Next Materials 9 (2025) 101289

Contents lists available at ScienceDirect

Next Materials

journal homepage: www.sciencedirect.com/journal/next-materials

Review article

Exploring the chemical reactivity and functionalization of sericin for advanced applications

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ARTICLE INFO

Keywords:
Sericin functionalization
Sericin industrial application
Bio-based materials
Covalent grafting
Covalent crosslinking

ABSTRACT

Sericin, a natural protein derived from silk, possesses highly desirable properties such as biocompatibility, biodegradability, antioxidant activity, and UV resistance. These attributes position sericin as a versatile material with potential across various fields, including biomedicine, textiles, cosmetics, and food packaging. However, its widespread adoption is currently hindered by inherent limitations, specifically high-water solubility and weak mechanical properties. To overcome these challenges, chemical derivatization strategies have emerged as a widely adopted approach to enhance and tune sericin's properties, thereby expanding its applicability. This review specifically focuses on covalent derivatization techniques, including crosslinking and grafting. By strategically leveraging its rich amino acid composition, sericin can undergo various chemical reactions to significantly improve its physical and functional characteristics. Investigations into crosslinking methods have shown particular promise in boosting its mechanical performances, leading to the development of advanced bio-based materials with precisely tailored functionalities. Despite extensive research into sericin's diverse applications, a comprehensive understanding and full resolution of its intrinsic limitations through derivatization remains an area requiring further exploration. This review aims to provide insights into sericin modification, underscoring its pivotal role in the development of sustainable materials, which unlocks new opportunities in engineering, materials science, and environmental applications, thereby setting the stage for future research and innovation in this promising field.

1. Introduction

Silk possesses desirable textile properties, strength, elasticity, coolness, softness, and dye affinity, but these are achieved only through human processing, including extraction, cleaning, twisting, and dyeing of fibers obtained from silkworm cocoons, most commonly Bombyx mori. These activities have a long history that dates back to the 27th century BC in China and spread throughout the Far East and Middle East, eventually arriving in Europe around the 6th century AD [1]. Although the history of silk lies beyond the scope of this review, it is important to note that silk production is deeply intertwined with human culture, and the procedures for obtaining threads and fabrics are well established. The initial steps include: (i) extracting continuous filaments from cocoons, (ii) performing raw silk quality control, and (iii) producing yarn and fabrics. At each stage, by-products are generated, most of which are discarded as discontinuous waste fibers composed of fibroin and sericin. Silk fibers are composed mainly of fibroin, a fibrous protein that accounts for 70–80 % [2] of the fiber, whereas the remaining part is composed of a glue-like protein, sericin, accounting for the 20–30 %, with some waxes, carbohydrates, pigments, and mineral salts. Unlike fibroin, sericin is water-soluble due to its high content of polar amino acids, exceeding 50 %. Sericin forms a layered coating around fibroin, which can be divided into inner, middle, and outer layers based on their solubility in water. From the inner fibroin core to the outer layers, hydrophobic amino acids decrease while hydrophilic ones increase, making the outermost layer the most water-soluble. Sericin imparts a dull color to silk and hinders carding, combing, spinning, and dyeing; therefore, it must be removed early in processing, before dyeing raw silk. In the initial phase of silk filament production, cocoons are boiled or steamed; this softens the natural protein sericin, which holds the fiber together in the cocoon. At this point, the softened cocoon is placed in water, allowing the silk fiber to be unraveled by locating the loose end of

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https://doi.org/10.1016/j.nxmate.2025.101289
Received 17 June 2025; Received in revised form 19 September 2025; Accepted 30 September 2025
Available online 6 October 2025

the silk fiber (in general, a single cocoon produces a continuous fiber that can be up to 900–1500 m long [2]). A single fiber is very fine and delicate, so to form a usable silk thread, multiple fibers (each coming from a different cocoon) are twisted together to obtain a single longer and stronger thread. After reeling and spinning, silk threads remain coated with sericin. The threads are washed, using hot water, soap, or a mild alkaline solution, to remove sericin [3], which gives silk a stiff, coarse texture. This process, known as degumming, softens the silk, enhances its smoothness and luster, and makes it more permeable to dyes, allowing for even coloration [3].

Degumming improves silk durability by exposing fibroin, its structural protein, thereby enhancing flexibility and strength. Once degummed, silk can be dyed or bleached and then wound onto cones or hanks for textile use. At this stage, sericin is removed, leaving by-products such as silk noils and scraps composed of short fibroin fibers. Additional waste arises from cocoons unsuitable for reeling or already used, which must also undergo degumming to separate varying amounts of sericin.

The global sericin market has experienced steady growth, with total sales volume increasing from approximately 4140 tons in 2018–4781 tons in 2023 [4]. The cosmetics and personal care sector is the largest consumer, accounting for nearly 3000 tons in 2023, followed by the pharmaceutical, textile, and food industries. In terms of market value, the sericin market grew from $276 million in 2018 to $319 million in 2023, with projections estimating it will reach $586 million by 2032, reflecting a compound annual growth rate (CAGR) of 6.4 % [4]. Despite sericin constituting 20–30 % of the silk cocoon's weight, a significant portion is discarded during silk processing.

Currently, the silk industry follows a linear economy model, focusing on continuous silk yarn while by-products are generally treated as secondary. However, these by-products can support the production of discontinuous yarns, either 100 % silk or blended with other natural fibers (wool, cashmere, alpaca, hemp, cotton, linen) or synthetic fibers (modal, micromodal, viscose). Recent initiatives aim to shift the industry toward a circular model, where side-stream products serve as the starting point for new mainstream materials. Specifically, the limitations encountered with sericin are related to issues from different aspects, such as extraction, purification, solubility, stability, and processability. Regarding solubility, native sericin is water-soluble, which makes it difficult to retain in long-term applications, especially in humid conditions. Moreover, unlike fibroin, sericin lacks strong mechanical properties, making it less favorable for applications requiring structural stability. Ultimately, native sericin has low film-forming ability and poor adhesion properties, limiting its use in coatings and films. Sericin requires modifications to improve its functional properties for industrial applications, as it is susceptible to microbial contamination and degradation, which require additional stabilization techniques.

To increase the industrial applicability of sericin, which is typically discharged in wastewater after the degumming process, various strategies have been developed, including chemical or enzymatic modifications, crosslinking with biopolymers, and the development of sericin-based blends. These approaches have been made possible by recent advancements in understanding the structure, properties, and processability of sericin, which have revealed its potential for use across industries such as cosmetics, biomedicine, food packaging, textiles, and more [5–7]. Notably, sericin's high water solubility makes it ideal for producing biomaterials such as fibers, films, hydrogels, microparticles, nanoparticles, and 3D scaffolds [8]. However, solubility also makes sericin susceptible to degradation in aqueous environments, resulting in sericin-based products with limited performance. To improve the functionality and durability of sericin-based materials, chemical or physical modifications are necessary to make the product suitable for different conditions and environments [9–11]. Various functionalization routes have been explored to optimize sericin's properties and overcome challenges such as instability, processing difficulties, and lack of standardization. Although growing interest in sericin functionalization has led to significant advances across diverse fields, its industrial use remains limited. This review compiles selected examples of chemical modifications aimed at enhancing sericin's performance, beginning with its origin and industrial relevance, followed by the inherent limitations of its native form. Functionalization is then discussed as the key strategy, with emphasis on functional group interconversion, covalent grafting, and crosslinking, along with their chemistries. Finally, the persistent challenges and resulting properties are analyzed, and future research directions are proposed.

This review addresses the critical need to bridge the gap between the promising inherent properties of sericin and its currently limited industrial adoption. Building on the previously published review by Aad et al. [12], which examined sericin's structure, properties, and general applications, the present work expands the discussion by providing a focused and detailed analysis of sericin's chemical reactivity and derivatization. Particular attention is given to the evaluation of functionalization strategies, the identification of research gaps, and the implications for industrial use. Relative to existing publications, this review will give an in-depth analysis of the chemistry beyond sericin covalent modifications, including:

- An overview of functionalization strategies for protein-based materials;
- Sericin modification strategies:
  - o Covalent functionalization;
  - o Covalent grafting;
  - o Covalent cross-linking;
- Limitations and challenges in sericin covalent modification.

The methodology for literature selection followed the following criteria:

(i) approach: OSINT with Web of Science, Google Scholar, Sci-Finder as main databases; ii) inclusion criteria: covalent chemical functionalization or modification of sericin, reaction conditions must be explicitly reported, as well as accurate characterization and properties features of the products; (iii) exclusion criteria: lack of experimental details, material characterization, non-covalent modification, class II hybrids; iv) search period: 2000–2025 (more than 50 % of the cited literature falls in the last five year period).

2. Sericin: from an industrial waste to a resource

As previously introduced, sericin accounts for approximately 20–30 % of raw silk, following fibroin. Given the annual global silk production of around 100,000 metric tons [13], the global industrial sericin waste can be estimated at about 50,000 metric tons of protein discarded in wastewater (equivalent to discarding 50,000 small cars into rivers every year) [6], contributing to global environmental issues [14, 15]. The urgent need to shift to a circular economy model requires sericin waste to be transformed into a valuable resource. In order to manage sericin as a feedstock, several considerations must be taken into account. First, sericin's characteristics, such as molecular weight, structure, and amino acid composition, which determine the final properties and uses of the recovered protein/peptides, as well as by-product contamination and yields, are strongly dependent on the degumming process, as summarized in the following table (Table 1). The upstream design of the degumming process is therefore a key step from which limitations on the use of sericin in specific industrial applications can derive.

Degumming processes fall into three main categories: physical (e.g., heat, microwave), chemical (e.g., alkalis, acids, surfactants), and biochemical (e.g., proteases). Heat extraction in water is the most energy-consuming, while chemical processes introduce pollutants into wastewater, making them environmentally unfriendly. The purest sericin is obtained through hot-water extraction, albeit with higher energy consumption.

Table 1
Main degumming processes, sericin outputs, and applications.

Method	Conditions	MW (kDa)	Reported application
Physical	Hydrothermal, 130 °C, high pressure	< 10	Anti-inflammatory
	Hydrothermal, 120 °C	> 10	Skin regeneration, cell proliferation and differentiation, tissue regeneration, wound healing, angiogenesis, antioxidants, biocomposites, fibers, drug delivery, UV protection, antibacterial, cosmetics, surfactant, heavy ions absorption, and electrode modification.
	Hydrothermal, 130 °C, high pressure	25–150	Improve hypercholesterolemia
	Hydrothermal, 80–100 °C	> 30	Osteogenesis, myocardial repair, drug delivery, seed cover, biocomposites, electrode modification, textiles, and sensors.
	Sonication (37°C–60°C)	> 30	Osteogenesis.
	Hydrothermal, 120 °C	45–230	Anti-tyrosinase.
Chemical	Na_2CO_3/K_2CO_3	> 5	Cell proliferation, chondrogenesis, osteogenesis, wound healing, antibacterial, Antioxidant, haemostasis, biocomposites, drug delivery, electrodes modification, Bioink, Cryopreservation, flexible electronics.
	Urea	10–225	Antioxidant, anti-tyrosinase, anti-inflammatory, and drug carrier.
	LiBr	> 100	Nerve regeneration, antiviral, myocardial repair, and electrode modification.
	Citric acid	> 5	Bioimaging
	Citric acid	50–100	Antioxidant
Enzymatic	Neutrase	0.2–10	Antidiabetic, hypotensive
	Alcalase	25–150	Antioxidant, anti-tyrosinase

2.1. A chemical perspective for sericin industrial applications

Sericin contains a high proportion of serine and threonine with hydroxyl groups in their side chains (46 % of total residues), plus additional polar amino acid chains (42 % of total residues) [16], and its molecular weight can range from 20 to 400 kDa [17]. Its wide range of molecular weight distribution can hamper some applications, for example leading to inconsistencies in product formulations. From a chemical standpoint, the amino acid composition of sericin imparts several functional properties. The presence of highly hydrophilic side chains enhances its affinity for water, making it useful as a moisturizing agent in cosmetics and pharmaceutical formulations, as well as a stabilizer for nanoparticle dispersions [18]. Additionally, this hydrophilicity gives sericin gelling properties, ideal for hydrogel production [19]. Its amphipathic nature and pH-responsiveness, due to the presence of both acidic and basic side chains, also make sericin a promising candidate for drug delivery systems and for supramolecular multilayer constructs [20]. At the same time its sensitivity to pH can cause denaturation or degradation during processing. Moreover, the functional groups present in the side chains (hydroxyl, amino, carboxyl) allow for facile chemical modifications, including cross-linking, bioconjugation with low molecular weight compounds, and grafting to natural or synthetic polymers. These processes enhance the mechanical and biological properties of sericin-based materials (*vide infra*). For instance, cross-linking or grafting sericin onto natural fibers (like cotton or wool) or synthetic fibers (like polyesters) brings sericin back to the textile industry, where it can serve as a functional agent (e.g., for moisture absorption or antistatic properties), coating, or binder [21,22].

2.2. A biological perspective for sericin industrial applications

Sericin's biological properties are heavily influenced by its molecular weight, amino acid composition, and structure (particularly β-sheet content), which are determined by the degumming process [23]. When properly extracted, sericin retains 18 amino acids, including 8 essential ones. Although it lacks tryptophan, one of the 9 essential amino acids required in the human diet, sericin is still a valuable dietary supplement in high-protein food [24].

However, some challenges must be addressed to expand its use in the food industry:

i) Control over the extraction process, which affects sericin structure, sustainability, cost, food-grade purity, and bioactivity
ii) Purification and fractionation for molecular weight uniformity, crucial for ensuring specific biological activity
iii) Removal of unpleasant odor [25]
iv) A deeper understanding of the structure–activity relationship

In addition to being biocompatible and biodegradable, sericin exhibits antioxidant activity through radical scavenging, making it useful in cosmetics (e.g., anti-aging), food, and packaging applications. Research indicates sericin has anti-inflammatory, antibacterial, antihypertensive, anti-constipation, antidiabetic, hypocholesterolemic, and metabolic regulatory effects. Its cytotoxic properties could be harnessed for anticancer treatments [26], while its ability to promote cell adhesion, proliferation, differentiation, and collagen synthesis supports its application in wound healing and regenerative medicine [27,28], along with a wide range of biomedical uses [29,30]. Additionally, sericin has been proposed as a cryopreservation supplement [31]. It should be emphasized that sericin's biological activity is tightly linked to the extraction method, which affects molecular weight, amino acid composition, and 3D structure [32]. Therefore, further work is required to optimize extraction methods tailored to specific applications, alongside a more comprehensive understanding of structure–activity relationships [16].

2.3. A physical perspective for sericin industrial applications

The primary structure (i.e., amino acid composition) of sericin underlies its photoluminescent properties, making it a promising biopolymer for use as a fluorescent probe in *in vivo* tracking, biosensing, cell imaging, trace ion detection, screen printing, and anti-counterfeiting applications [33]. Sericin's secondary structure consists mainly of random coils and β-sheets, representing its amorphous and crystalline regions, respectively. The predominance of random coils gives dry sericin a brittle texture and low mechanical strength, which poses a major limitation for the development and commercialization of pure sericin products. On the contrary, β-sheet structures in sericin and its peptides are responsible for properties such as mechanical stability, adhesion, emulsification, and moisturization [34]. Since sericin shows neither immunogenicity nor toxicity, it is approved for use in the food industry as both an emulsifier and antioxidant in salad dressings [35], as anti-hardening agent in protein bars [36], and as an anti-freeze agent in frozen potato and bread dough [37]. Increasing attention is being given to developing sericin-based materials in various forms, including scaffolds, membranes, hydrogels, conduits, bioelectronics, inks, sensors, nano- and microparticles, edible films for food preservation, and sustainable packaging [23].

Subsequent sections will place particular emphasis on strategies for the chemical modification of sericin, accompanied by selected examples of its applications.

3. Chemical modification of sericin

3.1. General overview

Although many papers in the literature treat the concepts of chemical modification and chemical functionalization as interchangeable or overlapping, this review is based on a clear distinction between the two, as illustrated in Table 2 and Fig. 1, to enhance clarity and consistency.

Chemical modification of a molecule refers to any process that alters the covalent bonding or composition of a (bio)(macro)molecule, resulting in a new chemical entity with different properties or functions. This can involve adding new atoms or functional groups to the existing molecular structure, removing atoms or functional groups, rearranging existing atoms within the molecule (isomerization), breaking existing bonds, and/or forming new ones. This process causes a modification of physicochemical properties. Chemical modification may be performed "artificially" with suitable chemical reactions, or biochemically *in vivo* (i.e., protein post-translational modification, Fig. 1). On the other hand, chemical functionalization is a more targeted form of chemical modification where the primary goal is to introduce or enhance specific chemical functionalities or properties to a (bio)(macro)molecule, typically by attaching specific functional groups to its structure. This includes both the addition of new functional groups and/or the transformation of existing ones with low reactivity into more responsive forms. These functional groups are chosen precisely because they impart a desired characteristic to the starting molecule; by enhancing or introducing specific desirable properties , such as improved stability, solubility, reactivity, biological activity, or material performance; by enabling or changing interactions to a specific target (e.g., receptors, ligands); by adding a detectable label (e.g., a fluorescent tag); or by improving its mechanical, electrical, or optical properties.

Because chemical modification encompasses a broader range of changes, the two terms are used in overlapping contexts. Nevertheless, from a chemical perspective, recognizing their differences can be useful for clarity. Building upon this distinction, functionalization is especially significant in the field of biopolymers, where it plays a crucial role in improving material performance [1]. It plays a crucial role in enhancing the properties of biopolymers, making them more versatile and suitable for a wider range of applications. In recent years, extensive research has focused on the chemical functionalization of biopolymers, which involves the introduction/interconversion of functional groups or molecules to modify their structure and improve their performance. There are several approaches to achieving chemical functionalization, which can be carried out through reactions such as esterification, etherification, acetylation, or amidation, which alter the biopolymer's existing chemical structure. *In vivo* examples of chemical modification in proteins include phosphorylation, glycosylation, ubiquitination, methylation, lipidation, proteolysis, and crosslinking [1,38,39]. Alternatively, functionalization includes grafting or chemical crosslinking, where new molecules or polymers are covalently bonded to the biopolymer's backbone. While grafting involves attaching functional groups/moieties or polymers to the biopolymer's backbone, chemical crosslinking creates intra- and intermolecular covalent bonds between polymer chains, forming a network structure.

Table 2
Differences in the scope, focus, and method between chemical modification and chemical functionalization.

	Chemical Modification	Chemical Functionalization
Scope	Broad, any alteration of chemical structure.	Specific, introducing/enhancing functionality
Focus	Change in chemical identity/ composition.	Imparting a new/improved property or function
Method	Can be any reaction (addition, removal, isomerization).	Often involves the introduction of specific functional groups

3.2. Functionalization strategies

3.2.1. Protein functionalization chemistry

3.2.1.1. General considerations. Proteins are composed of a diverse array of amino acids, each imparting specific chemical properties through its respective side chains; however, only a limited subset of these residues is amenable to chemical modification. Specifically, the functional groups most commonly targeted include hydroxyl groups on serine, threonine, and tyrosine; amine groups on lysine and histidine; the guanidinium group on arginine; carboxylic acid groups on aspartic and glutamic acid, and thiol groups on cysteine. Additionally, other side chains may serve as auxiliary sites for functionalization, albeit with greater difficulty functionalized as illustrated in Fig. 2. While each of these FGs exhibits chemical characteristics and reactivity profiles, they collectively provide valuable starting points for the chemical modification of proteins [40].

Furthermore, it is essential to recognize that these functional groups may interact/react with one another under specific reaction conditions, and such interactions can markedly influence the protein's physicochemical properties, including its conformation, reactivity, and solubility.

3.2.1.2. Residue-specific functionalization strategies

3.2.1.2.1. Carboxylic side chains. The carboxyl group is known to typically exhibit poor reactivity, especially when compared to more reactive functional groups such as hydroxyl, amine, and thiol groups, due to factors such as electron delocalization, inductive effects, the absence of a good leaving group (LG), and its acidity [41]. As a result, the formation of esters from carboxyl groups generally requires a catalyst or prior chemical activation.

Indeed, one of the most common reactions that occurs between a carboxylic acid and an alcohol is the esterification, which is performed under acidic conditions to promote the reaction (such as the *Fischer esterification* [42] - with inorganic acids or Lewis acids, Scheme 1), where water acts then as a discrete leaving group (LG).

Unfortunately acidic catalysis is not always viable (e.g. due to lability of the biomolecule); thus a well known alternative consists in converting the carboxyl group into a more reactive group through prior modification [43] or by using coupling agents [44]. In the first case, acyl or anhydride groups, for example, convert carboxylic acid into a more reactive moiety. The reaction of these groups with a hydroxyl group forms esters more readily, as the leaving group in both cases is the conjugate base of a particularly strong acid. In the second case, coupling agents are required to generate a more reactive site by converting the hydroxyl group into a better leaving group, such as through the use of urea derivatives (e.g., *Steglich esterification* [45], Scheme 1). For instance, solid-phase peptide synthesis (SPPS), a crucial method used in the synthesis of peptidic pharmaceutical ingredients or drug conjugates, largely relies on coupling agents in combination with protecting groups [46].

It is important to note that there are cases where carboxylic acids can act as nucleophiles, despite their generally low reactivity. As a fact, in a basic environment, in the presence of highly reactive substrates, such as acyl halides or dicarbonates, despite the strong delocalization, the carboxylate (i.e. the conjugated base of a carboxylic acid) can react with an acyl halide to form the corresponding anhydride (as happens in the first step of *Yamaguchi's Esterification* [47]), which can be considered then another "activated" carboxylic moiety. However, as mentioned earlier, when dealing with biopolymers, it is preferred to improve the reactivity through the use of coupling agents, even to afford better control of the general process.

As a matter of fact, carbodiimide coupling is one of the most well-known and common techniques to effectively modify carboxylic acids to form amide bonds and esters, as shown in Scheme 3, (B-5). The

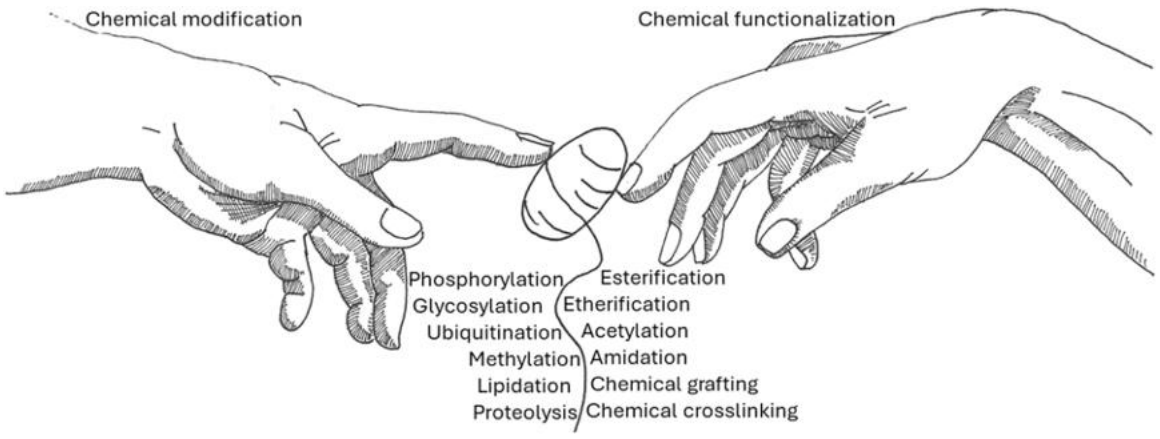


Fig. 1. Conceptual overlap and distinction between chemical modification and chemical functionalization, referring to biological systems. The cocoon symbolizes biopolymers in general. Chemical modification is represented by God's hand as the broader term, encompassing chemical functionalization, which is symbolized by Adam's hand. The sketch is mirrored relative to the traditional iconography to emphasize that the two terms are sometimes used interchangeably.

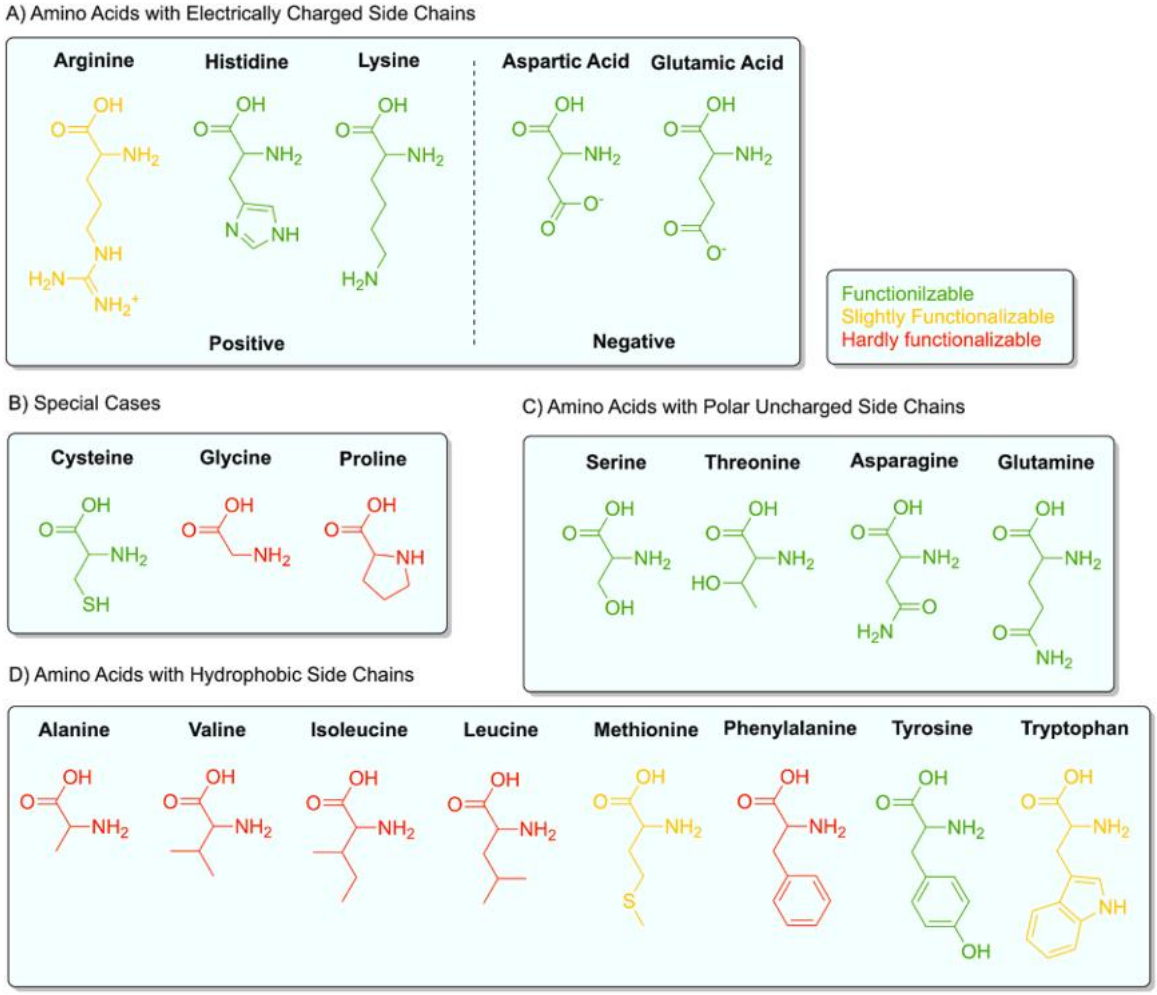
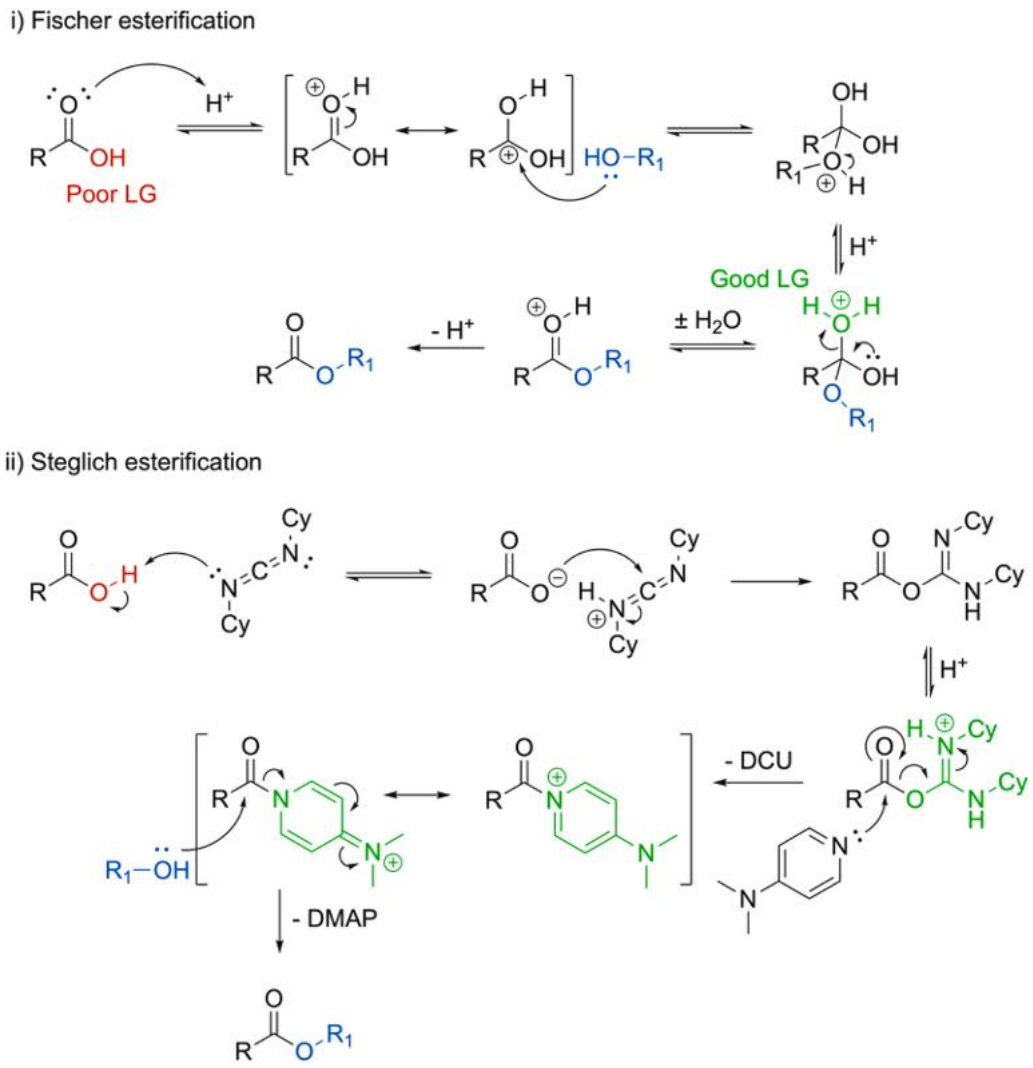


Fig. 2. Amino acids classification according to side chains' functionalization opportunities.

reaction can be performed with a wide spectrum of choice regarding the coupling agents, such as 1-ethyl-3-(3-dimethylaminopropyl)- carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) in aqueous media at a fixed pH [48], *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) [45] and *N*,*N*′-diisopropylcarbodiimide (DIC) [49]. In the first part of the reaction, the acid is first converted into a more reactive group; in particular, the water LG is converted into a stable iso-urea derivative, which then converts into an NHS-ester. This activated intermediate spontaneously/readily reacts with free primary amino groups, or other nucleophilic FGs, leading to a condensation reaction that forms, for instance, an amide as the final product.

Among the reaction panorama, carbodiimide coupling represents an



Scheme 1. Esterification mechanisms promoted by good leaving groups.

extremely strong and reliable procedure; not surprisingly, it is used for the incorporation of several biomolecules. It is clear that, since the protagonists of the coupling are acids and residues such as amines, alcohols, and thiols, internal (intrachain and interchain) crosslinking might occur during the modification in such complex systems as proteins [50].

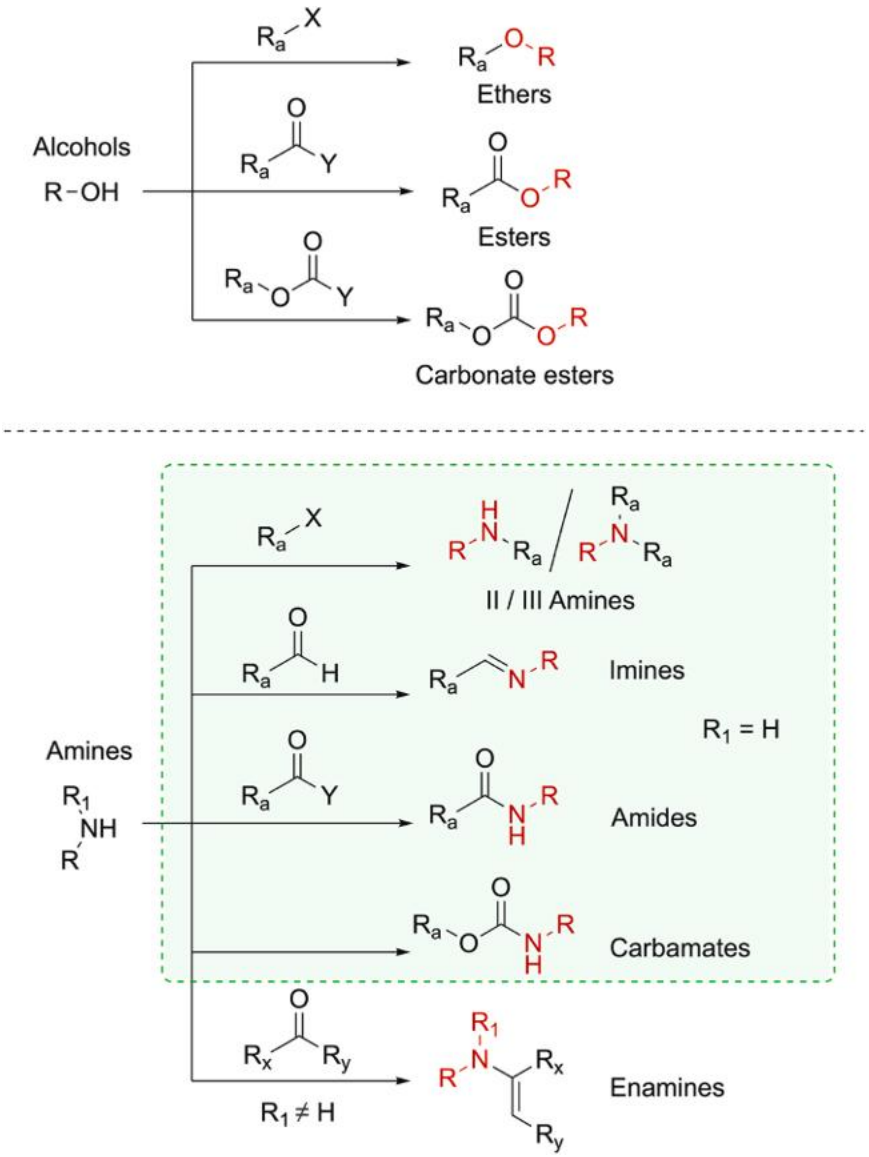
3.2.1.2.2. Nucleophilic side chains. The discussion regarding hydroxyl and amine groups differs slightly from what was previously reported, as they exhibit much higher nucleophilicity, especially in a basic environment, compared to carboxylic acids due to the absence of delocalization and inductive phenomena. Nonetheless, it is important to emphasize that the reactivity of these functional groups is highly dependent on their stereoelectronic features. Specifically, primary groups are usually more reactive than secondary groups, while tertiary groups are even less reactive [51].

In the case of alcoholic moieties (Scheme 2), the main products that can be formed are ethers, esters, and carbonates, depending on the adopted conditions and substrates. Meanwhile, amines can lead mainly to the formation of secondary and/or tertiary amines (with caution to avoid exhaustive alkylation [52] when alkyl halides are used), imines (Schiff's bases), enamines, amides, or carbamates (Scheme 2). Despite the theoretical potential to introduce a diverse array of functional groups and their corresponding reactivities, it is crucial to recognize that the concurrent natural presence of multiple distinct FGs within a complex molecular architecture in polyfunctionalized proteins, as sericin, can markedly influence the chemo- and regioselectivity of the functionalization processes [53]. Furthermore, these effects are extremely dependent on the specific reaction conditions employed, ultimately impacting the properties and characteristics of the final product [40].

By thoroughly analyzing the amino acid composition of sericin and assessing the reactivity of its principal FGs, it becomes possible to envision a variety of functionalization strategies. These approaches may involve either the introduction of new FGs or the modification of existing ones, based on reactions that have been discussed or proposed in the literature. However, such studies generally address silk proteins as a whole, without specific focus on sericin itself [54–56]. This gap then presents opportunities to develop targeted and optimized methodologies tailored specifically to this protein, aiming to improve its functional properties through customized advanced chemical modifications.

A common chemical variation in hydroxyl groups is carboxylation, which involves the introduction of a carboxylic group. This is typically carried out using an α-halo carboxylic compound, as shown in Scheme 3 (A-1), such as chloroacetic acid [55], where the hydroxyl is deprotonated under harsh alkaline conditions (pH > 13) and acts as a nucleophile in the substitution; however, under these conditions chain fragmentation may occur, leading to a lower final molecular weight (MW) [57].

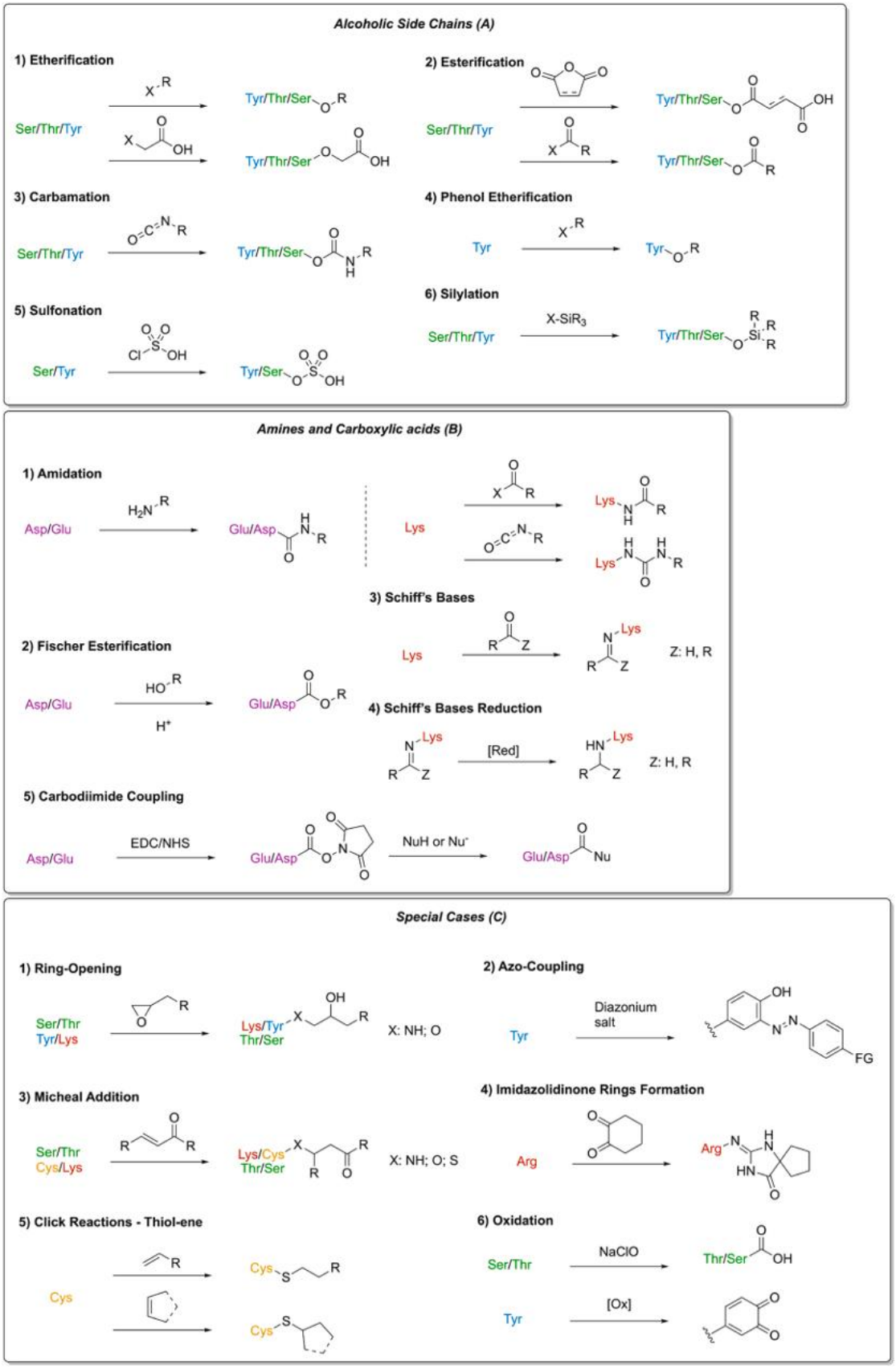
Another strategy involves reacting with acyl derivatives [58] or organic anhydrides, such as succinic anhydride [59] or maleic anhydride [60], in an ionic liquid as the reaction medium, as reported in



Scheme 2. Alcohol and amine moieties reactivity.

Scheme 3 (A-2); the latter offers better carboxylation efficiency and reduced protein degradation compared to the previously cited case, but requires harsher conditions, such as high temperatures (100 °C) and organic solvents, which might raise some concerns about the general safety of the process [61]. Apart from the classical reactivity of hydroxyl groups of serine and threonine based on their intrinsic nucleophilicity, another possibility stems from the oxidation of those moieties to carboxylic acid using oxidants such as sodium hypochlorite (as shown in Scheme 3, C-6), which also leads to structural protein modification [62]. Additionally, the hydroxyl groups can be silylated using silylating agents (Scheme 3, (A-6) [63]. Despite its specificity, this strategy has already been applied to silk proteins to enable further grafting of organic dyes, polymers, and other systems [63].

Tyrosine is one of the few amino acids with an aromatic side chain; thereby, alongside the hydroxyl group and its reactivity, which is inevitably influenced by the benzene ring, it is also capable of utilizing the chemical reactivity of its aromatic ring. In particular, it can be subjected to functionalization via azo coupling [64] (Scheme 3, C-2), through an electrophilic aromatic substitution (S_E^{Ar}) mechanism, also theoretically promoted by the inductive effects of the free -OH group [65]. Conversely to serine carboxylation, this coupling does not promote backbone fragmentation, thus preserving the chain's length. The reaction occurs between a typically pre-synthesized diazonium salt and the phenolic moieties of sericin; alternatively, it is feasible to conjugate the salt directly onto the protein, thereby enabling additional functionalization or the incorporation of specific functional groups that would otherwise be challenging to access [64]. A notable advantage of this approach lies in its capacity to facilitate the simultaneous or stepwise introduction of multiple FGs, including carboxylic acids, sulfonic acids, amines, aliphatic chains, ketones, and other functionalities. This versatility enables substantial modifications of the protein's physicochemical properties, thereby enhancing its suitability to achieve specific functional objectives [65]. Azo coupling reactions typically proceed within minutes under aqueous conditions at ambient temperatures, which are optimal operating conditions for proteins, demonstrating high efficiency. Nevertheless, despite the methodological advantages, certain limitations are present; for instance, an excess of nitrous acid can induce a deamination of free amine groups, thereby reducing their availability [66], which can limit further modifications. While tyrosine is primarily targeted for such modifications, histidine residues can also serve as effective substrates for the coupling process; however, it must be taken into account that the imidazolic ring presents slightly different reactivity



Scheme 3. Possible Routes for the Chemical Modification of Sericin [84].

due to its electronic effects, in addition to the nucleophilicity shown by the nitrogen atoms. Additionally, as briefly cited above, the phenolic moiety can exhibit nucleophilic properties, enabling subsequent reactions with electrophilic agents [67–71]. Unlike serine, the phenolic hydroxyl group of tyrosine presents a lower pKa (~10) and can consequently be deprotonated at relatively mildly basic pH values (pH ~ 9). This behavior is attributable to the conjugated π-electron system inherent to its aromatic ring. For instance, extremely depauperated substrates such as cyanuric chloride, which is characterized by the presence of several electron-withdrawing chlorine substituents on the triazine ring, which is already naturally electron-deficient, are capable of undergoing successive nucleophilic substitution, thereby facilitating the incorporation of various functional moieties, such as polymers or sugars, such as monosaccharides, into the protein structure [72]. Furthermore, this coupling mechanism is also applicable to lysine, where the nucleophile group is the free primary amine [69,72].

Moreover, the functionalization of tyrosine residues has recently been achieved efficiently through triazolinedione ene-type chemistry, representing a novel and versatile click chemistry approach applicable to various proteins [73]. In addition to this chemically driven strategy, enzymatic methods have also been employed for tyrosine modification. Specifically, tyrosinase, in the presence of oxygen, catalyzes the oxidation of tyrosine residues into reactive *o*-quinone intermediates, which can subsequently react with nucleophilic species or through other reactions, such as Michael additions, and Diels-Alder cycloadditions (Scheme 3, C-6) [74].

Finally, sulfonation of both tyrosine and serine residues can be achieved using chlorosulfonic acid, as depicted in Scheme 3 (A-5). However, this reaction may induce partial degradation of the protein backbone. Moreover, in the presence of ionic liquids, the sulfonation process may additionally involve the amino groups of lysine and arginine, potentially leading to further functional modifications [75,76].

Regarding the free amine groups, predominantly represented by the lysine residues, it is important to emphasize that these functional groups possess the capacity to participate not only in nucleophilic substitution, but also in acylation reactions (to form amides) and the formation of Schiff's bases and/or enamine derivatives with aldehyde or ketones, based on the substitution grade of the amino group (Scheme 3, B-1 and B-3) [5,77,78]. Schiff's bases can be subsequently reduced to their corresponding amines, which are generally more stable and resistant to hydrolysis compared to the initial imine structure [41]. An innovative interconversion involves converting amines into thiols via reaction with thiobutyrolactone [79], a process that holds potential for further modification utilizing click chemistry techniques or exploiting the HSAB characteristics of the thiol moiety.

As anticipated, the free amino groups of lysine residues can act as nucleophiles, capable of attacking various electrophilic substrates, such as isocyanates. When these electrophiles also bear additional functional groups, this approach provides a versatile strategy for including supplementary reactive moieties, such as vinyl [80] or azido groups [39]. Due to their high reactivity, isocyanates can also modify other nucleophilic amino acid residues, including serine, threonine, and tyrosine.

In the case of arginine, which contains a guanidinium group, it can react with 1,2-dicarbonyl derivatives such as 1,2-cyclohexandiones to form stable imidazolidinone rings, as shown in Scheme 3 (C-4) [55], or glyoxals [81].

In the realm of biopolymers, click chemistry emerges as a highly advantageous approach, offering numerous benefits such as rapid reaction rates, high product yields, mild reaction conditions, and exceptional selectivity [82]. Although an in-depth analysis of click chemistry is intentionally omitted here, its relevance is noteworthy. In biomaterial applications, copper-catalyzed azide-alkyne cycloaddition (CuAAC) has been extensively utilized and thoroughly reviewed [83], despite ongoing concerns regarding the potential toxicity of azide reagents and the *in vivo* effects associated with copper catalysts [81]. Alternatively, thiol-ene chemistry presents a viable, metal-free option (indicated in Scheme 3, C-5) characterized by rapid kinetics and biocompatibility [54], making it a promising approach for biopolymer modification.

This section has outlined a part of a theoretical spectrum of chemical reactions that could be employed to functionalize sericin, as depicted in Scheme 3. However, at the practical level, not all of the methods indicated before are actually implemented or pursued within the sericin context. The main limitations might concern the protein's solubility and stability under the conditions compatible with the desired modifications, sericin's sensitivity to certain reagents and practical conditions (such as extreme pH, oxidants, harsh chemicals), and considerations related to safety, biocompatibility, and potential impacts on the desired functional properties. Moreover, many applications of sericin require use in settings that are sensitive from regulatory or sustainability standpoints, where the deployment of "aggressive" reagents may be unacceptable. Consequently, the practical landscape is strongly guided by trade-offs between modification efficiency, preservation of the protein's physicochemical properties, and the requirements of the intended applications, making only a subset of theoretically available routes both feasible and useful. The following section will critically examine which approaches have been explored given these constraints, highlighting unresolved issues, if any.

3.2.2. *Sericin functionalization strategies*

Nowadays, some of the chemical reactions/modifications performed on silk-derived substrates in solution (i.e., in a homogeneous state) have proven to be a valuable synthetic pathway to a wide range of materials. Nevertheless, due to noncovalent interactions among chains and protein folding processes, reactive amino acids tend to be protected from external reagents; thus, solution-phase reactions can suffer from significant reactivity limitations. The introduction of specific FGs into the protein structure could alter the spatial conformations of amino acids, consequently exposing the internal side chains that were previously protected by the folding. In this context, protein chemical modification not only influences the final material properties, but might also be essential to reveal further FGs, enabling additional modifications that were initially inaccessible [84].

Within silk-based substrates, sericin has attracted growing interest due to its unique properties and potential applications. However, like many other biopolymers, sericin exhibits inherent limitations that have prompted efforts to enhance its functionality through various modification strategies. Over the years, sericin has been incorporated into various blends and composites, among others. Additionally, it has been subjected to physical and chemical crosslinking, as well as chemical modifications. However, despite these advancements, research on the chemical functionalization of sericin remains limited, leaving several areas underexplored and presenting opportunities for further investigation.

While serine is the predominant amino acid in its primary structure, sericin also contains 17 other amino acids, as shown in Fig. 3, including alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine [85].

Several potential routes exist for the chemical functionalization of sericin, which can be achieved by targeting various reactive groups present in its structure. The functionalization of sericin starts by taking advantage of its amino acid residues, and this presents a promising avenue for enhancing its properties and expanding its range of applications. The diverse functional groups, especially hydroxyls, amines, and carboxyls, offer multiple sites for modification, although each comes with unique reactivity challenges.

Building on the general strategies for protein functionalization discussed in the previous section, this section explores in detail the specific approaches used to functionalize sericin. A number of studies have explored diverse chemical functionalization strategies for sericin.

The following section outlines selected examples of functionalization approaches that target specific amino acids. This excludes methods

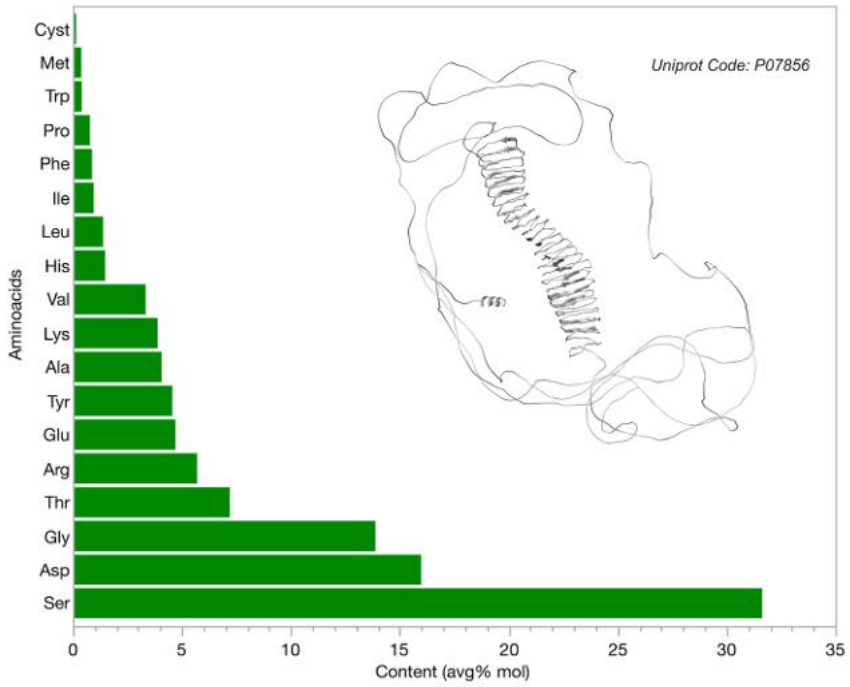
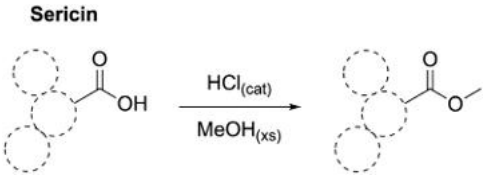


Fig. 3. Amino-acid content of silk sericin [85]. Protein graphics were obtained with UCSF Chimera (1.15 build), developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco [86].

classified as covalent grafting or crosslinking, which will be discussed in subsequent sections.

Simple chemistry was proposed by the research group of Lee [87] targeting carboxyl groups: methylated sericin was obtained by classical *Fischer* esterification (Scheme 4), in the context of drug delivery carriers preparation. The author specifically focused on a pathogenic bacterium (*Helicobacter pylori*) known to colonize gastric mucosa and be a primary etiological agent of duodenal and gastric ulcers. The necessity for this research arises from the limitations associated with conventional antimicrobial therapies, which frequently exhibit suboptimal efficacy due to factors such as insufficient resistance to gastric acidity and limited residence time within the gastric environment, thereby restricting their therapeutic potential. Consequently, the development of targeted, localized drug delivery systems has become imperative. In this context, sericin was identified as a promising material for such applications. The research team successfully synthesized methylated sericin-based beads to serve as nanocarriers. To do this, the authors suspended 10 g of sericin in 100 mL of methanol containing 0.1 mol of HCl, as catalyst. The batch was stirred for 24 h at room temperature, and the final product was recovered by centrifugation.

The final beads were obtained by dissolving the methylated sericin (MeSS) in DMSO/LiCl, followed by coagulation in methanol. The resultant beads underwent comprehensive characterization and *in vitro* evaluation. To verify and confirm the occurrence of esterification, the authors employed various analytical techniques, including FTIR spectroscopy and elemental analysis. Specifically, analysis of the FTIR spectra of MeSS revealed the disappearance of the absorption bands associated with the carboxylic OH group, concomitant with the emergence of characteristic stretching vibrations indicative of the newly formed ester functionalities. Additionally, by assessing the ratio of the absorption bands corresponding to methyl (CH_3) and methylene (CH_2) groups, the researchers observed an increased ratio, which was attributed to the correct incorporation of methyl groups following the esterification process. Regarding the elemental analysis, the observed changing ratio of C, N, and H following the reaction further corroborated the successful methylation process, evidenced by an increased carbon and hydrogen content compared to nitrogen; which does not participate. The researchers then estimated the "point of zero charge" (pH_{pzc}), which shifted from 5.0 for neat sericin to 8.6. This is in agreement with the methylation process that involved the consumption of free carboxylic acid groups; consequently, the surface of MeSS exhibited an increased density of positively charged sites. This shift in surface charge density contributed to the observed pH_{pzc} value. Beyond the characterization of the beads, the authors conducted a thorough evaluation of the swelling behavior of the final product, given its direct relevance to the intended biomedical application. The MeSS demonstrated increased swelling behavior in highly acidic conditions, while exhibiting limited swelling in both neutral and alkaline environments. This behavior starkly contrasts with that of pristine sericin and is notably influenced by the composition of ionic groups within the material. Importantly, this swelling profile aligns with the observed *in vitro* drug release kinetics. Under physiological conditions, the MeSS beads exhibited efficacy in drug release in acidic conditions, simulating the gastric environment, which perfectly matches the initial objectives of the study.



Scheme 4. Sericin carboxylic acid methylation/esterification.

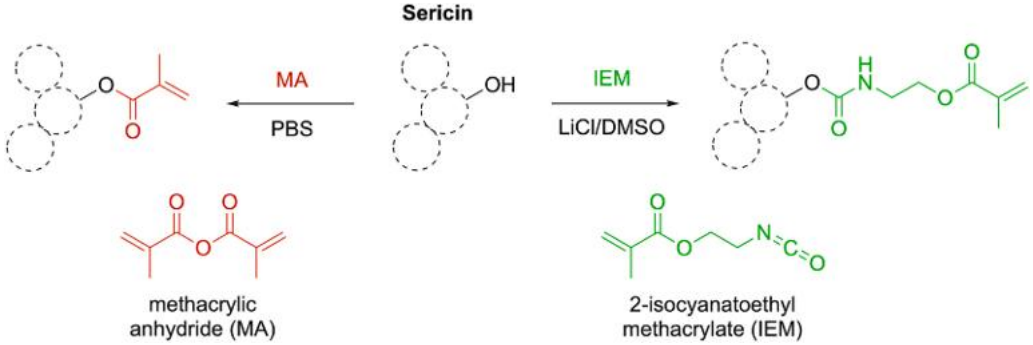
The research conducted by Jabbari *et al.* [8] centered on the development of sericin-based hydrogels for biomedical applications. Owing to their intrinsic properties, such as biocompatibility and biodegradability, these hydrogels represent a promising material for tissue regeneration that aims to emulate the biological extracellular matrix. Although natural-based hydrogels have already exhibited significant efficacy in regenerative medicine [88–90], there remains a pressing need to innovate and optimize novel hydrogel matrices with improved and tunable mechanical properties to better meet the demands of diverse therapeutic contexts. In particular, the authors decided to exploit the abundant free hydroxyl groups of sericin for functionalization purposes. Previous

studies have demonstrated that sericin can serve as a precursor for hydrogel scaffold synthesis following functionalization with methacryloyl groups, with its physicochemical and mechanical properties being notably influenced by the nature of the introduced functional groups [91,92]. Consequently, the researchers functionalized sericin with 2-isocyanatoethyl methacrylate (IEM) and systematically compared the resulting hydrogel's properties to those of a hydrogel derived from functionalized sericin with methacrylic anhydride (MA) (Scheme 5). Both hydrogels were subsequently obtained through photopolymerization processes, allowing for an assessment of how different functional groups impact the structural and functional characteristics of the final systems.

Sericin carbamate methacryloyl (SerAte-CM) was obtained by dissolving 1.0 g of sericin (2 % wt) in a solution of anhydrous LiCl/DMSO at 60 °C for 45 min under an inert blanket. Then, IEM was added dropwise into the solution at different ratios in function of the desired serine functionalization. The reaction was left for 24 h. The final product was collected by dialysis and centrifugation. Instead, sericin methacryloyl (SerAte-M) was prepared by dissolving 1.0 g of sericin in 7.0 mL of PBS (pH 8.5) for 2 h at 35 °C. Subsequently, MA PBS solution (10 mL) was added to the main batch dropwise, and the reaction was left stirring overnight at room temperature. The final product was isolated by dialysis and then lyophilized. The authors employed a range of spectroscopic techniques to characterize their products and confirmed the successful functionalization of sericin. Primarily, ^{1}H NMR was utilized to identify the presence of characteristic peaks corresponding to the vinyl and methyl protons associated with the introduced FGs. These signals were also instrumental in quantifying the degree of functionalization (DoF) by comparing their integrated areas to those peaks attributed to the amino acid residues of the protein backbone. Due to the inherently hygroscopic nature of sericin, a common challenge when working with this protein, the NMR spectra were acquired using specific protocols to totally suppress the water residual peak. This approach facilitated the clear observation of sericin-related signals, thereby ensuring accurate visualization of the functionalized product. These observations were further substantiated by FTIR, which revealed the emergence of characteristic absorption bands associated with the acrylate moiety. Additionally, a notable reduction in the intensity of the OH stretching band, typically attributed to sericin and residual water, was observed. It is important to acknowledge that sericin's propensity to absorb substantial quantities of water can adversely impact not only the characterization step, but even the functionalization process, as water reacts readily with isocyanates, leading to the formation of byproducts and to the consumption of the reagents involved. This side reaction underscores the necessity for nearly anhydrous conditions during such chemical modifications. However, the authors did not address or experimentally investigate this aspect, leaving it as a potential variable influencing the efficiency of the functionalization process. In addition to the aforementioned spectroscopic analyses, the authors assessed the impact of functionalization on the secondary structure of sericin through circular dichroism (CD). Notably, the results indicated that there were no significant alterations in the protein's secondary structure following chemical modification, as evidenced by the comparison of the CD before and after the functionalization. Regarding the physicochemical properties, rheological evaluations of the storage (G') and loss (G'') moduli demonstrated that the gelation kinetics were strongly influenced by the DoF. Specifically, a higher density of reactive methacrylate groups (i.e., increased crosslinking potential) was associated with a shorter gelation time. This parameter is critical for this application, as it must be sufficiently rapid to minimize cellular exposure to the photoinitiator and unreacted methacrylate groups, whose presence can potentially induce inflammation and tissue destruction. Furthermore, an elevated DoF corresponds to increased crosslinking density, which can adversely affect cell behaviors such as spreading and attachment. In this context, a DoF of approximately 31 % was identified as optimal, balancing structural integrity with biocompatibility. Morphologically, the hydrogel exhibited a stable honeycomb-like microstructure characterized by high porosity, with porosity levels increasing proportionally with the DoF. These characteristics are essential to establishing a consistent set of benefits conducive to optimal cell proliferation. Crosslinking had a notable impact on hydrogel swelling capacity, which decreased as the DoF increased. Additionally, the limited mass loss (< 25 %) during the incubation period of 21 days indicated that the hydrogels maintained sufficient structural stability, rendering them suitable for their intended biomedical application.

Although the aforementioned study presents a valid methodology for introducing methacrylate moieties via reactions with isocyanates or anhydrides to further modify the biopolymer, the authors, despite showing considerable interest in the resulting material's physical properties, did not evaluate the chemoselectivity of the functionalization processes. This aspect is critical for a comprehensive assessment of the reaction outcome, as it provides valuable insights into the selectivity and specificity of the modifications. The investigations were primarily limited to conventional spectroscopic analyses, operating under the assumption that most sericin's functional groups would be functionalized. However, the studies did not explicitly determine whether the reactions proceeded chemoselectively or whether competing side reactions occurred, factors that can significantly influence the final properties of the material.

In 2008, Yoon *et al.* [93] also published a study concerning the functionalization of sericin via reaction with isocyanates. Their research specifically aimed to repurpose industrially discarded sericin as a bio-based additive in polyurethane (i.e., polycarbamates) formulations, which are extensively employed across diverse applications. The primary objective was to address the biodegradability of these materials by leveraging the inherent biocompatibility of sericin, thereby contributing to the development of more environmentally sustainable polyurethane systems. Specifically, the authors functionalized sericin through reactions with 2-(methacryloyloxyethyl) isocyanate (MOI) and 2-(acryloyloxy) ethylisocyanate (AOI), yielding MOI- and AOI-modified sericin,



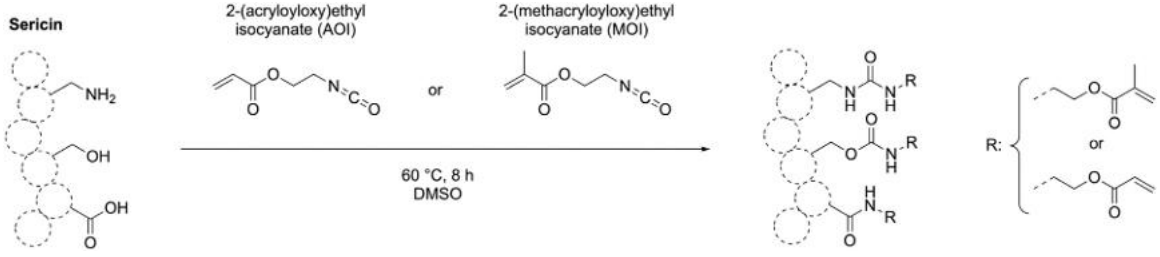
Scheme 5. Sericin hydroxyl groups functionalization with commercial isocyanate and anhydride.

herein referred to as MOISS and AOISS, respectively (Scheme 6). The synthesis of AOISS was intentionally conducted to serve as a comparison with less hindered isocyanate derivatives. The resulting modified sericin were subsequently employed to fabricate a polymeric film via polymerization of the introduced acrylic FGs.

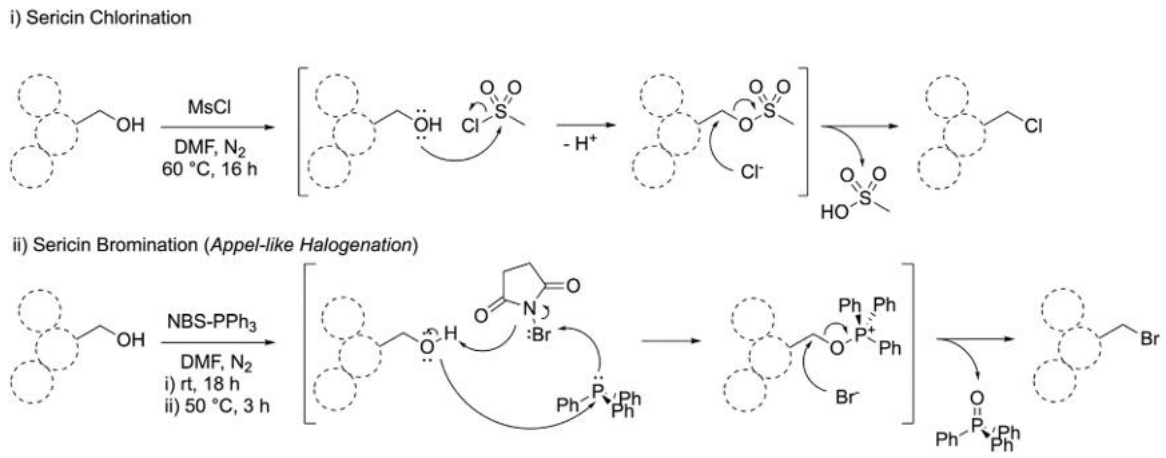
To functionalize sericin, the authors dissolved 2 g of sericin in 30 mL of DMSO at 110 °C using an oil bath for 2 h to eliminate the protein moisture. The solution was then cooled down, MOI was added (0.5/1.0/1.5/2.0 mol for every mol of polar residues of sericin), the temperature was raised to 60 °C, and stirred for 8.5 h. At the end of the reaction, 200 mL of THF was added, and the final product was recovered by precipitation in hexane. The same procedure was adopted to functionalize sericin with AOI. Despite the similarity in the underlying reaction type to previously reported studies, the research group of Yoon placed greater emphasis on elucidating the reaction mechanisms and progression, without neglecting thorough characterization of the resulting compounds. Initially, they analyzed the composition of the employed sericin by determining the mol% of amino acids containing hydroxyl groups, as well as the total mol% of polar amino acids, to assess the protein's suitability for functionalization. Subsequently, FTIR spectroscopy was employed to confirm the occurrence of reactions and identify characteristic spectral features. Both MOISS and AOISS displayed characteristic stretching vibrations corresponding to methacrylic and acrylic ester functionalities, respectively, along with the out-of-plane deformation vibrations of the vinyl groups, indicative of successful chemical modification. The formation of carbamate bonds was confirmed by the appearance of characteristic spectral bands, alongside the disappearance of the serine OH stretching vibration. Additionally, the authors proposed a plausible reaction pathway involving the nucleophilic attack of free amine groups in sericin (e.g. lysine residues) on the isocyanate, leading to the formation of urea linkages. Although some of the resulting bands overlapped with amide vibrational modes, the carbonyl stretching vibration peak was distinctly observable, thereby indicating that under the reaction conditions employed, chemoselectivity toward serine OH was practically unachievable. The same spectroscopic technique was further utilized to confirm polymerization, evidenced by a decrease in the intensity of the vinyl group absorption band. The authors did not substantiate their findings through complementary analytical techniques such as NMR or elemental analysis. It is important to acknowledge that, given the inherent complexity of such protein-based systems, comprehensive characterization remains a significant challenge. Proteins often represent difficulties in terms of analysis, as many conventional methodologies are insufficient to definitively identify or elucidate specific structural or compositional features. Concerning the physicochemical properties, both MOISS and AOISS demonstrated a reduction in water solubility relative to unmodified sericin, which can be attributed to the chemical modifications and subsequent crossliking. Among the two modified derivatives, MOISS demonstrated greater solubility, attributable to the steric hindrance imposed by the methyl groups, which reduced the packing efficiency of the protein matrix. As expected, the modifications adversely affected the swelling behavior; specifically, the sericin film modified with MOI preserved its structural integrity following water immersion, whereas the unmodified sericin exhibited disorganization. From a mechanistic perspective, both tensile and strength moduli increased post-modification, in dry and wet states, while elongation at break decreased accordingly. When compared to commercially available polymers such as HDPE and Nylon 6–6, the resultant films displayed intermediate mechanical performance between these two benchmark materials.

Sakamoto *et al.* [94] in 1997 published a study that diverges significantly in its conceptual approach from the predominant literature on sericin functionalization. Most existing research primarily treats sericin as a nucleophile that reacts with electrophilic substrates, which may then possess additional FGs and participate in various subsequent reactions. Conversely, Sakamoto *et al.* explored the alternative perspective of transforming sericin as an electrophile to be reacted with a complementary nucleophilic substrate. The electrophilic sericin derivatives can, in principle, be isolated and employed separately. This contrasts with conventions in certain experimental procedures (e.g. esterification with *in situ* activation by coupling agents) where electrophilic intermediates are transient. Specifically, the authors demonstrated sericin halogenation under heterogeneous conditions and subsequently introduced a simple nucleophile (propyl amine) into the halogenated protein as a proof of concept, illustrating the potential application. In particular, methanesulfonyl chloride (i.e. mesyl chloride, MsCl) was employed to achieve chlorination, while the couple *N*-bromosuccinimide--triphenylphosphine (NBS-PPh_3) was utilized for bromination (Scheme 7). Under varying reaction conditions, these procedures led to the formation of 3-haloalanines originating from the serine and threonine residues.

For the chlorination, 200 mg of sericin were dispersed in 2 mL of DMF at room temperature for 18 h under inert atmosphere, then methanesulfonyl chloride (mesyl chloride, MsCl) with 6 mL of DMF were added, with a ratio of 1:9 for MsCl:serine, and the mixture was stirred at 60 °C for 16 h. The product was recovered by precipitation in excess of methanol, then washed with methanol, DMF, and finally dried at reduced pressure. Meanwhile, for the bromination, NBS-PPh_3 (*N*-bromosuccinimide - Triphenylphosphine) couple was opted as a reagent system. Thus, 200 mg of sericin was dispersed in 2 mL of DMF for 2 days under a nitrogen blanket at room temperature. The mixture was then ice-cooled, and NBS-PPh_3 DMF solution (3 mL) was added to the batch (from 2 to 5 times the quantity of serine residues). The reaction was left at room temperature for 18 h and then heated at 50 °C for 3 h. The final product was recovered by the same method outlined earlier. The topic is highly intriguing, and the authors primarily assessed the reaction progress and efficiency through amino acid analysis via GC-MS following acid hydrolysis, without the application of additional analytical techniques.Concerning the chlorination process, multiple mechanistic pathways are conceivable. One plausible mechanism, depicted in Scheme 7, involves the nucleophilic attack of the hydroxyl group of serine and/or threonine residues on MsCl, resulting in the formation of a sulfonic ester intermediate. This is then susceptible to nucleophilic substitution by chloride ions, leading to the formation of chlorinated derivatives. According to the authors, the reaction mechanism might also proceed via a *Vilsmeier-Haack*-like pathway [95], wherein DMF reacts with MsCl to generate an iminium chloride ion active species; which is subsequently attacked by the hydroxyl group of serine, leading



Scheme 6. Sericin isocyanate functionalization.

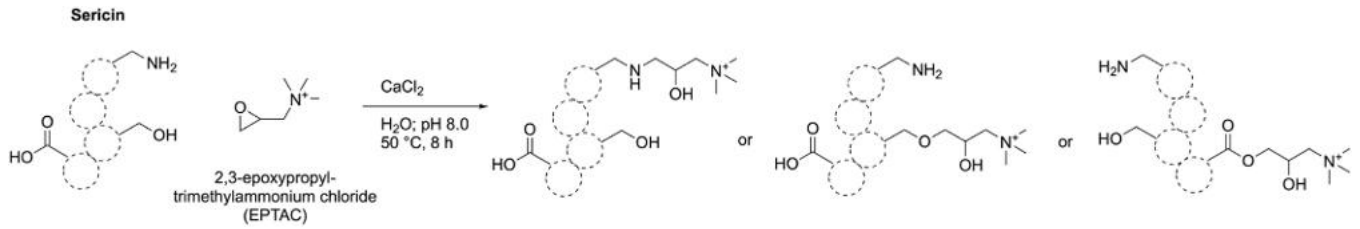


Scheme 7. Sericin Halogenation.

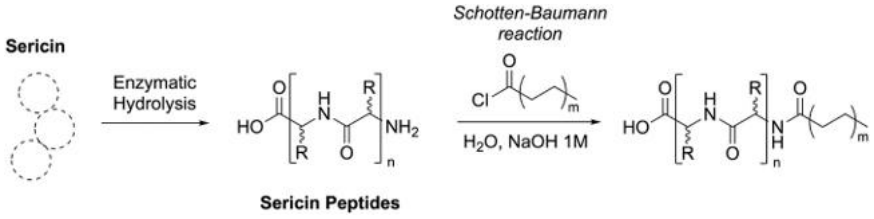
to the formation of an oxonium ion intermediate that undergoes nucleophilic substitution. As a result, serine residues are converted into 3-chloroalanine (Cl-Ala). However, following the functionalization process, the yield of Cl-Ala was directly affected by the characterization (0.397 mmol/g compared to Δ serine: 1.239 mmol/g), due to partial decomposition of the modified serine residues during hydrolysis. The subsequent substitution with propyl amine was successful; nonetheless, the reaction conditions were not optimal for a complete conversion, as some Cl-Ala residues persisted. To achieve more complete substitution, the employment of stronger nucleophiles would be necessary. Regarding threonine, a decrease of 0.222 mmol/g (22.4 % of the initial Thr content) was deducted by GC-MS analysis, indicating that the employed chlorination conditions are sufficiently reactive to facilitate the substitution at secondary hydroxyl groups. As previously discussed, achieving strict chemoselectivity under these conditions remains challenging, given that MsCl exhibits highly reactive behavior towards various nucleophilic FGs without inherent discriminatory capabilities. The authors reported a significant reduction in lysine content (0.105 mmol/g), which was attributed to the formation of lysinoalanine (2,9-diamino-4-azadecanoic acid) derivatives, resulting from the reaction of lysine with Cl-Ala. Concerning the bromination process, the mechanistic pathway corresponds to the one depicted on Scheme 7. Due to the steric hindrance of PPh_3, it is expected that only the primary OH groups are accessible and subjected to functionalization in this reaction. In comparison to the Cl-Ala, the brominated counterparts (Br-Ala) proved to be exceedingly difficult to quantify, as they rapidly decompose under the analytical conditions; the overall yield of Br-Ala, estimated after subsequent steps, was marginally lower than Cl-Ala. This reduction was attributed to the decreased nucleophilicity of the bromide ion in polar aprotic solvents, such as DMF. Conversely, the Br-Ala derivatives exhibited greater reactivity than the corresponding Cl-Ala, reflecting the enhanced reactivity of C-Br bonds when compared to C-Cl ones. In summary, Chlorination mediated by MsCl shows higher conversion efficiency at the expense of chemoselectivity, regioselectivity, and the reactivity of the chloro-sericin, while bromination mediated by NBS-PPh_3, which is less efficient in terms of conversion, is more selective, and the derivatives obtained are more reactive Consequently, the couple NCS-PPh_3 (NCS: *N*-chloro succinimide) may offer a viable strategy to address the issue of limited selectivity.

In an interesting study published by Saleem et al. [96], the authors sought to develop an improved and economical dyeing process for silk fabrics. To achieve this, they introduced functionalized biopolymers onto the surface of the silk textiles.This necessity was born from the increasing requests by the industry due to the lack of dyeing effectiveness. Typically, silk is dyed with acid dyes; however, these dyes are characterized by inadequate color fastness. As a consequence, reactive dyes have increasingly supplanted acid dyes due to their improved performance [97]. Nonetheless, reactive dyes also present significant limitations, particularly under high pH conditions, which is a mandatory environment for dye fixation [98]. Additionally, to maximize the exhaustion process, substantial quantities of electrolytes are required, which can pose environmental and economic challenges. Nevertheless, it was noted how the fabric surface modification with natural biopolymers actually represents an economical and more environmentally friendly alternative [99]. It is important to consider that natural fibers may facilitate the growth of bacteria and viruses owing to their inherent structural characteristics and proximity to human skin, potentially leading to various health and performance-related issues [100]. Therefore, the development of antibacterial textiles represents a viable approach to mitigating these concerns. Knowing that, the authors functionalized sericin with 2,3-epoxypropyl-trimethylammonium chloride (EPTAC, Scheme 8) to enhance its dye uptake, and activity against microorganisms and its silk fabric affinity.

6.75 g of low molecular weight sericin ($M_w < 5000$ g mol^{-1}) was dissolved in 100 mL of water together with 1 g of $CaCl_2$ and 2.25 g of EPTAC. Under stirring conditions, the temperature was raised to 50 °C, pH was adjusted to 8.0 for 5 h. Afterwards, the cationized sericin was coagulated in ethanol. The authors opted to investigate the modification of silk fabrics utilizing a range of spectroscopic techniques. Morphologically, the treated textiles exhibited a notably rougher surface, attributable to the deposition of reacted particles. These morphological observations were corroborated by FTIR and XPS analyses. In the FTIR spectra, in addition to characteristic signals associated with the protein backbone, an absorption band corresponding to the alkyl constituents of the quaternary ammonium groups was identified. Concurrently, XPS analysis revealed an increase in nitrogen content following treatment. Furthermore, peak deconvolution provided additional confirmations of the presence of quaternary ammonium functionalities in the fabric. The most notable findings pertained to the evaluation of the final physicochemical properties of the treated fabrics, whereby functionalized sericin was covalently crosslinked to the textile substrates, in comparison to untreated fabrics. These comparative analyses were conducted within the context of a dyeing process; notably, the modified fabrics demonstrated superior performance relative to conventional salt-assisted dyeing of untreated silk. Specifically, the treated textiles exhibited a higher dye uptake and achieved a dye fixation of 96.5 %, in contrast to 73.8 % observed in the untreated counterpart. Additionally, the modified ones displayed enhanced color strength while obviating the need for salt additives. The increased dye affinity was attributed to the presence of positively charged groups on the fabric surface, as well as the inherent affinity of sericin, which is rich in nitrogen-containing FGs, facilitating improved interactions with the dye molecules. The enhanced fixation and color strength observed were attributed to the increased availability of polar FGs resulting from the incorporation of sericin.



Scheme 8. Sericin's functionalization by ring-opening nucleophilic attack.



Scheme 9. Acylation of sericin peptides.

Conversely, both treated and untreated silk fabrics exhibited comparable levels of color fastness. From a mechanistic perspective, the modification imparted a significant improvement in tensile strength in both wrap and weft directions. These enhancements were ascribed by the authors to the numerous intermolecular interactions, such as hydrogen bonds and electrostatic forces, arising from the FGs present in sericin, silk, and the introduced quaternary ammonium groups. Furthermore, the antibacterial efficacy was notably enhanced as a result of the modification; as a fact, the introduced quaternary ammonium salts can easily penetrate the microbial cell walls. This interaction induces membrane disruption and leakage of cellular contents, subsequently leading to growth inhibition and cellular death. In conclusion, the authors developed a modified sericin-based silk fabric that maintains comparable color strength white exhibiting improved functional properties and minimizing process residues, thereby positively influencing wastewater treatment. Additionally, the resulting composite facilitates a more efficient dyeing process that consumes reduced amounts of water, energy, and chemical reagents compared to the most common industrial processes.

Currently, surfactants constitute some of the most extensively utilized molecules across various aspects of daily life and numerous industrial sectors. Nonetheless, their synthesis often necessitates harsh conditions, which are recognized for their suboptimal safety profiles and environmental impact [101]. Consequently, the pursuit of greener, more sustainable alternatives has gained considerable momentum over recent decades. In this context, bio-based materials have emerged as particularly promising candidates for the development of novel amphiphilic molecules [102]. Among these, proteins are considered highly suitable due to their intrinsic foaming and emulsifying properties [103]. However, their inherently high MW significantly constrains their practical applications. A fundamental design principle involves conceptualizing amino acids or peptides derived from proteins as the hydrophilic component, which can be functionalized with hydrophobic residues, such as long alkyl chains, to afford amphiphilic characteristics. To achieve this, the grafting of hydrophobic moieties onto proteins is commonly conducted under the *Schotten-Baumann* reaction conditions [104]. This reaction involves the nucleophilic attack of an amine group (or other nucleophiles) on an acyl chloride, mediated by a base. Notably, this methodology is typically performed under two-phase conditions, wherein the reaction mixture comprises an aqueous phase and an organic solvent. The aqueous phase generally contains the base, which serves to neutralize the acid generated during the acylation process, thereby facilitating the formation of the desired bond. In this context, the research group led by Nesterenko [58] published a study focusing on the functionalization of sericin through the attachment of long alkyl chain acyl chlorides to synthesize innovative bio-based surfactants. Recognizing that the inherently high MW of native sericin significantly limits its applicability, and noting the scarcity of the literature regarding the utilization of low molecular MW sericin obtained via enzymatic hydrolysis, the authors chose to explore this avenue further. They proceeded to functionalize enzymatically hydrolyzed sericin and conducted a characterization of the resulting lipopeptides.

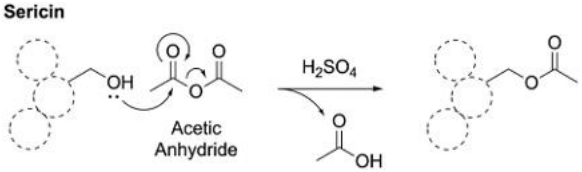
Specifically, the sericin peptides were dissolved in DI-water (0.5 % w/w), heated to 50 °C with a fixed pH of 9.0 using a 1 M NaOH solution. Then the long-chain fatty acid acyl chloride was added to the main batch with a 1:1 molar per terminal -NH_2, maintaining the pH at 9. After completion, the solution was neutralized, and the product was recovered by freeze-drying.

Despite the novelty and the thematic relevance of the study, certain aspects warrant further consideration and discussion. The authors successfully employed enzymatic hydrolysis to generate a peptide distribution based on different MW. These peptide fragments were subsequently acylated using acyl chlorides with alkyl chains of varying lengths (10, 12, and 14 carbons). As discussed in the section on general protein functionalization, the use of acyl chlorides is an effective strategy for selectively modifying the diverse functional groups inherent to sericin; however, their high reactivity introduces limitations in chemoselectivity, resulting in non-specific reactions, eventually compromising the process. In this regard, the authors did not thoroughly address the issue of FGs selectivity. The study primarily focused on the targeting of specific FGs, such as amines, without investigating or considering the potential for subsequent modifications of other amino acids, such as serine or threonine. Additionally, the reaction was conducted in an aqueous medium without the inclusion of organic solvents. Although acyl chlorides are liquid, their quantities were not explicitly specified, and without discrete volumes, it is unlikely that the reaction system presented a two-phase system characteristic of the *Schotten-Baumann* method. Under these conditions, it is more plausible that the acyl chlorides were dispersed as droplets within the aqueous phase. Moreover, the validity of the last assumption hinges on the premise that acyl chlorides do not react with water, which is inherently impossible. In practice, incorporating an organic solvent is advantageous also for mitigating the exposure of the reagents to water. Consequently, the reported procedure is possibly missing relevant details regarding the overall effectiveness of the methodology. The choice to omit organic solvents might be attributed to the desire to utilize solely aqueous

media, potentially to address solubility issues associated with peptides, since the resulting peptides exhibited high solubility in water. NMR and FTIR were used to confirm the successful acylation of the peptides. However, the reported spectra exhibited significant signal overlap, rendering the confident identification of primary amide challenging. The NMR revealed two peaks suggestive of the alkyl chains; yet, in the spectrum of the purified lipopeptides, these peaks were present at very low intensities, raising questions regarding their definitive assignment. Conversely, it was observed that the modified peptides demonstrated a reduction in surface tension, an effect that correlated with the functionalization of sericin with the alkyl groups. Nonetheless, there remains a lack of consensus within the existing literature concerning the general trends associated with such modifications. Therefore, although the authors proposed the synthesis of innovative bio-based surfactants, an endeavor that indeed represents a meaningful contribution towards advancing the field, the limitations observed in the final properties, coupled with uncertainties in the characterization data, undermine definitive conclusions regarding the overall effectiveness of the approach. Nonetheless, this study serves as an important illustrative example, highlighting the critical importance of operational conditions when working with complex systems such as sericin or its peptides. It underscores that meticulous optimization of experimental parameters is essential to realize the full potential of such biopolymers in surfactant development and other fields.

In an effort to enhance the intrinsic physical and mechanical properties of silk fibers, the research group of Haque [105] conducted a study in which they functionalized silk via treatment with acetic acid anhydride. This approach capitalized on the abundant presence of FGs, particularly polar side chains, present in the components of silk, notably sericin, despite its comparatively low proportion within the silk fiber.

They immersed 10 g of raw silk fibers in 150 mL of glacial acetic acid and kept them for 1 h at 25 °C. The fibers were then pressed out on a Buchner funnel, then soaked in 180 mL of acetic anhydride solution (5–15 wt%), added with 1 mL of concentrated H_2SO_4 at 25 °C. The mixture was then shaken vigorously for 1 min, after which 10 mL of the anhydride solution was added. The last step was repeated twice. After holding the mixture for 5 min, the fibers were washed with distilled water and dried at 80 °C for 6 h (Scheme 10). Regrettably, the authors did not provide comprehensive experimental evidence confirming the occurrence of acetylation beyond the assessment of certain physicochemical properties; however, the complexity of the characterization is a crucial and objective limit, especially for such simple introduced moieties. It is well established that organic anhydrides serve as a prominent class of reagents for acylation reactions across a broad spectrum of FGs, largely owing to their high reactivity. However, achieving precise selectivity among the various nucleophilic sites involved remains a considerable challenge, especially under the reaction conditions employed, which may promote non-specific modifications. Regrettably, Haque's research group did not explore this aspect in their investigation. Although they acknowledged the presence of various potentially reactive amino acid side chains, they assumed that the acylation predominantly targeted the free hydroxyl group, without further exploring the influence of other moieties. Conversely, the researchers initially subjected the silk fibers to treatment with glacial acetic acid (also produced as a byproduct during acetylation). Given that some of the FGs within the silk are basic, it is plausible that these residues were converted into their corresponding acetic salt derivatives,



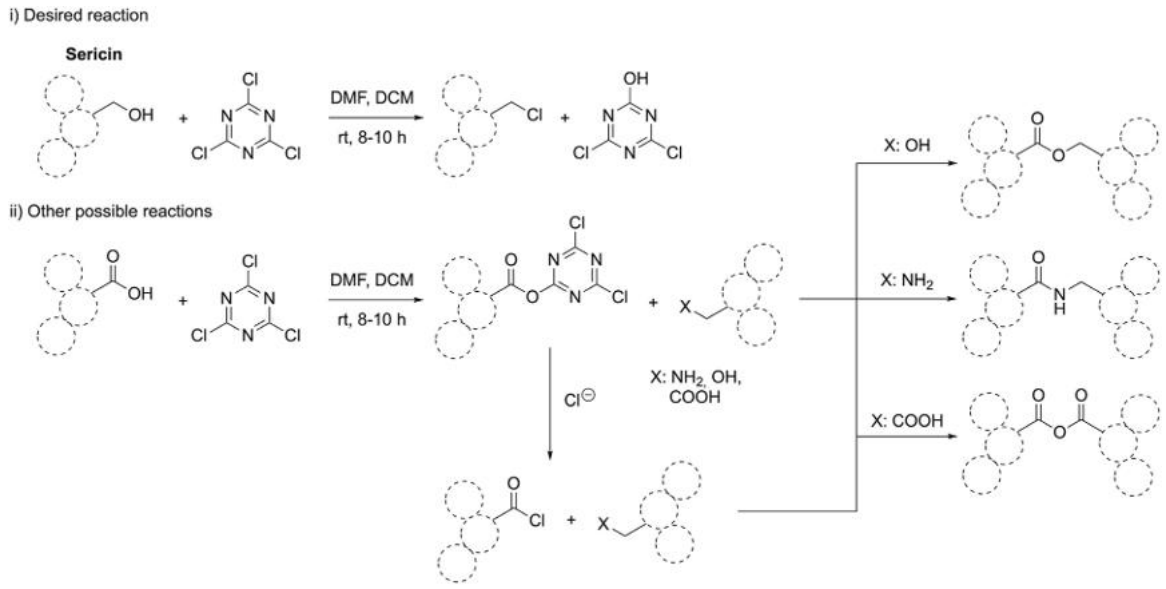
Scheme 10. Sericin acetylation with acetic anhydride.

mitigating their reactivity. Concerning the mechanical properties, the study reported an increase in the elastic modulus of the treated fibers correlating with higher concentrations of acetic anhydride; on the other hand, the elongation at break diminished following a similar trend. Furthermore, the acetylated fibers demonstrated enhanced thermal stability relative to the untreated counterparts. The introduction of acetyl groups not only influenced the discussed mechanical properties but also affected the moisture content of the fibers. Specifically, the acetylated fibers exhibited reduced hydrophilicity. The authors attributed this behavior to the successful acetylation of the native polar groups inherent to silk, which naturally possess affinity for water; as a result, the modification increased the overall hydrophobicity.

In 2024, the research team of Pattanaik [71] published a study concerning bio-based polymers, aimed at enhancing the dyeing properties of textiles. Specifically, they investigated the functionalization of sericin with cyanuric chloride to introduce chlorine atoms onto the protein backbone. The modified sericin was subsequently employed directly during the dyeing process of fabrics of various materials, with the objective of improving dye fixation and colorimetric performance.

They added 2 g of sericin and 2 g of cyanuric chloride to a mixture of 8 mL of DMF and 60 mL of DCM. The mixture was stirred for around 8 h at room temperature. Sericin chloride, with a brown color, was obtained then and filtered after precipitation.

As illustrated in Scheme 11, sericin was functionalized with cyanuric chloride, a compound that, despite its conventional nomenclature, comprises a triazine ring substituted with three chlorine atoms. The core of the reaction mechanism hinges on the reactivity of this aromatic reagent; specifically, the highly electron-deficient nature of the triazine ring substantially enhances its susceptibility to nucleophilic aromatic substitution (S_N^{Ar}). According to the authors, in addition to the hydroxyl groups, other FGs present in sericin may participate in the reaction, not only through direct reaction with cyanuric chloride but also via intermediates formed during chlorination of the chlorinated derivative, potentially resulting in internal crosslinking. However, aside from mentioning this possibility, the discussion regarding such crosslinking remained inconclusive. If internal crosslinking indeed occurs, it is important to consider that the spatial arrangement of amino acids and the conformational structure of sericin could impose steric constraints, thereby limiting crossliking phenomena and ultimately leading to the formation of a stable chlorinated protein conformation. Using FTIR, the authors confirmed the successful modification by identifying the characteristic absorption band attributable to chlorine atoms. However, no further analytical investigations were conducted. Although visual inspection of the FTIR spectra (comparing native and modified sericin) revealed observable differences, a more detailed analysis could have provided valuable insights, particularly in the context of the previously discussed hypotheses. Additionally, XRD analysis indicated an increase in both the number and intensity of diffraction peaks, suggesting an enhancement in crystallinity likely attributable to an increased content of β-structures. From a morphological perspective, the fabric fibers exhibited increased surface roughness following treatment with the chlorinated sericin, attributable to the formation of a fine coating derived from the modification process. Subsequent dyeing procedures revealed that treated fabrics exhibited enhanced color strength, likely as a consequence of the protein's interaction with the dye molecules; however, no significant differences were observed in color fastness when compared to untreated fabrics. Additionally, these modified materials demonstrated antimicrobial activity against both gram-positive and negative bacterial strains. These functionalization strategies pave the way for further exploration of sericin's applications. The following sections delve into two more specific methods, namely grafting and crosslinking, which offer powerful tools to modify sericin's structure for diverse bio-based material applications.



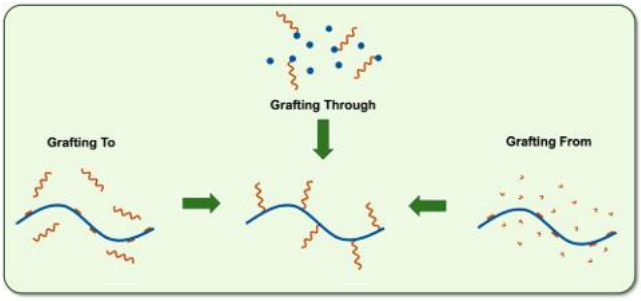
Scheme 11. Reaction of sericin with cyanuric chloride.

3.3. *Covalent grafting of sericin*

Chemical grafting refers to the process of obtaining a macro-system by attaching several functionalized fragments to a main chain through chemical reactions. Specifically, chemical covalent grafting is a technique for surface or external modification that involves the covalent bonding of a functional group, functionalized side chain, or a polymer (generally referred as the pendant) to the substrate's surface or backbone. However, the term "surface" could be misunderstood when applied to macromolecules, as it may not necessarily refer to the outermost layer in this context [106,107].

Grafting typically requires the presence of complementary FGs on both systems, the backbone and the pendant. Then, the covalent attachment is typically performed via dedicated chemical reactions such as acylic nucleophilic substitution (S_N^{Ac}), free radical reactions, or click chemistry (*Diels-Alder*, 1,3-dipolar cycloaddition/CuAAC, tensioned rings/aziridine-epoxide opening, thiol-ene, and others) [108–111].

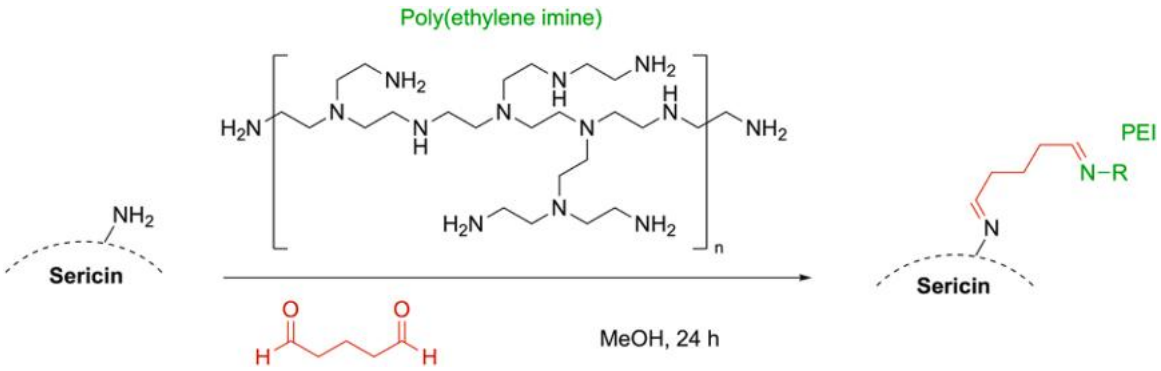
The pendant does not have to be a long chain initially; it can also be formed through a subsequent reaction. This approach is commonly used in grafting copolymerization, where simple monomers are grafted onto the main backbone, and then copolymer pendants are formed through further polymerization. The grafting strategy can be classified into three main methodologies, which differ in how the final grafted system is obtained and how the chains grow (Scheme 12): "grafting-onto," "grafting-from," and "grafting-through", where the first two are the most used for proteins. Due to the wide range of applications in biomaterials and biomedical fields, the possibility of covalently grafting proteins onto a specific material surface is frequently discussed.



Scheme 12. Grating strategies.

The group of Won Kwak and Hoon Lee [112] published a work in 2018 regarding the sericin beads functionalization with a synthetic polymer (Scheme 13), the polyethylenimine (PEI), to obtain a bio-based material capable of adsorbing dangerous metal ions from aqueous media (such as Cu^{2+}, Cd^{2+}, Hg^{+}, Zn^{2+}, and especially Cr^{6+}). The same authors previously tried to use unfunctionalized sericin macro-sized beads as "metal ions sponge", but they were unsatisfied with the performance [113]. Thus, in order to enhance the adsorption ability of the material by covering (i.e., grafting) the surface of the beads with PEI by exploiting the amino groups present in the sericin. Since both PEI and the sericin possess the amino functional group, to connect the two systems, they opted for glutaraldehyde as a grafting agent via a grafting-through approach.

Specifically, they dispersed 2.0 g of sericin in 10 % w/w PEI in methanol, stirring at 100 rpm for 24 h. The mixture was then diluted in 100 mL of methanol, followed by the addition of 4 mL of a 25 % w/v glutaraldehyde (GA) solution. The mixture was then stirred for an additional 24 h to promote the crosslinking reaction. The final product was washed with DI water to remove the unreacted glutaraldehyde and stored in DI water at 5 °C until use. Following the sericin grafting procedure with PEI, the researchers characterized the resulting modified beads employing a suite of spectroscopic techniques. SEM coupled with elemental analysis EDX (Energy-dispersive X-ray Spectroscopy) revealed an increase in bead diameter relative to the unmodified material, indirectly indicating successful grating. Furthermore, in elemental analysis EDX spectrum it was demonstrated a marked increase in carbon and nitrogen content, concomitant with a decrease in oxygen proportion. These findings were substantiated by FTIR; the spectra of the modified beads displayed characteristic absorption bands corresponding to PEI, confirming its successful incorporation. Notably, the three amide bands characteristics of sericin remained unaltered, implying that secondary structure of the protein was preserved during the modification process. Given the compositional profile of sericin, it would be pertinent to investigate whether serine residues actively participate in the grafting reaction, considering their abundance relative to free amine groups. This hypothesis could be substantiated by the elemental analysis conducted by the authors; however, it appears that this possibility was not explicitly addressed in the discussion. Furthermore, a critical consideration regarding the work by Lee's research group is that their grafting methodology primarily relies on imine formation, presumably involving only lysine residues. It is well established in the literature that the synthesis of imine-containing compounds necessitates the removal of



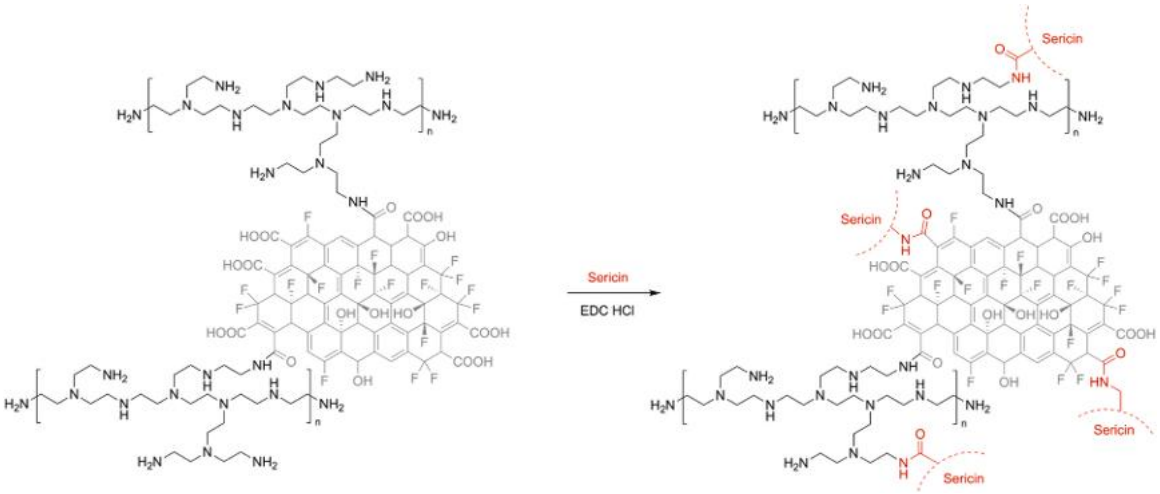
Scheme 13. Sericin grafted with poly(ethylene imine).

water (commonly achieved through the use of inorganic salts or azeotropic distillation) since the formation of imines proceeds via equilibria that are sensitive to water presence. The existence of residual water can readily shift these equilibria, potentially inhibiting or reversing imine formation. The susceptibility of imine-grafted compounds to hydrolysis presents notable challenges, particularly with regard to their storage stability and long-term integrity. In the present study, the authors did not specify whether the reagents used were anhydrous nor did they detail any protocols implemented to minimize moisture exposure. Instead, they employed aqueous washing procedures and stored the modified material in DI-water, conditions that are potentially detrimental to the stability of the imine bonds. Such practices may facilitate hydrolysis cleavage of the imine linkages, thereby compromising the efficacy and reliability of the grating strategy and undermining the stability of the final product. Notwithstanding these considerations, the modified beads were evaluated for their efficacy as adsorbent for chromium ions. Initially, the authors examined the behavior of PEI-modified sericin beads across a range of pH conditions, recognizing that both acidic and alkaline environments can profoundly influence the structure and charge of chromium ions. The adsorption performance was therefore assessed from highly acidic to highly basic pH levels. Results demonstrated a generally enhanced capacity for Cr(VI) removal by the modified beads compared to unmodified ones, particularly at lower pH values. This improved performance is attributable to the increased availability of positively charged amine groups of the PEI-modified beads, which enhance electrostatic interactions with negatively charged metal species. Conversely, at alkaline pH, the accumulation of negative charges on both the adsorbent surface and the metal ions led to electrostatic repulsion, thereby impairing the efficacy. The chromium removal efficacy of the modified material was markedly superior to that of the unmodified one. The authors further observed that the adsorption capacity was affected by competitive binding from other monovalent to trivalent anions, which are likely to be inherently present in wastewater samples. Consequently, a gradual decline in removal efficacy was noted as the concentration of these ions increased. Concerning the underlying adsorption mechanism, the authors demonstrated that the process could not be adequately described by a simple adsorption model alone. XPS analysis revealed the concurrent presence of both Cr(VI) and Cr(III) species, in a ratio of approximately 75:25, indicating that chromium reduction occurs simultaneously with adsorption during the process. This phenomenon has been previously documented and extensively discussed in the literature, with studies indicating that the OH groups inherent to sericin and PEI-modification play a role in the reduction of chromium [114,115]. Regardless of whether the reduction occurs directly on the surface of the absorbent or through an indirect mechanism, the observed decrease in Cr(VI) concentration in the solution was accompanied by a modest increase in Cr (III) levels, both in the wastewater and on the bead surface. Finally, regarding the desorption of various chromium ions from the beads, the authors observed that acidic agents significantly influenced this process, likely by competing with the adsorbed ions for binding sites. In the end, they obtained a bio-based reusable material, born from the union of natural and synthetic sources, with an improved adsorption capacity when compared to pure sericin.

With a similar concept, though with different underlying chemistry, Mehrali *et al.* [116] developed a novel method for the synthesis of a water-soluble fluorinated graphene oxide (FGO) modified with PEI anchored to sericin as a drug's nanocarrier. The scope of the work arises due to some critical issues of FGO in terms of medical applications. It was noted that the presence of amino groups could resolve the problems briefly mentioned earlier, and the use of sericin as a biodegradable component in FGO led to the definition of a new and interesting "apparatus". Furthermore, due to sericin's properties, such as its active pH response, biological activities (antioxidant, antibacterial, anticancer, anticoagulant), and the availability of various functional groups that can be exploited to form novel structures, the protein has been found to be an optimal candidate for this goal. In the end, they successfully designed and obtained an FGO nanocarrier modified with PEI and sericin polypeptide for the nuclear delivery of the antitumor drug curcumin. Grafting onto is the strategy used in this example.

Specifically, after the oxidation of the fluorinated graphene sheet and the PEI amidation (FP), 25 mg of the FP copolymer and 2.4 g of EDC·HCl were dispersed in Milli-Q water using a sonicator at a controlled pH of 5.0. The resulting solution was then added to a 1 mg/mL solution of sericin, stirred overnight, and then dialyzed. After freeze-drying, the final product (FPS) was obtained (Scheme 14).

Following the functionalization process, the authors employed a variety of analytical techniques to characterize their products. Given the UV-sensitive nature of certain components, UV-Vis spectroscopy was utilized to confirm successful incorporation of sericin. This was evidenced by a characteristic blue shift in the absorption peak of the PEI-modified graphene oxide, alongside the detection of a distinct protein-specific peak attributable to the π-π* transition of peptide bonds containing aromatic side chains. Additionally, UV-Vis spectroscopy proved instrumental in evaluating the loading of curcumin onto the system, achieving by monitoring the characteristic peaks associated with π-π* stacking interactions between curcumin and the FPS. The successful incorporation of sericin was further substantiated by FTIR, which identified characteristic protein-associated bands. These findings were corroborated by TGA, wherein the observed weight loss corresponding to specific thermal degradation stages provided evidence of successful grafting. Additionally, XPS analysis, including peak deconvolution (particularly of the nitrogen core-levels peaks) served as supplementary confirmation of the functionalization process. Ultimately, the authors developed a system featuring hydrolyzable amide bonds that are sensitive to the mildly acidic tumor microenvironment; this pH-responsive behavior facilitates cellular penetration and promotes the dissociation of the nanocarriers, enabling rapid drug release. The resulting product demonstrated drug loading capacity, drug release under acidic conditions, cytotoxicity, cellular uptake and nuclear delivery, as well as

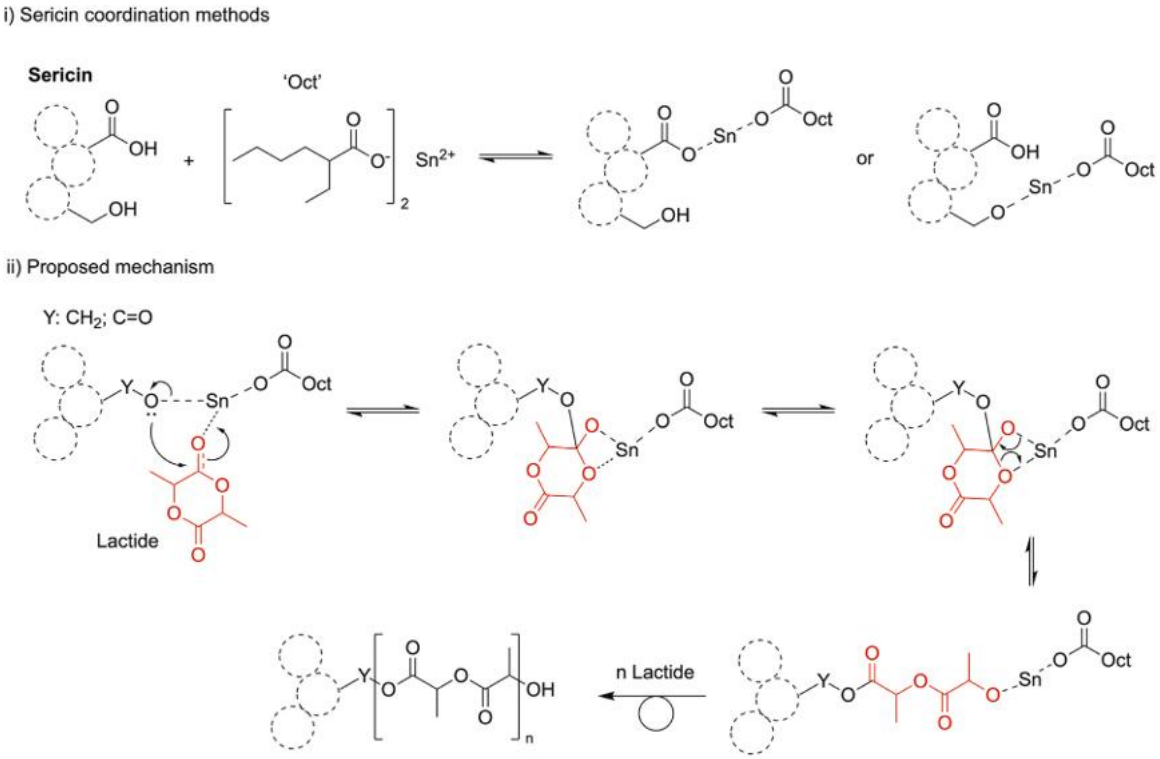


Scheme 14. Sericin grafted on fluorinated PEI graphene sheet.

apoptotic effects.

In 2019, Boonpavanitchakul *et al.* [117] published a paper, where they used sericin as a bio-initiator to perform a ring-opening polymerization (ROP) of polylactide directly on the backbone of the protein, effectively grafting polylactic acid (PLA), a polymer, on sericin (Scheme 15). Nowadays, PLA is widely used in many fields of applications since it's a bio-derived compound and biodegradable polyester, often employed as a matrix in biocomposite materials. However, pure PLA suffers from some limitations, such as strong hydrophobicity, high crystallinity, and low degradation rate. To address these issues, in recent years, many researchers have dedicated time and resources to improving the properties of PLA by incorporating it with natural macromolecules. In this context, one of the most promising systems is sericin, owing to its high polar amino acids' side chains, which can serve as anchors for polymerization reactions. To graft a polymer onto the surface of a protein, an appropriate technique must be selected. Among the most classical grafting approaches, "grafting-onto", "grafting-from", and "grafting-through," the second method is particularly noteworthy, as it enables the active modification of the chemical and physical properties of the grafted polymer for a specific application. Many research groups have explored the use of natural sources to obtain grafted polymers [118–120], reinforcing the idea that sericin is an optimal candidate for developing polymer-protein conjugates suitable for biomedical applications. In particular, in the work published by the authors, sericin is not only the "substrate" on which a polymer is grafted, but also the reaction macro-initiator. This is due to its free hydroxyl groups, which interact with the catalyst (Tin(II) 2-hexylethanoate) to catalyze the ROP of lactide.

To produce the sericin grafted with PLA (SS-g-PLA), *L*-lactide, powdered sericin, and $Sn(Oct)_2$ were added to a round-bottom flask,



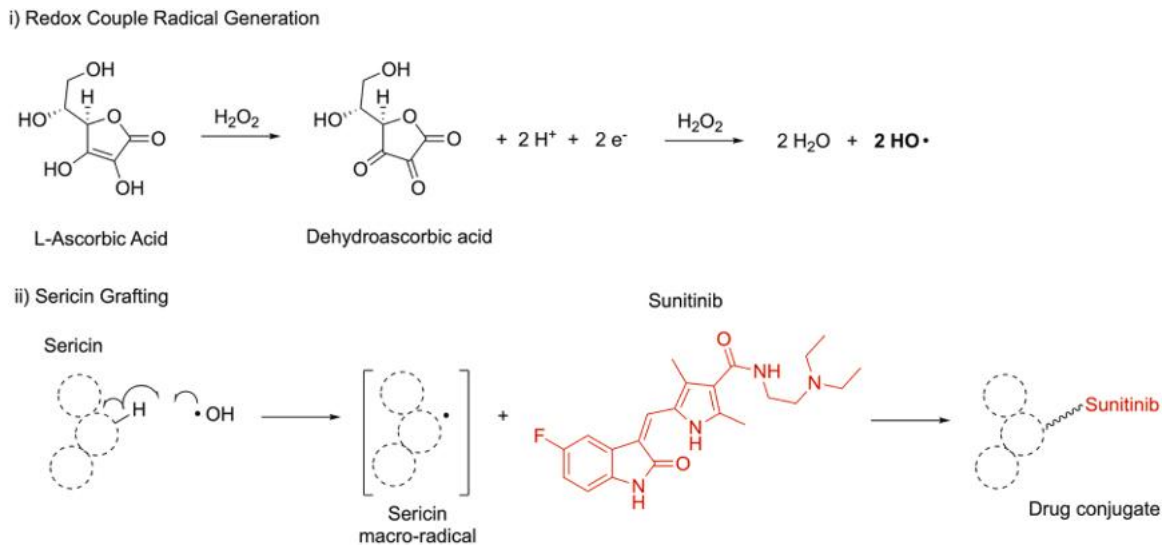
Scheme 15. Sericin-poly lactic acid grafting.

which was then purged with inert gas under vacuum and equipped with a stirrer. The flask was heated to 140 °C for 4 h while being magnetically stirred at 400 rpm to promote polymerization. The final product was then washed with several solvents to remove impurities and unreacted sericin, followed by centrifugation and drying at 40 °C. The proposed mechanism of the reaction is illustrated in Scheme 15. Regardless of the specific coordination geometries that the catalyst may adopt when interacting with sericin, the hydroxyl groups of serin residues are hypothesized to coordinate with the metal center. Subsequently, Tin(II) 2-ethylhexanoate engages with the carboxyl group of the *L*-lactide monomer, bringing the hydroxyl group into close proximity with the electrophilic carboxyl carbon. This spatial arrangement facilitated the hydroxyl group's nucleophilic attack on the carboxyl, leading to the formation of an intermediate wherein the metal center coordinates with the oxygen atoms of the former carboxyl and the adjacent geminal hydroxyl. Through a rearrangement that restores the carboxyl functionality, the six-membered lactide ring opens, allowing the polymerization to proceed. This mechanism results in a graft copolymer (SS-*g*-PLA) with a significantly lower MW compared to PLA obtained via conventional ROP methods. It is well established that this type of polymerization can be initiated by other FGs, such as primary amines of amino acids. However, as discussed by the authors, considering the compositional profile of sericin, it is hypothesized that the primary OH groups of serine play a predominant role as the initiation site, owing to its higher reactivity relative to the other FGs. Furthermore, an increase in sericin content correlates with a concomitant rise in the number of initiating sites. The observed reduction in MW was attributed to potential cleavage of shortened PLA chains that preferentially interact with sericin rather than forming extended copolymer chains. The final composite underwent a comprehensive characterization process, employing a variety of analytical techniques. The grafted sericin was initially characterized using one- and two-dimensional NMR spectroscopy. The authors successfully assigned each observed peak to its corresponding proton or carbon atoms, including the identification of the carboxyl carbon associated with the grafted PLA, thereby providing definitive confirmation of the successful chemical modification. These NMR findings were corroborated by XPS) and FTIR. In the case of XPS, the presence of the grafted polymer was evidenced through analysis of the relative ratio intensities among specific molecular moieties. Concurrently, FTIR spectroscopy demonstrated the increasing presence of grafted PLA by the progressive intensification of characteristic absorption bands, which were absent in pristine sericin, thus further confirming the successful grafting process. From a thermal perspective, TGA and DSC demonstrated that the grafted systems exhibited lower degradation temperatures and glass transition temperatures compared to pristine PLA. This behavior was primarily attributed to the presence of sericin, which facilitates greater chain mobility relative to neat PLA, thereby enabling chains to reorganize into crystalline regions at reduced temperatures. Furthermore, the degree of crystallinity was observed to notably decrease as the amount of sericin increased. Interestingly, the resulting SS-g-PLA copolymer displayed solubility in a range of organic solvents with varying polarities, such as DMSO, DMF, chloroform, and tetrahydrofuran, contrasting with the insolubility or limited solubility of both unmodified sericin and pure PLA in these solvents. The distinctive behavior of the final PLA-sericin macromer can be attributed to the combined influence of polar FGs originating from sericin and the inherent hydrophobicity of the grafted polymer. Additionally, it is posited that the presence of these functional groups may enhance properties such as biocompatibility, moisture absorption, and degradability, thereby contributing to the overall performance and potential applicability of the material.

In the work published by Scrivano *et al.* [121], the authors demonstrated that sericin could be an optimal candidate for the construction of bioconjugates for drug delivery systems (DDSs). These systems have gained significant attention over the years due to the advantageous properties of biopolymers to which the drug is attached. Various studies in the literature support that conjugating small anticancer drugs with natural or synthetic polymers is a reliable strategy to achieve a more stable drug-conjugate while maintaining its activity [122,123]. In this work, the authors chose sericin as a substrate for grafting to obtain a bioconjugate, using sunitinib (SUT) as a model drug. SUT is a small molecular tyrosine kinase inhibitor (smTKI) with anticancer and anti-angiogenic activities. However, SUT has limited solubility in water and ethanol, an important factor that significantly impacts both bioavailability and cell permeability. Thus, the protein-drug bioconjugate could improve solubility.

To obtain the conjugate, 40 mL of water and 15 mL of ethanol were mixed with 200 mg of sericin and 40 mg of sunitinib malate in a beaker. Then, 2.5 mL of H_2O_2 (30 % v/v) containing 83.5 mg of *L*-ascorbic acid was added. The mixture was stirred at room temperature for 1 day. The final product was then purified by dialysis and lyophilization. The research group obtained the macromolecular system (SER-SUT) via a single-step free radical grafting reaction of sunitinib onto sericin through a click reaction (Scheme 16). As a redox initiation system, the hydrogen peroxide/*L*-ascorbic acid couple was selected due to its solubility in aqueous media, biocompatibility, and capacity to operate effectively under aqueous conditions without producing toxic by-products. Regarding the reaction mechanism, the authors did not conduct a detailed investigation into the selectivity of radical formation and subsequent addition. Instead, they based their assumptions on existing literature [121], which suggests that the reactive sericin macro-radical is likely generated from polar, amino acid–based functional groups susceptible to hydrogen abstraction. These radicals are presumed to subsequently form covalent bonds with the drug molecule. However, in the absence of direct experimental validation of the specific radical pathways and site selectivity within the sericin structure, the proposed mechanism remains speculative. It is important to highlight that, within this domain of application, the molecular structure of the drug typically remains largely unaltered, as it must retain specific structural features and FGs essential for effective interaction with the target binding site. Consequently, despite the proposed formation of a covalent bond between the drug and sericin, it is plausible that SUT did not undergo significant structural modifications. To verify the grafting process, the authors employed FTIR and UV-Vis. In the first case, the assessment of reaction success was primarily based on the detection of the C-F stretching band; however, given the uncertainty surrounding the reaction mechanism, it is challenging to identify additional reliable spectral indicators. Similarly, UV-Vis corroborated the findings through the detection of two characteristic adsorption peaks corresponding to sericin and SUT within the grafted system. Furthermore, this analytical technique enabled quantification of the amount of SUT successfully graded onto the conjugate, which was determined to be approximately 6.5 mg per gram of the product. These relatively low grafting yields highlight some limitations in the characterization of the final product and suggest a suboptimal efficiency of the process. Nevertheless, the resultant bioconjugate demonstrated enhanced gastrointestinal bioavailability and membrane permeability, alongside the sericin-SUT conjugate exhibits a threefold increase in efficacy compared to unmodified SUT.

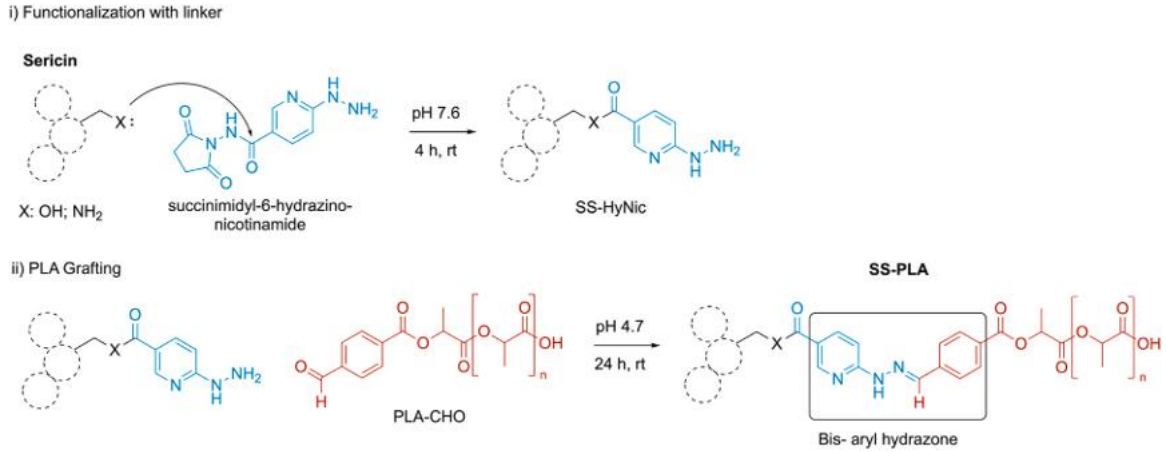
In the same context of DDS based on sericin, in 2020, the group of Magaraphan [125] published a paper focusing on the synthesis of bioconjugates. In this context, in recent years, amphiphilic protein-polymer conjugates have attracted attention as novel DDS materials because they have the property of self-assembling into a wide range of nanoscale morphologies, such as micelles. Another interesting point is that drugs can be loaded into these materials and released in response to a stimulus, such as the pH of a specific environment. To obtain a bioconjugate with sericin, a hydrophobic polymer must be covalently bonded to the hydrophilic protein to achieve the amphiphilic property. Since the final goal is to develop a DDS, the polymer ideally needs to be biocompatible, such as polylactic acid (PLA). PLA was previously discussed and, not surprisingly, is present again in another work on sericin. The authors, to



Scheme 16. Sericin radical activation [124] and grafting with sunitinib.

link PLA to sericin (SS), opted for a more complex synthesis using a different linker, such as the bis-aryl hydrazone linker (Scheme 17). This choice was made because the presence of aromatic rings provides UV absorption at 380 nm, which allows quantification of the conjugation between the protein and the polymer. The final SS-PLA conjugates self-assembled into spherical micelles in aqueous solution, where doxorubicin (DOX) was loaded as the drug.

To do this, sericin is first treated with the linker succinimidyl-6-hydrazinonicotinamide in a buffer solution for 4 h at room temperature. Then, the "labeled" sericin (500 μL of 6.3 μM in a buffer solution) is treated with a solution of functionalized PLA (PLA-CHO) containing terephthalaldehyde (500 μL of 5.4 μM) in anhydrous DMF at a controlled pH of 4.7 and room temperature for 24 h. The final product is obtained by centrifugation and lyophilization. Compared to other studies, the authors employed a multistep synthetic strategy that integrates markedly different chemical reactions and reactivities to achieve the final system. Broadly speaking, the construction of the bioconjugate can be delineated into two principal stages: the preparation of the functionalised polymer and the subsequent conjugation step. The PLA was functionalized via esterification with terephthaldehydic acid, facilitating the introduction of the terminal aldehyde group (PLA-CHO), which serves as the anchoring point for subsequent reactions. This transformation was thoroughly monitored using NMR, enabling the authors to quantify the reaction yield, which was determined to be approximately 80 %. On the other side, sericin's lysines were functionalized with succinimidyl-6-hydrazino-nicotinamide (S-HyNic), introducing thereby a hydrazine linker (SS-HyNic). The ensuing chemoselective reaction, between the hydrazine moiety and the aldehyde on PLA, provides the foundation for a click chemistry-based conjugation approach. The formation of the hydrazone bond between the two components was monitored spectroscopically, leveraging the characteristic absorption at 380 nm associated with bis-aryl hydrazone linkages in the UV-Vis spectrum. The experimental results demonstrated that the molar substitution ratio was directly proportional to the quantities of reagents employed, consistent with the mechanistic expectations of the reaction. Given that the number of hydrazine linkers determines the number of polymer chains that can be conjugated to each protein molecule, this approach enables the synthesis of protein-polymer conjugates with diverse architectures. Notably, despite variations in the molar substitution ratio of SS-HyNic, the conjugation process consistently achieved extremely near-complete utilization of hydrazine moieties, with the molar ratio between the two reagents about 1:1. This observation indicates an almost quantitative reaction efficiency, further corroborated by UV-Vis analysis, which confirmed the extent of the conjugation. To assess the conformational integrity of the conjugate, circular dichroism (CD) spectra of unmodified sericin and SS-PLA were compared. The results revealed only minor differences, suggesting that the modified protein retained its ability to adopt secondary structures



Scheme 17. PLA grafting on functionalized sericin.

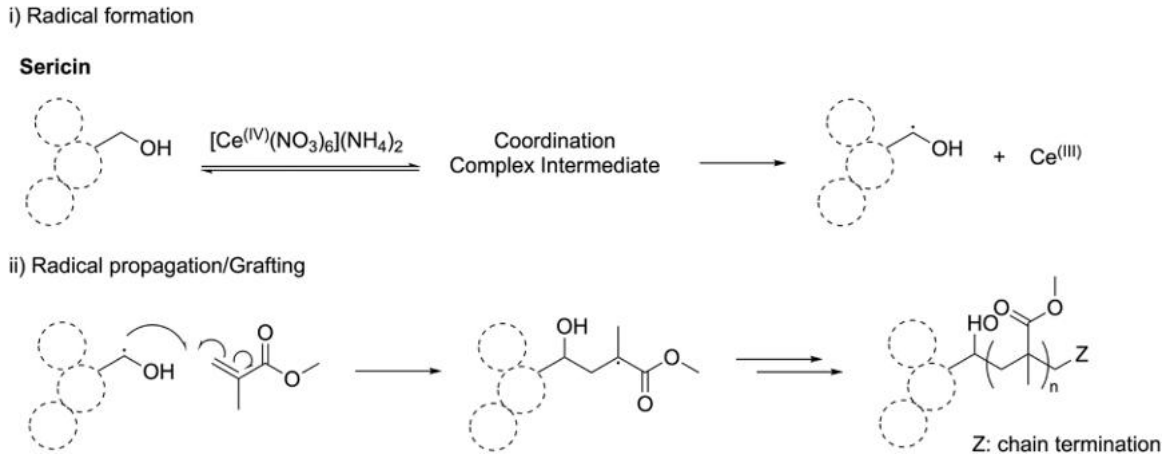
such as random coils and β-sheets. However, the signal intensities were notably low, a phenomenon that the authors ascribed to the presence of the PLA moiety, which may influence the overall CD response. Ultimately, the synthesized system exhibited intrinsic self-assembly into "multicompartiment" micelles, characterized by a morphology described by the authors as "raspberry-like", with particle sizes below 100 nm. Cytotoxicity assays confirmed that these micelles were non-toxic under the tested conditions. Moreover, the micelles demonstrated capacity for drug loading, with doxorubicin (DOX) incorporated during the self-assembly process. The system was shown to facilitate pH-response drug release under acidic environments, thereby highlighting its potential as an effective nanocarrier for targeted antitumor therapy.

In the discussion about grafting synthetic copolymers onto sericin backbone Song *et al.* [126] published a work on the grafting copolymerization of methyl methacrylate (MMA) onto sericin, paralleling similar conceptual processes extensively discussed in the literature involving ethyl acrylate, acrylic acid, acrylamide, and styrene. The focus of the work was to improve the properties of pristine sericin, such as its instability in water, poor solubility in common organic solvents, and low resistance to microbial attacks. These characteristics significantly affect the applicability of the native protein in various fields. As a result, chemical modification was considered an effective methodology to address these drawbacks and enhance other properties. In this context, one of the most important procedures for chemically modifying sericin, suggested by the authors, is the grafting of vinyl monomers to incorporate the desired properties without compromising the original nature of the protein. To do this, the research group chose MMA as the monomer and ceric ammonium nitrate (CAN) as the initiator due to its properties (Scheme 18).

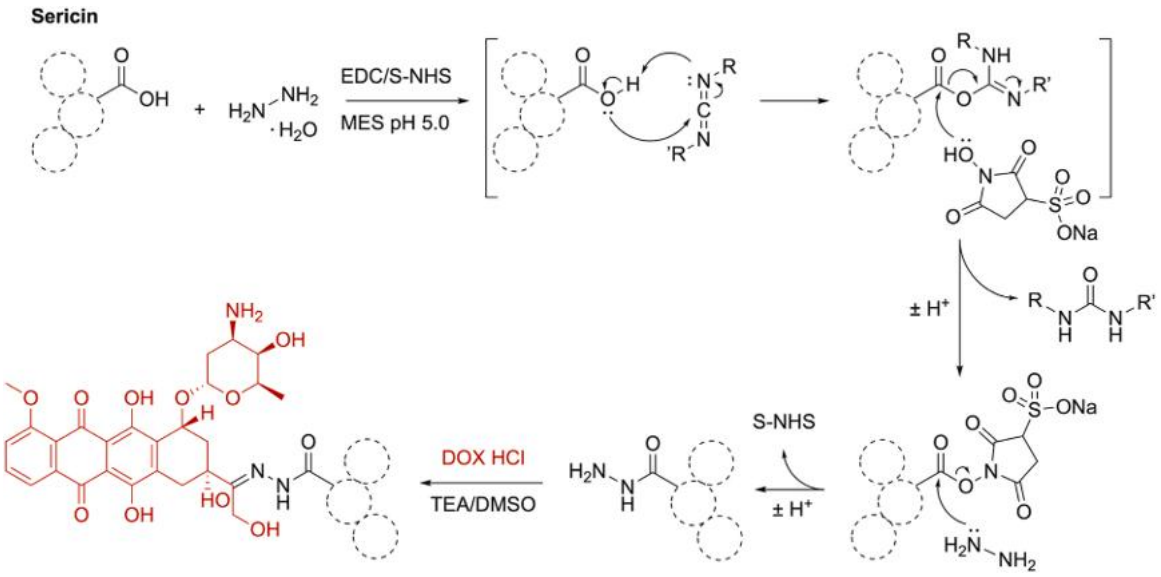
Specifically, to perform the "grafting from" process, sericin was first dissolved in hot water and then cooled to room temperature. MMA was then added to the solution, ensuring an inert environment by introducing an inert gas. The mixture was heated, and the initiator (dissolved in 0.01 M nitric acid) was added. After a defined time, the reaction was stopped by introducing an inhibitor, and the final product was obtained and purified through filtration and washing steps. The hypothesized mechanism was based on a single-electron transfer, which generated the radicals directly on the protein's backbone. Regrettably, the authors did not provide a detailed elucidation of the reaction mechanism. Instead, they extrapolated their proposed mechanism from a separate study focused on cellulose. Based on this reference, they assumed that radical formation occurs predominantly at serine residues within the substrate. However, they did not conduct experimental investigations to validate this hypothesis or consider alternative reactive sites, as discussed in the work by Scrivano *et al.* [121], thereby limiting the mechanistic understanding of the process. Initially, the grafting copolymerization was monitored gravimetrically, and these findings were subsequently corroborated through FTIR. The application of additional spectroscopic techniques, such as NMR, was constrained by the physical properties of the grafted sericin, as discussed by the authors. In essence, during the progression of the reaction, an increase in the mass of the extracted fractions was observed over time, indicative of successful grafting. Furthermore, the grafted sericin exhibited insolubility in acetone, a solvent known to dissolve pMMA; conversely, its derivative obtained after acidic hydrolysis demonstrated complete solubility. As anticipated, FTIR confirmed the successful grafting process through the identification of characteristic bands of methyl methacrylate, which intensified progressively with ongoing grafting reactions. Additionally, FTIR analysis provided evidence of altered solubility properties following acidic hydrolysis; post-treatment spectra closely resembled those of pure pMMA. Morphological examination revealed a transition from a smooth surface to a rougher texture, which is attributable to the grafting phenomena. Regrettably, the authors did not include a comprehensive investigation into the physicochemical properties of the grafted sericin, despite providing a general recommendation for future studies to explore these systems in greater depth.

Despite the various therapies developed for cancer treatment, chemotherapy remains the most common approach. Unfortunately, despite its unique "methodological efficacy," it presents severe limitations. As a consequence, the scientific community has sought better alternatives, and nano drug delivery vehicles could represent an optimal candidate also in this field. Thus, the group of Huang [127] studied a sericin-based nano drug delivery system (DDS) for cancer-targeted therapy, which had a dedicated cancer drug grafted onto its surface. Specifically, since their goal was to prepare biocompatible nanoparticles that would be stimuli-responsive and release the drug at a specific target, the authors exploited a pH-sensitive chemical bond to connect the bio-based scaffold and the drug through a characteristic protein functionalization. In particular, they functionalized sericin carboxylic acids with hydrazine to obtain the respective acyl hydrazides, which would then react with the drug (doxorubicin, DOX) to form the corresponding acyl hydrazone (Scheme 19). Compared to the previously discussed works about Magaraphan [125] and Scrivano [121], the authors opted for the same concept of labile FG in specific pH ranges, grafting the drug directly onto sericin, not by radical reaction, but through hydrazone coupling; in general, it can be seen as a hybrid of the two aforementioned.

Focusing on protein modification, sericin (600 mg) was dissolved in a 4-morpholineethanesulfonic acid (MES) solution (pH 5.0, 60 mL). Then, EDC·HCl (3.83 g, 20 mmol) and sulfo-NHS (N-hydroxysulfosuccinimide sodium salt - S-NHS, 1.09 g, 5 mmol) were added to the main batch. Subsequently, 1 mL of hydrazine hydrate (85 % v/v)



Scheme 18. Sericin free radical polymer grafting.



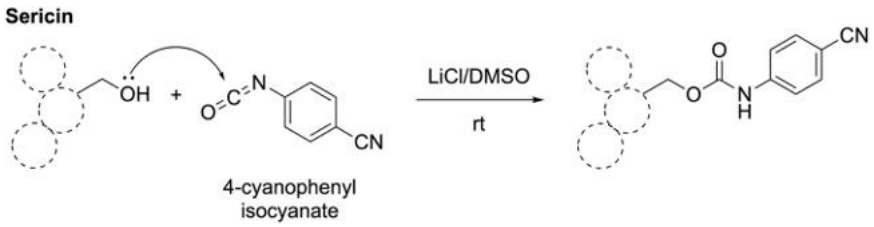
Scheme 19. Doxorubicin grafted on functionalized sericin.

was added dropwise. The reaction was carried out at room temperature in the dark for 24 h. The final product was recovered by dialysis and lyophilization. DOX was then grafted onto the sericin surface by dissolving the functionalized sericin (100 mg) in 10 mL of anhydrous DMSO, followed by the addition of triethylamine (20 µL) and DOX·HCl (10 mg). The mixture was stirred for 24 h in the dark, and the final product was recovered by dialysis and lyophilization.

To effectively achieve the functionalization of sericin, the authors leveraged the high abundance of glutamic acid and aspartic acid residues within the protein. The initial step of the synthesis involved the formation of an acyl hydrazide, accomplished through the generation of an activated succinimide ester, which facilitated efficient conjugation of hydrazine to the carboxylic acid groups of sericin. Subsequently, doxorubicin (DOX) was conjugated via the formation of acyl hydrazone bonds, a linkage characterized by its high specificity for the intended application, resulting in the desired product (SND). The progression of these functionalization steps was monitored using UV-Vis spectroscopy and FTIR. The presence of characteristic absorption peaks corresponding to sericin and DOX at specific wavelengths, along with distinctive vibrational bands associated with the hydrazide linkage and functional groups present in DOX, confirmed the successful covalent attachment of the drug to the protein matrix. Using the same analytical techniques, the authors assumed that the formation of undesired byproducts was negligible beyond the formation of the target compound without discussing it. However, these techniques do not provide definitive evidence to conclusively rule out the presence of side-products, such as diacylated derivatives, thereby limiting the certainty regarding the selectivity and completeness of the synthesis. Nonetheless, the synthetic strategy should intrinsically minimize intra-molecular side reactions, aided by considerations of sericin's conformational properties. The resulting SND conjugates demonstrated self-assembly capabilities, as evidenced by the formation of spherical nanoparticles approximately 40 nm in diameter, a size characterized by both atomic force microscopy (AFM) and dynamic light scattering (DLS). In addition, the authors appropriately emphasized the assessment of the biological behavior of their system. The nanoparticles demonstrated low cytotoxicity and exhibited a favorable profile in terms of hemocompatibility, showing a predisposition at minimal hemolytic activity *in vivo*. As initially designed, the nanoparticles maintained stability at neutral pH, with limited doxorubicin (DOX) release over a 52-hour incubation period. Conversely, at acidic pH levels, the drug release increased by more than fivefold, confirming the pH-sensitive lability of the hydrazone bond. This pH-responsive behavior suggests that the carriers would remain stable during circulation under physiological conditions and would effectively release the therapeutic agent upon internalization into target organelles, where the local acidic environment facilitates bond cleavage. Such design strategies enhance the selective delivery of chemotherapy agents to diseased tissues, thereby confining the therapeutic effects and minimizing off-target side effects. Furthermore, considering the numerous functional groups inherent to sericin, the selection of appropriate functionalization techniques could enable the further modification of sericin-based nanoparticles to accommodate a wide range of therapeutic applications, thereby facilitating the development of adaptable and biocompatible personalized drug delivery systems.

In a separate investigation conducted by Teramoto *et al.* [128], sericin was functionalized under non-aqueous conditions utilizing a DMSO/LiCl solvent system. The functionalization was achieved through the employment of 4-cyanophenyl isocyanate, a reagent characterized by its high reactivity towards hydroxyl groups, as depicted in Scheme 20. The authors not only documented the synthesis of the grafted sericin but also undertook a comprehensive characterization of the resulting product, evaluating parameters such as solubility, hygroscopicity, thermal stability, and structural properties. This thorough analysis provided valuable insights into the modifications induced by the functionalization process.

They started with drying 100 mg of sericin under vacuum at 70 °C for 24 h, then 1 M LiCl/DMSO (10 mL) was added. The mixture was heated to 60 °C for 45 min under stirring in an argon atmosphere to ensure homogeneity. Residual water was removed, then the 4-cyanophenyl isocyanate was added to the solution. The reaction was stirred at room temperature for 5 h under argon, then it was poured into excess cold ethanol to precipitate the product. Regarding the reaction process, the researchers dedicated considerable effort to identifying an appropriate reaction medium by evaluating the solubility of sericin in various organic solvents, including dimethylacetamide (DMAc), dimethylformamide (DMF), and dimethyl sulfoxide (DMSO), both in their pure forms and in the presence of lithium salts (specifically LiCl and LiBr). Their findings indicated that DMAc was ineffective in dissolving sericin, even at varying concentrations of lithium salts. Conversely, DMF and DMSO demonstrated a notable capacity to solubilize the protein, with DMSO exhibiting superior performance. Furthermore, in both solvents, the solubility of sericin increased proportionally with the concentration of lithium salts, with LiCl showing greater efficacy than LiBr. The solvent DMSO, owing to its ability to solvate lithium salts



Scheme 20. 4-cyanophenyl isocyanate grafting onto sericin.

effectively, facilitates interactions between halide ions and the protic polar groups of sericin. This interaction disrupts interchain hydrogen bonds, thereby enhancing the overall solubility of the protein. The observed disparity in performance between LiCl and LiBr salts may be attributed to the higher polarizability and larger molecular size of bromide ions relative to chloride ions. Consequently, bromide ions engage more with the solvent, thereby influencing the dissolution process. This phenomenon, stemming from differences in polarity and molecular dimensions, plays a fundamental role in various chemical reactions, including nucleophilic substitution mechanisms and others. Therefore, understanding the dissolution behavior of sericin under different solvent conditions is of critical importance and warrants further exploration. The suitability of the chosen solvent system was also evaluated in terms of sericin stability; subsequent analyses, replicating aspects of the experimental conditions, revealed no evidence of protein degradation, thereby confirming the solvent's inertness under these parameters. As previously mentioned, the authors selected 4-cyanophenyl isocyanate as the electrophilic reagent owing to its inherent high reactivity and its distinct infrared absorption characteristics, which facilitate spectroscopic monitoring. The underlying reaction mechanism is relatively straightforward, involving the nucleophilic attack of the hydroxyl group of serine on the electrophilic carbon of the isocyanate, resulting in the formation of a carbamate linkage. The research group also provided additional insights regarding the chemoselectivity associated with the reaction; they also acknowledged the presence of various nucleophilic amino acid residues within the sericin structure, which could potentially facilitate the selective grafting to amino acid side chains beyond serine itself, despite its abundance. They suggested the formation of multiple linkage types, including ureic bonds, alongside the expected carbamate linkages. However, FTIR spectroscopic analyses, used to assess the efficiency of grafting based on the characteristic nitrile absorption band, revealed only spectral features indicative of carbamate bond formation. No significant signals corresponding to alternative linkages, such as ureic bonds, were detected, suggesting a high degree of chemoselectivity toward carbamate formation under the experimental conditions. The authors rationalized these findings by attributing the observed selectivity primarily to serine high abundance within the protein structure [129,130]. However, it is important to consider that the reagent ratios and equivalents employed can significantly influence this observed selectivity. Furthermore, the conclusions are based predominantly on analyses conducted using a limited set of spectroscopic techniques, which may not fully capture the complete features of the reaction specificity. The efficiency of the functionalization reaction was evaluated using high-performance liquid chromatography (HPLC) analysis of hydrolyzed derivatives of the grafted sericin. Under acidic conditions, both carbamate and cyanide groups are susceptible to hydrolysis, resulting in the formation of amino acids and 4-aminobenzoic acid, respectively. These hydrolysis products can be detected and quantified by HPLC, with their concentrations serving as theoretical indicators of the reaction yield. The results demonstrated an increase in yield concomitant with higher amounts of isocyanate used, with modification levels ranging from 8.5 % to 31 % in terms of amino acid residues. However, these findings did not correspond proportionally to the excess of isocyanate employed. The observed low efficiency was further investigated via gel permeation chromatography (GPC), which revealed that a significant portion of the isocyanate reacted with itself, forming oligomeric species. Additionally, chain degradation of sericin was observed when reactions were conducted at 60 °C, indicating thermal instability under these conditions. The precise cause of the observed degradation remains unclear. However, as discussed by the authors in the experimental section, both sericin and DMSO are highly hygroscopic, necessitating stringent control of moisture content to prevent the hydrolysis of the isocyanate. Despite the researchers' efforts to remove residual water from the reagents and solvent, trace amounts may still have been present, potentially contributing to protein hydrolysis. Additionally, DMSO itself may participate in side reactions with the isocyanate, leading to the formation of unintended by-products. Overall, this underscores that optimizing reaction conditions is a critical consideration and represents a significant limitation in the functionalization of sericin, as the operational parameters directly influence the efficiency and stability of the process.

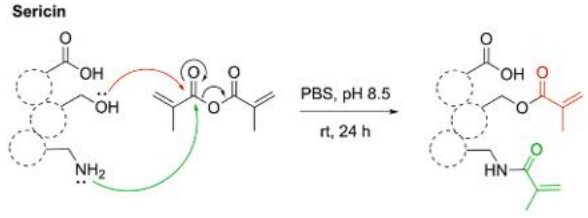
Following the modification, sericin exhibited insolubility in aqueous environments; conversely, its solubility in pure DMSO was enhanced, as was its solubility in other organic solvents such as hexafluoroisopropanol (HFIP). Additionally, the required amount of LiCl decreased in proportion to the increase in substitution yield. These observations were supported by TGA, which revealed a decrease in moisture content correlating with higher degrees of substitution. Nevertheless, the intrinsic hydrophilic nature of sericin imparted a slight hygroscopicity to the system. A reduction in decomposition temperatures was observed, likely attributable to the destabilizing influence of the 4-cyanophenyl substituent. As anticipated, FTIR was employed to monitor the progression of the reaction by identifying characteristic absorption bands associated with the carbamate, aromatic ring, and cyanide residues. Moreover, this analytical technique was not solely utilized for reaction monitoring but was also employed to investigate the structural characteristics of sericin post-modification. Specifically, the authors examined the amide I absorption band, which is sensitive to alterations in secondary structure, as intermolecular interactions directly influence the presence of β-sheet and α-helical conformations, thereby affecting the IR signal. The results indicated that, following modification, the amide I bands gradually shifted toward higher wavenumbers in correlation with increasing reaction yields. This shift suggests a progressive disruption of robust intermolecular hydrogen bonds, likely due to the steric hindrance introduced by the bulky 4-cyanophenyl groups, which compromised the native secondary structural arrangements of sericin. These structural modifications are likely directly responsible for the observed increase in solubility within organic solvents and the concomitant decrease in thermal stability. In conclusion, the authors extensively characterized and analyzed the effects of chemically modifying sericin with 4-cyanophenyl isocyanate. Their findings, coupled with the potential to utilize a broad spectrum of differently functionalized isocyanates, substantially contribute to the development of a novel conceptual framework for the modification and tailored tuning of sericin's physicochemical properties.

In a distinct research context, Zhu *et al.* [131] conducted a study focusing on the utilization of modified sericin as a precursor for the fabrication of highly specific and efficient cryogels, intended for application in hemorrhage management. Hemorrhages remain a significant cause of mortality; consequently, the development of hemostatic

materials endowed with antibacterial properties holds considerable importance for patient survival rates. The adoption of bio-based materials such as sericin is driven by the limitations associated with conventional commercial hemostatic agents, which often exhibit drawbacks including impediments to wound healing, elicitation of adverse immune responses, thermal damage, and insufficient biocompatibility (Scheme 21).

5 g of sericin and 2.93 g of methacrylic anhydride were added to phosphate-buffered saline (PBS, pH 8.5) and stirring the mixture for 24 h. The resulting solution was then dialyzed (MWCO 3500 Da) against PBS (pH 7.4) for 2 days, followed by deionized water for 3 days. After lyophilization, the Sericin-MA (SMA) powder was obtained. The obtained product was subsequently utilized by the authors to synthesize a cryogel via a specialized process called "freezing polymerization". This technique involves the polymerization reaction carried out in the presence of a radical initiator at sub-zero temperatures ($\leq -20\ ^{\circ}C$). The low-temperature conditions are critical, as they promote the formation of a highly porous structure thanks to the presence of solid crystals, which is essential for the material's intended application. Following this, silver nanoparticles were incorporated into the system through an *in situ* reduction of silver nitrate. The resulting composite was then subjected to lyophilization to obtain the final cryogel. With regard to material characterization, the authors predominantly concentrated on evaluating the performance of their sericin-based cryogel through *in vitro* and *in vivo* assessments. These evaluations primarily addressed parameters directly related to its efficacy as a hemostatic agent, including blood absorption capacity, coagulation kinetics, and antibacterial activity. Consequently, the study provided only minimal information concerning the comprehensive physicochemical characterization of the synthesized systems. Using SEM, the researchers highlighted the presence of the targeted interconnected porous architecture. However, techniques such as XPS and, notably, FTIR were employed primarily to confirm the presence of silver nanoparticles (XPS for elemental analysis and FTIR for chemical characterization) and to compare various synthesis intermediates. These approaches resulted in a discussion predominantly focused on the absence of significant alterations in the amide absorption bands, thereby limiting the depth of structural or chemical insights derived from these analyses. Beyond the limited chemical characterization, which may be only partially justified by the primary focus of the published study, the quantities of functionalization agents employed by the authors suggest the feasibility of detecting characteristic FTIR bands corresponding to the methacrylic groups. Specifically, if the reactive sites are lysine residues, the associated amide signals might be obscured due to spectral overlaps; however, considering the presence of serine residues, the formation of ester linkages should be readily identifiable within the IR spectra. Although the reported spectra display certain peaks that could be attributed to these functional groups, no explicit discussion or interpretation of these features was provided. Zhu's research team employed the sericin/Ag-based cryogel as a hemostatic agent, leveraging its excellent performance in arresting bleeding. This efficacy was attributed to the facilitation of coagulation processes and the enhanced adhesion properties, which are thought to arise from the collagen-like activity of sericin.

Sericin was utilized to modify 3D-printed poly(ε-caprolactone) (PCL) to enhance its bioactivity. Despite its wide application in tissue engineering, PCL's hydrophobicity and biological inactivity limit its potential uses. Iranpour *et al.* [132] leveraged sericin and its numerous biological properties to modify the scaffold's surface, as sericin has previously been shown to be effective as a coating for titanium scaffolds in bone tissue engineering. Thus, the authors functionalized sericin following carbodiimide chemistry.



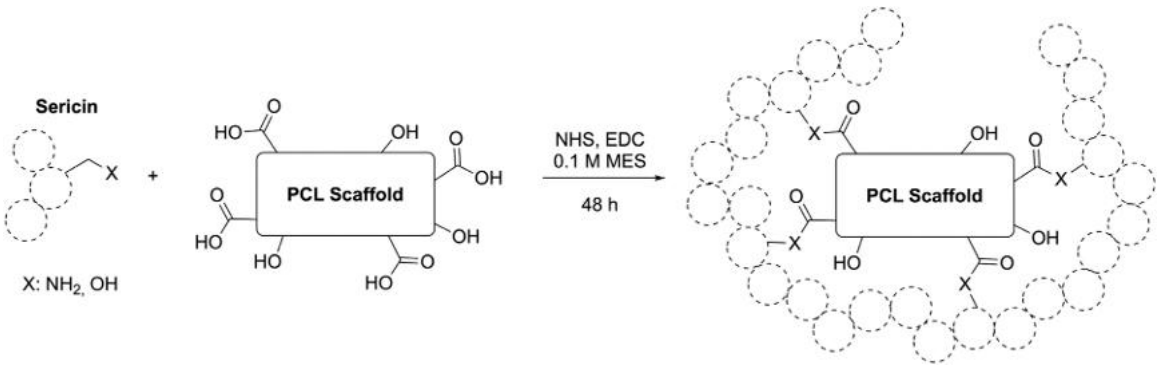
Scheme 21. Methacrylation of sericin.

5.7 mg of NHS and 3.8 mg of EDC were added to 10 mL of MES, then the already prepared poly PLC scaffolds were immersed in the solution, after being subject to surface modification with NaOH treatment and exposure to oxygen plasma, for 6 h at room temperature (Scheme 22). Then the scaffolds were placed in a solution of 0.1 M MES containing 0.1 g of sericin for 48 h, and washed lastly with PBS. The SEM analysis revealed the presence of sericin on the surface of the PCL scaffold, manifested as a subtle increase in surface roughness, without notable alterations in fiber diameter or pore size. FTIR further validated the successful grafting process, evidenced by the detection of characteristic amide bands attributable to sericin, in addition to the spectral features of PCL. Gravimetric assessment was employed to quantify the amount of sericin coating on the scaffold surface, resulting in an approximate deposition of 23 % by weight. Moreover, the incorporation of sericin influenced the hydrophilic properties of the PCL scaffold; specifically, the contact angle measurements demonstrated a statistically significant reduction in the contact angle of the modified material relative to unaltered PCL, indicating enhanced surface hydrophilicity. By integrating various spectroscopic methodologies, including SEM, EDX, and XRD, the scaffold's functional behavior was elucidated. Notably, its bioactivity was demonstrated through the formation of bone-like tissue, characterized by the development of a hydroxyapatite (HA) layer on the scaffold surface, which was corroborated by the detection of calcium and phosphate crystalline deposits. The amino acid residues of sericin served as nucleation sites, facilitating hydroxyapatite crystallization. Importantly, the overall crystallinity of the scaffold's structure was preserved following sericin coating, albeit with a reduction in the intensity of diffraction peaks. Additionally, the study emphasized the scaffold's capacity, modified with sericin, to effectively promote cellular adhesion without inducing cytotoxic effects, underscoring its potential for biomedical applications. The resulting coated scaffold is characterized by a significantly improved bioactivity, as evidenced by its reduced or negligible toxicity and its ability to promote cell differentiation and proliferation. Furthermore, this material held considerable promise for applications in orthopedic fields.

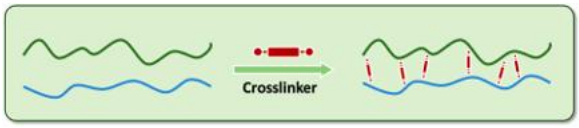
3.4. Covalent crosslinking of sericin

Covalent crosslinking is a chemical process that involves forming covalent bonds between two or more molecules, typically long-chain polymers, biomolecules (like proteins), or supramolecular constructs. These covalent bonds act as "bridges" that connect the individual chains, creating a larger, more robust, and often three-dimensional network structure. This process significantly changes the physical and chemical properties of the material. For example, in (bio)polymers, covalent crosslinking can lead to i) increased mechanical strength and rigidity [77,78]; ii) enhanced thermal stability [77]; iii) improved (bio)chemical resistance; iv) reduced solubility [133]. Specifically, the chemical junctions are provided by specific/dedicated coupling molecules, called crosslinking agents, which exhibit two or more reactive ends that react with target molecules, creating a network of bridges in the new structure (Scheme 23).

The crosslinking functionalities can stem from pre-existing functional groups on the main structure, or they can be introduced by an external molecule. This external molecule must possess at least two functional groups. Based on the functional groups involved, crosslinking is generally classified as either homo-crosslinking or hetero-crosslinking, achieved by selecting appropriate homo-functional and/or hetero-functional linkers. Rubber vulcanization by Charles Goodyear is probably "par excellence" [134] the historically most representative



Scheme 22. Sericin functionalization using EDC/NHS for PLC scaffolds.

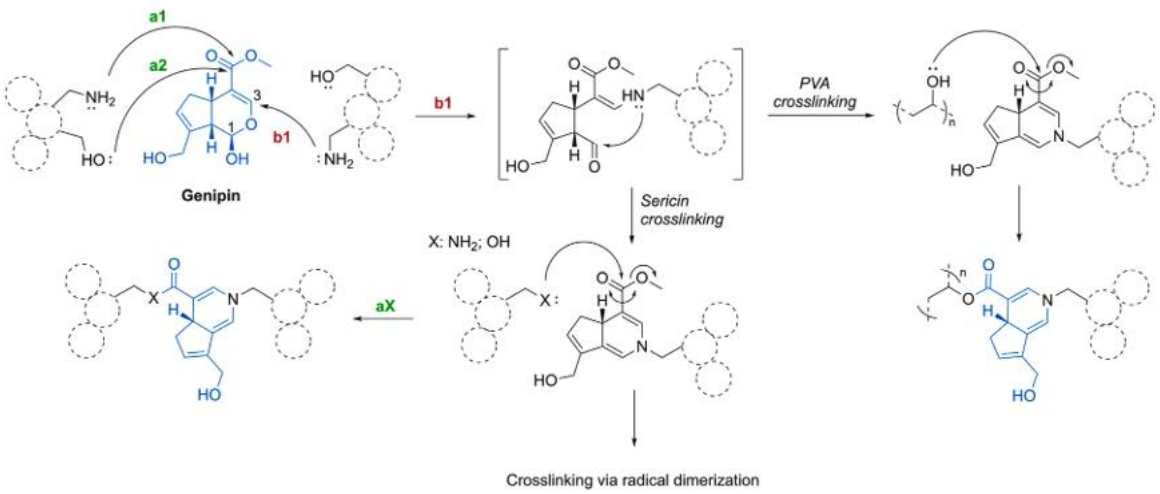


Scheme 23. Crosslinking general concept.

case of covalent crosslinking performed by sulfur as an external crosslinking agent. The latter importantly differs from non-covalent crosslinking, often referred to as "physical crosslinking," where weak intermolecular forces act as a "glue" among the interacting molecules, giving reversibility to the network formation. To those forces belong: hydrogen bonds, ion-dipole, dipole-dipole, ion-induced dipole forces, cation-π, σ-π, and π-π bonding, *Van der Waals* forces, salt-bridge interactions, and even supramolecular interactions. As an example, the formation of hydrogels due to hydrogen bonds and/or electrostatic forces perfectly represents a non-covalent crosslinking case, where the intramolecular interactions among the already present functional groups "regulate" the process towards achieving a thermodynamically stable rearrangement.

Within the realm of chemical crosslinking of sericin, an interesting example is the work by Aramwit *et al.* [5] focused on the characterization and formulation of a sericin-based scaffold for use in the biomedical field. In cases of serious wounds, appropriate skin substitutes need to be employed, but preparing suitable materials is quite challenging. Natural biomaterials have already attracted the scientific community's interest in this field, but they often do not meet all the requirements for the final application. To address this, polymer blending has been proposed as a strategy to enhance the properties of individual polymers. In this study, sericin was selected as the primary biopolymer due to its rich composition of amino acids like serine and aspartic acid, which facilitate copolymerization and blending. Polyvinyl alcohol (PVA) was chosen as a complementary polymer, and genipin was used as a crosslinking agent to improve the scaffold's mechanical integrity and permeability. The resulting sericin/PVA-based construct aims to serve as a promising candidate for wound healing applications due to its biocompatibility, structural stability, and potential to support tissue regeneration.

To achieve this, a solution of sericin (3 % w/v), PVA (2 % w/v), and glycerol (1 % w/v, in certain formulations) was mixed with a genipin solution (0.01–0.1 % w/v) at room temperature and stirred for 5 min. The mixture was then poured into a petri dish and frozen at −20 °C for lyophilization. Genipin is a natural crosslinking agent that exploits the presence of primary amino groups to react with proteins and/or amino acids. Regarding the reaction, the precise mechanism remains incompletely elucidated in current scientific literature. The authors referenced a previously published study [136], briefly supported by their FTIR analyses, which proposed a mechanism involving methylamine as the nucleophile. Based on that, the proposed pathway involves a nucleophilic attack by a primary amine at the C3 position of genipin, leading to the opening of the dihydropyran ring, followed by the attack of the resulting secondary amine on the aldehyde group. The final step of the mechanism may involve radical-mediated dimerization or additional nucleophilic attacks on genipin (Scheme 24). Aside from the discussion concerning the reaction mechanism, the authors base several of their results on the assumption that each reaction component can be assigned a singular, unambiguous definition. For example, genipin is referred to as the crosslinker, whereas glycerin is described as the plasticizer. It is important to recognize that these two designations denote fundamentally different roles: a "crosslinker" is defined as a substance or molecule that facilitates the formation of covalent bonds between two or more polymer chains, thereby effectively linking them as described at the beginning of this section [137]. Conversely, a "plasticizer" is typically an organic compound incorporated into a material, most commonly a polymer, to improve its flexibility, processability, and elongation properties. These additives act by disrupting intermolecular interactions within the polymer matrix, thereby facilitating increased molecular mobility and deformations [138]. Nonetheless, despite the distinct definitions, it is not uncommon for certain compounds, such as glycerol, to exhibit characteristics of both categories, functioning simultaneously as a plasticizer and, under specific conditions, as a crosslinker. Therefore, given the uncertainties associated with the reaction mechanism, particularly when radical processes are involved, the practice of pre-assigning rigid, singular definitions to the reaction components may prove misleading. To assess the effects of genipin addition, the authors employed FTIR. The analysis revealed no significant alterations in the amide I and II bands, except for a reduction in their intensity, which was attributed to potential interference from glycerol's presence, possibly disrupting intermolecular hydrogen bonds. Additionally, minor differences were observed following the incorporation of genipin, notably a further decrease in the spectral intensity of characteristic bands. The extent of crosslinking was estimated by quantifying the free amino groups through a secondary reaction of the final system with 2,4,6-trinitrobenzenesulfonic acid (TNBS), a reagent that is readily detectable under UV radiation. Notably, an increase in the amount of genipin corresponded to a higher percentage of crosslinking, as expected. However, given the unclear reaction mechanism, this estimation raises concerns regarding its reliability and the specific amino acids involved, which may not have been accurately characterized. The incorporation of glycerin and genipin both contributed to an increase in the moisture absorption capacity of the final formulations. Additionally, the swelling was observed to increase concomitantly with higher concentrations of genipin; paradoxically, these parameters are typically expected to decrease at elevated crosslinking levels due to the anticipated reduction in hydrophilicity. Furthermore, the authors investigated the release profile of sericin in an aqueous buffer solution maintained at pH 7.4. Results indicated that formulations containing higher quantities of genipin exhibited reduced sericin release. Such a limited release profile can present potential limitations that could impede the efficacy of the treatment. Consequently, adjusting the amount of crosslinker present in



Scheme 24. Genipin-sericin/PVA blend crosslinking proposed mechanism [135].

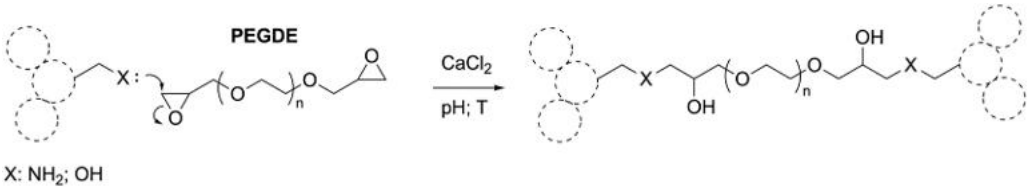
the formulation may facilitate the development of an optimized system. In conclusion, despite the ambiguities and limited clarity inherent in the findings of this study, it underscores the importance of thoroughly investigating reaction mechanisms and attaining a comprehensive understanding of the underlying processes. Such insight is fundamental for accurately interpreting and describing the system's behavior and outcomes. In the context of sericin research, it is not uncommon to encounter studies characterized by limited evidence supporting the results. This phenomenon not only hampers the overall understanding of the subject matter but also impedes the advancement of future research in this area.

The research group of Ma *et al.* [139] investigated strategies to enhance the properties of sericin in natural colored silks (NCS) through crosslinking, aiming to address a key limitation: pigment loss during washing. NCS are silks naturally colored by genetically predisposed silkworms capable of transferring plant-derived pigments, such as carotenoids, carotenoid derivatives, and flavonoids, into the silk fibers without altering the structure or inherent properties of the silk compared to conventional varieties. These silks hold great potential for biomedical and textile applications due to their favorable biological characteristics. However, the natural pigments are primarily associated with the sericin coating on the silk fibers, which is highly water-soluble. As a result, repeated washing leads to sericin dissolution and, consequently, color fading. To overcome this, the study focused on crosslinking sericin *in situ* within the silk fibers, aiming to reduce its solubility, improve its durability, and effectively preserve the embedded pigments during washing. Common crosslinkers, such as glutaraldehyde, genipin, citric acid, dimethyloldihydroxyethyleneurea, and cyanuric chloride, are often used but are not suitable for this application due to their drawbacks, low efficiency, and high toxicity. In this context, epoxy resins (poly(ethylene glycol) diglycidyl ether - PEGDE) can react with the hydroxyl, amine, and carboxylic groups of sericin in an aqueous environment, leading to a more environmentally friendly process (Scheme 25).

Thus, the research group prepared the crosslinked sericin by simply immersing the NCS filaments in an aqueous solution of PEGDE (n ~ 400) and $CaCl_2$ with the pH adjusted using $NaOH_{(aq)}$ or $HCl_{(aq)}$ in a liquor ratio of 20:1. The reaction's variables were studied and it was found that optimal results were achieved with a pH of 7.0, a temperature of 50 °C, 4.7 wt% $CaCl_2$, and a reaction time of 10 h. The crosslinking process was monitored using FTIR. Beyond the characteristic absorption bands of sericin, additional signals attributable to the PEGDE chain were observed. These signals exhibited slight shifts in comparison to the absorption bands of the unmodified starting material yet remained consistent with the structural features of aliphatic epoxy resins. Analysis of the amide bands indicated that the chemical structure of the protein was preserved following crosslinking. To quantitatively assess the extent of the reaction, the authors measured the weight gain resulting from the treatment, thereby enabling an estimation of the degree of crosslinking. This parameter was also evaluated under different reaction conditions. The results indicated that an appropriate neutral pH environment is optimal to minimize protein degradation and promote crosslinking. Additionally, variations in the concentrations of PEGDE and $CaCl_2$, as well as the reaction duration, significantly enhanced the efficiency of the modification process. Conversely, the temperature exhibited a plateau at approximately 50 °C; beyond this point, the fixation efficiency gradually declined, likely due to the dissolution of sericin.

Consistent with the researchers' expectations, the incorporation of PEGDE into sericin resulted in NCS fibers that exhibited minimal damage following degumming and washing procedures, thereby demonstrating notable durability. These enhancements also positively influenced the color fastness, as the crosslinked material maintained an acceptable color appearance even after 15 washing cycles, with a color intensity that was 32.5 % higher relative to the non-modified NCS. Although the antioxidant properties of the NCS were adversely affected by the crosslinking process, their retention levels remained superior compared to those of unmodified pristine NCS after several washes.

Perteghella *et al.* [140] studied the crosslinking of sericin with a goal conceptually different from the previous ones, but still within the biomedical field. Over the years, drugs used in the treatment of many neurological disorders have suffered from reduced effectiveness due to the presence of the blood-brain barrier (*BBB*), a natural regulatory



Scheme 25. Sericin crosslinking by ring-opening nucleophilic substitution.

"shield" in our skull. To bypass this barrier, the "nose-to-brain" delivery pathway has been considered an alternative since the 90 s, exploiting the olfactory epithelium to deliver drugs directly to the brain. In this specific field, many improvements have already been made in terms of formulation, especially with nano-drug delivery systems; sericin could find an application. The latter has been considered a potential candidate due to its intrinsic biological properties; nevertheless, on its own, it forms unstable nanoparticles due to its hydrophilicity; thus, crosslinking was required. In the work published by Perteghella's group, the authors opted for crocin as a crosslinking agent (Scheme 26), as it is a natural carotenoid found in saffron and offers an outstanding list of important therapeutic effects.

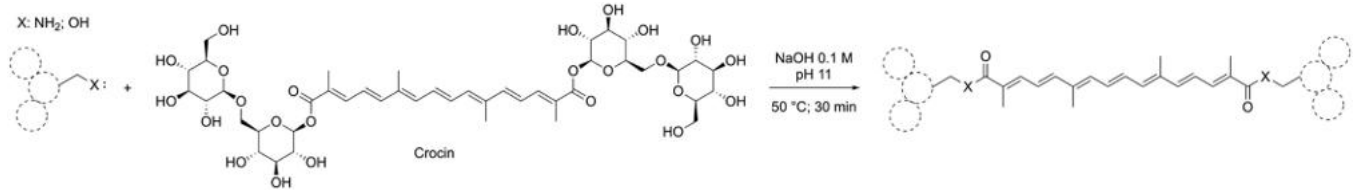
To obtain the sericin crosslinked nanoparticles, the authors added 48 mg of crocin to 2 mL of sericin solution (5 mg/mL) under magnetic stirring. Then, 4 mL of ethanol was added dropwise, and the pH of the solution was adjusted to 11 using a NaOH (0.1 M) solution. The flask (covered from light) was heated to 50 °C for 30 min to produce crocetin *in situ*. Afterward, 1 mL of glutamine (5 mg/mL) was added dropwise, and the obtained suspension was centrifuged. The addition of glutamine through a prepared solution was claimed to play a crucial role in the overall stabilization strategy of the obtained system. Specifically, its addition was necessary to effectively neutralize any residual free crosslinking agent that may have remained post-synthesis. By acting as a quenching agent, glutamine mitigated the potential of residual reactive species to compromise the structural integrity of the nanoparticles. Furthermore, this step was fundamental in enhancing the colloidal stability of the latter within suspension media. The authors did not investigate in the details the reaction mechanism and the actual bonds formed (covalent versus ionic). Moreover, given the alkaline condition used, concerns may arise regarding the overall efficiency of the crosslinking reaction and potential degradation of the protein.

Despite the aforementioned concerns, the authors presented interesting observations related to nanoparticle features. In terms of particle size, a morphological distinction was observed: NPc particles were slightly larger than NPg particles, though both maintained dimensions suitable for use as nanocarriers, including potential applications in drug delivery to the brain [141,142]. Morphologically, NPc particles exhibited a well-defined, nearly spherical shape, as confirmed by AFM, in contrast to the less uniform morphology observed for NPg particles. Compared to NPg, NPc exhibited suboptimal physical stability, as indicated by a continual increase in particle diameter over several days of storage. Notwithstanding this limitation, the incorporation of crocetin markedly enhanced the *in vitro* reactive oxygen species (ROS)-scavenging capacity of NPc, which also demonstrated complete cytocompatibility with human fibroblast and Caco-2 cells. Moreover, NPc conferred a notable degree of protection against oxidative stress-induced cellular damage. In summary, these findings support the potential application of NPc as a nanocarrier for nose-to-brain drug delivery. Nonetheless, the concerns raised regarding the crosslinking mechanism and the adequacy of the employed characterization techniques necessitate further comprehensive investigation.

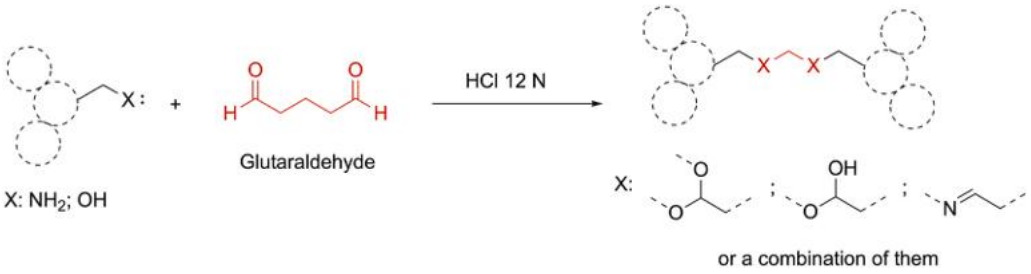
Previously, it was discussed that sericin can be grafted with other polymeric materials using glutaraldehyde (GA) as a "bridge" between the two macrosystems. The group of Kundu [77], however, published a study where GA is used as sericin crosslinker to achieve bidimensional (2D) membranes that can be used in skin tissue engineering. The authors opted for sericin due to its numerous advantages in terms of reactivity, solubility, and biological properties, making it suitable for skin wound healing (antibacterial, UV, and oxidation resistance, moisture absorption, etc.). Fortified by the fact that sericin-based 2D and 3D scaffolds have already found applications as potential tissue engineering materials, the authors decided to evaluate the capability of GA crosslinked sericin to be used in this field.

To obtain the 2D membranes, the researchers mixed 5 mL of sericin solution (2 % w/v), 100 μL of glutaraldehyde (25 % w/v), and 20 μL of 12 N HCl as a catalyst (Scheme 27). Following the membrane fabrication via the casting process, the resulting materials were subjected to comprehensive characterization across multiple parameters. Visually, the membranes exhibited a brownish, dense appearance. Morphologically, SEM images revealed a notably rough surface topology. This surface roughness was primarily attributed to the ethanol washing procedure employed prior to characterization. The ethanol treatment appeared to induce crystallinity within the membranes, which, in turn, generated tensile stresses responsible for the development of nanoscale surface roughness. FTIR analysis of the crosslinked membranes revealed prominent absorption bands, which were more intense compared to those observed in untreated sericin, along with subtle spectral shifts. These findings suggest that the crosslinking treatment influenced the secondary structure of the protein, with the authors specifically identifying the presence of stable β-sheet conformations. The adoption of this particular secondary structure confers increased rigidity to the protein matrix, thereby enhancing its insolubility and resistance to aqueous environments. These results imply that the introduction of an appropriate crosslinker can profoundly alter the molecular architecture of sericin. Complementary analysis via XRD further supported this observation, demonstrating an increase in crystallinity attributable to covalent bond formation during crosslinking. Additionally, the data indicated a transition from a predominantly random coil conformation to more organized β-sheet structures, contributing to the improved structural stability of the treated sericin. These assertions were substantiated through empirical evidence provided by the authors. DSC analysis demonstrated that the crosslinked membranes exhibit increased thermal stability relative to their uncrosslinked counterparts, primarily due to decreased molecular mobility. Additionally, mechanical testing indicated significant improvements in properties; while native sericin is inherently too fragile to form stable membranes, the crosslinked systems displayed demonstrable mechanical strength and elongation at break. The swelling capacity of the crosslinked membranes was also assessed via immersion in aqueous solutions and PBS at pH values of 3, 7.4, and 11, to evaluate their responsiveness to different environments. In all experimental conditions, the swelling behavior was characterized by an initial rapid increase, eventually reaching a plateau. Notably, higher swelling values were observed in PBS compared to pure water, particularly at pH 7.4 and 11, indicating a pH-responsive behavior of the membranes. The maximum swelling was achieved after approximately 6 h, suggesting that the more constrained molecular architecture of the crosslinked sericin limited fluid penetration, thereby promoting a controlled swelling process.

Regarding *in vitro* stability assessments, the membranes demonstrated notable stability in PBS at pH 7.4, exhibiting no significant degradation but a slight initial weight loss attributed to the release of uncrosslinked sericin. Conversely, exposure to lysozyme resulted in a



Scheme 26. Sericin-crocin crosslinking.



Scheme 27. Sericin-glutaraldehyde crosslinking proposed products.

slow degradation process, with only around 5 % weight loss observed after 5 days of incubation, indicating long-term enzymatic susceptibility. Cell culture studies further confirmed biocompatibility; fibroblasts seeded onto the membranes displayed excellent adhesion, morphology, and spreading. The crosslinked sericin supported cellular proliferation and growth effectively, demonstrating high cytocompatibility and suitability for tissue engineering applications. Within the field of Schiff's bases chemistry, Wang *et al.* [78] published a paper where they exploited imine bonds to crosslink sericin. It is known that sericin membranes are fragile and brittle after air drying and are easily solubilized in aqueous environments. Specifically, Wang's research group evaluated whether crosslinking sericin with another bio-based macromolecular system would help achieve this goal. As a reinforcement, carboxymethyl cellulose (CMC) was studied: a water-soluble, biodegradable, and biocompatible polymer that already has various applications in drug delivery and tissue engineering [143,144]. Moreover, CMC is widely available and economical, but it cannot be used as it is due to its moderate reactivity. To increase it, CMC can then be treated with sodium periodate ($NaIO_4$) to oxidatively cleave the C2-C3 bond, yielding a ring-opened dialdehyde (DCMC), which serves as an alternative natural-derived crosslinker compared to formaldehyde and glutaraldehyde (Scheme 28).

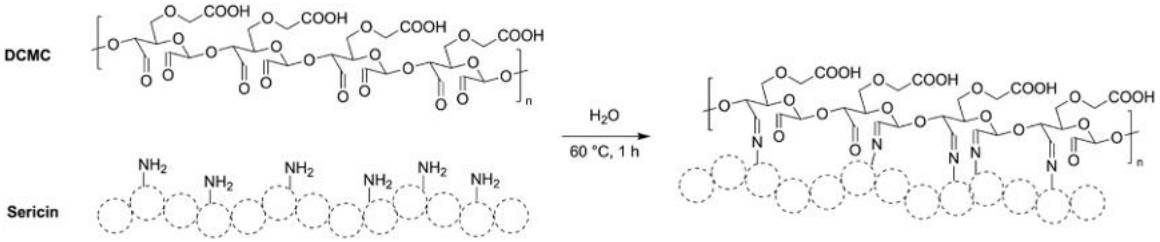
To synthesize the crosslinked system, DCMC was dissolved in 2 mL of water at 60 °C (6–24 % w/v). Subsequently, a sericin solution (3.75 % w/v, 8 mL) was blended with the DCMC solution at 60 °C and stirred for 1 h. Finally, the mixture was dried at 37 °C in a Petri dish to yield the crosslinked product.

The authors proposed that the primary reaction occurred between aldehyde groups and the free amino groups of lysines to the corresponding imines. However, considering the high abundance of serine residues, the formation of hemiacetals and acetals should also be considered. Regardless of which product predominates, both types of linkages are susceptible to hydrolysis. To verify the occurrence of crosslinking, the authors employed FTIR; initially, they confirmed the successful oxidation of CMC to DCMC. Subsequently, the authors inferred successful crosslink formation from the disappearance of the characteristic aldehydic C═O absorption band. However, they explicitly noted the absence of the imine (C═N) band, which is typically indicative of Schiff's base formation; this absence was likely due to overlapping spectral signals, complicating direct detection. Nevertheless, this type of covalent bond is generally characterized by a prominent absorption band, which raises questions regarding the reaction efficiency. Such discrepancies may also suggest that the concentration of available aldehyde groups was limited at the onset of the reaction, potentially attributable to the reagent ratio, necessitating the reaction with sericin's free amino groups, which are themselves present in relatively low abundance. FTIR was additionally employed to assess the impact of crosslinking on the protein's secondary structure by deconvoluting the Amide I band. This analysis facilitated the identification of various conformational states within the modified sericin. Despite the incremental increases in DCMC content, no substantial alterations in secondary structure were observed, aside from a modest enhancement in β-sheet content.

Morphologically, SEM images revealed that an increase in DCMC concentration within the final system led to the formation of small pores, which are considered fundamental for promoting effective cell adhesion. Consistent with expectations, crosslinking positively influenced the mechanical properties of the modified sericin by enhancing tensile strength and Young's modulus, while concurrently reducing elongation at break.

The resulting films demonstrated excellent retention of hydrophilicity despite the crosslinking process; notably, an increase in DCMC content progressively decreased the swelling ratio. Furthermore, water solubility was evaluated at different pH levels (4.0, 7.4, and 10.0). Results indicated that the films exhibited stability under neutral conditions, whereas under both acidic and alkaline conditions, the total soluble matter approximately doubled, highlighting pH-dependent variations in solubility behavior. The authors attributed this behavior to the increased charge density of the films under these conditions. However, if the selective formation of imine bonds is assumed, it is more plausible that hydrolytic reactions occurred, leading to the partial release or dissociation of the crosslinked DCMC. Regarding water interaction, the wettability of the films was assessed through contact angle measurements. As the DCMC content in the samples increased, the contact angle progressively rose; nonetheless, it did not reach values characteristic of hydrophobic surfaces. This suggests that some of the sericin and DCMC functional groups remain available for interaction with water.

The blood compatibility of the DCMC-SS films was assessed by measuring the hemolysis ratio following incubation with red blood cells. According to established standards for biomedical materials [145], a hemolysis index below 5 % is acceptable; the materials obtained in this work exhibited less than 2.5 %, showing excellent hemocompatibility. The results demonstrated a gradual decrease in hemolysis ratio with



Scheme 28. Sericin-DCMC imine crosslinking.

increasing DCMC content, which can be attributed to the crosslinking process reducing the availability of functional groups capable of interacting with erythrocytes. Ultimately, the DCMC-SS films also exhibited excellent cytocompatibility and promoted cell proliferation activity.

In 2016, Liu *et al.* [146] published a study focused on the preparation and characterization of a sericin-based injectable hydrogel as an optically trackable drug delivery system (OT-DDS). In the field of target-specific drug delivery, particularly for diseases like cancer, hydrogels have gained significant attention, especially injectable crosslinked ones, which can serve as *in situ* storage, reducing the need for invasive delivery methods, surgeries, infection risks, etc. Since hydrogels are highly influenced by the surrounding microenvironment, it is critical to have a non-invasive monitoring method to optimize therapy. Unfortunately, hydrogels often lack *in vivo* real-time tracking, and fluorescence dyes are frequently incorporated into them, raising significant concerns. This issue could be addressed if the starting material is already biocompatible and fluorescent. Consequently, the authors proposed a crosslinked sericin-based hydrogel using two biocompatible backbones: dextran (a polysaccharide) and a silk protein, with hydrazone-linking bonds (Scheme 29).

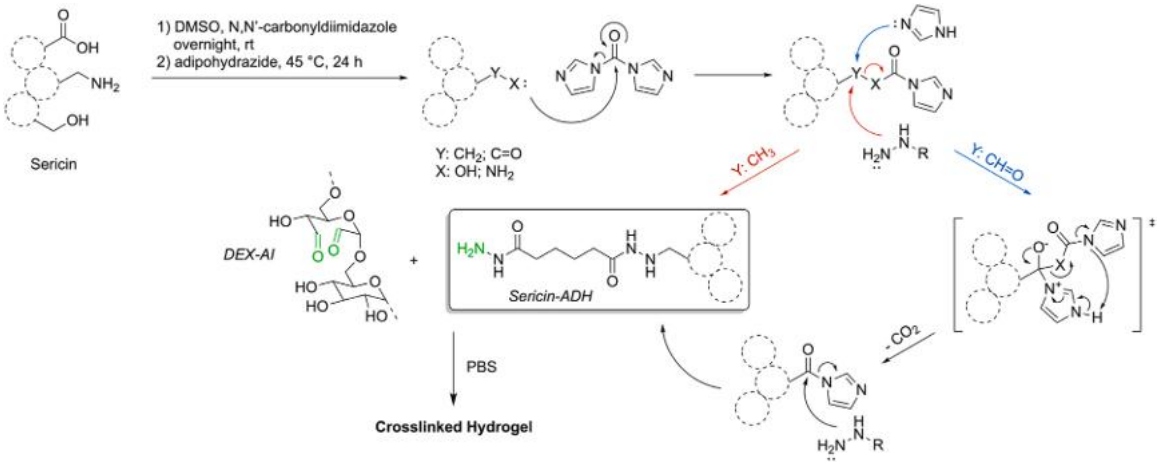
To achieve this, the researchers dissolved sericin (5.6 g) in 100 mL of DMSO at 35 °C for 2 h. To this solution, 4.1 g of N,N'-carbonyldiimidazole in 50 mL of DMSO was added. After stirring at room temperature overnight, the main batch was added dropwise to a solution of adipohydrazide (43.55 g in 200 mL DMSO) and stirred for 24 h at 45 °C. The functionalized sericin (sericin-ADH) was then obtained through dialysis and lyophilization. The hydrogel was fabricated by mixing equal volumes of sericin-ADH PBS solution (20 % w/v) with dextran dialdehyde (DEX-AI) PBS solution (10–35 % w/v).

The modification strategy employed encompasses a series of synthetic steps rooted in the fundamental concept previously reported by Wang *et al.* [78], which involves the utilization of imine-like bonds, specifically, acyl hydrazones in this case. However, the approach adopted by Liu's group deviates from traditional methodologies by transforming various native sericin functional groups into a uniform reactive entity, thereby enhancing the versatility of reactivity across multiple amino acid residues rather than restricting it to a limited subset. Initially, hydrazide groups are introduced into sericin (sericin-ADH) through the reaction of serine, lysine and/or glutamic acid residues, with N,N′-carbonyldiimidazole. This conjugation proceeds via two distinct mechanisms, as depicted in Scheme 29.

Post-reaction analysis demonstrates a 2.5-fold increase in -NH_2 groups, as determined by ninhydrin colorimetric assays, indicating the successful attachment of hydrazide functionalities. Subsequently, the sericin-ADH conjugate is crosslinked with chemically modified dextran (DEX-Al) to form hydrogels with varying degrees of DEX-Al concentrations. Following the crosslinking process (gelation), a significant reduction in the concentration of free -NH_2 groups was observed, correlating with the DEX-Al concentration. This decline indicates that the hydrazide groups effectively reacted with aldehyde functionalities, confirming successful crosslinking. FTIR was employed to characterize the hydrogels, revealing that the secondary structure of sericin remained largely unaltered post-crosslinking, with the exception of a slight conformational shift from a random coil to a β-turn. The kinetics of gelation were evaluated in PBS (pH 7.4) at multiple temperatures (4, 25, and 37°C). Results demonstrated that gelation time was both temperature- and DEX-Al content-dependent; specifically, an increase in either temperature or DEX-Al concentration resulted in a shortened gelation time. These findings suggest that fine-tuning these parameters could enable the customization of gelation properties to achieve desired system characteristics. While rapid gelation can offer advantages for *in vivo* applications, excessively fast reaction kinetics, occurring within seconds, are generally suboptimal for such systems.

SEM analysis of the sericin/dextran hydrogels revealed a highly ordered porous architecture, with pore size inversely correlated to the DEX-Al content; this is attributable to the increased prevalence of hydrazone bonds that promote denser crosslinking. Additionally, the extent of crosslinking influenced the swelling behavior of the hydrogels: increased crosslink density resulted in reduced water uptake, likely due to decreased pore size. This effect was more pronounced under acidic conditions, which may have compromised the stability of the acyl hydrazone bonds.

Following the initial phase centered on the physicochemical characterization of the materials, the researchers proceeded to evaluate properties pertinent to the intended application of the hydrogels. *In vitro* degradation studies conducted across different pH conditions over time revealed that the hydrogels degraded more rapidly in alkaline environments compared to slightly acidic (pH 6.0) and neutral (pH 7.4) conditions. The authors attributed this increased degradation rate primarily to protein hydrolysis rather than the hydrolysis of hydrazone linkages. *In vitro* assessments demonstrated favorable cytocompatibility, indicating the material's biocompatibility with cellular systems. Complementary *in vivo* studies (on mice) showed complete material degradation after 70 days, accompanied by minimal inflammatory response. The implantation site exhibited only a limited infiltration of inflammatory cells, providing strong evidence that sericin-based hydrogels elicit low immune reactions and supporting their potential for biomedical applications. Finally, the hydrogels demonstrated effective performance in the controlled release of both macromolecular and small-molecule drugs, with a notable inverse correlation between DEX-Al content and the cumulative release profiles. This trend is attributed to the decreased pore size and porosity associated with higher crosslinking density.



Scheme 29. Sericin-based crosslinked hydrogel preparation and previous protein functionalization strategy.

An important innovative aspect of this study, compared to existing literature, lies in the utilization of sericin's intrinsic photoluminescent properties as a novel methodological approach for characterizing and monitoring the functional attributes of the hydrogels. The findings indicated that sericin's photoluminescence was preserved in its functionalized form as well as within the assembled sericin/dextran hydrogel and scaffold constructs, thereby providing an additional, non-invasive means of tracking material properties and integrity. This technique enabled the verification of crosslinking through comparative analysis of the absorption and emission spectra of the synthesized compounds, facilitating the identification of specific wavelengths suitable for noninvasive monitoring of the hydrogels. Notably, despite being implanted subcutaneously, the hydrogels could be distinctly detected via their emitted photoluminescence, allowing for effective transdermal transmission. This capability enabled straightforward monitoring of *in vivo* and *in vitro* degradation processes, drug release kinetics, and porous structural characteristics. Ultimately, the authors successfully designed and developed a novel, crosslinked, sericin-dextran injectable hydrogel that is biostable and photoluminescent, establishing a versatile platform with potential applications in cancer therapy.

Regarding the synthesis of biomaterial for endothelialization of tissue-engineered heart valves, Wang *et al.* [60] published a paper in which sericin was chosen as the optimal candidate for constructing a biocompatible scaffold. In this context, tissue-engineered heart valves are exceptional systems, as they offer outstanding potential in terms of self-repair, growth promotion, and reconstruction, which can overcome the issues associated with prosthetic valves. In this field, animal-derived decellularized heart valves (DHV) are widely used as natural scaffolds. However, most commercially available DHVs are crosslinked with glutaraldehyde, raising concerns about its toxicity and the possible release of residues. To address this issue and avoid the use of particularly costly biomolecules for tissue engineering, the authors believed that sericin, appropriately modified, could provide a solution. In the study, sericin was acylated with maleic anhydride; meanwhile, the DHVs were initially chemically modified with maleic anhydride, and subsequently with 4-arm polyethylene glycol thiol, as depicted in Scheme 30.

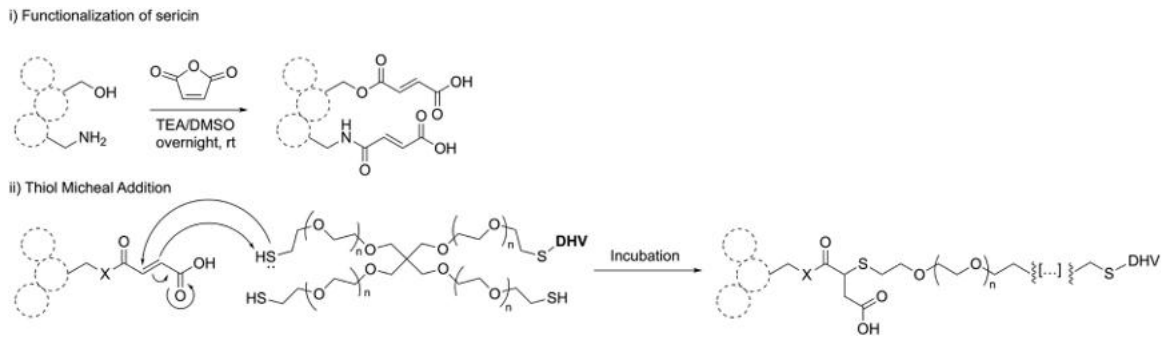
Specifically, sericin was dissolved in DMSO (4 % w/v), and an equal volume of maleic anhydride solution (0.8 % w/v) in DMSO was added, along with triethylamine as a catalyst (0.55 % v/v). The mixture was stirred overnight at room temperature, and the final product was recovered by dialysis and lyophilization. Subsequently, thiolated DHV valves were incubated in a 3 % (w/v) solution of functionalized sericin overnight.

The crosslinking process was based on a Michael 1,4-addition click reaction, whereby both modified DHV and sericin (MSS) participate. Specifically, the sulfur atom of the thiol FGs selectively attacks the electrophilic carbon at the fourth position of the Michael acceptor, a preference dictated by the soft acid-soft base interactions outlined by HSAB theory. Although this reaction exhibits high intrinsic selectivity, it is important to consider that maleic anhydride may also induce intramolecular crosslinking within sericin molecules. Such occurrences, while generally limited, are not entirely implausible. The successful synthesis of MSS was corroborated through spectroscopic analyses: ^{1}H NMR spectra revealed signals characteristic of maleic double bond moieties, and FTIR spectroscopy identified bands corresponding to the out-of-plane bending vibrations of =C-H groups associated with the introduced moieties. In addition to these observations, the authors further substantiated their claim by reporting a substantial reduction (~95 %) in the concentration of free amino groups in sericin post-reaction, as determined by a colorimetric assay. This finding suggests an almost quantitative degree of functionalization. However, as extensively discussed earlier in this review, lysine residues are not the sole nucleophilic functional groups present within the protein matrix; consequently, the involvement of other nucleophilic moieties should have been examined, though such investigations were not reported.

The functionalization of DHV and the subsequent crosslinking process were monitored using analogous methodologies. Beyond the previously described data, the disappearance of the thiol group, initially present in the thiolated DHV and absent in the final system (SCMV - sericin covalently modified valve), was particularly noteworthy, indicating successful crosslinking and chemical modification during the final reaction step. As documented in the study by Liu *et al.* [146], even in this case, the authors utilized the inherent photoluminescence properties of sericin to monitor the extent of chemical modification. The observed fluorescence intensity was notably strong, which served to further confirm the presence of sericin on the valve surfaces.

SEM and AFM analyses revealed that the surface of the SCMV exhibited a smooth and continuous topography, in contrast to the more irregular surface observed in the unmodified DHV. The measured surface roughness values were comparable between the two samples, indicating minimal or no significant alterations to the surface topology induced by sericin coating. Additionally, the application of sericin was found to enhance the hydrophilicity of the valve surfaces, a critical parameter in biomaterials that facilitates improved cell adhesion and tissue integration. The final material demonstrated a low hemolysis rate, suggesting favorable hemocompatibility. Moreover, the results obtained for SCMV indicated that sericin modification could potentially mitigate chronic inflammatory responses following tissue-engineered heart valves (TEHV) implantation. Finally, the modified valves demonstrated successful adhesion of seeded cells, which were observed to actively proliferate, migrate, and differentiate on the valve surface. Additionally, cellular infiltration and the formation of new capillaries were detected in the SCMV, attributed to the pro-migratory and pro-angiogenic properties of sericin. These findings indicate that covalent modification of the valve surface can effectively promote endothelialization both *in vitro* and *in vivo*. In conclusion, sericin-coated valves exhibit considerable potential for application in regenerative medicine, as they confer favorable biocompatibility and facilitate endothelialization while exhibiting high resistance to thrombogenesis. Consequently, such systems may serve as promising candidates for application in blood-contacting medical devices.

In 2016, Vulpe *et al.* [48] published a paper focused on the



Scheme 30. Sericin-thiol crosslinking by Michael addition.

rheological study of *in situ* crosslinkable hydrogels based on biocompatible materials, specifically collagen, hyaluronic acid (HA), and sericin. In the field of tissue engineering, collagen and hyaluronic acid have been widely studied and discussed [147,148], but sericin has not been investigated as extensively as the two previously mentioned macromolecules. Moreover, introducing sericin into a collagen-hyaluronic acid-based network could represent a key factor in exploring and achieving additional material properties such as swelling, particle size tuning, and degradability. To crosslink these systems, the authors decided to exploit carbodiimide coupling, using both ester- and amide-activating agents to covalently bond the three biopolymers (Scheme 31).

To do this, the researchers used 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). Specifically, the hydrogels were obtained by immersing the biopolymers in an aqueous solution (40 % v/v, ethanol/water), under magnetic stirring for 24 h. Then, EDC and NHS were added with a molar ratio of COOH:EDC: NHS= 1:10:2.

Despite the considerable efforts demonstrated by the authors in assessing the rheological properties of the various hydrogels derived from their methodology and during the reaction, this analytical approach alone does not unambiguously confirm the occurrence of the reaction process. It is noteworthy that the majority of the substrate functionalization studies proved their successes in their objectives through the application of diverse spectroscopic techniques, complemented by thermal analysis and other methods, providing more comprehensive insights. Regarding the reaction efficiency, it was determined gravimetrically, thereby offering a limited chemical insight into the reaction process and outcome. Nonetheless, it was observed that the hydrogels prepared in the presence of hyaluronic acid (HA) exhibited higher mass yields compared to all other formulations.

Water uptake assessments were conducted to evaluate the hydrogels' capacity for water retention, revealing that those composed solely of biopolymers that bonded through ester bonds exhibited the highest swelling ratios. The authors also noted that, owing to its relatively low MW compared to the other constituents, sericin plays a limited role in influencing the swelling behavior within the system. Hydrogels incorporating collagen demonstrated significantly reduced swelling ratios, which was attributed to the distinct morphological features observed. In fact, SEM imaging confirmed the 3D structure of the crosslinked hydrogels, characterized by an interconnected pore network whose dimensions were directly dependent on the specific components and their ratios. *In vitro* biocompatibility tests further indicated that the hydrogels were non-cytotoxic, with cell viability values comparable to the control group.

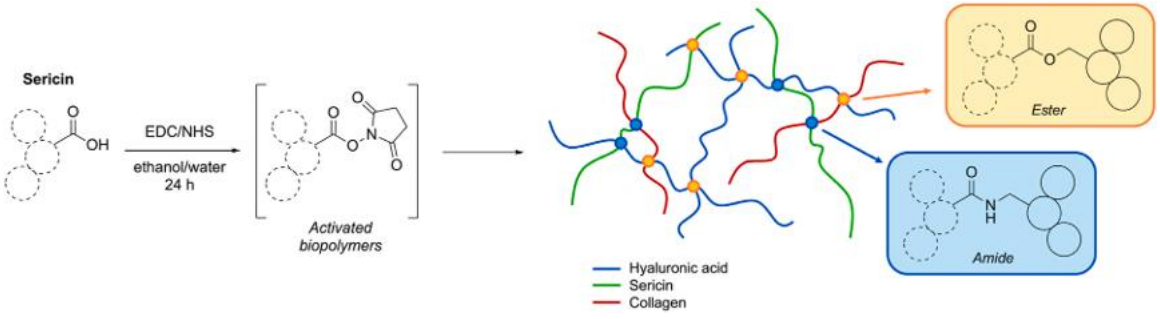
In the work published by Liang *et al.* [133], the group reported the preparation of a smart washable electronic textile by dyeing conventional textiles with sericin-graphene ink. Electronic textiles represent an area of interest for the development of wearable devices, as they would enable the collection of useful data for various applications simply by wearing them. However, the fabrication of these materials with adequate comfort and durability still represents a major challenge. Similarly, the use of graphene ink to "e-dye" the textile is not straightforward, as it suffers from water solubility issues. Therefore, the use of hydrophilic and aqueous, non-toxic, environmentally friendly graphene ink is highly desired, as opposed to non-aqueous alternatives. As a result, the authors used sericin as an amphiphilic agent in the preparation of graphene ink (Scheme 32), creating a subsequent crosslinkable system to secure the dyes, thanks to its intrinsic properties.

To perform the crosslinking reaction, the research group, after having treated a textile with the sericin-graphene ink, immersed the textile in a mixture of acetone and DMSO (1:1). Then, hexamethylene diisocyanate (HDI) was added (1:2 molar ratio to the free hydroxyl group of sericin), and the mixture was heated to 60 °C for 6 h.

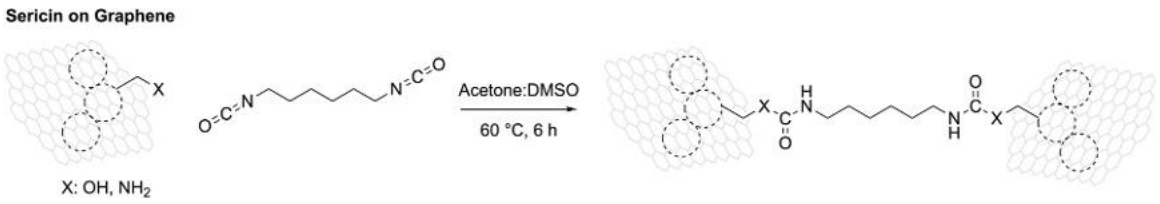
The comprehensive synthetic approach outlined by the authors encompasses several interesting strategies aimed at maximizing the potential of sericin. As a preliminary step, a stable and homogeneous aqueous ink composed of sericin-graphene (SG) was prepared. This was achieved by leveraging the aromatic residues of sericin to establish non-covalent interactions (likely π-π stacking) with the graphene layers, thereby effectuating a form of non-covalent grafting. Additionally, given that more than half of the amino acids in sericin possess hydrophilic side chains, the resulting ink demonstrated inherent stability in aqueous environments without the need for supplementary artificial additives. It is well-documented that sericin exhibits a high capacity to disperse carbon nanomaterials in water [149], further contributing to the stability and dispersibility of the formulation.

Raman spectroscopy was utilized to elucidate the successful deposition of sericin onto exfoliated graphene layers, primarily evidenced by shifts in the characteristic 2D band. These spectroscopic results were further validated by transmission electron microscopy (TEM), which revealed multilayered graphene surfaces distinctly encapsulated by amorphous regions corresponding to sericin. Additionally, AFM measurements indicated a film thickness of approximately 4 nm, further supporting the conclusion that sericin effectively coated the graphene substrate.

Subsequently, a commercial polyester textile was dyed with the synthesized sericin-graphene (SG) ink and subjected to *in situ* crosslinking using hexamethylene diisocyanate (HDI), resulting in the final-treated textile designated as HSG. HDI exhibits moderate reactivity toward various FGs present on sericin, making chemoselectivity challenging to achieve; however, this aspect does not constitute a critical issue within the scope of the study. Notably, unreacted functional groups on sericin may offer opportunities for further tailoring of SG properties. To follow the crosslinking procedure, the authors employed FTIR, with background subtraction of the absorbance of graphene contributions. In addition to the characteristic protein bands, the spectra revealed the presence of linear methylene absorption signals and prominent "ester moiety" carbamate bond bands. Following the crosslinking process, the modified SG-coated textile demonstrated enhanced hydrophilicity, as evidenced by decreased contact angles, attributable to the incorporation of sericin. This increase in hydrophilicity underscores the successful



Scheme 31. Sericin crosslinking by esterification and/or amidation.



Scheme 32. Sericin-hexamethylene diisocyanate crosslinking reaction.

enhancement of the textile's affinity for water. Notably, the SG composite retained its electrical conductivity post-crosslinking, thus preserving its suitability for potential applications in electronic devices.

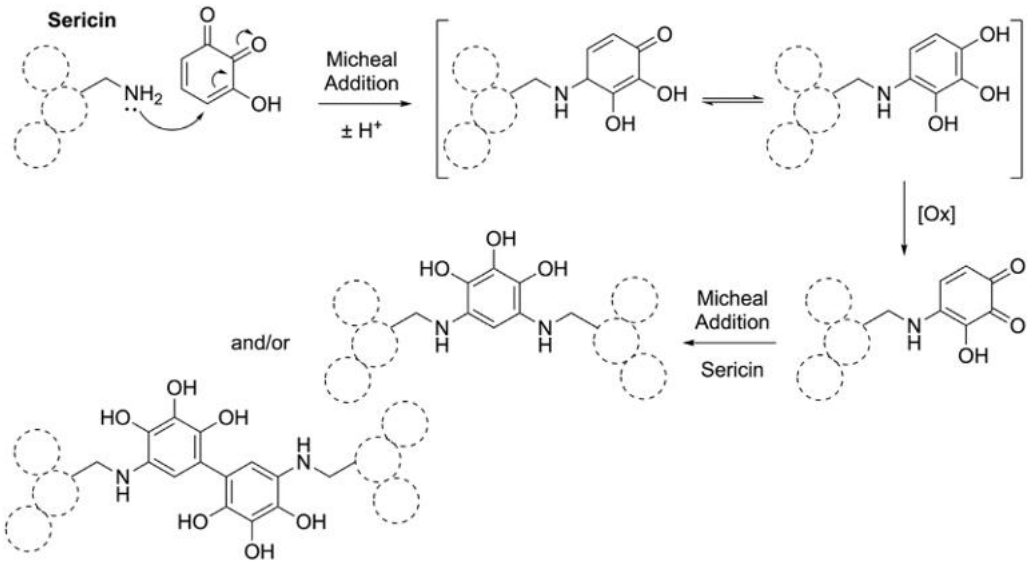
Given the potential for direct skin contact, the biocompatibility of the treated textile was assessed through *in vitro* incubation of human endothelial cells (HEC) on pristine graphene, SG, and HSG samples. The results demonstrated that in all cases, the seeded cells retained normal morphology, indicating favorable biocompatibility.

To evaluate wearing comfort, two key parameters, hydrophilicity and breathability, were examined. Hydrophilicity was assessed by measuring the vertical water-wicking rate, revealing that the HSG sample exhibited rapid and complete water absorption, in stark contrast to the untreated textile. Meanwhile, breathing performance was evaluated through the water vapor transmission rate (WVTR), with findings indicating that the crosslinking process did not adversely affect this parameter. The results also suggest that the textile's original knitted structure was preserved, maintaining its breathability while enhancing hydrophilicity, thereby supporting its suitability for practical wearable applications.

Considering the high stability of the SG ink in aqueous systems, concerns regarding its washing durability were addressed through empirical testing. The results demonstrated that electrical conductivity was effectively retained in the HSG samples, whereas it was substantially diminished in the SG samples, thereby emphasizing the critical role of the crosslinking process in preserving functional properties. These findings were further substantiated by SEM analysis, which revealed that ink particles remained clearly observable on the fibers of the HSG-treated textile, in contrast to their absence in the untreated SG samples. Additionally, SEM imaging confirmed that the HSG coating was uniformly distributed across the textile surface, conforming precisely to the underlying fiber structure. Importantly, this coating did not interfere with the textile's native properties, supporting the effectiveness of the crosslinking approach in maintaining both the functional and structural integrity of the material. Building upon these findings and leveraging the inherent flexibility of SG sheets, the authors demonstrated a proof-of-concept by fabricating a textile-based electronic circuit incorporating their ink, an LED, and a power source. Remarkably, the system maintained continuous functionality, with the LED remaining illuminated even under conditions of bending, twisting, and stretching of the textile substrate. Furthermore, the authors advanced this work by developing an integrated multimodal sensing platform composed of handcrafted electromyogram (EMG) textile electrodes in conjunction with strain sensors. This system successfully recognized complex hand movements with high accuracy, thereby illustrating the significant potential of HSG textiles for the development of integrated electronic components within smart wearable devices.

In 2022, Omar *et al.* [150] conducted a study aimed at evaluating the covalent interactions between sericin and phenolic compounds, with a particular emphasis on their structural and physicochemical properties, due to the limited existing literature on this subject. The researchers selected two widely occurring natural phenolic compounds, hydroquinone and pyrogallol, and systematically assessed the resulting alterations in their respective properties following respective interactions.

In their study, 10 g of cocoons were suspended in 500 mL of distilled water and heated at 121 °C for 60 min in an autoclave. The extracted sericin was then centrifuged for 20 min at 4 °C. The pH of the supernatant was adjusted to pH 9 with 1 M $NaOH_{(aq)}$. Hydroquinone and pyrogallol were added to the supernatant in a 1:10 (phenol/sericin) ratio. The pH was adjusted again to 9, and the mixture was stirred for 24 h at ambient temperature. Finally, the unbound phenolic compounds were removed by dialysis. The proposed reaction mechanism is illustrated in Scheme 33, with particular emphasis on the lysine residues of sericin, as the authors primarily focus on these amino acid sites. However, it is important to acknowledge that other FGs within the protein may also participate in similar reactions. Phenolic compounds are inherently reactive and can be readily oxidized to form quinones, which are highly reactive intermediates capable of covalently interacting with proteins [151,152]. Following the reaction with sericin, the phenolic moiety can undergo further oxidation, enabling subsequent reactions with additional protein molecules or facilitating intra- or intermolecular crosslinking. Regarding the mechanistic perspective, previous studies have demonstrated that the selectivity of similar reactions can be interpreted using the (HSAB) theory [153]. Consequently, applying the HSAB framework in this context may provide valuable insights into the specific pathways and selectivity patterns governing the reaction process. Although nitrogen is frequently characterized as a hard base, as is oxygen, the plausible reaction mechanism presented in Scheme 33 predominantly involves a 1,4-Michael addition, despite the fact that the carbon atom in the β-position relative to the carbonyl is typically considered a soft site. According to the HSAB theory, such an attack would be generally disfavored in favor of a direct nucleophilic attack on the more electrophilic, hard carbon, such as the carbonyl carbon via a 1, 2-addition. However, it must be acknowledged that kinetic factors, thermodynamic stability, and specific reaction conditions critically influence the reaction pathway. In particular, nucleophilic attack at the carbonyl carbon (1,2-addition) is typically associated with kinetically controlled pathways, especially when highly reactive nucleophiles or bases are involved. Conversely, the reported 1,4-Michael addition represents a thermodynamically favored pathway, reflecting a preference for the more stable conjugated system under certain conditions [154]. Furthermore, a key distinction between the two mechanisms pertains to the nature of the formed intermediate, which is intrinsically linked to the site of nucleophilic attack. The intermediate generated via the kinetic pathway generally exhibits lower stability compared to the enone intermediate produced through the 1,4-Michael addition. Additionally, the reaction conditions employed do not prohibit reversibility in either pathway; consequently, under equilibrium conditions, the thermodynamically more stable product, in this case, the 1,4-addition adduct, is favored. The authors conducted commendable work in tracking the reaction progression and thoroughly characterizing the resulting products. The total phenolic content (TPC) and free amino group content (FAC) were quantified via UV spectroscopy, employing Folin reagent and *o*-phthalaldehyde, respectively, to assess the chemical modifications. The TPC of the modified samples was significantly higher than that of pristine sericin, indicating successful incorporation of phenolic groups. Conversely, the FAC decreased post-reaction, confirming the occurrence of amino group consumption during the process. Size-exclusion chromatography coupled with high-performance liquid chromatography (SEC-HPLC) was utilized to analyze the MW distribution of the sericin derivatives modified with hydroquinone (HS) and pyrogallol (PS). In both cases, the elution times were shorter than those of the native starting material, suggesting a slight increase in MW attributable to



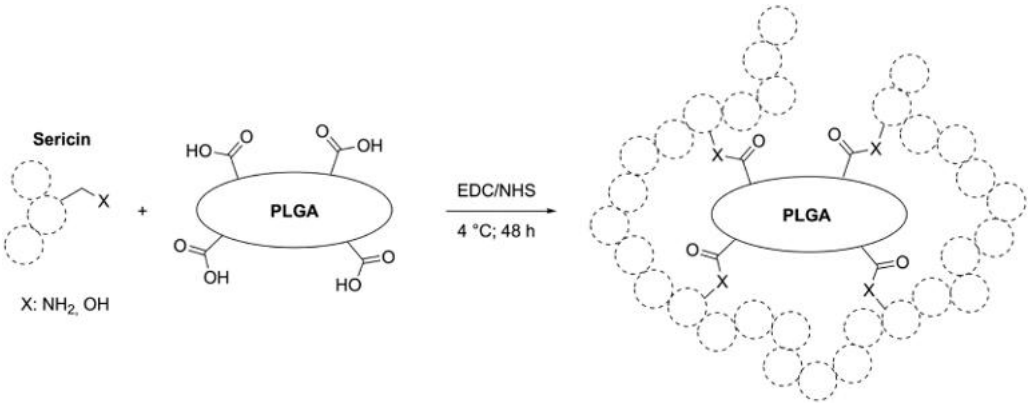
Scheme 33. Proposed mechanism of the reaction of sericin with phenolic compounds (specifically, pyrogallol) and subsequent crosslinking.

crosslinking. Furthermore, the broadened elution peaks indicated an increased heterogeneity in MW distribution, consistent with network formation and crosslinking within the samples. The researchers further substantiated these findings through UV spectroscopic analysis. The modified sericin samples exhibited, in addition to their characteristic absorption peak, supplementary signals attributable to the incorporated phenolic compounds, albeit with a slight redshift. Notably, the intensity of these newly observed peaks correlated proportionally with the amount of phenolic fractions introduced. The observed redshift may be ascribed to an extension of the π-conjugated system resulting from the chemical modification, or potentially from interactions between the phenolic moieties and the aromatic side chains of sericin. Considering the specific absorption wavelengths within the UV-A and UV-B ranges, these modified systems may also possess potential applications as UV-filtering agents. FTIR revealed only subtle spectral changes post-reaction, notably a slight redshift in the bands corresponding to amide A, amide B, and amide I. These shifts were attributed to increased hydrogen bonding interactions and alterations in the availability of free amino groups, thereby providing additional evidence supporting the occurrence of the proposed reaction. Although FTIR has been traditionally employed to assess secondary structure variations following crosslinking procedures, the authors opted to employ far-UV circular dichroism (CD), citing the significant overlap of spectral bands as a primary limitation in IR. Overall, the CD spectra of native sericin and its modified derivatives, HS and PS, exhibited similar profiles, with a common dominant band near 200 nm. Nonetheless, the reduced peak intensities observed for HS and PS suggest subtle modifications in the secondary structural elements, particularly an increase in β-turn configurations. In addition to employing spectroscopic methods, the authors assessed the thermal stability of the HS and PS samples, which demonstrated enhanced resistance to thermal degradation. The covalent crosslinking modification also influenced the surface hydrophobicity, resulting in increased surface hydrophobicity due to the presence of non-polar aromatic rings and the covalent attachment of functional groups. This heightened hydrophobicity contributed to greater repulsion toward water, which was corroborated by the improved oil-in-water emulsifying properties, as the surfaces of HS and PS more readily interacted with oil dispersed within aqueous media. Lastly, the study investigated the biological activities of the sericin-phenolic conjugates, which exhibited notable inhibitory effects on nitric oxide (NO) production, a marker associated with inflammatory responses under pathological conditions. Additionally, both HS and PS displayed enhanced free radical scavenging capacities and metal chelating activities, indicating their potential as biofunctional agents with antioxidant properties. In conclusion, the results obtained demonstrate that the modified sericin exhibits significant potential as a multifunctional additive, owing to its interesting physicochemical and biological properties. In the context of biomedical applications, sutures are a widely used material consisting of fibres of fibrous structures generally classified as absorbable or not. For these materials physical, mechanical, and biological properties are crucial. To improve wound healing, sutures can also be used to locally deliver cells, growth factors, and active biomolecules, such as antibiotics; however, given the rapid worldwide rise of resistant bacteria there is also the need of finding alternatives. In this framework, the silver-based products gathered attention since the low propensity of bacteria to develop resistance to them. As a consequence, Gallo *et al.* [155] published a study where they focused on the development of silver/sericin coatings for commercially available poly (lactic-co-glycolic acid) (PLGA) sutures to obtain a system that merges the different advantages of the single components.

The surface-exposed carboxylic acid groups of hydrolyzed PLGA were activated using EDC/NHS chemistry, followed by immersion in a 6 % sericin solution for 48 h at 4 °C (Scheme 34). After washing and drying, the sericin-coated sutures were treated with a silver nitrate solution and subjected to *in situ* photoreduction using methanol as the reducing agent under UV light (λ = 365 nm), forming silver clusters on the surface.

The synthesis can be then divided into three main steps: activation of PLGA by initial exposure of carboxylic acid through hydrolysis and subsequently conversion of them into *N*-succinimide ester; conjugation with sericin (Scheme 34); silver deposition onto sericin-PLGA by UV photoreduction of silver nitrate. Regarding the mechanism, in this case, in contrast with other reported studies, sericin acted as nucleophile and not as the substrate to be functionalized. Meanwhile, the photoreduction of silver was conducted with the addition of methanol which acted as a reducing agent. The reaction mechanism is discussed in the literature [156]; however, the authors did not further add any information regarding the participation of other FGs in the redox system, which might lead to unexpected behaviors or performances. Moreover, since methanol is oxidized and the material is thought to be used in the biomedical field, the presence of formic acid or in the worst-case scenario formaldehyde should be at least considered and evaluated. In addition to this, the authors did not report any further characterization to assess the reactions' occurrence.

By SEM imaging, the authors confirmed that the suture fibers perfectly maintained their braided structure despite having achieved the



Scheme 34. Carbodiimide-mediated crosslinking of sericin with PLGA linkers.

deposition of sericin and silver nanoparticles, which quantity was estimated by EDX. ICP-MS analysis revealed the amount of silver released during suture degradation over the time. The biocompatibility and the antibacterial capability of the silver treated material were confirmed in physiological degradation conditions without inducting cytotoxic effects related to silver release and recording significantly reduced bacterial growth on any bacterial strains that were tested. The silver-sericin-PLGA sutures showed a lower release of silver compared to the untreated sericin-PLGA system; the authors attributed this behavior to a shielding effect of silver which retards the degradation of sericin. The results also demonstrated that cell viability and proliferation were not affected by the presence of silver. In conclusion, the authors highlighted the synergistic effect of silver and sericin which improved cell migration and proliferation in the wound area confirming the general concept of their study. Nonetheless, the lack of chemical information about the process and the characterization severely limits the topic, especially in the optic of further sericin modification and properties tuning.

4. Unsolved challenges in sericin functionalization

In the previous sections selected examples of chemistries applied to sericin functionalization have been explored by affording sericin derivatives featured with different properties, resumed in Table 3.

Overall, it can be said that the functionalization of silk sericin presents significant potential but is still hampered by several notable limitations. Analyzing the functionalization/modification methods, it is evident that sericin's reactivity is often constrained due to its heterogeneous amino acid composition and the limited accessibility of functional groups. This results in challenges related to reaction selectivity and variability in outcomes, which are further influenced by solubility issues in both aqueous and organic solvents. Sericin's solubility is heavily dependent on environmental conditions and prior processing treatments, complicating the preparation of homogenous and controlled functionalized derivatives. Additional concerns include chemical stability and the lack of standardized protocols, which hinder cross-study comparability. Moreover, many studies overlook the structural alterations of sericin that may occur during chemical reactions, in turn affecting its functional properties. Another persistent challenge lies in the characterization of sericin and its derivatives; even today, completely comprehensive and reliable analytical methods of such complex systems remain a significant hurdle in the field. Inadequate chemical and physical characterization approaches or techniques often limit the understanding of the detailed relationship between chemical modifications and products properties. Even though it's extremely rich, the literature to date shows a lack of detailed, reproducible procedures, and standardized characterization techniques; in some cases oversimplified chemical methodologies are reported. These inaccuracies can mislead other research teams and hampers the progression of research. Addressing these issues through more rigorous characterization and methodological accuracy is crucial for advancing the effective functionalization of sericin and unlocking its full potential.

In order to reduce the gap between potentialities and applications, some hints follow.

Regarding the functionalization of silk sericin, several strategies grounded in current chemical research can be proposed. Firstly, improving reactivity and selectivity may be achieved through site-specific modification techniques. For example, exploiting the unique reactivity of certain amino acid residues, such as lysine, tyrosine, or cysteine, via selective chemical derivatization, taking advantages of click-chemistry strategies, or by enzymatic approaches can enhance control over functionalization and eventually exploit those FGs to further introduce interesting moieties for orthogonal reactions. Reaction conditions should be accurately designed, considering potential side reactions and by-products that may influence chemical structure of the products and purification steps. Secondly, to overcome solubility issues and heterogeneity, employing solvent systems such as aqueous or organic media supplemented with additives or ionic liquids can facilitate more homogeneous reactions. Thirdly, addressing characterization challenges requires the application and the complementation of advanced analytical techniques, such as mono and bidimensional NMR, mass spectrometry, FTIR, to cite a few, combined with chromatographic methods, such as SEC-HPLC. Solid-state NMR could also provide detailed insights into post-functionalization structural changes, while HRMS, alone or combined, can confirm the incorporation of desired FGs. Standardization of these analytical protocols will improve reproducibility across studies. Furthermore, enhancing methodological reproducibility involves detailed reporting of reaction conditions supported by comprehensive characterization data. Finally, to prevent the dissemination of inaccurate or misleading information, the scientific community should emphasize the need for rigorous chemistry and reproducibility. Implementing validation experiments, such as control reactions and stability assessments, can help verify the reported procedures. These combined efforts are essential steps to overcome current limitations and advance the reliable functionalization of silk sericin.

Finally, the use of water as a solvent in chemical processes has been frequently advocated as a sustainable and environmentally benign alternative to the most classical organic solvents. However, water can trigger undesired reactions, imposing the use of large excess of chemicals; moreover, water is anticipated to become an increasingly vital and highly demanded resource in the coming years. In this framework, the assertion that using water as a solvent is inherently green is fundamentally misleading if it fails to incorporate rigorous protocols for water purification and contaminant control, particularly when the reactions involve hazardous or toxic reagents. Without a clear, effective water reuse strategy, using it as a solvent isn't truly sustainable. This reduces its ecological and safety benefits, making it an inadequate substitute for

Table 3
Summary of the presented strategies of sericin chemical modification, along with the respective reaction types, reagents used, modified sericin's properties, and applications/improvements.

General Functionalization

Scheme	Reaction type	Reagents	Modified Sericin properties	Conceptual Application	Ref.
4	Esterification	$MeOH_{(xs)}$; $HCl_{(cat)}$	• MeSS beads were spherical with smooth surface • Swelling pH-dependant; increased under acidic environments	SS-beads as drug nanocarriers resistant to gastric acid	[87]
10		i) AcOH ii) Ac_2O; H_2SO_4	• Hydrophilicity significantly reduced • Increased elastic modulus. Elongation at break decreased • Enhanced thermal stability	SS properties general improvement	[105]
5	Isocyanate coupling	IEM; DMSO/LiCl; N_2	• Honeycomb-like morphology • Increased hydrophilicity compared to pristine SS • Swelling inversely proportional to DoF. Initially intense to reach plateau • Compressive modulus dependant on DoF; increased with DoF • Gelation process affected by DoF • No changes on structure; CD showed slightly increase of β-sheet	Cytocompatible hydrogels for tissue regeneration	[8]
6		MOI or AOI; DMSO	• Water solubility decreased, dependant on the packing efficiency after the reaction • Swelling negatively affected • Strength and tensile moduli increased; elongation break decreased	SS as bio additive into poly urethanes	[93]
7	Halogenation	a) MsCl; DMF, N_2 b) NBS-PPh_3; DMF, N_2	• nd	Reactivity study/change	[94]
11	Chlorination	TCT; DMF, DCM	• Surface roughness increased • Increased crystallinity • Antibacterial behavior increased after the modification	Improving dyeing of textiles	[71]
8	Epoxide ring opening	EPTAC; $CaCl_2$, H_2O; pH 8	• Surface roughness increased • Increased tensile strength • Antibacterial behavior increased after the modification	Improving dyeing of silk fabrics	[96]
9	*N*-Acylation	Fatty acid acyl chlorides; H_2O; pH 9	• Highly water-soluble	SS lipopetide-based surfactants	[58]

Covalent Grafting

Scheme	Reaction type	Reagents	Modified sericin properties	Conceptual application	Ref.
13	Imine formation	PEI; GA; MeOH	• Water insoluble	SS-based metal biosorbent	[112]
14	Amide or ester coupling	EDC·HCl; H_2O; pH 5	• Water soluble	Preparation of water soluble FGO	[116]
15	ROP	$Sn(Oct)_2$; *L*-lactide	• Insoluble in water, but soluble in organic solvents • Hypothetically increased swelling • Low degradation temperatures and Tg • Decreased degree of crystallinity	Evaluation of SS as ROP initiator	[117]
18	Free radical	MMA; CAN; H_2O; N_2	• Surface roughness increased	Improving SS properties	[126]
16		*L*-ascorbic acid/H_2O_2; SUT; H_2O/EtOH	• nd	Bioconjugated drug nano delivery systems	[121]
17	"Acylation" then hydrazone formation	i) S-HyNic; PBS ii) PLA-CHO; DMF	• Raspberry-like self-assembled micelles • Water soluble • Slight conversion of random coil to β-sheet		[125]
19	Acyl hydrazone formation	i) EDC/S-NHS; MES (pH 5) ii) DOX·HCl; TEA/dry-DMSO	• Self assembled nanoparticles • Water soluble		[127]
20	Isocyanate coupling	4-CN-PnNCO; DMSO/LiCl	• Insoluble in water, but soluble in organic solvents • Decreased hydrophilicity • Reduction decomposition temperatures • Structure changes due to the steric hindrance of the introduced moieties	Evaluation of SS properties after the modification	[128]
21	"Acylation"	i) MA; PBS (pH 8.5) ii) APS/TEMED freeze polymerization	• nd	Cryogels as hemostatic agent	[131]
22		NHS/EDC; MES (pH 5–5.5)	• Surface roughness increased • Hydrophilicity increased	Enhancing tissue engineering scaffolds properties	[132]

Covalent Crosslinking

Scheme	Reaction type	Reagents	Modified sericin properties	Conceptual application	Ref.
24	Mixed	PVA; Glycerol; Genipin; EtOH	• Increased moisture absorption • Swelling directly proportional to crosslinking degree	SS-based scaffolds for wound healing	[5]

(*continued on next page*)

Table 3 (*continued*)

Covalent Crosslinking					
Scheme	**Reaction type**	**Reagents**	**Modified sericin properties**	**Conceptual application**	**Ref.**
25	Epoxy ring opening	PEGDE; $CaCl_2$; H_2O (pH 8)	• nd	Enhance natural colored silk properties	[139]
26	*In situ* acylation	Crocin; NaOH; H_2O (pH 11)	• Well-defined morphology, almost spherical nanoparticles	Development of nose-to-brain drug carrier	[140]
27	Imine/Acetal/Hemiacetal formation	GA; $HCl_{(cat)}$; H_2O	• Surface roughness increased • Initial rapid swelling increase to plateau; improved at alkaline env. • Demonstrable strength and elongation at break • Increased thermal stability • Increased content of stable β-sheet	2D membranes for tissue engineering	[77]
28	Imine formation	DCMC; H_2O	• Formation of pores • Water soluble under lower and higher pH • Hydrophilicity decreased; but higher compared to SS • Decreased swelling by the crosslink • Enhanced tensile strength and reduced elongation at break • Slight formation of β-sheet	Improving SS properties	[78]
29	Hydrazide to Hydrazone conjugation	i) CDI, DMSO; R-$NHNH_2$ ii) DEX-Al; PBS	• Highly ordered morphology and porous; pore size inversely correlated to crosslink • Swelling gradually reduced by crosslink; worse at low pH • Slight conversion of random coil to β-sheet	Development of optical trackable DDS	[146]
30	Acylation into Thiol-ene click	i) MA; DMSO; $TEA_{(cat)}$ ii) DHV-SH	• Smooth and continuous topography • SS enhanced the material hydrophilicity	Construction of biocompatible scaffolds	[60]
31	Amide or ester coupling	EDAC/NHS; HA; collagen; H_2O	• Interconnected pore network • High swelling due ester-bond containing materials	Hydrogels for tissue engineering	[48]
32	Isocyanate coupling	HDI; acetone:DMSO	• No changes to textile structure, e-ink present as distributed particles • E-ink water soluble; but insoluble after the crosslink • SS increased the textile affinity to water • Treated textile rapidly swelled water	Preparation of smart wearable electronics	[133]
33	Michael addition	Hydroquinone or Pyrogallol; H_2O, pH 9	• Decreased hydrophilicity • Increased thermal resistance • Increased content of β-turn	Study of SS interaction with phenolic compounds	[150]
34	Amide or ester coupling	i) EDC/NHS; activated PLGA ii) Ag deposition	• No changes of the suture fibers • Significantly reduced bacterial growth	Sutures properties improvement	[155]

*SS = silk sericin
*nd = not discussed

other green or traditional solvents used safely and responsibly.

5. Conclusion and future perspectives

As a biopolymer salvaged from industrial silk fiber production, sericin aligns perfectly with the global move towards circular economy strategies, bioeconomy, and the repurposing of waste materials. It holds myriad opportunities that, to date, have only been minimally investigated.

Sericin's applications span various sectors, including food, packaging, tissue regeneration, pharmaceuticals for novel drug delivery strategies, functional textile materials, and electronic devices like "e-ink". It offers several advantages, such as biocompatibility, biodegradability, antibacterial, anti-inflammatory, and antioxidant properties. However, sericin also has inherent limitations, primarily its extreme solubility in aqueous environments and limited mechanical properties. To mitigate these drawbacks, chemical modification offers a viable solution. Sericin's rich array of functional groups supports a wide range of modification strategies, which can be optimized based on the final application. While numerous studies have incorporated sericin over the years, this review specifically aimed to address its applicability perspective from a chemical point of view.

Sericin's remarkable versatility as a bio-based resource, demonstrated through the various functionalization, grafting, and crosslinking strategies outlined, has opened diverse avenues for numerous applications. These modifications have resulted in improved properties including enhanced moisture absorption, swelling capacity, metal-chelating activity, mechanical strength, color fastness, thermal stability, bioactivity, and antioxidant activity, enabling applications in biomedical scaffolds, drug delivery systems, biosorbents, tissue engineering membranes, injectable hydrogels, and smart wearable textiles, e-inks, as discussed and referenced in the previous sections.

The chemical modification of sericin, whether through grafting, crosslinking, or other methods, clearly expands its applicability by introducing new functionalities and properties. This opportunity to tailor sericin's characteristics based on specific objectives drives the exploration of diverse reaction routes and results in innovative applications. Sericin is a highly promising candidate at the intersection of engineering, materials science, and environmental fields, with strong potential to contribute significantly to the development of sustainable bio-based materials across a broad spectrum of applications.

Despite its immense potential, in the most significant sectors from an industrial perspective, some issues are still present:

- Biomedical Field: Deeper investigations into its biological properties and structure-activity relationships are essential. There is a pressing need for an increase in clinical studies and robust clinical data to support its therapeutic applications.
- Food Industry: Challenges related to its inherent smell need to be overcome. Developing safe extraction procedures suitable for

obtaining food-grade sericin with desired nutritional properties (if intended as a supplement) is critical. Additionally, its filming and hydrophilic properties require improvement for effective use in food packaging applications.
- Textile Industry: Enhancing the mechanical properties of sericin-based materials remains a key area for development to meet the demands of durable textiles.

Collectively, the presented examples strongly affirm the potential of utilizing the silk industry by-product, sericin, to advance a wide range of sectors. Addressing the outlined limitations through continued research and development will be crucial to fully unlocking sericin's full commercial potential and supporting a more sustainable future.

CRediT authorship contribution statement

Rony Aad: Conceptualization, Methodology, Data collection, Data analysis, Writing–original draft, Writing – review & editing, Visualization. **Luca Leuzzi:** Conceptualization, Methodology, Data collection, Data analysis, Writing–original draft, Writing – review & editing, Visualization, **Silvio Mandelli:** Writing- original draft. **Laura Cipolla:** Conceptualization, Supervision, Writing – original draft, Reviewing, and Editing. **Simone Vesentini:** Supervision, Writing- original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

Acknowledgements

We acknowledge the Circular Economy Lab for Life Sciences-CELLS within the MUSA–Multilayered Urban Sustainability Action–project, funded by the European Union–NextGeneration EU, under the National Recovery and Resilience Plan (NRRP) Mission 4 Component 2 InvestmentLine 1.5: Strengthening of research structures and creation of R&D "innovation ecosystems", set up of "territorial leaders in R&D". This study has received funding from the European Union's Horizon Europe Research and Innovation Program under grant agreement No. 101070167 and from Fondazione Cariplo under the project RITESSERE No. 2022-0529.

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