Effects of oxidized cellulose nanocrystals on the structure and mechanical properties of regenerated collagen fibers

Changkun Ding
  Tiangong University   https://orcid.org/0000-0002-3127-7841
Chengfei Yue (✉ chengfei.yue@foxmail.com)
  Tiangong University   https://orcid.org/0000-0003-3870-2799
Jieliang Su
  Tiangong University
Hua Wang
  Tiangong University
Ning Yang
  Tiangong University
Bowen Cheng
  Tiangong University

Abstract

The mechanical properties of regenerated collagen composite materials are of significant importance for promoting its diverse applications. However, the efficient strategy reported so far for enhancing the mechanical properties of regenerated collagen fibers remains limited. Herein, the oxidized cellulose nanocrystals (oCNCs) were successfully prepared in aqueous media by oxidation of the hydroxyl groups on the surface of cellulose nanocrystals (CNCs) using ammonium persulfate (APS) as an oxidant, and collagen composite fibers were prepared by introducing oCNCs into the collagen matrix via the dry-jet wet spinning technique. The morphology, structures and properties of prepared collagen composite fibers were carefully investigated. These results indicated that oCNCs and CNCs can induce collagen microfibril orientation during the formation process of fibers and protect the possibility of their interactions with collagen molecules. Compared with CNCs, oCNCs offered higher levels of enhancing efficiency and the as-prepared collagen/oCNCs composite fibers exhibited extraordinary improvements in both the tensile strength and toughness with only a 0.05 wt% oCNCs loading. The reason for this improvement is that the hydroxyl groups and carboxyl groups of oCNCs can form stronger hydrogen bonds and electrostatic interactions with collagen molecules, resulting in the synergistic enhancement of the tensile strength, toughness and thermal stability of collagen composite fibers. This work provides an efficient and facile method to achieve collagen composite fibers with high mechanical properties for their broad applications.

1. Introduction

Collagen is a natural polymer that constitutes the main extracellular matrix (ECM) protein in various tissues, e.g. the skin, tendon, ligament, cartilage and bone (Gordon and Hahn 2010; Sorushanova et al. 2019). Collagen molecules contain at least one triple helix domain and can self-assemble into cross-striped fibrils in a quarter of a staggered arrangement in vivo to support cell growth and be responsible for the mechanical elasticity of connective tissue (Gordon and Hahn 2010; Ricard-Blum 2011). Up to now, the type of collagen has expanded to 29, and the most common of which is type I collagen (Orgel et al. 2001; Sorushanova et al. 2019). Amino acids are amphiphilic molecules, and collagen composed of amino acids is also amphoteric (Yue et al. 2021c). In its molecules, the dissociable groups mainly come from the side chains, such as amino ($-NH_2$) groups and carboxyl (-COOH) groups at both ends of the peptide chains (Sherman et al. 2015). Therefore, collagen is positively charged by its dissociation under acidic conditions. The desirability of collagen as a biomaterial principally depends on the fact that it is a naturally abundant extracellular matrix component, and it is also perceived as an endogenous constituent of the body and not as a foreign matter (Sherman et al. 2015). On the other hand, the appeal of collagen composite materials for biomedical applications stems from the fact that many proteolytic enzymes capable of degrading commonly used collagen composite materials are already present in the body (DeFrates et al. 2018). Thus, due to its excellent biocompatibility and biodegradability, collagen has increasingly been used as a promising biomaterial in tissue engineering and nanomedicine.

However, natural collagen also suffers from some defects, such as poor mechanical properties and thermal stability, and it is difficult to meet the application requirements without modification. The mechanical properties of collagen materials can be easily modified by traditional physical or chemical crosslinking, but this has been shown to affect their biocompatibility (Luo et al. 2018). Since traditional crosslinking methods always cannot satisfy the requirements of practical applications, other biological materials have been explored to be integrated into collagen and fabricate composite materials. With the development of nanoscience, in attempts to better improve the mechanical properties of collagen to expand the range of possible applications, various types of functional nanofillers (e.g., carbon nanotubes, hydroxyapatite) have been incorporated into collagen to pursue nanocomposites with better mechanical properties and thermal stability (Cheng et al. 2017; Grishkewich et al. 2017; Klemm et al. 2011; Naduparambath et al. 2018; Pracella et al. 2014; Prathapan et al. 2020). Green et al. demonstrated the utility of carbon nanotubes for the fabrication of collagen composite fibers through a gel-spinning approach to study whether or not rigid nano-inclusions could affect and facilitate fibril alignment by acting as a template (Green et al. 2017). They found that owing to their structural similarity and the rigid features of carbon nanotubes, these fillers can facilitate the self-assembly as well as the alignment of collagen fibrils in the process of fiber fabrication. Choi et al. used a new self-assembly-induced gelation method to synthesize collagen/hydroxyapatite composite porous microspheres featuring a nanofibrous structure (Choi et al. 2017). It is demonstrated that collagen nanofibers with a uniform size of several tens of nanometers were created in the whole composite microspheres regardless of the hydroxyapatite content. Nonetheless, the improved performance of composite materials with the incorporation of nanomaterials works at the expense of the high cost of raw materials, for instance, graphene oxide and carbon nanotubes, as well as a complicated preparation process (Cheng et al. 2017). Accordingly, a simple, low-cost method for the development of functional nanofillers with excellent mechanical properties and satisfactory biological properties is desirable.

As the most abundant crystalline polysaccharide nanomaterial on Earth, intensive studies have been devoted to nanocellulose, and especially to its specific form which has been revealed to be an interesting model filler in various polymer matrices (Heise et al. 2021). Generally, the term "nanocellulose" encompasses flexible, fiber-like cellulose nanofibrils (CNFs) and rigid, rod-shaped CNCs; dimensions and morphologies of nanocellulose typically vary as a function of their sources and extraction methods (Peng et al. 2020). Specifically, mechanical shearing techniques result in micron-long and flexible CNFs, and acid hydrolysis leads to nanometer-long and highly crystalline CNCs. So, the evident differences between CNCs and CNFs lie in their dimension and crystallinity (Grishkewich et al. 2017). Particularly, owing to their attractive crystallinity, chiral structure and self-assembly features, CNCs are often utilized to control and fabricate structured materials with highly ordered and aligned nanoparticles (De France et al. 2020; Thomas et al. 2018). However, CNCs tend to self-aggregate, and it remains challenging to obtain nanocomposites with high mechanical performance due to their interfacial compatibility with the polymer matrix for (Peng et al. 2020). So surface modification of CNCs is usually a necessity, particularly for hydrophobic matrices (Tortorella et al. 2020). Previous studies have shown that CNCs can be easily modified due to a large number of hydroxyl groups on the surface (Prathapan et al. 2020). The main

chemical modification classes of the reported CNCs are oxidation, esterification, urethane, silylation and amidation (Bespalova et al. 2017; Eyley and Thielemans 2014). These chemical modifications improve the dispersion, thermal stability, mechanical properties and biocompatibility of CNCs. Therefore, an in-depth study on the preparation method, surface modification and practical application of CNCs is of great significance for new green composite materials.

In the present work, to enhance the interactions between CNCs and collagen, and improve the reactivity of CNCs, we modified CNCs by one-step oxidation method with APS and characterized it in terms of morphologies and microstructures. Collagen composite fibers were prepared by dry-jet wet-spinning technique, and the effects of CNCs types on the self-assembly process, mechanical properties and thermal stability of collagen fibers were investigated. Thus, the objective of this work is to directly disperse CNCs and oCNCs in commercial collagen and use their good thermal stability and mechanical properties to improve the mechanical performance of collagen composite fibers without sacrificing any biological activity. This work not only broadens the application fields of modified CNCs but also provides a new insight for improving the properties of collagen fibers.

2. Experimental

2.1 Materials

CNCs were purchased from Huzhou Sciencek New Material Rice Technology Co., Ltd., China. Pure collagen (Bovine achilles tendon collagen, type I) was supplied by Tianjin Sannie Bioengineering Technology Co., Ltd., China. Except where otherwise noted, all other reagents were purchased from Tianjin Fengchuan Chemical Reagent Co., Ltd., China, and were of analytical grade and used without further purification.

2.2 Surface oxidation modification of CNCs

In this part, according to the previous study (Leung et al. 2011), we have used a one-step oxidation method for oxidation of CNCs (Scheme 1) that would carry significant benefits in terms of sustainability and low costs. Oxidation was performed in a three-necked round bottom flask equipped with a condenser. The CNCs (2 wt%) were pre-dispersed in deionized water by magnetic stirring and then treated with an ultrasonic cleaning bath (250 W) at 22 ~ 26 °C for 30 min. A 2.4 mol/L APS solution was added in CNC suspension with magnetic stirring for 30 min and transferred to the flask. The water bath was used to heat the blend solution to 70 °C for 30 min under mechanical stirring to obtain a white oCNC suspension. When the reaction was done, the suspension was centrifuged for 10 min, the supernatant was decanted, and then deionized water was added to the bottom colloid, followed by vigorous mixing for 5 min and repeated centrifugation. Repeat the centrifugation/washing cycle several times until the supernatant conductivity was ~ 5 $\mu S \cdot cm^{-1}$ (pH = 4), which is close to the conductivity of deionized water. Finally, the product was lyophilized to obtain oCNCs as a white powder.

2.3 Preparation of collagen composite fibers

The collagen/oCNCs composite fibers were prepared by using the dry-jet wet-spinning method (Scheme 2). First, a proper amount of oCNCs was pre-dispersed in water for 20 min by magnetic stirring and ultrasound treatment was performed at 22 ~ 26 °C for 1 h using an ultrasonic cleaning bath (250 W). Then, 0.9 mL of acetic acid was added to aqueous oCNC suspension (30 mL), and 0.45 g of lyophilized collagen was added to the acidic suspension and mechanical stirring was continued for 8 h. The collagen/oCNC solution was transferred to a refrigerated centrifuge and centrifuged for 40 min (9 000 rpm). Throughout the preparation and centrifugation, the solution temperature was maintained at 4 °C. The spinning solution was then incubated in the refrigerator for 12 h. The spinning solution was then transferred to the dry-jet wet-spinning apparatus (injection pump speed, 0.5 mL/min). The spinning jet traveled 25 cm inside the coagulation bath after crossing an air gap of 6 ~ 10 mm. The coagulation bath consisted of acetone, ammonium hydroxide, and deionized water in the volume ratio of 60:1:0.2, and the temperature of the coagulation bath was controlled at 25 ~ 35 °C. Finally, the gel state fibers produced in the coagulation bath were hung to dry at room temperature and drawn by applying a 5 g weight to align the collagen fibrils along the direction of the fiber axis. The preparation method for pure collagen fibers and collagen/CNCs composite fibers was the same. A series of collagen composite fibers with different oCNC or CNC contents were designated as F0, F1, F2, F3, F4 and F5 in Table 1.

Table 1
Parameters of collagen composite fibers.

Fiber code	Collagen/wt%	oCNCs/wt%	CNCs/wt%
F0	1.5	-	-
F1	1.5	0.01	-
F2	1.5	0.03	-
F3	1.5	0.05	-
F4	1.5	0.07	-
F5	1.5	-	0.05

2.4 Sample characterization

An H7650 transmission electron microscope (TEM) (Hitachi Company, Japan) was used to observe the morphologies of CNCs and oCNCs with an accelerating voltage of 100 kV. TEM samples were prepared by putting the CNC and oCNC aqueous suspensions on copper grids and then excess water was removed by placing a piece of filter paper at the edge of the grid. Prior to observation, samples were dried in a DZF-6050 vacuum drying chamber (Yuhua Instrument Co., Ltd., Gongyi, China) at 60 °C for 6 h.

AFM (Bruker Icon, Bruker Company, Germany) was used to observe the morphologies of CNCs and oCNCs in smart mode. The suspension was dropped on freshly cleaved mica and dried at room temperature to obtain samples for the AFM measurements.

A D/MAX-2550 type X-ray diffractometer (XRD) (Rigaku Corporation, Japan) was used to determine the crystalline structures of CNCs, oCNCs and fibers. Cu/Kα radiation with a wavelength of 0.15418 nm at 40 kV was used. Data were collected from 2θ of 5 to 40°. The XRD data were analyzed by area integration method using the analysis software Jade.6 to calculate the crystallinity of the sample. The crystallinity of the sample is the ratio of the area of the crystallization area to the total area of the XRD pattern.

A Tensor 37 Fourier-transform infrared (FT-IR) spectrometer (Bruker Company, Germany) was used to determine the chemical structures of CNCs, oCNCs and fibers. All spectra were recorded in ATR mode from 400 to 4000 cm^{-1} at a resolution of 2 cm^{-1} at room temperature.

The zeta potential was tested by using a zeta potential analyzer (Malvern Panalytical Company, UK). 200 µg/mL collagen solution was prepared using a 0.5 mol/L acetic acid solution. The collagen solution was stirred with different concentrations of CNCs and oCNCs at 4°C for 30 min. To avoid precipitation, the measurements were carried out immediately when the aforementioned blend solutions were obtained. Three measurements were applied to get an average value.

The fiber morphology was observed by an S-4800 field-emission scanning electron microscope (SEM) (Hitachi Company, Japan) and an accelerating voltage of 10 kV was used. Before observation, the samples were dried and then sputter coated with gold to avoid charging during observation.

A LLY-06 electronic fiber strength tester (Electronic Instrument Co., Ltd., Laizhou, China) was applied to measure the mechanical properties of the fibers. The tensile speed was 10 mm/min, and the clamping distance was 10 mm with a pre-tension of 0.2 cN and relative humidity of 65% at room temperature. Ten individual fiber samples were tested from each group and measurements are reported as the mean ± standard deviation.

Thermogravimetric analysis (TG) was performed using an STA 449 F3 thermogravimetric analyzer (Netzsch Company, Germany). About 10 mg of sample in a ceramic crucible was heated from 25 to 800 °C at a heating rate of 10 °C/min under a nitrogen atmosphere.

3. Results And Discussion

3.1 Surface oxidation modification of CNCs.

In this part, we characterized the oCNCs prepared by ASP oxidation and compared them with unmodified CNCs. Figure 1 shows the comparison results between oCNCs prepared under experimental conditions and unmodified CNCs. In Fig. 1a and b, the micromorphology and size distribution of oCNCs and CNCs were studied utilizing TEM and AFM. As can be seen from the TEM and AFM images, oCNCs and CNCs are both rod-shaped, with basically the same micromorphology (Grishkewich et al. 2017). However, the length of the oCNCs is slightly smaller than that of CNCs. This is because oxidation of the APS led to transversal cleavage of the amorphous domains of CNCs and it can also partially reduce the diameter of the crystalline regions (Souza et al. 2015). Figure 1c shows the XRD patterns of oCNCs and CNCs. CNCs

display two diffraction peaks at 2θ of 15.5°, 16.2°, and one peak at 22.7°, indicating the typical XRD pattern of cellulose I. After oxidation modification, the crystallinity of CNCs increased from 72.23–80.62%. A previous literature has reported that the carboxylate groups formed by oxidation are selectively present on cellulose microfibril surfaces without any internal crystalline regions (Isogai et al. 2011). As can be seen from the XRD pattern of oCNCs, the crystallinity and the crystal sizes of CNCs increased only slightly after oxidation, which demonstrates that an appropriate amount of APS mediated oxidation not only removed the amorphous regions of CNCs but also improved the crystalline structure of CNCs (Naduparambath et al. 2018). Furthermore, Fig. 1d shows the FT-IR spectra of oCNCs and CNCs. The changes of functional groups of CNCs and its oxidized counterpart were demonstrated by FTIR spectra. It is noted that for CNCs, the characteristic peaks at 3341 and 1370 cm^{-1} are assigned to stretching vibrations of hydroxyl groups and the symmetric bending of -CH_2, respectively. Also, CNCs have no absorption band from 1700 to 1900 cm^{-1} (de Oliveira et al. 2019; Pracella et al. 2014). In comparison, oCNCs have a stronger O-H stretching vibration absorption peak at 3237 cm^{-1}, indicating that there is a stronger intermolecular or intramolecular force in the oCNCs. Besides, the stretching vibration peak of oCNCs at 1735 cm^{-1} is a carboxyl peak obtained from the hydroxyl group at the C_6 position of the CNCs oxidized by APS (Habibi et al. 2006; Yao et al. 2017). These results demonstrate that APS can effectively transform the hydroxyl groups on the surface of CNCs into carboxyl groups, resulting in uniform oCNCs with nanoscale dimensions.

3.2 Zeta potential analysis

The zeta potential measurement represents a valuable approach to control the stability of a colloidal system (Yue et al. 2021a). It is evident that the electrical potential at the surface of the charged particles increases with increasing the absolute values of the zeta potential, leading to higher electrostatic repulsion forces between the charged particles, which are higher than the Van der Waals attractive forces. Therefore, the particles repel each other, resulting in a stable colloidal system. When the absolute values of zeta potential drop and tend to zero, the opposite is true (Guerra-Rosas et al. 2016). The zeta potential measurement of the solution was performed to evaluate the relationship between the stability of the collagen solution and the concentration of CNCs and oCNCs. Previous literature has reported that the CNCs have a major impact on the morphology, stability and fibril formation of collagen by forming comprehensive interactions involving electrostatic interactions and hydrogen bonds (Klemm et al. 2011; Rodrigues et al. 2017; Yan et al. 2018). As can be seen from Fig. 2, the zeta potential of collagen solutions is positive because collagen is positively charged under acidic conditions (Yue et al. 2021a). The experiment results showed that as the content of oCNCs increased from 0 to 0.07 wt%, the zeta potential of the solution decreased from 35.8 mV to 12.2 mV. However, under the same conditions, the potential of the collagen/CNCs solution only decreased to 15.5 mV. This is because, at the same content, the carboxyl groups of oCNCs can provide more negative charges, which makes the zeta potential of collagen/oCNCs is lower than that of collagen/CNCs. With the increase of oCNCs content, the zeta potential of collagen/oCNCs gradually decreases, leading to the stability decrease of the colloidal system

(Li et al. 2015). Overall, oCNCs are promising for the reinforcement of synthetic or natural polymer matrices at low loading levels.

3.3 SEM observation of fibers morphology

The surface and cross-section of the fibers were observed using SEM to study the distribution of oCNCs in the collagen matrix and their mutual interactions. Figure 3 displays SEM images of the surface and cross-section of the fibers. As shown in Fig. $3a_1$-f_1, the surface of all fibers shows small wrinkles due to the shrinkage of molecular chains during the formation process of fibers in a coagulation bath (Ding et al. 2019). Previous studies have found that the small wrinkles on the surface of regenerated collagen fibers are conducive to the adhesion and growth of rat tissue connective fibroblasts (Wang et al. 2016). Figure $3a_2$-f_2 illustrates cross-sectional SEM images of the collagen composite fibers. As can be seen, there is the lowest degree of fibril alignment and a looser structure in the cross-section microstructure of the F0 (Fig. $3a_2$). With the increase of oCNCs content, the cross-section microstructure of the composite fibers is more compact and orderly, and the oriented microfibril network structure is more obvious. In addition, oCNCs displayed good adhesion with collagen and no separation from the matrix was observed. This good adhesion facilitates the stress transfer from the matrix to the oCNCs under stress conditions effectively, thereby promoting the effective improvement of the mechanical properties of the composite fibers (Yue et al. 2021b). At the same time, the F5 also displays the obvious microfibril orientation structure along the fiber axis in the cross-section microstructure, while the oriented fibrils arrangement is similar to F3 and F4, which indicates that oCNCs and CNCs can induce collagen microfibril orientation during the formation process of fibers and protect the possibility of their interactions with collagen molecules (Green et al. 2017). Collagen molecules and nanofillers form an entanglement network that can better resist external forces, and the negative charge of oCNCs is more, so the electrostatic attraction between oCNCs and collagen molecule is stronger. Moreover, the physical entanglement of nanofiller will not be formed due to the existence of rod shape structure. Overall, these SEM results suggest that CNCs and oCNCs have a positive impact on the formation of oriented microfibril network structures, which is attributed to the hydrogen bonds between nanofillers and collagen molecules, enhancing the fibrillation ability of collagen molecules.

3.4 Mechanical properties of fibers

The material system, the dispersion state of nanofillers and the preparation process have a significant impact on the mechanical properties of the composite materials. The mechanical properties of collagen fibers are shown in Fig. 3, and the influence of oCNCs on the mechanical properties of collagen fibers is highlighted. As a natural collagen material, F0 presents a tensile strength of 1.10 cN/dtex and a elongation at break of 20.2%. Obviously, CNCs and oCNCs exhibited a strong reinforcement effect on all the collagen composite fibers because of their rigid nature. Amongst all the collagen composite fibers, F3 showed the highest tensile strength of 1.97 cN/dtex and elongation at break of 44.4%, which were higher than those of F0, exhibiting the significantly positive effect of oCNCs on the mechanical property of collagen fibers. Compared with F0, the tensile strength and elongation at break of F3 are increased by 95.0% and 44.5%, respectively. At low addition contents, oCNCs have a good dispersion in the collagen

matrix, and the self-assembly of collagen can occur effectively during the dry-jet wet spinning process (Yang et al. 2018; Yue et al. 2021b), mainly via hydrogen bonds of parallel chains between the native collagen fibrils and crystalline domains of CNCs (Green et al. 2017). Due to this assembled structure of the composite fibers, the load can effectively transfer to the hard reinforcing phase when a tensile force was subjected to the composite fibers, then the mechanical properties of the composite fibers are similar to that of randomly oriented rigid CNCs with high strength and modulus (Thomas et al. 2018). Consequently, the tensile strength and elongation at break of collagen composite fibers are improved. However, when the oCNCs content was above 0.05 wt%, the tensile strength and elongation at break of the F4 decreased simultaneously, which may be caused by the agglomeration phenomena of the oCNCs at high loadings (Pracella et al. 2014; Ten et al. 2012). Furthermore, it should be pointed out that the mechanical properties of F3 are evidently better than those of F5. For composite fibers, the crystallinity of oCNCs or CNCs is very important as it is a key factor to determine the reinforcing capability and tensile strength of nanofillers to be utilized in composite applications (Naduparambath et al. 2018). Compared with CNCs, the oCNCs can more effectively promote the crystallization of the collagen matrix owing to the increase of crystallinity, while improving its plastic response (Fortunati et al. 2013). On the other hand, there are stronger electrostatic interactions and hydrogen bonds between oCNCs and collagen molecules owing to the negatively charged carboxyl groups of oCNCs. Therefore, composite fibers prepared by collagen and oCNCs will possess higher intermolecular interactions (Naduparambath et al. 2018). Thus, considering the mechanical properties of fibers, the collagen composite fibers with 0.05 wt% nanofillers (F3 and F5) were selected for the basis of subsequent structure and performance studies.

3.5 FTIR spectroscopy analysis

The interactions among the components of the collagen composite fibers were investigated by FT-IR. The FT-IR spectra of F0, F3 and F5 are shown in Fig. 5. It can be observed that the characteristic peaks of F0 at 3305, 3066, 1631, 1542 and 1238 cm^{-1} corresponded to amide A and amide B, amide I, amide II and amide III bands, respectively (Yan et al. 2018). The characteristic peaks of collagen composite fibers are roughly consistent with F0 because the main contribution is from the collagen groups due to their higher content in the fibers. To study the possibility of the interaction between the components of the system, the change of the absorption intensity and position of amide A band was compared (Rodrigues et al. 2017). This band was chosen because it represents hydrogen bonds, including changes in the strength of hydrogen bonds and the formation of intermolecular hydrogen bonds (Mitra et al. 2013). It is worth noting that, compared with F0, the amide A peak in the spectrum of the collagen composite fibers is significantly blue-shift and the band strength is enhanced, which indicates that the intermolecular hydrogen bonds are formed between the nanofillers (CNCs and oCNCs) and the collagen molecules. Especially, the amide A band of F3 is stronger than that of F5, and this is because the oCNCs have a higher degree of crystallinity and some newly generated surface carboxyl groups so that it can form more hydrogen bonds with collagen molecules. Additionally, the wavenumbers of the amide I, II, and III bands are directly related to the triple helix structure of collagen (Kumar et al. 2015). While the amide I, II and III bands of the composite fibers showed little shift in the wavenumber, manifesting that the triple helix structure of the collagen did not change.

3.6 XRD analysis

The mechanical properties of materials are strongly influenced by the crystallinity of the material (Chatterjee et al. 2013). As shown in Fig. 6, XRD patterns were used to analyze the crystalline or amorphous structure of collagen fibers. There are two strong characteristic peaks of F0 at 7.6° and 20.5°. The first diffraction peak at 7.6° is a sharp diffraction peak that reflects the lateral spacing between collagen molecules (Green et al. 2017; Yue et al. 2021b). The second diffraction peak at 20.5° is a broad diffraction peak that represents diffuse scattering caused by the numerous structural layers within the collagen fibers (Labaki et al. 1991). It can be seen that the diffraction peak intensities of the collagen composite fibers with CNCs and oCNCs are greater than those of F0. This suggests that the CNCs and oCNCs can excellently induce the orientation of collagen molecules during the formation process of composite fibers, thus resulting in greater spacing of collagen microfibrils (Fortunati et al. 2013). Specifically, during the collagen composite fibers forming process, nanofillers act as nucleation sites causing the development of new crystallites contributing to a rise in the overall crystallinity of the composite fibers. And especially, the effective nucleation ability of CNC and oCNCs is usually attributed to their excellent crystallinity. Therefore, because of its high crystallinity, oCNCs are easy to establish hydrogen bonds and electrostatic interactions with collagen molecules, thereby stabilizing the alignment of collagen molecules and fibrils.

3.7 Thermal stability analysis

The decomposition behavior of collagen fibers as a function of temperature was analyzed by TG. In Fig. 7a, the TG curves of F0, F3 and F5 showed an initial weight loss at around 50 °C attributing to the loss of the free water within the fibers (Nomura et al. 1977). Next, the second decomposition was followed at around 200 °C, which resulted from the breaking of collagen molecule chains (Burjanadze and Kisiriya 1982). The initial temperature of the third process was about 320 °C and may correspond to further degradation of the short chains (Caballero et al. 1998). It can be seen from Fig. 7a that collagen composite fibers have a higher thermal decomposition temperature compared with F0. That could be attributed to the formation of stable hydrogen bonds between the oxygenic groups on the CNCs and oCNCs surface and collagen molecules resulting in better thermal stability compared with F0 (Liu et al. 2015). DTG analysis allowed to determine the effects of the inclusion of different types of CNCs in the collagen matrix. In the DTG curves of Fig. 7b, compared with F0, the peak value of the first peak of the DTG curves of the collagen composite fibers slightly decreased, which may be caused by the increase of the binding water between collagen molecules due to the addition of CNCs and oCNCs. The peak value of the second peak on the DTG curves increased significantly, which proved that the thermal stability of the collagen composite fibers was significantly higher than that of the F0. The addition of CNCs and oCNCs enhanced the hydrogen bonds and electrostatic interaction between molecules, which made the oriented microfibril network structures of collagen composite fibers more stable, and the thermal degradation temperature of fibers increased (Liu et al. 2015). In comparison to CNCs, oCNCs are easier to enhance their interaction with collagen molecules by electrostatic attraction. Thus, the electrostatic attraction

between oCNCs and collagen molecules makes the collagen molecules chimerism more dense, which makes the thermal degradation temperature of F3 reach the maximum.

4. Conclusions

In summary, we used APS to oxidize CNCs by a one-step oxidation method, and successfully prepared oCNCs and investigated their structures and performance. Collagen composite fibers with high mechanical properties were prepared by facile dry-jet wet-spinning with CNCs and oCNCs as a reinforcing phase. Zeta potential analysis exhibited that the oxidation of CNCs improved the reactive activity of CNCs in the collagen matrix and enhanced the interactions with collagen molecules. Meanwhile, the effect of oCNCs contents on the mechanical properties of collagen fibers was systematically evaluated in the contents range of 0 to 0.07 wt%. Keeping the contents of the CNCs and oCNCs unchanged at 0.05 wt%, through the analysis of structures and properties, collagen/oCNCs composite fibers were confirmed to exhibit extraordinary synergistic enhancement in both the structures, thermal stability and mechanical properties. The reason for this enhancement is that oCNCs and collagen molecules had stronger hydrogen bonds and electrostatic interactions, which arranges collagen microfibrils more denser during the dry-jet wet-spinning process. The excellent thermal stability and mechanical properties of collagen-based biomaterials provide more imagination for the preparation of products for biomedical equipments, which will also broaden the application scope of regenerated collagen fibers.

Declarations

Acknowledgment

Declaration of Competing Interest

The authors declare no conflict of interest.

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Figures

Figure 1

The comparison results between oCNCs prepared under experimental conditions and unmodified CNCs: (a) TEM images, (b) AFM images, (c) XRD patterns, (d) FT-IR spectra of CNCs and oCNCs.

Figure 2

Effect of CNCs and oCNCs on zeta potential of collagen solutions.

Figure 3

SEM images of the collagen fibers (a-f: F0-F5; a_1-f_1 are the SEM images of the fibers surface; a_2-f_2 are the SEM images of the fibers cross-section profiles; a_3-f_3 are the higher magnification of the fibers cross-section profiles).

Figure 4

Stress-strain curves of collagen fibers.

Figure 5

FT-IR spectra of collagen fibers.

Figure 6

XRD patterns of collagen fibers.

Figure 7

(a) TG curves and (b) derivative curves of collagen fibers.

Supplementary Files

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- Scheme1.png
- Scheme2.png