ADVANCED FUNCTIONAL MATERIALS
www.afm-journal.de

Dragline, Egg Stalk and Byssus: A Comparison of Outstanding Protein Fibers and Their Potential for Developing New Materials

*Eileen S. Lintz and Thomas R. Scheibel**

This feature article discusses three remarkable protein fibers: spider dragline, lacewing egg stalk and mussel byssus. Sequence–structure–function relationships of selected proteins in the fibers are highlighted and the potential of these proteins for use as new materials is summarized.

1. Introduction: Fibrous Proteins

Proteinaceous fibers are ubiquitous throughout biological systems and display varied mechanical properties and underlying structures (see **Table 1**). To find ways of producing novel biocompatible functional materials, fibrous proteins have become subjects of intense study, both to understand the molecular principles underlying the macroscopic behavior of natural fibers and to learn how to process fibrous proteins into useful materials. One observation has been that common structural motifs, such as the β-pleated sheet, can give rise to manifold properties depending on slight differences in underlying sequences. Secondly, processing plays a decisive if not equal role in the material properties of fibrous proteins as the sequences of the proteins themselves. Thirdly, the presence of auxiliary proteins and matrix components, hitherto relegated to the back bench of research on protein fibers, may be essential for proper assembly and the resulting characteristics of natural as well as synthetic protein materials. Finally, the tendency of protein materials to change their properties with factors such as hydration states and temperature can be either a blessing or a curse depending on the intended application, which must be chosen with care. These complexities make it challenging to produce synthetic functional protein materials that perform at the level of their natural blueprints for a given purpose. Nevertheless, researchers are making inroads into difficult territory with recombinant proteins, in particular with silks. This review discusses proteins from three natural fibers: spider (e.g. *N. clavipes, A. diadematus*) dragline silk, lacewing (*Chrysopidae*) egg stalk silk and mussel (*Mytilus* sp.) byssus (**Figure 1**) and highlights advances in producing novel materials inspired by these remarkable proteins.

E. S. Lintz, Prof. T. R. Scheibel
Lehrstuhl Biomaterialien
Universität Bayreuth
Universitätsstr. 30, Bayreuth 95440 Germany
E-mail: thomas.scheibel@bm.uni-bayreuth.de

DOI: 10.1002/adfm.201300589

2. Silks

Silks are generally (but not exclusively) understood to be proteins secreted by arthropods into fine (~2–~200 μm) threads to fulfill numerous purposes including prey capture, dispersal, reproduction, adhesion, and building cocoons, nests, egg sacs and stalks.[1,2] Biochemically, silks are diverse but do have a few defining elements. With some exceptions, they are large proteins (>100 kDa[3]) that contain long stretches of highly repetitive amino acid sequences rich in alanine, serine and/or glycine. These sequences usually yield a regular and aligned pattern of a dominant secondary structure such as coiled coil motifs, crystalline β-sheet regions or α-helices (e.g. in honeybee silk), which play significant roles in the material properties and function of the silk.[1,2,4,5] As fibers, silks tend to show high tensile strength for their weight (some even outperforming steel) and enough elasticity to yield remarkable toughness.[5–7] Historically, natural silks have been used for medical purposes such as for sutures and wound coverings.[8]

2.1. *Bombyx Mori* Silk

As a textile fiber with millennia of cultural history and technical uses, silkworm silk (from *B. mori*) is considered *the* paradigmatic silk. Silkworm silk is well characterized and a noteworthy body of scientific study has yielded a number of reviews that discuss *B. mori* silk structure,[9,10] material properties[11] and catalogue its use as a biomaterial,[12,13] so only a brief overview for the sake of comparison to other fibers is given here.

To weave cocoons for metamorphosis, *B. mori* fifth instar larvae secrete silk from a pair of labial glands in their heads. The silk emerges as a double thread consisting of the protein fibroin with a coating of glue-like glycoproteins called sericin that is removed in commercial silk production before processing into textiles. Fibroin consists of a 390 kDa heavy chain connected via a disulfide bond to light chain fibroin (25 kDa) linked with the purported chaperone P25 (30 kDa) through hydrophobic interactions[14] in a 6:6:1 ratio.[15] After preprocessing of this basic polymer unit in the endoplasmic reticulum and the formation of microfibrils in the Golgi apparatus, fibroin is secreted and transported in granules into the lumen of the silk glands where it is stored in the middle section of the gland in gel-like high concentrations (20–30%)[16] along with sericins. Thread assembly proceeds roughly as follows: as silk proteins move from the middle to the anterior silk gland, changes in pH

and ionic strength of the silk solution occur. Together with the removal of water and the shear forces imposed by spinning, the units of heavy chain fibroin align, cross-link and polymerize into a thread. The stretching action caused by the movement of the silkworm's head further aligns the molecules, strengthening the thread. The sericins enveloping each thread, called brins, dry more gradually, gluing the two silk brins which emerge from the paired labial glands into a single bave and allowing adhesion between threads to form a cocoon.[14] The natural processing of silkworm silk contributes significantly to the mechanical properties of the threads. As shown in forced reeling experiments, the strength, stiffness and extensibility of *B. mori* silk can be altered by controlling the speed of thread spinning.[17] However, the amino acid sequence of *B. mori* heavy chain fibroin is ultimately considered to determine the properties of silk.

Heavy chain fibroin contains highly repetitive glycine- and alanine-rich stretches dominated by the motif GAGAS, which in concert with periodically dispersed non-repetitive loop-forming regions, allow the assembly of anti-parallel β-sheets.[7] (See **Table 2** for a comparison of sequence motifs and the corresponding structures of proteins discussed in this review.) The anti-parallel β-sheet crystals in *B. mori* silk are loosely aligned parallel to the long axis of the thread and occupy 40–50% of the total volume of silk fibers, while the rest may be a more amorphous phase of β-turns and loops.[11] The strength of *B. mori* fibroin arises from the crystalline regions while elasticity is ascribed to the more amorphous portions of the silk.[7] The combination of crystalline and amorphous regions results in a fiber that is relatively stiff (7 GPa) and strong (0.6 GPa) yet extendible (18%) and with considerable toughness (70 MJ m^{-3}).[11]

A thread of insoluble spider dragline silk is produced at high speed from a highly concentrated aqueous solution under physiological conditions yielding a fiber with excellent mechanical properties. Particularly when considering the large proportion of hydrophobic stretches in heavy chain fibroin, this production is an astounding feat. Thus, the question arises: how does fibroin remain soluble in the lumen of the silk gland until it is ready to be spun? Some convincing evidence has been provided in the form of clustered acidic residues in the amino-terminal domain of heavy chain silk fibroin which provide charge repulsion at neutral pH, preventing aggregation. Charge repulsion is reduced with acidification as silk proteins move along from the middle to the anterior silk gland, allowing the formation of micelles where increasing hydrophobic interactions and the formation of β-sheet structures can occur.[18] Other researchers reason that the hydrophobic regions of heavy chain silk fibroin are too large in comparison to charged or hydrophilic regions to remain stable in solution without the aid of auxiliary proteins. While the amino-terminal domain may help trigger assembly, the ability of the fibroin complex to stay hydrated and avoid aggregation in the lumen of the posterior silk gland may likely be due to its association with light chain fibroin and the action of glycosylated P25, which stabilizes and enhances hydration of the fibroin complex so that the charged residues of its highly conserved termini and the hydrophilic regions of the molecules are exposed to water.[19] A further argument for the importance of the heavy and light chain fibroin complex with P25 is that this structure has been retained in the evolution of Lepidoptera, the order of moths and insects that includes the silkworm, for over 150 million years.[20] In this context, P25 might be considered a critical matrix component of silkworm silk fibers (**Table 3**).

Thomas Scheibel is Full Professor of Biomaterials (chair) at the Universität Bayreuth in Germany. He received both his Diploma of biochemistry and a Dr. rer. nat. from the Universität Regensburg and his habilitation from the Technische Universität München in Germany. He was a Kemper Foundation postdoctoral fellow and a DFG postdoctoral fellow at the University of Chicago. He has received several awards including the Biomimetics award of the German Bundesministerium für Bildung and Forschung (BMBF).

Eileen Lintz studied chemistry at the University of Massachusetts, Boston and at the Technische Universität München, where she received her M.Sc. She is currently working on her PhD thesis under the supervision of Thomsas Scheibel at the University of Bayreuth. Her research focuses on structure–function relationships of mussel byssus and byssus-inspired protein materials.

2.2. Spider Silk

Orb web weaving spiders, such as *Nephila clavipes* or *Aranaeus diadematus* can produce several different types of silk, up to five of which may be found in their webs.[21] Dragline silk, used as an ever-ready lifeline and to construct the frame and spokes of the spider's web, has garnered the most interest among biochemists and material scientists. Dragline silk is an unusually tough (see Table 1) yet lightweight material that shows slow biodegradation and low immune response, making it an attractive choice for many applications, particularly in the field of medicine.[22] The body of research on spider silk is large. Several reviews summarize the biology,[23,24] structure[25,26] and mechanical properties[11] of dragline silk. Reviews highlighting the use of spider-silk inspired materials[21,27,28] also exist. Here, we will highlight relevant facts regarding dragline silk structure, mechanics and assembly as useful for comparison to egg stalk silk and mussel byssus.

Table 1. Overview of Selected Fibrous Proteins.

Protein Fiber	Dominant Secondary Structure/ cross-links	Defining mechanical properties	Selected values for mechanical properties[a]				
			Young's modulus [MPa]	Strength [MPa]	Extensibility [%]	Toughness [MJ m^{-3}]	Resilience [%]
Elastomeric							
Elastin[167,108]	Disordered/ desmosine, iso-desmosine	Rubber-like elasticity, high resilience, high durability	1.2[153]	0.2	150	1.6	90
Resilin[167,108]	Disordered/ di- and tri-tyrosine	Rubber-like elasticity, very high resilience, high durability	0.0025[b][154] 2[c][167]	0.007 4	300 190	—— 4	97 92
Abuctin (Bivalvia)[157]	Disordered/ unknown	Rubber-like elasticity, high durability	4.7[158]	1–2[159]	100–200	8–18	82–97[160]
Flagelliform silk (*A. diadematus*)[11,161]	Disordered/ unknown, possibly hydrogen bonds, hydrophobic interactions	Extensibility, low-stiffness, high toughness, low resilience	3[11]	500	270	150	35[167]
β-Crystalline silks							
Bombyx mori cocoon[11]	Amorphous, ~50% loosely aligned β-sheets/ β-sheets	High tensile strength and toughness	7000[11]	600	18	70	29[162]
Spider dragline (e.g. *N. clavipes, A. diadematus*)[11]	Amorphous, ~ 11–15% (*N. clavipes*) ~35%[36] β-sheet content (*A. diadematus*)/ well-aligned β-crystallites	High tensile strength and very high toughness	10 000[11]	1100[m][11] 1256[n][36]	30[m][11] 27[n][36]	160[11]	35%
Lacewing egg stalk (e.g. *M. signata, C. flava*)[71]	Accordion pleated stacks (cross β-sheet)/ disulfide links	Flexural rigidity, high extension	5800[d] 1300[e]	68,[f] 70[g] 42,[f] 232[g]	3 434	1 110	——
α-Helical coiled coil silks							
Vespoidea (ants)[163]	Tightly packed coiled coils/ unknown	Extensibility	——	——	109[163]	——	——
Apoidea (hornets, bees, wasps)[163]	Tightly packed coiled coils, β-sheets/ evidence of oxidative cross-linking	Intermediate strength and high extensibility (drawn); stiff, brittle (spun)	——	132[163]	204	——	——
Collagens							
Collagen I (e.g. rat tail tendon)[164–167]	Triple helix/ intra-chain hydrogen bonding, inter-helix lysine cross-links	Relatively stiff and strong highly resilient elastomer	1200[167]	2	13	1.6	90
Mussel byssus (*Mytilus*)[167,108]	Triple helix/ likely as in collagen, includes sacrificial metal-ligand cross-links[94]	Tough elastomer, recovery after yield	870[c,h][167] 1900[d,h][108] 16[i][167]	75[c,h][167] 300[d,h][108] 35[i]	109[c,h][167] 40[d,h][108] 200[j]	45[c,h][167] 80[d,h][108] 35[j]	28[h] 53[i]
Keratins							
α-keratin (dry human hair)[168]	α-helical coiled-coils/ disulfide bonds	Relatively stiff and strong, recovery after load-induced α-helix to β-sheet transition	3051[169]	211	532	——	——
β-keratin (dry feather rachis)[170]	β-sheets/ disulfide bonds	Hardness, rigidity	2500[j][171]	130[172]	10.4	000531[k][173] 0.01514[l]	——

[a]Values are taken from the first referenced value in a row or from the reference immediately preceding; [b]recombinant protein; [c]natural protein; [d]measured in air; [e]measured in water; [f]engineered stress at break; [g]true stress at break; [h]distal thread; [i]proximal; [j]flexural stiffness; [k]longitudinal fracture toughness; [l]transverse fracture toughness; [m]*N. clavipes* thread cut from webs; [n]*A. diadematus* threads forced silked at 200 mm/s. fibers highlighted in yellow are discussed in this work.

Spider dragline silk is produced by a pair of major ampullate glands in the spider's abdomen and spun through spinnerets into a solid thread, often as a double threaded bave as in *B. mori*.[21,29,30] The major ampullate gland is subdivided into 4 regions: the tail, lumen, spinning duct and spigot. Dragline silk proteins are produced in columnar epithelial cells in the posterior sections of the gland and secreted into the lumen where they are stored in gel-like high concentration (up to 50% w/v).[31] During spinning, the proteins move into the spinning duct, which is further divided into three "limbs" of

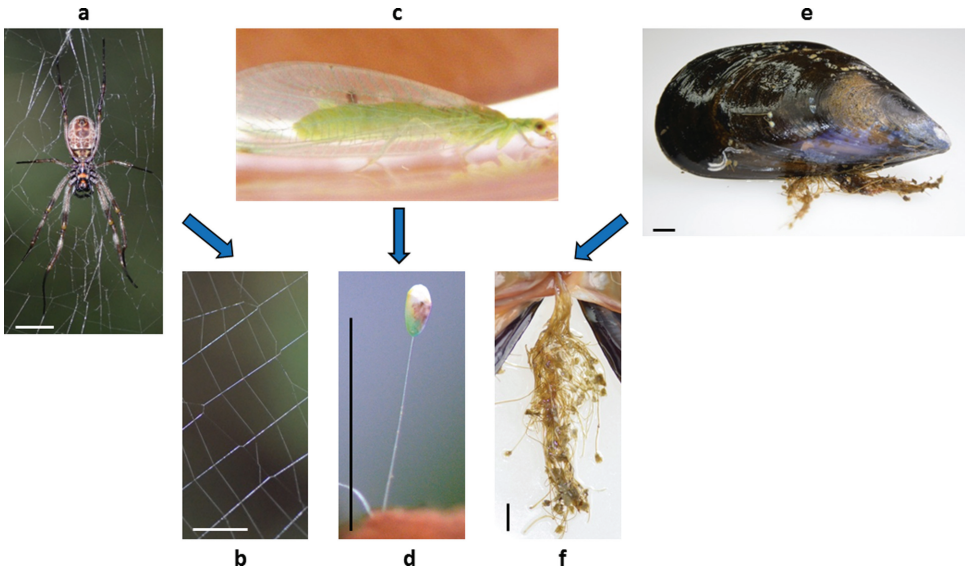

Figure 1. a) The golden orb weaver (*Nephila edulis*) and b) its web. The frame and radii of the web are made of spider dragline silk, an unusually tough and strong yet lightweight fiber. c) Green lacewings (*Chrysopa carnea*) and d) an egg suspended from a silken egg stalk that shows remarkable bending strength. e) The blue mussel (*Mytilus galloprovincialis*) and f) its byssus, a tangle of collagenous threads, each a tough tether with self-healing properties. Scale bars are approximately 50 mm.

its S-shaped curvature. In the first limb, an exchange of chaotropic (PO_4^{2-}) for kosmotropic (Na^+, Cl^-) ions commences, and the silk dope molecules align parallel to each other and perpendicular to the epithelium of the duct.[6,31] Further movement into the second limb causes a reorientation of the molecules which elongate and stack parallel to the epithelium. At this point, a transition in secondary structure takes place from disordered and polyproline-II helix conformations to primarily β-sheet structures, a process aided by the slight acidification of the silk dope which neutralizes charge repulsion and promotes hydrophobic interactions.[6,32–35] In the third limb of the duct, the progressively more viscous solution solidifies, water is removed and a draw down taper is formed. Rapid extensional flow further aligns the silk molecules and residual water is removed as the thread is pulled through the constricting lips of the spigot.[6,7]

As with *B. mori* silk, processing affects the mechanical properties of spider dragline silk. In forced silking experiments, higher reeling speeds led to higher modulus and tensile strength and lower elongation, presumably due to greater alignment of silk molecules.[36–39] In the case of naturally spun dragline silk, there is considerable variability in mechanical properties both within a species and between threads from a single individual. In light of this fact, it is possible that spiders are able to tune the mechanical properties of their silk to fit a specific purpose.[37] They certainly can regulate the rate of spinning, as

Table 2. Repetitive sequence motifs of selected proteins and their corresponding structures. The underlined residues are absolutely conserved.

Protein	Repetitive sequence motifs	Secondary structure	Selected higher-order structures
B. mori Fibroin[9]	GAGAS	Anti-parallel β-sheets	β-sheet nanocrystals loosely aligned along thread axis
MaSp1[174]	A GGX	Anti-parallel β-sheets	β-sheet crystallites well aligned with thread axis
MaSp2[21,175,176]	A_n GPGXX	Anti-parallel β-sheets, Elastin-like turns	β-sheet crystallites, possibly disordered (elastin-like)
MaXB1[5]	XSXAXAXXXSAGASSX XXXSXAXXXXASGSSX	Anti-parallel β-stacks in 8 residue register	Rows of cross β-stacks with β-sheets perpendicular to thread axis
MaXB2[5]	XSXAXAXKGSAXAXSX	Anti-parallel β-stacks in 8 residue register	Rows of cross β-stacks with β-sheets perpendicular to thread axis
PreCoID flanks[92,93]	N terminal: A GGX C terminal: A GGX GPGXX	Likely anti-parallel β-sheets, possibly others as well	β-sheet crystallites aligned with thread axis

Table 3. Matrix components of selected protein fibers.

Protein Fiber	Matrix Protein	Comments
Bombyx mori cocoon silk	P25	Aids in stabilizing the heavy and light fibroin chain complex, prevents aggregation in silkworm gland, may assist assembly[18–20]
Spider dragline silk	None known	The role of MaSp1s in *C. moluccensis* dragline silk is under investigation.[44,45]
Lacewing egg stalk	None known	Egg stalk dope from *C. carnea* contains more than the two proteins described from *Mallada signata*,[70] some of which may function as matrix proteins
Mussel byssus	PTMP1	Binds strongly to collagen and causes a loss of ordered structure in collagen fibrils, may have an effect on the mechanical properties of proximal thread[96,97]
	TMP	May separate and/or lubricate preCol fibrils or aid in thread maturation[95]

they possess muscular control of their spinnerets and also use this as well as their legs as a friction brake to change the speed of spinning.[40,41]

With the exception of a thin cuticle, spider dragline silk is composed almost entirely of two proteins called spidroins (a portmanteau of spider and fibroin). The major ampullate spidroins 1 and 2 (MaSp1 and 2) are classified according to their sequences and differ mainly in proline content.[42] Spidroins contain around 100 tandem repeats that account for over 90% of their sequences. Within these repetitive domains, both MaSp1 and 2 contain polyalanine stretches that have been shown by a variety of methods to form intramolecular anti-parallel β-sheets as strong cross-linking elements.[7,21] MaSp1 also contains GGX motifs whose structure is unclear, while Masp2 is also characterized by GPGXX motifs that, on the basis of sequence similarities and NMR evidence, are considered to form elastin-like β-turns[21,43] (see Table 2). Flanking the repetitive cores of MaSp 1 and 2 are non-repetitive domains that are highly conserved and crucial for the storage and assembly of the proteins,[3,21] although, as with *B. mori* silk, the terminal domains alone may not be sufficient for this purpose. Very little is known about other proteins in spider dragline silk and the roles they may play in the formation of threads. The presence of hitherto unidentified proteins has been confirmed in the outer layers of dragline threads,[44] but no known matrix proteins have been found (Table 3). Recently, a novel, quite short (only 1320 bp) MaSp1 gene (called MaSP1s) was identified in *Cyrtophora moluccensis* that contains the sequences for the conserved N and C termini of other MaSp1 proteins but only a short repetitive core domain.[45] It is surmised that, given the known role of the amino and carboxy termini in storage and assembly of spidroins, and the fact that MaSp1s is so short and occurs in relatively low abundance, would not likely contribute to the mechanical properties of dragline silk. Rather, it is conceivable that MaSp1s could function much the same way as P25 in *B. mori* silk. However, much experimental evidence remains to be gathered before any conclusions may be drawn about the function of MaSP1s.

Like *B. mori* silk, spider dragline silk can be considered a composite material of crystalline β-sheet regions embedded in an amorphous matrix (see **Figure 2**), but the degree of crystallinity differs at 40–55% for *B. mori* vs. 11–30% for *N. clavipes* spider dragline silk[7,46] and 34–35% for *A. diadematus*.[36] Moreover, β-sheet crystallites are more aligned along the thread axis in spider silk. It is also worth mentioning that there are differences between the hierarchical structure of *B. mori* and spider dragline silks. Both threads are composed of bundles of nano- and micro-fibrils but in spider dragline silk the bundles are embedded in the center of a core/shell structure,[47] with the shell being composed of glycosylated proteins, lipids, silk and possibly other proteins.[44] Also in contrast to *B. mori* silk, spider silk lacks the sericin coating which can cause an adverse immune response.[29] For this reason alone, dragline silk is attractive as a biomaterial, but its mechanical properties are also of great interest (**Figure 3**).

The molecular structure and hierarchical composition of spider silk results in excellent mechanical properties.[48] Spider dragline outperforms *B. mori* silk in tensile strength (1.1 GPa vs. 0.6 GPa), thus, it is the strongest non-mineralized biological material currently known.[11] It is also more extensible (30% vs. 18%) and over twice as tough (160 MJ m^{-3} vs. 70 MJ m^{-3}), making it one of the toughest fibrous materials surpassing nylon, rubber, Kevlar 49, and carbon fibers.[7,11,12] Spider dragline silk is also among the stiffest (Young's modulus: 10 GPa) known natural proteinaceous materials.

Although the tensile properties of dragline silk are impressive, its non-linear, viscoelastic behavior also sets it apart, showing how it is perfectly adapted for its role in aerial webs. The mechanical integrity of a spider's web is determined by the frame and radii made of major ampullate silk, whereas the viscid silk spiral threads play non-structural roles in prey capture.[49,50] At low strains that might be caused, for example, by light breezes, frame and radii threads perform elastically. When an insect flies into a web, the frame and radii absorb the kinetic energy of the impact through internal dissipation through heat, the breakage of bonds, or increases in entropy rather than storing energy as elastic deformation. The latter would lead to high recoil forces that would make a web more of a trampoline than a trap.[11,50] Thus, major ampullate silk shows a large hysteresis of approximately 65% (*Araneus*).[51] To prevent uncontrolled oscillations set off by impacts, these silks also exhibit material damping of 40–50% at high strain under repeated loading, a feature that major ampullate silks of varied species has in common.[50] As is typical of viscoelastic materials, the rate of loading is critical to the mechanical response of spider silk. Young's modulus, strength, extensibility and toughness all increase as strain rate increases.[11,52] As a material meant to absorb impact energy on short time scales, it is no wonder that the material properties of frame and radii threads are optimized for higher strain rates.

Dragline silk is also optimized to take advantage of changes in humidity and wetting such as when it rains or during dewfall. When wetted or exposed to humidity above 60%, water interpenetrates dragline silk and disrupts hydrogen bonds, allowing a rearrangement of peptide chains to configurations of higher entropy.[53] This process is termed supercontraction, as it causes dramatic shrinkage (up to 50%)[54] of unattached

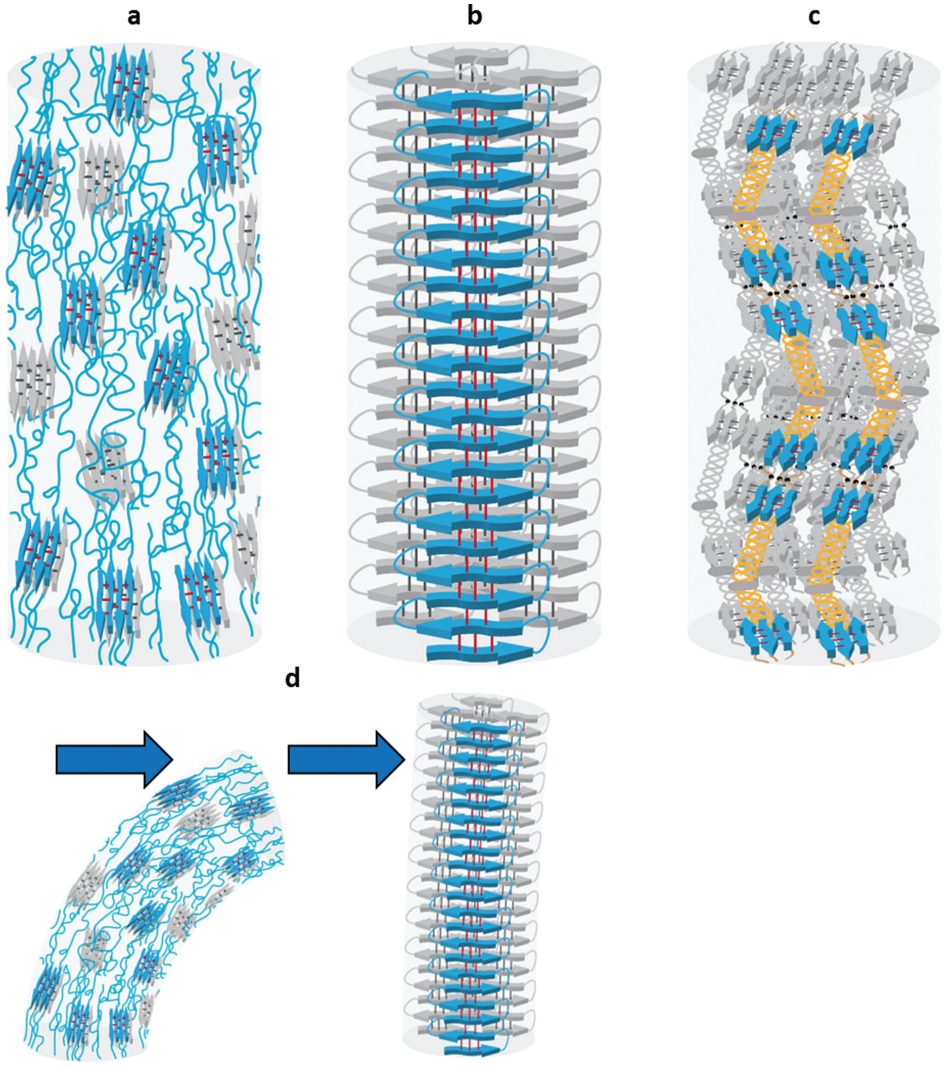

Figure 2. The dominant secondary structures in three proteinaceous threads a) β-sheet crystallites (blue with red hydrogen bonds) embedded in an amorphous matrix (blue squiggly lines) in the core of spider dragline silk. b) The cross β structure (blue with red hydrogen bonds) of the dominant proteins in lacewing egg stalk silk. c) Collagen fibers (yellow) with β-sheet containing flanks (blue) and sacrificial histidine bonds (black dots) in the core of distal mussel byssus. d) A comparison of the bending stiffness of spider dragline and lacewing egg stalk. The stiffness of lacewing egg stalk is nearly 3 times that of spider dragline.[5] For clarity, structures are simplified, not drawn to scale and only structures in the foreground are in color.

threads and changes their material properties to rubber-like as long as they are wet.[36,55] Threads under tension, as found in webs, show less change in material properties but develop considerable stress. It is thus supposed that supercontraction is a mechanism by which silks maintain tension in webs.[56–58]

2.3. Lacewing Egg Stalk Silk

Lacewings are insects that belong to the family *Chrysopidae*. Their larvae are voracious predators of many common agricultural pests including aphids and members of Lepidoptera. When laying its eggs, the female lacewing secretes a drop of concentrated silk dope from its colleterial glands onto a substrate (usually on the underside of a leaf) and then pulls its abdomen away, trailing a thin (15–20 µm) and short (ca. 1 cm) thread upon which an egg rests (Figure 1c,d). After waiting a moment, the egg is fully released and it is perched atop a silken pedicel (egg stalk) of unusual bending stiffness, (See Figure 2), which functions to protect eggs from predators and cannibalism.[5,59,60]

There are no studies on the natural production and assembly of egg stalk dope that compare in scope and detail to those of spider or silkworm silk. What little is known can be summed up as follows: Colleterial glands, also known as accessory genital glands, are part of the female adult lacewing reproductive tract. They can be conspicuously large, and morphologically distinct parts are evident. The main portion is delicate and sac-like, with finger or ribbon-like accessory glands trailing off distally.[61] The gland contains columnar secretory cells, the luminal surface of which is convoluted, forming "microvilli" known as the end apparatus. While this has not been shown for lacewings, in other

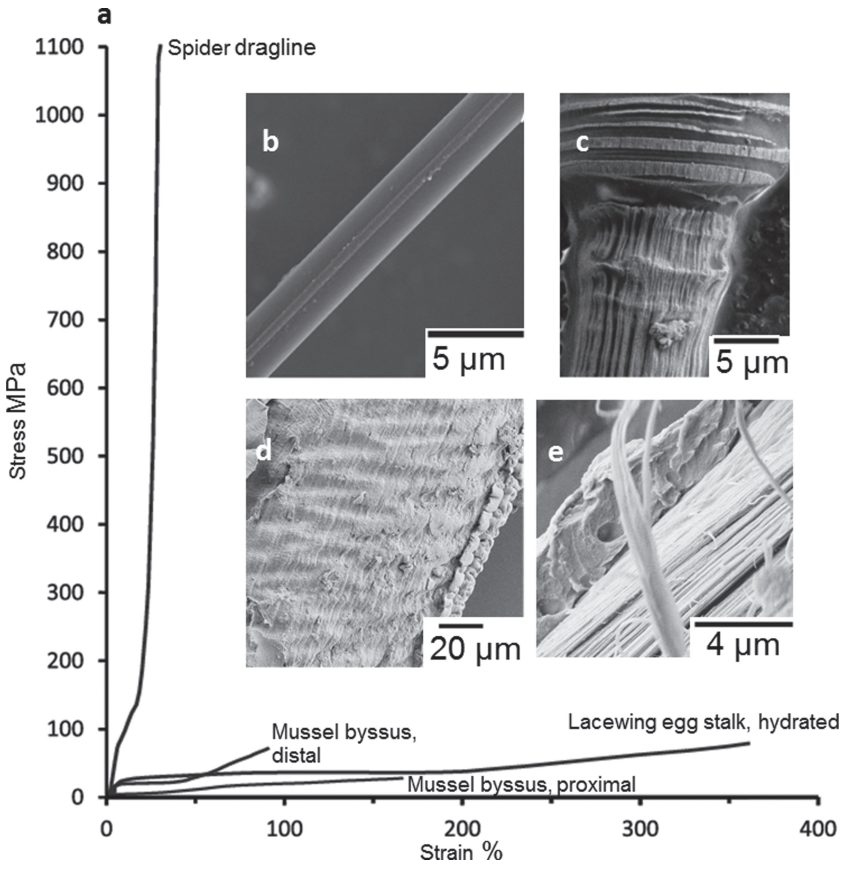

Figure 3. a) A comparison of stress strain curves of spider dragline silk, hydrated lacewing egg stalk silk and mussel byssus both proximal and distal thread. Spider dragline shows extreme strength compared to the other fibers whereas hydrated lacewing egg stalk shows extreme extension. Curves sketched after Gosline et al. 2002[167] and Bauer et al. 2012[71] to show relative differences in tensile properties. b) Spider dragline silk (*Araneus diadematus*). The two brins are clearly visible. c) lacewing egg stalk silk after tensile testing beyond the yield point. A change in the morphology of the thread (necking) is evident. d) Proximal byssal thread with cuticle partially removed, the wavy structure may be caused by softening of collagen fibrils by the matrix protein PTMP1. e) Distal byssal thread with cuticle partially removed, the striated tightly packed and aligned fibrils are clear.

insects, structural proteins pass through the end apparatus and aggregate into large globules in the colleterial gland lumen.[62,63] Attached to the main chamber of the gland is a rounded pouch, which may serve as a reservoir for storage and release of egg stalk proteins.[64] A narrow duct connects the reservoir to the gonapophyses from which the silk dope is deposited during the process of oviposition. When the lacewing secretes a droplet of soluble[62] viscous silk dope onto a surface, the thread drawn from the dope solidifies within seconds; presumably there is a change in secondary structure of the proteins induced by shear forces during drawing which alters the solubility of the proteins and aligns the molecules into a characteristic cross-β structure (Figure 2). The simplicity of thread formation in lacewings is in stark contrast to the complex spinning process of spider and silkworm silk, making biomimetic processing of recombinant egg stalk proteins relatively easy.[71]

Although first investigations into egg stalk structure[59] and composition[65] date back to the 1950's, research on the silk of Chrysopidae has not kept pace with that of spider or silkworm silk. The research group of Tara Sutherland has picked up where these first studies left off, analyzing the stalks of the Australian lacewing *Mallada signata*. They identified two proteins, which they dubbed MalXB1 (109 kDa) and MalXB2 (67 kDa). Evidence from the contents of *M. signata* colleterial glands, cDNA libraries, and the amino acid composition of the egg stalks from a related species (*Chrysopa flava*) indicate that MalXB1 and MalXB2 are likely present in the stalks in a 7:1 ratio.[5,66] Both proteins are over 70% repetitive with non-repetitive amino and carboxy termini. MalXB1 also has one non-repetitive intervening sequence. The core regions of the proteins consist entirely of 16-residue repeats; around half of the amino acids in the recurring sequences are absolutely conserved[5] (see Table 2). The tandem repeats of MalXB1 are serine-, alanine- and glycine-rich and interspersed with threonine and charged residues at regular intervals. The repetitive region of MalXB1 shows a similar amino acid composition and pattern but also exhibits a lysine-containing KGSA motif that is 88% conserved, as well as high amounts of valine and asparagine residues.[5]

Despite documented similarities in amino acid composition, egg stalk structure and mechanical properties, it is safe to say that virtually nothing is known about interspecies diversity in egg stalk proteins. While the Sutherland group has identified two proteins from cDNA libraries of colleterial silk glands,[5] SDS-PAGE shows that the silk dope of *Chrysopa carnea* contains five abundant proteins, and FTIR spectra of stalks suggest that one of them may contain poly-alanine motifs.[70] However, the identity, sequence, structure and function of the proteins in *C. carnea* silk dope have not yet been determined (Table 3).

The first investigations of the structure of lacewing egg stalks by X-ray diffraction revealed that *C. flava* stalks possess an unusual silk structure, called "cross-β," which had at that time in the 1950s not been known to exist naturally in protein materials. β-pleated sheets are oriented with the β-sheet backbone perpendicular to the main fiber axis rather than parallel as with *B. mori* or spider dragline silk.[59] (see Figure 2). Rare in functional proteins, cross-β structure is well-known from amyloid proteins which can cause neurodegenerative diseases such as Alzheimer's, Parkinson's and spongiform encephalopathies.[67] Both a model from the 1960s[68] to explain the X-ray diffraction evidence of egg stalk silk and a more recent study[5] strongly suggest that the protein chains fold back on themselves in eight residue register into long strands of accordion-like pleats connected by hairpin β-turns. The stabilizing force behind the cross-β structure derives from interactions between amino acid side chains of neighboring β-sheets

which protrude alternately on the upper and lower face of the β-sheet.[69] In MalXB1 and 2, the amino acid side chains make one face of the β-sheet more hydrophilic than the other.[5] This may facilitate folding of the accordion pleated structure. On the edges of the β-sheet stacks there are charged residues which may help the proteins align end to end. Additionally, MalXB1 contains seven and MalXB1 five cysteine residues. It is conjectured that these form cross-links that further stabilize the structure and enhance the rapid hardening and lateral stiffness of egg stalk silk.[5,70,71]

Atomic force microscopy measurements indicate that *M. signata* egg stalks exhibit high flexural rigidity with a bending modulus calculated to be nearly 3 times greater than that of silkworm fibers.[5] (see Figure 2) This property makes egg stalk silk an interesting candidate for materials that perform well under lateral loads. In contrast, tensile testing parallel to the main axis of the fiber reveals stress-strain curves with an initial elastic region showing a pronounced low-strain yield point, followed by a large region of plastic deformation before final stiffening and fracture.[71,72] The tensile behavior of egg stalk silk is highly dependent upon water content (Figure 3). At 30% humidity, the silk ruptures at low extensions (ca 3%), is stiffer (5.8 GPa), and exhibits little or no yield. At high humidity (100%), Young's modulus decreases to 1.3 GPa, the yield point takes place at lower stresses and a large plastic deformation of the fibers allows the material to reach 500% extension or more.[5,71] Stress at break, better measured as real stress rather than engineered stress due to reduced cross-sectional area from necking, increases from 70 MPa to 232 MPa. Correspondingly, toughness increases dramatically from 1 MJ m^{-3} at 30% relative humidity to 110 MJ m^{-3} at 100% relative humidity.[71]

A molecular model has been proposed to explain the performance of egg stalk silk under tensile testing, in particular its remarkable extension and dependence upon humidity. At low humidity, the threads are more crystalline,[72] stiffer and brittle.[71] Failure may occur at cysteine cross-links which are weaker than the sum of the many hydrogen bonds between β-sheets. At higher humidity, water competes with hydrogen bonding between β-sheets, weakening the cohesion of the cross-β structure so that the stacked β-strands can unfold and flatten against one another,[71] undergoing a non-reversible molecular rearrangement of the protein chains from cross-β to parallel-β. The transformation at the molecular level is also evident in the morphology of the thread (see Figure 3). When the peptide chains have unfolded, the thread begins to stiffen, and the peptide bonds become load bearing until final rupture.[59,71] There have been no attempts to assign biological relevance to the extreme extension of egg stalk silk; it may simply be a result of the cross-β structure of egg stalk proteins which provides lateral stiffness to threads rather longitudinal strength.

3. Collagens

3.1. Collagen in General

Collagens represent the most abundant class of animal proteins; they form the basis of load-bearing tissues such as tendon and bone and are the principle component of the extracellular matrix where their function is chiefly mechanical: to provide support and impart strength and elasticity.[73] The subject of collagen is complex. Collagens are often found in composites such as in connective tissues with elastin and proteoglycans or in bone with hydroxyapatite, where the mechanical properties of the fibers differ depending upon the other substances in the composite.[74,75] Collagenous tissues also have an elaborate hierarchical structure and perform differently at different levels of scale.[76] On the molecular level, the defining motif of collagen is the triplet repeat of GXY where X is often proline and Y 4-hydroxyproline. The combination of sterically small glycines at every third position and the presence of imino acids with constrained bond angles causes the collagen peptides (called alpha chains) to wind into left-handed polyproline type-II coils, three of which assemble into tightly packed right-handed triple helices staggered by one amino acid residue with glycine residues pointing inward.[77] Collagens may often integrate multiple functional domains containing globular or other structures. An unusual example of this type of multi-domain collagen is found in mussel byssus.

3.2. Mussel Byssus

The byssus of marine mussels such as *Mytilus galloprovincialis*, *M. edulis* or *M. californianis* is a bundle of dozens of tough yet elastic attachment threads for firm anchorage in tidal environments. Each thread is around 2–3 cm long and 100–200 μm in diameter and emerges on one end from a stem in the mussel's tissue and terminates on the other end in an adhesive pad or "plaque" that glues the thread to its point of attachment. Byssus, which is completely acellular, is remarkable for its enduring mechanical integrity in the face of a variety of stresses including cyclic mechanical loading, periodic drying and wetting and exposure to sun and seawater.[78,79]

The production of byssal proteins and the assembly of threads have been studied since the 1950s[80] although many aspects of byssogenesis still remain to be explained. The site of byssogenesis is the mussel foot, a muscular, tongue-like organ that contains several different glands: the stem, collagen, phenol, accessory and mucous glands.[81] As their names suggest, certain glands are associated with particular byssal precursors, although little definitive evidence for localizing the production of specific proteins to specific glands exists. The byssus gland is considered responsible for forming rings which attach each thread to the byssal stem that extends into living tissue and back to the byssal retractor mussels which pull the whole byssal apparatus taut. The collagen glands likely produce the collagenous material of the core of byssal threads while it is probable that phenol glands produce 3,4-diphydroxyphenylalanine (DOPA)-rich cuticle and adhesive proteins (up to 30 mol%)[82]) that form the plaque. The accessory glands may be responsible for the production of catecholoxidases and o-diphenol precursors, whereas the mucus glands seem to secrete acidic glycosaminoglycans and glycoproteins to perhaps either coat the threads, prepare surfaces or aid in the release of fully formed threads.[81,83] There is clear evidence that at least some byssal proteins are stored in high concentrated, well-ordered states in secretory granules.[79,84–86] There is also evidence that the collagenous proteins are present as smectic

polymer liquid crystals in the secretory granules before they are released for byssal formation.[87]

In an elegantly orchestrated process that is completed in a matter of minutes, a mussel begins making a thread by first feeling along surfaces with its foot to find an appropriate substrate for adhesion. It likely cleans the surface to remove films that would impede adhesion. The mussel then presses its foot against the surface and seals the edges of the distal groove (the site of thread secretion)[81] presumably creating a microenvironment differing in pH and ionic strength to that of seawater, thus allowing proper adhesion of the plaque, as well as assembly of the thread.[88] In what has been described as a method similar to polymer injection molding, plaque proteins are secreted into the distal depression of the foot while the thread may be formed in a process like extrusion molding[81] or reaction injection molding[89] with muscular or ciliated action to massage the thread into form.[81] The thread is held in the distal groove for a few minutes while presumably cross-links are formed although the nature of cross-linking in byssal threads is still unclear.[90] Once protein assembly is complete, the plaque and thread are released and the mussel foot retracts quickly, possibly drawing the thread to further align byssal components,[83] however, researchers are not in agreement on this point.[81]

Byssus is a complex and highly ordered composite material composed primarily of proteins (about 95% of dry weight),[78] the most abundant of which are the so-called preCols D, P and NG. The concentrations of preCol-D and P form what is roughly a cross-gradient with PreCol-D at its highest concentration in the distal end tapering off into the proximal end, while preCol-P reaches its maximal concentration in the proximal end and tapers off toward the middle. PreCol-NG is uniformly distributed along the thread. It is surmised that this protein gradient is responsible for the mechanical gradient of byssal threads, which exhibit a smooth transition from elastic at the proximal end to stiff at the distal end.[91]

All preCols show similar protein domain structure. A triple-helical collagen core is flanked on either end by stretches that show sequence homologies to silk (preCol-D), elastin (preCol-P) and polyglycine peptides/amorphous silk (preCol-NG). Capping these flanks are histidine and dihydroxyphenylalanine (DOPA) enriched sequences that are capable of metal complexation.[79] Recent studies using X-ray diffraction, FT-IR spectroscopy and NMR spectroscopy[92–94] confirm several predictions of byssal protein secondary structure based on sequence homologies. As in fiber forming collagens, the central domains of preCols form triple helical strands. These strands are aligned along the thread axis.[92,93] PreCol-D flanks, which contain polyalanine stretches and GGX motifs homologous to the β-sheet forming and amorphous regions of MaSp1 in spider silk, form anti-parallel β-sheet structures aligned parallel to the longitudinal axis.[92] While evidence for the secondary structure of preCol-P flanks remains inconclusive, it has been shown that preCol-NG flanks contain parallel β-sheets, although their orientation with respect to the whole thread is unknown.[93] There is also evidence of α-helical content in proximal byssal thread.[92]

Of the proteinaceous material in byssus, the preCols constitute nearly 90% of the distal thread and around 70% of proximal thread.[78] The remaining protein material includes at least two interesting matrix proteins, the importance of which may be illustrated by fibers drawn from purified PreCols which differed significantly to natural threads in size and mechanical characteristics.[79,95,96] The first, thread matrix protein (TMP), is glycine-, tyrosine- and asparagine-rich, repetitive and distributed evenly along the thread. TMP is present in several variants, the diversity of which may be due to alternative gene splicing, multiple gene copies, hydroxylation or the tendency of the TMPs toward spontaneous deamidation. The precise function of TMP is not known but TMP may act as a lubricant or spacer between preCol fibrils (as supported by significant charge repulsion resulting from deamidation of asparagine residues that form iso-aspartate). Or, TMPs may aid in maturation of threads as deamidation occurs rapidly as pH changes from 5 in the secretory granules to 8 in seawater.[96]

A second matrix protein, proximal thread matrix protein (PTMP1), is a glycine-rich, glycosylated 50 kDa protein that is found at highest concentration in the proximal region of the thread.[97] Recent research in our lab aims to clarify the roles of matrix components in determining the properties of protein fibers (Table 3). In an attempt to shed some light on byssal matrix proteins, the crystal structure of PTMP1 from *M. galloprovincialis* was solved, showing a novel two-domain structure containing Willebrand factor type A-like domains connected by a highly rigid linker stabilized by a β-sheet hydrogen pattern, three disulfide bonds and presumably at least one complexed zinc ion (paper submitted). The collagen binding capacity and the effects of PTMP1 on collagen were studied in vitro, revealing a high binding affinity as shown in enzyme-linked immunosorbent assay-like experiments in agreement with another study[97] and surface plasmon resonance (paper submitted). PTMP1 had drastic effects on the assembly of collagen in solution. Rather than the straight, well-aligned and packed fibrils of collagen in control samples, collagen assembled in the presence of PTMP1 is loosely packed, less oriented and wavy (paper submitted). PTMP1 may have an impact on not only the morphology of collagen fibrils in the proximal portion of byssus, but also on the mechanical properties. It is conceivable that it acts to soften the structure of the collagen, reducing modulus and increasing extendibility by physically crimping the fibrils. Given the high binding affinity of the protein, it may also act as a linker that prevents slippage between softened fibrils, thus contributing to the strain-induced stiffening of proximal byssus.[97] Beyond such speculation, a matrix protein capable of adjusting the assembly and mechanics of collagen has a wealth of potential applications in biomaterials research such as in tuning the properties of collagen-based gradient materials inspired by the mussel byssus.[98]

Another feature of byssus that bears mentioning is its cuticle composed entirely of a DOPA-rich protein, mussel foot protein 1 (mpf-1) that is 4–5 times stiffer and harder than the byssal core yet flexible enough to reach strains of 70%.[99] Three DOPA residues form bi-dentate metal ion complexes with Fe^{3+} that are responsible for the hardness of the cuticle. The unusual extensibility of such a hard substance results from its granule/matrix structure where the formation of micro tears in the matrix, which contains fewer DOPA cross links, prevent large scale cracks that would cause catastrophic failure.[100] Mfp-1 has been discussed as being suitable for providing a hard coating for soft substrates,[101] and as inspiration for materials that feature pH-induced metal-ligand cross-links with self-healing capacity.[102]

Byssus has evolved as both a tough tether and a shock absorber, able to withstand repeated irregular loads from waves in tidal areas. Investigations into the mechanics of byssal threads have analyzed the properties of proximal and distal regions alone as well as those of whole threads (Figure 3). Quasi-static tensile testing has shown that proximal byssal thread is characterized by low modulus (16 MPa) low ultimate stress (35 MPa) and high extensibility up to 200% with relatively constant stiffness over the full range of extension.[103] Cyclic testing of proximal threads to 50% extension showed pronounced strain-induced stiffening from 30 to 60 MPa, and increased resilience,[104,105] while changes in strain rate seem to bring about an increase in stiffness and loss of resilience.[103,105]

Whereas proximal thread shows essentially elastomeric behavior in static tensile tests, distal thread is more of an elastoplastic material. Distal thread exhibits a much higher modulus (870 MPa) compared to proximal thread, with a pronounced yield point at around 15% extension followed by large-scale deformation where stress remains virtually constant and lastly, stiffening before rupture at 109% extension at an ultimate strength of 75 MPa.[78,106–108] The behavior of distal thread under cyclic loading depends upon whether the thread is loaded below or above the yield point. At low strains, distal threads show significant hysteresis (70%) to dissipate energy, (although the proximal part shows hysteresis as well (40%)).[78] As in proximal threads, these properties are strain-rate dependent.[105,109] Beyond the yield point, cycles after plastic deformation show low stiffness and reduced energy dissipation.[105] Unlike in most elastoplastic polymers, this plastic deformation is reversible ("self-healing") in a time dependent matter due to the presence of sacrificial bonds provided by histidine metal-ion coordination complexes.[94,110]

Whole threads show a blending of proximal and distal properties. The initial response to tension is elastomeric, an attribute ascribed fully to the proximal portion of the thread. As stress increases, strain stiffening commences in the proximal region. However, before the proximal portion can fail at low ultimate stress, the load is transferred to the distal region which begins to yield. The substantial plastic deformation of the distal region serves to absorb a large amount of energy, preventing thread breakage and dislodgement of the mussel. It is argued that the yield behavior of byssal threads allows realignment along the axes of greatest load in the tidal zone as well as the possibility to spread load among other threads.[105] The slow (many hours to days) re-formation of sacrificial cross-links in distal thread would seem to support this hypothesis, as there is no need for speedy recovery when thread realignment and load redistribution must take place.

The performance of whole threads under cyclic loads can be summed up as follows: At very low loads, whole threads are relatively resilient, which is attributed to straining in the proximal portion of the thread. At sub-yield loads closer to the yield point, byssal threads exhibit significant hysteresis to dissipate energy.[105] When cycled beyond the yield point, the plastic deformation of the distal region which also undergoes stress-induced softening contributes to low overall stiffness and reduced toughness.[105,111]

4. Fibrous Protein-Based Materials and their Applications

In order to exploit the manifold properties of fibrous proteins, it is necessary to produce and process them for new materials. The most direct method of obtaining proteins is to isolate them directly from natural sources and employ them with or without modification. This approach is limited by the low solubility of most fibrous proteins, their recalcitrant cross-links and, in many cases, their low availability. Alternatively, synthetic analogues can be produced chemically or recombinantly, allowing targeted modifications to produce desired material characteristics.[112] Many examples of fibrous protein chimera have also been produced this way.[113–117] Natural or recombinant proteins can be used to make blends or other hybrid materials such as peptide/polymer amphipiles, (see reviews[118,119]) further extending the range of material properties. Control of protein assembly processes allows the production of diverse structures for a variety of applications from medicine to food science to biologically compatible electronic sensors and conducting materials. For an overview of selected materials based on proteins found in the fibers discussed in this review, their processing and potential (or actual) uses see **Table 4**.

4.1. Biomaterials from *B. Mori* Silk

While natural *B. mori* silk threads have long been used as textiles and for sutures, other materials and forms are desirable for broader applications in technical and medical fields. *B. mori* silk fibroin can be dissolved in a suitable solvent (usually highly concentrated aqueous salt solutions) and the silk solution used to create gels, films, foams, non-woven mats, coatings, particles and capsules or other materials for tissue scaffolds, drug delivery vesicles, bio-mineralization scaffolds, solid supports for catalysts, and many others. An exhaustive review of silk processing approaches is available.[13] Regenerated *B. mori* fibroin may also be used in blends for diverse purposes. Again, the authors would like to refer you to a thorough review of materials derived from *B. mori* fibroin blends.[120]

4.2. Biomaterials Inspired by Spider Dragline Silk

The main obstacle to producing materials from natural spider dragline silk is the sheer impracticality of harvesting threads from webs or the spiders themselves. Not only does each spider produce only milligrams of silk, spiders are also territorial and tend toward cannibalism when kept in close quarters, effectively foiling any attempt to farm them.[22,30,121,122] Efforts to produce full-length natural MaSps fail at the cloning stages due to the large, repetitious, guanine- and cytosine-rich genes. The best approach has been to construct synthetic sequences based upon natural motifs and adapt gene sequences to the codon usage of host systems.[22,123–125] Various synthetic constructs, one even approaching the molecular weight of natural dragline silk proteins, have been successfully cloned and expressed in a variety of host systems. A comprehensive

Table 4. Overview of materials based on *B. mori* silk, dragline, egg stalk, and byssus and their potential or actual applications.

Protein	Processed into	Sample applications, References
Bombyx mori fibroin		
Natural silk	Threads	Textiles,[13] sutures,[12,177] tissue scaffolds, tissue grafting, ligament replacement[13,177,178]
Regenerated	Non-woven mats	Tissue culture, scaffolds, skin grafts, bone regeneration[13,177,179]
	Particles, microcapsules	Cell encapsulation,[180] wound healing,[181] drug delivery, carrier systems, controlled release[182–187]
	Foams	Scaffolds,[12] bone regeneration[188]
	Hydrogels	Scaffolds, tissue engineering (esp. bones),[189,190] controlled release[191,192]
	Films, membranes, coatings	Stem cell-based tissue engineering (review 179 also for other fibroin structures), drug delivery/carrier systems/ controlled release,[193–195] wound coverings, skin replacement[196–198]
Spider dragline silk		
Natural *Nephila* spec.	Threads	Nerve regeneration,[130,131] skin replacement[199]
Recombinant *N. clavipes* MaSp1 analogues	Threads, films, non-woven mats, scaffolds	Cell scaffolds, tissue engineering,[135] artificial blood vessels[137]
	Threads, films	Biomineralization, bone regeneration[140,200]
	Films	Cell scaffolds[201,202]
		Wound dressings[203]
		Coatings[204]
		Targeted gene therapy[138,139,205]
Recombinant *E. australis* MaSp1 analogue	Films	Cell scaffolds,[134] tissue engineering[136]
Recombinant ADF4 (*A. diadematus* spidroin) analogue	Particles, capsules, vesicles	Cell encapsulation, drug delivery, cosmetics[133,206,207]
	Hydrogels	Cell scaffolds[208]
	Films	Cell scaffolds[209]
	Coatings	Implants(unpublished)
Egg stalk silk		
recombinant *M. signata* MaIXB1 analogue	Threads, films	Cell scaffolds[210]
Byssal proteins		
Mussel adhesive proteins (natural extract mixture from *M. edulis*)	Adhesive coatings	Commercially available adhesives: Adhesive Protein (Sigma-Aldrich), MAP (Swedish BioScience Laboratory), Cell-Tak (BD Biosciences Clontech)[149]
Mefp-1 (*M. edulis*)		Compliant coatings[101]
Recombinant Mefp-1 analogue,		Wettable bioadhesives[144]
Recombinant Mefp-5 (*M. edulis*)		Wettable bioadhesives[145]
Mefp-1 and *5* fusion protein		Wettable bioadhesives[147,148]
Mefp-1, 3 and 5 fusion protein	Hydrogel	Commercially available extracellular matrix mimic functionalized with peptide motifs (MAPTrix)

review[126] and a particularly useful table[29] cover this information in detail.

Recombinant dragline silk proteins have also helped clarify important structural and assembly information that may aid in processing the proteins into useful materials. The role of the non-repetitive carboxy-terminal domain during assembly of spidroins has been studied, both solving the structure of the domain and showing that the carboxy-terminus of a recombinant *Araneus diadematus* fibroin aids correct alignment and fibril formation.[127,128] The structure of the amino-terminal domain has also been solved and shown to be highly sensitive to pH changes that are known to occur during the spinning process.[128,129]

Recombinant spider silk proteins can be processed into threads, particles, microcapsules, films, mats, and hydrogels (see **Figure 4**). The resulting materials have been tested for their suitability in nerve regeneration,[130,131] drug release,[132] encapsulation,[133] tissue engineering,[134–137] targeted gene therapy[138,139] and biomineralization.[140] The proteins or the materials made thereof have also been functionalized to fulfill specific tasks such as cell adhesion and proliferation (as summarized in Table 4). Recombinant spider silk is currently being tested as a coating for silicone breast implants and has shown less encapsulation and a marked reduction in inflammatory markers in pre-clinical studies in rats (paper submitted).

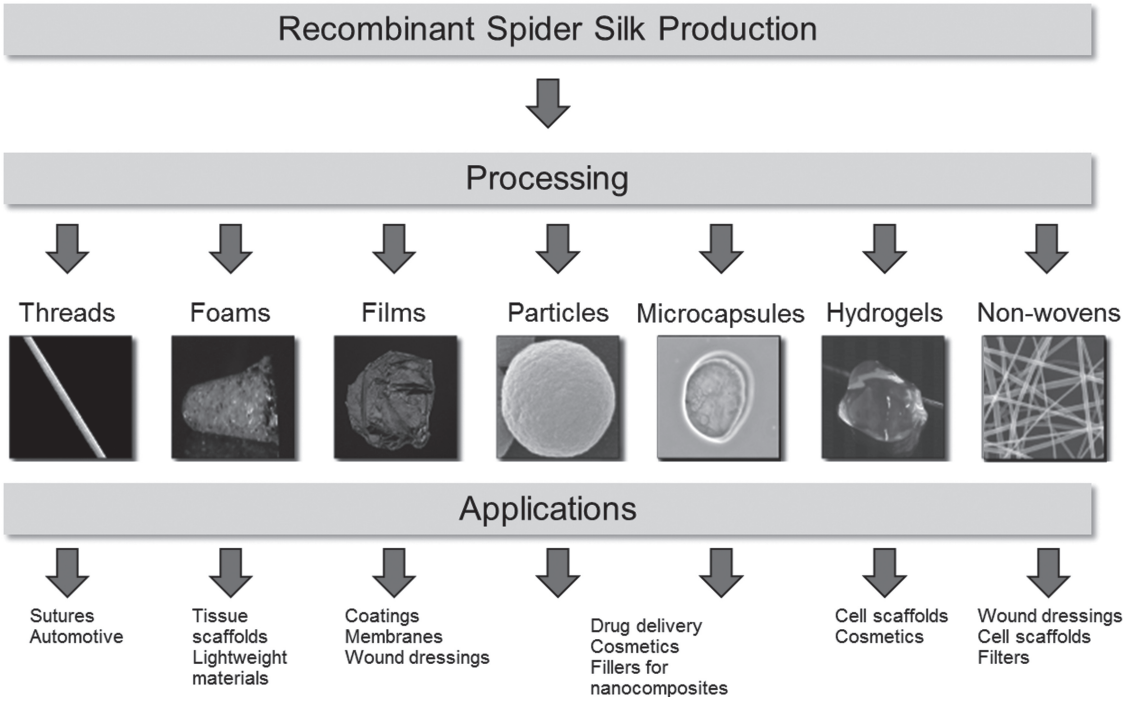

Figure 4. Recombinant spider silk can be processed into various forms for a variety of applications.

4.3. Biomaterials Inspired by Lacewing Egg Stalk Silk

Investigations into biomaterials of lacewing egg stalk silk are still very much in their infancy but first steps have been taken. The recombinant production of a 53 kDa engineered protein based upon the sequence of MalXB1 has been achieved.[70] DNA encoding a 48 amino acid long consensus motif was optimized for *E. coli* codon usage and multimerized 8 times using a seamless cloning technique similar to that used for producing recombinant spider dragline silk.[124] The repetitive core was ligated with the natural amino and carboxy termini of MalXB1. The resulting protein could be drawn into fibers, showed birefringence and had bending modulus, strength and toughness similar to that of natural egg stalk at low relative humidity (30%). At higher relative humidity (70% or above), however, the recombinant threads did not show the same extension as natural egg stalk silk, and morphological analysis by SEM revealed a porous rather than solid structure.[70] Recombinant egg stalk silk is currently being tested as films for use in cell culture.[210] In general its potential applications could encompass cell supports, coatings or threads where lateral stiffness is desirable.

4.4. Biomaterials Inspired by Mussel Byssus

Byssus is a highly chemically cross-linked composite material, and thus, byssal proteins are not amenable to extraction from threads, although they can be purified from mussel feet. A mixture of preCols was prepared in this manner by the research group of Herbert Waite and drawn into threads which in TEM and AFM experiments showed the same filamentous morphology as seen in natural byssus, if not the same mechanical properties.[79] Extraction and purification of preCols from mussel feet is arduous at best, impractical on a large scale and requires sacrificing a large number of mussels for milligrams of protein. To date, production of preCols remains beyond our reach; the challenges involved in molecular biology and the demands of the host system are even greater than those for silks. Not only are preCols large and highly repetitive, they also require post-translational modifications (for example hydroxylation of proline and presumably tyrosine residues) which are likely necessary for proper thread assembly and mechanical properties.[141] A fusion protein containing collagen and silk domains that models the block-copolymer design of preCol-D has been produced,[142] and this approach is likely a better starting point for investigating the properties of the preCols and their potential for new materials.

Other mussel foot proteins involved in adhesion, however, have been extracted from mussels or produced recombinantly. Although these are not strictly fiber-forming proteins, they are worth at least a cursory mention because they show remarkable promise as underwater glues and coatings particularly for medical applications (see Table 4) as they show strong adhesion to a variety of surfaces both hydrophobic and hydrophilic under wet and dry conditions, are biocompatible, non-immunogenic, environmentally benign and show controlled degradation under physiological conditions.[143] The byssal cuticle protein Mfp-1 is a potential compliant coating,[101] and has been produced recombinantly, albeit in low yields.[144] The adhesive protein Mefp-5 (*Mytilus edulis* foot protein-5) has been purified in full length from *E. coli*,[145] and a variant of a second adhesive protein Mfp-3 (mussel foot protein-3) from *Mytilus coruscus* was also successfully produced in *E. coli*.[146] A

fusion protein of partial Mefp-1 and Mefp-5 constructs has also been achieved.[147,148] A bio-compatible wettable adhesive comprised of a mixture of adhesive proteins from byssal extracts is already available for general use (e.g. CellTak).[149] A commercially available fusion protein of mfp-1,-3 and-5 (MAPTrix™) incorporating various bioactive peptides is also currently being marketed as an extracellular matrix mimic for cell culture studies.

A burgeoning field of research essentially reduces the principle of mussel adhesion to DOPA chemistry. Although the precise mechanism is still being elucidated, DOPA is believed to be responsible for both the cohesive (when DOPA is oxidized to DOPA-quinone) and adhesive (non-oxidized DOPA) properties of mussel foot proteins.[150] Thus, DOPA-modified polymers such as poly(ethylene glycol)[151] can serve as adhesives or coatings that are economical, simple to synthesize and easily scalable.[152]

Taking the mechanical gradient of byssus as inspiration and building upon previous work with synthetic polymers, large centimeter scale gelatin/fibroin gradient films have been produced using a precision pump system.[98] The films exhibit a fully reproducible range of modulus similar to that of their natural models (ca. 160–570 MPa). Although the films do fall short of the strength and toughness of byssal threads, the establishment of a protein gradient system may prove useful for directed cell attachment in cell culture or in the reinforcement of damaged tendons.

5. Outlook

The groundwork has been laid for advances in protein biomaterials inspired by or based on proteins from spider silk, lacewing egg stalk silk, and mussel byssus. Starting with the study of the natural proteins and continuing through recombinant production of cleverly designed analogues, useful protein materials can be fabricated for a variety of applications (see Table 4). The final and perhaps largest obstacle to overcome remains the processing of recombinant proteins. Although recombinant fibrous proteins such as spider silk can be processed into a variety of morphologies for a range of uses (see Figure 4), much work remains to achieve the fabrication of threads that rival the superior mechanical properties of natural protein fibers.

Acknowledgements

The authors thank Kristina Spieß for scouring the literature of protein-based biomaterials, Anja Hagenau for providing vector graphics material and SEM images, Felix Bauer for the image of the lacewing (Figure 1c), Martin Humenik for help with figures and the manuscript and Michael Suhre for numerous helpful critical comments. We gratefully acknowledge funding from the German Research Foundation (DFG SCHE 603/7–1).

Received: February 15, 2013
Revised: April 29, 2013
Published online: August 14, 2013

[1] L. Craig, *Annu. Rev. Entom.* **1997**, *42*, 231.
[2] T. D. Sutherland, J. H. Young, S. Weisman, C. Y. Hayashi, D. J. Merritt, *Annu. Rev. Entomol.* **2010**, *55*, 171.
[3] J. E. Garb, N. A. Ayoub, C. Y. Hayashi, *BMC Evol. Biol.* **2010**, *10*, 243.
[4] T. Scheibel, L. Serpell, in *Protein Folding Handbook* (Eds: J. Buchner, T. Kiefhaber) Wiley-VCH Verlag GmbH, Weinheim, Germany **2005**, 197.
[5] S. Weisman, S. Okada, S. T. Mudie, M. G. Huson, H. E. Trueman, A. Sriskantha, V. S. Haritos, T. D. Sutherland, *J. Struct. Biol.* **2009**, *168*, 467.
[6] M. Heim, D. Keerl, T. Scheibel, *Angew. Chem. Int. Ed.* **2009**, *48*, 3584.
[7] C. Fu, Z. Shao, F. Vollrath, *Chem. Commun.* **2009**, *43*, 6515.
[8] J. Newman, C. Newman, *Int. J. Dermatol.* **1995**, *34*, 290.
[9] C. Z. Zhou, F. Confalonieri, J. Jacquet, R. Perasso, Z. G. Li, J. Janin, *Proteins* **2001**, *44*, 119.
[10] C. L. Craig, C. Riekel, *Comp. Biochem. Phys. B* **2002**, *133*, 493.
[11] J. M. Gosline, P. A. Guerette, C. S. Ortlepp, K. N. Savage, *J. Exp. Biol.* **1999**, *202*, 3295.
[12] G. H. Altman, F. Diaz, C. Jakuba, T. Calabro, R. L. Horan, J. Chen, H. Lu, J. Richmond, D. L. C. Kaplan, *Biomaterials* **2003**, *24*, 401.
[13] J. G. Hardy, L. M. Römer, T. R. Scheibel, *Polymer* **2008**, *49*, 4309.
[14] F. S. Sehnal, T. Sutherland, *Prion* **2008**, *2*, 145.
[15] S. Inoue, K. Tanaka, F. Arisaka, S. Kimura, K. Ohtomo, S. Mizuno, *J. Biol. Chem.* **2000**, *275*, 40517.
[16] A. Matsumoto, A. Lindsay, B. Abedian, D. L. Kaplan, *Macromol. Biosci.* **2005**, *8*, 1006.
[17] Z. Shao, F. Vollrath, *Nature* **2002**, *418*, 741.
[18] Y.-X. He, N.-N. Zhang, W.-F. Li, N. Jia, B.-Y. Chen, K. Zhou, J. Zhang, Y. Chen, C.-Z. Zhou, *J. Mol. Biol.* **2012**, *418*, 197.
[19] F. Sehnal, M. Zurovec, *Biomacromolecules* **2004**, *5*, 666.
[20] N. Yonemura, F. Sehnal, *J. Mol. Evol.* **2006**, *62*, 42.
[21] L. Eisoldt, A. Smith, T. Scheibel, *Mater. Today* **2011**, *14*, 80.
[22] C. Vendrely, T. Scheibel, *Macromol. Biosci.* **2007**, *7*, 401.
[23] F. Vollrath, *Int. J. Biol. Macromol.* **1999**, *24*, 81.
[24] S. Winkler, D. L. Kaplan, *J. Biotechnol.* **2000**, *74*, 85.
[25] L. Römer, T. Scheibel, *Prion* **2008**, *2*, 154.
[26] L. Eisoldt, C. Thamm, T. Scheibel, *Biopolymers* **2012**, *97*, 355.
[27] M. Humenik, A. M. Smith, T. Scheibel, *Polymers* **2011**, *3*, 640.
[28] M. Heim, L. Römer, T. Scheibel, *Chem. Soc. Rev.* **2010**, *39*, 156.
[29] A. Rising, M. Widhe, J. Johansson, M. Hedhammar, *Cell. Mol. Life Sci.* **2011**, *68*, 169.
[30] J. G. Hardy, T. R. Scheibel, *Biochem. Soc. Trans.* **2009**, *37*, 677.
[31] F. Vollrath, D. P. Knight, *Nature* **2001**, *410*, 541.
[32] E. Terry, D. P. Knight, D. Porter, F. Vollrath, *Biomacromolecules* **2004**, *5*, 768.
[33] C. Dicko, J. M. Kenney, F. Vollrath, in *Advances in Protein Chemistry*, Vol. *73* (Eds: A. Kajava, J. M. Squire, D. A. D. Parry), Elsevier, Academic Press, New York, USA **2006**, pp.1–320.
[34] F. Vollrath, D. P. Knight, X. W. Hu, *Proc. R. Soc. London Ser. B* **1998**, *265*, 817.
[35] D. P. Knight, F. Vollrath, *Proc. R. Soc. London Ser. B* **1999**, *266*, 519.
[36] D. Keerl, T. Scheibel, *Bioinsp. Biomim. Nanobiomat.* **2012**, *1*, 83.
[37] B. Madsen, Z. Z. Shao, F. Vollrath, *Int. J. Biol. Macromol.* **1999**, *24*, 301.
[38] R. W. Work, P. D. Emerson, *J. Arachnol.* **1982**, *10*, 1.
[39] J. Perez-Rigueiro, M. Elices, G. Plaza, J. I. Real, G. V. Guinea, *J. Exp. Biol.* **2005**, *208*, 2633.
[40] M. A. Garrido, M. Elices, C. Viney, J. Perez-Rigueiro, *Polymer* **2002**, *43*, 1537.
[41] C. S. Ortlepp, J. M. Gosline *Biomacromolecules* **2004**, *5*, 727.
[42] K. N. Savage, J. M. Gosline, *J. Exp. Biol.* **2008**, *211*, 1937.

[43] J. E. Jenkins, M. S. Creager, E. B. Butler, R. V. Lewis, J. L. Yarger, G. P. Holland, *Chem. Commun.* **2010**, *46*, 6714.
[44] A. Sponner, W. Vater, S. Monajembashi, E. Unger, F. Grosse, K. Weisshart, *PLoS ONE* **2007**, *2*, e998.
[45] L. Han, L. Zhang, T. Zhao, Y. Wang, M. Nakagaki, *Int. J. Biol. Macromol.* **2013**, *56*, 156.
[46] T. Lefèvre, M.-E. Rousseau, M. Pézolet, *Biophys. J.* **2007**, *92*, 2885.
[47] O. Hakimi, D. P. Knight, M. M. Knight, M. F. Grahn, P. Vadgama, *Biomacromol.* **2006**, *7*, 2901.
[48] A. Nova, S. Keten, N. M. Pugno, A. Redaelli, M. J. Buehler, *Nano Lett.* **2010**, *10*, 2626.
[49] S. W. Cranford, A. Tarakanova, N. M. Pugno, M. J. Buehler, *Nature* **2012**, *482*, 73.
[50] S. P. Kelly, A. Sensenig, K. A. Lorentz, T. A. Blackledge, *Zoology* **2011**, *4*, 233.
[51] M. Denny, *J. Exp. Biol.* **1976**, *65*, 483.
[52] M. Hudspeth, X. Nie, W. Chen, R. Lewis, *Biomacromolecules* **2012**, *13*, 2240.
[53] T. A. Blackledge, C. Boutry, S. C. Wong, A. Baji, A. Dhinojwala, V. Sahni, I. Agnarsson, *J. Exp. Biol.* **2009**, *212*, 1981.
[54] R. W. Work, *J. Arachnol.* **1981**, *9*, 299.
[55] Y. Liu, Z. Z. Shao, F. Vollrath, *Nat. Mater.* **2005**, *4*, 901.
[56] G. V. Guinea, M. Elices, J. Pérez-Rigueiro, G. Plaza, *Polymer* **2003**, *44*, 5785.
[57] M. Elices, J. Pérez-Rigueiro, G. Plaza, G. V. Guinea, *J. Appl. Polym. Sci.* **2004**, *92*, 3537.
[58] J. Perez-Rigueiro, M. Elices, G. V. Guinea, *Polymer* **2003**, *44*, 3733.
[59] K. D. Parker, K. M. Rudall, *Nature* **1957**, *179*, 905.
[60] P. Duelli, *Experientia* **1986**, *42*, 624.
[61] R. Philippe, *Ann. Soc. Ent. Fr.* **1972**, *8*, 693.
[62] K. M. Rudall, W. Kenchington, *Annu. Rev. Entomol.* **1971**, *16*, 73.
[63] U. Slotta, K. Spieß, T. Scheibel, in *The Functional Fold. Useful Amyloid Structures in Nature* (Eds: S. P. Jarvis, A. S. Mostaert), Pan Stanford Publishing, Singapore **2008**, 82.
[64] J. C. Hwang, W. E. Bickley, *Ann. Entomol. Soc. Am.* **1961**, *54*, 422.
[65] F. Lucas, J. T. B. Shaw, S. G. Smith, *Nature* **1957**, *179*, 906.
[66] F. Lucas, K. M. Rudall, in *Comprehensive Biochemistry*, Vol *26B* (Eds: M. Florkin, E. H. Stotz), Elsevier, Amersterdam, Netherlands **1968**, 475.
[67] F. Chiti, C. M. Dobson, *Annu. Rev. Biochem.* **2006**, *75*, 333.
[68] J. Geddes, K. D. Parker, E. D. Atkins, E. Beighton, *J. Mol. Biol.* **1968**, *14*, 343.
[69] R. Nelson, M. R. Sawaya, M. Balbirnie, A. Ø. Madsen, C. Riekel, R. Grothe, D. Eisenberg, *Nature* **2005**, *435*, 7043.
[70] F. Bauer, T. Scheibel, *Angew. Chem. Int. Ed.* **2012**, *51*, 6521.
[71] F. Bauer, L. Bertinetti, A. Masic, T. Scheibel, *Biomacromolecules* **2012**, *13*, 3730.
[72] H. R. Hepburn, H. D. Chandler, M. R. Davidoff, *Insect Biochem.* **1979**, *9*, 69.
[73] P. Fratzl, *Collagen: Structure and Mechanics*. Springer, New York, USA **2008**.
[74] D. Pearle, R. F. Warren, S. A. Rodeo, *Clin. Sports Med.* **2005**, *24*, 1.
[75] F. S. A. Cavalcante, S. Ito, K. Brewer, H. Sakai, A. M. Alencar, M. P. Almeida, J. S. Andrade, A. Majumdar, E. P. Ingenito, B. Suki, *J. Appl. Physiol.* **2005**, *98*, 672.
[76] A.Gautieri, S. Vesentini, A. Redaelli, M. J. Buehler, *Nano Lett.* **2011**, *11*, 757.
[77] B. Brodsky, A. V. Persikov, *Adv. Protein Chem.* **2005**, *70*, 301.
[78] H. J. Waite, E. Vaccaro, C. Sun, J. M Lucas, *Phil. Trans. R. Soc. Lond. B* **2002**, *357*, 143.
[79] M. J. Harrington, J. H. Waite, *Biomacromolecules* **2008**, *9*, 1480.
[80] C. H. Brown, *L. Q. J. Microsc. Sci.* **1952**, *98*, 487.
[81] J. H. Waite, in *Structure, Cellular Synthesis and Assembly of Biopolymers*, Vol. *19* (Ed.: S. P. Case), Springer Verlag, Berlin, Germany **1992**, 27.
[82] H. Zao, N. B. Robertson, S. Jewhurst, J. H. Waite, *J. Biol. Chem.* **2006**, *281*, 11090.
[83] H. G. Silverman, F. F. Roberto, in *Biological Adhesive Systems: From Nature to Technical and Medical Applications*, Vol. *18* (Eds: J. von Byern, I. Grunwald), Springer, New York, USA **2010**, 273.
[84] L. Vitellaro-Zuccarello, *J. Ultrastruct. Res.* **1980**, *73*, 135.
[85] A. Tamarin, P. Lewis, J. Askey, *J. Morphol.* **1974**, *142*, 321.
[86] V. Benedict, J. H. Waite, *J. Morphol.* **1986**, *189*, 171.
[87] T. Hassenkam, T. Gutsmann, P. Hansma, J. Sagert, J. H. Waite, *Biomacromolecules* **2004**, *5*, 1351.
[88] D. S. Hwang, W. Wie, N. R. Rodriguez-Martinez, E. Danner, J. H. Waite, in *Polymer Adhesion, Friction, and Lubrication* (Ed: H. Zeng), John Wiley & Sons, Inc., Hoboken, USA **2013**, 319.
[89] X. X. Qin, J. Herbert Waite, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 10517.
[90] C. A. Klug, L. A. Burzio, J. H. Waite, J. Schaefer, *Arch. Biochem. Biophys.* **1996**, *333*, 221.
[91] H. J. Waite, H. C. Lichtenegger, G. D. Stucky, P. Hansma, *Biochemistry* **2004**, *24*, 7653.
[92] A. Hagenau, H. A. Scheidt, L. Serpell, D. Huster, T. Scheibel, *Macromol. Biosci.* **2009**, *9*, 162.
[93] A. Arnold, F. Byette, M. O. Séguin-Heine, A. Leblanc, L. Sleno, R. Tremblay, C. Pellerin, I. Marcotte, *Biomacromolecules* **2013**, *14*, 132.
[94] M. J. Harrington, J. S. Gupta, P. Fratzl, J. H. Waite, *J. Struct. Biol.* **2009**, *167*, 47.
[95] M. J. Harrington, J. H. Waite, *Adv. Mater.* **2009**, *21*, 440.
[96] J. Sagert, J. H. Waite, *J. Exp. Biol.* **2009**, *212*, 2224.
[97] C. Sun, J. M. Lucas, J. H. Waite, *Biomacromolecules* **2002**, *3*, 1240.
[98] K. U. Claussen, E. S. Lintz, R. Giesa, H.-W. Schmidt, T. Scheibel, *Macromol. Biosci.* **2013**, DOI: 10.1002/mabi.201300221.
[99] N. Holten-Andersen, G. E. Fantner, S. Hohlbauch, J. H. Waite, F. W. Zok, *Nat. Mater.* **2007**, *6*, 669.
[100] M. J. Harrington, A. Masic, N. Holten-Andersen, J. H. Waite, P. Fratzl, *Science* **2010**, *328*, 261.
[101] N. Holten-Andersen, J. H. Waite, *J. Dent. Res.* **2008**, *87*, 701.
[102] N. Holten-Andersen, M. J. Harrington, H. Birkedal, B. P. Lee, P. B. Messersmith, K. Y. Lee, J. H. Waite, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 2651.
[103] E. Bell, J. Gosline, *J. Exp. Biol.* **1996**, *199*, 1005.
[104] C. Sun, E. Vaccaro, J. H. Waite, *Biophys. J.* **2001**, *81*, 3590.
[105] E. Carrington, J. M. Gosline, *Am. Malacol. Bull.* **2004**, *18*, 135.
[106] M. J. Harrington, J. H. Waite, in *Fibrous Proteins* (Ed: T. Scheibel), Landes Bioscience, Austin, USA **2008**, 30.
[107] N. Aldred, T. Wills, D. N. Williams, A. S. Clare, *J. R. Soc. Interface* **2007**, *4*, 1159.
[108] A. Hagenau, P. Papadopoulos, F. Kremer, T. Scheibel. *J. Struct. Biol.* **2011**, *175*, 339.
[109] E. Vaccaro, J. H. Waite, *Biomacromolecules* **2001**, *2*, 906.
[110] J. H. Waite, X. X. Qin, K. J. Coyne, *Matrix Biol.* **8**, *17*, 93.
[111] K. Bertoldi, M. C. Boyce, *J. Mater. Sci.* **2007**, *42*, 8943.
[112] K. Sanford, M. Kumar, *Curr. Opin. Biotechnol.* **2005**, *16*, 416.
[113] A. Bracalello, V. Santopietro, M. Vassalli, G. Marletta, R. Del Gaudio, B. Bochicchio, A. Pepe, *Biomacromol.* **2011**, *12*, 2957.
[114] F. Teulé, Y. G. Miao, B. H. Sohn, Y. S. Kim, J. J. Hull, M. J. Jr. Fraser, R. V. Lewis, D. L. Jarvis, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 923.
[115] B. An, J. E. Jenkins, S. Sampath, G. P. Holland, M. Hinman, J. L. Yarger, R. Lewis, *Biomacromol.* **2012**, *13*, 3938.
[116] J. A. Gustafson, H. Ghandehari, *Adv. Drug Deliv. Rev.* **2010**, *62*, 1509.
[117] F. Teulé, B. Addison, A. R. Cooper, J. Ayon, R. W. Henning, C. J. Benmore, G. P. Holland, J. L. Yarger, R. V. Lewis, *Biopolymers* **2012**, *97*, 418.
[118] M. Zelzer, R. V. Ulijn, *Chem. Soc. Rev.* **2010**, *39*, 3351.
[119] F. Versluis, H. R. Marsden, A. Kros, *Chem. Soc. Rev.* **2010**, *39*, 3434.

[120] J. G. Hardy, T. R. Scheibel, *Prog. Polym. Sci.* **2010**, *35*, 1093.
[121] K. Spiess, A. Lammel, T. Scheibel, *Macromol. Biosci.* **2010**, *10*, 998.
[122] A. Leal-Egaña, T. Scheibel, *Biotechnol. Appl. Biochem.* **2010**, *55*, 155.
[123] T. Scheibel, *Microb. Cell Fact.* **2004**, *3*, 14.
[124] D. Huemmerich, C. W. Helsen, S. Quedzuweit, J. Oschmann, R. Rudolph, T. Scheibel, *Biochemistry* **2004**, *43*, 13604.
[125] S. Rammensee, U. Slotta, T. Scheibel, A. R. Bausch, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6590.
[126] A. Heidebrecht, T. Scheibel, in *Advances in Applied Microbiology*, Vol. *82* (Eds: G. M. Gadd, S. Sariaslani), Academic Press, Waltham, USA **2013**, *82*, p.115.
[127] L. Eisoldt, J. G. Hardy, M. Heim, T. R. Scheibel, *J. Struct. Biol.* **2010**, *170*, 413.
[128] F. Hagn, C. Thamm, T. Scheibel, H. Kessler, *Angew. Chem. Int. Edit.* **2010**, *50*, 310.
[129] G. Askarieh, M. Hedhammar, K. Nordling, A. Saenz, C. Casals, A. Rising, J. Johansson, S. D. Knight, *Nature* **2010**, *465* 236.
[130] C. Allmeling, A. Jokuszies, K. Reimers, S. Kall, C. Z. Choi, G. Brandes, C. Kasper, T. Scheper, M. Guggenheim, P. M. Vogt, *Cell Proliferat.* **2008**, *41*, 408.
[131] C. Radtke, C. Allmeling, K. H. Waldmann, K. Reimers, K. Thies, H. C. Schenk, A. Hillmer, M. Guggenheim, G. Brandes, P.M. Vogt, *PLoS ONE* **2011**, *6*, e16990.
[132] A. Lammel, M. Schwab, M. Hofer, G. Winter, T. Scheibel, *Biomaterials* **2011**, *32*, 2233.
[133] K. D. Hermanson, M. B. Harasim, T. Scheibel, A. R. Bausch, *Phys. Chem. Chem. Phys.* **2007**, *9*, 6442.
[134] M. Widhe, H. Bysell, S. Nystedt, I. Schenning, M. Malmsten, J. Johansson, A. Rising, M. Hedhammar, *Biomaterials* **2010**, *31*, 9575.
[135] C. Fredriksson, M. Hedhammar, R. Feinstein, K. Nordling, G. Kratz, J. Johansson, F. HussA. Rising, *Materials* **2009**, *2*, 1908.
[136] I. Agapov, O. Pustovalova, M. Moisenovich, V. Bogush, O. Sokolova, V. Sevastyanov, V. Debavov, M. Kirpichnikov, *Dokl. Biochem. Biophys.* **2009**, *426*. 127.
[137] P. Xiang, M. Li, C. Y.Zhang, D. L. Chen, Z. H. Zhou, *Int. J. Biol. Macromol.* **2011**, *49*, 281.
[138] K. Numata, M. R. Reagan, R. H. Goldstein, M. Rosenblatt, D. L. Kaplan, *Bioconjugate Chem.* **2011**, *22*, 1605.
[139] K. Numata, A. J. Mieszawska-Czajkowska, L. A. Kvenvold, D. L. Kaplan, *Macromol. Biosci.* **2012**, *12*, 75.
[140] C. W. P. Foo, S. V. Patwardhan, D. J. Belton, B. Kitchel, D. Anastasiades, J. Huang, R. R. Naik, C. C. Perry, D. L. Kaplan, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 9428.
[141] A. Hagenau, T. Scheibel, *J. Adhesion* **2010**, *86*, 10.
[142] B. An, T. M. DesRochers, G. Qin, X. Xia, G. Thiagarajan, B. Brodsky, D. L. Kaplan, *Biomaterials* **2013**, *34*, 402.
[143] H. J. Cha, D. S. Hwang, S. Lim, *Biotechnol. J.* **2008**, *3*, 631.
[144] R. L. Strausberg, D. M. Anderson, D. Filpula, M. Finkelman, R. Link, R. McCandliss, S. A. Orndorff, S. L. Strausberg, T. Wie, in *Adhesives from Renewable Resources* (Eds: R. W. Hemingway, A. H. Conner, S. J. Branham), American Chemical Society, Washington DC, USA **1989**, p.453.
[145] D. S. Hwang, H. J. Yoo, J. H. Jun, W. K. Moon, H. J. Cha, *Appl. Environ. Microbiol.* **2004**, *70*, 3352.
[146] N. Li, L. Tan, L. Yang, G. Shi, Z. Wang, Z. Liao, *Protein Pept. Lett.* **2011**, *18*, 1265.
[147] Y. S. Choi, D. G. Kang, S. Lim, Y. J. Yang, C. S. Kim, H. J. Cha, *Biofouling* **2011**, *27*, 729.
[148] J. M. Hong, B. J. Kim, J. H. Shiim, K. S. Kang, K. J. Kim, J. W. Rhie, H. J. Cha, D. W. Cho, *Acta Biomater.* **2012**, *8*, 2578.
[149] C. V. Benedict, P. T. Picciano, in *Adhesives from Renewable Resources* (Eds: R. W. Hemingway, A. H. Conner, S. J. Branham), American Chemical Society, Washington DC, USA **1989**, p.465.
[150] S. C. T. Nicklisch, J. H. Waite, *Biofouling* **2012**, *28*, 865.
[151] B. P. Lee, J. L. Dalsin, P. B. Messersmith, *Biomacromolecules* **2002**, *3*, 1038.
[152] B. P. Lee, P. B. Messersmith, J. N. Israelachvili, J. H. Waite, *Annu. Rev. Mater. Res.* **2011**, *41*, 99.
[153] B. B. Aaron, J. M. Gosline, *Biopolymers* **1981**, *29*, 1247.
[154] B. van Eldijk, C. L. McGann, K. L. Kiick, J. C. van Hest, *Top. Curr. Chem.* **2012**, *310*, 71.
[155] T. Weis-Fogh, *J. Mol. Biol.* **1961**, *3*, 648.
[156] C. M. Elvin, A. G. Carr, M. G. Huson, J. M. Maxwell, R. D. Pearson, T. Vuocolo, N. E. Liyou, D. C. C. Wong, D. J. Merritt, N. E. Dixon, *Nature* **2005**, *437*, 999.
[157] G. A. Kahler, F. M. Fisher, R. L. Sass, *Biol. Bull.* **1976**, *151*, 161.
[158] R. M. Alexander, *J. Exp. Biol.* **1966**, *44*, 119.
[159] W. Denny, *Biology and the Mechanics of the Wave-Swept Environment*, Princeton University Press: Princeton, NJ **1988**.
[160] G. A. Kahler, F. M. Fisher, R. L. Sass, *Biol. Bull.* **1976**, *151*, 181.
[161] F. Vollrath, D. T. Edmonds, *Nature* **1989**, *340*, 305.
[162] T. K. Maji, D. Basu, C. Datta, A. Banerjee, *J. Appl. Polym. Sci.* **2002**, *84*, 969.
[163] T. D. Sutherland, S. Weisman, A. A. Walker, W. T. Mudie, *Biopolymers* **2012**, *97*, 446.
[164] M. P. E. Wenger, L. Bozec, M. A. Horton, P. Mesquida, *Biophys. J.* **2007**, *93*, 1255.
[165] S. Ricard-Blum, *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a004798.
[166] M. D. Shoulders, R. T. Raines, *Annu. Rev. Biochem.* **2009**, *87*, 929.
[167] J. Gosline, M. Lillie, E. Carrington, P. Guerette, C. Ortlepp, K. Savage, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2002**, *357*, 121.
[168] J. W. S. Hearle, *Int. J. Biol. Macromol.* **2000**, *27*, 123.
[169] D. A. Greenberg, D. S. Fudge, *Proc. R. Soc. B.* **2013**, *280*.
[170] M. Toni, L. D. Valle, L. Alibardi, *J. Proteome Res.* **2007**, *6*, 3377.
[171] R. H. C. Bonser, P. P. Purslow, *J. Exp. Biol.* **1995**, *189*, 1029.
[172] M. Taylor, R. H. C. Bonser, J. W. Farrent, *J. Mater. Sci.* **2004**, *39*, 939.
[173] R. H. C. Bonser, L. Saker, G. Jeronimidis, *J. Mater. Sci.* **2004**, *29*, 2895.
[174] G. Bratzel, M. J. Buehler, *J. Mech. Behav. Biomed. Mater.* **2012**, *7*, 30.
[175] E. Brooks, S. R. Nelson, J. A. Jones, C. Koenig, M. Hinman, S. Stricker, R. V. Lewis, *Nanotech. Sci. Appl.* **2008**, *1*, 9.
[176] S. Keten, M. J. Buehler, *J. R. Soc. Interface* **2010**, *7*, 53.
[177] C. Vepari, D. L. Kaplan, *Prog. Polymer Sci.* **2007**, *32*, 991.
[178] R. L. Horan, A. L. Collette, C. Lee, K. Antle, J. Chen, G. H. Altman, *J. Biomech.* **2006**, *39*, 2232.
[179] Y. Z. Wang, H. J. Kim, G. Vunjak-Novakovic, D. L. Kaplan, *Biomaterials* **2006**, *27*, 6064.
[180] X. Wang, J. A. Kluge, G. G. Leisk, D. L. Kaplan, *Biomaterials* **2008**, *29*, 1054.
[181] R. Silva, H. Ferreira, A. Vasconcelos, A. C. Gomes, A. Cavaco-Palo, *Adv. Exp. Med. Biol.* **2012**, *733*, 155.
[182] X. Wang, E. Wenk, X. Hu, G. R. Castro, L. Meinel, X. Wang, C. Li, H. Merkle, D. L. Kaplan, *Biomaterials* **2007**, *28*, 4161.
[183] E. Wenk, A. J. Meinel, S. Wildy, H. P. Merkle, L. Meinel, *Biomaterials* **2009**, *30*, 2571.
[184] E. Wenk, A. J. Wandrey, H. P. Merkle, L. Meinel, *J. Control. Release* **2008**, *132*, 26.
[185] C. Bessa, E. R. Balmayor, H. S. Azevedo, S. Nürnberger, M. Casal, M. van Griensven, R. L. Reis, H. Redl, *J. Tissue Eng. Regen. Med.* **2010**, *5*, 349.
[186] C. Bessa, E. R. Balmayor, J. Hartinger, G. Zanoni, D. Dopler, A. Meinl, A. Banerjee, M. Casal, H. Redl, R. L. Reis, M. van Griensven, *Tissue Eng. Part C Methods* **2010**, *5*, 937.
[187] X. Wang, E. Wenk, A. Matsumoto, L. Meinel, C. Li, D. L. Kaplan, *J. Control. Release* **2007**, *117*, 360.
[188] N. Kuboyama, H. Kiba, K. Arai, R. Uchida, Y. Tanimoto, U. K. Bhawal, Y. Abiko, S. Miyamoto, D. Knight, T. Asakura,

N. Nishiyama, *J. Biomed. Mater. Res. B. Appl. Biomater.* **2013**, *101*, 295.
[189] A. Motta, C. Migliaresi, F. Faccioni, P. Torricelli, M. Fini, R. Giardino, *J. Biomater. Sci.-Polym. Ed.* **2004**, *15*, 851.
[190] F. Ak, Z. Oztoprak, I. Karakutuk, O. Okay, *Biomacromolecules* **2013**, DOI: 10.1021/bm3018033
[191] N. Guziewicz, A. Best, B. Perez-Ramirez, D. L. Kaplan, *Biomaterials* **2011**, *32*, 2642.
[192] T. Diab, E. M. Pritchard, B. A. Uhrig, J. D. Boerckel, D. L. Kaplan, R. E. Guldberg, *J. Mech. Behav. Biomed. Mater.* **2012**, *11*, 123.
[193] D. J. Hines, D. J. Kaplan, *Biomacromolecules* **2011**, *12*, 804.
[194] X. Wang, X. Hu, A. Daley, O. Rabotzagova, P. Cebe, D. L. Kaplan, *J. Control. Release* **2007**, *121*, 190.
[195] S. Hofmann, C. T. W. P. Foo, F. Rossetti, M. Textor, G. Vunjak-Novakovic, D. L. Kaplan, H. P. Merkle, L. Meinel, *J. Control. Release* **2006**, *111*, 219.
[196] T. L. Liu, J. C. Miao, W. H. Sheng, Y. F. Xie, Q. Huang, Y. B. Shan, J. C. Yang, *J. Zhejiang. Univ.-Sc. B.* **2010**, *11*, 10.
[197] A. Sugihara, K. Sugiura, H. Morita, T. Ninagawa, K. Tobouchi, R. Tobe, M. Izumiza, T. Horio, N. G. Abraham, S. Ikehara, *P. Soc. Exp. Biol. Med.* **2000**, *225*, 58.
[198] C. Cassinelli, G. Cascardo, M. Morra, L. Draghi, A. Motta, G. Catapano, *Int. J. Artif. Organs* **2006**, *29*, 881.
[199] H. Wendt, A. Hilmer, K. Reimers, J. W. Kuhbier, F. Schäfer-Nolte, C. Allmeling, C. Kasper, P. M. Vogt, *PLoS One* **2011**, *6*, e21833.
[200] J. Mieszawska, L. D. Nadkarni, C. C. Perry, D. L. Kaplan, *Chem. Mater.* **2010**, *22*, 5780.
[201] E. Bini, C. W. P. Foo, J. Huang, V. Karageorgiou, B. Kitchel, D. L. Kaplan, *Biomacromolecules* **2006**, *7*, 3139.
[202] W. Morgan, K. E. Roskow, S. Lin-Gibson, D. L. Kaplan, M. L. Becker, C. G. Simon, *Biomaterials* **2008**, *29*, 2556.
[203] L. Baoyong, Z. Jian, C. Denglong, L. Min, *Burns* **2010**, *36*, 891.
[204] S. T. Krishnaji, W. Huang, O. Rabotzagova, E. Kharlampieva, I. Choi, V. V. Tsukruk, R. Naik, P. Cebe, D. L. Kaplan, *Langmuir* **2011**, *27*, 1000.
[205] K. Numata, B. Subramanian, H. A. Currie, D. L. Kaplan, *Biomaterials* **2009**, *30*, 5775.
[206] A. S. Lammel, X. Hu, S. H. Park, D. L. Kaplan, T. R. Scheibel, *Biomaterials* **2010**, *16*, 4583.
[207] U. K. Slotta, S. Rammensee, S. Gorb, T. Scheibel, *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 4592.
[208] K. Schacht, T. Scheibel, *Biomacromolecules* **2011**, *12*, 2488.
[209] S. Wohlrab, S. Müller, A. Schmidt, S. Neubauer, H. Kessler, A. Leal-Egaña, T. Scheibel, *Biomaterials* **2012**, *33*, 6650.
[210] F. Bauer, S.Wohlrab, T. Scheibel, *Biomater. Sci.* **2013**, DOI: 10.1039/c3bm60114e.