ACCSCIENCE PUBLISHING

Materials Science in Additive Manufacturing

REVIEW ARTICLE

Unraveling the roles of fibrous silk in biomedical applications: A review

Mingzheng Zhao[1†], Shixuan Guo[1†], Fengqi Cheng[1†], Wenhan Tian[2], Weishi Liang[1,3], Jing Su[1], Yong Hai[1,3*], Juan Guan[2*], and Yuzeng Liu[1*]

[1]Department of Orthopedic Surgery, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, 100020, China

[2]School of Materials Science and Engineering, Beihang University, Beijing 100083, China

[3]Joint Laboratory for Research and Treatment of Spinal Cord Injury in Spinal Deformity, Laboratory for Clinical Medicine, Capital Medical University, Beijing 100069, China

Abstract

Biomedical materials have become essential for diagnosing, treating, and repairing diseased tissues, with applications ranging from hard dental implants to soft artificial blood vessels. Among these, fibrous silk (FS) – a naturally assembled material with exceptional mechanical and biological properties – has recently emerged as a promising candidate for advancing biomedical technologies, particularly with the advent of additive manufacturing and three-dimensional (3D) printing. This review comprehensively explores the advancements in FS-based materials for biomedical applications over the past two decades (2004 – 2024). FS, a unique material derived from silkworm silk fibers, exhibits exceptional mechanical properties, biocompatibility, controlled biodegradability, and antimicrobial characteristics, positioning it as a versatile candidate for various biomedical applications. The review begins with a detailed analysis of FS structure and morphology, covering natural FS, derived FS, and assembled FS. It then delves into the critical properties relevant to biomedical applications, such as mechanical resilience, biointegration, controlled degradation profiles, and antimicrobial performance. Subsequently, the review examines the extensive applications of FS-based materials across various biomedical fields, particularly in tissue engineering and regenerative medicine. Special emphasis is placed on the role of additive manufacturing and 3D printing in enhancing the design complexity and functional performance of FS-based scaffolds, highlighting their potential for developing customized implants and tissue-engineered constructs. Finally, the review provides insights into the future potential of FS-based materials, addressing current limitations and proposing strategies to further optimize their functionality in biomedical contexts.

Keywords: Fibrous silk; Biomedical applications; Additive manufacturing; 3D printing

†These authors contributed equally to this work

***Corresponding authors:**
Yuzeng Liu
(beijingspine2010@163.com)
Juan Guan
(juan.guan@buaa.edu.cn)
Yong Hai
(yong.hai@ccmu.edu.cn)

Citation: Zhao M, Guo S, Cheng F, *et al*. Unraveling the roles of fibrous silk in biomedical applications: A review. *Mater Sci Add Manuf.* 2025;4(2):025130020
doi: 10.36922/MSAM025130020

Received: March 26, 2025

1st revised: April 10, 2025

2nd revised: April 26, 2025

Accepted: April 28, 2025

Published Online: May 30, 2025

1. Introduction

Biomedical materials are specialized materials used to diagnose, treat, or repair diseased tissues and organs, thereby improving patients' quality of life.[1] At present, biomedical materials span a wide range of applications, including tissue engineering for both hard tissues (such as dental and bone implants) and soft tissues (such as artificial blood vessels

and heart valves).[2-4] In addition, they have been extensively used in drug delivery, gene therapy, and related fields.[5,6] With the rapid development of molecular biology, cell biology, clinical medicine, and other disciplines, as well as cross-disciplinary adventures, biomedical materials are playing an increasingly vital role in shaping the future directions of medical research.

As a historically significant and iconic material in the textile industry, silk fibers produced by silkworms have garnered significant attention throughout human history owing to their unparalleled luster and exceptional mechanical attributes.[7,8] In this review, we deliberately refer to silk in its fiber form as fibrous silk (FS) to distinguish it from the constituent silk fibroin (SF) proteins found in other forms. The production of FS begins with the *in vivo* biosynthesis of fibrous SF proteins by specialized epithelial cells within the silkworm gland, followed by transport through a long duct for structural condensation before final spinning.[9] At the molecular level, FS is characterized by highly ordered β-pleated structures (β-sheets), primarily arising from hydrophobic domains composed of GAGAGS segments (where G = glycine, A = alanine, and S = serine) in its primary sequence.[10] The interconnected crystalline regions, alongside a non-crystalline continuous phase containing less-ordered diffused chains, collectively contribute to the remarkable mechanical performance of silk fibers.[11]

Concerning the biological properties, FS exhibits non-immunogenicity, controllable biodegradability, non-toxic degradation products, compatibility with a wide variety of tissues, and renewability.[12,13] As understanding of the intricate structure and remarkable properties of FS has deepened, research focus has rapidly shifted from traditional textile applications to the realm of biomedical materials, encompassing nearly all aspects of medicine.[14-18]

In recent years, additive manufacturing (AM) and 3D printing have emerged as powerful tools for fabricating FS-based biomedical materials with highly complex and customized architectures. AM techniques, including selective laser sintering, stereolithography, and extrusion-based printing, enable precise control over the microstructure, porosity, and mechanical properties of FS-based scaffolds. These technologies have significantly expanded the application of FS in tissue engineering and regenerative medicine by facilitating the creation of patient-specific implants and functionalized biomaterials with enhanced bioactivity and tailored degradation rates.[19] The integration of AM and 3D printing with FS-based materials holds great promise for advancing personalized medicine and improving the efficacy of tissue repair and regeneration.

Although approximately 20 review articles have been published on the fabrication, structures, properties, and applications of SF-based biomaterials, it is important to note that SF is merely a fibrous protein sharing the primary sequence structure with FS. Natural FS exhibits a far more intricate and ordered assembly of SF chains.[20] In contrast, the number of research articles specifically focused on FS remains relatively small. Given the in-depth knowledge now available regarding FS's comprehensive material properties and the growing breadth of its biomedical applications, a timely review of recent advances in FS research is warranted.

This review focuses on FS-based materials for biomedical applications, covering research progress over the past two decades (2004 – 2024). First, the structure and morphology of FS are described, including natural FS, derived FS, and assembled FS. Next, critical properties relevant to biomedical applications – including mechanical performance, biocompatibility, biodegradation, and antimicrobial characteristics – are discussed. Subsequently, the applications of FS-based materials across various biomedical fields are examined, underscoring the advantages of FS. Finally, future prospects, current limitations, and possible directions for optimizing FS-based materials for biomedical applications are tentatively proposed.

2. The source, composition, and structure of FS

Natural silk fibers are fibrous materials commonly produced by certain species of arthropods and insects, including the domestic silkworm, spider, scorpion, mite, and bee. These species share a specialized set of organs adapted for silk production.[21-23] Silk protein fibers are either spun continuously (i.e., by silkworms) or produced upon demand (i.e., by spiders) during their life cycle. The intricate process of silk formation, occurring through the posterior, middle, and anterior parts of the silk gland duct, is regulated by variations in pH as well as potassium and calcium ion concentrations.[24-26] These conditions are critical for controlling the transport and conformational transition of SF.

Although spiders can produce significant quantities of silk – reaching up to 137 m at a time – their cannibalistic behavior limits large-scale silk harvesting and, consequently, the practical application of spider silk.[27,28] In contrast, silkworms are dedicated to silk producers, generating silk lengths ranging from 600 m to 1,500 m, relative to their body weight, to construct robust woven cocoons. Since the domestication of silkworms, silk fibers have been widely used in the textile industry, prized for

advantages such as luster and fineness over other textile fibers.[29,30]

FS accounts for about 70 – 80% of the total mass of silk fiber, while sericin makes up about 20 – 30%; minor constituents include wax, pigments, and inorganic matter.[31] FS is primarily composed of heavy (H) and light (L) chains, along with the P25 glycoprotein.[32] These components adhere to a molecular ratio of H: L: P = 6:6:1.[33] The H and L chains are interconnected through disulfide bonds located at their C-termini, forming a stable H–L complex, while the P25 glycoprotein associates non-covalently to further stabilize this assembly.[34] The primary sequence of the H-chain features repetitive GAGAGS segments, which constitute more than 80% of the FS composition.[35]

Bombyx mori silk, renowned for its intricate structure, comprises heavy chain (Fib-H), light chain (Fib-L), and the P25 glycoprotein.[36] Fib-H and Fib-L are covalently linked by disulfide bonds, forming a unique branched polymer,[34,37] while P25 physically interacts with this system, enhancing overall structural integrity.[33] Fib-H, the core structural component, consists of a 5,263-residue amino acid sequence (391.6 kDa), whereas Fib-L is shorter (262 residues, 27.7 kDa).[32] The P25 glycoprotein (30 kDa) also plays a crucial stabilizing role. The structure of Fib-H is characterized by a glycine-rich repetitive core and distinct terminal regions.[32] The core further divides into GAGAGS, tyrosine-rich (Y), GAAS, and non-repetitive domains. GAGAGS and Y domains form β-sheet nanocrystals with unique arrangements, while GAAS sequences create tetrapeptide β-turns that interrupt (GX)n crystalline domains.[38] Interestingly, the hydrophilic non-repetitive segments fold into a distorted Ω-shape, enhancing protein chain flexibility and facilitating 180° reversals that promote antiparallel β-sheet formation, ultimately strengthening the stability and mechanical properties of *B. mori* silk.[39]

Species of the genus Antheraea (family Saturniidae), including *Antheraea pernyi* and *Antheraea yamamai*, are essential for tussah silk production.[40] Tussah silk, similar in amino acid composition to Nophila silk, is characterized by elongated poly(A) repeats. This region alternates between (A)n sequences (where n = 11 – 13) and glycine-rich domains, containing characteristic motifs such as GGYG, GSGA, and GGAG. This extended poly(A) region enhances the crystallinity of Tunnah silk relative to Nophila silk, though both remain less crystalline than mulberry silk.[41] In terms of β-sheet domain content, the crystallinity hierarchy is mulberry silk > tussah silk > Nophila silk.

Spider silk's enormous molecular weight (~350 kDa) endows its secondary structure with remarkable complexity.[42] Research indicates that over 30,000 spider species possess the ability to produce silk, with each species capable of secreting up to seven distinct types of silk, each characterized by unique sequences that remain largely unexplored.[43] Among these, Nephila silk stands out as a well-studied model. This review focuses on the secondary structure of Nephila silk, whose amino acid sequence features repetitive core segments alternating between hydrophilic and hydrophobic regions, flanked by conserved N- and C-terminal domains. The hydrophobic cores, rich in (A)n motifs (n = 4 – 6), promote crystal formation,[44] while the hydrophilic regions, abundant in glycine and tyrosine residues, favor random coil or helical conformations that transform into β-sheet nanocrystals during spinning. These nanocrystals act as cross-linkers, binding amorphous segments into a robust network that impart Nephila silk with exceptional mechanical and biological properties.[45,46]

Spun FS exhibits a distinctive triangular cross-sectional morphology and semi-crystalline characteristics within its condensed structure. The sequences of Fib-H and Fib-L differ, and this sequence divergence is a key factor in regulating the crystallinity and secondary structure of SF.[47] The β-sheet configuration within the crystalline domains serves as a pivotal determinant of silk's mechanical performance, primarily contributing to its exceptional strength and elastic modulus.[48] The secondary structure and hierarchical arrangement of SF ultimately define the properties of the resulting biomaterials.[41] In particular, hydrophobic sections composed of repetitive amino acid sequences aggregate to form β-folded nanocrystals.

3. Properties of FS

FS represents a distinctive class of structural proteins, renowned for its excellent biocompatibility, tunable degradability, and unparalleled mechanical characteristics. These unique characteristics render FS highly suitable for a diverse array of processing techniques, encompassing both aqueous and organic solvent-based methods. Moreover, its chemical modifiability allows adaptation to a broad spectrum of biomedical applications. The following sections elaborate on these superior properties.

3.1. Mechanical properties

FS exhibits remarkable stiffness and superior tensile strength along its longitudinal axis, while maintaining a considerable level of ductility.[49] FS displays a well-balanced combination of modulus, elongation at break, and tensile strength, conferring outstanding ductility and toughness.

To better contextualize the superior mechanical properties of FS compared to other materials, Table 1 and Figure 1 present a comparison of the mechanical properties of various fibrous materials, including both

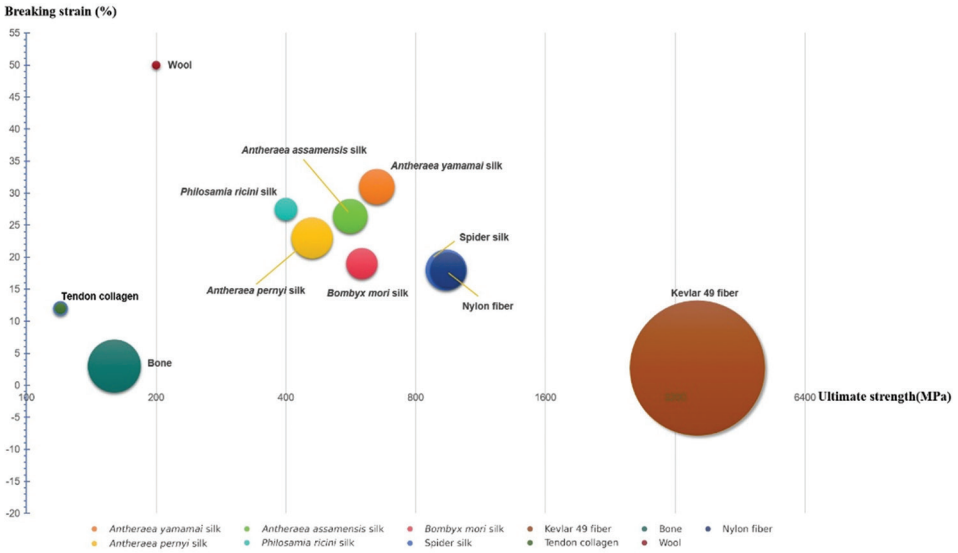

Figure 1. Mechanical properties of silk fibers and other fibrous materials
Note: The coordinates of each circle's center represent ultimate strength (X-axis) and breaking strain (Y-axis), while the area indicates the modulus value

Table 1. Mechanical data of silk fiber and other fibrous materials

Materials	Modulus (GPa)	Ultimate strength (MPa)	Breaking strain (%)	Toughness (MJ/m^3)	References
Bombyx mori silk	7	600	19	70	80
Antheraea yamamai silk	9	650	31	113	81
Antheraea pernyi silk	12	460	23	65	81
Antheraea mylitta silk	8	513	26	79	82
Antheraea assamensis silk	8.5	564	26.4	95	82
Philosamia ricini silk	3.6	400	27.5	71	82
Spider silk	11 – 13	875 – 972	17 – 18	-	
Araneus dragline silk	10	1100	27	160	59
Araneus viscid silk	0.5	500	270	150	59
Nephila clavipes dragline silk	11 – 13	880 – 970	0.17 – 0.18	110	59
Polylactic acid	1.2 – 3.0	350	56	-	59
Nylon fiber	5	950	18	80	80
Kevlar 49 fiber	130	3600	2.7	50	59
Silicone rubber	0.001	850	0.001	100	59
Carbon fiber	300	4000	1.3	25	59
Tendon collagen	1.2	120	12	6	59
Elastin	0.0011	2	150	2	80
Bone	20	160	3	4	59
Wool	0.5	200	50	60	59
Wood	6 – 20	60 – 100	-	5 – 9	41

natural and synthetic fibers such as spider silk, Kevlar 49 fiber, nylon fiber, and bone tissue. Natural silkworm FS demonstrates a tensile strength of approximately 0.5 – 0.6 GPa with a breaking elongation ranging from 10% to 40%.[50,51] Reported values include a breaking strain reaching up to 26%, an ultimate strength of 300 – 740 MPa, and a toughness of 70 – 78 MJ/m^3.[52,53] Among natural materials such as wool, cotton, elastic cellulose,

and elastin, FS stands out with superior mechanical performance.[54]

In addition, the strength of FS surpasses that of common degradable polymer biomaterials such as collagen and poly(L-lactic acid) (PLLA). Collagen bulk materials typically exhibit a strength range of 0.9 – 7.4 MPa and polylactic acid between 15 MPa and 26 MPa.[55-57] In terms of toughness, FS also outperforms many synthetic fibers, including Kevlar (50 MJ/m^3), carbon fiber (25 MJ/m^3), wool (60 MJ/m^3), and some collagen fibers like tendon collagen (7.5 MJ/m^3).[58] It is particularly noteworthy that the exceptional stretchability and resilience of FS far exceed those of Kevlar fiber, which has long served as a benchmark for high-performance fiber technology.[52,59] In addition, wild species-derived silks – so-called Tussah silks, including *A. pernyi* – have been extensively studied over the past decade. Guan *et al.*[60] reported that *A. pernyi* cocoon exhibited superior mechanical properties, achieving a tensile strength of 55 MPa and an elongation rate of 25%, compared to 25 MPa tensile strength and approximately 16% elongation for the domesticated *B. mori* cocoon.

However, compared to natural FS, most regenerated FS-based materials produced from SF solutions remain relatively fragile and prone to breakage. For instance, regenerated FS membranes crafted from silkworm SF solutions exhibit a dry tensile strength of only 0.02 GPa with a breaking elongation of <2%. These inferior mechanical properties are attributed to the disruption of the secondary and hierarchical structures compared to native FS.[61,62] To improve the mechanical properties of regenerated FS, researchers have investigated precise control over the size, quantity, distribution, alignment, and nanoscale spatial arrangement of crystalline and non-crystalline domains.[11,44,63] Yazawa *et al.*[64] introduced a cutting-edge spinning methodology, employing tetrahydrofuran as the coagulation solvent to inhibit the rapid formation of β-sheet structures. Subsequent post-stretching treatments facilitated the controlled formation and alignment of β-sheets, substantially enhancing ductility and toughness, even surpassing that of natural FS. Such regenerated FS may potentially serve as fibrous reinforcement materials.

To further expand FS's mechanical capabilities, diverse physical and chemical methods have been explored. Fang *et al.*[58] demonstrated that controlled-speed winding during FS production yielded fibers outperforming natural FS. Wet spinning techniques produced artificial FS with 0.42 GPa strength, 47.1% fracture strain, and 154.8 MJ/m^3 fracture energy. Yao *et al.*[65] applied rapid Bayesian optimization technology to refine FS processing, achieving a 2.20-fold increase in tensile strength, a 2.16-fold increase in modulus, and a 2.75-fold improvement in toughness compared to conventional methods. This framework extends to optimizing spinning flow rates, coagulation conditions, and fiber drawing processes to enhance FS properties.[66,67]

In addition to process optimization, researchers have explored the integration of functional materials to tailor FS properties. Cheng *et al.*[68] used molecular simulations to study graphene-peptide interactions, revealing significant enhancements in silk strength, elasticity, and overall mechanical performance.

In addition, studies have demonstrated that the mechanical performance of FS can be enhanced by incorporating specific substances into artificial feed, resulting in materials with superior strength characteristics.[69] Zhang *et al.*[70] reported that the incorporation of multi-walled carbon nanotubes, single-walled carbon nanotubes, lignosulfonate calcium, and graphene into mulberry leaves significantly improved FS's breaking stress, elongation, toughness, and tensile strength. These findings were further supported by independent studies.[71-73] Further additives – such as ionic precursors, nano-hydroxyapatite, feather fibers, and metal nanodroplets – have also been employed to enhance FS properties.

For instance, Guo *et al.*[74] coated mulberry leaves with $CaCl_2$, NaH_2PO_4, and nano-hydroxyapatite, which promoted an increase in α-helix and random coil content within the FS secondary structure, thereby improving mechanical properties. At low concentrations, the combination of Ca^{2+} and PO_4^{3-} yielded FS with a breaking strength of 0.49 GPa, elongation of 13.01%, and toughness of 0.023 GPa. At higher concentrations, these values increased to 0.62 GPa strength, 17.42% elongation, and 0.022 GPa toughness. Both formulations exceeded the tensile strength of natural silk (0.3 GPa), although with slightly reduced elongation. Gao *et al.*[75] encapsulated liquid metal nanodroplets in sodium alginate and sprayed the mixture onto mulberry leaves, resulting in FS with a tensile strength of 0.81 GPa and an elongation of 70%. Similarly, Lu *et al.*[76] incorporated rare earth ions (La^{3+}/Eu^{3+}) into the silkworm diet, which significantly enhanced the mechanical properties of silk by increasing β-sheet orientation and reducing fiber diameter. The resulting silk exhibited a tensile strength of 0.97 GPa and toughness of 188 MJ·m^{-3}, approaching the performance of spider dragline silk.

Owing to their distinctive attributes – such as ease of cultivation, short breeding cycles, and the ability to accumulate up to 25% pure protein – silkworms are considered ideal candidates for recombinant product delivery. Recent advances in genetic engineering have enabled the transformation of silkworm FS into a platform

for producing recombinant proteins, peptides, genes, and other bioproducts. This facilitates large-scale transgenic production and enhances FS's mechanical properties.[77] Genetic modifications not only introduce novel functions but also enhance material strength.[78] For instance, Japanese researchers by Teramoto *et al.*[79] developed transgenic silkworms with an expanded genetic code capable of incorporating azido groups into SF, thereby enabling the large-scale production of functionalized silk fibers. This approach provides a promising strategy for developing high-performance silk materials through click chemistry-based modifications.

3.2. Biosafety and immune interaction

FS demonstrates superior biocompatibility, biodegradability, and non-toxic, non-irritating properties compared to synthetic polymer compounds, such as polyetheretherketone and polylactic acid, which are widely employed in clinical applications. Additional investigations have demonstrated that properly degummed and sterilized FS exhibits biocompatibility comparable to other widely used biomaterials, including polylactic acid and collagen.[28] Table 2 shows that FS supports human fibroblast proliferation, exhibits low hemolytic activity, and elicits only a mild foreign body response, indicating excellent biocompatibility relative to other fiber materials.

Historically, FS has been widely used as a suture material for centuries due to its proven biosafety profile.[15] However, the combination of SF and sericin can trigger inflammatory responses, a natural defense mechanism against foreign bodies. Delayed allergic reactions to FS sutures have been attributed to sericin, which elevates IgE levels and induces hypersensitivity reactions.[28] Degumming, which removes sericin from FS, significantly enhances its biosafety by promoting cell adhesion and differentiation. Studies by Meinel *et al.*[83] and Boni *et al.*[84] have confirmed that degummed FS becomes immunologically inert and does not trigger an immune response. Consequently, degumming – often performed through protease digestion – is a critical step to improve FS biocompatibility and expand its applications beyond those of raw FS. Complete sericin removal from FS is crucial for producing clinically suitable FS biomaterials.

Multiple studies have affirmed the superior biosafety of silk proteins compared to synthetic polymers. The immunogenicity and antigenicity of FS scaffolds have been thoroughly evaluated, with silk-fibronectin conjugates demonstrating favorable outcomes in musculoskeletal treatments. For example, a study by Zhou *et al.*[85] using a rabbit model showed no infection despite some immune cell accumulation, although sericin's adverse effects on immune responses remain inconclusive. Further research is needed to comprehensively verify FS biosafety and immune interactions for biomaterial applications.

Due to its excellent biocompatibility, modified FS sutures have been widely used in tissue engineering for bone, tendon, and ligament repair. Kardestuncer *et al.*[77] cultured human tendon cells on Arg-Gly-Asp (RGD)-modified FS sutures. The modified sutures exhibited enhanced tendon cell adhesion, proliferation, and differentiation compared to both unmodified FS sutures

Table 2. Biocompatibility and biodegradability of silk and other common fibrous materials

Materials	Supported tissues or cell type	Key findings	Foreign body response[a]	Biodegradation time	References
Silkworm silk fiber (without sericin)	Human fibroblasts	Supports cell proliferation with low hemolysis	Mild	Slow (>12 weeks)	85
Spider silk	Subcutaneous tissue in rats	No impairment in wound healing; no infection or ectopic inflammation	Mild	Slow (>12 weeks)	85
Kevlar	Paravertebral muscles in rabbits	Slight fibrotic tissue reaction; foreign body giant cells observed near fibers	Severe	Non-biodegradable	184
Nylon	Rat tendon cells	Comparable to Kevlar in cellular response and growth rate	Moderate	Slow (several years)	185,186
Collagen	Bovine tendon fibroblasts	Facilitates tendon fibroblast migration, attachment, and proliferation	Mild	Moderate (4 – 12 weeks)	187
Polylactic acid	Bone mesenchymal stem cell	High cell viability and cytocompatibility for bone repair	Mild to moderate	Fast (2 – 12 weeks)	85

Notes: [a]Classification of foreign body response:
- Mild: Characterized by slight inflammation and minimal infiltration of monocytes and macrophages, with no significant tissue damage or fibrosis.
- Moderate: Characterized by moderate inflammation, increased infiltration of monocytes, macrophages, and some neutrophils, potentially resulting in minor fibrosis and localized tissue damage.
- Severe: Characterized by extensive inflammation, pronounced infiltration of macrophages and neutrophils, marked tissue fibrosis, and necrosis, potentially leading to severe tissue damage.

and tissue culture plastic. Specifically, the adhesion strength of tendon cells on FS-RGD increased by 1.3-fold relative to tissue culture plastic. After 6 weeks, collagen type I and decorin transcription levels were significantly higher on FS-RGD compared to unmodified FS and tissue culture plastic. Northern blotting analysis showed that mRNA levels were increased by 2 – 3 times on FS-RGD and FS compared to tissue culture plastic.

In addition, FS has been explored as a biological alternative for ligament reconstruction. Liu *et al.*[86] constructed FS cables with mechanical properties similar to the human anterior cruciate ligament (ACL), successfully cultivating human bone marrow stromal stem cells (BMSCs) and ligament fibroblasts on these FS cables to achieve robust cell adhesion and proliferation.

In another study, Zhou *et al.*[85] developed an FS/calcium phosphate cement (FS/CPC) biocomposite, exhibiting excellent biocompatibility and osteogenic activity for bone defect repair. In a rabbit radius defect model, imaging analysis, histological examination, and scanning electron microscopy revealed that the FS/CPC group exhibited early trabecular bone formation at 4 weeks, along with significantly higher maximum bending strength compared to other groups.

Although the current research is encouraging, concerns remain regarding the long-term safety of FS biomaterials in the human body. First, most biocompatibility studies to date have focused on short- to mid-term periods (3 – 6 months). Extended studies are necessary to assess the long-term immune responses to FS biomaterials following prolonged contact with human tissues. Such studies should take into account the implantation site and the construct type and utilize appropriate *in vivo* models for comprehensive analysis. Second, the immune reactions triggered by degradation products of FS biomaterials – strongly influenced by the size and structure of these products – require further investigation. It is well known that particulate debris is a major cause of biomaterial implant failure due to the activation of immune responses. Reports by Gellynck *et al.*[87] suggest that certain FS materials can induce mild proinflammatory cytokine production and enhance phagocytosis. Similarly, digestion of the C-terminus of *A. pernyi* silk by α-trypsin has been shown to reduce cell adhesion and restrict growth, indicating that degradation can adversely affect FS biocompatibility. Furthermore, studies by Lundmark *et al.*[83] have suggested that silk protein degradation products may potentially contribute to amyloidosis. Silk fiber solutions have been found to accelerate the accumulation of amyloid-like substances, leading to tissue degradation.

Therefore, comprehensive and long-term investigations into the degradation behavior and immune interactions of FS biomaterials are urgently required to fully address safety concerns regarding their clinical applications.

3.3. Biodegradation

The degradation of FS predominantly occurs through protein hydrolysis and prolonged absorption within the body, resulting in a relatively gradual degradation rate.[28] This gradual degradation allows for sufficient mechanical support during cell regeneration and tissue repair. Compared with other biological materials, FS offers significant advantages in terms of biodegradability. Table 2 presents the biodegradation characteristics of FS, exhibiting a slow degradation rate (exceeding 12 weeks), which ensures long-term mechanical support for tissue healing. After enzymatic hydrolysis, FS can be absorbed and utilized by the human body, making it an excellent carrier material for the controlled release of therapeutic agents. Lee *et al.*[84] found that when regenerated FS membranes were implanted subcutaneously in rats, the membrane thickness gradually decreased, with approximately 65% of the original thickness remaining after 19 months. In contrast, widely used synthetic biological materials such as polyglycolic acid and polylactic acid produce acidic by-products upon degradation, which can be harmful after metabolism and absorption by the body. In addition, these synthetic materials often experience an early decline in mechanical properties during degradation. By contrast, the slow degradation rate of FS allows it to maintain good mechanical strength over an extended period.

Research indicates that the degradation rate of FS is primarily determined by its secondary structure, particularly the β-sheet content, which accounts for its slow degradation.[88] In an *in vitro* experiment, protease XIV was used to degrade FS for more than 70 days. It was found that while the enzyme could degrade FS dissolved in water, it could not degrade FS that had formed a film.[89] This resistance is attributed to the β-sheet structure shielding enzymatic cleavage sites, thereby extending the degradation time of FS in the body. Similarly, Lu *et al.*[90] demonstrated that FS samples with higher β-sheet content degraded more slowly, whereas FS with fewer β-sheets degraded more rapidly. Furthermore, factors beyond secondary structure – including the FS type, implantation site, and *in vivo* environment – also influence degradation rates.[91]

As a medical biomaterial, the degradation rate of FS must align closely with the regeneration and repair rates of specific tissues, ensuring seamless integration with damaged tissues or organs and serving either as a substitute or catalyst for regeneration.[13] By adjusting factors such as β-sheet quantity, FS concentration, and implantation site,

the degradation behavior of FS can be fine-tuned to meet the distinct biological requirements of various tissues and organs.[92] However, degradation is also influenced by FS's inherent physical and chemical properties, such as molecular weight, β-sheet content, and hydrophobicity.[93]

Despite FS's adjustable degradability, more in-depth investigations are imperative to achieve a comprehensive comprehension of its degradation and clearance mechanisms. Rigorous research into the *in vivo* degradation behavior of FS under various conditions is necessary to propel the development of FS as a prominent biodegradable and biocompatible material.

4. Applications of FS -based biomaterials

FS is a versatile biomaterial with excellent mechanical properties, biocompatibility, and antimicrobial activity, making it highly suitable for various tissue regeneration applications. As illustrated in Figure 2, FS-based materials have been successfully applied in the regeneration of skin, cartilage, tendon, ligament, vascular, and bone, demonstrating substantial potential in tissue repair and healing enhancement.

4.1. Skin tissue regeneration

The skin, the largest organ of the human body, plays crucial roles in protection, temperature regulation, and sensory perception. Skin damage, a common historical and contemporary issue, can result from external factors affecting the epidermis or dermis, leading to inflammation, pain, infection, and disruption of natural immune defenses, ultimately prolonging healing processes.[94] Microbial infections, particularly bacterial infections, further complicate wound healing.

An effective wound dressing must (i) shield the wound, (ii) prevent dehydration, (iii) permit gas exchange, (iv) be biocompatible, and (v) deliver therapeutic agents to prevent infections and accelerate healing.[95] Traditional cotton or linen dressings have notable limitations, including

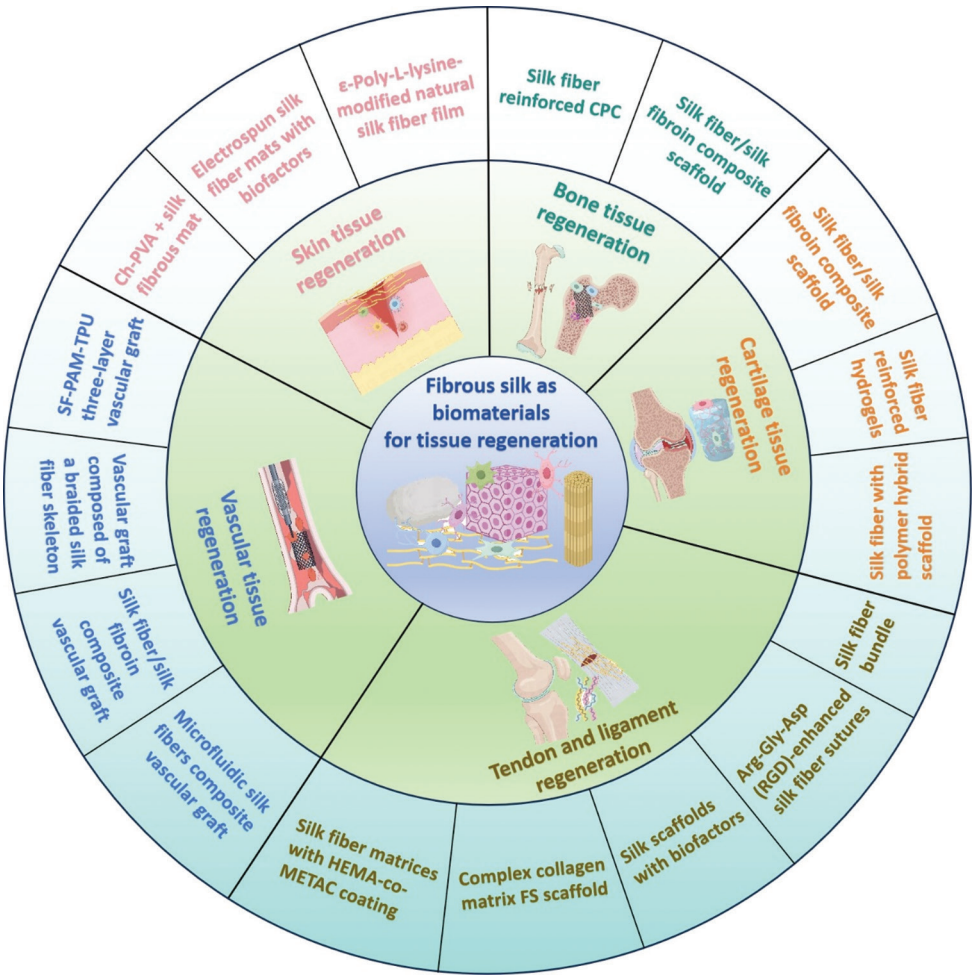

Figure 2. Applications of fibrous silk-based biomaterials

a high risk of infection, poor biocompatibility, lack of antibacterial properties, inadequate wound adherence, and poor moisture management. Limited biocompatibility also increases the risk of rejection reactions (e.g., skin redness, itching), further escalating infection risks.[96] Consequently, there is an urgent need for safer, more effective wound dressing materials. The current research focuses on developing new dressing materials with antibacterial properties, enhanced skin compatibility, and accelerated healing capabilities.[95,97]

FS offers superior mechanical strength, biocompatibility, and cost-effectiveness. Its high structural and morphological plasticity makes it an ideal biomaterials.[28] Notably, FS effectively mimics the skin microenvironment, aids in scar reduction, and treats atopic dermatitis.[98,99] Further, it promotes cell migration, proliferation, and growth factor expression, enhancing wound healing through nuclear factor-κB signaling pathways.[100] Thus, FS represents a valuable material for skin repair, significantly enhancing anti-infection defenses and wound healing outcomes.

For skin tissue repair, FS can be processed using physical, chemical, or genetic methods into hydrogels, films, electrospun pads, and sponges suitable for wound dressings. For example, Li *et al.*[17] fabricated FS films through hot pressing, enhanced with ε-polylysine, significantly accelerating wound healing, promoting granulation tissue formation, and increasing collagen deposition. Schneider *et al.*[101] developed electrospun FS pads loaded with epidermal growth factor, effectively promoting wound healing, especially for chronic wounds. Fathi *et al.*[102] co-electrospun polyvinyl alcohol, chitosan, and silk fibroin to produce hybrid fibers that exhibited superior mechanical and swelling properties and created a hydrophilic microenvironment conducive to cell adhesion and proliferation *in vitro*, as well as wound healing and tissue regeneration *in vivo*.

Moreover, FS dressings serve not only as basic antibacterial barriers but also as drug delivery systems. For instance, Qin *et al.*[103] fabricated a porous silk-based patch through ice templating, enabling both wound protection and controlled antibiotic (e.g., rifamycin) delivery. Sapru *et al.*[104] developed a silk-serine nanofiber matrix with enhanced cell compatibility, blood compatibility, and reduced immune reactivity, also boosting antibiotic delivery to minimize infection and inflammation risks.

Genetic engineering further expands FS's functionality by producing transgenic silk with embedded bioactive factors. For example, Wu *et al.*[105] used the piggyBac system to generate silkworms expressing truncated heavy chains of human epidermal growth factor protein, boosting cell proliferation with minimal cytotoxicity, ideal for wound dressing applications. Wang *et al.*[106] developed dual-functional FS expressing fibroblast growth factor-2 and transforming growth factor-beta 1 for enhanced cell growth and anti-inflammatory responses. Wang *et al.*[107] used a sericin-based system to engineer silkworms producing human acidic fibroblast growth factor-1, offering great promise for skin wound healing through stimulated cell growth.

4.2. Cartilage tissue regeneration

Human cartilage tissue consists of chondrocytes, matrix, and fibers. It is categorized into hyaline cartilage, fibrous cartilage, and elastic cartilage based on matrix composition.[108] Lacking nerves, blood, and lymphatics, cartilage exhibits limited self-repair capabilities due to its water-rich matrix.[109] Damage to articular cartilage disrupts joint function, impacting daily life and causing balance disorders in the human body. Surgical intervention is often needed, with microfracture and autologous chondrocyte transplantation commonly employed for minor injuries.[110] At present, research focuses on the development of biocompatible materials for cartilage repair. Critical requirements for these materials include biocompatibility to avoid immune rejection, controlled degradation, and mechanical properties that match the host tissue, with adjustability for various repair needs.[111] In addition, materials must support cell attachment, proliferation, and integration with host tissue.[112] A porous structure with interconnected pores is vital for nutrient transport and cell growth.

FS, a natural polymer, outperforms numerous natural and synthetic materials, especially for functional tissue replacements. Its superior mechanical properties, biocompatibility, controlled biodegradability, and adjustable porosity render it a prime candidate for cartilage repair. However, the current research primarily utilizes degummed silk treated with Na_2CO_3 and dissolved in LiBr to form a discontinuous SF solution. Researchers typically blend the SF solution with other materials and utilize 3D printing to fabricate cartilage bioscaffolds that foster cell growth, proliferation, and differentiation.[113-116] Conversely, direct use of FS remains limited. This review focuses on FS applications in cartilage repair to boost understanding and further its potential in this field.

Numerous studies confirm that SF solution combined with chopped FS can fill cartilage defects and boost chondrocyte regeneration. Singh *et al.*[117] fabricated a composite scaffold by combining chopped FS with SF solution in a 2:1 (w/w) ratio. The resulting material exhibited superior swelling (25 – 30%) and degradation rates (10 – 30%) due to its porosity. The addition of FS

enhanced the compressive modulus and stiffness nearly eightfold. Biochemical analyses showed increased DNA, sulfated glycosaminoglycan (1.5-fold), and collagen (1.4 fold) content compared to pure SF solution scaffolds (p<0.01). Furthermore, cartilage-specific gene markers, such as collagen II, Sox-9, and aggrecan, were upregulated by approximately 1.5-fold. These findings suggest that the FS-enhanced material holds potential for cartilage tissue engineering. Similarly, Kazemnejad *et al.*[118] developed a chondrocyte-seeded scaffold. Subcutaneous implantation experiments in mice demonstrated that the scaffold exhibited an appropriate *in vivo* degradation rate and regeneration capacity. The chondrocyte-seeded scaffolds effectively repaired most cartilage defects after 36 weeks, underscoring the potential for cartilage engineering.

Combining FS with hydrogels merges their respective material and biological advantages, enhancing cartilage repair scaffolds. Weitkamp *et al.*[119] created a porous 3D FS matrix embedded with chondrocytes, which was then immersed in a tyrosine-modified hyaluronic hydrogel, enhancing cartilage induction and biomechanics. Mirahmadi *et al.*[120] incorporated chopped FS and electrospun FS into thermosensitive chitosan/glycerophosphate hydrogels, fabricating a transparent scaffold for cartilage regeneration. FS enhancement notably improved the mechanical properties of the scaffolds. Both scaffold types preserved the chondrocyte cartilage phenotype, with significantly increased glycosaminoglycan content in the FS-hydrogel and notably higher collagen II expression in electrospun FS-hydrogel. Kim *et al.*[121] devised a bilayered polyethylene glycol (PEG) hydrogel for cartilage repair. The composite hydrogel featured a high-density PEG top later with a compression modulus of 700.1 kPa and a 3D FS-reinforced low-density PEG bottom layer with a compression modulus of 13.2 kPa. FS incorporation ensured robust interfacial bonding. The 3D FS constructs achieved a modulus of 567 kPa, with covalently bonded layers ensuring stability against torsion. The bottom layer promoted controlled degradation and cartilage formation. This research advances the potential of composite hydrogels for joint cartilage reconstruction, with ongoing animal studies exploring further applications.

Finally, studies confirm the potential of FS-based scaffolds for osteochondral defect repair. Yao *et al.*[122] innovatively integrated PLLA porous microspheres into FS scaffolds, forming millimeter-scale channels through physical drilling. This technique was applied to ear cartilage regeneration. The composite scaffold exhibited remarkable mechanical strength. *In vitro* experiments demonstrated that the FS+PLLA PMs porous microspheres (CMAF) scaffold enhanced cartilage cell proliferation, migration, and extracellular matrix (ECM) production. Furthermore, *in vivo* experiments confirmed that CMAF scaffolds exhibited superior cartilage formation ability and evenly deposited specific ECM components.

4.3. Soft tissue regeneration: tendon and ligament

Tendons and ligaments are vital dense connective tissues characterized by exceptional strength and toughness.[123] Tendons, as extensions of muscle, connect muscles to bones and are primarily composed of tightly packed collagen fibers surrounded by cells. Their primary function is to transmit muscle-generated forces to bones, thereby enabling movement.[124] Conversely, ligaments are located between bones and surrounding joints, where they enhance joint stability. Ligaments are composed of intricate fibrous tissue that restricts the direction of joint movement and absorbs mechanical shocks. Both tendons and ligaments can be damaged by leverage, excessive stress, muscle contraction, joint activity, or trauma, causing joint instability and abnormal movement.[125]

Altman *et al.*[62] employed FS to fabricate artificial ligaments by bundling single fibers to mimic the structure of ACL. Notably, the mechanical strength of these constructs matched that of natural ACLs. Further experiments demonstrated that FS scaffolds supported the proliferation, differentiation, and migration of BMSCs and exhibited key protein markers characteristic of natural ACLs, including collagen I, collagen III, and tenascin C.[126] Teuschl *et al.*[127] fabricated FS scaffolds, seeded them with cells, and performed ACL resections on 33 goats, randomly divided into two groups. Histological analysis at 6 and 12-month post-surgery revealed connective tissue growth around the FS scaffold. After 6 months, the seeded-cell group exhibited reduced FS material and increased tissue growth compared to the unseeded group. After 12 months, FS density had significantly decreased in both groups, accompanied by pronounced inward tissue growth. Notably, FS degradation and tissue regeneration were comparable between groups.

Fan *et al.*[128] successfully established an ACL regeneration model in pigs using mesenchymal stem cells (MSCs) seeded on FS scaffolds. *In vitro*, MSCs proliferated effectively, differentiated into fibroblast-like cells, and expressed ligament-specific genes (collagen I, collagen III, and tenascin C). Post-implantation, fibroblast-like morphology was observed at 24 weeks, along with significant production of ligament-specific ECM. In addition, a ligament-to-bone insertion comprising bone, Sharpey's fibers, and ligament zones was formed. Despite scaffold degradation, the regenerated ligament maintained tensile load at 24 weeks, indicating the promising potential of FS-based, cell-seeded scaffolds for ligament repair and regeneration.

In another study, Panas-Perez *et al.*[129] investigated ACL reconstruction using a collagen-FS scaffold. When FS content was ≥14% and collagen content ≤86%, the scaffold's initial tensile strength matched or surpassed that of human ACL. After 8 weeks in a rabbit model, the scaffold's strength reduced by 84 – 92%, while its volume decreased by 22 – 26%. Mechanical testing indicated that scaffolds with an ultimate tensile strength ≥129 MPa and an FS-to-collagen volume ratio of approximately 48:52 met the requirements for ACL reconstruction. Chen *et al.*[130] further applied collagen-FS scaffolds for medial collateral ligament repair, demonstrating enhanced medial collateral ligament tissue regeneration. In addition, DiSCAFF technology was employed to coat FS fibers with a HEMA-co-METAC hydrogel, enhancing their mechanical properties and improving stem cell adhesion. Coated FS fibers also upregulated the mRNA expression levels of collagen I and III.[131]

Fang *et al.*[132] developed an FS-based tendon scaffold and confirmed its effectiveness through *in vitro* and *in vivo* studies. The scaffold promoted cell adhesion, proliferation, and new tendon formation after 16 weeks in mice. Furthermore, the internal collagen bundles of the newly formed tendon tissue exhibited uniformity and alignment. Chen *et al.*[133] introduced an FS/collagen scaffold loaded with human embryonic stem cells and BMSCs. Both *in vitro* and *in vivo* studies demonstrated that these cells differentiated into tendon lineages, successfully generating artificial tendon tissue, thus offering a promising approach for tendon tissue engineering. Zheng *et al.*[134] created a 3D macroporous FS scaffold specifically designed for shoulder muscle–tendon regeneration. The scaffold fostered a highly structured, tissue-like environment, boosting cell infiltration and differentiation into tendon cells. Finally, Kardestuncer *et al.*[77] cultured tendon cells on RGD-modified FS sutures. Compared to tissue culture plastic and unmodified FS, FS-RGD enhanced tendon cell adhesion, proliferation, and differentiation. Specifically, the adhesion force of tendon cells on FS-RGD sutures increased by 1.3-fold relative to control, and the transcription levels of collagen I and decorin were significantly higher after 6 weeks. Northern blotting analysis further confirmed that mRNA levels were 2 – 3 times greater on FS-RGD compared to tissue culture plastic.

4.4. Vascular tissue regeneration

Vascular diseases, including hypertension and myocardial infarction, pose a major threat to human life. The global burden and mortality from these diseases are projected to surge, with an estimated 23.4 million deaths forecasted by 2030.[135] At present, replacing diseased or narrowed vessels remains the most effective treatment, particularly highlighting the urgent demand for small-diameter vascular grafts.[136] Although autologous vessels are ideal, their use is limited by scarcity and donor site morbidity.[137-139]

To address the limitations of autologous grafts, research has increasingly focused on developing artificial blood vessels through vascular tissue engineering, particularly for small-diameter replacements.[140,141] Key design considerations for engineering grafts include resilience to cyclic blood pressure, compatibility with host vessels, and anti-thrombogenicity. Synthetic materials such as polyester and expanded polytetrafluoroethylene have been widely used in vascular surgery; however, they suffer from high hydrophobicity, promoting platelet and plasma protein adsorption while hindering endothelial cell adhesion and growth. In addition, their inability to grow, repair, or remodel elevates the risk of thrombosis and may induce immune responses, limiting their suitability for small-caliber (<6 mm) artificial blood vessels.[142,143] Compared to synthetic biomaterials, natural materials such as silk fibers, elastin, and collagen exhibit superior biocompatibility and cell compatibility, owing to their functional molecules and intricate structures.[28] These natural materials demonstrate anti-thrombotic properties and enhanced adaptability.[144] Specifically, FS promotes endothelial cell attachment, while SF provides anti-thrombotic surfaces capable of enduring high shear stress and blood flow pressure.[145]

In recent years, significant progress has been made in developing composite vascular grafts based on natural FS. Mi *et al.*[146] devised a novel three-layer vascular graft comprising an FS-woven inner layer, a polyacrylamide hydrogel middle layer, and a thermoplastic polyurethane nanofiber outer layer. FS's intricate weave mimics the structure of vascular collagen, while polyacrylamide and thermoplastic polyurethane replicate the elasticity of elastin, thereby enhancing biocompatibility with endothelial cells and mechanical strength and addressing issues of rejection and leakage observed with traditional grafts. Nakazawa *et al.*[147] created an alternative vascular graft by weaving and compressing FS onto polymer tubes, followed by an SF coating, to foster cell adhesion and proliferation during vascular repair. Ding *et al.*[148] employed finite element analysis to simulate graft deformation and compliance, developing vascular grafts with woven FS skeletons of three pore sizes. His study found that grafts with intermediate pore sizes provided optimal mechanical properties and smooth muscle cell regeneration. Li *et al.*[149] successfully assembled silk nanofibers in formic acid using a microfluidic chip that mimics the geometry of a silkworm gland. These fibers exhibited aligned structures, enhanced mechanical properties, and promoted cell proliferation. In addition, they demonstrated the ability to modulate

cell behavior, highlighting their potential for extensive applications in vascular tissue regeneration.

In summary, natural FS-based composite vascular grafts possess immense potential due to their superior biocompatibility and mechanical properties. These advancements offer promising solutions for the fabrication of small-diameter artificial blood vessels and address challenges such as graft scarcity and donor site morbidity.

4.5. Bone tissue regeneration

Bone is a specialized connective tissue composed of a calcified matrix, consisting of approximately 70% inorganic material (primarily hydroxyapatite) and 30% organic material (mainly collagen I).[150] It provides structural support for the body extremities and protection for internal organs.[151] Bone defects, resulting from trauma, infections, tumors, and other conditions, present significant clinical challenges by impairing physiological functions.[152] Timely and effective repair of bone defects is therefore crucial.

Autologous bone grafting remains the gold standard for bone repair; however, its clinical application is limited by graft availability and infection risk.[153] Although allogeneic bone is more readily available, it is associated with antigenic issues, high costs, immune rejection, and safety concerns.[154] Artificial bone substitutes have thus emerged as alternatives, aiming to restore normal anatomical and physiological functions.[155]

Bone tissue engineering seeks to develop materials that meet several key requirements:[156] (i) mechanical properties comparable to human bone, (ii) adaptability to diverse bone shapes, (iii) osteoinductivity to stimulate stem cell differentiation, (iv) osteoconductivity to support new bone growth, and (v) seamless integration with existing bone tissue. Although many materials partially fulfill these criteria, silk fibers, with their unique biocompatibility, mechanical strength, and structural plasticity, are increasingly recognized as promising candidates for bone tissue engineering.[28] Future work is expected to further unlock the potential of silk-based materials in this field.

Compared to SF, the application of FS in bone repair remains underutilized. Researchers often convert FS into a solution for scaffold printing. Studies have revealed that incorporating FS into CPC boosts mechanical strength by leveraging the principle of fiber reinforcement. Zhou *et al.*[85] explored the application of SF in bone repair by mixing FS and CPC at a 1:20 ratio to fabricate bio-composite cylinders (4 mm in diameter and 15 mm in length) for animal testing. The results indicated that FS significantly enhanced the mechanical and biological properties of the composite, promoted osteogenesis, and effectively repaired bone defects.

Similarly, Mobini *et al.*[157] incorporated FS into a regenerated SF matrix and produced composite scaffolds through freeze-drying. Evaluations through electron microscopy, mechanical testing, and *in vitro* cell assays were conducted to assess the properties of these composites. Scanning electron microscopy revealed an interconnected porous structure that fostered cell adhesion and growth. Mechanical experiments revealed that FS addition increased the compression modulus and compressive stress. Furthermore, human MSCs cultured on the composite scaffolds exhibited enhanced adhesion, proliferation, and osteogenic differentiation, positioning the material as a promising candidate for bone tissue engineering research. Moreover, undegummed raw FS has shown research value in the development of apatite-organic polymer hybrids. Takeuchi *et al.*[158] explored apatite deposition on organic surfaces, including raw and degummed FS, under physiological conditions. Their results indicated that the sericin present in raw FS promoted greater apatite deposition compared to degummed FS, suggesting its potential for developing bone-mimicking polymers with improved bonding and mechanical properties through biomimetic approaches.

4.6. Antimicrobial activity

FS exhibits high plasticity, exceptional breathability, safety, non-toxicity, and wound-healing properties, making it suitable for use in skin wound dressings.[95] Prior research has confirmed FS's antimicrobial properties, demonstrating its ability to inhibit microbial growth.[159] Academic studies have further highlighted FS's notable antifungal properties. Key bioactive components – such as Kunitz-type BmSPI 51, TIL-type BmSPI 38, BmSPI 39, and phosphatidylethanolamine-binding protein – have been shown to significantly inhibit fungal spore growth *in vitro*.[160-165] In addition, FS demonstrates broad-spectrum antimicrobial efficacy against bacteria, viruses, and other pathogens. FS contains two serotonin derivatives (serotonin 1 and serotonin 2) that hinder pathogen growth, thereby broadening its antimicrobial spectrum.[31,166-169] Enzymes such as phenol oxidase and peroxidase contribute to pathogen elimination through the generation of reactive by-products.[170] In addition, non-organic acids, alkaloids, and flavonoids present in FS contribute to its robust antimicrobial activity.[166,171,172] Crude sericin extracted from FS, especially from the outer cocoon layer, has demonstrated potent antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*.[173]

Furthermore, FS serves as a versatile platform for antimicrobial material design, enabling tailored functionalization. Researchers have enhanced FS's antimicrobial capacity through various chemical and

physical approaches to produce composites and modified silk materials for medical applications.[174-176] These techniques aim to improve FS's antimicrobial efficacy, opening new avenues for medical material applications.

In the preparation of antimicrobial silk composites, a combination of synthetic and natural agents (e.g., quaternary ammonium compounds, inorganic nanomaterials, chitosan, and bioactive substances) has been incorporated with FS to enhance antimicrobial performance. For example, Zhang *et al.*[177] used ionic interactions to coat FS with 0.5% tannic acid, resulting in durable antibacterial activity suitable for medical applications. Similarly, Zhao *et al.*[178] demonstrated that FS chemically modified with sodium alginate–AgNPs exhibited robust antibacterial activity against *E. coli* and *S. aureus*. Li *et al.*[179] developed a de-gummed FS/nano-hydroxyapatite/polylactic acid scaffold infused with nanosilver, which exhibited both mineralization potential and notable antibacterial properties *in vitro*. Liu *et al.*[180] utilized FS as a biotemplate for *in situ* integration of Fe_3O_4 and Ag nanoparticles, producing Ag–Fe_3O_4–SF composites with strong antibacterial effects against *E. coli* and *S. aureus*, offering the potential for water disinfection applications. In a separate study, Li *et al.*[181] enhanced FS's antibacterial properties by chemically attaching nano TiO_2–Ag particles, suggesting that both nano TiO_2–Ag and nano zinc particles could further improve FS's antibacterial attributes. Valarmathi and Sumathi[182] employed electrospinning to fabricate FS-based fiber composites incorporating methyl cellulose and zink–hydroxyapatite, with tests confirming that zink–hydroxyapatite significantly improved the composite's antibacterial activity.

Medical supplies derived from antibacterial FS composites offer valuable solutions for hospital sterilization, textile production, and environmental purification. These materials, endowed with inherent antibacterial properties, can also serve as effective skin wound dressings. Li *et al.*[17] introduced natural antibacterial FS membranes – also referred to as flat silkworm cocoons (FSCs) – tailored for wound care applications. They integrated ε-polylysine (EPL) onto FS membranes through hot pressing, thereby enhancing their antibacterial efficacy. Testing revealed that FSC/EPL exhibited potent antibacterial activity against *E. coli* and *S. aureus* without the use of antibiotics, thereby hindering bacterial growth and mitigating the risk of antibiotic resistance. In another study, Li *et al.*[181,183] introduced an effective technique for chemically bonding pre-modified TiO_2 and TiO_2@Ag nanoparticles onto FS fabrics. The modified fabrics, particularly those enhanced with TiO_2@Ag nanoparticles, exhibited robust UV-blocking capabilities and antibacterial activity against *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa*. These findings offer valuable insights into the development of next-generation antibacterial medical textiles and the continued advancement of medical supply innovation and hospital infection control practices.

Moreover, FS modified through artificial feeding has shown enhanced antibacterial performance. Zhang *et al.*[183] encapsulated glucose around silver nanoparticles, applied the solution to mulberry leaves, and fed them to silkworms. The resulting FS exhibited antibacterial rates ranging from 72.5% to 95.9% against *E. coli* and from 50.8% to 95.9% against *S. aureus* when cultured with bacteria. Notably, the antibacterial effect was positively correlated with the concentration of silver nanoparticles, especially against *E. coli*, indicating a dose-dependent response. The study demonstrated that artificial feeding can alter the secondary structure of FS, significantly improving its antibacterial performance.

5. Three-dimensional printing of FS -based biomaterials

Three-dimensional printing, also known as AM, is defined as "a process of creating objects by joining materials layer by layer, based on 3D model data, as opposed to traditional subtractive manufacturing methods."[188] In recent years, 3D printing technology has rapidly advanced, finding applications across diverse fields ranging from automotive engineering to organ transplantation.[189-192] Due to its excellent biocompatibility, biodegradability, and integration with host tissues, FS has been extensively studied as a scaffold material in tissue engineering and regenerative medicine. The advent of 3D bioprinting has further revolutionized these fields by enabling the high-precision and repeatable fabrication of intricate biological structures. FS-based bioinks exhibit tremendous potential in 3D bioprinting due to their favorable printability, mechanical robustness, and cytocompatibility.[14] Over the past four decades, various 3D printing methods have been adapted for FS processing.[14,193] The three primary techniques currently used in bioprinting include: (i) inkjet 3D printing, (ii) extrusion-based 3D printing (e.g., fused deposition modeling and direct ink writing), and (iii) light-based 3D printing (e.g., stereolithography and digital light processing [DLP]).

5.1. Inkjet 3D printing

Inkjet printing is a liquid-phase deposition technique that operates by ejecting picoliter-scale droplets from a nozzle to precisely coat a substrate.[194-196] As early as 2006, Limem *et al.*,[197] from the group led by Kaplan, utilized inkjet printing to deposit a 0.6% (w/w) FS solution onto

transparent vinyl substrates in parallel line patterns. Human MSCs seeded on these patterns aligned along the FS lines and differentiated into osteoblasts, with cell bridging observed between lines spaced <1.25 mm apart after 4 weeks.

However, inkjet printing faces significant limitations in 3D bioprinting, primarily due to the narrow nozzle diameter and strict viscosity requirements of the bioinks. These constraints pose challenges for efficient cell loading and can result in thermal or mechanical stress, reducing cell viability.[198] Consequently, the range of suitable bioinks for inkjet printing remains limited. While FS's naturally low viscosity allows it to be printed without prior modification, post-treatment is typically required to stabilize the printed architecture. Rider *et al.*[199] developed an FS-based dental barrier membrane using inkjet printing and methanol treatment to induce rapid β-sheet formation. However, methanol exhibited cytotoxic, making it unsuitable for direct cell encapsulation.

To overcome this issue, Compaan *et al.*[200] introduced a methanol-free crosslinking strategy, combining FS with alginate to produce a low-viscosity bioink. Calcium ions were used to induce temporary alginate gelation, followed by horseradish peroxidase-mediated covalent crosslinking of FS, resulting in stable 3D scaffolds. This method enabled successful cell encapsulation and proliferation, significantly enhancing the feasibility of FS-based inkjet bioprinting. Despite being one of the earliest bioprinting methods, the use of FS bioinks in inkjet printing remains limited due to functional crosslinking challenges and nozzle-induced cell viability concerns.

5.2. Extrusion 3D printing

Extrusion-based 3D printing is a widely used method for fabricating both non-biological and biological structures, particularly due to its compatibility with bioinks across a broad viscosity range. This technique often involves co-printing two materials: a highly viscous component for mechanical support and a low-viscosity component to foster cell growth and proliferation.[201] The printing process entails loading the bioink or printing solution into a reservoir connected to a nozzle, from which the material is extruded layer by layer under controlled temperature conditions and solidified on the printing platform. Similar to inkjet printing, rapid and controlled solidification is required to maintain the structural integrity of the printed constructs.[202]

Ghosh *et al.*,[203] from the group led by Lewis, was among the first to apply extrusion-based printing to FS, successfully printing 28% – 30% FS solutions into square and mesh-like architectures. However, FS solutions exhibit shear-thickening behavior at shear rates above 100 s^{-1} due to β-sheet transitions, which can lead to nozzle clogging. To address this, materials with shear-thinning properties are preferred.[201] To mitigate shear-thickening during the printing process, researchers have explored various rheological modifications. Chawla *et al.*[204] developed FS–gelatin composites with optimized flow properties, while Das *et al.*[205] screened multiple FS–gelatin formulations to identify those that balance flowability with rapid gelation, minimizing clogging under high shear conditions.

A critical concern in extrusion-based 3D printing is whether functional additives introduced into FS bioinks compromise cell viability. Zheng *et al.*[206] addressed this concern by incorporating low molecular weight PEG 400 to promote β-sheet formation, thereby stabilizing FS bioinks. Their findings demonstrated that the modified bioink maintained biosafety without adversely affecting cell viability. Furthermore, FS–PEG 400 composites co-printed with hMSCs supported sustained cell proliferation within the 3D constructs for up to 15 days. In another study, Jose *et al.*[207] enhanced the stability of FS bioinks by incorporating glycerol and calendula alcohol during the printing process, achieving excellent cellular viability. Similarly, Rodriguez *et al.*[208] developed a novel freeform printing strategy using a PEG 400–laponite support bath. In this system, PEG 400 facilitated β-sheet formation, while synthetic laponite stabilized the ink and maintained structural integrity. Notably, synthetic laponite exhibited no cytotoxicity and supported over 90% cell viability.[206]

Collectively, these studies demonstrate that functionalized and additive-enriched FS bioinks can retain high biocompatibility, validating their utility in cell-laden extrusion-based 3D bioprinting.

5.3. Light-based 3D printing

Light-based bioprinting has recently emerged as an innovative technique in the field of 3D bioprinting, garnering significant attention from materials science researchers due to its ability to fabricate structures with high spatial resolution and accuracy. This technique involves the use of a light source within the printer to cure photosensitive resins, allowing for layer-by-layer or point-by-point construction of 3D models.[209-211] The two principal light-based 3D printing techniques currently employed are laser-induced forward transfer (LIFT) and DLP.

LIFT operates through single-pulse laser irradiation that triggers photochemical crosslinking within bioinks and ejects individual droplets onto a receiving substrate.[212] In contrast, DLP projects patterned light through a digital micromirror device to initiate spatially controlled photopolymerization.[213] In LIFT, the laser pulse

instantaneously heats a thin film beneath the donor layer, propelling droplets of high-viscosity biomaterials – such as FS hydrogels – with submicron precision. This enables the construction of intricate microarchitectures and multi-cellular composite constructs while preserving high cell viability. DLP, by comparison, offers rapid, spatially controlled solidification of photosensitive FS-based bioinks, achieving fast printing speeds and highly accurate scaffold geometries. It is particularly suitable for producing bone, cartilage, and neural tissue scaffolds.

LIFT offers several unique advantages for complex tissue engineering. For example, in neural tissue applications, LIFT has been used to deposit neural stem cell-laden FS bioinks to generate aligned scaffolds that promote directional axonal growth and support functional recovery. In addition, LIFT has been applied to fabricate microvascular networks by precisely patterning endothelial cells and FS, achieving a synergistic integration of biological activity and structural fidelity. A key advantage of LIFT is its nozzle-free mechanism, which avoids shear stress and nozzle clogging – common issues in conventional nozzle-based printing – making it especially advantageous for printing highly viscous or cell-laden FS-based inks.[214]

Since unmodified FS lacks photocrosslinkable functional groups, it is not directly suitable for DLP printing.[215] To meet the photocuring requirements of DLP, FS has been chemically modified through methacrylation to produce silk methacrylate (Sil-MA), significantly enhancing its printability in light-based systems. Sil-MA bioinks exhibit favorable rheological behavior and tunable crosslinking kinetics, resulting in excellent shape fidelity and structural stability during printing. Experimental studies have demonstrated that Sil-MA scaffolds effectively support the encapsulation, proliferation, and functional expression of various cell types, including chondrocytes, osteoblasts, and endothelial cells. These properties make Sil-MA a promising FS-based platform for applications in bone, cartilage, corneal, and vascular tissue engineering.

5.4. Technical challenges and future directions

Despite the significant promise of FS-based 3D printing in regenerative medicine, several challenges hinder its clinical translation. First, the current range of FS bioinks remains limited in both diversity and function, restricting their application in the engineering of multicellular and architecturally complex tissues. To address this, future efforts must focus on chemical modifications, incorporation of ceramic or conductive components, and the development of gradient formulations to enhance mechanical performance and biological function. Second, the degradation kinetics of FS scaffolds must be precisely synchronized with the rate of tissue regeneration to prevent premature material failure or prolonged scaffold persistence, which may lead to chronic inflammation or fibrosis. Further, achieving high print fidelity, maintaining structural integrity post-printing, and ensuring long-term shelf stability remain key technical hurdles.

On the commercialization front, regulatory pathways are often slow, fragmented, and inconsistent. Although over 700 FS-related studies are published annually, only a small fraction progress to clinical trials. To date, few FS-derived products – such as Silk Voice – have received approval from the United States Food and Drug Administration, and these are typically in powder form, limiting their suitability for implantable tissue-engineered constructs.

To advance FS bioprinting toward clinical translation, three key areas must be addressed: (i) scalable and standardized bioink production, with modular design and reproducible print performance; (ii) establishment of unified regulatory frameworks and expedited approval processes; and (iii) integration with cutting-edge technologies such as stem cell engineering, organ-on-a-chip platforms, and microfluidics. Through innovations in these areas, FS-based biomaterials may enable a paradigm shift from structural repair to true biological regeneration.

6. Limitations and prospects of FS -based biomaterials

To provide a balanced view of the biomedical advantages and challenges of FS materials, we summarize their key strengths and weaknesses in Table 3. For further context, Table 4 offers a detailed comparison of FS with other commonly used biomaterials, including collagen and synthetic polymers, based on parameters such as mechanical properties, biocompatibility, degradation behavior, cost, and scalability. These tables serve as a foundation for the following discussion on the current limitations and future prospects of FS-based biomaterials.

6.1. Limitations of FS -based biomaterials

The long-term *in vivo* stability of FS scaffolds is critically influenced by their degradation behavior and compatibility with tissue regeneration timelines. Although the β-sheet structure of FS confers slow degradation (typically exceeding 12 weeks), exposure to dynamic mechanical environments (such as joints or blood vessels) can cause material fatigue or disruption of crystalline domains, ultimately compromising structural integrity. For example, FS scaffolds implanted in the ACL of goats exhibited significant mechanical strength degradation after 12 months, despite incomplete material resorption. In addition, the size and chemical composition of FS

Table 3. Summary of advantages and disadvantages of fibrous silk in biomedical applications

Advantages	Disadvantages
Excellent and tunable mechanical properties (high tensile strength and ductility)	Insufficient mechanical strength in certain contexts compared to synthetic materials such as polylactic acid or polyetheretherketone
Superior biocompatibility and low immunogenicity	Unclear long-term *in vivo* safety; potential toxicity from degradation products requires investigation
Good biodegradability with adjustable degradation rates	Precise degradation control is challenging due to environmental and structural variability
Excellent antibacterial and antiviral properties	Antibacterial properties may diminish when combined with other materials or under long-term applications
Structural diversity and functional tunability	Lack of standardization and certification pathways hinders regulatory approval, particularly in AM-based products
Good compatibility with various biomaterials	Processing complexity in additive manufacturing may result in issues such as incomplete crystallization, insufficient crosslinking, and unsatisfactory material uniformity.
Broad potential in biomedical applications (e.g., tissue engineering, drug delivery, wound healing)	High production cost relative to synthetic alternatives, especially for purification and functionalization steps
Eco-friendliness and sustainability	Raw material supply is limited by silkworm species and feeding conditions, impacting yield consistency and stability

Table 4. Comparison of fibrous silk, collagen, and synthetic polymers (polylactic acid [PLA]/polycaprolactone [PCL]) in biomedical applications

Criteria	Fibrous silk	Collagen	Synthetic polymers (PLA/PCL)
Mechanical properties	High tensile strength, good ductility	Low tensile strength, rapidly degradable	Broadly tunable, moderate strength
Degradation rate	Slow (usually > 12 weeks), suitable for long-term support	Fast (days to weeks), suitable for short-term applications	Tunable (weeks to months), varies with structure and copolymer ratio
Biocompatibility	Excellent, with low inflammatory response	Excellent, naturally derived, minimal immune rejection	Good; may cause mild to moderate inflammation
Immunogenicity	Low post-degumming, though sericin residues or degradation products may elicit responses	Extremely low (especially in human-derived forms)	Potential risk depending on synthesis and degradation byproducts
Antibacterial properties	Lacks intrinsic activity but can be enhanced (e.g., with ε-polylysine)	None; typically requires antibiotics	Typically non-antibacterial; requires functional additives
Formability (e.g., 3D Printing)	Compatible with hydrogels/composites; under exploration	Poor; not suitable for complex structures	Good thermal processability; suitable for 3D printing, injection molding, etc.
Cost	Medium to high; limited by extraction/purification/recombinant techniques	Low; mature extraction technology	Low to medium; inexpensive raw materials and mature scale-up production
Scalability	Technical bottlenecks exist; recombinant fibrous silk production is still under development	High industrial maturity; established raw material supply	Industrialized and mature; stable supply chain
Structural modifiability and functionality	High; easy to modify (e.g., growth factors, conductivity)	Moderate; limited by structure stability	High; molecular design flexibility, easy functional integration
Clinical prospects	Broad potential, especially in soft tissue and bone regeneration	Extensively used (e.g., skin grafts, soft tissue, cardiac tissue engineering)	Already implemented in degradable scaffolds, sutures, drug delivery systems, etc.

degradation byproducts may trigger chronic inflammation or amyloid deposition, highlighting the need for further investigation into their long-term biosafety.

From a mechanical standpoint, natural FS has inherent limitations. While its tensile strength surpasses that of collagen and polylactic acid, it remains inferior

to synthetic materials such as polyether ether ketone or Kevlar. Regenerated FS materials, such as films or solution-derived scaffolds, demonstrate even poorer mechanical performance (elongation at break <2%) due to the disruption of native secondary structures during processing. Although advanced techniques such as genetic engineering and nanocomposite reinforcement (e.g., graphene enhancement) have shown promise in improving FS performance, the complexity and cost of large-scale manufacturing remain substantial barriers to clinical translation.

Although degummed FS is generally considered immunologically inert, residual sericin or degradation fragments can still elicit mild inflammatory responses. The long-term toxicity of FS in the human body remains insufficiently characterized, particularly in cases involving prolonged implantation, where degradation fragments may trigger chronic inflammation, fibrosis, or amyloid deposition. Most current *in vivo* studies are limited to small animal models – such as rodents (mice and rats) and rabbits – with experimental durations typically spanning several weeks to months, focusing primarily on early-stage tissue regeneration. For example, Fan *et al.*[128] conducted a 24-week study using FS scaffolds for ACL repair in pigs, while Zhou *et al.*[85] reported bone regeneration outcomes after just 4 weeks. Although these studies support the initial biocompatibility and regenerative potential of FS, longer-term experimental data (e.g., over 1 year), especially from large animal models such as goats or dogs, remain scarce. Therefore, further exploration of the long-term safety and toxicity of FS in the human body is essential.

Standardization and certification of FS materials for medical applications present additional challenges, particularly within the context of AM technologies. The lack of a unified regulatory framework contributes to prolonged and complex approval processes. In addition, natural silk production is subject to biological variability due to differences in silkworm species and breeding conditions, leading to inconsistent material quality. While the incorporation of FS with other materials (such as conductive carbon nanotubes or growth factors) can enhance functionality, the long-term compatibility and stability of these multi-material interfaces require further optimization. For instance, silver nanoparticle-loaded FS dressings may exhibit diminished antibacterial efficacy over time due to uneven silver ion release.

6.2. Future prospects of FS-based biomaterials

FS materials offer substantial potential for clinical translation, as evidenced by promising results in various animal models. Studies utilizing FS combined with CPC composites for rabbit bone defect repair have demonstrated excellent bone regeneration. FS also exhibits significant advantages in tendon, ligament, and soft tissue regeneration. For example, Zhou *et al.*[85] applied FS/CPC composites to treat rabbit radial bone defects and, after 4 weeks, observed new trabecular bone formation with significantly improved mechanical properties compared to the control group. In another study, Fan *et al.*[128] used FS scaffolds loaded with MSCs to repair ACL in pigs. After 24 weeks, the regenerated ligament exhibited collagen alignment and mechanical strength comparable to that of natural tissue. Li *et al.*[17] developed ε-polylysine-modified FS membranes that demonstrated excellent antibacterial and wound-healing properties in animal models, without the need for antibiotics.

Optimizing the mechanical properties, degradation rates, and biological activity of FS materials through biofunctional modifications, composite strategies, and tailored designs for specific medical applications has expanded their potential in bone repair, nerve regeneration, and skin wound healing. However, future research should focus on designing application-specific FS-based biomaterials and conducting long-term *in vivo* studies in large animal models to comprehensively evaluate their safety and effectiveness, thereby supporting clinical translation.

Advancements in AM and 3D printing technologies will be critical for enhancing FS's mechanical properties, structural design, and bioactivity. Integrating FS with other biomaterials (such as hydrogels, nanomaterials, polycaprolactone, or graphene) can yield multifunctional composites with enhanced clinical performance. Furthermore, the precision enabled by 3D printing facilitates the development of personalized implants and smart medical devices (such as electronic textiles) based on FS, providing tailored solutions across clinical needs. This approach can expand FS's applications in tissue engineering, drug delivery, wound healing, and smart healthcare technologies.

7. Conclusion

FS has emerged as a highly versatile biomaterial due to its unique combination of mechanical strength, biocompatibility, tunable biodegradability, and antimicrobial activity. This review systematically examined the structure, properties, and biomedical applications of FS, with a special focus on its integration with AM technologies for tissue engineering, wound healing, and regenerative medicine. FS-based materials have demonstrated promising results in pre-clinical models for the regeneration of skin, cartilage, tendon, ligament, vasculature, and bone. Despite these advances, several limitations remain, including the need for improved

mechanical properties in regenerated forms, incomplete understanding of long-term biosafety, and challenges in standardization and large-scale production. Future research should prioritize the development of multifunctional, application-specific FS composites, long-term *in vivo* validation in large animal models, and regulatory pathways to facilitate clinical translation. With continued innovation in biofunctionalization and 3D printing, FS is poised to play a significant role in the next generation of personalized and regenerative medical therapies.

Acknowledgments

None.

Funding

This study was supported by the National Clinical Medical Research Center of Orthopedics and Sports Rehabilitation Innovation Fund (2021-NCRC-CXJJPY-17) and the Clinical Research Incubation Program of Beijing Chao-Yang Hospital (CYFH202316).

Conflict of interest

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

Author contributions

Conceptualization: Yong Hai, Juan Guan, Yuzeng Liu, Mingzheng Zhao, Shixuan Guo, Fengqi Cheng, Wenhan Tian

Data curation: Mingzheng Zhao, Shixuan Guo, Fengqi Cheng, Wenhan Tian

Visualization: Fengqi Cheng, Wenhan Tian

Writing – original draft: Mingzheng Zhao, Shixuan Guo

Writing – review & editing: Yong Hai, Juan Guan, Yuzeng Liu

All authors approved the final version of the manuscript, contributed to the article, and approved the submitted version.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The original data from this study are publicly available on Google Scholar and can be accessed using the reference titles or DOI numbers.

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