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Surface modification of high density polyethylene tubes by coating chitosan, chitosan hydrogel and heparin

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Summary

Chitosan and chitosan hydrogel were immobilized on both the inside and outside surfaces of high density polyethylene (HDPE) tubes with 2.5x4 mm diameters. First, acrylic acid was grafted on the surfaces of HDPE by electron beam (2.5 Mrad) preirradiation method. Then chitosan/HC1 and chitosan/lactic acid solutions were coated on the modified hydrophilic HDPE surfaces, the latter could form a pH-sensitive hydrogel layer on the surfaces. The tube surfaces were further modified with heparin by surface interpenetrating method to improve blood compatibility. ATR-FTIR and ESCA methods were used to characterize the coated surfaces. The morphology changes were monitored by Scanning Electron Microscope (SEM).

Indroduction

Surface properties of polymers have become progressively important in the field of biomaterials, since polymers can contact with physiological components such as blood and living tissues. Surface modification has the advantage of improving the surface properties without altering the bulk physical and mechanical properties[1,2]. Hydrogels are often coated on polymer surfaces to improve biocompatibility. The hydrogel layer not only provides smooth, slippery surface, but also prevents bacterial colonization and lowers the interaction between the artificial surface and surrounding tissues[3,4]. Recently, various stimuli sensitive hydrogels on polymer surfaces have been developed. The hydrogels on the surface vary in their properties, such as film thickness, hydrophilicity, surface charge and flexibility, in response to changes in environmental conditions, such as pH, temperature and ionic strength. These responses can be very sensitive, as the hydrogel surface coatings can be very thin[5,6]. Chitosan, a random copolymer of ß(1→4)-D-glucosamine and acetyl-ß(1→4)-D-glucosamine, has been the subject of intense study during the last decade as it is one of the few abundantly available and naturally derived biocompatible cationic polysaccharides. It has been reported to inhibit bacterial growth by suppressing the bacterial metabolism when chitosan sticks to the bacterial cell wall[7,8]. In spite of its excellent film forming property, there is little publication concerning surface modification of polymers by chitosan, especially by its derivatives such as pH-sensitive chitosan hydrogels for biomedical applications.

In the present study, chitosan and pH-sensitive chitosan hydrogels were covalently immobilized on both the inside and outside surfaces of high density polyethylene (HDPE) tubes, which have 4 mm diameters for the potential use as urinary catheter. The chitosan hydrogel layers were further modified with heparin by surface interpenetrating method to improve blood compatibility. The surfaces were characterized by ATR-FTIR and ESCA methods and the morphology changes were monitored by Scanning Electron Microscope (SEM).

Experimental

Materials

High density polyethylene (HDPE) tubes with 4 mm diameters provided by Vygon Laboratories (France) were used as base substrate. Acrylic acid (99%) from Aldrich (Germany) was distilled under reduced pressure to remove the stabilizer. Chitosan (Mw =70,000) from Fluka (Switzerland) and D, L-lactic acid (99%) from Lancaster (England) were used for the preparation of chitosan hydrogel. The degree of deacetylation (DD =88%) of chitosan was determined by IR spectroscopy[9]. Heparin with an activity of 185 IU/mg (APTT) and Mn=15,000 was purchased from Pharmacia-Upjon (Sweden). Unionized water (Milli Q) was used in all aqueous solutions.

Irradiation and grafting acrylic acid

HDPE tubes were first washed with ethanol and dried at room temperature, and then were irradiated by electron beam with 2.5 Mrad dose at ambient temperature in the air. The samples were immediately stored in liquid N_2. The grafting experiment was performed in a glass ampoule containing 30 wt% acrylic acid and 0.05 wt% Mohr's salt [$FeSO_4(NH_4)_2SO_4$] in aqueous solution at 50°C. Prior to the grafting, the oxygen in the solution was purged by bubbling argon at 50°C for 10 minutes.

Surface modification by chitosan and chitosan hydrogels

Modified HDPE tubes were carefully washed with distilled water to remove the remaining monomer and homopolymer. Then the tubes were immersed in the chitosan/HC1 (0.5%) and chitosan/lactic acid (Weight ratio Chit/LA=1/2) solutions (0.5%) for 10 seconds. The dip-coated tubes were heated at 80°C for 2 hours, and repeatedly washed in 1M NaCl solution followed by distilled water.

Surface interpenetrating of heparin

Modified HDPE tubes were immersed in pH 2.2 buffer containing 2 wt% heparin for certain period of time. Then the samples were transferred into pH 7.4 buffer. Increasing pH of the buffer led to the collapse of swollen hydrogel layer and entrapment of heparin on the surface. The samples were carefully washed by distilled water and then dried. The chemical structures of chitosan, chitosan hydrogel and heparin are shown in Fig. 1.

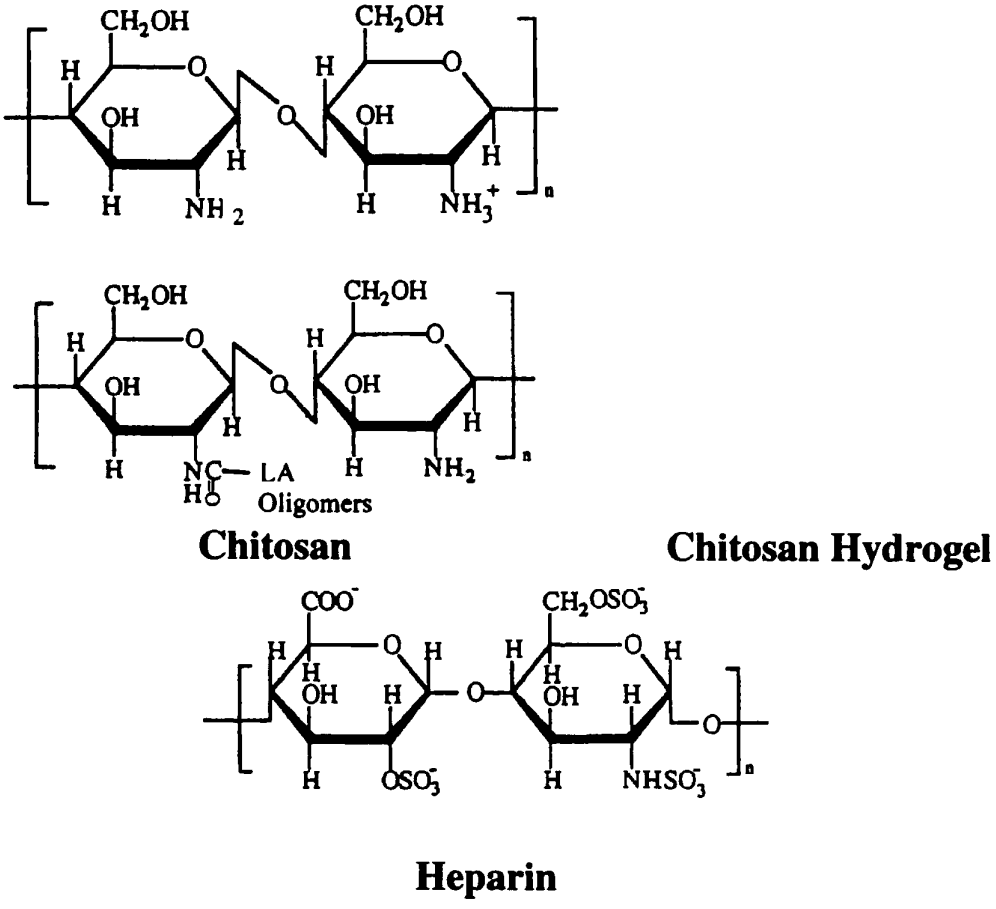

Figure 1. Chemical structures of chitosan, chitosan hydrogel and heparin

ATR-FTIR

Chitosan and chitosan hydrogels coated HDPE tubes were analyzed on a Perkin-Elmer 2000 Infrared Fourier transform spectrometer. Analyses were preformed using the attenuated total reflection technique (ATR).

ESCA (XPS)

Photoelectron spectra were acquired with a Kratos Axis HS instrument using a Mg-Kα radiation source and 90° take-off angle with respect to the sample surface. The operating conditions were as follows: X-ray source, 12kV, 20mA, pass energy 80eV, base pressure in the chamber below $2x10^{-7}$ Torr.

Scanning electron microscope (SEM)

The samples were broken in liquid nitrogen. The morphology of surfaces and intersection parts were studied using a SEM Jeol JSM-5400. Samples were mounted on metal stubs and sputter-coated with gold-palladium (Denton Vacuum Desc II).

Results and discussion

As shown in Fig.2, preirradiation method was used to graft acrylic acid on the surface of hydrophobic HDPE tubes. HDPE tubes were irradiated in the air so that the macroradicals formed were converted to peroxides and hydroperoxides. The advantage of this process was that the intermediate oxidized polymer could be kept for long periods of time before performing the final grafting reaction. When the irradiated HDPE tubes were heated in the presence of acrylic acid solution, the peroxides decomposed to generate radicals that served as active sites for the grafting reaction. The dissociation of the hydroperoxide led to hydroxy radicals ($^{\bullet}OH$), which were the direct cause of homopolymerization. Therefore 0.3 wt% Mohr's salt [$FeSO_4(NH_4)_2SO_4$] was used to reduce the homopolymer and to increase the grafting yield, since Fe^{2+} in Mohr's salt is oxidized to Fe^{3+} and thus reduces $^{\bullet}OH$ to OH^- [10,11].

Chitosan could not be attached to the untreated HDPE surfaces since untreated HDPE is hydrophobic. After the acrylic acid grafting, functional groups such as -COOH were created. Thin chitosan solutions could retain on the surfaces after the tubes were dip-coated. The immobilization of chitosan on the HDPE tubes could be expected due to the hydrogen bonding, the ionic bonding, and possibly the covalent bonding (-NHOC-) between the $-NH_2$ groups of chitosan and the -COOH groups on the surfaces. Similar results have been obtained by surface grafting chitosan on the plasma treated polypropylene for improving dyeing behavior[12]. In the case of chitosan/lactic acid solution, the dehydration of chitosan lactate salt also occurred to form amide groups during heating. Simultaneously, the polycondensation of lactic acid occurred to form lactic acid side chains. The formation of chitosan hydrogel was due to the physical crosslinking through hydrophobic side chains aggregation and intermolecular interactions by hydrogen bonds between side and main chains, which eventually led to a corresponding decrease of chitosan chain mobility in the aqueous solutions. [13].

In the blood contacting applications, chitosan promotes surface induced thrombosis and embolization. Thus heparin has been used to modify chitosan membranes for resisting complement activation and platelet adhesion in the hemodialysis[14,15]. In the present study, the semi-interpenetrated network of heparin was generated on the surface of chitosan hydrogel. The heparin molecules were partly entrapped inside the chitosan hydrogel matrix due to the strong ionic bonds ($-NH_3^+$ $^-O_3S-$) between chitosan and heparin. The amount of heparin on the surfaces increased with the swelling time in the heparin solution.
The ATR-FTIR spectra of HDPE and modified samples surfaces were shown in Figure 3. Compared to the spectrum of untreated HDPE, the acrylic acid grafted surface has several new peaks at 1740cm^{-1}, 1270cm^{-1} and 1201cm^{-1}, which could be assigned to the carboxylic groups on the surface. These peaks disappeared after the coating by chitosan and chitosan hydrogel. The spectrum of chitosan coated surface shows peaks assigned to the saccharide structure at 897 cm^{-1} and 1153 cm^{-1} and a strong amino characteristic peak at around 1591 cm^{-1}. In the case of chitosan hydrogel (LA/Chit=2) coated surface, no significant difference was found in the spectrum as

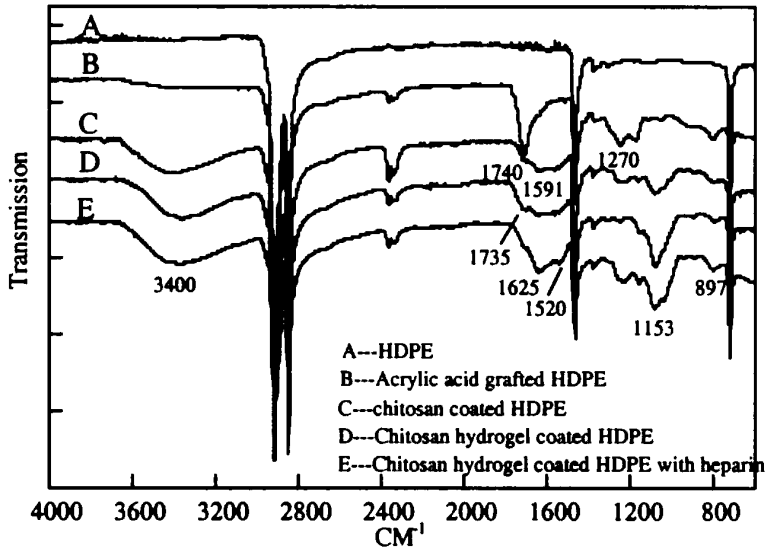

Figure 2. Surface modification of HDPE by chitosan and chitosan hydrogel and heparin

compared to the chitosan coated sample except a small peak at 1735 cm^{-1}, which could be assigned to the ester group of the LA side chains. Both chitosan and chitosan hydrogel coated samples are hydrophilic and have a peak at 3400 cm^{-1} corresponding to the hydroxyl groups of chitosan. Surface interpenetrating of heparin into chitosan hydrogel will form a polyelectrolyte complex due to the different charged groups. Two new absorption bands appearing at 1625 cm^{-1} and 1520 cm^{-1} are related to the deformation of the NH_3^+ groups in chitosan. However, no characteristic absorption peaks corresponding to heparin could be distinguished in the IR spectrum, since both chitosan and heparin are polysaccharides and have similar chemical structure as shown in Fig. 1.

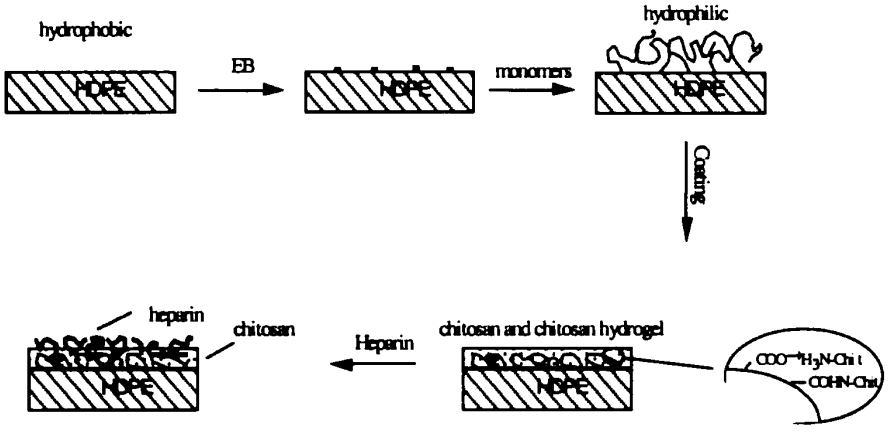

Figure 3. ATR-IR spectra of HDPE and surface modified HDPE

Results of the ESCA surface analysis of HDPE and modified HDPE tubes are presented in Table 1. The surface elemental composition of acrylic acid grafted HDPE tube shows characteristic peaks of C (70.2%), O (25.4%) and N (4.4%) with atomic percentage. The existence of N in the grafted surface could be due to the Mohr's salt on the surface, which had not been completely washed out. The chitosan coated HDPE tube shows a lower percentage of C (64.6%), and higher percentage of O (28.2%) and N (7.2%) than grafted tube, while this composition is significantly different from the predicted values of 55% C, 36% O and 9% N for pure chitosan sample. The difference in the elemental composition could be due to the predominant presence of acetylglucosamine residues on the membrane surface in dry state or due to environmental contamination. Chitosan hydrogel coated HDPE tube has similar element composition as chitosan coated sample, since only about 10% of the $-NH_2$ groups of chitosan were grafted by lactic acid. There is a decrease in the composition of C, N and an increase of O on heparin modified sample. Furthermore, the presence of S (1.45%) confirms that heparin is present on the surface.

Table 1. ESCA results of surface coated HDPE tubes

Atomic Conc. (%)	Acrylic acid grafted HDPE	Chit coated HDPE	Chit hydrogel coated HDPE	Chit hydrogel coated HDPE+heparin
C	70.25	64.61	63.27	62.52
O	25.38	28.20	29.35	29.58
N	4.37	7.19	7.38	6.45

Surface topography of chitosan hydrogel coated HDPE tubes was investigated by scanning electron microscope (SEM). As shown in Fig.4(A), a smooth layer with 1μm thickness still remained on the surface even after the tube was broken. It also illustrates that strong bonding existed between the chitosan layer and the HDPE surface. As shown in Fig.4(B), the fibrous heparin-chitosan network was formed on the sample surface after 5 minutes immersion in the heparin solution.

Conclusions

Chitosan, chitosan hydrogel and heparin were used to modify the inside and outside surfaces of high density polyethylene (HDPE) tubes with 4 mm diameters. The surfaces were further modified by heparin to improve their blood compatibility. A heparin network partly, entrapped in the chitosan matrix, was formed on the surface. This method could be used to improve the antibacterial and blood compatible properties of biomedical devices made from polymers.

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