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β-Chitin nanofibrils for self-sustaining hydrogels preparation via hydrothermal treatment

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ABSTRACT

A transparent nanofibril suspension could be readily obtained by treating purified squid pen powder in water with ultrasonic irradiation. The obtained suspension is consisted of β-chitin nanofibrils (CNF) with 3–10 nm in width and several micrometers in length. The degree of acetylation (DA) of CNF was found to be 84% which is about 10% lower than that of untreated sample. The CNF suspension could be transformed into a durable 3-D hydrogels (CH) by simply heating to 180 °C for 1–4 h in an autoclave. Hydrophobic interaction between CNF was believed to play the major role for CNF self-assembling into hydrogels, since the as-prepared chitin hydrogels readily dissolved in a typical chaotropic solution (8 M urea) under room temperature. The hydrothermal duration and CNF concentration (0.3–2% (w/v)) strongly affected the physical properties of CH. The suspension of 1% (w/v) CNF treated with 4 h, 180 °C hydrothermal heating generated a CH with 99.3% water content, CNF with 87% crystallinity and an mechanical strength of 0.7 N breaking force.

1. Introduction

Chitin, the second most abundant organic materials in nature after cellulose, mainly presents in crab and shrimp shells, outer skins or cuticles of other arthropods, and molluscan shell of squid (so-called squid pen). Due to its different origin, chitins have α- and β-type crystalline structure. Chitins from crab and shrimp are known to have a rigid β-structure while squid pen consists of less rigid β-structure. Both type of chitins are insoluble in general organic solvents because of the crystalline structure resulted from the strong hydrogen bonding between the acetamide group, hydroxyl group, and carbonyl group (Austin, 1975; De et al., 1992; Gardner & Blackwell, 1975; Kaifu, Nishi, & Tokura, 1981; Minke & Blackwell, 1978; Muzzarelli, 2012; Tamura, Nagahama, & Tokura, 2006). The less rigid β-chitin is more susceptible to enzymatic degradations or chemical reactions (Fan, Saito, & Isogai, 2008; Kurita, Kaji, Mori, & Nishiyama, 2000). Usually, chitin solutions could be obtained by dissolving chitin in dimethylacetamide (DMAc)/lithium chloride (LiCl) or *N*-methyl-pyrrolidinone (NMP)/LiCl solvents. By employing these chitin solutions, chitin beads and hydrogels were prepared by phase inversion method using water as a nonsolvent (Yilmaz & Bengisu, 2003; Yusof, Lim, & Khor, 2001). Recently, several novel solvents such as ionic liquid (Wu, Sasaki, Irie, & Sakurai, 2008), calcium chloride saturated methanol (Tamura, Furuike, Nair, & Jayakumar, 2011), low temperature NaOH/urea solution (Abe & Yano, 2011) have been developed to prepare chitin solution. Based on these chitin solutions, hydrogels were readily prepared via solvent exchange or chemical cross-linking for biomedical and other applications (Chang, Chen, & Zhang, 2011; Ding et al., 2012; Muzzarelli, 2011a, 2011b; Muzzarelli & Muzzarelli, 2005; Muzzarelli et al., 2007; Tamura et al., 2011; Tang, Zhou, & Zhang, 2012). In addition to chemical method for obtaining dissolved chitin suspension, crab and squid pen chitins could be ground (Mikhailov & Lebedeva, 2007) and ultrasonicated (Fan et al., 2008) into nanofibrils to form a transparent nanofibrils suspension. Chitin nanofibrils suspension prepared by mechanical methods have been used to produce transparent oxygen barrier dry film (Yusof, Lim, & Khor, 2004) and reinforce nanocomposite structure (Ifuku, Morooka, Morimoto, & Saimoto, 2010; Mathew & Oksman, 2007). To the best of our knowledge, the direct application of chitin nanofibrils suspension for hydrogels preparation has never been reported.

In this work, the direct transformation of chitin nanofibrils suspension obtained from squid pen into durable chitin hydrogels via hydrothermal process was studied. The preparation of hydrogels could be achieved without employing chemical solvents

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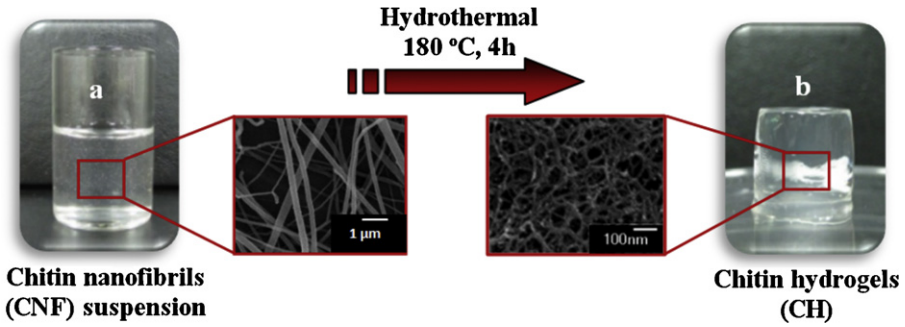

Scheme 1. Preparation of chitin hydrogels, chitin nanofibrils (CNF) suspension after ultrasonication (a) and chitin hydrogels (CH) obtain CNF suspension after hydrothermal treatment (b).

and cross-linkers. The effects of CNF concentration, hydrothermal temperature, and durations on the structure and properties of as-prepared chitin hydrogels were investigated.

2. Experimental

2.1. Materials

Squid pen was collected from local fish market, sodium hydroxide (NaOH), hydrochloric acid (HCl) and Triton® X-100 ($C_{34}H_{62}O_{11}$), urea ($CO(NH_2)_2$), genipin ($C_{11}H_{14}O_5$) were obtained from ACROS, NJ, USA. Deionized (DI) water and all other chemicals were analytical grade used without further purification.

2.2. Preparation of chitin nanofibrils suspension

Chitin nanofibrils were produced from purified squid pen by mechanical treatment according to the method described previously (Fan et al., 2008; Nata, Wang, Wu, & Lee, 2012). Briefly, squid pens collected from fish market were washed thoroughly with tap water and dried before ground into fine powder by a high speed blender. Powdered squid pen (30 g) was dispersed in 500 mL of 0.5% Triton X-100 by stirring for 24 h at room temperature to remove lipoproteins. After thorough wash with water, the wet cake was suspended in 500 mL of 1 N NaOH solution and stirred for 24 h to eliminate the remaining proteins. After washing thoroughly with water to neutral pH, the collected chitin wet cake (0.3, 0.5, 1, and 2 g) was dispersed in 100 mL water and adjusted pH to 3–4 with dilute HCl. The wet cake itself contained about 74% of water. The transparent CNF suspension were obtained by ultrasonicating the dispersed chitin cake with power of 10 W for 30–60 min at 4 °C.

2.3. Preparation of chitin hydrogels

To prepare chitin hydrogels, the as-prepared CNF suspension (12 mL) was transferred into a glass vial and sealed in a stainless steel autoclave reactor. The reactor was heated and maintained at 180 °C for 1–4 h. After cooling at room temperature, the reactor was opened and hydrogels was removed from the glass vials. After thorough washing with deionized water, the hydrogels were kept in water for further characterization.

2.4. Cross-linked with genipin and stability in 8 M urea

The as-prepared hydrogels was further cross-linked by immersing in a 2.5 mg/mL genipin solution, pH 8.5 at 37 °C for 48 h. The original clear and transparent CH gel turned into brown color after cross-linking reaction. Both the hydrothermally prepared and cross-linked hydrogels were immersed in 8 M urea for 24 h to observe their structural integrity.

2.5. Characterization

Field emission scanning electron microscopy (FE-SEM) images were taken using a scanning electron microscope (JOEL, JSM-6500F, Japan). Critical point drying (Samdri PVT-3D) was employed to prepare the chitin hydrogels for FE-SEM observation. The chitin nanofibrils suspension was produced by using sonicator irradiation (Qsonica sonicator, Newton, CT). The average degree of acetylation (DA) was determined from elemental analysis using EA000200 Elementar varioEL III-CHNS and calculated based on the ratio of carbon–nitrogen content by $\overline{DA} = (((C/N) - 6.16)/1.72)$ (Lavall, Assis, & Campana-Filho, 2007). The CNF (W_{CNF}) content of CH was calculated by $W_{CNF} = (W_{CH}/W_{CNFo}) \times 100$, where W_{CH} is dry weight of chitin hydrogels and W_{CNFo} is dry weight of CNF suspension. The X-ray diffraction (XRD) measurement was performed on Rigaku D/MAX-B X-ray diffractometer by using Copper K-alpha (Cu K_α) radiation. The operation voltage and current were kept at 40 kV and 100 mA, respectively. The crystallinity index (CrI) was determined by $CrI_{110} = (I_{110} - I_{am}) \times 100/I_{110}$, where I_{110} is the maximum intensity around 20° and I_{am} the intensity of amorphous diffraction at 16° (Zhang, Xue, Xue, Gao, & Zhang, 2005). Mechanical strength of the as-prepared hydrogels was evaluated by a testometric strength test machine (M500-25AT) using a load cell of 1 kg operating at a deformation rate of 1 mm s^{-1} performed under ambient conditions. Force at break was calculated using the Win-Test software.

3. Results and discussion

3.1. Hydrogels formation via hydrothermal reaction

Transparent CNF suspension was readily obtained by ultrasonicating the purified squid pen powder suspension under mild acidic condition as previously reported (Fan et al., 2008). As shown in Scheme 1, the transparent suspension is mainly consisted of nano-sized straight fibers of 3–10 nm in width and several micrometers in length. The DA of CNF was determined to be approximately 84% which is about 10% lower than that of chitin cake before ultrasonication treatment, as shown in Table 1. In other words, the intensive ultrasonication employed not only disrupted chitin powder into nanofibrils but also deacetylated some of the N-acetylated groups in the β-chitin structure. After 180 °C, 4 h hydrothermal treatment, the CNF suspension could be tranformed into a 3-D free standing

Table 1
Elemental analysis and degree of acetylation of β-chitin before and after ultrasonicating purified squid pen chitin powder.

Sample	N (%)	C (%)	DA (%)
β-Chitin powder (before)	5.85 ± 0.012	39.56 ± 0.139	93.91
Chitin nanofibrils (after)	5.88 ± 0.014	38.81 ± 0.124	84.33

Table 2
Effect of CNF concentration of 12 mL suspension and hydrothermal duration on hydrogels volume obtained at 180 °C.

Volume of as-prepared CH (mL)	CNF concentration (%, w/v)			
	0.3 (CH1)	0.5 (CH2)	1 (CH3)	2 (CH4)
1 h	0	0	0	9.99 ± 0.01
2 h	11.93 ± 0.04	11.88 ± 0.04	11.88 ± 0.03	8.96 ± 0.06
3 h	8.10 ± 0.14	7.98 ± 0.03	9.05 ± 0.07	8.25 ± 0.07
4 h	7.45 ± 0.07	7.53 ± 0.04	7.55 ± 0.07	7.55 ± 0.07

hydrogels. As observed by FE-SEM, the hydrogels is consisted of kinky and entangled nanofibrils with an average diameter of ca. 1/3 of the original straight nanofibrils observed in the CNF suspension (Fig. 1). The CNF suspension prepared from 0.3%, 0.5%, 1% and 2% (w/v) of purified squid pen cake were employed to obtain the hydrogels CH1, CH2, CH3, and CH4, respectively. The CH4 could be formed after 1 h hydrothermal duration (CH4-1) was set as an ultimate goal for hydrothermal temperature selection. Temperature at 100 °C, 121 °C, 140 °C were tested but no hydrogels formation could be observed. The temperature was increased up to 180 °C, the most common temperature employed for hydrothermal carbonization of polysaccharides (Sevilla & Fuertes Antonio, 2009), to study the possibility of hydrogels formation under hydrothermal condition. In addition to temperature, chitin concentration and hydrothermal duration also significantly affect the size and CNF content of the as-prepared hydrogels. As shown in Table 2, only CH4 could form as a 10 mL hydrogels after 1 h hydrothermal treatment. No apparent hydrogels formation could be observed for 0.3%, 0.5%, and 1% of CNF suspension. Extending the hydrothermal duration allows CNF to have more chance to interact with each other and facilitates hydrogels formation. After 4 h incubation, hydrogels with size ca. 7.5 mL were formed out of 12 mL of CNF suspension despite of their initial concentrations. As shown in Fig. 1, the CNF recovered in the hydrogels increased with hydrothermal duration and reached 100% after 4 h. In other words, a fraction of CNF in the original CNF suspension was not included in the gel and existed in supernatant during the hydrogels formation process. The CNF recovery yield increased with CNF concentration and hydrothermal duration. This indicates that the initially well-dispersed CNF need time to diffuse and interact with each other to form a 3-D free standing hydrogels during a static hydrothermal condition. The higher initial CNF suspension concentration will provide and promote more frequent interactions for CNF to entangle with each others so that a gel can be formed. With a higher chance to entangle with each others, the higher CNF concentration suspension therefore results in a higher CNF recovery yield after hydrogels formation. However, the long duration at elevated temperature will also possibly induce the degradation of CNF and the dehydration of the degraded products (Sevilla & Fuertes Antonio, 2009) probably leads to the formation light brownish but transparent hydrogels as observed in CH4-3 and CH4-4 hydrogels (Fig. S1, supporting information).

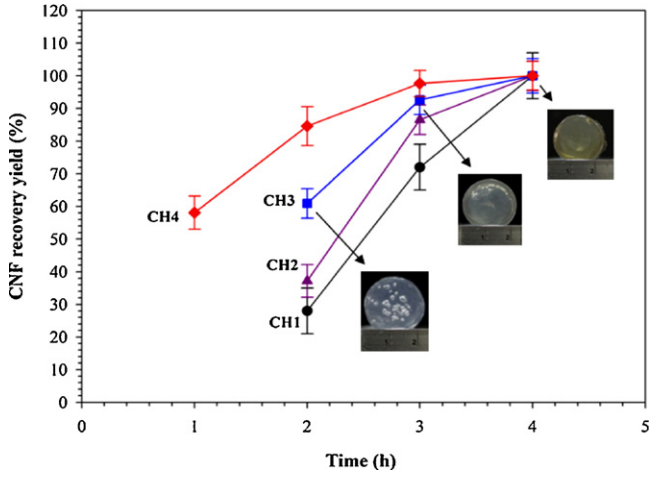

Fig. 1. Effect of CNF concentration and hydrothermal duration on CNF recovery yield in the hydrogels prepared at 180 °C. Photographs are CH3-2, CH3-3 and CH3-4 gels (1% (w/v) of CNF suspension) obtained after 2, 3, and 4 h hydrothermal treatment.

Hydrophobic interaction is known as an entropy-driven process and becomes pronounced at elevated temperature. Evidently, the hydrogels formation at elevated hydrothermal temperature can attribute to the hydrophobic interaction between the CNF which may contain appreciable hydrophobic domains in its chain structure. Similarly, hydrogels of graphene oxide (Xu, Sheng, Li, & Shi, 2010), hydrophobically modified chitosan (St. Dennis et al., 2011), and carbonaceous chitin hydrogels (Nata et al., 2012) have also been reported to be formed under hydrothermal condition with elevated temperature. The presence of hydrophobic interactions between CNF of the as-prepared chitin hydrogels was demonstrated by incubating the hydrogels (CH1–4) in 8 M urea solution since urea is a well-known chaotropic compound which disrupts hydrophobic interaction very effectively. As shown in Fig. 2, CH1–4 was completely dissolved in 8 M urea after 24 h incubation at room temperature. In contrast, the hydrogels chemically cross-linked by genipin well maintained their original shape in 8 M urea. Genipin is a mild and low cyto-toxic cross-linker has been used to cross-link chitosan via the amino groups (Muzzarelli, 2009). Since the obtained CNF has a DA about 84%, there still has an appreciable amount of amino groups in the structure of CNF available for cross-linking by genipin. After 48 h incubation with genipin, the hydrogels instead of developing a commonly reported blue color as for genipin cross-linked chitosan gel, brownish color was observed as shown in Fig. 2. The brownish color intensity increased with CNF content was also noticed. The brownish color was probably resulted from the alkaline pH (~8.5) involved in cross-linking the as-prepared chitin hydrogels because similar brownish color has also been reported before for genipin cross-linking in the strong alkaline condition (Levinton-Shamuilov, Cohen, Azoury, Chaikovsky, & Almog, 2005).

3.2. Characterization of as-prepared hydrogels

As observed by FE-SEM (Fig. 3), anisotropic contraction of CNF in the as-prepared hydrogels caused by the elevated hydrothermal temperature results in the kinky and shrunken nanofibrils. The kinky and fibrous network of CH1–4 hydrogels observed are quite similar to that of cellulose nanofiber hydrogels prepared by incubating in strong NaOH solution (Abe & Yano, 2011). It was also observed that the porosity of the hydrogels increased with the CNF concentration. To identify the chemical and physical structure of the as-prepared hydrogels, FT-IR and XRD studies have been carried out. As shown in Fig. 4, very strong stretching vibration bands of O—H, N—H (asymetric) and N—H (symmetric) at 3460 cm^{-1}, 3270 cm^{-1}, and 3110 cm^{-1}, respectively (Lavall et al., 2007) were observed for the β-chitin purified from squid pen. Also, the specific β-chitin adsorption bands involving C=O···H—N

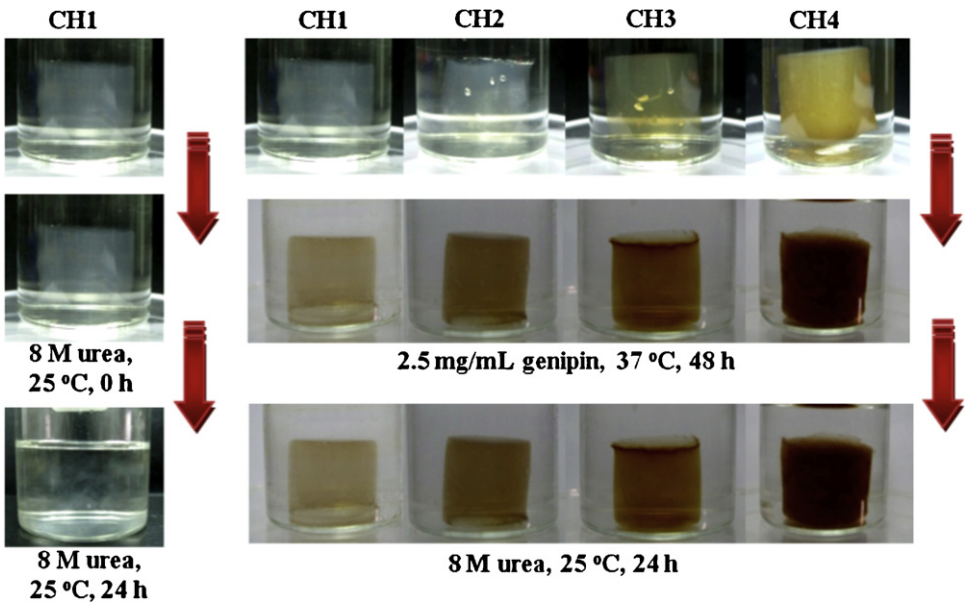

Fig. 2. Effect of chaotropic solution (8 M urea) on dissolving as-prepared chitin hydrogels and further genipin cross-linked hydrogels.

intermolecular hydrogen bonding were observed in the regions of 3264 cm^{-1}, 1300 cm^{-1} and 1520 cm^{-1} (Focher, Naggi, Torri, Cosani, & Terbojevich, 1992). In contrast, the bands involving N—H, O—H stretching vibration, and C=O···H—N intermolecular hydrogen bonding for CH1, CH2, CH3 and CH4 hydrogels were rather weak and shifted significantly. This indicates that the inter- and intra-molecular hydrogen bonding of original β-chitin powder were disrupted after ultrasonication and hydrothermal treatments.

As shown in XRD pattern (Fig. 5), the intensity characteristic crystalline peaks of β-chitin at 2θ indexed as (0 2 0) at 9° and (1 1 0) at 20° were observed for the purified β-chitin powder (Lavall et al., 2007). The intensities of those peaks decreased after ultrasonication treatment. The original 78.53% crystallinity of β-chitin powder decreased significantly to 54.22% for the 0.3% (w/v) CNF prepared by ultrasonication (Fig. 5, inset). Evidently, the high energy intensity employed in ultrasonication effectively disrupts some of the

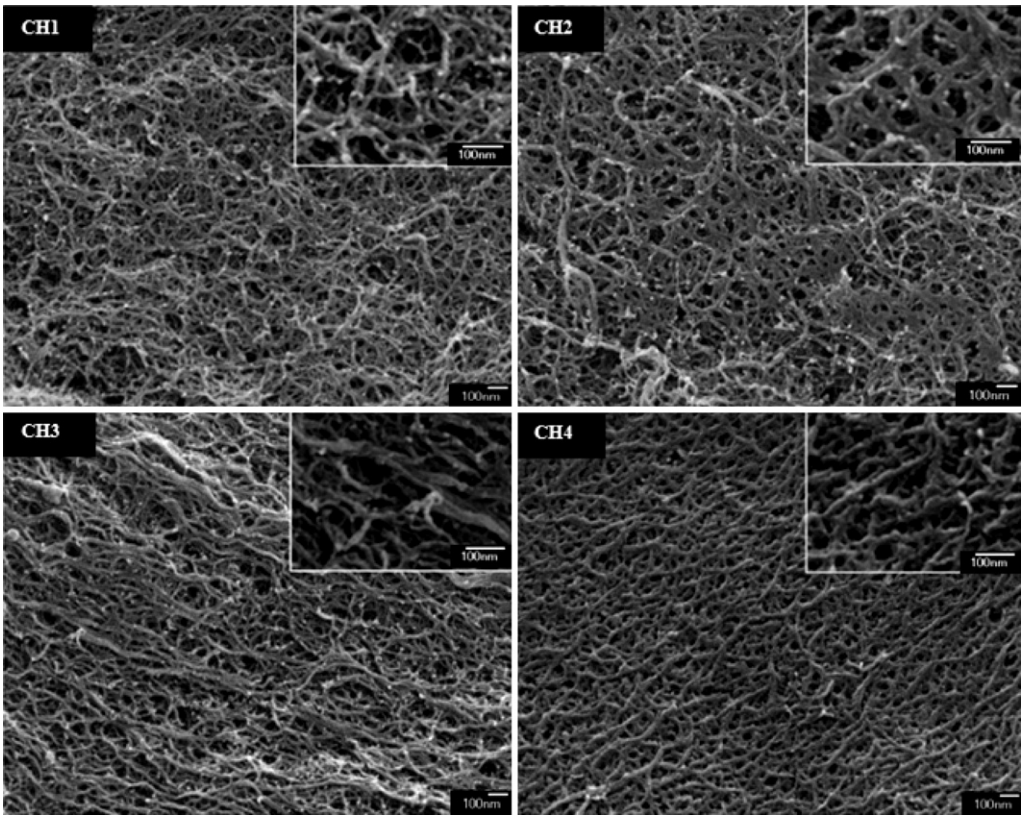

Fig. 3. FE-SEM images of chitin hydrogels prepared from suspension of different CNF concentration. 0.3%, w/v (CH1), 0.5%, w/v (CH2), 1%, w/v (CH3), and 2%, w/v (CH4) after 180 °C for 4 h hydrothermal treatment.

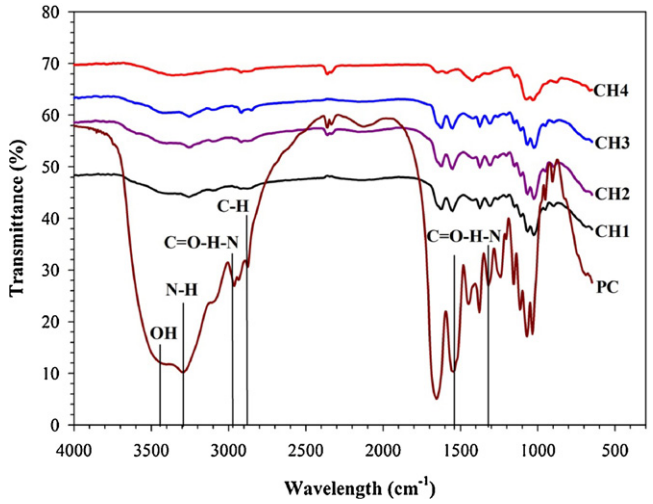

Fig. 4. IR spectra of β-chitin powder purified from squid pen (PC), hydrogels prepared from different concentration of CNF suspension under 180 °C for 4 h hydrothermal treatment.

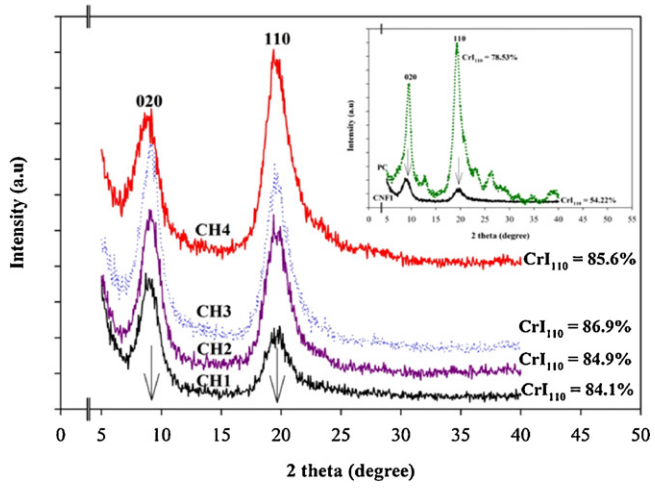

Fig. 5. XRD pattern of β-chitin powder purified from squid pen (PC) (inset), CNF prepared by ultrasonication (inset), and hydrogels obtained from different concentration of CNF suspension under 180 °C for 4 h hydrothermal treatment.

original crystalline structure of β-chitin powder. On the other hand, the crystallinity of as-prepared hydrogels could be increased to approximately 85% due to the amorphous part of chitin nanofibrils was re-crystallized by annealing effect with the hydrothermal treatment at 180 °C.

The mechanical strength of as-prepared hydrogels was evaluated by measuring the force that breaks the gel. As shown Table 3, the force at break of CH1-4, CH2-4, CH3-4 and CH4-4 was around 0.16 N, 0.38 N, 0.68 N and 0.65 N, respectively. Since all the hydrogels reached the same volume (~7.5 mL) and 100% CNF recovery yield after 4 h hydrothermal treatment as shown in Table 1 and Fig. 1, the CNF content of CH3-4 and CH4-4 should be about 2-fold higher than that of CH1-4 and CH2-4. Evidently, the significantly higher mechanical strength obtained for CH3-4 and CH4-4 is resulted from their higher CNF content. Interestingly, when as-prepared hydrogels were chemically cross-linked by genipin (Fig. 2), the force at break, as shown in Table 3, decreased about 30% except for CH1–4 which prepared from lower CNF concentration. Evidently, genipin chemical cross-linking enhances the rigidity of the CH gels but not the resilience. The physically cross-linked CH obtained by elevating temperature is rather spongy and resilient as compared with the further chemically cross-linked CH.

Table 3
Force at break of chitin hydrogels prepared from 0.3%, w/v (CH1), 0.5%, w/v (CH2), 1%, w/v (CH3) and 2%, w/v (CH4) at 180 °C, for 4 h hydrothermal treatment before and after genipin cross-linking.

CNF concentration (%, w/v)	Force at break (N)	
	Before cross-linking	After cross-linking
0.3	0.162 ± 0.01	0.156 ± 0.01
0.5	0.387 ± 0.01	0.250 ± 0.06
1	0.677 ± 0.03	0.529 ± 0.05
2	0.652 ± 0.02	0.446 ± 0.06

4. Conclusions

The chitin hydrogels with a high water-holding capacity based on β-chitin nanofibrils were obtained by direct hydrothermal treating the chitin nanofibrils suspension at 180 °C for 4 h. The hydrophobic interactions induced at elevated hydrothermal temperature transformed the well dispersed CNF suspension into a resilient 3-D hydrogels. The mechanical strength of the as-prepared hydrogels can be greatly enhanced by increasing CNF concentration to 1% (w/v). Since chitin is a biodegradable and cytocompatible biopolymer, the chitin hydrogels molded into many shapes and prepared directly by elevating hydrothermal temperature without employing any chemical cross-linkers should have very potential biomedical applications such as for the wound dressing and scaffolds in tissue engineering.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.07.022.

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