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FEATURE ARTICLE

Spider silk as a load bearing biomaterial: tailoring mechanical properties *via* structural modifications

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Spider silk shows great potential as a biomaterial: in addition to biocompatibility and biodegradability, its strength and toughness are greater than native biological fibres (*e.g.* collagen), with toughness exceeding that of synthetic fibres (*e.g.* nylon). Although the ultimate tensile strength and toughness at failure are unlikely to be limiting factors, its yield strain of 2% is insufficient, particularly for biomedical application because of the inability to mimic the complex ultrastructure of natural tissues with current tissue engineering approaches. To harness the full potential of spider silk as a biomaterial, it is therefore necessary to increase its yield strain. In this paper, we discuss the means by which the mechanical properties of spider silk, particularly the yield strain, can be optimized through structural modifications.

Spider silk is known for its exceptional mechanical properties. With an ultimate tensile strength (UTS) of around 1.2 GPa and toughness of 158 J cm^{-3},[1] in addition to biodegradability and biocompatibility, it is a highly promising material for medical, military and industrial applications.[2,3] There is, however, an important aspect of spider silk mechanics that tends to be overlooked when citing these types of figures: that of yield. In nature, spider silk is a single-use material, for which its high UTS and energy processing capabilities are critical. Used as a load bearing biomaterial, however, whether for orthopaedic,

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cardiovascular, or almost any application outside of biomedicine, spider silk would be expected to withstand months to years of cyclic stress. It would therefore need to operate in its elastic region (below 2% strain), where the UTS and toughness cannot manifest. Although a certain amount of "plastic" deformation can be recovered by immersion in water,[4–6] which may be useful under extreme, intermittent conditions, the post-yield characteristics are of little value in normal function (Fig. 1).

The very small useable extensibility of spider silk is perhaps its greatest mechanical limitation as a potential biomaterial, particularly because of our inability to apply it, or any other fibre, to mimic the complex structure of natural tissues. Through the hierarchical structuring of relatively weak base materials, bulk materials such as cartilage, bone and blood vessels can perform far beyond what could be expected from a man-made structure with the same components. Articular cartilage, for example, owes its mechanical properties to two main levels of structure. At the ultrastructural level, the highly negatively charged proteoglycans swell against a constraining collagen meshwork to provide the intrinsic stiffness in the material.[7] At the architectural level of the tissue, a dense layer of collagen oriented tangential to the articular surface forms a strain limiting superficial layer that converts to a less dense radial orientation in the deep layers forming an 'arcade' structure.[8] This controls the movement of water by both allowing a greater osmotic stress due to a higher proteoglycan density and also a directional permeability, ensuring a gradual, controlled transfer of stress from the fluid to the solid structure.[7,9,10] Each of these levels of hierarchy cannot be replicated with current tissue engineering technology, prompting the necessity for superior base materials to compensate for the reduced role of ultrastructure.

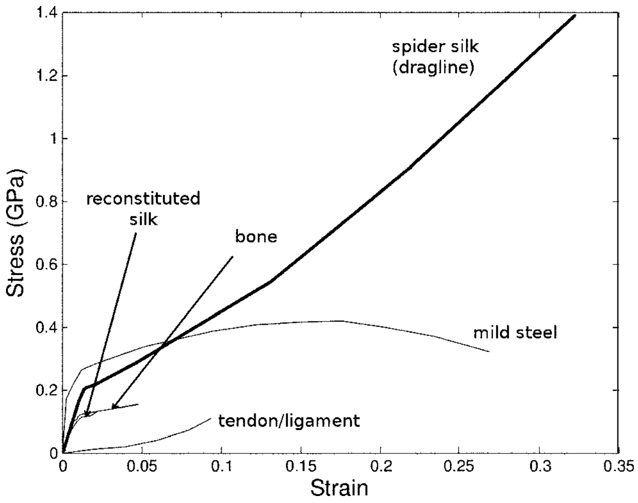

Fig. 1 The stress–strain characteristics of spider silk compared with other common materials.

Although this discussion focuses primarily on spider silk's potential in biomedicine, the points raised here may also apply to its industrial potential in applications requiring high resilience, and in the development of new composites. Outside of biomedicine, the ability to tune the mechanical properties of spider silk[11] for a particular task allows it to be utilized in a range of applications requiring light weight and exceptional energy processing capability, including ropes, body armour, parachutes and seat belts. In multi-use applications, the ability to extend the elastic region will allow more energy to be dissipated in each cycle of use, improving the 'real-world' performance of the material.

The functional characteristics of spider silk originate from the complex hierarchical interactions within and between two proteins: the highly ordered, hydrophobic Spidroin I and the

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less-ordered, hydrophilic Spidroin II.[12,13] The combination of these proteins can be simplified into 40% ordered domains (two hydrogen bonds per amide group), 15% permanently disordered domains (one hydrogen bond per amide group) and 45% intrinsically disordered domains which have a potential for order.[11] These domains predominantly occur in β-sheet, α-helical and pseudo-amorphous chain configurations[14] to form a complex, glassy polymer (see Fig. 2b). The central problem in spider silk mechanics, however, is not identifying the constituents, but understanding how they interact to confer silk's exceptional mechanical properties. A number of models have been proposed to describe these interactions[11,14,15,16] (Fig. 2), yet detailed structure–function relationships remain elusive.

Based on the prevailing models and the established understanding of structure,[14–16] we can conceive a number of phenomena occurring during elastic deformation. The disordered domains, though predominantly glassy and under pre-tension, will show a very small degree of 'lock-in' as they align with the direction of applied stress, pull the β-sheets into alignment,[17] and thus decrease the entropy of the system. Under sufficient stress these less-ordered structures will break their hydrogen bonds in series and reconfigure. Cooperative hydrogen bond rupture is unlikely to occur in the disordered domains, allowing local 'plastic' deformation at stresses corresponding to the energy required to rupture individual bonds[18,19] which when grouped together, possess a combined cohesive energy of 42 kJ mol^{-1} in a disordered domain compared to 52 kJ mol^{-1} in an ordered domain, based on calculations for poly(alanine) using 10 kJ mol^{-1} per hydrogen bond, due to fewer bonds.[15] In these more-ordered β-sheet and helical structures, the entropic elasticity of the protein backbone and related cooperative rupture of hydrogen bonds provide an intrinsic strength and local elasticity, which allows them to withstand up to three or four times the stress required to break a single bond.[18] After yield, the glassy characteristics are thought to give way to a rubber-like behaviour.[11]

Despite the vague understanding of the structure–function relationships, it has already been suggested that by controlling and/or reinforcing the nanoscale folding structure, a new class of super-tough materials may be within reach.[11,20] Proline[21] and glycine[22] content, and the speed of spinning[23] have been shown to affect the mechanical characteristics of spider silk by controlling the structure on this level. A recent article by Lee *et al.*[24] showed that the stiffness, extensibility, strength, and therefore toughness

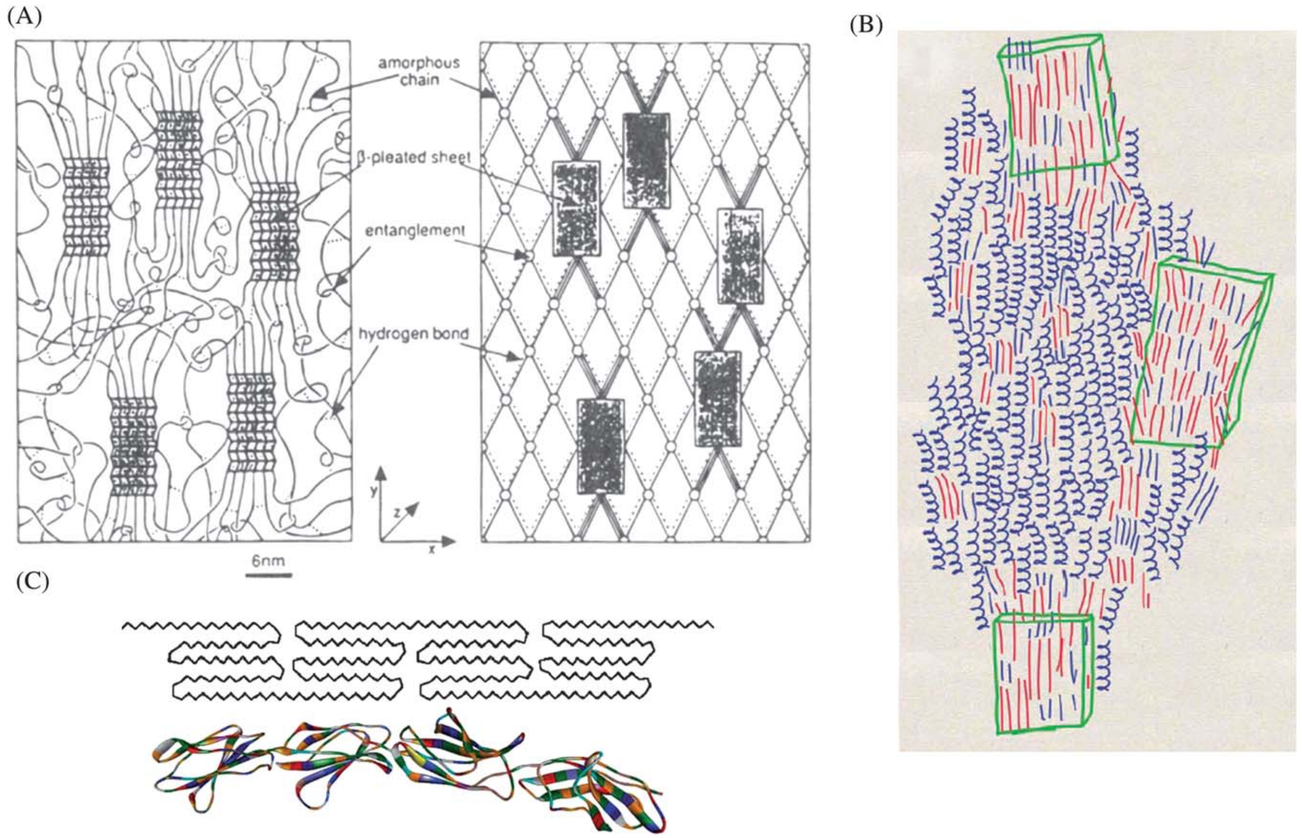

Fig. 2 The prevailing models of spider silk structure. (A) is reproduced with permission from ref. 16, and considers spider silk to be a network of rubber-like chains reinforced by β-sheet crystals. This model fits closely to mechanical data but not to structural studies. (B) shows a glassy structure, reproduced with permission from ref. 14, based on their NMR findings. Although this model shows the presence and possible distribution of secondary structures, we are yet to establish how they interact. The molecular structure shown here consists of β-sheet regions, containing alanine (red lines) and glycine (blue lines), interleaved with predominantly 3_1-helical parts (blue curls), which do not contain alanine. (C), reproduced with permission from ref. 11, has a simplified interpretation of structure, showing a series of beads with varying degrees of order, as defined by the level of hydrogen bonding between the folds.

can be increased substantially by infiltrating metal impurities into the secondary and tertiary structure. This was achieved through multiple pulsed vapour-phase infiltration of zinc, titanium, or aluminium. Long-term exposure to the precursor vapour was argued to lead to the infiltration of Al^{3+}, Ti^{4+}, or Zn^{2+} to create metal–protein complexes with metal-coordinated or covalent bonds. Although the high stiffness resulting from this particular modification may be detrimental for many biomedical applications due to stress shielding effects,[25,26] the study provides an interesting insight into the methods by which spider silk could be modified to become a more effective biomaterial. This leads to consider the mechanisms involved in yield, and particularly how yield can potentially be extended to allow higher strains while maintaining its elastic modulus (Fig. 3), which is possibly the best mechanical feature of spider silk for biomaterial applications as it has the potential to replace 'native' fibres such as collagen and elastin in tissue constructs. The controllable stiffness, in addition to its desirable chemistry and morphology, may also be useful in directing biological responses.[27,28]

Several theories describe the mechanism of yield in glassy polymers. Although these assume a simpler structural configuration than that of silk, they show that both intra-[29] and inter-molecular[30] processes are important, with inter-molecular processes being dominant at temperatures more than 80 K below the glass transition point.[31] From this classical perspective, inter-molecular processes are likely to be more important for biomedical applications, as physiological temperature (310 K) is far below the glass transition point of spider silk (471 K).[32] Recent spider silk-specific models,[15] however, have predicted that yield may be related to a mechanically induced glass transition, with a local strain energy equivalent to the thermal energy required to break hydrogen bonds and would be likely to involve both inter- and intra-molecular processes.

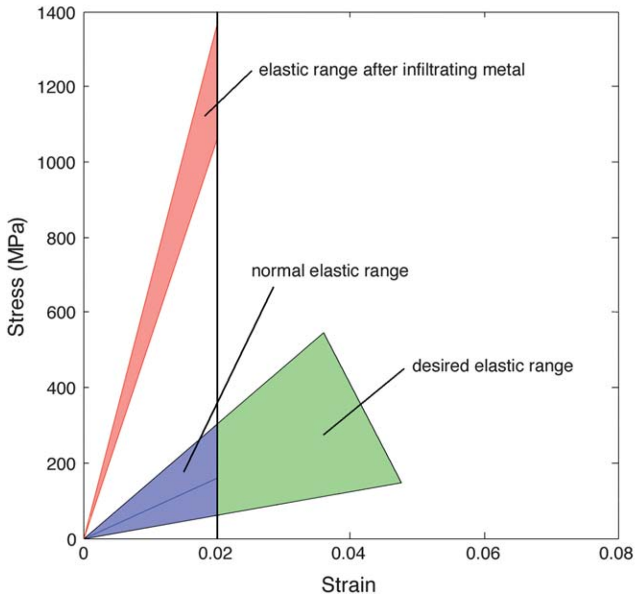

Fig. 3 The desired range of elastic behaviour for the biomedical application of spider silk, compared with normal (untreated) and the reported behaviour after structural modification by infiltrating metal,[24] which are currently limited to approximately 2% strain.

The concept of a mechanically rather than thermally driven transition is similar to the transition points in a classical phase diagram, which involve a combination of 'pressure' (*i.e.* mechanical stimulus) and temperature. Based on these interpretations, it can be reasonably postulated that yield in spider silk occurs as the conformation changes and the disordered domains in the material dissipate elastic energy by breaking hydrogen bonds. By breaking the bonds in the disordered domains, essentially freeing the protein chains, the material reverts to a reinforced rubber type structure[11] that allows large post-yield deformation.

The question remains, however, of how the yield strain can be increased. Lee *et al.*[24] (Fig. 4) propose, based on Termonia's model[16] (Fig. 2A) that the overall increase in extensibility they observed in infiltrated fibres was due to an increased proportion of rubber-like amorphous domains, and a reduction in the size of β-sheets from the water vapour in the infiltration process. This interpretation does not take into account the glassy rather than rubber-like nature of the material[14] (Fig. 2B). A more structurally and thermodynamically relevant interpretation may be found based on the work of Porter *et al.*[11,15] (Fig. 2C), who have taken a simplified approach using group interaction modelling with a fraction of ordered and disordered domains that are defined by the number of hydrogen bonds between amide groups.

Although the magnitude of the change in mechanical properties reported by Lee *et al.* is somewhat surprising, the inclusion of metal and the replacement of hydrogen bonds with metal–organic coordinated bonds or covalent bonds will naturally increase stiffness and UTS. Toughness and yield, though intrinsically related to strength in biological materials, are quite different and more difficult matters. Toughness is related to nanometre-scale properties[33] but is also likely to involve interfibrillar interactions (tens-to-hundreds of nanometre scale).[34] An increase in yield strain may involve changes on either of these levels. A number of modifications are therefore likely to affect yield, but by increasing yield strain may concurrently reduce the

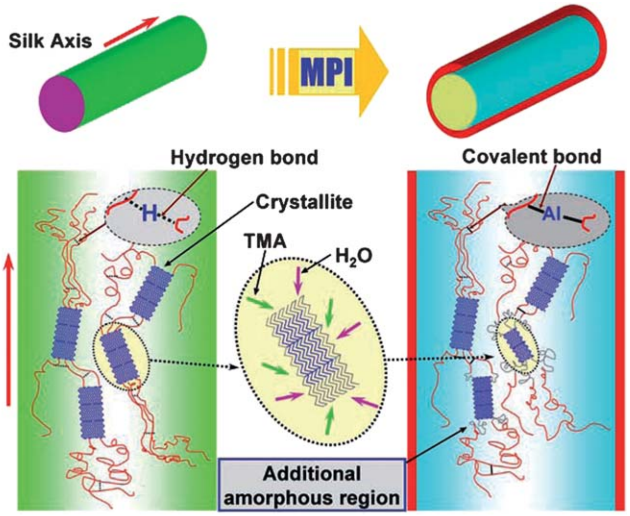

Fig. 4 Changes after infiltration of metal as interpreted by Lee *et al.* including smaller crystallites, additional amorphous regions and the replacement of hydrogen bonds in the amorphous domains with covalent bonds. Reproduced with permission from ref. 24.

elastic modulus and UTS. Such modifications could be made by increasing the fraction of disordered domains and their potential for modification under strain; loosening bonds between the protein chains in the permanently disordered domains to allow a larger deformation before the stress is transferred to the ordered domains; changing the interactions between the two proteins, Spidroin I and II; and allowing a greater unwinding of the fibrils under applied load.

We argue that the key to increasing yield strain, without sacrificing strength, stiffness and toughness, is to slow down the transmission of stress from larger to smaller hierarchical levels, and also the transmission from disordered to ordered domains. These processes may involve interactions between the fibrils in the core of the fibre, the elastic motion of protein chains before locking into a rigid configuration, and the strain-induced ordering of disordered domains.[14,20] Although such conformational changes are likely to reduce the post-yield toughness of the material, we consider this to be of limited importance in a cyclic loading scenario in which the material must operate in its elastic region.

As a portion of the intrinsically disordered domains has the potential to change their configuration to collect an additional hydrogen bond under strain,[11] an increase in the fraction of these domains is perhaps the simplest target for increasing yield strain. By increasing the proportion of disordered domains, while maintaining a potential for order, a larger deformation will be required to effect this yield mechanism as more material will need to be forced into an ordered configuration before the hydrogen bonds are broken. The progressive ordering of these domains under stress would have the added benefit of more closely representing the stiffening of native biological fibres with deformation. A problem with these changes, however, would be the associated decrease in initial elastic modulus, which may require further modifications to offset.

To maintain high, linear stiffness and a high yield strength, the cooperative breaking of hydrogen bonds is critical. As shown by Keten and Buehler,[18,19] three to four hydrogen bonds per fold in β-sheets, or per turn in helical structures, provide optimal mechanical performance in any biological material. Therefore, the decrease in stiffness and strength resulting from modifications that increase elasticity may potentially be offset by optimizing β-sheet size to correspond to four hydrogen bond lengths in the process of increasing the fraction of disordered domains. In addition to controlling crystal size effects, reinforcement with infiltrated ceramic particles[20] or metal ions[24] may improve the ability to tune the mechanical properties. These techniques, however, are likely to strengthen only the disordered domains as they are more easily penetrated[5] and which, by strengthening, may reduce the elasticity gained by other techniques.

The proper modification of spider silk can potentially lead to an exceptional class of fibre-based natural materials, in addition to aiding the development of new synthetic materials. We have argued that the low useable extensibility of spider silk limits its potential as a biomaterial, and suggests a combination of two structural modifications that may overcome this limitation without sacrificing its other desirable properties. The first of these modifications is an increase in the fraction of those disordered domains that retain the potential for order under strain. The second is to offset the softening caused by the first modification by increasing the efficiency of the β-sheets, which can be achieved by optimising their geometry to encourage the cooperative rupture of hydrogen bonds in groups of three to four.

A potential method for applying these structural modifications may rely on pulsed laser irradiation, selectively tuned to the resonances of particular secondary structures during the spinning or extrusion process. By targeting the vibrations that correspond to particular features of the secondary structure with infrared laser pulses, these features can potentially be either encouraged/reinforced or broken up. If, for example, the random coil vibrations at 1642 cm^{-1},[35] α-helices at 1658 cm^{-1},[36] β-sheets at 1620 cm^{-1} and β-sheet bends/turns at 1691 cm^{-1}[37] can be manipulated during spinning to influence the final secondary structure. With sufficiently high intensity pulses, certain features may be broken up, for example the inter-β-sheet vibrations at 1620 cm^{-1} can be broken up while retaining the connectivity of the bends at 1690 cm^{-1}, a decrease in order may be possible while maintaining the potential for order under strain. In addition to the amide I vibrations described above, which are properties of the entire macromolecule, specific alanine and glycine moieties can be targeted at lower wavenumbers. It has been shown that side- and main chain stretching in the β-sheet forming alanine sequences occur at around 963 cm^{-1},[38] with glycine moieties vibrating at 1014 and 1026 cm^{-1} for coils and sheets respectively,[38] yet it is unclear what effect these specific wavenumbers will have on structure and consequently on mechanical function.

The manipulation of silk conformation during spinning would be particularly important for synthetic or recombinant silks which, though very effective in non load-bearing applications such as drug delivery, wound healing and scaffolds for nerve tissue regeneration,[2,39,40] do not yet show the exceptional mechanical properties of their natural counterparts.[41] Even in these non load-bearing applications, the local substrate stiffness is important in determining cell response. The provision of a low stiffness 'toe in' region in the stress–strain curve being useful in encouraging proliferation in cells such as fibroblasts and neurons,[28] while cells such as osteoblasts prefer stiffer substrates.[42] The control of mechanical stiffness is also critical for mesenchymal stem cell differentiation. Soft matrices that mimic brain are neurogenic (forms nerve tissue), stiffer matrices that mimic muscles are myogenic (forms muscle tissue), and comparatively rigid matrices that mimic collagenous bone prove osteogenic (forms bone tissue).[43] Similar stiffness-dependent behaviour has also been observed in cardiac stem cells.[44] Industrial and biomedical applications requiring sheets or other non-spun morphologies may also benefit from laser-based conformation control as they lack the mechanical environment that assists the formation of secondary and tertiary structure.

Despite the tremendous potential of manipulating spider silk properties, there is a significant gap between the ability to infer the mechanical effects of certain structural changes, and the capability to make those changes without disrupting other, finely balanced interactions in the fibre. To achieve this, it is necessary to develop a deeper understanding of the structure–function relationships in spider silk over its full range of hierarchical levels, from the cooperative breaking of hydrogen bond groups to protein–water interactions, protein–protein interactions, the interplay between secondary structures and the role of the core fibrils. A number of techniques have been employed in an attempt to gain insights into the structure and structure–function relationships, such as nuclear magnetic resonance,[14,45] birefringence,[46,47] X-ray scattering,[17,23] infra-red spectroscopy,[38,48] near-edge X-ray absorption fine structure[49] and a suite of mechanical tests[1,4,6,50,51] and have identified constituents or extrusion conditions that control mechanical properties.

The insights that can be gained through these experiments are invaluable to the extension of established models *e.g.* ref. 15 and the development of new models such as the recent series of atomistic models by Keten and Buehler.[52–54] These massively parallelized models can be used to predict the outcome of low-level changes to β-sheet or helical structures on the nanoscale strength, stiffness and toughness. They further provide an interesting approach by which the changes to mechanical performance can be predicted based on structural modifications, emphasizing the effects of crystal size on the mechanical properties. These models will be particularly important for guiding structural modifications for *in vivo* applications, in which the complications caused by immersion of the fibre in water must be considered. A large number of investigations have described these effects in the bulk material properties, and attempted to explain the underlying changes to secondary structure *e.g.* ref. 4,6,55 and 56 and it is generally accepted that water infiltrates the pseudo-amorphous domains, decreasing the order, leading to supercontraction and a weakening of the fibre. The role of water in yield processes is not well understood, and changes to the structure may have very different effects under wet conditions to those in ambient air.

Due to the considerable interspecific, intraspecific and intra-individual variations in spider silk properties,[57] a consistent picture of structure–function relationships has not yet been achieved. Such cohesion would require the simultaneous measurement of a range of transient changes, spanning the full structural hierarchy, ideally while the deformation occurs, and under varying humidity. These measurement techniques should include wide angle X-ray scattering and infra-red spectroscopy to describe the changes to the secondary structures, small angle X-ray scattering to describe the interactions between ordered domains, and birefringence and second harmonic generation microscopy to describe the fibril behaviour. Although it is not feasible to perform each of these measurements simultaneously, a coherent insight may be gained through the careful selection of similar samples and sufficient sample numbers. An emerging technique that shows great potential for use with spider silk is combined atomic force microscopy and transmission electron microscopy[58] to describe the nanometre-scale mechanics and local deformation mechanisms in the fibre and fibrils. The combination of these measurement techniques is challenging, yet it is a critical step forward for a fundamental understanding of this exciting material, for its modification, and for biomedical application, particularly in load-bearing tissues.

Acknowledgements

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