Surface properties and *in vitro* analyses of immobilized chitosan onto polypropylene non-woven fabric surface using antenna-coupling microwave plasma

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Antenna coupling microwave plasma enables a highly efficient and oxidative treatment of the outermost surface of polypropylene (PP) non-woven fabric within a short time period. Subsequently, grafting copolymerization with acrylic acid (AAc) makes the plasma-treated fabric durably hydrophilic and excellent in water absorbency. With high grafting density and strong water affinity, the pAAc-grafted fabric greatly becomes feasible as an intensive absorbent and as a support to promote chitosan-immobilization through amide bonds. Experimental result demonstrated that surface analyses by FTIR-ATR have shown that R–CONH–R′, amide binding were emerged between pAAc and chitosan. The XPS measurements on C_{1s} 286.0 eV (C–OH), 286.5 eV (C–N) and 288.1 eV (O=C–NH) also could be found. Bioactivity assessments on the chitosan-immobilized surfaces were anticipated by activated partial thromboplastin time (aPTT), thrombin time (TT), and fibrinogen concentration. By means of cell counter we counted the ratio of blood cell adhesion on the modified fabric matrix. After human plasma incubated with the chitosan-immobilized PP fabrics, the required time for aPTT and blood cell adhesion increased significantly, while fibrinogen concentration and TT did not change. Due to the capability of anticoagulation and cell adhesion, the chitosan-immobilized PP fabric can be used as the substrate for cell culturing and then developed the wound-dressing substitute for second-degree burn.

Introduction

Chitosan and chitin, next to cellulose, are the second most plentiful nature and nontoxic, biodegradable cationic polymers. The major source of commercial chitosan is the exoskeleton of marine crustaceans such as crabs, lobsters, krill, and shrimps [1]. As such, chitosan is an abundant natural polymer available from a renewable resource. Chitosan, a mucopolysaccharide having structural characteristics similar to glycosamines, is a linear β(1 → 4)-D-glucosamine and aceyl-β(1 → 4)-D-glucosamine, which can be obtained by alkaline N-deacetylation derivative of chitin [2]. Thus, chitosan is usually not a homopolymer of D-glucosamine, but a copolymer containing less than 40% N-acetyl-D-glucosamine residues. Chitosan has both reactive amino and hydroxyl groups, which can be used to chemically alter these properties under mild reaction conditions. Therefore, there are many interesting chitosan derivatives, especially for biomedical applications [1, 3, 4]. Chitosan has been proposed for the development of membranes and fibers for hemodialysis and blood oxygenators, skin substitute, wound-dressing and sutures materials, as a matrix for immobilization of enzymes and cells, for binding with bile and fatty acids, and as a vehicle for

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drug and gene delivery [5–9]. Although chitosan is suitable for medical applications, for those applications that involve blood-contact such as hemodialysis membranes, chitosan promotes surface induced thrombosis and embolization [5, 7]. It is indicated in the literature that chitosan has the capacity to activate both complement and blood coagulation system [10, 11]. Therefore, to use chitosan derivatives for biomedical applications, a test for evaluating biocompatibility must be performed.

Polyethylene (PP) non-woven fabrics can be fabricated easily without knitting, weaving or other traditional processes. The versatility of non-woven formation techniques such as melt-blown, hydrentangled methods enables the fabrics to be developed into different types of multi-layer bonded structures, with a variety of bulk properties suitable for hygienic, family and medical uses [12]. By mixing with cotton and nylon, and forming a composite non-woven fabric can obtain special properties. It usually holds a permeable characteristic, i.e. functionalized products are specially used as separators, high efficient gas filtering materials or other percutaneous access devices. Since the 1960s, the fabrics are also widely used for disposable matters in orthopedics or wound-healing base substrates, their applications for bacterium resistant, health care related products increase drastically [13]. PP non-woven fabrics demonstrate a hydrophobic character. To meet specific demands for sophisticated applications, modifications of the non-woven surface have received much attention in creating new chemistries that allow promoting superficial functionabilities such as hydrophilicity, biomedical-related affinities, permeability or selectivity, on or through the fabric surface [14–17].

To activate a hydrophobic PP non-woven fabric surface, plasma pretreatment is widely recognized as a clean and effective method [18]. Plasma processing involves reactive chemistry derivatives from electrons, ions, and free radicals [19]. Low temperature plasma processing is progressively applied to activate the outermost surface of a polymer, likewise, not to affect its structural dimensions [20]. Both ionized species and free radicals in the reactive plasma may interact with molecular chains of the substrate [21]. Previous studies have shown that plasma density is a crucial factor to create thermal decomposable hydro-peroxide on the polymer surface [22]. Plasma pre-treated fabrics are further grafted with hydrophilic monomer to raise its long-term hydrophilicity. A variety of studies have used acrylic acid (AAc) as the monomer to graft copolymerization with the plasma-treated substrate and to form a steadily hydrophilic surface [18, 22, 23].

This study utilizes a specific microwave plasma system [22, 23] to initiate and activate PP non-woven fabric, followed by grafting with AAc and coupling with chitosan. Our intention is to promote pAAc-grafting density for bonding with chitosan molecules. The objective of this study is to elucidate interactions between chitosan and AAc, and also relate the bioactivities of chitosan. To understand the immobilization process, it is of importance to identify the chemical species and to do quantitative analysis using surface-sensitive techniques such as soft X-ray photoelectron spectroscopy (XPS) and Fourier-transformed infrared with attenuated total reflection (FTIR-ATR). To assess the bioactivity of immobilized chitosan, biological tests such as activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen concentration and blood cell adhesion have been employed. This study utilizes specific treatments combined with physical and biological evaluations to optimize the modified surface. Our aim is to widen the application of this porous and disposable material for biomedical use.

Materials and methods

Substrate, chemicals and apparatus

Pure PP non-woven fabric had an average of $50\,g \cdot m^{-2}$ and 0.15 mm thickness, provided by the Laboratory of Non-Woven Fabrics, Industrial Technology Research Institute (Taiwan). The main chemicals included:

chitosan: [2-Amino-2-deoxy-(1 → 4)-β-D-glucopyranan],
Mr ∼ 150 000 (Low molecular weight, Fluka 22741),
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
(EDC, Sigma), and
AAc (Merck, inhibitor removal under oxygen free
evaporation before use).

Antenna-coupling microwave is distributed by 12 × 12 array-elements onto a quartz plate and extended to an effective area of ca. $40 \times 40\,cm^2$ in the reactive chamber [24]. The operating parameters were: O_2 gas plasma (600 W, 0.2 Torr) with a treatment time less than 10 s. The plasma-treated samples (with peroxide concentration of ca. 10^{-8} mole cm^{-2} [25]) were immersed into 30 wt % AAc aqueous solution, added with a concentration of 10^{-4} M Fe(II) and concurrently drained N_2 gas on and into the reactive solution. The reaction proceeded at 70 °C for 1 h. The sample was rinsed at 70 °C for 12 h [22, 25, 26]. The amounts of grafted pAAc and immobilized chitosan on PP non-woven fabric were measured by an estimation of [27, 28]:

$$\text{Grafting density } (\mu g\,cm^{-2}) = (W_f - W_i)/A,$$
$$\text{Immo-bilized chitosan } (\mu g\,cm^{-2}) = (W_c - W_f)/A,$$

where A is the dimensional area of the PP fabric;
W_f, W_i and W_c are the weights of PP non-woven fabric after and before AAc grafting and after chitosan immobile process.

For comparison, the graft-copolymerized samples were immersed in 50 ml, 0.01 N NaOH solution for 2 h, and then titrated with 0.01 N HCl [29]. The titrated amount was calculated as the reference quantity of the grafted pAAc divided by the same dimensional area.

Chitosan immobilization

The pAAc-grafted PP fabrics were immersed in $10\,mg\,ml^{-1}$ EDC aqueous solution which was buffered to pH 4.8 (in sodium citrate) at 4 °C for 30 min. Water-soluble EDC was used for activating O=C–OH group on the pAAc-grafted samples [17, 22]. The EDC-coupled samples were then immersed in chitosan-containing solutions, which included $3\,mg\,ml^{-1}$ and buffered to pH 3.0 acetic acid-buffered solutions at 4 °C for 24 h. The chitosan-immobilized samples were washed and dried

mildly at 4 °C. This immobilization process was carried out in a clean environment.

Surface characterization

ATR-FTIR and surface sensitive XPS were utilized for identifying chemical species. ATR-FTIR spectra were recorded using Bomem DA8.3 with an ATR device containing a germanium crystal and Harrick KBr prism. The number of scans for ATR-FTIR spectra was 200 scans (resolution $2\,cm^{-1}$). XPS spectra were acquired with a Physical Electronics PHI 1600 spectrometer with a magnesium anode at 400 W and 15 kV–27 mA (MgK_{α} 1253.6 eV, type 10–360 spherical capacitor analyzer). The specimens were analyzed at an electron take-off angle of 70°, measured with respect to the surface plane. The operating conditions were as follows: pass energy 23.4 eV, base pressure in the chamber below 2×10^{-8} Pa, step size 0.05, total scan number 20, scan range 10 eV (for multiplex scan). The peaks were quantified from high-resolution spectra, obtained by using a monochromatic Mg X-ray source. Elemental compositions at the surface using C 1s, O 1s and N 1s core level spectra were measured and calculated from XPS peak area with correction algorithms for atomic sensitivity [30]. The XPS spectra were fitted using Voigt peak profiles and a Shirley background [21].

In vitro tests

Venous blood was obtained from six normal young individuals, ages in the range of 20–25, who had not consumed aspirin or other nonsteroidal anti-inflammatory drugs for at least two weeks; the blood was collected in glass vacutainer (5 ml) and mixed with the anticoagulant of 3.8% (w/v) and sodium citrate (9 vol. blood: 1 vol. citrate) in tubes. Fresh blood of 30 ml from each donor was sampled. Platelet-rich plasma (PRP) were prepared by centrifugation of whole blood at 160 G for 15 min at 4 °C [31], and human plasma from the upper part was obtained with a micropipette, possible vibrations were prevented from buffy coat (white blood cells with a part of platelets). Experiments and tests were completed within 4 h.

To explore the bioactivity of chitosan-immobilized surface, human plasma was incubated at 37 °C for 30 min and used for testing a specific activity such as the decay constant of thrombin or Factor X on a global test such as aPTT. The biological assay kits included: vacutainer containing sodium citrate for TT, aPTT and fibrinogen reagents (from Becton, Instrument Lab. and Biopool, respectively). Aliquots were taken from incubates 3 min after the addition of reagents. The Coatron M2 (TECO, semi-auto type) was used to determine aPTT by addition of cephaline and $CaCl_2$ to human plasma; the intrinsic factors (XI and XII) of coagulation were activated by ellagic acid on an extract of bovine cerebral tissue, a substitute of platelet factor III. In addition, coagulation (Factor X) was induced by addition of $CaCl_2$. The clotting method for TT was determined by adding bovine thrombin to human plasma, using 30 µl of human plasma incubated at 37 °C for 3 min; then 30 µl of Thrombin Reagent (Instrumentation Lab. 09758515) was added.

Blood cell adhesion was calculated by subtracting the residual amounts of blood cells after human plasma contacted with the modified PP fabrics. The platelet count of the sampled human plasma was about 3.0×10^5 platelets μl^{-1}. Samples were incubated with PRP and whole blood at 37 °C for 30 min and then taken out. The amount of blood cell adhered upon the chitosan-immobilized sample was calculated using the cell counter (Sysmex K-1000, auto type). The morphology of adhered RBC on the sample was investigated using Scanning Electron Microscopy (SEM; JSM-6300). Before taking photomicrographs, samples were gently rinsed with normal saline to remove the loosely adsorbed RBC and then fixed in 2.5% saline-buffered glutaraldehyde solution, and dehydrated by standard procedures: frequent methods to immobilize and to dry the biological samples for doing SEM measurement can be found elsewhere [22, 32]. Human blood obtained in six different batches usually customarily leads to somewhat different numbers of blood cell adhered; however, a general tendency of the data may remain.

Analytical results of coagulation tests and blood cell adhesion were expressed as mean $\pm$ standard error. Statistical significance was described using unpaired *t*-test.

Result and discussion

Hydrophilic treatment

The antenna-coupling microwave plasma exposed to the fabric surface results in uniform treatment and a macroscopically hydrophilic surface [25, 33, 34]. Plasma density, characterized by Langmuir probe [24], was estimated in the range of 10^9–10^{10} particles cm^{-3}. The specific design of the plasma system is regarded as a high-density plasma source, compared with commercially available radio-frequency plasma processing systems [24, 35, 36]. PP non-woven fabric surface with diverse pore sizes and large surface areas could become hydrophilic within 10 s of O_2 plasma treatment [22]. To intensify the derived peroxides capable of reacting with AAc monomers, an addition of 10^{-4} M Fe(II) as the redox agent was optimized [26]. The grafted amount of pAAc on the plasma-modified PP non-woven fabric was then controlled to ca. $236\,\mu g \cdot cm^{-2}$. Such grafted quantity was large, compared to similar treatments of solid surface polymers like films or sheets, or highly hydrophobic porous structure, such as expended polytetrafluoroethylene [18, 26].

This specific plasma processing enlarges the activated sites on the fabric surface for graft copolymerization with AAc monomers. Using FTIR-ATR, the IR absorption peak at $1705\,cm^{-1}$ (C=O or O=C–OH) was correlated with the plasma treatment and the grafted pAAc (comparing Fig. 1(a) with 1(b)) [37]. Similarly, it was also corresponding with XPS measurements; the increase of C 1s peak intensities at 286.0 eV (C–O), 288.1 eV (C=O), and 289.2 eV (O=C–O) binding energy (comparing Fig. 2(c) with 2(a) and (b)) can be related to the functional groups of the grafted pAAc. Variations of oxygen/carbon (O/C) ratio, shown in Table I, indicate that oxygen was enhanced after plasma pretreatment (~ 0.45), but declined as a result of pAAc graft

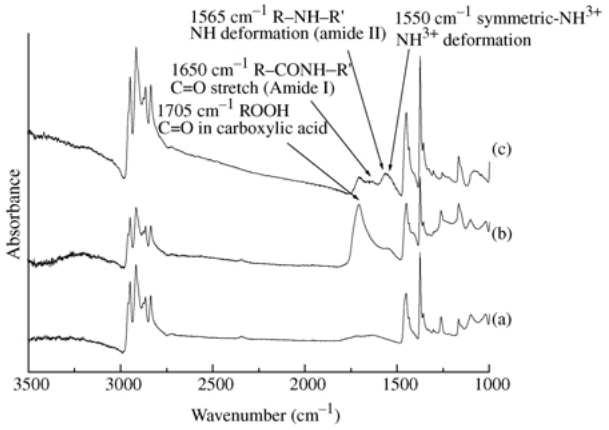

Figure 1 ATR-FTIR spectra: (a) non-woven PP fabric, (b) after O_2 plasma pre-treatment and then grafted with 236 μg cm^{-2} pAAc, (c) chitosan-immobilized PP.

copolymerization ($\sim$ 0.32). Plasma treatment on PP non-woven fabric surface produced highly oxidative structures, identified as –O* or –O–O* radicals [25, 38]. After thermal and redox reactions, the plasma pretreated PP non-woven fabric was able to graft copolymerization with AAc monomer. Owing to the porous matrix, the stoichiometrical calculation of pAAc was not provided using surface-sensitive XPS.

The pAAc-grafted sample was chemically hydrophilic and capable of regaining moisture. Alkaline solution or water absorbency of the plasma-modified pAAc-grafted fabric by our present process increased about five to seven times with respect to the net weight of the non-treated sample [25]. The advantage of high water absorbency has made the modified PP non-woven fabric potentially feasible as wound-dressing base substrate and intensive absorbents before thrombus formation occurs.

Chitosan immobilization: structural confirmation

The pAAc-grafted PP non-woven fabrics were utilized as links for bonding with chitosan molecules. In Fig. 1(c), compared with the pAAc-grafted sample (Fig. 1(b)), the IR-active band at 1565 and 1650 cm^{-1} were usually assigned to N-H deformation (amide II band) and C=O stretch (amide I band) [39–41]. This assignment is in good agreement with Ito *et al*. [42], Kang *et al*. [43], and Liao *et al*. [23]. The IR absorption peak appearing at 1550 cm^{-1} in the spectra of chitosan-immobilized sample was assigned as a symmetric-NH^{3+} deformation, which confirmed the presence of NH^{3+} in chitosan [44]. Thus, the poly-complex between chitosan and pAAc is

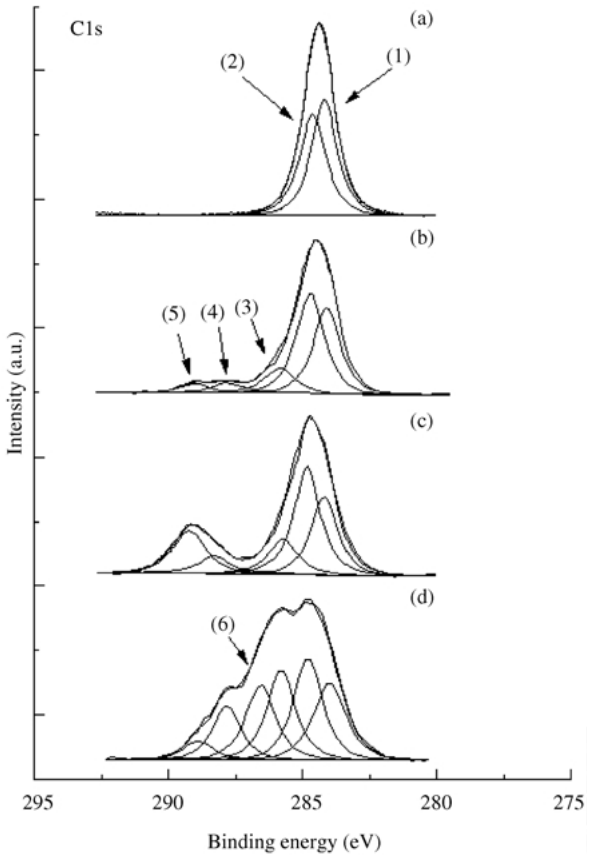

Figure 2 XPS C 1s core level spectra of the modified surfaces: (a) pure PP non-woven fabric, (b) plasma-treated PP, (c) pAAc-grafted PP, and (d) chitosan-immobilized PP. Peak assignment [45, 46]: (1) 284.6 eV and (2) 285.0 eV for C–C, C=C and C–H, (3) 286.0 eV for C–O, (4) 288.1 eV for C=O or amidebond, (5) 289.2 eV for O=C–OH, and (6) 286.5 eV for C–N.

formed, amino groups in chitosan form complexes with carboxyl groups in pAAc.

Typical chemical compositions of the immobilized chitosan were furthermore characterized using surface-sensitive XPS measurement. In Table I, the O/C ratios of the modified samples varied obviously with the sequential process of plasma treatment, pAAc grafting, and chitosan-immobilization. The XPS C 1s core level spectra for the chitosan-immobilized samples are shown in Fig. 2(d). The curves were fitted in accordance with references 45 and 46. Each of the spectrum was deconvoluted into seven peaks: 284.6 eV and 285.0 eV (hydrocarbon and carbon), 286.0 eV (C–O), 286.5 eV (C–NH_2), 288.1 eV (C with O, C=O group and, amide bond), and 289.2 eV (O=C–OH group in D-glucosamine or in pAAc), respectively. The chitosan-immobilized sample displayed a significant increase of NH_2, OH and amide group. These groups were corresponding with chitosan molecules (NH_2 and OH) or complex between

TABLE I The XPS measurements of C, O, and N elements from samples prepared in different treatment steps were expressed in atomic ratios with respect to C element. More than ten measurements for each sample were averaged

Samples	Atomic percent (%)			O/C	N/C
	C	O	N		
PP	97.94	2.06	—	0.02	—
Plasma-treated PP	68.74	31.26	—	0.45	—
AAc-grafted PP	76.10	23.90	—	0.32	—
Immobilized-chitosan PP (pH = 3.0)	66.93	29.19	3.88	0.44	0.06

TABLE II Results of major bioactivity tests on the chitosan-immobilized PP non-woven fabric surface (t-test, expressed as mean ± S.E.M., $N = 4$)

Specimen	Ratio of platelets adhesion (%)	Ratio of RBC adhesion (%)	Ratio of WBC adhesion (%)
pAAc-grafted PP	16.5 ± 3.76	1.9 ± 0.12	7.3 ± 2.47
Chitosan-immobilized PP	62.7 ± 0.06[a]	4.1 ± 0.04[a]	19.3 ± 9.44[a]
Specimen	**Fibrinogen conc. (mg/dl)**	**aPTT(s.)**	**TT(s.)**
Reference (human plasma)	294.4 ± 21.2	44.2 ± 2.74	15.2 ± 0.40
Non-treated PP	293.9 ± 12.8	43.7 ± 0.89	15.5 ± 0.20
pAAc-grafted PP	295.4 ± 13.9	44.8 ± 0.66	15.6 ± 0.51
Chitosan-immobilized PP	295.2 ± 16.4	49.6 ± 3.59[a]	15.0 ± 0.55
Heparin-immobilized PP [23]	289.3 ± 10.5	74.1 ± 2.84[b]	22.0 ± 0.46[b]

[a]$p < 0.01$, [b]$p < 0.05$.

chitosan and AAc (amide group). In Fig. 2(d), the binding energy at 286.5 eV was assigned to NH_2 groups bonded to the symmetric ring in chitosan. The O=C–OH group alter notably owing to the participation of functional group of the grafted-pAAc binding with chitosan as amide group.

Chitosan immobilization: quantity and blood clotting test

The immobilized quantity of chitosan can be affected by the grafted amount of pAAc owing to the reactive quantity of O=C–OH group with specific sequences in chitosan molecules and the addition of water soluble EDC. With the pAAc-grafted amount of ca. 236 $\mu g\,cm^{-2}$, the quantity of the immobilized chitosan was 21.8 $\mu g\,cm^{-2}$.

The extent of blood cell adhesion, activation, and coagulation time were used to assess the *in vitro* blood compatibility of chitosan-immobilized surface. In Table II, the coagulant activity and the ratio of blood cell adhesion for the chitosan-immobilized surfaces were examined. The ratios of platelet, RBC and WBC adhesion were increased (~ 62.7, 4.1 and 19.3% respectively) as compared with the hydrophilic interaction of the grafted-pAAc (~ 16.5, 1.9 and 7.3%, respectively). The chitosan-immobilized surface was noticeably increased the ratio. However, the hydrophilic characteristic of pAAc grafted surface obviously contributed to extend blood cell adhesion. The blood cell adhesion test implies that a hydrophobic fabric with hydrophilic pretreatment is capable of creating protein-containing and bioactive surface properties.

Fig. 3(b) illustrates SEM photomicrographs of RBC adhesion test on the chitosan-immobilized PP non-woven fabric surface; the chitosan-immobilized fabrics appeared to enhance surface from agglutination of blood cells. Chitosan can advance blood cells activation from cell adhering to the chitosan-immobilized surface [47]. The adhesions of red blood cells have lost their typical biconcave morphology, and they appear to have an unusual affinity toward to one another. The red blood cells, seen on the chitosan-immobilized surface, seemed to have coalesced into an erythrocyte clot or plug [48]. Note that the increase of platelet adhesion for the chitosan-immobilized fabric was attributed to the chitosan induced platelet activation on the chitosan-immobilized surface. The mechanism inducing the

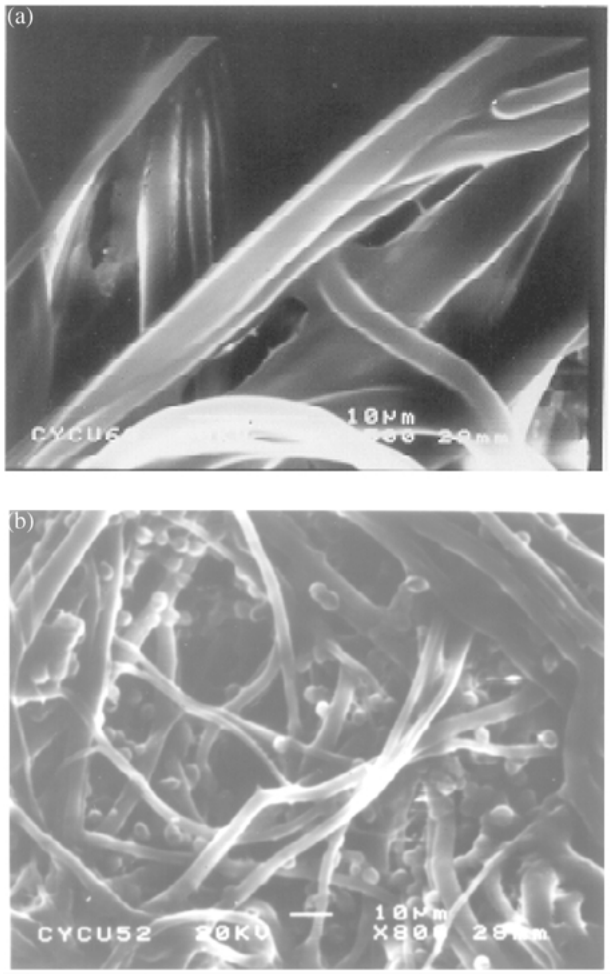

Figure 3 SEM photomicrographs: (a) non-treated PP non-woven fabric surface (2500 ×), (b) chitosan-immobilized PP (800 ×).

formation of coagulum of blood cells by chitosan has been shown to be due to the interaction of the positively charged chitosan molecule with receptors containing muraminic acid residues on the blood cell surface [49]. Upon platelet activation, the platelet secretes their granular contents serotonin (5′-HT), platelet-derived growth factor (PDGF), thrombospondin (TSP), etc. to activate other resting platelets or WBC as well as the adhesion reaction. For the pAAc-grafted surface, an analogous SEM photomicrograph of blood cell adhesion was also measured. For pure PP non-woven fabric surface, blood cell adhesion or entrapped in the fabric matrix was significantly lowered owing to the hydrophobic characteristic of the substrate (Fig. 3(a)).

Besides, the fibrinogen concentration was insignificantly changed with immobilized chitosan. The normal range of fibrinogen concentration in human plasma is around 200–400 mg dl^{-1}, which covers the values shown in Table II.

Coagulation test

Blood coagulation on the surface of material depends on several catalytic factors. Among them, the plasma coagulation factors–material interface reaction is primarily important in coagulation process. Table II shows the result of coagulation test evaluated by the aPTT and TT tests. The aPTT test was slightly increased with the chitosan-immobilized surface, but TT test was insignificantly changed. To compare the result of anticoagulability of heparin-immobilized PP (The immobilized quantity of heparin was $13.44 \pm 0.09\,\mu g\,cm^{-2}$, measured by Liao and Tyan [23]), the anticoagulability of chitosan-immobilized surface was extremely lower than heparin-immobilized surface. Comparably the aPTT test depicted relatively significantly changes in measuring the clotting time. The aPTT assessment corresponds to Factor X and initiates the inhibition of Factor XII. Both activations follow the intrinsic pathway of coagulation cascade. On the heparin-immobilized surface, it is noticeable for the inhibition of thrombin (common pathway) and Factor X in two different mechanisms [49–52]. Thus, the aPTT and TT were significantly increased with the heparin-immobilized surface. On the chitosan-immobilized surface, chitosan did not affect on thrombin but increase the aPTT to about 5.5 s. The result of aPTT suggested that chitosan contained a higher amount of glucosamine unit to prolong the aPTT to a lesser extent. The slight increase in the coagulation time on chitosan-immobilized surface suggested that the chitosan surface did not readily inhibit the intrinsic coagulation pathway [53], but this effect did involve the Factor XI and XII [54]. Although the aPTT of chitosan-immobilized surface was increased, but the slight increase did not have special meaning of physiology. The specific mechanism of chitosan-induced coagulation remains unknown.

Conclusion

Antenna-coupling microwave plasma system can provide high-density O_2 plasma. A highly oxidative treatment of the porous PP non-woven fabric within 10 s of treatment time has been achieved. Owing to the effective activation of a large surface area, the modified PP non-woven fabric can greatly enhance the grafting density of pAAc. The pAAc-grafted sample is chemically hydrophilic and capable of regaining moisture. Alkaline solution or water absorbency of the plasma-modified pAAc-grafted fabric by our present process increased about five to seven times with respect to the net weight of the non-treated sample that makes the modified PP non-woven fabric feasible as an intensive absorbent and as coupling support to promote chitosan-immobilization through amide bonds.

Although the specific mechanism of chitosan-induced hemostasis remains unclear, but the *in vitro* tests undertaken seem to indicate that the hemostatic effect of chitosan is independent of the normal clotting cascade and may be due to the cellular agglutination of the blood cell component. In this study, it seems that chitosan can bind directly onto the blood cell membrane, possibly via ionic binding, particularly with red blood cells and platelets independent of intrinsic coagulation cascade. Thus, chitosan binding to blood cell membranes may promote immediately binding to other blood cells, and format of hemostatic plug. The effect of chitosan on red blood cell morphology and behavior, shown in the SEM photomicrographs, supports the theory that it interacts with blood cells, involving blood cells cross linking to appearance a cellular aggregate or plug. To develop the wound-dressing substitute for second-degree burn, the relationship between the immobilized chitosan and the coagulation activity should be therefore further studied.

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