA analysis was carried out with a VG ESCA210 spectrometer (Thermo VG Scientific, West Sussex, United Kingdom) operated by the Tainan Regional Instrumentation Center, which is managed by the National Science Council of Taiwan.

In addition, the surface hydrophilicity of the inner surface of the tubing was examined with a contact-angle technique with both sessile drop and captive bubble methods (model CA-A contact-angle meter; Face Co., Tokyo, Japan). The inner surface of the plastic tubing was also evaluated with SEM (XL40 FE-SEM; Philips, Eindhoven, The Netherlands) to examine the surface morphology of these tubes before and after the chitosan coating as well as the bacterial perfusion testing.

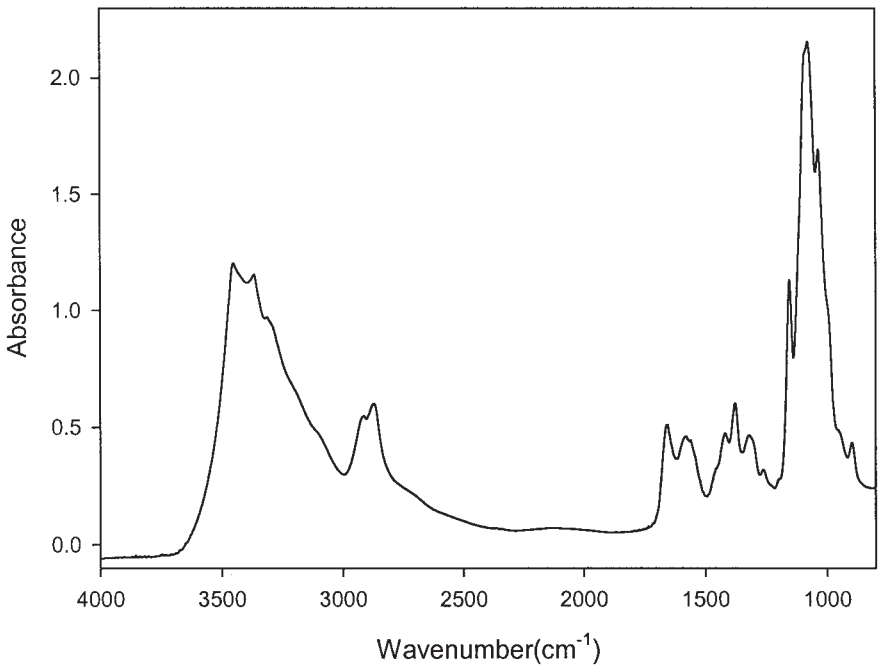

Figure 4 Fourier transform infrared spectrum of the bulk chitosan membrane.

In vitro closed stent perfusion testing[6,9]

Bile collected from patients who suffered from jaundice or cholelithiasis was centrifuged in an RC5CPlus centrifuge (Sorvall Products, Newtown, CT) at 7500 rpm for 40 min to remove the solid impurities. Then, the treated bile was filtered with grade 3 qualitative filter paper (particle retention > 6 μm; Whatman, Maidstone, England) followed by Coring (Coring, NY) 500-mL poly(ether sulfone) disposable sterile filters with a pore size of 0.22 μm to eliminate the bacteria. It was then stored in a glass serum bottle wrapped with aluminum foil to prevent degradation from light exposure at -80°C.

All the tools were first sterilized with an autoclave or UV light irradiation before the perfusion experiment. An *in vitro* perfusion system was set up, as shown in Figure 2. The peristaltic pump (Cole–Parmer, Chicago, IL) was used to keep the flow circulation at a rate of 2 mL/min during the testing. Two hundred fifty milliliters of PBS (pH 7.4; Sigma, St. Louis, MO) or human pretreated bile containing 10^7 CFU/mL *E. coli* (*E. coli* DH5α, a strain capable of producing β-glucuronidase) was placed in the reservoir, which was kept in a 37°C water bath. The tested tubes, including PE, OxPE, and Ox-CoatPE, were equilibrated in PBS for 12 h before the perfusion testing. After 24 h of perfusion, these stents were removed and rinsed gently with PBS. Then, the middle part of each tube was cut with a surgical blade into four 1-cm length sections: three for the quantitative analysis of the bacterial colony count on the surfaces of the stents by the spreading plate method[6] and one, which was fixed in 5% diethylene glycol diglycidyl ether (Fluka, Buchs SG, Switzerland) for 10 h, for SEM evaluation. *E. coli* was fixed in 5% diethylene glycol diglycidyl ether instead of glutaraldehyde, a widely used fixation agent for bacteria, because the glutaraldehyde-crosslinked chitosan coating on the OxPE surface exhibited weaker mechanical properties and that resulted in surface cracking after the SEM sample preparation.

RESULTS AND DISCUSSION

Surface characterization of the stents

Figure 3 shows the ATR–FTIR spectra of all the tested materials. With Chromerge oxidation, the relative absorbance of the C—H stretching (2970–2850 cm^{-1}) and —CH_3 symmetrical (1372 cm^{-1}) and asymmetrical (1463 cm^{-1}) bending decreased on the OxPE surface. In contrast, a few peaks that could be attributed to the O—H stretching vibration (3200–3500 cm^{-1}) and C—O—C stretching vibration (1100 cm^{-1}) could be observed for this oxidized PE tube.[28] On the chitosan-deposited PE surface (Ox-CoatPE), in comparison with the bulk chitosan membrane (Fig. 4), characteristic IR absorption peaks associated with chitosan were noticed: O—H and N—H stretching (3100–3500 cm^{-1}), primary N—H bending (1576 cm^{-1}), C—O—C stretching (1154 cm^{-1}), and C—O stretching (1080 cm^{-1}) vibrations.[15,18,25,29,30] In addition, peaks attributed to the PE substrate were noticed as well. This indicated that the thickness of the chitosan layer was

TABLE I
ESCA of Tested Tubing

Material	Atomic percentage				
	O	N	C	S	Si
PE	7.3	1.5	91.0	0.1	0.0
OxPE	16.6	3.1	78.2	1.3	0.8
Ox-CoatPE	31.5	6.0	61.6	0.3	0.6

TABLE II
Contact Angles of Tested Tubing

Material	θ	
	Sessile drop method	Captive bubble method[a]
PE	95.6 ± 1.09	88.5 ± 1.52
OxPE	86.1 ± 2.38	44.0 ± 3.63
Ox-CoatPE	68.2 ± 4.33	45.4 ± 4.54
Chitosan membrane	ND[b]	37.2 ± 3.89

[a] The sample was preimmersed in deionized water for 24 h.
[b] Not determined because of swelling of the chitosan membrane during the measurement.

less than the depth of the resolution of the ATR–FTIR technique, which was about 1–2 μm. Moreover, similar ATR–FTIR spectra were noticed between the non-PBS-perfusion Ox-CoatPE and the sample after 24 h of PBS perfusion (p-Ox-CoatPE). This suggests that the chitosan layer remained well attached to the inner surface of the PE tubing after perfusion.

Besides ATR–FTIR analysis, the ESCA technique, which has better surface sensitivity (i.e., ca. 10–250 Å in depth) than ATR–FTIR, was used to characterize the chemical configuration of the top surfaces of the tested tubing.[29,31] In comparison with untreated PE, the decrease in the C atomic percentage and the increase in the O atomic percentage of OxPE (Table I) indicated that some —CH_2 groups on PE reacted with the Chromerge solution and formed C—O, C—O—C, and/or O—H functional groups. This finding was further supported by the ESCA spectra of C1s peaks (Fig. 5). The C1s spectrum of OxPE tubing exhibited a tail at the higher binding energy, and this could be attributed to the formation of C—O and/or C—O—C groups on the surface.[31,32] Similar but more apparent findings were noted for the Ox-CoatPE surface; significant increases in the atomic percentages of N and O (Table I), as well as multiple peaks and peak broadening found on the C1s spectra (Fig. 5), were a result of the incorporation of C—O, C—N, C—O—C, and C═O groups associated with chitosan. The S atom noted on the OxPE sample might have been due to the Chromerge residuals after the oxidation reaction.

The hydrophilicity of the inner surface of the plastic tubing was evaluated with the sessile drop and captive bubble methods (Table II). Sessile drop measurements indicated that the surface hydrophilicity increased in the following order: Ox-CoatPE > OxPE > PE. This suggested that the incorporation of hydro-

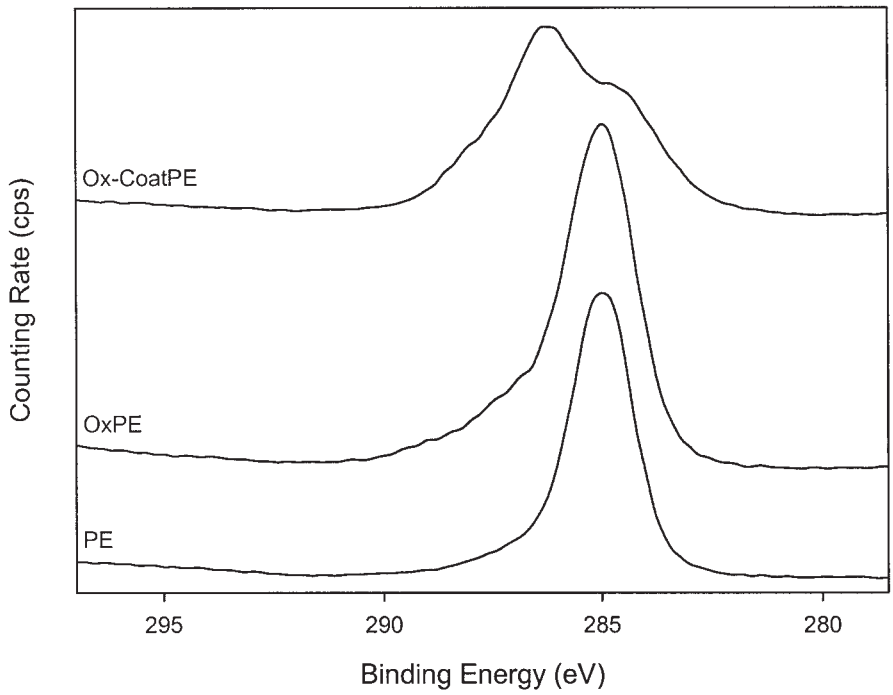

Figure 5 ESCA C1s spectra of the tested tubing.

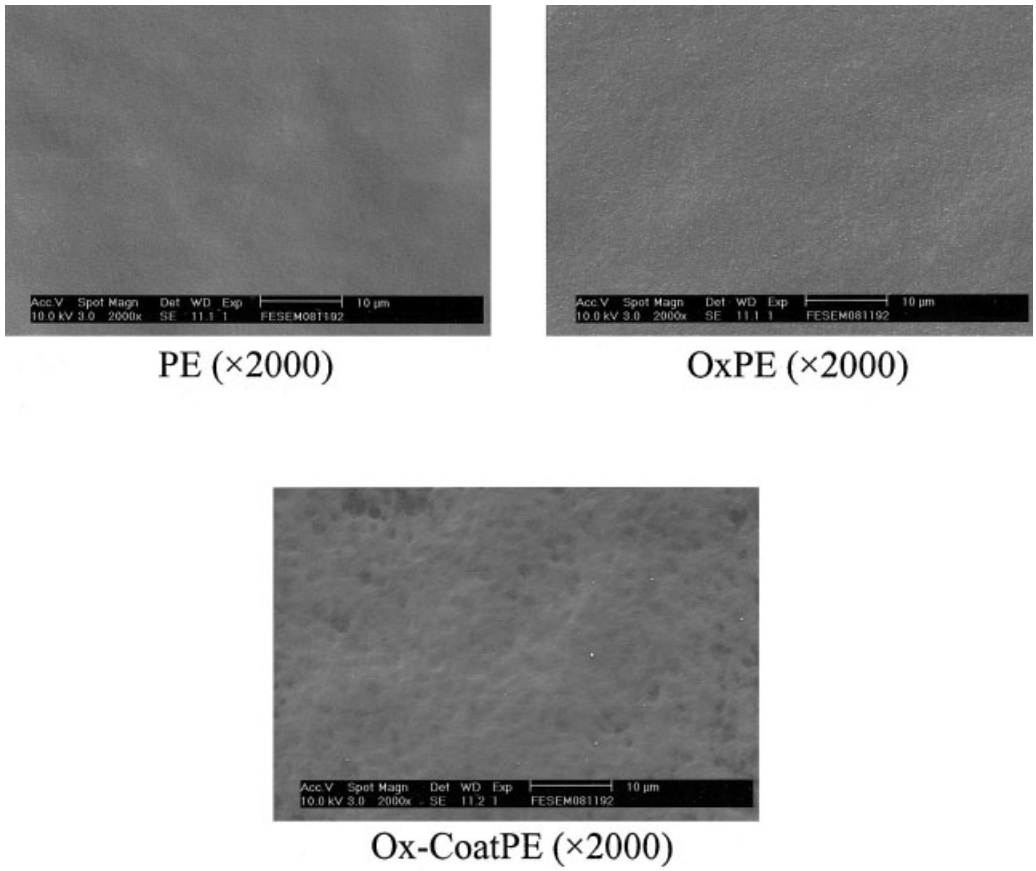

Figure 6 SEM images of the different tested plastic tubing samples.

philic functionalities (O—H, C—O, and C—O—C on OxPE and O—H, —NH_2, C—O, C═O, C—N, and C—O—C on Ox-CoatPE, as shown in Fig. 3) greatly increased the surface hydrophilicity of the modified PE tubing. Much lower contact-angle values were noticed when OxPE and Ox-CoatPE were analyzed by

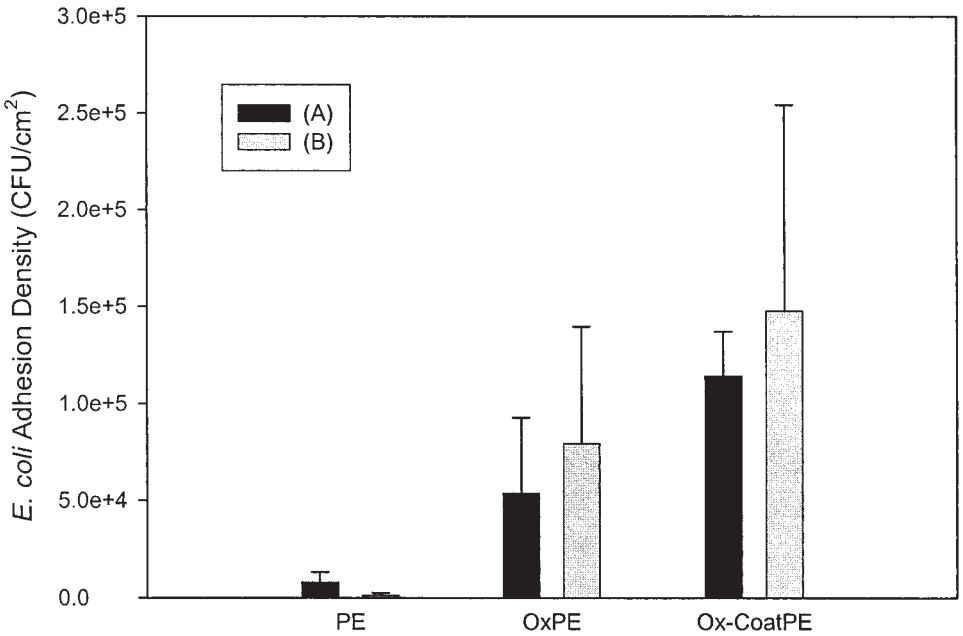

Figure 7 Surface density of living *E. coli* on the tested tubing after 24 h of (A) PBS or (B) bile perfusion.

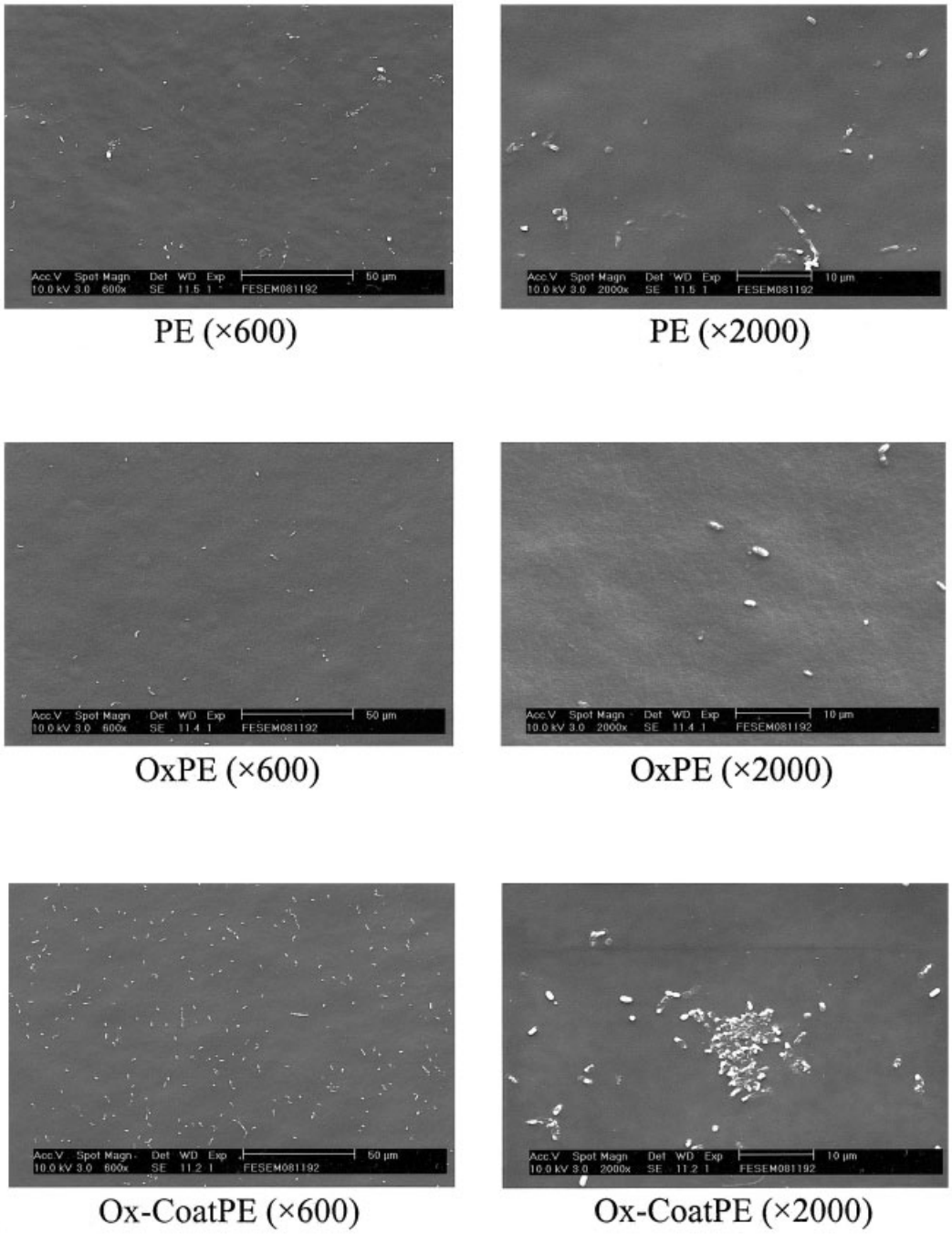

Figure 8 SEM images of different tested tubing samples after a 24-h PBS perfusion experiment.

the captive bubble method, in which the samples were preimmersed in deionized water for 24 h. This might be due to the thermodynamically driven chain rotation of the polymer backbone, which led to the appearance of more hydrophilic functionalities on the surface.

The inner surface of each plastic tube was examined with SEM to study the effect of chemical treatment on the surface morphology (Fig. 6). The PE surface was the smoothest, whereas a rougher surface morphology was noted after the surface oxidation with a Chromerge solution (OxPE). A slightly microporous swelled morphology was noticed after the chitosan coating (Ox-CoatPE).

In vitro stent perfusion testing

After 24 h of either PBS or bile perfusion, the amounts of living *E. coli* attached to different samples ranked the same: Ox-CoatPE > OxPE > PE (Fig. 7). This

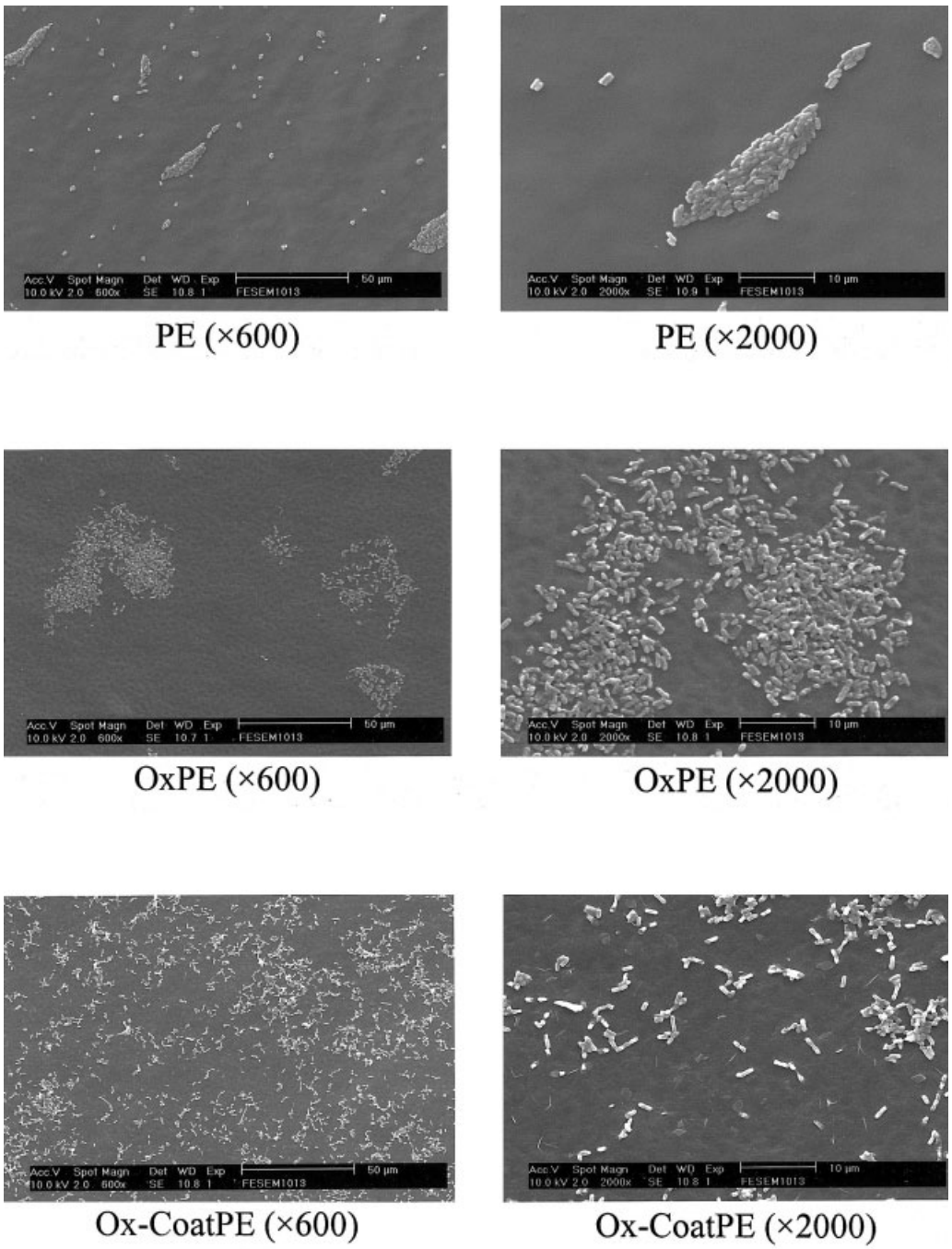

Figure 9 SEM images of different tested tubing samples after a 24-h bile perfusion experiment.

indicated that the coated chitosan on the PE surface could not inhibit the deposition of *E. coli* effectively. More *E. coli* was observed on the Ox-CoatPE surface than on PE and OxPE after 24 h of PBS and bile perfusion testing (Figs. 8 and 9). This observation is identical to the finding for the amount of *E. coli* quantified by the spreading plate method. Moreover, *E. coli* clustered together after bile perfusion but not after PBS perfusion. This might be attributed to the various proteins in bile that were adsorbed onto the chitosan coating before the bacterial adhesion.

Because the bacterial inhibition mechanisms of chitosan are not fully understood, some mechanisms have been proposed for its antibacterial ability. One mechanism indicates that the polycationic property of chitosan interferes with the negative charge on the surface of the bacteria so that the interaction between the chitosan and cell membrane changes the permeability of the bacteria or subdues bacterial growth by stopping the bacterial metabolism.[17,18,29,33] The antibacterial effect of chitosan (with an acid dissociation constant of 6.3) appears more remarkable in an acidic

environment because the —NH_2 groups of chitosan are protonated to —NH_3^+ groups that practice the aforementioned mechanism. Therefore, chitosan has weaker anti-bacterial-adhesion capacity at pH > 7, such as PBS and bile in this experiment.[17,18]

The adherence of bacteria to surfaces is an extremely sophisticated process because the bacterial surface contains hydrophobic and hydrophilic zones. In addition, the hydrophilic regions consist of cationic and anionic groups. In the PBS system, an increase in the hydrophilicity and the negative charges of the chitosan-coated surface suppress bacterial adhesion.[34,35] Although chitosan is a hydrophilic material, glucopyranose, the cyclic repeating unit of chitosan, bears —CH_3 hydrophobic groups; therefore, more *E. coli* may be able to attach to the Ox-CoatPE surface through such hydrophobic–hydrophobic interactions. Moreover, in the PBS environment, the —NH_2 groups of chitosan may partly remain in the form of —NH_3^+ on the Ox-CoatPE surface and generate a slightly positively charged surface that leads to the further adsorption of *E. coli*. Finally, the increase in the roughness caused by the swelling of chitosan in a liquid may augment the attachment of *E. coli*. In contrast, the PE surface is hydrophobic and perhaps smooth, and that is why there is less *E. coli* on the PE surface.[2,10] Similarly, although OxPE has a hydrophilic surface, its rougher morphology results in more *E. coli* adsorbed on the surface (in comparison with PE).

Similar rationales may be applied to the findings of bacterial adhesion within an infected human bile perfusion system. However, the proteins within human bile may be preadsorbed onto the material surface before *E. coli* adhesion, and this will definitely complicate our rationales. Further analyses of the preadsorbed protein composition, which are currently being undertaken, will certainly enhance our knowledge to prevent bacterial adhesion onto biliary stents.

CONCLUSIONS

The clogging of endoscopic plastic biliary stents by sludge deposition makes their replacement in 3–5 months necessary. In this study, chitosan-coated PE tubes were evaluated with the aim of enhancing the biocompatibility and antimicrobial ability of PE stents in a flow system in which the pH was higher than 7. The characterization results of ATR–FTIR spectra and ESCA showed the successful coating of chitosan onto PE tubing, and this coated chitosan could bear perfusion testing for 24 h *in vitro* without detachment. Contact-angle analysis further demonstrated the increased hydrophilicity of PE tubes with chitosan coatings. SEM evaluation revealed that Ox-CoatPE had a microporous swelled morphology that was rougher than the morphology of the noncoated control. After 24 h of PBS or bile perfusion, the chitosan coating on the inner surface of a PE tube could not effectively prevent *E. coli* adhesion in the simulated *E. coli* infected flow environment in which the pH was greater than 7. This may be attributed to the rougher and slightly positively charged surface of Ox-CoatPE tubing. In addition, the —CH_3 hydrophobic groups of chitosan may have partially contributed to this finding, although chitosan itself is hydrophilic in bulk. Moreover, the preadsorbed bile protein layer within the infected human bile system may also modulate the increased bacterial adhesion. Although chitosan could not reduce the adhesion of living *E. coli* onto the stents in the flow system at pH > 7, these Ox-CoatPE tubing may still have potential for biliary stent use because of chitosan's good biocompatibility and antimicrobial ability, which could be increased by sulfonation[6,36] or quaternization[37] of the chitosan surface. Further research is currently proceeding.

The technical assistance and academic advice of Chia-Wen Lin, Mei-Chaun Peng, Shen-Hsiu Hung, and the laboratory staff of the authors' groups (J.-C. L., C.-Y.C., and X.-Z.L.) are acknowledged.

References

1. Wenderoth, D. F.; Ferslev, B.; Macarri, G.; Molinari, G.; Lünsdorf, H.; Timmis, K. N. Environ Microbiol 2003, 5, 859.
2. Leung, J. W. C.; Ling, T. K. W.; Kung, J. L. S.; Vallance-Owen, J. Gastrointestinal Endoscopy 1988, 34, 19.
3. Coene, P. P. L. O.; Groen, A. K.; Cheng, J.; Out, M. M. J.; Tytgat, G. N. J.; Huibregtse, K. Gut 1990, 31, 913.
4. Costamagna, G.; Pandolfi, M. J Clin Gastroenterology 2004, 38, 59.
5. Jansen, B.; Goodman, L. P.; Ruiten, D. Gastrointestinal Endoscopy 1993, 39, 670.
6. Peng, M. C.; Lin, J. C.; Chen, C. Y.; Wu, J. J.; Lin, X. Z. J Appl Polym Sci 2004, 92, 2450.
7. Gitnick, G.; LaBrecque, D. R.; Moody, F. G. Diseases of the Liver and Biliary Tract; Mosby-Year Book: St. Louis, 1992; p 543.
8. Leung, J. W. C.; Lau, G. T. C.; Sung, J. J. Y.; Costerton, J. W. Gastrointestinal Endoscopy 1992, 38, 338.
9. Hoffman, B. J.; Cunningham, J. T.; Marsh, W. H.; O'Brien, J. J.; Watson, J. Gastrointestinal Endoscopy 1994, 40, 581.
10. McAllister, E. W.; Carey, L. C.; Brady, P. G.; Heller, R.; Kovacs, S. G. Gastrointestinal Endoscopy 1993, 39, 422.
11. Zhang, H. J.; Tsang, T. K.; Jack, C. A. J Lab Clin Med 2003, 142, 58.
12. Rösch, T. Endoscopy A 1998, 30, 247–252.
13. Ginsberg, G.; Cope, C.; Shah, J.; Martin, T.; Carty, A.; Habecker, P.; Kaufmann, C.; Clerc, C.; Nuutinen, J. P.; Törmälä, P. Gastrointestinal Endoscopy 2003, 58, 777.
14. van Berkel, A. M.; Boland, C.; Redekop, W. K.; Bergman, J. J. G. H. M.; Groen, A. K.; Tytgat, G. N. J.; Huibregtse, K. Endoscopy 1998, 30, 681.
15. Tang, R. P.; Du, Y. M.; Fan, L. H. J Polym Sci Part B: Polym Phys 2003, 41, 993.
16. Anthonsen, M. W.; Vårum, K. M.; Smidsrød, O. Carbohydr Polym 1993, 22, 193.
17. Rabea, E. I.; Badawy, M. E. T.; Stevens, C. V.; Smagghe, G.; Steurbaut, W. Biomacromolecules 2003, 4, 1457.
18. Liu, X. F.; Guan, Y. L.; Yang, D. Z.; Li, Z.; Yao, K. D. J Appl Polym Sci 2001, 79, 1324.

19. Chung, Y. C.; Wang, H. L.; Chen, Y. M.; Li, S. L. Bioresour Technol 2003, 88, 179.
20. Matsuhashi, S.; Kume, T. J Sci Food Agric 1997, 73, 237.
21. Tsai, G. J.; Su, W. H.; Chen, H. C.; Pan, C. L. Fish Sci 2002, 68, 170.
22. Jeon, Y. J.; Kim, S. K. Carbohydr Polym 2000, 41, 133.
23. No, H. K.; Park, N. Y.; Lee, S. H.; Meyers, S. P. Int J Food Microbiol 2002, 74, 65.
24. Zheng, L. Y.; Zhu, J. F. Carbohydr Polym 2003, 54, 527.
25. Wang, X. H.; Du, Y. M.; Liu, H. Carbohydr Polym 2004, 56, 21.
26. Hu, S. G.; Jou, C. H.; Yang, M. C. Biomaterials 2003, 24, 2685.
27. Qin, C. Q.; Du, Y. M.; Xiao, L.; Liu, Y.; Yu, H. G. J Appl Polym Sci 2002, 86, 1724.
28. Silverstein, R. M.; Webster, F. X. Spectrometric Identification of Organic Compounds, 6th ed.; Wiley: New York, 1998; p 81.
29. Qu, X.; Wirsén, A.; Olander, B.; Albertsson, A. C. Polym Bull 2001, 46, 223.
30. Peng, T.; Yao, K. D.; Yuan, C.; Goosen, M. F. A. J Polym Sci Part A: Polym Chem 1994, 32, 591.
31. Andrade, J. D. In Surface and Interfacial Aspects of Biomedical Polymers; Andrade, J. D., Ed.; Plenum: New York, 1985; Vol. 1, p 105.
32. Beamson, G.; Briggs, D. High Resolution XPS of Organic Polymers: The Scienta ESCA300 Database; Wiley: Chichester, England, 1992; p 277.
33. Helander, I. M.; Nurmiaho-Lassila, E. L.; Ahvenainen, R.; Rhoades, J.; Roller, S. Int J Food Microbiol 2001, 71, 235.
34. Kodjikian, L.; Burillon, C.; Roques, C.; Pellon, G.; Freney, J.; Renaud, F. N. R. Invest Ophthalmology Visual Sci 2003, 44, 4388.
35. James, N. R.; Jayakrishnan, A. Biomaterials 2003, 24, 2205.
36. Lin, C. W.; Lin, J. C. J Biomater Sci Polym Ed 2001, 12, 543.
37. Jia, Z. S.; Shen, D. F.; Xu, W. L. Carbohydr Res 2001, 333, 1.