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Fibrillation of dried chitin into 10–20 nm nanofibers by a simple grinding method under acidic conditions

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ABSTRACT

Chitin nanofibers were prepared from dried chitin by fibrillation using a grinding apparatus. The fibrillated chitin samples were observed by FE-SEM and were found to have a fine nanofiber network; the structure was highly uniform with a width of 10–20 nm and a high aspect ratio. Mechanical treatment under acidic conditions is the key to fibrillating dry chitin. The cationization of amino groups on the chitin fiber facilitates fibrillation into nanofibers by electrostatic repulsions. Even though the degree of substitution of amino groups was only 3.9%, it was enough to break the strong hydrogen bonds between the nanofibers. Furthermore, optically transparent nanocomposites using chitin nanofibers with acrylic resin were prepared to examine the fibrillation properties of dry chitin and nanofiber homogeneity. Since dried chitin was fully fibrillated, the optical losses of these composites were less than 2% even after the drying process. Moreover the films were characterized by X-ray diffraction, and TGA.

1. Introduction

Chitin is the main component of the exoskeleton of arthropods such as crabs, prawns, and insects. These exoskeletons have a fine hierarchical organization consisting of α-chitin nanofibers and various types of proteins and minerals (Chen, Lin, McKittrick, & Meyers, 2008; Raabe, Sachs, & Romano, 2005). Since chitin nanofibers are considered to have great potential, various methods have been employed to extract chitin nanofibers from these natural nanocomposites (Fan, Saito, & Isogai, 2008a; Min et al., 2004; Revol & Marchessault, 1993; Zhao, Feng, & Gao, 2007). We have recently succeeded in isolating α-chitin nanofibers from crab and shrimp shells with a uniform width of 10–20 nm and a high aspect ratio by simple process (Ifuku et al., 2009). Since each chitin nanofibers is encased in proteins and minerals, the nanofibers can be isolated by the removal of these matrix components, followed by a quite simple grinding treatment for fibrillation. In the grinding treatment, an acidic condition is also the key to fibrillating the chitin effectively (Fan, Saito, & Isogai, 2008b). A small number of amino groups in the chitin are cationized by the addition of an acid, which promotes the fibrillation of chitin into nanofibers by electrostatic repulsion. Electrostatic repulsion force is applied for the fibrillation of cellulose to obtain nanofibers through TEMPO-catalyzed oxidation too (Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006).

In general, the drying process of chitin and cellulose fibers generates strong hydrogen bonding between these fibers after removal of the matrix, which makes it difficult to fibrillate them to nanofibers. Therefore, it is commonly understood that these natural polysaccharides must be kept wet after removal of the matrix for nanofiber preparation (Abe, Iwamoto, & Yano, 2007; Fan, Saito, & Isogai, 2009; Ifuku et al., 2009; Iwamoto, Abe, & Yano, 2008; Saito et al., 2006). However this requirement presents a strong disadvantage in the commercial application of nanofibers. If chitin nanofibers could be obtained from dry chitin, we could prepare nanofibers easily and immediately whenever we like. Therefore, preparation of nanofibers from a dried pure chitin is an important goal for expanding the use of nanofibers as an environmentally friendly natural nanomaterial. If the electrostatic repulsion force of amino cations can break the hydrogen bonds between the chitin bundles, chitin nanofibers could be obtained from pure dry chitin. Accordingly, we have studied the fibrillation of dried chitin into nanofibers by a grinding method under acidic conditions.

2. Experimental section

2.1. Materials

Dried crab shell powder of *Paralithodes camtschaticus* (Red king crab) and chitin powder from crab shells was purchased from Kawai Hiryo and Nacalai Tesque, respectively. The other chemicals

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were purchased from Aldrich or Kanto chemical and used without further purification.

2.2. Purification of chitin from crab shell

Crab shell powder was purified according to the general method (Shimahara & Takiguchi, 1998). Crab shell powder was treated with 2 N HCl for 2 days at room temperature to remove the mineral salts. The suspension was filtered and washed thoroughly with distilled water. The sample was refluxed in 2 N NaOH for 2 days to remove the proteins. After filtration and rinsing with distilled water, the pigment and lipid compositions in the sample were removed by ethanol for 6 h under reflux followed by filtration and rinsing with water.

2.3. Fibrillation

Purified dry chitin was dispersed in water at 1 wt.%, and acetic acid was added to adjust the pH value to 3 to facilitate fibrillation. The chitin was roughly crushed with a domestic blender. Finally, the slurry was passed through a grinder (MKCA6–3; Masuko Sangyo Co., Ltd.) at 1500 rpm. Grinder treatment was performed with a clearance gauge of −1.5 (corresponding to a 0.15 mm shift) from the zero position. The position was determined as the point of slight contact between the two grinding stones.

2.4. Fabrication of chitin nanofiber composites

Fibrillated chitin nanofibers were dispersed in water at a fiber content of 0.1 wt.%. The suspension was vacuum filtered using a hydrophilic polytetrafluoroethylene membrane filter (Millipore, pore size: 0.2 μm). The obtained chitin nanofiber sheets with 50 mm thick, were cut into 2 × 3 cm and were impregnated with neat acrylic resin (tricyclodecane dimethanol dimethacrylate (TCDDMA), Shin-Nakamura Chemical Co., Ltd.) with 2-hydroxy-2-methylpropiophenone photo-initiator under reduced pressure overnight. The refractive index of TCDDMA resin was 1.532. The resin-impregnated sheets were cured using UV curing equipment (SPOT CURE SP-7, Ushio Inc.) for 8 min at 40 mW/cm^2. The chitin nanofiber composite sheets thus obtained were approximately 70 μm thick, and the fiber content was approximately 58%.

2.5. Scanning electron microscopy (SEM)

The chitin suspension was placed in a Teflon petri dish, and ethanol was added. The diluted suspension was dried at 105 °C in the oven, and the obtained sheets were coated with an approximately 2-nm layer of platinum by an ion-sputter coater and observed with a field emission scanning electron microscope (JSM-6700F; JEOL, Ltd.).

2.6. Elemental analysis

The DS values of the amino groups of the chitin nanofibers were calculated from the C and N content in the elemental analysis data using an elemental analyser (Elementar Vario EL III, Elementar).

2.7. Regular light transmittances

UV–visible transmittance spectra were recorded on a JASCO V-550 photometer measured at 200–1000 nm.

2.8. X-ray diffraction

Equatorial diffraction profiles were obtained with Ni-filtered Cu Kα from an X-ray generator (Shimadzu XRD-6000) operating at 40 kV and 30 mA.

2.9. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was carried out on DTG-50 (Shimadzu). Samples were placed in an open aluminum pan. Temperature programs were from 50 to 500 °C at a heating rate of 10 °C min^{-1} under an air condition.

3. Results and discussion

3.1. Fibrillation of dry chitin into nanofibers

First, we tried to prepare cellulose nanofibers from dry pulp fibers, as a preliminary experiment. Fig. 1 shows SEM images of cellulose fiber (a and b) before and (c) after one pass through the grinder with a concentration of 1 wt.% for fibrillation of pulp fibers into cellulose nanofibers. In Fig. 1a and b, it can be seen that pulp fibers are made up of a bundle of cellulose nanofibers. Fig. 1c shows that the pulp fibers were not successfully fibrillated into nanofibers after the grinder treatment. This is obviously because the drying process for the pulp-making causes strong hydrogen bonding between the nanofibers after the removal of matrix substances of hemicellulose and lignin, thus making it hard to obtain cellulose nanofibers. So in general, the sample should never-dry out completely for bionanofiber preparation after the removal of matrix (Abe et al., 2007; Iwamoto et al., 2008).

We next tried to fibrillate dried chitin into nanofibers. Dried chitin was prepared from a crab shell with an α-chitin crystal structure, which is commercially available as a fertilizer at low cost. The chitin was purified by the removal of matrix components of proteins and calcium carbonate according to the conventional method, followed by drying in an oven. It is well known that almost all proteins and minerals (mainly calcium carbonate) can be removed by treatment with NaOH and HCl solutions (<0.1%) (Shimahara & Takiguchi, 1998). On the other hand, since complete removal of hemicelluloses from wood fiber is difficult, considerable amount of hemicelluloses remain in cellulose by alkali treatment (Iwamoto et al., 2008). Fig. 2a shows an SEM image of a crab shell after removing proteins and minerals, followed by drying. It can be observed that the chitin was made up regularly structured bundles of nanofibers. The image is from the endocuticle part of the shell, which makes up approximately 90 vol.% of the crab exoskeleton (Chen et al., 2008). The slurry of purified dry chitin was passed through a grinder with a concentration of 1 wt.% with and without acetic acid to fibrillate the bundles of chitin nanofibers. Interestingly, in the case of treatment under acidic condition, the obtained chitin slurry had high viscosity, similar to the chitin nanofibers prepared by the previous method (Ifuku et al., 2009). This result suggests that dried chitin was successfully fibrillated and homogeneously dispersed in acidic water with a high surface-to-volume ratio. Fig. 2b–d shows SEM images of chitin fibers after one pass through the grinder treated (b) without and (c and d) with acetic acid. In the case of grinder treatment without acetic acid, dried chitin could not be fibrillated at all, and thick bundles of chitin nanofibers were observed, as shown in Fig. 2b. This lack of fibrillation also occurred because the drying process of purified chitin causes strong hydrogen bonding between hydroxyl, acetamide, and amino groups with strong dipole moments on the chitin fiber surface, which make it difficult to fibrillate bundles of chitin nanofibers as well as dry pulp fibers. On the other hand, chitin slurry treated with acetic acid at pH 3 was successfully fibrillated, as shown in Fig. 2c and d. The chitin sample has a very fine nanofiber network; the structure is highly uniform with a width of 10–20 nm and a high aspect ratio, including a 2 nm thick platinum coating layer, and thicker fibers were not observed within the extensive area. The appearance of the fibers was very similar to nanofibers prepared with the never-dry process used in the previous method

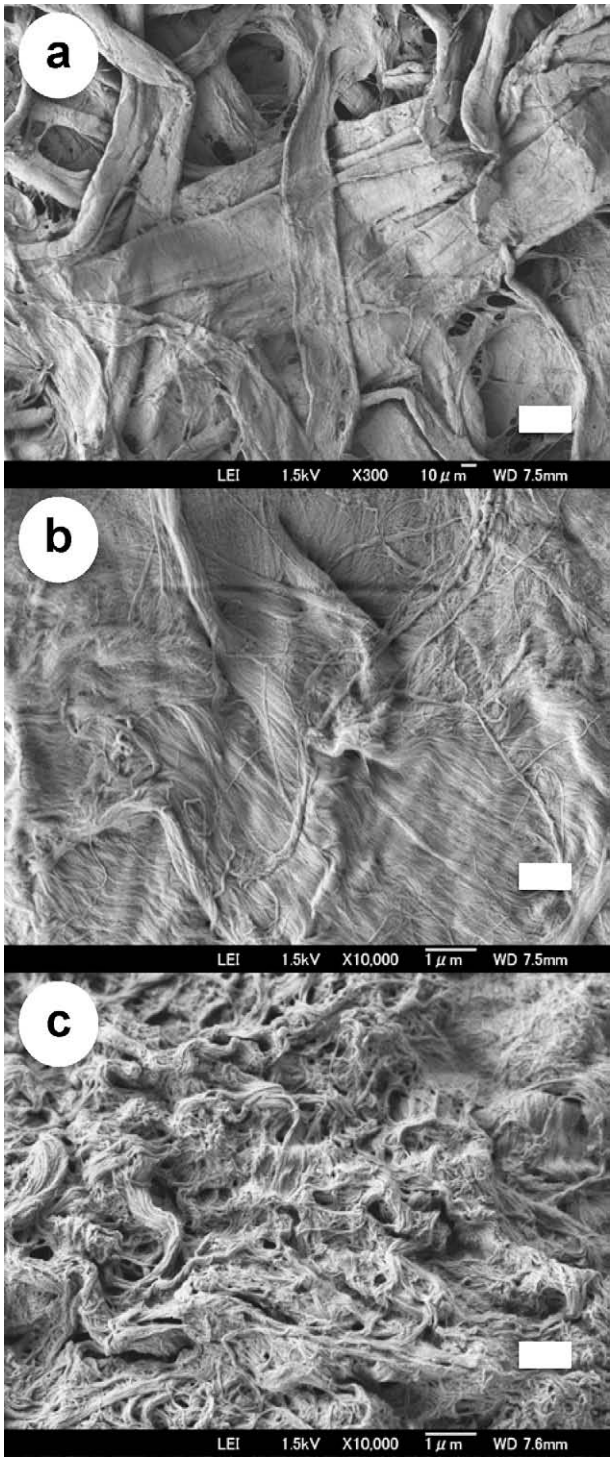

Fig. 1. FE-SEM micrographs of cellulose fibers from wood pulp (a and b) before and (c) after one pass through the grinder. The length of the scale bar is (a) 30000 nm, and (b and c) 1000 nm, respectively.

(Ifuku et al., 2009). The success of this new method is obviously due to the electrostatic repulsions between the nanofibers. That is, slight C2 amino groups on the chitin fiber surface were cationized under acidic conditions, which facilitated fibrillation into nanofibers by electrostatic repulsions. It should be emphasized that even though the degree of substitution of the amino groups was only 3.9%, the electrostatic repulsion force arising from the cationic surface charges was enough to break strong hydrogen bonds between the nanofibers. Thus, grinder treatment under acidic conditions allowed us to obtain chitin nanofibers with a uniform width of 10–20 nm from purified dry chitin easily and immediately. This method could provide a significant advantage for industrial application in terms of a stable supply, storage stability, transportation costs, storage space, and so on, since chitin nanofibers can be prepared by a simple method from light, low-volume, and nonperishable dried chitin. Thus, use of the chitin nanofibers could be quite different from that of cellulose, which does not have amino groups to cause electrostatic repulsions. Since other acidic chemicals are also available to facilitate fibrillation, including ascorbic acid, lactic acid, and so on, we can select acidic additives in accordance with their applications.

The preparation method for chitin nanofibers was also found to be applicable to dry chitin powder purchased from a chemical reagent company. In Fig. 3a, we can see well by SEM that commercially available dry α-chitin powder from crab shell is also made up of nanofibers with a variety of thicknesses from tens to hundreds of nanometers. Moreover, these thicker fibers are also known to be made up of bundles of thinner nanofibers (Chen et al., 2008; Raabe et al., 2005). Fig. 3b and c shows SEM images of chitin fibers after one pass through the grinder without and with acetic acid, respectively. In Fig. 3b, it can be seen that the chitin powder was not fibrillated at all due to the strong interfibrillar hydrogen bonding interaction. On the other hand, in Fig. 3c, the aggregates have clearly been fibrillated into homogeneous nanofibers with a width of 10–20 nm by grinder treatment at pH 3, even though the degree of substitution of amino groups was only 3.9% and the slurry also formed a gel. This is also due to the electrostatic repulsion caused by cationization of amino groups on the chitin fiber surface. In this way, since commercially available dry chitin powder is also made up of bundles of nanofibers, chitin nanofibers could easily be prepared from the dry chitin powder by using the repulsion force, thus breaking the hydrogen bonds. Chitin nanofibers from commercial pre-purified dry chitin is advantageous for laboratory-scale investigations because a large amount of chitin could be immediately and easily obtained by a simple fibrillation process under acidic conditions without any purification processes (removal of proteins, minerals, lipids, and pigments), which generally require at least 5 days (Shimahara & Takiguchi, 1998).

3.2. Characterization of chitin nanofibers/TCDDMA resin nanocomposite

Recently, Yano et al. reported composite films reinforced with cellulose nanofibers (Yano et al., 2005). Due to the size effect, the nanocomposite was optically transparent even with a high fiber content. This study could be applicable for chitin nanofibers for transparent composite preparation, and it is known that this transparency strongly depends on the fiber thickness (Nogi, Handa, Nakagaito, & Yano, 2005). Here, to examine the fibrillation properties of dry chitin as well as nanofiber homogeneity, we prepared optically transparent nanocomposites using obtained chitin nanofibers with acrylic resin according to the previously reported procedure (Ifuku et al., 2007; Yano et al., 2005). The thickness and fiber content of the prepared nanocomposites conformed to 70 μm and 60 wt.%, respectively. Fig. 4 shows the regular light transmittance spectra of acrylic resin without fibers, nanocomposites reinforced with chitin nanofibers from crab shell fibrillated with the conventional never-drying process (Ifuku et al., 2009), nanofibers fibrillated under acidic condition after the drying process, and nanofibers made from commercial dry chitin powder. And appearance of optically transparent nanocomposite, prepared from dry chitin are shown in Fig. 5. Compared with the acrylic resin film of the same thickness, these nanocomposites, even with a high fiber content had high transparency in the range of visible light of

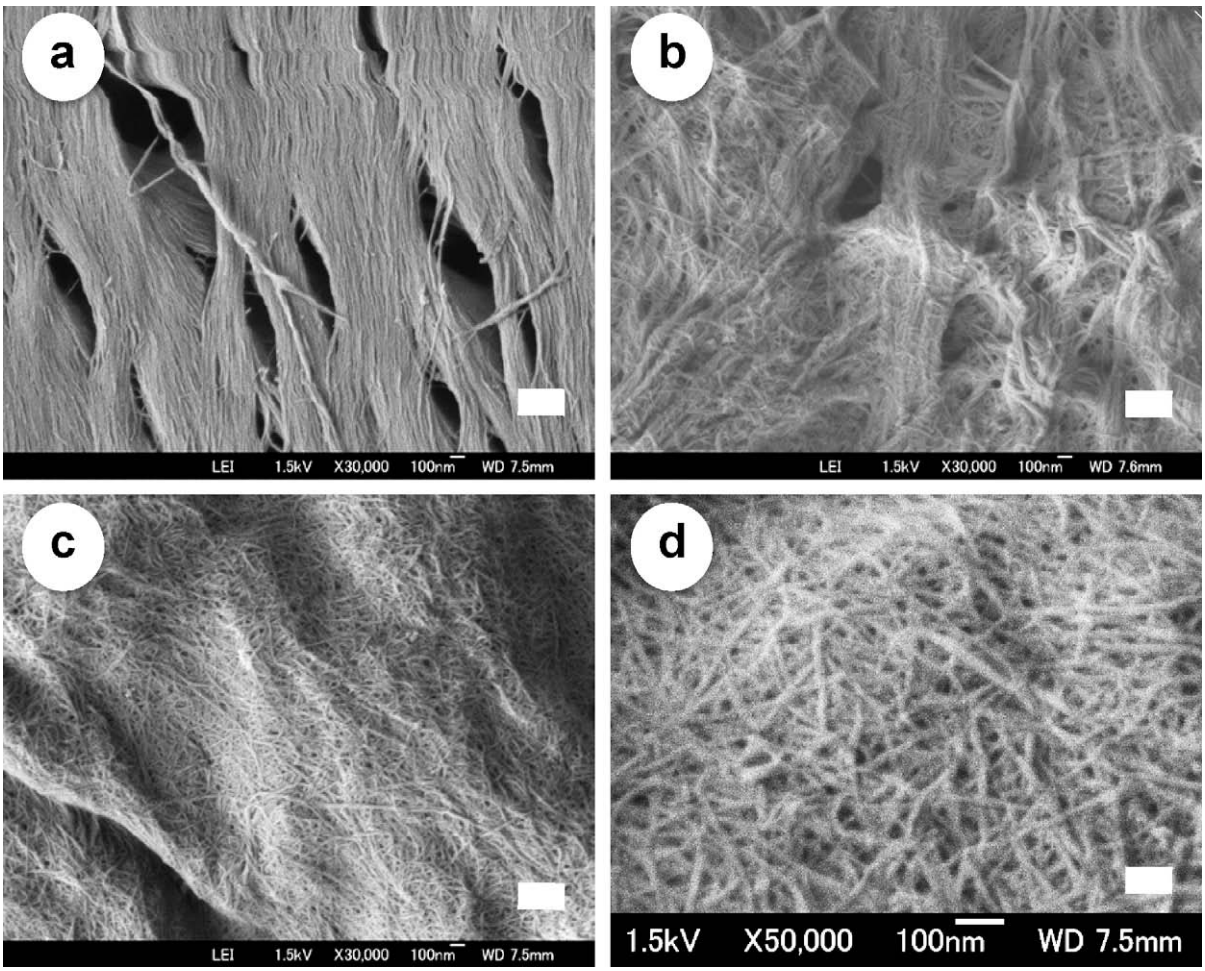

Fig. 2. FE-SEM micrographs of (a) crab shell after removing matrix components, and chitin fibers after one pass through the grinder treated (b) without and (c and d) with acetic acid. The length of the scale bar is (a–c) 300 nm, and (d) 100 nm respectively.

400–800 nm, indicating that each chitin nanofiber was far thinner than the wavelength of visible light as shown in Figs. 2c, d and 3c. Remarkably, the regular transmittances of these nanocomposites were 89.8%, 88.7%, and 87.9%, respectively at a 600-nm wavelength, which is the center of the visible light spectrum. Therefore, the optical losses of the composites caused by the drying process were less than 2%. These results obviously indicate that the chitin nanofibers obtained from these dried chitin samples were fully fibrillated under acidic conditions just as well as ground in a never-dried state. Since in the case of dried cellulose nanofibers, optical loss of the cellulose/resin nanocomposite was considerably high, this feature distinguishes chitin nanofibers from cellulose nanofibers (Iwamoto et al., 2008).

Fig. 6 shows X-ray diffraction profiles of (a) acrylic resin without fibers, (b) transparent chitin/TCDDMA nanocomposites, (c) chitin nanofibers, and (d) dry chitin powder. In Fig. 6d, the four diffraction peaks of chitin nanofibers observed at 9.3°, 19.3°, 20.8°, and 23.4°, which correspond to 020, 110, 120, and 130 planes, respectively, are typical crystal patterns of α-chitin. The diffraction profile of the chitin nanofibers (Fig. 6c) is in excellent agreement with the profile of dry chitin powder, indicating that crystal structure of the obtained nanofibers did not change after one pass through the grinder. The TCDDMA resin showed a diffraction pattern of a mainly amorphous material. The X-ray profile of the nanocomposite clearly showed diffraction peaks of both chitin nanofibers and TCDDMA components. This indicates that each crystal structure of the nanocomposite did not change after composition process.

Thermogravimetric analyses (TGA) of nanofibers from dry chitin, TCDDMA resin, and chitin/TCDDMA nanocomposite were carried out to evaluate their degradation profiles and thermal stability (Fig. 7). In the thermogram of chitin nanofibers, there was one degradation step, which starts from 250 °C with a major degradation peak at 350 °C, determined by the derivative TGA curves. TCDDMA resin showed the two mass losses in the temperature range of 215–455 °C with the major degradation peaks at 305 and 427 °C, respectively. Although the TGA curves of chitin/TCDDMA nanocomposite showed a combination of thermal degradation of TCDDMA resin and chitin nanofibers, the major degradation temperature of the chitin component increased from 350 to 365 °C. The slight increase in the thermal stability could be due to the composition-effect. That is, since chitin nanofibers were completely embedded in TCDDMA resin, the nanofibers were not exposed to the air, which inhibited thermal degradation.

4. Conclusion

Chitin nanofibers were prepared from dried chitin, which was extracted from crab shell. The nanofibers from dried chitin had a uniform width of approximately 10–20 nm, the same as nanofibers prepared in a never-dried state. Mechanical treatment under acidic conditions is the key to fibrillating dried chitin. The cationization of amino groups on the chitin fiber surface broke the strong hydrogen bonds between the nanofibers by an electrostatic repulsion. These

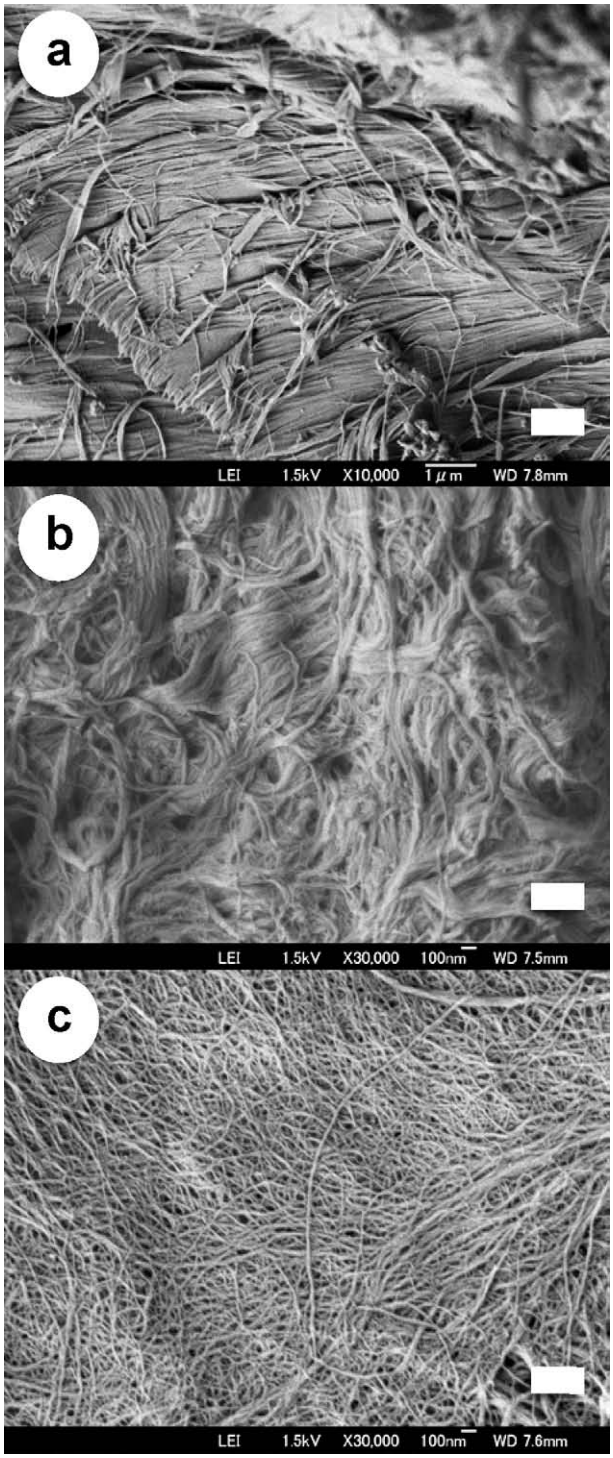

Fig. 3. FE-SEM micrographs of (a) commercially available dry α-chitin powder, and chitin fibers after one pass through the grinder (b) without and (c) with acetic acid. The length of the scale bar is (a) 1000 nm and (b and c) 300 nm, respectively.

results indicate that the never-dried process is not necessary for chitin nanofiber preparation; this finding is quite different than that for cellulose nanofibers, since cellulose does not have ionic charges without chemical modification. Nanofibers from dry chitin are advantageous for commercial applications in terms of storage, supply, transportation, and so on. Since this method allows us to obtain homogeneous chitin nanofibers with a high surface-to-volume ratio effectively, easily and immediately, we expect that this

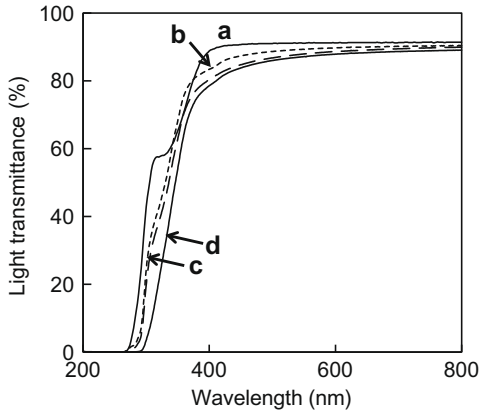

Fig. 4. Regular light transmittance spectra of (a) acrylic resin without fibers, and nanocomposites reinforced with (b) chitin nanofibers from crab shell fibrillated by the conventional never-drying process, (c) chitin nanofibers fibrillated under acidic condition after the drying process, and (d) nanofibers made from commercial dry chitin powder.

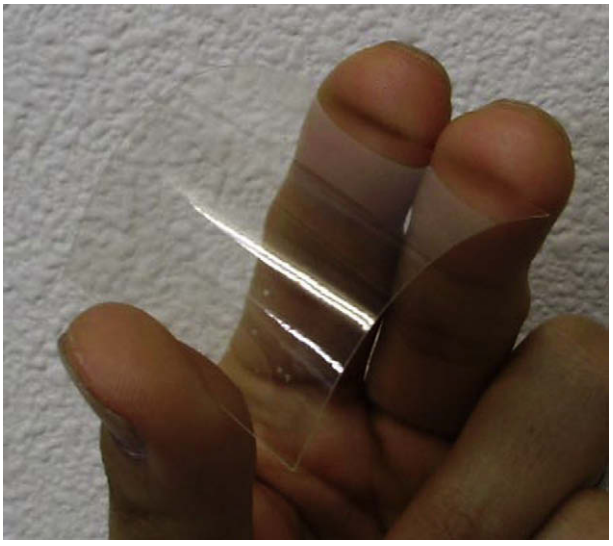

Fig. 5. Appearance of optically transparent nanocomposite prepared from dry chitin.

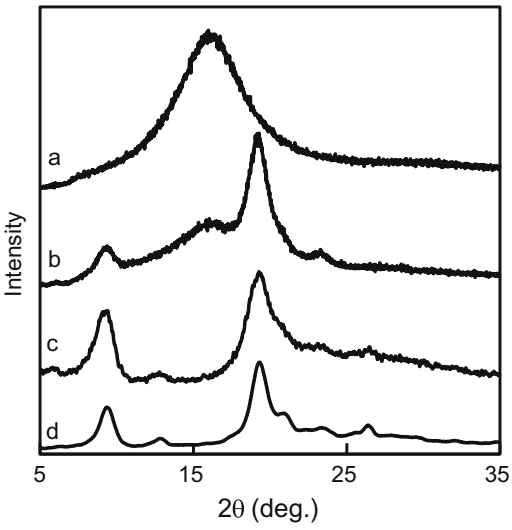

Fig. 6. X-ray diffraction profiles of (a) TCDDMA resin, (b) chitin/TCDDMA nanocomposite, (c) chitin nanofibers, and (d) dry chitin powder.

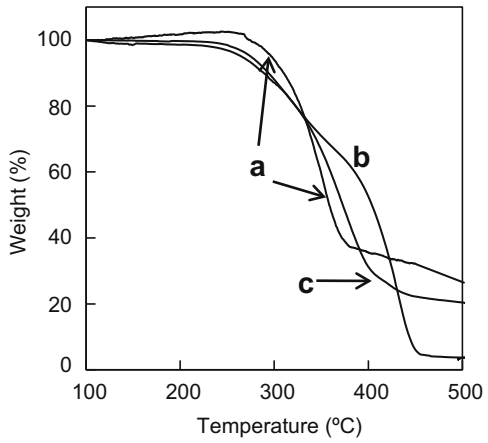

Fig. 7. TGA curves of (a) chitin nanofibers, (b) TCDDMA resin, and (c) chitin/TCDDMA nanocomposite.

study will expand the potential application of chitin nanofibers into novel green nanomaterials.

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