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Robust Biological Fibers Based on Widely Available Proteins: Facile Fabrication and Suturing Application

Jinrui Zhang, Jing Sun, Bo Li, Chenjing Yang, Jianlei Shen, Nan Wang, Rui Gu, Daguang Wang,* Dong Chen, Honggang Hu,* Chunhai Fan, Hongjie Zhang, and Kai Liu*

Lightweight and mechanically strong protein fibers are promising for many technical applications. Despite the widespread investigation of biological fibers based on spider silk and silkworm proteins, it remains a challenge to develop low-cost proteins and convenient spinning technology for the fabrication of robust biological fibers. Since there are plenty of widely available proteins in nature, it is meaningful to investigate the preparation of fibers by the proteins and explore their biomedical applications. Here, a facile microfluidic strategy is developed for the scalable construction of biological fibers via a series of easily accessible spherical and linear proteins including chicken egg, quail egg, goose egg, bovine serum albumin, milk, and collagen. It is found that the crosslinking effect in microfluidic chips and double-drawn treatment after spinning are crucial for the formation of fibers. Thus, high tensile strength and toughness are realized in the fibers, which are comparable or even higher than that of many recombinant spider silks or regenerated silkworm fibers. Moreover, the suturing applications in rat and minipig models are realized by employing the mechanically strong fibers. Therefore, this work opens a new direction for the production of biological fibers from natural sources.

functionalities due to the protein's hierarchical structures, such as α -helix or β -sheet.^[4,5] The superior mechanical properties of those silks have inspired scientists to create biomimetic protein-based fibers for various biomedical applications.^[6,7] To date, many groups have constructed biological fibers based on the regeneration of silkworm proteins and recombination of spider silk proteins.^[8–11] However, by the two methods, the hierarchical structure of proteins cannot be preserved well or some functional protein domains are absent.^[12–14] Consequently, most of the existing engineered protein fibers show weak mechanical performance in comparison to natural spider silk or silkworm fibers. Furthermore, the impossibility for farming spiders and the high cost for producing recombinant spider silk proteins limit their massive production and applications. Meanwhile, some other fibrous proteins including collagen,^[15,16]

1. Introduction

In nature, structural protein is one of the main components for the formation of high-performance biomaterials, such as spider silks and silkworm fibers.^[1–3] Those natural fibers exhibit extraordinary mechanical properties together with sophisticated

elastin,^[17] phage virus,^[18] and hagfish^[19] have been investigated for fiber production. However, the mechanical strengths of those artificial fibers are not strong as well. Thus, it is still challenging to construct strong fibers from fibrous proteins. This also reminds us to develop new structural proteins and efficient spinning technology to fabricate biological fibers. Especially,

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the fabrication of fibers with robust mechanical behaviors by employing cheap and easily accessible proteins from natural resource is an attractive goal.

It is known that fibrous proteins consist of rigid-folded domains (α -helix or β -sheet) together with flexible unfolded structures.^[14] In general, the rigid domains and flexible structures in the fibrous proteins are responsible for the resulting fiber's stiffness and toughness, respectively. On the contrary, unlike those classic natural fibrous proteins, globular proteins are neither rigid nor flexible and thus are not regarded as a good choice for the fabrication of robust fibers.^[20] In addition, due to the spherical structure, the hydrophobic units of globular proteins are encapsulated by the hydrophilic residues ($-\text{NH}_2$, $-\text{SH}$, $-\text{COOH}$, etc.), leading to the weakened supra-molecular interactions between the protein particles. As a result, plenty of defects among proteins might be produced in the fiber spinning process, finally leading to the weak mechanical performance.^[21,22] Thus, the construction of mechanically strong fibers through appropriate globular proteins by a facile strategy remains a great challenge.

Recently, microfluidic spinning is becoming one of the most attractive methods to fabricate protein fibers.^[23–26] Other technique such as electrospinning, it will destroy protein folded structures due to the applied high voltage, thus decreasing fibers' mechanical performance. Microfluidics are simple and cost-effective, allowing the construction of various continuous functional microfibers.^[27–29] In this method, the convenient control of spinning parameters, including flow rates, channel size and types, and shear force, is important to improve molecular structure and orientation of produced fibers. Furthermore, the mass production of protein fibers with diverse microstructures and uniform size can be easily realized by microfluidics. Therefore, it is meaningful to develop a microfluidic strategy to fabricate protein-based fibers.

Herein, we present a facile microfluidic method for the scalable production of biological fibers based on a series of spherical and linear proteins including chicken egg, quail egg, goose egg, bovine serum albumin (BSA), milk, and collagen. It is found that the crosslinking of spherical proteins by glutaraldehyde (GA) and double-drawn processing are crucial to increase the mechanical performance of the protein fibers. In particular, the obtained fibers from BSA spherical protein exhibited extraordinary mechanical performance, which is comparable or even higher than the fibers originated from spider silk or silkworm proteins. Furthermore, we demonstrated that the as-spun fibers are promising for suturing skin and organs in rat and minipig models. Therefore, this work suggests that widely available proteins in nature are promising to fabricate robust fibers for future biomedical applications.

2. Results and Discussions

2.1. General

As illustrated in **Figure 1**, a series of widely available proteins including chicken egg, quail egg, goose egg, BSA, milk, and collagen were used to produce continuous protein fibers by a microfluidic device. In a typical experimental setup, the microfluidic device was constructed by coaxially assembling two tapered glass capillaries on a glass slide, featuring the outer inlet channel for the protein solution and the inner inlet channel for the GA crosslink reagent (**Figure 1**). The aqueous protein spinning solution and the 5% GA solution were then pumped into the inlet and outlet of the microfluidic device, respectively. Then, the fluids reached the cross intersection where crosslinking reaction occurred between the two solutions. The tapered outlet provides a shearing force on the fluid

Figure 1. Schematic of the protein fiber fabrication by a microfluidic technique. The used spherical and linear proteins in this work are A) BSA, B) egg ovalbumin, C) milk casein, and D) collagen. E) Schematic of the microfluidic spinning setup: 1, microfluidic chip; 2, coagulation bath; 3, protein fiber; 4, rotation collector. F) A photo of the two-channel microfluidic wet spinning device. The scale bar is 2 cm. G) The collected protein fibers, for example, BSA fibers. The scale bar is 1 cm.

Figure 2. Characterization of the fibers fabricated from globular proteins. A) SEM image of the surface morphology of the protein fibers. The fibers are smooth and have a uniform size in diameter. The scale bar is 50 μm . B) POM analysis of the fiber. The observed birefringence suggested an ordered structure in the fiber. The scale bar is 100 μm . C) SR-SAXS 2D pattern and the corresponding structure model of the fiber. Here chicken egg white-based fiber as an example. The purple ellipse spheres represent the ovalbumin proteins, the green rods represent the crosslinking GA, and the pink and brown particles represent other proteins and minerals in the egg white. D) Stress–strain curves of the egg white fibers with GA crosslinking treatment. E) Stress–strain curves of the GA crosslinked BSA fibers with double-drawn treatment. F) Stress–strain curves of the milk casein fibers with GA crosslinking treatment. The curves in D–F) with different colors represent three parallel tensile tests. G–I) The corresponding mechanical performance of three types of fibers (egg white fibers with GA crosslinking treatment, crosslinked BSA fibers with double-drawn treatment, and milk fibers with GA crosslinking treatment).

to spin the fiber, which is a key step to induce the alignment of protein molecules. Thus, the fibers were then collected after the treatment in the coagulation ethanol bath (Figure 1G). By this microfluidic spinning technique, we could realize the massive production of naturally sourced protein fibers.

2.2. Fabrication of Egg Ovalbumin-Based Fibers

To generate ovalbumin fibers, chicken, quail, and goose eggs were used as protein sources. The egg white was separated from the eggs, and then it was used directly as a spinning solution without any further treatment. Through the microfluidic device, the ovalbumin fibers were successfully constructed

with or without GA crosslinking. Scanning electron microscopy (SEM) investigation showed that the fibers have fibrillary structure, smooth surface, and uniform size in diameter (Figure 2A and Figure S1, Supporting Information). Moreover, the egg white fiber is birefringent under polarized optical microscopy (POM) analysis, indicating an ordered arrangement of molecules in the egg white fiber (Figure 2B). Further characterization of egg white fibers was conducted by Synchrotron Radiation Small-Angle X-ray Scattering (SR-SAXS). As shown in Figure 2C, the SR-SAXS 2D pattern revealed that the ovalbumin protein particles are stacked in the fiber.

Next, mechanical properties of the egg white fibers were investigated by uniaxial tensile tester under ambient conditions. The typical stress–strain curves of egg white fibers were shown

in Figure 2D and Figure S3 in the Supporting Information. Before crosslinking treatment with GA, the egg white fibers exhibited considerable mechanical performance, including breaking strength of ≈ 60 MPa, Young's modulus of ≈ 2.6 GPa, and toughness of ≈ 1.8 MJ m $^{-3}$, respectively (Figure S4, Supporting Information). After crosslinking with GA, the mechanical performance of egg white fibers was similar to the non-crosslinked fibers (Figure S4, Supporting Information and Figure 2G–2). This indicates that the crosslinking reaction between aldehydes of GA and amine groups of the egg white protein might be not active due to the present impurities in egg white. In a comparison of the SR-SAXS 2D patterns of fibers before and after crosslinking, both of them have similar stacking of ovalbumin molecules (Figure S2, Supporting Information), which also indicate that there is no significant structural difference in the fibers after GA treatment.

To broaden the scope of ovalbumin-based fibers, we also investigated other sources from quail and goose eggs. The fiber preparation procedure based on quail and goose egg white is the same as the chicken egg white fibers. SEM results indicated that those ovalbumin fibers have smooth surface morphology and uniform size in diameter (Figures S5 and S6, Supporting Information). The birefringent in the POM images revealed that both fibers have an ordered molecular structure (Figures S7 and S8, Supporting Information). In a comparison of the SR-SAXS 2D pattern of the fibers (Figures S9 and S10, Supporting Information), the ovalbumin fibers have similar stacking of ovalbumin molecules before and after GA crosslinking treatment.

The mechanical properties of those ovalbumin fibers were then investigated (Figures S11 and S12, Supporting Information). Before the GA crosslinking treatment, Young's modulus of ≈ 2.4 GPa was recorded in the elastic region of 0–3% applied strain (Figures S11A and S13B, Supporting Information) for quail egg white fibers, and Young's modulus of ≈ 2.2 GPa was recorded in the elastic region of 0–1.5% applied strain for goose egg white fibers (Figures S12A and S14B, Supporting Information). After crosslinking with GA, the moduli decreased to ≈ 1.2 GPa (Figures S11B and S13B, and Table S1, Supporting Information) and ≈ 1.6 GPa (Figures S12B and S14B, and Table S2, Supporting Information), respectively. On the contrary, the breaking strength and toughness of those ovalbumin fibers were enhanced by GA treatment. In their pristine state, the average breaking strengths of the quail and goose egg white fiber were ≈ 44.50 and ≈ 27.83 MPa, respectively. Those values slightly increased to ≈ 47.84 and ≈ 29.25 MPa after crosslinking with GA (Figures S13A and S14A, Supporting Information). Analogous observations on fibers toughness showed that after GA crosslinking for the two ovalbumin fibers, the toughness slightly increased from an average of 0.73 to 1.73 MJ m $^{-3}$, and from 0.19 to 0.28 MJ m $^{-3}$, respectively (Figures S13C and S14C, Supporting Information).

2.3. Fabrication of BSA-Based Fibers

Regarding the production of BSA fibers, 600 mg mL $^{-1}$ BSA solution together with 5% GA solution were pumped into the microfluidic device and coagulated in the ethanol bath. SEM studies suggested that BSA fibers have smooth surface morphology,

fibrous structure, and uniform size in diameter (Figures S15, Supporting Information). The POM analysis showed that the BSA fibers are birefringent, indicating an ordered arrangement of BSA molecules in the fiber (Figure S16, Supporting Information). Furthermore, the SR-SAXS 2D pattern that revealed the BSA proteins are stacked in a long-ranged ordering in the fibers (Figures S17 and S18, Supporting Information), which is in agreement with the POM results.

The stress–strain curves of the fabricated BSA fibers were investigated (Figure S19, Supporting Information and Figure 2E). Remarkably, there is a significant increase in the mechanical performance of the BSA fibers after the GA crosslinking treatment. The breaking strength and the toughness of the BSA fiber increased from ≈ 55.24 to ≈ 99.28 MPa, and from ≈ 1.01 to ≈ 99.8 MJ m $^{-3}$, respectively (Figures S19 and S20, Supporting Information). The results indicated that the highly condensed BSA network crosslinked by GA plays an important role in increasing the fibers' strength and toughness. Notably, this outstanding toughness is already at the same level as the spider dragline silk and is even superior to that of many recombinant spider silks and regenerated silkworm fibers.^[30,31] Meanwhile, the Young's moduli of the fibers are ≈ 3 GPa (Figure S20, Supporting Information), indicating the preserved stiffness in the BSA fibers. In the next step, double-drawn treatment was introduced to maximize the tensile performances (Table S3, Supporting Information). It was found that the breaking strength increased significantly to ≈ 270 MPa and the Young's modulus was up to ≈ 4 GPa (Figure 2). Meanwhile, the corresponding extensibility and toughness are $\approx 50\%$ and ≈ 60 MJ m $^{-3}$, respectively. The enhanced mechanical strength can be attributed to the formed long-range ordered structure in the BSA fibers, which is confirmed by POM investigation. The birefringent texture of the double-drawn BSA fiber is uniform and much brighter than that of the pristine sample (Figure S16, Supporting Information). Moreover, in a comparison of the SR-SAXS 2D patterns (Figure S17, Supporting Information), the BSA proteins were deformed after GA crosslinking and double-drawn treatment, resulting in a tight stacking and decreased defects among the BSA proteins.

It is known that typical proteins for the formation of lightweight and mechanically strong biological fibers are composed of folded rigid and unfolded flexible units. In stark contrast to them, although BSA lacks of rigid protein domains, the BSA fibers treated by crosslinking and double-drawn exhibit an outstanding breaking strength and toughness, which have exceeded over that of several regenerated silkworm fibers and recombinant spider silks,^[32–35] as well as the hagfish slime thread protein fibers^[36] and human recombinant collagen fibers.^[15] In addition, the BSA fiber is superior to the pseudoprotein and phage virus fibers in breaking strength.^[18,37] Additionally, the robust mechanical performance in the present BSA system is significantly stronger than that of any other reported globular protein-based fibers.^[21,38]

2.4. Fabrication of Milk Casein-Based Fibers

To produce milk casein-based fibers, commercially available milk was used. After centrifugation, the bottom part of

milk was used as a spinning solution. The milk fibers were fabricated by the same microfluidic device at a flow rate of $5 \mu\text{L min}^{-1}$. SEM images showed a distinctively fibrous structure with a rough surface (Figure S21, Supporting Information). POM analysis revealed that the milk fiber is birefringent, indicating an ordered arrangement of casein molecules in the fiber (Figure S22, Supporting Information). Furthermore, in comparison to the SR-SAXS 2D patterns of fibers without and with GA crosslinking treatment (Figures S23 and S24, Supporting Information), the milk proteins were stacked in fiber after GA crosslinking treatment. This may result in decreased defects in the fibers.

The uniaxial tensile test was applied to investigate the mechanical properties of the milk casein fibers (Figure 2F and Figure S25, Supporting Information). In the absence of GA crosslinking, the milk fibers showed a breaking strength of $\approx 48 \text{ MPa}$. Then, this value increased to $\approx 75 \text{ MPa}$ after GA crosslinking treatment (Figure 2F and Figure S26A, Supporting Information). Similarly, the Young's moduli of milk fibers increased from ≈ 2.5 to $\approx 4.0 \text{ GPa}$ (Figure 2H and Figure S26B, Supporting Information). We also observed a similar trend for the toughness of the milk fibers, which increases from 0.75 to 1.25 MJ m^{-3} (Figure 2 and Figure S26C, Supporting Information). All the results indicated that the milk fibers have robust mechanical properties due to the GA-induced covalent network of the casein nanoparticles. Notably, in the present milk fiber system, the strength is significantly stronger than that of other reported milk-based fibers (Table S3, Supporting Information).

2.5. Fabrication of Collagen-Based Fibers

To produce collagen fibers by the microfluidic device, the spinning solution of collagen (15 mg mL^{-1}) was prepared by dissolving porcine skin with acetic acid ($\text{pH} = 3$). The collagen fibers were then collected after the treatment in the coagulation ethanol bath (adjusted to $\text{pH} = 9$ using NaOH). In our system, due to the low solubility of the used collagen in water, it cannot form fibers using a single-channel microfluidic device (without GA treatment). Only collagen fibers by GA crosslinking treatment were fabricated by a dual-channel microfluidic chip. In order to characterize the collagen fibers, SEM, POM, and SR-SAXS were performed. The SEM images revealed that the collagen fibers have a fibrous structure, smooth surface morphology, and uniform size in diameter (Figure 3A and Figure S27, Supporting Information). In addition, the POM analysis showed that the collagen fiber is birefringent, indicating an ordered molecule structure in the fiber (Figure 3B). Furthermore, the SR-SAXS 2D pattern of the collagen fibers revealed that the collagen proteins are stacked in the fibers after GA crosslinking treatment (Figure 3C).

Similarly, the uniaxial tensile tests were performed to investigate the mechanical properties of collagen fibers (Figure 3D,E). After GA crosslinking treatment, the fibers showed a breaking strength of $\approx 45 \text{ MPa}$, a Young's moduli of $\approx 2 \text{ GPa}$, and a toughness of $\approx 10 \text{ MJ m}^{-3}$, respectively (Figure 3F–H). To further reinforce the mechanical behaviors of the collagen fibers, a “secondary crosslinking” strategy was applied, in which coagulation containing 5% GA was prepared to realize secondary

crosslinking for the collagen fiber. To our surprise, the mechanical properties of collagen fibers increased significantly after secondary crosslinking with GA, including the breaking strength of $\approx 160 \text{ MPa}$, the Young's moduli of $\approx 4 \text{ GPa}$, and the toughness of $\approx 15 \text{ MJ m}^{-3}$, respectively (Figure 3F–H). The present mechanical properties of the collagen fibers with secondary crosslinking treatment are comparable with some regenerated silk fibers^[35] and even superior to that of many collagen fibers (strength of 36 MPa and modulus of 359 MPa , Table S3, Supporting Information).^[39] This behavior may be associated with the GA crosslink degree in the fibers. All the results indicated that the highly condensed network by secondary crosslinking is important to increase the fibers' mechanical performance.

2.6. Surgical Applications by the Biological Fibers

To further demonstrate the versatile nature of the biological fibers, their surgical applications were explored. For this purpose, the alginate/BSA composite fiber was fabricated from the designed microfluidic device. To produce the composite fibers (Figure 4A), a mixture of alginate and BSA (9:1) were used as the spinning solution, and a 1% CaCl_2 solution was used as the coagulation bath. First, we assessed the mechanical properties of the alginate/BSA composite fiber by uniaxial tensile testing. The composite fiber has a high breaking strength of $\approx 420 \text{ MPa}$, a Young's modulus of $\approx 10 \text{ GPa}$, and a toughness of $\approx 40 \text{ MJ m}^{-3}$ (Figure 4B). Notably, these composite fibers demonstrated a higher mechanical performance when compared to the BSA fiber studied above. This may be ascribed to the high crystallinity of alginate in the composite fiber. Then, the biocompatibility of the fibers was studied. We loaded A549 cells in the alginate/BSA composite fibers and investigated the cell morphology and viability. The composite fibers were immersed in a solution containing the tripeptide Arg-Gly-Asp solution (30 mg mL^{-1}) and sterilized (121°C , 40 min). Figure 4C illustrated a micrograph of A549 cells loaded onto the composite fibers after 48 h in the CO_2 incubator. The results revealed that A549 cells anchor and spread on the smooth fibers (Figure 4C and Figure S28, Supporting Information), indicating that the alginate/BSA composite fibers are cytocompatible.

The biosafety nature of the alginate/BSA composite fibers together with their high mechanical performances indicated their potential applications in surgical sutures. To evaluate the capability of the fibers as surgical sutures, wound closing experiments using rat and minipig models were performed. A single bundle of 100 pieces of alginate/BSA fibers, named (alginate/BSA)₁₀₀, were used to suture skin and organs (Figure 4D–4). We first performed the wound suture experiments on rat skin and liver. It showed that the (alginate/BSA)₁₀₀ suture has an admirable mechanical strength and flexibility to close the incision. Further wound suture experiments on porcine skin (Figure 4G,H), liver (Figure 4), and stomach (Figure S29, Supporting Information) proved that the (alginate/BSA)₁₀₀ suture has the ability to close the wound incision without breakage. From those results, we can conclude that the biological fibers might be used as a new candidate for surgical suturing due to the combination of robust mechanical performance and biodegradability.

Figure 3. Characterization of the collagen fibers. A) SEM image of the surface morphology of the collagen fiber. The fibers are smooth and have a uniform size in diameter. The scale bar is 5 μm . B) POM analysis of the collagen fiber. The observed birefringence suggested an ordered structure in the collagen fiber. The scale bar is 20 μm . C) SR-SAXS 2D pattern and the corresponding structure model of the collagen fiber. The orange rods represent the collagen proteins and the green rods represent the crosslinking of GA. D,E) Stress–strain curves of collagen fibers with single and double GA crosslinking treatment, respectively. The curves with different colors represent three parallel tensile tests. F–H) The corresponding mechanical performance of the collagen fibers, including breaking strength, modulus, and toughness.

3. Conclusions

Although many recombinant and regenerated fibrous proteins have been extensively investigated for fiber production, the high cost limited their massive production, and high-tech applications. Here, we have successfully constructed protein fibers from a series of spherical and linear proteins in nature including chicken egg, quail egg, goose egg, BSA, milk, and collagen by a coaxial capillary microfluidic device. Those easily accessible proteins were employed for the scalable production

of protein fibers. To the best of our knowledge, it is the first time that ovalbumin fibers were extensively constructed by microfluidics. This strategy is effective to construct spherical proteins fibers by controlling molecular packing and orientation and reducing defects. Interestingly, the BSA-related fibers exhibited extraordinary mechanical performance which is comparable or even higher than the reported recombinant spider silks and regenerated silkworm fibers. In addition, the milk and collagen fibers were also successfully fabricated by this microfluidic technique, exhibiting robust

Figure 4. Suturing applications by the biological fibers. A) A photo of protein fiber bundles. Here, a single bundle of 100 pieces of alginate/BSA fibers, named (alginate/BSA)₁₀₀, were used for suturing. The scale bar is 3 cm. B) Stress–strain curves of alginate/BSA composite fibers. The curves with different colors represent three parallel tensile tests. C) Confocal microscopy image of the (alginate/BSA)_n composite fibers co-cultured with Adenocarcinomic human alveolar basal epithelial cells (A549) cells. The scale bar is 20 μ m. A549 cells are stained with Calcein-AM (green)/PI (red) in phosphate-buffered saline buffer solution. D) A photo of wound suture on a rat and magnified in E). The scale bar is 1 cm. The scale bar is 0.5 cm. F) Wound suture on a rat liver. The scale bar is 0.3 cm. G) A photo of wound suture on a minipig and magnified in H). The scale bar is 15 cm. The scale bar is 1 cm. I) Wound suture on the porcine liver. The scale bar is 1 cm. White and black arrows indicate the suturing positions by the alginate/BSA fiber bundle.

mechanical properties. Therefore, this strategy is universal for the construction of protein fibers from widely available proteins in nature. Furthermore, due to the natural biocompatibility, those protein fibers exhibit admirable mechanical strength and flexibility, which are successfully used in surgical suturing using rat and minipig models. Thus, we believe that this strategy is general and efficient for fiber construction based on naturally sourced proteins. It will definitely be further extended to construct other protein fibers and offer great potentials for the applications of mechanically strong fibers in biomedicine.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biological fibers, microfluidics, proteins, suturing

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