Syntheses of Novel Chitosan Derivative with Excellent Solubility, Anticoagulation, and Antibacterial Property by Chemical Modification

Hengquan Liu,[1,2] Yuancong Zhao,[2] Si Cheng,[1,2] Nan Huang,[1,2] Yongxiang Leng[1]

[1]*Key Laboratory of Advanced Technology of Materials, Chinese Education Ministry, Chengdu 610031, China*
[2]*School of Material Science & Engineering, Southwest Jiaotong University, Chengdu 610031, China*

Received 10 September 2010; accepted 4 May 2011
DOI 10.1002/app.34889
Published online 2 November 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The soluble and antibacterial chitosan derivative was prepared on the basis of the regioselective chemical modification. The *N*-(2-phthaloylation) chitosan was obtained via the reaction of chitosan with phthalic anhydride in *N*,*N*-dimethylformamide (DMF) at 130°C, and *O*-(3,6-hydroxyethyl) chitosan was produced using chlorohydrins as grafting agent and hydrazine hydrate as reductant. The structure of hydroxyethyl chitosan (HC) was characterized by X-ray diffraction (XRD), Fourier Transform infrared spectroscopy (FTIR), and gel permeation chromatography (GPC) respectively. The solubility, anticoagulation, and antibacterial property were assessed separately. The result shows that amine I of chitosan is replaced and the amide II disappears during chemical modification, and the functional groups of C6-OH and -NH2 are also reacted. The water-solubility of the novel chitosan derivative was enhanced relatively; it could even slightly soluble in methanol. The results of platelet adhesion and the activated partial thromboplastin times (APTTs) indicate that grafting hydroxyethyl could improve anticoagulation of chitosan. The antibacterial activity of HC against *Enterococcus* and *E. coli* had been much better owing to enhancing the degree of protonation. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 2641–2648, 2012

Key words: chitosan derivatives; solubility; anticoagulation; antibacteria; chemical modification

INTRODUCTION

Antithrombogenic and antibacterial biomaterials have been of great interest as one of the ultimate objectives for developing artificial organs and medical devices.[1–3] Surface modification via increasing hydrophilicity is believed to be a useful method for improving blood compatibility,[4–6] now many modified polymer biomaterials have been used to prevent plasma protein adsorption, platelet adhesion, and thrombus formation. On the other side, prevention of biomaterials-related infections remains a major dilemma in clinic practice, and the problem causes high rates of mortality and morbidity and significant increases in health care costs.[7–9] The surface adhesion is mediated by physicochemical interactions between the bacteria and biomaterials. So the surface modification of biomaterials or devices is a relatively straightforward strategy for creating the desirable surfaces which will decrease the susceptibility to bacterial adhesion.[10,11]

Chitosan is a polysaccharide composed of *N*-glucosamine and *N*-acetyl- glucosamine units, in which the number of *N*-glucosamine units exceeds 50%.[12–14] Chitosan has found several applications due to its excellent chemical, physical, and biological properties, such as biocompatibility, biodegradability, nontoxicity, adsorptive properties, film-forming ability, and antimicrobial activity.[15,16] However, despite its desirable characteristic, its actual use is limited because of its poor solubility in water. Clearly, one of the reasons for the chitosan lies in the rigid crystalline structures, and the acetamido or primary amino groups of (*N*-acetyl-)D-glucosamine residues have an important role in the formation of peculiar conformational features through intra- and/or intermolecular hydrogen bonding. Therefore, removal of the two hydrogen atoms of amino groups of chitosan and introduction of some hydrophobic nature by chemical modifications will cause destruction of its inherent crystalline structure, resulting in the improvement of the water solubility, so its

Correspondence to: N. Huang (huangnan1956@163.com).
Contract grant sponsor: Key Basic Research; contract grant number: 2005CB623904.
Contract grant sponsor: NSFC; contract grant number: RGC30831160509.
Contract grant sponsor: Program for New Century Excellent Talents in University; contract grant number: 06-0800.

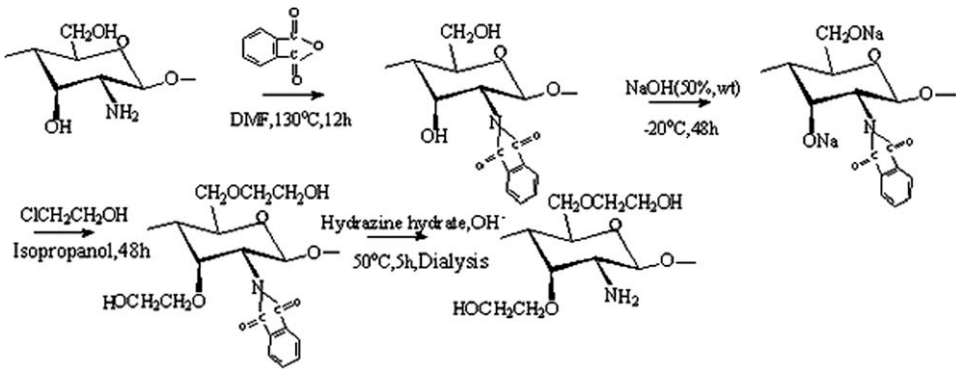

Figure 1 The schematic diagram of synthesis of N-chitosan and HC.

derivatives could widen their applications.[17–22] Several efforts have been reported to prepare solubility by chemical modifications, even a few examples attained solubility in general solvents.[23,24] However, antibacterial activity of most chitosan derivatives decreases or disappears owing to substitution of $-NH_2$ of chitosan.[25] Some studies have found several factors about bactericidal activity of chitosan and its derivatives, and the charge character of chitosan have been proposed: the positive charge would be produced through protonation of -NH_2 at acid condition ($NH_2 \xrightarrow{H^+} NH_3^+$), and the cationic nature of chitosan and its derivatives helps them to bind with sialic acid in phospholipids, and then restrains the movement of microbiological substances; another interaction between positively charged chitosan derivative and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents. In the case of blood contacting applications, chitosan has been shown to be highly thrombogenic.[26–30] In fact, the positively charged chitosan tends to attract plasma proteins, which were adsorbed on the material surface. This protein adsorption in turn results in platelet adhesion and activation on the surface of the material, thrombus formation, and ultimately device failure.

This work describes the preparation of chitosan derivatives, in which the $-NH_2$ of chitosan was protected by phthaloylation, and the $-NH_2$ would be recovered using hydrazine hydrate. The results of this work could improve some properties of chitosan related medical devices.

EXPERIMENTAL

Material

Chitosan, its degree of deacetylation was determined by potentiometric titration and its viscosity molecular weight (M_w) was 2.2×10^5, was purchased from Sigma, Chemical Reagent Co. Ltd. The chlorohydrin, phthaloylation, DMF, and sodium hydroxide was purchased from the chemical reagent of Changzheng in Chengdu, excellent grade. The *E. coli* and *Enterococcus* were supplied from West China School of Preclinical and Forensic Medicine in Chengdu.

Preparation of chitosan derivative

As shown in Figure 1, a mixture of chitosan (5 g, 30 mmol) and phthalic anhydride (13.5 g, 90 mmol) in DMF (100 mL) was heated and stirred at 130°C under a nitrogen atmosphere. After 12 h, the mixture became a clear and viscous solution. The precipitates were obtained by pouring the solution into ice-water and collecting by filtration, those residues were successively washed completely by Soxhlet's extraction with ethanol, and dried over P_2O_5 to give *N*-chitosan.[31] *N*-chitosan (5 g) and sodium hydroxide aqueous solution 20 g (50.0%, wt) were added to a double-neck bottle. The mixture was cooled to −20°C and was frozen for 48 h. To extrude superfluous sodium hydroxide, then chlorohydrin was dropwised to the mixture of *N*-chitosan and isopropanol (150 mL), and allowed to react for 48 h. The mixture was separated through centrifugal settling, after that, hydrazine hydrate (90 mmol) was added to resultant dreg, and the pH of above blending was adjusted to 8.0 with hydrochloric acid solution, ensuring it to react at 50°C for 5 h under stirring. The dreg was collected by filtration, and washed by ethanol completely; the novel chitosan derivative was obtained. The dialysis method was used to purify HC with bag filter ($M_w = 3000$) in water.

Structure of chitosan derivative

X-ray diffraction patterns of chitosan and HC were obtained using a X'Pert Pro MPD (Philips, Netherlands) diffractometer with Cu Kα irradiation ($\lambda = 0.154060$ nm). The crystallization index (CrI) of samples were evaluated according to the relevant literature[32]:

$$\mathrm{CrI}\% = \frac{I_{110} - I_{\mathrm{am}}}{I_{110}} \times 100\% \quad (1)$$

where I_{110} is the maximum intensity ($2\theta = 20^0$) of the (110) lattice diffraction, and I_{am} is the intensity of amorphous diffraction at $2\theta = 10^0$.

The FTIR spectrum of HC was obtained with a Nicolet 5700 spectrometer. After being dried completely at 50°C, the powders could be used for FTIR analysis with ordinary KBr pellet method. Degrees of acetylation (DAs) of chitosan were determined with the method as the literature.[33] The —OH band at 3450 cm^{-1} as a reference, the acetyl content (%) was determined from the ratio of absorbances:

$$\mathrm{DAs} = \left(1 - 1.15 \times \frac{A_{1655}}{A_{3450}}\right) \times 10,065,285 \qquad (2)$$

In addition, Weight-average molecular weights (M_w) of the samples were measured by GPC according to literature.[34]

Characterization of chitosan derivative

Chitosan films were prepared by dissolving chitosan in 1% acetic acid creating a 2% w/v chitosan solution. An amount of 65 mL of the solution was poured into a 60 mm diameter polystyrene Petri dish and dried at 50°C until a film was formed. The film was removed from the Petri dish and punched to obtain 15 mm diameter circles. The contact angles of the test liquids on the sample surface were measured by the sessile drop technique using a contact angle geniometer (JY-82, China). Double distilled water and diiodomethane were used. The results shown in this article represent the statistical averages of six measurements on different regions of the sample surface.

The zeta potential was measured using a Zetasizer (Malvern, UK). The chitosan was dispersed in100 mM/L sodium acetate buffer and the HC was suspended in double distilled water, and measured under the automatic mode.

One gram powder of chitosan and HC were dispersed in 9 g H_2O respectively, then stirred at 25°C for 12 h. Undissolved section was separated and washed with acetone, then dried in an oven in a vacuum. We defined solubility (S):

$$S = \frac{1 - M}{1} \times 100\% \qquad (3)$$

M, the weight of undissolved section (g)

The experiment was carried out three times and we draw the average number.

Anticoagulation

The whole blood was centrifuged at 1000 rpm for 15 min to obtain platelet rich plasma (PRP). Chitosan and HC solution were deposited on 316 L stainless steel plate and the surface was incubated with 500 μL of PRP in 24 well plates at 36°C ± 1°C. At the end of 2 h, the PRP was aspirated from the wells, and surface was rinsed with phosphate buffered saline (PBS) to remove nonadherent platelets. They were then fixed for 1 h in 1% paraformaldehyde (PFA). Blood platelet adhesion specimens were observed by SEM (S450, Japan Hitachi). The size of all of the samples applied to *in vitro* experiments was 10 mm × 10 mm, and the amounts of the samples used for statistical count are not less than four.

For the APTTs measurement, the sample (diameter: 1 cm) was attached to a silcaned tube (diameter: 3 cm; height: 2 mm). The fresh human platelet-poor plasma (PPP) (100 μL) and actin-activated cephthaloplastin reagent (100 μL) were added, followed by the addition of a 0.03M $CaCl_2$ solution (100 μL) after a 3-min incubation at 37°C. The clotting time of the plasma solution was measured by a coagulometer (Clot 1A, Innova).

Antibacterial assess

Antibacterial activity of samples against *E. coli* and *Enterococcus* was evaluated using method described as follows: A representative bacteria colony was picked off, placed in a nutrient broth (peptone 10 g, beef extract 3 g, NaCl 5 g in distilled water 1000 mL; pH 7.0–7.2), and incubated at 37°C for 24 h. The obtained fresh culture where bacteria cells grew luxuriantly was ready for antibacterial test. 0.2 mL of the fresh culture was inoculated to the plate covered chitosan derivatives and incubated in a shaking bed (150 rpm) at 37°C for 24 h. After being rinsed, fixed and critical point dried, the specimens were coated with gold-palladium and observed by SEM (S450, Japan), the quantitative assay of both bacteria was accounted at the same surface area and magnification.

Statistical analysis

The results were expressed as mean standard error, with $n > 3$ per group for all comparisons. Statistical significance was determined by using Student's t-test for three groups of data. The level of significance was selected as $P < 0.05$.

RESULTS AND DISCUSSION

Structure of HC

Figure 2 shows the X-ray diffraction patterns of chitosan and HC. A sharp peak at $2\theta = 20°$ (amine II of chitosan) and a broad peak with a maximum at $2\theta = 10°$ (amine I of chitosan) were observed separately. To the HC, besides the amine II peak, a sharp peak

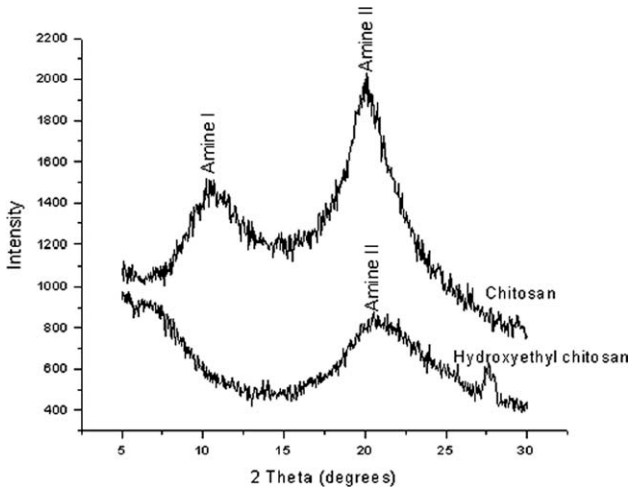

Figure 2 The X-ray diffraction patterns of chitosan and HC.

at $2\theta = 10°$ disappeared. The CrI of chitosan was 28.2% as formula (1), showing a slightly higher of 20.1% of HC, which indicates that the crystallinity of chitosan decrease relatively through chemical modification.

The FTIR spectrum indicates some differences in the absorption intensities from Figure 3. As expected, *N*-deacetylation is associated with a progressive weakening of the band occurring at 1665 cm^{-1} (amide I) Furthermore, the vibrational mode of amide II at 1550 cm^{-1} for chitosan was observed, but the amide II disappeared owing to chemical modification. The weak absorptions around 3500 cm^{-1} in HC indicated that there are minor free -OH groups. A marked change of the shape and the intensity of CH_2 at 1455 cm^{-1} stretching bands of HC were found, which suggests a different arrangement of primary hydroxyethyl, but the bending mode of $-CH_2$ groups present in the 1425 cm^{-1} region. Both shifts and intensity changes of the bands have been related to -OH of

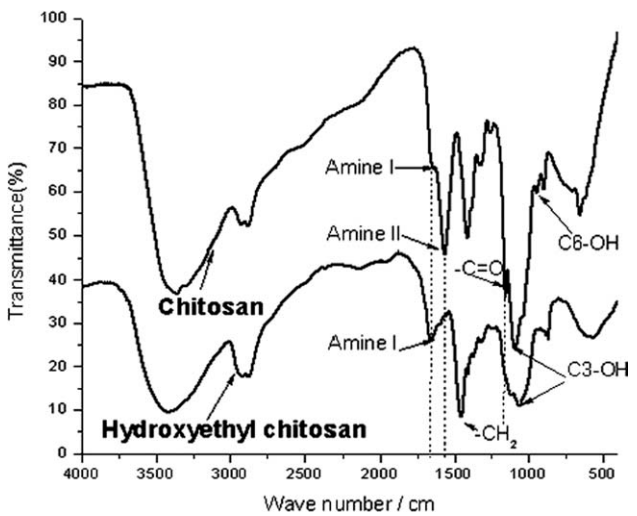

Figure 3 The FTIR spectra of chitosan and HC.

TABLE I
Contact Angles and Surface Energy Components

Sample	θ_{water}	$\theta_{diiodomethane}$	γ_s (nJ/cm^2)	γ_s^{LW} (nJ/cm^2)	γ_s^{AB} (nJ/cm^2)
316L SS	82.9	41.4	39.14	35.86	3.28
Chitosan	79.0	46.0	37.69	32.1	5.59
HC	39.4	52.4	60.02	30.75	29.27

modifications at C3 and C6 position of chitosan, the grafting hydroxyethyl($-CH_2CH_2OH$) in this region makes it easy to assess the kind of chain packing. The Degree of acetylation (DAs) of chitosan was 76.65% as formula, (2) relatively a lower of 84.10% of HC, which shows that the DAs of chitosan increase relatively via chemical modification.

Contacting angle and interface free energy

A series of contact angle data on different surfaces was shown in Table I. The surface free energy components were presented by the Lifshitz-van der Waals/acid-base approach (LW-AB).[35] It can be seen that contact angle with water of HC is much less than 316L SS and chitosan, and the interfacial interaction energy increased obviously. Therefore, the wettability of chitosan was better than the HC.

Solubility and molecular weight

Table II shows the solubility and molecular weight of chitosan and HC. The results show that chitosan derivates with $M_w = 1.96 \times 10^5$ can dissolve in H_2O, it was even slightly soluble in methanol. It suggests that HC is a good water-soluble chitosan derivate due to decreasing intermolecular interactions,[36] such as van der Waals forces, and then increased water solubility. The result also shows that the molecular weight of chitosan relatively decrease owing to the breakdown molecular chain during chemical modification.

The chitosan has poor water solubility and can only swell in deionized water or under acidic condition, which perhaps should be attributed to its high crystallization degree and high molecular weight of chitosan.[37,38] This has been preliminarily demonstrated to improve solubility of chitosan by using appropriate chemical modification such as introducing water-soluble entities, hydrophilic moieties, bulky

TABLE II
The Solubility and Molecular Weight of Chitosan and HC

Samples	Water (*S*, %)	Methanol (*S*, %)	M_w	M_n
Chitosan	–	–	2.21×10^5	4.50×10^4
HC	92% ± 4.5%	16% ± 5.2%	1.96×10^5	4.30×10^4

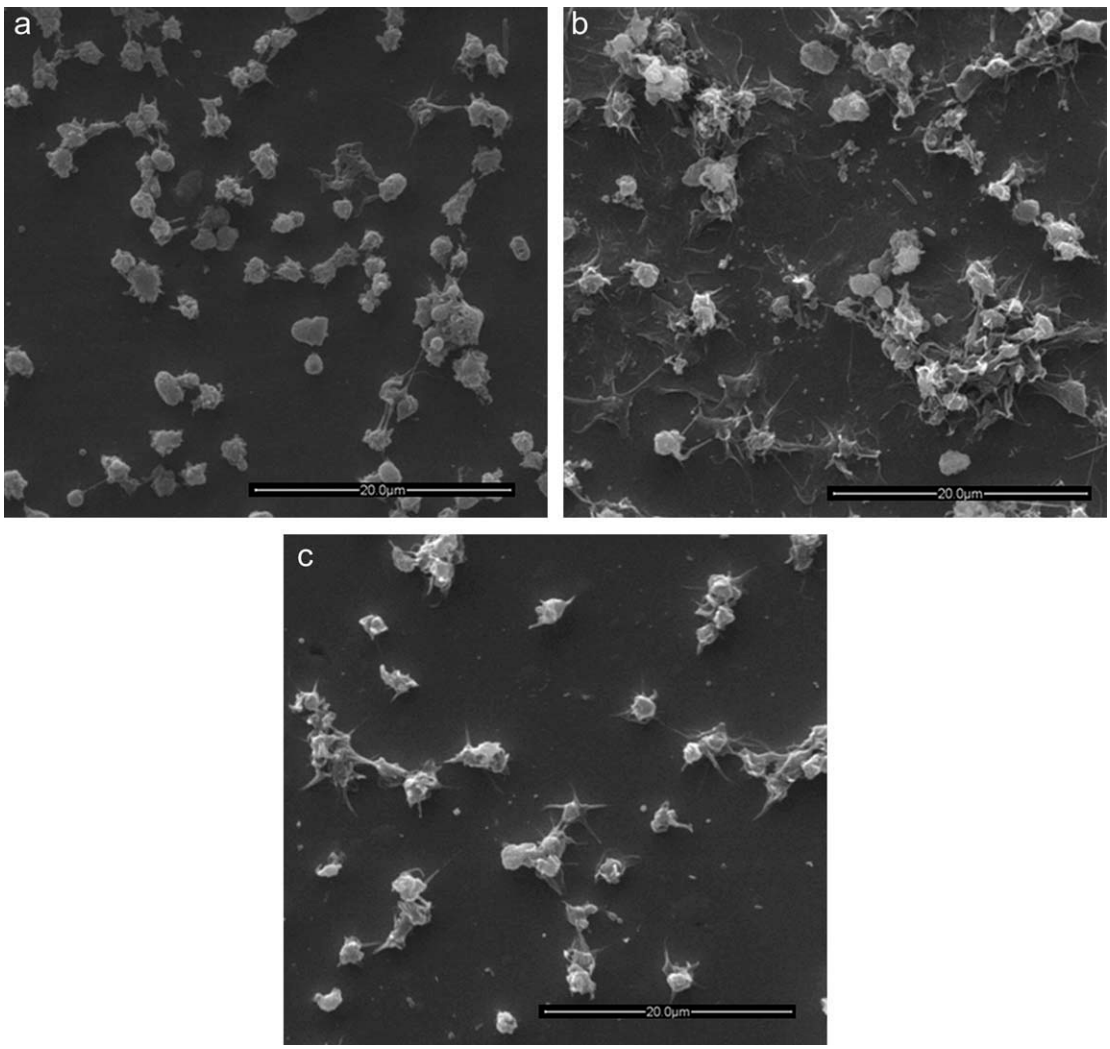

Figure 4 The SEM photographs of *in vitro* platelet adhesion: (a) 316L SS, (b) chitosan, and (c) HC.

and hydrocarbon groups, etc.[37–39] Comparing with chitosan, the CrI of HC reduces to 20% and the molecular weight also deceases as shown in Figure 2 and Table II, respectively. The incorporation of hydroxyethyl would decrease intermolecular hydrogen bond effectively. As expected, the chitosan copolymer grafted hydroxyethyl has well water solubility.

Anticoagulation

Figure 4 shows the SEM morphology of adhered platelets on various surfaces after incubation for 2 h. The amount of adhered platelets is significantly reduced on the surface of HC sample, and the adherent platelets exhibit minimum aggregation, and morphological changes of the platelets is also less than chitosan. The platelets of 80% are in the aggregation and pseudopodium state on the surface of chitosan sample. At the same magnification, there is an average of 7000 to 7500 contact-adherent platelets per 1 mm^2 area on the 316L SS surface. In contrast, about

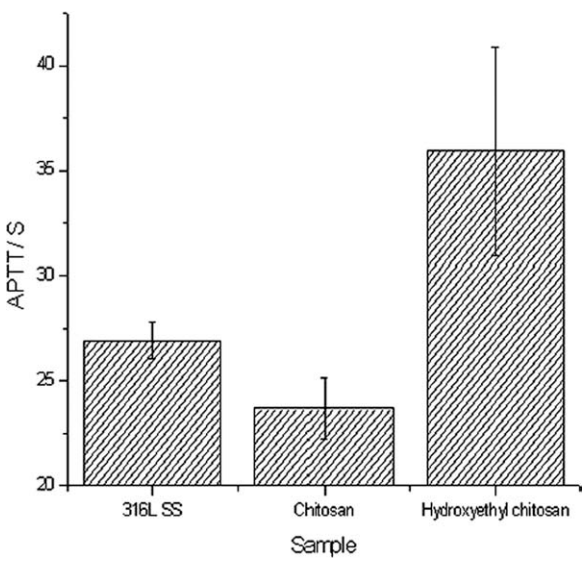

Figure 5 The activated partial thromboplastin times (APTTs) of various samples.

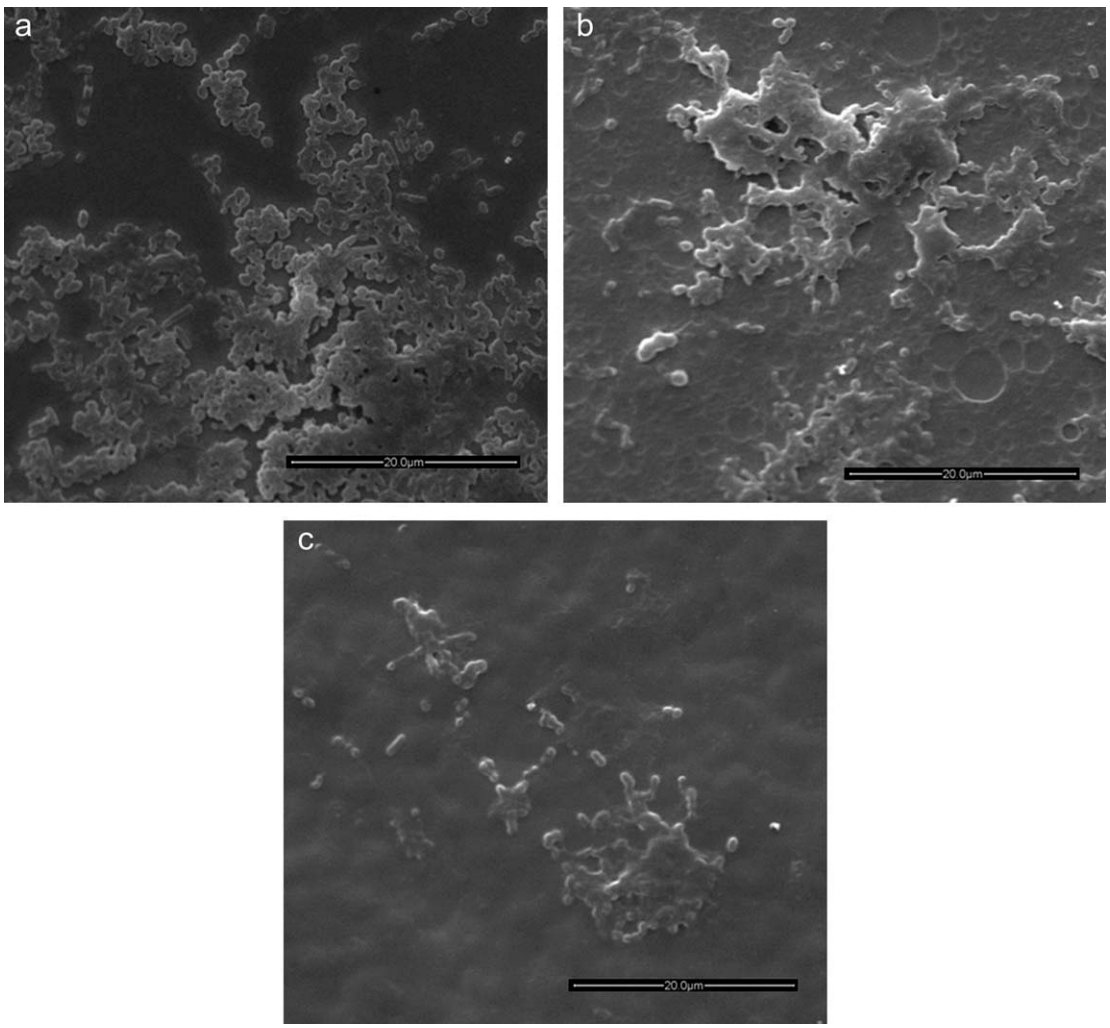

Figure 6 The SEM morphologies of the bacterial colonization: (a)316L SS, (b) chitosan, and (c) HC.

8500 platelets are present on the same area of the chitosan sample, while platelets adhered on HC are only as half as on chitosan. Our data suggest that adhesion of platelets can be significantly suppressed by grafting hydroxyethyl onto the -OH of chitosan and the number of adhered platelets depends on the substitution degrees.[11]

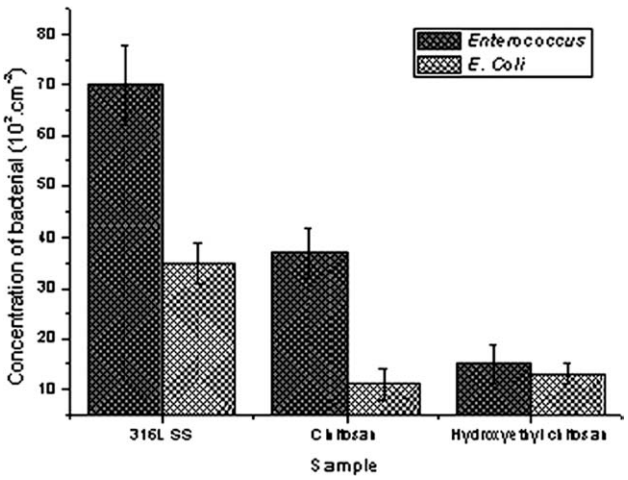

Figure 7 The amount of both bacteria adhered to the various surfaces.

To examine bioactivity of the HC *in vitro*, the APTTs which show the bioactivity of intrinsic blood coagulation factors is examined, and the results are depicted in Figure 5 The APTTs of HC are prolonged comparing 316L SS and chitosan samples. On contrast, the APTTs of chitosan are decreased from 26.9 to 23.7 s, which indicates that activation of the intrinsic blood coagulation system is increased relatively.

When blood contacts a foreign material, plasma proteins are adsorbed onto the material surfaces immediately, and provoke the adhesion of platelets,

TABLE III
The Zeta Potential of Different Samples

Samples	Chitosan	HC
Zeta potential (mV)	21.7	36.5

white blood cells and some red blood cells onto the plasma protein layer. Adherent, aggregated platelets release materials such as ADP, ATP, thereby inducing more platelet aggregation on the surface. In the final phase, thrombus, a nonsoluble fibrin network is formed.[40–42] In this work, the chitosan derivative of containing hydroxyethyl displayed the fewest number of adherent platelets, the least amount of platelet aggregation (Fig. 4), and the longest APTT of any of the surfaces examined (Fig. 5). Platelet adhesion and activation of polymers is a complex process that is dependant on a variety of properties including surface chain mobility, hydrophilicity, hydrogen bonding properties, charge density, and steric barrier among chains.[39] In this work, the hydroxyethyl was inducted result in breakdown molecular chain and rearrangement of chitosan chains, which could produce a steric barrier among different chains. The anticoagulation of polymer is affected with the steric barrier and the surface charge density, but the former is more than latter.[40] So the effect of steric barrier produces undesirable protein adsorption, the blood compatibility is also improved simultaneously. The platelet adhesion behavior is only one better indication of anticoagulation, the other aspects of anticoagulation, such as coagulation factors, should be done later on.

Antibacterial property

The SEM morphologies of the bacterial colonization of various sample is shown in Figure 6 The surface of 316L SS is completely covered with bacteria, the amount of bacteria on the surface of chitosan sample reduces evidently, and the surface of HC is minimum bacteria adhesion. The result shows that chitosan has the lower antimicrobial activity against the tested bacteria primarily due to its poor solubility in the neutral condition, but the HC presents excellent antibacterial activity *in vitro*.

As shown in Figure 7, the highest number of *Enterococcus* and *E. coli* adhesion was observed on 316L SS surface. In contrast, two kinds of bacteria have only minor adherence on the surface modified by chitosan. In particular, the least amount of bacteria can be observed on HC sample surface. This demonstrates that the chitosan can slightly inhibit the adhesion of two types of bacteria. The amounts of *E. coli* on HC surface were slightly greater than that of chitosan sample, and *Enterococcus* was one-third of chitosan. It was demonstrated that the chitosan derivative against *E. coli* was much better than *Enterococcus*, owing to differences in charge of cell walls between *Enterococcus* and *E. coli*.[43]

The cationic nature of chitosan has been suggested the main factor contributing to its interaction with the negatively charged microbial cell surface and producing an impairment of bacterial activity.[44] In fact, the antibacterial mechanism of chitosan is generally considered due to the amino group at the C-2 position of the glucosamine residue,[45] that is, the cationic nature of chitosan at acidic condition, so the antimicrobial activity of chitosan was shown in Figure 6. The OH at C-3 and C-6 position were replaced by hydroxyethyl, which results in deviation of electron cloud in polymer chain, and the degree of protonation of NH_2 is enhanced relatively. The surface charge amounts can be assessed on the basis of their zeta potential. As shown in Table III, HC exhibits a cationic nature, which allows for electrostatic interactions with the aforementioned negatively charged *E. coli*, so the adsorption and binding of cationic group are effective to explain its antibacterial mechanism against *E. coli*, the antibacterial activity of HC against *E. coli* was more effective than chitosan sample [Fig. 6(b,c)].

CONCLUSIONS

Hydroxyethyl chitosan with good water solubility was prepared via chemical modification. The The chitosan derivative shows a good anticoagulation owing to provide a steric barrier to inhibit protein adsorption. Comparing to the chitosan, HC shows much better antibacterial activity against *Enterococcus* than chitosan, it indicates some synergistic effects of the antibacterial activity between hydroxyethyl group and the degree of protonation of NH_2.

References

1. Olsson, P.; Sanchez, J.; Mollnes, T. E.; Riesenfeld, J. J Biomed Sci Polym Ed 2000, 11, 1261.
2. Kang, I. K.; Kwon, O. H.; Lee, Y. M.; Sung, Y. K. Biomaterials 1996, 17, 841.
3. Kang, I. K.; Kwon, O. H.; Kim, M. K.; Lee, Y. M.; Sung, Y. K. Biomaterials 1997, 18, 1099.
4. Jacobs, H.; Grainger, D.; Okano, T.; Kim, S. W. Artif Organs 1988, 12, 506.
5. Sharma, C. P. Bull Mater Sci 1994, 17, 1317.
6. Lee, J. H.; Lee, H. B.; Andrade, J. D. Prog Polym Sci 1995, 20, 1043.
7. Matin, M. A.; Pfaller, M. A.; Wenzel, R. P. Ann Intern Med 1989, 110, 9.
8. Pittet, D.; Tarara, D.; Wenzel, R. P. JAMA 1994, 271, 1598.
9. Arnow, P. M.; Quimosing, E. M.; Brech, M. Clin Infect Dis 1993, 16, 778.
10. Davenas, J.; Thévenard, P.; Philippe, F.; Amau, M. N. Biomol Eng 2002, 19, 263.
11. Sodhi Rana, N. S.; Sahi, V. P.; Mittelman, M. W. J Electron Spectrosc Relat Phenom 2001, 121, 249.
12. Yan, R. X. Water-Soluble Polymer; Chemical Industry Press: Beijing, China, 1998; Chapter 11.
13. Yuan, Z. Paper and Pulp Analysis; Shanghai Science and Technology Press: Shanghai, China, 1959; p 247.
14. Zhao, T. D. Chitosan. Chemical Industry Press: Beijing, China, 2001; Chapter 1.
15. Gerasimenko, D.V.; Avdienko, I. D.; Bannikova, G. E.; Zueva, O. Yu.; Varlamov, V. P. Appl Biochem Microbiol 2004, 40, 253.
16. Qun, G.; Ajun, W. Carbohydr Polym 2006, 64, 29.

17. Keisuke, K.; Hiroyuki, L.; Shinichii, L.; Hitoshi, F.; Yoshio, L. Macromol Chem 1982, 183, 1161.
18. Alexandrova, V. A.; Obukhova, G. V.; Domnin, N. S.; Topchiev, D. A. Macromol Symp 1999, 144, 413.
19. Sridhari, T. R.; Dutta, P. K. Indian J Chem Technol 2000, 7, 198.
20. Sugimoto, M.; Morimoto, M.; Sashiwa, A.; Saimoto, H.; Shigemasa, Y. Carbohydr Polym 1998, 36, 49.
21. Takemono, K.; Sunamoto, J.; Akasi, M. Polymers and Medical Care. Mita: Tokyo, 1989; Chapter IV.
22. Terada, N.; Morimoto, M.; Saimoto, H., Okamoto, Y.; Minami, S.; Shigemasa, Y. Chem Lett 1999, 12, 1285.
23. Kochkina, Z. M.; Chirkov, S. N. Microbiology 2000, 69, 217.
24. Kumar, M. N. React Funct Polym 2000, 46, 1.
25. Muzzarelli, R.; Tarsi, R.; Filippini, O., Giovanetti, E.; Biagini, G.; Varaldo, P. E. Antimicrob Agents Chemother 1990, 34 2019.
26. Amiji, M. M. Carbohydr Polym 1997, 32, 193.
27. Hagiwara, K.; Kuribayashi, Y.; Iwai, H.; Azuma I.; Tokura, S.; Ikuta, K.; Ishihara, C. Carbohydr Polym 1999, 39, 245.
28. Hirano, S.; Zhang, M.; Nakagawa, M.; Miyata, T. Biomaterials 2000, 21, 997.
29. Liu, H. Q.; Huang, N.; Leng, Y. X.; Yu, H. L.; Le, L.; Li, K. Z. J Wuhan Univ Tech 2010, 25, 795.
30. Lee, K. Y.; Ha, W. S.; Park, W. H. Biomaterials 1995, 16, 1211.
31. Nishimura, S.; Kohgo, O.; Kurita, K.; Kuzuhara, H. Macromolecules 1991, 24, 4745.
32. Prashanth, K. V. H.; Kittur, F. S.; Tharanathan, R. N. Carbohydr Polym 2002, 50, 27.
33. Miya, M.; Iwamoto, R.; Yashikawa, S.; Mima, S. Int J Biol Macromol 1980, 2, 323.
34. Muzzarelli, R. A. A.; Tanfani, F.; Emanuelli, M.; Pace, D. P.; Chiurazzi, E.; Piani, M. Carbohydr Res 1984, 126, 225.
35. Sharma, P. K.; Rao, K. H. Adv Colloid Interface Sci 2002, 98, 341.
36. Kubota, N.; Tatsumoto, N.; Sano, T.; Toya, K. Carbohydr Res 2000, 324, 268.
37. Zohuriaanr, M. J. Iran Polym J 2005, 14, 235.
38. Mourya, V. K.; Inamdar, N. N. React Funct Polym 2008, 68, 1013.
39. Kurita, K. Mar Biotechnol 2006, 8, 203.
40. Goosen, M. F. A.; Sefton, M. W. Thromb Res 1980, 20, 534.
41. Huang, N.; Yang, P.; Leng, Y. X.; Chen, J. Y.; Sun, H. Biomaterials 2003, 24, 2177.
42. Byun, Y.; Jacobs, H. A.; Kim, S. W. ASAIO J 1992, 38, M649.
43. Sagnella, S.; Kwok, J.; Marchant, R. E.; Liu, Q. Mater Res 2001, 57, 419.
44. Xie, W.; Xu, P.; Wang, W. Carbohydr Polym 2002, 50, 35.
45. Raafat, D.; Bargen, K.; Haas, A.; Sahl, H. G. Appl Environ Microbiol 2008, 74, 3764.