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Surface states of PVA/chitosan blended hydrogels

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Abstract

Hydrogels were prepared from blends of poly(vinyl alcohol) (PVA) and chitosan in varying proportions. Electron spectroscopy analysis of the resulting hydrogel membranes, cast on glass plates, revealed that the chitosan component was concentrated on the surface of the air-surface side of the membranes and was nearly constant from 10 to 40 wt% of chitosan content of the blends. The increasing water-contact angle on the hydrogel surface with increasing chitosan content indicated that the chitosan molecules were more hydrophobic than were PVA molecules. The zeta-potential in the air-surface side of the hydrogels increased to reach a constant value at more than 10 wt% chitosan content at all experimental pH values (3.0, 5.0, and 7.2). Electron-probe micro-analyzer results showed that the two-dimensional distribution of the chitosan component on the surface of the air-surface side of the Chitosan-15 sample was heterogeneous when it was compared with that on the surface of the air-surface side of the Chitosan-40 sample; the chitosan component on the surface of the Chitosan-15 sample was localized in islands. Observations of the Chitosan-15 sample by confocal laser-scan microscope gave the same results.

Keywords: Poly(vinyl alcohol); Chitosan; Hydrogel

1. Introduction

Naturally produced polysaccharides, such as chitin [poly-β(1-4)-*N*-acetyl-d-glucosamine] and chitosan [poly-β(1-4)-d-glucosamine], have recently been re-evaluated and found to be useful resources and functional materials [1,2]. However, their homopolymers are inadequate to meet the diversity of our demands for materials. Polymer blending is one of the most effective methods for providing new, desirable polymeric materials for practical applications.

Chitosan membranes blended with poly(vinyl alcohol) (PVA) have already been reported to have good mechanical properties [3] because of the specific intermolecular interactions between PVA and chitosan in the blends. Hydrogels composed of such blends have high blood compatibility [4,5] and are good candidates for use as matrices for the controlled delivery of drugs [6].

Recently, we found that PVA/chitosan hydrogels blended in an autoclave, without any further chemical reactions with crosslinking reagents such as glutalaldehyde or paraformaldehyde, exhibited high attachment to and promoted growth of cultured fibroblast cells (L-929), depending on the chitosan content [7]. Hydrogels are soft rubbery membranes with high elasticity, resembling body tissue. The reason for their high cell attachment and promotion of cell growth is not yet understood. In general, this behavior is known to be affected by the nature of the two-dimensional distribution of functional molecules on the matrices and the surface properties of the matrices, such as electrical charge and water-contact angle [8]. Blends of polymers form unique heterogeneous structures in such a way as to minimize the interfacial or surface free energy [9].

The purpose of the present study was to determine the surface concentration and distribution pattern of chitosan on PVA/chitosan blended hydrogel membranes, using electron spectroscopy for chemical analysis (ESCA), an electron-probe micro-analyzer (EPMA) and a confocal laser-scan microscope (CLSM), and also measuring contact angle and zeta-potential, because these surface states should affect cell attachment and growth behavior.

2. Experimental

2.1. Materials

PVA powder of molecular weight 75 kDa and a saponification degree of 99.85% was supplied by Kuraray Co. Ltd,

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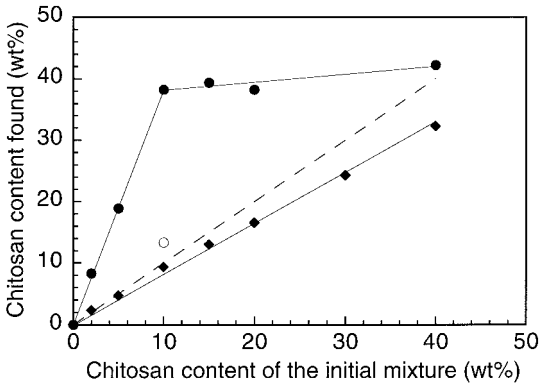

Fig. 1. Chitosan content measured by elementary analysis and ESCA as a function of chitosan content of the initial mixture. ◆, from elementary analysis; ●, in the air-surface side and ○, in the glass-surface side from ESCA.

Japan. Chitosan powder was supplied by Katakura Chikkarin Co. Ltd, Japan, and had a molecular weight of 250 kDa and an *N*-acetylation degree of 14% estimated from elementary analysis. Both powders were used without further purification.

PVA/chitosan blended hydrogel membranes were prepared by the following procedure [7]. The chitosan powder was dissolved in an aqueous solution of acetic acid (chitosan:acetic acid = 5:4). PVA powder was added to the chitosan solution to form an aqueous solution of 10 wt% in total polymer concentration. This solution was autoclaved under nitrogen gas at 2×10^5 Pa and 120°C (Taiatsu Techno Co. Ltd, Japan, TEM-V100). To make sure that the polymer solution obtained was homogeneous, it was irradiated with laser light. The clear solution was cast on a glass plate, gradually dried in an atmosphere of about 60% relative humidity and room temperature (~20°C) for 24 h, and subsequently dried under vacuum at 30°C for 24 h. To neutralize the acetic acid contained in the membrane, the latter was immersed in a 4% NaOH aqueous solution for 6 h, and finally washed thoroughly with deionized water. The initial chitosan contents in the PVA/chitosan blended hydrogels were 2, 5, 10, 15, 20, 30 and 40 wt%. Each hydrogel was abbreviated to Chitosan-X (where X means wt% of chitosan). A pure PVA hydrogel membrane was prepared by the same procedure. These samples were stored in sterilized, deionized water in a refrigerator.

2.2. Surface and elementary analyses

Spectra from electron spectroscopy for chemical analysis (ESCA) were obtained using a PHI model 5600ci spectrometer with a monochrome AlKα X-ray source. Prior to the measurement, the hydrogel samples were attached to a copper block cooled in liquid nitrogen to fix the surface state of the hydrogels instantaneously, lyophilized and analyzed without any pretreatment. The C_{1s} peak for hydrocarbon species was used as reference and assigned the value of 284.6 eV to compensate for charging effects.

The distribution pattern of chitosan on the surface of the hydrogels was observed by a Nippon Denshi electron-probe micro-analyzer (EPMA) JXA-8800M. The hydrogel samples were immersed in a 5 wt% $CuSO_4$ aqueous solution for 6 days at room temperature and then washed with deionized water. Prior to the measurement, the $CuSO_4$-stained samples were attached to a copper block cooled in liquid nitrogen to fix the surface state of the hydrogels instantaneously, lyophilized and analyzed with treatment of carbon deposition.

The air-surface side of the cast membranes was used for surface analyses.

Elementary analysis was carried out by an element analyzer (model CHNS-O EA1108, Calro Erba Instruments, Co. Italy) to determine the nitrogen and carbon content of the hydrogels.

2.3. Confocal laser-scan microscope

The confocal laser-scan microscope was a MRC-1024 (BIO RAD Lab. Co. Ltd) with an Ar ion laser source of 488 nm at 400× scale.

Hydrogel pieces were immersed in a 10 mM phosphate buffer solution containing Fluorescein-4-isothiocyanate (FITC) of 4.4×10^{-4}% for 64 h at 4°C in darkness. The FITC-stained samples were washed with 10 mM phosphate buffer solution of pH 7.2, and then with 10 mM phosphate buffer solution containing 10% methanol to remove the unreacted FITC. The samples were observed using an excitation wavelength of 490 nm and fluorescent wavelength of 520 nm.

2.4. Contact angle

Water-contact angle experiments were performed using a Kyowa Kaimen Science Instrument CA-X. In order to measure a surface property of the hydrogels in the wet state, the procedure used was to attach air to the hydrogels while keeping them in water.

2.5. Surface charge

The electric charge on the hydrogel surface, zeta-potential, was measured using a plane cell, which was attached to an electrophoretic light-scattering instrument (ELS-800, Otsuka Electronics Co. Ltd, Japan). Prior to measurement, the hydrogel membranes were immersed in 10 mM phosphate buffer solutions of pH 7.2, 5.0 and 3.0 for 24 h. The zeta-potential of the air-surface side of the hydrogel membranes was measured in the buffer solutions, which included latex particles that have a slightly negative charge and play a monitoring role.

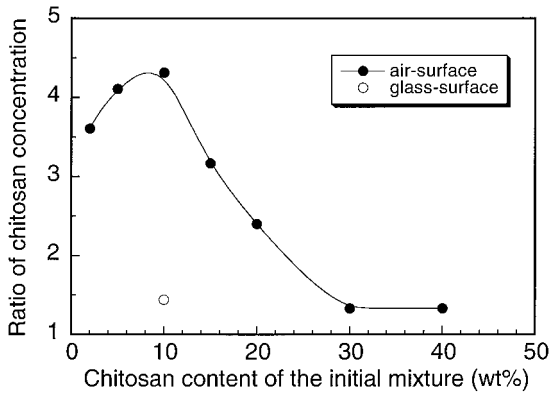

Fig. 2. Ratio of surface concentration of chitosan to bulk concentration of chitosan in hydrogels as a function of chitosan content of the initial mixture. ●, air-surface side and ○, glass-surface side.

3. Results and discussion

The proportion of chitosan contained in the PVA/chitosan blended hydrogels was investigated. The chitosan contents calculated from the results of the elementary analysis were lower than those calculated from the initial amount of chitosan added during the preparation process of the hydrogels (Fig. 1).

The proportion of chitosan on the hydrogel surfaces was then investigated. Specifically, the amounts of both nitrogen and carbon in both the air- and glass-surface sides of the hydrogels were measured by ESCA. In the air-surface side of all the samples, the chitosan contents calculated from ESCA were much higher than those calculated from elementary analysis (Fig. 1). However, the chitosan content in the glass-surface side of the Chitosan-10 sample was nearly the same as that calculated from elementary analysis (Fig. 1). These results suggest that the chitosan components were concentrated on the surface of the air-surface side of the hydrogels. Furthermore, the chitosan contents in the air-surface side were nearly constant at about 40 wt% in hydrogels of 10–40 wt% of chitosan content. If the air-surface were covered with chitosan molecules only, the calculated chitosan content would be 100 wt%, much higher than found in our hydrogels (about 40 wt%). Measurements using differential scanning calorimetry (DSC) [10], X-ray [10,11], Fourier transform infrared FT-IR [11,12], and ^{13}C-CP/MAS NMR [12] have shown that chitosan mixes with PVA at the molecular level in blends and that strong hydrogen bonding between the OH group of PVA and the OH and NH_2 groups of chitosan in the blends takes place. If both PVA and chitosan molecules were co-existing in a molar ratio (per monomer unit) of 5:1 on the air-surface, the calculated chitosan content would be 40 wt%.

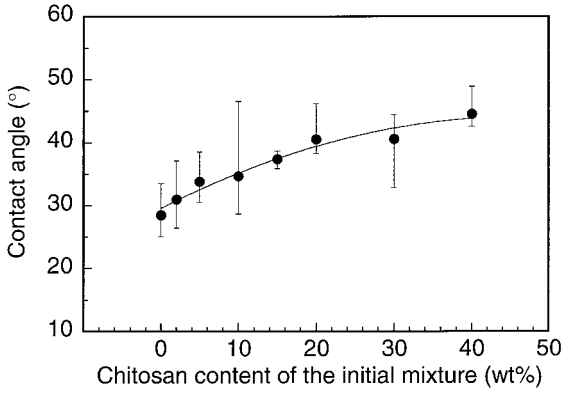

Fig. 3. Relationship between water-contact angle and chitosan content of the initial mixture. The values are means and show error bars.

The ratio of surface concentration of chitosan on the air-surface side to overall concentration of chitosan was calculated by dividing the chitosan content in the air-surface side by that obtained from elementary analysis. At lower chitosan contents of the initial mixture, the surface concentration of chitosan in the air-surface side was about four times higher than the overall concentration would suggest (Fig. 2). With increasing chitosan content the ratio of surface concentration gradually decreased. At 40 wt% chitosan, the difference between surface and bulk concentrations of chitosan became very small. This pattern reveals how the surface chitosan concentration of the Chitosan-10 sample in the air-surface side can be nearly the same as that of the Chitosan-40 sample (see Fig. 1). The result suggests that the chitosan component is concentrated on the surface of the air-surface side during the preparation of the hydrogel membranes.

The contact angle in the air-surface side of the hydrogels to water was investigated as a function of chitosan content of the initial mixture in the blended hydrogels. Water-contact angle increased, i.e. the surface became more hydrophobic, with increasing chitosan content (Fig. 3). The result indicates that chitosan molecules are more hydrophobic than PVA molecules, and migrate to the air-surface side during the preparation of the hydrogel membranes (in the drying process) to minimize surface energy. However, we do not know why the water contact angle increases gradually with increasing chitosan content, even at concentrations higher than 10 wt% chitosan, in spite of the nearly constant surface concentration of chitosan (see Fig. 1).

The chitosan molecules have amino groups that generate positive charges in an aqueous (especially acidic) solution by protonation; thus, the zeta-potential on the air-surface side of the hydrogels was investigated in 10 mM phosphate buffer solutions of pH 7.2, 5.0 and 3.0. The zeta-potential increased with chitosan content and reached a constant value at every pH in hydrogels with more than 10 wt% chitosan content (Fig. 4). The nature of the dependence of the zeta-potential on chitosan content agrees well with that of the chitosan content in the air-surface side of the hydrogel (see Fig. 1), obtained from ESCA. The results prove that the chitosan molecules were concentrated on the surface of the air-surface side of the hydrogels and that the amino groups played a role in the increase in zeta-potential. The pK_a value of the amino group in the chitosan molecule is reported to be

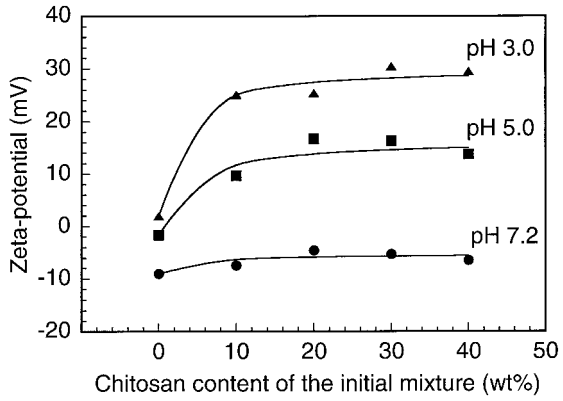

Fig. 4. Relationship between zeta-potential on the air-surface side of the hydrogels and chitosan content of the initial mixture.

6.3–6.6 [13–15]. When the experimental conditions for zeta-potential measurement were more acidic (pH 5.0 and 3.0) than pH 6.3, the zeta-potential values became more positive because of the protonation of the amino groups. However, at pH 7.2 all the blended hydrogel membranes had negative zeta-potential values, because PVA molecules have negative charges.

A linear relationship was observed in all hydrogels between zeta-potential values and experimental pH. From this relationship, the iso-electric point on the surface of each hydrogel was obtained: pH 6.2 (Chitosan-10), pH 6.9 (Chitosan-20), pH 6.7 (Chitosan-30) and pH 6.5 (Chitosan-40).

The two-dimensional distribution of the chitosan components on the surface of these hydrogels was investigated by analyzing, using EPMA, the distribution pattern of $CuSO_4$-complexed amino groups in the chitosan-containing hydrogels. When the distribution of chitosan in the Chitosan-15 sample (Fig. 5b) was compared with that in the Chitosan-40 sample (Fig. 5a), that in the Chitosan-15 sample was heterogeneous, being mainly localized in islands of about 0.020–0.050 mm diameter in the air-surface side. The chitosan components in the glass-surface side of the Chitosan-15 sample (Fig. 5c) were distributed much more uniformly, and similar to the air-surface side of the Chitosan-40 sample. The distribution pattern of chitosan in the Chitosan-40 sample was similar to that of the four-times magnified distribution pattern.

The observations by confocal laser-scan microscope also suggest that the chitosan components in the Chitosan-15 sample are localized in islands in the air-surface side (Fig. 6). The size of the islands in Fig. 6 seems to be similar to that in Fig. 5. In the case of Chitosan-40 and non-blended samples, such a distribution pattern was not observed.

We investigated the miscibility of the component polymers in the blends by DSC and FT-IR spectrophotometry [16]. FT-IR spectra of the dried hydrogel samples indicated specific intermolecular interaction between the component polymers, as reported by other researchers [11,12]. The melting temperature of the samples slightly shifted to the lower temperature region by about 4°C with increasing chitosan content, but the glass transition temperature was not observed clearly. The degree of crystallinity of PVA in the samples, which was estimated from the DSC data, decreased with increasing chitosan content. These results suggest that the distribution of chitosan in the samples with higher chitosan content (Chitosan-40) seems to be uniform at the molecular level. However, in the case of the samples with lower chitosan content such as Chitosan-15, PVA molecules gathered together to form a crystal during the membrane preparation, while PVA molecules that interacted with chitosan molecules did not join the crystal formation and were excluded from forming islands.

Two-dimensional distribution patterns of the chitosan

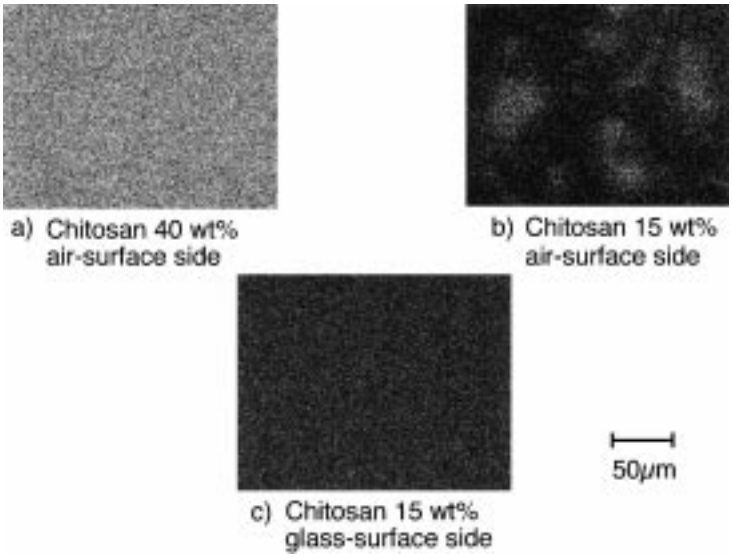

Fig. 5. Two-dimensional distribution of copper on the air-surface side of hydrogels stained by $CuSO_4$: (a) air-surface side for Chitosan-40; (b) air-surface side for Chitosan-15; and (c) glass-surface side for Chitosan-15. White dots indicate the presence of copper.

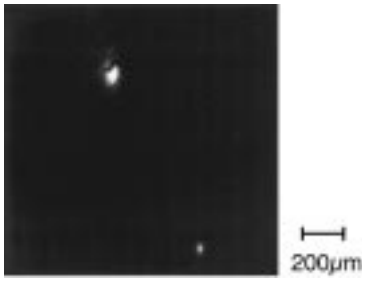

Fig. 6. Confocal laser-scan microscope data for the air-surface side of hydrogels stained by FITC. White dots indicate chitosan molecules that have reacted with FITC.

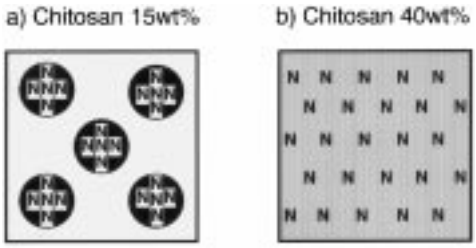

Fig. 7. Schematic representation of the distribution pattern of chitosan components in the air-surface side of the blended hydrogels: (a) Chitosan-15; and (b) Chitosan-40. The chitosan component is abbreviated as N.

components on the air-surface side of the Chitosan-15 and Chitosan-40 samples are schematically represented in Fig. 7 on the basis of the above results. During the drying process of the cast membranes, chitosan is concentrated on the air-surface side of the membranes because chitosan is more hydrophobic than PVA is (see Fig. 3). The total amount of chitosan in the air-surface side of the Chitosan-15 sample is shown to be nearly the same as that in the Chitosan-40 sample (see Fig. 1). The distribution of chitosan molecules in the Chitosan-15 sample is heterogeneous, while that in the Chitosan-40 sample is uniform (see Figs. 5 and 6). Therefore, in the Chitosan-15 sample, the chitosan molecules are localized in high-density islands, and the concentration of chitosan molecules in these islands is higher than their concentration in the air-surface side of the Chitosan-40 sample. This change from heterogeneous to uniform distribution of chitosan molecules, although the two samples had nearly the same amount of chitosan in their air-surface side (see Fig. 1), may be responsible for the increase in the water-contact angle (see Fig. 3).

4. Conclusions

Chitosan components were found to be concentrated on the surface of the air-surface side of the PVA/chitosan blends because chitosan is more hydrophobic than PVA. The surface-concentration effect of chitosan was also clear from the results of the zeta-potential measurements. The distribution of chitosan molecules on the surface of the air-surface side of the Chitosan-40 sample was uniform, while that in the Chitosan-15 sample was heterogeneous. The concentration of chitosan molecules in the localized high-density islands of the Chitosan-15 sample was higher than their concentration on the surface of the air-surface side of the Chitosan-40 sample.

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