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New Opportunities for an Ancient Material

Fiorenzo G. Omenetto and **David L. Kaplan**[*]
Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA.

Abstract

Spiders and silkworms generate silk protein fibers that embody strength and beauty. Orb webs are fascinating feats of bioengineering in nature, displaying magnificent architectures while providing essential survival utility for spiders. The unusual combination of high strength and extensibility is a characteristic unavailable to date in synthetic materials yet is attained in nature with a relatively simple protein processed from water. This biological template suggests new directions to emulate in the pursuit of new high-performance, multifunctional materials generated with a green chemistry and processing approach. These bio-inspired and high-technology materials can lead to multifunctional material platforms that integrate with living systems for medical materials and a host of other applications.

Mechanically, silks are outstanding material systems. The toughness of silk fibers is superior to any of the best synthetic high-performance fibers available today, including Kevlar (Dupont Advanced Fiber Systems). Although important insights into silk protein self-assembly have been achieved over the past 10 years (1, 2), the mechanisms by which these proteins achieve metastable states in the glands of the spinning organisms remain unclear. This is a remarkable processing achievement that allows the concentration of protein in the glands to reach >30 weight % in water (1), whereas at this concentration most proteins, globular or fibrous, aggregate and precipitate. In the case of silk, this would result in the premature formation of β sheets (crystallization), resulting in insolubility of the spinning dope and blockage of the spinning apparatus, events that would be catastrophic to the silk-spinning organism (2). The lack of full comprehension of these processing steps has limited the ability to spin reconstituted silk solutions into fibers with properties comparable to those of native fibers.

Relationships between silk protein processing and fiber properties suggest that material features are controlled by a combination of the chemistry and the spinning process. Mechanical properties of spider silks are modulated on the basis of spinning conditions, including temperature, reeling rate, and drawing rate, as well as the specific type of silk (Fig. 1) (3, 4). Silkworm silk fibers are also influenced by process conditions such that properties can be achieved to match those of spider silks (5). Silks from *Bombyx mori* obtained at different reeling rates result in stronger but more brittle fibers. Wet spinning with postspinning treatments can also be used to generate spider silk–like properties and microstructures from silkworm silks (6).

The concentration of silk protein increases during the progression from synthesis to spinning in the glands, with changes in chemistry (cations and decrease in pH) and physical features (sol-gel phase transition), along with mechanical alignment in shear flow (1). Chain alignment is involved in later processing stages, and the internal drawdown of fibers—

[*]To whom correspondence should be addressed. david.kaplan@tufts.edu.

perhaps including active transport of water, addition of hygroscopic polymers such as sericin (in the silkworm), or other unknownmechanisms—plays an important role in the critical final steps that lead to fiber formation. Silks are not "squeezed out" of the glands but are mostly pulled out, either by the legs of the spider or by the "figure eight" movement of the head of the silkworm during spinning. Rheological studies into the different structures in the spinning gel formed in vivo versus in vitro are instructive in terms of how much native silk material (harvested directly from the gland) differs from reprocessed (fibers resolubilized into solution) silks (7, 8). This disparity in solution behavior, as discerned from rheological assessments, suggests that there are still substantial knowledge gaps in understanding how to reverse engineer silk protein fibers. Morphological differences in the fiber cross sections among species originate at the spinerettes of spiders and can provide important clues on how processing within the gland, married to the spinning system, can coexist to control morphological features of the final fibers. Thin ribbons, such as those produced by tarantulas, to circular cross sections and other morphologies have been reported depending on the specific spider species. The influence of gland-specific features and process variables have not been studied in detail related to fiber morphology, yet could provide interesting insight into modes to regulate such fiber features.

Silk chemistries have been selected through evolution, and therefore structure-function relationships have been optimized for survival related to the needs of the silk-producing organisms (9–11). Among silk variants, particular interest is devoted to the silkworm silk of *B. mori* as the commodity silk protein used in textiles and medical sutures and to the dragline silks of spiders such as *Nephila clavipes* and *Araneus diadematus*. Silks are modular in design, with large internal repeats flanked by shorter (~100 amino acid) terminal domains (N and C termini). Silks have high molecular weight (200 to 350 kD or higher) with transcripts of 10,000 base pairs and higher and >3000 amino acids (10, 12). The larger modular domains are interrupted with relatively short spacers with hydrophilic charge groups in the case of silkworm silk. N and C termini are involved in the assembly and processing of silks, including pH control of assembly (12). The N and C termini are highly conserved, in spite of their relatively small size compared with the internal modules (N terminus around 130 amino acids and C terminus around 100 amino acids). Accordingly, the inclusion of such modules in cloned silks may be essential when the emulation of native properties is the goal (Fig. 2).

Because native silkworm and spider populations already display variations in the sizes of silk genes and, as a result, in the proteins generated, it is perhaps important to relate size and modular unit distribution to silk protein assembly and material functions. The roles of the N and C termini in processing related to pH, cations, or protein concentration may be responsible for only a subset of the functional features of these domains (13). It is conceivable that their role is to help stabilize silk during synthesis and processing in the gland, further underscoring the critical need to include such domains in genetically engineered silks (14, 15). Some dragline spider silks consist of two key proteins, such as Masp1 and Masp2 in the case of *N. clavipes* or *A. diadematus*, with one of the proteins usually containing proline as a putative entropic spring (16). Yet silkworm silks generally have one main protein.

The competition for water between the modular domains in silk proteins has not been quantified. This has implications for processing silks in aqueous and organic solvents. The silk-spinning dope in the glands of spiders and silkworms is stable to perturbations, such as when spiders are flicked off the ceiling by an arachnophobe or during the handling of silkworms. In contrast, high concentrations of silk protein in water readily crystallize from their metastable state, with almost any energy input resulting in rapid crystallization. Under natural conditions, silks can be stored for days and weeks in the producing organisms at

ambient temperatures in preparation for spinning without premature crystallization. Conversely, careful control of these processes is essential in laboratory conditions in order to avoid premature crystallization. Many of the above factors lead to questions about how silks can be used as design templates in order to build synthetic polymer chemistry analogs that would recapitulate some or all of the useful functional features that exist in naturally occurring silks (17). For example, the ability to design synthetic block copolymers with modular designs that can be processed in water under ambient conditions, and yet achieve water-stable robust mechanical properties without the need for chemical crosslinking, would seem to be an enviable outcome from the lessons offered from the designs of silk chemistries and processing.

The main source of silkworm silk is through sericulture, the domesticated production of silk from *B. mori*. This production approach provides the textile world and the medical suture world with an abundance of material for current demands. Additionally, this production process is a source of support for microeconomies in many parts of the world. In contrast to the established supply chain available for silkworm silk, there are no commercial options for spider silks, largely because of the more aggressive nature of spiders and the more complex and smaller quantities of silk mixtures generated in orb webs. Silks have been cloned and expressed in a variety of heterologous hosts, from bacteria and fungi to plants and mammals, as well as via transgenic silkworms (18–22), yet the material properties of these cloned silks are inferior to the native materials. This difference can be traced to molecular weight and the modular domains. With few exceptions, most silks when cloned and expressed include only some of the core modular domains; in some cases, these modular domains plus the C terminus are truncated. These truncated silks may be sufficient for some applications, but only where mechanical properties are not critical.

Some silks are encoded by single exons, whereas others have conserved introns, features that are unique because in most eukaryotes it is unusual to have proteins encoded by single exons, particularly when the encoded proteins are so large (>3000 amino acids) (23). Silk genetic domains are remarkable for their repetitive and modular nature, their size, and the highly evolved host cell requirements to meet the extreme demands of just a few amino acids (e.g., glycine and/or alanine in the case of silkworm silk) (24). Further, the level of silk protein generated in the silkworm during transition from the worm to the moth is remarkable, prompting research into the use of transgenic silkworms as production factories for cloned genes and the encoded proteins (25).

Whether transgenic animals or plants, transgenic silkworms themselves, *Escherichia coli*, or other hosts will become the most efficient system remains to be determined. Regardless, issues of secretion, stabilization of large modular gene structures, and determining metabolic demands during production still need to be resolved. These insights will not only drive improvements in silk expression but also provide important clues for the broader biotechnology community for protein expression needs. The role of processing, in combination with sequence chemistry, will play a critical role in silk material properties. Improved processing approaches for solutions and fibers, such as postspinning draw, water annealing, and the infusion of metals, can help to achieve outcomes that mimic native silks (26–28).

Future Directions

Down the road, efficient transgenic plants could be used to crop silks, much in the same way that cotton is harvested today. This would add to the all-green process involved in silk protein technologies and lay the foundation for continued propagation of the themes presented here. These approaches will need to be matched with scaled-up processing

schemes to emulate or control material properties, from mechanical performance and degradation lifetimes to control of optical clarity. Further, with the above insights as a foundation, aside from pure silk proteins for materials, a new generation of silklike proteins can also be envisioned. Extending these design concepts to a broader range of chemistries, such as other types of protein block copolymers and other types of fusion or chimeric proteins, would all be more approachable with the advances outlined for pure silks. Although each of these systems would require answers to many of the above questions, each could find a place in the applications of polymeric materials in the future because of the compelling native functions of silks and the ability to conduct these processes in a green chemistry mode. This interest would be driven not only by the practical features of the products but also by the fascination with the process of silk spinning and the beauty and utility of silk materials.

Silk-based materials have been transformed in just the past decade from the commodity textile world to a growing web of applications in high-technology directions (Fig. 3) (29). Silk sutures have been used for thousands of years, along with other natural materials like animal gut, cotton, and linen. Most of these natural materials were replaced with nylon starting in the 1930s and later with degradable polymer systems from polyesters (e.g., polylactic acid and polylactic-co-glycolic acid). Today silk sutures are used primarily in eye and lip surgery, intraoral surgery, and for some skin wounds, mainly because of their excellent handling and tying capabilities. Silk sutures sold commercially today are defined by the U.S. Pharmacopeia (USP) as nonadsorbable, primarily because of wax coatings that negate proteolytic digestion in vivo. The new generation of silks with controllable degradation rates based on processing, biomaterial scaffolds, and related systems for a range of medical needs is anticipated. In the next few years, silk sutures, drug delivery systems, and fiber-based tissue products that exploit the mechanical properties of silks can be envisioned for ligament, bone, and other tissue repairs. Follow-on applications for silks could include degradable and flexible electronic displays for improved physiological recording and function as well as environmentally compatible systems, and implantable optical systems for diagnosis and treatment (30–32). These systems would offer a new generation of devices with sensitivity and function unattainable with current materials. These silk-based medical devices would exploit controlled degradation lifetime, optical clarity, conformal contact with underlying substrates, and biocompatibility, all yielding novel functional materials from one system. Progress in these areas should also prompt polymer chemists to pursue synthetic mimics of silk as additional options to exploit the functional features and green chemistry and processing.

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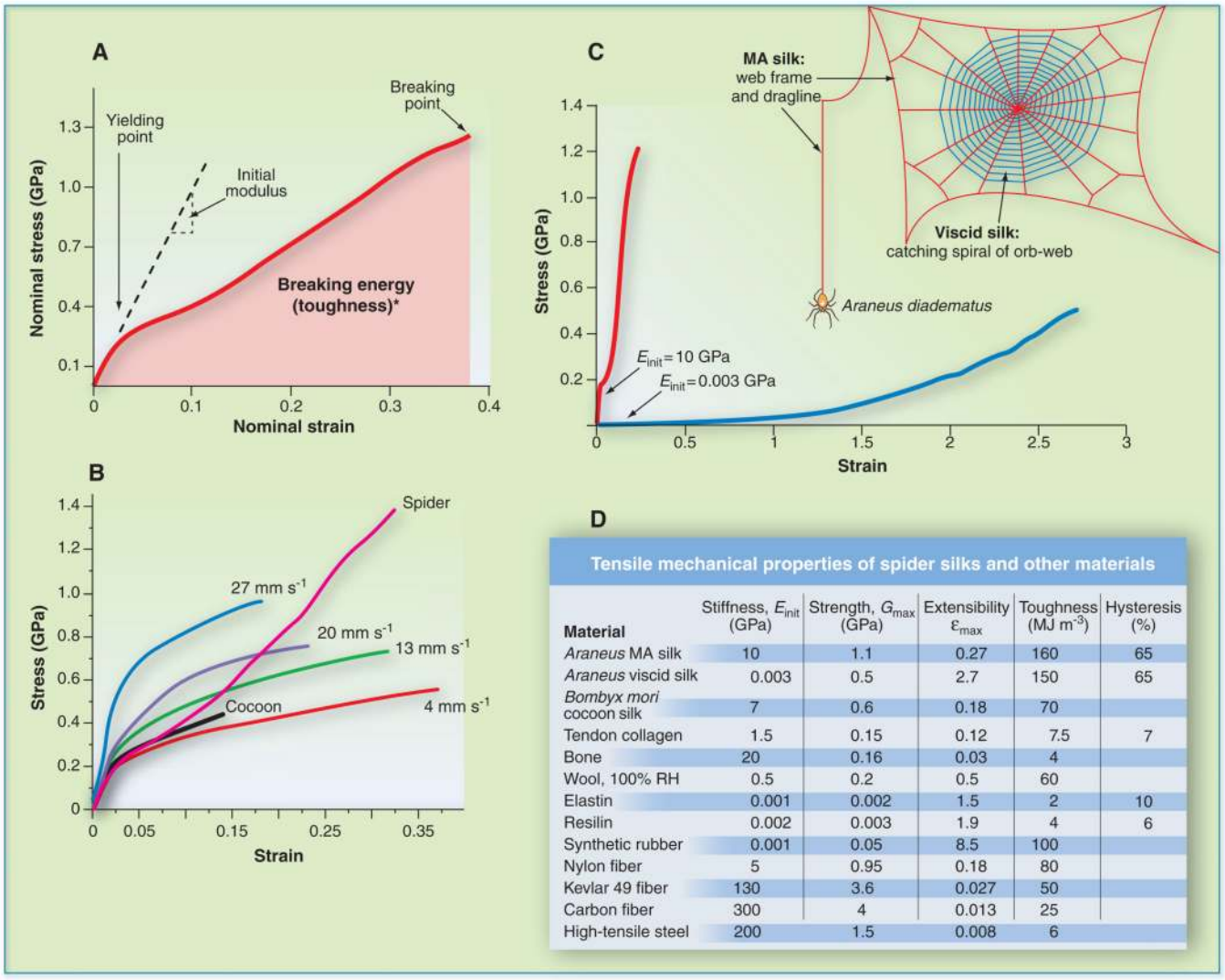

Tensile mechanical properties of spider silks and other materials

Material	Stiffness, E_{init} (GPa)	Strength, G_{max} (GPa)	Extensibility ε_{max}	Toughness (MJ m^{-3})	Hysteresis (%)
Araneus MA silk	10	1.1	0.27	160	65
Araneus viscid silk	0.003	0.5	2.7	150	65
Bombyx mori cocoon silk	7	0.6	0.18	70	
Tendon collagen	1.5	0.15	0.12	7.5	7
Bone	20	0.16	0.03	4	
Wool, 100% RH	0.5	0.2	0.5	60	
Elastin	0.001	0.002	1.5	2	10
Resilin	0.002	0.003	1.9	4	6
Synthetic rubber	0.001	0.05	8.5	100	
Nylon fiber	5	0.95	0.18	80	
Kevlar 49 fiber	130	3.6	0.027	50	
Carbon fiber	300	4	0.013	25	
High-tensile steel	200	1.5	0.008	6	

Fig. 1.

Mechanical properties of silks. (**A**) Impressive toughness and relative strength of reeled spider dragline silk. The area under the curve shown indicates fiber toughness or the energy taken up by the material before breaking. In terms of strength-to-weight ratio, the spider silk strength (1.1 GPa) is about equivalent to high-tensile engineering steel (1.3 GPa), yet spider silk has a relative density of 1.3 compared with that of steel at 7.8, when reeled at 20 mm s^{-1} at 25°C for *Nephila edulis.* In terms of toughness, spider silk is 165 ± 30 kJ kg^{-1}, which is substantially higher than that of Kevlar 81 (33 kJ kg^{-1}) (1, 5). (**B**) Spinning silk from *B. mori* silkworms at different speeds illustrates control of fiber mechanical properties resulting from processing inputs to complement the importance of chemistry. The data show that the properties can match those of spider silks when spun from the worms at higher rates than native processes. The speeds shown for the lines reflect the rate at which the silk was drawn from the silkworm under controlled conditions at 25°C and are compared with standard degummed silk from cocoons, which are spun from the glands at a natural speed of 4 to 15 mm s^{-1} at 20°C (3). (**C**) Stress strain curves for major ampullate (MA) gland silk (red line) and viscid silk (blue line) from the spider *A. diadematus*. E_{init} = initial stiffness (4). (**D**) Compilation of data from multiple sources and based on data from the spider *A. diadematus* (4). RH indicates relative humidity.

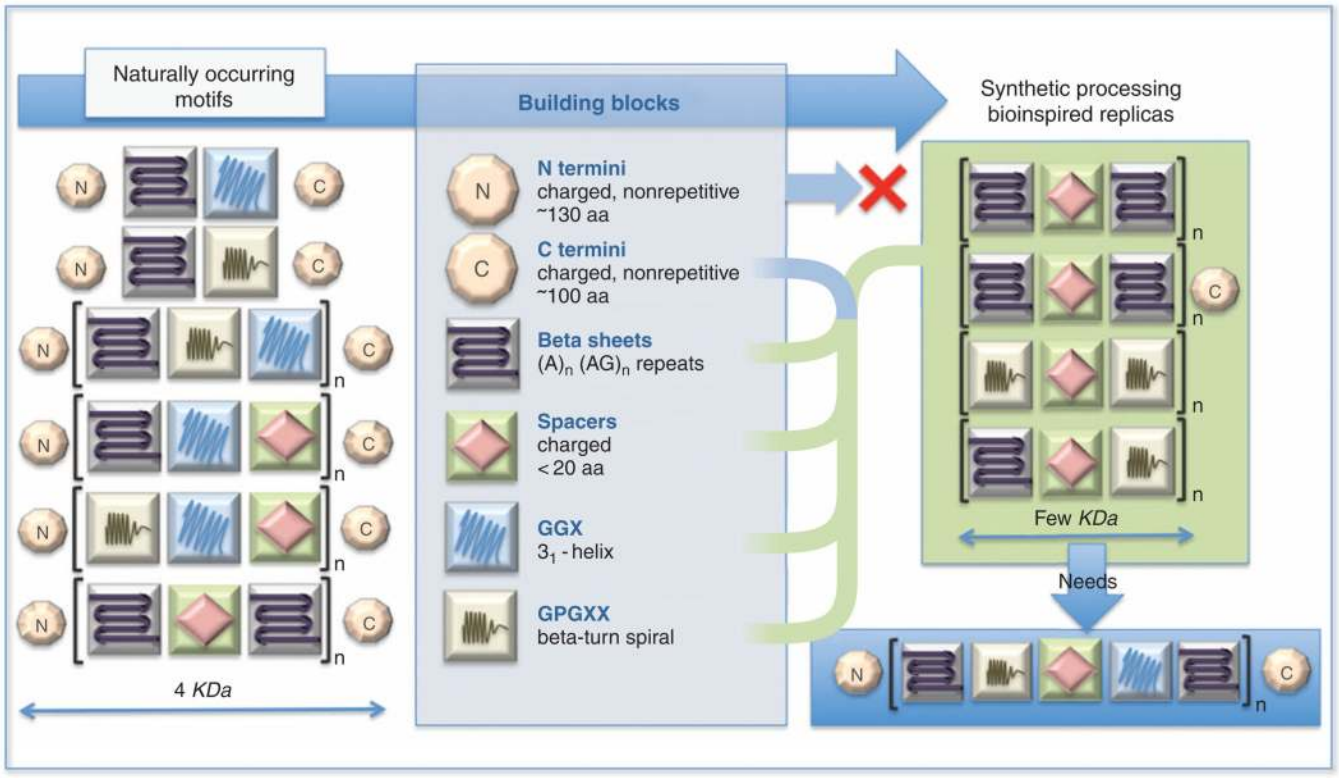

Fig. 2.

Modular designs of silk proteins. Silks are fibrous proteins and are characterized by modular units linked together to form high molecular weight, highly repetitive proteins. These modular units or domains, each with specific amino acid sequences and chemistries, provide specific functions. In particular, sequence motifs such as polyalanine (polyA) and poly alanine-glycine (polyAG) (β sheet–forming), GXX (31-helix), GXG (stiffness), and GPGXX (β spiral) are key components in different silks whose relative positioning and arrangement are intimately tied with the end material properties. These domains are linked together to generate high molecular weights and also include characteristic and highly conserved N and C termini. Charged amino acids are strategically located at the chain ends and in spacers to optimize water interactions related to processing and assembly. Current modes of expression of silks in heterologous hosts are generally confined to a limited number of thesemodular units and lack the inclusion of both N- C-terminal domains. The incorporation of all of these key domains, along with issues of size (number of modular units), will facilitate improved material properties from silks generated by recombinant DNA techniques.

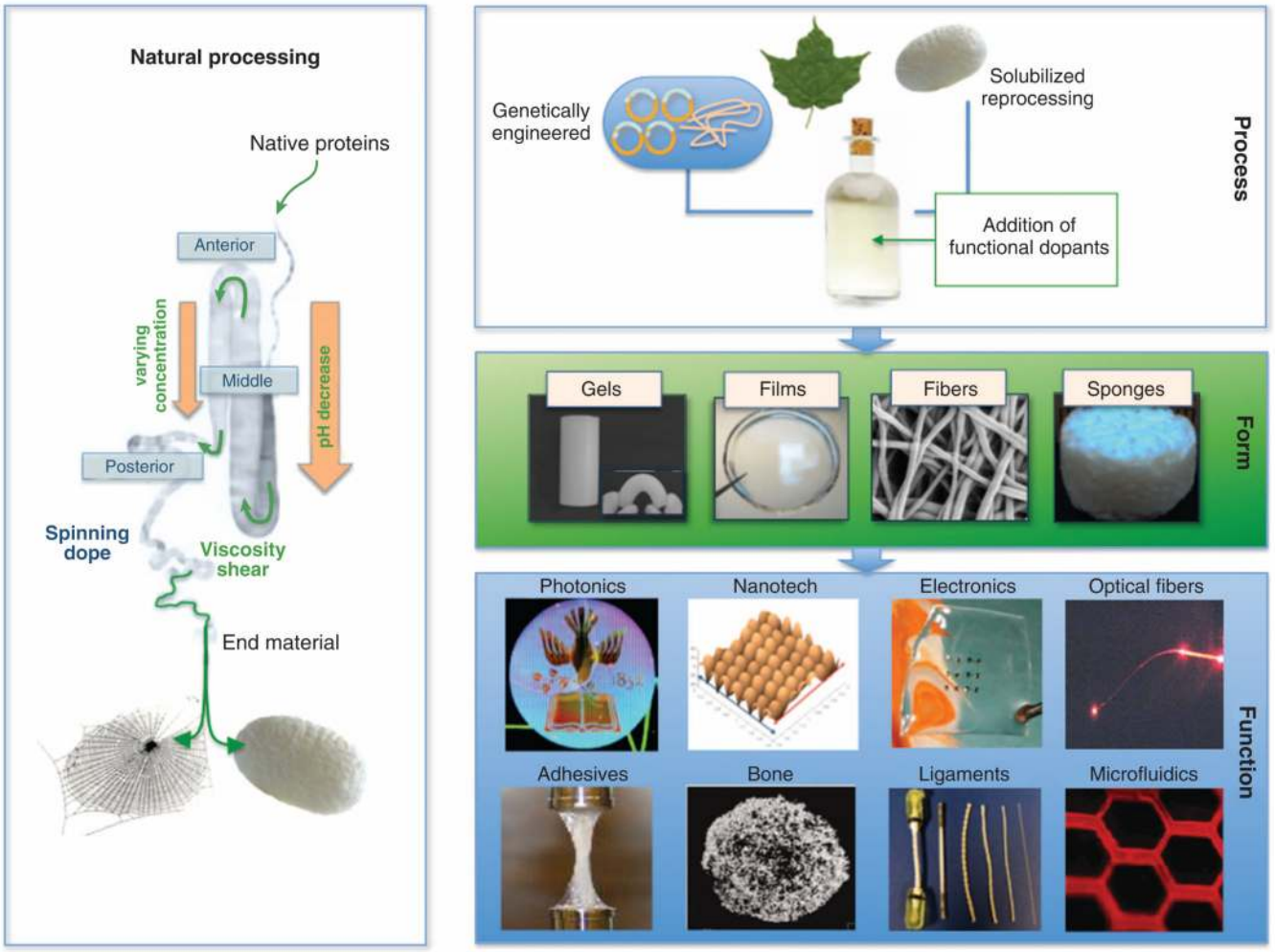

Fig. 3.

Generating new materials from silks. Native silk processing on the left illustrates the transition from synthesis, storage, and spinning related to protein concentration, pH, salts, and physical phenomena. On the right, analogous processes with source materials from reconstituted native silk proteins or genetically engineered silks lead to new materials and technology platforms with control of mechanical, morphological, and structural features. A range of materials can be generated from silks through processing into hydrogels, fibers, sponges, films, and other forms. The properties of these systems can bemodified (e.g., mechanical, degradation profile, and optical clarity) depending on the processing modes used and then generated into functional devices and technology platforms.