ARCHETYPES OF SPIDER SILK

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ABSTRACT

Spider dragline silk is well-known for their excellent strength, extensibility, toughness and biological properties which overwhelmingly exceeds most of the engineered materials available today. Development of spider silk inspired materials is gaining interest due to their wide range of application potential. Various strategies like synthesis of recombinant spider silk, synthetic peptide polymers and polymer silk composites have been used to produce artificial spider silk materials. In our research group, we had demonstrated all these approaches to develop spider silk inspired fibers and materials.

Key Words: artificial spider silk; recombinant; synthetic peptides

Spider silks are one of the extensively studied natural protein materials due to their outstanding mechanical behavior. Chemical building blocks in spider silks are hierarchically organized in a way to fulfil the functional needs of spider[1]. Interestingly, this special organisation of natural materials lead to their mechano-functionalities often exceed what is achievable using man-made approaches. Besides the incredible mechanical properties, spider silks are biocompatible and display smart properties like supercontraction[2], directional water collection[3] and phononic bandgap[4]. Additionally, the behavior of spider silks is also highly associated with the proportion, structure and orientation of β-sheet nanocrystals within them. Our research team has made significant inroads in developing materials inspired by spider silk for various applications. Our first spider silk inspired material was produced by introducing short chain of polyaniline (P(A)) into multiblock biopolymers with poly(Ɛ-caprolactone) via coupling reaction. As shown in Figure 1, proposed polymers displayed excellent shape recovery and fixity due to strong β-sheet structures produced by P(A). The crystalline regions produced by them act as net points providing a new insight for the design of novel shape memory polymers[5].

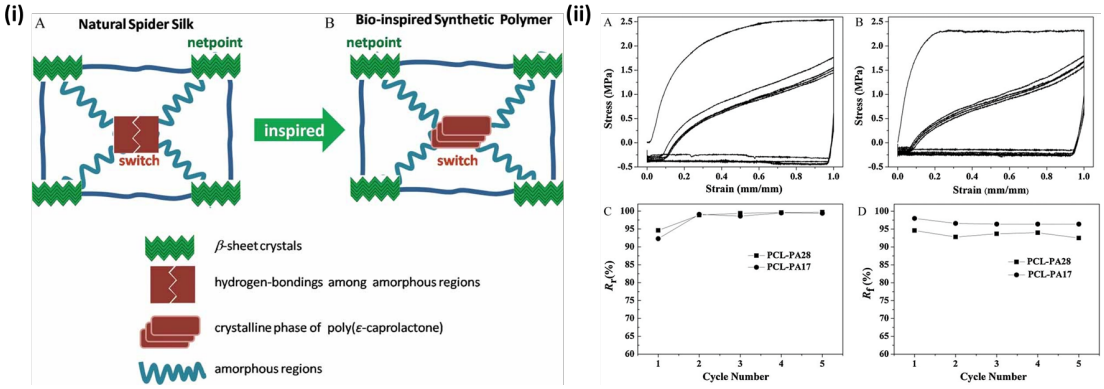

Fig. 1 (i) Shape-memory structural models of: A) natural spider silks and B) bioinspired synthetic polymers. (ii) Shape-memory properties of bioinspired copolymers. A,B) Typical cyclic tensile tests for PCL-PA17 and PCL-PA28, respectively. C) Shape recovery ratio (Rr) and (D) shape fixing ratio (Rf) for five cycles when em ¼ 100%

Similarly, Stretchable peptide-polyurethane were synthesized containing short β-sheet forming peptide blocks of poly(γ-benzyl-l-glutamate)-b-poly(propylene glycol)-b-poly(γ-benzyl-l-glutamate) (PBLG-b-PPG-b-PBLG), isophorone diisocyanate as the hard segment,

and polytetramethylene ether glycol as the soft phase(See Figure 2). Mechanical tests revealed the spider silk inspired polyurethanes displayed medium tensile strength (0.25–4.6 MPa), high stretchability (>1600%), human-tissue-compatible Young's modulus (226–513 KPa)[6].

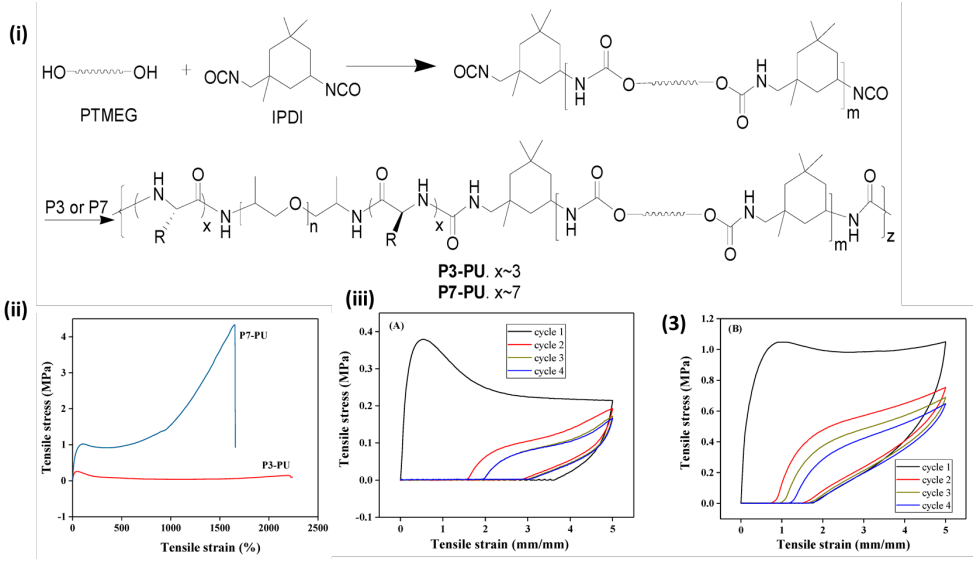

Fig. 2 (i) Synthesis of the obtained peptide-polyurethane/ureas (P3-PU and P7-PU) (ii)Stress-strain curves of (P3-PU and P7-PU) (iii) Cyclic Tensile curves of P3-PU(A) and P7-PU(B) at 500% strain)

Spider silk fibers are extremely sensitive to relative humidity above 70% and contract about 50% of its original length. This has inspired us to synthesize a spider silk like polymer composite by inducing β-sheet by blending regenerated silk fibroin(Figure 3). The hybrid composite displayed two-way shape memory effect in water, where HBs and β-sheet crystals act as switch units and netpoints, respectively[7].

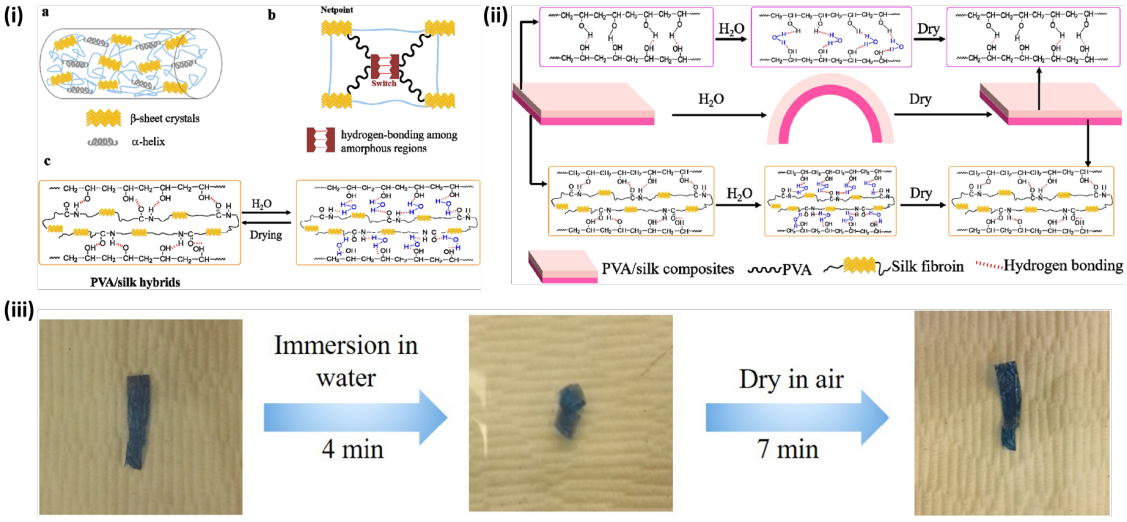

Fig. 3 (i) (a) Structural model of silk (spider silk, silkworm silk) and (b) proposed water-sensitive shape memorymechanical model of spider silk. (c) Cleavage and reforming of hydrogen bonds between PVA and silk under effect of water (ii)Schematic illustration of the two-way shape-memorybehavior of PVA/silk hybrids actuated with water (iii) Photography showing water-sensitive two-way shape-memory effect of PVA/10% silk hybrid

Real artifical counterparts of spider silks can be produced using gene recombinant technology. We have opted this approach to produce spider silk based drug carriers(Figure 4). Inspired by the natural process of micelle formation silk spheres based on eTuSp1 spidroin were formed using HFIP-on Oil method without any surfactants[4].

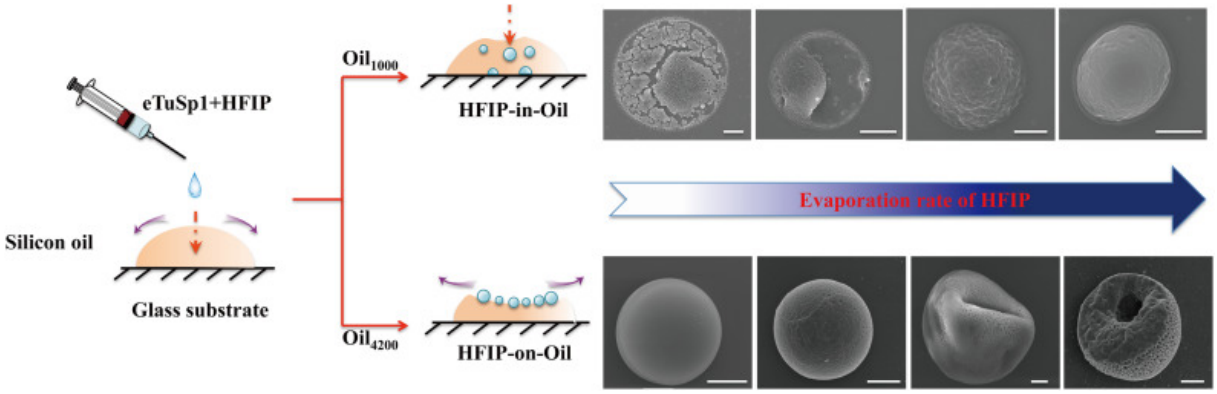

Fig. 4 Fabrication of spider silk spheres in an HFIP-Oil system. Oil_{1000}: silicon oil with M_w of 1000; Oil_{4200}: silicon oil with M_w of 4200. Scale bar is 10 μm. Accompanied by evaporation of HFIP, various silk morphologies could be observed in two different modes.

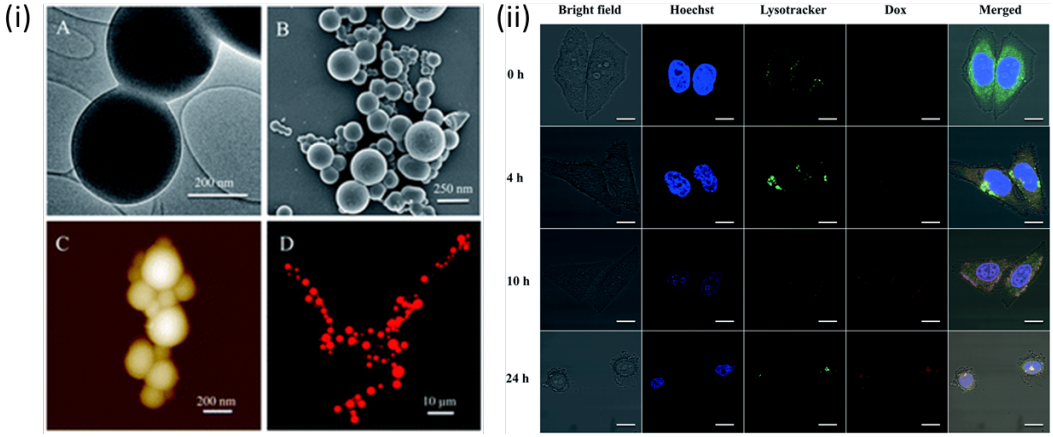

Fig. 5 (i) The morphology structure of silk spheres and their potential in drug loading. (A) TEM image of compact eTuSp1 spheres. (B) FE-SEM image of dried eTuSp1 spheres. (C) AFM image of eTuSp1 spheres. (D) CLSM image of eTuSp1-Dox spheres. (ii) ysosomal delivery of eTuSp1-Dox spheres in Hela cells. Hoechst and Lysotracker are used to strain the cell nucleus (blue) and lysosomes (green), respectively. The trafficking of the Dox in the intracellular environment is recorded in 24 h by the aid of fluorescence detection.

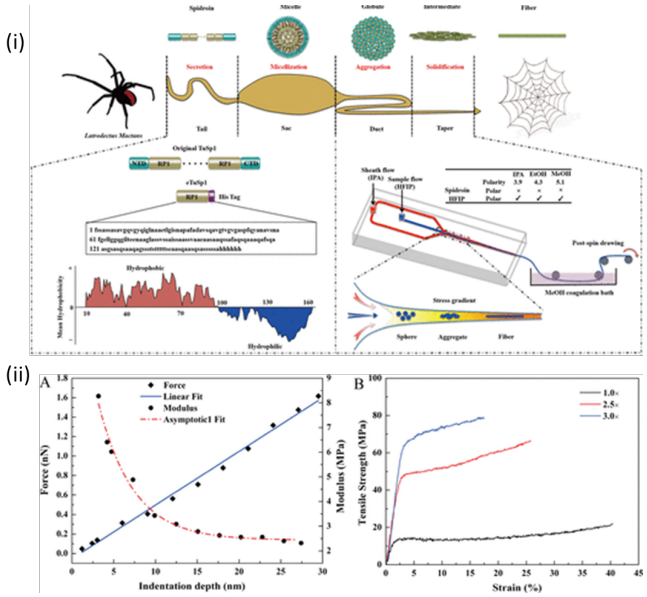

Fig. 6 (i) Schematic of the silk fiber assembly by a biomimetic microfluidic device. A spider's gland, consisting of a tail, sac, duct, and tapper, is illustrated with corresponding function of protein secretion, micellization, induced aggregation and

solidification. In the lower left of this figure, the recombinant protein eTuSp1 consists of ~60% hydrophobic and ~40% hydrophilic moieties. In the lower right of this figure, a microfluidic chip is designed with two inlet channels and one outlet channel, where isopropanol (IPA) is taken as the sheath flow and HFIP with spidroins dissolved is used as the core flow. The table inset shows the miscibility features of IPA, ethanol (EtOH), and methanol (MeOH) toward the HFIP and spidroins. (ii) Mechanical properties of eTuSp1 spheres and fibers: (A) compressive modulus of silk spheres is measured by AFM indentation (both the force and modulus data are well-fitted by linear and asymptotic equations); (B) tensile modulus of silk fibers, tested under various post-spin draw ratios.

Drug loaded spheres of eTuSp1 spidroin was used for lysosomal drug delivery (Figure 5). The spheres were loaded with Doxorubicin with maximum 35% loading and 30% loading efficiency. The spheres demonstrated excellent hemocompatibility and showed good control in PBS solutions[8]. We have made attempt to spin fibres using eTuSp1 spidroin with help of biomimetic microfluidic spinning device (Figure 6). The fibers were formed by the nonsolvent induced phase separation in place of conventional ion-exchange and acidification. Because of the applied shear and elongational flow these intermediates were forced to form fibrillar assemblies. Interestingly, the fibers were formed by the assembly of spheres/aggregates in the microfluidic channel. The eTuSp1 fibers displayed maximum strain of 40% and a tensile strength of 78.3 MPa at ratios of 1.0x and 3.0x respectively. The fiber showed high elastic modulus of 1.88 ± 0.88 GPa[9].

Spider silk inspired materials are versatile shows a great potential for wide range of applications. Better understanding of their microstructure and further advancing routes to produce spider silk like macromolecules will pave way to produce innovative materials for various fields including textile, electronics and biomedicine.

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