

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(8): 1501-1506
www.biochemjournal.com
Received: 02-05-2024
Accepted: 06-06-2024

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Transgenic silkworms producing functionalized silk with medical applications: A comprehensive review

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i8s.3777>

Abstract

Silk, traditionally prized for its biocompatibility, strength, and versatility, has gained significant attention in the field of biomedical applications. Recent advancements in genetic engineering have led to the development of transgenic silkworms capable of producing functionalized silk with enhanced properties, making it suitable for a variety of medical applications. These innovations leverage the unique ability of silkworms to spin silk fibers and modify the protein structure to incorporate functional biomolecules such as antimicrobial peptides, growth factors, and anticancer agents. This review explores the state-of-the-art progress in creating transgenic silkworms that produce functionalized silk for medical applications. We focus on the genetic modifications that enable these advancements, the properties of the resulting silk, and the potential uses in wound healing, drug delivery, tissue engineering, and regenerative medicine. Furthermore, challenges related to scalability, safety, and regulatory approval are discussed, along with future directions for further development in this promising field.

Keywords: Silk, transgenic silkworms, biomedical applications, genetic engineering, functionalized silk, medicine

Introduction

Silk has long been valued in both traditional textiles and modern biomedical applications, owing to its remarkable biocompatibility, biodegradability, and mechanical properties. Its versatility as a biomaterial is particularly apparent in applications such as tissue engineering, wound healing, drug delivery, and regenerative medicine. Traditionally, silk has been produced by the domesticated silkworm (*Bombyx mori*), which naturally spins high-quality fibres primarily composed of fibroin proteins. However, there is considerable potential to enhance the medical utility of silk by engineering its properties to meet specific biomedical needs.

Silk fibres, once primarily used in textiles, have demonstrated significant advantages for biomedical purposes. These include biocompatibility (Meinel *et al.*, 2005) [17], material versatility (Shao and Vollrath, 2002) [24], mechanical robustness (Tansil *et al.*, 2012) [27], and controllable degradability (Lawrence *et al.*, 2009) [14]. These properties have made silk an ideal material for a variety of medical applications, including surgical sutures (Altman *et al.*, 2003) [2], vascular grafts (Nakazawa *et al.*, 2011) [19], drug delivery systems, and tissue engineering scaffolds (Tao *et al.*, 2012) [28]. Sericin, a by-product previously discarded during the degumming process, has emerged as a promising material in biological applications. Its properties include biocompatibility, UV protection, antibacterial activity, antioxidant effects, anti-tyrosinase activity, and coagulant and moisturising capabilities (Aramwit *et al.*, 2012) [5]. Notably, sericin has been shown to stimulate cell migration by promoting c-Jun phosphorylation (Martinez-Mora *et al.*, 2012) [15] and has been proven to be non-inflammatory (Aramwit *et al.*, 2009; Panilaitis *et al.*, 2003) [4, 23]. Furthermore, sericin can form hydrogels, gel films, and sponges, making it suitable for wound dressing applications (Teramoto *et al.*, 2008; Nishida *et al.*, 2010) [29, 22]. When combined with other polymers, sericin can form membranes or scaffolds with enhanced properties, offering potential for use in cosmetics and pharmaceuticals (Aramwit *et al.*, 2012; Akturk *et al.*, 2011) [5, 1].

Moreover, sericin can also be used to improve the functional properties of both natural and synthetic fibres, fabrics, and materials (Aramwit *et al.*, 2012; Zhang, 2002) [5, 39]. Expanding the use of silk materials for biomedical purposes will largely depend on the ability to engineer silk proteins with specific biological functions.

Advances in genetic engineering have enabled the generation of transgenic silkworms, particularly through the use of the piggyBac transposon from the lepidopteran species *Trichoplusia ni*. This system has become a valuable tool for both fundamental and applied research into silk production (Tamura *et al.*, 2000; Xia *et al.*, 2014) [26, 37]. Several transgenic expression systems, which utilise promoters such as fib-H, fib-L, P25, and sericin 1, have been established to produce exogenous proteins in the silk of genetically modified silkworms (Wang *et al.*, 2013; Tomita, 2011) [35, 31]. These advances allow for the creation of silk with enhanced mechanical properties and novel biofunctionalities. For instance, a recombinant silk spun by transgenic silkworms, combining silk and spider silk proteins, was found to be significantly tougher than natural silkworm fibres (Teule *et al.*, 2012) [30]. In another example, transgenic silkworms have been engineered to produce coloured fluorescent silk, useful for textile applications, through the fusion expression of fluorescent proteins with fibroin (Iizuka *et al.*, 2013) [12, . Additionally, a fusion protein combining fibroin L chain with human basic fibroblast growth factor (bFGF) was shown to have biological activity, making it a promising material for tissue engineering after refolding via the glutathione redox system (Hino *et al.*, 2006) [10]. Furthermore, silk films containing collagen or fibronectin-derived peptides, produced by transgenic silkworms, demonstrated improved cell-adhesive properties (Yanagisawa *et al.*, 2007) [38]. These genetically engineered silk materials have greatly expanded the potential applications of silk in biotechnological fields.

Recent advances in genetic modification have made it possible to develop transgenic silkworms that produce silk fibres with added bioactive molecules. These modified fibres retain the structural properties of natural silk but also offer enhanced functionalities, such as antimicrobial, anticancer, and growth-promoting properties. As a result, functionalised silk is increasingly being explored for a range of medical applications. This review aims to highlight the development and potential of transgenic silkworms producing silk for medical uses, focusing on the genetic techniques employed, the functional properties of the silk, and its various applications in the field of biomedicine.

Genetic Modifications of Silkworms for Silk Functionalization

1. Genetic Engineering of Silkworms

Genetic modification of silkworms has significantly advanced the production of silk with tailored functionalities. This process typically involves inserting foreign genes into the silkworm genome, enabling the expression of specific proteins within the silk fibres. Various techniques, such as transposon-based systems, viral vectors, and CRISPR/Cas9 gene-editing technology, are commonly employed for this purpose. The most widespread method involves introducing genes encoding bioactive molecules directly into the silk-producing cells (spinning glands) of the silkworm. For instance, antimicrobial peptides (AMPs) like defensins, lysozyme, and cecropins can be incorporated into fibroin

proteins, granting the silk antimicrobial properties. Similarly, genes encoding growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic proteins (BMPs) have been successfully introduced to promote cell proliferation and tissue regeneration, making the modified silk highly suitable for applications in wound healing and tissue engineering.

2. Advancements with CRISPR/Cas9 for Precision Functionalization

The advent of CRISPR/Cas9 gene-editing technology has dramatically enhanced the precision and efficiency of genetic modifications in silkworms. This technique allows for the direct modification of the silkworm genome, enabling the integration or knockout of specific genes that influence silk production and quality. CRISPR/Cas9 has been employed to engineer silkworms that produce silk with enhanced mechanical strength, elasticity, and bioactivity. For example, silkworms that express collagen and elastin in their silk have been developed, producing bioactive fibres more suitable for tissue engineering. Additionally, CRISPR/Cas9 facilitates the fine-tuning of silk composition, enabling the incorporation of multiple bioactive components into a single silk strand. Such multi-functional silk can be precisely tailored for various medical applications, including controlled drug delivery and tissue regeneration.

3. Development of Recombinant Silk for Biomedical Applications

Wang *et al.*, 2013^[35] produced an advanced silk material by incorporating the recombinant human fibroblast growth factor (hFGF1) specifically within the sericin layer of silk. This genetically engineered silk is designed to be directly applicable as a biomedical material, and can be easily produced through large-scale breeding of transgenic silkworms. We constructed a transgenic silkworm, designated hSFSV, which could efficiently synthesise recombinant hFGF1 proteins using a modified sericin 1 expression system. The recombinant protein was successfully expressed in the silkworm's major silk gland cells (MSG) and secreted into the lumen of the gland, where it was spun into the sericin layer of silk. The expression of recombinant hFGF1 was confirmed by SDS-PAGE, with an estimated yield of 20 ng μL^{-1} of the total cocoon protein and 0.07% of the cocoon shell weight. These results suggest that the modified sericin1 expression system is an efficient tool for the recombinant production of proteins within the silk gland.

4. Mechanical and Biological Properties of Recombinant Silk

Scanning electron microscopy (SEM) and stress tests were conducted to assess the mechanical properties of the hSFSV raw silk and the impact of the foreign hFGF1 protein on its structure. The hSFSV silk exhibited a similar substructural morphology to wild-type (WT) silk. However, slight alterations in the diameter and mechanical properties were observed, likely due to the incorporation of the recombinant hFGF1 protein into the sericin layer. These changes are consistent with findings from previous studies where the presence of foreign proteins, such as fluorescent proteins, in the fibroin or sericin layers of silk affected the mechanical properties (Iizuka *et al.*, 2013; Ware and Simons, 1997) [12, 36]. Moreover, the hSFSV raw silk significantly promoted the growth and proliferation of NIH/3T3 cells, both with and

without the addition of heparin. This suggests that the sericin layer may be an ideal environment for maintaining the biological activity of recombinant hFGF1 over extended periods, potentially stabilised by heparin analogues present in the silk. No diffusion of hFGF1 from the sericin layer into the culture medium was observed, indicating that the recombinant protein may be tightly immobilised within the silk's meshwork structure, preventing its release into the medium during culture periods. Similar findings were reported in a previous study, where a genetically engineered fibroin protein fused with human bFGF was immobilised on polystyrene and retained at the surface, promoting cell growth without releasing the protein into the medium (Hino *et al.*, 2006) ^[10].

5. Large-Scale Production and Future Directions

Transgenic silkworms can be easily maintained and reared on a large scale once the transgenic line is established, facilitating the efficient production of functional silk materials. Future work will focus on the development of strategies for reeling the hSFSV silk without compromising the bioactivity of the recombinant hFGF1 protein, to enable the production of various biomedical materials. This genetically engineered silk, with recombinant hFGF1 incorporated into the sericin layer, holds great potential for tissue engineering applications, such as wound dressing. Additionally, the methods used here are broadly applicable to the synthesis of other functional recombinant proteins in the sericin layer of silk, providing new insights into creating silks with diverse biological functions and potential applications (Wang *et al.*, 2014) ^[33].

Properties of Functionalized Silk

1. Mechanical Properties

Silk is widely recognised for its exceptional mechanical properties, including tensile strength, elasticity, and flexibility, which make it an ideal material for use in biomedical devices. Through genetic modification, these properties can be further enhanced, tailoring the silk for specific medical applications. For example, silk can be engineered to possess increased strength for sutures or wound dressings, providing durability and support during healing. Conversely, silk can be made more flexible to suit soft tissue engineering, where pliability and resilience are essential for integrating with natural tissues.

2. Biocompatibility and Biodegradability

A key reason for silk's popularity in medical applications is its excellent biocompatibility with human tissues. Silk proteins facilitate cell adhesion and proliferation while minimising inflammatory responses, making them ideal for use in regenerative medicine and tissue engineering. Furthermore, silk is biodegradable, which is a critical attribute for scaffold materials, as they need to degrade gradually as new tissue forms. Functionalisation of silk can enhance these properties by incorporating growth factors or peptides that promote healing and tissue regeneration. Additionally, the rate of degradation can be precisely controlled, allowing silk to break down at a desired pace that matches the needs of the medical application.

3. Antimicrobial Properties

Functionalised silk, produced by transgenic silkworms, can be embedded with antimicrobial peptides (AMPs) to offer protection against bacteria and fungi, making it highly

effective for wound healing applications. The incorporation of AMPs into silk fibres provides localised antimicrobial action at the wound site, which helps prevent infection and accelerates the healing process. Bioengineered silks, functionalised with peptides such as human neutrophil defensin 2 (HNP-2), human neutrophil defensin 4 (HNP-4), and hepcidin, have shown significant antimicrobial activity against common pathogens like *Escherichia coli* and *Staphylococcus aureus* (Gomes *et al.*, 2011) ^[9]. These modified silks not only retain their self-assembly properties but also demonstrate minimal cytotoxicity, making them safe for use in biomedical applications. *in vivo* studies have indicated that silk films containing hepcidin elicit a mild immunological response, comparable to standard biomaterials, supporting their potential in clinical applications such as vascular grafts and tissue engineering scaffolds (Gomes *et al.*, 2012) ^[8]. Beyond biomedicine, antimicrobial silk could also have applications in cosmetics and food packaging, where infection prevention and preservation are critical.

4. Drug Delivery Systems

Silk functionalisation also opens up new opportunities for drug delivery. Silk fibroin can be engineered to encapsulate therapeutic agents, such as anticancer drugs or antibiotics, and release them in a controlled manner. This ability to modulate drug release rates allows for sustained therapeutic effects over extended periods, making silk-based systems promising for chronic wound treatment, cancer therapy, and other conditions that require continuous drug delivery. For instance, silk spheres functionalised with poly-lysine peptides have been successfully used to load nucleic acids, such as CpG-siRNA, providing efficient delivery and protection from serum degradation. These silk spheres not only protect the drug from early degradation but also ensure its sustained release into target cells, offering potential for prolonged therapeutic effects. This approach has demonstrated delayed silencing of target molecules, such as STAT3, which are involved in cancer cell proliferation (Kozłowska *et al.*, 2017) ^[7, 13]. The versatility of silk-based drug delivery systems allows them to be adapted for various nucleic acid-based therapeutics, expanding their potential to target different cells or silencing various genes, thereby offering wide-ranging applications in precision medicine (Deptuch and Dams-Kozłowska, 2017) ^[7, 13].

Medical Applications of Functionalized Silk

1. Wound Healing

Functionalised silk has shown significant potential in enhancing wound healing, particularly for chronic or non-healing wounds. Silk-based dressings, which can be embedded with antimicrobial agents, help prevent infection while simultaneously promoting tissue regeneration. Additionally, by incorporating growth factors into silk fibres, wound healing can be accelerated, and scarring can be minimised. Silk fibroin membranes have been developed as scaffolds for tissue regeneration, including applications in skin, bone, and cartilage repair. These scaffolds offer the structural support needed for new tissue growth and have demonstrated efficacy in improving the healing process in animal models.

2. Tissue Engineering

Functionalised silk scaffolds are increasingly being explored in the field of tissue engineering. Silk's natural

biocompatibility, biodegradability, and mechanical strength make it a suitable material for creating scaffolds that support the regeneration of various tissues, including bone, cartilage, and nerve tissues. The incorporation of growth factors or peptides into silk scaffolds can further enhance tissue regeneration and improve the integration of the scaffold with the surrounding host tissue. Silk-based scaffolds have shown success in engineering vascular tissue, promoting bone regeneration, and aiding nerve repair, thus demonstrating their broad potential for clinical use.

3. Drug Delivery

As previously discussed, functionalised silk has promising applications in drug delivery. Silk fibres can be engineered to encapsulate both small molecules and biologics, providing a controlled and sustained release of therapeutic agents. This ability to control drug release rates makes silk ideal for targeted drug delivery, reducing systemic side effects and improving treatment efficacy. Furthermore, the inclusion of growth factors such as FGF2 and TGF- β 1 into functionalised silk can enhance the material's application in wound healing and skin repair. Nonwoven silk mats, produced from raw silk materials like natural cocoon shells, can release these growth factors along with native sericin proteins, both of which promote wound healing and tissue regeneration (Aramwit and Sangcakul, 2007)^[3].

Recent studies have also demonstrated the potential of silk-based biomaterials to serve as efficient drug carriers. These biomaterials have the advantage of prolonged, sustained drug release, making them ideal for therapeutic applications where continuous drug delivery is required. For example, silk-based carriers have been engineered to release growth factors such as TGF- β 1, SDF-1, BMP-2, FGF2, FGF1, EGF, PDGF, and NGF over extended periods (Bienert *et al.*, 2017; Wang *et al.*, 2014)^[6, 35]. In one study, FT silk was shown to release 11.9 ± 0.2 μ g of FGF2 and 4.2 ± 0.1 μ g of TGF- β 1 over one week, which was sufficient to demonstrate biological activity (McGee *et al.*, 1988; Mustoe *et al.*, 1987)^[16, 18]. By using genetic engineering to express specific drug proteins in silkworms, it is possible to create silk-based drug carriers that provide a controlled, slow release of therapeutic agents. This approach offers a straightforward and efficient method for fabricating silk drug carriers, further expanding the medical potential of functionalised silk.

Challenges

1. Scalability and Production Efficiency

The large-scale production of genetically modified silk presents a challenge due to the complexities involved in breeding transgenic silkworms and harvesting silk fibres. Currently, production processes are costly and require significant investment in infrastructure, such as facilities for silkworm cultivation and silk spinning. Increasing the scalability of these processes will be essential for mass production, which includes the development of automated systems for silk processing and weaving, reducing time and labour costs.

2. Regulatory and Safety Concerns

Functionalised silk, especially when genetically modified, must undergo rigorous testing to ensure its safety and regulatory compliance. While silk itself is known for its biocompatibility, incorporating foreign proteins or bioactive molecules raises concerns over immunogenic responses and

toxicity. Detailed safety assessments are necessary to determine the long-term effects of these materials within the human body. Additionally, the regulatory approval process for medical devices and materials involving genetically engineered organisms is complex and must be navigated carefully.

3. Environmental Impact

Although silk is biodegradable, the production of genetically engineered silk may involve environmentally harmful processes, such as chemical treatments or the release of certain by-products. These issues need to be addressed through sustainable practices in the production and processing of silk materials, ensuring that their ecological footprint remains minimal. Research on alternative, environmentally friendly techniques for silk functionalisation and harvesting will be important for making this technology more sustainable in the long term.

4. Customization and Multi-functionality

The potential for customising silk's properties for specific medical applications is a key strength, but achieving the desired balance between different functionalities remains challenging. The integration of multiple bioactive molecules—such as growth factors, drugs, or peptides—into silk requires precise engineering to ensure that the silk maintains its mechanical properties while exhibiting the necessary bioactivity. Further developments in genetic engineering and silk processing will enable more versatile silk materials that cater to a wide range of medical needs.

5. Long-term Performance and Degradation Control

While silk is biodegradable, the rate at which it degrades in the body must be carefully controlled for different applications. In tissue engineering, for example, scaffolds must degrade at a rate that allows for tissue growth without compromising structural integrity. Similarly, drug delivery systems must ensure a controlled release of therapeutic agents over time. The challenge lies in fine-tuning these degradation rates to match the specific needs of various medical applications, which will require continued research and refinement in material design and production.

6. Integration with Other Biomaterials

To enhance silk's functionality, it often needs to be combined with other biomaterials, such as hydrogels or synthetic polymers. The challenge here is to ensure that the silk and other materials interact seamlessly without compromising the desired properties of either. Developing hybrid materials that combine silk's biocompatibility, biodegradability, and bioactivity with the mechanical strength and durability of other materials will expand its potential applications in regenerative medicine and drug delivery.

7. Personalised Medicine

With the rise of personalised medicine, there is an increasing demand for medical materials that can be tailored to individual patients. Silk-based biomaterials offer the potential for such personalised treatments, especially in areas like tissue engineering and drug delivery. Customising silk materials to match a patient's unique genetic or medical profile could significantly enhance the effectiveness of treatments. However, achieving this level of customisation

will require further advances in both silk production and diagnostic technologies.

Way Forward

1. Advancing Genetic Engineering Techniques

Ongoing improvements in genetic engineering, particularly with tools like CRISPR/Cas9, will make it possible to create more efficient and functional silk-based biomaterials. These advancements will allow for greater precision in controlling the expression of genes in silkworms, leading to silk fibres with enhanced properties tailored to specific medical applications.

2. Optimising Silk Processing Methods

Innovations in silk spinning, weaving, and fabrication will be essential to scale up production and enhance the properties of functionalised silk. Streamlining the production process through automation and new fabrication techniques will be crucial for reducing production costs and ensuring that silk-based biomaterials are commercially viable.

3. Collaborative Research

Collaboration across multiple disciplines—including material science, biotechnology, and clinical medicine—will be critical to pushing forward the application of functionalised silk in healthcare. Cross-disciplinary research will help translate theoretical concepts into practical, real-world solutions and facilitate the integration of these materials into clinical practices.

4. In vivo and Clinical Testing

To ensure that functionalised silk is safe and effective for medical use, comprehensive *in vivo* and clinical testing must be carried out. These tests will provide valuable data on the performance of silk-based materials in human subjects and guide the regulatory approval process for their use in medical treatments.

Conclusion

Transgenic silkworms producing functionalized silk represent a significant advancement in the field of biomaterials. By incorporating bioactive molecules such as antimicrobial peptides, growth factors, and anticancer agents into silk fibers, researchers are creating novel materials with enhanced properties for a range of medical applications. These materials hold great promise for use in wound healing, tissue engineering, drug delivery, and regenerative medicine. However, challenges related to scalability, safety, and regulatory approval must be addressed to fully realize the potential of functionalized silk in the medical field. With continued research and development, transgenic silk could revolutionize medical treatments and contribute to significant advancements in biotechnology and biomedicine.

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