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Short communication

Mechanical properties of electrospun collagen–chitosan complex single fibers and membrane

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ABSTRACT

Collagen and chitosan blends were fabricated into ultrafine fibers to mimic the native extracellular matrix (ECM). So far less mechanical property investigation of electrospun fibers has been reported because of the small dimensions of micro and nanostructures that pose a tremendous challenge for the experimental study of their mechanical properties. In this paper, the electrospun collagen–chitosan complex single fibers and fibrous membrane were collected and their mechanical properties were investigated with a nano tensile testing system and a universal materials tester, respectively. The mechanical properties were found to be dependent on fiber diameter and the ratio of collagen to chitosan in fibers. Fibers with a smaller diameter had higher strength but lower ductility due to the higher draw ratio that was applied during the electrospinning process. For the electrospun single fibers, the fibers demonstrated excellent tensile ductility at chitosan content of 10% and 20% and the highest tensile strength and Young's modulus at chitosan content from 40% to 60%. For the electrospun fibrous membrane, the ultimate tensile strength of the fibrous membrane decreased with the increase of chitosan content in fibers and the trend in the ultimate tensile elongation is similar to that of the single fiber.

1. Introduction

The objective of tissue engineering is to regenerate native tissues from living cells to replace defective or lost tissues and organs. Its typical method is to produce synthetic tissues by incorporating isolated living cells into porous scaffolds and create conditions for cells to proliferate, organize and develop into the desired tissues or organs. The challenge and difficulty of tissue engineering is how to create an excellent scaffold. Here, the scaffold as analogue of the native extracellular matrix (ECM) found in tissues not only provides initial biomechanical substrate for the cells but also regulates their proliferation, differentiation and morphogenesis until cells produce an adequate ECM of their own. Such a scaffold, therefore, needs to be developed for *in vitro* tissue reconstruction as well as for cell-mediated tissue regeneration *in vivo*.

The native ECM is a molecular complex made up of proteins and polysaccharides and comprises 3-dimensional hierarchical fibrous structures of nanometer scale dimensions [1]. It can be mimicked from the components and the microstructure by fabricating proteins–polysaccharides complex ultrafine fibers to develop excellent scaffold for tissue engineering.

Collagen is the most abundant structural protein found in the animal body such as in skin, tendon, cartilage and bone [2]. It is also the principal structural elements of the native extracellular matrix (ECM). Owing to a wealth of merits such as its biological origin, non-immunogenicity, excellent biocompatibility and biodegradability, collagen has been widely used as biomaterials in the pharmaceutical and medical fields as sealants for vascular grafts [3], carrier for drug delivery [4], dressings for wound healing [5] and tissue engineering scaffold [6]. Chitosan is only a basic natural polysaccharide derived from chitin, which is the second natural resource only inferior to the cellulose. Because of its abundant production in nature, excellent biocompatibility, appropriate biodegradability, excellent physico-chemical properties, and commercial availability at relatively low cost, it has also been widely used as biomaterials in the pharmaceutical and medical fields [7,8].

Chitosan can form complex with collagen [9,10], which can cause the complementary performance and synergy. The collagen–chitosan complex is expected to mimic the components of the native ECM in designing tissue-engineering scaffolds. The collagen and chitosan blends have also been widely used as biomaterials in the pharmaceutical and medical fields recently [11,12]. They were fabricated into

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fibers and porous scaffolds in the macroscopic scale by solvent casting [13], wet/dry spinning [14] and freeze drying [15]. However, the native ECM is in the nanoscale fibrous network structure [16]. Recently, it has been found that the nanofibrous scaffolds can improve the regeneration of tissues *in vitro* including bone, cartilage, cardiovascular tissue, nerve, and bladder and minimize the scars in the regenerated tissues as human cells can attach and organize well around the fibers with diameters smaller than those of the cells [17]. At present, electrospinning technique has been used as an efficient processing method to manufacture nanofibrous structures for a number of applications [18–20]. Recently, the collagen–chitosan complex nanofibers have been prepared by electrospinning to mimic the native ECM from the components and the nanofibrous structure in our lab [21,22].

As a tissue engineering scaffold, the mechanical property of biomaterials is very important, for example, not only the scaffold provides initial biomechanical profile for the cells but also structural integrity is also required for the scaffold before new tissue can be formed [23]. The small dimensions of micro and nanostructures pose a tremendous challenge for the experimental study of their mechanical properties. So far less mechanical property investigations of electrospun fibers have been reported. The tensile test is a simple and reliable method for measuring their mechanical properties but it can also be difficult to implement at the micro and nanoscale. In this paper, the electrospun collagen–chitosan complex single fibers and fibrous membrane were collected and their tensile behavior was tested with a nano tensile testing system and a universal materials tester respectively.

2. Materials and methods

2.1. Materials

Collagen I (mol. wt, 0.8–1 × 10^5 Da) was purchased from Sichuan Ming-rang Bio-Tech Co. Ltd (China) while Chitosan (85%, deacetylated, M_η, about 10^6) was purchased from Ji Nan Hai-de-bei Marine Bioengineering Co. Ltd (China). Two kinds of solvents, 1, 1, 1, 3, 3, 3-hexafluoroisopropanol (HFP) from Fluorochem Ltd. (United Kingdom) and Trifluoroacetic acid (TFA) from Sinopharm Chemical Reagent Co., Ltd (China) were used to dissolve the collagen, chitosan and their blends.

2.2. Electrospinning

Collagen, chitosan and the collagen–chitosan blend with various chitosan content were dissolved in HFP/TFA (V/V, 90/10) at a concentration of 8% (g/ml). These prepared solutions were then used in the electrospinning experiments.

The electrospinning experiments were performed at room temperature. The polymer solution was placed into a 1 ml syringe with a needle having an inner diameter of 0.46 mm. A clamp connected the high voltage power supplier (which can supply positive voltage from 0–30 kV) to the needle. A piece of aluminum foil was placed at about 130 mm directly below the needle and acted as grounded collector. The polymer jets generated from the needle by the high voltage field formed the nanofibers and membrane at the grounded collector. The schematic illustration of electrospinning was shown in Fig. 1. The applied voltage and feed rate of the solution were fixed at 16 kV and 0.8 ml/h, respectively. The electrospun fibrous membrane was stored in the vacuum oven at normal room temperature.

2.3. Collection of a single fiber

A single electrospun polymer fiber is so weak that any direct touch on the fibers during manipulation can damage the fiber. As such, extreme care must be taken in collecting and testing the tensile specimens in order to avoid any damage. In this study, the specimens were collected as follows: a rectangular aluminum or copper frame was placed 60° to the horizontal surface between the spinneret and the grounded collector. During electrospinning, several strands of fibers were deposited across the frame as shown in Fig. 2. The frame (a) was attached to the aluminum frame for collection of a single fiber. The double-sided tapes on the frame allowed the fiber to be adhered to the frame. The frame (a) was then separated into the plastic frame (b) and the metal frame (c). The single fiber on the plastic frame (b) was used to perform the tensile test and the single fiber on the metal frame (c) was used to measure the diameter of the fiber.

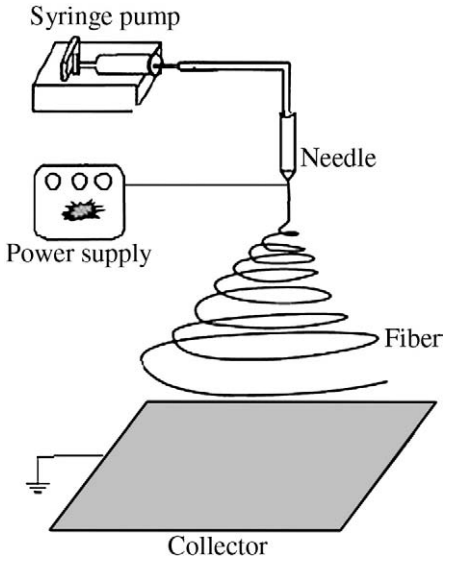


Fig. 1. Schematic illustration of electrospinning.

2.4. Scanning electron microscopy analysis

The morphologies of the electrospun fibers and membrane were observed under a Scanning Electron Microscope (SEM) (Quanta FEG 200, FEI Company, The Netherlands) at an accelerating voltage of 10 or 15 kV. Prior to scanning under the SEM, the samples were sputter coated for 90 s with gold using a JEOL JFC-1200 fine coater. Basing on the SEM photographs, the diameters of fibers were analyzed using image visualization software Adobe Photoshop.

2.5. Tensile test of a single fiber

The tensile tests and collection of fibers were carried out on the same day to minimize the effects of environment. A commercial nano tensile testing system (Nano Bionix System, MTS, TN, USA) was used to conduct the tensile test. The test was performed in ambient temperature at 20 °C and humidity of 65%. The gauge length (10 mm) of the electrospun fibers was determined by the gap between the parallel strips of the plastic frame. The samples were mounted on the nano tensile tester. The plastic frame was cut along the discontinuous lines before stretching the fiber as shown in Fig. 1. Samples were stretched to failure at a low strain rate of 1%/s. The machine-recorded data were used to plot the tensile stress–strain curves of the specimens.

2.6. Tensile test of fibrous membrane

Rectangular membrane specimens with a dimension of 10 mm × 50 mm were prepared according to the method reported in the literature [24]. First, a white paper template was cut as shown in

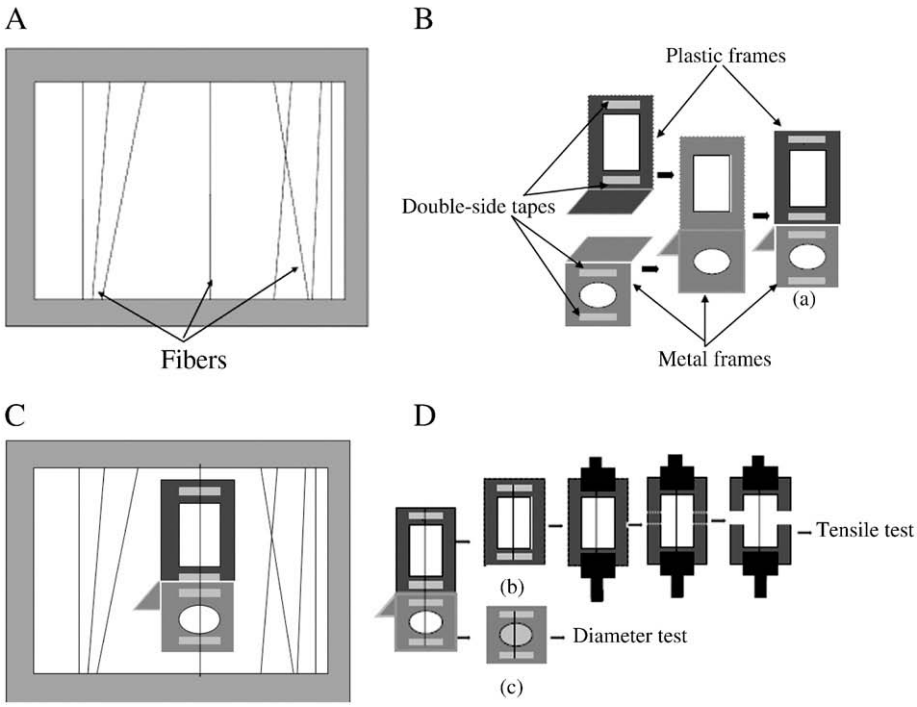


Fig. 2. Collection and tensile test of a single fiber.

Fig. 3(A), and double-side tapes were glued onto the top and bottom areas of one side. The template was then glued onto the topside of the fibrous membrane, and was cut into rectangular pieces along the discontinuous vertical lines. After the aluminum foil was carefully peeled off, single side tapes were applied onto the gripping areas as end-tabs. The resulting specimens had a planar dimension of 10 mm (width) × 30 mm (gauge length) as shown in Fig. 3(B). The specimen thicknesses were measured using a micrometer with a precision of 0.01 mm.

The tensile testing of membrane was performed using a universal materials tester (H5K-S, Hounsfield, UK) with a load cell of 50 N at ambient temperature of 20 °C and humidity of 65%. A cross-head speed of 10 mm/min was used for all of the specimens tested. The machine-recorded data were used to plot the tensile stress–strain curves of the specimens.

3. Results and discussion

3.1. Morphology of a single electrospun fiber and membrane

The collagen–chitosan complex fibers with diameter ranging from nanometer to micrometer size were produced through electrospinning. Fig. 4 showed SEM micrograph of a typical single collagen–chitosan complex fiber with chitosan content of 60% and Fig. 5 showed SEM micrographs of collagen–chitosan complex fibrous membranes. The diameter of fiber is at the scale from tens of nanometers to several micrometers. Pure collagen fibrous membrane have higher average fiber diameter (810 ± 580 nm) than that (415 ± 286 nm) of pure chitosan nanofibers. The collagen–chitosan complex fibrous membranes have different average fiber diameters with different chitosan content in fibers. They are 691 ± 376 nm, 515 ± 253 nm and 434 ± 263 nm with

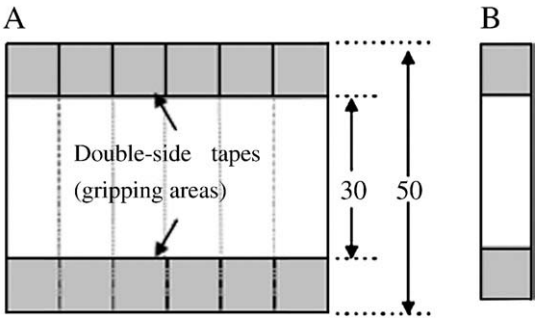


Fig. 3. A paper template model used to prepare tensile specimens of the electrospun nonwoven fiber membrane and a tensile specimen model. (A) a paper template model; (B) a tensile specimen model.

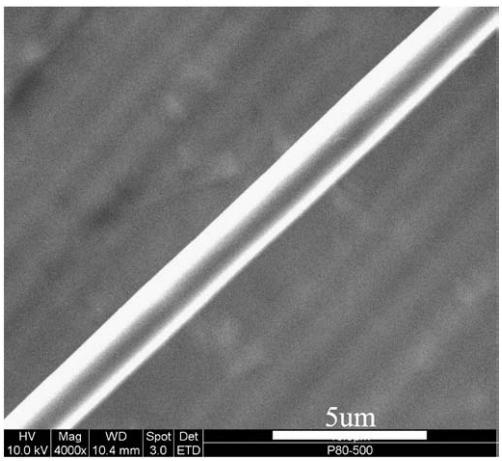


Fig. 4. SEM micrograph of a single fiber.

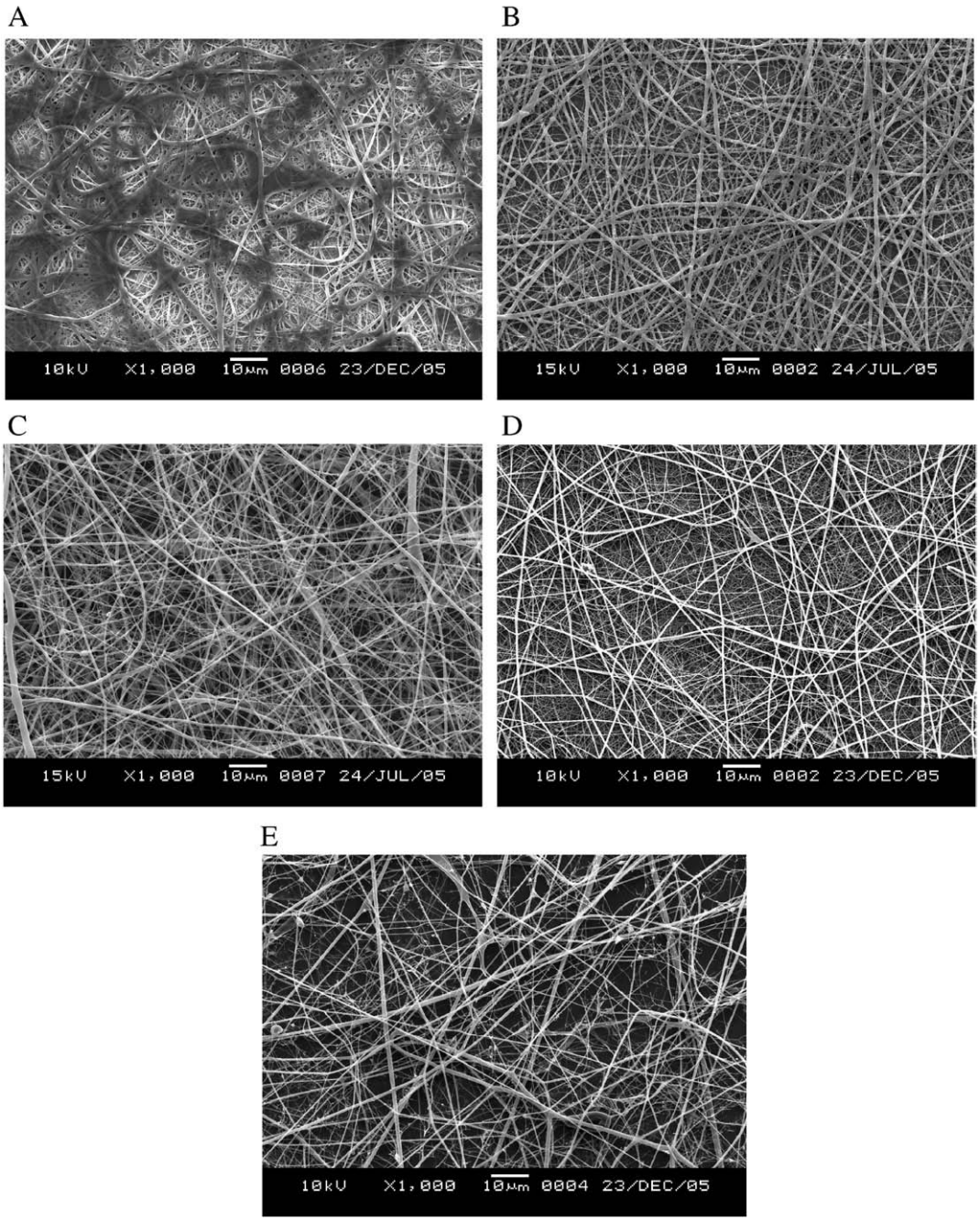


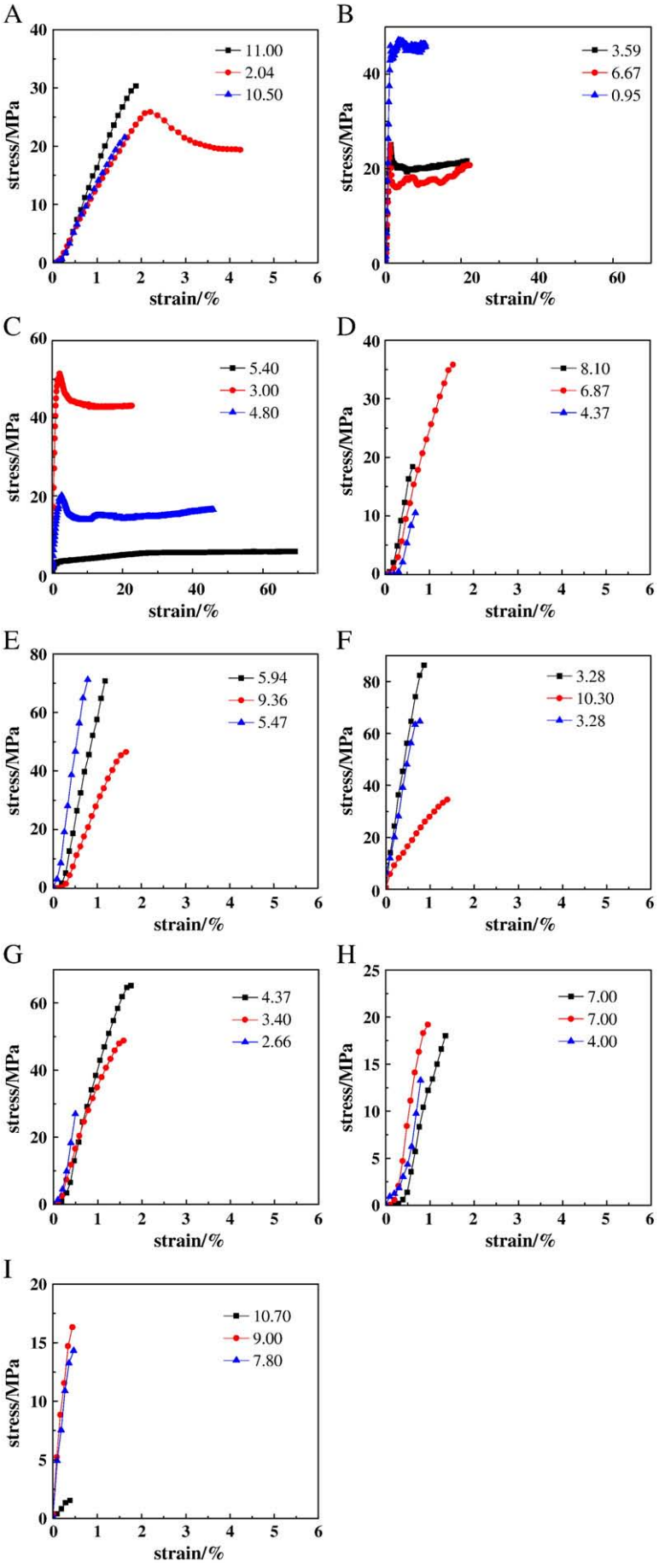
Fig. 5. SEM micrographs of fibrous membrane with different chitosan content. (A) 0%; (B) 20%; (C) 50%; (D) 80%; (E) 100%.

chitosan content of 20%, 50% and 80%, respectively. Fiber diameters decrease with the increase of chitosan content. Because applied voltage, collecting distance, solution feed rate and solution concentration were fixed, fiber diameters are mainly dependent on the ratio of chitosan to collagen. The variety of fiber diameter may be that the organic salt formed between TFA acid and the amino groups on chitosan increase the charge density of the electrospun polymer solution, which result in higher draw ratio in electrospinning process [22].

3.2. Mechanical characterization of single electrospun fiber

The tensile stress–strain curves of the collagen–chitosan complex fibers with different chitosan content and fiber diameter were shown in Fig. 6. The tensile properties of the fibers were found to vary with the fiber diameter and chitosan content.

From Fig. 6(B) or (C), all the tensile strength, yield stress and Young's modulus decreased with increase in fiber diameter to some extent whereas the strain at break was found to increase with increase in fiber diameter. From Fig. 6(A) and (D) to (I), it was found that no apparent correlation between tensile properties and fiber diameter due to poor strain at break. In conclusion, the variation of mechanical properties with the fiber diameter was observed in this study. The drawing process in electrospinning reduces fiber diameter and provides a higher molecular chain orientation along the fiber axis. Certain portions of the polymer jet could have traveled a longer distance before reaching the grounded frame since the bending instability is not a static process. The draw ratio increased as a result of



this longer distance traveled. The process of drawing usually results in higher strength and stiffness but lower ductility. The smaller fibers exhibited higher strength and lower ductility, indicating that a higher draw ratio was applied [23].

From Fig. 6(A) to (I), all the tensile properties of the fibers varied with various chitosan content. Pure collagen fibers have been broken mostly before yield point, which implies that collagen fibers are hard and brittle. With a little addition of chitosan at 10% of the electrospun blends, the fibers become tough. The fiber is elongated to reach a larger strain at about 20% and a clear yield point appears on the tensile stress–strain curve. That means that chitosan is a plasticizer in the collagen fibers and weakens the intermolecular interaction among collagen molecule chains, which make the molecular chains slide more easily under the tensile force. With the further increase of chitosan content to 20%, the plasticizing improves and the break strain reaches about 40%. When chitosan content was increased to 30%, the tensile stress–strain curves of the fibers showed brittle mechanical behavior again. The reason can be that chitosan is enough to isolate collagen in collagen continuous phase. The bicomponent structure gives the brittle mechanical behavior of the fibers. With the further increase of chitosan content to 40%, 50% and 60%, that is, when chitosan and collagen have near content in the fibers, strong intermolecular interactions such as hydrogen bond, even ionic bond can occur between collagen and chitosan molecules [22]. The interaction causes the break strength to increase to more than 60 Mpa, which is 3 times as high as that of pure collagen fibers. When chitosan content increases to more than 70% in the electrospun blends, chitosan as a continuous phase controls the mechanical properties of the fibers and shows brittle tensile stress–strain behavior with less break elongation, even less than 1% for the fiber at chitosan content of 80%. No single fiber can be collected when chitosan content increases to 90% and 100%.

The dependence of the average ultimate tensile strength, the average ultimate tensile elongation and the average Young's moduli on chitosan content in the fibers were summarize in Fig. 7. The highest average ultimate tensile strength occurs when chitosan content ranges from 40% to 60%. Pure collagen fibers have lower Young's modulus. With the addition of chitosan in the electrospun blends, the Young's modulus of fibers increases. The collagen–chitosan complex fibers also give the highest Young's modulus when chitosan content is located at 40% to 60%. The reason is due to the strong intermolecular interaction between collagen and chitosan when chitosan is about equal to collagen in weight. The average ultimate tensile elongation increases with a little addition of chitosan in the electrospun blends and reaches the highest value at chitosan content of 20%. The reason is that a small amount of chitosan acts as a plasticizer in the collagen fibers, which weakens the intermolecular interaction among collagen molecule chains and makes the molecular chains slide and elongate more easily under the tensile force. So the mechanical properties of collagen–chitosan complex fibers can be improved to some extent by adjusting the ratio of chitosan to collagen in the fibers.

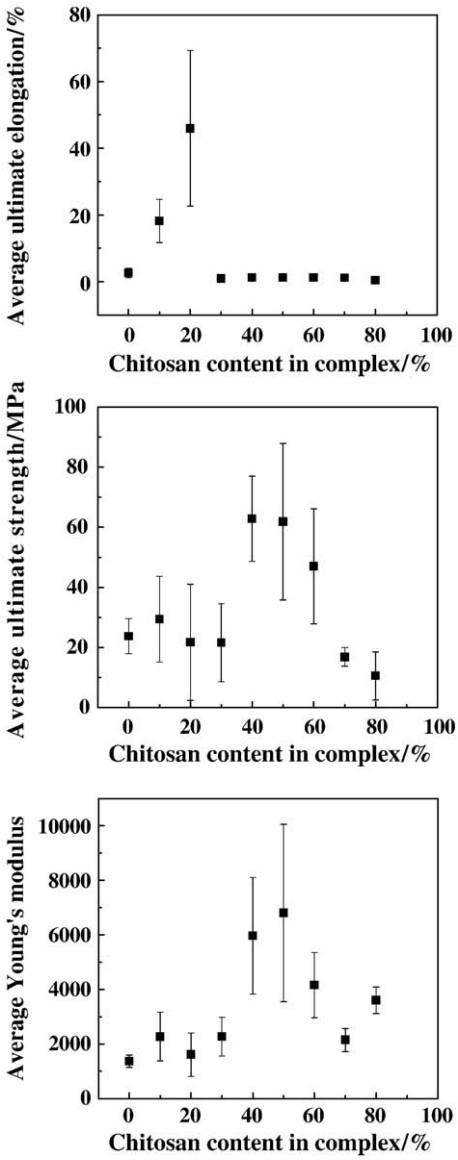


Fig. 7. Tensile properties of electrospun collagen–chitosan complex single ultrafine fibers with different chitosan contents.

3.3. Mechanical characterization of fibrous membrane

The tensile stress–strain curves of the collagen–chitosan fibrous membrane with different chitosan content were given in Fig. 8. Comparing with the tensile stress–strain curves of the single fibers in Fig. 6, there are some similarities between them, such as, pure collagen fibrous membrane has better tensile stress–strain behavior than chitosan fibrous membrane, the highest ultimate tensile elongation of the collagen–chitosan fibers can be seen at chitosan content of 20% (Fig. 8B).

Collagen fibrous membrane shows tough and flexile tensile stress–strain behavior as shown in Fig. 8(A), its stress–strain curve shows yield point at about 4.5 MPa and break elongation at about 12%. Different from the tensile behavior of collagen fibrous membrane, the tensile stress–strain curve of chitosan fibrous membrane does not show yield point and gives much lower value on both stress and strain (Fig. 8E).

With the addition of a little chitosan (20%) in the electrospun blends, the collagen–chitosan fibrous membrane becomes softer and flexile and break elongation reaches about 75% (Fig. 8B). With the further increase of chitosan content to 50% of the electrospun blends, the tensile stress–strain behavior of fibers is similar to that of pure collagen fibrous membrane again, but break stress reduces a lot to be only about 1.5 MPa (Fig. 8C). When chitosan content is higher than 50% and at about 80%, both the break stress and break strain of fibers reduce further to about 0.8 MPa and 10%, respectively (Fig. 8D).

Fig. 6. Tensile stress–strain curves of electrospun collagen–chitosan complex fibers with different diameter in different chitosan content. (A) 0%; (B) 10%; (C) 20%; (D) 30%; (E) 40%; (F) 50%; (G) 60%; (H) 70%; (I) 80%. (The legends in the figures represent the diameters of fibers in um).

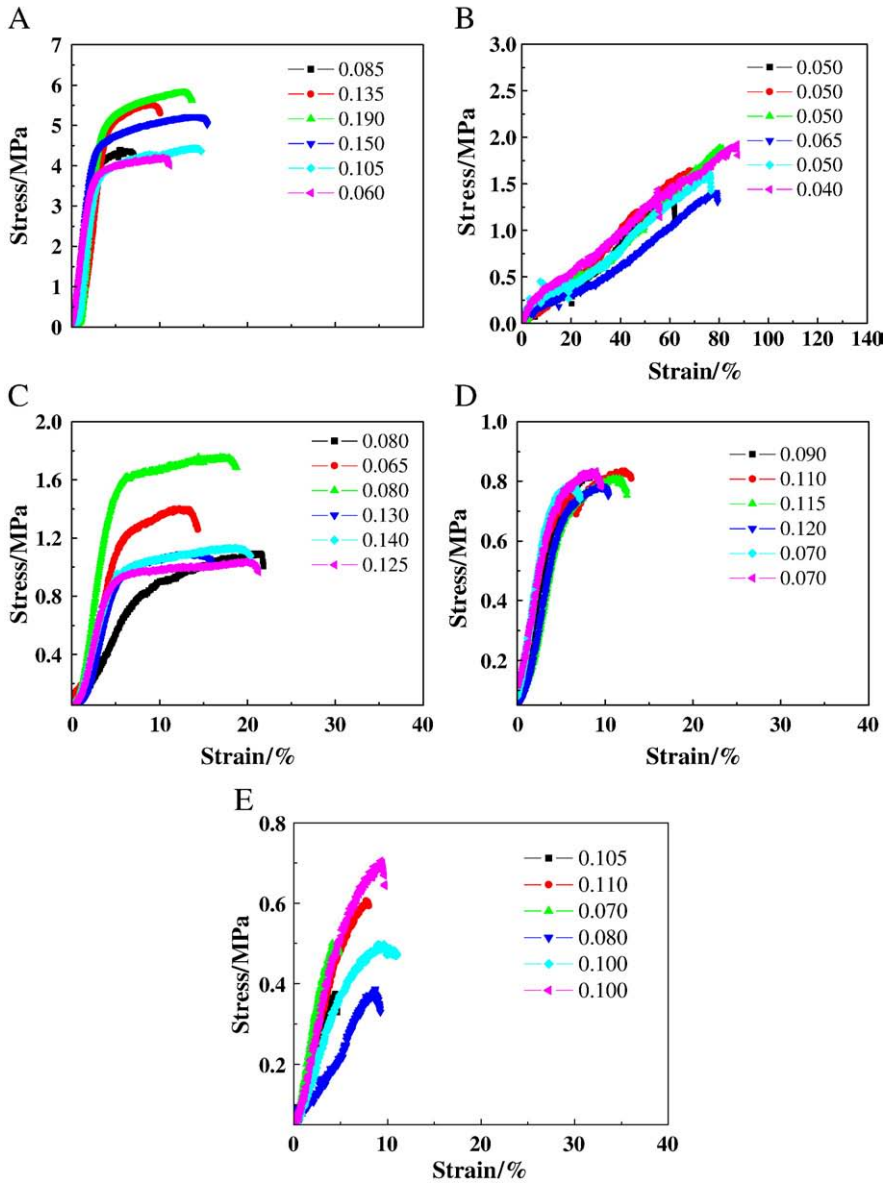


Fig. 8. Tensile stress–strain curves of electrospun collagen–chitosan complex membrane with various chitosan contents. (A) 0%; (B) 20%; (C) 50%; (D) 80%; (E) 100%. (The legends in the plots represent different thicknesses of the membranes in mm).

The dependence of the average ultimate tensile strength, the average ultimate tensile elongation and the average Young's moduli of fibrous membrane on chitosan content in the fibrous membrane were summarized in Fig. 9.

From Fig. 9, the average ultimate tensile elongation has the similar results with that of single fibers and the average ultimate tensile strength decreases with the increase of chitosan content in the fibers. The average Young's moduli of fibrous membrane also decrease with the increase of chitosan content in the fibers, but the fibrous membranes with chitosan content of 20% have lowest Young's moduli due to the plasticizing of chitosan mentioned previously. Comparing with the average tensile strength and the average Young's module of a single fiber, the fibrous membrane has the much lower tensile strength. The reason is due to the different stretching and failure mechanism between the single fiber and fibrous membrane. The mechanical properties of fibrous membrane depend on both the mechanical properties of the single fibers making up the membrane and the structure of membrane. If the fibers are aligned in fibrous membrane, the tensile behavior of the fibrous membrane is anisotropic; if the fibers are collected at random in fibrous membrane, the tensile behavior of the fibrous membrane is isotropic. All fibrous membranes here are isotropic. The mechanical properties of fibrous membrane still depend on how tight the fibers are packed. In the experiments, pure collagen fibrous membrane is found to be packed more tightly than pure chitosan fibrous membrane, which can be due to the different effect of deposition and solvent volatilization during electrospinning process. So collagen fibrous membranes give the higher tensile strength and Young's module than chitosan fibrous membrane. With the increase of chitosan content in the fibrous membrane, the collagen–chitosan fibrous membrane also becomes less and less compact, even though it could be a little confusing. Here, the tight degree of the fibrous membrane packed has become the main factor in the break of fibrous membrane with most chitosan in the fibrous membrane. All these result in the decrease of the ultimate tensile strength and Young's module of fibrous membrane with the increase of chitosan in the fibrous membrane.

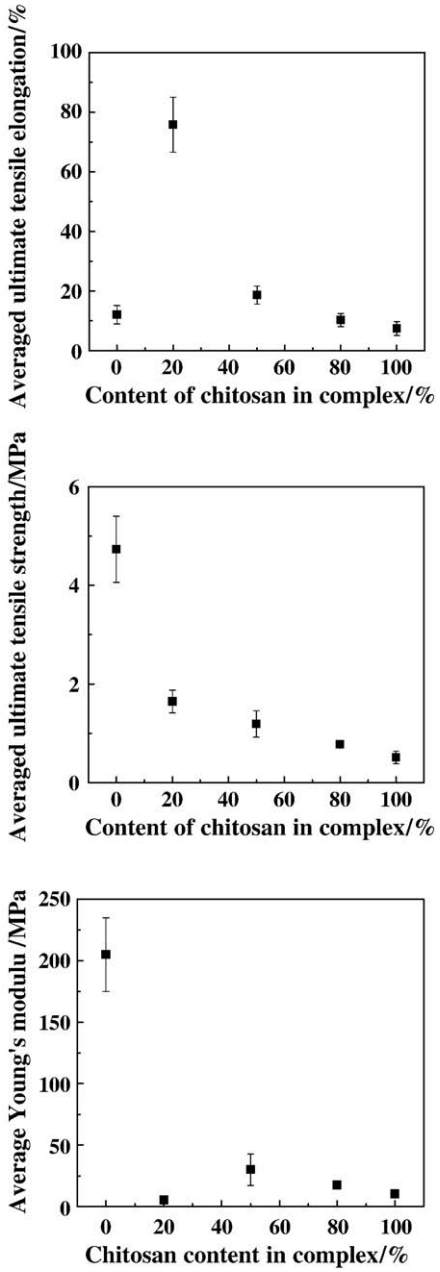


Fig. 9. Tensile properties of electrospun collagen–chitosan complex fibrous membrane with different chitosan content.

Comparing Fig. 9 with Fig. 7, though both the fibrous membrane and the single fiber have the largest ultimate tensile elongation at chitosan content of 20%, the fibrous membrane has the larger ultimate tensile elongation than the single fiber. It is easy to understand it, when the fibrous membrane is stretched, even though a single fiber making up the fibrous membrane has been broken, the fibrous membrane can still keep the shape and continuously be elongated under the tensile force until all the fibers making up the membrane have been broken.

4. Conclusion

The tensile behavior of electrospun collagen–chitosan complex single fibers and fibrous membrane was investigated. The mechanical properties were found to be dependent on fiber diameter. Fibers with a smaller diameter had higher strength but lower ductility due to the higher draw ratio that was applied. In addition, it was found that the ratio of collagen to chitosan in fibers would affect the tensile behavior of the fibers and the fibrous membrane. For the electrospun single fibers, the fibers demonstrated excellent tensile ductility at chitosan content of 10% and 20% and the highest tensile strength and Young's modulus at chitosan content from 40% to 60%. For the electrospun fibrous membrane, the ultimate tensile strength of the fibrous membrane decreased with the increase of chitosan content in spun fibers and the trend in the ultimate tensile elongation is similar to that of the single fiber. This interesting observation could be of importance to the design of tissue engineering scaffolds, which will be helpful for producing fibers with the desired mechanical properties for various tissue engineering scaffolds. Further studies are under way to optimize the electrospun fibers as well as to understand the interaction between cells and the synthetic nanofibrous extracellular matrix in order to develop a perfect tissue engineering scaffold.

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