Scalable Spider-Silk-Like Supertough Fibers using a Pseudoprotein Polymer

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Spider silks are tougher than almost all other materials in the world and thus are considered ideal materials by scientists and the industry. Although there have been tremendous attempts to prepare fibers from genetically engineered spider-silk proteins, it is still a very large challenge to artificially produce materials with a very high fracture energy, not to mention the high scaling-up requirements because of the extremely low productivity and high cost levels. Here, a facile spider-silk-mimicking strategy is first reported for preparing scalable supertough fibers using the chemical synthesis route. Supertoughness ($\approx$387 MJ m^{-3}), more than twice the reported value of common spider dragline silk and comparable to the value of the toughest spider silk, the aciniform silk of *Argiope trifasciata*, is achieved by introducing β-sheet crystals and α-helical peptides simultaneously in a pseudoprotein polymer. The process opens up a very promising avenue for obtaining excellent spider fibers.

Spider silks have been one of the long-term research topics of increased interest in science in the past half century, mainly due to their remarkable mechanical properties compared with steel and Kevlar, which are strong and stiff but heavy and strong and soft but with limited stretchability, respectively. Spider silks are strong, soft, light, and very stretchable and thus have an extraordinary toughness, namely, a high specific energy to failure, making spider silks the most desirable materials for high-performance applications such as energy absorption, space suits, bullet-proof applications, balloon parachutes, medical devices, and specialty ropes.[1–8] The power of spider silk is demonstrated by one particular silk called aciniform silk, which is used for wrapping prey and lining eggcases. This silk is extremely thin ($\approx$1 μm in diameter) but can control animals such as bats and birds.[9,10] However, spiders are cannibals, and thus farming is impossible and natural spider silks cannot meet human demands, so artificial spider silk has become a booming market.[11,12] Tremendous worldwide efforts have been made, particularly in genetically engineering spider-silk proteins (spidroins),[4,13] but it is still a very large challenge to scale up due to the low efficiency, high cost, and uncontrollable quality of the process. Specifically, the toughness of recombinant spidroin fibers, except for one report (still not consolidated by other studies), has seldom been accomplished those of natural spider silks because of molecular weight limitations and self-assembly difficulties.[14]

As the most resilient protein fiber in nature, the tensile toughness of spider dragline silk can reach 180 MJ m^{-3}, which is two to three times that of man-made fibers such as Kevlar or nylon.[5,15] Such excellent performance is inseparable from its structure, which contains β-sheet crystals, α-helices, and random coils within its small fibrils.[16,17] It is generally accepted that the high fiber strength is largely provided by aligned β-sheet nanocrystallites, while its 3_{10}-helical and surrounding amorphous regions and random coils, contribute to the fiber extensibility.[5,16,18] More importantly, aciniform silk, which spiders use for wrapping prey and lining eggcases, is actually the toughest biological material with a value of $\approx$376 MJ m^{-3}, whose extensibility is approximately twice that of dragline silk.[19–21] This phenomenon is most certainly due to its protein secondary structure.[22] As stated in ref. [22]: "*Argiope argentata* prey-wrapping silk (aciniform silk) show that native, freshly spun aciniform silk are dominated by α-helical (50% total) and random coil (35% total) secondary structures, with minor β-sheet nanocrystalline domains (15% total)." Based on this understanding, scientists wanted to mimic the structure of spider silks by manufacturing artificial silks using chemical synthesis processes,[23,24] but progress has been very slow. To overcome this problem, Tsuchiya and Numata recently reported the synthesis of multiblock co-polypeptides that can mimic spider silk's secondary structure containing both β-sheet crystals and α-helical peptides by two-step chemical synthesis.[11] Although this work represented a milestone forward in terms of a more realistic synthetic structure of artificial spidroins, the resulting co-polypeptide has a very low molecular weight and thus has extremely unsatisfactory mechanical properties in addition

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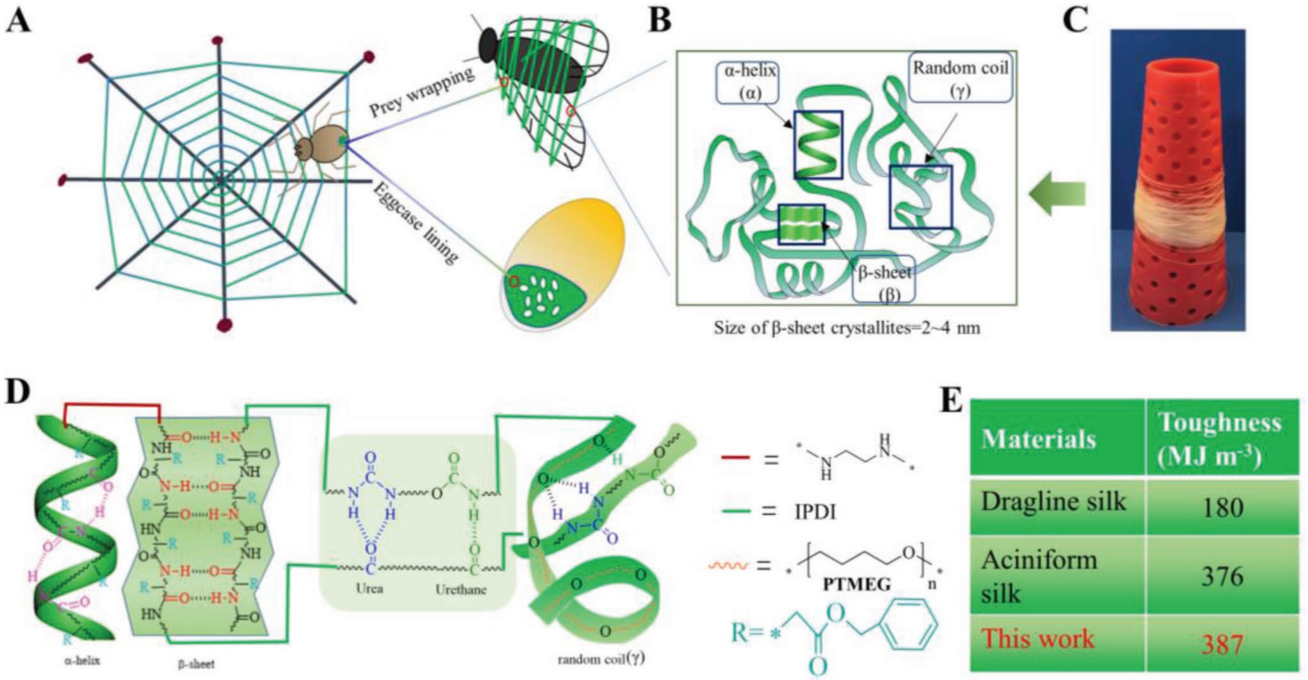

Materials	Toughness (MJ m^{-3})
Dragline silk	180
Aciniform silk	376
This work	387

Figure 1. Spider aciniform silk: structure, toughness, and its pseudoprotein polymer design. A) Aciniform silk of spiders and its application in nature. B) Protein secondary structure of aciniform silks. C) The obtained pseudoprotein polymer fiber with a similar structure to that of aciniform silk in this work. D) Design of the aciniform silk secondary structure in scalable supertough fiber materials. E) The obtained pseudoprotein polymer fiber's toughness compared with values from natural fibers.

to its low efficiency rooted in the postcondensation stage. Therefore, making a readily processable polypeptide material is a very large challenge for scalable spider-silk-like tough fibers, in which α-helical, nanosized β-sheet, and amorphous-region/random-coil structures can be formed simultaneously.

In this study, we present a spider aciniform-silk-mimicking structural design (**Figure 1**) and synthesis of high-performance pseudoprotein polymers for fibers to address the above challenge. Alpha-helical and nanosized β-sheet structures formed from an amine-terminated peptide poly(γ-benzyl-L-glutamate) (PBLG) are connected by a small diamine and further linked to random-coil-like chains of spider-silk proteins using urea linkage. The resulting fiber materials exhibit supertoughness (≈387 ± 35 MJ m^{-3}) and a significantly higher tensile strength (≈100 MPa, which is three times higher than values of 20-30 MPa[25]) of its counterpart polyurethane (PU). The toughness of this fiber is more than twice that of spider-dragline silk and comparable to that of the toughest spider silk, aciniform silk of *Argiope trifasciata*. This is the first report ever on the production of scalable spider-silk supertough fibers that are at least 30 times more inexpensive via a facile method, thereby opening up promising and profitable doorways to realize the human dream of obtaining extraordinarily high-performance materials, particularly fibers of protein origins.

Initially, the molecular structure of PBLG with nanosized β-sheet and α-helical conformability was synthesized by ring-opening polymerization of BLG-*N*-carboxyanhydride (Figure S1, Supporting Information). In recent publications, the authors reported that PBLG could form α-helical and β-sheet structures simultaneously in their polymers depending on the average degree of polymerization (DP),[26,27] which could affect the secondary structure of the polypeptide.[27,28] Generally, polypeptides with a DP > 18 are composed only of α-helical secondary structures, while in polypeptides with a DP < 18, nanosized β-sheet and α-helical secondary structures are present.[28] In this work, the DP was controlled to be ≈15 (Figure S2, Supporting Information). ^{1}H nuclear magnetic resonance (^{1}H NMR), solid-state ^{13}C NMR, and Fourier transform infrared (FTIR) spectroscopy results confirmed the molecular structure of the new peptide diamine (**Figure 2**C; Figure S3, Supporting Information), whose secondary structure contained β-sheet and α-helical structures, as shown in Figure 1. Gel permeation chromatography (GPC) trace images (Figure S4, Supporting Information) showed a low polydispersity index (PDI = 1.21), and the molecular weight (M_n = 3700) was consistent with the ^{1}H NMR results. The pseudoprotein polymers were regulated via control of the molecular structures incorporating nanosized β-sheet, α-helical, and random coil structures to obtain polymers with both supertoughness and a high tensile strength.

The synthetic route to pseudoprotein polymer samples is shown in Figure S2 (Supporting Information), where poly(tetramethylene ether glycol) (PTMEG) was used as the soft segment and random coils, and isophorone diisocyanate (IPDI) and peptide diamine were used as the linkers. Several polymers with different molar ratios of PTMEG/IPDI/peptide were synthesized (Table S1, Supporting Information). The peptide content of these pseudoprotein polymer samples was calculated using Equation S1 (Supporting Information) and is summarized in Table S1 (Supporting Information). The structure

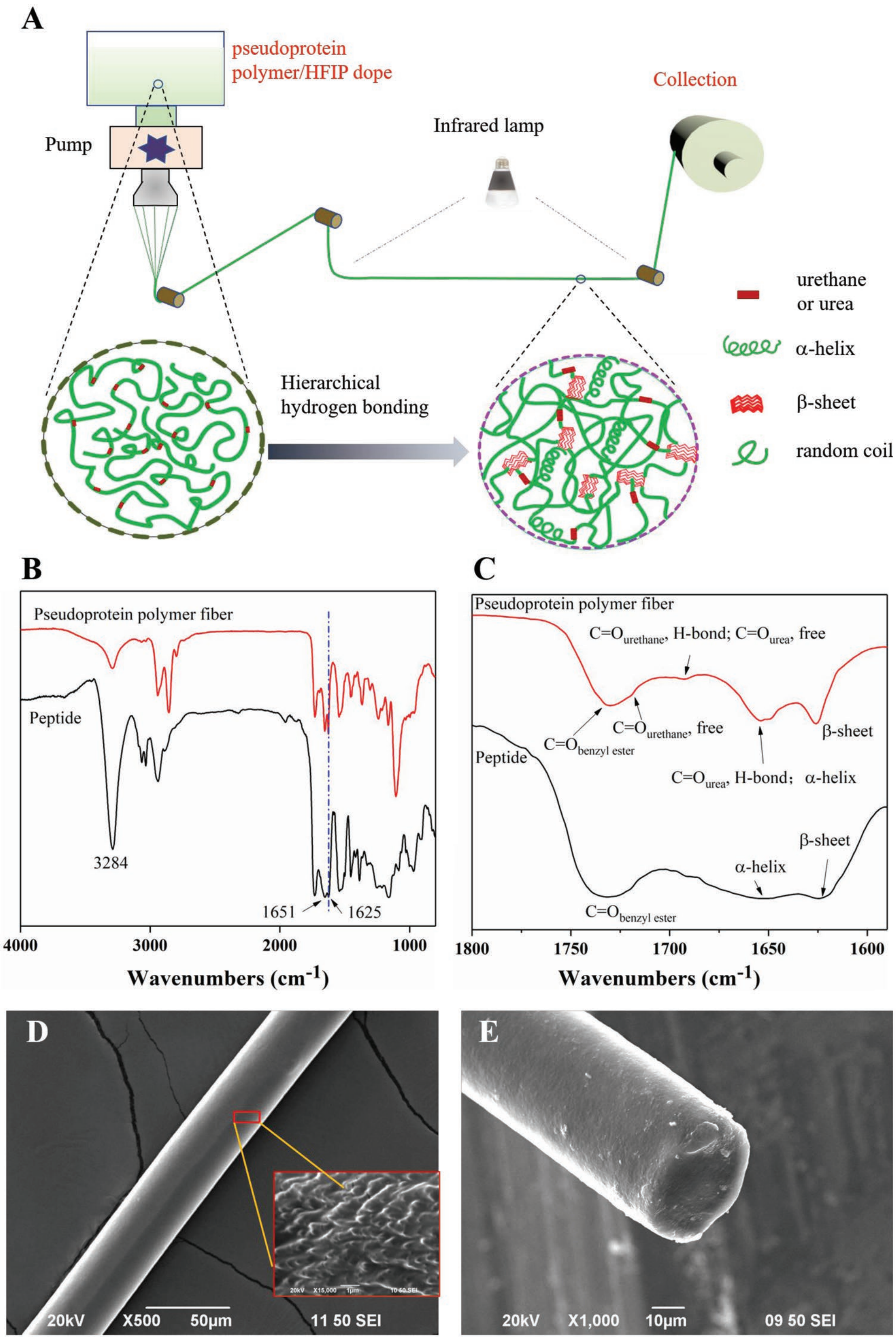

Figure 2. Fabrication and characterization of the pseudoprotein polymer fiber. A) Fiber preparation scheme. B,C) FTIR spectra of peptide and pseudoprotein polymer fiber. D,E) SEM images of the pseudoprotein polymer fiber (peptide content: 41.5 wt%): surface and cross section.

of the obtained pseudoprotein polymer was confirmed by ^{1}H NMR as shown in Figure S3 (Supporting Information).

The pseudoprotein polymer fibers were prepared by dry spinning (Figure 2A), which mimics the natural spinning process of spider silks.[29] The pseudoprotein polymer dope was solidified into a fiber immediately leaving the spinneret due to evaporation of the hexafluoroisopropanol (HFIP) solvent under infrared radiation. After spinning, aciniform-silk-like structures containing nanosized-β-sheet and α-helical structures linked by urethane/urea with PTMEG as random coils were formed in the pseudoprotein polymer fibers. Figure 2B,C shows the FTIR spectra of the specimen, where the peaks between 1600 and 1750 cm^{-1} are attributed to different C=O stretching vibrations. The peaks at 1625 and 1651 cm^{-1} represent the

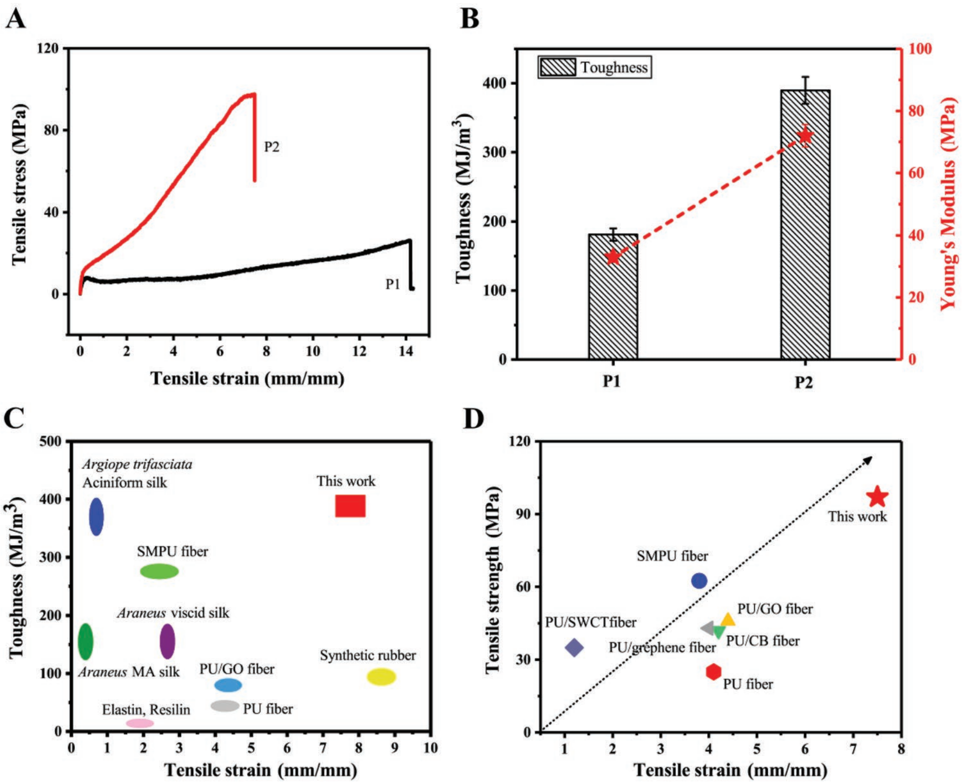

Figure 3. Mechanical properties of the pseudoprotein polymer fibers. A) Stress–strain curves of the pseudoprotein polymer fibers by P1 and P2 polymers with different peptide contents P1: 22.6%, P2: 41.5% (see Table S1, Supporting Information), and B) their toughness and Young's modulus values. C) Ashby plot of the toughness versus extensibility of the pseudoprotein polymer fibers and other materials reported in references. D) Comparison of the tensile strength and extensibility of the pseudoprotein polymer fibers with PU and PU nanocomposite fibers with different nanofillers (SMPU: shape memory polyurethane; SWCNT: single-walled carbon nanotubes; GO: graphene oxide; CB: carbon black).

β-sheet and α-helical structures and H-bonded C=O in urea, respectively.[27] The free C=O in urethane is located at higher wavenumbers (1718 cm^{-1}) compared to the free C=O in urea and H-bonded C=O in urethane (1692 cm^{-1}).[30] Moreover, the SEM images show that the pseudoprotein polymer fiber had protein-like self-assembled sub-micrometer wrinkles on the surface (Figure 2D) and a diameter of ≈30 µm (Figure 2E).

The mechanical properties of the pseudoprotein polymer fibers were evaluated by tensile tests. **Figure 3**A shows their stress–strain curves, and the derived mechanical parameters (the Young's modulus and toughness) are shown in Figure 3B. It is clearly observed that the modulus and stiffness of the pseudoprotein polymer fibers increase with the peptide content, which is due to the increase in nanosized β-sheet content. In particular, P2 has a tensile strength of ≈100 MPa, an elongation at break of ≈750% and thus a toughness of 387 MJ m^{-3}, which is more than twice the toughness of spider dragline silk (≈160 MJ m^{-3}) and is comparable to that of the toughest spider silk—*Argiope trifasciata* aciniform silk (≈320 MJ m^{-3}) (Figure 3C; Figure S7A, Supporting Information). After prestretching P2 with 400% stretching level (similar to post-drawing) before testing, the tensile strength and modulus reach ≈200 and 230 MPa, respectively, and its toughness is still up to 190 MJ m^{-3} (Figure S6, Supporting Information). To date, if not none, it is difficult to find synthetic fibers that exhibit the toughness of spider silks because, generally, improvements in the tensile strength of synthetic fibers are associated with a decrease in elongation at break and vice versa.[31] Similarly, Koeppel and Holland pointed out that, despite various attempts, surpassing the properties of spider dragline silks is still an area of considerable effort.[32] Although some of existing work can reach a high value in strength or stiffness, no one can have an extensibility (strain at break) more than 300%, neither a toughness more than 260 MJ m^{-3}.

Additionally, a comparison of the mechanical properties of the pseudoprotein polymer fibers and polyurethane (PU) nanocomposite fibers reinforced with single-walled carbon nanotubes (SWCT), graphene, graphene oxide (GO), and carbon black (CB) is shown in Figure 3D and Figure S7B (Supporting Information).[33,34] Compared with pure PU, the PU nanocomposite fibers always showed an enhancement in mechanical strength with a much reduced or similar strain at break, but notably, while maintaining an excellent strain at break, the pseudoprotein polymer fibers exhibited the highest tensile strength among all samples. To be precise, the pseudoprotein polymer fiber, even though sharing a similar structure to PU, has much higher performance than its all PU counterparts in terms of both breaking strain (≈2 times of PU fiber) and strength (≈3 times of pure PU fiber), thus leads to its super-toughness equal to that of the toughest material in nature. All these are clear indicators of the great potential and effectiveness of the chemical synthesis approach used in this work due

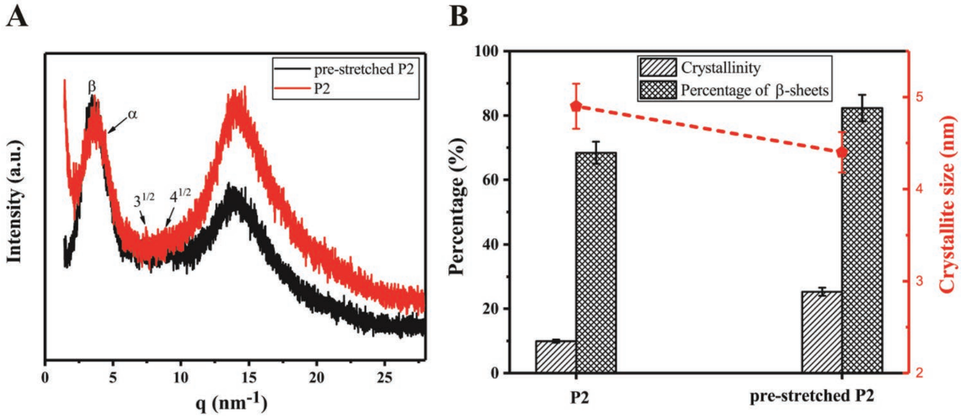

Figure 4. Influence of prestretch. a) WAXD profiles of the pseudoprotein polymer fibers and B) their crystallinity, crystallite size, and percentage of β-sheets.

to its capability in the formation of spider-silk-like α-helices, 4–5 nm β-sheet crystals and random coils within the pseudoprotein polymer fiber.

To further confirm the relationship between the properties and structure of the pseudoprotein polymer fibers, wide-angle X-ray diffraction (WAXD) was conducted. **Figure** 4A shows the WAXD profiles of P2 before and after prestretching, and the diffraction pattern of P2 displays the presence of two secondary structures (β-sheet and α-helical structures), which is in agreement with the FTIR results. The first diffraction peak at 3.5 nm^{-1} corresponds to the antiparallel nanosized β-sheet secondary structure, while the three signals at higher wavenumbers, with relative positions of $1{:}3^{1/2}{:}4^{1/2}$, are attributed to the formation of the α-helical secondary structure.[28] The α-helical structure is not as tightly constrained as the β-sheet nanocrystallites, which thus gives rise to the extensibility of the P2 fiber.[16] The strength of the fibers directly correlates with their β-sheet contents due to the large amount of hydrogen bonds in these nanocrystallites.[16] The relative nanosized β-sheet contents of P2 before and after prestretching were calculated by fitting the WAXD profiles with Gaussian functions (Figure S5, Supporting Information) and are illustrated in Figure 4B. After prestretching P2, the nanosized β-sheet content increased, resulting in an increase in the crystallinity and tensile strength. Furthermore, the crystallite sizes of P2 before and after prestretching were evaluated according to Scherrer's equation (Equation (S2) and Table S2, Supporting Information), which is in the range of 4–5 nm and decreases after prestretching. A small crystallite size could guarantee uniform deformation and concerted failure.[2] It has been reported that β-sheet crystallites with a size of 2–4 nm would result in maximum strength stiffness and mechanical toughness.[2]

In summary, a spider-silk-mimicking strategy is reported for supertough fibers (≈387 ± 35 MJ m^{-3}) involving urethane/urea-linking of α-helical and nanosized β-sheet structures to amorphous random coils, which are elastomeric molecular chains in peptide-polyurethane/urea. The fiber toughness is more than twice the value of spider dragline silk, which has the highest toughness value in the world. Moreover, the cost-effective, widely available feedstocks and facile preparation method of the pseudoprotein polymers make it possible for large-scale production for high-performance applications.

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

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