1. Introduction

Polysaccharides are widely distributed in nature and are regarded as structural materials and reservoirs of water and energy [1]. Among the natural polysaccharides, chitin, comprising main chains of β(1→4)-linked N-acetyl-D-glucosamine (GlcNAc) residues, is an important biomass resource because it is one of the most abundant polysaccharides present in nature, mainly prevalent in the exoskeletons of crustaceans [2‒4]. However, chitin remains largely underutilized because of its poor solubility and processability, owing to its intractable bulk structure composed of numerous intra- and inter-molecular hydrogen bonds.

The fabrication of nanoscale polymeric assemblies (e.g., nanofibers and nanowhiskers) is a useful method for the functionalization of chitin [5–7] because of the remarkable properties of bio-based nanomaterials, such as low weight, high tensile strength, and biocompatibility [8–14]. Efficient procedures for the preparation of chitin nanofibers (ChNFs) and nanowhiskers have been developed using a top-down approach that breaks down the starting bulk materials from native chitin sources [5, 6, 15–18].

Based on another approach—the bottom-up technique—we previously developed a facile method to fabricate ChNFs with a width of approximately 20‒60 nm and length of several hundred nanometers. Self-assembling regeneration at the nanoscale from an ion gel of a chitin/ionic liquid, namely, 1-allyl-3-methylimidazolium bromide (AMIMBr), was achieved using methanol [19, 20]. This was based on our previous findings that AMIMBr efficiently dissolves and swells chitin [21]. Isolation of the resulting ChNFs from the methanol dispersion via filtration produced a ChNF film possessing a heavily entangled nanofiber morphology. Furthermore, the self-assembled ChNFs had a bundle-like structure consisting of an assembly of thinner fibrils [22]. The treatment of the ChNF film with an aqueous NaOH induced the partial generation of amino groups on the chitin chains (i.e., partially deacetylated chitin nanofibers (PDA-ChNFs)). This led to the successful disentanglement of the bundles by cationization and electrostatic repulsion in 1.0 mol/L aqueous acetic acid with ultrasonication, yielding
individual thin fibril materials. The average width and length of the obtained fibrils, calculated from scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images, were distinctly smaller than those of the parent ChNFs and PDA-ChNFs. Furthermore, thin fibrils were isolated from the resulting dispersion via filtration to form a film with a highly condensed morphology. The film demonstrated superior flexibility, higher tensile strength, and greater elongation at break than those of the PDA-ChNF film. As the highly condensed nanostructure from the thin fibrils likely contributed to the superior mechanical properties, the materials were particularly named as “scaled-down ChNFs (SD-ChNFs)” (Fig. 1 (a)) [23].

The formation of hydrogels from ChNFs has been achieved through the preparation of ChNF networks using several approaches. For example, network structures have been fabricated by the treatment of ChNFs with aqueous acidic and alkaline solutions, such as aqueous NaOH resulting in the formation of chitin hydrogels [24‒26]. The cross-linking of ChNFs with other components has also been conducted to produce hydrogels [27]. In our previous investigation, poly(2-methyl-2-oxazoline) (PMeOx)—a hydrophilic and highly polar polymer—was grafted onto the abovementioned PDA-ChNF film via the grafting-to approach by reaction of the living propagating end of PMeOx with the amino groups in dimethyl sulfoxide. As the reaction progressed, a gel-like structure was formed. This was successfully converted into a hydrogel with high water content through the exchange of the disperse media [28, 29]. The nanofiber disentanglement that occurred during the grafting of PMeOx on the ChNFs to construct a network structure resulted in the formation of the hydrogel.

Based on the above findings that hydrogelation from ChNFs was achieved by the development of efficient methods for the formation of ChNF networks, in this study, we report that the modification of monosaccharide residues, such as D-xylose (Xyl), with SD-ChNFs by reductive amination, gives rise to a network structure, leading to the formation of

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**Fig. 1** Procedures for (a) preparation of scaled-down ChNF (SD-ChNF) dispersion, (b) reductive amination of monosaccharide residues on SD-ChNFs, and (c) SEM image of lyophilized sample from D-xylose (Xyl)-modified SD-ChNF hydrogel.
hydrogels (Fig. 1 (b)). Furthermore, the reductive amination of other monosaccharide residues (D-glucose (Glc) and N-acetyl-D-glucosamine (GlcNAc)) on SD-ChNFs also results in hydrogelation.

2. Materials and Methods

2.1 Materials

Chitin powder obtained from crab shells was purchased from Wako Pure Chemicals (Tokyo, Japan). An ionic liquid, AMIMBr, was prepared by the reaction of 1-methylimidazole with 3-bromo-1-propene according to a method reported elsewhere [30]. Other reagents and solvents used in this study were commercially available and were used without further purification.

2.2 Preparation of PDA-ChNF films

A mixture of chitin (0.120 g, 0.59 mmol) and AMIMBr (1.00 g, 4.92 mmol) was allowed to stand at room temperature for 24 h and subsequently heated with stirring at 80 °C for 24 h to obtain a chitin ion gel (10 wt.%). The gel was soaked in methanol (30 mL) at room temperature for 48 h for regeneration, followed by ultrasonication (Branson 1510, 42 kHz, 70 W) for 10 min to yield a self-assembled ChNF dispersion with methanol. The dispersion was filtered to isolate ChNFs, which were treated with methanol by Soxhlet extraction for 48 h and dried at 50 °C for 2 h under reduced pressure to obtain a ChNF film.

A mixture of the resulting ChNF film (0.117 g, 0.58 mmol) with 30 wt.% aqueous NaOH (20 mL) was maintained at 80 °C for 5 h. The prepared film was separated via filtration. The film was immersed in water (50 mL) for 10 min with ultrasonication, filtered, and washed with water. The procedure was repeated once, following which the resulting film was washed with methanol and dried at 50 °C for 2 h under reduced pressure to obtain a ChNF film.

2.3 Preparation of Xyl-modified ChNFs (entry 1, Table 1)

A mixture of the PDA-ChNF film (degree of deacetylation (DDA): 23.0%, 0.0146 g) with 1.0 mol/L aqueous acetic acid (5.0 mL) was ultrasonicated using a homogenizer (Branson Advanced-Digital Sonifier 450, 20 kHz, 400 W) at room temperature for 10 min to obtain an SD-ChNF dispersion. Subsequently, D-xylose (0.577 g, 3.84 mmol, 200 equiv. with amino groups) and NaBH₃CN (0.234 g, 3.72 mmol, 200 equiv. with amino groups) were added to the dispersion, and the mixture was stirred at room temperature for 72 h to produce a hydrogel. The mixture was centrifuged to isolate the product, washed with water, and lyophilized to yield the Xyl-modified ChNFs (0.0152 g).

1H NMR (DCl/D2O): δ 2.30 (m, CH₃, 2.47 H), 3.15–3.32 (br, -N-CH2-, 0.31 H), 3.32–4.05 (m, H 2-6 of GlcN, GlcNAc, and Xyl moieties, 6.67 H), 4.58 (m, β (1→4)-linked GlcNAc-H1), 4.74 (m, GlcNAc-H1β, GlcN-H1β, β (1→4)-linked GlcN-H1), 4.80 (br, β (1→4)-linked Xyl-modified GlcN), 5.13 (m, GlcNAc-H1α) (H1 protons; total 1H).

2.4 Preparation of Glc- and GlcNAc-modified ChNFs (entries 3 and 4, Table 1)

The reductive amination of D-glucose and N-acetyl-D-glucosamine (200 equiv. with amino groups) on SD-ChNFs was conducted in the presence of NaBH₃CN (200 equiv. with amino groups) in 1.0 mol/L aqueous acetic acid following the same procedure as above to produce the Glc- and GlcNAc-modified ChNF hydrogels.

2.5 Measurements

1H NMR spectra were recorded on JEOL ECA 600 and JEOL ECX 400 spectrometers. The NMR measurements were conducted after complete dissolution of the samples in 15 wt.% DCl/D2O by acidic hydrolysis for 72 h. SEM images were obtained using a Hitachi S-4100 H scanning electron microscope with an accelerating voltage of 5 kV. Prior to recording the SEM images, the samples, which were obtained by lyophilization (24 h) of the isolated hydrogels and spin-coating of the dispersions or aggregated mixtures, were first placed on the sample stubs, followed by coating with platinum using a magnetron sputter. Average nanofiber widths were calculated based on 50 fibers in each SEM image. Dynamic viscoelastic measurement was conducted using a rheometer (Rheosol-G 1000, UBM) using a 19.98 mm-diameter parallel plate with a 1.0 mm gap over a frequency range of 0.1 to 10 Hz.

3. Results and discussion

Prior to reductive amination, we prepared the SD-ChNF dispersion in aqueous acetic acid from the
PDA-ChNF film according to a previously reported procedure (Fig. 1 (a)) [23]. The PDA-ChNF film was first obtained via regeneration from a chitin/AMIMBr ion gel using methanol, followed by partial deacetylation at 80 °C for 5 h in 30 wt.% aqueous NaOH. From the integrated ratio of signals assignable to acetamido protons to that of total anomeric (H1) protons (2.24:1) in the ¹H-NMR spectrum of the sample hydrolyzed from the as-prepared PDA-ChNF film in DCl/D₂O (Fig. 2 (a)), the DDA value was calculated to be 23.0% for the total repeating units of chitin. A mixture of the obtained PDA-ChNF film with 1.0 mol/L aqueous acetic acid was then ultrasonicated using a homogenizer at room temperature for 10 min to obtain the SD-ChNF dispersion.

The reductive amination of d-xylose on SD-ChNFs was then performed under the conditions listed in entry 1 in Table 1 to produce Xyl-modified ChNFs, according to the procedure shown in Fig. 1 (b). D-Xylose and NaBH₃CN as reducing agent (200 equiv. with amino groups, each) were first added to the SD-ChNF/aqueous acetic acid dispersion, following which the mixture was stirred at room temperature. With the reaction time, the mixture gradually became turbid, and consequently, a hydrogel was produced after 72 h, as shown in Fig. 3 (a, right). The hydrogel was purified by immersion in water, and was further subjected to lyophilization to isolate the produced Xyl-modified ChNFs. The SEM image of the lyophilized sample illustrates the network

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**Fig. 2** ¹H NMR spectra of (a) PDA-ChNFs and (b) Xyl-modified ChNFs (entry 1, Table 1) in DCl/D₂O.

**Table 1** Reductive amination of monosaccharides on ChNFs under various conditions

| Entry | Monosaccharide | Acetic acid (mol/L) | Equiv. b) | Time (h) | Hydrogelation | DS c) (%) | Yield d) (%) | Water content e) (%) |
|-------|----------------|---------------------|-----------|----------|---------------|-----------|-------------|---------------------|
| 1     | Xyl            | 1.0                 | 200       | 72       | +             | 15        | 91          | 20.1                |
| 2     | Xyl            | 1.0                 | 50        | 168      | -             | 4         | 45          |                     |
| 3     | Xyl            | 0.1                 | 50        | 168      | -             | 15        | 98          |                     |
| 4     | Glc            | 1.0                 | 200       | 72       | +             | 15        | 94          | 35.2                |
| 5     | GlcNAc         | 1.0                 | 200       | 72       | +             | 14        | 87          | 36.6                |

a) Reaction was carried out at room temperature.
b) Equivalents of monosaccharide and NaBH₃CN with amino groups.
c) Degrees of substitution of monosaccharide residues for total repeating units of chitin.
d) Weights of lyophilized samples.
e) After re-hydrogelation.
The 1H NMR spectrum of the sample hydrolyzed from the lyophilized material in DCl/D2O (Fig. 2 (b)) demonstrated signals similar to those in the spectrum of PDA-ChNFs (Fig. 2 (a)), and a new signal assignable to the methylene protons in the Xyl residues (δ 3.1–3.3) generated by reductive amination. The anomic signal (H1), ascribed to Xyl-modified GlcN units, was also detected at δ 4.80. Based on the integrated ratio of the signals from the methylene protons to those of the total anomic (H1) protons (0.31:1), the degree of substitution (DS) of the Xyl residues for the total repeating units of chitin was calculated as 15%. By considering the weight of the lyophilized product, the yield was estimated as 91%. When re-hydrogelation of the lyophilized product was attempted by immersion in water, a hydrogel with a water content of 20.1% was obtained, and was characterized by dynamic viscoelastic measurements (Fig. 4 (a)). The storage (G’) moduli were obviously higher than the loss moduli (G”) in the larger frequency region. Besides, G’ decreased with decreasing frequency in the lower frequency region and the difference between the two moduli gradually decreased in accordance with the decrease of frequencies. The decreasing of G’ with decreasing frequency implies that some relaxation motions in the gel arise in the corresponding time region. However, G’ was still higher than G” in the lower frequency region. In addition to this, G’ became a straight line with a constant slope less than 0.5 at the lower frequency. Those tendencies of G’ and G” in the lower frequency region suggests that the sample shows a behavior of so-called weak gel [31–33]. Moreover, the sample apparently did not flow and its shape almost maintained even after 6 h, also suggesting its weak gelling state.

For comparison, lower ratios of D-xylose and NaBH3CN to amino groups (50 equiv. with amino groups, each) were employed for the reductive amination of the SD-ChNF dispersion in 1.0 mol/L aqueous acetic acid (entry 2, Table 1). However, even after 168 h (7 days), the reaction solution was mostly intact, and hydrogelation was not observed. The reaction mixture was neutralized with aqueous NaOH (1.0 mol/L) to precipitate the product by aggregation. The as-obtained product was isolated by centrifugation, washed with water, and lyophilized. From the 1H NMR analysis in DCl/D2O, the DS of the

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**Fig. 3** (a) Photographs and (b) SEM images of samples spin-coated from reductive amination mixtures from D-xylose and SD-ChNFs in accordance with reaction times and (c) plausible mechanism for hydrogelation via formation of network structure.
Xyl residues for the total repeating units of chitin was calculated as 4%. This result indicates a lower efficiency of the reductive amination of the Xyl residues on SD-ChNFs for lower ratios of D-xylose and NaBH3CN to amino groups, resulting in no hydrogelation from the Xyl-modified ChNFs.

We previously reported that SD-ChNFs were not formed efficiently when aqueous acetic acid was diluted to concentrations exceeding 1.0 mol/L, owing to the insufficient disintegration of the bundles of the parent PDA-ChNF films [23]. Therefore, hydrogelation was attempted by the reductive amination of D-xylose with NaBH3CN (50 equiv. with amino groups, each) after the treatment of the PDA-ChNF film in diluted solution, i.e., 0.1 mol/L aqueous acetic acid (entry 3, Table 1). After 7 days, aggregates were produced in the mixture, but not in the gelling form. The aggregates were isolated by centrifugation, washed with water, and lyophilized. 1H NMR analysis of the sample in DCl/D2O indicated that the DS of the Xyl residues for the total repeating units of chitin was 15%, which was comparable to that of entry 1. These data indicate that reductive amination efficiently occurred even in 50 equiv. of D-xylose and NaBH3CN with amino groups in 0.1 mol/L aqueous acetic acid, probably owing to less protonation of such groups under weaker acidic conditions. These findings strongly suggest that thicker ChNFs, formed by insufficient disintegration of the bundles, did not efficiently form network structures at the nanoscale, thereby not leading to hydrogelation but causing the aggregation of the product instead.

When D-glucose and N-acetyl-D-glucosamine were used for the reductive amination of SD-ChNFs under the same conditions as entry 1 (entries 4 and 5, Table 1, respectively), hydrogels were formed in both cases. The SEM images of the lyophilized samples from the hydrogels showed a network morphology similar to that of the sample in entry 1 (Fig. 3 (b, right)). Based on the 1H NMR analysis of the lyophilized samples in DCI/D2O, the DSs of the Glc and GlcNAc residues for the total repeating units of chitin were 15% and 14%, respectively. Re-hydrogelation of the lyophilized samples was observed by immersing them in water (water content from Glc- and GlcNAc-modified ChNFs: 35.2 and 36.6%, respectively). The dynamic viscoelastic measurements of the resulting hydrogels (Fig. 4 (b) and (c)) showed profiles similar to those of the Xyl-modified ChNF hydrogel (Fig. 4 (a)), which supported their weak gel behaviors.

To evaluate the mechanism responsible for the formation of hydrogels, morphological changes through the reductive amination of D-xylose on SD-ChNFs were thoroughly investigated under the conditions of entry 1 (Fig. 3). Aggregates were gradually formed in the mixture from SD-ChNFs with increasing reaction times, as shown in Fig. 3 (a, left and center). The SEM image of a sample spin-coated from the mixture, obtained at a reaction time of 6 h, showed a thicker nanofiber morphology (Fig. 3 (b, center)) compared with that of the parent SD-ChNFs as depicted in the SEM image of a sample spin-coated from the SD-ChNF/aqueous acetic acid dispersion (Fig. 3 (b, left)) (average widths: 27 and 17 nm, respectively). Thus, it was speculated that the
aggregates were formed by the assembly of the SD-ChNFs. The DS of the Xyl residues for the total repeating units of chitin in the aggregates was 7%, as evaluated from the $^1$H NMR measurements in DCI/D$_2$O. The formation of the hydrogel in the mixture was observed after 72 h (Fig. 3 (a, right)), as described above. The SEM image of the sample spin-coated from the gel-like aggregates demonstrated a network morphology from long nanofibrils, where the average width was calculated as 48 nm. Overall, with increasing DS values of the Xyl residues in accordance with reaction times, the average nanofiber widths increased accordingly (Fig. 3). Moreover, these results suggest that the efficiency of reductive amination and the nanofiber size in the starting dispersion strongly affect the hydrogelation behavior of the products. Based on these findings, a mechanism for the formation of hydrogels by the reductive amination of monosaccharide residues on SD-ChNFs is proposed as follows (Fig. 3 (c)). In 1.0 mol/L aqueous acetic acid, disintegration of the bundles of PDA-ChNFs occurred by cationization and strong electrostatic repulsion to produce SD-ChNFs. The electrostatic repulsion among SD-ChNFs was gradually weakened by the modification of Xyl, leading to the assembly of SD-ChNFs. With increasing reaction time, further interaction of the assembled SD-ChNFs occurred to form longer nanofibers. The nanofibers then stacked each other to construct the network structure, giving rise to the hydrogel in the mixture. The effect of the ionic strength on the hydrogelation behavior of anionic cellulose nanofibers (2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO)-oxidized cellulose nanofibers) has been similarly discussed [34, 35].

4. Conclusions

In this study, facile hydrogelation via the formation of a network structure from monosaccharide-modified SD-ChNFs was achieved by reductive amination in an SD-ChNF/aqueous acetic acid dispersion. Several monosaccharides, such as D-xylose, D-glucose, and N-acetyl-D-glucosamine, were used for the reductive amination of SD-ChNFs in the presence of NaBH$_3$CN as the reducing agent. The results under different conditions suggested that the efficiency of the reductive amination as well as the nanofiber size in the starting dispersion strongly affected the hydrogelation behavior of the products. Consequently, with the progress of reductive amination, hydrogelation occurred via the formation of a network structure at the nanoscale through hierarchical assembly from SD-ChNFs. This study provides a new approach for hydrogel processing from ChNF sources, and is promising for application in the fabrication of new functional chitin-based materials in future research.

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