Nanostructurally Controlled Hydrogel Based on Small-Diameter Native Chitin Nanofibers: Preparation, Structure, and Properties

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Chitin nanofibers of unique structure and properties can be obtained from crustacean and fishery waste. These chitin nanofibers have roughly 4 nm diameters, aspect ratios between 25–250, a high degree of acetylation and preserved crystallinity, and can be potentially applied in hydrogels. Hydrogels with a chitin nanofiber content of 0.4, 0.6, 0.8, 1.0, 2.0, and 3.0 wt % were successfully prepared. The methodology for preparation is new, environmentally friendly, and simple as gelation is induced by neutralization of the charged colloidal mixture, inducing precipitation and secondary bond interaction between nanofibers. Pore structure and pore size distributions of corresponding aerogels are characterized using auto-porosimetry, revealing a substantial fraction of nanoscale pores. To the best of our knowledge, the values for storage (13 kPa at 3 wt %) and compression modulus (309 kPa at 2 wt %) are the highest reported for chitin nanofibers hydrogels.

Introduction

Materials from renewable resources have the potential to be more environmentally friendly than polymer materials of petrochemical origin. The annual production of chitin in nature is estimated to be in the range of 10–100 trillion tones, and the seafood industry generates large amount of chitin waste. The disposal of this bioresource is an environmental problem and this resource is simply underutilized. Chitin is a nanocomponent embedded in the shells of crustaceans such as lobsters, crabs, shrimps in the form of mechanically functional chitin microfibrils (nanofibers). Chitin microfibrils share many characteristics with cellulose microfibrils, including high stiffness, low density, high surface area, and high aspect ratio, making chitin interesting also for nanotechnology applications.

The literature on chitin-based nanocomponents is dominated by chitosan, regenerated chitin, and chitin nanocrystals. Chitosan polymers are deacetylated derivatives of chitin. A recent development is electrospun chitin nanofibers or microfibers produced from dissolved chitin or derivatives including chitosan. Electrospun nanofibers form randomly interwoven networks and are attractive as biomedical textiles for wound dressings. However, toxic chemicals such as DMAc (N,N-dimethylacetamide) or NaOH/urea are used during processing. Although chitosan nanofibers can be prepared through green routes, the resulting fiber diameter is much larger than the desired in the presented work. Environmental challenges are also present in chitin nanocrystal preparation due to the use of strong acid hydrolysis, strong base or (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO)-mediated oxidation.

Chitin nanocrystals have mainly been used as reinforcement in polymer nanocomposites. It has also been added to bacterial cellulose films to provide bactericidal activity. An interesting application of chitin is as a component in hydrogels. For example, as chitin nanocrystals were combined with a polymer hydrogel, the storage modulus (a measure for the stiffness of a hydrogel network structure) increased from 34 to 1700 Pa on addition of 0.5% chitin nanocrystals. The polymer hydrogel itself was a mixture of cyclodextrin and a triblock co-polymer of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) in water. A limitation of acid-hydrolyzed chitin nanocrystals is often the short aspect ratio (length/diameter ratio). Recent advances in disintegration of cellulose nanofibers from wood pulp fibers have inspired isolation of high-aspect-ratio chitin nanofibers. Such chitin nanofibers have particularly small diameters (ca. 4 nm). They were used to prepare "nanopaper" structures with novel characteristics such as high optical transparency and high specific nanofiber surface area. Transparent
chitin nanofiber and polymer matrix nanocomposites were thus prepared. Critical factors included good stability of the chitin–water hydrocolloid as well as the nanoscale dimensions of the chitin nanofiber cross-sections. Apart from providing excellent mechanical properties, chitin nanofiber materials also have advantages such as gas-barrier properties, optical transparency, and slow thermal expansion.

Colloidal suspensions of chitin or cellulose nanofibers will form gels after mechanical homogenization at relatively low concentrations. One gelation mechanism is simply formation of physical entanglements between nanofibers due to the high surface area and high aspect ratio. Chitin hydrogels are particularly interesting for applications in water purification, tissue scaffolds, drug delivery, and 3D printing.[17] For hydrogels prepared using chitosan or regenerated chitin, chemical modification or mechanical reinforcement are necessary to achieve favorable mechanical properties. Chitosan has been combined with clay to form hydrogels,[18] as well as with inorganic–organic frameworks to form aerogel microspheres.[19] In another example, chitin nanocrystals were combined with chitosan, with the storage modulus reaching 169 kPa at 13% chitin content.[14] It is also possible to prepare regenerated chitin hydrogels from chitin solutions in NaOH/urea[20] or chitosan hydrogels through base-induced precipitation.[19] Gel properties are developed by chitin precipitation in organic solvent and a freezing–thawing approach.[21] However, such regenerated chitin hydrogels had lower mechanical properties compared to cross-linked hydrogels from chitin or cellulose nanofibers. By cross-linking with epichlorohydrin, mechanical properties were improved. The storage modulus was roughly 70 Pa at 1% chitin content.[22] Chemically cross-linked hydrogels based on chitin nanocrystals were also prepared by heating in an autoclave but no mechanical properties were reported.[20] Recently, chitin hydrogels in the form of nanofiber networks were also prepared from regenerated α-chitin nanofibers. The procedure involved conversion of thin β-chitin nanofibers (3–4 nm in width and length in micrometers) isolated from squid[23] to α-chitin nanofibers through treatment in NaOH/urea followed by quenching in ethanol;[22] thus, β-chitin nanofibers could be converted to α-chitin and then trapped before dissolution. Hydrogel properties were mechanically enhanced due to the mixture of dangling polymer chains and partially dissolved nanofibers. Tensile properties were reported.[22]

The chitin nanofibers used in the present study were analyzed previously.[14,15] Chitin powder was treated with 2 M HCl, 20% NaOH, and ethanol to remove calcium carbonate particles, protein matrix, and pigments, respectively; subsequent mechanical treatment was performed at pH 3 to facilitate nanofiber liberation through electrostatic repulsion. The obtained chitin nanofibers show some structural analogies to TEMPO-oxidized cellulose nanofibers in terms of charged surfaces, high aspect ratio, and uniform nanofiber width. The aspect ratio of the chitin nanofibers presented herein was in the range of 25–250, with diameters of 3–4 nm and lengths reaching several micrometers.[14] Positively charged nanofiber surfaces improve colloidal stability due to electrostatic repulsion between fibrils. This serves to counteract nanofiber aggregation. In a previous study, a freestanding hydrogel with a water content of 99.9% was successfully produced from a colloidal water suspension of TEMPO-oxidized cellulose nanofibers.[23] This was accomplished by careful adjustment of the pH value and evaporation of water to increase concentration.[23] Herein, we develop a green process for the preparation of hydrogels from water suspensions of chitin nanofibers. No toxic chemicals or organic solvents are used. The novelty of the hydrogel is the unique properties of the chitin nanofibers developed in our lab[14,16] and the preparation procedure, which results in a substantial fraction of nanoscale pores. The procedure involves suspending chitin nanofibers in water, where stability is achieved through electrostatic repulsion effects. This makes it possible to increase concentration without induction of gelation. Then gelation is induced by neutralization of the suspension so that electrostatic repulsion effects are removed. The unique structural, rheological, and mechanical properties of the chitin nanofiber hydrogels are discussed. Moduli and strengths of the chitin nanofiber hydrogels are much higher than for regenerated chitin-based hydrogels or hydrogels based on short rod-like chitin nanocrystals. Reasons include the nanostructurally controlled network structure and its nanoscale porosity.

Results and Discussion

Hydrogel preparation and rheological characteristics

Chitin nanofibers (ChNFs) have positively charged surfaces in acidic solution due to the protonation of amino groups obtained from partial deacetylation of chitin molecules present on the fibril surface during the extraction procedures. For this reason, ChNFs can be electrostatically stabilized at low pH values in acetic acid. The charge density depends on the degree of acetylation (DA) and pH value, which is therefore important for gel formation. ChNF hydrogels were prepared through a new and environmentally friendly route. The positively charged ChNF surfaces were neutralized either by dialysis or by addition of NaOH solution to the ChNF suspension to produce freestanding hydrogels (Scheme 1). Hydrogels with a ChNF content in the range of 0.4–3.0 wt% were produced through dialysis method. In addition, NaOH neutralization was used to prepare ChNF hydrogels with ChNF contents from 0.4 to 2.0 wt%. For the dialysis method, higher concentrations could be achieved through solvent exchange in a dialysis membrane. Dialysis removes acetic acid as well as hydronium ions, leading to deprotonation of amino groups, with the pH value increasing to neutral. The gel formation mechanism involves gradual build-up of stronger fibril–fibril interactions with secondary bonding of individual nanofibers to each other on the nanoscale, as was described for cellulose nanofibers of similar geometry.[24] At much higher ChNF concentrations, mechanical entanglement and even capillary condensation take place between ChNFs, leading to the formation of a giant supramolecular agglomerate “hydrogel”. As a test, the resulting hydrogels were soaked in deionized water at pH 7 and pH 3 for several days; in both cases their physical properties were...
retained. This is an important achievement as neither cross-linking nor chemical modification was required for hydrogel formation. The total charge of ChNFs was roughly 315 μmol g⁻¹ as measured by titration using a particle charger analyzer (Stabino), which is much lower than the carboxylate content in TEMPO-oxidized cellulose nanofibers (1500 μmol g⁻¹). In addition to the aspect ratio, it seems reasonable to assume that the gelation concentration may be also related to the nanofiber surface charge, which is essential for the stabilization of nanofiber colloids to prevent agglomeration. A similar phenomenon was reported for colloidal suspensions based on TEMPO-oxidized cellulose nanofibrils, in which gelation was possible at much lower concentrations (i.e., 0.1%).

Dynamic storage modulus ($G'$) is a measure of the stiffness of the hydrogel networks. ChNF hydrogels of 1 wt% solid content produced through dialysis were much stiffer ($G' = 2.6$ kPa) compared to hydrogels prepared by NaOH neutralization ($G' = 2.0$ kPa), see Figure 1a and b. There is a linear relationship between frequency or shear rate and storage modulus in the frequency range of 1–100 Hz at 10% min⁻¹ strain rate, although the dependence is not so strong for NaOH-neutralized hydrogels. $G'$ increased steadily with frequency at all concentrations for dialyzed hydrogels. A steady increase in $G'$ against ChNF concentration is also apparent. The loss modulus ($G''$) is related to the energy dissipated due to permanent deformation of polymer hydrogel, a viscoelastic material. For the NaOH-neutralized hydrogel samples with concentrations of 0.4–0.8 wt %, $G''$ was higher than $G'$, which may relate to a lack of gelation in local regions caused by incomplete neutralization. As the nanofiber geometry is the same, the reason may be a more efficient bonding mechanism between the nanofibers.

![Scheme 1](image)

**Scheme 1.** Hydrogel preparation from water suspension of chitin nanofibers: (a) dialysis neutralization and (b) NaOH neutralization.

![Figure 1](image)

**Figure 1.** Storage modulus ($G'$) and loss modulus ($G''$) as a function of frequency for ChNF hydrogels prepared from aqueous suspensions with various ChNF contents by (a) dialysis and (b) neutralizing with NaOH. (c) Effect of ChNF concentration on $G'$ of the hydrogels. (d) Rheological behavior of ChNF suspension with 0.8 wt% solid content at pH 3.
The effect of aspect ratio is important for nanofiber hydrogels. \(^{24a}\) It influences the concentration at which gelation occurs and also the storage modulus. Cellulose nanocrystals with an aspect ratio of about 50 required as much as 30 g L\(^{-1}\) solid content for gelation to occur.\(^{24a}\) Herein, gelation occurred already at a ChNF concentration of 0.4 wt% for both preparation routes. The highest value of storage modulus was achieved at 3.0 wt% for the dialyzed ChNF hydrogel (\(G' = 13\) kPa). For TEMPO-oxidized cellulose nanofibers, Saito et al. reported a higher \(G'\) value at a lower concentration (ca. 20 kPa at 0.8 wt%).\(^{22}\) Fe\(^{3+}\)-induced cellulose hydrogels prepared from TEMPO-oxidized cellulose nanofibers had an even higher value (\(G' = 31\) kPa at 1.27 wt% cellulose content).\(^{26}\) Reasons for property differences include nanofiber network structure (i.e., extent of dispersion), intrinsic nanofiber properties and dimensions, extent of defects in nanofibers, and nature of nanofiber–nanofiber bonds. Chitin nanofibers is positively charged whereas TEMPO-oxidized cellulose is negatively charged. The amount of charge in TEMPO-oxidized cellulose nanofibers is about 1500 \(\mu\)mol g\(^{-1}\)\(^{24a}\) whereas that of the ChNFs discussed herein is 315 \(\mu\)mol g\(^{-1}\). In carboxymethyl cellulose nanofibers obtained from disintegrated wood pulp fibers, the charge is 600 \(\mu\)mol g\(^{-1}\).\(^{24a}\) In TEMPO-oxidized cellulose and ChNFs, the total charge is critical for the strong gel-forming characteristics.

Figure 1c shows the changes in \(G'\) as a function of ChNF concentration. Both dialyzed and NaOH hydrogels show a linear increase with ChNF concentration and the reason may be more efficient bonding mechanisms between the nanofibers, which increases network stiffness. The measurements shown in Figure 1c was done based on study for TEMPO-oxidized cellulose hydrogel which was adopted from disintegrated wood pulp fibers, the charge is 600 \(\mu\)mol g\(^{-1}\).\(^{24a}\) In TEMPO-oxidized cellulose and ChNFs, the full papers.

Structural characterization of chitin nanofiber hydrogels

ChNF aerogels were produced to improve the understanding of the hydrogel structure. Freeze-drying was carried out after changing the solvent to tert-butanol: Freeze-drying in water tends to disturb the original network structure as the growth of ice crystals often creates a different pore structure than was present in the hydrogel.\(^{21,26}\) The SEM micrographs shown in Figure 2 reveal the nanofiber network structures of ChNF hydrogels with different ChNF concentrations. ChNFs are present as long swirled nanofibers, many of them with diameters around 10 nm. ChNFs with larger diameters (20–100 nm) are aggregates formed due to incomplete fibrillation or agglomeration during freeze-drying. The images show a gradual increase in pore size with decreasing ChNF concentration. According to the micrographs, most pores have a characteristic size below 1 \(\mu\)m. More uniform pore sizes seem to form as the ChNF concentration is increased. Many chitin scaffolds in the literature obtained through freeze-drying from water have a characteristic pore size much larger than 1 \(\mu\)m. Chitin-whisker–hyaluronan–gelatin scaffolds showed pore sizes of 139–166 \(\mu\)m.\(^{29}\)

Auto-porosimetry measures pore sizes by recording changes in pressure obtained when liquid is pushed out from pores with different sizes during adsorption–desorption cycles. The liquid is first adsorbed to equilibrate the sample and then desorbed. The volume of the liquid adsorbed per gram of chitin (ChNF) was assumed to correspond to the total volume of pores. For the pore size distribution, reference is made from the fact that samples with many large pores adsorb more liquid compared to those with many small pores per gram of chitin (ChNF). Figure 3a shows the cumulative pore volume against pore size obtained from the first cycle of an auto-porosimetry measurement. The cut-off pore size specified for the auto-porosimetry membrane allowed pore size measurements between 5 and 500 \(\mu\)m. However, we were interested in the volume of pores below 5 \(\mu\)m to be able to compare pore size distributions. The volume of pores below 5 \(\mu\)m in size was estimated from the volume of hexadecane retained after the first cycle. Hexadecane was first adsorbed in small pores below 5 \(\mu\)m to equilibrate the sample during the adsorption cycle and was not desorbed in the subsequent measurements. In Figure 3a, the pore volume of pores smaller than 5 \(\mu\)m is indi-
cated by box A and is roughly 60% of the total pore volume at 2.0 wt% ChNFs. Data shown in Figure 3b present the volume of hexadecane retained per milligram of chitin (ChNF) after desorption during the first cycle. A large fraction of small pores is observed for aerogels with high ChNF concentrations. The liquid adsorbed in large pores was desorbed in the next desorption cycle. However, the large volume of liquid retained in small pores at high ChNF concentrations makes it tempting to speculate that a large fraction of the pores were below 5 µm. The high-resolution micrographs of the nanofiber gel structure shown in Figure 2 confirm the sub-micrometer scale of the nanofiber network structure in the material. This study is a first report of the importance of nanofiber and network characteristics of chitin hydrogel properties, but future work should include more detailed characterization of the pore size distribution below 5 µm using Brunauer–Emmett–Teller (BET) gas adsorption measurements. In a previous study, the pore sizes in aerogels prepared from cellulose nanofibers after solvent exchange to tert-butanol ranged was on the nanometer scale (10–16 nm). However, the small pore size was achieved by centrifugation, a more tedious preparation procedure than our method.

Compression properties

Mechanical behavior of ChNF hydrogels is influenced by the pH value. Figure 4 shows the compressive stress–strain curves for ChNF hydrogels. The stress–strain curve typically consists of an initial linear region followed by a steep increase in slope above 40% compressive strain. This region is characterized by strain hardening. The nanofiber network is deformed so that structural network damage is taking place. The ChNF network collapses to some extent and, therefore, becomes more resistant to compression because collapsing nanofibers begin to touch and interact. Liquid is still present in the material, and hydraulic effects on the stress–strain behavior is expected (water may also contribute to strain hardening). Strain hardening increased gradually with ChNF concentration. At 0.4 wt%, the slope was essentially linear over the entire range. The hydrogels deformed to a maximum compressive strain of around 80% at which point the compressive stress was maximum. At higher deformation, the network structure was increasingly damaged. The origin of discontinuities in the compression curve at 2 wt% is not entirely clear (Figure 4a), perhaps it can be assigned to artifacts during testing or a hydromechanical modification of the hydrogel at high compressive stress. Compressive strength and modulus increase with ChNF concentration (Figure 4b). Uniaxial compression data are summarized in Table 1, together with data for other hydrogels reported previously in literature. With the exception of hydrogels ob-

Figure 3. Data from auto-porosimetry measurements as a function of ChNF concentration for adsorption–desorption cycles of ChNF aerogels: (a) cumulative pore volume (%) as a function of pore size and (b) volume of hexadecane (mm$^3$/mg of chitin (ChNF)) adsorbed in pores with a characteristic size below 5 µm.

Figure 4. (a) Compression stress–strain curves of ChNF hydrogels prepared by NaOH neutralization. (b) Uniaxial compressive properties as a function of ChNF concentration.
tained from cellulose nanofibrils prepared by TEMPO-mediated oxidation, the hydrogel presented herein shows better mechanical properties than most soft water-based bionanofiber hydrogels. Furthermore, the preparation procedure is very simple and environmentally friendly.

Conclusions

A new solvent-free procedure was developed for the preparation of strong and stiff hydrogels based on native chitin nanofibers (ChNFs). These ChNFs are unique in terms of preserved native structure, high aspect ratio (length/diameter ratio of ca. 25–250), small diameter, high purity, and high molar mass. Despite the high aspect ratio, colloidal suspensions of ChNFs are stable liquids at low pH values (pH 3) and low concentrations due to electrostatic repulsion between cationic chitin nanofibers. Hydrogel formation is then simply induced by neutralization so that repulsion is removed and individual smooth nanoscale ChNF form strong physical bonds by secondary forces. Physical entanglements provide additional resistance to deformation. Moduli and strengths are much higher than for regenerated chitin-based hydrogels or hydrogels based on short rod-like chitin nanocrystals. The reasons are the excellent intrinsic strength and stiffness of the unique ChNFs, the dense physical cross-linking in the three-dimensional nanofiber network, and physical entanglements due to the very high ChNF aspect ratios. Furthermore, the prepared ChNF hydrogels, especially at high ChNF contents, can have more than 60% of the pore volume in pores below 5 μm in size (sub-micrometer scale). This is due to the preparation scheme, where first electrostatic repulsion (before gelation), then (after gelation) strong physical ChNF–ChNF cross-links prevent ChNF agglomeration. It may be concluded that the nanostructure control mechanisms ensure that the hydrogel is dominated by a large fraction of individual but strongly connected chitin nanofibers rather than ChNF agglomerates, and that there is a strong influence on the macroscopic mechanical properties although structural organization on the sub-micrometer scale is likely random. The strong improvements in mechanical properties of ChNF hydrogels reported here may facilitate ChNF use in applications such as tissue engineering, water purification, 3D printing, drug delivery, or wound healing devices.

Experimental Section

Materials. Native low-protein chitin nanofibers (ChNFs) from lobster Homarus americanus of Northwest Atlantic were prepared as described in our previous publication.13,14 The concentration of ChNFs in its colloidal suspension was 1.0 wt%. Protein content in the ChNFs was 4.7% as determined by performing ninhydrin–hydroxidatin test. Degree of acetylation (DA) was in the range of 86–87% as determined by solid-state cross-polarization magic angle spinning 13C nuclear magnetic resonance (CP/MAS 13C NMR) spectroscopy. Aspect ratio (length to width ratio) of the ChNFs was ca. 250 as measured by atomic force microscopy (AFM).

Preparation of hydrogels. The dependence of gelling behavior of ChNFs in water as effect of pH and concentration on ChNF agglomeration was studied. Hydrogels of different ChNF concentrations were prepared by neutralizing acetic acid present in the water suspension of ChNFs. Neutralization was achieved through either dialysis or addition of NaOH. The colloidal suspension was dialyzed in deionized water under ambient conditions using a regenerated cellulose dialysis membrane with a molecular weight cut-off of 12 kDa until neutrality was reached. The concentration of ChNFs in the hydrogel was varied from 0.4 to 3.0 wt%. To prepare hydrogels with 3.0 wt% ChNFs, the hydrogel in the dialysis membrane was dehydrated in ethanol and then rehydrated in deionized water. Through dialysis, ChNF neutralization, dehydration, or rehydration was achieved through osmosis. For the preparation of NaOH-neutralized hydrogels, typically 2.0 mL 20% NaOH solution was carefully added dropwise on top of a 75 mL ChNF suspension. Colloidal suspensions with low concentrations were prepared by diluting a 1.0 wt% suspension whereas higher concentration such as 2.0 wt% were obtained slow evaporation of water in air from the 1.0 wt% suspension.

Rheological tests. Rheological tests of ChNF hydrogels were performed using a flat parallel plate configuration with a diameter of 25 mm and a gap thickness of 1 mm on a Rheometer (MCR 501, Anton Paar, Austria). Dynamic rheological properties were measured in frequency-sweep mode over the range of 1–100 rad s−1 at a strain rate of 10% and ambient temperature (25 °C). A transducer detected the change in torque and recorded storage modulus and tan delta. Storage modulus and tan delta were recorded for hydrogels with ChNFs concentration of 0.4, 0.6, 0.8, 1.0, 2.0, and 3.0 wt%. For each concentration, three samples were prepared. Rheological characterization of ChNF hydrogel sample with 0.8 wt% ChNFs content at pH 3 was performed as control in parallel plate configuration using a Malvern Rheometer (Kinexus, Malvern Instruments Ltd, UK).

Structural characterization of chitin nanofiber hydrogels. Aqueous ChNF hydrogel was solvent exchanged with tert-butanol and

| Table 1. Compressive mechanical properties of ChNF hydrogels (strain at highest compressive stress was 80%).[6] |
|---|---|---|---|---|
| Material | ChNF conc. [wt %] | Modulus [kPa] | Yield strength [kPa] | Yield strain [%] | Strength [kPa] |
| this work | 0.8 | 213.3 (2.5) | 3.1 (0.6) | 39.1 (1.3) | 17 (2.0) |
| | 1.0 | 216.5 (4.7) | 7.8 (5.0) | 46.4 (1.8) | 24 (3.5) |
| | 2.0 | 309.1 (4.0) | 10.3 (2.0) | 31.2 (2.1) | 81 (4.2) |
| bacterial cellulose[30b] | -2 | 7 | -50 | _IM_ | _IM_ |
| gelatin[30b] | 15 | 160 | -50 | _IM_ | _IM_ |
| regenerated chitin[7] | 2.0 | < 5 | 22 | _IM_ | _IM_ |
| cellulose nanofibrils[30a] | 3.0 | 541 | | | |

[a] Values in parentheses are the sample standard deviations. [b] No data available.
then freeze-dried to produce ChNF aerogels. Solvent exchange was first performed in 50% tert-butanol in water twice at an interval of 2 h and then in 100% tert-butanol overnight. More time was given for thicker samples to ensure complete solvent exchange. Pore size and size distribution of the aerogel was measured using a TRI/Auto-porosimeter version 2008-12 (TRI/Princeton, USA). The aerogel sample was placed in a chamber and then equilibrated in liquid hexadecane after the chamber was vacuumed. Pore size measurement specification was according to the membrane cut-off pore size of 1.2 μm. The minimum pore size was 5 μm and maximum 500 μm. The morphology of ChNF aerogels was characterized by using field-emission scanning electron microscope (FE-SEM, Hitachi S-4800 Japan). The samples (ChNF aerogels) were conditioned in a desiccator overnight to remove moisture and then sputtered with a 5 nm thick layer of platinum/palladium using an Agar HR sputter coater. FE-SEM images were recorded at 1.0 kV from secondary electrons.

**Compression test.** Mechanical properties of the ChNF hydrogel were measured under compression mode using a universal testing machine (Instron Model 5944) equipped with a 50 N load cell. Circular specimens were prepared with diameter 25 mm and thickness was measured. The stress–strain curves of the hydrogels were recorded at room temperature at a strain rate of 10% min⁻¹. Measurements were performed on at least two specimens for each concentration. Compressive modulus, strength, and strain were measured. Compressive modulus (E) was determined as a slope in the elastic region. Yield stress was calculated as stress value corresponding to the intersection of the tangent between the elastic region and the initial plastic deformation region.

**Acknowledgements**

The authors thank the Swedish Research Council Formas (Carbo-chem, 2009-1687) for supporting this work.

**Keywords:** chitin · compression · hydrogel · nanofibers · rheology

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