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

Changkun Ding · Chengfei Yue · Jieliang Su · Hua Wang · Ning Yang · Bowen Cheng

Abstract The mechanical properties of regenerated collagen composite materials are of use in diverse applications. However, there are few efficient strategies to enhance the mechanical properties of regenerated collagen fibers. Herein, oxidized cellulose nanocrystals (oCNCs) were successfully prepared in aqueous media via oxidation of the hydroxyl groups on the surface of cellulose nanocrystals (CNCs) using ammonium persulfate (APS) as an oxidant. Collagen composite fibers were then prepared by introducing oCNCs into the collagen matrix via a dry-jet wet-spinning technique. The morphology, structures, and properties of prepared collagen composite fibers were carefully investigated. The results indicate 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. The oCNCs offered better enhancement efficiency than CNCs, and the as-prepared collagen/oCNC composite fibers exhibited extraordinary improvements in both 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, thus resulting in a 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 broad applications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10570-022-04750-z.

C. Ding · C. Yue · J. Su · H. Wang · N. Yang · B. Cheng
Tianjin Key Laboratory of Advanced Fibers and Energy Storage, School of Materials Science and Engineering, Tiangong University, Tianjin 300387, China
e-mail: dingchangkun@tiangong.edu.cn
Graphical abstract

Keywords  Collagen · Cellulose nanocrystals · Oxidation modification · Fibers · Mechanical properties

Introduction

Collagen is a natural polymer that constitutes the main extracellular matrix (ECM) protein in various tissues, e.g., 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. Collagen supports cell growth and underlies the mechanical elasticity of connective tissue (Gordon and Hahn 2010; Ricard-Blum 2011). There are currently 29 types of collagen, and the most common is type I collagen (Orgel et al. 2001; Sorushanova et al. 2019). Amino acids are amphiphilic molecules, and collagen is composed of amino acids and thus is also amphoteric (Yue et al. 2021). The dissociable groups are 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 value of collagen as a biomaterial principally depends on the fact that it is a naturally abundant extracellular matrix component. It is an endogenous constituent of the body and is not foreign matter (Sherman et al. 2015). The value appeal of collagen composite materials for biomedical applications stems from the fact that many proteolytic enzymes can degrade common collagen composite materials 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. Thus, it is challenging 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). Traditional crosslinking methods cannot always satisfy the requirements of practical applications; other biological materials can be integrated into collagen to fabricate composite materials. Various types of functional nanofillers (e.g., carbon nanotubes and hydroxyapatite) have been incorporated into collagen to pursue nanocomposites with better mechanical properties and thermal stability (Klemm et al. 2011; Pracella et al. 2014; Cheng et al. 2017; Grishkewich et al. 2017; Naduparambath et al. 2018; Prathapan et al. 2020). Green et al. (2017) demonstrated the utility of carbon nanotubes in collagen composite fibers through a gel-spinning approach to study whether or not rigid nano-inclusions could affect and facilitate fibril alignment when acting as a template. They found that carbon nanotubes can facilitate the self-assembly and alignment of collagen fibrils due to their structural similarity and rigid features. Choi et al. (2017) used a new self-assembly-induced gelation
method to synthesize collagen/hydroxyapatite composite porous microspheres featuring a nanofibrous structure. Collagen nanofibers with a uniform size of several tens of nanometers were created across the entire composite microspheres regardless of the hydroxyapatite content. The improved performance of composite materials with the incorporation of nano-materials works at the expense of the high cost of raw materials, e.g., graphene oxide and carbon nanotubes; this also requires a complicated preparation process (Cheng et al. 2017). Accordingly, a simple and low-cost method for the development of functional nanofillers with excellent mechanical properties and satisfactory biological properties is desirable.

Nanocellulose is the most abundant crystalline polysaccharide nanomaterial on Earth, and it is an interesting model filler in various polymer matrices (Heise et al. 2021). Generally, the term “nanocellulose” encompasses flexible, fiber-like cellulose nanofibrils (CNFs) as well as rigid and rod-shaped CNCs. The 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. Acid hydrolysis leads to nanometer-long and highly crystalline CNCs. Thus, the evident differences between CNCs and CNFs lie in their dimensions and crystallinity (Grishkewich et al. 2017). CNCs have attractive crystallinity, chiral structure, and self-assembly features; thus, CNCs are often utilized to control and fabricate structured materials with highly ordered and aligned nanoparticles (Thomas et al. 2018; De France et al. 2020). However, CNCs tend to self-aggregate, and it remains challenging to obtain nanocomposites with high mechanical performance due to their interfacial compatibility with polymer matrices (Peng et al. 2020). Thus, the 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 (Eyley and Thielemans 2014; Bespalova et al. 2017). 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 critical for new green composite materials.

Here, to enhance the interactions between CNCs and collagen, and improve the reactivity of CNCs, we modified CNCs through a one-step oxidation method with APS. We characterized it in terms of morphologies and microstructures. Collagen composite fibers were prepared using a dry-jet wet-spinning technique, and the effects of CNC 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 new insights for improving the properties of collagen fibers.

**Experimental**

**Materials**

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

**Surface oxidation modification of CNCs**

We used a one-step oxidation method for the oxidation of CNCs according to the literature (Leung et al. 2011); this approach has significant benefits in terms of sustainability and low costs (Scheme 1). 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 into the 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 complete, 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 with repeated centrifugation. The centrifugation/washing cycle was repeated several times until the supernatant conductivity was ~5 μS·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.

| 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     |

Scheme 2 Process of preparing collagen composite fibers using the dry-jet wet-spinning technique
Preparation of collagen composite fibers

The collagen/oCNCs composite fibers were prepared using a dry-jet wet-spinning method (Scheme 2). First, the oCNCs were pre-dispersed in water for 20 min via magnetic stirring, and ultrasound treatment was performed at 22–26 °C for 1 h using an ultrasonic cleaning bath (250 W). Next, 0.9 mL of acetic acid was added to the aqueous oCNC suspension (30 mL), and 0.45 g of lyophilized collagen was added to the acidic suspension (pH ≈ 2.5) with continued mechanical stirring for 8 h. The collagen/oCNC solution was transferred to a refrigerated centrifuge and centrifuged for 40 min (9,000 rpm). The solution temperature was maintained at 4 °C throughout the preparation and centrifugation process. The spinning solution was then incubated in a 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 at volume ratios of 60:1:0.2. The temperature of the coagulation bath was controlled at 25 ºC. 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.

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; excess water was then 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 AFM.

A D/MAX-2550 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 20 values of 5°–40°. The calculation method of crystallinity index in the Supplementary Information shows specific procedures.

A Nicolet iS50 Fourier-transform infrared (FT-IR) spectrometer (Thermo Fisher Scientific, America) was used to determine the chemical structures of CNCs, oCNCs, and fibers. Samples from each fiber were ground and mixed thoroughly with potassium bromide at a ratio of 1:5 (sample: KBr), and an aliquot of the mixture was made into a pellet. All of the spectra were recorded from 400 to 4000 cm⁻¹ at a resolution of 0.1 cm⁻¹ at room temperature. To avoid the influence of water content, the test was carried out immediately when the dried fibers were obtained.

The zeta potential and size of CNCs, oCNCs, collagen/CNCs, and collagen/oCNCs dispersion were tested with a zeta potential analyzer (Malvern Panalytical Company, UK). CNCs and oCNCs were pre-dispersed in deionized water via magnetic stirring; they were treated with an ultrasonic cleaning bath (250 W) at 22–26 °C for 1 h to CNC and oCNC dispersions. A 200 μg/mL collagen solution was then prepared using 0.5 mol/L acetic acid. 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 with 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.

An LLY-06 electronic fiber strength tester (Electronic Instrument Co., Ltd., Laizhou, China) was measured 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; the relative humidity was 65% at room temperature. Ten individual fiber samples were tested from each group,
and measurements were reported as the mean ± standard deviation.

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

Results and discussion

Surface oxidation modification of CNCs

We characterized the oCNCs prepared by APS oxidation and then compared them with unmodified CNCs. Figure 1 compares oCNCs prepared under experimental conditions and unmodified CNCs. The micromorphology and size distribution of oCNCs and CNCs were studied with TEM and AFM (Fig. 1a, b). The oCNCs and CNCs are both rod-shaped with very similar micromorphology (Grishkewich et al. 2017). However, the length of the oCNCs is slightly smaller than CNCs. This is because oxidation of the APS led to transversal cleavage of the amorphous domains of CNCs; this partially reduces the diameter of the crystalline regions (Souza et al. 2015). The crystallinity of CNCs is an important factor in controlling their rigidity and thermal stability. Therefore, changes in the crystalline structure and crystallinity were evaluated via a comparison of XRD patterns. Figure 1c shows the XRD patterns of oCNCs and CNCs. oCNCs have two diffraction peaks at 2θ of 15.5° and 16.2° and one peak at 22.7°, thus indicating the typical XRD pattern of cellulose I (Park et al. 2019). These results suggest that the APS oxidation system did not alter the original crystal integrity of cellulose (Liu et al. 2020). Previous literature has reported that the carboxylate groups formed by oxidation are selectively present on cellulose microfibril surfaces without affecting cellulose molecules of the crystal interior (Isogai et al. 2011; Bashar et al. 2019). Moreover, the crystallinity index of oCNCs increased from 72.2% for pulp to over 80.6% after hydrolysis under the action of APS. This indicates that the amorphous region of CNCs undergoes oxidization to form oCNCs (Liu et al. 2020).
The XRD pattern of oCNCs indicates that the crystallinity and crystal sizes of CNCs increased only slightly after oxidation, which demonstrates that an appropriate amount of APS-mediated oxidation removed the amorphous regions of CNCs and improved the crystalline structure of CNCs (Naduparambath et al. 2018). Figure 1d shows the FT-IR spectra of oCNCs and CNCs. The changes in functional groups of CNCs and their oxidized counterparts are demonstrated by FT-IR spectra. In the CNCs, the characteristic peaks at 3341 and 1370 cm\(^{-1}\) are assigned to stretching vibrations of hydroxyl groups and symmetric bending of –CH\(_2\), respectively. CNCs have no absorption band from 1700 to 1900 cm\(^{-1}\) (Pracella et al. 2014; de Oliveira et al. 2019). In comparison, oCNCs have a stronger O–H stretching vibration absorption peak at 3237 cm\(^{-1}\), thus indicating stronger intermolecular or intramolecular forces in the oCNCs. 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).

The changes in the zeta-potential and size of the CNC (0.01 wt%) and oCNC (0.01 wt%) dispersions under different pH are shown in Table S1 (Supplementary Information). The zeta potential of oCNCs is higher than CNCs at the same pH, which indicates that the oxidation of APS destroys the amorphous structure of CNCs and leads to more negative charges on the surface of CNCs. There was an obvious increase in the zeta-potential of CNC and oCNC dispersions when the pH value shifted from 2.0 to 6.0. Although the surface charges of CNCs and oCNCs are affected by the change in pH, CNCs and oCNCs are negatively charged under acidic conditions (Wang et al. 2018). Furthermore, the size of the oCNCs is slightly smaller than CNCs, which is consistent with the results mentioned above. These results demonstrate that APS can effectively transform the hydroxyl groups on the surface of CNCs into carboxyl groups, thus resulting in uniform oCNCs with nanoscale dimensions.

Zeta potential analysis

The zeta potential is a useful metric to control the stability of a colloidal system (Yue et al. 2021). The electrical potential at the surface of the charged particles increases with increasing absolute values of the zeta potential, thus 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, thus resulting in a stable colloidal system. The opposite is true when the absolute values of the zeta potential decrease and approach zero (Guerra-Rosas et al. 2016). Thus, the zeta potential was measured 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). Figure 2 shows that the zeta potential of collagen solutions is positive because collagen is positively charged under acidic conditions (Yue et al. 2021). The results show that as the content of oCNCs increased from 0 to 0.07 wt%, the zeta potential of the solution decreased from 35.8 to 12.2 mV. However, the potential of the collagen/CNCs solution only decreased to 15.5 mV under similar conditions because the carboxyl groups of oCNCs can provide more negative charges at the same content. This makes the zeta potential of collagen/oCNCs lower than collagen/CNCs. With increasing of oCNCs content, the zeta potential of collagen/oCNCs gradually decreases, thus decreasing the stability of the colloidal system (Li et al. 2015). In addition, the change in pH was marginal with increasing amounts of CNCs and oCNCs, thus suggesting a minor effect of CNC and oCNC addition on pH for these systems (Wang et al. 2018). More detailed size
data of collagen/CNCs and collagen/oCNCs colloidal particles are listed in Table S2 (Supplementary Information). With increasing nanofiller (CNCs or oCNCs) contents, the sizes of collagen/CNCs and collagen/oCNCs colloidal particles increased significantly suggesting that the increase in CNC and oCNC promotes collagen fibrillogenesis. In particular, at the same content, the size of collagen/oCNCs colloidal particles is larger than that of collagen/CNCs colloidal particles, which demonstrates that oCNCs can better promote collagen fibrillogenesis. Overall, oCNCs are promising for the reinforcement of synthetic or natural polymer matrices at low loading levels.

SEM observation of fibers morphology

The surface and cross-section of the fibers were studied with SEM. We evaluated 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. Figure 3a1–f1 show that the surfaces of all fibers have small wrinkles due to the shrinkage of molecular chains during the formation 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 3a2–f2 illustrate cross-sectional SEM images of the collagen composite fibers. Sample F0 has the lowest degree of fibril alignment and a looser structure in the cross-section microstructure (Fig. 3a2). The cross-section microstructure of the composite fibers is more compact and orderly with increasing oCNCs content. The oriented microfibril network structure is more obvious. In addition, oCNCs displayed good adhesion with collagen; no separation from the matrix was observed. This good adhesion effectively facilitates the stress transfer from the matrix to the oCNCs under stress conditions, thus improving the mechanical properties of the composite fibers (Yue et al. 2022). Sample F5 has obvious microfibril orientation structures along the fiber axis in the cross-section microstructure. The oriented fibril arrangement is similar to F3 and F4, which indicates that oCNCs and CNCs can induce collagen microfibril orientation during the formation of fibers. This can protect their interactions with collagen molecules. Collagen molecules and nanofillers form an entangled network that can better resist external forces (Green et al. 2017). The negative charge of oCNCs is higher, and thus the electrostatic attraction between oCNCs and collagen molecules is stronger. Moreover, the physical entanglement of nanofillers is not formed due to the rod-shaped 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, thus enhancing the fibrillation of collagen molecules.

Fig. 3  SEM images of the collagen fibers. a–f: F0–F5; a1–f1 are the SEM images of the fibers’ surfaces; a2–f2 are the SEM images of the fibers’ cross-section profiles; and a3–f3 are higher magnification fiber cross-section profiles

![SEM images of collagen fibers](image-url)
Mechanical properties of fibers

The material system shows that the dispersion of nanofillers and the preparation process significantly impact the mechanical properties of the composite materials. The mechanical properties of collagen fibers are shown in Fig. 4, and the influence of oCNCs on the mechanical properties of collagen fibers is highlighted. (Please see more detailed mechanical data in Table S3, Supplementary Information).

As a natural collagen material, F0 presents a tensile strength of 1.10 cN/dtex and elongation at a breakage of 20.2%. CNCs and oCNCs exhibited a clear and strong reinforcement effect on all collagen composite fibers because of their rigid nature. Of the collagen composite fibers, F3 showed the highest tensile strength of 1.97 cN/dtex; the elongation at breakage was 44.4%, which is higher than F0. These data illustrate the significant positive effect of oCNCs on the mechanical properties of collagen fibers. The tensile strength and elongation at the break of F3 increased by 95.0% and 44.5% versus F0, respectively. At low addition contents, oCNCs have a good dispersion in the collagen matrix. The self-assembly of collagen can occur effectively during the dry jet/wet-spinning process mainly via hydrogen bonds between parallel chains in the native collagen fibrils and crystalline domains of CNCs (Green et al. 2017; Yang et al. 2018; Yue et al. 2022). 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 (Thomas et al. 2018). The mechanical properties of the composite fibers are then similar to randomly oriented rigid CNCs with high strength and modulus. The tensile strength and elongation at the break of collagen composite fibers were consequently improved. However, the tensile strength and elongation at the break of the F4 decreased simultaneously when the oCNCs content was above 0.05 wt%. This may be caused by the agglomeration phenomena of the oCNCs at high loadings (Ten et al. 2012; Pracella et al. 2014). The mechanical properties of F3 are clearly better than F5. The crystallinity of oCNCs or CNCs is important in the composite fibers because it is a key factor that determines the reinforcing capability and tensile strength of nanofillers to be utilized in composite applications (Naduparambath et al. 2018). Versus CNCs, the oCNCs can more effectively promote the crystallization of the collagen matrix due to the increase in crystallinity while improving the plastic response (Fortunati et al. 2013). There are also stronger electrostatic interactions and hydrogen bonds between oCNCs and collagen molecules due to the negatively charged carboxyl groups of oCNCs. Therefore, composite fibers prepared by collagen and oCNCs have higher intermolecular interactions (Naduparambath et al. 2018). A comparison of the collagen composite fibers’ mechanical properties with the literature data on collagen fibers indicated a marked improvement in mechanical properties (Table S4, Supplementary Information). Thus, considering the mechanical properties of fibers, collagen composite fibers with 0.05 wt% nanofillers (F3 and
F5) were selected for subsequent structure and performance studies.

FT-IR spectroscopy analysis

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. The characteristic peaks of F0 at 3300, 3076, 1631, 1541, and 1236 cm\(^{-1}\) correspond to amide A, 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 with higher concentrations in the fibers. The changes in the absorption intensity and position of amide A, amide I, and amide II bands were compared next to study the possibility of the interactions between the components of the system (Rodrigues et al. 2017). These bands were chosen because they represent hydrogen bonds including changes in the strength of the hydrogen bonds and the formation of intermolecular hydrogen bonds (Mitra et al. 2013). In the collagen composite fibers, the characteristic peak of the amide A band showed some obvious red-shift versus F0. This shift is attributed to the formation of strong hydrogen bonds between the nanofillers (CNCs and oCNCs) and the collagen molecules—this is widely reported in the literature (Wang et al. 2014; Wenko et al. 2015; Long et al. 2018). The amide A band of F3 is stronger than that of F5 because the oCNCs have a higher degree of crystallinity and some newly generated surface carboxyl group; thus, it can form more hydrogen bonds with collagen molecules. However, the low content of nanofillers makes it difficult to detect the hydrogen bonding signal of the collagen composite fibers at the amide I band and amide II band. To further confirm hydrogen bonding between collagen molecules and nanofillers, collagen composite fibers with a higher content of nanofillers were prepared and analyzed by FT-IR (Figure S1, Supplementary Information). Figure S1 shows a shift to the lower wavenumber of the composite fibers for the amide I band and a reverse shift for the amide II band (Ding et al. 2020). These both resulted from the formation of hydrogen bonds between the nanofillers (CNCs and oCNCs) and the collagen molecules.

XRD analysis

The mechanical properties of materials are strongly influenced by the crystallinity of the material (Chatterjee et al. 2013). Figure 6 presents XRD patterns 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. 2022). 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). The diffraction peak intensities of the collagen composite fibers with CNCs and oCNCs are greater than F0. This suggests that the CNCs and oCNCs can modulate the orientation of collagen molecules during the formation of composite fibers, thus resulting in the greater spacing of collagen microfibrils (Fortunati et al. 2013). Calculations with Jade 6.5 show that F0 has a crystallinity index of 24.3%. The crystallinity index of F3 and F5 increased to 31.2% and 28.4%, respectively, upon the introduction of CNCs and oCNCs. Specifically, nanofillers act as nucleation sites during the collagen composite fiber formation process, thus leading to the development of new crystallites contributing to a rise in the overall crystallinity of the composite fibers. The effective nucleation ability of CNCs and oCNCs is usually attributed to their excellent crystallinity. Therefore, oCNCs can easily establish hydrogen bonds and electrostatic interactions with collagen molecules due to their high
crystallinity. This in turn stabilizes the alignment of collagen molecules and fibrils.

Thermal stability analysis

The decomposition behavior of CNCs, oCNCs, and collagen fibers as a function of temperature was analyzed by TG. Figure 7 shows TG and DTG curves of CNCs, oCNCs, and collagen fibers. The decomposition of CNCs and oCNCs had an initial slight weight loss near 80 °C, which is due to the evaporation of adsorbed or bound water on CNCs and oCNCs. The weight of CNCs and oCNCs drops sharply near 280 °C, which is the primary thermal degradation stage and is mainly related to the depolymerization and degradation processes of the cellulose chains (Lin et al. 2009; Bashar et al. 2019). The oCNCs have worse thermal stability than CNCs. This agrees well with other reports of cellulose nanoparticles decorated with carboxylic groups (Bashar et al. 2019; Park et al. 2019). The reduced stability might be attributed to the smaller particle size, higher specific surface area, and increased surface carboxyl groups of oCNCs, thus resulting in low resistance to heat versus CNCs (Cheng et al. 2014; Sehaqui et al. 2017). The TG curves of F0, F3, and F5 showed three main weight loss steps. The first one, around 50 °C, is related to the loss of the free water within the fibers, and the second decomposition was followed at around 200 °C, which resulted from the breakage of collagen molecular chains. The initial temperature of the third process was near 320 °C and may correspond to further degradation of the short chains (Ma et al. 2018; Ding et al. 2019). Importantly, the second thermal decomposition stage is the most important one for collagen fibers and is related to the interactions between collagen fibrils and CNCs or oCNCs (Ma et al. 2018). The DTG curves show that collagen composite fibers have a higher thermal decomposition temperature than pristine collagen fibers. The peak temperatures of the second decomposition stage for F3 and F5 were significantly increased versus F0 (Fig. 7b). This is attributed to the formation of hydrogen bonds between CNCs and oCNCs as well as the collagen molecules mentioned above in the FT-IR analysis section. This in turn leads to more stable and oriented microfibril structures in collagen composite fibers (Liu et al. 2015). Moreover, the decomposition temperature of F3 was higher than that of F5, which reflected the additional electrostatic attractions between oCNCs and collagen molecules. Meanwhile, the decomposition temperature in the third stage remained similar and is responsible for further degradation of the shorter collagen molecular chains.

Conclusions

In this study, we used APS to oxidize CNCs via a one-step oxidation method and successfully prepared oCNCs and investigated their structures and performance features. Collagen composite fibers with high
机械性能的纤维通过干湿纺纱过程制备。作为这样的热稳定性和机械性能的改善，原因在于通过化学氧化处理了CNCs和oCNCs而增强了相互作用，使得在干湿纺纱过程中的干湿纺纱过程中的微纤维束更密。因此，这种氧化处理使得这两种材料对于生物医疗设备有显著的热稳定性和机械性能。由于这些增强，使得这些热稳定性能和机械性能对于结构和材料的增强对结构和材料的增强在底朝上制造中具有更广泛的应用。

**Acknowledgments** We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript. We would like to thank the Analytical & Testing Center of Tiangong University for structured illumination microscopy work.

**Funding** This work was supported by the Scientific Research Project of Tianjin Education Commission under [Grant No.: 2019ZD04] and TGU Grant for Fiber Studies under [Grant No.: TGF-21-B4].

**Declarations**

**Competing interests** The authors declare no conflict of interest.

**References**

Bashar MM, Zhu H, Yamamoto S, Mitsuishi M (2019) Highly carboxylated and crystalline cellulose nanocrystals from jute fiber by facile ammonium persulfate oxidation. Cellulose 26(6):3671–3684. https://doi.org/10.1007/s10570-019-02363-7

Bespalova Y, Kwon D, Vasanathan N (2017) Surface modification and antimicrobial properties of cellulose nanocrystals. J Appl Polym Sci 134(18):44789. https://doi.org/10.1002/app.44789

Chatterjee S, Nueesch FA, Chu BTT (2013) Crystalline and tensile properties of carbon nanotube and graphene reinforced polyamide 12 fibers. Chem Phys Lett 557:92–96. https://doi.org/10.1016/j.cplett.2012.11.091

Cheng M, Qin ZY, Liu YN, Qin YF, Li T, Chen L, Zhu MF (2014) Efficient extraction of carboxylated spherical cellulose nanocrystals with narrow distribution through hydrolysis of lyocell fibers by using ammonium persulfate as an oxidant. J Mater Chem A Matter 2(1):251–258. https://doi.org/10.1039/c3ta13653a

Cheng Q, Ye D, Chang C, Zhang L (2017) Facile fabrication of superhydrophilic membranes consisted of fibrous tunicate cellulose nanocrystals for highly efficient oil/water separation. J Membr Sci 525:1–8. https://doi.org/10.1016/j.memsci.2016.11.084

Choi JW, Kim JW, Jo IH, Koh YH, Kim HE (2017) Novel self-assembly-induced gelation for nanofibrous collagen/hydroxyapatite composite microspheres. Materials 10(10):1110. https://doi.org/10.3390/ma1011110

De France K, Zeng ZH, Wu TT, Nystrom G (2020) Functional materials from nanocellulose: utilizing structure-property relationships in bottom-up fabrication. Adv Mater 33(28):2000657. https://doi.org/10.1002/adma.202000657

de Oliveira JP, Bruni GP, Mello el Halal SL, Bertoldi FC, Guerra Dias AR, Zavareze EdR (2019) Cellulose nanocrystals from rice and oat husks and their application in aerogels for food packaging. Int J Biol Macromol 124:175–184. https://doi.org/10.1016/j.ijbiomac.2018.11.205

DeFrates KG, Moore R, Borgesi J, Lin G, Mulderig T, Beachley V, Hu X (2018) Protein-based fiber materials in medicine: a review. Nanomaterials 8(7):457. https://doi.org/10.3390/nano8070457

Ding C, Du J, Cao Y, Yue C, Cheng B (2019) Effects of the aspect ratio of multi-walled carbon nanotubes on the structure and properties of regenerated collagen fibers. Int J Biol Macromol 126:595–602. https://doi.org/10.1016/j.ijbiomac.2018.12.144

Ding C, Tian M, Feng R, Dang Y, Zhang M (2020) Novel self-healing hydrogel with injectable, pH-responsive, strain-sensitive, promoting wound-healing, and hemostatic properties based on collagen and chitosan. ACS Biomater Sci Eng 6(7):3855–3867. https://doi.org/10.1021/acsbio.9b00588

Eleye S, Thielemans W (2014) Surface modification of cellulose nanocrystals. Nanoscale 6(14):7764–7779. https://doi.org/10.1039/c4nr01756k

Fortunati E, Puglia D, Luzi F, Santulli C, Kenny JM, Torre L (2013) Binary PVA bio-nanocomposites containing cellulose nanocrystals extracted from different natural sources: Part I. Carbohydr Polym 97(2):825–836. https://doi.org/10.1016/j.carbpol.2013.03.075

Gordon MK, Hahn RA (2010) Collagens. Cell Tissue Res 339(1):247–257. https://doi.org/10.1007/s00441-009-0844-4

Green EC, Zhang Y, Li H, Minus ML (2017) Gel-spinning of mimetic collagen and collagen/nano-carbon fibers: understanding multi-scale influences on molecular ordering and fibril alignment. J Mech Behav of Biomed Mater 65:552–564. https://doi.org/10.1016/j.jmbbm.2016.08.022

Grishkewich N, Mohammed N, Tang JT, Tam KC (2017) Recent advances in the application of cellulose...
nanocrystals. Curr Opin Colloid Interface Sci 29:32–45. https://doi.org/10.1016/j.cocis.2017.01.005

Guerra-Rosas MI, Morales-Castro J, Ochoa-Martinez LA, Salvia-Trujillo L, Martin-BellosO O (2016) Long-term stability of food-grade nanomulsions from high methoxyl pectin containing essential oils. Food Hydrocolloids 52:438–446. https://doi.org/10.1016/j.foodhyd.2015.07.017

Habibi Y, Chanyz H, Vignon MR (2006) TEMPO-mediated surface oxidation of cellulose whislers. Cellulose 13(6):679–687. https://doi.org/10.1007/s10570-006-9075-y

Heise K, Kontturi E, Allahverdiyeva Y, Tammelin T, Lindstrom T, Ankerfors M, Isogai A, Saito T, Fukuzumi H (2011) TEMPO-oxidized cellulose nanocrystals prepared from a novel family of nature-based materials. Angew Chem Int Ed Engl 50(24):5438–5466. https://doi.org/10.1002/anie.201001273

Labaki LC, Torriani IL, Grigera JR (1991) Humidity-dependent structural changes in native collagen studied by X-ray diffractometry. Braz J Med Bio Res 24(1):115–122. https://doi.org/10.1007/BF02175102

Leung ACW, Hrapovic S, Lam E, Liu Y, Male KB, Mahmoud L, Liu L, Wang K, Zhang H, Yuan Y, Wei H, Wang X, Lin YC, Cho J, Tompsett GA, Westmoreland PR, Huber GW, Li W, Lan Y, Guo R, Zhang Y, Xue W, Zhang Y (2015) Klemm D, Kramer F, Moritz S, Lindstrom T, Ankerfors M, Isogai A, Saito T, Fukuzumi H (2011) TEMPO-oxidized cellulose nanocrystals. Curr Opin Colloid Interface Sci 29:32–45. https://doi.org/10.1016/j.cocis.2017.01.005

Luo X, Guo Z, He P, Chen T, Li L, Ding S, Li H (2018) Study on structure, mechanical property and cell cytompatibility of electrospun collagen nanofibers crosslinked by common agents. Int J Biol Macromol 113:476–486. https://doi.org/10.1016/j.ijbiomac.2018.01.179

Ma YH, Wang WH, Wang YB, Guo Y, Duan SM, Zhao KX, Li SZ (2018) Metal ions increase mechanical strength and barrier properties of collagen-sodium polycrylate composite films. Int J Biol Macromol 119:15–22. https://doi.org/10.1016/j.ijbiomac.2018.07.092

Mitra T, Sailakshmi G, Gnanamani A, Mandal AB (2013) Exploring the dual role of alpha, omega-di-carboxylic acids in the preparation of collagen based biomaterial. J Porous Mater 20(4):647–661. https://doi.org/10.1007/s10934-012-9638-2

Naduparambath S, Jinita TV, Shaniba V, Sreejith MP, Balan AK, Purushothaman E (2018) Isolation and characterisation of cellulose nanocrystals from sago seed shells. Carbohydr Polym 180:13–20. https://doi.org/10.1016/j.carbpol.2017.09.088

Orgel J, Miller A, Irving TC, Fischetti RF, Hammersley AP, Wess TJ (2001) The in situ supermolecular structure of type I collagen. Structure 9(11):1061–1069. https://doi.org/10.1016/s0969-2126(01)00669-4

Park N-M, Choi S, Oh JE, Hwang DY (2019) Facile extraction of cellulose nanocrystals. Carbohydr Polym 223:115114. https://doi.org/10.1016/j.carbpol.2019.115114

Peng N, Huang D, Gong C, Wang YX, Zhou JP, Chang CY (2020) Controlled arrangement of nanocellulose in polymeric matrix: from reinforcement to functionality. ACS Nano 14(12):16169–16179. https://doi.org/10.1021/acsnano.0c08906

Pracella M, Hague MMU, Puglia D (2014) Morphology and properties tuning of PLA/cellulose nanocrystals bio-nano-composites by means of reactive functionalization and blending with PVAc. Polymer 55(16):3720–3728. https://doi.org/10.1016/j.polymer.2014.06.071

Prathapan R, Tabor RF, Garnier G, Hu J (2020) Recent progress in cellulose nanocrystal alignment and its applications. ACS Appl Bio Mater 3(4):1828–1844. https://doi.org/10.1021/acsabm.0c00104

Ricard-Blum S (2011) The collagen family. Cold Spring Harb Perspect Biol 3(1):a004978. https://doi.org/10.1101/cshperspect.a004978

Rodrigues APH, Pereira IM, de Souza SD, Brey Gil CS, Machado G, Carvalho SM, Pereira FV, Paiva PRP, de Oliveira LCA, Patricio PSiO (2017) Control of properties of nanocomposites bio-based collagen and cellulose nanocrystals. Cellulose 24(4):1731–1744. https://doi.org/10.1007/s10570-017-1218-9

Sehaqui H, Kulasinski K, Pfenninger N, Zimmermann T, Tin-gaut P (2017) Highly carboxylated cellulose nanofibers via succinic anhydride esterification of wheat fibers and facile mechanical disintegration. Biomacromol 18(1):242–248. https://doi.org/10.1021/acs.biomac.6b01548

Sherman VR, Yang W, Meyers MA (2015) The materials science of collagen. J Mech Behav Biomed Mater 52:22–50. https://doi.org/10.1016/j.jmbbm.2015.05.023

Sorushanova A, Delgado LM, Wu Z, Shologu N, Kshirsagar A, Raghunath R, Mullen Bayon Y, Pandit A, Raghunath M, Zeugolis DJ (2019) The collagen suprafamily: from biosynthesis to advanced biomaterial development. Adv
Souza NF, Pinheiro JA, Silva P, Saraiva Morais JP, de Souza M, Filho MdS, Santa Brigida AI, Muniz CR, Rosa MdF (2015) Development of chlorine-free pulping method to extract cellulose nanocrystals from pressed oil palm mesocarp fibers. J Biobased Mater Bioenergy 9(3):372–379. https://doi.org/10.1166/jbmb.2015.1525

Ten E, Bahr DF, Li B, Jiang L, Wolcott MP (2012) Effects of cellulose nanowhiskers on mechanical, dielectric, and rheological properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/cellulose nanowhisker composites. Ind Eng Chem Res 51(7):2941–2951. https://doi.org/10.1021/ie2023367

Thomas B, Raj MC, Athira KB, MH Rubiyah, Joy J, Moores A, Drisko GL, Sanchez C (2018) Nanocellulose, a versatile green platform: from biosources to materials and their applications. Chem Rev 118(24):11575–11625. https://doi.org/10.1021/acs.chemrev.7b00627

Tortorella S, Buratti VV, Maturi M, Sambri L, Franchini MC, Locatelli E (2020) Surface-modified nanocellulose for application in biomedical engineering and nanomedicine: a review. Int J Nanomed 15:9909–9937. https://doi.org/10.2147/ijn.S266103

Wang S, Ren J, Li W, Sun R, Liu S (2014) Properties of polyvinyl alcohol/xylan composite films with citric acid. Carbohydr Polym 103:94–99. https://doi.org/10.1016/j.carbpol.2013.12.030

Wang X, Wu T, Wang W, Huang C, Jin X (2016) Regenerated collagen fibers with grooved surface texture: physicochemical characterization and cytocompatibility. Mater Sci Eng C Mater Biol Appl 58:750–756. https://doi.org/10.1016/j.msec.2015.09.038

Wang W, Zhang X, Li C, Du G, Zhang H, Ni Y (2018) Using carboxylated cellulose nanofibers to enhance mechanical and barrier properties of collagen fiber film by electrostatic interaction. J Sci Food Agric 98:3089–3097. https://doi.org/10.1002/jsfa.8809

Yan H, Huang D, Chen X, Liu H, Feng Y, Zhao Z, Dai Z, Zhang X, Lin Q (2018) A novel and homogeneous scaffold material: preparation and evaluation of alginate/bacterial cellulose nanocrystals/collagen composite hydrogel for tissue engineering. Polym Bull 75(3):985–1000. https://doi.org/10.1007/s00289-017-1776-0

Yang X, Zhao Y, Mussana H, Tessema M, Liu L (2018) Characteristics of cotton fabric modified with chitosan (CS)/cellulose nanocrystal (CNC) nanocomposites. Mater Lett 211:300–303. https://doi.org/10.1016/j.matlet.2017.09.075

Yao JJ, Chen SY, Chen Y, Wang BX, Pei QB, Wang HP (2017) Macrofibers with high mechanical performance based on aligned bacterial cellulose nanofibers. ACS Appl Mater Interfaces 9(24):20330–20339. https://doi.org/10.1021/acsami.6b14650

Yue C, Ding C, Cheng B, Du X, Su J (2021) Preparation of collagen/aspatic acid nanocomposite fibers and their self-assembly behaviors. J Nat Fibers. https://doi.org/10.1080/15440478.2021.1973938

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.