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Silk Fibroin Modification: Methods and Applications

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Abstract Silk fibroin (SF) is a natural protein with good mechanical properties, biocompatibility, and biodegradability. These features make it useful in many fields, especially biomedicine and materials science. However, natural silk fibroin has some limitations that can affect its performance in different applications. Modification is an effective way to improve its properties and develop new materials for specific purposes. This review discusses the main methods used to modify silk fibroin, including chemical, physical, and biological approaches. It also describes how these methods affect the structure and properties of the resulting materials. Particular attention is given to cross-linking, surface functionalization, and the preparation of silk fibroin nanocomposites with metals, metal oxides, carbon-based materials, ceramics, and other components. These approaches can improve mechanical strength, stability, antimicrobial activity, and the ability to deliver drugs in a controlled manner. The review also examines the use of modified silk fibroin in wound dressings, tissue engineering, drug delivery systems, hydrogels, scaffolds, sensors, and other functional materials. The choice of modification method depends on the desired properties and the intended application of the final product. Despite significant progress, further research is needed to improve the reproducibility, safety, stability, and large-scale production of these materials. A better understanding of the relationship between modification methods, material properties, and practical applications will help expand the use of silk fibroin in biomedical and advanced materials.

Keywords Silk fibroin, Modification methods, Chemical modification, Nanocomposites, Biomaterials

1. Introduction

Silkworm silk, primarily produced by Bombyx mori, has been used for centuries in home textiles, apparel, and decorative materials owing to its low density, attractive luster, flexibility, and outstanding mechanical performance. The ability to cultivate B. mori at an industrial scale enables reliable and cost-effective access to silk, providing notable advantages over many natural and synthetic biomaterials. Beyond traditional textile applications, silkworm silk has long attracted biomedical interest because of its favorable biocompatibility and biodegradability. Historically, silk-based materials have been widely utilized in healthcare products, particularly as surgical sutures, and have also been incorporated into cosmetics and traditional medical practices. [1,2] Importantly, degummed natural silk was approved by the U.S. Food and Drug Administration in 1993, supporting its clinical translation and long-term use in medical devices and reconstructive applications. [3,4]

The silkworm lifecycle involves five larval instar stages before metamorphosis. During this period, silk proteins are synthesized and stored within specialized silk glands under aqueous physiological conditions. At the end of the fifth instar, the silkworm spins a continuous thread to form a cocoon. Raw silk consists of two fibroin filaments held together by an outer sericin coating that acts as a natural adhesive. [5,6] Following degumming to remove sericin, the remaining fibroin fibers exhibit a smooth, glossy surface and desirable handling properties, which contribute to their value in the textile industry. From a materials perspective, silk fibroin fibers offer a rare combination of high strength, toughness, thermal stability, controlled biodegradability, and excellent biological compatibility, often outperforming many conventional natural and synthetic fibers in terms of balanced performance. [7,8]

A major advantage of silk fibroin lies in its processing versatility. Native fibroin fibers can be manufactured into braided, knitted, or non-woven constructs. In contrast, regenerated silk fibroin (obtained by dissolution and reconstitution) enables the fabrication of diverse material formats, including sponges, hydrogels, films, electrospun mats, microparticles, and microneedle platforms. [8,9]

This flexibility has significantly expanded the use of silk fibroin in biomedical engineering, including tissue scaffolds and drug delivery systems. More recently, advanced processing and device integration have positioned silk fibroin as an attractive platform for emerging technologies, including edible sensors, organic electronic devices, photonic components, and implantable bio-trackers/detectors. [10,11] In these

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applications, silk fibroin demonstrates favorable interactions with biological systems, often supporting cell adhesion, proliferation, and function without triggering severe immune responses. [12]

Despite these advantages, native silk fibroin also presents limitations that restrict its performance in advanced biological and technological environments. Silk fibroin is rich in repetitive amino acid sequences (notably glycine, alanine, and serine), which form stable β-sheet crystalline domains responsible for its high tensile strength and slow degradation profile. However, this highly ordered structure can also reduce chemical reactivity and limit surface bioactivity, thereby limiting interfacial interactions with certain cells, biomolecules, or functional ligands. Moreover, the inherent hydrophobicity and limited availability of reactive functional groups may reduce wettability and constrain efficient chemical coupling, thereby narrowing its applicability in high-performance biointerfaces and engineered systems.

To overcome these limitations, a wide range of modification strategies has been developed to introduce new functionalities while preserving the intrinsic benefits of silk fibroin. Chemical modification can incorporate reactive groups that enable covalent immobilization of drugs, peptides, or signaling molecules, thereby enhancing biological specificity and performance. Physical surface treatments, such as plasma exposure or ultraviolet irradiation, can increase surface energy and hydrophilicity, improving cell attachment and interfacial compatibility. In addition, tailoring the balance between crystalline and amorphous domains provides an effective route to fine-tune mechanical behavior, thermal stability, and degradation rates. Such control is particularly important for applications requiring long-term stability under physiological conditions.

An especially powerful approach involves combining silk fibroin with nanoscale components to create functional nanocomposites. Incorporation of metal or metal-oxide nanoparticles can impart antibacterial activity, while carbon-based nanostructures may improve electrical conductivity for bioelectronic and sensing applications. Ceramic nanoparticles, in contrast, can enhance osteoconductivity and mechanical reinforcement for bone-related tissue engineering. Therefore, through rational design of modifications and composite architectures, silk fibroin can be transformed from a mechanically robust yet relatively inert protein fiber into a tunable multifunctional biomaterial suitable for regenerative medicine, drug delivery, flexible bioelectronics, and other advanced technologies.

In this review, we provide a comprehensive overview of recent research on silk fibroin modification and nanocomposite development, highlighting the major strategies reported by different investigators and summarizing the key functional outcomes achieved through advanced modification techniques. Silk fibroin has become a standout biomaterial because it offers an unusual mix of strength, biocompatibility, and flexible processing into many formats. The key message of this section is that SF is not just a “natural polymer”, but a tunable platform whose properties can be engineered through regeneration and modification. At the same time, its performance strongly depends on how it is processed, which explains why careful control of preparation steps matters.

1.1. Literature Search Strategy and Review Methodology

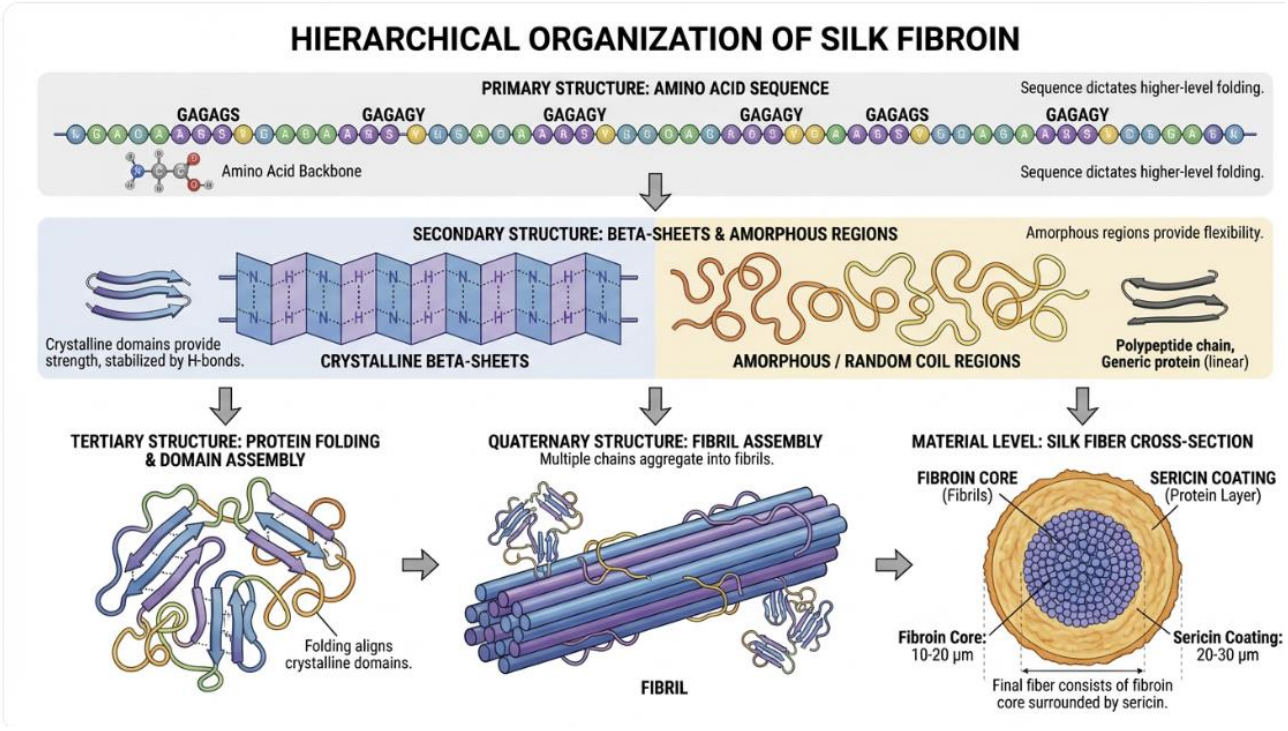

Figure 1. SF hierarchical structure

To capture both foundational work and the most recent advances in silk fibroin (SF) materials, we conducted a structured literature review covering publications through December 2025. The search was performed across major databases (Scopus, Web of Science, PubMed, and Google Scholar) using combinations of keywords such as "silk fibroin", "regenerated silk fibroin (RSF)", "degumming", "dissolution", "crosslinking", "functionalization/modification", "hydrogel", "scaffold", "electrospinning", "nanocomposite", "biosensor", and "wearable electronics".

Search results were screened by title and abstract, followed by full-text evaluation of relevant articles. We prioritized peer-reviewed English papers that reported clear processing conditions, structure–property relationships, and functional performance of modified SF systems. Duplicate records were removed, and studies with insufficient experimental detail were excluded where applicable. Because the field is rapidly expanding and highly interdisciplinary, this review follows a narrative approach, highlighting representative and influential studies while emphasizing critical challenges (reproducibility, biosafety, nanofiller stability, and scalability) that determine real-world translation.

2. Silk Fibroin

Silkworm silk is mainly composed of silk fibroin (70%), sericin (25%), and minor non-protein impurities (5%). Structurally, raw silk consists of two fibroin filaments coated with a sericin layer that also entraps small amounts of non-protein contaminants. [13] Sericin is a hydrophilic protein with a relatively loose and disordered conformation, functioning as a natural adhesive and protective "glue" that contributes to lubrication and cohesion within the cocoon.

Silk fibroin (SF) is a fibrous structural protein assembled from a heavy chain (H-chain, 390 kDa), a light chain (L-chain, 26 kDa), and a glycoprotein component (P25, 25-30 kDa), commonly reported in a molar ratio of 6:6:1. [14,15] SF contains 18 amino acids, with nonpolar residues - particularly glycine, alanine, and valine - accounting for approximately 70.5%, while polar residues such as serine and tyrosine make up the remaining 29.5%. This composition strongly contributes to SF crystallinity and mechanical robustness.

The H-chain is characterized by multiple large repetitive domains separated by shorter non-repetitive regions. The repetitive segments are enriched in Gly-X motifs (X = Ala, Ser, Thr, or Val). They can pack into β-sheet-rich microcrystalline domains stabilized by intermolecular and intramolecular interactions, including hydrogen bonding, van der Waals forces, and hydrophobic interactions. [16,17] In contrast, the non-repetitive regions contain irregular yet conserved sequences that act as flexible linkers between microcrystalline blocks. These regions are enriched with residues such as glutamic acid, aspartic acid, arginine, lysine, and proline, which contribute to local disorder and structural variability. [18,19] Proline residues can introduce conformational constraints by forming backbone rings, thereby influencing chain orientation and β-sheet packing. [19] Additionally, the N-terminal (151 residues) and C-terminal (50 residues) sections of the H-chain are largely non-repetitive and are typically considered more amorphous compared to the crystalline core regions. [19,20]

Compared to the H-chain, the L-chain is relatively more hydrophilic and elastic, which is generally attributed to its predominantly non-repetitive sequence and reduced tendency to form highly crystalline structures. This hierarchical crystalline–amorphous architecture is central to silk fibroin's unique combination of strength and processability, and it also provides multiple opportunities for chemical modification and nanoscale reinforcement. This section highlights that the unique behavior of silk fibroin stems directly from its molecular architecture, especially the heavy/light chain structure and β–sheet–rich crystalline domains. These structural features explain both the impressive mechanical performance and the challenges in solubility and reprocessing. Importantly, the presence of reactive amino acid residues also creates clear opportunities for chemical functionalization and advanced composite design.

3. Extraction of Silk Fibroin

Silk fibroin extraction is not a simple "preparation step" - it is a decisive stage that controls molecular weight, secondary structure, and ultimately the reliability of final materials. Degumming and dissolution conditions shape the quality of regenerated silk fibroin and its ability to form stable films, hydrogels, and scaffolds. A major challenge is that inconsistent extraction protocols can lead to substantial differences in properties across studies.

3.1. Degumming

The bicomponent nature of raw silk requires the removal of sericin to obtain purified silk fibroin (SF). This separation step, commonly known as degumming, primarily aims to remove the sericin coating (SS) surrounding fibroin filaments. [21] Sericin is generally water-soluble, and its removal can be promoted by treatment in hot water baths. [22,23] However, mild water-based degumming may still leave measurable amounts of sericin on the fibroin surface, which can be undesirable for high-performance applications.

In biomedical and advanced material systems, complete sericin extraction is often critical because residual sericin may alter the resulting material properties, affecting fibroin crystallization behavior, mechanical and thermal stability, and even cytocompatibility. [24,25] More intensive approaches, such as high-temperature/high-pressure (HT-HP) degumming, can improve sericin removal but may also cause partial degradation of fibroin chains. Although water-based methods offer the advantage of producing sericin-rich aqueous solutions without requiring additional chemicals, achieving highly purified fibroin while preserving molecular integrity remains a key challenge. [26,27]

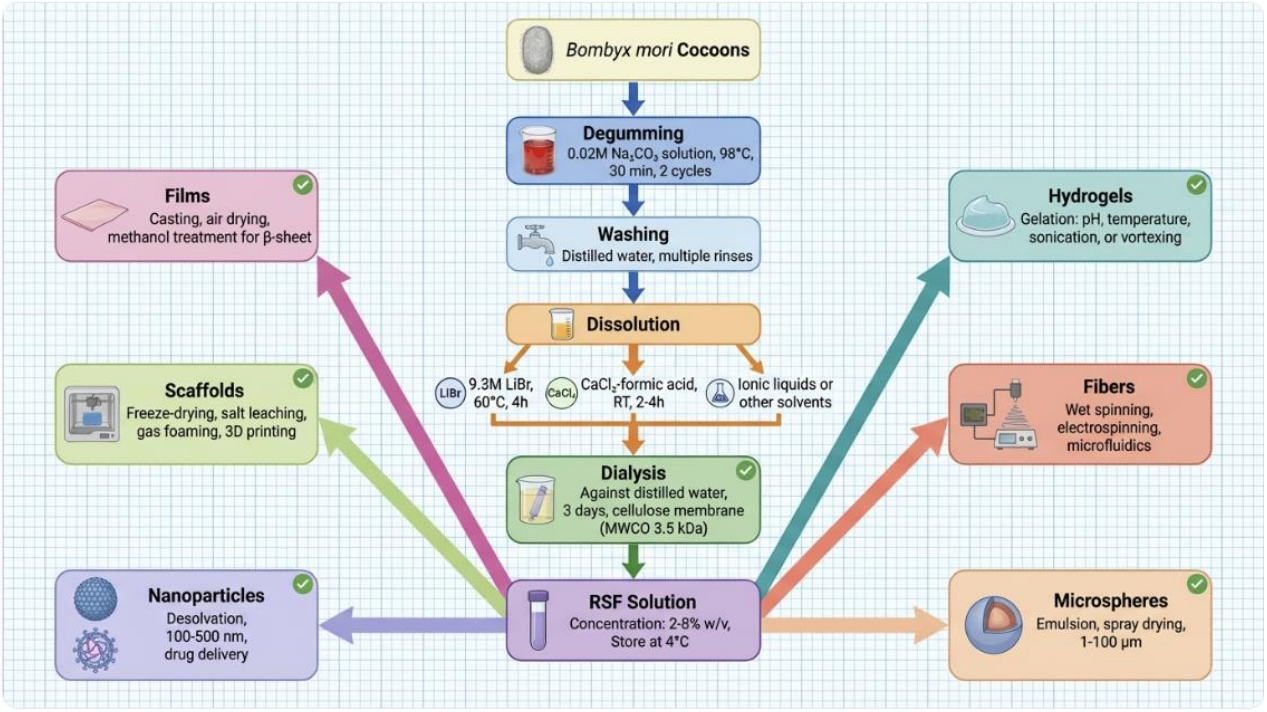

Figure 2. Overview of the RSF Processing Steps

Degumming strategies differ considerably in efficiency, selectivity, cost, and environmental impact. Alkaline degumming is widely used because it effectively hydrolyzes sericin and provides high removal yields. Nevertheless, this approach may damage the fibroin structure, lowering the average molecular weight and, consequently, influencing downstream mechanical properties. In contrast, urea-based degumming can be gentler toward fibroin but often requires high urea concentrations to reach complete purification. Since urea acts as a strong hydrogen-bond disruptor (denaturant), it may also induce significant conformational changes in protein chains. [28]

Waste streams generated during degumming should be appropriately treated to reduce environmental risks, including potential eutrophication associated with wastewater discharge. [29,30] Enzymatic degumming provides an alternative route for selective sericin hydrolysis with minimal fibroin damage and reduced contamination. However, the high cost of enzymes remains a major limitation for large-scale industrial adoption. [22]

Among conventional industrial approaches, alkaline (soap) degumming, typically using sodium carbonate (Na_2CO_3) or sodium phosphate solutions, remains one of the most practical and cost-effective methods. During treatment, Na_2CO_3 mainly disrupts weaker hydrogen-bond interactions associated with sericin, and conversion of sericin carboxyl groups (-COOH) into sodium carboxylates (-COONa) increases its solubility and facilitates extraction. [26] Temperature plays a critical role: values below 80 ℃ are often insufficient for complete sericin removal, whereas temperatures above 100 ℃ may damage the fibroin surface and promote polypeptide chain cleavage, producing low-molecular-weight fragments (30 kDa) [21,24,28] Several reports suggest that short degumming times and low Na_2CO_3 concentrations can effectively remove sericin while minimizing fibroin degradation. [26,31]

The addition of wetting agents, such as sodium dodecyl sulfate (SDS), may further reduce surface tension and enhance sericin extraction. [24] However, such variations remain less explored in systematic studies. Other emerging techniques-such as infrared heating, plasma pre-treatment, or supercritical CO_2 processing-have also been proposed. [32] However, challenges including fibroin degradation, limited industrial scalability, low efficiency, and high operational cost have restricted broader implementation.

Recently, steam-based degumming has gained attention as an environmentally friendly alternative, with sericin extraction achieved using pressurized water vapor, potentially reducing water contamination and energy consumption while maintaining high sericin removal efficiency. [33] Following degumming, further dissolution and regeneration steps may be applied to obtain regenerated silk fibroin (RSF) solutions suitable for nanocomposite fabrication and chemical functionalization. Degumming is essential for removing sericin, but it must be balanced carefully to avoid damaging fibroin chains. Different approaches (alkaline, enzymatic, steam-based) offer trade-offs among efficiency, molecular preservation, cost, and sustainability. Overall, successful degumming is best viewed as an optimization problem in which purity and chain integrity must be

achieved simultaneously.

3.2. Dissolving

Silk fibroin (SF) is characterized by a highly ordered β-sheet-rich architecture, which makes it insoluble in water and many common solvents. To obtain regenerated silk fibroin (RSF) solutions, SF is typically dissolved using concentrated neutral salts under hydrothermal conditions. Common dissolution systems include LiBr, LiSCN, $CaCl_2$, and $Ca(NO_3)_2$, which disrupt the intermolecular hydrogen bonding and hydrophobic packing that stabilize fibroin. [34–36]

Dissolution is a critical step because SF's strong β-sheet packing makes it naturally resistant to solvents and difficult to process. Salt-based solvent systems remain the most practical route to obtain RSF solutions, but each solvent choice affects molecular weight retention and reassembly behavior. In practice, solvent selection should be based on the desired end material - whether the goal is rapid gelation, high mechanical strength, or stable long - term performance.

3.3. Films, Hydrogels, and Scaffolds

Regenerated silk fibroin (RSF) can be processed into a broad range of material formats, including films, electrospun fiber mats, hydrogels, and three-dimensional porous scaffolds. These platforms are widely explored for wound dressings, soft tissue repair, drug delivery, and increasingly for flexible bioelectronic and biointerface applications. Importantly, RSF-based constructs also serve as convenient model systems for studying cell-material interactions, either in their native state or after surface functionalization. Once RSF is obtained, it can be engineered into multiple material formats that match different application needs. The ability to switch between films, electrospun mats, hydrogels, and 3D scaffolds makes SF a true "platform material." Importantly, the final structure and performance depend not only on the format but also on how β-sheet formation and porosity are controlled during processing.

4. Modification Strategies of Silk Fibroin

Modification strategies are central to turning SF from a strong biopolymer into a multifunctional engineered material. Chemical, physical, and biological approaches each provide different levels of control over stability, bioactivity, and responsiveness. The main takeaway is that smart modification should enhance performance without compromising safety, reproducibility, or structural integrity.

4.1. Chemical Modification of Silk Fibroin

Chemical modification enables precise tuning of SF networks via covalent bonding and the introduction of functional groups. These methods can significantly improve stability and add new capabilities, such as targeting, adhesion, or metal binding. At the same time, chemical complexity may introduce toxicity risks or reproducibility issues if reactions are not carefully controlled.

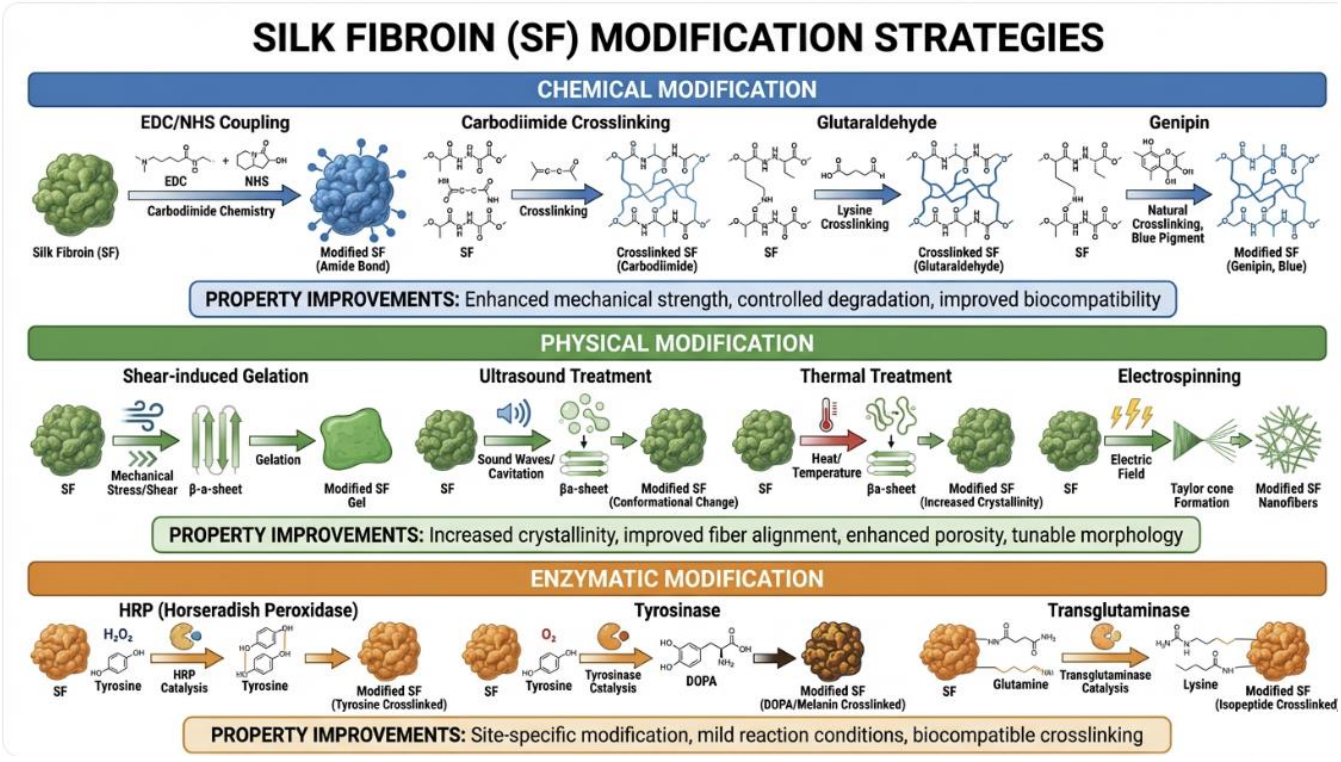

Figure 3. Modification strategies overview

Table 1. Comparison of chemical, physical, and biological modification strategies applied to silk fibroin materials

Strategy type	Example approach	Main target groups/mechanism	Main benefit	Main limitation	Applications
Chemical crosslinking	EDC/NHS coupling	–COOH ↔ –NH_2 (amide bonds)	Strong network, stable properties	Requires pH control; reaction efficiency varies	Scaffolds, stable hydrogels
Chemical crosslinking	Genipin crosslinking	–NH_2 (amine-rich sites)	Better biocompatibility vs harsh agents	Slow gelation; cost is higher	Tissue engineering
Chemical crosslinking	Glutaraldehyde	–NH_2	Very strong crosslinking	Cytotoxicity concern; residue risks	Mostly non-implant or well-washed systems
Photo-crosslinking	Methacrylated SF (UV/Vis curing)	Added vinyl groups → radical curing	Fast, spatially controlled	Requires modification step; photoinitiator safety	Bio-printing, patterned gels
Monofunctionalization	Sulfonation/ carboxylation/ thiolation	Introduces-SO_3H/ -COOH/-SH	Charge tuning, better binding/adhesion	Over-modification may weaken the structure	Interfaces, adsorption, functional coatings
Physical modification	Ultrasound/ shear/vortex	β-sheet induction via assembly	Mild, no chemical residues	Sensitive to parameters (reproducibility)	Injectable gels, soft interfaces
Solvent-induced	MeOH/EtOH treatment	β-sheet stabilization	Rapid strengthening of films	Not always cell-friendly during processing	Films, mats, robust coatings
Biological/enzymatic	HRP/H_2O_2, transglutaminase	Enzyme-driven crosslinking	Biomimetic, mild conditions	Enzyme cost; reaction variability	Cell-laden hydrogels

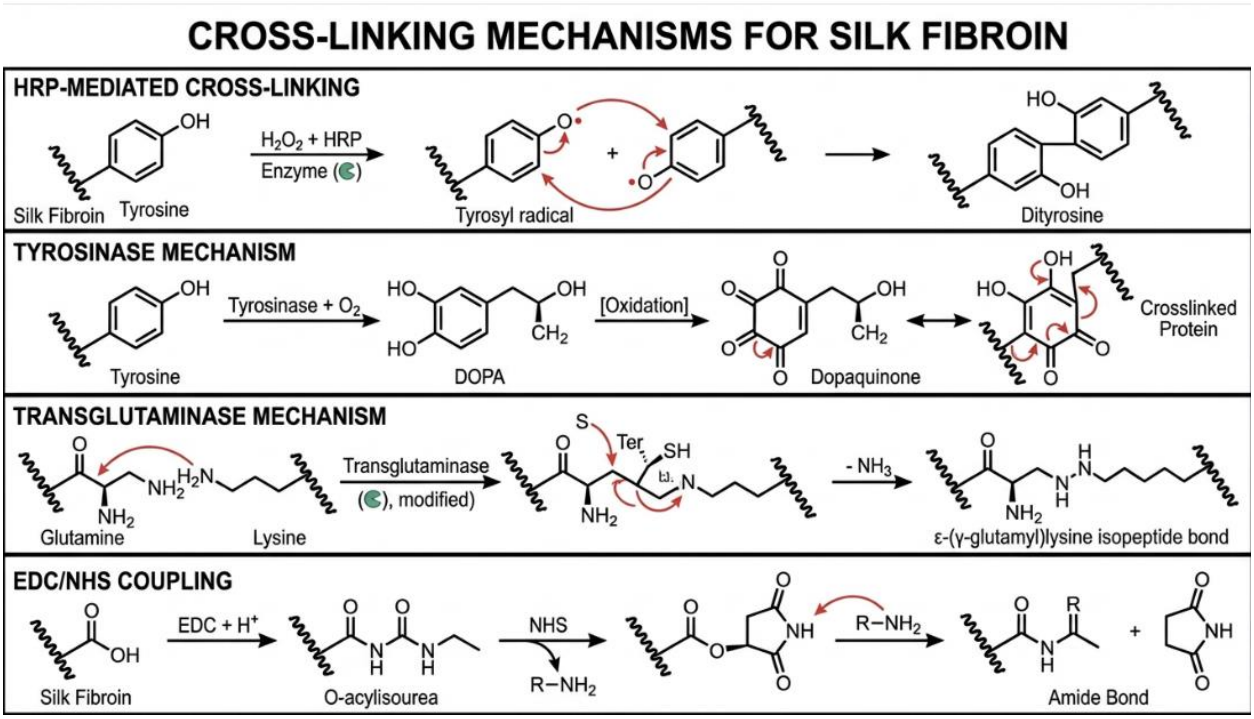

Figure 4. Crosslinking mechanisms

4.1.1. Chemical Cross-Linking

Chemical cross-linking refers to the formation of covalent bonds between silk fibroin (SF) chains, generating stable junction points that produce a three-dimensional network. Compared with physically crosslinked systems, chemically crosslinked SF hydrogels typically exhibit higher structural stability, improved mechanical strength, and enhanced resistance to dissolution. Current cross-linking methods commonly include small-molecule crosslinkers, photo-initiated reactions, irradiation-based approaches, and enzyme-mediated cross-linking.

Small-molecule crosslinkers react with functional groups naturally present in SF, such as hydroxyl (-OH), amino (-NH_2), and carboxyl (-COOH) moieties, promoting network formation. Genipin, a relatively low-toxicity crosslinker, is frequently used to fabricate SF-based hydrogels. For instance, Haohao Zhou et al. prepared a poly(vinyl alcohol)/ silk fibroin/nano-hydroxyapatite composite hydrogel using genipin as the crosslinking agent. [37] FTIR analysis revealed an enhanced tertiary amine C–N signal upon genipin incorporation, suggesting the formation of new covalent

bonds involving free amino groups in SF.

In addition to genipin, glutaraldehyde, and carbodiimide, chemical methods are also widely used for SF cross-linking. [38] Glutaraldehyde can react with protein side chains, particularly the ε-amino group of lysine and, in some cases, the tyrosine side chain. Yinan Li et al. reported a silk fibroin-based hydrogel formed by covalent modification and glutaraldehyde cross-linking. [39] In another study, Qiang Lv et al. used carbodiimide-mediated coupling to cross-link SF with collagen, producing a hybrid hydrogel network. Nevertheless, toxicity concerns may arise for certain crosslinkers, and minimizing exposure to potentially cytotoxic agents is important during hydrogel fabrication. In this context, genipin is generally considered less cytotoxic than glutaraldehyde. [40]

Photo-cross-linking represents another practical strategy in which ultraviolet or visible light triggers covalent network formation. A representative approach involves the formation of dityrosine bonds between tyrosine residues using photoredox catalysts. Whittaker et al. introduced a rapid and mild ruthenium (Ru)-catalyzed visible-light cross-linking method for generating SF hydrogels. [41,42] In this system, tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate enables visible-light activation and promotes dityrosine bond formation, yielding stable hydrogel networks. Similarly, riboflavin-mediated photo-cross-linking can also proceed via dityrosine formation under mild conditions. [43,44]

An additional photo-cross-linking pathway is enabled by chemically modifying SF chains with polymerizable groups. Methacryloyl functionalization provides reactive vinyl moieties that can undergo efficient free-radical photopolymerization, offering a versatile route to fast-forming photo-crosslinked SF hydrogels. In recent studies, glycidyl methacrylate has been widely investigated for introducing methacryloyl groups onto silk fibroin, thereby enabling tunable crosslinking density and mechanical properties. [45]

Chemical crosslinking strengthens SF networks by forming covalent bonds, improving durability and mechanical integrity. Crosslinkers such as genipin, glutaraldehyde, carbodiimide chemistry, and photo-crosslinking systems each offer distinct advantages, but safety profiles differ significantly. In practice, selecting crosslinkers requires balancing performance improvement against biocompatibility and long-term stability.

4.1.2. Monofunctionalization

Monofunctionalization describes direct chemical strategies that introduce new functional groups onto silk fibroin by targeting native amino acid side chains. In many cases, these modifications are designed to increase the density of reactive sites for subsequent conjugation or coupling reactions, while maintaining the overall structural integrity of SF.

Diazonium coupling is frequently used to modify tyrosine residues and can increase the availability of reactive aromatic sites, thereby enhancing opportunities for downstream functionalization. [46,47] [48,49] This reaction proceeds via electrophilic aromatic substitution between the phenolic ring of tyrosine and diazonium salts, typically producing azobenzene-type derivatives. [50]

Sulfation provides an effective route to introduce negatively charged sulfate groups into SF, typically by treating SF constructs with sulfating reagents under controlled conditions. Substitution of hydroxyl groups in tyrosine and serine residues can yield sulfated functionalities, and the resulting negative charge facilitates the adsorption or binding of positively charged molecules. [51,52]

Arginine masking can be achieved using 1,2-cyclohexanedione under basic aqueous conditions, converting arginine residues into uncharged imidazolidinone derivatives and thereby modulating surface charge characteristics. [53]

Carboxylation strategies aim to increase the density of carboxylic acid groups on SF, most commonly through succinic anhydride-based modification of amino-containing residues. This approach can improve the overall negative charge of SF with high modification yields, while limiting excessive structural disruption under optimized conditions. [54,55]

Finally, thiolation introduces sulfhydryl (-SH) groups onto primary amines in SF, often via reagents such as 2-iminothiolane, enabling subsequent coupling reactions or the introduction of metal-binding functionality. [56,57]

Overall, cross-linking strategies mainly regulate network stability and mechanics. In contrast, monofunctionalization expands the chemical ‘toolbox’ of silk fibroin by introducing charged or reactive groups for advanced conjugation and nanocomposite integration. Monofunctionalization introduces specific chemical groups onto SF to tailor surface charge, reactivity, and binding behavior without entirely restructuring the network. Strategies such as diazonium coupling, sulfation, carboxylation, and thiolation expand SF functionality for advanced conjugation and interfacial control. These approaches are especially valuable when fine “chemical tuning” is needed rather than significant structural alteration.

4.2. Physical Modification of Silk Fibroin

Silk fibroin (SF) solutions can be transformed into hydrogels with three-dimensional network structures through physical interactions, without the addition of chemical cross-linking agents. [58] Such physically formed hydrogels are typically generated under mild and cytocompatible conditions, making them attractive for biomedical applications. A variety of processing parameters have been reported to trigger or accelerate gelation, including changes in concentration, temperature, shear stress, ultrasonication, pH adjustment, organic solvent induction, surfactant exposure, salt addition, and the application of electric fields. [59]

Physical modification offers a chemistry-free route to tune SF assembly by promoting β-sheet formation through external triggers. These methods are appealing because they reduce reagent-related toxicity concerns and often allow mild, cell-compatible processing. However, achieving

consistent and predictable properties may be challenging because gelation kinetics can be sensitive to slight variations in conditions.

4.2.1. Ultrasonication-Induced Gelation

Ultrasonication is one of the most effective physical triggers for rapid SF gelation because it accelerates intermolecular interactions and promotes conformational transitions associated with β-sheet formation. [60–62] Mechanistically, ultrasonication can generate localized heating, increase shear forces, and disturb gas–liquid interfacial equilibrium, collectively enhancing silk chain aggregation. [63] Natalia Gorenkova et al. reported that short sonication times (15-45 s) were sufficient to initiate gel formation in SF solutions. [64]

In practice, the final hydrogel properties are primarily governed by pre-gel concentration, ultrasound power, and sonication duration, enabling relatively precise control over gelation kinetics and network structure.

Ultrasonication is a fast and efficient method for triggering SF gelation by accelerating molecular interactions and β-sheet formation. It allows practical control over gelation time and final stiffness by adjusting sonication power and duration. Still, careful parameter tuning is essential because excessive energy can compromise chain integrity or introduce batch-to-batch variability.

4.2.2. Shear- and Vortex-Induced Gelation

SF solutions are highly sensitive to mechanical stress. In vivo, native fibroin experiences carefully regulated flow conditions within the silk gland to prevent premature gelation before spinning. [65] In vitro, however, externally applied shear stress can effectively promote β-sheet formation and accelerate gelation. [66] Both stirring and flow can drive conformational changes from less-ordered helical structures toward more-ordered β-sheet-rich domains. Vortexing, in particular, provides controllable shear gradients and allows modulation of gelation dynamics by adjusting vortex time, which can be beneficial for time-sensitive applications such as cell encapsulation. [67] This approach is operationally simple and avoids the use of potentially harmful chemical reagents.

Shear and vortex forces can drive SF self-assembly with simple mechanical inputs, making this approach attractive for injectable or in situ-forming materials. The method is operationally straightforward and avoids the use of chemical additives. However, gelation can be highly sensitive to mixing intensity and time, which may limit reproducibility without standard protocols.

4.2.3. Temperature-Triggered Self-Assembly

Temperature is another key factor controlling silk gelation. At elevated temperatures, increased molecular collision frequency and enhanced hydrophobic interactions promote SF aggregation and self-assembly into β-sheet-stabilized networks. [58,68] Because β-sheet domains are thermodynamically stable, temperature-induced gelation is generally irreversible. SF solutions are often stable for short-term storage at 4 ℃ (approximately one week), whereas gelation typically proceeds faster at ambient temperatures. [59,69]

Temperature strongly influences SF gelation by controlling chain mobility and the rate of β-sheet formation. Higher temperatures generally accelerate gelation, while lower temperatures slow assembly but may support better structural control. This method is scalable and straightforward, but its practical success depends on stable temperature control during processing.

4.2.4. Organic Solvent Induction

Water-miscible organic solvents, particularly alcohols such as methanol and ethanol, can accelerate SF gelation by promoting dehydration of hydrated domains and facilitating β-sheet nucleation. [68,69] Solvent polarity plays a central role by sequestering water molecules from silk chains, thereby strengthening intermolecular interactions and enabling the formation of β-sheet-rich segments. [63] Different alcohols exhibit distinct induction strengths; for example, longer-chain alcohols, such as n-butanol, may induce β-sheet formation more gradually. [70] While solvent-triggered gelation offers rapid and convenient processing, residual solvents may limit direct cell encapsulation and in situ biomedical applications.

Organic solvents like methanol or ethanol can rapidly induce β-sheet formation and stabilize SF structures. This is effective for strengthening films and some scaffold forms, but solvent exposure can limit direct biological compatibility during fabrication. Therefore, solvent-induced strategies often require additional washing and validation steps before biomedical use.

4.2.5. pH Adjustment and Electric-Field-Assisted Gelation

Adjusting pH is a widely used method to control SF gelation. When the solution pH approaches the isoelectric point of silk fibroin (pH 3.8–4.0), electrostatic repulsion decreases, and SF assemblies become more prone to aggregation, leading to hydrogel formation. [58] CO_2 bubbling has been reported as an additional route to modulate pH and promote gelation. [71,72] Electric fields can also accelerate gelation by inducing localized proton migration and pH gradients. In an applied electric field, proton movement toward the anode can decrease local pH toward the isoelectric region, causing fibroin molecules to aggregate near the anode and form a hydrogel network. [73], [74] In addition to accelerating gelation, shear and electric-field triggers may induce directional structures within hydrogels, potentially improving mechanical strength and guiding directional cell growth.

pH adjustment modulates SF chain charge and interactions, providing a convenient way to control gelation behavior. Electric field assistance can further guide assembly and accelerate network formation in some systems. These approaches are promising for controlled gelation, but they require careful optimization to avoid structural instability under extreme conditions.

4.2.6. Surfactant-Assisted Gelation

Surfactants such as sodium dodecyl sulfate, sodium lauroyl sarcosinate, sodium lauryl sulfate, and poloxamers have been explored to modulate gelation rate and promote hydrogel formation. [75,76] Due to their amphiphilic nature, surfactants can interact with the hydrophobic segments of SF, reduce surface tension, and promote intermolecular interactions that favor β-sheet formation. [77]

Subsequent FTIR and X-ray diffraction (XRD) studies have consistently shown that SF hydrogels contain higher β-sheet content than the corresponding solutions or membranes, supporting the central role of β-sheet formation in gelation. [78]

Overall, physically induced gelation strategies primarily aim to accelerate the transition of SF from less ordered conformations to β-sheet-rich networks. By adjusting key physical parameters—such as temperature, shear, ultrasound, pH, electric fields, and solvent/surfactant conditions-the sol-gel transition of silk fibroin can be effectively regulated, enabling tunable hydrogel formation for diverse biomedical and functional material applications. [79]

Surfactants can promote SF gelation by improving chain exposure and driving intermolecular interactions that favor β-sheet formation. This provides an additional lever for controlling assembly in otherwise stable SF solutions. However, surfactant residues may impact biocompatibility, so purification and safety evaluation are essential.

4.3. Biological Modification of Silk Fibroin

Enzymatic cross-linking is a bio-mediated strategy that relies on enzyme catalysis to activate specific reactive groups on silk fibroin (SF) side chains, thereby enabling covalent network formation under mild conditions. Compared with conventional chemical cross-linking, enzymatic approaches typically avoid harsh reagents, organic solvents, and potentially hazardous cross-linkers, which makes them highly attractive for biomedical applications that demand excellent cytocompatibility. Commonly explored enzymes include horseradish peroxidase (HRP), transglutaminase, carbonic anhydrase, alcohol oxidase, tyrosinase, and laccase. [80,81] Among these, HRP is one of the most frequently used catalysts for SF hydrogel formation and hybrid network design. [82–84]

In a representative study, Nicole R. Raia et al. employed HRP-mediated cross-linking to fabricate hydrogels by combining silk fibroin with tyramine-substituted hyaluronic acid (HA). [85] In this system, enzymatic reactions promoted the coupling of tyrosine residues in SF with tyramine groups in modified HA, forming tyramine–tyrosine linkages and generating a hydrogel with mechanical properties and biocompatibility comparable to those of native tissues. Similarly, Lin Wang et al. reported laccase-mediated cross-linking between tyramine-modified HA and SF, producing hydrogels with improved structural stability and enhanced formability. [86] These enzyme-driven strategies provide promising routes toward flexible, biologically compatible hydrogel architectures, including enzyme-enabled double-network designs.

Although physical cross-linking methods are operationally simple and do not require chemical reagents or extreme reaction conditions, physically gelled SF hydrogels may suffer from brittleness and limited mechanical performance. [87] Chemical cross-linking methods can address these limitations by improving network stability and enabling broader tuning of strength, porosity, swelling behavior, and biodegradability. However, the use of photoinitiators and certain chemical cross-linkers may introduce cytotoxicity risks depending on cross-linker type, reaction conditions, and residual content, which can restrict biomedical translation unless carefully controlled. In this context, enzymatic cross-linking offers a favorable balance between network stability and biocompatibility, and is widely considered one of the most practical cross-linking routes for cell-compatible silk hydrogels.

In practice, high-performance SF hydrogels are often achieved by integrating multiple cross-linking strategies to refine network architecture and introduce application-specific functionality. By combining bio-mediated and physicochemical modification routes, silk fibroin hydrogels can be engineered with tailored mechanical behavior, tunable degradation, and diverse biological functions to meet the requirements of regenerative medicine, tissue engineering, and advanced biointerfaces.

Overall, enzymatic cross-linking is a key enabling platform for injectable, cell-compatible silk fibroin hydrogels, particularly when combined with complementary physical or chemical strategies to achieve tunable mechanics and multifunctionality. Biological modification, especially enzyme-mediated crosslinking, provides mild, biomimetic routes to form SF networks. Enzymatic systems can offer better biocompatibility and more controlled chemistry than harsh synthetic crosslinkers. The main limitation is that enzymes can increase costs and complexity, and results can vary depending on enzyme activity and reaction conditions.

4.4. Nanocomposites and Advanced Modifications

In recent years, the integration of nanomaterials into silk fibroin (SF) matrices has emerged as a powerful route to overcome intrinsic limitations of native SF and to introduce multifunctionality for advanced biomedical and technological applications. Depending on nanoparticle chemistry and architecture, SF nanocomposites can be engineered to provide antibacterial activity, improved mechanical strength, enhanced electrical conductivity, controlled drug delivery, and bioactive microenvironments. Below, we summarize the most widely investigated categories of nanomaterials used for SF-based nanocomposites and highlight representative advances reported in the literature.

Nanocomposite integration pushes SF beyond conventional biomaterials by adding conductivity, antimicrobial effects, optical function, and stimulus responsiveness. The real performance advantage often depends on nanoparticle dispersion quality and strong interfacial interactions with the

SF matrix. At the same time, long-term stability and leaching risks must be considered for realistic translational use.

4.4.1. Metallic Nanoparticles

Metallic nanoparticles are among the most extensively studied nanofiller classes in SF nanocomposites, mainly due to their strong antimicrobial effects and broad applicability in wound management and tissue engineering. Among them, silver nanoparticles (AgNPs) are the most frequently used. Multiple studies have demonstrated that AgNP incorporation can confer robust antibacterial activity to SF scaffolds, making them promising for wound-dressing applications. For example, Paladini et al. evaluated silver-treated SF scaffolds and confirmed both antibacterial performance and regenerative potential in relevant biological tests. [88] The antibacterial effect of AgNPs is commonly attributed to the release of Ag ions, which can disrupt bacterial membranes, interfere with intracellular proteins, and impair replication processes, thereby acting against both Gram-positive and Gram-negative bacteria.

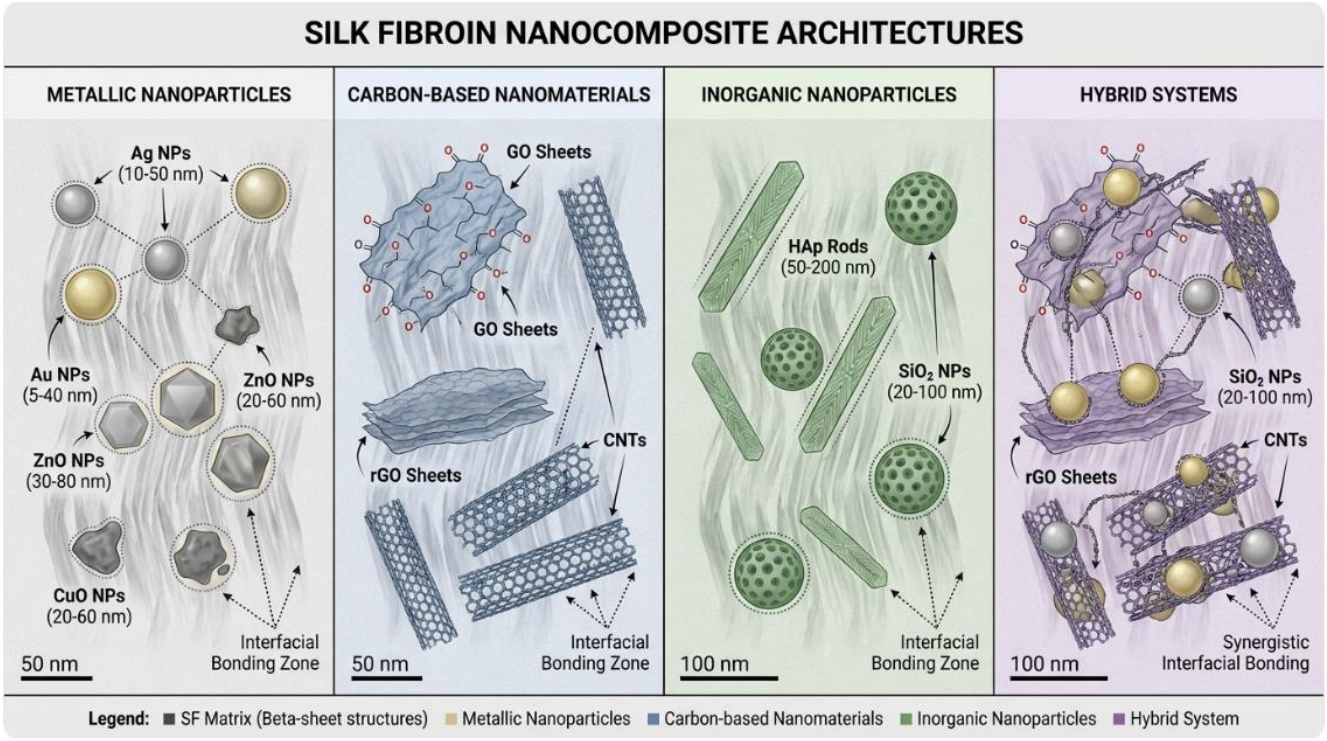

Figure 5. Nanocomposite architectures

Table 2. Representative silk fibroin nanocomposites, functional contributions of nanofillers, and translational considerations

Nanofiller category	Representative examples	Main function added	Typical improvement	Potential limitations	Suitable applications
Metal nanoparticles	AgNPs, AuNPs, CuNPs	Antimicrobial, plasmonic	Infection control, enhanced healing	Ion release, cytotoxicity at high dose	Wound dressings, coatings
Metal oxides	ZnO, TiO_2, Fe_3O_4	Antibacterial, photocatalytic, magnetic	Stability, activity, imaging potential	ROS generation, long-term safety uncertain	Wound care, responsive systems
Carbon-based	CNTs, GO/rGO, graphene	Conductivity, strength	Wearable electronics, sensors	Aggregation, biocompatibility concerns	Biosensors, flexible devices
Bioactive inorganic	Hydroxyapatite, bioactive glass	Osteoinduction	Bone integration, stiffness	Brittleness if overloaded	Bone tissue engineering
Silica / nanoclays	SiO_2, montmorillonite	Reinforcement, barrier effect	Mechanical and thermal stability	Dispersion challenges	Packaging, scaffolds
MOFs	ZIF-8, UiO-type MOFs	High drug loading, responsive release	Controlled delivery, multifunctionality	Metal leakage, stability in aqueous media	Drug delivery, wound healing
Hybrid/polymeric nano	Nanocellulose, polymer NPs	Strength, flexibility	Tunable mechanics, greener composites	Interface compatibility issues	Scaffolds, wearable materials

Gold nanoparticles (AuNPs) are also widely used owing to their favorable biocompatibility and optical properties, as well as their amenability to surface functionalization. Arumugam et al. designed a dual therapeutic system by incorporating both silver and gold nanoparticles into SF/collagen nanofiber scaffolds, achieving enhanced antibacterial efficacy and accelerated wound healing. [89] In this strategy, silver primarily contributed antimicrobial activity, while gold supported cytocompatibility and potentially modulated inflammatory responses. In addition, Agudelo et al. functionalized electrospun scaffolds with gold or silver nanoparticles for cardiovascular tissue engineering, highlighting the versatility of these metal-based nanomaterials across different biomedical domains. [90]

Beyond noble metals, zinc oxide nanoparticles (ZnO NPs) have attracted significant interest as multifunctional alternatives due to their antibacterial properties, biocompatibility, and additional benefits, such as UV protection and support for wound healing. Yang et al. developed SF/tannin/ZnO nanocomposite hydrogels and demonstrated that ZnO incorporation improved both mechanical stability and antibacterial performance. [91] In a related report, the same group introduced hemostatic functionality to SF/tannin/ZnO hydrogels, suggesting that ZnO can promote rapid blood coagulation by supporting platelet activation and aggregation. [92] Moreover, Cheng et al. reported injectable SF hydrogels containing pH-responsive ZnS nanoparticles for synergistic redox regulation in diabetic wound healing, illustrating how nanoparticle chemistry can be tailored toward targeted therapeutic outcomes. [93]

In addition to these widely used metal-based nanomaterials, other systems have been explored for specialized functions. Lartigue et al. investigated SF foams incorporating metallic nanoparticles as scaffolds for soft tissue regeneration. [94] Cojocaru et al. developed advanced 3D magnetic scaffolds containing magnetic nanoparticles for tumor-associated bone defects, enabling potential therapeutic and imaging capabilities under external magnetic fields. [95]

Metal nanoparticles, especially silver-based systems, are widely used to introduce antimicrobial activity into SF materials. These composites can effectively target both Gram-positive and Gram-negative bacteria, supporting wound healing and infection control. However, metal ion release and dose-dependent cytotoxicity remain essential concerns for long-term applications.

4.4.2. Carbon-Based Nanomaterials

Carbon-based nanomaterials - especially carbon nanotubes (CNTs) and graphene derivatives—have been incorporated into SF matrices primarily to introduce electrical conductivity and mechanical reinforcement, which are particularly relevant for bioelectronics and electrically responsive tissue engineering. Rathnayake et al. reported that integrating functionalized CNTs into electrospun SF fibers increased electrical conductivity by 13.7-21.8-fold, enabling effective electrical stimulation of fibroblasts and enhanced collagen production. [96] Such conductivity enhancement is valuable for applications where electrical cues regulate cell behavior, including neural and cardiac tissue engineering.

From a mechanical perspective, carbon nanomaterials reinforce due to their high intrinsic strength and aspect ratio, enabling efficient load transfer when uniformly dispersed and aligned. In electrospun architectures, CNT alignment may also induce anisotropic mechanical behavior, which is desirable when mimicking aligned tissues such as tendons and ligaments.

Beyond CNTs, graphene oxide (GO), reduced graphene oxide (rGO), and other two-dimensional materials have been increasingly explored. Rana et al. reviewed SF composites incorporating MXene and carbonaceous materials, highlighting the growing potential of 2D nanomaterials for multifunctional biomedical platforms. [97] These nanomaterials offer large surface areas for biomolecule loading, tunable electrical/thermal properties, and reinforcement effects, supporting applications ranging from biosensors to drug delivery systems.

Carbon nanomaterials such as CNTs and graphene derivatives provide strong mechanical reinforcement and enable electrical conductivity in SF composites. This makes them highly attractive for wearable electronics, biosensors, and electroactive tissue interfaces. The key challenge is ensuring safe, uniform dispersion because aggregation can reduce performance and raise biocompatibility concerns.

4.4.3. Ceramic and Mineral Nanoparticles

Ceramic and mineral nanofillers are widely used to enhance osteogenic activity, interfacial bioactivity, and mechanical stability of SF scaffolds, particularly for bone tissue engineering. Bioactive glass nanoparticles are notable examples because they can release therapeutic ions and promote the formation of hydroxyapatite-like layers, supporting bone regeneration. Niknafs et al. demonstrated that bioactive glass nanoparticles incorporated into SF/bacterial nanocellulose composite scaffolds significantly enhanced osteogenic performance, including increased alkaline phosphatase activity and calcium deposition. [98]

Silica nanoparticles are also attractive due to their biocompatibility, accessibility, and modifiable surface chemistry. Mart ń-Moldes et al. investigated how silica nanoparticle size influences osteoinduction in biomineralized silk–silica composites, showing that nanoscale dimensions can impact cellular differentiation pathways. [99] Importantly, biomineralization-based integration can create intimate organic-inorganic interfaces that resemble natural bone structure.

A particularly unique ceramic class is cerium oxide nanoparticles (CNPs), which exhibit antioxidant activity and neuroprotective potential due to their ability to scavenge reactive oxygen species. Saremi et al. developed tissue-engineered nerve grafts using SF/polycaprolactone fibrous mats functionalized with cerium oxide nanoparticles, reporting improved cell adhesion and proliferation along

with controlled release over prolonged time scales. [100] Such antioxidant activity is especially relevant in neural regeneration, where oxidative stress can inhibit repair.

In wound-related systems, magnesium-based nanoparticles have also attracted interest for their antibacterial effects and hemostatic properties. Mao et al. incorporated magnesium hydroxide into collagen/SF sponges and observed improved mechanical performance, accelerated healing, and antibacterial activity in infected wound models. [101] Released Mg^{2+} species can modulate the microenvironment and may support tissue regeneration.

4.4.4. Metal-Organic Frameworks

Metal-organic frameworks (MOFs) represent a rapidly expanding class of porous nanomaterials for SF nanocomposites, owing to their high loading capacity, tunable pore structures, and controlled release behavior. Zhu et al. introduced a multifunctional modification strategy using MOFs as intermediates to improve SF hydrogel performance, reporting enhanced mechanical strength together with antibacterial and pro-angiogenic functions. [102] Their work suggested that MOF–SF interactions at the secondary-structure level could substantially reinforce the composite network, making these systems promising for surgical adhesives and wound-closure applications.

Rahman et al. developed a dual-encapsulation system combining ZIF-8 MOFs with SF/polycaprolactone nanofibers for curcumin delivery and accelerated wound healing. In this design, the pH-responsive ZIF-8 framework provided high drug-loading capacity and triggered release, while SF/PCL nanofibers offered mechanical support and cytocompatibility. The composite demonstrated strong antibacterial performance against E. coli and S. aureus and achieved near-complete wound closure in animal models, attributed to improved collagen deposition and re-epithelialization. [103]

Beyond drug delivery, MOFs can serve as structural reinforcers and multifunctional carriers. By selecting appropriate metal nodes and organic linkers, MOF properties such as pore chemistry, degradation profile, and surface interactions can be tailored to match application requirements, enabling SF nanocomposites for complex wound care and advanced therapeutic environments.

MOFs offer high surface area and strong drug-loading capacity, enabling SF composites with responsive release behavior. These systems are promising for controlled delivery and multifunctional wound applications. Still, MOF stability and potential metal-ion leakage must be addressed through careful design and long-term testing.

4.4.5. Polymeric and Hybrid Nanomaterials

Polymeric nanomaterials and hybrid organic-inorganic architectures offer additional opportunities for constructing multifunctional SF nanocomposites. Bacterial nanocellulose (BNC) is a naturally derived nanofibrous reinforcement that improves mechanical strength and supports cytocompatibility. Niknafs et al. showed that SF/BNC scaffolds provide an effective host matrix for incorporating bioactive glass nanoparticles, in which nanocellulose provides reinforcement and bioactive glass promotes osteogenesis. [98]

Another distinctive reinforcement class is silk fibroin nanowhiskers, typically produced by controlled acid hydrolysis of silk fibers. Because they preserve chemical compatibility with SF, nanowhiskers can provide efficient reinforcement without introducing interface mismatch. Lin et al. reported that SF films incorporating acid-hydrolyzed SF nanowhiskers exhibited improved swelling behavior, mechanical performance, hydrophilicity, and antibacterial activity, while maintaining high cytocompatibility. [104]

Hybrid systems combining multiple nanofillers have also become increasingly popular to achieve synergistic property enhancement. Spessot et al. developed methacrylated SF-based nanocomposite scaffolds for bone tissue engineering by incorporating various functional components to improve mechanical and biological outcomes simultaneously. [105] Such multicomponent designs enable independent tuning of different functionalities - for example, one nanofiller contributing mechanical reinforcement while another provides biological activity or therapeutic delivery.

At the cutting edge of advanced architectures, spherical polyelectrolyte brushes loaded with metal nanoparticles have been used to reinforce SF hydrogels for wearable electronics. Li et al. reported robust wearable sensors based on SF hydrogels reinforced with nanoparticle-containing spherical polyelectrolyte brushes, demonstrating that sophisticated nanoparticle design can yield composites with high mechanical resilience, conductivity, and sensing capability. [106]

Collectively, SF nanocomposites enable a broad toolbox for tailoring antibacterial activity, electrical conductivity, mechanical reinforcement, and therapeutic functionality. However, the performance and safety of these systems strongly depend on nanoparticle dispersion, interfacial compatibility, potential leaching, and long-term stability. Therefore, careful selection of nanomaterials and structural design remains essential to balance multifunctionality with cytocompatibility and translational feasibility.

Hybrid and polymer-based reinforcements enable SF composites to achieve multi-property enhancements, including strength, bioactivity, and tailored degradation. Combining SF with nanocellulose, bioactive glass, or similar fillers creates synergistic mechanical and biological benefits. The remaining challenge is maintaining interface compatibility while keeping fabrication scalable and reproducible.

5. Applications of Modified Silk Fibroin

Silk fibroin (SF) - based nanocomposites and advanced modifications have enabled a broad spectrum of biomedical and technological applications by combining intrinsic cytocompatibility, tunable biodegradation, and versatile processability with nanomaterial-driven functionalities such as antibacterial activity, controlled drug release, electrical conductivity, and bioactive signaling. Key application

domains include smart drug delivery and wound therapy, bone tissue engineering, and emerging bioelectronic and photonic platforms.

Modified SF systems have expanded from traditional scaffolds into innovative therapeutic platforms and functional devices. Across applications, the key advantage is that SF can provide a safe structural backbone while modifications deliver targeted functionality. The most successful designs are those that connect processing choices and material chemistry directly to application-specific performance needs.

5.1. Smart Drug Delivery and Wound Healing

SF nanocomposites are widely investigated for drug delivery due to their excellent biocompatibility and flexible fabrication routes, while nanofillers can introduce high loading capacity and stimulus-responsive release behavior. In particular, MOF-integrated systems have attracted significant interest because MOFs offer highly porous architectures with tunable chemistry. Rahman et al. developed a dual cooperative encapsulation strategy combining ZIF-8 MOFs with silk fibroin/polycaprolactone nanofibers for curcumin delivery and accelerated wound healing. [103] The pH-responsive nature of ZIF-8 enabled enhanced drug release under acidic wound environments associated with infection or inflammation, supporting localized treatment while minimizing unnecessary release in healthy tissues.

Similarly, Zhu et al. employed MOFs as drug-loading intermediates within SF hydrogels and demonstrated that MOF-SF interactions at the secondary-structure level improved both mechanical performance and drug-loading capacity. [102] The photocurable hydrogel format further enabled minimally invasive administration (injection) and in situ gelation for sustained drug release at the target site. Beyond MOFs, responsive inorganic nanomaterials have also been developed for the treatment of chronic wounds. Cheng et al. reported injectable SF hydrogels containing pH-responsive ZnS nanoparticles that release zinc ions in acidic environments of diabetic wounds, providing antibacterial action and redox regulation in a condition-dependent manner. [93] These studies highlight the potential of SF-based systems to serve as "intelligent" wound therapeutic platforms that dynamically respond to pathological microenvironments.

Importantly, modern SF nanocomposites increasingly serve as multifunctional therapeutic platforms, integrating structural support, antimicrobial protection, and controlled therapeutic release within a single material system to address multiple stages of wound repair simultaneously.

SF composites are highly suited for drug delivery and wound healing because they can combine controlled release with bioactive and antimicrobial properties. Stimuli-responsive designs further improve efficiency by releasing therapeutics primarily when needed. Future progress depends on enhancing stability and translating these concepts into clinically reliable, scalable systems.

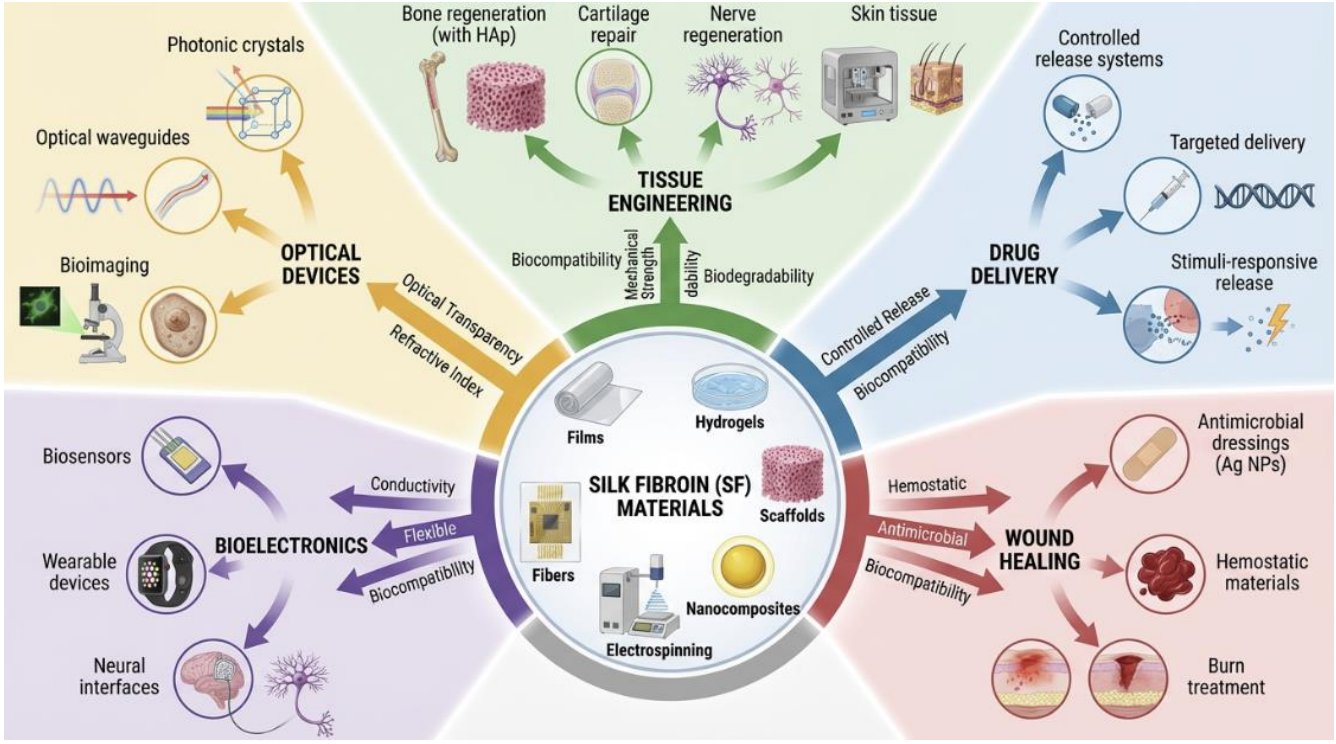

Figure 6. Decision tree illustrating application-driven method selection and subsequent testing

5.2. Bone Tissue Engineering and Osteogenic Regeneration

In bone tissue engineering, modified SF nanocomposites are commonly designed as scaffolds that provide mechanical support, promote osteogenic differentiation, and integrate well with host bone. Ceramic and mineral nanoparticles are particularly prevalent due to their ability to enhance both mechanical performance and osteoinductive capacity. Niknafs et al. demonstrated that incorporating bioactive glass nanoparticles into silk fibroin/bacterial nanocellulose composite scaffolds significantly improved osteogenic outcomes, including enhanced alkaline phosphatase activity and calcium deposition. [98] This performance is often attributed to the release of calcium, silicon, and phosphate -containing ions that support osteoblast function, combined with the mechanically reinforcing role of nanocellulose and the cytocompatible SF matrix.

Silica-based nanocomposites have also been investigated for osteoinduction, and nanoparticle size can strongly influence biological responses. Mart ń-Moldes et al. examined biomineralized silk-silica systems and reported that nanoparticle dimensions affect cellular differentiation pathways, emphasizing the importance of precisely controlling nanofiller properties to optimize osteogenic signaling. [99]

Nanoclays provide another promising category due to their mechanical reinforcement effect and potential to modulate osteogenic environments. Saurav et al. reported enhanced performance of regenerated SF composites reinforced with laponite and organomodified montmorillonite, demonstrating simultaneous improvements in mechanical behavior and osteogenic differentiation. [107] In addition, photocrosslinkable platforms allow spatial and mechanical control over scaffold performance. Spessot et al. developed methacrylated SF-based nanocomposite scaffolds for bone tissue engineering and highlighted how nanoparticle incorporation can tune mechanical properties and degradation rates to match bone healing timelines. [105]

Infection prevention remains a critical challenge in orthopedic applications, where implant-associated infections such as osteomyelitis can lead to severe complications and device failure. Xiang et al. reported sandwich-like electrospun SF nanocomposite membranes that simultaneously promoted osteogenesis and exhibited antibacterial activity, supporting the concept of dual-function scaffolds for regeneration and infection control. [108] Finally, magnetic nanocomposite scaffolds represent a specialized strategy for tumor-associated bone defects. Cojocaru et al. developed advanced 3D magnetic scaffolds that may enable imaging and externally controlled therapeutic modalities (drug guidance or hyperthermia), while maintaining structural support for bone regeneration after tumor resection. [95]

In bone regeneration, SF nanocomposites benefit from reinforcement fillers that improve stiffness while supporting osteogenic signaling. Mineral-like components and bioactive reinforcements help promote cell adhesion and bone-like matrix formation. The main design challenge is aligning mechanical strength, porosity, and biological cues into a single stable scaffold.

5.3. Biosensors, Wearable Electronics, and Biointerfaces

SF nanocomposites have recently expanded into wearable electronics and biosensing platforms, leveraging SF's flexibility, biocompatibility, and processability together with nanoparticle-driven electrical, optical, or sensing functions. For wearable devices that monitor physiological signals, the required materials must combine conductivity, mechanical robustness, and skin-compatible performance.

Li et al. developed durable wearable sensors based on SF hydrogels reinforced with spherical polyelectrolyte brushes containing metal nanoparticles. [106] These nanocomposite hydrogels exhibited combined strength, conductivity, and sensing capabilities, enabling the detection of physiological cues, such as strain or pressure, while maintaining conformability for skin contact. Carbon nanomaterial reinforcement provides another major route for conductive SF systems. Rathnayake et al. reported that incorporation of functionalized CNTs into electrospun SF fibers increased conductivity by 13.7-21.8 -fold, supporting applications in biosensing and signal transmission while preserving flexible form factors suitable for wearable interfaces. [96]

Conductive SF composites enable soft, skin-compatible sensors and wearable platforms that can combine mechanical flexibility with stable electrical response. These systems highlight SF's unique ability to operate in both biological and electronic environments. Long-term stability, hydration control, and repeatable signal performance are key for real-world deployment.

5.4. Optical and Photonic Applications

In addition to electrical conductivity, SF nanocomposites may provide optical functionalities relevant to photonic sensors and light-guiding devices. Zhang et al. demonstrated that in situ mineralization can generate silk strands that guide light along their length. [109] Such optical fiber-like constructs could be adapted for wearable phototherapy platforms, optical sensing, or optogenetic stimulation, illustrating the expanding scope of SF nanocomposites beyond classical biomedical scaffolds.

Overall, modified SF nanocomposites provide a versatile materials platform spanning smart drug delivery, multifunctional wound therapy, osteogenic scaffolds, and emerging wearable or photonic technologies. Future progress will depend on optimizing nanofiller dispersion, interface stability, long-term safety, and scalable manufacturing while preserving SF's inherent biocompatibility and degradability.

SF nanocomposites also support optical and photonic functions due to their transparency, tunable structure, and compatibility with functional nanomaterials. This opens emerging opportunities in photonics beyond classical biomedical scaffolding. Continued innovation will rely on precise structural control and integration with stable optically active components.

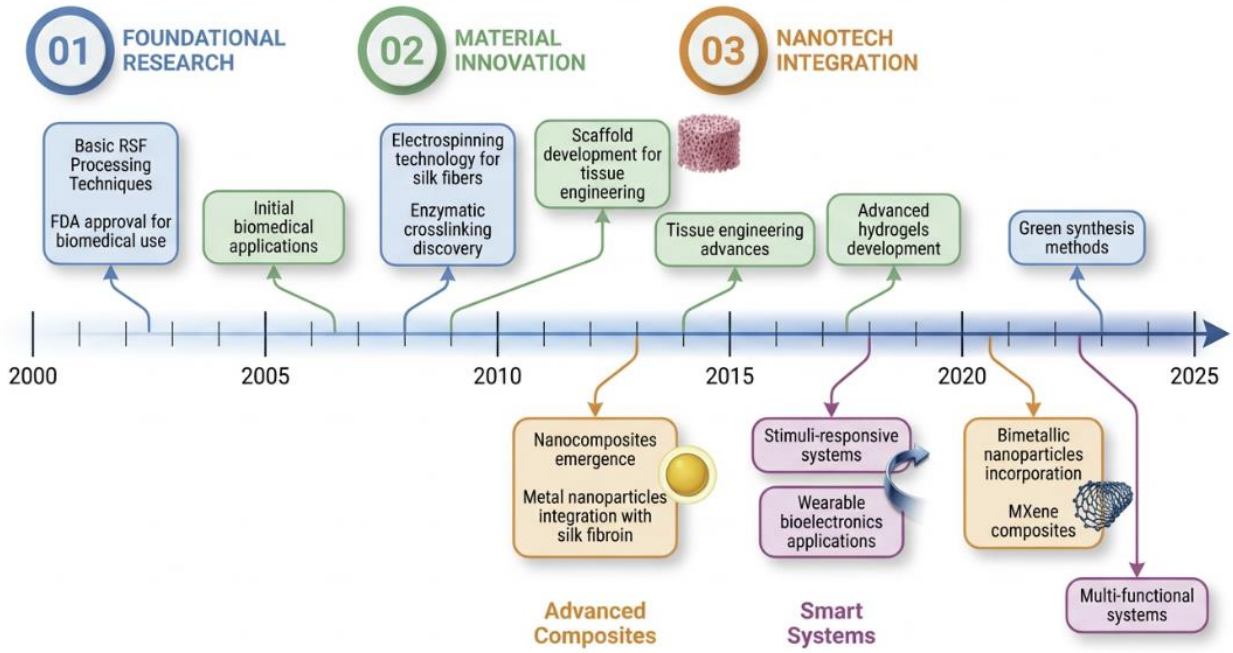

Figure 7. Timeline (2000–2025) highlighting frontier advances from 2022 to 2025

6. Conclusions

Silk fibroin (SF) has transitioned from a traditional natural fiber into a highly adaptable biomaterial platform capable of meeting the complex requirements of modern biomedical and functional technologies. This progress is rooted in SF's intrinsic strengths - biocompatibility, biodegradability, mechanical robustness, and wide-ranging processability - and has been amplified by advances in extraction, regeneration, and rational modification.

As emphasized in this review, the ultimate performance of regenerated silk fibroin (RSF) is tightly governed by upstream processing decisions. Degumming severity and dissolution chemistry directly shape molecular weight distribution, chain integrity, secondary-structure recovery, and the stability of the resulting constructs. Once properly controlled, RSF can be reliably assembled into films, electrospun mats, hydrogels, and porous 3D scaffolds, forming a unified materials toolkit for wound repair, tissue regeneration, drug delivery, and biointerface engineering.

Most importantly, chemical, physical, and biological modification strategies, together with nanocomposite and hybrid architectures, have transformed SF from a mechanically strong yet relatively inert protein into a programmable, multifunctional system. By integrating metallic nanoparticles, carbon nanomaterials, ceramics/minerals, MOFs, and advanced hybrid nanostructures, SF-based platforms can simultaneously deliver antimicrobial protection, controlled therapeutic release, enhanced osteogenic cues, improved conductivity, and mechanically reinforced networks. Collectively, these advances position modified silk fibroin as a key candidate for next-generation regenerative materials, smart therapeutic systems, and emerging wearable or photonic bio-devices.

This review shows that SF has evolved into a highly adaptable platform material, shaped by extraction methods, regeneration quality, and targeted modification strategies. The combined toolkit of chemical, physical, biological, and nanocomposite approaches makes SF relevant for both biomedical and advanced functional technologies. The strongest future impact will come from designs that are not only high-performing but also reproducible, safe, and scalable.

7. Outlook and Future Perspectives

Despite substantial progress, the next phase of silk fibroin research must shift from demonstrating isolated functions to delivering reproducible, safe, and scalable multifunctional platforms. Achieving this goal will require addressing several critical scientific and translational challenges.

The next stage of SF research will depend on solving practical barriers that limit reproducibility and translation. Standardized processing, safer chemistries, and long-term stability testing are necessary to move from promising lab demonstrations to real devices and therapies. Ultimately, sustainability and regulatory readiness will define how far SF-based technologies can scale into real-world applications.

7.1. Standardization and Reproducibility of RSF Processing

A persistent limitation across the literature is variability introduced by differences in degumming conditions, dissolution solvents, dialysis protocols, and storage history. Future studies should standardize and report key metrics, including molecular weight distribution, β-sheet content, rheological behavior, gelation kinetics, and residual salt/

solvent content. Establishing clear processing-structure-property maps will enable reliable comparisons between studies and support industrial-scale reproducibility.

A significant gap in the field is that RSF preparation varies significantly between studies, making comparisons difficult. Establishing standardized protocols will strengthen reliability and accelerate industrial adoption. Without reproducible baselines, even strong modification strategies may produce inconsistent outcomes.

7.2. Safer and More Precise Functionalization Chemistries

While cross-linking and monofunctionalization have greatly expanded SF performance, future approaches must emphasize precision and cytocompatibility. Enzyme-mediated cross-linking and visible-light systems are emerging as promising approaches for injectable and cell-laden hydrogels. Still, careful control of catalyst/initiator residues and long-term biological fate is essential. Next-generation strategies should aim to maximize functional group density while preserving chain integrity and avoiding uncontrolled degradation.

Functionalization must increasingly prioritize safety, control, and minimal structural damage to SF chains. Moving toward milder chemistries and better reaction specificity will reduce toxicity risks and improve consistency. The best advances will be those that deliver functionality while maintaining SF's biocompatibility.

7.3. Nanocomposite Stability, Interfacial Design, and Long-Term Biosafety

The benefits of SF nanocomposites depend strongly on nanoparticle dispersion, interfacial compatibility, and resistance to aggregation or leaching. Moving beyond simple blending toward controlled immobilization and interface engineering will be critical, particularly for metallic nanoparticles and MOFs used in chronic wound therapy or implantable systems. Long-term studies must also evaluate immunological response, oxidative stress, and degradation pathways to ensure biosafety under clinically relevant conditions.

Nanocomposites deliver impressive performance, but their benefits depend on stable filler retention and strong SF-nanoparticle interfaces. Leaching and long-term biosafety remain major concerns, especially for clinical translation. Future work must include realistic long-term evaluation, not only short-term functionality tests.

7.4. From Multifunctionality to Theranostic Silk Platforms

The most impactful SF systems will likely integrate therapy and diagnostics within a single material design. Conductive carbon-based composites, MOF-enabled drug reservoirs, magnetic scaffolds, and responsive inorganic nanoparticles provide a foundation for theranostic platforms that can monitor infection, inflammation, redox state, or mechanical loading while simultaneously guiding regeneration or delivering therapeutics. Such systems could redefine SF from a passive scaffold into an active biointerface capable of real-time intervention.

The most impactful SF systems will likely combine sensing, response, and therapy into a single integrated platform. Rather than being passive scaffolds, these materials can become active biointerfaces that monitor and intervene in real time. Achieving this goal will require careful integration of electronics, bioactivity, and stability into a single design.

7.5. Scale-up Manufacturing and Regulatory Readiness

To translate SF nanocomposites from laboratory studies to real-world deployment, scalable fabrication methods (electrospinning, 3D printing, photocurable hydrogels, coating-based manufacturing) must be coupled with robust quality control frameworks. Regulatory readiness will demand consistent composition, sterilization compatibility, predictable degradation products, and validated safety profiles. Therefore, translational research should increasingly prioritize standardized manufacturing protocols and clinically relevant evaluation models.

Translation will require moving beyond lab-scale fabrication into consistent, scalable manufacturing routes. Regulatory requirements will demand standardized quality control, validated safety, and clinically relevant performance testing. Building "manufacturable" SF platforms is just as important as demonstrating multifunctionality.

7.6. Sustainability and Circular Bio-Based Materials

Silk waste streams are abundant and underutilized in many regions, creating an opportunity to couple high-value biomedical innovation with sustainable resource utilization. Future work should emphasize greener dissolution systems, solvent recycling, and energy-efficient processing, positioning silk fibroin as a flagship example of circular, bio-based advanced materials.

Silk waste represents an underused resource that can be converted into high-value advanced materials. A circular approach can make SF technologies more sustainable and economically attractive. Future SF innovation will benefit from aligning performance improvements with green processing and waste reduction.

7.7. Final Perspective

In summary, silk fibroin research is entering a stage where success will be defined not only by novelty but by predictability, safety, and practical scalability. The field is shifting from materials preparation toward materials programming, where molecular-level modification and nanoscale engineering yield controlled, application-specific outcomes. With continued progress in structure standardization, cytocompatible functionalization, and stable nanocomposite integration, modified silk fibroin systems are poised to deliver clinically meaningful regenerative therapies, infection-resistant scaffolds, intelligent wound

platforms, and next-generation wearable or photonic biointerfaces.

Silk fibroin research is entering a mature phase in which success will be defined by intelligent design choices rather than novelty alone. The strongest SF systems will be those that balance performance, safety, reproducibility, and scalability. With continued progress, SF is well-positioned to support next-generation functional biomaterials, devices, and biointerfaces.

Declaration

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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