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Improving the mechanical and thermal properties of gelatin hydrogels cross-linked by cellulose nanowhiskers

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ABSTRACT

This study demonstrates the preparation of a renewable and biocompatible hydrogel with superior mechanical properties consisting of a gelatin matrix cross-linked with oxidized cellulose nanowhiskers. We found an increased degree of chemical cross-linking (0.14–17%) between gelatin and nanowhiskers with the increased amount of aldehyde contents (0.062–0.230 mmol g^{-1}). ^1H nuclear magnetic resonance (NMR) T_2 relaxation experiments on D_2O swollen hydrogels demonstrated systems consisting of both gelatin and cellulose nanowhiskers displayed a higher percentage of "ridge" protons, attributed in part to increasing chemical cross-linking junction points between gelatin and nanowhiskers. This increase in hydrogel rigidity not only modified local chain dynamics but also influenced gel swelling, showing relatively reduced water uptake ability than that of the neat gelatin. Rheological measurements confirmed a 150% improvement in storage modulus (G') of the cross-linked hydrogels compared to neat gelatin. Chemical cross-linking also increased the resistance of the gels towards thermal degradation above the melting temperature of gelatin as observed by thermal scanning experiments.

1. Introduction

Hydrogels represent an interesting class of polymer networks, which have received a substantial amount of interest in pharmaceutical and biomedical applications including contact lens materials, artificial tendons, matrices for tissue engineering and drug delivery systems. Development of hydrogels is a rapidly growing research arena and a number of synthetic, as well as, naturally derived materials have been studied and reported in the literature to form well-characterized hydrogels (Jagur-Grodzinski, 2010; Van Vlierberghe, Dubruel, & Schacht, 2011). Among those, gelatin is a widely used material to form hydrogels for numerous biomedical applications such as wound dressing, plasma expander, adhesive and adsorbent material, vascular prostheses and in drug delivery as hard or soft capsules, hydrogels, or microspheres due to its high water content capacity, biocompatibility, biodegradability and non-immunogenicity (Chen, Leu, Fang, Chen, & Fang, 2011; Dragusin et al., 2011; Huang & Fu, 2010; Silva, Mano, & Reis, 2010).

Gelatin is a protein obtained by de-naturing the triple-helix structure of collagen into single strain molecules. Upon cooling the aqueous solution of gelatin below 35 °C, it forms physical thermo-reversible gels due to partial recovery of collagen triple-helix structure by disorder–order rearrangement (Bode, da Silva, Drake, Ross-Murphy, & Dreiss, 2011; Liao, Zhang, & Chen, 2009; Pena, de la Caba, Eceiza, Ruseckaite, & Mondragon, 2010). However, the application of gelatin is typically limited at higher temperature (above 35 °C), where breaking of the secondary bonding structure destroys the physical network. This leads to poor thermal and mechanical properties, and has thus far limited gelatin, a readily available and relative inexpensive material from further application. Therefore, chemical cross-links between the protein chains of gelatin are used to stabilize these gels, often refer to a chemical gelatin gels (Draye, Delaey, Van de Voorde, Van Den Bulcke, Bogdanov, et al., 1998). Various cross-linking agents including carbodiimide (Kuijpers et al., 1999), formaldehyde (de Carvalho & Grosso, 2004), glutaraldehyde (Leo, Vandelli, Cameroni, & Forni, 1997; Liu, De Yao, Wang, & Li, 2000), oxidized polysaccharides such as dextran (Draye, Delaey, Van de Voorde, Van Den Bulcke, Bogdanov, et al., 1998; Draye, Delaey, Van de Voorde, Van Den Bulcke, De Reu, et al., 1998) chondroitin sulfate (Dawlee, Sugandhi, Balakrishnan, Labarre, & Jayakrishnan, 2005; Kuijpers et al., 2000), and starch (Mu et al., 2010) have been used to chemically cross-link gelatin chains in order to improve the gel stability, increasing the resistance to thermal degradation and improving mechanical properties.

Recently, rod-like polysaccharide nanoparticles known as cellulose nanowhiskers, have gained considerable interest as a promising biomaterial due to their outstanding properties such as high surface area, high mechanical property, hydrophilicity, biocompatibility, and biodegradability (Eichhorn, 2011; Eichhorn et al., 2010). Cellulose nanowhiskers are usually obtained by the

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controlled acid hydrolysis of native cellulose fibers, where the size and mechanical characteristics of the resulting nanowhiskers depend on the source and hydrolysis conditions of cellulose fibers. The typical dimension of wood-based nanowhiskers is 5–10 nm in width and 100–300 nm in length (Habibi, Lucia, & Rojas, 2010; Peng, Dhar, Liu, & Tam, 2011; Siqueira, Bras, & Dufresne, 2009). Because of their unique characteristics, cellulose nanowhiskers have been incorporated as fillers in several polymeric hydrogel matrices. For instance, supramolecular hydrogels based on cyclodextrin/polymer inclusion was prepared by Zhang et al. (2010) and it was shown that incorporation of cellulose nanowhiskers into these hydrogels enhance gelation, mechanical strength and facilitates sustained release of drugs. Goetz, Mathew, Oksman, Gatenholm, and Ragauskas (2009) synthesized hydrogels by co-cross-linking a poly(methyl vinyl ether co maleic acid) (PMVEMA)–polyethylene glycol (PEG) matrix with cellulose nanowhiskers, again resulting in a significant improvement in mechanical and swelling properties. Other examples of composite hydrogels include CNW-reinforced with various polymer matrices such as agarose (Osorio-Madrazo et al., 2012), regenerated cellulose (Wang & Chen, 2011), poly(acrylamide-co-acrylate) (Spagnol, Rodrigues, Neto, et al., 2012; Spagnol, Rodrigues, Pereira, et al., 2012), hemicellulose (Karaaslan, Tshabalala, Yelle, & Buschle-Diller, 2011) and chitosan-graft-poly(acrylic acid) copolymer (Spagnol, Rodrigues, Pereira, et al., 2012). Nevertheless, there has been no attempt so far on the employment of cellulose nanowhisker as a renewable, non-toxic and inexpensive cross-linker in gelatin matrix to enhance the material's hydrogel properties.

In presence of periodic acid, cellulose nanowhiskers undergo oxidative cleavage at the C_2—C_3 glycol bond resulting in dialdehyde groups at the respective carbon atoms. These aldehyde groups could act as a potential cross-linker since they will react with free amine groups of gelatin through Schiff's base formation. In this study, we present chemical cross-linking of gelatin by periodate oxidized nanowhiskers containing different amounts of aldehyde groups. To investigate the effect of cross-linking on the gel properties, physicochemical, thermal and mechanical properties of the formulated hydrogels were studied using a variety of advanced techniques. We observed a significant improvement in mechanical and thermal properties of the cross-linked gels compared to neat gelatin gels, which could broaden the use of gelatin hydrogels in various biomedical applications.

2. Materials

A fully bleached commercial softwood Kraft pulp was used as a source for cellulose nanowhiskers. Gelatin (type A, Bloom 300) and all chemicals and solvents were purchased from VWR International.

3. Experimental

3.1. Preparation of cellulose nanowhiskers

Cellulose nanowhiskers were prepared by sulfuric acid hydrolysis of a bleached softwood pulp based on a literature procedure (Bondeson, Mathew, & Oksman, 2006). In brief, 60.00 g (oven dried weight) of the pulp was mixed with H_2SO_4 solution (64%, w/w, 1:10 g/mL) with continuous stirring at 45 °C for 45 min. The hydrolysis reaction was stopped by adding excess (10-fold) of distilled water followed by the removal of acidic solution by successive centrifugation at 12,000 rpm for 10 min until the supernatant became turbid. The sediment was collected and dialyzed (MWCO: 12–14,000) against tap water until the solution pH became neutral. After dialysis, the content was sonicated for 10 min and centrifuged for 5 min at 10,000 rpm. The cloudy supernatant, containing nanowhiskers, was collected and the remaining sediment was again mixed with water, sonicated and centrifuged to obtain additional nanowhiskers; this procedure was repeated till the supernatant was clear. Cellulose nanowhiskers were obtained in 20–30% yield.

3.2. Sodium periodate oxidation of cellulose nanowhiskers

An aqueous mixture of cellulose nanowhiskers (100.00 mL, 1.74 wt.%, w/v) and sodium periodate (0.17 g, 0.79 mmol) was stirred for 2 days in absence of light at room temperature. The product was then placed into dialysis membranes (MWCO: 12–14,000) and dialyzed against DI water for 2 days to remove the spent oxidant, and then freeze-dried providing a gravimetric yield of 98%. The same procedure was then repeated using 2.44, 4.07 and 5.70 mmol of sodium periodate. These samples were named as DAC1, DAC2, DAC3 and DAC4, where DAC, dialdehyde cellulose and 1, 2, 3, and 4 corresponds to 0.1, 0.3, 0.5 and 0.7, weight ratio of sodium periodate to nanowhiskers, respectively.

3.3. Synthesis of cross-linked hydrogels

A solution was prepared by adding gelatin (4.708 g) to water at 40 °C with slow stirring and the solution was kept at this condition for 3–4 days to remove all entrained air-bubbles. The oxidized nanowhisker suspension (30 mL, 1.74 wt.%, 0.523 g) was degassed to get rid of any bubbles using a water aspirator. Then the nanowhisker suspension was warmed to 40 °C and slowly added to gelatin solution and mixed for 20 min under constant stirring. The mixed gel was poured onto a Teflon mold at room temperature and after drying for 24 h at room temperature the gels were stored at 4 °C for 10 days. The gels were punched into samples of 14 mm diameter and 2 mm thickness for further characterization. The same procedure was followed to prepare cross-linked gels with four different levels of oxidized nanowhiskers.

3.4. Determination of carbonyl groups by copper titration

The carbonyl group content of the starting and oxidized whiskers was determined following Tappi standard method T430 (Tappi Standard T430). Briefly, DAC nanowhiskers (1.00 g) were treated with an aqueous $CuSO_4$ solution (3.50 mL, 0.40 N) and a carbonate–bicarbonate solution (63.5 mL, 2.40 N, 1.04 N). The mixture was heated to 100.00 °C for 3 h, cooled, filtered, and washed with 5% aqueous Na_2CO_3 solution (70.00 mL, w/v) and hot water (150.00 mL). The whiskers along with the filter paper were dispersed in 5% phosphomolybdic acid (16.60 mL, w/v), stirred, filtered, and then washed thoroughly with water. The filtrate was diluted with deionized water (450.00 mL) followed by the titration with 0.05 N $KMnO_4$ to a faint pink end point. A blank test was also performed following the same procedure. For each sample, the experiment was repeated three times to obtain the copper number and the data were reported with an error of less than ±3.0%. The copper number and carbonyl group content was calculated by following the expressions (Rohrling et al., 2002).

$$\text{Copper number(Cu\#)} = \frac{6.36(V - B)N}{W}$$

$$\text{Carbonyl group content(mmol/100 g)} = \frac{\text{Cu\#} - 0.07}{0.6}$$

V is the volume of $KMnO_4$ solution to titrate the filtrate from the specimen, mL; B is the volume of $KMnO_4$ solution to titrate the blank filtrate, mL; N is the normality of $KMnO_4$, 0.05 N, W is the weight of the CNWs, g.

3.5. FT-IR spectroscopy

The starting whisker and oxidized samples were dried at 105 °C for 6 h and then cooled to room temperature for FT-IR analysis. The oven dried CNWs, periodate oxidized CNWs and freeze dried cross-linked gels were pressed into KBr pellets (1:200). Transmission mode FT-IR spectra were collected with a Nicolet Magna-IR™ 550 spectrometer. Spectra were obtained in 400–4000 cm^{-1} range and for each sample 64 scans were taken at a resolution of 4 cm^{-1}.

3.6. Determination of degree of cross-linking

The degree of cross-linking of the hydrogels was determined by Ninhydrin (2,2-dihydroxy-1,3-indanedione) assay (Wu et al., 2011). Lyophilized gels were ground to powder and to 5 mg of the samples in a test tube, 1.00 mL of Ninhydrin solution (1.5% in ethanol, w/v) were added followed by heating the mixture for 25 min at 80 °C. After cooling down to room temperature for 1 h, it was diluted with water and the optical absorbance was measured using PerkinElmer Lambda 35 UV–visible spectrometer at λ 570 nm against a blank solution without gels, which had been treated exactly the same way as the cross-linked gels. The experiment was repeated three times for each sample and the data were reported with an error of less than ±5.0%. Degree of cross-linking was determined by following the equation:

$$\text{Degree of cross-linking}(\%) = \left\{1 - \left(\frac{\text{Absorbance of cross-linked gel}}{\text{Absorbance of non-cross-linked gel}}\right)\right\} \times 100$$

3.7. Swelling properties

Swelling properties of the chemically cross-linked hydrogels were studied by incubating in water for 2 days at room temperature and then the gels were gently blotted and finally weighed. The dry weight of the gel was determined by drying the gel in vacuum oven at 50 °C till constant weight was obtained. The experiment was repeated three times for each sample and the data were reported with an error of less than ±2.0%. Then the equilibrium fluid content was calculated from the following equation:

$$\text{Equilibrium fluid content}(\%) = \left\{1 - \left(\frac{\text{weight of dry gel}}{\text{weight of swollen gel}}\right)\right\} \times 100$$

3.8. NMR experiments

^1H spin–spin (T_2) NMR experiments on samples swollen in 100% D_2O were performed on a Bruker DSX-400 spectrometer, operating at a frequency of 399.875 MHz for ^1H NMR in a Bruker double-resonance MAS probe head at spinning speed of 2 kHz at room temperature. A standard Carr–Purcell–Meiboom–Gill (CPMG) sequence with a $\tau = 500$ μs, utilized a 5 μs (90°) proton pulse, 10 s recycle delay and 128 scans. The CPMG experiment was collected 26 data points between 0.002 and 10 s. The resulting T_2 decay profiles were analyzed using a two-component Gaussian-exponential model in IgorPro® software.

3.9. Rheological study

Hydrogels were equilibrated in water at room temperature for 2 days before the rheological measurements were taken. The rheological experiments at oscillatory shear deformation of the gelatin hydrogels were carried out with AR2000 rheometer parallel plates of 14 mm diameter. The storage (elastic) G' and loss (viscous) G'' moduli were recorded at constant temperature (27 °C) and at shear strain of 0.05% in a range of frequency from 0.1 to 10 Hz (Draye, Delaey, Van de Voorde, Van Den Bulcke, Bogdanov, et al., 1998; Fonseca Silva, Habibi, Colodette, & Lucia, 2011). The temperature dependence of the storage modulus was determined by oscillatory shear deformation and temperature ramp of 27–50 °C with a heating rate of 1.5 °C/min at a constant frequency 1 Hz and constant shear rate of 0.05.

Table 1
Carbonyl content of oxidized cellulose nanowhiskers.

Samples	$NaIO_4$/CNWs (w/w)	Carbonyl content (mmol g^{-1})
CNWs	0.00	0.006
DAC1	0.10	0.060
DAC2	0.30	0.114
DAC3	0.50	0.150
DAC4	0.70	0.231

3.10. Scanning electron microscopy (SEM)

Swollen gels were quickly frozen using liquid nitrogen and freeze-dried for 3–4 days. Surfaces of all the samples were coated with gold in a Quorum Q150T ES sputter coater for 10 min. Surface morphology of lyophilized non-cross linked and cross-linked hydrogels was studied by Hitachi S-800 scanning electron microscope at 500× magnifications.

4. Results and discussion

Cellulose nanowhiskers were oxidized in presence of sodium periodate to yield the corresponding C_2/C_3 dialdehyde product and the carbonyl content was quantified by Cu^{2+} titration (Table 1). The increase in sodium periodate equivalent for the oxidative treatment of cellulose nanowhiskers resulted in a corresponding increase in carbonyl content, which was evident in the transmission mode FT-IR spectra, as shown in Fig. 1(A). The characteristic C=O stretching vibration band of the dialdehyde cellulose nanowhisker appeared at 1740 cm^{-1} and this spectral data confirms a gradual oxidation of nanowhiskers as the intensity of the carbonyl band increases with increased oxidant.

Aqueous solutions of gelatin were then cross-linked with oxidized cellulose nanowhiskers containing varying amount of aldehydes to obtain the chemical hydrogels. It was observed that the hydrogels composed of only gelatin are transparent, whereas the cross-linked hydrogels are slightly yellowish in color and semi-transparent. As shown in FT-IR spectra (Fig. 1(B)), the presence of cellulose nanowhisker in the cross-linked gel can be evidenced from the appearance of nanowhisker bands in the region 3300–3500 cm^{-1} and 1000–1200 cm^{-1}. The cross-linking reaction occurs between the aldehyde group in nanowhiskers and the free ε-amino groups of the lysine and hydroxylysine residues present in gelatin as shown in Fig. 1(C). The coupling was favored by the disappearance of the carbonyl band of oxidized nanowhisker indicating the occurrence of chemical interaction between nanowhisker and gelatin.

In order to determine the reaction efficiency between gelatin and dialdehyde cellulose nanowhiskers, the amount of the unreacted free amines in the chemically cross-linked samples was measured by spectrophotometrically following a Ninhydrin assay with the function of oxidized nanowhisker. The degree of cross-linking was calculated by comparing the absorbance of cross-linked samples with the control sample, i.e. gelatin gels in the absence of

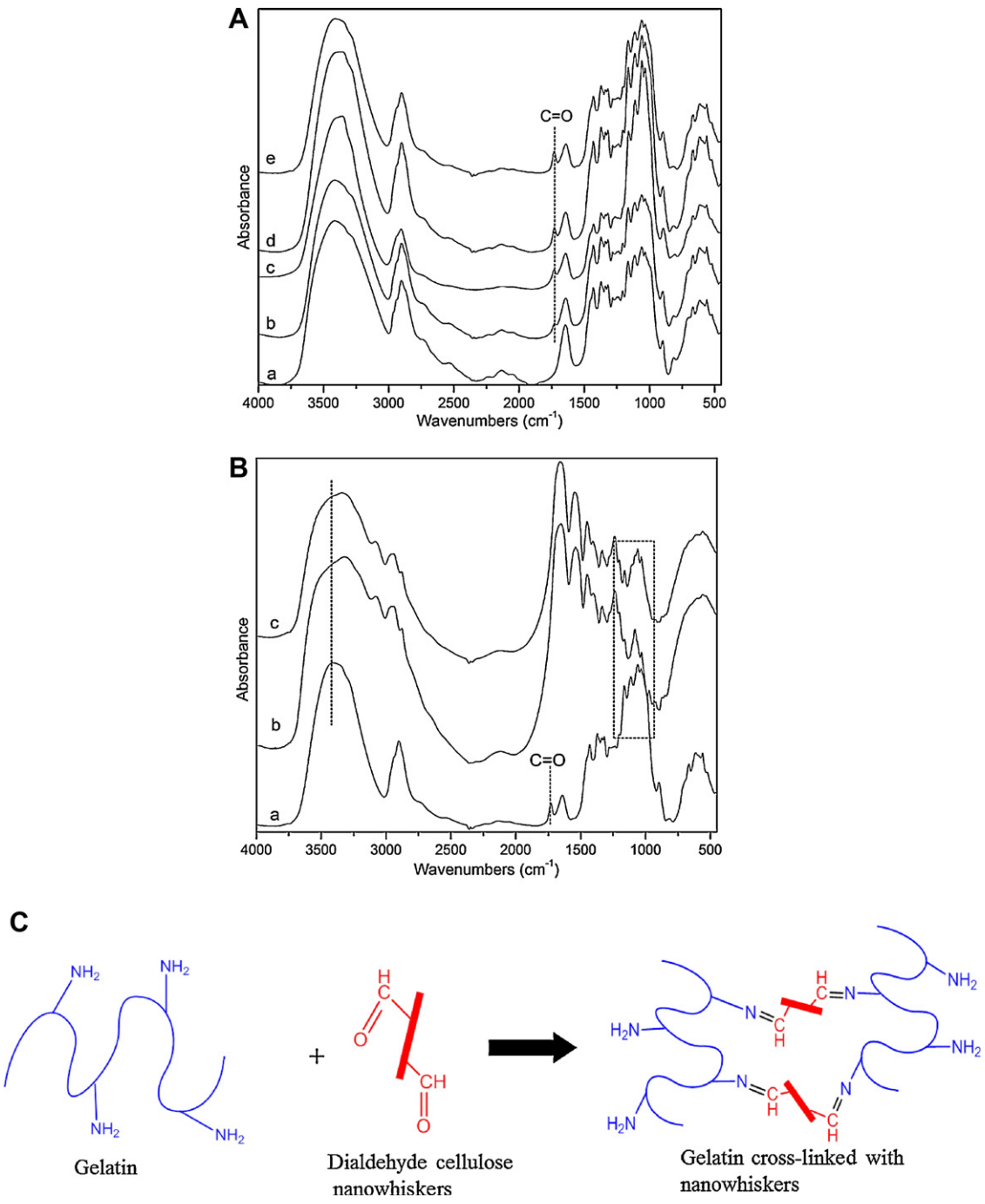

Fig. 1. (A) FT-IR spectra of (a) nanowhiskers, (b) DAC1, (c) DAC2, (d) DAC3 and (e) DAC4. (B) FT-IR spectra of (a) DAC4, (b) gelatin and (c) cross-linked hydrogels. (C) Schematic representation of cross-linked hydrogels.

oxidized nanowhiskers. The degree of cross-linking was found to increase from 0.14 to 17% (Fig. 2) as a function of the oxidation level due to the presence of an increased amount of aldehyde groups to chemically react with the available free amines.

^{1}H spin–spin relaxation (T_2) NMR experiments were carried out to analyze the effect of cross-linking on molecular mobility in an effort to further characterize gel properties. Since T_2 relaxation decay intensity is sensitive to local chain dynamics in the polymer/gel matrix, the T_2 relaxation behavior can be used to describe molecular motion, determining the relative amounts of rigid and mobile components within the hydrogel network before and after cross-linking (Goetz, Foston, Mathew, Oksman, & Ragauskas, 2010). In general, the faster the rate of T_2 relaxation the more rigid or fewer degrees of freedom the chemical group associated with that chemical shift has. In this experiment, T_2 data were collected using a Carr–Purcell–Meiboom–Gill (CPMG) sequence, which utilizes the application of a train of 180° pulses subsequent to the initial 90° excitation pulse, designed to eliminate chemical shift, *J*-coupling and magnetic field inhomogeneity effects on T_2 relaxation (Duer, 2002). Since typical Gaussian relaxation behavior is associated with rigid polymer systems and exponential behavior with more mobile structures, the T_2 relaxation behavior was analyzed using the

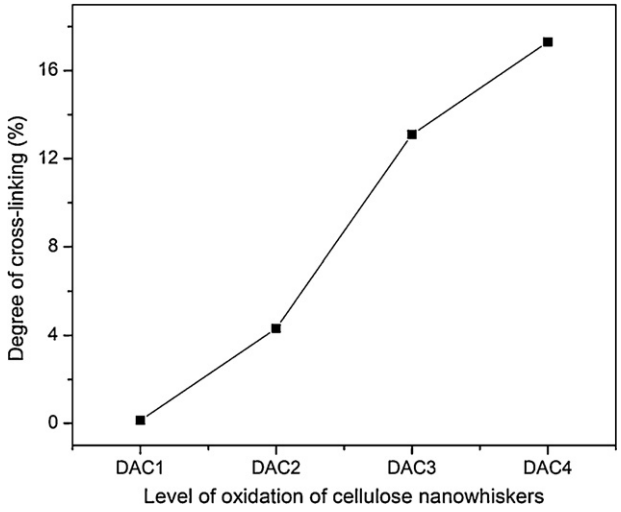

Fig. 2. Degree of cross-linking of hydrogels as a function of oxidized nanowhiskers.

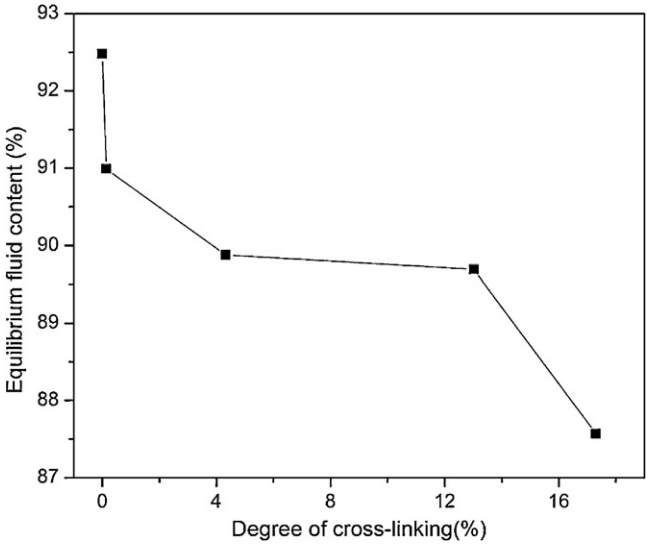

Fig. 3. Equilibrium fluid content of cross-linked hydrogels.

two-component Gaussian-exponential model shown below (Eq. (1)) (Leisen, Beckham, & Sharaf, 2004).

$$I(t) = \%\text{Ridge} \times \exp\left(\left[\frac{-t}{T_{2,\text{Rigid}}}\right]^2\right) + \%\text{Mobile} \times \exp\left(\frac{-t}{T_{2,\text{Mobile}}}\right) + \text{Offset} \quad (1)$$

In utilizing this two component model, the Gaussian component should indicate the relative proportion of protons belonging to rigid repeat units in cross-linking junction points, while the exponential component should describe the amount of more mobile repeat units belonging to chains outside or between junctions of cross-linking points.

The ^{1}H MAS NMR spectra obtained for the cross-linked hydrogels composed of gelatin and dialdehyde cellulose nanowhiskers swollen in D_2O and spun at 2 kHz, showed a series of fairly resolved peaks between ~4.75 and 0.8 ppm. The appearance of the pure gelatin is identical to that of the hydrogel with dialdehyde cellulose nanowhiskers (see Supplemental Fig. 1), which was expected based on a rough calculation showing ~90% of the total protons in the gelatin/cellulose nanowhisker system belong to gelatin. In addition, the spectrum of pure cellulose nanowhiskers displays a broad peak, due to crystalline nature of the cellulose and extremely fast T_2 relaxation, centered at ~3.50 ppm (Goetz et al., 2010) (not shown), manifesting as a minor background feature of the hydrogel spectrum.

Table 2 represents the resulting percent rigid and mobile proton in the cross-linked hydrogels based on the ^{1}H spectral integrations from ~4.75 to 0.8 ppm and defined by non-linear least squared fit of the T_2 relaxation model in Eq. (1) (see Supplemental Fig. 2). The hydrogels composed of both gelatin and cellulose nanowhisker in general displays a relatively higher chain rigidity, greater relative number of cross-linking junction points and a corresponding lower proportion of mobile protons. The consistent ~30–35% increase in rigid proton content is greater than the ~10% proton due to the addition of cellulose nanowhiskers which indicates the existence of chemical linkages between the gelatin matrix and nanowhisker and demonstrates this cross-linking affects the local molecular mobility of the chains. An average T_2 value of 4.4 ms and 21.0 ms was calculated for the rigid and mobile components respectively for all the hydrogels.

Table 2
Relative rigid and mobile component intensities based on a Gaussian-exponential model of the hydrogels determined by ^{1}H spin–spin relaxation (T_2) NMR experiments.

Degree of chemical cross-linking (%)	% rigid	% mobile
0	35	65
0.14	44	56
4.32	42	58
13.02	50	50
17.30	50	50

The swelling properties of the hydrogels prepared from the interaction of dialdehyde cellulose nanowhisker and gelatin were evaluated from their water uptake value. It was found that all the gels attained the equilibrium swelling in 2 days. As shown in Fig. 3, the equilibrium water content was decreased with the increase in the level of oxidation of cellulose nanowhiskers, which is attributed to the increased degree of cross-linking. Similar behavior was also observed for gelatin gels cross-linked with glutaraldehyde and plant polyphenols, periodated alginate (Liao et al., 2009; Lou & Chirila, 1999; Strauss & Gibson, 2004). The swelling behavior was further explained as a result of the formation of rigid network after cross-linking, observed by NMR experiments, which consequently has less ability to uptake water. Equilibrium fluid content was calculated to be decreased from 92.50 to 87.60% with the increase in degree of cross-linking. The small difference in the water uptake value could be due to the presence of characteristic gelatin network resulting from gelatin–gelatin physical structuring in the cross-linked gels.

Hydrogel formation of gelatin below its melting temperature primarily involves the gelatin–gelatin physical interaction as a result of structuring of helical configuration. However, hydrogel formation between gelatin and dialdehyde cellulose nanowhisker involves two different interactions; one is associated with the chemical cross-links between gelatin and oxidized nanowhisker and the second one is due to the formation of physical cross-links between the gelatin chains. Viscoelastic properties of the hydrogels were measured from the mechanical response of the samples as they are deformed under periodic strain. The elastic modulus G' and the viscous (also imaginary or loss) modulus G'' of the physically cross-linked gels at room temperature are presented in Fig. 4(A). It was found that G'' component is considerably smaller than G' component, exhibiting the formation of an elastic network. However, there is a significant increase in storage modulus of chemically cross-linked hydrogels compared to the physical gel and the increase in degree of cross-linking led to an increase in

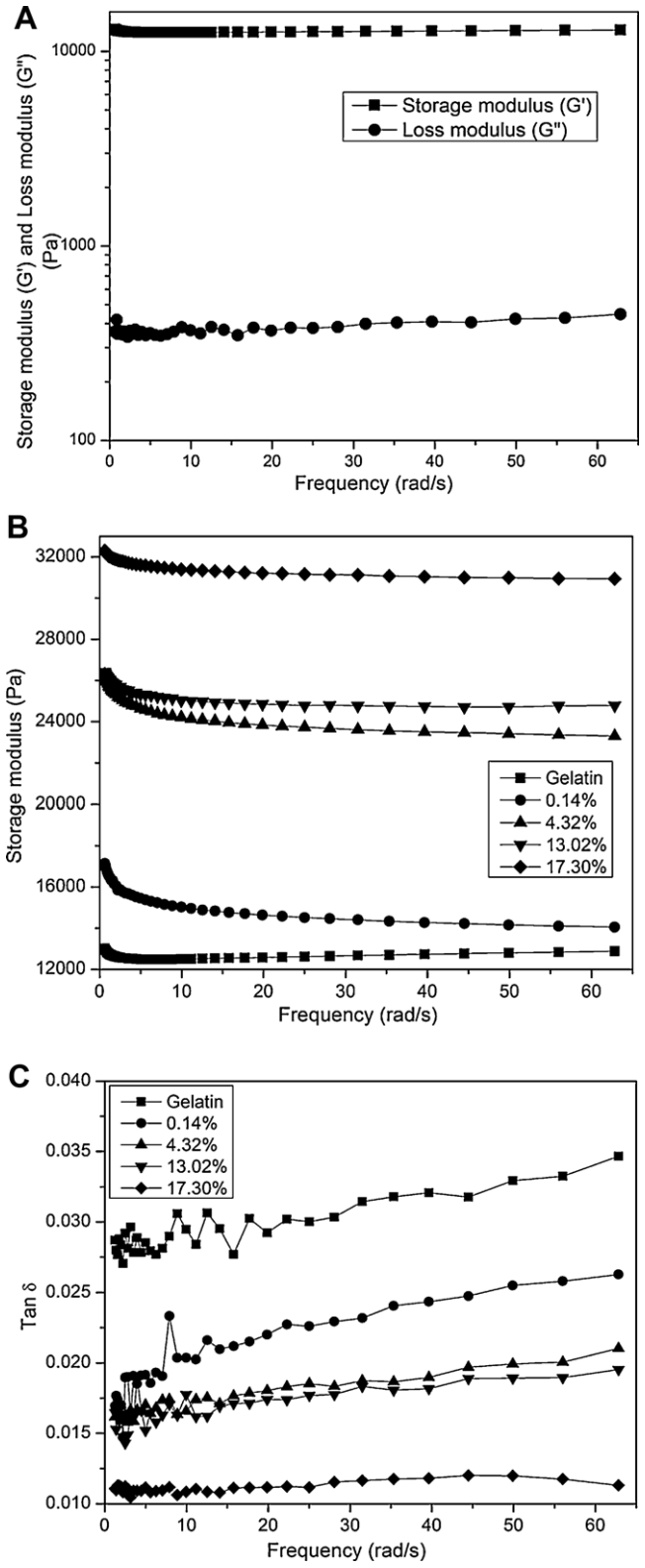

Fig. 4. (A) Dynamic rheological observations of the gelatin gels. (B) Effect of chemical cross-linking on the storage modulus of the gelatin gels. (C) Tan δ value of the hydrogels.

storage modulus of the cross-linked gels as shown in Fig. 4(B), which is also reflected in tan δ plot Fig. 4(C). This is mainly attributed to the influence of chemical interaction between gelatin and dialdehyde nanowhiskers. Schacht et al. prepared hydrogels from gelatin and dextran dialdehyde (1:1 w/w) and the degree of

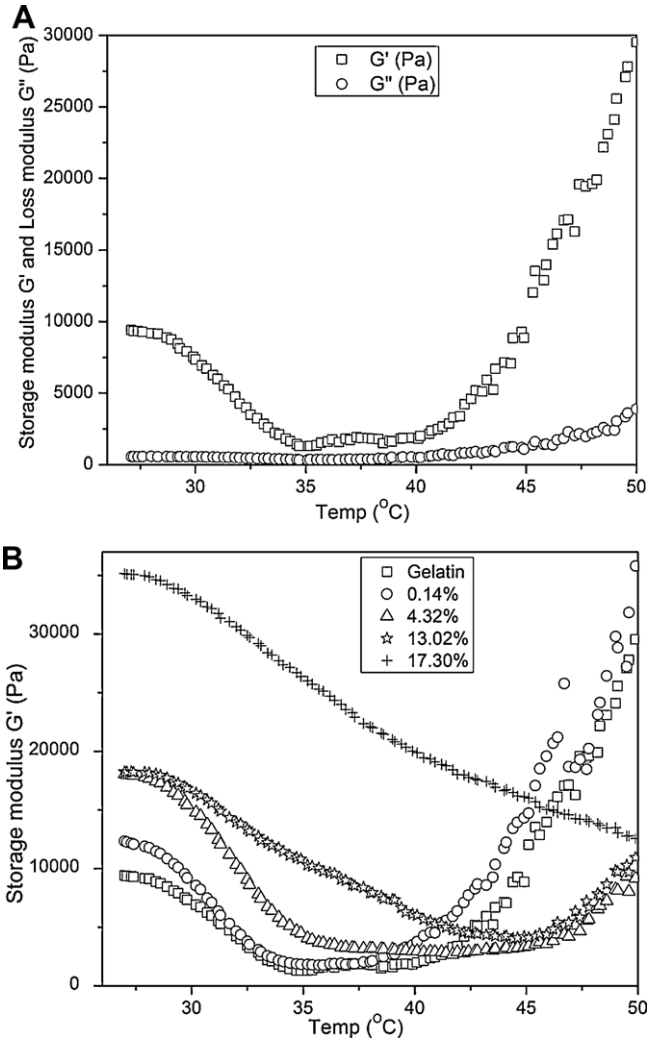

Fig. 5. (A) Effect of temperature on dynamic rheological behavior of the physically cross-linked gelatin gels. (B) Effect of temperature on storage modulus of chemically cross-linked gelatin gels.

oxidation of dextran was varied from 5 to 20% (Schacht, Bogdanov, VandenBulcke, & DeRooze, 1997). They had shown that when the degree of oxidation was below 20%, the storage modulus of cross-linked gels were lower than the gelatin hydrogels. In case of cellulose nanowhiskers, we found a large increase (150%) in storage modulus of the gels for ~4% degree of oxidation.

Thermal scanning experiments were performed from 27 to 50 °C in order to study and differentiate the contribution of physical and chemical cross-linking below and above the melting point. As shown in Fig. 5(A), the storage modulus of gelatin gels decreases rapidly to very low values until the melting point (35 °C) indicating the breaking of the physical linkages in gelatin. However, above the melting point there is a rapid increase in G', which could be explained due to the melting of gelatin, subsequently affecting the area between the plates of the rheometer and this leads to a sharp increase in G' to above its initial value. At the same time the viscous or loss modulus (G'') clearly increases with temperature, suggesting that the gel becomes more liquid like due to melting of gelatin.

Fig. 5(B) shows the effect of temperature on storage modulus of chemically cross-linked gels with the degree of cross-linking compared to gelatin gels. The initial decrease in G' value before the melting point is possibly due to the breaking of the physical attachments. It can be observed that there is a gradual improvement in the melting behavior and G' of the hydrogels with the degree of

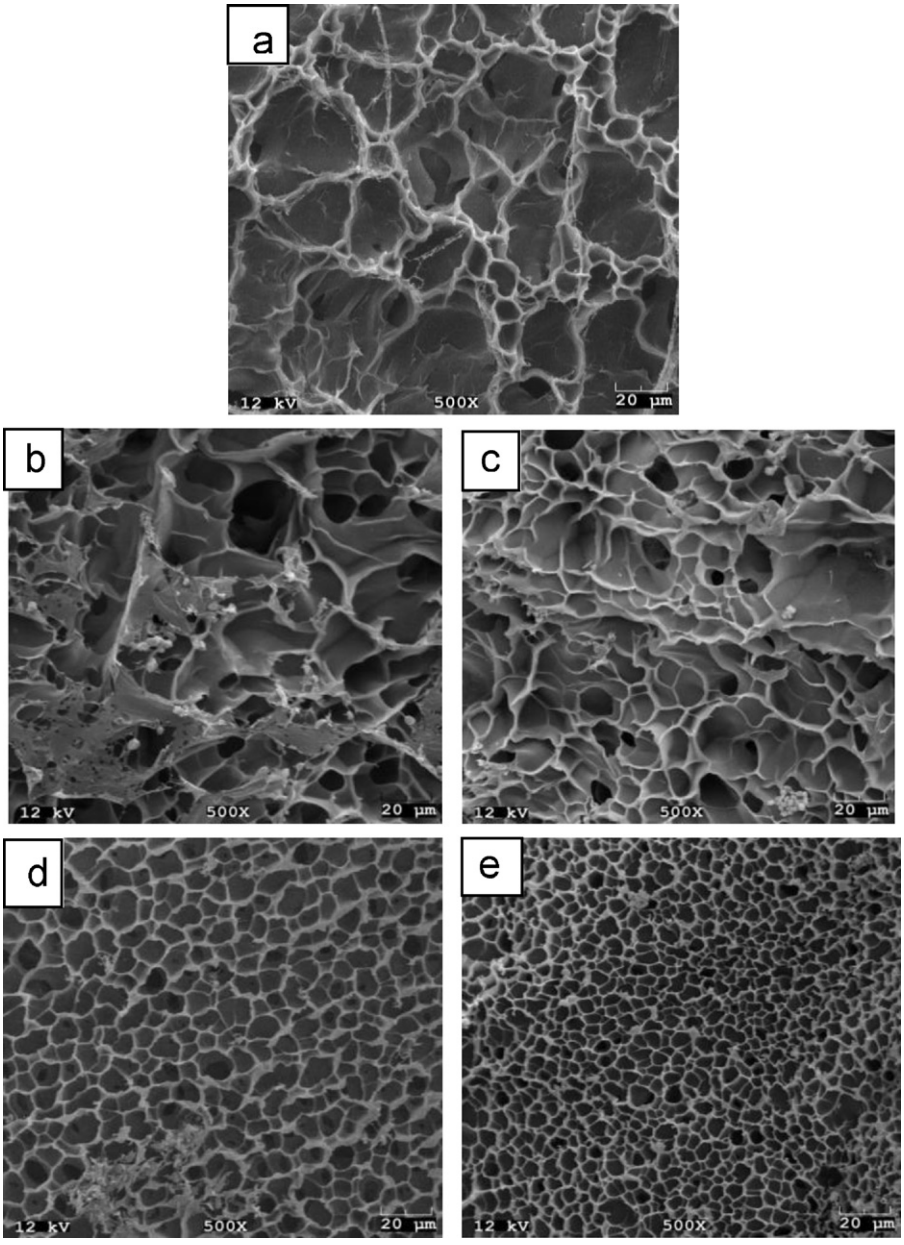

Fig. 6. Cross-sectional morphologies of (a) gelatin and (b) 0.14%, (c) 4.32%, (d) 13.02%, and (e) 17.30% cross-linked gels (scale bar 20 μm).

cross-linking. For lower degree of cross-linking (0.14%) samples exhibited similar thermal behavior as gels prepared from gelatin, whereas for 4 and 13% cross-linked samples, the elastic modulus value stays higher than the gelatin samples even above the melting point of gelatin and they became stable until 45 °C, suggesting a significant contribution from chemical cross-linking. Samples with 17% degree of cross-linking were found to be stable until 50 °C with a high storage modulus due to the presence of more chemical bonds resulting from higher degree of cross-linking. The higher G' value of chemical gels at below and above the melting temperature of gelatin indicates the existence of a stable elastic network at higher temperature because of the chemical cross-linking between gelatin and oxidized CNWs.

The changes in morphology of gelatin hydrogels cross-linked by oxidized nanowhiskers were investigated through SEM images. In order to preserve better morphology, the swollen hydrogels were quickly frozen in liquid nitrogen and then freeze dried as reported in the literature and this technique has been found to be very useful to reveal the interior morphology of the swollen hydrogels with minimal artifact (Liao et al., 2009; Su, Chen, & Lin, 2010). Fig. 6 shows the images of the transverse cross-sectional surfaces of the gels with different degree of cross-linking and pore structures are observed to be different depending on the degree of cross-linking. A relatively open network structures with interconnected pores of various size were observed for gelatin samples. However, cross-linking affected the openness of the network structure as the pore size of the cross-linked gels became smaller and more regular in shape than the control samples. For 17% cross-linking, the interior appeared to be more compact with the smallest pore size. The gradual decrease in pore size and increase in compactness of the cross-linked gels is attributed to the effect of increased degree of cross-linking with additional support from our NMR experiments illustrating the formation of rigid structure after cross-linking. Similar findings were reported by Kim and Chu (2000) and Liao et al. (2009) that the cross-linking offers more intermolecular association forming more number of junction points, which in turn affects the pore structure, size and its distribution.

5. Conclusions

This work reports the first successful study on the synthesis and characterization of gelatin hydrogels chemically cross-linked by dialdehyde cellulose nanowhiskers containing varying amounts of aldehyde groups. The increase in aldehyde groups resulted in an increase in degree of cross-linking leading to the formation of a rigid dense network, observed by T_2 NMR experiments, which reduced the water uptake ability of the hydrogels. Further, the increase in degree of cross-linking improved the mechanical properties of hydrogels by 150% and increased the thermal stability of the gels, as the gels did not degrade until 50 °C. These findings on this work would broaden the biomedical applications of the chemically cross-linked gelatin hydrogels in wound dressing, tissue engineering and sustained release applications.

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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.2012.08.080.

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