Stephen Strassburg, Kai Mayer and Thomas Scheibel*

Functionalization of biopolymer fibers with magnetic nanoparticles

Abstract: Hybrid fibers consisting of biopolymers and inorganic nanoparticles are receiving increasing attention due to their unique properties. Commonly, the nanoparticles are chosen for their intrinsic properties such as magnetic, thermal, or electrical conductivity. The biopolymer component of the hybrid fiber is chosen for its mechanical properties and ability to act as a scaffold or matrix for the nanoparticles. While there are many fiber-forming synthetic polymers, there has been a recent interest in replacing these systems with biopolymers due to their sustainability, biocompatibility, nontoxicity, and biodegradability. Fibers made from biopolymers have one additional benefit over synthetic polymers as they make good scaffolds for embedding nanoparticles without the need of any additional bonding agents. In particular, naturally occurring biopolymers such as proteins exhibit a myriad of interactions with nanoparticles, including ionic, H-bonding, covalent, Van der Waals, and electrostatic interactions. The diverse range of interactions between magnetic nanoparticles and biopolymers makes resulting hybrid fibers of particular interest as magnetic-responsive materials. Magnetically responsive hybrid biopolymer fibers have many features, including enhanced thermal stabilities, strong mechanical toughness, and perhaps most interestingly multifunctionality, allowing for a wide range of applications. These applications range from biosensing, filtration, UV shielding, antimicrobial, and medical applications, to name a few. Here, we review established hybrid fibers consisting of biopolymers and nanoparticles with a primary focus on biopolymers doped with magnetic nanoparticles and their various putative applications.

Keywords: electrospinning, magnetic nanoparticle, microfluidics, polysaccharide, silk, wet spinning

***Corresponding author: Thomas Scheibel**, Department of Biomaterials, Universität Bayreuth, Prof.-Rüdiger-Bormann-Straße 1, 95447 Bayreuth, Germany; Bayreuth Center for Colloids and Interfaces (BZKG), Universität Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany; Bayreuth Center for Molecular Biosciences (BZMB), Universität Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany; Bayreuth Center for Material Science (BayMAT), Universität Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany; and Bavarian Polymer Institute (BPI), Universität Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany, E-mail: Thomas.Scheibel@bm.uni-bayreuth.de. <https://orcid.org/0000-0002-0457-2423>

Stephen Strassburg and Kai Mayer, Department of Biomaterials, Universität Bayreuth, Prof.-Rüdiger-Bormann-Straße 1, 95447 Bayreuth, Germany

1 Introduction

Biopolymers are a family of macromolecules containing natural or recombinant proteins, polysaccharides, and synthetic biodegradable polymers (Figure 1). Characteristics of biopolymers may include biocompatibility, biodegradation, and renewability, which make these materials excellent candidates to be used in different fields of research and applications [1–5]. Despite this, synthetic polymers are simply easier and cheaper to mass produce and thus have been subsequently more commonly used as fiber materials [6]. Recently that trend appears to be changing as advances in protein and polysaccharide extraction as well as better methods of producing recombinant proteins have significantly dropped the cost of these biopolymers [7–10]. As environmental concerns grow and more renewable materials are necessary, there appears to be a growing shift from synthetic polymeric materials to renewable biopolymers [9].

Figure 1: (A) Overview of different biopolymers including synthetic (biodegradable) and naturally derived polymers. Biopolymers include polymers derived from natural materials or synthetic polymers that are biodegradable. Natural biopolymers include polysaccharides and proteins. An example of a biodegradable synthetic polymer, (poly(lactic acid)), a polysaccharide, (cellulose), and the protein eADF4(C16), a recombinant major ampullate silk derived from *Araneus diadematus* is shown on the bottom of (A). (B) Structural properties found in biopolymers. Synthetic polymers and polysaccharides often have multiple structural properties (i.e., semicrystallinity: amorphous and crystalline domains) as do proteins (helical and β -sheet conformations).

2 Biopolymer-based materials

Biopolymers are highly adaptable and offer more functionality than traditional synthetic polymers. For example, many proteins have evolved over billions of years to carry out a myriad of diverse tasks such as catalysis, molecular recognition, and the storage of energy or information. Chances are, if there is a material needed for a specific application, nature has already designed a protein or polysaccharide for the job. For example, spider silk fibers outperform most of their synthetic polymer counterparts in toughness [5, 11, 12]. Proteins such as hemoglobin and ferritin are excellent binders of metal ions such as iron. Ferritin has even been used as a scaffold for the synthesis of gold nanoparticles [13]. Chitin and cellulose are often used to make thermally stable films [1]. In addition, more recent studies have used cellulose for optically clear glasses and papers. [14, 15]. These are but a few examples and do not even begin to scratch the surface of the myriad of functionalities biopolymers can have. But that does not mean nature is perfect, since natural materials have evolved to fulfill multiple criteria. In fact, many of these systems can be improved for specific applications simply by adding synthetic materials, such as inorganic nanoparticles. Using biopolymers as scaffolds offers a diversity that cannot be matched by traditional synthetic polymer systems; and the addition of nanoparticles into biopolymer fibers allows a tunability of the material that would not otherwise be realized. These hybrid composites allow for antimicrobial, thermally, electrically, magnetically, and/or light-responsive materials with unmatched renewability, biocompatibility, and mechanical properties.

Despite the diversity of biopolymers there are still drawbacks, the main one being their processability. The difficulty with processability is different for proteins compared to polysaccharides. Typically, proteins are difficult to obtain in high yields due to recovery methods being costly and time-consuming, while polysaccharides tend to be insoluble, often requiring harsh organic solvents to fully dissolve. More recently, advances in protein recovery and unique variants of solvents (such as ionic liquids and deep eutectic solvents) for solubilizing polysaccharides have led to biopolymers becoming an increasingly more appealing option compared to traditional crude-oil-based polymers [8, 9, 16].

2.1 Polysaccharides for material's applications

The two most commonly studied and also the most abundant polysaccharides in nature are cellulose and chitin [17]. Chitin is derived from many crustaceans, insects, mollusks, and fish and is most commonly treated with a deacetylation process to form chitosan [2]. Conversely, cellulose typically is derived from plants, such as wood from trees, cotton, algae, and even can be secreted by bacteria, making it the most abundant biopolymer on Earth [17]. Both cellulose and chitosan can be processed into fibers,

films, and gels with good mechanical properties, and it should come as no surprise that they are common materials for use in bionanocomposites [1, 2, 15].

2.2 Proteins for material's applications

Many natural proteins derived from animal or plant organisms are used for producing fibers. Protein-based composite materials can be made from a wide range of differing proteins. Globular proteins such as bovine serum albumin, lysozyme, and cytochrome *c* are all used in conjunction with nanoparticles for drug delivery applications [18–20]. Conversely, many fibrous and structural proteins such as collagen, silk, and keratin are common materials for fibers, films, and gels used in material applications [1, 21–23]. The most abundant of these proteins examined in literature is collagen. Despite this abundance of literature, there are only a few examples of collagen nanoparticle composite materials examined and most of these studied systems use a collagen–Ag nanoparticle composite for antimicrobial applications [24, 25].

Natural silk fibers are produced by insects, arachnids, and even some crustaceans [26]. Silk is a well-known natural fiber used in textile industry and other applications. Specifically, silkworm silk has become a multibillion-dollar textile business, a long way from the initial silk textile beginnings several thousand years ago in China [27].

Most of the silk produced today comes from domesticated *Bombyx mori* silkworms [28]. While not as strong as dragline spider silk ($16 \times 10^4 \text{ J kg}^{-1}$), it is more abundant and easily usable for textiles [29]. There is a large portion of literature working with silkworm silk and nanoparticles. Much like collagen and other protein-based materials, much of the biopolymer–nanoparticle composite literature focuses on antimicrobial textiles and nonwoven meshes [30].

Figure 2: Schematic of a spider silk web from (A) *Araneus diadematus*, (B) the macroscopic structure of dragline spider silk, and (C) the microscopic molecular structure of the MAS protein dragline proteins. Crystalline regions are predominately β -sheets while the amorphous regions consist of α -helices and random coils. Originally published by Heidebrecht et al. [38] and slightly adapted with permissions.

A very interesting and highly complex proteinaceous material from nature is spider silk [34]. Spiders produce a variety of elaborate proteins among which major ampullate silks (MAS) are the basis for a fiber with outstanding mechanical properties combining a high tensile strength and moderate elasticity [22]. MAS fibers are used by spiders as a scaffold upon which to attach other silks during the creation of a web and as a lifeline when it is necessary to escape from predators. The combination of strength and stretchiness is mainly derived from the domains of crystalline β -sheets and flexible helices/amorphous regions within the polypeptide chain (Figure 2), imparting a toughness that is greater than that of bone, synthetic rubber, Kevlar, and high tensile steel or other man-made fibers [11, 12, 35–37].

3 Magnetic nanoparticles

Nanoparticles are small particles ranging between 1 and 100 nm in size and can form a variety of shapes such as, spherical, cubic, conical, rod-like, and many others [39, 40]. They can be made from organic, metallic, or ceramic molecules, and often their nanoscale properties differ from their bulk properties (even between materials of the same composition) [40]. Nanoparticles are of particular scientific relevance because of the diverse range of interactions they can experience. These interactions include: ionic, H-bonding, covalent, Van der Waals, and electrostatic interactions, which allow for a highly robust material that can be used for many applications [41].

Magnetic nanoparticles are a subset of nanoparticles that exhibit a magnetic response when exposed to a magnetic or electric field. Magnetically responsive materials may appear a niche topic when compared to nanoparticle research in anti-microbial, thermally, or electrically responsive biocomposites, but their application

Table 1: Examples of magnetic nanoparticles, their shape, and application.

Examples	Size	Shape	Application
Fe_3O_4 [50, 51]	12 nm– μm	Sphere, spindle, cube, rods octahedral,	Drug delivery, medical applications, thermal coatings
$\text{BaFe}_{12}\text{O}_{19}$ [52]	<100 nm	Sphere	Medical applications
$\text{SrFe}_{12}\text{O}_{19}$ [53]	30–70 nm	Sphere, spindle, cube, rods	High-frequency devices, permanent magnets
ZnFe_2O_4 [54, 55]	<100 nm	Sphere	Medical applications
CoFe_2O_4 [50]	8.5 nm	Sphere	Drug delivery
$\text{InCu}_2\text{Fe}_2\text{O}_4$ [56]	28–37 nm	Sphere, cube	Electronic applications
CuFe_2O_4 [56]	28–37 nm	Sphere, cube	Electronic applications
Ni/NiO [54, 57]	13 nm	Sphere	Medical applications
MnFe_2O_4 [50, 54]	<100 nm	Sphere, cube	Medical applications

introduces a diverse field of responsive materials where morphology and topology can be controlled via magnetic fields [42]. Furthermore, tunable morphology invites a diverse range of applications from membranes, to filtration, and even for biosensor and electronic applications [21, 45–49].

Typically, magnetic nanoparticles used for biocomposite materials are a variant of iron oxide [58–62]. However, nanoparticles of cobalt [47, 63, 64], platinum [65], manganese–zinc [54], and yttrium–iron [66] can also be used as magnetic materials. The myriad of differing magnetic nanoparticles not only offers diverse chemical interactions but also comes in a wide range of sizes and shapes. Table 1 shows the diversity of magnetic nanoparticle composition, size, shape, and examples of biocomposite material applications found in literature.

4 Nanoparticle-doped biopolymer fibers

4.1 Processing techniques

There are three main processing techniques for biopolymer–nanocomposite based fibers, wet spinning, electrospinning and microfluidics. Each technique produces fibrous materials but the size of the materials will vary depending on the spinning method. Choosing the correct technique will largely depend on the biopolymer–nanoparticle composite that is being examined as well as the final application for the material.

4.1.1 Wet spinning

The most commonly used method of producing biopolymer fibers with diameters in the micrometer or larger range is wet spinning [67]. Wet spinning is conducted by extruding an aqueous or organic biopolymer solution into a coagulation bath. Upon contact with the coagulation bath, the biopolymer precipitates into a continuous fiber while the solution solvent diffuses to the bulk of the coagulation bath (Figure 3A) [67]. The fiber can then be collected or post-treated/post-stretched in a continuous or batch process. The diameters of fibers produced by wet spinning can be controlled by the concentration of the spinning dope, needle diameter, and posttreatment [67]. Typical solvents used for coagulation baths of biopolymers are polar organic solvents such as methanol, ethanol, acetone, propanol, or isopropanol [68], as well as aqueous solutions with varying pHs, ionic strengths, and salt compositions [69].

Figure 3: Schematics of (A) wet spinning, (B) electrospinning, and (C) microfluidics. The green color represents the biopolymer, the black dots represent nanoparticles, and the orange/brownish color represents coagulation bath or solvent. Wet spinning produces larger fiber diameters than both electrospinning and microfluidics with electrospinning producing the smallest fibers. Wet spinning and microfluidics use a coagulation bath that precipitates the dissolved biopolymer while the solvent in format mismatch! electrospinning is dried during the stretching and whipping process on the way to the collector plate.

4.1.2 Electrospinning

Electrospinning is an electrostatically driven process allowing for the production of fibers with diameters ranging from micrometers down to a few nanometers [70]. The basic setup comprises a needle, which is charged ($\pm 0\text{--}30\text{ kV}$) and through which a biopolymer solution is extruded. [71] The high electric field induces electrostatic repulsion at the surface of an extruded droplet and leads to the formation of a Taylor cone through coulombic forces (Figure 3B). When the electrostatic forces overcome the solvent surface tension and the chain entanglement in the biopolymer solution is sufficiently high, a droplet is formed into a continuous jet [4]. At a certain distance from the Taylor cone, the jet experiences whipping instabilities and is stretched and dries on its path to the oppositely charged collector plate [72]. Fibers are “randomly” deposited on the collector plate as a nonwoven mesh. The diameters of fibers produced

by electrospinning can be well controlled, typically in the micro- or nanometer range, by adaption of the spinning dope properties (biopolymer concentration, viscosity, conductivity, solution volatility, and surface tension of the solvent) and process parameters (voltage, distance to deposition plate, flow rate, temperature, humidity) [4]. Fibers produced via electrospinning tend to have smaller diameters than those produced through wet spinning [73].

4.1.3 Microfluidics

Microfluidics, while not as well studied as wet spinning or electrospinning, is an increasingly common method of fiber formation explored in literature [74]. Similar to wet spinning, microfluidic spinning allows for decreasing the scale of the input and coagulation solvent feeds (typically in the submilliliter range). The smaller size of the inlet streams leads to precise control of the fluid dynamics as well as the introduction of capillary forces that are often ignored in traditional wet spinning. Precise control offers advantages including small volumes of solvents, small size of the spinning set up, narrower fiber diameters than wet spinning and low energy consumption but with the trade-off of lower fiber yield [75]. There are many types of microfluidic setups including both batch and continuous flow processes [74, 76]. An example of one such batch setup is shown in Figure 3C.

4.2 Examples of nanoparticle-doped fibers

There is a growing literature on nanoparticle fiber doping using noble metals. Using biopolymers as scaffolds for the growth or adhesion of noble metal nanoparticles offers many advantages over traditional synthetic polymers. One unique consideration for interactions with metal nanoparticles is the structure of the protein or polysaccharide. Proteins in particular, with their diverse amino acid sequences, can adopt different secondary structures, such as random coil, β -sheet, or α -helix, which have been shown to have a major effect on the capture and release of noble metal nanoparticles [77]. This consideration leads to a finely tunable system but also adds an additional layer of complexity to the material production.

Work with *B. mori* silk fibers by Calamak et al. and *Pholcus phalangioides* spider silk by Singh et al. are two excellent examples of the effect protein structure has on biocomposite materials [43, 77]. In the first case, Calamak et al. examined the release profiles of Ag nanoparticles when embedded in *B. mori* silk scaffolds consisting of either a random coil or β -sheet conformation. The results showed a clear distinction in the release profiles, with the crystalline β -sheet structure showing a cumulative lower release rate [77]. Similarly, Singh et al. used spider silk, harvested from webs of *P. phalangioides* spiders, as a scaffold for growth of gold nanoparticles, which could later be used for vapor detection via changes in

conductivity. Not only did the gold nanoparticles grown in this manner showed a rapid response time in conductivity on exposure to methanol, the nanoparticles showed excellent cycling efficiency indicating a strong binding to the silk fiber, likely a result of the β -sheet conformation of the spider silk protein [43]. Similarly, Tang et al. saw similar strong adhesion of Ag nanoparticles to commercial silk fabrics that could withstand many washing cycles [78]. Thus it appears for applications where nanoparticle–protein adhesion is important, β -sheet conformation is desired; while for applications where nanoparticle release is important, random coil or helix conformations are more suitable.

A subset of nanoparticle hybrid fibers, using magnetic nanoparticles, are of growing interest because of their magnetically responsive behavior allowing their use in many applications such as in medical devices [1], as antimicrobial surfaces [79], or to exploit their advantageous thermal and electrical properties [48, 82–86]. Nevertheless, the design and modification of these responsive materials are dependent upon the biopolymer and nanoparticle interactions. Often that requires choosing the correct biopolymer and nanoparticle combination and there are several to choose from [15, 87]. In some cases, modification of the biopolymer can be involved, which can be a nontrivial process. Even after an adequate system is chosen, a correct solvent and spinning method may be difficult to find. Still, the rewards for finding a system that successfully fits all of the criteria necessary to obtain biocomposite hybrid materials are vast, and there is an increasing amount of literature discussing magnetic nanoparticle–biopolymer interactions and processing techniques [11, 42, 44, 47, 58, 60, 64, 66, 88, 89].

One such reward involves a lesser known characteristic of metallic nanoparticles, their ability to absorb light, specifically, they have remarkable UV shielding. This is well known in the sunscreen industry where nanoparticles are readily used as additives [90]. Using similar nanoparticles as in sun screen, such as TiO_2 and CeO_2 , several studies have shown enhanced UV shielding could be applied to silk textile fibers [31, 32]. In addition, not only did the TiO_2 and CeO_2 nanoparticles show remarkable adhesion to the silk fabrics tested, they also provided antibacterial capabilities and good thermal stabilities. Of particular interest, Li et al. reported a strong correlation in the decrease of transmitted UV light with increased TiO_2 content. Equally interesting, upon exposure to UV illumination, a photolytic self-cleaning was observed for the TiO_2 -loaded silk fabrics [31].

The plethora of magnetic nanoparticle characteristics, ranging from thermal stability to electrical and magnetic responsiveness to UV shielding and antimicrobial properties, make magnetic nanoparticle biocomposites a rich field for exploring many different applications. These applications of magnetic nanoparticle biocomposites include medical devices, membranes, biosensors, and even bioseparations and filtration. Several interesting applications for nanoparticle–biopolymer composite systems are examined in section 6.

5 Biopolymer–nanoparticle composite properties

5.1 Morphology

Homogeneity of the fiber, density and distribution of nanoparticles, uniformity of fiber diameters, surface quality, and spinning artifacts are but a few of the important morphological features that are necessary to examine. There are different techniques for examination of these properties including: scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), and atomic force microscopy (AFM), to name a few of the more commonly used techniques.

For evaluating surface morphology and interfaces of hybrid fibers, SEM and AFM are the most popular techniques [91, 92]. Briefly, SEM uses back-scattered electrons to detect differences in electron densities [91]. The combination of high magnification, large depth of focus, great resolution, ease of sample observation, and high contrast between metal nanoparticles and biopolymers makes SEM a ubiquitous characterization technique in the field of biopolymer–nanoparticle fibers. Most, if not all, of the different applications examined in section 6 use SEM to characterize the presence of nanoparticles and examine the interface between biopolymer and nanoparticle. While a qualitative technique, the addition of an EDS detector allows for the probing of elemental information that is not apparent in the SEM image [91]. EDS exploits the unique interactions between elemental atomic structure and their emission of X-rays. Since each element has a unique atomic structure, the emitted X-rays likewise have a unique energy spectra allowing for precise determination of elemental makeup of the surface [91]. EDS is particularly useful for distinguishing between nanoparticles (higher energy emissions) and biopolymers (lower energy emissions) and can help to elucidate nanoparticle density and distribution [91].

The less used, but more powerful technique, AFM, allows the user to examine many aspects of the surface of the hybrid material that could not otherwise be noticed in SEM [92]. AFM uses a force sensitive cantilever to tap or drag on the surface of the fiber creating a 2D surface map that is sensitive to the surface roughness, stiffness, and phase (delayed force response). As an example, recent work by Carapeto et al. used AFM to examine films of 1,6-hexanediamine-*graft*-cellulose doped with Ag nanoparticles [93]. They examined the surface of the hybrid film using height, amplitude, and phase images of the material. There they found no large aggregates and a random distribution of the Ag nanoparticles, which could have also been observed in SEM. However, what makes this work particularly interesting is by combining information on the three surface images, and estimating the shape of the nanoparticles to be a sphere, the authors were capable of quantifying the volume of each individual nanoparticle as well as the total volume fraction of the nanoparticles at the surface of

the material [93]. Using a quartz crystal microbalance as a comparison, the quantitative estimations were only ~7% different [93].

Perhaps the most powerful technique, but also the most difficult to work with is TEM. Unlike SEM, TEM requires electrons passing through the material (thus samples are required to be quite thin: 1–100 nm in thickness). Electrons passing through the sample experience a slight loss of energy, which can arise from differences in thickness, density, atomic element, crystal structure, and/or orientation [94]. TEM can be used for many diverse characterization methods. Uses of TEM include determining particle size or examining the cross-sectional area of fibers [77, 79], probing fibril formation [4, 95, 96], and examining electron diffraction patterns, to name a few. The main advantage of using TEM is the ability to probe the bulk of the material that the aforementioned techniques cannot replicate.

5.2 Thermal properties

Thermal gravimetric analysis (TGA) and dynamic scanning calorimetry (DSC) are the two most common methods of thermal analysis [97]. TGA measures the change (loss) of mass of a sample with increasing temperature. DSC examines the change in heat capacities of a sample compared with that of a control (air) at varying temperatures.

Figure 4: TGA schematic curves of a pure biopolymer (short dashed line), pure magnetic nanoparticle (long dashed line), and magnetic nanoparticle-doped biopolymer (solid line). The biopolymer doped with magnetic nanoparticles exhibits enhanced thermal properties compared to that of the pure biopolymer. The difference of mass loss at very high temperature (shown by double arrow) is directly proportional to the concentration of nanoparticles in the doped fiber.

Biopolymer fibers with and without magnetic nanoparticles demonstrate a different behavior under varying thermal conditions. Unlike mechanical properties, increasing the concentration of magnetic nanoparticles has often been reported in literature as increasing the thermal stability of the material [32, 33, 77, 78, 98, 99]. A schematic of a TGA plot including pure nanoparticles, pure biopolymers, and nanoparticle–biocomposite hybrid fibers is shown in Figure 4. The pure biopolymer fibers lose mass at a much lower temperature than pure nanoparticles. When a composite material of nanoparticles and biopolymers is produced, the composite material loses mass at a temperature and rate somewhere in the middle that of pure biopolymer and pure nanoparticles [100].

DSC can characterize the material glass transition temperature, crystallinity, crystallization/melting temperatures, and transitional enthalpies. Magnetic nanoparticles can influence many of these transitions. As mentioned earlier, Calamak et al. found that increasing the crystallinity (β -sheet) of silk fibers lowers the total cumulative Ag release compared to amorphous (random coil) fibers [77]. The authors also found that the thermal degradation temperature increased slightly with the addition of Ag nanoparticles and was higher for the crystalline structures as opposed to the amorphous fibers. Only the amorphous fibers showed a glass transition (190 °C) [77]. These results show that understanding the different thermal transitions in DSC is an important factor in designing nanoparticle–biopolymer composite materials.

5.3 Mechanical properties

Mechanical properties describe the physical properties of a material under the application of a force (i.e. resistance to shear or strain). Examples of mechanical properties include the modulus of elasticity, tensile strength, elongation at break, hardness, fatigue limit, and toughness. Biopolymers are known to have a wide range of mechanical properties. For example, natural dragline spider silk has extraordinary mechanical properties due to a combination of strength and extensibility [22, 101]. Using biopolymers, such as dragline spider silk, for their strong mechanical properties is an appealing prospect for many applications, but there is no guarantee that after the addition of magnetic nanoparticles the extraordinary mechanical properties would remain.

Work by Mayes et al. examined how the mechanical properties from dragline spider silk from *Nephila edulis* behaved after the addition of magnetite nanoparticles [11]. Magnetite nanoparticles were added via dipping fibers in colloidal suspension of FeCl_3 for 30 min followed by air-drying overnight. The authors found that the dragline silk retained its flexibility and its toughness even after the addition of magnetic nanoparticles. Hysteresis cycles were performed at ambient temperature and humidity using a custom-built stress–strain gauge. The fact that there was no discernible difference between unmodified spider silk (no nanoparticles added) and magnetite-doped silk

fibers even after repeated loading and unloading of the fibers was a promising sign that nanoparticles situated on a fiber's surface did not adversely affect the mechanical properties of the biopolymer fibers [11]. However, this is not always the case. Work by Li et al. examined cellulose fibers soaked in an AgNO_3 aqueous solution at 80 °C for 24 h. The cellulose–AG nanocomposite fibers showed a decrease in stiffness and elasticity with increasing wt% of Ag NP [99]. Another consideration when doping biopolymers with magnetic nanoparticles is the minimum amount of magnetic ingredients in the composite fiber to obtain a magnetic effect. Zhou et al. showed that the strength of a wet-spun single-walled carbon nanotube–permalloy nanoparticle–poly(vinyl alcohol) (PVA) fiber exhibited a failure strength of around 0.7 GPa when at saturation load of magnetic nanoparticle. This failure strength was decreased significantly from ~25 GPa for the pure single-walled carbon nanotube–PVA mixture without the permalloy MNP [102]. Though the work by Zhou did not use a biopolymer, it highlights an important trade-off between mechanical strength and magnetic properties that must be considered when designing magnetically responsive fibers.

5.4 Magnetic properties

Magnetic properties of hybrid fibers can be tested using different physical methods, though all experimental setups feature an external magnetic field, which elicits a reaction of the magnetic material. The simplest test of the magnetic behavior is the manual detection of the influence of an external magnet on the sample. More advanced techniques such as a vibrating sample magnetometer (VSM) and superconducting quantum interference device (SQUID) are far more sensitive and capable of detecting minute changes in magnetic fields [103–105].

It is rare in literature that more advanced setups are used for magnetic responsiveness characterization. One such example is work by Munaweera et al., which used yttrium iron garnet nanoparticles embedded in cellulose acetate nonwovens (more information further) [66]. Before embedding into the nonwoven material, the yttrium iron garnet nanoparticle powder was repeatedly cycled using the SQUID to saturation magnetization at room temperature. The authors found that no magnetic hysteresis was observed, but the particle size had a strong effect on the saturation magnetization [66].

6 Applications

6.1 Antimicrobial

The most abundant use of biopolymer–nanocomposite materials is for antimicrobial applications. Silver nanoparticles are the most commonly used ones due to the natural

Figure 5: (A) Antimicrobial effects of uncoated and CeO₂ nanoparticle-loaded silk fabrics on *E. coli* and *S. aureus* cultured in LB medium under constant shaking at 37°C overnight. The media was then exposed to 2 mm × 2 mm squares of untreated and CeO₂-loaded silk fabrics for 2 h. The contents were plated onto agar plates and cultured for another 24 h. The CeO₂-treated solutions indicated a significant decrease in bacterial colony survival compared to the control (blank) and the untreated silk fabrics. Adapted with permissions from Lu et al. [32]. (B) SEM image of the yttrium iron garnet nanoparticle-loaded cellulose nonwoven mesh attached to cellulose filter paper. The electrospun nonwoven retains its magnetic responsiveness even after attachment to the cellulose filter paper. (C) Images of unwashed and washed filter papers from (B). The unwashed filter paper shows a strong fluorescence when exposed to UV irradiation at 245 nm indicating that the FITC-BSA has been successfully bound to the filter paper. Upon washing with deionized water, the fluorescence disappears indicating that the FITC-BSA has been removed. (B) and (C) were reproduced with permissions from Munaweera et al. [66].

antimicrobial characteristics of the silver ions, but the use of the biopolymer can vary. Examples include: silk (spider or silk worm) [33, 78, 80], cotton [106], cellulose [81, 99, 107], chitosan [108, 109], and even biocompatible synthetic fibers [110, 111].

Silver nanoparticles are generally either embedded into the biopolymer fiber or grown on the fiber itself regardless of the biopolymer in use. Differing approaches can be used for the embedding of particles. Lu et al. successfully developed an efficient green method for attaching Ag nanoparticles to polydopamine-functionalized silk that resulted in a uniform distribution [112]. The strong absorption to the polydopamine-functionalized silk was induced by using a reducing agent. This process is not unique to silk either. A similar method was developed by Chen et al. They demonstrated that using cotton fibers grafted with glycidyl-methacrylate–iminodiacetic acid could strongly adsorb and bind Ag nanoparticles [106]. For both of the aforementioned cases, the nanoparticles were attached via metal coordination bonds, but Ag nanoparticles can also be attached covalently [31]; a process that is especially useful for protein–Au nanocomposites because of the high affinity for Au nanoparticles to bind to cysteine amino acid residues in many proteins [113].

Alternative to embedding, nanoparticles can be grown onto the fiber. Both Lu et al. and Chang et al. successfully grew Ag nanoparticles on silk fibers via UV- or γ -irradiation, respectively [33, 80]. For either of the aforementioned cases, the silk fibers were soaked in AgNO_3 solution and irradiated for at least 1 h, resulting in uniformly distributed Ag nanoparticles on the fiber surface. The process is highly tunable allowing for the density and size of the nanoparticles to be controlled by the initial concentration of AgNO_3 in the starting solution and irradiation time. Furthermore, these nanoparticles were strongly adhered to the surface and showed strong stability even after repeated washing cycles [33, 80]. In addition to their work with Ag nanoparticles, Lu et al. also showed similar antimicrobial results using CeO_2 nanoparticles immobilized on the surface of silk fabrics [32]. These fabrics showed strong antimicrobial behavior to *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *B. subtilis* (Figure 5A). Perhaps equally interesting, these fabrics also exhibited excellent UV-shielding properties, paving the way for multifunctional biopolymer–nanocomposite fibers.

Regardless of how nanoparticles are attached, their main purpose is to release ions to prevent bacterial growth on the fiber and/or destroy bacteria. The required strength of the adhesion of nanoparticles will vary depending on the application, but this is also what makes the biopolymer–nanocomposite fiber so robust. Take, for example, the work by Xu et al. with Ag–poly(lactic acid) nanocomposite fibers [110]. Their work showed a steady release of Ag nanoparticles over time indicating weak adhesion to the poly(lactic acid) fiber. The fibers did show very strong antimicrobial characteristics against *S. aureus* and *E. coli* (as high as 94–98% prevention of bacterial growth) for up to 20 days when even the poly(lactic acid) fiber degraded [110]. A short-term biodegradable nanofiber composite would be particularly useful in wound dressings or as a skin/surface drug delivery system. In contrast, examining work by He et al.,

development of Ag nanoparticles synthesized in porous cellulose was found to be especially stable over a much longer time period [107]. In addition, work by He et al. showed a remarkable antimicrobial effect on to *E. coli*, *S. aureus*, *Penicillium glaucum*, and *Saccharomyces cerevisiae*. This long-term stability is useful for applications involving implants or biological scaffolds. Simply by tuning the strength of adhesion between biopolymer and nanoparticle, a plethora of different applications can be found.

6.2 Magnetically responsive materials

Cellulose is a common polysaccharide used for magnetic-responsive materials. Notably, Biliuta et al. examined cellulose fibers doped with Fe_3O_4 nanoparticles (both uncoated and coated with oleic acid) using 2,2,6,6-(tetramethylpiperidin-1-yl)oxyl (TEMPO) as an oxidation method for attaching the nanoparticles to the fibers [88]. The authors found that oxidation of the fibers increased the activity and attachment of the magnetic nanoparticles even in the absence of the oleic acid coating, which prior to this discovery was often deemed necessary for attaching nanoparticles to cellulose fibers. This methodology indicates a robust method of attachment of magnetic nanoparticles without the need for stabilizers such as oleic acid. While the attachment density of noncoated Fe_3O_4 nanoparticles was lower than those of the coated particles, the uncoated particles showed a much higher magnetization value [88], thus paving the way forward for new methods of nanoparticle attachment in the absence of a stabilizer.

As another example of cellulose-based hybrid materials, cellulose acetate fibers were doped with yttrium iron garnet nanoparticles [66]. Munaweera et al. electrospun novel magnetic nonwoven mats using cellulose acetate and yttrium iron garnet nanoparticles. Nonwovens produced in this manner showed a magnetic response and could easily be picked up by an external magnet. Interestingly, when electrospun onto cellulose filter paper (Figure 5B), the mats retained their magnetic responsiveness and were capable of filtering fluorescently labeled bovine serum albumin protein from aqueous carbonate solutions (Figure 5C). These filter papers were found to separate from 0.0039 to 0.0069 μmol of protein per 1 cm^2 of filter paper. Furthermore, bovine serum albumin could then be recovered by washing the magnetic filter paper with deionized water [66]. While the amount of protein recovered may be small, this example highlights the more promising applications for magnetically responsive biopolymer meshes.

In a similar study using chitosan, the reverse effect was elucidated. Electrospun cross-linked chitosan nanofibers were added with Fe_3O_4 for targeted protein release. Bovine serum albumin was again used as a model protein, this time for drug delivery, and the release rate was found to be controllable by varying the cross-link density (by increasing the molarity of the cross-linker) and magnetic stimulation [60]. What is interesting is that both of these examples show opposite effects by changing the

magnetic nanoparticle chemistry, thus indicating a potential application in magnetically assisted bioseparations and/or drug delivery simply by choosing a specific nanoparticle for the necessary application.

For protein hybrid materials, there appears to be only one recent example of magnetic nanoparticles used as composite materials in conjunction with collagen fibers. Therein, cross-linked collagen fibers were embedded with oleic-acid-coated iron oxide nanoparticles. These embedded fibers were found to be only weakly magnetic but remained stable in organic solutions of heptane. Perhaps most interesting, the collagen–iron oxide nanocomposite fiber was capable of rapid, efficient absorption of used motor oil from water [58], thus making these materials a potentially promising new absorption material for applications in environmental protection and cleanup industries. Aside from collagen, hemolysin protein derived from *P. aeruginosa* was used as a glue to connect $\text{Co}_2\text{Fe}_2\text{O}_4$ nanoparticles. These materials could later be lyophilized from solution and formed a fiber-like structure that aligned when exposed to a magnetic field [47, 64].

Silk-based proteins from silkworms and spiders also show scant literature (though more than most other proteins) [11, 42, 44, 89]. A recent publication by Zhou et al. reported that Fe_3O_4 nanoparticles could be embedded in silk fabrics through a swelling-fixing method [44]. The authors reported that these fabrics showed a decrease in crystallinity after nanoparticle embedding but their thermal and mechanical stability remained unaffected. Furthermore, the composite fabrics showed good magnetic responsiveness but the responsiveness decreased by ~15% after several washing cycles [44]. Despite the decrease in magnetic responsiveness after washing, the authors believe this was a good first step in determining a facile method of functionalizing silk textiles with magnetic nanoparticles.

A more commonly examined protein–nanoparticle biocomposite fiber found in literature is spider silk. Recombinant spider silk makes an ideal material for use with hybrid magnetically responsive nanoparticles, but only recently has a recombinant spider silk fiber been produced with a toughness matching that of naturally spun spider silk [22, 101]. It should come then as no surprise that after this breakthrough, the literature of spider silk–nanoparticle biocomposites has begun to grow. One of the more recent examples includes work by Liu et al. where they report a facile, environmentally friendly method to prepare Ag- Fe_3O_4 silk fiber nanocomposites with high antimicrobial activities against both *E. coli* and *S. aureus*. These materials also appear to be responsive at relatively low magnetic strengths (actuated with a small household magnet) [42]. What makes this work of particular interest is the ability to “regenerate” or recycle the released antimicrobial Ag ions. The authors suggest that due to the synergistic nature of the Ag- Fe_3O_4 nanoparticles, ions of Ag released upon contact with bacteria are later reabsorbed by the remaining Fe_3O_4 nanoparticles allowing for a regenerative material that is also magnetically responsive [42].

Similar work with the goal of environmentally friendly composite materials is ongoing within our group. A recent, 2018 paper by Herold et al. examined Au

nanoparticle coupling with recombinant spider silk proteins for use in sustainable hydrogen production [114]. The authors demonstrated that spider silk with Au or TiO_2 binding moieties could be processed into films. These films could then be used for light-induced hydrolysis of water to produce H_2 gas [114]. Not only are these films responsive to external stimuli (in this case light) but also use materials that have been shown in literature to be antimicrobial (a topic touched upon previously). These results mark a seminal first step in the production of biopolymer–nanoparticle composite materials and have spawned several new projects examining different methods of producing biopolymer–nanoparticle composites.

Many of the other examinations of silk–magnetic nanoparticle biocomposites in literature still use natural spider silk fibers, typically obtained from the webs of spiders in nature or harvested from spiders themselves. Perhaps one of the earliest example of spider silk–magnetic biocomposites is work with MAS from *Nephila edulis* and superparamagnetic magnetite (Fe_3O_4) nanoparticles. The inaugural work indicated that spider silk fibers could be easily coated with magnetic (and nonmagnetic) nanoparticles via dip coating the fibers in a colloidal solution of nanoparticles. Furthermore, these silk fibers retained their mechanical toughness and elasticity even after addition of magnetite particles to the fiber scaffold. However, it was also noted that the coating of Fe_3O_4 nanoparticles was susceptible to delamination upon mechanical flexing, which may explain why there is no adverse effect to the mechanical properties of the dragline spider silk fiber [11]. While techniques and methodology for nanoparticle attachment have improved over the past 20 years, this remains a seminal starting point to magnetic nanoparticle biocomposites.

More advanced methods of attaching magnetic nanoparticles to natural spider silk are demonstrated in the work by Singh et al., which used spider silk webs from *Crossopriza lyoni* and Fe_3O_4 nanoparticles [89]. Their work is unique in that it uses ionic liquids and deep eutectic solvents for the dispersion of spider silk and magnetic nanoparticles. The dispersions of silk and magnetic nanoparticles indicated nanoscale structural distribution and rapid attachment of the Fe_3O_4 nanoparticles. Regenerated fibers from these dispersions did not appear to inhibit the growth of mammalian cells *in vitro* and appear to show antimicrobial activity against *E. coli*, *Pseudomonas stutzeri*, and *Bacillus licheniformis* [89].

Similar, ongoing work by Grill et al. successfully electrospun meshes of recombinant spider silk protein (derived from *Araneus diadematus*, see also Figure 1A) in a hexafluoroisopropanol, HFIP, solution (Figure 6A) and successfully attached Au-coated magnetic nanoparticles to the fiber mesh via dip coating (Figure 6B). A high affinity between the recombinant spider silk and the Au magnetic nanoparticles was observed in the fiber meshes. Unfortunately, the low solubility of the Au nanoparticles in the HFIP dope solution prevented electrospinning from a single dope solution. In an effort to circumvent this low solubility, other functionalized magnetic nanoparticles with various shapes and sizes were explored. These included but are not limited to: hematite iron oxide spindles synthesized by the Wagner group (University of Rostock), biophosphate-coated

Figure 6: SEM images of electrospun fiber meshes of recombinant spider silk (derived from *Araneus diadematus*) (A) without Au-coated magnetic nanoparticles and (B) with Au-coated magnetic nanoparticles following dip coating. The bright clusters located on the fiber mesh indicate high affinity between the recombinant spider silk and the gold-coated magnetic nanoparticles. Because the Au nanoparticles were insoluble in the HFIP solutions, attempts at improving solubility were tested with other coated nanoparticles. The SEM images of electrospun fiber meshes of recombinant spider silk (C) with hematite iron oxide spindles indicate a somewhat successful single pot production of nanoparticle-doped spider silk meshes. Building upon these results a recombinant spider silk mesh was applied to a chitosan film. The resulting film indicated no loss of rollability after the mesh was applied (D). The inset of (D) indicates that the electrospun spider silk mesh is present on the inner layer of the chitosan roll, but it is not possible at this magnification to determine the presence of nanoparticles.

magnetite spheres synthesized by the Dutz group (University of Ilmenau), and gelatin-coated nickel magnetic rods synthesized by the Tschöpe group (University of Saarland). Each nanoparticle was electrospun into a magnetic mesh to varying degrees of success. The hematite iron oxide spindles did cluster on the spider silk mesh but only sparsely and as large aggregates (Figure 6C). This is likely due to the low solubility of the hematite iron oxide in the HFIP solutions. The biophosphate-coated magnetite and gelatin-coated nickel magnetic nanoparticles did show higher solubility in the dope solution but did not adhere to fiber meshes. While not completely solving the issues of solubility, a magnetic mesh from a single dope solution did allow for applications with previous materials developed by Aigner et al. [115]. Using “rollable” chitosan films previously described in literature, spider silk–magnetic nanoparticle meshes were successfully electrospun onto the surface of chitosan films (Figure 6D) though it is unclear from the SEM images if the nanoparticles remain adhered after rolling. The thickness of the mesh varied depending

on the spinning time, which ranged from 30 s to 7 min. Surprisingly, the mesh thickness did not have an effect on the rolling ability of the chitosan films, thus providing a potential posttreatment method for producing magnetically responsive materials without sacrificing the initial material elasticity.

It is important to note that the aforementioned examples are the first few of a rapidly growing field. Whether it be for use as therapeutic, structural, or many of the other applications, spider silk–nanoparticle biocomposites are quickly becoming an interesting case study for magnetic-responsive materials.

7 Summary and future directions

In addition to antimicrobial properties, nanoparticles offer many other benefits in combination with biopolymer fibers, most notably their thermal absorption characteristics. In fact, many of the mentioned examples not only showed enhanced antimicrobial or magnetic properties of the biopolymer–nanocomposite fibers, but also the thermal stability was significantly increased in the presence of metal nanoparticles [32, 33, 77, 78, 98, 99]. In addition to high thermal stabilities, many nanoparticles have a wide range of dielectrics. This makes them appealing materials for use in organic electronics. Recently, work by Deshmukh et al. has examined SiO₂ nanoparticle reinforced poly(vinyl alcohol) and poly(vinyl pyrrolidone) blends for use in flexible organic electronics [49], adding to the growing list of uses for biopolymer–nanoparticle composites.

Due to the many advantageous properties of biopolymer nanocomposites, it should come as no surprise that addition of nanoparticles brings with it multiple benefits. These benefits allow for a variety of applications into many different fields, some of which yield surprising results. A niche but growing application for nanoparticles is their using in catalytic systems. As an example, Ouyang et al. created a hollow polyethersulfone fiber with Au nanoparticles embedded inside the hollow fiber for use in catalysis reactions [116]. Though polyethersulfone is not a biopolymer, similar work has been examined using biopolymers and nanoparticles. In addition to work on antimicrobial fibers, both He et al. and Xu et al. examined attached metallic nanoparticles as catalytic reaction sites [107, 110]. The embedded Au nanoparticles could then act as a catalytic reduction site for 4-nitrophenol when soaked in a solution of NaBH₄. More recently, Marks et al. and Kamal et al. have developed systems of cellulose fibers embedded with metal nanoparticles for the removal of contaminants in water [98, 117]. For the former case, Marks et al. used the weakly magnetic Pd nanoparticles embedded in hollow cellulose fibers, which later were added into a hydrogel membrane as channels for water treatment applications [117]. The latter case, Kamal et al. successfully embedded stabilized ferromagnetic Co nanoparticles in bacterial cellulose, which showed a very high affinity for test pollutants, 2,6-dinitrophenol, and methylene blue dye. These nanoparticles embedded in bacterial cellulose fibers

continued to be very stable after five catalytic cycles, showing no significant difference in performance after each cycle [98].

Responsive materials, purification, filtration, antimicrobial properties, and bioreactors are but a few of the growing applications for magnetically doped biopolymer composites. The applications for magnetically responsive biocomposite materials will only grow as we learn more about the interaction between biopolymers and nanoparticles. Given the fact that many nanoparticles (and biopolymers) have multiple desired material properties, it is likely that literature will start to see more multipurpose materials. With an increased emphasis on biocompatibility, recyclability, and “green” environmentally friendly materials, we expect to see a continued shift away from synthetic polymer materials. As we have described in this review article, while still early in development, magnetically responsive biocomposite materials can be used for many applications, and the future looks very promising for their continued study.

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