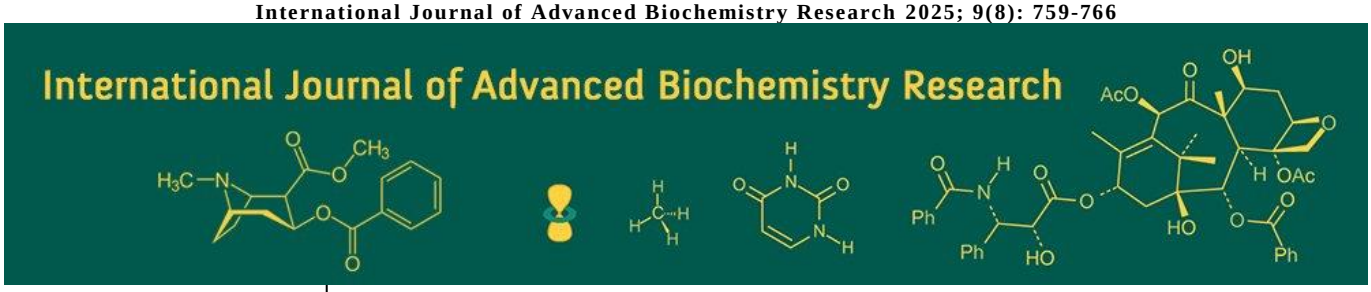

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(8): 759-766
www.biochemjournal.com
Received: 02-06-2025
Accepted: 06-07-2025

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Biomimetic silk production: Advances in synthetic silk gland models and *in vitro* spinning systems

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i8j.5309

Abstract
Silk has long been revered as a natural fiber with exceptional mechanical strength, elasticity, biodegradability, and biocompatibility, making it highly desirable for diverse applications ranging from textiles to biomedical engineering. However, traditional silk production through sericulture is labour-intensive, season-dependent and environmentally demanding. In response to these limitations, scientific efforts have increasingly focused on replicating the silk production process *in vitro* through synthetic silk gland models that emulate the physiological and biochemical mechanisms of native silk glands in *Bombyx mori* and spiders. This review provides a comprehensive overview of the biomimetic strategies employed to develop artificial silk-spinning systems, including microfluidic devices that replicate the gland's tapering structure and fluid shear environment and modular bioreactors that integrate protein expression with spinning. It also discusses innovations in wet-spinning, electrospinning and advanced spinneret technologies such as multi-lumen nozzles and 3D-printed extrusion systems that have enabled the fabrication of silk fibers with properties approaching those of natural silk. The role of recombinant protein expression in microbial hosts like *E. coli*, *Pichia pastoris*, and *Bacillus subtilis* is explored, highlighting advances in the scalable production of spidroins and fibroins tailored for biomimetic processing. Challenges such as achieving native-like hierarchical structures, improving fibre uniformity and mechanical properties and replicating the controlled chemical gradients of the natural gland are critically analyzed. Overall, synthetic silk gland technologies hold immense promise for revolutionizing silk production by offering eco-friendly, controllable and scalable alternatives to sericulture. These systems not only pave the way for high-performance artificial silk fibers but also establish a platform for producing customizable biomaterials for future use in tissue engineering, drug delivery, bioelectronics, and smart textiles.

Keywords: Synthetic silk gland models, *Bombyx mori*, Spider silk, biomimetic silk production, recombinant spidroins, microfluidic devices, electrospinning, tissue engineering, smart textiles

1. Introduction
Silk obtained from the domesticated silkworm (*Bombyx mori*) has earned global recognition for its outstanding physical and biochemical properties, including high tensile strength, flexibility, and superior biocompatibility. These attributes make silk a valuable material not only in textiles but also in medical, cosmetic, and bioengineering industries (Altman *et al*., 2003; Kundu *et al*., 2012) [1, 15]. The silk fiber primarily consists of two proteins fibroin, which forms the core structural filament, and sericin, a glue-like protein that binds the fibers. Despite its benefits, conventional silk production *via* sericulture presents several limitations. The process is labor-intensive, time-consuming and susceptible to climatic variability, pest outbreaks and disease occurrences. Moreover, ethical concerns regarding the killing of silkworm pupae during cocoon processing have prompted the search for more sustainable and animal-friendly alternatives (Sehnal & Zurovec, 2004; Vepari & Kaplan, 2007) [19, 26]. To address these concerns, recent research has focused on the development of synthetic silk gland systems that emulate the structure and function of natural silk glands. These systems aim to reproduce the process of silk spinning in a laboratory setting, eliminating the dependency on live silkworms. The silk gland in *B. mori* consists of a highly organized tri-sectional structure posterior, middle and anterior glands each contributing specific roles in the synthesis, modification and solidification of silk proteins (Jin & Kaplan, 2003) [12]. Reproducing these intricate physiological and biochemical processes artificially is a

significant challenge. Nonetheless, advancements in synthetic biology, protein engineering and fluid dynamics have enabled the creation of artificial spinning devices and microfluidic systems that mimic the silk-spinning mechanism. These synthetic platforms are designed to simulate critical factors such as shear stress, pH gradient, ionic environment, and molecular alignment key elements in converting liquid silk dope into solid fibers (Koeppel & Holland, 2017; Andersson *et al.*, 2017) [13, 2]. Moreover, recombinant DNA technologies have made it possible to produce silk-like proteins in microbial hosts, offering a scalable and eco-friendly alternative to sericulture (Heidebrecht & Scheibel, 2013) [6].

The advent of synthetic silk gland models marks a promising frontier in biomaterials research. These biomimetic systems have potential applications in diverse fields, from biodegradable textiles to implantable medical devices and smart materials. By replicating the natural silk production pathway *in vitro*, they offer a sustainable and ethically sound platform for next generation silk fiber development (Guo *et al.*, 2020; Zhang *et al*., 2023) [4, 33].

2. Structure and Function of Natural Silk Glands

The silk gland of the silkworm (*Bombyx mori*) is a highly specialized organ that facilitates the synthesis, storage and extrusion of silk proteins in a precisely coordinated manner. This gland, extending nearly the entire length of the larva's body, is divided into three distinct but interconnected regions: the posterior silk gland (PSG), middle silk gland (MSG) and anterior silk gland (ASG). Each region plays a unique biochemical and physiological role in the production of silk fibers.

2.1 Posterior Silk Gland (PSG)

The posterior region of the silk gland is primarily responsible for the synthesis of **fibroin**, the main structural protein of silk. Fibroin is composed of two polypeptide chains a heavy chain and a light chain linked by disulfide bonds and further stabilized by an associated glycoprotein, P25. These three components assemble in a specific molar ratio, forming a high-molecular-weight complex that is secreted into the gland lumen. The PSG maintains the fibroin solution in a soluble state at high concentrations without premature aggregation, a task made possible by the tightly regulated intracellular environment.

2.2 Middle Silk Gland (MSG)

In the middle section of the silk gland, sericin proteins are synthesized and secreted. Sericin acts as a natural adhesive, enveloping the fibroin filament and enabling the binding of individual fibers during cocoon formation. Apart from its adhesive role, the MSG contributes to maintaining the fluid properties of the silk solution, influencing its pH, ion concentration, and overall rheology. These adjustments prepare the protein solution for its transition into solid fibers as it progresses toward the anterior section.

2.3 Anterior Silk Gland (ASG)

The anterior portion of the silk gland is a narrow duct through which the silk solution passes before being extruded through the spinneret. As the fibroin-sericin complex travels through the ASG, it experiences a range of mechanical and chemical stimuli. These include decreasing pH, ion exchange, increasing shear force and water removal, all of which promote molecular alignment and the transition of silk proteins from random coils to organized β-sheet structures. This phase transition is crucial for the formation of solid, mechanically strong silk fibers.

2.4 Significance for Biomimetic Applications

Understanding the anatomical and functional features of the natural silk gland is essential for designing synthetic silk-spinning systems. Artificial models must replicate the gradual changes in chemical composition, fluid dynamics, and protein structure that occur throughout the silk gland. Mimicking these parameters is fundamental to producing silk fibers with comparable strength, elasticity and durability to those produced biologically.

3. Challenges in Mimicking Silk Gland Physiology

Replicating the silk-spinning process of *Bombyx mori in vitro* is a significant scientific and engineering challenge. The natural silk gland is a complex, highly efficient biological system where the conversion of a protein-rich liquid (silk dope) into a solid fiber occurs under ambient conditions, without the use of harsh chemicals or extreme temperatures. This remarkable transformation is orchestrated by a delicate interplay of biochemical gradients, mechanical forces, and molecular alignment, which current synthetic models struggle to replicate fully (Jin & Kaplan, 2003; Guo *et al*., 2020) [12, 4].

3.1 Control of Protein Solubility and Aggregation

In the posterior silk gland, fibroin proteins are stored at very high concentrations (up to 30% w/v) in a metastable, soluble state. Maintaining this level of concentration without premature aggregation requires a precise ionic and pH environment. In synthetic systems, preventing unwanted aggregation or gelation of silk proteins before fiber formation is a major obstacle. Achieving the correct molecular conformation and maintaining protein solubility during storage and processing are essential for successful spinning (Holland *et al.*, 2012) [8].

3.2 Simulating Biochemical Gradients

The transformation of silk dope into a solid fiber is triggered by gradual changes in pH, ion concentration, and hydration along the silk gland. In particular, the shift from a neutral to slightly acidic environment, combined with an increase in potassium and calcium ions, promotes the formation of β-sheet structures. Recreating these precise gradients in a synthetic setting requires advanced microfluidic or bioreactor systems with finely tuned delivery mechanisms, which are technically challenging and often cost-prohibitive (Askarieh *et al*., 2010; Guo *et al*., 2020) [3, 4].

3.3 Shear-Induced Molecular Alignment

One of the most critical factors in natural silk spinning is the application of shear and elongational stress as the protein solution passes through the narrowing anterior silk gland. These mechanical forces help align fibroin molecules in the direction of flow, encouraging ordered β-sheet crystallization that gives silk its exceptional mechanical properties. *In vitro* systems must replicate this flow-induced alignment using precise control over channel geometry and

flow rates, a task that is still under active investigation (Jin & Kaplan, 2003; Koeppel & Holland, 2017) [12, 13].

3.4 Recombinant Protein Expression and Yield

The production of silk-like proteins using microbial expression systems—such as *E. coli*, yeast, or insect cells faces limitations in yield, molecular weight, and post-translational modifications. Native fibroin proteins are very large (over 300 kDa) and their repetitive sequences often lead to instability or low expression in heterologous systems. Synthetic biology has made progress in producing chimeric or truncated spidroins, but matching the quality and performance of native silk remains difficult (Andersson *et al*., 2017; Heidebrecht & Scheibel, 2013) [2, 6].

3.5 Fiber Spinning and Post-Processing

Even if the protein solution is successfully generated and stabilized, the final step spinning continuous, mechanically robust fibers poses another set of challenges. Artificial spinning techniques such as wet spinning, dry spinning and electrospinning require solvents or coagulation baths, many of which do not mimic the benign aqueous conditions of natural silk spinning. Achieving fibers with similar toughness, extensibility and uniformity to natural silk requires optimization of spinning parameters, post-spinning stretching, and sometimes chemical treatments, all of which complicate scalability (Numata & Kaplan, 2010) [18].

4. Synthetic Silk Gland Models: Design and Technologies

The quest to replicate the natural silk-spinning process has led to the development of synthetic silk gland models, which aim to simulate the physiological, biochemical and mechanical environment of the native silk gland. These engineered systems are designed to enable the *in vitro* production of silk fibers by mimicking the sequence of events that occur inside a silkworm's gland from protein synthesis and storage to molecular alignment and final fiber extrusion. The success of these models depends on how effectively they reproduce the key structural and functional features of the biological gland, including gradient-driven transitions and flow-induced self-assembly.

4.1 Microfluidic Silk Spinning Systems

One of the most widely explored technologies for mimicking silk glands is microfluidics. These systems use micron-scale channels to replicate the narrowing geometry of the anterior silk gland, where silk proteins align and transition into fibers. By carefully controlling fluid flow, shear stress, and pH gradients, microfluidic devices can reproduce the directional alignment of proteins, which is essential for achieving fiber strength and elasticity (Koeppel & Holland, 2017) [13]. Some microfluidic devices also incorporate salt and ion gradients (e.g., potassium, phosphate, and calcium ions) to induce β-sheet formation and promote solidification of the silk dope (Guo *et al.*, 2020) [4].

4.2 Biomimetic Bioreactors

In addition to microfluidics, biomimetic spinning reactors have been developed to facilitate continuous fiber formation. These bioreactors are engineered to maintain a controlled environment similar to the natural silk gland, including appropriate temperature, pressure, ionic strength, and protein concentration. For example, Kojic *et al.* (2018) [14] designed a biomimetic wet-spinning reactor that successfully mimicked silk gland conditions, while Xia *et al.* (2021) [29] developed a continuous bioreactor system using engineered *Bacillus subtilis* to produce fibers. Although more scalable than microfluidic setups, these systems often require post-spinning modifications to improve fibre quality.

4.3 Artificial Spinneret Designs

Some research groups have explored artificial spinnerets modelled after the morphology of silkworm or spider spinnerets. These spinnerets are fabricated using advanced materials and precision engineering to create narrow, converging channels that impose shear and extensional flow on the protein solution. Teulé *et al.* (2012) [22] demonstrated that recombinant silk proteins extruded through artificial spinnerets could form fibers, although they often required coagulation baths or solvent exchange processes to solidify the fibers, depending on the spinning technique used (e.g., wet spinning, dry spinning).

4.4 Use of 3D Printing and Soft Lithography

Emerging technologies such as 3D printing and soft lithography have enabled the fabrication of customized silk gland analogs with intricate internal geometries. Heidebrecht and Scheibel (2013) highlighted how soft lithography and related fabrication techniques can be used to design flow channels that precisely control residence time, turbulence, and gradient application, thereby enhancing the fidelity of synthetic spinning processes.3D-printed silk spinning units also offer the advantage of rapid prototyping and adaptability, making them suitable for experimental optimization of different silk types and spinning conditions.

4.5 Integration with Recombinant Protein Systems

All synthetic gland models rely on the availability of recombinant silk proteins, typically produced in microbial hosts. Andersson *et al.* (2017) [2]. demonstrated that engineered chimeric spidroins could be expressed in microbial systems and processed into fibers with improved spinnability, while Tian *et al.* (2021) [24]. reported the design of functionalized silk proteins with novel bioactive properties, expanding the potential applications of synthetic fibers. When these recombinant proteins are introduced into artificial glands under controlled flow and chemical conditions, they can undergo a similar self-assembly process as observed in nature, although fiber quality and consistency still require improvement.

5. Recombinant Silk Protein Production and Expression Platforms

The efficient production of silk proteins is a critical prerequisite for the development of synthetic silk gland systems. Natural extraction from *Bombyx mori* or spiders presents limitations due to low yield, labor intensity, and animal dependence. Consequently, researchers have turned to recombinant DNA technology to biosynthesize silk proteins in heterologous hosts, enabling scalable, controllable, and ethical production. These recombinant systems aim to replicate the native sequence, solubility, and

self-assembly behavior of fibroin or spidroin proteins, which are central to the silk fiber's mechanical properties.

5.1 Choice of Expression Systems

A variety of expression platforms have been explored to produce silk proteins, each with distinct advantages and challenges.

- **Bacterial systems (*Escherichia coli*)** are frequently used due to their rapid growth and low-cost cultivation. However, the repetitive and high-molecular-weight nature of silk genes often causes plasmid instability and protein degradation in prokaryotes (Teulé *et al*., 2012) [22-23]. Moreover, post-translational modifications essential for proper folding and solubility are absent in bacterial systems.
- **Yeast systems (*Pichia pastoris*)** offer eukaryotic processing machinery and higher yields than bacteria. They can handle moderately repetitive sequences and perform glycosylation, although not always in the native form (Huemmerich *et al*., 2004) [9, 10].
- **Insect cell lines** such as those based on baculovirus vectors, have demonstrated high expression efficiency and proper folding of silk proteins. These systems are particularly useful for producing spider silk proteins with high molecular weights, although they are more expensive and time-consuming (Winkler *et al*., 2000) [28].
- **Transgenic plants** such as tobacco and potato, have been engineered to express silk proteins. While scalable and safe, plant systems face challenges related to purification, yield variability, and codon optimization (Menassa *et al.*, 2004) [17].
- **Transgenic animals**, including goats and silkworms, have been developed to secrete silk proteins in milk or cocoon fibers, respectively. Though capable of high-yield production, these systems raise ethical and regulatory concerns (Xu *et al.*, 2007) [31].

5.2 Optimization of Gene Design

Due to the highly repetitive nature of silk genes, synthetic gene design is essential for stable expression. Techniques such as codon optimization, removal of unstable motifs and modular construction using shorter tandem repeats are commonly employed. In particular, mini-spidroin genes encoding only the terminal and repetitive core domains have shown promise in enhancing solubility and yield (Andersson *et al*., 2017) [2]. Fusion with solubility tags (e.g., MBP, GST) and use of secretion signals also improves expression in both microbial and eukaryotic systems.

5.3 Purification and Refolding

Once expressed, recombinant silk proteins must be purified and refolded into their native-like conformation to be suitable for spinning. This typically involves chromatographic purification (e.g., affinity, ion exchange), followed by dialysis, pH adjustment and controlled concentration to mimic the silk dope environment. Maintaining solubility during this phase is crucial as premature aggregation can hinder fiber formation.

5.4 Functionalized and Engineered Silk Proteins

Recent advancements have enabled the creation of engineered silk proteins with novel functionalities, such as cell-binding motifs, antimicrobial peptides, or stimuli-responsive elements. These designer proteins can be produced recombinantly and integrated into synthetic spinning systems, offering tailored mechanical and biological properties for medical or textile applications (Tian *et al.*, 2021) [24].

6. Challenges in Mimicking the Natural Silk Spinning Process

Replicating the natural silk-spinning process outside a living organism presents formidable scientific and engineering challenges. The native silk gland, particularly in *Bombyx mori* and spiders, functions as a highly specialized biological microreactor. It transforms a concentrated protein solution (known as silk dope) into solid fibers through a finely controlled physiological and biochemical process. The *in vitro* replication of this system demands precise control over multiple parameters such as protein concentration, shear stress, pH, ion gradients, and mechanical pulling, all of which are tightly regulated *in vivo*.

6.1 Protein Solubility and Stability

One of the primary hurdles in synthetic silk spinning is maintaining the solubility of silk proteins at high concentrations. In nature, fibroin or spidroin proteins are stored in the silk gland at concentrations exceeding 20-30% (w/v), yet remain remarkably soluble due to their amphiphilic domains and controlled ionic environment (Jin & Kaplan, 2003) [12]. *In vitro*, recombinant proteins tend to aggregate or precipitate prematurely, making it difficult to maintain a metastable dope suitable for fibre formation (Slotta *et al.*, 2007) [20] Stabilizing additives and optimized pH buffers have been tested, but results often lack reproducibility and scalability.

6.2 Shear-Induced Structural Transitions

In native silk glands, the transition from soluble to insoluble protein involves shear-induced alignment of protein chains and conformational switching, particularly from random coils to β-sheet structures (Jin & Kaplan, 2003) [12]. Artificially reproducing this mechanical trigger is complex. Bioreactors and microfluidic channels have been designed to apply shear stress or elongational flow to mimic the glandular architecture, but they often fail to recreate the precise orientation and alignment necessary for strong fibre formation.

6.3 pH and Ionic Gradient Regulation

The physiological silk spinning duct maintains a distinct pH gradient (from ~7.6 to 5.7 in *Bombyx mori*) along with ion gradients of potassium, calcium, and phosphate. These gradients regulate the conformational transformation of proteins and fibre assembly (Askarieh *et al.*, 2010; Hagn *et al*., 2011) [3, 5]. Replicating these conditions synthetically requires precise microenvironmental control that is challenging in current *in vitro* systems. Any deviation in pH

or ion concentration can lead to incomplete β-sheet formation or unstable fibre morphology (Guo *et al*., 2020) [4]

6.4 Flow Channel Geometry and Device Design

The geometry of the natural silk gland tapers from a storage sac to a narrow duct, gradually increasing shear and aligning protein chains. Mimicking this geometry in synthetic devices involves complex microfabrication techniques. Attempts using capillaries, tapered microtubes, and electrospinning nozzles have yielded fibers with varying degrees of success, but few match the structural regularity and strength of native silk (Koeppel & Holland, 2017; Heidebrecht & Scheibel, 2013) [13, 6].

6.5 Post-Spinning Processing and Fiber Properties

In natural systems, post-spinning steps such as water removal, fibre stretching, and chemical stabilization are inherently built into the process. *In vitro*, these steps must be recreated through drying, post-drawing, or crosslinking. Inadequate post-processing often results in brittle or non-uniform fibers. Achieving mechanical properties similar to native silk such as tensile strength >500 MPa and toughness >150 MJ/m³ remains a key challenge (Numata & Kaplan, 2010; Vollrath & Knight, 2001) [18, 27].

7. Recent Advances in Biomimetic Silk-Spinning Devices and Bioreactor Systems

The development of biomimetic silk-spinning devices and bioreactor systems marks a transformative phase in the quest for scalable *in vitro* silk production. These innovations aim to replicate the intricate structural, biochemical, and mechanical conditions of the natural silk gland, which are essential for producing high-performance silk fibers. Recent advances, integrating synthetic biology, microfluidics, materials science, and bioprocess engineering, have enabled significant strides in replicating natural silk spinning mechanisms.

7.1 Microfluidic Spinning Systems

Microfluidic platforms offer a highly controlled environment to simulate the geometry and physicochemical gradients present in the native silk gland. These systems are capable of reproducing shear stress, pH gradients, and ion exchange critical for silk protein alignment and β-sheet formation. Heidebrecht and Scheibel (2013) [6] designed a microfluidic chip using a layered flow channel system that enabled the spinning of recombinant spider silk proteins into continuous fibers. By manipulating flow rates and channel geometry, they successfully induced structural transitions necessary for fiber assembly under near-physiological conditions.

7.2 Bioinspired Wet-Spinning Bioreactors

Wet-spinning technologies, inspired by natural silk extrusion processes, utilize controlled coagulation environments to solidify silk dope into fibers. These systems often incorporate gradients of pH and salts to mimic the spinning duct of spiders or silkworms. Kojic *et al*. (2018) [14] reported a wet-spinning bioreactor that employed recombinant *MaSp1* proteins and incorporated ion exchange mechanisms, enabling the formation of robust, elastic silk fibers with enhanced mechanical uniformity. The introduction of controlled draw ratios and extended spinning channels helped in achieving better alignment and improved crystallinity.

7.3 Modular Bioreactors for Scalable Production

To address the challenge of scalability, modular bioreactors have been engineered to combine upstream expression and downstream fiber formation processes. Xia *et al*. (2021) [29] developed a continuous flow bioreactor system using genetically engineered *Bacillus subtilis* to express silk proteins. Their bioreactor maintained a stable environment with controlled shear forces and temperature, facilitating the extrusion of silk fibers with desirable properties. Such systems offer a promising route to industrial-scale synthetic silk production.

7.4 Electrospinning and Nanofiber Technology

Although not a direct biomimicry of the silk gland, electrospinning is widely used to fabricate silk-based nanofibers. This method uses electrostatic force to draw silk protein solutions into ultrafine fibers. Zarkoob *et al*. (2004) [32] demonstrated that electrospun silk nanofibers, while lacking the crystalline alignment of natural silk, showed high surface area and customizable porosity, making them suitable for applications in wound dressings and tissue scaffolds. Advances such as coaxial electrospinning and patterned collectors have helped improve fiber orientation and mechanical strength.

7.5 Multi-Lumen Spinnerets and 3D Printing

Recent innovations in 3D printing and microfabrication have enabled the development of multi-lumen spinnerets capable of mimicking the complex morphology of silk extrusion organs. These spinnerets allow for the simultaneous extrusion of different silk proteins or additives, resulting in core-shell or composite fiber architectures. Teulé *et al.* (2012) [22-23] constructed a multi-channel spinning nozzle that successfully combined different recombinant spider silk proteins, resulting in fibers with customizable tensile properties and enhanced toughness. Such advances point toward customizable synthetic silk architectures with multifunctional applications.

8. Comparative Analysis of Synthetic vs. Natural Silk Fibers

Synthetic silk fibers produced through biomimetic approaches have drawn increasing interest due to their potential to replicate the exceptional mechanical and biophysical properties of natural silk. However, despite advances in recombinant protein expression and fiber spinning technologies, significant differences still exist between synthetic and native silk fibers, particularly in terms of structural hierarchy, molecular alignment, and mechanical performance.

8.1 Structural Properties

Natural silk, especially that from *Bombyx mori* and orb-weaving spiders, exhibits a highly organized hierarchical structure comprising crystalline β-sheet domains interspersed with amorphous regions. This nanoscale

organization contributes to the outstanding tensile strength and toughness of the silk fiber (Altman *et al*., 2003) [1]. In contrast, synthetic fibers often exhibit lower β-sheet crystallinity and irregular alignment due to challenges in replicating the physiological spinning environment (Humenik & Scheibel, 2018) [11].

8.2 Mechanical Performance
The mechanical behavior of silk fibers is determined by factors such as tensile strength, elasticity, and toughness. Native *Bombyx mori* silk typically exhibits tensile strength ranging from 300 to 600 MPa, while spider dragline silk can exceed 1000 MPa with remarkable extensibility (Vollrath & Knight, 2001) [27]. Biomimetically spun synthetic silk, although promising, generally achieves lower tensile strength and elasticity, often due to inadequate protein orientation and incomplete mimicry of spinning duct conditions (Teulé *et al*., 2012; Kojic *et al*., 2018) [22-23, 14].

8.3 Protein Composition and Post-Translational Modifications
Natural silk proteins, such as fibroin and spidroins, are synthesized with precise amino acid sequences and undergo co- and post-translational modifications during secretion and fiber formation. Recombinant proteins used in synthetic silk production often lack such modifications or are expressed in heterologous systems that cannot fully replicate the native processing environment (Lewis, 2006) [16]. This results in fibers with inferior folding, self-assembly, and mechanical properties.

8.4 Environmental and Processing Conditions
One key difference lies in the spinning process itself. Natural silk is spun at ambient temperature and pressure using water as a solvent, making it a highly sustainable process. Synthetic spinning often involves organic solvents or artificial coagulation baths, which can introduce defects or alter the molecular conformation of the protein (Jin & Kaplan, 2003) [12]. However, recent efforts in aqueous spinning and microfluidic systems have helped bridge this gap (Huemmerich *et al.*, 2004) [9, 10].

8.5 Biocompatibility and Functional Performance
Both natural and synthetic silks exhibit biocompatibility, which has led to their exploration in biomedical applications. Nonetheless, natural silk generally demonstrates superior functional performance *in vivo*, attributed to its native structure and processing (Kundu *et al.*, 2012) [15]. Synthetic silk's performance can be enhanced through composite formation, surface modification, or blending with bioactive molecules.

9. Future Perspectives and Challenges in Synthetic Silk Production
Despite significant advancements in the biomimetic synthesis of silk, several scientific, technological and industrial challenges remain before artificial silk production can fully replace or complement natural sericulture. Future directions focus on enhancing protein yield, improving fiber quality, reducing production cost, and achieving industrial scalability, while also exploring new functional applications of synthetic silk.

9.1 Enhancing Recombinant Protein Yield and Quality
One of the primary bottlenecks in synthetic silk production is the limited yield and quality of recombinant spidroins or fibroins. Current microbial expression systems, such as *Escherichia coli*, *Pichia pastoris*, and *Bacillus subtilis*, often struggle with the production of high-molecular-weight repetitive proteins due to translational stalling or proteolytic degradation (Teulé *et al*., 2009) [21]. Advances in metabolic engineering and codon optimization, as well as the development of novel host organisms (e.g., transgenic silkworms or plants), offer promising avenues to overcome these limitations (Xu *et al*., 2021) [30].

9.2 Replicating Native Silk Gland Conditions
Fully mimicking the complex microenvironment of the silk gland characterized by shear flow, pH and ionic gradients, and dehydration is essential to achieve native-like fiber properties. Although microfluidics and bioinspired bioreactors have advanced significantly, replicating the dynamic and compartmentalized nature of the native spinning duct remains challenging (Holland *et al*., 2012) [7]. Future research is expected to focus on integrating sensor technologies and AI-based feedback systems to achieve better process control and precision.

9.3 Fiber Functionalization and Customization
Synthetic silk offers the unique advantage of molecular-level customization. Functional groups, bioactive peptides, or hybrid polymers can be incorporated into recombinant silk proteins to yield fibers with desired properties such as antimicrobial activity, enhanced cell adhesion, or drug delivery capabilities (Tokareva *et al*., 2014) [25]. This functionalization opens up new opportunities in biomedical engineering, smart textiles and responsive materials.

9.4 Economic and Environmental Sustainability
A major challenge remains the economic feasibility of synthetic silk at an industrial scale. The costs associated with recombinant protein production, purification, and fiber spinning are currently higher than those of traditional silk production. However, continuous bioprocessing, use of low-cost feedstocks, and renewable energy-powered systems could make artificial silk more sustainable and cost-effective in the future (Xia *et al*., 2021) [29].

9.5 Regulatory and Market Acceptance
For biomedical and textile applications, synthetic silk must meet stringent regulatory standards for safety, biodegradability, and performance. Standardization of production protocols, quality control metrics, and regulatory approval pathways will be critical for market acceptance. Public perception and ethical considerations related to genetically modified organisms (GMOs) used in silk production may also influence future adoption.

10. Conclusion
The quest to replicate nature's silk-spinning prowess through synthetic gland models and biomimetic systems represents a transformative frontier in materials science and biotechnology. Mimicking the intricate processes of silk protein synthesis, alignment, and extrusion *in vitro* not only enables the production of high-performance fibers but also

offers a sustainable and customizable alternative to conventional sericulture.

Progress in microfluidics, modular bioreactors, and wet-spinning techniques has brought researchers closer to reproducing the complex physicochemical environment of the native silk gland. Coupled with advances in genetic engineering and protein expression systems, it is now feasible to generate recombinant silk proteins with controlled molecular weights and tailored functional groups. This convergence of synthetic biology, mechanical engineering, and material design has opened up new possibilities for producing artificial silk with desirable mechanical, biological, and chemical properties.

Nevertheless, significant challenges remain particularly in scaling up production, improving fiber mechanical properties, and reducing costs. The full replication of natural silk's hierarchical architecture and spinning environment is still under development. Moreover, regulatory and environmental considerations will play a key role in determining the industrial viability and acceptance of these technologies.

Looking forward, interdisciplinary collaborations and emerging tools such as AI-guided process optimization, smart bioreactor systems, and CRISPR-based host engineering are expected to drive the next generation of synthetic silk platforms. Ultimately, biomimetic silk production holds enormous promise for revolutionizing multiple industries from regenerative medicine and sutures to high-performance textiles and biodegradable composites heralding a new era of eco-friendly and functionally advanced biomaterials.

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