Feature Article

Silks as ancient models for modern polymers

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ABSTRACT

Silks have a great potential as sustainable, ecologically benign commercial polymers. Here we discuss this fascinating bio-material by merging the biologist's with the polymer scientist's views i.e. combine insights into the characterisation and understanding of evolved structure, property and function in natural silk proteins with the broad scope of applied disciplines ranging from molecular modelling to rheology and mechanical testing. We conclude that silk cannot be defined simply by only its origin or material composition but any meaningful designation must include the key feature of formation by extrusion spinning. We further conclude that silk 'spinning' largely depends on a highly specific denaturation process dependent on competing molecular-level interactions of hydrogen bonding between water and main chain amide groups in the silk protein chains. Finally we conclude that silks have a bright future not only as archetype models to guide our understanding of highly adapted and energy efficient bio-polymers but also as prototype models to guide the design of totally novel polymer systems.

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1. Introduction

Silk, especially spider silk, is often sold to the public as a super material with a wide range of special and amazing features. Undeniably, some silks such as the draglines of the golden orb spider *Nephila* do indeed have remarkable strength and toughness. Yet many other silks have mechanical properties that are far from extraordinary when compared with man-made polymers. Indeed, it must be remembered that every silk has evolved for a very specific task, or range of tasks and without doubt (given the power of Darwinian selection) all silks are extremely well adapted each for its own task. Most importantly, silks (like all other biological products) also have evolved to give good value for money with 'money' being the euphemism for 'energy', which is (after all) the common currency of life.

Natural silks have undergone many millions of years of tuning by stringent selection processes that have shaped the forms and performances of all contemporary silks. Clearly, design by evolution has led performances to be highly adapted to the criteria likely to be encountered. Hence silks are extremely effective as well as supremely efficient materials with a massive range of properties ready to satisfy a wide range of biological functions selected to promote survival chances. Nevertheless, in essence silks are structural polymers and can be analysed as such. In the following

we will outline what this means for both the silk fraternity and the applied-polymers community. Natural silks have evolved many tricks that provide astonishing (and sometimes startling) insights into advanced polymer design. Thus much can be learned from studying such silks – without prejudice – as both archetypes and prototypes for polymer design.

Commercially, silks not only have a shining past but also a bright future. After all, silks can be used (or studied) in their native forms (Vollrath and Knight [1]) but, importantly, they can also be chemically modified and can even be totally disassembled in preparation for re-spinning re-assembly under artificial conditions (Zhou et al. [2]). In addition, the genetic and protein blueprints of specific silks can be analysed with the view of either copying them into microbes or of chemically synthesising them, both with the view of creating a spider-less silk-production system (Lazaris et al. [3]). Importantly, in the light of new research (Holland et al. [4]), we must ask: Are these reconstituted or synthetic materials really silks? Indeed, what makes a 'silk'? Is it the most basic chemical composition of the material in question? Is it the physical behaviour of the spinning dope? Is it is the mechanical behaviour of the fibre? Or is it the natural origin of the material in all its permutations? These are questions we will explore in our essay in order to get to the heart of this fascinating material. Interestingly, a reconstituted silk tends to have the mechanical property profile of a very ordinary polymer material and thus has lost (despite still having all building blocks of the native material) the key features that make a silk special. To date, no reconstituted silk even spins like a silk, which may be a bit of a give-away (Holland et al. [4]). Clearly, having the correct amino

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acids and also having them in the correct sequence of mers does not make a silk, if our definition for a silk combines behaviour and mechanical properties rather than just chemical composition. However this may be, the simple fact that so far all reconstituted silks have lost the magic of their origin gives us some tantalising cues as to what it is that may make the natural silks so special (Fig. 1).

Many excellent, recent review articles are available on silk, for example see (Kluge et al. [5], Grunwald et al. [6], Vollrath et al. [7], Holland and Vollrath [8], Porter and Vollrath [9]) to name but a few. The purpose of this article is not to add to this body of literature. Instead we aim to outline the insights we have gained from studying natural silks and convey the lessons they are continuing to teach us. In addition we will explore the directions that in our view silk-polymer researchers will need to take in order to make 'synthetic' analogues of natural silk a realistic possibility. Occasionally in our essay we will go into considerable detail of the biology of the system as this, we think, is important to fully understand the design criteria as well as the processing and material constraints under which this specific class of materials has evolved. After all, some of these natural constraints do pose limitations also for the industrial use of techno-silks – to put a simple name to the bio-inspired silk-materials we all would like seen being invented on the back of natural silks. For the purpose of clarity we will explore the ancestral evolutionary relationships under the heading of 'archetypes' and then explore their applicability to industrial uses under the heading of 'prototypes'.

2. Background

Our approach to silk combines the biological characterisation and understanding of structure, property and function in natural silk materials with a broad range of polymer science 'tools' ranging from molecular modelling to rheometry and the wide gamut of other mechanical testing techniques. While sounding rather obvious, this approach does require some quite radical adaptation and evolution of pivotal ideas from both sides.

Traditionally, silk is a specialist agricultural product with an impressive history in many cultures and civilisations. Perhaps no longer having the influence it had in times gone by, silk is still produced in impressive quantities and commands excellent prices – both, in fact, not very far from other specialist fibres such as e.g. KEVLAR. But silk has more to offer than a notable history, it also has, in our view, a great future. It will be a future as a fibre with extraordinary properties that is produced sustainably and ecologically, that is more of a carbon sink rather than a carbon source, and that can be recycled easily, consisting 99% of protein. For this great

Fig. 1. Comparison plot of natural silk with other natural and synthetic polymers.

future, as we envision it, silks will no longer be used only as they are now (i.e. principally in fashion) but instead also deployed as a fully integral components of composite materials, whether in engineering or in medicine.

Most importantly, natural silk fibres as well as biology's highly evolved production 'technology' can act as templates and inspiration for the development of novel 'techno' silks. Such materials would be designer polymers (probably also principally proteinaceous) that can be manufactured industrially in bulk from, perhaps, bio-reactor products or straight from genetically modified agricultural precursors such as e.g. soy bean proteins. To aid the advance of such materials there is much we must first learn from natural silks. Thus they have great potential as prototypes in a world that is becoming ever more resource-limited and which, therefore, is putting ever more emphasis on the characteristics renewable and energetically efficient. Naturals silks are masters at this kind of thing, as we shall demonstrate.

But silks are not only prototypes, i.e. models, for future development, but they are also archetypes, i.e. classical examples of past development. As archetypes, silk fibres are representative not only of dead silk-type materials but also of a wide range of other biological elastomers, which are live i.e. are maintained by the animal in a steady-state equilibrium. Moreover, in silks we are beginning to find all sorts of molecular mechanisms that impart their specific properties to other bio-elastomers (as we will show), such as collagen, elastic, keratin, and resilin (Porter and Vollrath [9]).

"What is silk?" At first thought one might think that such a question would have a straight and easy answer such as: "silk is the fine filament or fibre that silkworms (or spiders) secrete to make their cocoons (or webs)" and/or "silk is the thread and fabric made from such fibres". However, when we dig deeper into the subject, we see that there are more silk filaments than just the threads made by silkworms or by spiders. For example, there are 'silk' exudates spun by beetle larva, lace-wing larva, mites, pseudoscorpions, flees, ant and bee larva and several other arthropod taxa (Craig [10]). And these silks are far from identical to one another (with many having evolved independently) – although they also share many, often very specific, similarities.

Thus, silks are not only separated by different 'inventive' processes but, in addition, are also isolated by hundreds or tens of millions of years of distinct evolutionary tuning and honing for specific uses. Moreover, spiders (for example) make more than one silk in the same individual, with some spiders, like the common garden spider *Araneus* sp. or the golden silk spider *Nephila* sp. spinning 7 different silks from 7 sets of different silk glands, often several used together at the same time (Vollrath [11]). Interestingly, one of these silks, the product of the aggregate glands, would never be called a silk (looking merely at its consistency and composition which is liquid and made up of neurotransmitters) were it not for the fact that it is produced by a gland that clearly has evolved from a silk producing gland (Vollrath and Knight [12]). Another set of silks, produced by lace-wing and beetle larvae is not produced by specialist silk glands from silk proteins, but is produced in the hind gut by firmly aggregated faecal matter (Mello [13]). Finally, the long cellulose 'hairs' that grow on maize corn cobs are, confusingly, also called silks. However, we will ignore any botanical 'silks', which in any case are grown not spun, in order to focus on the 'real' (i.e. zoological) natural silks. These silks are primarily protein-based, although they tend to incorporate a wide range of additional compounds, generally in minor quantities.

At this point, alarm bells are probably ringing loud to the polymer scientists as they hear the biologist's deceptively innocent term 'protein-based'. The 20 possible peptide mer unit segments in such polymers are strung together in an almost infinite possible number of combinations, but are radically different at an individual
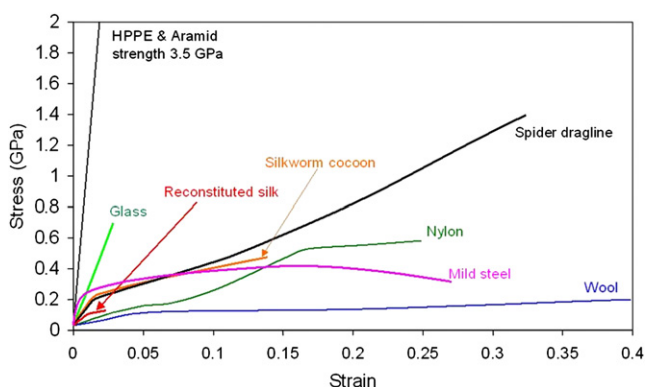
level. Their common feature is the Nylon 2 polymer chain backbone (−CHR−CO−NH−), but their differences are in the wide range of side chain groups, R, that can have almost any possible chemical characteristic.

The first simplifying constraint to be applied derives from the natural origin of the material. Silks are mainly used as structural engineering materials in nature, but with a wide range of possible combinations of the core mechanical properties of stiffness, strength, and toughness required for a diverse range of applications, from capture and storage of prey through to protection of eggs or pupae in a range of environments from hot deserts to underwater. These functional design constraints show up well in the different silks. It is possible to capitalise on these differences by reconciling the biological imperative of efficient energy management (to optimise the chances of survival) with the physics of the mechanical properties of polymers. This can be done by identifying the molecular energy 'management' that controls a silk's material properties (Vollrath and Porter [14]). Indeed, as we will show, the approach of understanding energy-management at the molecular level can be extremely useful to outline ways forward to the overall goal of emulating silks. The insights gained from such a model can be expanded further in order to allow the prediction of mechanical properties of any polymer type (Porter and Gould [15]). And such predictions strongly support our argument that understanding the mechanisms involved in silk could be very useful indeed in guiding development in polymers across a broad front.

The second major simplification derives from the polymeric functionality of the material. Silk structure can be 'reduced' to that of ordered and disordered domains at the nano-meter scale due to the dominance of hydrogen bonding in determining the semi-crystalline morphology of the polymers (Porter et al. [16]). The complexity of infinite possible variations in structure is thereby reduced to the standard generalities of the science of macromolecular chains.

Deploying these two simplifications will allow us to suggest ways forward in the development of futuristic silk materials by using the parallel themes of energy management and order-disorder which are equally important in both biology and polymer science. This allows us to bridge the deep gulf that still exists between the materials and engineering scientists (who would love to be able to re-create silk technology and make techno-silks for the 21st century) and the zoologists (who would love to make use of the extensive knowledge and box-of-tools of the polymer scientists – if only they knew about them).

3. Definitions

Clearly, as outlined in the introduction, we cannot answer the simple question "What is silk?" with a plain and ready reply describing the material. Hence let us pose a slightly more complex question: "What are silks?" in order to include the full diversity of the zoological materials outlined above. After introspection and much discussion we have come to the conclusion to define the class of materials typically called silks by their production process rather than by their material composition. After all, the silks mentioned above, and indeed all natural silks that we know about, are extruded i.e. "spun". Hence, we here put forward the definition for protein silks that they are "filaments that are spun at the point-of-delivery from feedstocks, which can differ widely in detail but are protein based". Thus our definition places the emphasis firmly on the coupling of the extrusion spinning aspect with the composition of the feedstock material. Accordingly silks can have chemical compositions and material properties that are rather similar to other bio-elastomer materials such as collagens, for example (Gosline et al. [17]). These non-silk bio-materials, however, differ

from silks in their developmental history as they all are "grown" rather than spun. Interesting cases for hybrid spun-grown biomaterials may be the dog–fish eggcase (Knight and Vollrath [18]) and the mussel byssus thread (Waite [19]).

Why emphasise this difference between spinning and growing? Extrusion spinning is a process that is well established both in industrial applications and in commercial polymer research. Thus, extrusion spinning with its huge body of both theory and practice behind it can form a valuable link here between the polymer industry and biology. On the other hand, and importantly for the industrial production of synthetic bio-materials, growing is the typical way for Nature to make her materials and structures. But growing is also a process that so far has largely eluded mankind as a way of making materials (other than perhaps crystals). Growing implies the assembly in an orderly fashion by bringing together at the site of construction, often under tight cellular control and typically sequentially, the various components and combining them 'block-by-block' to create complex polymer structures that rely on emergent properties in order to generate the forms which make-up the morphology. In biology such a growth process is called 'morphogenesis' or 'ontogeny' with the organisation of the spatial and temporal distribution being of paramount importance for the functionality of the final product. Examples of bio-materials that are grown are the collagen of, for example, the joint-cartilage, the meniscus and the spinal disks, or the elastin of the aorta, or the resilin of the insect wing mechanism, or the titin of our muscles, or the keratins of hair, hoof and nails, or the collagen//keratin composites of skin and scales, or the collagen/mineral composites of bones and mussel shell, or the *byssus* filaments of mussel attachments (Shewry and Tatham [20]).

4. Biology and polymer science

The best studied of all spider threads is the drag-line of the *Nephila* spider, which is also a key component of this animal's orb web (for details and references see Vollrath [21]). Each filament has a perfectly circular cross section and a complex hierarchical construction with a core and multiple skin hierarchy. The core and inner layer consists of fibrous proteins (spidroins with molecular weights in the hundreds of kilo Daltons) while the outer layers consist of glycoproteins, lipids and other coating compounds. The core itself is mechanically isotropic (Ebenstein and Wahl [22]) although it is highly structured, being made up of micro-filaments and containing numerous micro-channels. These give the silk the appearance of a filled rubber and act, perhaps, as self-healing inclusions as well as aiding in crack deflection (Shao et al. [23]).

Similar filled channels have evolved independently in some commercially important moth silks (Robson [24]). Otherwise, however, moth silks such as those of the mulberry silkworm *Bombyx mori* are rather different in structural appearance to spider silks. Firstly, they are not round in cross-section but sharply triangular or flattened ovoid. Secondly, the paired filaments (fibroin proteins without an obvious skin-core structure) tend to be surrounded by a thick coat of sericin proteins. The fibroins of these silks, like the spider spidroins, also have high molecular weights while the sericins have rather small weights.

While the silks of spiders and moths are well known, there are many other arthropods that also have evolved silks independently (Craig [10]). Very little is known about them in comparison, and we suspect that valuable insights into bio-polymer are hidden in their folds, structures and production processes; and indeed a number of research groups worldwide are now beginning to focus on such silks.

For all silks, the conversion of aqueous solution to solid fibre can be interpreted as a controlled de-naturation by de-hydration

during a phase transition which in turn is driven by the combination of mechanical (flow field) and chemical (changing pH) forces; for details and references see (Vollrath and Knight [1]). Interestingly, the spinning of the multilayered ply-like walls of the dogfish egg-capsule is following a similar principle, albeit in a divergent rather a convergent die (Knight and Vollrath [25]). The principal thread of a spider is formed by drawing protein dopes from within the internal lumen of a gland, where they are stored often for days or weeks, through a hyperbolic die, followed by a duct for curing and coating of the fibre and ending in a spigot where the thread exits the animal (Vollrath and Knight [12]). In some cases co-extrusion of multiple feedstocks, stored concentrically, can occur and in some cases inclusions can be co-extruded in special vesicles that elongate in the extensional flow field of the convergent geometry of the duct-die. This flow field in the draw-down taper also helps to re-arrange the molecules of the fibrous proteins by aligning them. Whether the molecules also refold during this process is still an open question. In any case, during spinning the feedstock undergoes a sol-gel phase transition driven by the combination of shear flow and a shift in pH. Curiously, during conversion the fibre pulls away from the wall of the die leading to internal draw-down without die-swell and the fibre exiting the spigot is already hardened, although it benefits from further post-draw extension. The thread of a silkworm is formed more or less in the same way, although in this case the internal draw-down is into an aqueous solution of proteinaceous coating compounds which after extrusion harden slowly and thus become the matrix of the cocoon composite.

While the biology gives us the frame parameters, polymer theory will be key to unblock our understanding by providing crucial analytical 'tools'. In particular, polymer theory will allow us to examine, analyse and model the different states of matter found in silks, specifically the crystallite-amorphous hierarchy and the various degrees of order and their underlying principles. Indeed, hydrogen bonded order, scaling and hydration, together with the highly regulated production process that control them, are the key parameters that set silks aside from man-made polymers. Understanding these parameters, and being able to transfer them into industrial production processes will be vital on the road to designing silk-replacements and, furthermore, also to conceive and create totally novel silk-based materials.

Silks have structural properties that are extremely attractive. But, in addition, silk is for a biomaterial also unusually accessible experimentally since it has evolved to function *ex vivo* and hence can be studied without any complicated preparation away from the animal. This accessibility allows us to examine particular aspects of the material and test both specific and general hypotheses. For example, we can analyse the silk's nanometer-scale structure in great depth (and without consideration for difficult *in vivo* conditions) in order to consider its implications for the interactions between the constituent proteins and the water molecules that surround them. Water is the key, since all biological systems are built around 'wet-engineering' and bio-elastomers rely for their functionally on their fully integrated hydration properties. In fact, the characteristic of embedded hydration properties built into the molecular structure is a key feature that differentiates natural silks from man-made polymers and even from reconstituted silk materials. Such properties are built into the material not only by the molecular structure of the proteins but are tuned by and during the extrusion-spinning process.

Rather than simply reviewing silk as an interesting natural polymer, we are presenting a framework for dialogue between the biological and polymer perspectives by looking firstly at the possible structures of natural silk, secondly at the spinning process by which silks are formed and thirdly at the mechanisms that control the solid silk material properties. These aspects are inextricably linked, but provide convenient categories for discussion.

5. Structure

In order to piece together a mechanism for silk properties and to link the spinning process and the final fibre properties, one could do worse than starting with the structure of the biological feedstock material. Here some 'unique' characteristics distinguish silk from most other polymers, forcing us to invoke and combine both the rules of biological protein characterisation and polymer science.

Rather than being a relatively simple homo-polymer or copolymer of one or two characteristic mer units, proteins can be built from any permutation and combination of amino acid (peptide) segments with a composition –NH–CO–CHR–, where R is one of 20 different side groups that have almost any possible chemical functionality. This 'primary structure' is the first level of structure in a protein. There are many thousands of different silk compositions, with the more interesting engineering polymers being perhaps those most recently evolved in nature. These have high fractions of the simplest amino acid, peptide-group building-block called Glycine (G) with R=H, which makes it a very flexible group that can adopt a wide range of folded structures. An interesting conclusion from a study of the co-evolution of spinning and silk protein structure is that the more advanced functional silks with more complex spinning apparatus seem to require much simpler and more flexible chain segments (Dicko et al. [25]). Other very prolific peptide groups are the next simplest structures of Alanine (A) with R=CH$_3$, and Serin (S) with R=OH, which, respectively, confer hydrophobicity and polarity. The other important segment in engineering silks is Proline (P), which is odd among the peptides in that it does not have the highly polar main-chain amide group and intrinsically disorders the chains by twisting them out of their usual highly regular conformer torsional angles (Rauscher et al. [26]).

To illustrate the biological primary structure consider three important silk protein sequences i.e.:

- *Bombyx mori* fibroin: commercial textile silk -GA-GA-GS-
- *Nephila* Spidroin I: the ordered spider silk protein for stiffness -GRG-
- *Nephila* Spidroin II: the disordering spider protein for toughness -GPGRR-

Such primary protein structures assemble to form secondary structures of semi-crystalline morphology of ordered, nanometer scale, hard domains in a softer matrix of more disordered polymer chains, which is the classic 'Termonia' morphology of spider silks (Termonia [27]). The secondary structures are held together by a very high density of strong hydrogen bonds between the main-chain amide groups, which is another key difference from most synthetic polymers. This is where the polymer scientist and biologist usually differ at an unconscious level.

Fig. 2a shows schematically the general silk morphogy picture of a hard domain in an amorphous matrix. Now ask a polymer scientist and a biologist to sketch the way this morphology might be filled with polymer chains. Fig. 2b shows the polymer scientist sketch of separate chains converging and aligning in the crystal domain, surrounded by separate disordered chains. Fig. 2c shows an extreme biological perspective composed of a single folded chain beta sheet crystal domain surrounded by helical structures, also from single chains.

Our own discussions have come to the conclusion that the strongest spider dragline silks have a hybrid structure comprising about 45–65% hard domains of beta sheet folds in a matrix of mixed helical conformer chains that do not actually form extended helix
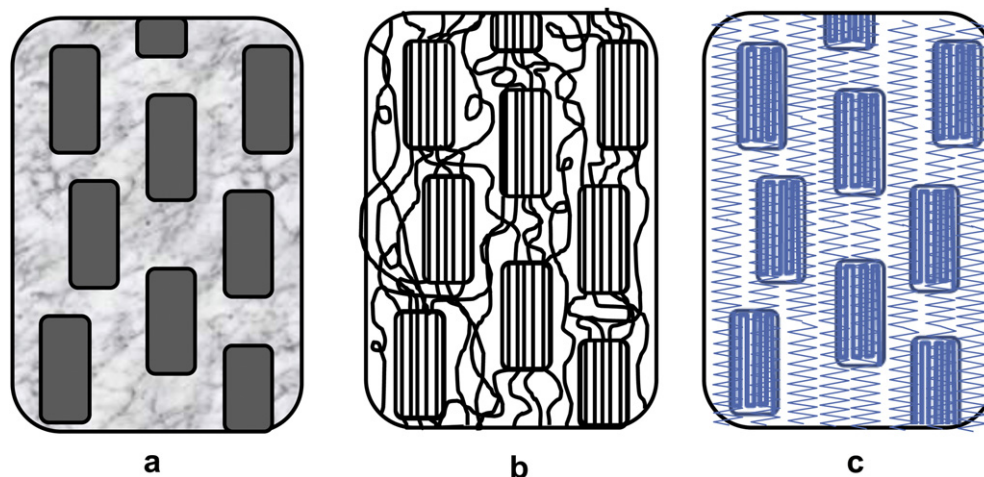
Fig. 2. Schematic comparison of ways to visualise silk morphology: a. is simply hard crystal domains in an amorphous matrix. b. is a polymer sketch with separate chains converging into the crystal domains. c. is a biologist sketch of folded beta sheets in a helical matrix.

structures, thereby looking like an amorphous matrix. Due to the lack of consensus between analytical approaches to defining crystal structure in silks, such as x-ray scattering, FTIR and UV spectroscopy, and NMR, we have quantified silk in terms of ordered and disordered fractions. A biologist might call these beta sheet and helical fractions respectively. We shall see later that this is a key pragmatic simplifying step for predicting silk properties from such an apparently complex chain structure.

As polymeric fibres, silk fibres are an unusual combination of being seemingly highly oriented (from birefringence data, for example) but having a moderately high elastic modulus (about 10 GPa) that is essentially isotropic (Ebenstein and Wahl [22]). Also, as biological materials, the silk feedstock solutions are almost gels with quite high viscosities that need be stable in concentrated solutions for extended periods of inactivity, yet flow instantly on demand through very small ducts and transform spontaneously from solution into solid fibres by expelling the water solvent. Again, through a process of polymer-biology dialogue, we have suggested a simple macromolecular morphology as an example structure that is consistent with these property observations, and is also based upon a protein (fibroin) structure (Vollrath and Porter [28]). Fig. 3 shows the fibroin 'string-of-beads' structure, which consists of 'beads' of beta folds with a length in the chain axis of about 4 nm each. The 'string' has the characteristics of a liquid crystal, with limited flexibility in the chain axis, which is consistent with observation (Vollrath and Knight [1]) and which is important for both processing and mechanical properties, as discussed below.

How do we arrive at this hypothetical string-of-beads morphology as a working model for a macromolecular structure? We start with the fold-size of the beta domains and the realistic assumption that the beta morphology is typically about 10 units long. In the dope, hydrated domains naturally form into symmetrical beads of about 4 nm domain size to minimise drag during flow,

which shrink to about half that diameter when water is lost during spinning. Such a model and view would allow us to link spinning rheology and structure using domain-size calculations.

After this introduction to silk morphology we next discuss how structure relates to processing and properties based on insights we have gained from our many dialogue iterations focusing on self-consistency between the polymer and the biological views of silk structure.

6. Processing: how is silk made?

Remember that we have defined silk specifically as a material that is spun. Hence, in order to fully understand silk, we need to fully appreciate its production process, which 'runs' at ambient temperature and very low pressures with water as solvent. Ultimately, as we argue, processing is the key to the material and its tremendous success with a wide diversity of animals. For the biologist, processing imparts flexibility because it is – in effect – behaviour. Thus, processing allows the animal to rapidly adapt a core material to a wide range of conditions and environmental challenges. Hence processing provides the selection arena of evolution with the tool to tune a silk to perform optimally under very specific and potentially highly variable conditions. For the spider, for example, who may build a new web every day in a constant arms race with cunning prey, the adapted material properties through processing conditions may matter very much. The polymer scientist will be entirely familiar with the process of solution spinning of fibres, and input from the science and technology of spinning will be invaluable for understanding and eventually emulating the spinning of silk-like fibres.

How, then, is this functional flexibility achieved and why does it uncover principles of protein polymer processing? The typical silk is spun from a highly concentrated (30-50%) solution of large
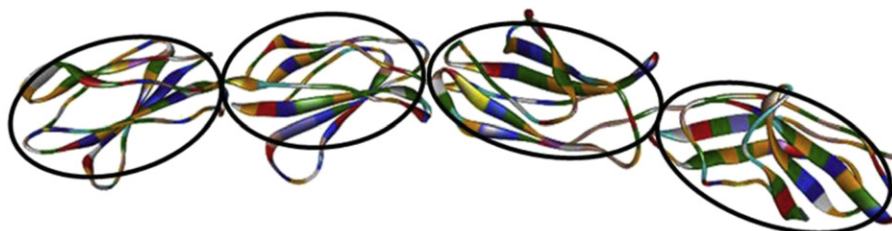

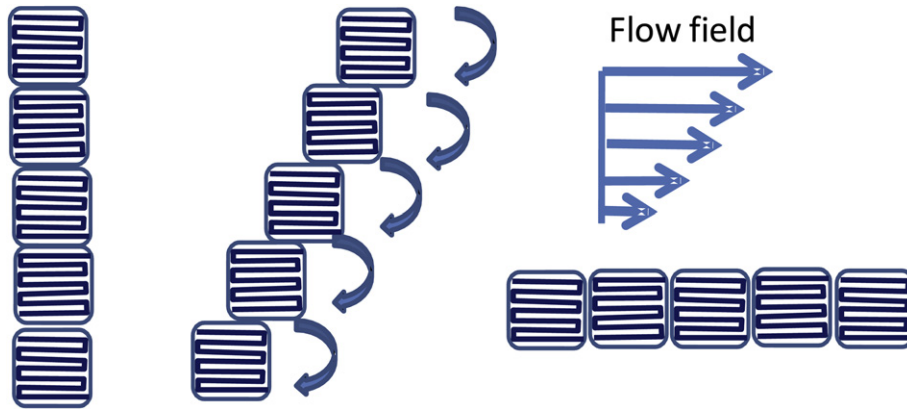**Fig. 3.** 'String-of-beads' suggestion for silk hairpin folding morphology.

Fig. 4. Schematic suggestion of morphology development from stored protein feedstock to final fibres under shear flow.

(several hundred kDa) molecules. As discussed under structure, the molecular structure is highly repetitive and consists of alternating sequences of motifs encoding for both rigid and flexible elements. These sequences appear to be folded into a hydrated nanoscale string-of-beads structure that in turn shows liquid crystalline flow behaviour.

What is the key and unique step in silk spinning that differentiates it from synthetic polymers as well as from re-constituted silks? This 'step' procedure seems to be invoked during the transition from a concentrated solution or gel feedstock (or 'dope') to a solid fibre product. It is a procedure that is effected by mechanical (shear-flow) processing in combination with chemical (pH-shift) processing. We have modelled the denaturation process quantitatively at a fundamental level of competing molecular-level interactions of hydrogen bonding between water and main chain amide groups in the protein (Porter and Vollrath [29]). Both, modelled and observed processing conditions lead to spontaneous but controlled dehydration or denaturation of the protein with the water being 'pushed out' of the fibres by assemblies of hydrophobic groups in the polymer. Thus both rate and ratio of dehydration affect the formation (and thus conformation) of the hydrogen bonds that hold the macromolecules folded at the nano-scale and interlinked by intermolecular hydrogen bonds, and thus determine the solid fibre properties.

The geometry and flow rates in the natural spinning ducts studied so far will seem quite familiar and reasonable to a polymer scientist, and indeed natural spinnerets often have apparently sophisticated, hyperbolic geometries that ensure a gradual and linear increase in the shear rate through the duct. This reinforces our view that the special characteristics of silks are imparted by the synergy between the spinning conditions and the properties of the natural feedstock. The spinning dope must be stable and is stored for quite long times, but must potentially be ready for instant use. We can assume that the complex folded protein secondary structure of the solid fibre morphology is already embodied somehow in the feedstock in the suggested string-of-beads morphology, for example, since a reliable refolding operation under the complex and unstable denaturing conditions seems improbable. An interesting analysis of the evolution of protein composition with the increasing specialism and complexity of spinning glands shows the single most important correlation that the fraction of the simplest and most flexible possible peptide group Glycine increases with gland complexity (Dicko et al. [25]), which again highlights the synergy between spinning conditions and properties through the evolution of composition.

Since stability and control are important aspects of silk production, the morphological transition from stored feedstock to processed fibre is an interesting potential interface between biology and polymer science. One suggested transition route, shown schematically in Fig. 4, is that the protein is stored as folded stacks with an amyloid structure (important in degenerative diseases due to protein misfolding) normal to the flow axis, giving gel-like properties (Knight and Vollrath [30]). As flow is switched on, the stacks are gradually oriented into the flow axis, with segments of the stacks also rotating individually as symmetrical units, eventually becoming the 'string-of-beads'.

Holland and collaborators have measured the rheological properties of natural and reconstituted silk solutions over a wide range of concentrations and made the remarkable observation that the viscosity of natural silk solutions is about 5 orders of magnitude greater than that of re-constituted silk that has been dissolved from solid silk fibres using strong chaotropic agents such as LiBr or ionic solvents (Holland et al. [4]; [31]). One might argue that the reconstituted silk is not fully dissolved or that it has been degraded significantly. However, this does not appear to be the case, and the viscosity of the reconstituted silk solutions is comparable to aqueous Polyvinylalcohol solutions at the same concentration; for example, at 4% concentration natural silk has a low shear viscosity of about 500 Pa s, relative to regenerated silk and fully hydrolysed Polyvinylalcohol with about 0.01 Pa s. Indeed, the viscosity-concentration relation for natural silk dope is in the square of concentration, rather than the power five dependence observed in polymers such as Polyvinylalcohol, suggesting a totally different molecular level mechanism: see Fig. 5. Qualitatively, the natural silk
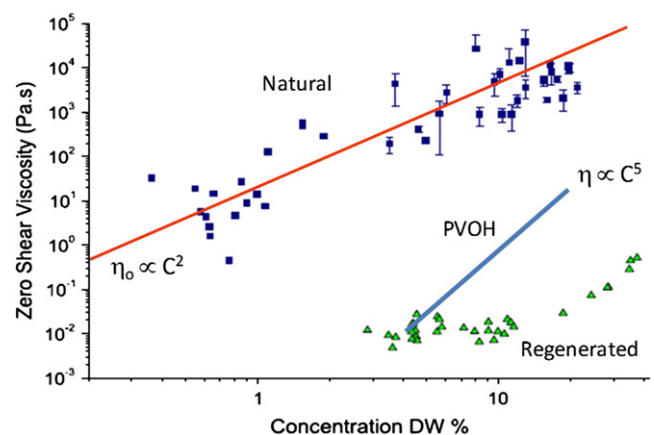

Fig. 5. Solution viscosity for natural and reconstituted silks, with full hydrolysed PVOH solution shown for reference (blue line) (adapted from Holland et al. [4]).

dope 'feels' more like a gel than a viscous fluid, suggesting that there is something special about the natural dope that is not simply due to chemical composition.

Importantly, this viscosity effect appears to be a strong quality indicator of silk 'spinnability' to produce good fibre properties, with a strong correlation between the two aspects; higher viscosity gives silk fibres with higher strength and toughness (Holland et al. [4]). Again, the first reaction of a polymer scientist might be to assume that the higher viscosity spinning dope induces a higher degree of molecular chain orientation in the silk fibres during spinning, thereby increasing strength in the fibre axis. Birefringence experiments suggest that the molecular alignment in natural silk fibres is very strong, but mechanical testing of elastic properties at the microscale (and even the macroscale) tells us that natural silk is actually isotropic with an unremarkable elastic modulus of about 10 GPa (Ebenstein and Wahl [22]).

One way to reconcile these observations at the polymer level is for the natural silk macromolecules to be liquid crystalline, with the string-of-beads morphology suggested previously. The macromolecules in solution might be folded into 4 nm diameter hydrated 'beads', each with about 7 folds of about 10–12 peptide segments, and these beads aligned into liquid crystalline 'strings'; this would be consistent with some silks having hard and soft chain segments of that length that is genetically encoded into the silk structure. These beads could then align into the flow axis to give high orientation and the accompanying birefringence, but the segmentation into 'beads' does not give a continuous chain along the fibre axis, thereby making the fibres essentially isotropic. The natural silk dope would then be a liquid crystalline solution, and a theoretical analysis of the rheological properties of such a solution that includes interchain and inter-string hydrogen bonding would be very useful and also be self-consistent with polymer science concepts.

In summary, we conclude that natural silk dope is intrinsically very different (in some yet to be explained way) from a conventional polymer solution and also from reconstituted silk solutions. Understanding this difference in terms of the synergy between the spinning process and the natural silk dope rheological characteristics and properties will be the key to producing good synthetic silk analogues. In a truly interdisciplinary fashion the biologist's studies of evolved archetypal properties provide the polymer chemist with the keys to the development of novel polymer prototypes.

7. Silk fibre properties

Given the massive diversity of functions for silk in Nature, even quantifying a 'straightforward' mechanical property like tensile modulus for silk is rather pointless without specifying its purpose. Biological materials evolve on a 'fit for purpose' basis, with survival and propagation being the main selection criteria that generally require optimised use of energy and material resources. Strong and stiff spider dragline silks that support the weight of a spider or form the structural framework for a web require very different engineering material properties to those of the softer, tougher, and sticky capture threads in a web. As a rough guide, silk fibres have tensile modulus values in the range 10 kPa for a highly hydrated gel to about 20 GPa for the stiffest dragline silk, and strengths from almost zero values of yield stress to about 1.6 GPa respectively. A good natural textile silk fibre has a modest modulus of about 10 GPa and a strength of 400 MPa. An important strength parameter is fibre diameter, which might range from about 20 μm down to a few tens of nanometers.

Just as nature imposes evolutionary constraints, so the polymer scientist and engineer have selection rules for desirable properties. These would define which and what silks might be most useful and interesting to understand and develop as new engineering materials, for example, in addition to the drive for environmental sustainability. Polymer science already understands and makes highly oriented polymer fibres that are stiffer and stronger than silk, and can design polymer systems with massive viscoelastic damping capability and which work in extreme environments. But silk offers a wide range of combinations of stiffness and toughness that could offer attractive opportunities in engineering design, probably based on biomimetic principles.

For the sake of simplicity, let us focus here on the attractive balance of stiffness and toughness offered by a range of natural silks from conventional textile silks (derived from silkworm cocoons) to spider silks (with their tremendous range of mechanical functions from simple support of weight to capture and confinement of prey). For example, a dragline of *Nephila clavipes* needs to support a weight of about 2.27 g (force = 0.022 N) and the fibre has a diameter of about 6 μm, such that a strength of about 0.8 GPa would be fit-for-purpose. The measured strength to break is about 1.6 GPa maximum. Here it is interesting also that the estimated yield stress of about 0.2–0.3 GPa for a modulus of 10 GPa means that the spider must invoke post-yield plastic flow and strain hardening to dampen its movement in a sophisticated combination of viscoelastic properties. In some cases this might even lead to a kind of shape-memory material, which in itself can lead to interesting and potentially important insights into protein fibrils per se (Emile et al. [32]).

To model silk properties quantitatively, we started from the biological requirement in nature to control and optimise energy use, as in the spider dragline example above (Vollrath and Porter [14]; Porter et al. [16]; Porter and Vollrath [28]). For mechanical properties, stiffness is energy storage, toughness is energy dissipation, and strength is energy to break molecular bonds. From the polymer side, these properties are controlled by the molecular dynamics of interchain bonding between characteristic groups of atoms. In silk, this bonding is a combination of van der Waal's forces and (most importantly) hydrogen bonding between peptide segments.

Our own attempts to simulate mechanical properties directly using molecular dynamics produced interesting insights but provided poor agreement with the fine details of typical profiles of stress–strain to break. Many different combinations of protein folding motifs and polymer morphologies were tried in simple tensile elongation simulations at a range of temperatures, with remarkably similar results in most cases. Fig. 6 shows the example simulation of a model 'string-of-beads' poly(glycine) structure illustrated in Fig. 3 using periodic boundary conditions with an energy minimised structure with a realistic density of 1.3 g/cc at 300 K. The low strain modulus of 14 GPa agrees quite well with observation (shown as a line for comparison), but the yield process is not reproduced. The modulus remains approximately constant until large scale breaking of hydrogen bonding is observed at about the observed failure stress, and is shown in the graphics of the structure evolution under strain alongside the stress–strain plots.

In order to develop a quantitative model for silk properties, we used the biological principle of energy management to refine an established polymer modelling approach called group interaction modelling, GIM, which uses a mean field potential function method for viscoelastic properties based upon energy storage and dissipation in interactions between characteristic groups of atoms in a polymer (Porter [33]; [34]). In brief, a reference purely elastic bulk modulus, B_e, is calculated from a potential energy-well of inter-group interactions. All the other components of the viscoelastic stiffness matrix (such as tensile and shear modulus) are the result of thermo-mechanical energy dissipation that reduces the modulus from the elastic upper limit. Details of the new model are given elsewhere for silk (Vollrath and Porter [14]; Porter et al. [16]; Porter
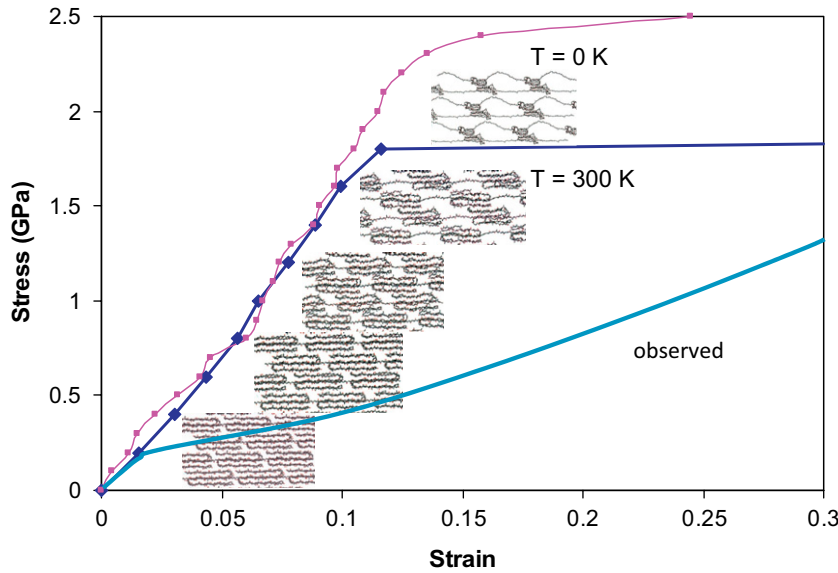
Fig. 6. Molecular dynamics simulation of stress-strain in dragline silk using the string-of-beads morphology, with snapshots of the deforming structure and a typical observed profile shown for reference.

and Vollrath [29], [35]) and also for synthetic polymers (Porter and Gould [15]; Foreman et al. [36]). This set of models is able to predict the highly nonlinear mechanical properties of any polymer type as a function of temperature, strain, and strain rate in the form of analytical equations using a very small number of independently calculated structural parameters to describe composition and morphology. Thus, a model originally developed for simple amorphous polymers was adapted and extended by investigating silks in order to be reapplied more effectively to synthetic polymers. Clearly, this model (and its derivation) is an example of the benefits of biology-polymer dialogue.

Without going into details, it is perhaps useful to show briefly how the general mechanical properties of a dragline silk relate to structure. A reference purely elastic modulus B_e is given by the cohesive binding energy density, with a proportionality constant of 18 from the Lennard-Jones potential function, so a cohesive energy of about 40 kJ/mol and a volume of 50 cc/mol for a generic silk peptide segment gives $B_e \approx 14$ GPa. The energy dissipated through the broad secondary relaxation due to hydrocarbon segments results in an isotropic tensile modulus of about 9 GPa, giving a Poisson's ratio of about 0.4, which agrees well with our own measurements and is characteristic of a tough polymer such as polycarbonate. Finally, the post-yield strain hardening of the strongest silks is critical for their strength-toughness balance, and is the single most important differentiator between natural silk and synthetic polymers. We have shown how the gradual post-yield regeneration of hydrogen bonding under strain can absorb large amounts of energy of deformation and increase the elastic modulus to its low strain initial value, thereby imparting the massive toughness to some natural silks such as spider draglines (Vollrath and Porter [14]).

Some important general principles were formulated using the bio-GIM approach to make prediction of silk properties both practical and physically meaningful in terms of structural features. First is the reduction of complex polypeptide structures simply into 'ordered' and 'disordered' fractions, since the more rigid ordered domains deform far less than the disordered domains; and the disordered domains can then be treated as relatively conventional amorphous viscoelastic polymer that is mainly responsible for energy dissipation, and therefore toughness. Second, the properties of silk can be fine-tuned by water that is active only in the disordered fraction, and acts in a similar way to conventional plasticiser

in polymers. Water essentially binds specifically to polar groups in the silk and reduces the glass transition temperature of those segments. Since the accepted glass transition temperature, T_g, at about 200 °C in dry silk is due specifically to polar amide-amide bonding, the thermal property of T_g is reduced by more water, and modulus and yield stress at any temperature are reduced in an easily quantified manner (Fu et al. [35]).

A third important principle is that strong-tough silks are nanostructured, like many natural materials (Porter and Vollrath [37]). The building blocks of protein secondary structure are all of nanometer size and we suggested that this is a key attribute for strength, just as small grains are important for strength in metals. Until recently, we attributed the poor performance of regenerated silk fibres to the loss of nanostructure after solvation in a strong chaotropic solvent. However, a recent paper suggests that regenerated silks can be as strong and tough as natural silkworm fibres (Zhou et al. [2]). Careful inspection of these results suggests that the improved strength may be due to the small fibre dimensions, in the same way that brittle glass fibres become stronger as diameter is reduced in the classic Griffith experiments (Porter [34]). This raises the more general question of how the hierarchy of structures in natural silk affects strength and toughness, and is an important issue for future biology-polymer collaboration.

If, in analogy, energy is the currency of life then water is its primary commodity – first and also foremost. Without water nothing 'moves' in biology, and water is the key to understanding all biological interactions. Accordingly, the silk molecules are formed in water, they are stored in an aqueous solution and they are largely dehydrated in order to form the fibre. Finally, in the fibre the molecular interaction with either residual or taken-up water tunes mechanical properties. While water is both a solvent and a facilitator, it is its interaction with the hydrogen bonds as well as with occupied and potential bond-sites that controls material properties. Another potentially important effect of water is the reversible large-scale shrinkage of some silks on exposure to water, called supercontraction.

Sensitivity to water is a key descriptor of silk properties (Guinea et al. [38]). While some silks shrink and swell considerably when submerged in water, others shrink little or not at all. Silks that shrink tend to also respond to smaller changes in humidity, thus self-tuning the silk to environmental conditions (Vehoff et al. [39]). Importantly, these silks also tend to stiffen-up when re-dried and

then show a significant drop in elastic modulus together with a significant increase in strain-to-failure. We have shown that these changes are directly linked to the loss of a meta-stable form of order on exposure to water, which is significantly linked to the fraction of the disordering peptide group proline (Liu et al. [40]). Cyclical loading of these silks demonstrates that degree of order is reversible i.e. that it is, to some extent, plastic even in the finished fibre. This is due to the segments around proline being forced and then fixed by drying into a state of hydrogen-bonded order under strain. Water can then access the protein at the proline site and loosen the hydrogen bonds and relax the frozen-in strain for supercontraction.

We conclude that a fuller understanding will be necessary of the diverse roles played by water molecules as well as by hydrogen bonds before we can say that we understand silk as bulk material. However, when we do understand these details then we should be able to put our hypotheses to the test by designing and developing synthetic silks, albeit at first probably with only small molecular weights, for a wide range of medical, mechanical and bio-mimetic functions.

8. Outlook

Silks have a tremendous potential as sustainable, ecologically benign commercial polymers. Indeed, the remarkable soil enhancement properties of the mulberry bush in the natural silk production cycle (Pauli [41]) combined with the bio-degradability of the end product (Horan et al. [42]) make silks environmentally beneficial polymers. In addition to having the potential of being carbon sink (rather than carbon source) materials natural silks also have beneficial socioeconomic components that are important especially for the developing world. After all, their production carries very low set-up costs and low skills while being highly labour-intensive. At the same time the ca 100,000 ton of present day annual production word-wide (down from many times this tonnage at the beginning of the 20th century) match (at raw fibre costs) those of high-end synthetic polymers but with only a fraction of their carbon and pollution costs.

Clearly, today's concerns about both the ecological and the social 'costs' of most of our industrial products make silks a very attractive polymer following its time-honoured, semi-agricultural production methods. However, personally we also see silks as having further and novel functionalities as well as great potential not only for modern production methods more appropriate for developed nations but also as inspiration for totally new materials, which we now outline in concept and idea. As we have shown in our essay, silks provide valuable insights into the production methods and structure-function relationships of a wide class of protein bio-polymers.

In the short term, we foresee that studies into the re-processing of silk materials will provide important key information on re-modelling and re-designing silk materials. By re-processing we mean the solvation of natural, spun silks and their re-constitution first into spinning feedstock and then on through adapted extrusion into fibres or films as well as by moulding into more complex shapes. For such mediated reconstitution it is important that the chaotropic solvents used for solvation are not too strong in order to avoid destroying important and original intra-chain folding patterns while at the same time fully unlocking the molecules from their present networks and thus preparing them for re-extrusion. After all, the work by Holland et al. (Holland et al. [4], [31]) has shown that natural silks, as yet un-spun, have very specific flow characteristics that are quite different (by orders of magnitude) from those of silks re-constituted by traditional methods, which suggest that novel re-constitution techniques will be one key to using natural silks as the basis for re-processing.

In the medium term we expect that we will be able to not only solve the problem of re-solvation of natural silks but also of tuning natural and reconditioned silks into novel semi-synthetic fibres with bulk properties that will not only match those of natural fibres but in specific characteristics excel them.

Finally, in the longer term we foresee that the combination of biological and technical polymer studies (based on intimate collaborations between biologists and polymer physicists/chemists) will lead to novel understanding of polymerisation. This, perhaps using enzymatic and catalyst chemistry, will ultimately have to rely on 'bucket' chemistry in order to provide commercially viable routes to bio-polymer production, always keeping in mind the importance of sustainability of the full production, use and decommissioning processes. Key to this will be a solid understanding of the ability to de-and re-nature the core materials without total de-hydration in order to control both the nano-scale structure and the elastomeric properties that make silks such special materials.

What kind of materials could be made from and with silks? Public opinion, poorly served by the press and some injudicious researchers often views spider silks as a perfect material for bullet-proof vests. This, clearly, is a non-starter. Nevertheless, silk composites, made for example from the stiffer *Bombyx* silks bound into tight networks can be used in tough composites that initially could, perhaps, be modelled on the original silkworm cocoons from which the natural fibres are un-ravelled. On the other hand, silks can be made magnetic or semi-conductive without loss of key material properties (Mayes et al. [43]), which gives some indication about commercially interesting silk materials.

Alternatively, silks are already well known as old materials for medical uses (Heslot [44]) and recent developments have seen a wide range of potentially important advances in this field ranging from ligaments [www.serica.com] and meniscus [www.orthox.com] to nerve repair and placement [www.neurotex.com] extending all the way to more esoteric uses such as hypothetical artificial muscles (Agnarsson et al. [45]) or cigarette filter tips (Tian et al. [46]). Finally, modern infusion techniques such as plasma treatment of silks seem to offer further avenues of modifying the core bulk material (Lee et al. [47]). In summary, we are confident that the astonishing increase in researchers interested in silks will lead to many more ways of not only probing but also modifying and commercialising this diverse and truly wonderful material thus further emphasising the importance of learning from Nature's archetype materials in order to develop prototypes and products fit for the challenges posed by the 21st century.

Acknowledgements

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