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Review

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Application of silk fibroin coatings for biomaterial surface modification: a silk road for biomedicine

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Abstract: Silk fibroin (SF) as a natural biopolymer has become a popular material for biomedical applications due to its minimal immunogenicity, tunable biodegradability, and high biocompatibility. Nowadays, various techniques have been developed for the applications of SF in bioengineering. Most of the literature reviews focus on the SF-based biomaterials and their different forms of applications such as films, hydrogels, and scaffolds. SF is also valuable as a coating on other substrate materials for biomedicine; however, there are few reviews related to SF-coated biomaterials. Thus, in this review, we focused on the surface modification of biomaterials using SF coatings, demonstrated their various preparation methods on substrate materials, and introduced the latest procedures. The diverse applications of SF coatings for biomedicine are discussed, including bone, ligament, skin, mucosa, and nerve regeneration, and dental implant surface modification. SF coating is conducive to inducing cell adhesion and migration, promoting hydroxyapatite (HA) deposition and matrix mineralization, and inhibiting the Notch signaling pathway, making it a promising strategy for bone regeneration. In addition, SF-coated composite scaffolds can be considered prospective candidates for ligament regeneration after injury. SF coating has been proven to enhance the mechanical properties of the substrate material, and render integral stability to the dressing material during the regeneration of skin and mucosa. Moreover, SF coating is a potential strategy to accelerate nerve regeneration due to its dielectric properties, mechanical flexibility, and angiogenesis promotion effect. In addition, SF coating is an effective and popular means for dental implant surface modification to promote osteogenesis around implants made of different materials. Thus, this review can be of great benefit for further improvements in SF-coated biomaterials, and will undoubtedly contribute to clinical transformation in the future.

Key words: Silk fibroin; Coating; Surface modification; Notch signaling pathway

1 Introduction

Silk fibroin (SF) is a natural polymeric protein mainly derived from the cocoons of *Bombyx mori*. This protein has been widely applied in conventionally fabricated films, hydrogels, and scaffolds (Kumar and Singh, 2017). In essence, SF is a high-molecular weight protein complex comprising heavy chain (about 325 kDa) and light chain (about 25 kDa) that are held together with a disulfide bond at the C-terminus, encapsulating a glycoprotein (Lujerdean et al., 2022). The heavy chain is the major component of SF protein consisting of 12 hydrophobic crystallizable domains and 11 hydrophilic amorphous domains. The amino acid sequence of the heavy chain consists of repetitive Gly–Ala/Ser/Tyr dipeptides and hydrophobic blocks, packing into crystalline β-sheet domains, which are stabilized by inter-strand hydrogen bonding (Wenk et al., 2011). SF can self-assemble into larger fibrous structures by reforming the weak hydrogen bonds (Melke et al., 2016); this intramolecular fibroin self-assembly leads to strong physical interactions, which are responsible for the excellent mechanical properties of SF (Yucel et al., 2014). In addition to its great mechanical properties, silkworm SF has been proved to be highly biocompatible both in vitro and in vivo (dal Prà et al., 2004). Meanwhile, SF is susceptible to proteolytic degradation by various enzymes, and the

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resulting amino acids can be absorbed, which is favorable in biomedical applications (Melke et al., 2016). The SF coating is known to improve the hydrophilicity and chemical activity of the substrate material with minimal immunogenicity, thereby increasing its biocompatibility. The porosity of SF favors cell adhesion and induces cell migration into the substrate material pores, promoting wound healing, angiogenesis, and osteogenesis. Moreover, the SF coating enhances the corrosion resistance of metallic materials, owing to the better preservation of the conformation of β-sheet filaments and the ion-induced structural transition from random loops to Type I α-helices (Yang et al., 2015). Additionally, SF coatings can load other components onto the surface of the substrate material to increase its performance (Elia et al., 2015; Rnjak-Kovacina et al., 2015; Saha et al., 2019). The crystallizable domains provide a major control point for SF coatings. By manipulating the crystal form and content of these domains, the properties of SF-coated biomaterials may be controlled (Yucel et al., 2014). Thus, SF has significant advantages as a coating material for biomedical applications because it exhibits several desirable properties, such as slow degradability, remarkable mechanical strength and thermal stability, versatility in processing, and easy modification (Šišková et al., 2021).

In the past few years, SF-coated biomaterials have attracted increasing attention. By compiling the statistics of the articles searched from the PubMed database (https://pubmed.ncbi.nlm.nih.gov) using the keywords of "silk fibroin coating" and "biomaterials," it was found that the first report appeared in 2003, and the annual number of publications was less than 10 before 2012. The number increased continually after 2012 and reached 30 in 2021 (Fig. 1). This trend indicates that SF-coated biomaterials are becoming more popular in the field of biomedicine. Indeed, many recent studies have focused on applying SF coatings in bone, ligament, skin, mucosa, and nerve regeneration, and dental implant surface modification. In this review, we searched the databases of PubMed, Embase (https://www.embase.com), and Web of Science (https://www.webofscience.com) from Jan. 2016 to Oct. 2022 to identify eligible studies using the keywords "silk fibroin," "coating," and "biomaterial." Literature that used SF as a substrate material or blends rather than as a coating was excluded. The publication language was limited to English only. As shown in Table 1, we summarized 31 studies on SF coatings conducted over the past six years. It can be seen that SF coatings have been applied in several kinds of materials (metals and polymers usually) through different methods, such as dipping,

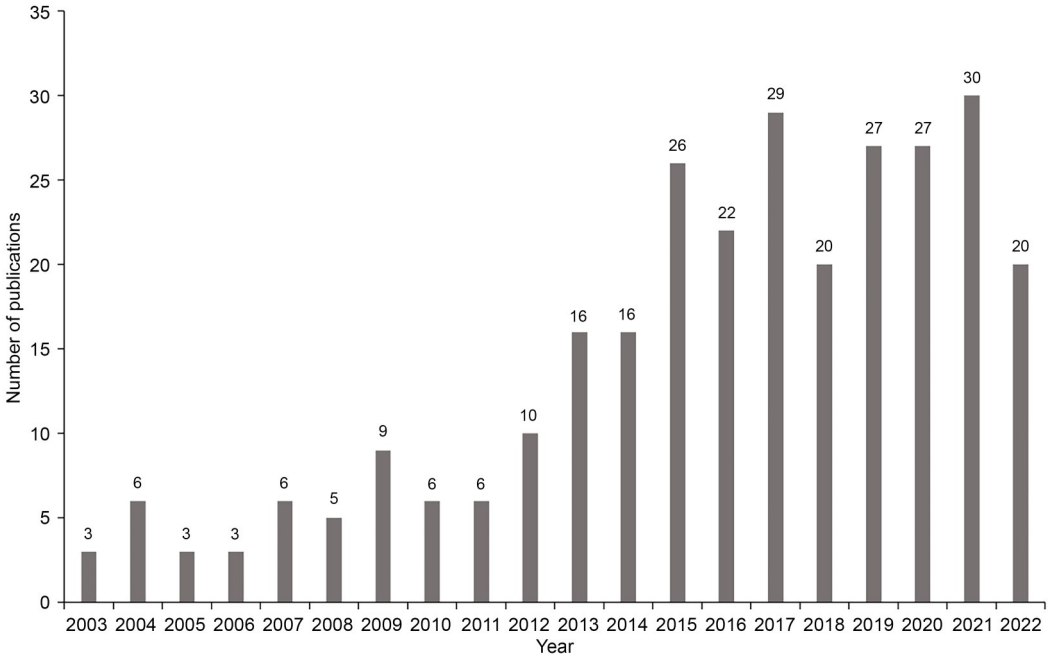

Fig. 1 Annual publications on SF-coated biomaterials since Jan. 1, 2003 to Dec. 31, 2022, with PubMed as the search engine and "silk fibroin coating" and "biomaterials" as the search terms.

Table 1 Coating composition, substrate material type, modification method, animal model, and application in studies on SF coatings

Coating composition	Substrate material type	Modification method	Animal model	Application	Reference
SF	Metal	Dip coating	Bone-defect diabetic sheep model	Biomedical implants	Ma et al., 2021
SF	Metal	Dip coating	NA	Bone regeneration	Watanabe et al., 2021
SF	Metal	Dip coating	NA	Bioresorbable stent	Xu et al., 2020
SF	Polymer	Dip coating	NA	Breast prosthesis implantation	Joseph et al., 2021
SF	Polymer	Dip coating	NA	Nerve regeneration	Ziemba et al., 2020
SF	Polymer	Dip coating	NA	Bone regeneration	Unalan et al., 2016
SF	Polymer	Dip coating	NA	Ligament regeneration	Jiang et al., 2016
SF	Polymer	Electrospinning	NA	Breast prosthesis implantation	Carrasco-Torres et al., 2019
SF	HAM	Electrospinning	NA	Skin regeneration	Arasteh et al., 2016
SF	SIS	LBL assembly	Subcutaneous-implantation rat model	Skin, mucosa, and ligament regeneration	Bi et al., 2020
SF	PULL microcarrier	Reductive amination	NA	Bone regeneration	Aydogdu et al., 2016
SF	Metal	EPD	NA	Dental implant surface modification	Qu et al., 2019
SF and TiO_2 nanotubes	Metal	EPD	NA	Dental implant surface modification	Saha et al., 2019
SF, polyglutamate, and polylysine	Cell surface	LBL electrostatic deposition	NA	Cell therapies, 3D printing, and preservation	Hasturk et al., 2020
SF and heparin disaccharide	Polymer	LBL assembly	Subcutaneous-implantation rat model	Wound healing	Qian et al., 2018
SF and gentamicin	Metal	LBL assembly	NA	Dental implant surface modification	Sharma et al., 2016
SF and polypyrrole	Polymer	Electrospinning	Sciatic nerve-dissected rat model	Nerve regeneration	Sun BB et al., 2019
SF and polyethylene oxide	Polymer	Electrospinning	NA	Breast prosthesis implantation	Valencia-Lazcano et al., 2018
SF and peptide	Metal	Spin coating	NA	Biomedical implants	Zhou et al., 2021
SF, HA, and a natural oxide	Metal	Spin coating	NA	Biomedical implants	Rahman et al., 2021
SF and peptide	Metal	Spin coating	NA	Bone regeneration	Sun YM et al., 2019
SF and peptide	Polymer	Spin coating	NA	Bone regeneration	Wang CY et al., 2018a
SF, fluoride, and silk-phytic acid	Metal	Spin coating	NA	Biomedical implants	Xiong et al., 2019a
SF, fluoride, and K_3PO_4	Metal	Spin coating	NA	Biomedical implants	Xiong et al., 2019b
SF, fluoride, Ca, and Sr/P	Metal	Spin coating	NA	Biomedical implants	Xiong et al., 2018
SF, AgNPs, and gentamicin	Metal	Dip coating	Bone-defect rabbit model	Biomedical implants	Zhou et al., 2020
SF and tibolone	Metal	Dip coating	Osteoporotic rat model	Dental implant surface modification	Barik et al., 2020
SF and VEGF	Polymer	Dip coating	ACL-reconstruction rabbit model	Nerve regeneration	Ai et al., 2017
SF, EDC, and NHS	Polymer	Dip coating	NA	Bone regeneration	Yao et al., 2016
SF and DOPA	Ceramic scaffold	Dip coating	NA	Bone regeneration	Wang et al., 2016
SF and PEI	Cellulose nanofibrous	Dip coating	NA	Tissue engineering	Ye et al., 2017

SF: silk fibroin; NA: not available; LBL: layer-by-layer; HAM: human amniotic membrane; SIS: small intestinal submucosa; PULL: pullulan; EPD: electrophoretic deposition; 3D: three-dimensional; HA: hydroxyapatite; AgNPs: silver nanoparticles; VEGF: vascular endothelial growth factor; ACL: anterior cruciate ligament; EDC: *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; NHS: *N*-hydroxysuccinimide; DOPA: dopamine; PEI: polyethylenimine.

spinning, electrospinning, layer-by-layer (LBL) assembly, and electrophoretic deposition (EPD) techniques, which are widely used in the field of biomedicine.

For application in bone regeneration, Wang et al. (2016) prepared a promising scaffold, wherein dopamine (DOPA) was introduced onto a strontium-doped calcium polyphosphate scaffold and subsequently modified by SF. The resulting SF-coated scaffold presented suitable biomechanical properties, angiogenesis-stimulating ability, and biocompatibility. For the application of ligament regeneration, Jiang et al. (2016) coated polyethylene terephthalate (PET) ligaments with SF, which enhanced the "ligamentization" process in vivo. Ye et al. (2017) developed a membrane based on cellulose nanofibrous substrates that were modified with multiple layers of positively charged polyethylenimine (PEI) and negatively charged SF. The results of mouse lung fibroblast cell culture indicated that the PEI/SF-coated membrane supported the adherence and spreading of cells, which demonstrated that this new membrane may be an ideal substitute for tissue engineering (Ye et al., 2017). Moreover, Ziemba et al. (2020) provided a novel method to increase the neuroregenerative capacity of electrospun scaffolds using thin-film SF coatings. In addition, Wang CY et al. (2018a) reported an osteopromotive polyetheretherketone (PEEK) implant coated with SF and bone-forming peptide, and their results showed that composite coatings can hasten the osteogenic differentiation and maturation of osteoblasts, suggesting the potential of SF coatings in orthopedics and dentistry.

Due to its tunable drug loading and release properties, SF has been used as a carrier for bioactive drug delivery in several therapeutic applications (Bayraktar et al., 2005; Tan et al., 2019). Notably, SF coating acts as a carrier of antibiotics for drug delivery and controlled release, and enhances the anti-inflammatory and anti-infective properties of composites, which is beneficial for the application of biomaterials. However, designing coatings that can accurately control the drug release kinetics remains a major challenge. Cheng et al. (2020) proposed a simple assembly strategy for EPD polymer coatings to improve drug release, which could prevent infections around transdermal orthopedic percutaneous orthopedic implants. Furthermore, a two-layer SF-coating system was constructed using sequential EPD technology. Dissolved *B. mori* SF molecules and pre-made SF nanospheres were used as substrates and then covered by a top layer of *Antheraea pernyi* SF molecules that are rich in arginine to improve the cellular response of the two-layer coating. The results demonstrated that the dose and kinetics of the loaded drugs can be controlled quantitatively by nanoparticle concentration and deposition time as the main processing parameters (Cheng et al., 2021). Overall, the above results indicate the high utility of SF as a novel controlled-release coating material (Cao et al., 2017).

Based on these data, SF coatings have great potential for the surface modification of biomedical materials. In this review, we demonstrated the isolation process of SF from *B. mori* cocoons, followed by the discussion of various methods for coating SF onto the substrate biomaterials. Furthermore, we reviewed the applications of SF coatings in biomaterial surface modification, which can be beneficial for the better transformation of SF coating into clinical practice (Fig. 2).

2 Preparation methods of SF coatings

2.1 Isolation of SF from *B. mori* cocoons

SF-coating biomaterials are fabricated using regenerated SF solutions by dissolution in aqueous or organic solvents (Qu et al., 2019). Silk is mainly composed of two proteins, SF (fibrous protein) and sericin (globular protein) (Melke et al., 2016). It has been verified that sericin can facilitate the formation of β-sheet (hydrophobic region), with a strong affinity for adsorption proteins to induce the long-term recruitment of inflammatory cells (Zhang et al., 2021; Boni et al., 2022). Hence, the preparation of SF from silk requires a degumming process to remove the sericin, in order to decrease the undesired immune response. Due to its higher hydrophilicity compared to SF, sericin can be easily removed by boiling silk in alkaline solutions (Altman et al., 2003). This degumming step eliminates inflammatory reactions and enables successful cell culture and biomaterial implantation. Further processing through dissolution in aqueous solutions or other solvents permits the production of regenerated SF (Yucel et al., 2014). A common method to isolate SF from *B. mori* cocoons and the way of SF coating facilitating bone regeneration are shown in Fig. 3. Nowadays, many researchers have improved and innovated ways of obtaining SF (Yamano et al.,

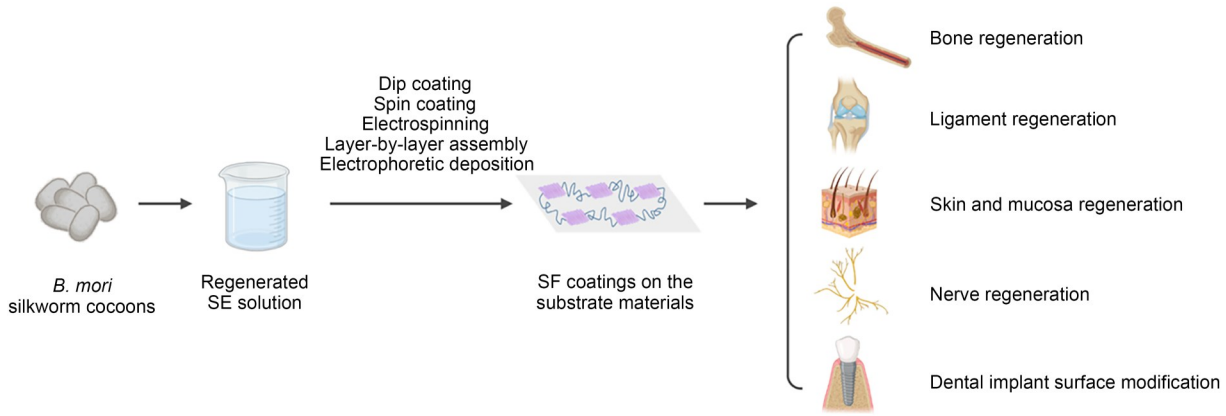

Fig. 2 The overview of application of SF coatings in the modification on biomaterial surface. *B. mori*: *Bombyx mori*; SF: silk fibroin.

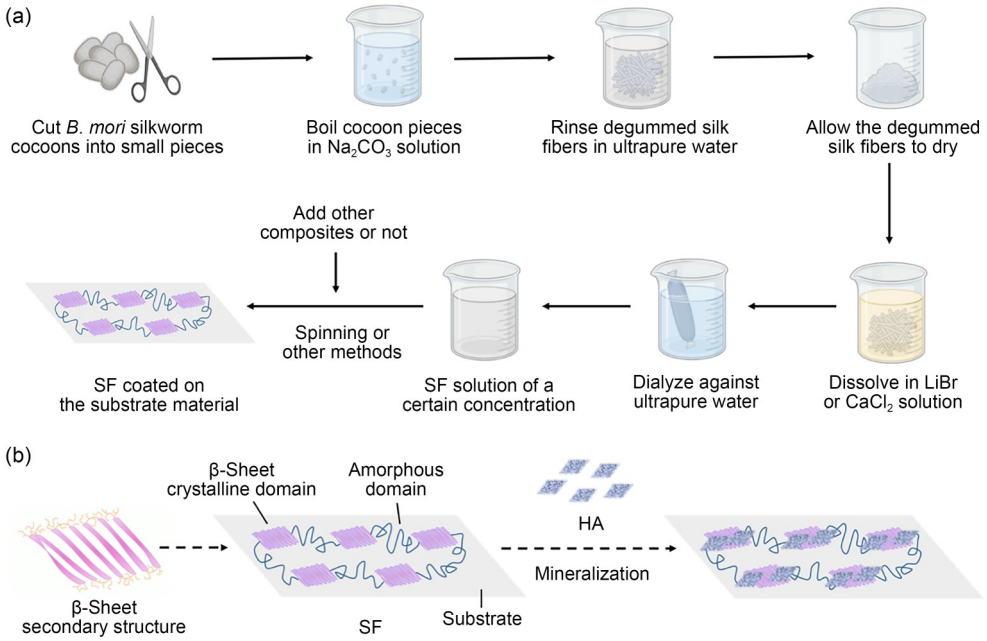

Fig. 3 A common method to isolate SF from *Bombyx mori* cocoons and the way of SF coating facilitating bone regeneration. (a) A typical method isolates SF from *B. mori* cocoons. (b) SF coating facilitates bone regeneration by promoting matrix mineralization owing to the β-sheet domains that provide nucleation sites for the deposition of HA crystals. SF: silk fibroin; HA: hydroxyapatite.

2022). A previous report presented silk powder fabrication from *B. mori* cocoons through cryogenic milling, and the results indicated that silk powders retained the dominant β-sheet structure. This new milled form of silk can form coatings without using harsh solvents, making them more biologically friendly (Baranowska-Korczyc et al., 2021). Zhu et al. (2022) reviewed environmentally friendly and green degumming processes of silk, including enzymes, supercritical CO_2, acids, steaming, and ultrasonic degumming. The applications of such degumming techniques, however, still carry major challenges. At the same time, reducing production cost and lower environmental impact will be crucial factors in widening the applicability of this material.

2.2 Methods for coating SF onto substrate biomaterials

Several techniques are available for coating SF onto the substrate biomaterials, including dipping, spinning, electrospinning, LBL assembly, and EPD techniques (Table 1), with each process having its

own advantages and drawbacks (Fan et al., 2018; Wang SG et al., 2018).

Although dip coating is a simple operation that can keep the active ingredient when the substrate material is a type of polymer, this method has several limitations, such as inadequate coating rate and the lack of precise control over the coating outcome (Barik et al., 2020; Ziemba et al., 2020; Joseph et al., 2021).

The traditional spinning technique is convenient for coating on metal substrates, particularly when other components are added to co-functionalize the substrates. Only a small amount of solution is required for spinning, the coating formation speed is higher compared to dip coating, and the fibrous structure is uniform. However, the strength and elasticity of the coating are lower than those of the electrospinning, and fiber breakage is common in the process, so that the continuity is relatively poor (Xiong et al., 2019b).

Electrospinning is a controllable process capable of spinning diverse materials to produce coatings with good uniformity, continuity, and porosity. SF coatings can be obtained as electrospun nanofibers through this technique (Valencia-Lazcano et al., 2018). However, the influencing factors of the electrospinning technique are much more complicated than those of the traditional spinning method, and the formation process may be unstable. That is, many parameters could affect the morphology and properties of the electrospun materials, including voltage, the distance between the spinneret and collector, the solution feed rate, the rotating speed of the drum collector, and ambient parameters such as temperature and humidity (Arasteh et al., 2016; Ojah et al., 2020).

Alternatively, SF molecules can be enriched on the biomaterial surface through hydrophobic–hydrophobic interactions to form an LBL self-assembled coating (Qiao et al., 2017). SF coatings can resist enzymatic hydrolysis, thereby defending the bioactivity of biomolecules and lengthening the residence time of the substrate materials. For example, Bi et al. (2020) observed that controlling the layer number (ten layers) of the SF-LBL coating provided a small intestinal submucosa (SIS) membrane with tunable degradation and mechanical retention properties. The LBL technique is easy to operate but is time-consuming, and the bonding strength of the two layers has yet to be verified (Hasturk et al., 2020).

EPD is advantageous for producing SF-coated biopolymers, because it homogeneously deposits SF on surfaces with complex geometries and efficiently controls the coating thickness and morphology (Luo et al., 2019). However, it may produce inner stress when preparing multilayer coatings; the coating produced is relatively thick and the adhesion to the substrate material is rather poor, which makes it easy to peel off the coating (Qu et al., 2019; Saha et al., 2019).

In recent years, some scholars have proposed new methods of SF-coating preparation. For example, Arkhangelskiy et al. (2021) developed a versatile room-temperature process for the deposition of SF on different materials. The coatings of thin SF films were achieved using an atmospheric plasma torch and the aerosol solution of SF as working gas. This technique enhances the adhesion of the protein to the substrate and can even be deposited on non-planar and flexible substrates (Arkhangelskiy et al., 2021).

Based on the nature of the substrate materials and the applications of the products, it is necessary to try different preparation methods and either choose the best one or combine several techniques.

3 Applications of SF coatings

SF has been used in bioengineering via various techniques when fabricated into films, hydrogels, and scaffolds (Ealla et al., 2022). Herein, we focused on the surface modification of biomaterials using SF coatings, and then discussed the diverse applications of SF coatings for biomedicine, including bone, ligament, skin, mucosa, and nerve regeneration, and dental implant surface modification (Fig. 4).

3.1 SF coatings for bone regeneration

Bones are rigid tissues composed of organic components (collagen), inorganic constituents (carbonated hydroxyapatite (HA)), non-collagenous proteins, and growth factors. Employing artificial methods is a promising strategy for regenerating native bones. However, providing an appropriate bone extracellular matrix (ECM) that mimics the mechanical properties of natural bones and the microenvironment of bone cells for bone regeneration in vivo remains challenging (Chen et al., 2017; Holzapfel et al., 2017).

SF have shown favorable effects on bone regeneration, including biocompatibility, biodegradability, porosity, and ease of processability (Bharadwaz and

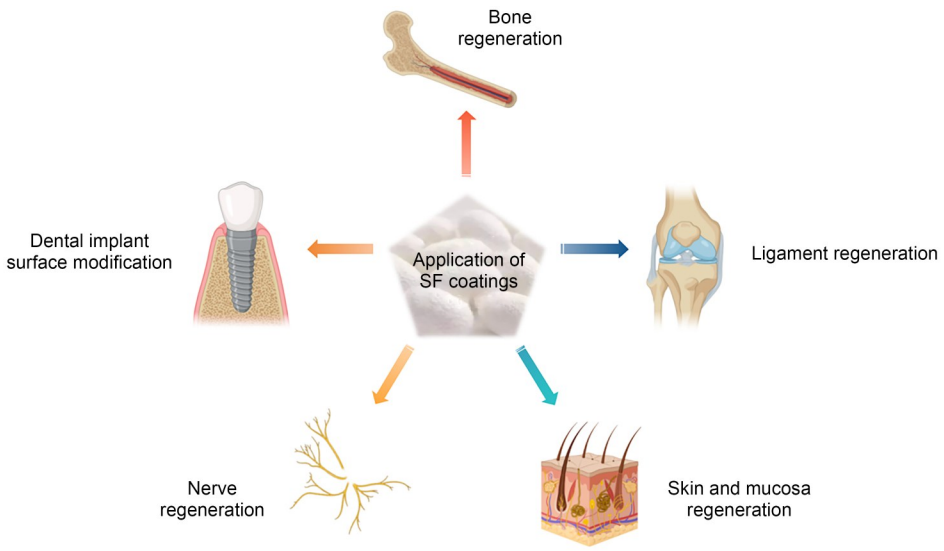

Fig. 4 Diverse applications of silk fibroin (SF) coatings for biomedicine, including bone, ligament, skin, mucosa, and nerve regeneration, and dental implant surface modification.

Jayasuriya, 2020). In bone regeneration, osteoprogenitor adhesion and mineralized matrix deposition on three-dimensional (3D) scaffolds are considered vital steps that the various properties of SF can promote (Midha et al., 2016). Sun YM et al. (2019) fabricated osteoid-like calcium titanate (CT) nanorods by anodization and a hydrothermal method. Subsequently, the functional fibroin and peptide layers on CT (PFCT) were used to provide binding sites and bioactive molecules for osteogenesis. These in vitro results suggested that PFCT can efficiently improve osteoblast adhesion, proliferation, and differentiation, making it a promising strategy for bone defect repair. For biomedical implants, another research proposed that SF-coated titanium implants (STIs) can alleviate diabetes-induced compromised osteointegration (Ma XY et al., 2021). To confirm this hypothesis, Ma XY et al. (2021) performed an in vivo study on diabetic sheep with crista iliaca defect using TIs or STIs. It was demonstrated that the SF coating improved the clinical treatment effect of TIs under diabetic conditions, possibly via the suppression of the nuclear factor-κB (NF-κB) pathway.

Microcarrier systems provide a simple method for bone regeneration as an injectable cell carrier. In a recent study, pullulan (PULL) microspheres were prepared as cell carriers for repairing bone defects (Bi et al., 2020). Coating with SF and biomimetic mineralization via incubation in simulated body fluid improved the cytocompatibility of PULL. As observed from the degradation analysis, SF-coated microspheres lost approximately 12% of their weight after 14 d, which indicated that the covalent bonding between SF and surface-oxidized PULL caused slower weight loss. Moreover, in vitro experiments with Saos-2 cells revealed that SF-coated PULL microspheres were biocompatible for bone regeneration. The cell viability was higher on SF-coated microspheres on the 7th day, and alkaline phosphatase activity was also higher than that on uncoated ones under dynamic conditions, which was achieved by an orbital shaker platform rotating at 120 r/min in a CO_2 incubator (Bi et al., 2020).

The characteristics of the biomaterial strongly influence the behavior of adherent cells on its surface. In the case of bone tissues, cells attach to the ECM-binding sites via integrins, which can mediate cell signaling and affect cell migration, proliferation, and differentiation. The porosity of SF favors cell adhesion and induces cell migration in the coating pores, thereby promoting osteogenesis. Midha et al. (2016) showed that human osteoblasts develop firm adhesions to SF films, and their in vitro study also reported cell adhesion, which was featured by extended filopodia and lamellipodia. As mentioned above, the stable β-sheets of SF proteins have amorphous links similar to the anionic nature of non-collagenous proteins, acting as nucleation sites for the deposition of HA-nanocrystals (Fig. 3b). Furthermore, SF downregulates the expression of Hairy/enhancer of split (Hes) and Hes-related repressor protein (Hey) by inhibiting the Notch signaling

pathway, thereby activating Runt-related transcription factor 2 (Runx2) and promoting osteogenic differentiation (Sethi and Kang, 2012; Jung et al., 2013). In this way, type I collagen (Col I), alkaline phosphatase (ALP), osteocalcin (OCN), and osterix (OSX) expression levels were upregulated (Fig. 5). In conclusion, due to their specific biomaterial characteristics, SF coatings facilitate bone regeneration by inducing cell adhesion and migration, promoting HA deposition and matrix mineralization, as well as inhibiting the Notch signaling pathway.

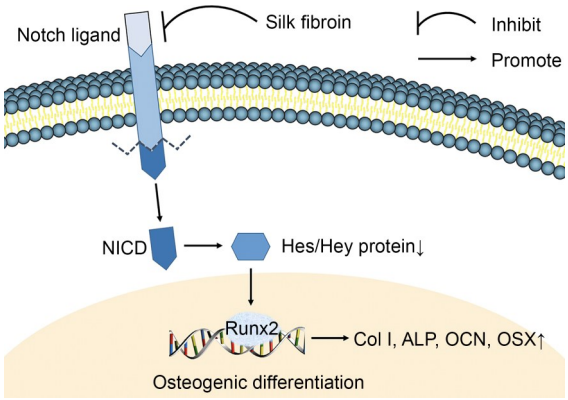

Fig. 5 Silk fibroin downregulated the expression of Hes/Hey protein by inhibiting the Notch signaling pathway, thereby activating Runx2 and promoting osteogenic differentiation, which led to the upregulation of the Col I, ALP, OCN, and OSX expression. Runx2: Runt-related transcription factor 2; NICD: Notch intracellular domain; Hes: Hairy/enhancer of split; Hey: Hes-related repressor protein; Col I: type I collagen; ALP: alkaline phosphatase; OCN: osteocalcin; OSX: osterix.

The addition of components other than SF can promote the osteogenic, antibacterial, or mechanical properties of the coated materials (Table 1). Specifically, Sharma et al. (2016) fabricated antibiotic (gentamicin)-loaded SF nanoparticles and deposited them on a titanium surface for sustained drug release in vitro, and their results confirmed that SF-coated surfaces were superior to bare titanium for osteoblast adhesion, proliferation, and differentiation. Barik et al. (2020) developed an SF-based localized drug delivery system on a titanium surface. Osteoporosis was artificially induced in rats by bilateral ovariectomy, followed by the placement of implants, to evaluate the effect of localized delivery of tibolone via STIs. Tibolone released from SF-coated implants could significantly facilitate peri-implant bone growth (Barik et al., 2020).

Accordingly, SF coating is a promising candidate for bone regeneration because of its biocompatibility, biodegradability, and osteoinductivity, and the performance of the coated materials can be improved by adding components other than SF.

3.2 SF coatings for ligament regeneration

Ligaments are hypocellular connective tissues that resist tension and stabilize joints during movement. They are subject to high physiological loads, making them prone to injury. Conventional treatments through surgical repair and grafting are ineffective, with high rates of reinjury. Thus, ligament regeneration via artificial approaches is a promising strategy for healing after injury (Lim et al., 2019).

PET is commonly utilized in reconstructing the anterior cruciate ligament (ACL) due to its optimal mechanical behavior in vivo; however, the poor biocompatibility and hydrophilicity of PET impede the "ligamentization" process. Thus, the surface modification of PET is considered suitable for ligament regeneration. A study by Jiang et al. (2016) showed that mouse fibroblasts displayed better adhesion and proliferation on the SF-coated PET (PET+SF) ligament than on the PET ligament. Moreover, the amount of deposited collagen on PET and PET+SF ligaments was determined at 1, 3, 5, and 7 d. The fibroblast cells of PET+SF ligament produced more collagen than those of PET ligament at the same time intervals (P<0.05). This indicated that the PET+SF ligament showed better biocompatibility and a higher quantity of collagen than the PET ligament. Based on these results, SF coating is recommended as an efficient strategy for PET ligament surface modification and the "ligamentization" process (Jiang et al., 2016).

The ultra-high-molecular-weight polyethylene (UHMWPE) has been a widely used material to prepare artificial ligaments in orthopedics due to its good mechanical properties and chemical stability. However, the wear particles of UHMWPE can induce postoperation osteolysis around the artificial ligament, and the lack of osseointegration may lead to a poor bone–graft healing interface. With the aim to overcome this problem, Ai et al. (2017) loaded vascular endothelial growth factor (VEGF) and SF on the UHMWPE surface. The chromic acid-treated UHMWPE was placed in VEGF/SF-mixed solution to obtain the coating, and the resulting composite material was assessed

using both in vitro and in vivo experiments. The findings showed that bone marrow mesenchymal stem cells cultured on the SF/VEGF-coated UHMWPE had better proliferation than the pristine UHMWPE when cultured for 3 and 7 d. Moreover, experiments with the ACL reconstruction rabbit model demonstrated that graft-bone healing could be significantly promoted in the SF/VEGF-coated UHMWPE group. This research highlighted that the SF coating plays an important role in the biological performance of UHMWPE and has great application potential in ACL reconstruction (Ai et al., 2017). Thus, new SF-coated composite scaffolds can be considered as a prospective candidate for ligament regeneration in the future.

3.3 SF coatings for skin and mucosa regeneration

Achieving full-thickness wound healing with minimal scarring and physiological restoration is difficult in clinical practice (Vig et al., 2017). SF coatings have been proven to enhance the mechanical properties of substrate materials and render integral stability to the dressing material during the regeneration of skin and mucosa, thus giving time to infiltrate and synthesize the surrounding tissue and ECM (Chouhan and Mandal, 2020).

Arasteh et al. (2016) fabricated a bilayer alternative to mimic the ECM microstructure of the skin by combining human amniotic membrane (HAM) and SF. De-epithelization increased the Young's modulus and ultimate tensile strength of the amniotic membrane from 68.46 to 108.03 MPa and 16.14 to 108.03 MPa, respectively. Also, the mechanical properties of the de-epithelized amnion were significantly improved due to the SF nano-fiber coating. Furthermore, in vitro study on mouse embryonic fibroblasts suggested that the HAM/SF scaffolds could sustain cell adhesion and proliferation. Thus, the HAM/SF nanofibrous bilayer composite can be a promising substitute for applications in skin regeneration (Arasteh et al., 2016).

Although naturally derived ECM scaffolds can accelerate tissue repair due to their excellent bioactivity, their rapid degradation in vivo limits their application. SIS is a type of ECM substitute widely utilized in regenerating tissues such as the bladder, abdominal wall, and skin, and has been popular in clinical trials. Bi et al. (2020) modified SIS by an SF-LBL assembly process to improve its mechanical and structural stabilities. From the experimental results, it was suggested that the mechanical properties and degradation rate of the SF-coated SIS scaffold can be adjusted by changing the number of SF-coating layers. Furthermore, the cultivation of NIH3T3 fibroblasts on the scaffolds showed that the SF coating did not affect the biocompatibility of SIS. The implantation of biomaterials into the host results in a foreign body reaction (FBR) that brings about material degradation. To evaluate the FBR and structural retention of membranes in vivo, SF-coated SIS and glutaraldehyde-crosslinked SIS membranes were applied in the subcutaneous implantation rat model. The results demonstrated that the SF modification could induce a more moderate FBR than the traditional glutaraldehyde chemical crosslinking, thus effectively prolonging the residence time of the biomaterial. This SF-LBL modification provides a potential strategy for applying high-performance ECM-based scaffolds with tunable biodegradability.

As seen above, SF coating has been extensively employed in skin and mucosa regeneration due to its good biocompatibility, hemostasis, and promotion of collagen formation in fibroblasts and tissue reconstruction.

3.4 SF coatings for nerve regeneration

Peripheral nerve repair remains among the greatest challenges in tissue engineering, for it is limited by tissue availability, donor-site morbidity, and scar tissue formation that insulates the electrodes. Hence, SF is a promising biomaterial to accelerate nerve regeneration, thanks to its biocompatibility, biodegradability, dielectric properties, mechanical flexibility, and promotion of angiogenesis (Muangsanit et al., 2018; Wang CY et al., 2018b).

Electrospun poly-L-lactic acid (PLLA) fibers are commonly used for tissue repair owing to their uniform morphology. Ziemba et al. (2020) aimed to promote neurite outgrowth along electrospun fibers with an SF coating. These thin-film SF coatings were found to offer a novel method to increase the neuroregenerative capacity of electrospun scaffolds. However, in recent years, more researchers would rather choose SF-based composites than SF-coated materials for the nerve conduit application because of the natural structure of SF fibers.

3.5 SF coatings for dental implant surface modification

The theory of osseointegration proposed by Brånemark (1983) laid the foundation of modern oral implantology. Since denture restoration by implantation became an alternative for patients with poor alveolar bone conditions, new developments and challenges have been proposed for bone augmentation and implant surface treatment. In recent years, implant surface modification technology to promote early osteogenesis around implants has become the main focus in implantology research (Schünemann et al., 2019; Sun BB et al., 2019).

Titanium is a commonly used material for manufacturing implants and has many clinical applications. Saha et al. (2019) coated nano-TiO_2-modified Ti6Al4V surfaces with SF to enhance the osteoconductive and osteogenic properties of Ti6Al4V to improve implant performance. Their in vitro experiments revealed that the SF-coated TiO_2 modified the Ti6Al4V surface, increasing the osteogenic potential for implant application.

Zirconia is a favored material in the field of dental implantology for its several benefits. Qu et al. (2019) developed SF electro-gel-coated zirconia implants for controlled drug delivery and evaluated the mechanical and biological properties of the coating. The wettability of the coated implants was close to that of standard sandblasted and acid-etched (SLA)-treated zirconia. Additionally, cell culture experiments showed that it was nontoxic to osteoblast-like cells. Therefore, SF electro-gel coating can be a potential drug delivery material for zirconia implants.

The two primary complications restricting biomedical implant applications are the lack of bone tissue integration and biomedical device-associated infections, for which solutions must be found. Zhou et al. (2017) built silver nanoparticles (AgNPs)/gentamicin (Gen)-embedded SF-based biomimetic coatings on orthopedic titanium with the assistance of polydopamine, and demonstrated the sustained release of Ag^+ (28 d) and a tenfold improvement in antibacterial efficiency for the novel AgNPs- and Gen-embedded SF coatings. Moreover, their team fabricated a structure-controlled drug-loaded SF coating (α-helices 32.7%), which could achieve the release control of AgNPs and highly efficient osteogenesis. These novel biofunctional coatings are expected to have promising applications in orthopedic and dental TIs because of their excellent antibacterial, biocompatible, and osteogenic activity (Zhou et al., 2020).

In conclusion, SF coating is a promising strategy for improving the biocompatibility and osseointegration of dental implants made from different materials.

4 Future perspectives

Although several advances have been made in the research of SF coatings, there are still many problems to be overcome (Fig. 6). Exploring the interaction between SF and the substrate material is important for explaining the effects that SF may have on the biological and physicochemical properties. However, few studies have attempted to explain the interaction and bonding strength between SF and substrate materials, and consider whether the bond is strong enough for tissue engineering applications. Besides, few researchers have regarded the thickness of the SF coating applied to the substrate material. Thus far, it seems that reducing the thickness of SF coating while ensuring the bonding strength is more conducive to product transformation.

In addition, most of the studies are still in the stage of novel material development and cytological experiments, and few studies have focused on the effects of these new materials in vivo. Existing studies are limited to small animal models, such as subcutaneous-implantation rat model, osteoporosis rat model, and ACL reconstruction rabbit model. Researchers should establish more suitable animal models especially with large mammals, conduct more in vivo experiments, and compare the new materials with existing ones to reflect their advantages in clinical applications.

The bone–implant interface and soft tissue integration at the trans-mucosal region are important for the success of dental implants. Many studies have shown that the surface of materials loaded with SF coatings has a reticular structure, with increased roughness and hydrophilicity, which is conducive to bone regeneration and peri-implant osseointegration (Saha et al., 2019; Sun BB et al., 2019). However, for soft tissue regeneration, a smooth interface is more favorable. Overcoming this contradiction or preparing a two-phase material may be one of the future research directions for SF-coated biomaterials.

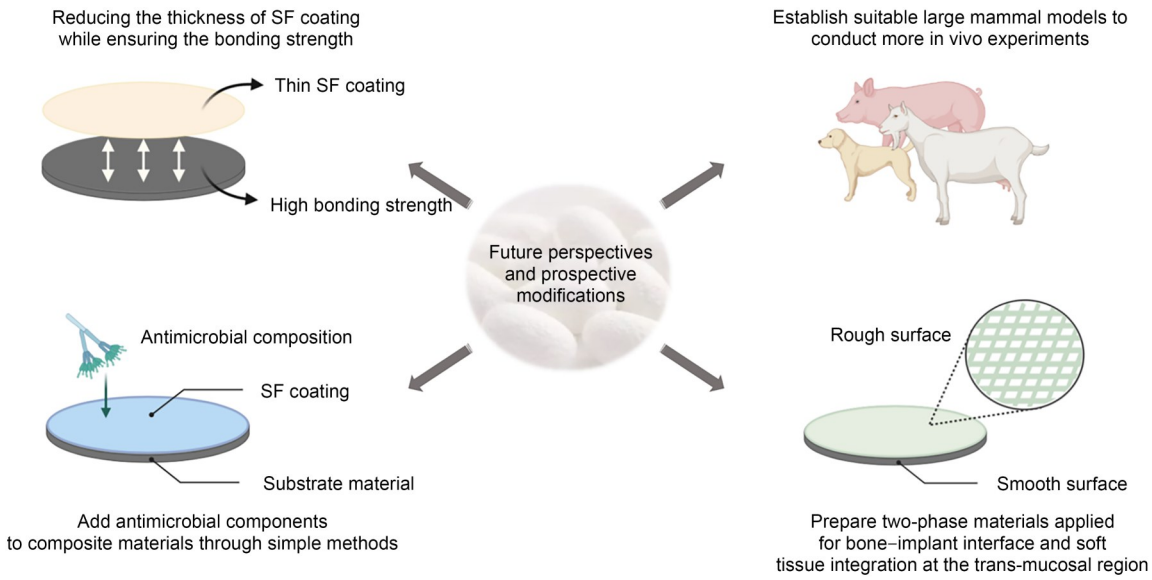

Fig. 6 Future perspectives and prospective modifications of silk fibroin (SF) coatings.

Moreover, SF itself has no antibacterial function, while for many biomaterials to be implanted in vivo, their anti-inflammatory and antibacterial properties must be taken into consideration. Some researchers have attempted to add antimicrobial components when preparing composite materials, but this makes the operation method cumbersome and not universally applicable. Therefore, the preparation of new SF-coated biomaterials with optimal advantages still requires further research.

5 Conclusions

This review focused on the applications of SF coatings in biomedicine where a wide group of researchers are working to develop appropriate biomaterials. SF is a natural biopolymer with good cell adhesion and cell growth performance, and excellent mechanical properties. Moreover, its minimal immunogenicity, tunable biodegradability, and high biocompatibility make it a potential material for biomedical applications. The variety of techniques for coating SF onto biomaterials include dipping, spinning, electrospinning, LBL assembly, and EPD coating. SF facilitates osteogenesis by promoting matrix mineralization owing to the β-sheet domains that provide nucleation sites for the deposition of HA crystals. Furthermore, SF can downregulate Hes/Hey protein expression by inhibiting the Notch signaling pathway, thereby activating Runx2 and promoting osteogenic differentiation. Although the clinical application of SF-coated biomaterials is still burdened with challenges, these kinds of materials have shown remarkable potential in biomedicine, and will continue to contribute to the dramatic new developments in bone, ligament, skin, mucosa, and nerve regeneration, and dental implant surface modification.

Data availability statement

All data supporting the findings of this study are available within the paper.

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Author contributions

Jinxing HU wrote and edited the manuscript. Zhiwei JIANG and Jing ZHANG edited the manuscript. Guoli YANG contributed to the conceptualization of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Zhiwei JIANG is a young scientist committee member for *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)* and was not involved in the editorial review or the decision to publish this article. Jinxing HU, Zhiwei JIANG,

Jing ZHANG, and Guoli YANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Ai CC, Sheng DD, Chen J, et al., 2017. Surface modification of vascular endothelial growth factor-loaded silk fibroin to improve biological performance of ultra-high-molecular-weight polyethylene via promoting angiogenesis. *Int J Nanomedicine*, 12:7737-7750. https://doi.org/10.2147/Ijn.S148845

Altman GH, Diaz F, Jakuba C, et al., 2003. Silk-based biomaterials. *Biomaterials*, 24(3):401-416. https://doi.org/10.1016/S0142-9612(02)00353-8

Arasteh S, Kazemnejad S, Khanjani S, et al., 2016. Fabrication and characterization of nano-fibrous bilayer composite for skin regeneration application. *Methods*, 99:3-12. https://doi.org/10.1016/j.ymeth.2015.08.017

Arkhangelskiy A, Maniglio D, Bucciarelli A, et al., 2021. Plasma-assisted deposition of silk fibroin on different surfaces. *Adv Mater Interfaces*, 8(13):2100324. https://doi.org/10.1002/admi.202100324

Aydogdu H, Keskin D, Baran ET, et al., 2016. Pullulan microcarriers for bone tissue regeneration. *Mater Sci Eng C*, 63:439-449. https://doi.org/10.1016/j.msec.2016.03.002

Baranowska-Korczyc A, Hudecki A, Kamińska I, et al., 2021. Silk powder from cocoons and woven fabric as a potential bio-modifier. *Materials*, 14(22):6919. https://doi.org/10.3390/ma14226919

Barik A, Ray SK, Byram PK, et al., 2020. Extensive early mineralization of pre-osteoblasts, inhibition of osteoclastogenesis and faster peri-implant bone healing in osteoporotic rat model: principle effectiveness of bone-specific delivery of Tibolone as evaluated *in vitro* and *in vivo*. *Biomed Mater*, 15(6):064102. https://doi.org/10.1088/1748-605x/abb12b

Bayraktar O, Malay Ö, Özgarip Y, et al., 2005. Silk fibroin as a novel coating material for controlled release of theophylline. *Eur J Pharm Biopharm*, 60(3):373-381. https://doi.org/10.1016/j.ejpb.2005.02.002

Bharadwaz A, Jayasuriya AC, 2020. Recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. *Mater Sci Eng C*, 110:110698. https://doi.org/10.1016/j.msec.2020.110698

Bi XW, Li LH, Mao ZN, et al., 2020. The effects of silk layer-by-layer surface modification on the mechanical and structural retention of extracellular matrix scaffolds. *Biomater Sci*, 8(14):4026-4038. https://doi.org/10.1039/d0bm00448k

Boni BOO, Bakadia BM, Osi AR, et al., 2022. Immune response to silk sericin-fibroin composites: potential immunogenic elements and alternatives for immunomodulation. *Macromol Biosci*, 22:2100292. https://doi.org/10.1002/mabi.202100292

Brånemark PI, 1983. Osseointegration and its experimental background. *J Prosthet Dent*, 50(3):399-410. https://doi.org/10.1016/s0022-3913(83)80101-2

Cao Y, Liu FQ, Chen YL, et al., 2017. Drug release from core-shell PVA/silk fibroin nanoparticles fabricated by one-step electrospraying. *Sci Rep*, 7:11913. https://doi.org/10.1038/s41598-017-12351-1

Carrasco-Torres G, Valdés-Madrigal MA, Vásquez-Garzón VR, et al., 2019. Effect of silk fibroin on cell viability in electrospun scaffolds of polyethylene oxide. *Polymers*, 11(3):451. https://doi.org/10.3390/polym11030451

Chen J, Zhuang A, Shao HL, et al., 2017. Robust silk fibroin/bacterial cellulose nanoribbon composite scaffolds with radial lamellae and intercalation structure for bone regeneration. *J Mater Chem B*, 5(20):3640-3650. https://doi.org/10.1039/c7tb00485k

Cheng X, Deng DM, Chen LL, et al., 2020. Electrodeposited assembly of additive-free silk fibroin coating from pre-assembled nanospheres for drug delivery. *ACS Appl Mater Interfaces*, 12(10):12018-12029. https://doi.org/10.1021/acsami.9b21808

Cheng X, Long DP, Chen LL, et al., 2021. Electrophoretic deposition of silk fibroin coatings with pre-defined architecture to facilitate precise control over drug delivery. *Bioact Mater*, 6(11):4243-4254. https://doi.org/10.1016/j.bioactmat.2021.03.046

Chouhan D, Mandal BB, 2020. Silk biomaterials in wound healing and skin regeneration therapeutics: from bench to bedside. *Acta Biomater*, 103:24-51. https://doi.org/10.1016/j.actbio.2019.11.050

dal Prà I, Petrini P, Chiarini A, et al., 2004. Silk fibroin-coated three-dimensional polyurethane scaffolds for tissue engineering: interactions with normal human fibroblasts. *Tissue Eng Part A*, 9(6):1113-1121. https://doi.org/10.1089/10763270360728026

Ealla KKR, Veeraraghavan VP, Ravula NR, et al., 2022. Silk hydrogel for tissue engineering: a review. *J Contemp Dent Pract*, 23(4):467-477. https://doi.org/10.5005/jp-journals-10024-3322

Elia R, Michelson CD, Perera AL, et al., 2015. Electrodeposited silk coatings for bone implants. *J Biomed Mater Res Part B Appl Biomater*, 103(8):1602-1609. https://doi.org/10.1002/jbm.b.33351

Fan YQ, Li X, Yang RJ, 2018. The surface modification methods for constructing polymer-coated stents. *Int J Polym Sci*, 2018:3891686. https://doi.org/10.1155/2018/3891686

Hasturk O, Sahoo JK, Kaplan DL, 2020. Synthesis and characterization of silk ionomers for layer-by-layer electrostatic deposition on individual mammalian cells. *Biomacromolecules*, 21(7):2829-2843. https://doi.org/10.1021/acs.biomac.0c00523

Holzapfel BM, Rudert M, Hutmacher DW, 2017. Scaffold-based bone tissue engineering. *Orthopade*, 46(8):701-710 (in German).

https://doi.org/10.1007/s00132-017-3444-0

Jiang J, Ai CC, Zhan ZF, et al., 2016. Enhanced fibroblast cellular ligamentization process to polyethylene terephthalate artificial ligament by silk fibroin coating. *Artif Organs*, 40(4):385-393. https://doi.org/10.1111/aor.12571

Joseph E, Rajput SS, Patil S, et al., 2021. Mechanism of adhesion of natural polymer coatings to chemically modified siloxane polymer. *Langmuir*, 37(9):2974-2984. https://doi.org/10.1021/acs.langmuir.1c00047

Jung SR, Song NJ, Yang DK, et al., 2013. Silk proteins stimulate osteoblast differentiation by suppressing the Notch signaling pathway in mesenchymal stem cells. *Nutr Res*, 33(2):162-170. https://doi.org/10.1016/j.nutres.2012.11.006

Kumar S, Singh SK, 2017. Fabrication and characterization of fibroin solution and nanoparticle from silk fibers of *Bombyx mori*. *Part Sci Technol*, 35(3):304-313. https://doi.org/10.1080/02726351.2016.1154908

Lim WL, Liau LL, Ng MH, et al., 2019. Current progress in tendon and ligament tissue engineering. *Tissue Eng Regen Med*, 16(6):549-571. https://doi.org/10.1007/s13770-019-00196-w

Lujerdean C, Baci GM, Cucu AA, et al., 2022. The contribution of silk fibroin in biomedical engineering. *Insects*, 13(3):286. https://doi.org/10.3390/insects13030286

Luo ZW, Li J, Qu J, et al., 2019. Cationized *Bombyx mori* silk fibroin as a delivery carrier of the VEGF165-Ang-1 co-expression plasmid for dermal tissue regeneration. *J Mater Chem B*, 7(1):80-94. https://doi.org/10.1039/c8tb01424h

Ma XY, Ma TC, Feng YF, et al., 2021. Promotion of osteointegration under diabetic conditions by a silk fibroin coating on 3D-printed porous titanium implants via a ROS-mediated NF-κB pathway. *Biomed Mater*, 16(3):035015. https://doi.org/10.1088/1748-605X/abaaa1

Melke J, Midha S, Ghosh S, et al., 2016. Silk fibroin as biomaterial for bone tissue engineering. *Acta Biomater*, 31:1-16. https://doi.org/10.1016/j.actbio.2015.09.005

Midha S, Murab S, Ghosh S, 2016. Osteogenic signaling on silk-based matrices. *Biomaterials*, 97:133-153. https://doi.org/10.1016/j.biomaterials.2016.04.020

Muangsanit P, Shipley RJ, Phillips JB, 2018. Vascularization strategies for peripheral nerve tissue engineering. *Anat Rec*, 301(10):1657-1667. https://doi.org/10.1002/ar.23919

Ojah N, Borah R, Ahmed GA, et al., 2020. Surface modification of electrospun silk/AMOX/PVA nanofibers by dielectric barrier discharge plasma: physiochemical properties, drug delivery and in-vitro biocompatibility. *Prog Biomater*, 9(4):219-237. https://doi.org/10.1007/s40204-020-00144-1

Qian YN, Li LH, Song Y, et al., 2018. Surface modification of nanofibrous matrices via layer-by-layer functionalized silk assembly for mitigating the foreign body reaction. *Biomaterials*, 164:22-37. https://doi.org/10.1016/j.biomaterials.2018.02.038

Qiao F, Zhang JJ, Wang JH, et al., 2017. Silk fibroin-coated PLGA dimpled microspheres for retarded release of simvastatin. *Colloids Surf B Biointerfaces*, 158:112-118. https://doi.org/10.1016/j.colsurfb.2017.06.038

Qu YY, Hong G, Liu L, et al., 2019. Evaluation of silk fibroin electrogel coating for zirconia material surface. *Dent Mater J*, 38(5):813-820. https://doi.org/10.4012/dmj.2018-228

Rahman M, Balu R, Abraham A, et al., 2021. Engineering a bioactive hybrid coating for *in vitro* corrosion control of magnesium and its alloy. *ACS Appl Bio Mater*, 4(7):5542-5555. https://doi.org/10.1021/acsabm.1c00366

Rnjak-Kovacina J, DesRochers TM, Burke KA, et al., 2015. The effect of sterilization on silk fibroin biomaterial properties. *Macromol Biosci*, 15(6):861-874. https://doi.org/10.1002/mabi.201500013

Saha S, Pramanik K, Biswas A, 2019. Silk fibroin coated TiO_2 nanotubes for improved osteogenic property of Ti6Al4V bone implants. *Mater Sci Eng C*, 105:109982. https://doi.org/10.1016/j.msec.2019.109982

Schünemann FH, Galárraga-Vinueza ME, Magini R, et al., 2019. Zirconia surface modifications for implant dentistry. *Mater Sci Eng C*, 98:1294-1305. https://doi.org/10.1016/j.msec.2019.01.062

Sethi N, Kang YB, 2012. Notch signaling: mediator and therapeutic target of bone metastasis. *Bonekey Rep*, 1:3. https://doi.org/10.1038/bonekey.2012.2

Sharma S, Bano S, Ghosh AS, et al., 2016. Silk fibroin nanoparticles support *in vitro* sustained antibiotic release and osteogenesis on titanium surface. *Nanomedicine*, 12(5):1193-1204. https://doi.org/10.1016/j.nano.2015.12.385

Šišková AO, Mosnáčková K, Hrůza J, et al., 2021. Electrospun poly(ethylene terephthalate)/silk fibroin composite for filtration application. *Polymers*, 13(15):2499. https://doi.org/10.3390/polym13152499

Sun BB, Zhou ZF, Li DW, et al., 2019. Polypyrrole-coated poly(L-lactic acid-*co*-ε-caprolactone)/silk fibroin nanofibrous nerve guidance conduit induced nerve regeneration in rat. *Mater Sci Eng C*, 94:190-199. https://doi.org/10.1016/j.msec.2018.09.021

Sun YM, Hu C, Yang YY, et al., 2019. Fibroin/peptide co-functionalized calcium titanate nanorods improve osteoinductivity of titanium *via* mimicking osteogenic niche. *Mater Sci Eng C*, 103:109836. https://doi.org/10.1016/j.msec.2019.109836

Tan MX, Liu WW, Liu FQ, et al., 2019. Silk fibroin-coated nanoagents for acidic lysosome targeting by a functional preservation strategy in cancer chemotherapy. *Theranostics*, 9(4):961-973. https://doi.org/10.7150/thno.30765

Unalan I, Colpankan O, Albayrak AZ, et al., 2016. Biocompatibility of plasma-treated poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) nanofiber mats modified by silk

fibroin for bone tissue regeneration. *Mater Sci Eng C*, 68:842-850.
https://doi.org/10.1016/j.msec.2016.07.054

Valencia-Lazcano AA, Román-Doval R, de la Cruz-Burelo E, et al., 2018. Enhancing surface properties of breast implants by using electrospun silk fibroin. *J Biomed Mater Res Part B Appl Biomater*, 106(5):1655-1661.
https://doi.org/10.1002/jbm.b.33973

Vig K, Chaudhari A, Tripathi S, et al., 2017. Advances in skin regeneration using tissue engineering. *Int J Mol Sci*, 18(4): 789.
https://doi.org/10.3390/ijms18040789

Wang CY, Wang SN, Yang YY, et al., 2018a. Bioinspired, biocompatible and peptide-decorated silk fibroin coatings for enhanced osteogenesis of bioinert implant. *J Biomater Sci Polym Ed*, 29(13):1595-1611.
https://doi.org/10.1080/09205063.2018.1477316

Wang CY, Jia YC, Yang WC, et al., 2018b. Silk fibroin enhances peripheral nerve regeneration by improving vascularization within nerve conduits. *J Biomed Mater Res Part A*, 106(7):2070-2077.
https://doi.org/10.1002/jbm.a.36390

Wang SG, Hu F, Li JC, et al., 2018. Design of electrospun nanofibrous mats for osteogenic differentiation of mesenchymal stem cells. *Nanomed Nanotechnol Biol Med*, 14(7): 2505-2520.
https://doi.org/10.1016/j.nano.2016.12.024

Wang X, Gu ZP, Jiang B, et al., 2016. Surface modification of strontium-doped porous bioactive ceramic scaffolds *via* poly(DOPA) coating and immobilizing silk fibroin for excellent angiogenic and osteogenic properties. *Biomater Sci*, 4(4):678-688.
https://doi.org/10.1039/c5bm00482a

Watanabe M, Bhawal UK, Takemoto S, et al., 2021. Biofunctionalized titanium surfaces with modified silk fibroin carrying titanium binding motif to enhance the ossific differentiation of MC3T3-E1. *Biotechnol Bioeng*, 118(7): 2585-2596.
https://doi.org/10.1002/bit.27777

Wenk E, Merkle HP, Meinel L, 2011. Silk fibroin as a vehicle for drug delivery applications. *J Controlled Release*, 150(2): 128-141.
https://doi.org/10.1016/j.jconrel.2010.11.007

Xiong P, Jia ZJ, Li M, et al., 2018. Biomimetic Ca, Sr/P-doped silk fibroin films on Mg-1Ca alloy with dramatic corrosion resistance and osteogenic activities. *ACS Biomater Sci Eng*, 4(9):3163-3176.
https://doi.org/10.1021/acsbiomaterials.8b00787

Xiong P, Jia ZJ, Zhou WH, et al., 2019a. Osteogenic and pH stimuli-responsive self-healing coating on biomedical Mg-1Ca alloy. *Acta Biomater*, 92:336-350.
https://doi.org/10.1016/j.actbio.2019.05.027

Xiong P, Yan JL, Wang P, et al., 2019b. A pH-sensitive self-healing coating for biodegradable magnesium implants. *Acta Biomater*, 98:160-173.
https://doi.org/10.1016/j.actbio.2019.04.045

Xu W, Yagoshi K, Asakura T, et al., 2020. Silk fibroin as a coating polymer for sirolimus-eluting magnesium alloy stents. *ACS Appl Bio Mater*, 3(1):531-538.
https://doi.org/10.1021/acsabm.9b00957

Yamano M, Hirose R, Lye PY, et al., 2022. Bioengineered silkworm for producing cocoons with high fibroin content for regenerated fibroin biomaterial-based applications. *Int J Mol Sci*, 23(13):7433.
https://doi.org/10.3390/ijms23137433

Yang MY, Zhou GS, Shuai YJ, et al., 2015. Ca^{2+}-induced self-assembly of *Bombyx mori* silk sericin into a nanofibrous network-like protein matrix for directing controlled nucleation of hydroxylapatite nano-needles. *J Mater Chem B*, 3(12):2455-2462.
https://doi.org/10.1039/c4tb01944j

Yao MZ, Huang-Fu MY, Liu HN, et al., 2016. Fabrication and characterization of drug-loaded nano-hydroxyapatite/polyamide 66 scaffolds modified with carbon nanotubes and silk fibroin. *Int J Nanomedicine*, 11:6181-6194.
https://doi.org/10.2147/Ijn.S106929

Ye XG, Li S, Chen XX, et al., 2017. Polyethylenimine/silk fibroin multilayers deposited nanofibrics for cell culture. *Int J Biol Macromol*, 94(Part A):492-499.
https://doi.org/10.1016/j.ijbiomac.2016.10.047

Yucel T, Lovett ML, Kaplan DL, 2014. Silk-based biomaterials for sustained drug delivery. *J Controlled Release*, 190: 381-397.
https://doi.org/10.1016/j.jconrel.2014.05.059

Zhang Y, Shi N, He L, et al., 2021. Silk sericin activates mild immune response and increases antibody production. *J Biomed Nanotechnol*, 17(12):2433-2443.
https://doi.org/10.1166/jbn.2021.3206

Zhou WH, Jia ZJ, Xiong P, et al., 2017. Bioinspired and biomimetic AgNPs/gentamicin-embedded silk fibroin coatings for robust antibacterial and osteogenetic applications. *ACS Appl Mater Interfaces*, 9(31):25830-25846.
https://doi.org/10.1021/acsami.7b06757

Zhou WH, Zhang T, Yan JL, et al., 2020. *In vitro* and *in vivo* evaluation of structurally-controlled silk fibroin coatings for orthopedic infection and in-situ osteogenesis. *Acta Biomater*, 116:223-245.
https://doi.org/10.1016/j.actbio.2020.08.040

Zhou WH, Yan JL, Li YY, et al., 2021. Based on the synergistic effect of Mg^{2+} and antibacterial peptides to improve the corrosion resistance, antibacterial ability and osteogenic activity of magnesium-based degradable metals. *Biomater Sci*, 9(3):807-825.
https://doi.org/10.1039/d0bm01584a

Zhu L, Lin JX, Pei LJ, et al., 2022. Recent advances in environmentally friendly and green degumming processes of silk for textile and non-textile applications. *Polymers*, 14(4):659.
https://doi.org/10.3390/polym14040659

Ziemba AM, Fink TD, Crochiere MC, et al., 2020. Coating topologically complex electrospun fibers with nanothin silk fibroin enhances neurite outgrowth in vitro. *ACS Biomater Sci Eng*, 6(3):1321-1332.
https://doi.org/10.1021/acsbiomaterials.9b01487