Effect of nano titanium dioxide on mechanical properties of regenerated silk

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Abstract. Silk and spider silk have excellent mechanical properties, and their superior physical properties are closely related to their complex structure. Here, we reported the wet spinning behavior of nanomaterials mixed with silk proteins and study the effect of the different sizes of TiO2 nanoparticles (NPs) on the mechanical properties of regenerated silk. Our study retained silk protein nanofibers in CaCl2-FA solution, and optimized the wet spinning process, and produced high-quality regenerated silk fiber (RSF) with excellent mechanical properties by using TiO2 NPs. Among them, when the TiO2 NPs of 5-10 nm was 4 mg/g(TiO2 NPs/Silk Fibroin(SF)), the tensile stress of the RSF reached at 312.44 ± 20.31 MPa, which is 113.11% higher than the control group; the tensile strain reached 46.55 ± 2.76%, which is 152.85% higher than the control group. And the secondary structure conformation of RSF tended to transform from silk I to silk II, and its β-sheet content was 47.27 ± 0.25%. We found that the mechanical properties of RSF decreased with the size of TiO2 NPs increased. This may be due to the smaller the size of TiO2 NPs and the larger the specific surface area, which can provide more binding sites between TiO2 NPs and silk proteins, and also transformed the RSF conformation from silk I to silk II. The characteristics of this spinning method are simple operation, environmentally friendly, low cost, and large-scale industrialization potential. The method of producing RSF has great potential in the production of textiles and multifunctional materials.

1. Introduction
In the past decades, The TiO2 nanoparticles (NPs) has attracted great interest as one of the most promising materials. TiO2 NPs are colorless, non-toxic, odorless, small-sized particles with small size effects, surface effects, and excellent optical, electrochemical, and catalytic properties [1], which can improve the thermal properties and mechanical properties. In the development of new textile materials, TiO2 NPs can improve the anti-ultraviolet, infrared, abrasion resistance, antistatic, antibacterial, self-cleaning functions of fiber materials, and have broad application prospects [2-4]. Some researchers have mixed TiO2 NPs with other materials to improve the mechanical strength of materials, so they are widely used in the field of improving the mechanical strength of biochemical materials.
In recent years, in order to improve the performance of regenerated silk fibers (RSF), a series of attempts have been made, including the dissolution of silk, the type and concentration of regenerated silk protein dope, spinning conditions such as coagulation baths and post processing. However, mixing silk solution with other materials (such as nanomaterials) is a commonly method to improve the properties of silk [5]. Chen et al [6] prepared a PSA/TiO$_2$ NPs composite spinning solution blending polysulfone amide (PSA) with TiO$_2$ NPs, and prepared composite fibers by wet spinning process. The results show that a small amount of TiO$_2$ NPs can be uniformly dispersed in the PSA matrix without significantly changing the molecular structure and chemical composition of the PSA. The essence of silk is fibrin, because of the excellent mechanical properties and biological affinity of natural silk, silk is widely used in traditional textile industry and biomedical fields. However, since people cannot modify the special physiological structure of the silkworm itself, it is difficult to further improve the mechanical properties of natural silk [7]. There are two main ways to improve the mechanical properties of silk. One is the endogenous method, which allows silkworms to directly spit out silk with enhanced mechanical properties, including genetic modification and feeding methods. However, due to the toxicological effects of materials on organisms, people cannot feed a large amount of materials to feed silkworms, which also limits the performance of silk to a certain extent [8-10]. The second is an exogenous method, which is prepared artificial regenerated yarn by wet spinning or dry spinning, and changed the spinning conditions or added other materials to improve the mechanical strength of the rayon. In recent years, with the development of people's production and life, the demand for the manufacture of functional materials required in specific environments had continuously increased, which makes it particularly urgent to artificially simulate the spinning process of living organisms to prepare regenerated fibers.

In this paper, CaCl$_2$-Formic Acid (FA) was used to prepare silk protein solution. The system can directly dissolve silk in CaCl$_2$-FA at room temperature, and the silk was nanofiber-like in CaCl$_2$-FA solution [11]. On this basis, TiO$_2$ NPs blend solution was prepared for the modification of RSF. In order to make full use of the functions of TiO$_2$ NPs, we studied the effect of the size effect of TiO$_2$ NPs on the mechanical properties and structure of the modified regenerated silk, laying a certain foundation for the further development of the theoretical basis of new materials for RSF.

2. Materials and methods

2.1. Materials and reagents
Mulberry silk was from Institute of Sericulture, Chinese Academy of Agricultural Sciences; sodium carbonate, formic acid and anhydrous calcium chloride were both from Shanghai Sinopharm Reagent Co., Ltd; TiO$_2$ NPs was from Shanghai Aladdin Biotechnology Co., Ltd.

2.2. Equipment and instrument
Collecting type constant temperature heating magnetic stirrer (DF-101S, Gongyi Yingying Yuhua Instrument Factory, China), Oven (DHG-9246A, Shanghai Rongfeng Scientific Instrument Co., Ltd., China). Mechanical tensile tester (3343, Instron Corporation, USA). Microscope (ECLIPSE E100, Nikon Instruments Co., Ltd., Japan).

2.3. Wet spinning
The experimental device in this article is built independently. All experiments were performed at room temperature. The silk fibroin (SF) solution was extruded through a syringe needle into a 75% ethanol coagulation bath using a syringe at room temperature, and then collected by stretching on a runner. Then, the regenerated fiber obtained on the collector was immersed in ethanol for 30 minutes to further solidify and remove FA and Ca$^{2+}$, and then the soaked regenerated silk was taken out and air-dried. Due to the high specific surface area and high surface energy, small particles of TiO$_2$ nanoparticles (NPs) can easily form large-sized agglomerates, it is difficult to disperse in liquid media in the agglomerated state, and TiO$_2$ NPs is difficult to fully exert its characteristics [12]. Therefore,
before using TiO$_2$, it must be dispersed by ultrasound. The agglomerates of TiO$_2$ NPs can be dispersed in a solvent to destroy the agglomerated structure so that it can be uniformly dispersed in the solvent, thereby improving the stability of the TiO$_2$ dispersion. Prepare a 15% (w/v) SF solution by method 2.3, 0.4, 0.8, 2.0, 4.0, 8.0, 16.0 mg/g of TiO$_2$ NPs powders of different sizes (5-10, 25, 40, 60, 100 nm) were added during the dissolution process. Spinning was performed using the method of 2.4. The spinneret was 27G (with an inner diameter of 0.21 mm) and the advance speed was 2 ml/h.

![Figure 1. TEM imaging of TiO$_2$ NPs with different sizes.]

2.4. TEM imaging
For electron microscope TEM imaging, after dispersing nanometer TiO$_2$ of various sizes in water (1 μg/ml), used a transmission electron microscope (T-120, Netherlands) to observe its distribution.

2.5. Mechanical test
The diameter of the regenerated filament was measured through a microscope (Eclipse E100. Japan), and a single fiber test was performed using a mechanical test instrument (Instron 3343. USA) with an 8 N load cell (temperature 20 ± 0.5 °C; relative humidity 40 ± 5%) Gage length was 6mm, stretching rate was 2 mm/min, and initial tension was 0 cN. 20 measurements were performed on each sample during the test.

2.6. FTIR test
The structural change of wet-spun fibers was analyzed by Fourier transform infrared spectroscopy (FTIR) using a microscope (Bruker Hyperion) and a Fourier transform infrared spectrometer (Bruker 66V) in the spectral range of 400-4000 cm$^{-1}$.

3. Results and discussion

3.1. Mechanical behavior
In this experiment, we selected five TiO$_2$ NPs materials of different sizes as shown in Figure 1, which are 5-10, 25, 40, 60 and 100 nm, respectively. We found that the TiO$_2$ NPs have a uniform morphology and meet the requirements of various sizes. As shown in Figure 2, with the nanomaterial blend content increased, the mechanical properties of RSF fibers gradually improved, and the experimental groups reached the optimum at 4 mg/g (TiO$_2$ NPs/SF), with the blend content continued to increase, the mechanical properties of RSF fibers gradually decreased. With the nano material size decreased, the mechanical properties of RSF showed a general trend of improving gradually. The 5-10 nm group had the largest mechanical properties, and its tensile stress and strain were 312.44 ± 20.31 MPa and 46.55 ± 2.76%, respectively, which were 113.11% and 152.85% higher than the control of
146.61 ± 20.41 MPa and 18.41 ± 1.31%, respectively. As the size gradually increased, there was no significant effect on diameter of the RSF, but the mechanical properties were gradually reduced, and the mechanical properties of 5-10 nm group have been significantly improved. The resulting blended RSF fiber exhibited significantly greater tensile strain and strength than the control RSF fiber, because the control RSF fiber could not reach the molecular chain orientation level of the natural silk, and the blended might make the original secondary structure of RSF similar to the natural silk. It is possible that the smaller size of TiO2 NP has a larger specific surface area, which is more likely to form a stable structure when combined with silk proteins. This will cause the conformation of the protein to change, thereby increasing the tensile stress and tensile strain of the regenerated silk and giving them good mechanical properties.

3.2 Secondary structure

Recent studies have found that the infrared spectrum test is performed in an open environment, and the sample cannot be placed in a vacuum state during the measurement. This also causes water vapor interference in the amide I and amide II regions, the amide III region was not affected by water vapor. In this paper, we analyzed the amide III region (1200-1300 cm⁻¹) to reduce the effect of water vapor on the FTIR spectrum. As shown in Figure 3, by analyzing the amide III region data of the FTIR spectrum, we found that the random coil is the main structure of RSF, whether it was the control group or the experimental group.

After adding TiO2 NPs, the conformation of RSF changed from Silk I to Silk II with the blend content increased, and the β-sheet gradually increased (Figure 3). When the blend content was 4.0 mg/g (TiO2 NPs/SF), the β-sheet structure with a highly ordered arrangement was the highest content at this time, which was consistent with the change in the mechanical properties of regenerated fibers found above. After the TiO2 NPs was added in silk fibroin solution, on the one hand, TiO2 NPs have fully diffused in the solution, and there was no agglomeration phenomenon to maintain its own size; on the other hand, this phenomenon also promoted the good combination of TiO2 NPs and silk fibroin, thus changed the structure of the protein itself.

When the TiO2 NPs blend content of 5-10 nm group was 4.0 mg/g, the β-sheet content was 47.27 ± 0.25%, which was an increase of 27.53% compared with 37.05 ± 0.85% in the control. When the blend content of TiO2 NPs/SF continued to increase, TiO2 NPs will aggregate in the solution, making their size far beyond the nanometer range, which is not conducive to binding with silk fibroin, and it cannot modify the protein, and the protein structure is also difficult to change. Therefore, the structure of the protein changed from Silk II to the more disordered original structure of Silk I, and the mechanical properties of the regenerated fiber began to decrease. These results indicated that TiO2 NPs have modified the silk fibroin, and the structure of the protein itself also changed, which directly promoted the mechanical properties of the RSF fiber within a certain blending range. We found that with the smaller-sized TiO2 NPs were added, the conformation of the RSF fiber changed from Silk I to Silk II. This may be related to the change in the structure of the nanomaterial itself as its size decreased. This phenomenon was first found on Fe2O3 materials. When its size was reduced to less than 30nm, its equilibrium crystal structure changed from corundum crystal structure to inverse spinal cord type. In summary, within a certain blending range, nano-sized TiO2 NPs with smaller size will make composite regenerated fibers have better mechanical properties. This may be due to the combination of silk fibroin and nanomaterials and their own structure. With this change, the regenerated fibers have different mechanical properties, but the TiO2 NPs with different sizes and blend content have no significant effect on the diameter of the regenerated fibers.

Figure 4 showed the FTIR spectrum of RSF fibers at 1100-1700 cm⁻¹. Random coils and spirals, β-sheet showed absorption peaks at 1235 cm⁻¹ (amide III) and 1265 cm⁻¹ (amide III), and β turns showed absorption peaks at 1680 cm⁻¹ (amide I) [13-14]. These peaks also showed that the RSF fiber structure was similar to control silk.
**Figure 2.** Effect of the size and concentration of nanomaterials on the mechanical properties of RSF

(a, b, c, d, e are 5-10 nm, 25 nm, 40 nm, 60 nm, 100 nm respectively).

**Figure 3.** Analysis result of amide III region of FTIR spectrum of RSF fiber

(a, b, c, d, e are 5-10 nm, 25 nm, 40 nm, 60 nm, 100 nm).
The size of a nanomaterial determines its surface curvature and also affects its surface area. The size and curvature of nanoparticles affect not only the amount of protein bound to nanoparticles, but also the type of protein bound to nanoparticles [15-16]. Therefore, the size is an important factor that affects the type and amount of proteins that combined with nanomaterials. The composition and structure of the protein in the protein corona are very important, when the size of the nanomaterial is close to that of the protein, high-curvature nanomaterials reduce protein interactions in protein corona to keep protein structure stable [17]. Studies have shown that when Polyacrylic acid-gold nanoparticles (PAA-GNPs) with different sizes interact with human fibrinogen, the larger particles have a slower binding speed with proteins, and the fibrinogen molecules were bound in a positive cooperation manner. When the diameter was less than 10 nm, a protein molecule can accommodate 2 nanoparticles [18]. Lynch et al. [19] studied the effect of the size and surface area of a polymer nanomaterial called NIPAM/BAM (50:50) on protein adsorption. They found that nanoparticles of the same mass and larger surface area bind more serum proteins, that is, smaller nanoparticles bind more proteins.

This series of studies showed that the particle size of the nanomaterial determines its effect on the conformational change of the protein when it is combined with the protein, and also determines the degree of change in the aggregation state before and after the action. The reason why nanoparticles can improve RSF may be that the smaller the size of TiO$_2$ NPs and the more edges, it can provide more key binding points for forming RSF fibers, and it can bind to proteins more easily. This caused the conformation of the protein to change from an irregular Silk I structure to a more regular, β-sheet-rich Silk II structure, thereby improving the mechanical properties of RSF. These conclusions have enriched our understanding of regenerated silk and its structure. The combination of nanomaterials and proteins is determined by the combined action of multiple forces, not just by a single factor. This is a complex process that requires further research to clarify this mechanism.

4. Conclusion

First, by simple controlling the silk fibroin (SF) solution concentration, spinneret diameter and injection speed, we found that silk spinning was most suitable with a 15% (w/v) SF solution, a spray with an inner diameter of 0.21 mm was used at injection speed of 2 ml/h. Then, a blended spinning dope of regenerated silk protein TiO$_2$ nanoparticles (NPs) was prepared by the blended method, and further wet-spun to obtain regenerated silk fiber (RSF) of blending TiO$_2$ NPs. Mechanical tests have shown that when blended with smaller of TiO$_2$ NPs(5-10 nm) and the blend content was 4 mg/g(TiO$_2$ NPs/SF), the tensile stress of RSF fibers was better, reaching 312.44 ± 20.31 MPa, and the strain was 46.55 ± 2.76%, which were 113.11% and 152.85% higher than the control group, respectively, and had good mechanical properties. It was shown that the addition of TiO$_2$ NPs enhanced the mechanical
properties of the hybrid fibers, especially for smaller sizes. Then, through infrared analysis of RSF fibers, it was found that the conformation of the RSF secondary structure had a tendency to change from Silk I structure to Silk II structure after blending TiO$_2$ NPs. Its β-sheet content reached a maximum of 47.27 ± 0.25%, which was an increase of 27.53% compared with the control group. We found that the mechanical properties of RSF fibers with small size of TiO$_2$ NPs were better, 5-10 nm group was 36.57% higher than 100 nm group, and β-sheet content was increased by 14.34%. The research of the size effect of regenerated fibers with nanomaterial has great potential in the production of textiles and multifunctional materials.

Acknowledgement
The authors gratefully acknowledge financial support from the Jiangsu Specially Appointed Professor Program Sujiashoshi ([2015]17).

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