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Protein-Based Structural Materials

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Protein-based materials, like many biomaterials, hold many opportunities for the future. First, materials from Nature exhibit many creative and unexpected implementations that present solutions to common and longstanding problems in materials science. Hence, engineers and scientists alike have much to learn via "bioinspiration." Second, biogenic materials have evolved slowly over time, always in a benign relationship with and as an integral part of the global ecosystem. For synthetic materials, this has not been the case, which has led to some of the greatest current societal problems, including pollution, excessive depletion of resources, and effects on the climate. From Nature, we can learn how to alleviate some of these adverse effects, and we can get ideas for how to make materials in a more sustainable way. In combination with synthetic biology, this presents a transformative opportunity to tackle some of our most pressing problems over the coming decades. Third, biogenic and bioinspired materials also have great prospects in biomedical applications, where game-changing solutions are also expected to have a large societal impact. Each of these three aspects of biomaterials are represented in this special topic on protein-based structural materials.

Protein-based materials occupy a special place in the realm of biogenic materials, where ceramics and biopolymers are key materials classes. Proteins represent the key structural biopolymers in mammals, including humans, and form some of the strongest biopolymers overall. Spider silk is an especially inspiring example of powerful protein materials, exhibiting mechanical performance at a level matching or beating some of the best synthetic materials systems. By weight, some spider silks are about five times stronger than steel and five times tougher than Kevlar, thus demonstrating that protein materials can achieve critically important materials properties in a fully sustainable way. Synthetic biology further adds the prospect of mass production and further optimization of such materials. The idea that synthetic polymers can be replaced by benign biopolymers with equal or superior properties is especially welcome at a time when it has become obvious that increasing pollution of the oceans with plastics comes at a very high long-term cost. Hence, studying such natural and synthetic proteins for engineering and medical applications is highly relevant.

In this special topic, four articles are presented: two address natural proteins, two address synthetic peptides—essentially proteins with a very short amino acid sequence—and three of the four make a connection to biomedical applications. The first article, "Nanofibrils as Building Blocks of Silk Fibers: Critical Review of the Experimental Evidence" by Wang et al., addresses the nanostructure of what is arguably *the* prototype structural protein material: spider silk. Silks have fascinated researchers for decades, featuring outstanding mechanical performance and vast potential as multifunctional materials. The development of synthetic fibers mimicking natural silk is a major goal but has been hindered by insufficient knowledge of the silk structure. Nanoscale fibrils have long been suggested to play a significant role in silk; in this review, the authors examine prior evidence of nanofibrils in spider and silkworm silks. They find the available data to be far from conclusive. The volumetric percentage of nanofibrils in silk fibers is unclear, and conflicting results have been reported regarding their physical dimensions, morphology, and spatial organization. Some works have proposed an entirely different, globular nanostructure of silk fibers. Hence, many of the structural models have been developed based on very incomplete evidence. This review highlights the gaps in our knowledge about the nanostructure of silk fibers and can act as a guide for future studies.

Hannes C. Schniepp is the *JOM* advisor for the Biomaterials Committee, part of the TMS Functional Materials Division and Structural Materials Division, and guest editor for the topic Protein-Based Structural Materials in this issue.

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The next manuscript, “Optimization of Gelatin–Potassium Phosphate Aqueous Two-Phase System for the Preparation of Hydrogel Microspheres” by Erkoc et al., also considers a natural protein, viz. gelatin, to make an aqueous two-phase system, providing a simple route towards preparation of gelatin emulsions. The authors present a simple method to generate water-in-water emulsions from an aqueous two-phase system, viz. gelatin and potassium phosphate (K_2HPO_4) salt. Liquid gelatin forms as the dispersed phase of the two-phase emulsion system, and gelatin microspheres can be retrieved after visible-light-induced crosslinking reaction. The authors investigated the effect of the continuous-phase volume ratio on the occurrence of phase separation and the emulsification process. They also studied the influence of the polymerization method on the size and morphology of the gelatin hydrogel particles. The results demonstrate that K_2HPO_4 is an appropriate phase-forming salt, where biodegradable gelatin particles obtained through this w/w emulsion system have potential for biomedical applications. In addition, sustained release of a model molecule, methylene blue, from gelatin particles was observed up to 5 days. This system is advantageous for providing an inexpensive and environmental platform for emulsion formation and in that K_2HPO_4 salt acts as a promising alternative to organic solvents or auxiliary polymers to form a continuous phase.

The next two manuscripts tap into the potential of protein-inspired synthetic materials. Both consider peptides that are tailored for materials science applications. The manuscript “Repeatedly Applied Peptide Film Kills Bacteria on Dental Implants” by Wisdom et al. uses peptides that were designed to adsorb on titanium and form a functional film. The rising use of titanium dental implants has increased the prevalence of peri-implant disease, which shortens their useful life. A growing view of peri-implant disease suggests that plaque accumulation and microbiome dysbiogenesis trigger a host immune inflammatory response that destroys soft and hard tissues supporting the implant. The incidence of peri-implant disease is difficult to estimate, but with over 3 million implants placed in the USA alone and the market growing by 500,000 implants/year, such extensive use demands additional interceptive approaches. The authors report a water-based, nonsurgical approach to address peri-implant disease using a bifunctional peptide film, which can be applied during initial implant placement and later reapplied to existing implants to reduce bacterial growth. Bifunctional peptides are based upon a titanium binding peptide (TiBP) optimally linked by a spacer peptide to an antimicrobial peptide (AMP). The authors show that dental implant surfaces covered with a bifunctional peptide film kill bacteria. Further, using a simple protocol for cleaning implant surfaces fouled by bacteria, the surface can be effectively recoated with TiBP–AMP to regain an antimicrobial state. Fouling, cleansing, and rebinding was confirmed for up to four cycles with minimal loss of binding efficacy. Rebinding after fouling of a water-based film extends control over the oral microbiome composition, providing a novel nonsurgical treatment for dental implants. Many proteins and peptides exhibit very interesting mechanical properties and are studied for applications in biomedicine and as sustainable engineering materials. This work encourages future contributions regarding the characterization of natural protein materials, synthesis efforts including synthetic biology approaches, and computer simulations or modeling of such materials across the relevant length scales.

Finally, the manuscript “Self-Assembled Recombinant Proteins on Metallic Nanoparticles as Bimodal Imaging Probes” by Esra Yuca and Candan Tamerler uses designer peptides to synthesize a nanomaterial with tailored, multimodal optical functionality. Such combination of multiple modalities lies at the center of the development of new methods for sensing and imaging that are required for comprehensive understanding of events at the molecular level. Various imaging modalities have been developed using metallic nanoparticles owing to their exceptional physical and chemical properties. Due to their localized surface plasmon resonance characteristics, gold and silver nanoparticles exhibit unique optoelectronic properties that are commonly used in biomedical sciences and engineering. Self-assembled monolayers or physical adsorption have previously been adapted to functionalize the surface of nanoparticles with biomolecules for targeted imaging. However, depending on differences among the functional groups used on the nanoparticle surface, wide variation in the displayed biomolecular property to recognize its target may result. In the last decade, the properties of inorganic binding peptides have been proven advantageous to assemble selective functional nano-entities or proteins onto the surface of nanoparticles. The authors explored formation of self-assembled hybrid metallic nanoarchitectures composed of gold and silver nanoparticles with fluorescent proteins, for use as bimodal imaging probes. They employed metal binding peptide-based assembly to self-assemble green fluorescence protein onto metallic substrates of various geometries. Assembly of the green fluorescent proteins, genetically engineered to incorporate gold- or silver-binding peptides onto metallic nanoparticles, resulted in the generation of hybrid bimodal imaging probes in a single step. Green fluorescent activity on gold and silver surfaces can be been monitored using both plasmonic and fluorescent signatures. The results demonstrate a novel bimodal imaging system that can be finely tuned with respect to nanoparticle size and protein concentration. The resulting hybrid probes may mitigate the limitation of depth penetration into biological tissues as well as providing high signal-to-noise ratio and sensitivity.

The following list summarizes the papers published under this topic of protein-based structural materials. To download any of the papers, follow the URL http://link.springer.com/journal/11837/71/4/page/1 to the table of contents page for the April 2019 issue (vol. 71, no. 4):

- "Nanofibrils as Building Blocks of Silk Fibers: Critical Review of the Experimental Evidence" by Qijue Wang and Hannes C. Schniepp.
- "Optimization of Gelatin–Potassium Phosphate Aqueous Two-Phase System for the Preparation of Hydrogel Microspheres" by Pelin Erkoc, Nihal Dogan, and Seda Kizilel.
- "Repeatedly Applied Peptide Film Kills Bacteria on Dental Implants" by Cate Wisdom, Casey C. Chen, Esra Yuca, Yan Zhou, Candan Tamerler, and Malcolm L. Snead.
- "Self-Assembled Recombinant Proteins on Metallic Nanoparticles as Bimodal Imaging Probes" by Esra Yuca and Candan Tamerler.

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