

Effect of Acidity on the Physicochemical Properties of α - and β -Chitin Nanofibers

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Abstract

We have investigated whether acidity can be used to control the physicochemical properties of chitin nanofibers (ChNFs). In this study, we define acidity as the molar ratio of dissociated protons from the acid to the amino groups in the raw chitin powder. The effect of acidity on the physicochemical properties of α - and β -ChNFs was compared. The transmittance and viscosity of the β -ChNFs drastically and continuously increased with increasing acidity, while those of the α -ChNFs were not affected by acidity. These differences are because of the higher ability for cationization based on the more flexible crystal structure of β -chitin than α -chitin. In addition, the effect of the acid species on the transmittance of β -ChNFs was investigated. The transmittance of β -ChNFs can be expressed by the acidity regardless of the acid species, such as hydrochloric acid, phosphoric acid, and acetic acid. These results indicate that the acidity defined in this work is an effective parameter to define and control the physicochemical properties of ChNFs.

Keywords: Chitin, Nanofiber, Dispersibility

1. Introduction

Chitin is a polysaccharide and it has hierarchical structures based on chitin nanofibers (NFs) (ChNFs) [1,2]. ChNFs can be obtained by acid hydrolysis [3], ultrasonic homogenization [4,5], and the electrospinning method [6], and they have attracted research interest in materials engineering [7,8]. In particular, chitin can be applied to medical supplies [9–11], cosmetics [12], and food [13] because the *N*-acetyl group at the C2 position has high biochemical significance [14,15].

There are two types of chitin depending on crystalline structure: α -chitin and β -chitin. α -Chitin has antiparallel molecular chains with hydrogen bonds between the chains [16]. β -Chitin has parallel molecular chains without significant hydrogen bonds between intermolecular sheets [17,18]. It is expected that the physicochemical properties of ChNFs strongly depend on their crystal structure (α - or β -chitin) because the amounts of hydrogen bonds in the crystals are different. Some of the properties of α - and β -ChNFs have been separately reported. For example, it has been reported that β -chitin can be disintegrated into β -ChNFs with 3–4 nm in widths at pH 3–4 using an ultrasonic homogenizer [19]. However, α -chitin cannot be disintegrated into α -ChNFs under the same conditions. Considering the past literature on ChNFs, it seems that β -ChNF dispersions are more transparent and viscous than α -ChNF dispersions [20,21]. Comparison of the two types of chitin in terms of the physicochemical properties of the NFs prepared under the same conditions is required.

Adding acid to raw chitin powder before disintegration is important to obtain fine homogeneous ChNFs because the protons from the acid bond to the amino groups of chitin. Protons bonding to the amino groups, namely, cationized chitin, result in electrostatic repulsion between ChNFs. The electrostatic repulsion improves the dispersibility of the ChNFs. Previously, we reported that when acid remained in β -chitin powder after β -chitin purification from squid pen, fine homogeneous β -ChNFs can be obtained [22]. However, the details of the effect of the amount of the acid on the physicochemical properties of β -ChNFs are not clear.

To evaluate the amount of added acid, the pH, which includes information about the aqueous solution, is generally used [23–25]. A parameter considering not only the aqueous solution but also the amount of amino groups in raw chitin should be used to control the physicochemical properties of ChNFs.

In this work, we propose a new parameter for the acidity: the molar ratio of the dissociated protons from the acid added to the amino groups in chitin. For this new parameter, we assumed that the amounts of (1) dissociated protons for cationization of chitin and (2) amino groups accepting protons are important factors to control the physicochemical properties of ChNFs. We investigated whether the acidity parameter can be applied to various acid species, such as hydrochloric acid (HCl), acetic acid (CH₃COOH), and phosphoric acid (H₃PO₄). We also compared the physicochemical properties of α - and β -ChNFs using the acidity parameter. We used a Star Burst system with a water jet to produce the NFs because it can produce ChNFs

even without acid, namely, under neutral pH. We checked the effectiveness of the new acidity parameter to control the physicochemical properties of ChNFs with and without acid.

2. Experimental Section

2.1. Materials

α -Chitin and β -chitin were used in this study. α -Chitin (<100 μm in diameter, 96% degree of *N*-acetylation) was obtained from Yaegaki Bio-industry, Inc. (Himeji, Japan) and used without further purification. β -Chitin was prepared from squid pens (*Todarodes pacificus*) according to the method reported in our previous study [22]. In brief, squid pens were treated with 0.1 mol L⁻¹ hydrochloric acid solution for 16 h at 15 °C to remove ash and then treated twice with 1 mol L⁻¹ sodium hydroxide solution for 2 h at 90 °C to remove protein. The purified β -chitin (95% degree of *N*-acetylation) was pulverized to approximately 100 μm particle size using a dry pulverizer (Nippon Steel & Sumikin Fine Technology Co., Ltd., Cyclone Mill, Osaka, Japan). Hydrochloric acid, phosphoric acid, acetic acid, sodium hydroxide, and *tert*-butyl alcohol were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used without further purification.

2.2. Preparation of ChNFs

The α - and β -chitin powders were separately suspended in distilled water at 0.1 w/v% or 1

w/v%. Dilute hydrochloric acid solution (or dilute acetic or phosphoric acid) was added to the chitin slurries to adjust their acidity before disintegrating into NFs. The pK_a values of phosphate are $pK_{a1} = 2.15$, $pK_{a2} = 7.20$, and $pK_{a3} = 12.33$, and that of acetate is $pK_a = 4.76$ [26]. The chitin slurries were disintegrated using a Star Burst system (Star Burst Mini, Sugino Machine Co., Ltd., Uozu, Japan) equipped with a ball-collision chamber (Fig. 1). The chitin slurries pressurized at approximately 235 MPa were ejected from the nozzle (100 μm aperture) and were made to collide with the ceramic ball. The disintegrated samples were cooled at 25 °C through a heat exchanger and then recovered. The number of collisions with the ceramic ball (passes) was set to 2, 5, or 10. The pH of the dispersion was measured before and after disintegration using a pH meter (KR5E equipped with LE407, As One Co., Ltd., Osaka, Japan).

2.3. X-Ray Diffraction (XRD)

The 1 w/v% α - and β -ChNFs were dried at 75 °C for 12 h. The dried α - and β -chitins were converted into pellets by pressing at approximately 750 MPa for 1 min using a hand-press machine (SSP-10A, Shimadzu Co., Ltd., Kyoto, Japan). The XRD patterns of the pellets were measured from diffraction angle $2\theta = 5\text{--}35^\circ$ using an X-ray diffractometer (RINT 2500HF/PC, Rigaku Co., Ltd., Tokyo, Japan) with Cu-K α radiation at 40 kV and 40 mA. The crystallinity index (CI) was determined with the following equation [27]:

$$CI = (I_{1-10} - I_{am}) / I_{1-10} \quad (1)$$

where I_{1-10} is the maximum intensity of the [1-10] mixed plane, I_{am} is the intensity of the amorphous diffraction at 16° . The d -spacing and crystal size (CS) were determined with Bragg's law and Scherrer's equation:

$$d\text{-spacing } [\text{\AA}] = \lambda / 2\sin\theta \quad (2)$$

$$\text{CS [nm]} = k\lambda / \beta_0\cos\theta \quad (3)$$

where λ is the wavelength of Cu-K α radiation ($\lambda = 1.5418 \text{ \AA}$), θ (in radians) is the scattering angle, k is the constant indicating shape factor (0.9), and β_0 (in radians) is the full width at half-height of the diffraction peak.

2.4. Fourier Transform Infrared (FT-IR) Spectroscopy

The dried chitins were blended with potassium chloride and converted into pellets using a mini hand-press machine (MHP-1, Shimadzu Co., Ltd.). FT-IR spectra of the pellets were recorded using an FT-IR spectrometer (FT/IR 4200, Jasco Co., Ltd., Hachioji, Japan) from 500 to 4000 cm^{-1} with 4 cm^{-1} resolution and 32 scans.

2.5. Optical Transmittance

α - and β -ChNFs (0.1 w/v%) were loaded into quartz cuvettes. The transmittance was measured in the range 200–700 nm at 25°C using a spectrophotometer (V530, Jasco Co., Ltd.) equipped with a Peltier-type temperature controller (ETC-505, Jasco Co., Ltd.) using distilled

water as the blank. The transmittance at 600 nm was used to compare different conditions.

2.6. Viscoelastic Measurements

α - and β -ChNFs (1.0 w/v%) were loaded into 50 mL glass bottles. The α and β -ChNFs were heated at 80 °C for 30 min using a constant temperature drier, stirred at 2000 rpm for 1 min, and then defoamed at 2000 rpm for 1 min using a mixer (ARE-250, Thinky Co., Ltd., Tokyo, Japan). The defoamed α - and β -ChNFs were placed in a thermostat chamber filled with water at 25 °C, and the viscosity was measured with a Brookfield viscometer (DV-1 Prime, Brookfield Asset Management Inc., Toronto, Canada). A #64 spindle was used at a viscosity above 1000 mPa s. A #62 spindle was used at a viscosity below 1000 mPa s. Next, the rotating speed was accelerated at 30, 50, and 100 rpm, and the viscosity at 600 s was used to compare the different conditions. Finally, the rotating speed was decelerated at 50 and 30 rpm, and the viscosity at 300 s was used to compare the different conditions.

2.7. Electrophoretic Mobility Measurements

α - and β -ChNFs (0.1 w/v%) were sonicated at 40 °C for 5 min with an ultrasonic washing machine under 0.02 MPa vacuum conditions to remove air bubbles, and they were then loaded into disposable polycarbonate cells. The electrophoretic mobility (EM) was measured at 25 °C using a laser-Doppler-electrophoresis-type apparatus (Zetasizer Nano, Malvern Instruments

Ltd., Malvern, UK). The average value of the EM was calculated by averaging the values obtained by performing the measurement three times with the same samples.

2.8. Field-Emission Scanning Electron Microscopy

α - and β -ChNFs (0.1 w/v%) were diluted with *tert*-butyl alcohol and precipitated with a centrifuge (MX-305, TOMY SEIKO Co., Ltd., Tokyo, Japan) at 20,000 \times g. After repeating this process several times, the solvent of the supernatant was changed from water to *tert*-butyl alcohol. The precipitated sample was frozen in a frozen glass bottle and dried under 0.02 MPa vacuum conditions for a few hours. The dried α - and β -ChNFs were coated with an \sim 2 nm thick osmium layer using an osmium coater (Neoc-STP, Meiwafoysis Co., Ltd., Tokyo, Japan). The sample was observed by a field-emission scanning electron microscope (S-5000, HITACHI Co., Ltd., Tokyo, Japan) operated at 5.0 kV. A histogram of the α - and β -ChNF widths was constructed from four field-emission scanning electron microscopy (FE-SEM) images (number of NF widths \approx 170) with Image-J (NIH, Bethesda, USA) [28].

3. Results

3.1. Physicochemical Properties of α - and β -ChNFs Prepared at Various Acidities

We define the acidity using the following equation:

Acidity [mol mol^{-1}] = amount of dissociated protons [mol]/amount of amino groups in chitin

[mol] (4)

The amount of dissociated protons of HCl was calculated from the concentration and the volume of the HCl aqueous solution because HCl completely dissociates at any pH. The amount of amino groups in chitin was calculated from the mass of chitin and the degree of *N*-acetylation. In this study, α - and β -ChNFs at 0.93 mol mol⁻¹ acidity are expressed as α -ChNFs [0.93] and β -ChNFs [0.93], respectively.

The degrees of *N*-acetylation of the α - and β -ChNFs [0–2.25] are the same as their powders. The XRD patterns and FT-IR spectra of the α - and β -ChNFs after 10 passes are shown in Fig. 2, and the CIs, *d*-spacings, and CSs of the ChNFs are shown in Table 1. These results indicate that the crystal and chemical structures are essentially unaffected by the acidity. The CIs of the α - and β -ChNFs are slightly higher than those of the corresponding α - and β -chitin powders. In addition, the peak of the [010] plane of the β -ChNFs shifts from about 8.1° to 9.3°. While the CSs of the β -ChNFs are higher than that of β -chitin powder, the CSs of the α -ChNFs are lower than that of α -chitin powder. It is notable that the crystal sizes of the β -chitin powder and β -ChNFs [0–1.86] are smaller than those of the α -chitin powder and α -ChNFs [0–2.25]. Photos of the α - and β -ChNFs (1 w/v%) after 10 passes are shown in Fig. 3 (top). β -ChNFs [0] and α -ChNFs [0–2.25] became turbid. The β -ChNFs turned clear with increasing acidity. Photos were taken two months after disintegration (Fig. 3, bottom), and the appearance of all of the α - and β -ChNFs did not change.

The pH of the 0.1 w/v% α - and β -ChNFs at various acidities and pass times are shown in Table 2. The pH decreases with increasing acidity for all of the α - and β -ChNFs. The pH of the β -ChNFs [0.23–1.85] change by approximately 1 with disintegration, while the pH of the α -ChNFs [1.13, 2.25] slightly increase with increasing pass time. The pH of the α - and β -ChNFs [0] are lower after disintegration than before disintegration.

The effect of the acidity on the transmittance of the α - and β -ChNFs after 2 and 10 passes is shown in Fig. 4(a). The spectra in the range 200–700 nm and the transmittance as a function of the pH before and after disintegration are shown in the Supplementary data. The transmittance values of the α - and β -ChNFs increase with increasing pass time. The transmittance of the β -ChNFs after 2 passes continuously increases from 25 to 85% with increasing acidity. In addition, the transmittance of the β -ChNFs [0] after 10 passes is 80%, and it reaches 96% with increasing acidity. In contrast, the transmittance of the α -ChNFs after 10 passes slightly increases with increasing acidity. The viscosities of the α - and β -ChNFs after 2 and 10 passes for various acidities are shown in Fig. 4(b). The viscosity as a function of the rotating speed is shown in the Supplementary data. The viscosity of the β -ChNFs [0.93] is approximately 10 times higher than that of the β -ChNFs [0]. Interestingly, the viscosity of the β -ChNFs [1.85] after 10 passes is lower than that of the β -ChNFs [0.93] after 10 passes. In contrast, the viscosity of the α -ChNFs after 10 passes slightly increases with increasing acidity. The viscosity decreases with accelerating rotating speed (see Supplementary data), indicating

that the α - and β -ChNFs have the properties of a pseudoplastic fluid. In addition, the viscosity decreases with time despite the same rotating speed, indicating that the α - and β -ChNFs are thixotropic. The EMs of the α - and β -ChNFs for various acidities are shown in Fig. 4(c). The EMs of the α - and β -ChNFs generally increase with increasing acidity. The EMs of the β -ChNFs are higher than those of the α -ChNFs. In particular, the β -ChNFs [0.46–1.85] after 2 passes have high EM values.

FE-SEM images of the α - and β -ChNFs are shown in Fig. 5, and the width distributions of the α - and β -ChNFs are shown in Fig. 6. The histogram of the β -ChNFs [0] after 2 passes shows a wide distribution, and thick fibers above 100 nm in width are often observed. For the β -ChNFs [0] after 10 passes, the distribution becomes narrow, and the average width is approximately half that after 2 passes. The distribution of the β -ChNFs [0.93] after 2 passes is the narrowest and all of the widths are less than 35 nm, and the distribution is essentially the same after 10 passes. The histogram of the α -ChNFs [0] is similar to that of the β -ChNFs [0]. However, for the α -ChNFs [1.13], a decrease of the NF width and a narrow distribution, such as that of the β -ChNFs [0.93], are not observed.

3.2. Influence of the Acid Species on the Transmittance of the β -ChNFs

We performed a further study of the influence of the acid species added to β -chitin because the transmittance of the β -ChNFs drastically changes with the acidity, as shown in Fig. 4. β -

Chitin is used in medical supplies, cosmetics, and food, so we chose two low toxicity acids: phosphoric acid and acetic acid, which are different in valence and pK_a . The pH for each condition is shown in Table 3. To determine the amount of dissociated protons, the amount of the dissociated anion as a function of the pH was calculated using the pK_a , and the valence for each acidity was calculated. The transmittance values of the β -ChNFs disintegrated after addition of these acids are shown in Fig. 7. The spectra in the range 200–700 nm and the transmittance as a function of the acidity using the total acid or the pH before and after disintegration are shown in the Supplementary data. The transmittance under acidic condition is higher than that under non-acidic conditions regardless of the type of acid, and it increases with increasing pass time. When hydrochloric, phosphoric, and acetic acid were added to β -chitin, the transmittance of the β -ChNFs shows almost the same tendency with the acidity.

4. Discussion

As reported in our previous study, the increase of CIs and the shift of [010] plane of β -ChNFs are likely due to rearrangement of the crystal structure and desorption of water from the β -chitin crystal structure, respectively [22,29]. The increase of CSs of β -ChNFs would be derived from rearrangement of the crystal structure during the drying process. On the other hand, the α -ChNFs would be formed in between disintegrating thick microfibrils into thin NFs because CSs of α -ChNFs decreases during disintegrating process.

Under acidic conditions, the pH of the ChNFs increases with progress of disintegrating into NFs, namely the pass time (Table 2). When chitin disintegrates into NFs, more amino groups are exposed to bulk water, and more protons bond to the amino groups. Thus, the number of protons in the bulk water decreases, and the pH becomes close to neutral. Conversely, under non-acidic conditions (i.e., α - and β -ChNFs [0]), the pH slightly decreases with increasing pass time, which is probably because of CO₂ from the atmosphere dissolving in the water. The gas–liquid contact area increases because the Star Burst system rapidly ejects the dispersion. The slight decrease of the pH is because of CO₂ dissolution in water.

The transmittance and viscosity of the β -ChNFs drastically and continuously change with the acidity, as shown in Fig. 4(a) and (b). The transmittance and viscosity of the α -ChNFs do not change as much with the acidity. The transmittance and viscosity of the α -ChNFs [1.13, 2.25] are slightly higher than those of the α -ChNFs [0]. The different sensitivity to the acidity for the α - and β -ChNFs is probably because of their crystal structures and abilities to be cationized by protons from the acid. α -Chitin has many hydrogen bonds between chitin molecular chains and has larger crystal sizes than β -chitin [16]. When a large chitin crystal contains many hydrogen bonds, it is difficult to disintegrate the crystal because of the strong attraction between crystals. In addition, the change of the pH of the α -ChNFs is small with increasing pass time (Table 2), indicating that a small number of protons bond to the exposed amino groups. Moreover, the cationized surface area of the α -ChNFs would be smaller than

that of the β -ChNFs because the EMs of the α -ChNFs are lower than those of the β -ChNFs (Fig. 4(c)). Thus, repulsion between the α -ChNFs is lower, and the α -ChNFs are thick with a wide width distribution. In contrast, β -chitin does not have significant hydrogen bonds between intermolecular sheets and can form a hydrate [17,18]. In addition, the pH of the β -ChNFs [0.23–1.85] changes by approximately 1 with disintegration. From these results, cationization inside the β -chitin crystal would progress under acidic conditions, as well as on the surface of the β -ChNFs, which can be attributed to an environment of high ion concentration inside the β -chitin crystal. The osmotic pressure inside the β -chitin crystal becomes high because of the high ion concentration in the β -chitin crystal, resulting in the β -chitin crystal swelling because of movement of bulk water to balance the osmotic pressure. When a mechanical shearing force is exerted on swollen β -chitin, it easily disintegrates into fine homogeneous NFs. The high EMs of the β -ChNFs [0.47–1.85] after 2 passes suggest complete cationization of β -chitin, and this significantly promotes disintegrate into fine NFs.

The narrow width distribution of the β -ChNFs [0.93] causes high transmittance because the homogeneous structure inhibits multiple scattering. In addition, the thin width of the β -ChNFs [0.93] causes high viscosity because entanglement between the β -ChNFs increases with increasing number of β -ChNFs in water. The decrease of the viscosity of the β -ChNFs [1.86] is probably because of shortening of the NF length, not the NF diameter. It has been reported that shortening of β -ChNFs starts by strong mechanical forces with the Star Burst system above

30 passes [30]. A higher acid concentration promotes shortening of the length of the β -ChNFs. These α - and β -ChNF dispersions consist of entangled α - and β -ChNFs, namely, physical cross-linking, rather than covalent bonding, namely, chemical cross-linking. When the rotating speed is increased or a shearing force is continuously exerted on the α - and β -ChNFs, these entanglements are forcibly relieved, so the viscosity decreases.

Even if the acid species is changed, the amount dissociated protons can be calculated using the pH after disintegration. The pH after disintegration shows the result of interaction between each acid and the amino groups in β -chitin. In other words, the amount of dissociated acid in the presence of β -chitin can be calculated. Thus, the transmittance values of the β -ChNFs after adding phosphoric and acetic acid are the same as that after adding hydrochloric acid. These results show that the amounts of dissociated protons and amino groups define the physicochemical properties of ChNFs.

5. Conclusions

In this study, the acidity was defined as the ratio of the amount of dissociated acid to the amount of amino groups in chitin to indicate the degree of cationization of chitin. When the acidity increases, the transmittance of the β -ChNFs after 2 passes changes from 20% to 80% and the viscosity of the β -ChNFs after 10 passes changes from 220 to 3650 mPa s, while the transmittance and viscosity of the α -ChNFs do not change as much. The difference between

the α - and β -ChNFs would be because of the ability of cationization of the ChNFs based on the rigidity of the crystal structure. Cationization of β -chitin, which does not have significant hydrogen bonds between intermolecular sheets and has smaller crystal sizes than α -chitin, rapidly progresses not only on the surface of the β -ChNFs but also inside the crystal under acidic conditions. The osmotic pressure inside the β -chitin crystal becomes high owing to the high ion concentration inside the β -chitin crystal, resulting in the β -chitin crystal swelling. When a mechanical shearing force is exerted on the swollen β -chitin, it easily disintegrates into fine homogeneous NFs. In addition, the transmittance increases with decreasing distribution of ChNF widths, and the viscosity increases with decreasing average width of the ChNFs. The transmittance of the β -ChNFs with addition of phosphoric, acetic, and hydrochloric acid can be expressed by the acidity. These results reveal that the acidity proposed in this work is an effective parameter to control the physicochemical properties of ChNFs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

References

- [1] D. Raabe, C. Sachs, P. Romano, The crustacean exoskeleton as an example of a structurally and mechanically graded biological nanocomposite material, *Acta Mater.* 53 (2005) 4281–4292. doi:10.1016/j.actamat.2005.05.027.
- [2] F.-C. Yang, R.D. Peters, H. Dies, M.C. Rheinstädter, Hierarchical, self-similar structure in native squid pen., *Soft Matter.* 10 (2014) 5541–5549. doi:10.1039/c4sm00301b.
- [3] M. Paillet, A. Dufresne, Chitin whisker reinforced thermoplastic nanocomposites, *Macromolecules.* 34 (2001) 6527–6530. doi:10.1021/ma002049v.
- [4] Y. Fan, H. Fukuzumi, T. Saito, A. Isogai, Comparative characterization of aqueous dispersions and cast films of different chitin nanowhiskers/nanofibers, *Int. J. Biol. Macromol.* 50 (2012) 69–76. doi:10.1016/j.ijbiomac.2011.09.026.
- [5] H.P. Zhao, X.Q. Feng, H. Gao, Ultrasonic technique for extracting nanofibers from nature materials, *Appl. Phys. Lett.* 90 (2007) 1–3. doi:10.1063/1.2450666.
- [6] B.M. Min, S.W. Lee, J.N. Lim, Y. You, T.S. Lee, P.H. Kang, W.H. Park, Chitin and chitosan nanofibers: Electrospinning of chitin and deacetylation of chitin nanofibers, *Polymer (Guildf).* 45 (2004) 7137–7142. doi:10.1016/j.polymer.2004.08.048.
- [7] I.F. Nata, S.S.S. Wang, T.M. Wu, C.K. Lee, β -Chitin nanofibrils for self-sustaining hydrogels preparation via hydrothermal treatment, *Carbohydr. Polym.* 90 (2012) 1509–

1514. doi:10.1016/j.carbpol.2012.07.022.
- [8] K. Abe, S. Ifuku, M. Kawata, H. Yano, Preparation of tough hydrogels based on β -chitin nanofibers via NaOH treatment, *Cellulose*. 21 (2014) 535–540.
doi:10.1007/s10570-013-0095-0.
- [9] Y. Maeda, R. Jayakumar, H. Nagahama, T. Furuike, H. Tamura, Synthesis, characterization and bioactivity studies of novel β -chitin scaffolds for tissue-engineering applications, *Int. J. Biol. Macromol.* 42 (2008) 463–467.
doi:10.1016/j.ijbiomac.2008.03.002.
- [10] R. Izumi, S. Komada, K. Ochi, L. Karasawa, T. Osaki, Y. Murahata, T. Tsuka, T. Imagawa, N. Itoh, Y. Okamoto, H. Izawa, M. Morimoto, H. Saimoto, K. Azuma, S. Ifuku, Favorable effects of superficially deacetylated chitin nanofibrils on the wound healing process, *Carbohydr. Polym.* 123 (2015) 461–467.
doi:10.1016/j.carbpol.2015.02.005.
- [11] S. Sowmya, P.T.S. Kumar, K.P. Chennazhi, S. V Nair, H. Tamura, R. Jayakumar, Biocompatible β -chitin Hydrogel / Nanobioactive Glass Ceramic Nanocomposite Scaffolds for Periodontal Bone Regeneration, *Trends Biomater. Artif. Organs*. 25 (2011) 1–11.
- [12] S. Gautier, E. Xhaufaire-Uhoda, P. Gonry, G.E. Piérard, Chitin-glucan, a natural cell scaffold for skin moisturization and rejuvenation, *Int. J. Cosmet. Sci.* 30 (2008) 459–

469. doi:10.1111/j.1468-2494.2008.00470.x.
- [13] L.K. Han, Y. Kimura, H. Okuda, Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet, *Int. J. Obes.* 23 (1999) 174–179. doi:DOI 10.1038/sj.ijo.0800806.
- [14] K. Madhumathi, N.S. Binulal, H. Nagahama, H. Tamura, K.T. Shalumon, N. Selvamurugan, S. V Nair, R. Jayakumar, Preparation and characterization of novel β -chitin-hydroxyapatite composite membranes for tissue engineering applications, *Int. J. Biol. Macromol.* 44 (2009) 1–5. doi:10.1016/j.ijbiomac.2008.09.013.
- [15] R. Jayakumar, V. V. Divya Rani, K.T. Shalumon, P.T.S. Kumar, S. V. Nair, T. Furuike, H. Tamura, Bioactive and osteoblast cell attachment studies of novel α - and β -chitin membranes for tissue-engineering applications, *Int. J. Biol. Macromol.* 45 (2009) 260–264. doi:10.1016/j.ijbiomac.2009.06.002.
- [16] P. Sikorski, R. Hori, M. Wada, Revisit of α -chitin crystal structure using high resolution X-ray diffraction data, *Biomacromolecules.* 10 (2009) 1100–1105. doi:10.1021/bm801251e.
- [17] Y. Nishiyama, Y. Noishiki, M. Wada, X-ray structure of anhydrous β -chitin at 1 Å resolution, *Macromolecules.* 44 (2011) 950–957. doi:10.1021/ma102240r.
- [18] D. Sawada, Y. Nishiyama, P. Langan, V.T. Forsyth, S. Kimura, M. Wada, Water in crystalline fibers of dihydrate β -chitin results in unexpected absence of intramolecular

- hydrogen bonding, *PLoS One*. 7 (2012) 4–11. doi:10.1371/journal.pone.0039376.
- [19] Y. Fan, T. Saito, A. Isogai, Preparation of chitin nanofibers from squid Pen β -chitin by simple mechanical treatment under acid conditions, *Biomacromolecules*. 9 (2008) 1919–1923. doi:10.1021/bm800178b.
- [20] S. Ifuku, K. Yamada, M. Morimoto, H. Saimoto, Nanofibrillation of dry chitin powder by star burst system, *J. Nanomater.* 2012 (2012) 1–7. doi:10.1155/2012/645624.
- [21] R. Kose, T. Kondo, Favorable 3D-network formation of chitin nanofibers dispersed in water prepared using aqueous counter collision, *Sen'i Gakkaishi*. 67 (2011) 91–95. doi:10.2115/fiber.67.91.
- [22] S. Suenaga, N. Nikaido, K. Totani, K. Kawasaki, Y. Ito, K. Yamashita, M. Osada, Effect of purification method of β -chitin from squid pen on the properties of β -chitin nanofibers, *Int. J. Biol. Macromol.* 91 (2016) 987–993. doi:10.1016/j.ijbiomac.2016.06.060.
- [23] C. Chen, H. Yano, D. Li, K. Abe, Preparation of high-strength α -chitin nanofiber-based hydrogels under mild conditions, *Cellulose*. 22 (2015) 2543–2550. doi:10.1007/s10570-015-0654-7.
- [24] N.E. Mushi, N. Butchosa, M. Salajkova, Q. Zhou, L.A. Berglund, Nanostructured membranes based on native chitin nanofibers prepared by mild process, *Carbohydr. Polym.* 112 (2014) 255–263. doi:10.1016/j.carbpol.2014.05.038.

- [25] S. Ifuku, M. Nogi, K. Abe, M. Yoshioka, M. Morimoto, H. Saimoto, H. Yano, Preparation of chitin nanofibers with a uniform width as α -chitin from crab shells, *Biomacromolecules*. 10 (2009) 1584–1588. doi:10.1021/bm900163d.
- [26] D.D. Perrin, B. Dempsey, *Buffers for pH and metal ion control*, New York: Chapman and Hall, 1974.
- [27] Y. Zhang, C. Xue, Y. Xue, R. Gao, X. Zhang, Determination of the degree of deacetylation of chitin and chitosan by X-ray powder diffraction, *Carbohydr. Res.* 340 (2005) 1914–1917. doi:10.1016/j.carres.2005.05.005.
- [28] NIH, Image J, (2016). <https://imagej.nih.gov/ij/>.
- [29] H. Nagahama, T. Higuchi, R. Jayakumar, T. Furuike, H. Tamura, XRD studies of β -chitin from squid pen with calcium solvent, *Int. J. Biol. Macromol.* 42 (2008) 309–313. doi:10.1016/j.ijbiomac.2007.10.011.
- [30] A.K. Dutta, H. Izawa, M. Morimoto, H. Saimoto, S. Ifuku, Simple Preparation of Chitin Nanofibers from Dry Squid Pen β -chitin Powder by the Star Burst System, *J. Chitin Chitosan Sci.* 1 (2013) 186–191. doi:10.1166/jcc.2013.1023.

Tables

Table 1. CI, d -spacing [\AA], and CS [nm] of the α - and β -chitin powders, and the α - and β -ChNFs after 10 passes for various acidities.

Sample	Acidity [mol mol ⁻¹]	CI	d -Spacing [\AA]		CS [nm]	
			[1-10] ^{*1}	[010] ^{*2}	[1-10] ^{*1}	[010] ^{*2}
β -Chitin powder	0	80	4.5	10.9	6.0	9.2
β -Chitin	0	82	4.5	9.9	6.4	10.5
	0.47	86	4.6	9.5	6.9	11.0
	0.92	88	4.6	9.5	7.1	10.4
	1.86	89	4.6	9.5	7.1	10.2
α -Chitin powder	0	86	4.6	9.5	13.1	22.5
α -Chitin	0	89	4.6	9.6	9.7	16.6
	1.13	89	4.6	9.6	9.2	17.0
	2.25	89	4.6	9.7	9.4	17.0

*1 For α -chitin, [110] plane

*2 For α -chitin, [020] plane

Table 2. pH of the 0.1 w/v% α - and β -ChNFs for various acidities and pass times.

Sample	Acidity [mol mol ⁻¹]	The pH at respective pass times		
		0 passes	2 passes	10 passes
β -Chitin	0	7.4	6.8	6.9
	0.23	5.2	5.6	5.9
	0.47	4.5	5.4	5.7
	0.93	4.1	5.0	5.3
	1.85	3.6	4.0	4.2
α -Chitin	0	6.5	6.2	6.4
	1.13	3.9	4.1	4.2
	2.25	3.5	3.7	3.6

Table 3. pH and acidities of the 0.1 w/v% β -ChNFs with various acids and pass times.

Acids	pH	pH	Acidity	pH	Acidity	pH	Acidity
	at 0 passes	at 2 passes	at 2 passes [mol mol ⁻¹]	at 5 passes	at 5 passes [mol mol ⁻¹]	at 10 passes	at 10 passes [mol mol ⁻¹]
Phosphoric acid	5.1	6.0	0.17	6.1	0.17	6.2	0.17
	4.7	5.6	0.32	5.8	0.32	5.9	0.33
	4.4	5.2	0.63	5.4	0.63	5.6	0.64
	4.0	4.4	1.23	4.6	1.24	4.7	1.24
	3.7	4.0	1.83	4.1	1.83	4.2	1.84
Acetic acid	5.4	6.1	0.22	6.2	0.22	6.3	0.22
	4.8	5.4	0.37	5.6	0.40	5.7	0.41
	4.5	5.1	0.63	5.3	0.71	5.5	0.78
	4.3	4.7	0.86	4.8	0.96	5.0	1.17
	4.2	4.5	1.2	4.5	1.2	4.6	1.4
	4.0	4.2	1.4	4.2	1.4	4.3	1.7

The pH at 5 passes is the average of the pH at 2 and 10 passes

Figure Captions

Fig. 1. Star Burst system equipped with a ball-collision chamber.

Fig. 2. XRD patterns and FT-IR spectra of (a) β -chitin powder, (b) β -ChNFs [0], (c) β -ChNFs [0.47], (d) β -ChNFs [0.92], (e) β -ChNFs [1.86], (f) α -chitin powder, (g) α -ChNFs [0], (h) α -ChNFs [1.13], and (i) α -ChNFs [2.25] after 10 passes.

Fig. 3. Photos of the 1 w/v% α - and β -ChNFs after 10 passes for various acidities. The upper photos were taken just after disintegration, and the lower photos were taken two months after disintegration.

Fig. 4. Effect of the acidity on the (a) transmittance at 0.1 w/v%, (b) viscosity at 1 w/v%, and (c) EM at 0.1 w/v% of the α - and β -ChNFs after 2 and 10 passes.

Fig. 5. FE-SEM micrographs of the β -ChNFs [0, 0.93] after 2 and 10 passes, and the α -ChNFs [0, 1.13] after 2 and 10 passes.

Fig. 6. Width distributions of the β -ChNFs [0, 0.93] after 2 and 10 passes, and the α -ChNFs [0, 1.13] after 2 and 10 passes.

Fig. 7. Transmittance of the β -ChNFs with various acids and acidities after 2 (left), 5 (middle), and 10 (right) passes.

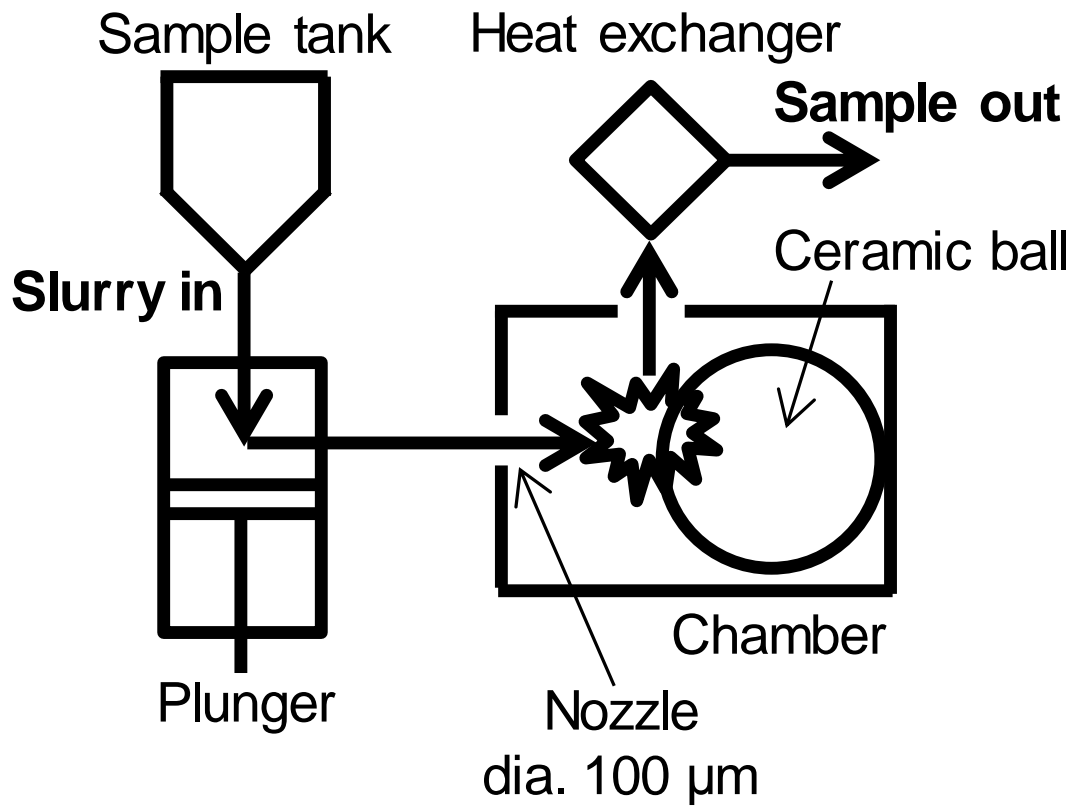


Fig. 1. Star Burst system equipped with a ball-collision chamber.

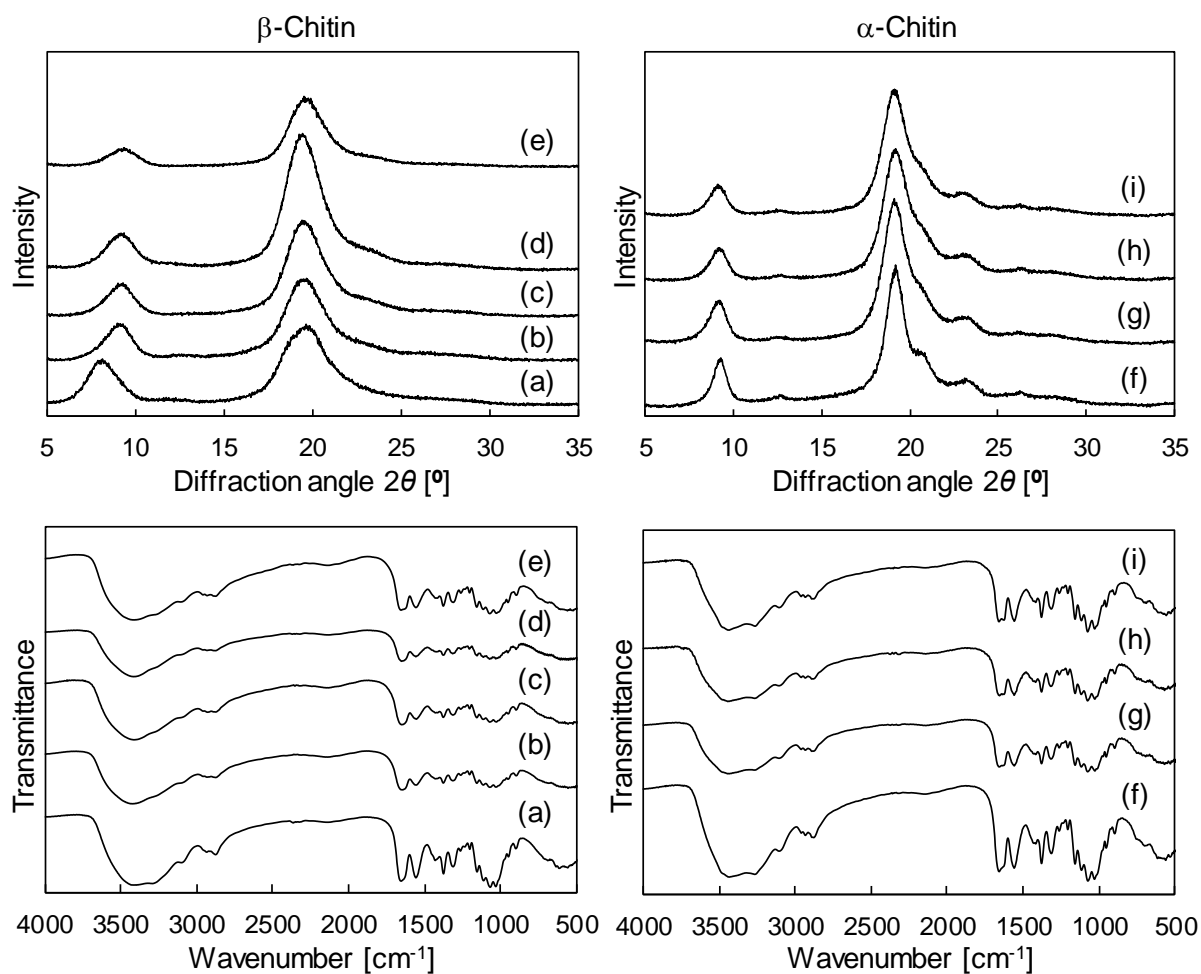


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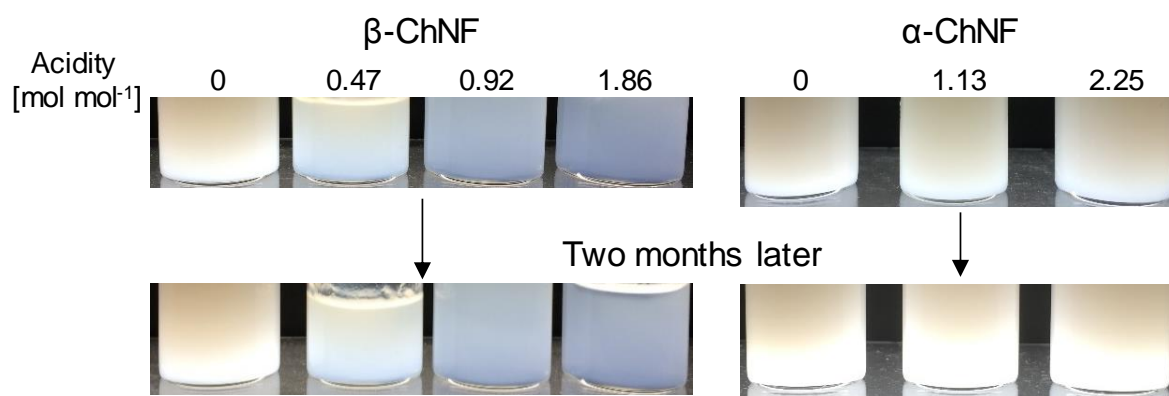


Fig. 3. Photos of the 1 w/v% α - and β -ChNFs after 10 passes for various acidities. The upper photos were taken just after disintegration, and the lower photos were taken two months after disintegration.

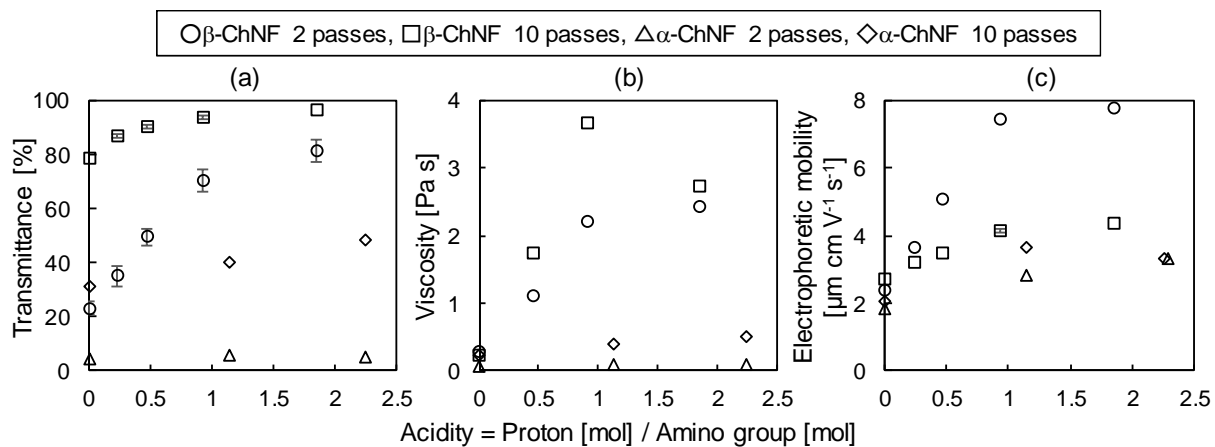


Fig. 4. Effect of the acidity on the (a) transmittance at 0.1 w/v%, (b) viscosity at 1 w/v%, and (c) EM at 0.1 w/v% of the α - and β -ChNFs after 2 and 10 passes.

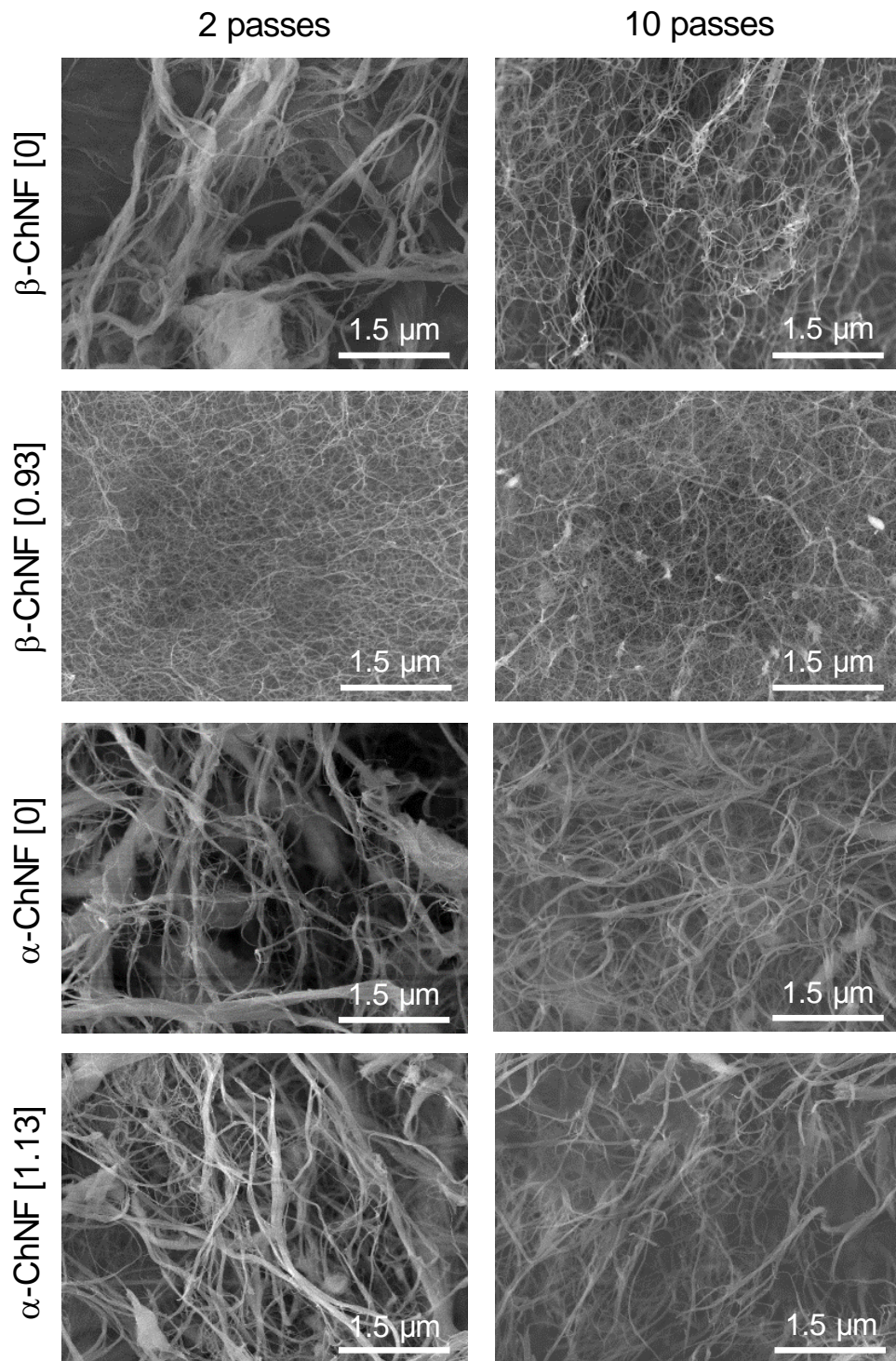


Fig. 5. FE-SEM micrographs of the β -ChNFs [0, 0.93] after 2 and 10 passes, and the α -ChNFs [0, 1.13] after 2 and 10 passes.

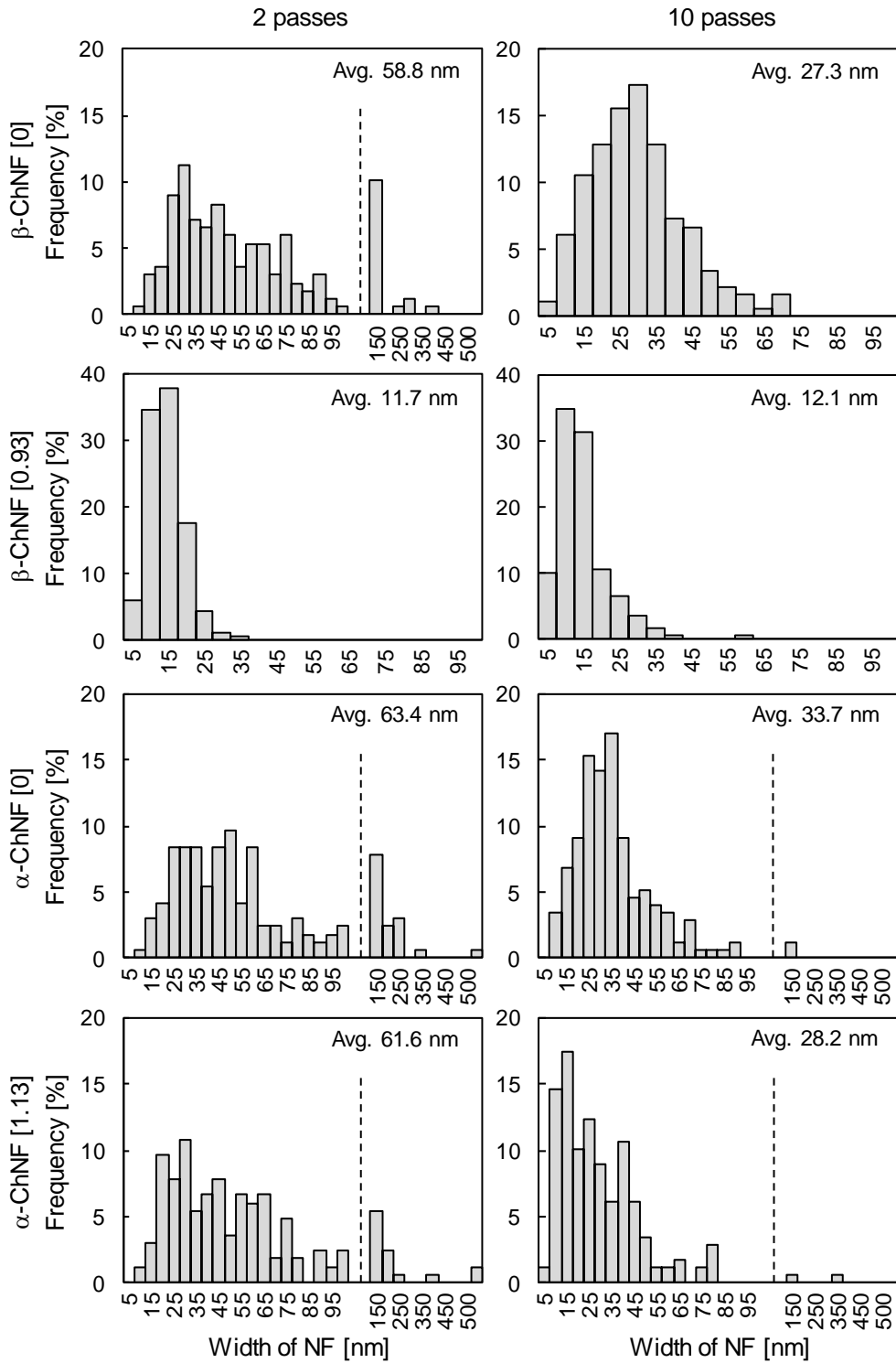


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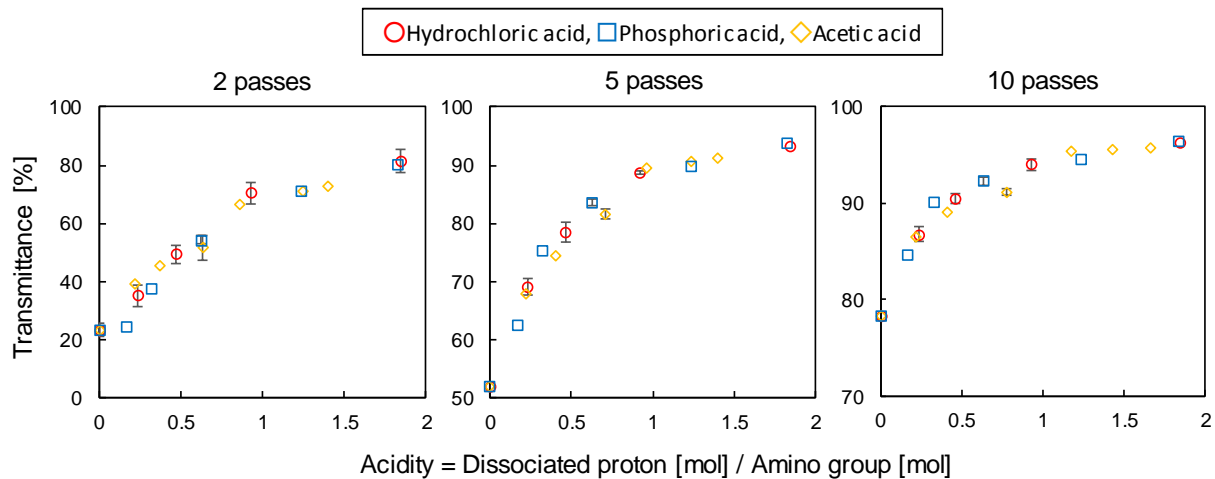


Fig. 7. Transmittance of the β -ChNFs with various acids and acidities after 2 (left), 5 (middle), and 10 (right) passes.