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NARRATIVE REVIEW

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Preparation of silk fibroin-derived hydrogels and applications in skin regeneration

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Abstract

Purpose: To compare different methods of preparing silk fibroin hydrogels, then summarize the applications of silk fibroin hydrogel-based scaffolds in skin regeneration and finally discuss about future prospects to inspire people interested in this field.

Methods: A narrative review of the relevant papers was conducted. Notably, for applications in skin regeneration, this review provides a categorized summary and discussion of studies from the past decade.

Results: Silk fibroin is a naturally occurring, biocompatible biomaterial that is easily producible. Thanks to its exceptional processability, silk fibroin has found diverse applications in skin regeneration. These applications encompass sponges, fiber fabrics, thin films, and hydrogels. Hydrogels, in particular, are noteworthy due to their water-containing network structure, closely resembling natural tissues. They provide a biomimetic three-dimensional growth environment for cells and have the capacity to incorporate growth factors. Consequently, there are abundant studies of silk fibroin hydrogel-based scaffolds in skin regeneration. Besides, some commercialized medical devices are also made of silk fibroin.

Conclusion: Silk fibroin hydrogel could be prepared with multiple methods and it is widely used in constructing scaffolds for efficient skin regeneration. In the future, silk fibroin hydrogel-based skin scaffolds could be more biomimetic and smart.

KEYWORDS

hydrogel, silk fibroin, skin regeneration, tissue engineering

1 | INTRODUCTION

Large-size skin defects resulting from trauma and tumor resections pose significant challenges for the body's natural repair mechanisms, impacting both the physical and mental well-being, as well as the overall quality of life for patients.[1] Clinical approaches commonly employed to address skin defects include autologous tissue transplantation, allogeneic tissue transplantation, and xenogeneic tissue transplantation, which may lead to complications at the donor site and cause rejection or pathogen transmission.[2–6] The advent of tissue engineering technology has ushered in new strategies to achieve functional skin tissue regeneration. Tissue engineering encompasses three pivotal components: biomaterials, cells, and growth factors.[2,7] Biomaterials form the cornerstone of tissue engineering scaffolds, providing mechanical support and sites for cell adhesion. An ideal tissue engineering scaffold not only facilitates tissue regeneration but also degrades as the tissue regenerates, ultimately achieving functional regeneration with the appropriate tissue structure.[7] Therefore, the selection of biocompatible

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and degradable biomaterials plays a pivotal role in constructing tissue engineering scaffolds for skin repair.

Materials used in fabricating tissue engineering skin scaffolds include both synthetic biomaterials and natural biomaterials. However, the biocompatibility and biodegradability of natural biomaterials surpass those of synthetic biomaterials.[8–13] As a result, researchers have increasingly turned to natural biomaterials or integrated them into synthetic ones to enhance cell adhesion and the biocompatibility of tissue engineering skin scaffolds. Natural biomaterials, including collagen, gelatin, sodium alginate, chitosan, cellulose, and silk fibroin, have gained prominence in this regard.[7,14] Among these natural biomaterials, silk fibroin derived from silkworms holds a prominent position in tissue engineering research due to its exceptional mechanical adjustability, biocompatibility, processability, and degradability.[14–16] Additionally, silk fibroin has received validation from both the Food and Drug Administration (USA) and the National Medical Products Administration (NMPA, China) for its application in medical device development.[17] Consequently, it emerges as a promising biomaterial with immense potential for large-scale production and utilization at the bedside.

Leveraging silk fibroin's excellent processability, researchers can employ various techniques to craft scaffolds in diverse forms, such as sponges, fiber fabrics, thin films, and hydrogels, all tailored for tissue regeneration.[18] With the elucidation of the structure and components of the natural extracellular matrix of tissues, hydrogel scaffolds with three-dimensional (3D) networks are considered to closely resemble human tissue architectures.[19] Hydrogel scaffolds can accommodate cells involved in tissue regeneration, providing them with a 3D water-containing network structure similar to the natural extracellular matrix.[20] They can also carry bioactive factors to achieve different biological functions.[21] Moreover, by adjusting the crosslinked network of hydrogels, control over their mechanical properties can be achieved, enabling biomimetic mechanical properties for different tissues.[22] Consequently, constructing tissue engineering skin scaffolds based on silk fibroin hydrogels has emerged as a more promising approach for therapy.

In this review, we first introduce the structure and properties of silk fibroin derived from silkworms, and systematically summarize and compare the existing techniques for preparing silk fibroin hydrogels. Subsequently, using examples from skin tissue engineering and wound healing, we recapitulate research from the past decade on tissue engineering skin scaffolds based on silk fibroin hydrogels for treating defects in skin tissues. Subsequently, we present the relevant clinical trials and commercial products to underscore crucial considerations in bedside applications, thereby highlighting their significance. Ultimately, we delve into a comprehensive discussion and offer valuable insights regarding the future development of tissue engineering skin scaffolds based on silk fibroin hydrogels.

2 | SOURCES AND PROPERTIES OF SILK FIBROIN

The silk fibroin discussed in this review is sourced from silkworms. Silkworm silk is secreted by the silkworm's silk gland and primarily composed of fibrous silk fibroin and globular sericin.[23,24] Silk fibroin is a fibrous protein, while sericin is a globular protein that binds the silk fibroin fibers together. Research suggests that the coexistence of silk fibroin and sericin may lead to inflammatory reactions in the body.[23,25] Therefore, in biomedical applications, silk fibroin and sericin are typically separated for different uses. This article mainly explores the properties and applications of silk fibroin derived from silkworms. There are different types of silkworms, including the Chinese silkworm (*Bombyx Mori* silkworm), Thai silkworm (*Bombyx Mori* silkworm), tasar silkworm (Antheraea mylitta silkworm), eri silkworm (Philosamia ricini silkworm), and muga silkworm (Antheraea assamensis silkworm).[26] Chinese silkworm silk is mainly white, and the cocoon is egg-shaped; Thai silkworm silk is bright yellow, with smaller cocoons that are spindle-shaped; tasar silkworm silk is deep brown, with larger cocoons; eri silkworm silk is white and coarser; muga silkworm silk is golden yellow and its cocoons have a looser structure (Figure 1A). It's important to note that the Thai silkworm and Chinese silkworm are the same species, but differences in climate and rearing conditions lead to variations in the appearance of the silk. Silk fibroin derived from different species of silkworms possesses variations in amino acid sequences, resulting in distinct physicochemical and biological properties.[28] Chinese silkworms are widely bred and stable, making silk fibroin derived from Chinese silkworms possess excellent mechanical properties, biocompatibility, and processability.[29] Consequently, silk fibroin sourced from Chinese silkworms is the most commonly used type in the field of biomedical research. Unless specified otherwise, the studies discussed in this article use silk fibroin sourced from Chinese silkworms.

Silk fibroin from Chinese silkworms consists of a heavy chain (MW = 390 kDa) and a light chain (MW = 26 kDa), linked by disulfide bonds.[27] The heavy chain contains approximately 12 repeated hydrophobic blocks (mainly composed of glycine, alanine, serine, valine, and tyrosine) and 11 hydrophilic blocks that separate the hydrophobic segments. These hydrophobic segments play a crucial role in determining the secondary structure of silk fibroin. Silk fibroin primarily exhibits two types of secondary structures: an amorphous random coil and a crystalline β-sheet (Figure 1B). Hydrogen bonding between repeated hydrophobic blocks leads to the formation of the crystalline β-sheet structure, allowing the tuning of silk fibroin's mechanical and biological properties.[30,31] Furthermore, the amino acids composing silk fibroin possess diverse side chains, enabling chemical modifications.[32] Silk fibroin also exhibits high plasticity, allowing it to be processed into various forms of tissue engineering scaffolds, including sponges, fiber fabrics, thin films, and the hydrogel form discussed in this article (Figure 1C).[18]

3 | PREPARATION OF SILK FIBROIN HYDROGELS

When the peptide chains of silk fibroin in a water solution form a crosslinked network, a silk fibroin hydrogel with a 3D structure can be obtained. The crosslinking of silk fibroin can be achieved through

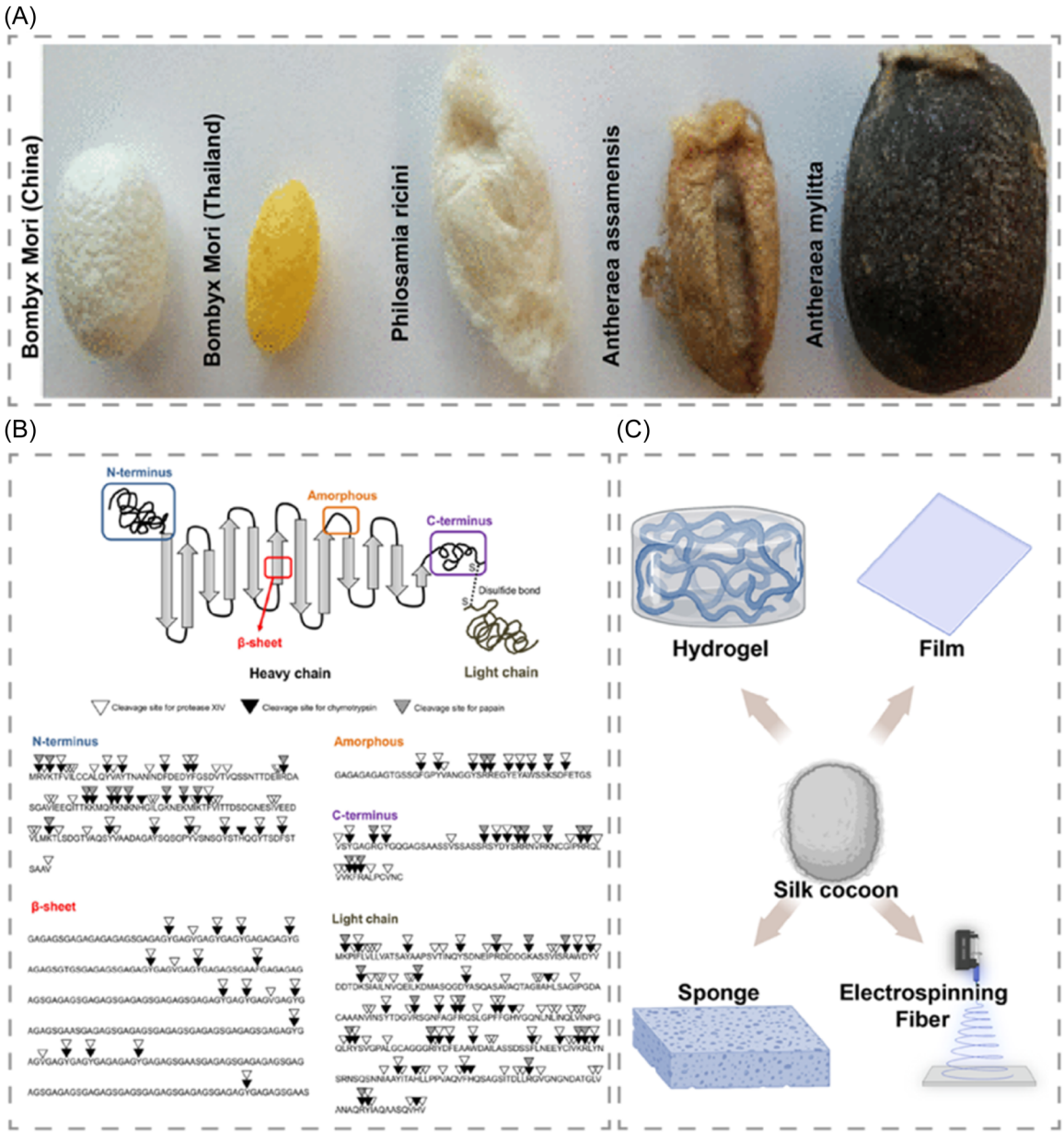

FIGURE 1 (A) Images of cocoons from different silkworm species.[26] (B) Secondary structure and amino acid composition of silk fibroin sourced from Chinese silkworms.[27] (C) Common forms of silk fibroin scaffolds.

two main methods: physical crosslinking and chemical crosslinking (Figure 2). Next, we will compare and summarize various crosslinking methods, explaining the advantages and disadvantages of each approach. Besides, the comparison of different methods is summarized in Table 1.

3.1 | Physical crosslinking

Crosslinking refers to the formation of a 3D network structure from the dispersed state of macromolecules. Physical crosslinking, on the other hand, entails the establishment of a 3D network structure through noncovalent interactions among macromolecules, encompassing hydrogen bonding, hydrophobic interactions, electrostatic attractions, ionic interactions, and intertwining of macromolecular chains. Physical crosslinking processes typically involve the transformation of fibrous protein secondary structures, wherein irregular coils or α-helices undergo a transition to β-folded crystalline structures, culminating in the creation of a hydrated 3D network architecture.[41,58–62] Common methods for achieving physical crosslinking involve molecular self-assembly, ultrasonic treatment, shear force application, electric field exposure, organic solvent treatment, thermal manipulation, pH adjustment, and utilization of surfactants, among others. In the subsequent sections, we will provide a comprehensive summary and comparative analysis of various techniques employed in physical crosslinking.

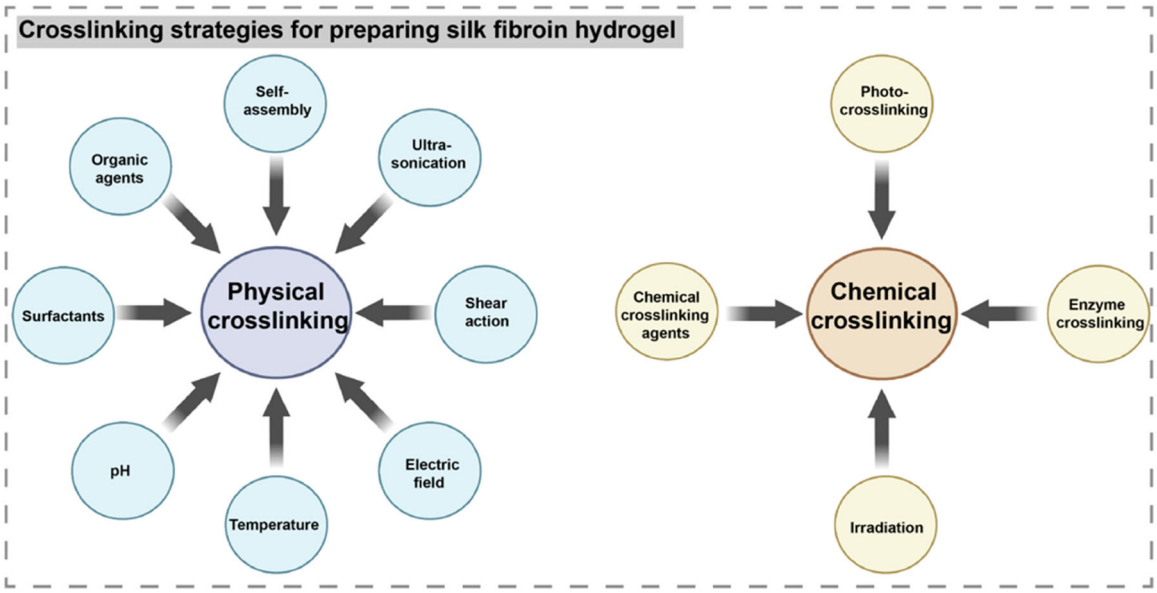

FIGURE 2 Common crosslinking methods for constructing silk fibroin hydrogels, primarily categorized as physical crosslinking and chemical crosslinking.

TABLE 1 Comparison of different crosslinking methods.

Crosslinking type	Method	Advantage	Main shortcoming	Ref.
Physical	Self-assembly	Mild procedure	Time-consuming	[33, 34]
	Ultrasonic	Effective, environmentally friendly	Not suitable for scaffolds loaded with cells or growth factors	[35–37]
	Shear	Stimulus responsiveness	Time-consuming and high gelation concentration	[38]
	Electric field	Intact structure of silk fibroin	Complicated operations	[39, 40]
	Heat	Shorter gelation time	Not suitable for cell-laden solutions; poor quality control	[31, 41–43]
	pH modulating	Stable hydrogel formation	Decomposition risks	[31, 41–44]
	Organic Solvent	High efficiency, remarkable stability	Toxicity of organic solvents	[45, 46]
	Surfactant	Reduced gelation time	Certain surfactants are nonbiodegradable	[47, 48]
Chemical	Chemical Reagent	Tailorable mechanical properties	Toxicity of chemical reagents	[49, 50]
	Enzyme	Flexible and nontoxic process	Restricted enzyme types, immunoreaction risks	[51–53]
	Irradiation	No additives, high-efficiency	Not suitable for cell-laden solutions	[54]
	Photo-Crosslinking	High efficiency, tailorable mechanical properties	Toxicity of certain photoinitiators	[55–57]

3.1.1 | Self-assembly

The self-assembly into a gel primarily capitalizes on the thermodynamic instability of fibrous proteins in aqueous solutions. Following treatment with lithium bromide or calcium chloride-ethanol-water systems, the predominant form of fibrous protein in aqueous solution is characterized as irregular coils. However, these irregular coils are inherently unstable, and through the process of solution evaporation and concentration at room temperature, they transform into the more stable β-folded configuration, consequently aggregating into silk protein hydrogels.[63] Chouhan et al. combined silk protein solutions (3% wt/v) obtained from silkworms of mulberry origin with silk protein solutions (3% wt/v) derived from tussar silkworms. The resulting mixture of silk protein solutions underwent gelation within the human body temperature range (15–20 min). This study harnessed the stronger hydrophobic interactions and hydrogen bonding between silk proteins from different silkworm species, facilitating the aggregation of silk proteins and the transition from irregular coils to β-folded crystalline structures.

Ultimately, this led to the formation of silk protein hydrogels.[64] Even after incubation in a drying oven at 37°C for 12 h, the hydrogel maintained 75% of its water content, demonstrating the stability of the gel network formed through this method and its remarkable water retention properties. Despite the prolonged timeframe required for the self-assembly process, it is capable of constructing silk protein hydrogels with a stable network structure.

3.1.2 | Ultrasonic

Ultrasound treatment, similarly, enhances the mutual collision of silk protein molecules through the action of ultrasonic waves. Molecular interactions driven by hydrogen bonding or hydrophobic regions lead to aggregation. Ultimately, under the influence of ultrasound, silk protein molecules undergo a transition in secondary structure (from irregular coiling to β-folded crystalline structure), culminating in the formation of silk protein hydrogels. Research has demonstrated that under ultrasound exposure, silk protein solutions can form hydrogels within 1 min.[65] Therefore, the gelation principles of ultrasound treatment and self-assembly are analogous, relying on intermolecular interactions of silk protein molecules and the transition of secondary structures. However, ultrasound treatment achieves gelation more rapidly, within 1 min.[35] It is worth noting that certain ultrasound frequencies can have damaging effects on cells, and ultrasound processes often generate considerable heat. In such cases, harnessing self-assembly becomes more suitable for constructing silk protein hydrogels containing cell-sensitive active components like cells or growth factors responsive to physical stimuli.[66,67]

3.1.3 | Shear

Under the influence of shear forces, the collision probability of silk protein molecules increases, and intermolecular interactions, likewise facilitated by hydrogen bonding or hydrophobic effects, lead to aggregation. Subsequently, silk protein molecules undergo conformational changes in their secondary structure, resulting in the formation of a hydrogel. In the study by Chen et al., shear forces were applied to silk protein solutions through mechanical stirring, leading to the preparation of silk protein hydrogels.[38] They further observed that, in this gelation process, the β-folded crystalline structures formed by silk proteins aligned along the direction of shear forces, ultimately yielding a hydrogel with anisotropic properties and a directed 3D framework. Additionally, they discovered that the incorporation of surfactants reduced the gelation time. We summarized how surfactants facilitate silk protein crosslinking in Section 3.1.8.

3.1.4 | Electric field

Electrofield processing leverages the principles of electrochemical cells, involving electrochemical reactions.[39] Within an electrolytic cell, the occurrence of electrochemical reactions leads to a localized decrease in pH within the solution, which falls below the isoelectric point of silk protein. As a result, silk protein acquires a negative charge and migrates toward the anode, where it aggregates in a micellar-like form. Subsequently, intermolecular physical entanglements occur, along with a transition in secondary structure, resulting in the formation of a hydrogel. Wang et al. employed this method to produce silk protein hydrogels containing graphene nanosheets after reacting under a voltage of 50 V for 30 min.[68] The use of an electrolytic cell to generate silk protein hydrogels requires external voltage application, which might potentially harm cells and hinder the creation of scaffolds for cell loading. Additionally, the time required for fabricating silk protein hydrogels through electrolytic cell methods is relatively prolonged. Consequently, research on the construction of silk protein hydrogels using electrolytic cells is limited and has not gained mainstream prominence.

3.1.5 | Heat

The essence of using a heating method to prepare silk protein hydrogels lies in intensifying the collision of silk protein molecules and enhancing the hydrophobic interactions between them, ultimately leading to the formation of a network structure through β-folded crosslinking of silk protein molecules.[69] Research has demonstrated that higher temperatures result in silk protein hydrogels with stronger mechanical properties and smaller pore sizes.[41] Consequently, by adjusting the concentration of silk protein and the heating temperature, the mechanical strength of silk protein hydrogels can be modulated. While the heating method allows for the creation of mechanically tunable silk protein hydrogels, it is limited when it comes to producing silk protein hydrogel scaffolds for cell loading or other bioactive factor incorporation.

3.1.6 | pH modulating

The charge carried by a protein is influenced by the pH of the solution it resides in, leading to alterations in its physical behavior. When the solution's pH approaches the isoelectric point of silk protein (pH 3.8–4), the total charge carried by the silk protein becomes close to zero. Consequently, the electrostatic repulsion between the molecules is minimized, facilitating aggregation and the transition in secondary structure, ultimately forming a gel.[70] In the study by Nagarkar et al., when the pH of the silk protein solution was reduced from 8.2 to 2, the formation of silk protein gel was observed, and the speed of gel formation was positively correlated with the rate of pH reduction.[71] This gelation process involves a change in the solution's pH, which can potentially impact cellular behavior. Therefore, when constructing cell-containing hydrogels, the influence of pH variations on cell activity must also be taken into consideration.

3.1.7 | Organic solvent treatment

Studies have reported that the use of methanol, ethanol, and other water-soluble organic reagents can rapidly dehydrate the hydrophobic regions of silk protein. This induction of hydrophobic interactions among silk protein molecules leads to the formation of β-folds, resulting in a 3D crosslinked network and the creation of silk protein hydrogels.[45,72,73] Shu et al. discovered that mechanically training ethanol-treated silk protein hydrogels could alter the orientation of β-fold crystallization within the gel, thereby modifying the hydrogel's mechanical and optical properties.[74] Although the preparation of silk protein hydrogels using organic solvents requires only a few minutes, the significant impact of these solvents on cell activity remains a limitation in the construction of cell-loaded hydrogel scaffolds.

3.1.8 | Surfactant treatment

Surfactants, being amphiphilic molecules, can envelop the hydrophobic regions of silk protein, thereby reducing surface tension and enhancing hydrophobic interactions. This ultimately encourages the formation of intermolecular β-folds, resulting in silk protein hydrogel formation. Common surfactants include sodium dodecyl sulfate and polysorbate.[47,75] Therefore, the incorporation of surfactants into silk protein solutions can expedite the formation of silk protein hydrogels. Given their potential cytotoxicity, the biocompatibility of surfactants must be considered when selecting appropriate agents.[47]

In summary, the physical crosslinking methods employed to generate silk protein hydrogels are fundamentally reliant on the formation of β-folds, leading to the creation of a water-based 3D molecular network. Thus, among the eight physical methods detailed in this article, all involve changes in the secondary structure of silk protein molecules. Research has shown that alterations in silk protein secondary structure can impact the behavior of rat bone marrow-derived mesenchymal stem cells (rBMSCs). An increase in β-fold crystallization promotes osteogenic differentiation of rBMSCs growing on the surface of silk protein hydrogels.[76] Consequently, when using physical crosslinking to construct silk protein hydrogels, the effects of secondary structure changes on silk protein's biological properties must be considered. Additionally, apart from the self-assembly method, the other seven methods introduce factors such as sound, heat, force, electricity, and chemical reagents, which may affect the biological activity of both cells and noncellular bioactive components. Therefore, for the fabrication of cell-loaded or growth factor-loaded silk protein hydrogel scaffolds, the effects of these physical factors on the aforementioned bioactive components need to be taken into account.

3.2 | Chemical crosslinking

Differing from physical crosslinking, chemical crosslinking involves the formation of covalent bonds between polymer chains, resulting in a stable water-based 3D network structure. Silk protein's amino acid side chains possess reactive functional groups like hydroxyl, amino, and carboxyl groups, rendering them amenable to chemical crosslinking or post-modification followed by crosslinking. Furthermore, chemical crosslinking doesn't rely on the intermolecular interactions of silk protein molecules to form β-folds, thereby retaining their original secondary structure. Common chemical crosslinking methods encompass chemical reagent crosslinking, enzyme crosslinking, irradiation crosslinking, and photo-crosslinking.

3.2.1 | Chemical reagent crosslinking method

Chemical reagent crosslinking primarily employs chemical agents as crosslinking molecules to connect the hydroxyl, amino, and carboxyl groups of silk protein, thereby forming a 3D network structure and producing silk protein hydrogels.[77] Common chemical agents include genipin,[78–80] glutaraldehyde,[81] and carbodiimide.[72,82] Genipin is a product of geniposide hydrolysis by β-glucosidase, and it can react with the ε-amino group of silk protein, forming a crosslinked network.[49] Glutaraldehyde is a widely-used protein crosslinking reagent that can react with the ε-amino and phenolic hydroxyl groups of silk protein, creating a crosslinked network.[83] Additionally, carbodiimide mainly reacts with carboxyl groups. Since silk protein has a low carboxyl content, carbodiimide is commonly employed to crosslink silk protein with other high carboxyl-containing macromolecules such as collagen or gelatin.[72,84] Chemical crosslinking reagents usually exhibit cytotoxicity and must be carefully dosed when used in tissue engineering scaffold preparation. Among these three chemical crosslinking reagents, genipin is relatively less toxic.

3.2.2 | Enzyme crosslinking method

Enzyme crosslinking utilizes enzyme-catalyzed reactions to chemically link functional groups between silk protein chains, forming covalent bonds. Horseradish peroxidase (HRP) is the most common enzyme used with H_2O_2 for silk protein hydrogel preparation.[85–87] With the reaction of HRP and H_2O_2, the phenolic hydroxyl groups of silk protein tyrosine residues could be crosslinked, leading to the preparation of silk protein hydrogels.[88] Moreover, enzyme crosslinking can be employed to construct composite hydrogels containing silk protein and other biomacromolecules. Raia et al. crosslinked silk protein and tyramine-modified hyaluronic acid using HRP and H_2O_2, resulting in a composite hydrogel with improved moldability and structural stability.[89] Glutamine transferase, carbonic anhydrase, and tyrosinase can all be employed based on similar mechanisms to crosslink silk protein with other biomacromolecules, resulting in hydrogels containing silk protein.[90–92] In comparison to chemical reagent crosslinking, enzyme-based crosslinking is more environmentally friendly.

3.2.3 | Irradiation crosslinking method

Irradiation crosslinking involves subjecting a silk protein solution to γ-rays, generating a large number of free radicals in both the silk

protein and water molecules.[93–95] The subsequent recombination of these free radicals leads to the formation of new chemical bonds between silk protein molecules, resulting in the formation of silk protein hydrogels. Under irradiation conditions, silk protein crosslinking occurs rapidly, making irradiation crosslinking suitable for preparing silk protein hydrogels with dispersed nanoparticles.[96] Kim et al. utilized rapid irradiation crosslinking to prepare silk protein hydrogels containing hydroxyapatite nanoparticles. However, the poor dispersion of hydroxyapatite nanoparticles within the silk protein hydrogel hinders the creation of a uniformly dispersed hydrogel system.[97] For the preparation of silk protein hydrogels loaded with cells or other bioactive components, the use of irradiation crosslinking may be restricted due to its potential harm to these active ingredients.

3.2.4 | Photo-crosslinking method

The photo-crosslinking method relies on chemically modifying silk protein to render it capable of crosslinking in the presence of a photosensitizer-induced free radical. The most common silk protein modification reagent in photo-crosslinking is glycidyl methacrylate (GMA).[55] GMA reacts with the ε-amino group of silk protein lysine residues. The modified silk protein undergoes an addition reaction when exposed to free radicals generated by the photosensitizer, forming a crosslinked network.[98] Currently, the most commonly used photosensitizer in biomedical research is lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), known for its high efficiency and low toxicity. The advent of photo-crosslinking has simplified the creation of silk protein hydrogels loaded with cells and growth factors.[99–102] Additionally, by combining digital stereolithography printing technology, researchers have constructed silk protein hydrogel scaffolds with specific 3D structures. This technique enables precise control over the spatial distribution of cells within the silk protein hydrogel.[103]

In summary, in contrast to physical crosslinking, the 3D network structure formed by chemical crosslinking is more stable. Furthermore, chemical crosslinking does not significantly impact the secondary structure of silk protein, imparting greater versatility to silk protein hydrogels. Among the four chemical crosslinking methods outlined in this review, photo-crosslinking has become the predominant method for preparing silk protein hydrogels in the field of tissue engineering. Photo-crosslinking does not introduce excessive chemical reagents, ensuring the biocompatibility of silk protein hydrogels. Moreover, due to its rapid gelation, it can be employed in the construction of hydrogel scaffolds loaded with cells or other bioactive components. The nature of photo-crosslinking also allows for digital stereolithography printing, enabling the creation of hydrogel scaffolds with specific 3D structures. Lastly, digital stereolithography printing technology can precisely control the distribution of cells within the hydrogel scaffold. Therefore, photo-crosslinked silk protein can be processed into tissue engineering scaffolds with both cell-type biomimicry and spatial distribution biomimicry.

4 | APPLICATION OF SILK FIBROIN HYDROGELS IN SKIN REGENERATION

The skin, as the largest organ of the human body, often suffers from large-scale defects due to factors such as trauma and tumor excision. Current clinical treatments for extensive full-thickness skin defects mainly involve autologous skin grafts, allogeneic skin grafts, and xenogeneic skin grafts.[2] These approaches carry risks of donor scarcity, immune rejection, and pathogen transmission, often leading to fibrotic scar formation during skin regeneration.[3,4,104] Therefore, achieving functional regeneration of skin defects remains challenging. Tissue engineering techniques offer new strategies for functional skin regeneration. By constructing skin tissue engineering scaffolds based on bioactive materials, the regeneration of skin tissue can be promoted. Recent research indicates that silk protein hydrogels have the ability to inhibit fibrotic scar formation, making them a trend in the field of skin tissue engineering.[25,105–107] Recent studies have shown that silk protein hydrogels exhibit a 3D network structure similar to the natural extracellular matrix and can carry cells and growth factors that promote tissue repair, thus demonstrating promising applications.[90,92] This review provides a categorized summary and discussion of skin tissue engineering studies based on silk protein hydrogels over the past decade (2013–2023).

4.1 | Pure silk fibroin hydrogel scaffolds

Pure silk protein hydrogel scaffolds are primarily composed of silk protein and do not contain other biomaterials or bioactive factors (including cells, growth factors, and small molecules). In the study by Chouhan et al., silk protein solutions (3% wt/v) sourced from mulberry silkworm and tussah silkworm were mixed to form gels through self-assembly within the human body temperature range (15–20 min) (Figure 3A).[64] They used self-assembly of different types of silk proteins to construct a silk protein hydrogel with thermo-responsive in situ gelation properties. Compared to collagen hydrogels, this silk protein hydrogel not only suppresses local inflammatory responses in skin tissue but also accelerates epithelialization, ultimately achieving functional skin regeneration for third-degree burns.

In another research, riboflavin (RF) and ammonium persulphate (APS) were used for preparing photopolymerized silk fibroin gel for advanced burn wound care.[112] With RF and APS, the tyrosine of the silk fibroin would be photo-oxidated when exposed to white light, leading to gelation. They applied the gel to second-degree burns in rodent models, and the results proved that the gel realized scarless healing. Notably, using RF, APS and white light for gelation will cost near 20 min, which means it is not suitable for in situ gelation. To achieve fast in situ gelation, we previously used GMA-modified silk fibroin to prepare silk gel.[113] Lithium phenyl (2,4,6-trimethylbenzoyl) phosphate (LAP) was used as photo-initiator; UV light at 380 nm was utilized for gelation within 10 s.

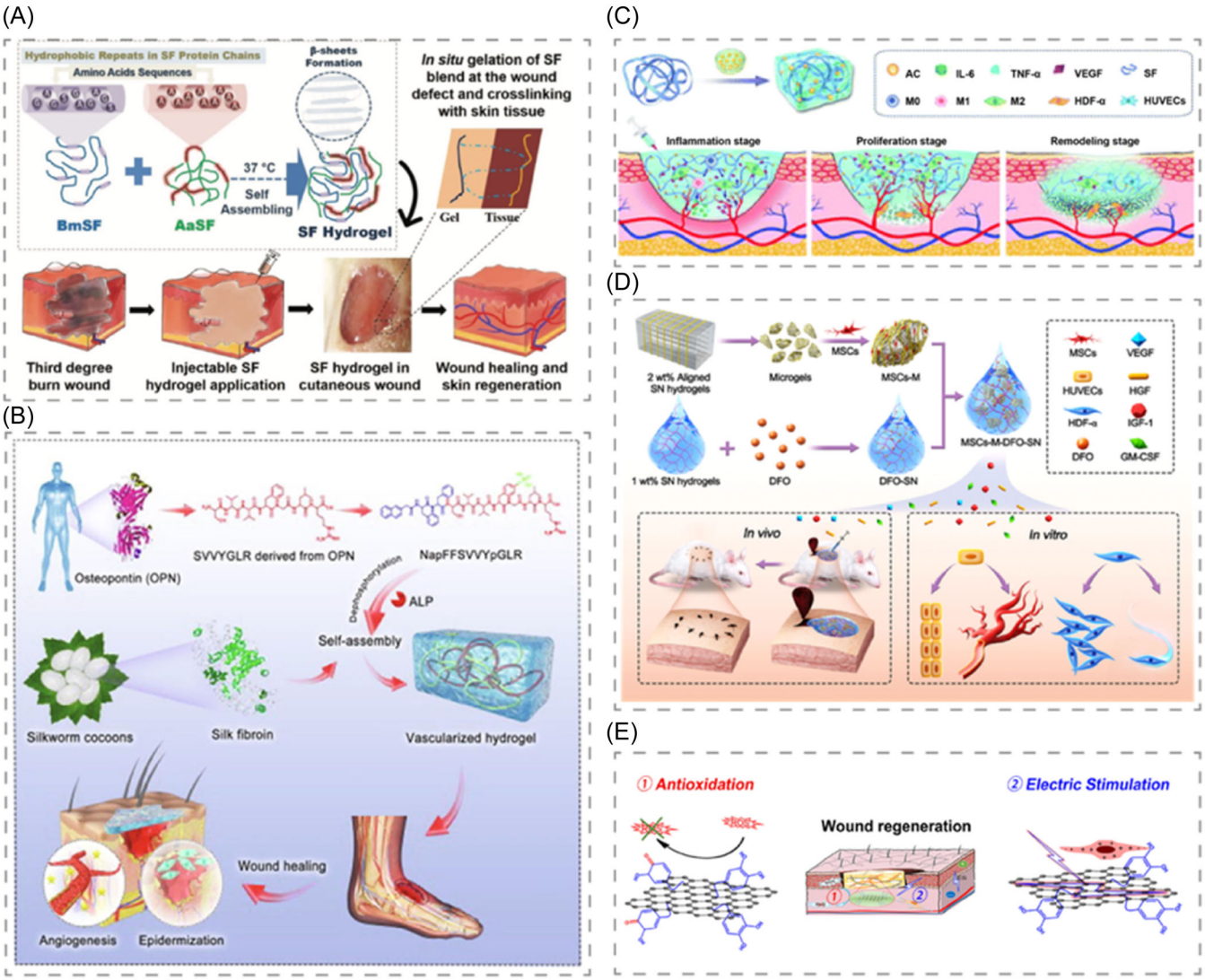

FIGURE 3 Applications of composite silk protein hydrogel scaffolds in skin tissue engineering. (A) Self-assembly of silk protein hydrogels. Mixing silk protein solutions sourced from mulberry silkworm and tussah silkworm results in the formation of hydrogels with thermoresponsive gelation properties within the human body temperature range. This hydrogel promotes functional skin regeneration for third-degree burns.[64] (B) Incorporation of angiogenic peptide into silk protein hydrogel. The bioactive peptide NapFFSVVYGLR, similar in function to vascular endothelial cell growth factor, is integrated into silk protein aqueous solutions, forming self-assembling hydrogels. These hydrogels accelerate neovascularization and achieve rapid skin defect functional regeneration.[108] (C) Silk protein nanofiber hydrogel as a drug carrier. Heat dialysis-prepared silk protein nanofiber hydrogels serve as carriers for asiaticoside, a drug. This hydrogel exhibits anti-inflammatory and proangiogenic effects, promoting skin defect repair.[109] (D) Introduction of mesenchymal stem cells (MSCs) into silk protein hydrogel. MSCs are introduced into desferrioxamine-containing silk protein hydrogels, promoting neovascularization and suppressing inflammatory reactions. This hydrogel enhances the therapeutic effects of desferrioxamine and optimizes skin flap repair.[110] (E) Enhanced scavenging of reactive oxygen species (ROS) by silk protein hydrogel. Polydopamine-reduced graphene oxide is incorporated into silk protein hydrogel, enhancing its ability to clear ROS. This hydrogel also facilitates electrical signal transmission, promoting cell growth and proliferation.[111]

Thus, it could be used for fast in situ gelation and digital light processing (DLP) 3D printing. Our study proved that in situ-gelated hydrogel and 3D printed scaffolds both performed well in promoting skin regeneration while scaffolds with 3D structure facilitated cells infiltration, suitable for large-size defects. Besides, single-cell transcriptome was used to reveal that the photo-gelated silk fibroin hydrogel created a favorable microenvironment for high-efficient skin regeneration.

4.2 | Silk fibroin hydrogel scaffolds loaded with bioactive agents

Researchers believe that vascularization during skin defect regeneration can improve the hypoxic environment at the defect site, facilitate nutrient supply, and support the migration of regenerating cells to the defect site. Therefore, researchers have incorporated bioactive factors that promote angiogenesis into silk

protein hydrogels to achieve rapid and effective skin regeneration. Wang et al. incorporated the angiogenic peptide NapFFSVVYGLR, which mimics the function of vascular endothelial cell growth factor, into silk protein aqueous solutions to form self-assembling stable hydrogels (Figure 3B).[108] Their experimental results showed that silk protein hydrogels with the incorporated angiogenic peptide could promote neovascularization as early as 3 days after subcutaneous implantation, achieving accelerated functional regeneration of skin defects. Liu et al. prepared silk protein nanofiber hydrogels through heat dialysis and used these hydrogels as carriers for the drug asiaticoside for skin defect repair (Figure 3C).[109] Their results demonstrated that the asiaticoside-loaded silk protein hydrogel exhibited anti-inflammatory and proangiogenic effects. Wu et al. introduced mesenchymal stem cells into desferrioxamine-containing silk protein hydrogels and applied them to skin flap repair (Figure 3D).[110] They found that introducing mesenchymal stem cells could promote neovascularization and suppress inflammatory reactions, optimizing the therapeutic effects of desferrioxamine-containing silk protein hydrogels.

The immune microenvironment also affects the outcomes of skin defect repair. Therefore, incorporating immune-regulatory substances into silk hydrogels can promote immune microenvironment modulation and skin defect regeneration. Inflammation at the defect site generates reactive oxygen species, hindering skin defect repair. Lee et al. integrated epigallocatechin gallate into silk protein hydrogels, endowing them with the ability to scavenge reactive oxygen species at the defect site, ultimately promoting skin regeneration.[114] Tang et al. used polydopamine-reduced graphene oxide to enhance the ability of silk protein hydrogels to scavenge reactive oxygen species (Figure 3E).[111] Moreover, they found that polydopamine-reduced graphene oxide could conduct electrical signals, promoting cell growth and proliferation. Bhar et al. incorporated *aloe vera* gel extract into silk protein hydrogels, further enhancing their immune-modulating effects.[115] Their results indicated that *aloe vera* gel extract-incorporated silk protein hydrogels significantly reduced inflammation levels at the defect site (downregulating expression of interleukin-1β and tumor necrosis factor-α) and promoted expression of anti-inflammatory factors (interleukin-10, transforming growth factor-β).

It is evident that silk protein hydrogels inherently possess the capability to modulate the immune microenvironment of skin defects and promote functional regeneration. Moreover, researchers can enhance their angiogenesis-promoting or immune-regulatory capabilities by incorporating functional components (growth factors, cells, small molecules, and so on) into silk protein hydrogels, thus achieving the goal of promoting skin defect regeneration. Compared to composite silk hydrogel scaffolds, pure silk protein hydrogel scaffolds are cost-effective, easy to quality control, and suitable for large-scale production, offering greater translational potential.

5 | AN UPDATE ON THE CLINICAL TRIALS ABOUT SILK FIBROIN-DERIVED DRESSINGS OR GRAFTS

The primary objective of clinical trials is to validate the therapeutic efficacy of bench-side technologies and to demonstrate their realistic advancements over conventional strategies. In the last decade, clinicians and scientists have extensively assessed the effectiveness of numerous wound dressings and skin grafts containing silk fibroin. While not all formulations are in hydrogel form, these studies collectively underscore silk fibroin's promise as a material for developing innovative wound dressings and skin scaffolds. Our aim is to synthesize the findings of pertinent clinical trials and inspire scientists working in this field.

A Chinese research team pioneered a physically crosslinked silk fibroin wound dressing.[116] Both their preclinical and clinical studies (involving 71 patients) substantiate the significant reduction in wound healing time and adverse events—such as inflammatory reactions—compared to two commercial dressings (Suprathel®, PMI, Denkendorf, Germany; Sidaiyi®, Healthtech) (Figure 4A). Notably, the absence of additives in the silk fibroin-derived dressing facilitates registration and industrial manufacturing. This innovation, now registered as SeriSkin® (ZHEJIANG XINGYUE BIOTECHNOLOGY CO. LTD), has received approval from the NMPA of China, specifically for managing skin graft donor sites.

In another study, the efficacy of a knitted silk fibroin wound dressing (Dressilk®, Prevor, France) was compared to a biosynthetic dressing, consisting of a nylon mesh covered by porcine type 1 collagen (Biobrane®, Smith and Nephew, USA).[117] Designed for superficial wounds, burns, and skin graft donor sites, the clinical trial involving 11 patients demonstrated that Dressilk® and Biobrane® exhibit similar functions in inhibiting scar formation (Figure 4B). This suggests that Dressilk® could be a promising alternative to biosynthetic options. Overall, these results highlight the potential of silk fibroin-based wound dressings and skin scaffolds in managing skin defects.

Furthermore, Hasatsri et al. developed a bilayered skin graft incorporating silk fibroin, silk sericin and gelatin.[118] Initially in hydrogel form, the composite layer was freeze-dried for application. Clinical studies involving 23 patients revealed that skin defects treated with the bilayered graft healed significantly faster than those treated with Bactigras®, a drug-loaded leno weave (Smith and Nephew, USA) (Figure 4C). Although the freeze-dried foam form was used in this study, the porous nature of the material was emphasized for its ability to absorb wound exudate, addressing a critical consideration often overlooked in hydrogel-based formulations.

Comparing the number of clinical trials to bench-side research, it becomes evident that only a limited number of research-based products have undergone clinical trials and demonstrated promising efficacy in patient outcomes. Thus, challenges associated with commercializing silk fibroin-derived medical products do exist. Firstly, for research, the source of silk fibroin might be flexible, while for medical

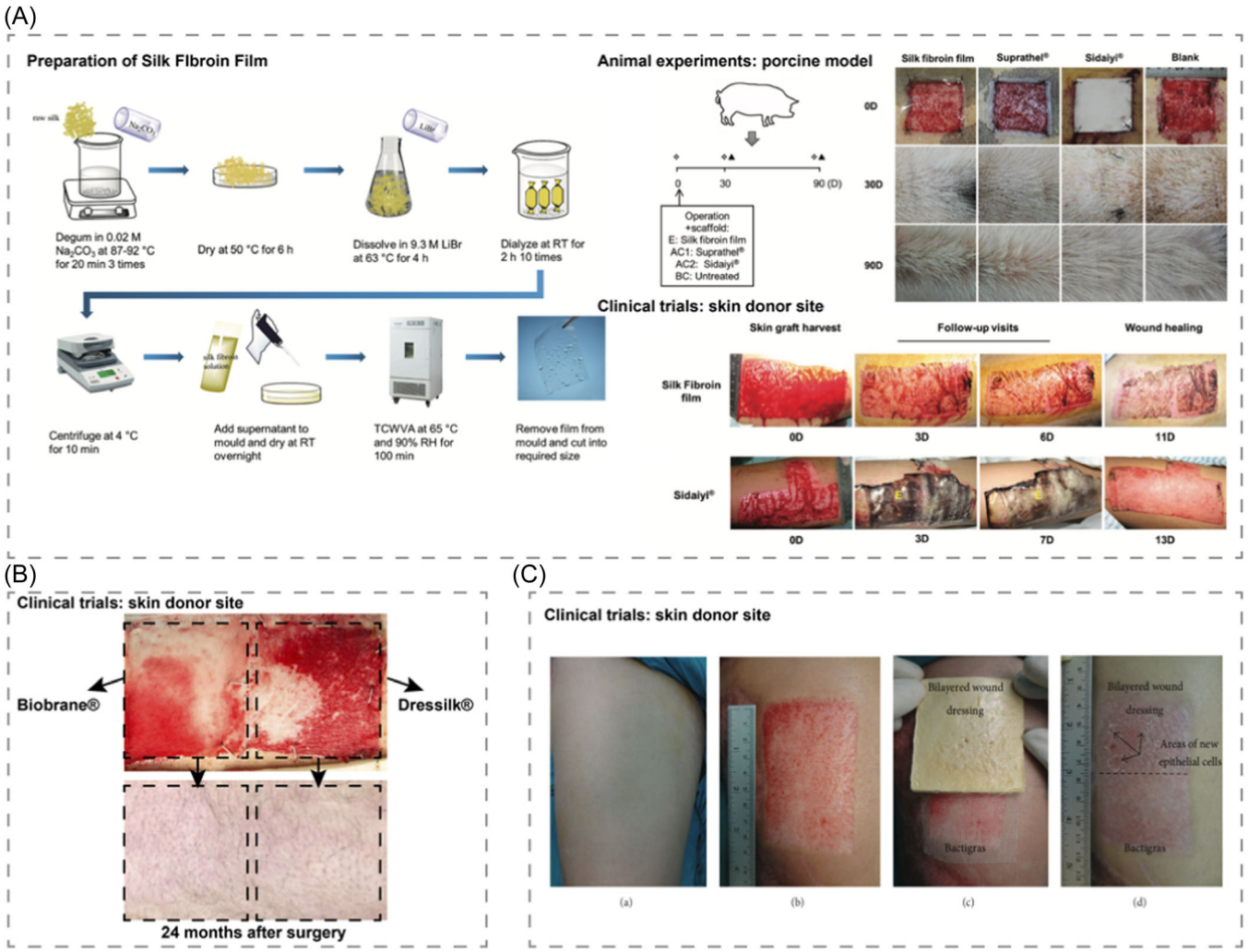

FIGURE 4 Clinical studies using silk fibroin-derived medical products. (A) Silk fibroin film represents better healing efficacy in comparison to polyurethane wound dressing-Suprathel® and silk-silicone composite dressing-Sidayi®.[116] (B) Dressilk® (a knitted silk fibroin wound dressing) represents similar healing efficacy to Biobrane® (a nylon mesh covered by porcine type 1 collagen).[117] (C) The bilayered wound dressing containing silk fibroin exhibits better therapeutic efficacy than Bactigras® (a drug-loaded leno weave).[118]

products, the source of silk fibroin should be medical grade, meeting many compulsory criteria, such as limited endotoxin, virus, bacteria, and so on. These criteria make only a few available suppliers of medical-grade silk fibroin in the market, which might affect the commercializing process. Secondly, for bench-side, many silk fibroin-based scaffolds might contain other bioactive agents, which was mentioned before. Although the added bioactive components could improve the therapeutic efficacy of the scaffolds, they might hinder the scalable production and registration process. The more complicated the scaffold is, the harder the scaffold could be commercialized. Thus, commercializing silk fibroin hydrogel scaffolds is challenging. Notably, according to current status, there is still a need for the development of dressings and scaffolds using silk fibroin as the raw material and hydrogel as the application form, as hydrogel is similar to our extracellular matrix and may favor skin regeneration.[119–122] Herein, scientists and physicians need to work together to commercialize more silk fibroin hydrogel-based medical products.

6 | SUMMARY AND FUTURE PROSPECTS

Due to its excellent biocompatibility and degradability, silk fibroin hydrogel has found extensive applications in the field of skin tissue engineering. Moreover, silk fibroin has been shown to possess anti-inflammatory and anti-fibrotic properties, further making it a valuable asset in skin tissue engineering endeavors.[25,28] Depending on the requirements of different tissue regeneration scenarios, researchers have incorporated various components into silk fibroin hydrogels. Over the past decade, researchers have explored the use of silk fibroin hydrogels to promote skin tissue regeneration, often by incorporating components that stimulate angiogenesis or inhibit inflammation for skin regeneration. However, the physical properties of biomaterials, such as mechanical strength and porosity, significantly influence tissue regeneration outcomes.[123–125]

For skin defects, hydrogel scaffolds with biomimetic mechanics and porous structures facilitate cell migration and skin tissue

regeneration. In previous studies, our research group developed a gelatin-hyaluronic acid hydrogel scaffold with a cantilever beam structure and mechanical properties (Young's modulus approximately 30.53 kPa) similar to human soft tissue, demonstrating its advantageous support for cell migration and tissue regeneration.[126] As previously mentioned, silk fibroin can be modified with GMA to form photo-crosslinkable methacrylated silk fibroin (MeSF), allowing for DLP 3D printing to create silk fibroin hydrogel scaffolds with biomimetic 3D structures.[113] DLP 3D printing is a gentle process that enables the fabrication of silk fibroin hydrogel scaffolds loaded with cells and growth factors. Moreover, by adjusting the concentration and crosslinking degree of MeSF, the mechanical properties of the scaffold can be tailored. Therefore, combining MeSF hydrogel with DLP 3D printing technology presents a promising strategy for constructing skin tissue engineering scaffolds or skin equivalents.

Moreover, smart hydrogels might also be attractive for scientists focusing on silk fibroin. Recently, smart hydrogels are becoming prevalent in tissue engineering.[127–129] Speaking of smart hydrogels, it basically means that they can response to endogenous or/and exogeneous stimuli.[130,131] For endogenous stimuli, they are usually provided by the microenvironment at the defect, such as reactive oxygen species (ROS), immune cells, electrical signals, etc. For exogenous, they are usually provided by the caregivers, such as ultrasound, heat, magnetic field, electric field, and so on. The smart hydrogels could response to these stimuli and modulate the microenvironment at the defect to promote tissue regeneration. Thus, chemical modification could be utilized to bring stimuli-responsive motifs to silk fibroin, making the silk fibroin hydrogel stimuli-responsive. Additionally, due to the advancement of recombinant DNA technology, recombinant silk fibroin is another promising alternative to fabricate smart hydrogels as recombinant DNA technology could insert functional sequences into silk fibroin as well.[132] Besides, functional nanoparticles, such as liposome, magnetic particles, and piezoelectric materials could be added to silk fibroin hydrogel to make it smart hydrogel with microenvironment modulatory ability.

Overall, to enhance the therapeutic effects of silk fibroin hydrogel-based tissue engineering skin scaffolds, future research can be focused on two aspects. Firstly, researchers need to know how the physical properties of the scaffolds influence the therapeutic efficacy, such as mechanical properties, 3D structure, etc. Then, they can use advanced technologies, for example 3D bioprinting, to fabricate scaffolds with customized physical properties. Secondly, researchers need to analyze the temporal and spatial dimensions of skin development and regeneration processes to understand key biological events and their regulatory factors. These knowledges will enable the intelligent loading of appropriate bioactive components into silk fibroin hydrogels or construct stimuli-responsive silk fibroin hydrogels for spatiotemporal control of the regeneration process. As a result, future silk fibroin hydrogel scaffolds should combine biomimetic physical properties with intelligent control over biological events to achieve rapid skin regeneration.

AUTHOR CONTRIBUTIONS

Dipeng Li: Funding acquisition; Writing—original draft. **Renjie Liang**: Conceptualization; Writing—original draft; Writing—review and editing. **Yirong Wang**: Writing—original draft. **Yanting Zhou**: Writing—original draft. **Weibang Cai**: Writing—original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data are available in the main text. All authors have read and approved the final version of the manuscript. Liang Renjie had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author Renjie Liang affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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