Studies of Sulfonated Polyethylene for Biliary Stent Application

Mei-Chuan Peng,[1] Jui-Che Lin,[1] Chiung-Yu Chen,[2] Jiunn-Jong Wu,[3] Xi-Zhang Lin[2]

[1]*Department of Chemical Engineering, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan, Republic of China*
[2]*Division of Gastroenterology and Department of Medicine, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan, Republic of China*
[3]*Department of Medical Technology, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan, Republic of China*

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ABSTRACT: Palliative treatment for obstructive jaundice by endoscopic biliary stent insertion is a recent commonly used method. Unfortunately, stent reobstruction may occur within 3 to 6 months as a result of bacterial adhesion and formation of biofilm. Bacterial adhesion was postulated as the initial step of stent clogging and the bacterial enzyme activity of β-glucuronidase led to the deposition of calcium bilirubinate. In this study, surface sulfonation of the polyethylene lumen was postulated to improve the patency of the biliary stent. Surface modification with sulfonated group formation was carried out with fuming sulfuric acid containing 20 wt % sulfuric trioxide (SO_3). The reaction time varied from 1 to 3 h at room temperature. ATR-FTIR and ESCA techniques showed that the surface amount of sulfonated functionalities increased with sulfonation time. The contact angle of the sulfonated PE, determined by the sessile drop technique, decreased compared to that of unmodified PE, but cannot be detected by the captive bubble method because of the high surface hydrophilicity. SEM micrographs indicated that the sulfonated PE inner lumen remained relatively smooth after extended sulfonation reaction. Adhesion of *Escherichia coli* to the sulfonated PE stents after 48-h bile perfusion was about 10- to 20-fold less than that to the unmodified PE, as observed by SEM and surface spreading method. These results indicated that the surface sulfonated groups could effectively decrease the adhesion of *E. coli* in human bile, probably attributable to the hydrophilic repellence between the bacterial cell membrane and sulfonated groups. This finding suggested that the sulfonated PE tubing could prolong the patency period of plastic stents and may be of great potential as a biliary stent in a real clinical setting. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 2450–2457, 2004

Key words: biomaterials; polyethylene (PE); biliary stent; surface modification; ESCA/XPS

INTRODUCTION

Endoscopic biliary stent insertion is a well-known palliative treatment for obstructive jaundice in the clinical setting. In addition to gallstone, pancreatitis or tumors of metastasis of cancer cells also cause the bile tract to narrow and to be occluded.[1–8] Because surgery cannot proceed or might be dangerous for patients, a biliary stent could be considered for treatment. However, a biliary stent needs to be replaced about every 3 to 6 months because of stent blockage.[2]

Two major materials, metal and plastic, have been used to build biliary stents. Self-expanding metal stents prop up the blockage duct wall by itself with mechanical tension to prevent occlusion of the bile duct. It is unfortunate that ingrowth of the tumor tissue through the wire mesh may cause reobstruction of metal stents. Endoscopic removal of the metal stent is impossible after a few weeks because of incorporation of the stent into the bile duct wall. Surgical removal is possible but difficult. To overcome the problem of tumor ingrowth, plastic-covered metal stents or plain plastic endoprostheses have been designed. The mechanical tension of a plastic stent is not as good as that of a metal one, so it has a shorter live. However, it could prevent the ingrowth of tumor tissue and make it easier to remove. Once a plastic endoprosthesis is blocked, the endoprosthesis can be removed either endoscopically or can be pushed into the duodenum transhepatically.[9,10]

Most plastic stent blockage is caused by biliary sludge that consists of a mixture of bacteria and amorphous materials (proteins, calcium bilirubinate, fibers, etc.).[3–6] It is postulated that the mechanism of stent occlusion is initiated by protein adsorption and bacteria adhesion, after which bacteria multiply and begin to encompass crystals, all of which lead to creation of biofilm; finally the stent tends to occlude with a buildup of material that contains mainly calcium bilirubinate, some calcium palmitate, cholesterol crys-

Correspondence to: J.-C. Lin (jclin@mail.ncku.edu.tw).
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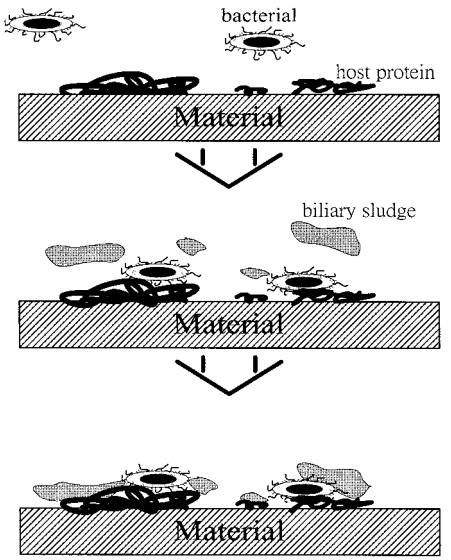

Figure 1 Schematic drawing for the possible mechanism leading to the occlusion of the biliary stent.

tals, and occasionally some retrograde deposition of ingested food in the matrix of the biofilm (Fig. 1).[7,8] It has been shown that live *Escherichia coli* in the bile is the probable factor producing the sludge that blocked biliary stents by production of β-glucuronidase, which can deconjugate bilirubin–glucuronide, resulting in precipitation of calcium bilirubinate.[3,11,12]

To decrease the rate of occlusion of plastic biliary stents, many studies have focused on preventing the first step of bacterial adhesion *in vitro* or *in vivo*. The earliest report was to add antibiotics, aspirin and doxycycline, or hydrophobic bile salt. Ampicillin–sulbactam was used in an *in vitro* model of stent occlusion with porcine gallbladder bile containing *E. coli* for 8 weeks and it decreased the formation of biofilm and occlusion.[7] Aspirin and doxycycline were given to reduce mucin secretion and suppress bacterial growth, the results of which investigation revealed that the appropriate antibiotic against the bacteria can effectively reduce surface bacterial adherence.[13] A hydrophobic bile salt, such as taurocholate (TCA) or taurodeoxycholate (TDCA), improves the solubility of sludge and reduce bacterial adhesion on plastic stents.[14] As for changing the design of a plastic biliary stent, common methods include the use of a larger diameter or creation of side holes around the stent.[1]

In clinical applications, polyethylene, polyurethane, and Teflon are the three most commonly used biliary stent materials. Recently, two new polymers, Vivathane® and Hydromer®, have come into the market, which were noticed on their ultrasmooth and hydrophilic surface properties, respectively. Both materials had been shown to reduce bacterial adhesion in the infected bile perfusion experiments *in vitro*.[15,16] Surface modification by silver coating on polyurethane led to a reduction of adherent bacteria compared with untreated, it was suggested that silver coating may have a potential benefit in preventing stent blockage.[17,18]

Device surface modified with heparin have attracted investigators' attention for development of nonthrombogenic blood-contact biomaterial surface. Heparin, a sulfonated glycosaminoglycan, depended on the biological properties of its molecular size (chain length) and electric charge (sulfonation).[19] Sulfonated groups have shown the anticoagulant activity and the pendent negatively charges expel blood component further by electrical repulsion.[20] Microbial attachment on sulfonated blood-contact biomaterial surface was also investigated by various research groups. Cooper and colleagues[21] investigated a series of functionalized polyurethanes, including pellethane, sulfonated pellethane, phosphonated pellethane, a zwitterionic phosphonated polyurethane, and quaternized amine polyurethane. Colonization assay was tested by *Staphylococcus aureus*, in which approximately 10^5 colony-forming units (cfu) in tryptic soy broth (TSB) or phosphate-buffered saline (PBS), were to be spread on polymer films for 1 h. Results showed no evident difference between pellethane and sulfonated pellethane.[21,22] Kim et al. grafted sulfonated groups onto polyurethane and poly(ethylene oxide) and tested the biological responses including platelet interaction and bacterial adhesion. *Staphylococcus epidermis* and *E. coli* were suspended in TSB or plasma and the polymer films were incubated in 10^8 cfu/mL for 24 h at 37°C for the adhesion study. All sulfonated polymer surfaces significantly reduced bacterial adhesion.[20,23,24] However, to our knowledge, none of the researchers explored the role of sulfonated groups in resisting microbial attachment onto polymeric biomaterials in human bile.

In this study, we attempted to prolong the patency of a biliary stent by surface modification with sulfonated functionalities on commonly used plastic stents. In previous studies, surface modification by sulfonation was carried out with a solution of fuming sulfuric acid, chlorosulfonic acid in dichloroethane, or a gaseous mixture of sulfur trioxide and nitrogen as sulfonating agents.[25–29] We chose fuming sulfuric acid as the surface sulfonation reagent to modify the inner surface of polyethylene (PE) tubing. Surface properties of the sulfonated PE tube were evaluated by Fourier transformed infrared spectroscopy in attenuated total reflectance mode (ATR-FTIR), electron spectroscopy for chemical analysis (ESCA), contact angle measurement, and scanning electron microscopy (SEM). In addition, a bile perfusion experiment within infected human bile was used to assess the capability in resisting bacterial adhesion.

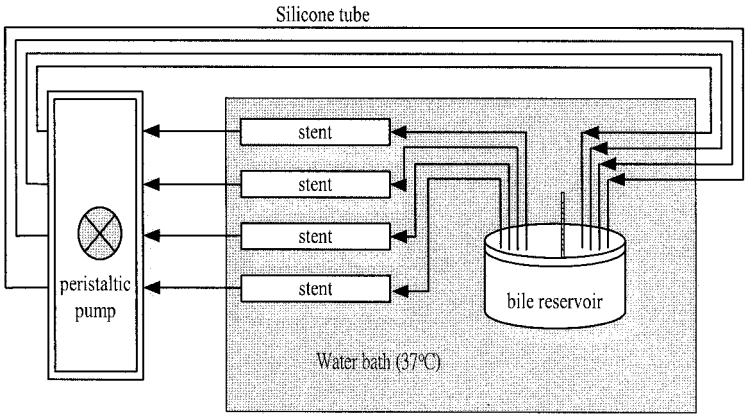

Figure 2 Schematic drawing for the continuous *in vitro* human bile perfusion model.

EXPERIMENTAL

Surface sulfonation of polyethylene tube

Polyethylene tubes (PE 330, Intramedic®, ID = 2.92 mm, OD = 3.73 mm, 20 cm long; Becton Dickinson, San Jose, CA) were first ultrasonically cleaned with detergent and distilled water for 2 h each to remove the surface contaminants, and then dried at 50°C in a vacuum oven for 24 h.

The cleaned PE tubes, while both ends were capped with glass tubes, were then carefully filled with fuming sulfuric acid (oleum, containing 20 wt % SO_3 in H_2SO_4; Riedel-de Haën, Seelze, Germany). The surface sulfonation reaction then proceeded at room temperature for 1 to 3 h. After surface sulfonation, the PE tubes were sequentially rinsed by 98% H_2SO_4, 50% H_2SO_4, distilled water, and acetone.[30] These sulfonated PE tubes were dried at 50°C in a vacuum oven for another 24 h. All dried tubes were then filled with 10*M* NaOH for 10 min to convert the surface acid functionality to its respective sodium salts (i.e., $R—SO_3^-Na^+$ and $R—COO^-Na^+$). After these procedures, all samples were preserved in distilled water, which was replaced daily, until being tested.

Surface characterization

The surface chemical configuration was characterized by ATR-FTIR and ESCA. The ATR-FTIR spectra were collected after 64 scans at a resolution of 4 cm^{-1} using an FTS 40A system (Bio-Rad, Cambridge, MA). The ESCA analysis was taken using a VG ESCA210 spectrometer (Thermo VG Scientific, West Sussex, UK) operated by Tainan Regional Instrumentation Center, managed by National Science Council, Taiwan.

In addition, the surface hydrophilicity of the inner surface of these sulfonated PE tubes, under ambient conditions as well as under an aqueous environment, was determined by the sessile drop method and the captive bubble technique, respectively (Model CA-A contact angle meter; Face Co., Tokyo, Japan). The inner section of all samples was also examined by scanning electron microscopy (SEM, JXA840; JEOL, Tokyo, Japan) to study the relative surface roughness before and after the surface sulfonation reaction as well as the bacterial adherence testing.

Bile perfusion experiment

All tools used in the perfusion experiment were either disposable or sterilized by UV radiation or autoclave before the experiment. Human bile, collected from patients in the National Cheng Kung University Hospital by the endoscopic nasal biliary drainage method, was first centrifuged at 8500 rpm for 40 min to remove the solid precipitates. The supernatant was further filtered by use of Whatman #5 filter paper (particle retention $> 2.5\mu m$; Whatman, Clifton, NJ). This bile solution was finally sterilized by Corning® (Corning, NY) disposable sterile filter with 0.22 μm pore size, and then preserved at -80°C in glass serum bottles wrapped with aluminum foil to prevent degradation by light exposure. This sterilized bile was cultured to ensure sterility and was generally used within 1 week.

A 25-mL solution of *Escherichia coli* (*E. coli* DH5α, 10^8 cfu/mL of sterilized normal saline) was resuspended in 225 mL of sterile human bile to a final concentration of 10^7 cfu/mL for the perfusion experiment. An *in vitro* flow model (Fig. 2) was set up to provide a continuous circulation of human bile for testing.[2] The PE tubes, with different degrees of surface sulfonation, were connected in parallel to the system by silicon rubber tubing. Because the content of human bile may vary from batch to batch, a nonmodified PE tube was used as the control in each experiment. The flow rate of the bile was set at 2 mL/min by a peristaltic pump (Cole-Parmer, Chicago, IL) while the temperature of the water bath was main-

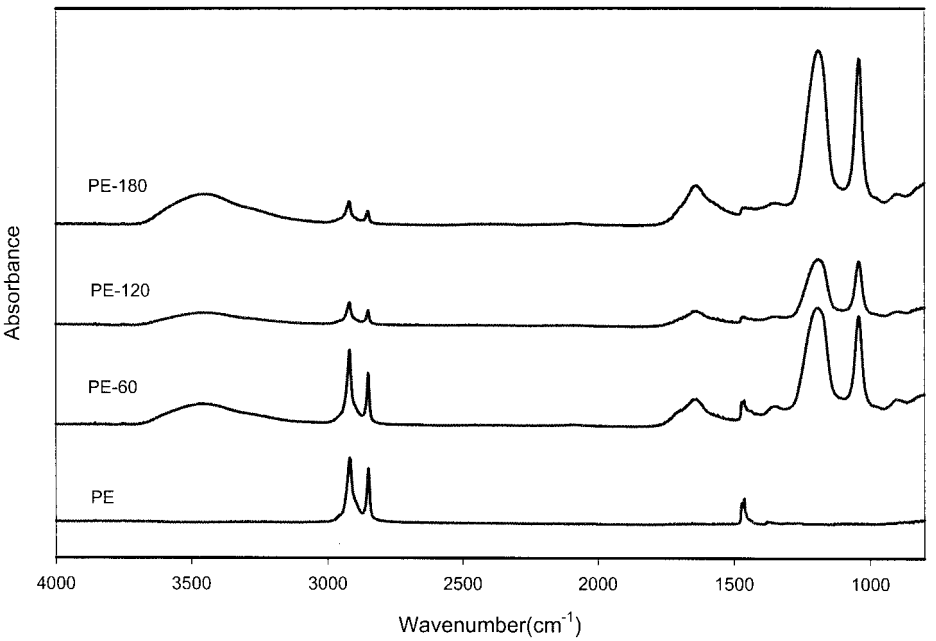

Figure 3 ATR-FTIR spectra for the sulfonated polyethylene tubing studied.

tained at 37°C throughout the testing. The polymer-coated tubing was equilibrated with sterilized deionized water for 12 h right before the bile perfusion experiment. After 48 h, the PE tubes were removed and rinsed gently with sterile PBS. The tubes were then cut into three sections: one was fixed in 5% glutaraldehyde (Sigma, St. Louis, MO) solution and dried for SEM evaluation, and the other two were left for counting the adherent bacterial.

Bacterial enumeration was completed by the surface spreading method.[32] The adherent *E. coli* was removed by either scraping with a spatula or ultrasonication in 4 mL of sterilized normal saline. Then a series (at least six) of 10-fold dilutions for the collected bacterial suspension were prepared. A 100-μL sample of each dilution was pipetted onto each agar plate and then spread over the whole surface using a flamed glass spreader. The plates were incubated at 37°C overnight, after which the colonies were counted. SEM analyses were also performed on these PE specimens after *E. coli* removal to verify the efficacy of these two bacterial removal techniques (i.e., spatula scraping and ultrasonication).

RESULTS AND DISCUSSION

Surface characterization

The sample nomenclature for the sulfonated PE tubing in the subsequent discussions is PE-*x*, where *x* indicates the sulfonation time in minutes. The surface chemical configuration of the sulfonated sample was first evaluated by ATR-FTIR analysis (Fig. 3). To avoid potentially ill-defined IR spectra related to the variations of the hydrogen bond formation and uncontrollable amounts of surface-embedded water caused by the sulfonic and/or carboxylic acid functionalities, the sulfonated PE tubing was transformed into the form of salts by immersion in 10*M* NaOH.[33]

ATR-FTIR spectra clearly indicate that the CH_2 bending peak (1472 cm^{-1}) and C—H stretching peaks (2850–2940 cm^{-1})[33,34] were gradually diminished with the sulfonation time. In contrast, the peaks associated with symmetric and asymmetric SO_3^- stretching modes (1045 and 1170 cm^{-1}) and sulfuric acid ester ($\sim$ 1230 cm^{-1}) were increased. In addition to the sulfonic/sulfonate functionalities found on the top few micrometers' depth of surface layer, a broad feature, centered around 1670 cm^{-1}, was noticed. This can be assigned to the combination of carbonyl functionality ($\sim$ 1700 cm^{-1}) and HOH bending (1640 cm^{-1}).[33,35] This indicated that surface oxidation also occurred on the SO_3/H_2SO_4-modified PE surface. A broad peak around 3500 cm^{-1} was also observed that can be attributed to the OH stretching mode. In combination with this OH band and the HOH bending peak found earlier, surface hydration on these modified PE tubes is evident.

Besides the ATR-FTIR analysis, the ESCA technique was performed to characterize the surface chemical configuration of the top 10- to 250-Å thickness, rather than the thickness of only a few micrometers by the ATR-FTIR technique, of these sulfonated PE tubes.[36] Results indicated that the sulfur atomic percentage was increased after reaction with the SO_3/H_2SO_4 (Ta-

TABLE I
ESCA Analysis for Various Sulfonated Polyethylene Tubing

	Atomic percentage (%)				Ratio	
Material	C	O	S	Si	O/C	S/C
PE	87.4	7.5	0	5.1	0.09	0
PE-60	65.1	25.5	4.5	4.8	0.39	0.069
PE-80	62.3	27.1	4.7	5.9	0.44	0.075
PE-120	60.4	29.1	5.0	5.5	0.48	0.082
PE-180	62.0	27.9	5.1	5.0	0.45	0.082

ble I). However, the extension of the treatment time did not significantly vary the S atomic percentage. In addition, the O atomic percentage was increased after the reaction, indicating that surface oxidation also occurred along with the surface sulfonation. This finding can be supported by the ESCA C1s spectra of various sulfonated samples (Fig. 4). A broadened C1s peak, with a tail at the higher binding energy region, was observed on the sulfonated samples. This can be attributed to the formation of carbonyl functionalities (C═O) and/or C—O groups on the surface.[37] Interestingly, the C1s peaks of these sulfonated samples are similar to each other, suggesting that the degree of surface oxidation was alike among the specimens tested. Further analysis of the S2p spectra (Fig. 5), which exhibited a broad peak ranging from 166.2 to 170.2 eV, revealed that the sulfur-containing groups created were mainly sulfur atom bound with three oxygen atoms (i.e., sulfonic acid functionality).[38]

Similar values for the Si atomic percentage were observed in this study and this might be attributed to either the additives used for PE tubing processing or the containments associated with the sample preparation. However, this finding would not alter our interpretation for the surface sulfonation and surface oxidation described above.

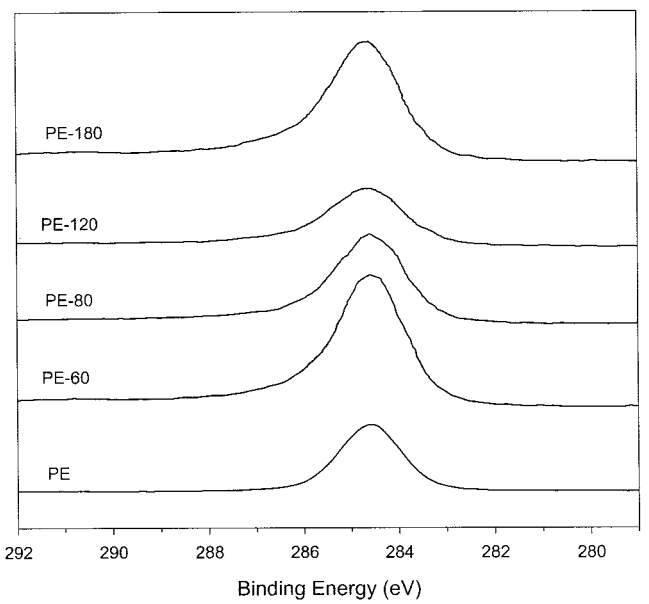

Figure 4 C1s spectra of various sulfonated polyethylene tubing and nontreated control.

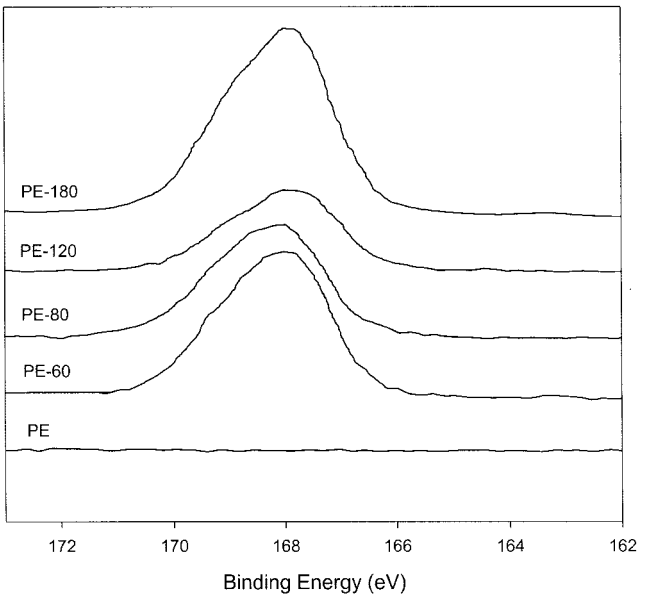

Figure 5 S2p spectra of various sulfonated polyethylene tubing and nontreated control.

The surface hydrophilicity of modified PE tubes was determined by the sessile drop and captive bubble techniques, which probe the top 3- to 20-Å–thick layer (Table II).[36] These results indicated that the surface hydrophilicity increased with sulfonation time. This could be related to the increase of hydrophilic functionalities, such as sulfonic acid and carbonyl groups, in the surface layer consisting of only the top few angstroms. However, based on the ATR-FTIR and ESCA analyses, the chemical composition of the deeper surface layer, ranging from about 250 Å to a few micrometers, instead did not vary significantly with the sulfonation time.

TABLE II
Contact Angle Results for Various Sulfonated PE Tubings Studied

	θ-value	
Material	Sessile drop method (water-in-air)	Captive bubble method (air-in-water)[a]
PE	102.0 ± 1.3	84.9 ± 1.9
PE-60	33.1 ± 2.7	ND[b]
PE-120	27.5 ± 2.8	ND[b]
PE-180	23.0 ± 2.8	ND[b]

[a] The sample was preimmersed in water for 24 h.
[b] Not determined because the air bubble cannot attach to the water-immersed surface.

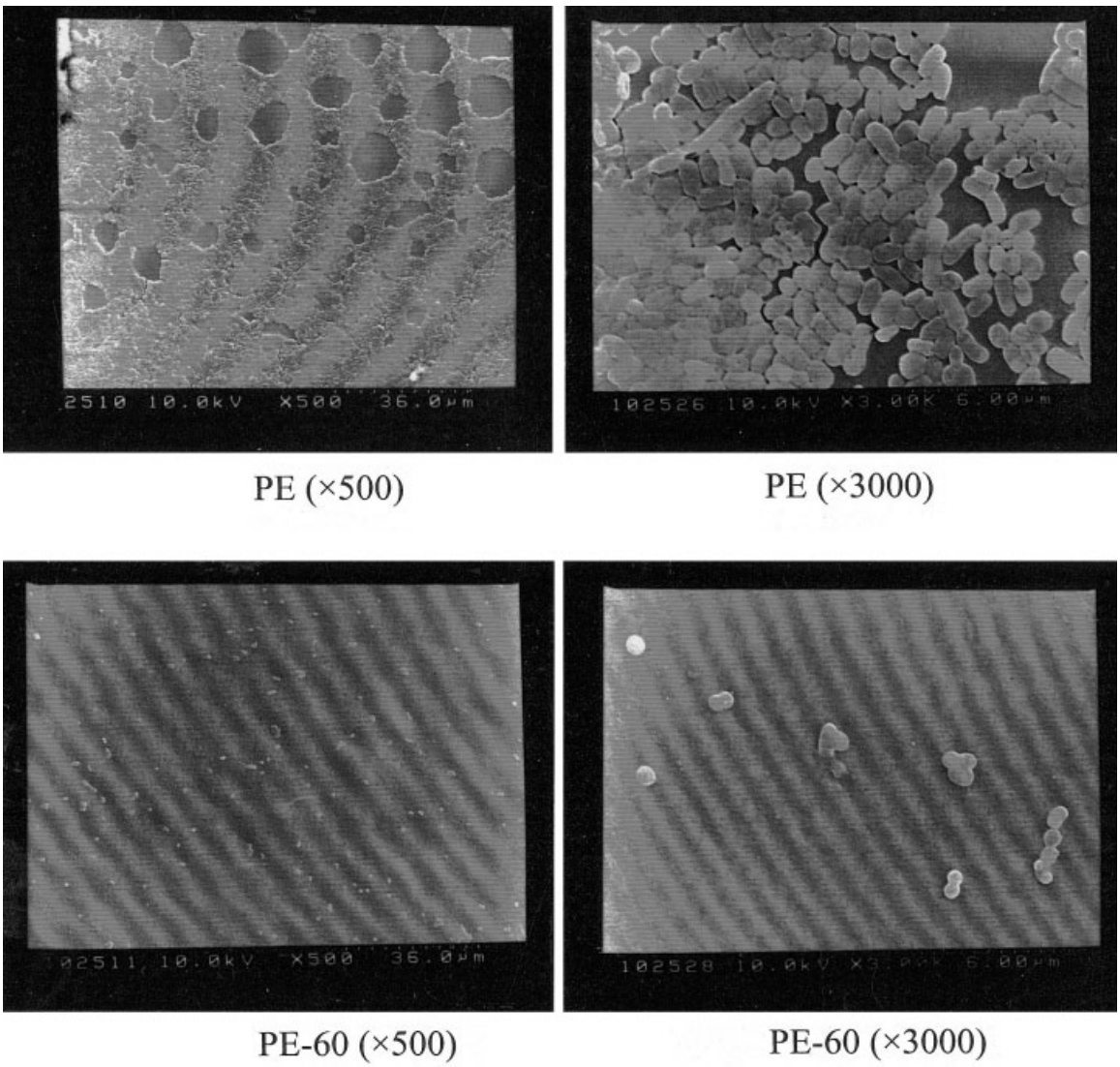

Figure 6 SEM micrographs for *E. coli* adhering to various polyethylene tubing after 48 h bile perfusion experiments.

The surface morphology of the sulfonated PE tubes was examined by SEM analysis, results of which indicated that the sulfonation reaction does not lead to any significant variation in surface morphology (data not shown).

Bile perfusion experiment

After 48 h of perfusion, biofilm formation was observed on all of the sulfonated PE surfaces as well as on the nontreated PE (Fig. 6). The image of the nontreated PE sample indicates a monolayer of biofilm deposition on some parts of the surface but no evident slime or amorphous materials. Further SEM morphological evaluation revealed that the sulfonated families had less bacteria adhesion than did the unmodified PE. However, no conspicuous difference in microbial adhesion among these sulfonated samples was observed. The *E. coli* adhesion density on various sulfonated polyethylene tubing, determined by ultrasonication and scratching method, is shown in Figure 7. The *E. coli* adhesion density on nontreated PE was about $51.8 \pm 59.2 \times 10^5$ cfu/cm^2 by the ultrasonication removal method and $63.9 \pm 69.8 \times 10^5$ cfu/cm^2 by the scratching technique ($n = 3$). PE-60, which was sulfonated for 60 min, adhered about $4.4 \pm 2.2 \times 10^5$ and $5.6 \pm 4.5 \times 10^5$ cfu/cm^2, which is approximately 10% of the unmodified PE. The bacterial adhesion amounts on PE-120 and PE-180 were similar to that of PE-60 and the lowest value, $2.7 \pm 1.7 \times 10^5$ cfu/cm^2 (which is only about 5% of the unmodified PE), was found on PE-180 by the scratching method. These results indicated that adhesion of *E. coli* to the sulfonated PE stents in human bile was about 10- to 20-fold less than rate of adhesion to unmodified PE. These experimental results indicated that the surface sulfonated groups

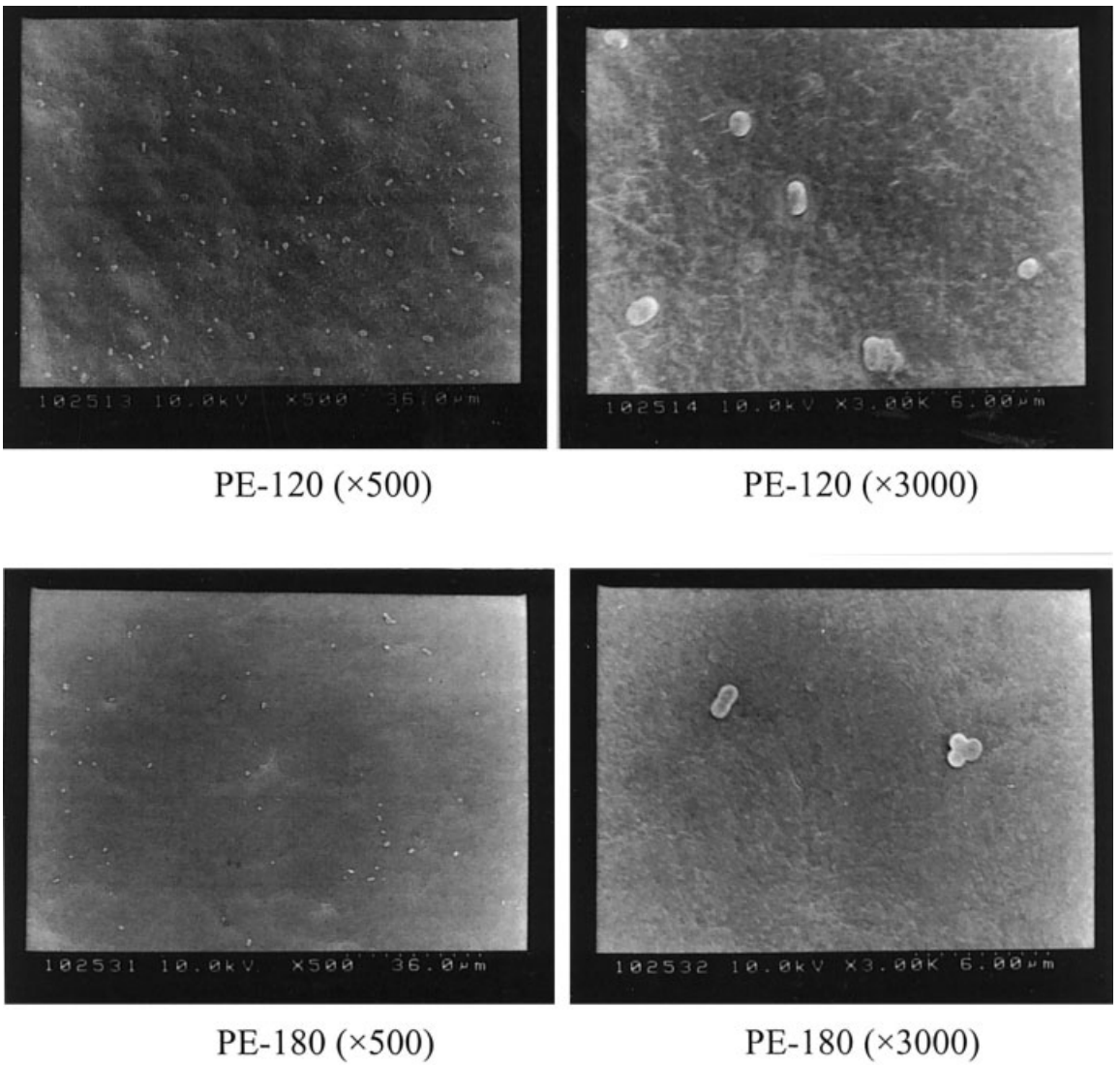

Figure 6 (*Continued from the previous page*)

could effectively decrease the adhesion of *E. coli* in human bile. The surface negative charge associated with the sulfonated groups probably induced the hydrophilic repellence between two hydrophilic surfaces, sulfonated PE and bacteria cell membrane, under an aqueous environment (i.e., human bile) and thus resulted in a decreased adhesion of bacteria on sulfonated PE. Moreover, the close similarity between the two bacterial adhesion density values, as determined by different microbial removal techniques, sonication or specula scratching, indicated that either one of the methods is effective in removing the surface-adhered *E. coli*.

It is worth noting that the human bile used in each experiment was collected from different patients. Therefore, the content in different batches of bile was then not exactly the same, although the treatment process was the same. This might be one of the reasons for the large standard deviation in the bacterial adhesion density on various PE tubes tested.

CONCLUSIONS

Stent clogging remains a serious problem in endoscopic biliary stenting: stents are blocked again generally within 3 to 6 months by sludge deposition in most patients. In this study, surface sulfonation of the inner lumen was used with the aim of improving the patency rate of the polyethylene biliary stent. Surface characterization results with ATR-FTIR and ESCA techniques indicated the surface amount of sulfonated functionalities increased with sulfonation time. Moreover, the contact angle of the sulfonated PE was decreased after being put in contact with fuming sulfuric acid. After 48 h of bile perfusion, adhesion of *E. coli* to the sulfonated PE stents in simulated infected human bile was less than that to unmodified PE. This finding can be attributed to the surface negative charge effect associated with the sulfonated groups that induced the hydrophilic repellence between sulfonated PE and the bacteria cell membrane. The sulfonated PE tubing

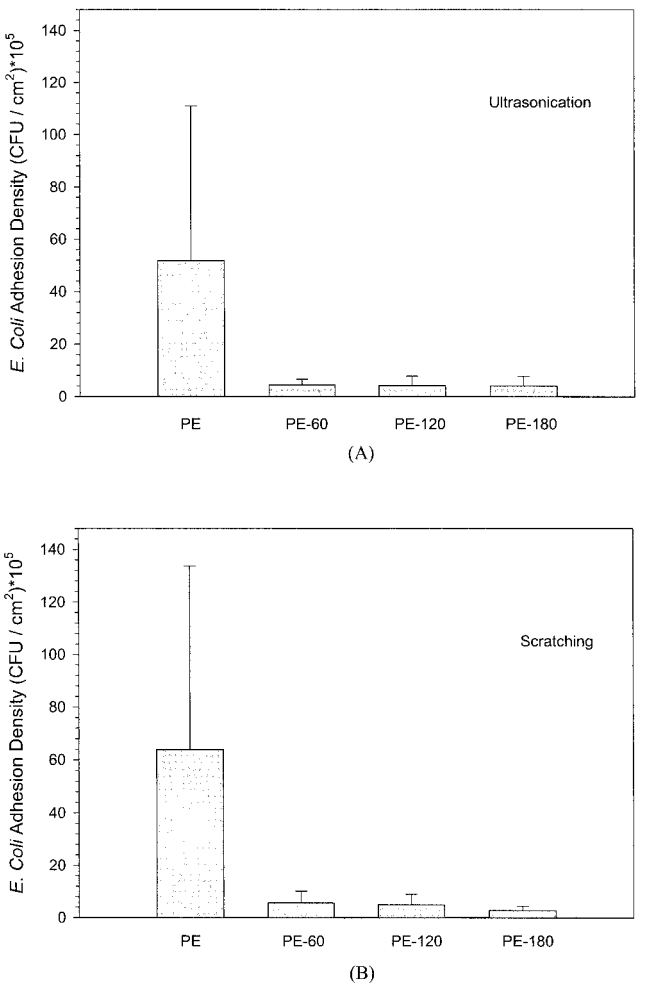

Figure 7 *E. coli* adhesion density on various sulfonated polyethylene tubing determined after (a) ultrasonication or (b) scratching the surface.

may be of potential as the biliary stent in real clinical settings and further *in vivo* animal experiments are currently in the planning stage.

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