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Complex film of chitosan and carboxymethyl cellulose nanofibers

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ABSTRACT

A polymer film composed of a mixture of chitosan (Ch) and carboxymethyl cellulose sodium salt (CMC) nanofibers was deposited on a glass surface. The thin film of the Ch–CMC mixture obtained was stable, and fibroblast adhesion to the film was lowest when the weight ratio of Ch to CMC was 4:6. The ζ-potential and contact angle of the mixture film indicated that a polyion complex of Ch and CMC was formed. The mechanical strength of the film composed of Ch–CMC nanofiber complexes was much higher than that of the film composed of Ch–water-soluble CMC complexes (non-nanofiber), likely because the entanglement of nanofibers was enhanced by electrostatic attractions. These results indicate that the charge-neutralized nanofiber film was highly effective in suppressing cell adhesion and therefore is a promising material for biomedical applications.

1. Introduction

Zwitterionic polymers have been designed to mimic phosphatidylcholine (lecithin), which is abundant in cell membranes [1,2], and their applicability in biomedical fields has extensively been investigated. For example, polymer films composed of apolar monomers such as *n*-butyl methacrylate (BMA) and zwitterionic monomers such as 2-methacryloyloxyethyl phosphorylcholine (phosphobetaine), 3-sulfo-*N*,*N*-dimethyl-*N*-(3-methacrylamidopropyl)propanaminium inner salt (sulfopropylbetaine), and 1-carboxy-*N*,*N*-dimethyl-*N*-(2-methacryloyloxyethyl)methanaminium inner salt (CMB, carboxymethylbetaine) were found to be highly biocompatible [3–16]. We previously reported that the amount of proteins adsorbed and the number of platelets adhered onto a film of a random copolymer of CMB and BMA were much lower than that on a polyBMA film [8,9].

Similarly, the charge-neutralized surface was found to be bio-inert. A self-assembled monolayer of a 1:1 mixture of positively and negatively charged alkyl mercaptans, for example, was found to be resistant to protein adsorption [17]. A terpolymer film composed of 76 mol% of BMA and equal amounts of negatively and positively charged monomer residues (methacrylic acid (MA) and 3-(dimethylamino)propyl methacrylamide (DMAPMA), respectively) was also found to be bio-inert [18].

Polyion complexes (PICs) have been extensively used in many bio-related applications and research fields, including as cell film carriers using PIC gel-modified membranes [19], bio-based food packaging polymers [20], cellulose wet wiper films [21], composite membranes for dehydration of ethanol/water azeotrope [22], ultralong polyion nanoladders facilitated by ionic recognition [23], ocular drug delivery for bioactive proteins [24], and preparation of submicrometer-sized unilamellar PIC vesicles for drug delivery systems [25].

Recently, nanofibers from biomass have been gaining attention because the nanofibers can be easily prepared from various types of biomass. For example, cellulose nanofibers consisting of kraft pulp have been applied to transparent paper (1) by polishing using emory paper (without any additives), (2) by the lamination of transparent plastics (polycarbonate films), and (3) by the deposition of transparent acrylic resins [26]. Acrylic resin nanocomposites combined with chitin nanofibers obtained from crab shells as mechanical-reinforcing agents are promising candidates as substrates for the manufacturing of optoelectronic devices such as bendable displays, flat panel displays, and solar cells [27].

The fibrillation of pulp fiber has mainly been performed by two methods, by using either a grinder or a high-pressure homogenizer. The former method results in the successful fibrillation of

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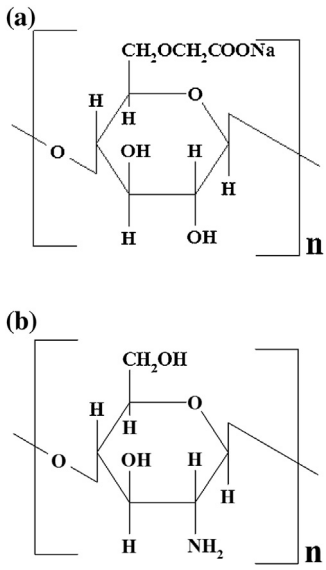

Scheme 1. Chemical structure of (a) carboxymethyl cellulose sodium salt (CMC) and (b) chitosan (Ch).

wood pulp fibers into nanofibers, and provides films with better transparency than that obtained by the latter method after the deposition of acrylic resins [28]. Recently, another fibrillation method has been reported; a water-jet method [29], which requires neither chemicals nor heat energy.

Composite materials composed of chitosan (Ch) or carboxymethyl cellulose nanofibers and other polymers such as alginate and poly(vinyl alcohol) have been developed and examined for biomaterial applications, such as in artificial extracellular matrices and wound dressing [30–32]. However, functional biomaterials using only chitosan and carboxymethyl cellulose nanofibers may be developed based on their advantageous mechanical and electrostatic properties. In this study, a thin film of PIC, composed of a mixture of Ch and carboxymethyl cellulose sodium salt (CMC) nanofibers (Scheme 1), was prepared on the surface of a glass substrate by using the cast method, and the adhesion behavior of cells to the film was investigated. Charge neutralization was found to be critical for biocompatibility of the surface of polymer, metal and ceramic materials.

2. Material and methods

2.1. Materials

CMC and Ch nanofiber suspension (2% in water), which had been prepared by using a unique atomizing system (Star Burst System) [29], were donated by Sugino Machine Ltd. (Uozu, Toyama, Japan). Water-soluble CMC and Chitosan 10 were purchased from Nacalai Tesque Inc. (Kyoto, Japan) and Wako Pure Chemical Industries Ltd. (Osaka, Japan), respectively. Other reagents used were commercially available. All aqueous solutions were prepared in ultrapure water (18 MΩ cm, EMD Millipore, Billerica, MA, USA).

2.2. Construction and characterization of PIC films

A slide glass (Matsunami Glass Ind., Ltd., Osaka, Japan) was cut into a 24 × 26 mm tip and pretreated with an ozone cleaner at room temperature for 1 h. In order to prepare the square PIC film, a silicon frame (thickness, 1.0 mm; square window, 21 × 23 mm; incubated in MeOH for 24 h to remove plasticizer prior to use) was placed on the glass, and a 0.5 wt% suspension of the nanofiber mixture (600 μL), which had been thoroughly dispersed in water by using a vortex mixer for 3 min, was poured into the frame. The glass tip was dried over silica gel in a desiccator at room temperature for 48 h and the silicone frame was removed. At the preparation of non-nanofiber complex PIC film, carboxymethyl cellulose sodium salt and chitosan 10 dissolved in 0.05 M HCl were used, and the PIC films were prepared by the same method as that for nanofiber PIC film.

Contact angles, θ, of pure water (3–4 μL) at 30 s after deposition on the surface of polymer films constructed on glass substrates were measured at 23 °C and 60% relative humidity by using the sessile drop method (CA-D, Kyowa Interface Science, Tokyo, Japan). The θ values were determined at 5 points, and the values were averaged as reported elsewhere [33,34]. All measurements were conducted in air at room temperature. The surface ζ-potentials of the nanofiber complex films constructed on glass substrates (35 × 15 mm) were measured in a 10 mM NaCl aqueous solution at 25 °C by using ζ-potential analyzer (ELSZ-2, Otsuka Electronics Co., Ltd., Osaka, Japan). The thickness of the complex film was evaluated by laser scanning microscopy (TCS SP8, Leica Microsystems, Tokyo, Japan). Moreover, the degree of deacetylation of pure Ch and Ch nanofibers and the degree of carboxymethylation of pure CMC and CMC nanofibers were determined by conductometric analysis. Dried samples (Ch: 10 mg and CMC: 30 mg) were uniformly dispersed in Milli-Q water (10 mL), and then 1.0 mL of 0.1 M HCl was added into the suspension. Fifty microliters of 0.1 M NaOH was added at appropriate intervals, and the conductivity of the suspension was measured at 25 °C. The degree of deacetylation and carboxymethylation were determined from the inflection points of the obtained conductivity curve.

2.3. Mechanical strength of non-fiber film

The mechanical strength of various polymer films was evaluated by using a high-sensitive tensile testing machine (KES-G1-SH, Kato Tech Co., Ltd., Kyoto, Japan). The nanofiber film was prepared from a 1 w/v% Ch-CMC mixture (1200 μL). The film could be easily peeled from the silicon film attached to glass plate. A slip of the film 5–10 mm in width was suspended by using a clamp. The distance between the clamps was set to 5 mm. The relationship between elongation and loading of these nanofiber or non-nanofiber films constructed with various contents of Ch and CMC were measured, and the maximum loading and stretch at fracture was determined. The tensile strength (MPa = N/mm^2) of each film was determined using the following equation.

$$\text{Tensile strength (MPa)} = \frac{\text{maximum load at break (gf)} \times 9.81 \times 10^{-3}\ \left(\text{N/gf}\right)}{\text{sectional area}\ \left(\text{mm}^2\right)}$$

2.4. Adhesion test of fibroblasts

NIH 3T3 cells were used for a cell adhesion assay. Single cells were obtained from culture at passage 141 by treatment with 0.25% trypsin-1 mM ethylenediaminetetraacetic acid solution. Cells suspended in culture medium (minimum essential medium (MEM) containing 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL)) were seeded onto the PIC-modified substrate (sterilized with 70% ethanol aqueous solution) at a density of 5×10^4 cells/cm^2. The seeded cells were incubated for 12 h at 37 °C and 5% CO_2 and washed with fresh culture medium to remove weakly adherent cells. The adhered cells were cultured for an additional 12 h at 37 °C in 5% CO_2; after incubation for 15 min with the culture medium containing 2 μg/mL Hoechst 33342 (Dojindo Laboratories, Kumamoto, Japan) and 1 μg/mL calcein-AM (Dojindo Laboratories), the cells were washed with PBS. Stained cells were observed by using an epifluorescence microscope (DP71, Olympus