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Review

Silk Fibroin and Its Nanocomposites for Wound Care: A Comprehensive Review

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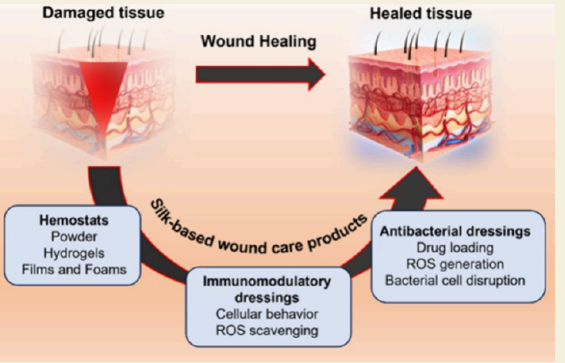
ABSTRACT: For most individuals, wound healing is a highly organized, straightforward process, wherein the body transitions through different phases in a timely manner. However, there are instances where external intervention becomes necessary to support and facilitate different phases of the body's innate healing mechanism. Furthermore, in developing countries, the cost of the intervention significantly impacts access to treatment options as affordability becomes a determining factor. This is particularly true in cases of long-term wound treatment and management, such as chronic wounds and infections. Silk fibroin (SF) and its nanocomposites have emerged as promising biomaterials with potent wound-healing activity. Driven by this motivation, this Review presents a critical overview of the recent advancements in different aspects of wound care using SF and SF-based nanocomposites. In this context, we explore various formats of hemostats and assess their suitability for different bleeding situations. The subsequent sections discuss the primary causes of nonhealing wounds, i.e., prolonged inflammation and infections. Herein, different treatment strategies to achieve immunomodulatory and antibacterial properties in a wound dressing were reviewed. Despite exhibiting excellent pro-healing properties, few silk-based products reach the market. This Review concludes by highlighting the bottlenecks in translating silk-based products into the market and the prospects for the future.



KEYWORDS: *Silk fibroin, wound care, hemostats, immunomodulation, antibacterial, tissue engineering, biopolymers, nanoparticles, proteins*

1. INTRODUCTION

The skin, the human body's largest organ, performs several critical roles, including thermal regulation, protection against invading pathogens, hydration, and initialization of vitamin D synthesis. Therefore, a severe injury and compromised wound closure can be debilitating and even life-threatening to the patient. The skin has an innate mechanism for wound closure involving well-regulated interactions among various cells, growth factors, and cytokines. The cascade of healing is classified into four overlapping phases (Figure 1): (i) hemostasis, (ii) inflammation, (iii) proliferation, and (iv) matrix remodelling.[1] These phases work together to achieve physiological restoration of the tissue. Wounds that fail to progress through these stages of healing exhibit impaired healing. Several factors can impede wound closure, such as the severity of the injury, underlying comorbidities (uncontrolled diabetes, old age, etc.), and pathophysiology of the wound. Such wounds often enter a state of pathologic inflammation due to incomplete, delayed, or uncoordinated healing processes and contribute toward the development of difficult-to-heal or nonhealing wounds. In 2018, an estimated 2% of the population in developing countries was affected by nonhealing wounds, causing an enormous healthcare burden estimated to exceed three billion USD annually.[2] These difficult-to-heal wounds (such as severe burn wounds, infected wounds, etc.) present an enormous unmet clinical need worldwide. Therefore, extensive research has been dedicated to developing effective wound therapies that facilitate accelerated healing.[3] In the following sections, the phases of wound healing and the existing unmet clinical needs pertinent to healing and soft tissue regeneration are discussed in detail.

1.1. Phases of Wound Healing

1.1.1. Hemostasis. The body's first response to an injury is hemostasis, a mechanism that controls bleeding at the site of

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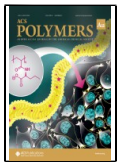

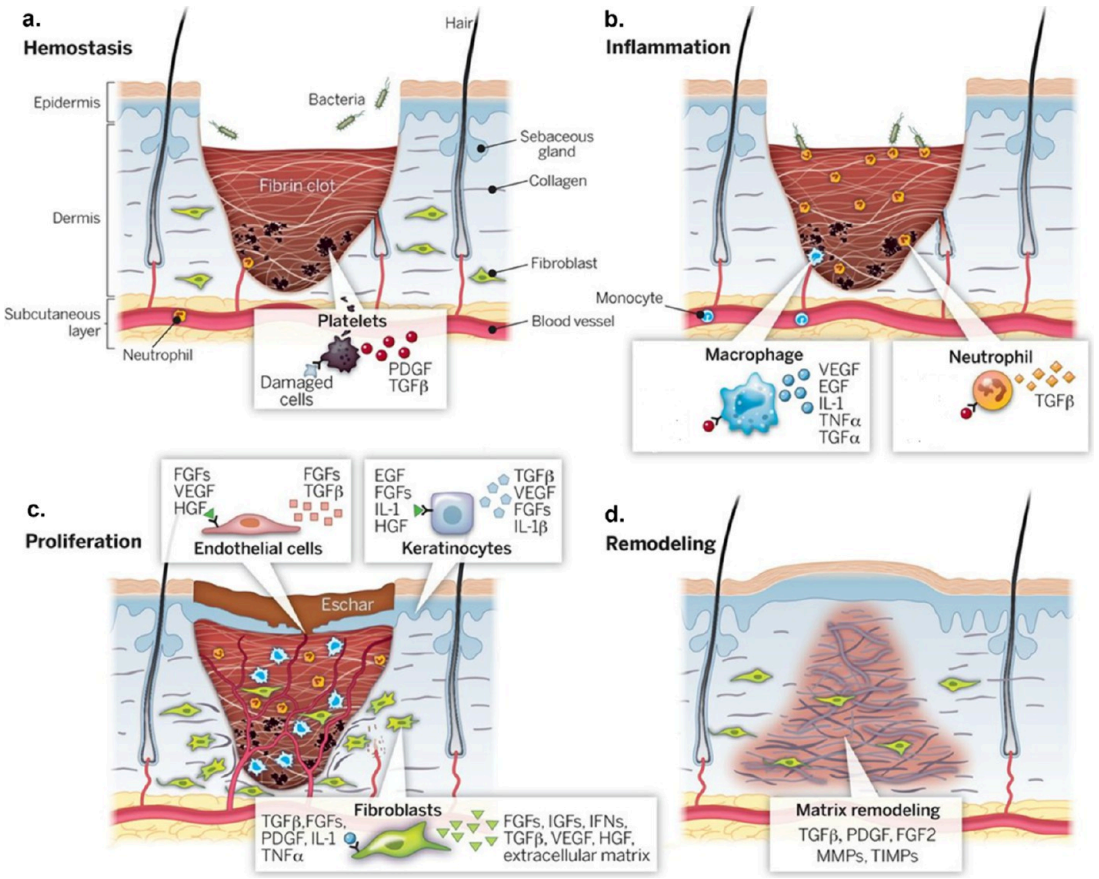

Figure 1. Phases of wound healing: (a) Hemostasis; (b) Inflammation; (c) Proliferation; and (d) Remodelling. (Adapted with permission from ref 4. Copyright 2014 The American Association for the Advancement of Science.)

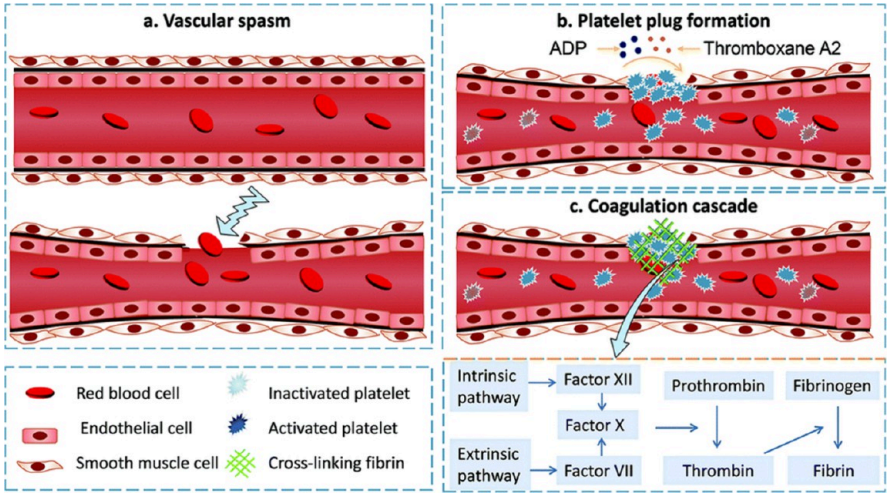

Figure 2. Schematic representation of hemostasis process: (a) Vascular spasm: vascular damage immediately stimulates vasoconstriction, restricting blood flow out of the vessel. (b) Platelet plug formation: platelets are activated that adhere to bleeding sites, forming an initial platelet plug. (c) Coagulation cascade: the extrinsic and intrinsic pathways are activated to convert fibrinogen into fibrin strands, reinforcing the platelet plugs. (Adapted with permission from ref 5. Copyright 2020 The Royal Society of Chemistry.)

injury. Hemostasis consists of multiple interlinked steps and is divided into four stages (Figure 2): (i) vascular spasm; (ii) formation of a temporary platelet plug; (iii) activation of the coagulation cascade; and (iv) formation of a fibrin mesh to produce a stable "blood clot".[5] The most immediate response to an injury is vasoconstriction (brief contraction of the blood vessel) to restrict the blood flow to the site of injury, thereby reducing blood loss. Vasoconstriction is triggered by local pain receptors and endothelial cells. Simultaneously, the process of platelet plug formation is initiated. In an intact blood vessel, the endothelial cells prevent clotting by expressing fibrinolytic heparin and thrombomodulin, which keeps the platelets in an inactive form. When blood vessels are damaged, the blood comes into contact with the extracellular matrix (ECM)

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Table 1. Cytokines Involved in the Inflammatory Phase

Cytokine	Cell source	Biological activity
	Pro-inflammatory cytokines	
Interleukin-1 (IL-1)	Macrophages, keratinocytes	Collagen synthesis; keratinocyte and fibroblast chemotaxis
Interleukin-2 (IL-2)	T lymphocytes	Promote infiltration of fibroblasts
Interleukin-6 (IL-6)	Macrophages, fibroblasts, PMNs	Enhance fibroblast proliferation, hepatic acute-phase protein synthesis
Interleukin-8 (IL-8)	Macrophages, fibroblasts	Macrophage and PMN chemotaxis; keratinocyte maturation
Tumor necrosis factor-α (TNF-α)	Macrophages	PMN migration; ±collagen synthesis
Interferon gamma (IFN-γ)	Macrophages, T lymphocytes	PMN and macrophage activation; suppress collagen synthesis; promote collagenase activity
	Anti-inflammatory cytokines	
Interleukin-4 (IL-4)	T lymphocytes, mast cells, basophils	Inhibition of IL-1, IL-6, TNF, fibroblast proliferation, and collagen synthesis
Interleukin-10 (IL-10)	T lymphocytes, keratinocytes, macrophages	Inhibition of: IL-1, IL-6, TNF, macrophages, and PMNs

components of the surrounding tissue, leading to the activation of platelets. The platelets are activated by binding to these ECM proteins (such as collagen, fibronectin, and von Willebrand factor) mediated by their receptors (e.g., glycoprotein VI). These activated platelets release messenger molecules such as thromboxane A2, adenosine diphosphate (ADP), and serotonin, causing aggregation of platelets at the site of injury. The platelet aggregate (platelet plug) physically seals the breaks in the blood vessel to prevent blood outflow; this is termed primary hemostasis.[6,7]

Aggregated platelets secrete several growth factors and chemokines to attract various cell types (fibroblasts and inflammatory cells) to initiate the healing process. The growth factors include platelet-derived growth factor (PDGF), transforming growth factor-β (TGF- β), insulin-like growth factor-1 (IGF-1), transforming growth factor alpha (TGF-α), and vascular endothelial growth factor (VEGF). These biomolecules serve to attract additional platelets, activating them in a positive feedback loop, accelerating the formation and propagation of the plug. Moreover, the surface of the activated platelets serves as a site for coagulation, facilitating the formation of a blood clot. The coagulation cascade is initiated to form a fibrin mesh that can strengthen the platelet plug and form a stable clot.[6,7]

A coagulation cascade is a sequential activation of a series of clotting factors in a multistep pathway. The coagulation cascade can be triggered by two different pathways: extrinsic and intrinsic, as shown in Figure 2c. The extrinsic pathway is activated when external trauma causes blood to escape circulation and come into contact with the tissue factor (TF). The intrinsic pathway is initiated by clotting factors within blood vessels and serves as a positive feedback loop to amplify coagulation. The two pathways converge into a common pathway to produce thrombin, which cleaves soluble fibrinogen to produce insoluble fibrin. Thrombin also activates platelets to generate a positive feedback loop for clot propagation. Once an adequate clot had formed, the coagulation process is deactivated to prevent excessive thrombosis. Subsequently, the clot is dissolved with fibrinolysis, a cascade that produces an enzyme (plasmin) that cleaves fibrin, dissolving the clot.[8]

1.1.2. Inflammation. The next phase of wound healing, inflammation, occurs within 24 h of injury and lasts up to 2 weeks in normal wounds but can persist significantly longer in nonhealing wounds. The primary function of the innate inflammatory phase is defense against pathogenic invasion and clearing debris in wounds.[9] The immune response is triggered by injury-induced signals: damage-associated molecular patterns (DAMPs) released by necrotic tissue and pathogen-associated molecular patterns (PAMPs) from the bacterial components. These DAMPs and PAMPs bind to pattern recognition receptors of the resident immune cells (mast cells, T cells, Langerhans cells, and macrophages), eliciting downstream inflammatory pathways.[10]

Mast cells at the wound site release granules containing enzymes (histamine and other active amines), which trigger the hallmarks of inflammation: redness, heat, swelling, and pain.[11] The pro-inflammatory chemokines and cytokines attract circulating leucocytes to the injury site. The roles of various cytokines in regulating the inflammatory phase are summarized in Table 1.

These soluble mediators recruit and activate fibroblasts and epithelial cells, preparing the body for the next phase of wound healing.

While an immune response is triggered to combat possible infections, it is crucial for the inflammation to subside to facilitate wound resolution and progress toward healing. In the absence of infection, the number of neutrophils declines within a few days after the onset of injury.[12] The majority of the neutrophils are extruded from the site as they adhere to the fibrin scab, while the remaining ones are removed by innate clearance mechanisms, including macrophage efferocytosis, apoptosis, phagocytosis, and necrosis.[13]

1.1.3. Proliferation. During the proliferative phase, the injured tissue undergoes structural and functional restoration. Key milestones include replacing the provisional fibrin matrix with a new matrix rich in collagen, proteoglycans, and fibronectin. Other critical processes are angiogenesis (formation of new blood capillaries) to restore circulation, formation of granulation tissue, and epithelialization. The proliferation phase is marked by extensive activation of keratinocytes, fibroblasts, and endothelial cells working together to promote matrix deposition, wound closure, and angiogenesis. Within 12 h postinjury, keratinocytes become activated as a response to various stimuli, such as changes in mechanical tension, electrical gradients, and exposure to pathogens, cytokines, and growth factors.[14] Consequently, the keratinocytes at the wound edge transform into a more invasive and migratory phenotype by undergoing a partial epithelial-mesenchymal transition. This allows the keratinocytes in the leading edge to migrate laterally across the wound, forming an epidermal layer (re-epithelialization).[15] The keratinocytes trailing behind the leading edge adjust their cell-adhesion properties, enabling them to reorganize their

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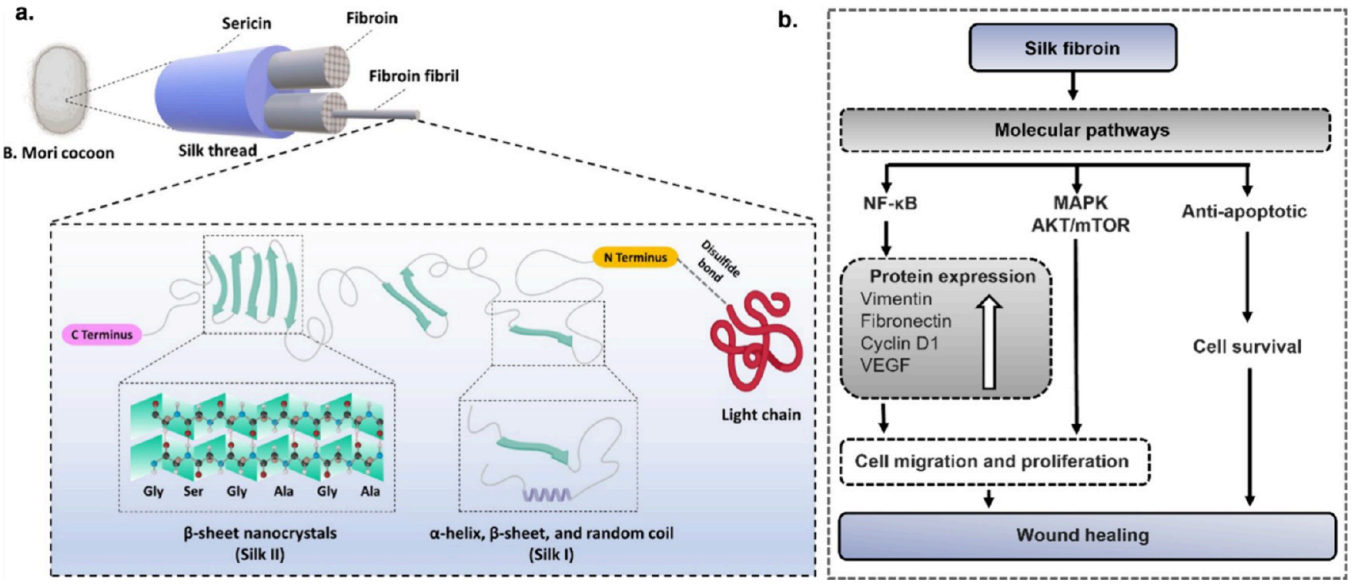

Figure 3. (a) Schematic representation of the structure of *B. mori* silk fibroin (SF). (Adapted with permission from ref 49. Copyright 2022 Elsevier.) (b) Molecular pathways activated by SF to promote wound healing.

arrangement to match with the migrating epithelial sheet.[14] Subsequently, the keratinocytes in the newly formed epidermis release matrix metalloproteinases (MMPs) to pave the way for migrating cells from the surrounding ECM into the wound site and facilitate the synthesis of new ECM proteins to restore the basement membrane. Migration of keratinocytes terminates when opposing edges meet, forming a thin epithelial layer and establishing new adhesions with the underlying matrix. Subsequently, keratinocytes differentiate, stratify, and regenerate the epidermis.[16]

Fibroblasts are the primary cells involved in replacing the provisional fibrin-rich matrix with granulation tissue. The newly formed granulation tissue serves as a scaffold to enable migration and differentiation of various cell types required for angiogenesis and deposition of ECM. Fibroblasts respond to various signaling molecules (including TGF-β, FGFs, HGF, and PDGF) from endothelial cells, platelets, and macrophages. These signals direct the cells to either adopt a pro-fibrotic role to deposit ECM proteins or differentiate into myofibroblasts to facilitate wound contraction.[15]

1.1.4. Matrix Remodeling. Matrix remodeling is the final phase of healing, during which granulation tissue matures into scar tissue and the tissue tensile strength is improved. The maturation of granulation tissue involves the aggregation of smaller capillaries into larger vessels and a decrease in the levels of glycosaminoglycans (GAGs), GAG-associated water content, and proteoglycans. Overall, a decrease in the metabolic activity of granulation tissue is observed during maturation. Fibroblasts play a key role in wound ECM remodeling and are primarily responsible for forming mature collagen fibrils. The tensile strength of the tissue is enhanced by changes in the amount, type, and organization of collagen. The composition of healthy skin is 80% type I collagen and 10% type III collagen. In contrast, granulation tissue primarily comprises 30% type III collagen with 10% type I collagen.[17] As the wound heals, type III collagen is gradually replaced by type I, resulting in increased tensile strength of the developing scar tissue.[18] However, the integrity and structural organization of the scar ECM are never fully restored. The collagen fibrils in a healthy tissue exhibit a basket weave orientation, whereas the scar ECM adopts a parallel arrangement of the fibrils. Therefore, scar tissue resulting from a wound typically possesses only up to 80% of the preinjury strength.[17,19]

These sequential changes in the ECM are achieved by optimizing collagen degradation and synthesis, which are regulated temporally by key MMPs. These collagenases, secreted by anti-inflammatory macrophages, keratinocytes, and fibroblasts, cleave the native helical collagen through the healing process.[20] Another critical aspect of remodeling is the restoration of skin elasticity with elastin. The degradation of a healthy dermal matrix releases elastin fragments, which serve as a signal for elastin synthesis from its precursor (tropoelastin).[21] Over time, the wound healing process subsides and concludes when endothelial cells, macrophages, and fibroblasts exit the injury site or undergo apoptosis.[22]

1.2. Silk Fibroin for Wound Care Applications

While wound healing is a crowded research area, there are several unmet clinical needs that are actively being addressed by researchers and clinicians. Some examples include personalized medicine, infection control, scar management, and, most importantly, cost-effective solutions. Addressing these limitations to improve patient outcomes requires interdisciplinary collaboration among researchers, clinicians, and industry partners.

In recent times, biomimetic wound dressings have been considered a promising candidate for soft tissue regeneration. Each wound type requires a specific set of properties in a dressing material; there is no universally ideal dressing for all wounds. Nonetheless, an optimal dressing typically comprises a biocompatible material with pro-healing capabilities. A non-adhesive dressing is often preferred to prevent damage to underlying healing tissue during removal.[23,24] Semiocclusive dressings allow aeration at the wound bed while serving as a protective barrier against microbial invasion.[25] There are clinically available skin replacement products; however, given the wide range of wound types and their anatomical locations, a broader range of products is warranted. The choice of biomaterial determines the quality of wound healing. In this

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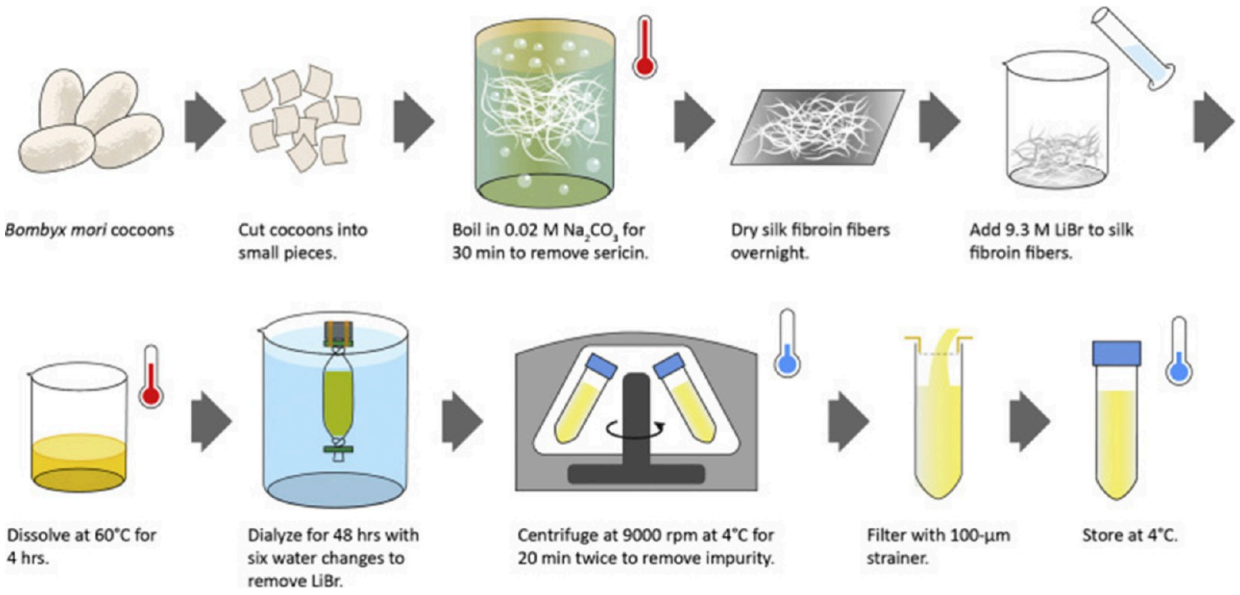

Figure 4. Extraction of SF from silk cocoons. Adopted with permission from ref 50. Copyright 2021 Elsevier with license under CC BY 4.0.

regard, dressings composed of natural polymers are of central interest owing to their low immunogenicity and bioactivity. Among them, silk fibroin (SF) has emerged as a potential candidate for wound dressings. SF-based dressings have also proven their efficacy as carriers for the local delivery of drugs, bioactive agents, and growth factors while supporting complete healing.[23,24,26]

As of today, collagen is the most popular biopolymer used in wound dressings; some examples are Integra (Integra Life-Sciences), Biobrane (Smith & Nephew), Kollagen (Eucare), etc. Collagen-based commercial dressings promote tissue growth and facilitate collagen deposition within the wound bed. Nonetheless, collagen is extracted from animal sources (bovine, porcine, etc.) and can lead to cross-contamination and provoke immune responses if not purified thoroughly. Invariably, the intensive extraction and purification processes render collagen-based products prohibitively expensive, particularly for patients from the lower-income population.[27] Thus, there is a pressing need to develop wound care products that meet the requirements of an ideal dressing while remaining cost-effective. In this regard, SF offers the added advantage of easy availability and affordability. To provide a rough comparison, 1 g of collagen costs USD 1575,[28] whereas SF (1 g) is priced at USD 300.[29] The price disparity is attributed to the rigorous processing involved in the collagen extraction.

Silk is a protein biopolymer commonly extracted from the cocoons of silkworms. Silkworms produce protective cocoons by spinning silk fibers during metamorphosis. SF has been extensively studied for applications in tissue engineering and regenerative medicine due to its distinctive properties, particularly the SF type isolated from domesticated silkworms (*Bombyx mori*). The availability of raw material and feasibility of SF extraction from cocoons using green technology have endorsed it as a viable biomaterial.[30,31] Moreover, the easy processability of SF makes it suitable for fabricating a diverse range of structural constructs such as hydrogels, porous scaffolds, thin films, nanofibrous mats, injectable systems, and 3D printed grafts.[32−34]

1.2.1. Structure and Properties of SF. Silk fibers are made up of two protein components, i.e., fibroin and sericin (Figure 3a). The major component is fibroin, which makes up the structural component of the silk filament and imparts mechanical strength, while sericin acts like an adhesive material, binding the fibroin filament together.[35] Reports suggest that the nanofibril (3.5 nm diameter) is the building element of silk, and a fibroin filament consists of a bundle of these nanofibrils. These nanofibrils intertwine and form into larger fibril units, termed microfibrils (20−200 nm diameter).[36] The primary structure of SF is composed of heavy (H−) polypeptide chains (390 kDa) and light (L-) polypeptide chains (26 kDa) connected by a single disulfide bond at the C-terminus of the H-chain to form H-L complexes. Moreover, glycoprotein P25 (30 kDa) is noncovalently connected to H-L chains, contributing to the overall structural integrity of the SF. The H-chain, L-chain, and P25 assemble with a ratio of 6:6:1. Moreover, SF is composed of both amorphous (∼33%) and crystalline (∼66%) regions. The H-chain primarily contributes to the fibrous properties of silk fibers with an amino acid composition of glycine (G) (46%), alanine (A) (30%), serine (S) (12%), Tyrosine (Y) (5.3%), and Valine (V) (1.8%).[37] Leucine, isoleucine, valine, and other amino acids are commonly found in the nonfibrous L-chains.[38]

The amino acid sequence of SF is approximately divided into four motifs, denoted as (i), (ii), (iii), and (iv), which alternate along the chain.[39] Motif (i) comprises a highly repetitive sequence of AGSGAG that arranges into β-sheets and forms the crystalline region. The AGSGAG sequence repeats 443 times, accounting for 2598 amino acid residues. This represents nearly half of the total amino acid residues present in the H-chain (5263).[40] Motif ii is characterized by a sequence that is comparatively less repetitive and includes hydrophobic and/or aromatic residues such as GAGAGY, GAGAGVGY, and GAGAGV. The sequence of motif iii is very similar to that of motif (i) except for the addition of an AAS motif. Motif (iv) consists of polar, negatively charged, bulky hydrophobic, and/or aromatic amino acid residues, making up the amorphous regions of the H-chains.[41,42]

1.2.2. Wound Healing Mechanism of SF. SF has been shown to modulate cellular behavior in most aspects of wound healing, on both physiological and molecular levels. SF is

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known to accelerate healing using the nuclear factor-kappa B (NF-κB) signaling pathway, which governs various cellular behaviors, including adhesion, proliferation, clearance of reactive oxygen species (ROS), and inflammation.[43] Consequently, NF-κB is considered to be an important mechanism during the healing of various wounds. In the SF-treated cells, an increased expression of two critical mediators of NF-κB was observed, i.e., Toll-like receptors (TLRs) and tumor necrosis factor receptor (TNFR). These mediators upregulated the expression of various target proteins such as epidermal growth factor (EGF), vimentin, cyclin D1, fibronectin, IL-10, transforming growth factor (TGF), and VEGF.[44,45] A recent study suggests that SF can modulate the expression of proteins involved in the proliferation and remodeling phases through NF-κB signaling.[43]

Another study reported that the *in vivo* application of SF (in β-sheet form) to burn wounds promoted the expression of MMP-12, fibronectin, integrin-β1, and type III collagen in the granulation tissue.[46] Moreover, SF promoted the upregulation of three distinct pathways: phosphoinositide 3-kinase (PI3K/AKT), mitogen-activated protein kinase kinase (MEK1), and c-Jun N-terminal kinase (JNK) resulting in the activation of induced c-Jun expression through downstream mechanisms and cell migration. Aykac et al. reported that SF has a protective effect in a burn wound (rat model) by stimulating the apoptotic pathway.[47] Various complex cellular pathways take place in a tightly coordinated cascade during the healing process, such as Wnt and Notch, TGF-β, AKT/mammalian target of rapamycin (mTOR), and mitogen-activated protein kinase (MAPK) signaling.[48] AKT/mTOR and MAPK are two critical cellular pathways in wound healing. A summary of the molecular pathways activated by SF is given in Figure 3b.

1.2.3. SF Extraction and Processing. To produce SF-based wound care products, the raw silk from the cocoons must undergo degumming to eliminate sericin and obtain pure fibroin (Figure 4).[50] Herein, cut cocoons (empty shells left after moths emerge) are cut into smaller pieces and boiled in an aqueous solution of 0.02 M sodium bicarbonate for 20 min with continuous stirring. Subsequently, the boiled cocoons are thoroughly washed using distilled water to remove the gum-like sericin protein, resulting in degummed silk fibroin fibers. SF solution is prepared by mixing degummed fibers (10 g) in 9.3 M lithium bromide (LiBr) solution and then keeping it at 40 °C for 4 h until it is fully dissolved. Besides LiBr, several solvent systems have been used for silk dissolution, such as Ajisawa's reagent (calcium chloride:ethanol:water with 1:8:2 molar ratio), calcium nitrate aqueous solution, ionic liquids etc. These solvents offer different solubility power, and hence, require different processing temperatures and duration.[51] Upon dissolution of fibroin, the salt (e.g., LiBr) is eliminated by dialyzing (cellulose membrane, 12 kDa cutoff) against deionized water for 3 days with a frequent water change. The SF solution was collected in 50 mL conical tubes and centrifuged at 4 °C at 12,700*g* for 20 min. The supernatant of centrifuged SF solution is filtered (100 μm filter) into new conical tubes. The final outcome is an aqueous solution of regenerated SF that can be used to fabricate various biomaterials. Alternatively, the solution can be lyophilized (freeze-dried) for long-term storage or to fabricate biomaterials in organic solvents.[52] The SF extraction process is the starting point for developing different formats of wound care products, such as hydrogels, foams, nanofibers, films, etc.[34,53−55]

The physicochemical properties of SF-based products rely on the properties of the extracted fibroin. For instance, the molecular weight of extracted SF depends on the boiling duration. A batch of silk subjected to 30 min of degumming exhibits a broad molecular weight distribution approximately centered at 100 kDa. The molecular weight tends to decrease when degummed for longer times and increase for shorter degumming times.[56] Prolonged boiling times lead to cleavage of the disulfide bonds between L- and H-polypeptide chains, along with the fragmentation of the amorphous sequences, resulting in a polydispersed molecular weight.[56] Moreover, the properties of the product can be influenced by the variability in the sterilization processes. Aqueous fibroin solutions can be sterilized by several methods, including γ radiation, filtration, and autoclaving. Herein, variability in irradiation dosing or steam sterilization duration can affect the molecular weight of the extracted SF. Hence, standardization of the sterilization protocol is essential. In some cases, sterilizing the final product before use proves to be a more convenient approach.[57,58] The choice of sterilization methods depends on the format of the SF-based product described below.

1.2.4. Fabrication of SF-Based Products. *1.2.4.1. Films.* SF films can be produced by spin coating, casting, or layer-by-layer technique.[59−61] For instance, nonpatterned SF films can be generated by depositing SF solution onto a plate and allowing it to dry overnight. Subsequently, β-sheet crystalline content is enhanced by immersing the film in an alcohol solution (ethanol or methanol) or by water annealing within a vacuum desiccator.[62] Conversely, patterned SF film can be produced by depositing fibroin solution on a preformed polydimethylsiloxane (PDMS) mold, overnight drying, and inducing β-sheet formation. The resulting patterned SF film is then peeled from the PDMS mold using a pair of tweezers.

1.2.4.2. Hydrogels. Physically cross-linked SF hydrogels can be prepared by promoting the self-assembly of SF polypeptides (the process of gelation) owing to increased hydrophobic interactions. Gelation via the self-assembly process can occur naturally, but it is typically lengthy at 37 °C, taking up to three months. A range of stimulating techniques can be employed to expedite the gelation process, such as high temperature, low pH, vortexing, lyophilization, ultrasonication, altering ion concentration, and incorporating dehydrating agents.[53] Moreover, chemically cross-linked SF hydrogels can be synthesized using functionalized SF and other precursors tailored to the specific design requirements. The most common approach is the formation of dityrosine cross-links through enzyme-catalyzed reaction using horseradish peroxidase-hydrogen peroxide or ammonium persulfate-riboflavin.[24,63,64]

1.2.4.3. Fibrous Mats. SF fibers are prepared using electrospinning, dry jet spinning, and wet-spinning.[55,65,66] While spinning methods produce microdiameter fibers, electrospinning can yield submicrometer to nanodiameter fibers, offering large surface areas and the capability to incorporate nanomaterials onto them. A spinning dope is formulated using concentrated SF solutions (25%). Consequently, silk proteins undergo self-assembly into micelles through hydrogen bonding and hydrophobic interactions. Subsequently, these micelles get aligned under shear stress and dehydration to generate fibers. Electrospun nanofibrous mats are developed by applying a positive voltage to a SF solution-loaded syringe, initiating a jet directed toward a grounded collector plate. The resulting fibrous mats are subjected to

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methanol treatment to induce the β-sheet formation, followed by a washing step (with water).[67]

1.2.4.4. 3D Structures. 3D structures of SF, i.e., foams, sponges, or scaffolds, are fabricated using well-established methods such as salt leaching (porogen leaching), freeze-drying, gas foaming, solid free-form construction, and 3D printing.[54,68,69] Salt-leaching involves pouring SF solution over an evenly distributed bed of salt, allowing the SF solution to transform into a gel, and subsequently removing the salt by immersing the system in water to obtain a salt-free porous 3D scaffold. Depending on the size of the salt crystal, concentration of SF solution, and gelation mechanism, the SF sponges can exhibit a range of mechanical strengths, degradation rates, surface smoothness, porosity, and pore interconnectivity.[70] In freeze-dried foams, the diameter of the pores can be varied by altering the freezing temperature, pH of the solution, and the solvent system.[71] The average pore diameter can be increased from 60 to 250 μm by introducing multiple cycles of freezing and thawing. Alternatively, employing gas-foaming can offer more control over the pore structure.[72] In recent times, 3D printed constructs of SF have gained much popularity due to ease of fabrication and shape fidelity. The physicochemical properties of the printed constructs rely on the bioink formulation and the cross-linking/gelation mechanism used.[64,69]

1.2.4.5. Microspheres and Nanoparticles. SF microspheres/nanoparticles show promise as delivery systems (drugs/gene/protein) and can be produced using self-assembly, polymer cocarrier, or lipid-based emulsifiers. SF micelles can self-assemble through the rearrangement of hydrophobic and hydrophilic chain segments of the SF polypeptides when ethanol is added, subsequently quenching below its freezing point.[73] To develop lipid-based silk microspheres, a film of lipid (e.g., 1,2-dioleoyl-*sn*-glycero-3-phosphocholine) is prepared in a tube and used to emulsify the silk/payload solution as it is added dropwise. The system is then subjected to lyophilization to yield lipid-coated silk vesicles, which are then suspended in methanol and centrifuged to remove the lipid, resulting in SF microspheres.[74] Moreover, a simpler water-based approach can be employed to prepare SF microspheres through phase separation using polymers, such as poly(vinyl alcohol) (PVA). Herein, the silk-PVA mixture is ultrasonicated to induce phase separation, cast into a film, and subsequently dissolved. The suspension is then centrifuged to remove PVA, yielding SF microspheres.[75] Several SF particulate systems can be achieved by spray drying, electrospraying, milling, microfluidics techniques, and laminar jet breakup.[76−78]

While all the aforementioned techniques are well established on a lab-scale, the transition to large-scale production requires additional considerations. Later sections discuss the translational bottlenecks of commercializing SF-based wound care products.

1.2.5. Silk Fibroin-Based Nanocomposites for Wound Care. While SF has shown promise in wound care applications, its potential can be substantially enhanced by incorporating nanoscale fillers to obtain SF nanocomposites. In this review, SF nanocomposites are defined as the combination of SF with one or more components (such as nanoparticles, nanophases, etc.) at the nanometer scale. Incorporating nanofillers presents a potent avenue for advancing personalized medicine, serving as diagnostic and therapeutic tools. This is achieved through their size similarity to biological entities and high surface-to-volume ratio, which affords easy surface modifications.[79] For example, recent studies introduced various biocompatible self-assembling nanoparticles that demonstrated enhanced wound recovery.[80−82] The nanoparticles, such as silica, gold, and zinc oxide, exhibited favorable characteristics, including minimal *in vivo* toxicity and antibacterial capabilities.[83−85] Additionally, these nanofillers yielded favorable outcomes in terms of physicochemical attributes, improved stability, and enhanced biological activity.[86] This review reveals that different nanofillers may elicit a range of biological responses, including pro-clotting behavior, antimicrobial properties, immunomodulatory activity, and enhanced cell proliferation. The following sections present a comprehensive overview of recent advancements in SF and SF-nanocomposites for various aspects of wound care, such as hemostasis and management of difficult-to-heal wounds.

2. HEMOSTASIS

The body's first response to a bleeding situation is hemostasis. For minor epidermal wounds, blood clotting is achieved via inherent coagulation mechanisms with sequential activation of clotting factors. However, in the case of massive hemorrhage, sole reliance on physiological clotting mechanisms is inadequate in facilitating hemostasis. Uncontrolled acute blood loss has fatal consequences, such as hypovolemic shock, hypotension, decreased cardiac output, and tachycardia. Deficient oxygen perfusion leads to acidosis and hypothermia from increased anaerobic metabolism.[87] Hence, ensuring prompt and effective hemorrhagic control is imperative for reducing mortality, thereby necessitating external interventions, such as the use of hemostats.[88]

SF and its nanocomposites are extensively used for hemostatic applications in various formats such as powders, films, hydrogels, and sponges. Composite hemostatic materials have gained interest, particularly in addressing the key differences between deep wounds and superficial bleeds.[89] Furthermore, hemostatic agents can be categorized as active and passive hemostats. Active hemostatic agents contain fibrinogen or thrombin that works by actively triggering the coagulation cascade to form a fibrin clot. Active agents are particularly important for patients with coagulation disorders.[90] Passive agents include mechanical hemostats of biopolymers such as collagen, gelatin, chitosan, silk fibroin, etc. These hemostats attain clotting by promoting platelet aggregation and activation, offering a matrix for blood clot formation. Hence, passive hemostatic agents are effective in patients with functional coagulation systems.[90] Most often, a combination of active and passive hemostats is preferred to cater to a wide spectrum of patients and ensure rapid clotting with acceptable biocompatibility and feasible manufacturing techniques. The following sections discuss various formats of hemostats and their suitability for different bleeding scenarios, as summarized in Figure 5.

2.1. Active Hemostatic Dressings

Fibrinogen is a primary coagulation factor that is depleted during massive blood loss. Therefore, the supplementation of fibrinogen is considered a critical step in hemorrhage management. The first choice of treatment is the transfusion of whole blood and blood components. However, allogenic blood transfusion carries an increased risk of infection and challenges related to lower availability, storage difficulties, and

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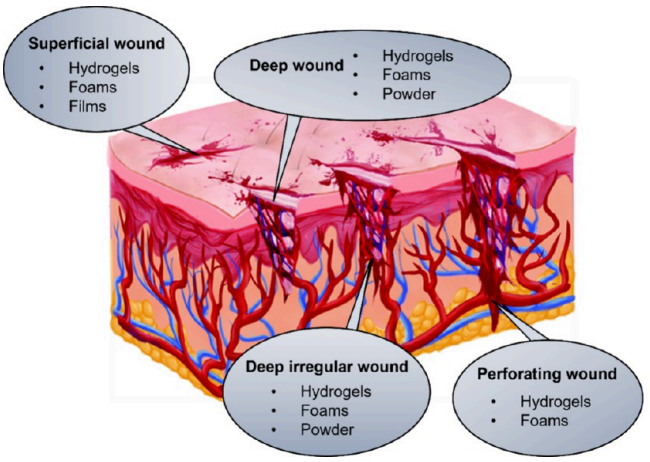

Figure 5. Illustration depicting different bleeding scenarios and the most suited forms of hemostats for each (Adapted with permission from ref 91. Copyright 2021 The Royal Society of Chemistry.)

higher costs. On the other hand, severe trauma is associated with the dysregulated generation of thrombin, leading to depleted local availability of thrombin.[92] Furthermore, supplementation of thrombin is also valuable for patients receiving anticoagulation drugs during vascular surgeries.[93−97] In this regard, local delivery of active hemostatic agents using biomaterial substrates is preferred. SF is being explored as a potential carrier for blood clotting agents.[98−100] Teuschl et al.,[99] presented a method to incorporate and codeliver coagulation substrate (fibrinogen) and catalyst (thrombin) in an SF sponge-like device. To prevent premature polymerization of thrombin and fibrinogen, the authors designed a hemostatic sponge such that both active agents were spatially separated. Thrombin was loaded in the SF core structure, surrounded by an SF/fibrinogen ring-like structure. This design facilitated the diffusion of fibrinogen upon hydration followed by the release of thrombin to initiate polymerization. Several research groups are exploring various silk-based systems for the local delivery of clotting agents.[98,100] However, some individuals are known to have adverse reactions against human blood components. Administering active agents increases the risk of thrombosis and thromboembolism, especially if the active agents are delivered intravascularly.[101] In such cases, passive hemostatic agents are preferred to mitigate the associated risks.

2.2. Passive Hemostatic Dressings

2.2.1. Powder-Type. Powder-type hemostatic agents are most suited for small, inaccessible, and irregular-sized wounds. The powder only affects the bleeding site and exhibits adhesive properties.[102,103] When in contact, these hemostatic agents absorb moisture from the blood and expand in size, thereby creating a physical barrier to stop the bleeding. Additionally, moisture absorption causes a rise in the local concentration of clotting factors that trigger clotting.[103,104] Huang et al.[105] use surface roughness of nonporous SF/alginate microspheres for accelerated blood clotting. The authors demonstrate that due to surface roughness, a higher number of red blood cells (RBCs) aggregated on the surface and the coagulation time was reduced. However, the molecular mechanism responsible for coagulation is not yet fully understood. Lei et al.[106] achieved effective hemostasis using low molecular weight SF (LMSF) prepared by hydrolysis of SF in a ternary solvent system ($CaCl_2$, EtOH, and H_2O) at different temperatures (50, 75, and 98 °C). The authors show that with an increase in hydrolysis temperature, the content of the β-sheet structure in the LMSF decreased. The LMSF hydrolyzed at 50 °C (LMSF-50) exhibited the most effective blood clotting by activating the intrinsic coagulation cascade. LMSF powder absorbed moisture (600% by weight) when in contact with the bleeding site and transformed it into a gel. The LMSF gel behaved like a physical barrier to blood loss and assisted in primary hemostasis by platelet aggregation. The authors demonstrate a significantly higher platelet adhesion on LMSF-50 compared with cotton gauze. Biswas et al.[107] developed alkaline (NaOH) hydrolyzed silk microfibers (AHSMf) (Figure 6a) for reducing clotting time (20−30 s). The authors attribute the pro-clotting behavior to several mechanisms, as shown in Figure 6b. These include enhanced platelet activation in alkaline pH, allosteric modulation of thrombin by Na^{+}, and a local rise in the concentration of clotting factors due to moisture absorption. Despite several advances, powder-type hemostatic agents are unsuitable for large bleeding wounds as they get washed away by the blood flow. In some cases, complete clearance of powder after clotting could pose a challenge and become a potential irritant during healing.

2.2.2. Hydrogels. Hydrogel-based hemostats have become the primary candidates for effective hemorrhagic control in a

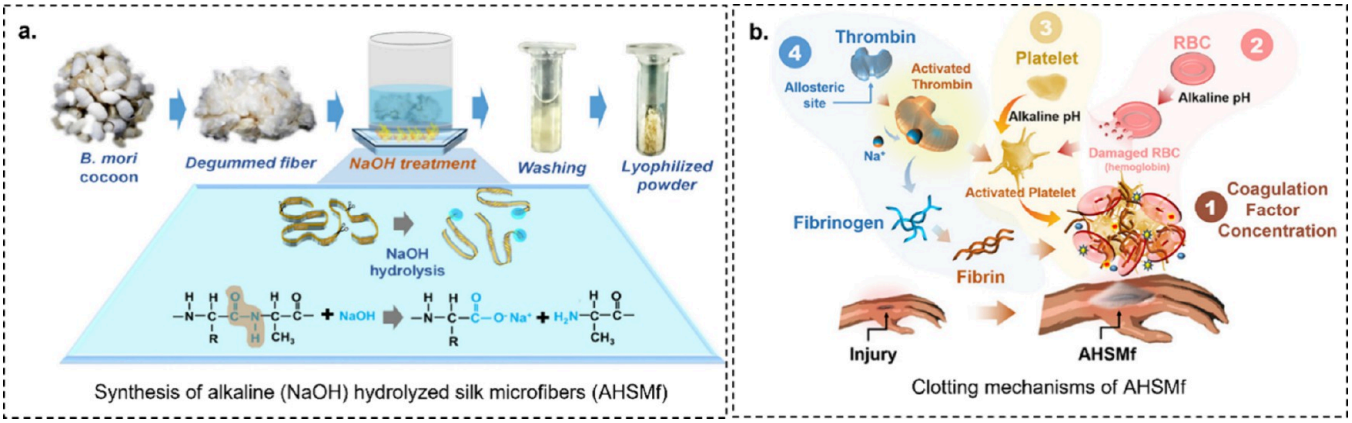

Figure 6. (a) Synthesis protocol for alkaline hydrolyzed silk microfibers (AHSMf) and (b) proposed clotting mechanisms of AHSMf. (Adapted with from ref 107. Copyright 2022 American Chemical Society).

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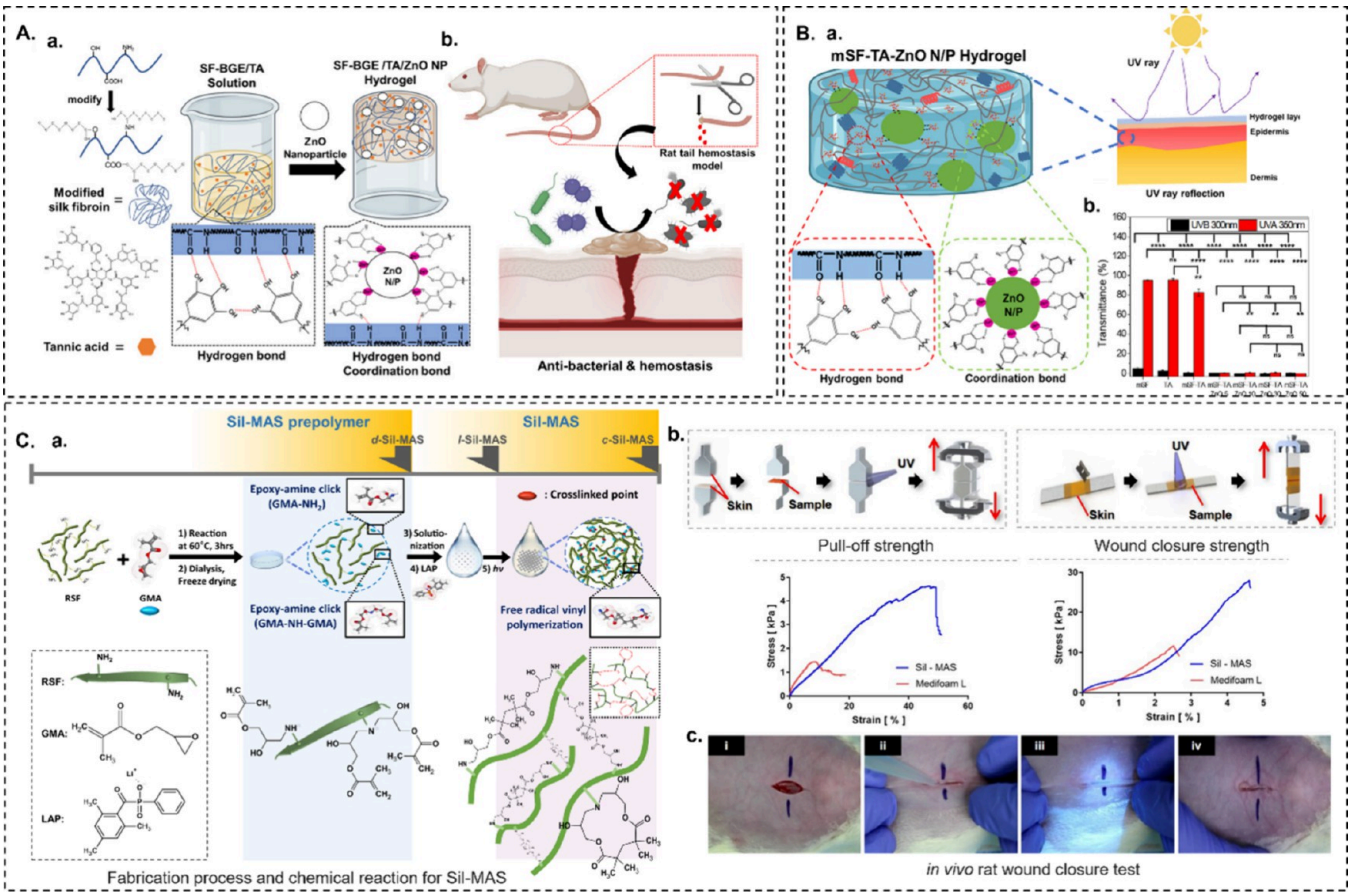

Figure 7. (A) (a) Schematic illustration of sol−gel transition of SF-BGE/TA/ZnO and (b) hemostatic efficacy on a rat tail amputation model (Adapted with permission from ref 119. Copyright 2022 MDPI with license under CC BY 4.0.) (B) UV blocking ability of the multifunctional hemostatic hydrogel (mSF-TA-ZnO N/P). (Adapted with permission from ref 114. Copyright 2022 Elsevier.) (C) (a) Fabrication and the chemical reaction for Si-MAS (3D chemical structure element color code: white: H; red: O; gray: C; blue: N). The reaction of modified regenerated SF (RSF) with glycidyl methacrylate (GMA) via epoxyamine click reaction (d-Sil-MAS) was used to obtain powdered Sil-MAS. d-Sil-MAS mixed with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) (l-Sil-MAS) for photo-cross-linking at 365 nm via free radical vinyl polymerization (c-Sil-MAS). b) Measurement of the pull-off and wound closure strength of l-Sil-MAS in comparison to the commercial product (Medifoam L) along with the corresponding stress/strain curves. c) Photographic images of the *in vivo* rat wound closure test (i-iv). (Adapted with permission from ref 116. Copyright 2020 Nature with license under CC BY 4.0.)

clinical setting. This is attributed to its adjustable physicochemical properties and its ability to match the mechanical properties of native tissues, creating a favorable microenvironment for cell response.[108−110] An added advantage of hydrogels is their ability to adapt to narrow and complex wounds, particularly by *in situ* gelation. Hydrogels take up large volumes of liquid to attain hemostasis in large arterial bleeds. Several research groups have developed SF-based adhesives by tannic acid (TA) modification[111−114] and photocuration.[115−117] The abundant phenolic moieties present in TA offer a strong binding affinity toward the mucosal layer. Bai et al.[111] show that TA-modified SF hydrogels possessed high adhesive strength (≈ 134.1 kPa) on wet tissues. The authors demonstrated rapid hemostasis (<30 s) owing to the superior wet-adhesion ability of tannins that can seal the bleeding site and create a physical barrier to blood flow. Zhu et al.[112] showcase the utility of TA-modified SF sealants in cavity bleeding wounds, particularly for the tooth extraction model. Therefore, SF-based sealants have gained attention for minimally invasive surgeries. Although fibrin-based sealants are available for clinical applications, they are ineffective under dynamic conditions (e.g., beating heart, shear of blood flow) due to their poor adhesive strength on bleeding tissues.[118]

Further, in an attempt to develop multifunctional hemostatic hydrogel, Yang et al.[119] combined modified SF with ZnO nanoparticles. Herein, the authors chemically modified SF by introducing butyl glycidyl ether (BGE) into its side chain to obtain a water-soluble derivative (SF-BGE) (Figure 7Aa). Further, a nanocomposite hydrogel was prepared using a combination of SF-BGA, tannic acid (TA), and ZnO nanoparticles. The sol−gel transition was triggered by the formation of coordination bonds between ZnO and TA (Figure 7Ab). Further, the hemostatic efficacy of the composite hydrogel was demonstrated in a rat tail amputation model (Figure 7Ac). In another study by the same research group,[114] a nanocomposite adhesive gel was obtained by loading zinc oxide (ZnO) nanoparticles into TA-modified SF (mSF-TA-ZnO N/P) (Figure 7B). Herein, the authors showed the blocking ability of the composite hydrogel. The plot in Figure 7Bb depicts an enhanced UV-blocking efficacy as the concentration of ZnO nanoparticles increased. ZnO particles serve as a shield to protect wounds from ultraviolet (UV) rays by absorbing and reflecting UV light, aiding in better cosmetic outcomes.[120] Nonetheless, the gelation of SF with TA modification is a time-consuming process, and hence, photocuration is being deployed. Herein, gelation is achieved by

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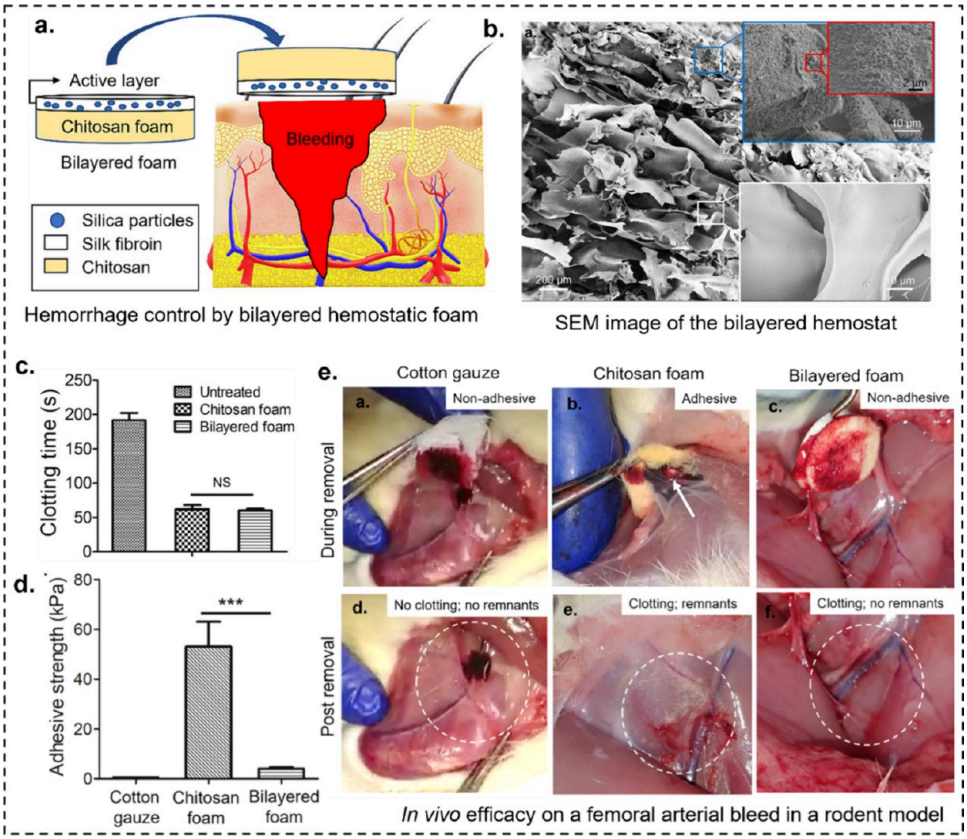

Figure 8. (a) Schematic of hemorrhage control using the bilayered hemostatic foam; (b) SEM micrograph of the hemostatic foam; blood clotting time (c) and mucoadhesive strength (d) of the bilayered foam in comparison to chitosan-based commercial product; and (e) *in vivo* study demonstrating minimal mucoadhesiveness and rebleeding. (Adapted with permission from ref 136. Copyright 2023 Elsevier.)

chemical cross-linking between neighboring functional groups upon irradiation with UV- or visible light. Kim et al.[116] used methacrylated SF sealant (Sil-MAS) to attain rapid gelation by exposure to UV light for 10−30 s at a distance of 5 cm (Figure 7Ca). The authors demonstrated the applicability of Sil-MAS as a laparoscopic medical glue in a rabbit laceration model of liver and stomach serosa. Figure shows a notably superior strength (pull-off and wound closure) of Sil-MAS adhesive in comparison to the commercial product (Medifoam L) (Figure 7Cb). The *in vivo* efficacy of the developed hydrogel was tested in a rodent model (Figure 7Cc). The hydrogel-based hemostats are more suitable for a surgical setting. For application in a prehospital setting (e.g., combat zone), the hydrogel must be endowed with additional capabilities, such as inherent antibacterial properties. As hydrogels are designed for long-term applications (retained at the bleeding site), future endeavors could focus on advancing multifunctional hydrogels. These could incorporate features such as controlled degradation and pro-healing properties. Such multifunctional capabilities in hydrogel-based hemostats would significantly enhance the overall wound healing at the site where they are deployed.

2.2.3. Nanofibrous Films and Foams. Nanofibers have gained popularity due to their ability to structurally mimic extracellular matrix (ECM), creating a favorable microenvironment for hemostasis and wound healing.[65] The electrospinning technique is used to produce a mat of nanofibers. Nanofibrous hemostats are most appropriate for long-term applications.[65,121] Electrospun mats have a high porosity that allows water and gaseous exchange.[122] Additionally, the high surface area-to-volume ratio enhances the loading efficacy of hemostatic agents or drugs for efficient healing.[123,124] In several studies, inorganic hemostatic agents (kaolin,[125] halloysite nanotubes,[126] etc.) were loaded into SF nanofibrous films. Kaolin initiates the intrinsic coagulation cascade due to its negatively charged surface that activates Factor XII.[127] Halloysite nanotubes (HNTs) are aluminosilicate clay minerals with a hollow tubular structure.[128] The outer surface of HNTs is negatively charged with siloxane groups, while the inner surface is positively charged with Al−OH groups.[129,130] This facilitates the effective binding of drugs for sustained release.[131] Karahaliloglu et al.[125] developed a bilayered electrospun hemostatic dressing wherein the kaolin was loaded into an active layer for rapid blood clotting (138 s). In the work reported by Ren et al.,[126] HNTs were loaded into electrospun fibers of SF and chitosan (CS) blend. The authors demonstrated the drug-loading efficacy of the developed dressing for local delivery at the wound site. Nanofibrous films have proven to be effective hemostats; however, electrospinning is the preferred fabrication technique that presents a significant industrial hazard during scale-up due to its requirement for high-voltage equipment.

Moreover, while several advances have been made for hemorrhagic control in a clinical setting, these approaches are rendered ineffective in a prehospital setting, particularly in remote and austere conditions. An austere condition is defined as "the environment where professional health care providers normally do not operate, and basic equipment and capabilities necessary for resuscitation are often not available."[132] Therefore, there is a global interest in developing effective first-line

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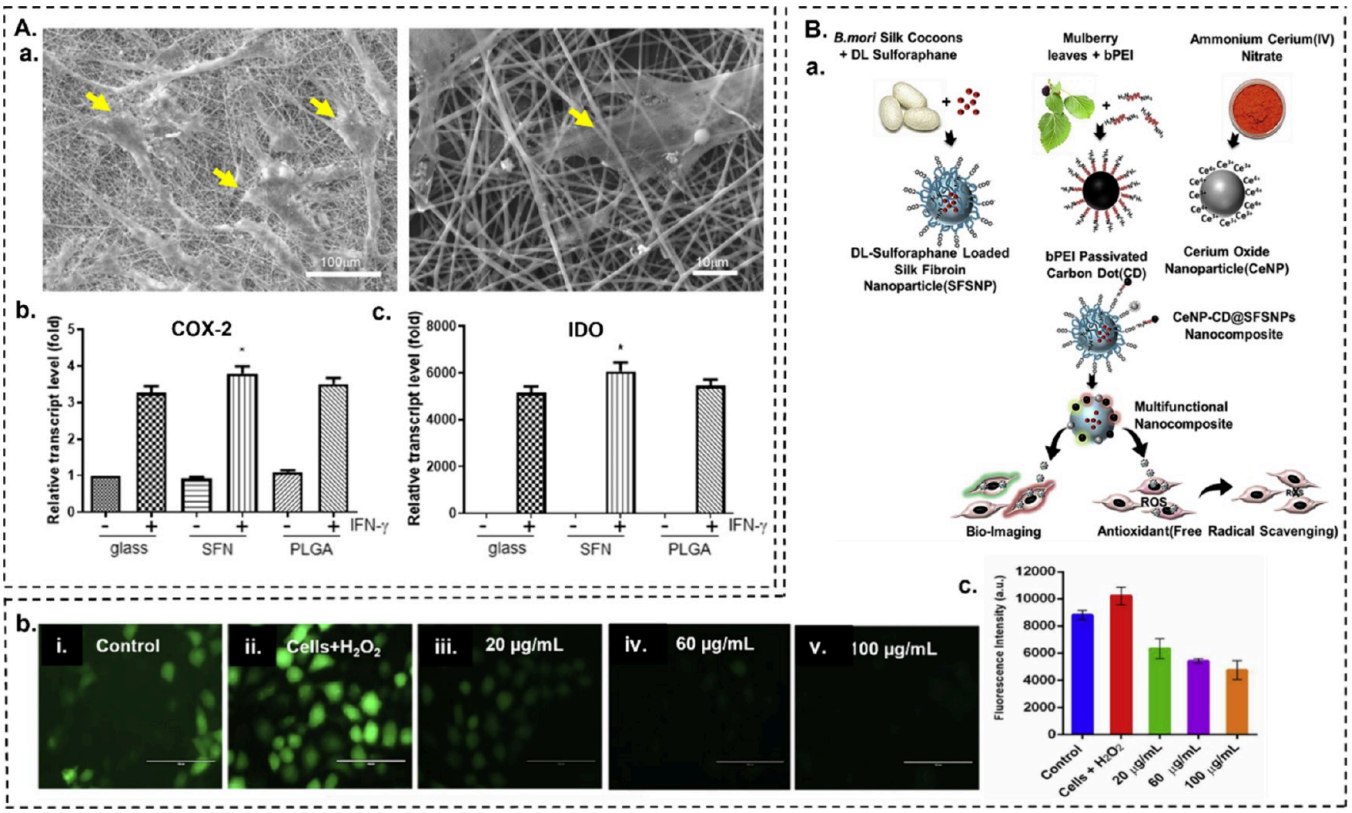

Figure 9. (A) (a) SEM micrographs revealing MSCs on SF nanosheets (cells are shown by yellow arrows); expression of immunomodulatory functional cytokines COS-2 (b) and IDO (c). (Adapted with permission from ref 140. Copyright 2019 Elsevier.) (B) (b) Fluorescence images of ROS levels in A549 cells: (i) untreated control, H_2O_2 treated cells with no nanoparticles (ii) and varying concentration of composite nanoparticles (iii–v). (c) Quantitative measure of ROS scavenging. (Adapted with permission from ref 142. Copyright 2020 Elsevier.)

hemostatic interventions for emergency coagulation management prior to the patient's arrival at the hospital.[133,134] An effective first-aid hemostatic dressing must attain rapid hemostasis and prevent rebleeding upon dressing removal. Additionally, the dressing must have an easy application procedure (e.g., needle-free and with no premixing of components) to allow care providers with little or no medical training to minimize bleeding until the patient is taken to definitive care.[135] In this regard, foam-based hemostats have gained popularity for achieving hemostasis through the absorption of moisture from the bleeding site. This results in a local increase in clotting factors, triggering the clotting cascade. Recently, Indrakumar et al.[136] developed a first-aid bilayered hemostatic foam composed of an active layer on top of a chitosan foam. Chitosan foams are currently employed in the clinic for hemostasis and constitute a substantial share of the hemostat market. Nevertheless, they are limited by mucoadhesiveness that causes rebleeding and leaves behind material residues that may serve as irritants during healing. Hence, the authors layered the chitosan foam with an interfacial active layer of silica particles and SF (Figure 8a,b). While the *in vivo* clotting time achieved by the bilayered foam was similar to that of the commercial product prepared from chitosan (Figure 8c), the active layer reduced the mucoadhesive strength by 10-fold compared to chitosan-based hemostats (53 ± 10 kPa) (Figure 8d), thereby minimizing rebleeding by 50%. Moreover, the authors achieved easy product removal without compromising the pro-clotting ability (Figure 8e) in a rodent model.

3. DIFFICULT-TO-HEAL WOUNDS

For most people, wound healing is a well-orchestrated process characterized by four distinct but overlapping phases of healing. While the wound may leave a visible scar, it is not accompanied by persistent pain, excessive exudate, distress, or odor. Contrarily, some patients experience delayed healing associated with symptoms that negatively impact their quality of life. Clinicians face the dual challenge of meeting patient expectations of seamless healing and promptly identifying cases where healing may be prolonged. Factors that determine the quality of healing could be patient-related (comorbidities, lifestyle, age), wound-related (size of the ulcer, anatomical location, wound bed conditions), clinical competency (skills and knowledge of the clinician), and treatment options (cost of treatment, availability, and treatment efficacy). Among these, persistent inflammation and susceptibility to microbial infections are the most prevalent factors contributing to nonhealing wounds.[137] These difficult-to-heal wounds are often characterized by persistently elevated levels of pro-inflammatory cytokines and reactive oxygen species (ROS) that prevent the wound from advancing toward the proliferative and remodeling phase, causing impaired healing. Consequently, these open wounds become susceptible to infections that lead to biofilm formation, exhibiting a high antibiotic resistance. The subsequent sections discuss wound dressings composed of SF and its nanocomposites engineered to incorporate two prominent desired attributes, i.e., antibacterial and immunomodulatory activities.

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3.1. Immunomodulatory Dressings

Chronic wounds get locked in the inflammatory phase, impeding the progression of healing toward the proliferative phase, and despite diligent wound management, the wounds remain intractable. As a result, wounds develop a hostile microenvironment characterized by an imbalance in chemokines, pro-inflammatory cytokines, ROS, and proteases.[138] The overproduction of ROS directly damages the ECM and cell membrane and induces premature cell senescence.[139] Therefore, there is a need for wound dressings with the ability to modulate immune responses, creating a pro-regenerative microenvironment.

In a recent study, Kim et al.[140] demonstrated that the immunomodulatory function of human mesenchymal stromal cells (MSCs) can be enhanced by culturing on SF nanofiber sheets (SFN) (Figure 9Aa). SFN-grown MSCs exhibited increased expression of immunomodulatory functional cytokines (IDO_1 and COX_2) when stimulated by interferon (IFN)-γ (Figure 9Ab,c). Another study investigated incorporating nicotinic acid into silk scaffolds as an immunomodulatory strategy.[141] The authors show that cultivating M1-macrophages on nicotinic acid-loaded silk scaffolds led to suppressed gene expression of pro-inflammatory markers, including TNF-α, CXCL10, and CD197.

In addition to altering cellular behavior, numerous studies have addressed the significant role of oxidative stress in nonhealing wounds by developing wound dressings with antioxidant capabilities.[142−144] SF, by itself, has a limited radical scavenging ability. Therefore, SF is often combined with various active ingredients, such as cerium oxide, melanin, anti-inflammatory drugs, etc. SF is composed of amino acids featuring numerous functional groups, including amines, alcohols, phenols, carboxylic groups, and thiols, facilitating the conjugation of various therapeutic biomolecules. Notably, SF carries an inherent ability to respond to pH changes, allowing for controlled drug release. The loading and release of biomolecules or drugs from SF occur via electrostatic interactions, facilitated by its negative charge (−24 to −26 mV), enabling strong interactions with positively charged drugs. This interaction prevents the typical burst release of drugs observed in various polymeric systems.[145] Working on these principles, Passi et al.[142] used SF in combination with cationic cerium oxide nanoparticles (CeNPs) to combat oxidative stress. Herein, the authors coupled CeNPs and carbon dots (CDs) with sulforaphane-loaded SF nanoparticles to obtain a multifunctional CeNP-CD@SFSNPs nanocomposite (Figure 9Ba). These composite nanoparticles exhibited ROS scavenging ability from H_2O_2 mediated oxidative stress, as analyzed by fluorescence-based DCFH-DA assay (Figure 9Bb,c) The antioxidant activity of CeNPs is attributed to its ability to switch between two oxidation states (+3 and +4) in response to the microenvironment, exhibiting enzyme mimetic activity. The authors demonstrated a significant reduction in ROS in the group treated with SF/CeNPs nanocomposite compared to the control group. The percentage of ROS levels decreased from 36.4% to 8.1% with an increase in the concentration of SF/CeNPs. Besides ROS reduction, the nanocomposite effectively scavenged hydrogen peroxide radicals generated from H_2O_2-induced stress. Similarly, Maity et al.[143] used melanin-conjugated silk to achieve antioxidant capabilities. Melanin readily interacts with free radicals and other reactive species due to its unpaired electrons, thus serving as an antioxidant.

3.2. Antimicrobial Dressings

Reports suggest that roughly 75% of chronic wounds result in infections, impeding the healing process.[2] Localized bacterial infections in wounds can escalate to systemic infections, sepsis, multiorgan failure, and other fatal outcomes. Furthermore, complications arise when bacteria form biofilms, a protective shield that adheres firmly to the wound bed, leading to persistently elevated inflammation.[146] In this scenario, the primary objective is to minimize the bacterial load, typically through surgical debridement. However, bacterial infections can extend to deeper layers within the wound bed, making complete eradication of bacteria challenging with debridement alone.[147,148] Therefore, the most common treatment regime is surgical debridement followed by the administration of antibiotics. Nevertheless, the compromised blood supply in chronic wounds renders systemic antibiotic therapy ineffective, and insufficient dosing can contribute significantly to the emergence of antimicrobial resistance.[149] Conversely, topical antimicrobials have limited therapeutic efficacy due to their poor penetrative capabilities into the skin.[150] To overcome these limitations, there has been increasing focus on developing wound dressings with inherent antibacterial properties and drug-loading abilities for improved efficacy of antibiotics. SF by itself lacks antibacterial ability;[49] therefore, several antibacterial approaches have been explored, including the incorporation of inherently antibacterial inorganic nanoparticles, polymers/peptides, and plant extracts[151−154] For example, Indrakumar et al.[155] incorporated SF modified with TA (TASF) as an additive in calcium sulfate dihydrate (CSD) to formulate multifunctional pellets for infection control. The addition of TASF imparts antioxidant and antibacterial properties to the pellet. Additionally, the authors illustrate that the inclusion of TASF in antibiotic-loaded pellets prolonged the antibacterial efficacy against Gram-positive bacteria (*S. aureus*), implying a potential reduction in the drug dosage for an effective bactericidal effect. This prolonged effect was attributed to the presence of TA, which exhibits a stronger bactericidal effect against *S. aureus* owing to its ability to inhibit NorA efflux pumps within the bacterial cell.[156] Overall, this nanocomposite system has been proposed for use in a point-of-care setting, enabling personalized antibiotic treatment.

Several inorganic nanomaterials, including metal (e.g., Cu, Ag, Au, and Ca) and metal oxide (e.g., TiO_2, ZnO, and CaO), graphene oxide (GO) nanosheets, and transition metal dichalcogenide (TMD) (e.g., $MoSe_2$) have been incorporated with SF for antibacterial applications.[157] Compared to antibiotics, the development of resistance in bacterial cells toward these nanomaterials is more challenging due to their multiple mechanisms of action. Inorganic nanomaterials primarily exert bactericidal activity by generating reactive oxygen species (ROS).[158] At low concentrations of ROS, the antioxidant defense mechanisms within bacterial cells can effectively neutralize the impact. However, higher concentrations of ROS can overcome the defense mechanisms, leading to oxidative damage of intercellular components such as DNA, proteins, enzymes, and lipids.[159] Apart from ROS generation, inorganic nanoparticles exhibit bactericidal activity by disrupting the cell wall, dysregulating the metabolic activity of bacteria, and damaging their DNA and chromosomes.[159]

Typically, these inorganic nanoparticles are synthesized by using physiochemical approaches, which require toxic chemicals and harsh conditions. Consequently, there has been a

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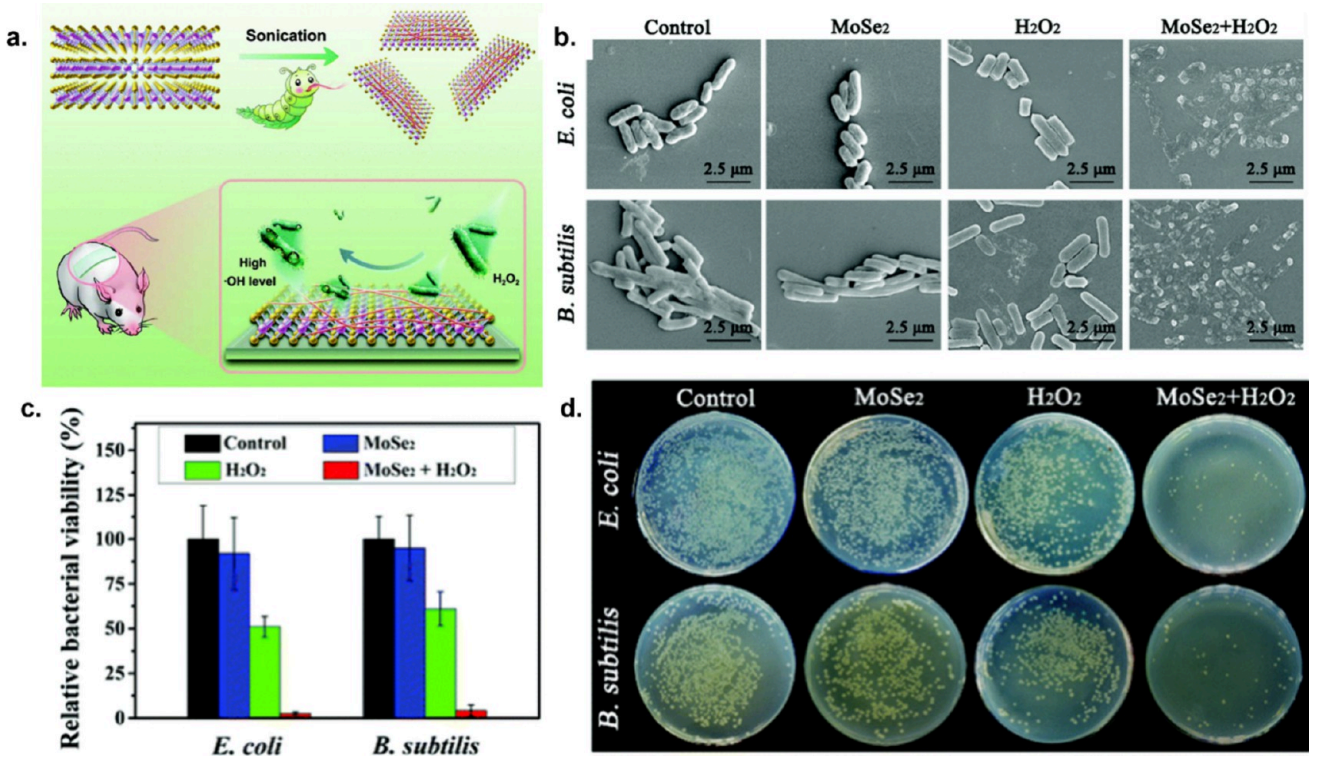

Figure 10. (a) Schematic representation of using SF as an exfoliating agent to prepare thin-layer TMD nanosheets that exhibit peroxidase-like activity and catalyze the decomposition of H_2O_2 into ·OH radicals; (b) SEM micrographs of *E. coli* and *B. subtilis* after different treatments; (c) relative percentage of viable bacterial cells after different treatments determined by plating counting method and corresponding agar plates (d). (Adapted with permission from ref 170. Copyright 2017 The Royal Society of Chemistry.)

growing preference for employing green methods for nanoparticle synthesis, using ecofriendly and biocompatible polymers.[160,161] SF is one such polymer that is often used as a reducing and stabilizing agent for the *in situ* fabrication of different inorganic nanoparticles.[162−164] The primary mechanism of reduction by SF is attributed to the ionization of its phenolic moieties (tyrosine) at basic pH, resulting in electron transfer to the metal ions.[165,166] Other reduction mechanisms include the chelation of metal ions and electron transfer by unprotonated carboxylic groups at basic pH and reduction via the methanolic hydroxyl groups in serine amino acids.[167,168]

Furthermore, reports suggest that the hydrophobic−hydrophilic segments in SF can facilitate its binding with graphene and TMDs.[169] In a study, Huang et al.[170] used carboxyl-modified SF (CMSF) as an exfoliating agent to prepare $MoSe_2$ nanosheets with long-term dispersion stability (Figure 10a). The authors demonstrate a high peroxidase-like catalytic activity toward the decomposition of peroxide (H_2O_2) into hydroxyl radicals (·OH). This approach led to a 3-fold reduction in the use of peroxide compared to conventional medical therapy to achieve an effective antibacterial property against Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) bacteria (Figure 10b−d). Under physiologically relevant levels of H_2O_2, the SF-$MoSe_2$ films demonstrate both pro-healing and bactericidal properties in an *in vivo* rodent model.

In addition to in situ fabrication techniques, antibacterial SF-based composites containing inorganic nanoparticles can be produced *ex-situ*. This involves the initial synthesis of nanoparticles followed by their incorporation into the SF substrate.[171,172] Incorporating inorganic nanoparticles into silk fibroin-based materials not only imparts antibacterial activity and enhances mechanical properties but also prevents excessive nanoparticle leaching. This leads to prolonged antibacterial efficacy, reduced cytotoxicity, and improved overall biocompatibility.[171,173] SF nanocomposites with GO,[174] TiO_2,[175,176] ZnO,[177,178] and CaO_2[179] nanoparticles have been employed as antibacterial wound dressings.

Despite their effective broad-spectrum antibacterial properties, the translation of inorganic SF nanocomposites in clinical practice is limited by unforeseen risks linked to their safety and stability in long-term applications. The nanoscale size of these particles allows them to cross biological barriers, infiltrate tissues, and interact with intracellular organelles and cells, resulting in membrane disruption, DNA alterations, mitochondrial apoptosis, and cell death. Moreover, based on their physicochemical attributes like charge, shape, and wettability, these nanoparticles tend to agglomerate and accumulate in tissues and organs, eliciting toxicity and adverse immune responses.[180,181] Therefore, it is crucial to carefully assess the toxicological properties of these nanoparticles and adopt green methods of synthesis to eliminate the use of hazardous chemicals.

4. CHALLENGES TRANSLATING SF-BASED PRODUCTS INTO THE MARKET

SF-based biotech companies dedicated to wound care and soft tissue regeneration have emerged worldwide, such as Fibroheal (India), SYLKE (USA), Sofregen (USA), KLISBio (Italy), etc. Nonetheless, scaling up a lab-scale prototype to a market-ready product poses significant challenges. Despite extensive research on SF-based biomaterials for medical and pharmacological applications, only a minimal number of these products advance to clinical trials. This can be attributed to variability in silk

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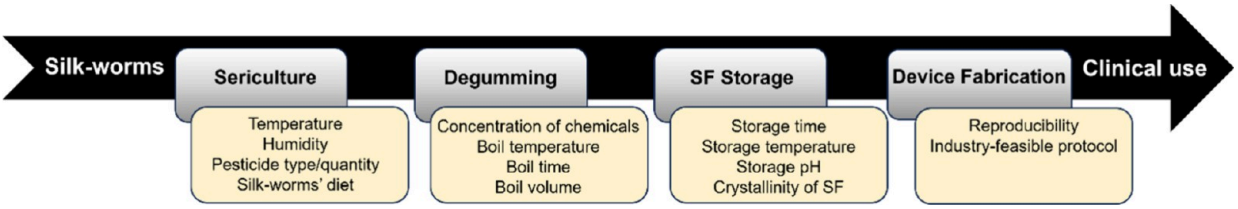

Figure 11. Sources of variability in SF from sericulture to clinical use.

Table 2. Comparison of Different Biopolymers for Wound Care Applications

Biopolymer	Collagen	Alginate	Silk fibroin
Source	Bovine, porcine	Brown seaweed	Silk cocoons
Advantages	Promotes collagen deposition; clinically well-accepted biopolymer; pro-healing	High exudate absorption capacity; Maintains a moist wound microenvironment	Easy availabilityPro-healingCost-effective
Limitations	Rigorous extraction and purification processes; loss of triple helical structure during processing; Antigenicity, if not purified thoroughly; expensive	Passive dressings, not pro-healing; not appropriate for heavily bleeding wounds; limited control over mechanical properties	Batch-to-batch variations; properties susceptible to environmental and storage conditions; processability or fabrication at industry-scale
Commercial products	Alloderm, Amniograph, Integra, Oasis	Algicell, Kaltostat, AlgiSite M	Fibroheal, SYLKE, Sofregen, KLISBio
References	27, 186	187, 188	184, 189

source, challenges in standardizing SF-extraction protocols, and a lack of industry-feasible product fabrication techniques.

Given that SF is derived from natural sources (*Bombyx mori* cocoons), it inevitably experiences variability that is directly reflected in the processed material.[182] The industrial production of SF from cocoons lacks standardization and automation, relying predominantly on manual handling, thereby rendering it susceptible to the uncontrollable factors summarized in Figure 11. During sericulture, several factors contribute to batch-to-batch variations in the outcoming cocoons.[56,183,184] This includes the seasonal availability of mulberry leaves, which influences the adoption of different diets, along with variations in environmental temperature and humidity, and the type and quantity of pesticides used. Similarly, during the degumming process, variations in the boiling parameters, such as the concentration of chemicals, temperature, time, and volume, contribute to the observed variability. Additionally, the difference in the storage time of the extracted SF can alter its physicochemical properties.

Another bottleneck in the mass production of fibroin lies in its processing and storage. Fibroin is extracted as an unstable aqueous solution, introducing several steps in the transition from raw material to medical product. Moreover, if not stored at the appropriate temperature and pH, the secondary structure of SF changes, altering its water solubility and thereby diminishing its processability.[185] For medical products intended for prolonged use from the time of production, the stability becomes crucial. This is especially true concerning resorbability, which tends to be extended for highly crystallized fibroin (with higher β-sheet content).[185] Crystallization resulting from aging under uncontrolled storage times and conditions contributes significantly to delayed resorbability, inconsistent data, and failure in clinical trials. Perhaps the first important step in procuring a raw material suitable for biomedical applications should be standardization of the sericulture and extraction processes. This implies sericulture in a controlled environment to yield medical-grade silk cocoons.

5. CONCLUDING REMARKS

Wound healing is an intricate process involving various cells, extracellular ECM components, and bioactive molecules to repair the damaged epidermis effectively. Identifying secure and convenient dressing materials (synthetic or natural) capable of generating a dermoepidermal layer in a single surgical procedure is of significant clinical interest. As discussed, the cellular interactions of SF are vital in accelerated wound healing. To date, SF-based dressings have been applied in several formats (powder, hydrogels, electrospun mats, films, and sponges) for different aspects of wound care, such as attaining rapid blood clotting, exhibiting immunomodulatory and antibacterial properties, and contributing to overall pro-healing effects. From a clinical perspective, SF-based substrates show promise as dressing materials and artificial skin grafts, holding great potential in plastic surgeries. Moreover, SF dressings loaded with bioactive agents (drugs, proteins, peptides, nucleic acids, and nanoparticles) exhibit multifunctional properties tailored for wound-specific treatment. It is important to recognize that there exists a diverse array of wound care products tailored to different aspects of wound healing, each with distinct design and material prerequisites. These products can be categorized based on various parameters: (1) the functions they fulfill (such as debridement, absorption, occlusion, antibacterial action, adherence, and hemostasis); (2) the physical format of the product (e.g., hydrogels, membranes, foams, or particulates); or (3) their mechanisms of action (whether passive or active). In Table 2, we compare three clinically accepted biopolymers (collagen, alginate, and silk fibroin) regarding the general biomaterial requirements of a wound care product, such as nontoxicity, nonallergenicity, pro-healing ability, and cost-effectiveness. No single wound dressing material suits all types of wounds; the physician determines the optimal treatment strategy and wound care product following the wound assessment.

6. FUTURE PERSPECTIVES

Over the past two decades, extensive research has been dedicated to developing functional biomaterials to modulate the microenvironment within chronic wounds. These efforts address challenges such as persistent inflammation and impaired regenerative capacity in chronic wounds. Nonetheless, the current clinically available dressings need to meet the needs of patients in terms of cost-effectiveness. Furthermore, reversing the progression of chronic wounds from the

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advanced stages poses a significant challenge. Thus, a critical research focus revolves around developing smart diagnostic dressings capable of early identification of wounds that can potentially become chronic. Application of these bioactive smart dressings could significantly improve therapeutic outcomes for chronic wounds and replace clinically invasive procedures.

In recent developments, SF-based smart dressings that are equipped with temperature and strain (pressure) sensors have emerged. These research efforts showcase the potential of next-generation smart dressings and wearable sensors for wound monitoring. Some studies have developed silk-based systems, such as transdermal patches using microneedles for on-demand drug delivery.[190,191] Moreover, 3D bioprinting is an evolving technology wherein SF-based bioinks are leveraged to develop skin grafts with controlled architecture and reproducibility, allowing large-scale production.[192] SF exhibits favorable biological and mechanical properties of 3D-bioprinted constructs with tunable degradation rates. 3D-bioprinting affords accurate placement of cells and biomacromolecules within silk-based constructs, yielding fully viable and functional full-thickness artificial skin grafts. Consequently, it presents the ability to bridge the gap between the high demand and availability of skin grafts. Nonetheless, there exists untapped potential for improvements in bioink formulation and understanding cell responses, particularly in the context of multicomponent and multicellular bioinks tailored for clinical applications.[193]

In recent years, while mulberry silk (*Bombyx mori*) continues to be the most explored source for wound healing applications, non-mulberry silk varieties like *Antheraea assama* and *Philosamia ricini* have gained significant traction. This is attributed to the abundant presence of Arg-Gly-Asp (RGD) sequences in these silk proteins, which play a crucial role in enhancing cellular responses.[194,195] Nonetheless, there are limited studies on nonmulberry silk-based nanocomposites, offering substantial scope for further investigation. Despite the promising capabilities exhibited by these nonmulberry silk proteins, the significant challenge lies in sourcing the raw materials. Consequently, scaling up production into a commercially viable product requires additional considerations.

Nevertheless, advancements in bioprocess engineering show potential for the large-scale production of recombinant silk varieties, such as silk-elastin-like protein (SELP) and recombinant dragline spider silk, which are artificially developed by using recombinant DNA technology. Advanced techniques in genetic engineering have led to the creation of transgenic silkworms capable of producing silk cocoons containing growth factor peptides.[196] While leveraging recombinant DNA technology provides numerous advantages, these advancements may raise ethical considerations. Taken together, silk protein is an inexpensive and abundant biopolymer that may afford cost-effective wound care solutions. Extensive research on silk-based products for wound care applications depicts their transition from the lab to the market. Nonetheless, to expedite the commercialization process, a standardized protocol for extracting medical-grade SF at an industry scale is essential, which would allow detailed and consistent clinical studies.

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CRediT: **Sushma Indrakumar** conceptualization, formal analysis, writing-original draft; **Tapan Kumar Dash** formal analysis, writing-review & editing; **Vivek Mishra** funding acquisition, project administration, writing-review & editing; **Bharat Tandon** funding acquisition, project administration, writing-review & editing; **Kaushik Chatterjee** conceptualization, funding acquisition, project administration, supervision, writing-review & editing.

Notes

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