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REVIEW

Silk Protein Gene Engineering and Its Applications: Recent Advances in Biomedicine Driven by Molecular Biotechnology

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Abstract: Silk protein, as a natural polymer material with unique structures and properties, exhibits tremendous potential in the biomedical field. Given the limited production and restricted properties of natural silk proteins, molecular biotechnology has been extensively applied in silk protein genetic engineering to produce novel silk proteins with specific properties. This review outlines the roles of major model organisms, such as silkworms and spiders, in silk protein production, and provides a detailed introduction to the applications of gene editing technologies (eg, CRISPR-Cas9), transgenic expression technologies, and synthetic biology techniques in silk protein genetic engineering. By analyzing the genetic factors influencing silk protein expression, this review further elaborates on the innovative applications of silk proteins in drug delivery systems, tissue engineering and regenerative medicine (eg, skin, bone, cartilage, and vascular repair), as well as antibacterial immune strategies. Notably, modified silk proteins expressed by transgenic silkworms demonstrate significant advantages in enhancing drug bioavailability and promoting cell proliferation and differentiation. In conclusion, silk protein gene engineering, through continuous innovations in molecular biotechnology, has provided an effective pathway for the production of high-performance silk protein materials. The extensive applications of these modified silk proteins in the biomedical field have not only expanded the functionality of silk proteins but also offered new approaches to address medical challenges. In the future, the development of silk protein gene engineering will further rely on interdisciplinary integration to promote in-depth research and the expansion of industrial applications of silk proteins.

Keywords: silk protein, genetic engineering, molecular biotechnology, performance optimization, biomedical applications, tissue engineering, regenerative medicine

Silk proteins are proteins synthesized by organisms, possessing special structures and properties, and often exist in a filamentous form within organisms, such as silkworm silk and spider silk.[1] Relying on their exceptional mechanical properties, biocompatibility, degradability, and many other characteristics, silk proteins exhibit tremendous potential and value for application in drug delivery systems, tissue engineering and regenerative medicine, as well as antibacterial and immune strategies, becoming promising biomedical materials.[2] However, the production of natural silk proteins faces limitations, primarily manifesting in the following two aspects:[3]

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Firstly, the yield of natural silk proteins is limited.[4] This is because the breeding and cultivation processes of organisms capable of producing high-quality silk proteins (such as silkworms and spiders) are complex and time-consuming, and are simultaneously affected by various factors such as the environment, feed, and diseases, resulting in silk protein yields that are difficult to meet the demand for large-scale applications.

Secondly, the properties of natural silk proteins may not meet all application requirements.[5] Although silk proteins possess various excellent properties, certain specific applications may require silk proteins with specific performance characteristics (such as specific mechanical strength, biodegradation rates, or biological activity). However, the properties of natural silk proteins are often influenced by the genetic characteristics and growth environment of their source organisms, making it difficult to meet the customized needs of all applications.

Therefore, these limitations have prompted researchers to explore the modification of silk protein genes through genetic engineering and molecular biotechnology to produce novel silk proteins with specific properties, thereby broadening the application scope of silk proteins and enhancing their application value (Figure 1). Early on, silk proteins have already found applications in medicine, such as using silkworm silk for wound suturing. These applications marked the initial exploration of silk proteins in biomedical applications.[6] With significant advancements in recombinant technologies, including the emergence of gene cloning and transgenic technologies, research on silk proteins has entered a new phase. These technologies have not only increased the production yield of silk proteins but also enhanced their scalability and sustainability, offering significant advantages compared to natural silk protein production.

Although gene engineering and molecular biotechnology have been applied in silk protein research, concise explanations of their specific effects on silk proteins still need to be strengthened.[7] For instance, through gene editing technologies such as CRISPR/Cas9, researchers are able to precisely modify silk protein genes, thereby altering the properties of the proteins they encode. Transgenic expression technology allows for the expression of silk protein genes in heterologous systems to achieve their mass production. Furthermore, the rise of synthetic biology technologies has made it possible to design silk proteins with entirely new properties.

This review systematically elaborates on the primary biosynthetic sources of silk proteins, particularly highlighting the contributions from silkworms and spiders. In addition, it briefly mentions other arthropods as secondary sources of

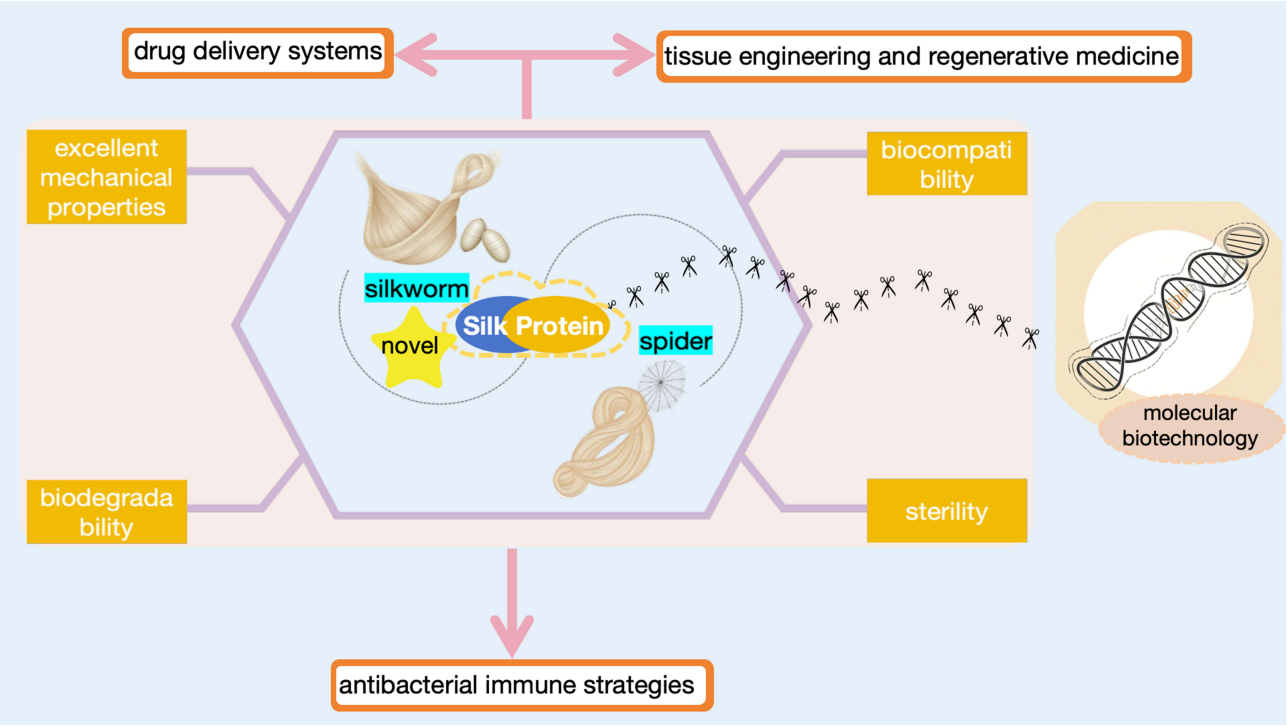

Figure 1 Modification and Production of Silk Proteins – Addressing New Demands for Biomedical Applications.

silk proteins. The latest advancements in molecular biotechnology within the field of silk protein genetic engineering are analyzed in detail, exploring the key genetic factors regulating silk protein expression. Meanwhile, the diverse applications of silk proteins in the biomedical field are also thoroughly discussed, with a special emphasis on their potential in antibacterial and immunoregulatory aspects. We focus on analyzing the recent progress in molecular biotechnology that facilitates the production of recombinant silk proteins and their applications in biomedicine. By reviewing past milestones and providing concise explanations of related technologies, this review aims to provide readers with a comprehensive perspective to explore the future directions and application potential of silk proteins.

Major Model Organisms for Silk Protein Production

Although chemical synthesis methods have attempted to mimic some structures of natural silk proteins using organic polymers, the resulting novel fiber materials still exhibit significant gaps in mechanical properties compared to natural silk proteins. Therefore, selecting appropriate host organisms for biotechnological production is a crucial approach to obtaining high-quality silk proteins.

Silkworm (Bombyx mori)

The silkworm, as a paradigm of economically important insects with a long history, has contributed to the brilliance of the silk industry through its exceptional silk-spinning ability.[8] As a significant lepidopteran insect, the silkworm not only produces silk for cocoon formation but also has its larval excretions applied in traditional Chinese medicine.[9] Furthermore, the silkworm serves as a key model organism in the fields of insect physiology, developmental biology, genetics, and pest control.[10] Its unique silk gland tissue, consisting of ASG, MSG, and PSG, is the core site for silk protein synthesis and secretion.[11]

ASG serves as the initial segment of the silk gland tissue in the Bombyx mori silkworm, responsible for receiving and initially processing the sericin protein precursor secreted from the corpora allata.[12] Sericin, a viscous substance surrounding the fibroin protein in silk, plays a crucial role in enhancing the strength and toughness of silk threads. Within the ASG, the sericin protein precursor undergoes a series of physical and chemical transformations, gradually converting into a sericin protein solution suitable for subsequent secretion. Throughout this process, the cellular structure and function of the ASG play a pivotal role.

MSG represents the core component of the silk gland tissue in the Bombyx mori silkworm and serves as the primary site for the synthesis and secretion of fibroin protein.[13] Fibroin, the main constituent of silk, exhibits properties such as high strength, high toughness, and excellent biocompatibility. Within the MSG, the fibroin precursor undergoes cellular synthesis and processing, resulting in the formation of fibroin fibers with specific structures and functions. These fibers gradually accumulate and align into orderly silk threads within the MSG, preparing them for subsequent secretion by the posterior silk gland.

PSG is the terminal segment of the silk gland tissue in the Bombyx mori silkworm, primarily responsible for mixing the fibroin fibers synthesized in the MSG with the sericin protein solution processed in the ASG and secreting them outside the body.[14] In the PSG, under the extrusion action of cells, the fibroin fibers and sericin protein solution gradually fuse and coagulate into silk threads. These threads are expelled through the silkworm's spinning orifice, gradually solidifying into silk in the air. The cellular structure and function of the PSG have a significant impact on the characteristics of the silk threads, including their thickness, gloss, and strength.

Silk fibers, an outstanding product of nature, is primarily structured by two key components: sericin and fibroin.[15] These two proteins play indispensable roles in the formation of silk, exerting a profound influence on its overall properties. Consequently, the Bombyx mori silkworm is not only a vital pillar of the silk industry but also an ideal model for studying the mechanisms of silk protein synthesis and regulation.

Sericin, serving as the outer coating material of silk, consists of a group of glycoproteins with molecular weights ranging from 20 to 400 kDa and rich in serine.[16] These glycoproteins are synthesized in the MSG and impart silk with notable hydrophilicity. The hydrophilicity of sericin originates from its specific amino acid sequences, which contain hydrophilic groups that enable silk to interact with water molecules, thereby exhibiting good wettability and softness. Additionally, sericin plays protective and adhesive roles. It acts like a protective layer covering the exterior of fibroin, preventing fibroin from damage by the external environment during silk formation. Through its adhesive properties, sericin tightly binds multiple fibroin fibers together, forming resilient and elastic silk fibers.

Fibroin is the core component of silk structure, comprising three key constituents: Fib-H, Fib-L, and P25 glycoprotein hexamer.[17] These constituents are tightly linked by disulfide bonds, forming a stable and robust protein network structure. Fibroin exhibits exceptional mechanical properties, imparting silk with high strength, toughness, and good elasticity. During silk formation, the fibroin fibers align along specific directions, forming a highly ordered microfibrillar structure. This structure enables silk to effectively disperse stress when subjected to external forces, thereby exhibiting outstanding tensile and tear resistance.

The developmental process of the silk gland in silkworms encompasses two critical stages: formation during the embryonic stage and growth during the larval stage. This process is precisely regulated by a complex network of hormonal signals.[18] Especially during the peak growth period, nutrients pass through the PI3K-Akt-SGF1-Dimm signaling pathway, significantly accelerating the massive synthesis of silk proteins.[19] Meanwhile, the transcription factor STAT plays a pivotal role in the immune response of silkworms, primarily by regulating the expression of antimicrobial peptide genes and enhancing resistance to pathogens, thereby improving the survival rate of silkworms.[20] The hormonal signal network and the transcription factor STAT jointly coordinate the development of the silk gland and immune response in silkworms, ensuring their healthy growth and efficient silk production.

During the embryonic stage, the silk gland, as a product of mitosis within the cell nucleus, emerges after undergoing ten cell divisions during egg-embryo development and forms a complete tissue during the blue-head stage. Cell cycle genes, particularly Cdc gene family, precisely control the division and proliferation of glandular cells by regulating the interactions between cyclins, CDKs, and CKIs. Among them, CyclinB and Cyclin3 are crucial for the division and quantity of silk gland cells during the embryonic stage.[21] The interactions between cell cycle genes and their associated proteins play a key regulatory role in the division and proliferation of silk gland cells during the embryonic stage.

Entering the larval stage, Cyclin D and Cyclin E take the lead in replicating genomic DNA, driving the continuous growth of silk gland cells. However, Cyclin B3 plays a pleiotropic role in the development of female reproductive organs and early embryogenesis in silkworms.[22] SCR gene exerts an inductive effect during this process, while nutrition and hormonal signals also have significant impacts on silk gland development.[23] Furthermore, the high expression of silk gland nuclear protease during specific developmental stages of the pupa reveals its crucial role in silk gland remodeling and degeneration.[24] Insulin finely regulates the nuclear replication of silk gland cells through the PI3K/AKT and Tor signaling pathways.[25] In summary, the development of the silk gland during the larval stage is the result of synergistic interactions between various genes, nutrition, and hormonal signals, ensuring the normal growth and differentiation of silk gland cells.

Silk proteins, as exemplary natural silk proteins, position their producing organism, the silkworm, at the core of eukaryotic silk protein research. The study of silk gland development and its functional mechanisms is of great significance for understanding biological processes such as cell proliferation, differentiation, and biomaterial synthesis.[26] TALENs are commonly used for gene modification in silkworms.[27] Research has confirmed that the silk gland, as a key tissue regulating silk protein synthesis, interacts with other tissues, such as the fat body and midgut. Through the biosynthetic pathway of glycine-serine, these inter-tissue interactions can effectively enhance the production of silk proteins.[28] The investigation into the synthesis mechanisms and regulation of silk proteins not only deepens our understanding of the biological characteristics of silkworms but also provides a theoretical basis for the efficient production of silk proteins (Figure 2).

Spiders

Apart from silk proteins derived from silkworms, spider silk proteins, originating from spiders of the class Arachnida within the phylum Arthropoda, also constitute an important category of silk proteins.[29] Spider silk proteins, however, possess unique structures and properties, exhibiting significant potential and value for application in various fields such as materials science and biomedical science.[30] Currently, recombinant spider silk proteins are widely used in various domains including biomedicine, cosmetics, and technology.[31] As a unique silk protein-producing host in nature, spiders yield spider silk proteins that exhibit substantial application potential across multiple fields.[32] Among them, the highly ordered hydrophobic MaSp1, the less ordered hydrophilic MaSp2, MaSp3 which is believed to be involved in silk formation during the spinning process, and MaSp4 and MaSp5 that are closely related to tenacity, are all intricately associated, collectively forming the complex system of spider silk proteins.[33]

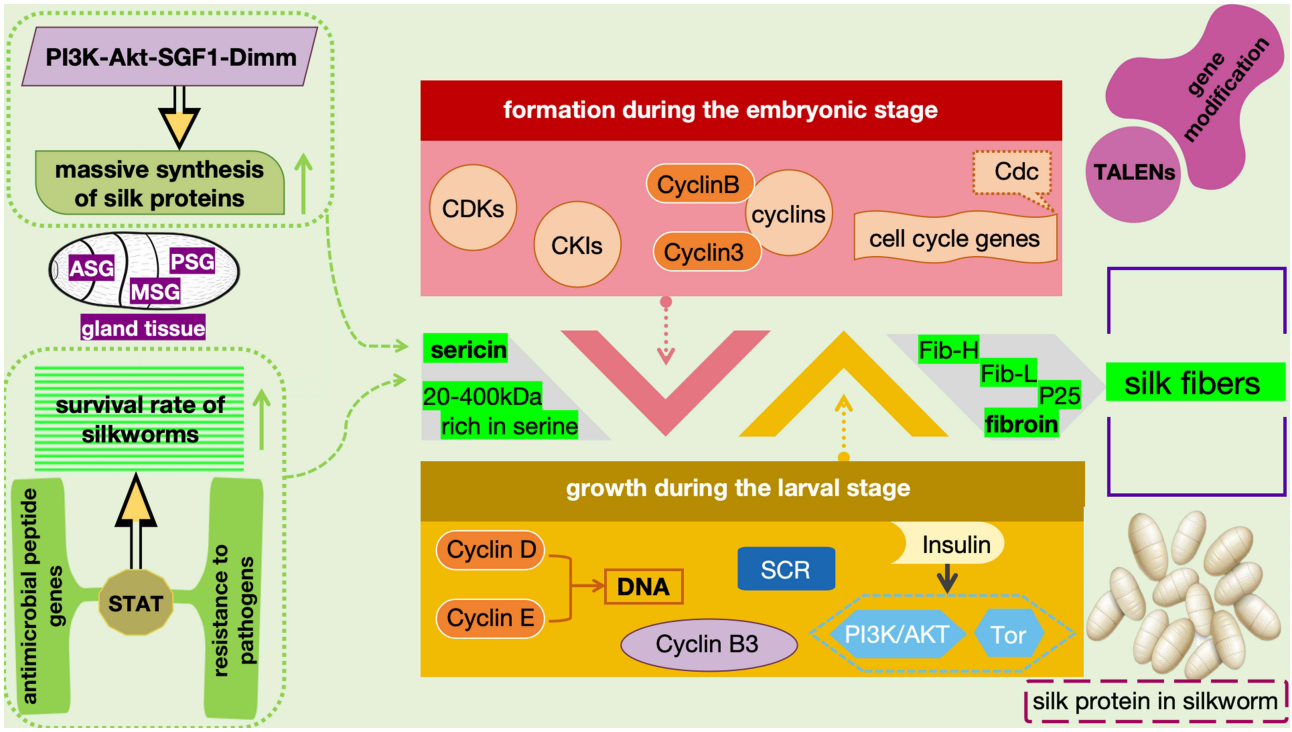

Figure 2 Development of Silk Glands in Silkworm (Bombyx mori), Synthesis of Silk Proteins, and Their Regulatory Mechanisms.

MaSp1 is one of the primary components in spider silk, featuring a highly ordered structure and hydrophobic properties.[34] It primarily consists of repetitive sequences of glycine and alanine, which can form stable β-sheet structures, thereby imparting high strength and high modulus characteristics to the spider silk. MaSp1 plays a crucial role in the formation process of spider silk, determining its mechanical properties and stability. In contrast to MaSp1, the structure of MaSp2 is relatively less ordered and exhibits certain hydrophilic properties.[35] MaSp2 contains numerous glycine-proline-glycine repetitive sequences, which allow it to play a role in elastic modulation within the spider silk. MaSp1 and MaSp2 usually coexist, and their interactions and synergistic effects contribute to the excellent comprehensive performance of spider silk. MaSp3 is another protein found in spider silk, although its exact function has not been fully elucidated; it is believed to participate in the formation of spider silk during the spinning process. MaSp3 may interact with MaSp1 and MaSp2, undergoing complex interactions and self-assembly processes to form spider silk with specific structures and properties.[36] MaSp4 and MaSp5 are proteins closely related to the toughness of spider silk.[37] They enhance the toughness and fracture resistance of spider silk through specific sequences and structural features. The presence of these proteins in spider silk enables it to not only possess high strength and high modulus but also exhibit good elasticity and toughness, thereby allowing it to adapt to various complex environments and stress conditions.

Spider silk proteins not only exhibit exceptional mechanical properties such as high strength and elasticity but also possess biodegradable, chemically stable, and low immunogenic and inflammatory response-inducing characteristics, making them highly attractive for use in biomaterials and biomedicine.[38] Spider silk proteins exhibit a wide distribution of molecular weights and possess intricate yet exquisite structures, comprising three distinct domains: a repetitive central core domain where highly repetitive crystalline regions alternate with a few crystalline peptides; a conserved N-terminal domain; and a C-terminal domain.[39] Natural spider silk proteins are rich in alanine, glycine, serine, and glutamine, while the content of acidic and basic charged amino acids is relatively low.[40] The core repetitive region contains specific sequence motifs, leading to variations in the secondary structure depending on spider species, silk type, and silk fiber state.[41] This unique structure endows spider silk with exceptional physical and chemical properties, making it a focal point in the fields of structural biology and biomaterials research, and providing extensive development space for applications in novel energetic materials, biomedical applications, and more.[42] Studies have confirmed that the egg sac silk from the Latrodectus hesperus species is a high-performance biomaterial, primarily

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secreted by the tubuliform glands, and contains the main component TuSp1 along with at least six minor components. Among these, ECP3 is a small 11.8 kDa protein that lacks the typical spider protein structure and has limited homology with known spider proteins.[43] The complex structure and composition of spider silk proteins offer abundant research potential and application prospects in the fields of high-performance biomaterials and biomedical applications.

In recent years, research on the nano-assembly mechanisms of spider silk proteins has revealed the crucial role of IDRs in this process.[44] Studies have confirmed that IDRs play a significant role in the nano-assembly of spider silk proteins.[45] Using the minor ampullate spider as a research subject, it was found that it can self-assemble into oligomers and form droplets, a process that relies on the aggregation-prone nature of both IDRs and folded repetitive domains. The formation and stability of the droplets are primarily influenced by tyrosine residues within the IDRs, while the terminal domains also impact pH- and salt-dependent self-assembly properties.[46] Therefore, droplets may represent an intermediate state between the solution state and the fiber state.[47] Additionally, research has demonstrated that the pyriform silk produced by pyriform spiders consists of PySp1, whose core repetitive domain comprises 234-residue Py units, exhibiting structural modularity. This structure is composed of a six-α-helical globular core surrounded by IDRs on both sides, forming a beaded structure. Notably, the N-terminal and C-terminal domains are highly conserved across silk types, while the diversity of repetitive region sequences among different spiders directly reflects their mechanical properties.[48] The structural diversity and assembly mechanisms of spider silk proteins provide extensive research space for their applications in fields such as biomaterials and nanotechnology, with the potential to develop novel materials with exceptional mechanical properties (Figure 3).

Others

In exploring alternative strategies for silk protein production, apart from the traditional silkworm and spider, other host organisms have demonstrated significant potential.[49] These hosts encompass prokaryotes (such as *Escherichia coli* and *Bacillus megaterium*),[50,51] eukaryotes (including yeast, transgenic plants, bovine mammary epithelial cells proficient in extracellular protein secretion, and hamster kidney cells adapted for producing large quantities of recombinant proteins, etc).,[52] transgenic animals (like mice and sheep, with the latter employing a liposome-mediated method to transfect sheep fibroblasts with plasmids containing spider protein genes, thereby producing recombinant spider proteins in sheep hair

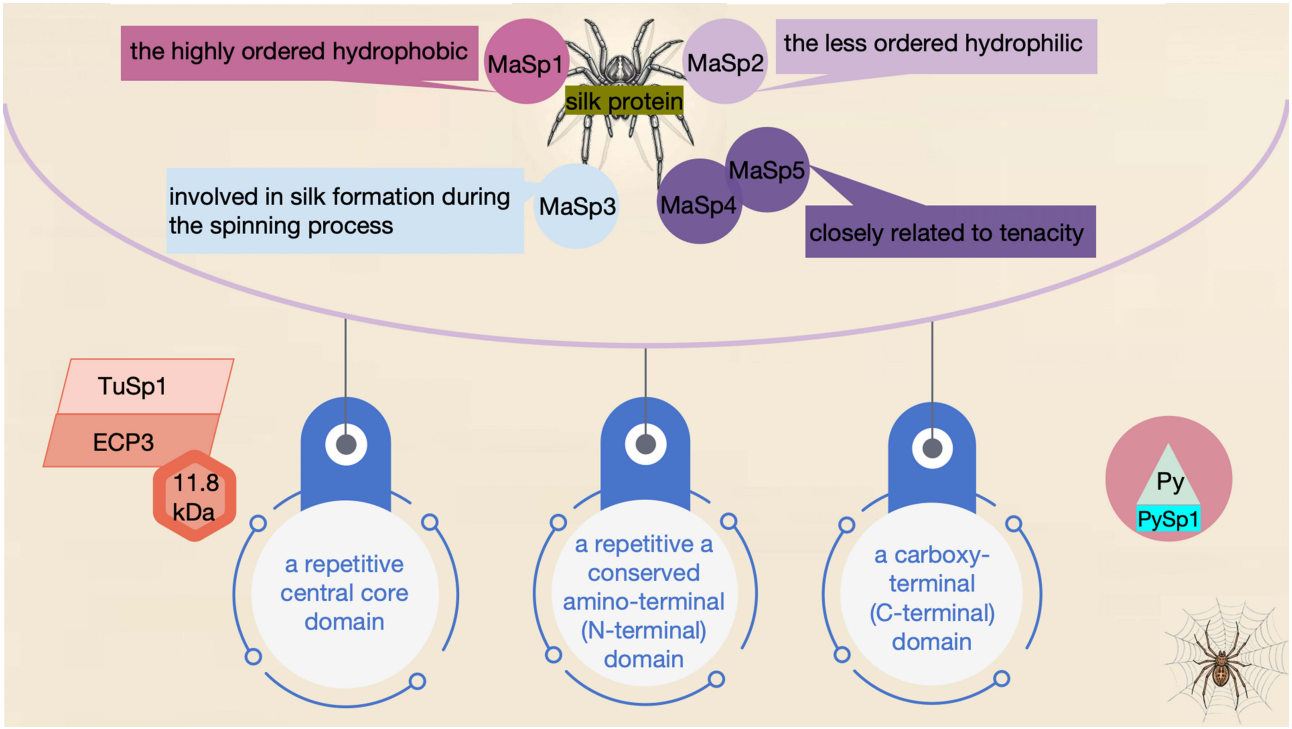

Figure 3 Structural Diversity and Assembly Mechanisms of Spider Silk Proteins.

follicles),[53] and plant hosts (such as tobacco leaves, Arabidopsis thaliana, Medicago sativa, and microalgae, including Chlamydomonas reinhardtii and Chlorella pyrenoidosa).[54] Utilizing these hosts for biotechnological production can effectively circumvent many of the limitations associated with directly obtaining silk proteins from silkworms and spiders.

It is particularly noteworthy that eukaryotic hosts, leveraging their complex cellular machinery and superior protein processing capabilities, can produce recombinant silk proteins that are highly similar to natural silk proteins.[55] Compared to prokaryotic hosts, eukaryotic hosts exhibit higher efficiency in synthesizing larger genes, although prokaryotic hosts are well-established in terms of cell lysis and protein purification techniques. Various silk proteins, such as MaSp1, MaSp2, and PySp1, have been successfully produced in prokaryotic hosts.[56] Similarly, multiple silk proteins, including DP-1B (MaSp), Z-4RepCT (fusion protein), Flagelliform silk, MaSp 1, MaSp 2, and ADF-3 (MaSp), have been produced in eukaryotic hosts.[57] Eukaryotic hosts also demonstrate robust silk protein production capabilities when compared to transgenic animals and plants. Silk proteins like MaSp1 and MaSp2 have been successfully produced in transgenic animals, while plant hosts have successfully produced various silk proteins including MaSp1, MaSp2, and DPIB-8p.[58] Therefore, eukaryotic hosts exhibit significant advantages in the production of recombinant silk proteins, providing a reliable platform for the wide application of silk proteins.

Application of Molecular Biotechnology in Silk Protein Genetic Engineering

With the rapid development of molecular biotechnology, its application in the field of silk protein genetic engineering has become increasingly extensive and profound, opening up new avenues for the modification, innovation, and large-scale production of silk proteins (Figure 4). These technologies not only significantly influence our understanding of the basic biological characteristics of silk proteins but also drive their potential applications in various fields such as biomedicine and materials science. Within this framework, gene editing technology, transgenic expression technology, and synthetic biology technology serve as the three pillars, collectively supporting the vast landscape of silk protein genetic engineering research.[59]

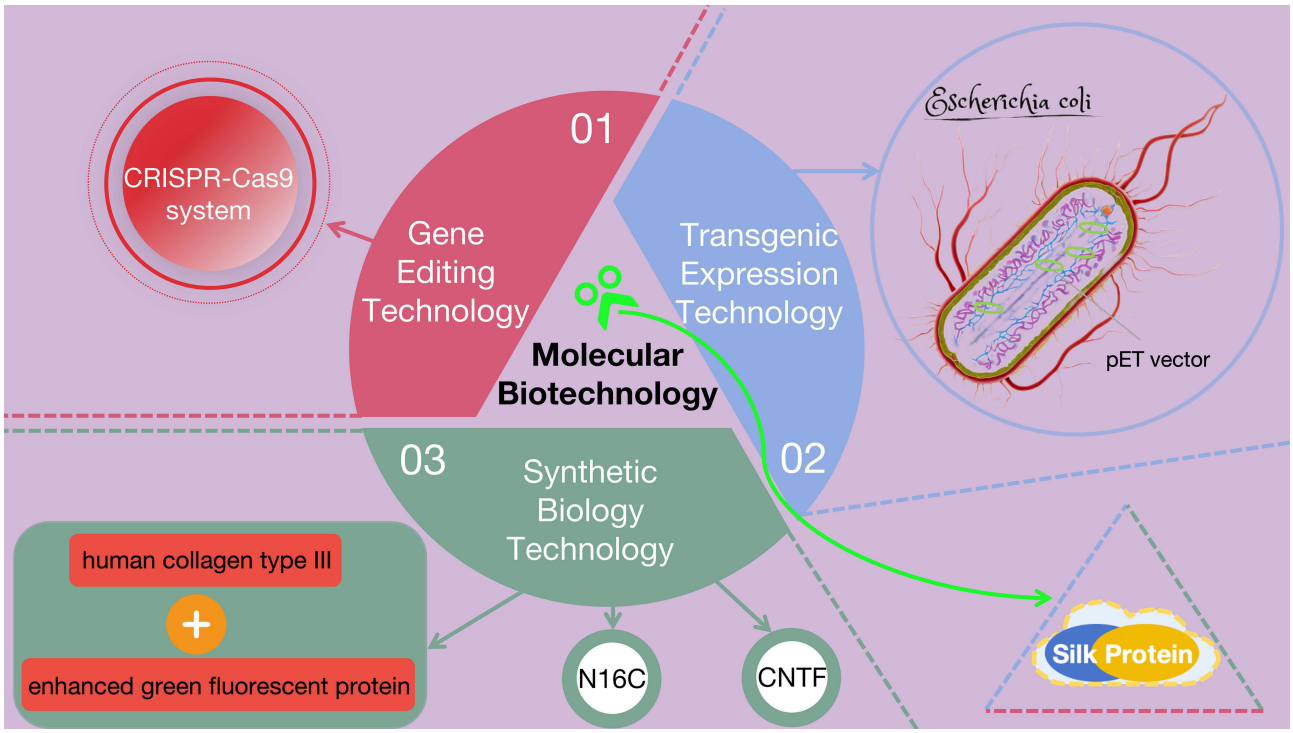

Figure 4 Extensive Application and Innovative Approaches of Molecular Biotechnology in Silk Protein Genetic Engineering.

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Gene Editing Technology

As a frontier field of molecular biotechnology, gene editing technology plays a crucial role in silk protein genetic engineering due to its high precision and flexibility.[60] The CRISPR-Cas9 system stands out among these technologies, utilizing its unique "scissors" function—designing specific single-guide RNA to guide the Cas9 endonuclease—to precisely "cut" the silk protein gene, enabling targeted gene mutations, deletions, or insertions.[61] This direct manipulation of gene sequences provides scientists with unprecedented capabilities to explore and modify the amino acid sequences, domain arrangements, and overall expression patterns of silk proteins, thereby creating silk protein materials with novel properties.[62]

For instance, using a CRISPR/Cas9 targeting strategy, natural spider silk protein genes up to 10 kb in size have been successfully integrated into the intron regions of the silk Fib-H or Fib-L in the silkworm genome, ensuring that the CRISPR/Cas9-induced sequence changes during editing do not affect normal protein production. The fibers spun by the resulting transgenic silkworms exhibit a tensile strength of up to 1.2 GPa, comparable to natural spider silk, opening up new avenues for the production and application of silk proteins.[63] The CRISPR-Cas9 gene editing technology provides a powerful tool for silk protein genetic engineering, driving the development and application of novel silk protein materials with excellent performance.

However, despite the immense potential demonstrated by CRISPR-Cas9 gene editing technology in silk protein genetic engineering, it also faces several challenges and limitations. Further discussion of these challenges and limitations will aid in achieving a more comprehensive understanding of the technology's applicability and constraints. For instance, the off-target effects of the CRISPR-Cas9 system are a non-negligible issue, as they may lead to unintended editing of non-target genes, thereby affecting the traits of transgenic silkworms and the quality of silk proteins. Additionally, the application of CRISPR-Cas9 technology in silk protein genetic engineering is constrained by the complexity of the silkworm genome and the regulatory mechanisms of silk protein gene expression. Therefore, future research needs to further optimize the CRISPR-Cas9 system to improve its editing efficiency and accuracy, and delve deeper into the expression regulatory mechanisms of silk protein genes, in order to provide stronger support for the development of silk protein genetic engineering.

Transgenic Expression Technology

Transgenic expression technology achieves heterologous expression of silk proteins by constructing recombinant vectors containing the target silk protein genes and introducing them into suitable host cells.[64] This method not only increases the yield of silk proteins but also broadens their sources.[65] For example, researchers have increased the repetitive gene sequence encoding spider silk protein MaSp2 to multiple contiguous units and transferred it into *Escherichia coli* for expression via the pET vector. This approach not only significantly enhances the yield and molecular weight of spider silk protein but also confers additional functions, such as improved blood compatibility and antibacterial properties.[66] In another study, transgenic technology was used to ectopically express pierisin-1A, a cytotoxin derived from Pieris rapae, in silkworms to inhibit sericin protein expression, successfully cultivating transgenic silkworms with modified middle silk glands capable of producing cocoons composed solely of silk fibroin.[67]

Furthermore, current research has successfully achieved transgenic expression of the artificially synthesized spider protein gene 4s in cloned sheep embryos to mimic the fibrosis mechanism of spider silk proteins.[68] Additionally, silk proteins with bioengineered characteristics and functionalities have been developed, which can promote wound healing processes in rat and human skin fibroblasts.[69] Transgenic expression technology provides an effective means for the high-efficiency production and functional modification of silk proteins, greatly advancing the application progress of silk protein materials in biomedicine and other fields.

Synthetic Biology Technology

Synthetic biology technology aims to create biological organisms or components with new functions by designing, constructing, and optimizing biological parts, devices, and systems.[70] In silk protein genetic engineering, this technology is applied to construct complex silk protein synthesis pathways for customized production of silk proteins.[71] Specifically, by designing and optimizing gene clusters related to silk protein synthesis through synthetic biology methods, silk proteins with specific properties can be efficiently expressed in specific hosts.[72] Researchers have successfully utilized this technology to modify the PSG of silkworms, enabling them to produce cocoons containing human collagen type III and enhanced green fluorescent

protein, achieving the co-weaving of silk protein and target protein within the cocoons.[73] Another study demonstrated the successful expression of CNTF protein in the MSG of silkworms, which can be secreted into silk at a concentration of 3.2 mg/g. Compared to natural silk proteins, the prepared CNTF-functionalized silk protein materials promote the proliferation and migration of neural cells.[74]

Furthermore, research has confirmed that the artificially constructed spider silk gene N16C, built using the golden gate assembly technique and comprising an N-terminus, 16 repeats of a 33-residue primary leg segment, and a C-terminus, has been successfully expressed, purified, and made into films in *Escherichia coli*.[75] Synthetic biology technology provides a powerful tool for the customized production and functional modification of silk proteins, promoting the wide application of silk protein materials in fields such as biomedicine and bioengineering.

It is noteworthy that, despite the significant progress made by synthetic biology techniques in the customized production and functional modification of silk proteins, there are still several bottlenecks to be addressed at the industrial application level. For instance, key challenges that urgently need to be tackled include how to further increase the yield and reduce the cost of silk proteins, as well as how to ensure the stability and bioactivity of functionalized silk proteins.

Genes Influencing Silk Protein Expression

The factors influencing silk protein expression are intricate and diverse, among which the role of genes is particularly pivotal. To systematically present these genes and their functional characteristics, we have meticulously compiled Table 1 for detailed elucidation. This table not only summarizes the core genes that impact silk protein expression but also briefly outlines related experimental approaches and research findings, providing invaluable insights for in-depth exploration and understanding of these genes.

In the field of molecular biotechnology, the primary methods for editing, expressing, and synthesizing silk protein genes encompass gene editing technology, transgenic expression technology, and synthetic biology technology. Building upon these methodologies, we have further investigated how these genes regulate silk protein expression, aiming to comprehensively uncover the mechanisms underlying silk protein production and regulation. Furthermore, the research findings concerning these genes have laid a theoretical foundation for the application of silk protein in biomedical fields, particularly in cutting-edge areas such as drug delivery systems, tissue engineering, and regenerative medicine, where an in-depth understanding and precise regulation of silk protein-expressing genes are of paramount importance.

Applications of Silk Proteins in the Biomedical Field

The widespread application of silk proteins in the biomedical field is rooted in their unique physicochemical properties and exceptional biological functions. These characteristics are largely attributed to advancements in molecular biotechnology, such as genetic engineering, which enable customized modification of silk protein production and functionalization, thereby meeting the diverse needs of biomedical applications. In drug delivery systems, genetically engineered silk proteins can precisely regulate drug release rates and targeting, achieving revolutionary technological breakthroughs. This capability for precise control significantly enhances the efficacy of drug treatment and reduces side effects. Similarly, genetically engineered silk proteins play a pivotal role in tissue engineering and regenerative medicine. By optimizing the structure and function of silk proteins, researchers have successfully developed scaffold materials with excellent biocompatibility, degradability, and mechanical properties, providing robust support for tissue repair and regeneration.

Application of Silk Proteins in Drug Delivery Systems

Genetically engineered silk proteins, serving as drug carriers, exhibit significant potential and important value in drug delivery systems. Through genetic engineering techniques, the structure and functions of silk proteins can be precisely designed and regulated to meet the diverse requirements of drug delivery systems, which include but are not limited to the formation of hydrogel, mats, and scaffolds. Their unique biocompatibility allows silk protein carriers to reduce rejection reactions with biological organisms and improve drug bioavailability. Additionally, the degradability and adjustable degradation rate of silk proteins enable drugs to be slowly released over a predetermined period, achieving precise control of drug release. These characteristics collectively endow silk protein-based drug delivery systems with important roles in controlling drug release, enhancing drug efficacy, and achieving targeted delivery. In the future, with

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Table 1 Genes Influencing Silk Protein Expression

Gene	Full Name/Description	Functions and Characteristics	Experimental Methods and Findings	References
eIF6	Eukaryotic Initiation Factor 6	Regulation of Silk Protein Expression; Ribosome Anti-Association Factor, Inhibiting the Association of 60S and 40S Subunits; Indirect Regulation of Protein Transmembrane Transport	Utilizing CRISPR/Cas9 technology to construct eIF6 gene deletion mutants, revealing its inhibitory effects on larval and silk gland development, and impacting cocoon shell ratio	[76]
eIF Family	*eIF2, eIF3f, eIF3s5, eIF4E2, eIF5*	Successful cloning and expression in silkworm; High expression of *eIF5* in silk glands promotes silk protein synthesis	Highlighting the indispensability of *eIFs* in the silk spinning physiological process of silkworms	[76]
BmSLC7A5	Solute Carrier Family 7 Gene (SLC7); Named as SLC7A5 in Silkworm	Involved in the activation of TORC1 signaling pathway and protein translation process; potentially involved in leucine transport	Twelve SLC7 homologous genes were identified, among which *BmSLC7A5* was significantly enriched in the silk gland; influencing silk protein synthesis and larval development; leucine treatment increased silk protein synthesis, whereas knockdown of *BmSLC7A5* decreased it	[77, 78]
BmGMC2	Ecdysone Oxidase	Plays a crucial role in silk production and silk protein synthesis in silkworms	Detecting spatiotemporal expression patterns and obtaining homozygous mutants (K-GMC2) using the CRISPR-Cas9 system; compared to the wild type, K-GMC2 larvae exhibited significantly reduced silk production and major silk protein levels, with decreased adhesion strength of natural silk proteins at the final stage. Proteomics data revealed a significant reduction in ribosomal protein abundance, with enrichment of DEPs (differentially expressed proteins) related to neurodegenerative diseases and genetic information processing, indicating that BmGMC2 gene knockout may lead to cellular stress and affect silk protein synthesis.	[79]
BmEcKL1	Important candidate genes for silk gland development and silk protein synthesis in silkworms	Plays a key role in silk gland development and silk protein synthesis in silkworms	By specifically knocking out the expression of the *BmEcKL1* gene in the middle and posterior silk glands using CRISPR/Cas9 technology, we cultivated Δ*BmEcKL1*-MSG and Δ*BmEcKL1*-PSG strains with improved silk glands and increased silk production.	[80]
BmMBF2	Multiprotein Bridging Factor 2	Key regulatory factor for silk protein synthesis; inhibits fibroin heavy chain; forms bridging complexes with multiple proteins; involved in the regulation of silk protein gene expression	*BmBrC-Z2* directly activates *BmMBF2* to inhibit silk protein synthesis; after specific knockout of *BmMBF2* using CRISPR/Cas9 technology, silk protein gene expression was significantly upregulated.	[81]
BmVps13d	Bombyx mori vacuolar protein sorting-associated protein 13d	Vacuolar Protein Sorting-associated Protein, playing a crucial role in the specific recognition, sorting, and transport of intracellular substances; involved in maintaining the integrity and homeostasis of cells and their organelles, essential for cell growth and development	Highly expressed in the midgut and silk glands; studies using the CRISPR/Cas9 system revealed that mutations in the *BmVps13d* gene led to a significant decrease in larval body weight and reduced silk production in both female and male silkworms.	[82]
Bmdimm	Key regulatory factor for the expression of FibH, a crucial component of silk protein	Directly regulated by the JH-BmMet/BmSRC-BmKr-h1 pathway; highly expressed in the silk glands and also distributed in the fat body	Knockout of *Bmdimm* leads to a shortened larval stage and decreased silk production by inhibiting Bmkr-h1 expression	[83]
P25	*P25* Fibroin Hexamer Protein	One of the components of silkworm silk protein; together with Fib-H and Fib-L, it forms the core of silkworm fibers; plays a crucial role in the silk formation process, contributing to the stability of the fibroin complex and influencing the morphology of fibroin secretion spheres in the PSG lumen.	This finding provides new insights into understanding the molecular mechanisms of silk formation.	[84]
let-7	A Key microRNA	Loss of function prolongs the fifth larval instar, increases wandering stage weight, but leads to developmental arrest during the pupal-to-moth transition.	Employing the CRISPR/Cas9 system to knock out *let-7* significantly promotes silk gland growth and enhances silk production; conditional knockout of *let-7* concurrently stimulates the growth of both the silkworm body and silk glands, substantially increasing silk yield.	[85]

Bmelo l 2	Key enzymes of the very long-chain fatty acid elongase family in the silkworm, Bombyx mori.	Belonging to the very long-chain fatty acid elongase family, it plays a central role in fatty acid metabolism and biosynthesis; not only is it involved in the fatty acid elongation process, but it is also closely related to silk production performance.	Research reveals that *Bmelo l 2* influences the fatty acid composition and content in the silkworm, Bombyx mori, by regulating very long-chain fatty acid synthesis; precise modulation of its enzymatic activity can effectively enhance silk production.	[86]
Bmgsb	Genes playing crucial roles in the transcriptional regulation of silk protein genes and the development of silk glands	Regulating the transcription of silk protein genes; influencing silk gland development.	After knockdown of *Bmgsb*, the expression of fibroin genes (including Fib-L, Fib-H, p25), cellular heat shock response genes, phenoloxidase genes, as well as genes related to silk fibroin and ubiquitin protein hydrolysis enzymes, were significantly upregulated in the anterior and middle sections of the silk gland.	[87]
BmJHBPd2	Belonging to the JH Binding Protein	Has a significant impact on silk synthesis; regulates transcription factors and hormone signaling pathways	Overexpression of *BmJHBPd2* significantly inhibits the upregulation of the key transcription factor BmKr-h1, leading to suppression of the JH signaling pathway and activation of the 20E signaling pathway, which subsequently suppresses silk protein gene expression and reduces silk yield.	[88]
BmSuc l	β-fructofuranosidase-encoding gene	Highly expressed in the midgut and silk gland; involved in the synthesis of silk fibroin; maintains the balance of silk protein content and mechanical properties in silk fibers.	Using the CRISPR/Cas9 system to induce gene mutations in *BmSuc1*, studies have shown that BmSuc1 mutations lead to larval developmental delay, weight loss, and a decrease in the molecular weight of certain sericin proteins in the silk gland. Furthermore, after knocking out the *BmSuc1* gene, the sericin protein content in the silk cocoon of the silkworm is reduced, and the mechanical properties of the silk fibers are also adversely affected.	[89]
Fzr	Genes regulating endoreplication and protein synthesis in the silkworm	Promotes endoreplication and protein synthesis in silkworm PSG cells; regulates the growth and silk production of PSG.	Double transgenic CRISPR/Cas9-mediated *Fzr* mutation in silkworm PSG cells. *Fzr* mutation leads to silk gland growth arrest and reduced silk production, which is associated with disrupted endoreplication processes due to dysregulation of cell cycle proteins and DNA replication-related regulatory factors, as well as inhibited ribosome biogenesis pathways and/or enhanced ubiquitin-mediated protein degradation pathways.	[90]

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in-depth research on the structure and properties of genetically engineered silk proteins, as well as the continuous development of novel preparation techniques, silk protein-based drug delivery systems are expected to be applied and promoted in more fields. These innovative applications will not only enrich the choices of drug delivery systems but also make greater contributions to human health (Figure 5).

(1) Advantages of Silk Proteins as Drug Carriers

Silk proteins, with their excellent human tissue compatibility, can effectively avoid severe immune responses and achieve progressive degradation in the body, thereby significantly reducing the long-term burden on the organism.[91] By precisely controlling their molecular structure, cross-linking degree, and composition with other materials, the degradation rate of silk proteins can be accurately regulated to match the specific release requirements of different drugs.[92] Furthermore, silk proteins exhibit outstanding mechanical properties, including excellent toughness and elasticity, which are crucial for maintaining the structural integrity of drug carriers and ensuring that drugs are not damaged during transport and release.[93]

Based on these unique advantages of silk proteins, researchers such as Song have successfully developed a novel, strong silk protein-based tissue adhesive, PHT-SP-Cu^{2+}, specifically designed for transdermal drug delivery system patches.[94] Experimental results demonstrate that this adhesive performs well in terms of adhesion, drug loading capacity, and drug release efficiency, providing a new and more effective solution for the field of transdermal drug delivery. Particularly noteworthy is that when silk proteins are prepared into micro-needle dressings with specific structures using the Kirigami method, their ductility is significantly improved, and their tensile strength is also significantly enhanced. This characteristic is of utmost importance for adapting to the dynamic changes of wounds and maintaining the integrity of the dressing.[95] Therefore, silk protein-based micro-needle dressings show great application potential in the field of smart wound management. Silk proteins, with their excellent biocompatibility, controllable degradation properties, and superior mechanical performance, have been widely utilized in the fields of drug delivery and wound management, demonstrating significant value.

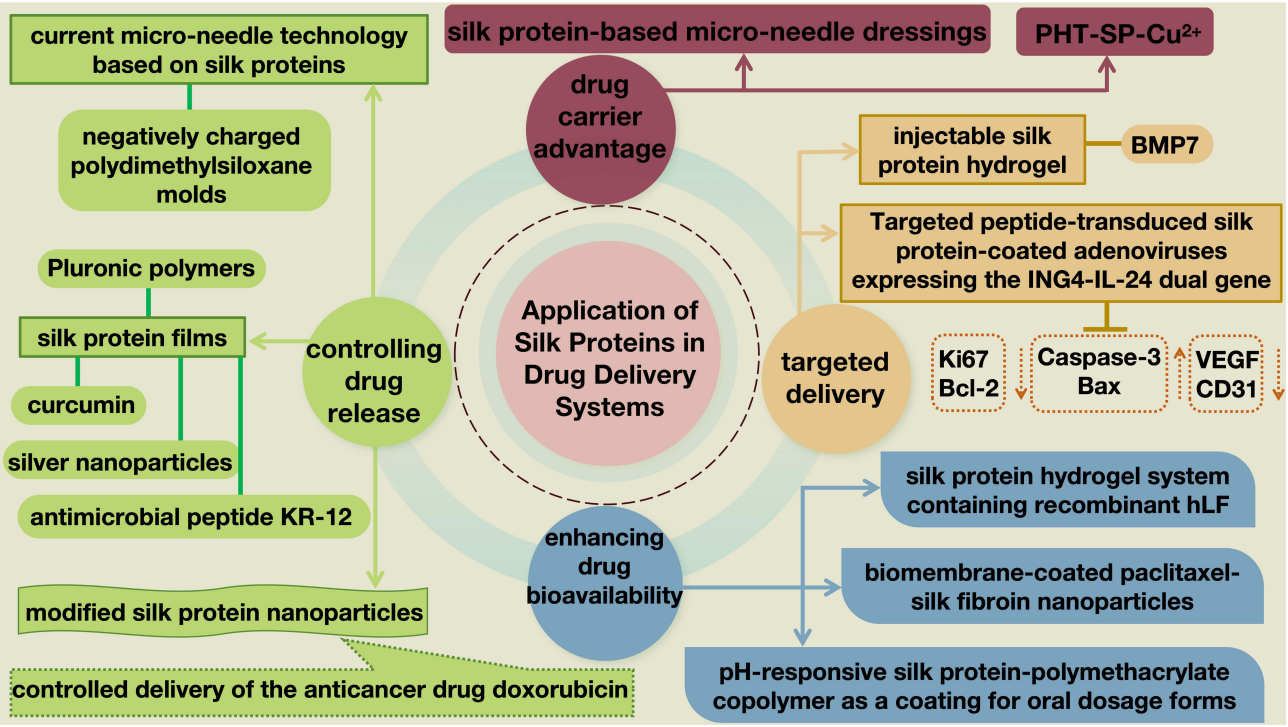

Figure 5 Application Prospects and Advantages of Silk Protein-Based Drug Delivery Systems.

(2) Precision Regulation and Innovative Applications of Silk Protein Drug Delivery Systems

The silk protein-based drug delivery system, with its remarkable ability for sustained and stable drug release, significantly prolongs the duration of drug action and effectively reduces dosing frequency and adverse drug reactions.[96] The core of this system's precise regulation of drug release rates lies in the diverse unique properties exhibited by silk proteins.[97] Specifically, by finely tuning the pore structure and cross-linking density of silk proteins, the diffusion pathways and rates of drugs can be influenced, thereby achieving initial control over drug release. On the other hand, the incorporation of intelligent responsive elements (such as temperature and pH-sensitive groups) into silk proteins enables them to sense changes in the external environment and dynamically adjust drug release behavior accordingly, achieving more precise control.

The controllable characteristics of drug release are of great significance for optimizing therapeutic efficacy and enhancing patients' quality of life.[98] Furthermore, through modification techniques such as doping with Pluronic polymers, not only are the mechanical properties, hydrophilicity, and light transmittance of silk protein films significantly enhanced, but also the sustained release of antibacterial agents (such as curcumin, silver nanoparticles, and the antibacterial peptide KR-12) is successfully achieved, bringing a novel strategy to the field of drug delivery.[99] Additionally, the development of modified silk protein nanoparticles offers new possibilities for controlled delivery of the anticancer drug doxorubicin.[100]

In the field of transdermal drug delivery, microneedle technology based on silk protein (prepared by embedding or mixing silk protein solution in a negative polarity polydimethylsiloxane mold) demonstrates unique advantages. These microneedles not only inherit many of the benefits of silk protein drug delivery systems but also provide painless and efficient solutions for sustained-release drug applications such as reducing scar formation and promoting wound healing.[101,102] In summary, the properties of silk protein and its pivotal role in controlled drug release open up new avenues for optimizing drug therapy, enhancing patients' quality of life, and exhibit tremendous application potential.

(3) Role of Silk Proteins in Enhancing Drug Bioavailability

Drug bioavailability refers to the speed and extent to which a drug is absorbed into the bloodstream by the body.[103] As a drug carrier, silk proteins can enhance drug bioavailability through the following mechanisms: firstly, by encapsulating or adsorbing drugs within the carrier, reducing drug degradation and first-pass effect in the gastrointestinal tract; secondly, by improving drug solubility and stability, increasing drug solubility and biomembrane permeability in the body; and thirdly, by adjusting the size and surface properties of the carrier to optimize drug distribution and targeting in the body.[104]

Studies have confirmed that using pH-responsive silk protein-polymethacrylate copolymer as a coating for oral dosage forms, encapsulating novel silk protein drug formulations in capsules, can effectively resist the acidic gastric environment, ensuring direct delivery of drugs to the intestine and facilitating the release of pancreas-dependent drugs.[105] To address the issue of low bioavailability of paclitaxel, self-assembly technology was used to conjugate paclitaxel with silk fibroin to form nanoparticles, which were further coated with *Escherichia coli* outer membrane vesicles, resulting in biomembrane-coated paclitaxel-silk fibroin nanoparticles that exhibit good antitumor effects and improve the bioavailability of paclitaxel.[106] To tackle the challenge of abundant yet inefficient delivery of iron, this study developed a silk protein hydrogel system containing recombinant hLF. This system aims to mitigate the side effects of chemotherapy drugs on silkworm cocoons carrying hLF, primarily relying on the construction of silk protein hydrogels with uniform porous microstructures dominated by β-sheet morphology.[107] Silk proteins, as drug carriers, enhance drug bioavailability through various mechanisms, providing new ideas and methods for the design and optimization of drug delivery systems.

(4) Role of Silk Proteins in Targeted Delivery

Targeted delivery refers to the precise delivery of drugs to diseased sites, reducing damage and side effects to normal tissues.[108] Silk proteins, with their hydrophobic and hydrophilic domains, can bind hydrophobic therapeutic agents and ligands, enabling targeted drug delivery.[109] Silk protein-based drug delivery systems can achieve targeted delivery through multiple means: firstly, utilizing the inherent bioadhesive properties of silk proteins to adhere drug carriers to specific tissues or cell surfaces; secondly, introducing targeting molecules (such as antibodies, ligands, etc.) onto the surface of silk protein carriers through chemical modification or physical adsorption to achieve active targeting; and

thirdly, combining external stimuli (such as light, magnetism, ultrasound, etc.) to trigger directed movement and drug release from the carriers, enabling remote control and precise delivery.[110]

Leveraging the inherent bioadhesive properties of silk proteins, an injectable silk protein hydrogel has been developed as a carrier for loading BMPs 7.[111] Studies have confirmed that delivering BMP7 directly to adipose tissue through this silk protein scaffold system constitutes a novel strategy aimed at promoting adipose browning and enhancing energy expenditure. This approach indicates a potential therapeutic option for achieving precise delivery of target substances to fat storage sites, exploring new avenues for the treatment of metabolic diseases. Targeted peptide-transduced silk protein-coated adenoviruses expressing the ING4-IL-24 dual gene have been used to achieve targeted gene therapy for lung cancer, effectively inhibiting lung tumor cell proliferation (downregulating Ki67 and Bcl-2), promoting cell apoptosis (upregulating Caspase-3 and Bax), and blocking tumor angiogenesis (downregulating VEGF and CD31).[112] Notably, research has shown that silk protein-based nanoporous microspheres formed by self-assembly of ionic liquids, as a novel drug delivery carrier, exhibit broad application potential, providing new perspectives and possibilities for the field of drug targeted delivery.[113] Therefore, targeted cationic silk protein modification on the surface of adenoviruses has been proven to be an effective strategy for overcoming the natural tropism and in vivo instability of adenoviral vectors, demonstrating clinical application potential.

Innovation of Silk Proteins in Tissue Engineering and Regenerative Medicine

Genetically engineered silk protein, as a naturally occurring polymer material that has undergone precise design and regulation, demonstrates immense potential for application in tissue engineering and regenerative medicine due to its exceptional biocompatibility, degradability, mechanical properties, and good cell affinity. Through genetic engineering techniques, the structure and function of silk protein can be customized to meet the diverse needs for biomaterials in tissue engineering and regenerative medicine. As a biological scaffold material, genetically engineered silk protein can mimic the microenvironment of natural tissues, providing a platform for cell attachment, growth, and differentiation. Its unique three-dimensional structure and porosity facilitate cell migration, nutrient transport, and waste removal, thereby promoting the repair and regeneration of damaged tissues. In particular, genetically engineered silk protein has exhibited outstanding performance in the repair and regeneration of critical tissues such as skin, bone, and cartilage (Figure 6).

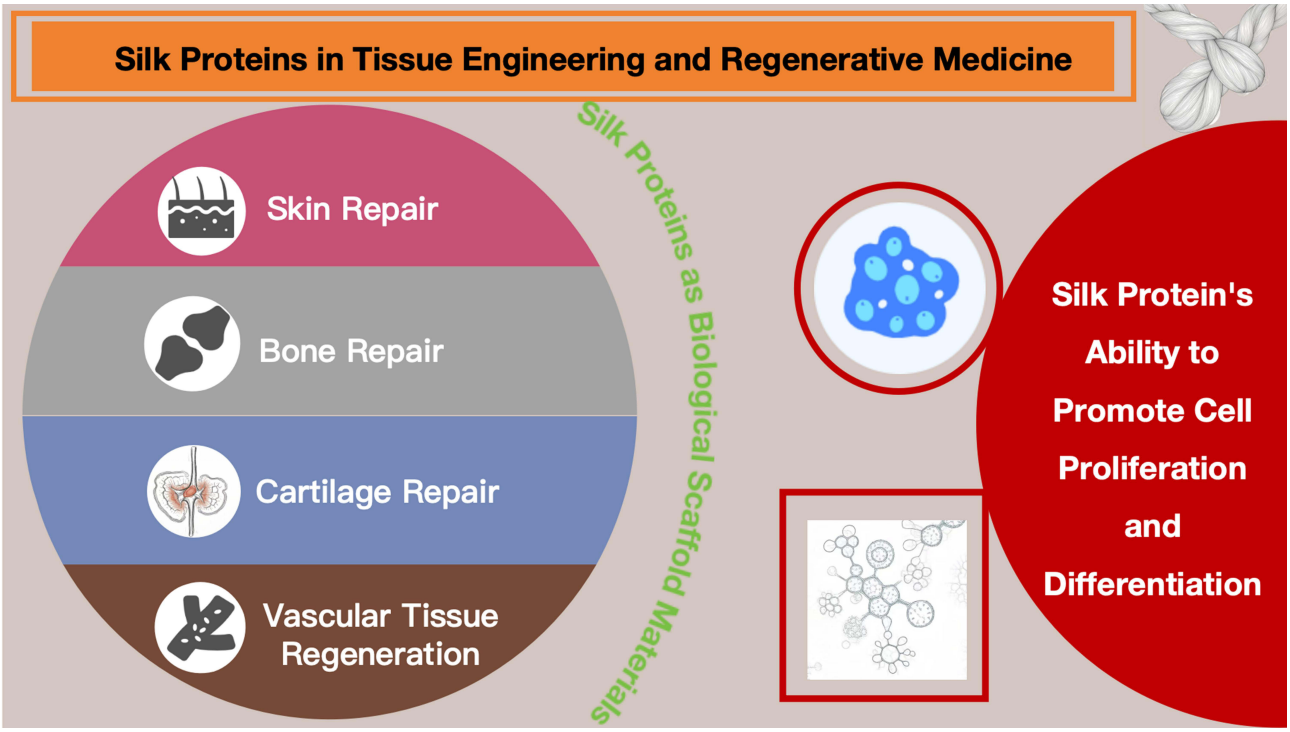

Figure 6 Application of Silk Protein in Biological Scaffold Materials and Its Unique Ability to Promote Cell Proliferation and Differentiation.

Innovative Applications of Silk Proteins as Biological Scaffold Materials

(1) Skin Repair

Silk protein films and fibrous scaffolds, with their excellent breathability and moisture retention properties, constitute an ideal substrate for skin cell growth.[114] Studies have shown that such scaffolds significantly promote skin cell proliferation and migration, thereby accelerating the wound healing process and effectively inhibiting scar formation. Importantly, silk protein scaffolds, as delivery systems, can significantly reduce stress peaks around the wound site when loaded with cytokines, bioactive components, cells, and tissues, not only providing necessary physical support but also optimizing the efficiency of wound care.[115] Specifically, collagen/silk protein composite scaffolds loaded with bone mesenchymal stem cells, with their outstanding skin affinity, breathability, and water permeability, further enhance the potential for wound healing, demonstrating the potential and advantages in the field of regenerative medicine (eg, wound dressings and skin grafting).[116]

Furthermore, research has confirmed that chitosan-based silk protein topical hydrogels exhibit significant efficacy in effectively controlling acute bleeding, showing good therapeutic effects on promoting wound healing and enhancing hemostatic function.[117] Additionally, the antibacterial properties exhibited by silk proteins provide a strong safeguard against wound infection. Studies have demonstrated that near-infrared responsive silk protein films prepared through electrospinning technology possess excellent mechanical properties and blood compatibility. Under near-infrared irradiation, they can rapidly generate reactive oxygen species, effectively killing Staphylococcus aureus, promoting M2 polarization of macrophages, and accelerating wound healing, thus providing a new strategy for the treatment of wound infections.[118] Silk protein films and fibrous scaffolds, with their unique properties, show significant advantages in the field of skin repair, offering innovative solutions for accelerating wound healing, inhibiting scar formation, and preventing infection.

(2) Bone Repair

In the field of bone tissue engineering, silk proteins have shown significant application potential as bone substitute materials and bone-guiding regeneration membranes.[119] Their unique three-dimensional porous structure provides an ideal environment for bone cell attachment, proliferation, and differentiation, and promotes the construction of vascular networks, thereby ensuring that the newly formed bone tissue receives adequate nutrition and oxygen supply.[120] Due to their excellent mechanical strength and biocompatibility, silk proteins have become an ideal material for 3D-printed bone tissue engineering scaffolds. They can promote osteogenic gene expression, collagen accumulation, and mineralization, thereby supporting bone tissue regeneration and modulating related immune responses.[121] Studies have shown that poly-L-lactic acid/silk fibroin composite nanofiber scaffolds, after being coated with ECM from osteoblasts, significantly enhance the osteogenic differentiation ability of bone marrow mesenchymal stem cells.[122]

Another study demonstrated that silk fibroin porous bone scaffolds reinforced with short-cut fibers and nano-HA, prepared by the freeze-drying method, not only optimized mechanical properties but also effectively reduced foreign body reactions, promoted anti-inflammatory responses, and significantly accelerated the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells. This scaffold, through precise regulation of Capns1 expression and calcium signaling pathways, exhibited excellent bone repair and regeneration effects in rat models.[123] Furthermore, the combined application of silk proteins with growth factors such as BMPs further enhances bone repair efficacy, providing new therapeutic strategies in the field of bone tissue engineering. Chitosan/silk protein composite double-layered PCL nanofiber mats combine enhanced antibacterial properties with osteogenic potential, and their osteogenic mechanism involves complexing Runx2 plasmids to enhance the expression of osteogenic-related genes and the formation of mineralized nodules.[124] Silk proteins, with their excellent properties and diverse application forms, exhibit great potential in the field of bone repair and regeneration, offering new materials and therapeutic strategies for bone tissue engineering.

(3) Cartilage Repair

Addressing the unique avascular and low cellular metabolic rate characteristics of cartilage tissue, silk proteins have demonstrated significant repair potential.[125] By precisely controlling mechanical properties and microstructure, silk protein scaffolds have successfully mimicked the natural cartilage matrix, effectively guiding chondrocyte proliferation

and differentiation, and promoting cartilage regeneration. Further studies have shown that silk protein hydrogel scaffolds combined with chitosan nanoparticles, through precise regulation of the release of TGF-β1 and BMP-2, exhibit excellent biocompatibility and significantly enhance the chondrogenic capacity of bone marrow stromal cells, providing an efficient strategy for the treatment of knee joint cartilage defects.[126]

Additionally, silk proteins serve as drug delivery platforms, carrying anti-inflammatory and repair factors, which is expected to further optimize repair effects. Specifically, silk fibroin scaffolds delivering tanshinone IIA (10 μg/mL) significantly promote cartilage matrix synthesis and regeneration by activating chondrocyte activity, inhibiting apoptosis, and reducing oxidative stress, showing strong potential and good biomechanical properties in the repair of articular cartilage defects.[127] Besides tanshinone IIA, silk proteins can also carry other drugs, such as etanercept and methylprednisolone.[128,129] The latest research confirms that the silk protein-gelatin-chondroitin sulfate-hyaluronic acid-aloe vera composite three-dimensional scaffold, with its high porosity, large pore size, excellent water absorbency, and mechanical strength, combined with anti-inflammatory properties, significantly promotes the proliferation and chondrogenic differentiation of bone marrow mesenchymal stem cells and effectively inhibits IL-1β expression, making it an ideal choice for cartilage tissue engineering.[130] Therefore, silk proteins, through their unique properties and diverse application strategies, exhibit significant potential in the field of cartilage repair, providing new solutions for cartilage tissue engineering.

(4) Vascular Tissue Regeneration

Leveraging its excellent biocompatibility and structural tunability, combined with biological cue strategies, silk protein has significantly optimized the growth, adhesion, survival, and proliferation of vascular tissue cells.[131] This characteristic positions silk protein as a highly effective and safe material with potential applications in the field of vascular repair and regeneration, providing a solid foundation for the development of vascular tissue engineering.[132] Studies have confirmed that silk protein/fibroin composite vascular scaffolds prepared using electrospinning technology exhibit outstanding mechanical properties, hydrophilicity, blood compatibility, biodegradability, and cell compatibility. Most importantly, they enhance the proliferation and adhesion of mesenchymal stem cells, making them an ideal candidate material for artificial blood vessels in vascular tissue engineering.[133]

Research has demonstrated that small-diameter silk protein artificial blood vessel implants exhibit high patency rates, good endothelial cell coverage, and remodeling capabilities after implantation in dogs, validating their clinical application potential as vascular substitutes for diameters less than 6mm.[134] Another innovative design—a double-layered microneedle composed of chitosan and a silk protein complex containing the angiogenic drug deferoxamine—significantly promotes angiogenesis, accelerates wound contraction, and effectively promotes cell proliferation and fibroblast migration by modulating cytokine expression, showing remarkable wound healing promotion effects.[135] Silk protein gels loaded with thymoproteasome-10 and ZIF-8 also exhibit good ability to promote angiogenesis and bone regeneration.[136] Through its excellent properties and diverse application strategies, silk protein shows great potential in the field of vascular tissue regeneration, offering new materials and methods for the development of vascular tissue engineering.

Silk Protein's Ability to Promote Cell Proliferation and Differentiation

The significance of silk protein in tissue engineering is primarily attributed to its notable ability to promote cell proliferation and differentiation.[137] This characteristic is largely due to the similarity between silk protein and ECM components, allowing it to mimic the natural tissue microenvironment and provide necessary signals and support for cells.[138] For instance, a biomimetic nanofiber scaffold combining natural silk protein nanofibers with glycosaminoglycan hyaluronic acid and a silk fibroin-based interpenetrating network hydrogel for corneal stroma regeneration fully leverage this property of silk protein.[139,140]

Furthermore, silk protein can further enhance its regulatory effects on cell behavior by adjusting its surface properties, chemical modifications, or binding with other biologically active molecules. For example, by introducing growth factors, cytokines, or drug molecules, stem cells can be induced to differentiate in a specific direction, accelerating the tissue repair and regeneration process. Studies have shown that a 3D-printed silk fibroin/collagen/HA bioscaffold loaded with

recombinant human erythropoietin, used for bone defect repair, exhibits excellent in vitro mechanical properties, and MC3T3-E1 cells easily adhere and proliferate on it.[141]

Additionally, metformin-loaded silk protein scaffolds with multi-scale, multi-layered anisotropic micro-nano composite topological structures can upregulate the expression of genes related to Schwann cell proliferation, adhesion, migration, and myelin sheath formation, effectively repairing damaged neural tissue.[142] Scholars have also confirmed that injectable silk protein/MXene conductive hydrogels combined with electrical stimulation promote the differentiation of neural stem cells into neurons, significantly enhancing axon growth density and length while effectively inhibiting their differentiation into undesirable astrocytes.[143]

Other research includes hydrogels based on recombinant spider silk protein that can stimulate the proliferation and migration of human corneal cells.[144] Silk protein occupies a central position in tissue engineering. Its characteristic of promoting cell proliferation and differentiation provides a solid biological foundation for tissue repair and regeneration processes, and demonstrates significant advantages and practicality.

Discussion on Alternative or Competitive Methods

In the biomedical field, besides silk protein-based materials, there exist various biomaterial-based approaches for applications such as drug delivery, tissue engineering, and regenerative medicine. These alternative or competitive methods each possess unique characteristics, and compared to silk protein-based materials, they exhibit both advantages and limitations.

Applications of Other Biomaterials

In the biomedical field, a variety of natural and synthetic biomaterials have been extensively and deeply studied, and have been applied in various medical practices. Natural biomaterials include collagen and hyaluronic acid, while PLGA belongs to synthetic biomaterials. These materials each possess unique characteristics, providing a wealth of options for fields such as drug delivery systems, tissue engineering, and regenerative medicine. In drug delivery systems, they exhibit good biocompatibility and controllable degradability. In tissue engineering and regenerative medicine, they are also commonly used as biological scaffold materials to promote cell adhesion, proliferation, and differentiation.

Collagen, as the most abundant protein in the human body, is widely utilized in the biomedical field due to its excellent biocompatibility, degradability, and cellular affinity.[145] In drug delivery systems, collagen serves as a carrier material, stabilizing drugs and controlling their release rates. In the fields of tissue engineering and regenerative medicine, collagen is commonly used as a biological scaffold material, providing a substrate for cell adhesion and growth, and promoting tissue repair and regeneration.

Hyaluronic acid is a natural high-molecular-weight polysaccharide with excellent water retention and biocompatibility.[146] In drug delivery systems, hyaluronic acid can serve as an adhesion enhancer, improving the stability and bioavailability of drugs in vivo. Additionally, it functions as a scaffold material in tissue engineering, providing cells with a moist microenvironment conducive to growth and differentiation.

PLGA is a biodegradable polymer synthesized through the copolymerization of lactic acid and glycolic acid.[147] It exhibits a controllable degradation rate and excellent mechanical properties, making it one of the commonly used synthetic materials in the biomedical field. In drug delivery systems, PLGA can be utilized in various carrier forms such as microspheres and nanoparticles for achieving sustained-release and targeted delivery of drugs. In the realm of tissue engineering and regenerative medicine, PLGA serves as a scaffold material, providing support and guidance to cells, and promoting tissue regeneration and repair.

Comparison of Advantages with Silk Protein-Based Materials

(1) Silk Protein

Silk protein, as a biological polymer material derived from nature, has received widespread attention due to its unique physical, chemical, and biological properties. In the fields of biomedicine and tissue engineering, silk protein has demonstrated exceptional performance.[148]

Silk protein possesses excellent mechanical strength, toughness, and elasticity, which enable it to withstand complex physiological environments and maintain a stable structure in vivo. Compared with other biomaterials, silk protein exhibits superior mechanical properties, meeting the demands of various tissues and organs for material strength.

Silk protein exhibits excellent biocompatibility with human tissues, without eliciting significant immune responses or rejection reactions. It supports cell adhesion, growth, and differentiation, providing an ideal microenvironment for tissue repair and regeneration. This biocompatibility endows silk protein with broad application potential in the biomedical field.

The editability of silk protein is another significant advantage. Through genetic engineering techniques, novel silk proteins with specific properties can be customized, such as altering their degradation rates, mechanical properties, or biological activities. This capability for customization offers more possibilities and flexibility for the application of silk protein in the biomedical field.

Silk protein demonstrates unique advantages over other biomaterials in terms of mechanical properties, biocompatibility, and editability. These attributes position silk protein as a promising candidate in biomedical and tissue engineering applications, including the fabrication of bioscaffolds, drug delivery carriers, and artificial organs.

(2) Other Biomaterials

Collagen and hyaluronic acid, due to their similarity to human tissue components, exhibit excellent biocompatibility and low immunogenicity. Synthetic materials such as PLGA possess controllable degradation rates and processing properties. Biomaterials like collagen, hyaluronic acid, and PLGA each demonstrate unique advantages in the biomedical field.[149] However, compared to silk protein-based materials, they may have certain limitations in some aspects. Therefore, when selecting biomaterials, comprehensive consideration and assessment are required based on specific application scenarios and patient needs.

Comparison with Disadvantages of Silk Protein-Based Materials

(1) Silk Protein

Despite its numerous advantages, silk protein is relatively high in production costs, and its preparation process may involve complex biotechnological procedures.[150] Furthermore, certain properties of silk protein, such as degradation rate, may need to be optimized according to specific applications.

(2) Other Biomaterials

Natural materials such as collagen and hyaluronic acid have limited sources and are expensive. Synthetic materials like PLGA may produce acidic products during degradation, affecting the microenvironment of surrounding tissues. Both natural materials such as collagen and hyaluronic acid, and synthetic materials like PLGA, exhibit certain disadvantages in their respective application fields.[151,152] These disadvantages become more prominent when compared to silk protein-based materials, necessitating sufficient attention and resolution during research and development.

The Potential of Silk Protein in Antibacterial and Immunomodulatory Applications

With the increasing severity of global public health issues, research on antibacterial and immunomodulatory materials has become a focal point in the scientific community. Silk protein, a biomacromolecular material derived from nature, plays a significant role in the fields of antibacterial and immune regulation due to its unique biological activity, good biocompatibility, and processability, demonstrating potent efficacy and advantages. Next, we will explore new findings and application potentials of silk protein and its modified products in antibacterial and immunomodulatory aspects, aiming to provide new ideas and directions for research and applications in related fields (Figure 7).

Antibacterial Properties of Silk Protein

(1) Natural Antibacterial Activity

Silk protein itself does not directly exhibit potent broad-spectrum antibacterial activity, but its unique fibrous structure and surface properties open avenues for functionalization with antibacterial properties.[153] The closely packed

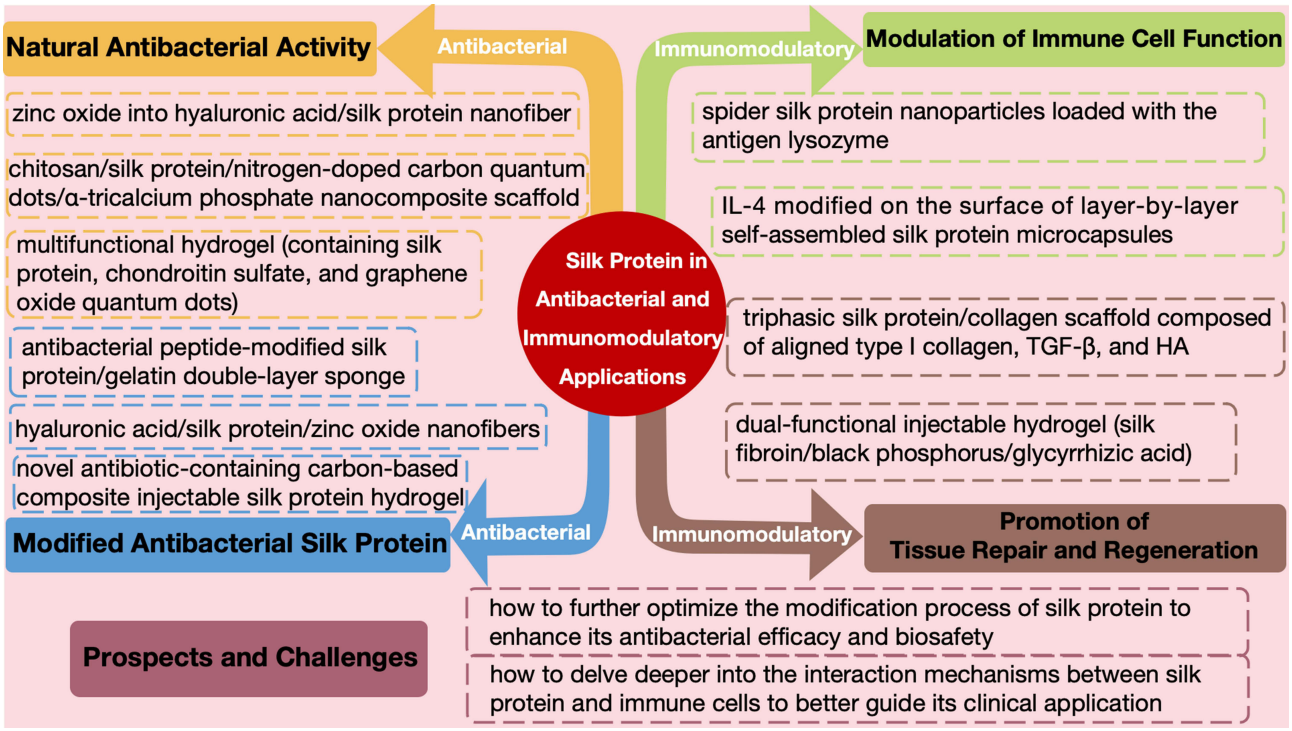

Figure 7 New Findings and Application Potentials of Silk Protein and Its Modified Products in the Fields of Antibacterial and Immune Regulation.

arrangement of silk protein fibers forms an effective physical barrier, hindering microbial attachment and invasion.[154] However, it is important to note that silk fibers are multifilamentary. Compared to monofilaments, the increased surface area and a wicking effect, to some extent, enhance bacterial adhesion. Therefore, when utilizing silk proteins for antibacterial functionalization, it is necessary to comprehensively consider the advantages and disadvantages associated with its fibrous structure.

Studies have shown that incorporating zinc oxide into hyaluronic acid/silk protein nanofiber wound dressings can enhance antibacterial efficacy; however, caution is needed regarding the potential cytotoxic effects of high concentrations of zinc oxide to ensure the safety and effectiveness of the material application.[155] Another study demonstrated a chitosan/silk protein/nitrogen-doped carbon quantum dots/α-tricalcium phosphate nanocomposite scaffold prepared by electrospinning, which exhibited minimum inhibitory concentrations against *Escherichia coli* and *Staphylococcus aureus*, excellent biocompatibility, and the ability to promote cell migration and proliferation. In vivo experiments further verified its significant efficacy in accelerating wound healing and promoting epithelial cell regeneration, providing a new type of high-efficiency material for the field of wound healing and skin tissue regeneration.[156]

Interestingly, addressing the challenge of chronic wounds combined with bacterial infections, researchers have successfully developed a multifunctional hydrogel dressing with both antibacterial and angiogenic properties (containing silk protein, chondroitin sulfate, and graphene oxide quantum dots). It exhibits excellent antibacterial performance and can effectively promote the differentiation of endothelial cells into H-type blood vessels, thereby accelerating the regeneration process of infected skin defects.[157] Although silk protein itself does not directly possess potent broad-spectrum antibacterial activity, through combination with other antibacterial components or functional modifications, it demonstrates significant application potential in the field of antibacterial materials, providing new solutions for wound care and tissue regeneration.

(2) Modified Antibacterial Silk Protein

Chemical modifications (eg, cross-linking and immobilization of the amino group of Actinomycin X2 with the carboxyl group of silk protein via carbodiimide reaction), physical modifications (eg, embedding antibacterial agents into silk protein materials through solution casting or soaking methods), and antibacterial material composite strategies have

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significantly enhanced the antibacterial efficacy of silk protein, while also promoting wound repair and optimizing the wound microenvironment.[158–160] Through molecular grafting of antibacterial peptides, zinc metal ions, and antibacterial polymers, silk protein has achieved efficient inhibition against a broad spectrum of bacteria and fungi.[161] Recent research has verified that a 32 μg/mL antibacterial peptide-modified silk protein/gelatin double-layer sponge exhibits excellent mechanical properties, high water absorption, a reasonable biodegradation rate, and controlled release performance without cytotoxicity, while demonstrating strong antibacterial activity against various bacteria (including drug-resistant bacteria), indicating its broad prospects as a wound dressing for promoting the healing of infected wounds; however, further in vivo studies are needed for comprehensive evaluation.[162]

Interestingly, a study on hyaluronic acid/silk protein/zinc oxide nanofibers pointed out that the addition of zinc oxide enhanced antibacterial performance, but caution is required regarding the potential cytotoxicity of high concentrations of zinc oxide. Furthermore, anchoring silk protein at a concentration of 8 mg/mL onto the surface of polyurethane scaffolds effectively inhibited the growth of Klebsiella pneumoniae, further highlighting its outstanding potential as an antibacterial material.[163] Additional research has shown that a novel antibiotic-containing carbon-based composite injectable silk protein hydrogel dressing not only exhibits excellent antibacterial performance and good cell compatibility but also integrates multiple functions such as sustained drug release, antioxidant properties, and self-healing, synergistically promoting rapid healing of burn wounds, specifically manifested as reduced wound contraction, increased collagen deposition, thickened granulation tissue, and accelerated angiogenesis.[164] Through various strategies such as chemical modification, physical modification, and antibacterial material composites, the antibacterial efficacy of silk protein has been significantly improved, while also promoting wound repair and optimizing the wound microenvironment, demonstrating its wide application prospects in the field of antibacterial materials.

Application of Silk Protein in Immune Regulation

(1) Modulation of Immune Cell Function

Silk protein and its modified products can influence the activity, differentiation, and function of immune cells, thereby regulating the immune response of the body.[165] For example, certain silk protein derivatives can promote the phagocytic activity of macrophages, enhancing the non-specific immune capacity of the organism; while others may inhibit excessive activation of T-cells, alleviating symptoms of autoimmune diseases.[166] Research has shown that spider silk protein nanoparticles loaded with the antigen lysozyme, as a novel protein delivery system, not only exhibit high-efficiency delivery and improved delivery efficacy but also significantly enhance antigen-specific immune responses in mouse models.[167] This enhanced effect is attributed to the depot effect of the antigen at the injection site, long-term persistence, efficient uptake by dendritic cells, and internalization in lymph nodes. Studies have confirmed that IL-4 modified on the surface of layer-by-layer self-assembled silk protein microcapsules can regulate the polarization of macrophages towards the M2 type, thereby promoting cartilage matrix repair and inhibiting inflammatory responses.[168] Through modulating the activity and function of immune cells, silk protein and its modified products demonstrate significant potential in immune regulation and disease treatment, providing new ideas and methods for the biomedical field.

(2) Promotion of Tissue Repair and Regeneration

In the processes of tissue injury and inflammation, silk protein can promote the repair and regeneration of damaged tissues by regulating immune cell behavior and secreted factors.[169] This mechanism not only facilitates accelerated wound healing but also may exhibit positive effects on the treatment of chronic inflammatory diseases and the promotion of tissue regeneration.[170] Studies have confirmed that the triphasic silk protein/collagen scaffold prepared by Geng et al, composed of aligned type I collagen, TGF-β, and HA, demonstrates good biocompatibility and can promote tendon formation. In vivo studies have shown that after implantation of this scaffold loaded with rat tendon-derived stem cells, tendon tissue regeneration occurred with the formation of a clear transition zone, highlighting its significant potential in the field of tendon-to-bone junction regeneration.[171] Furthermore, scholars have verified through research that by incorporating black phosphorus and glycyrrhizic acid into methacrylate-modified silk fibroin, a dual-functional injectable hydrogel (silk fibroin/black phosphorus/glycyrrhizic acid) was

successfully prepared.[172] This hydrogel possesses both suitable electrical conductivity and anti-inflammatory efficacy, effectively alleviating oxidative damage, optimizing the inflammatory microenvironment, and promoting the differentiation of neural stem cells and the recovery of neural signal transmission. By regulating immune cell behavior and secreted factors, silk protein demonstrates wide application potential in tissue repair and regeneration, as well as in the treatment of chronic inflammatory diseases, providing new materials and strategies for the development of the biomedical field.

Application Prospects and Challenges

Silk protein holds great potential in the fields of antibacterial and immune regulation, but its practical application still faces some challenges.[173] For instance, how to further optimize the modification process of silk protein to enhance its antibacterial efficacy and biosafety; and how to delve deeper into the interaction mechanisms between silk protein and immune cells to better guide its clinical application. With the continuous advancement of science and technology and the deepening of interdisciplinary research, it is believed that these issues will gradually be resolved, and silk protein will play a more significant role in the fields of antibacterial and immune regulation.

Prospects

Compared with the existing literature on silk protein genetic engineering, the specific contribution of this review lies in systematically examining the roles of major model organisms such as Bombyx mori and spiders in silk protein production, and delving into the latest applications of gene editing technologies (particularly CRISPR-Cas9), transgenic expression techniques, and synthetic biology in silk protein genetic engineering. Through comprehensive analysis, this review not only emphasizes the innovative applications of silk proteins in drug delivery systems, tissue engineering and regenerative medicine, as well as antibacterial and immune strategies, but also highlights the innovative aspects and distinctions within these applications.

Molecular biotechnology, particularly the continuous innovation and application of gene editing, transgenic expression, and synthetic biology, has significantly propelled the advancement of silk protein gene engineering. Specifically, CRISPR-based gene editing technology has achieved remarkable progress in silk protein gene engineering, opening up new avenues for the optimization and customization of silk protein properties. Furthermore, the outstanding performance of silk protein in innovative applications such as antibacterial wound dressings fully demonstrates its immense potential and value in the biomedical field.

With the continuous maturation and refinement of technology, the performance of silk protein will be further enhanced, and its application fields will continue to expand. Notably, research in silk protein gene engineering has surpassed the traditional scope of molecular biology, achieving deep integration with materials science, chemical engineering, computer science, and other disciplines. This interdisciplinary fusion trend introduces new perspectives and methodologies into silk protein research, particularly in fields such as regenerative endodontics. For instance, silk protein scaffolds incorporating graphene oxide and reduced graphene oxide can promote the differentiation of human dental pulp stem cells at the genetic level, enhance the expression of crucial osteogenic/odontogenic markers, and support ECM mineralization. These innovative applications further demonstrate the versatility of silk protein.[174]

However, despite the significant achievements, the field still faces several key challenges, such as ethical issues and scalability, which require more attention in future research. To enhance the impact of silk protein gene engineering research, we also need to continuously explore alternative or competitive approaches, for instance, the performance of other biomaterial-based methods in similar applications, in order to enrich and refine the knowledge system in this field.

Looking ahead, silk protein gene engineering research will delve deeper, and its industrial application prospects will broaden further. However, simultaneously, we need to continuously address and resolve new challenges and issues, particularly in terms of ethics and scalability. Through sustained innovation and interdisciplinary collaboration, we are hopeful of overcoming these challenges, further advancing silk protein gene engineering, and making greater contributions to human health and sustainable development. (The complete abbreviations are shown in Table 2).

Table 2 List of Abbreviations (According to Alphabetical Order)

Abbreviations	Full Name
ASG	Anterior silk gland
BmMBF2	Multiprotein bridging factor 2
BMPs	Bone Morphogenetic Proteins
Cdc	Cell division cycle
CDKs	Cyclin-dependent kinase
CKIs	Cyclin-dependent kinase inhibitors
CNTF	Ciliary Neurotrophic Factor
ECM	Extracellular matrix
ECP3	Eggcase protein 3
eIF6	Eukaryotic initiation factor 6
Fib-H	Fibroin heavy chain
Fib-L	Fibroin light chain
HA	Hydroxyapatite
hLF	Human lactoferrin
IDRs	Intrinsically disordered regions
JH	Juvenile Hormone
MaSp	Major ampullate spidroin 1
MSG	Middle silk gland
PLGA	Poly(lactic-co-glycolic acid)
PSG	Posterior silk gland
PySp1	Pyriform spidroin 1
SCR	Sex comb reduced
SLC7	Solute Carrier Family 7
TALENs	Transcription activator-like effector nucleases
TGF-β	Transforming growth factor β
TuSp1	Tubuliform spidroin 1

Consent for Publication

All authors have given their consent for the publication of this review article. The content of this article has not been published or submitted for publication elsewhere.

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Disclosure

The authors declare no competing interests in this work.

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