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Review

Blends of Silk Waste Protein and Polysaccharides for Enhanced Wound Healing and Tissue Regeneration: Mechanisms, Applications, and Future Perspectives

Devipriya Vasudevan and D. Sangeetha*

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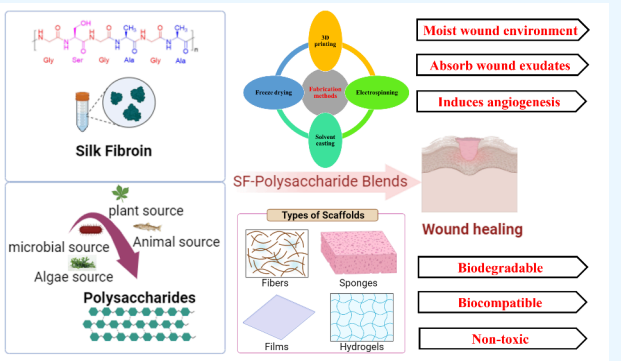
ABSTRACT: Wound healing is a highly sophisticated process, and therefore, a pioneering approach for designing excellent wound dressings with desirable characteristics vital for maintaining the external wound environment by assessing the inherent conditions of a patient for effective wound healing. Silk fibroin (SF), a versatile biocompatible material, has garnered significant attention for its potential in the field of wound healing and tissue regeneration. When SF is blended with polysaccharides, their synergistic properties can result in a material with enhanced bioactivity and tunable mechanical properties that facilitate the controlled release of therapeutic agents. This review explores how SF interacts with certain polysaccharides such as cellulose, chitosan, alginate, and hyaluronic acid (HA) and also delves into the underlying mechanisms through which these SF-polysaccharide blends induce processes such as cell adhesion, proliferation, and differentiation for enhanced wound healing and tissue regeneration. This review also emphasizes the potential of the aforementioned blends in diverse wound healing applications in conjunction with other treatment approaches, further addressing the current challenges in this domain and future directions for optimizing SF-polysaccharide blends for clinical research.



1. INTRODUCTION

Skin is the largest human body organ and the outermost layer involved in the defense mechanism, as it is a protective barrier from hazardous environments. Skin injuries or wounds that result in impairment in the skin functioning caused by any external factors can be healed through a series of physiological processes due to the highly regenerative properties of the skin.[1,2] Wounds that are accompanied by the risk of infections and prolonged healing periods have been a great concern for medical society since ancient years due to the challenges in wound monitoring and management.[3] The quest for functional and biocompatible wound dressings has grown significantly in biomedical research in response to the demand for both successful and rapid wound healing, as they serve an indispensable role when it comes to wound care and management. Conventional wound dressings frequently fall short of meeting the necessary criteria for improved wound healing, which complicates the entire process of recovery from wounds. There is an urgent need to develop wound dressings with materials that cater to all of the requirements for the enhanced healing of wounds.

Natural biomaterials have been explored by researchers owing to their inherent properties such as bioavailability, biodegradability, biocompatibility, inexpensiveness, and resemblance to the ECM environment of human tissues.[4] It is noticeable that there are several protein-based biopolymers, a category of natural biomaterials, found in nature abundantly that can be extracted from waste materials that cater to all those above-mentioned properties for their utilization in fabricating tissue engineering scaffolds. Silk is widely recognized as the "holy grail" of biomaterials and has shown its potential in a variety of biological applications.[5] SF is a naturally occurring fibrous protein polymer, extracted in considerable amounts from the waste of silk cocoons, which is a perfect fit for wound healing applications due to its abundance in nature and advantageous qualities including inexpensive manufacturing costs, excellent tensile strength, remarkable biocompatibility, biodegradability, low immunogenicity,[6] less toxic effects on the host tissue.[7,8] One of the main attractive features of SF is its ability to encapsulate and enable the sustained release of small biomolecules and drugs.[9,10] Silk

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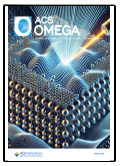

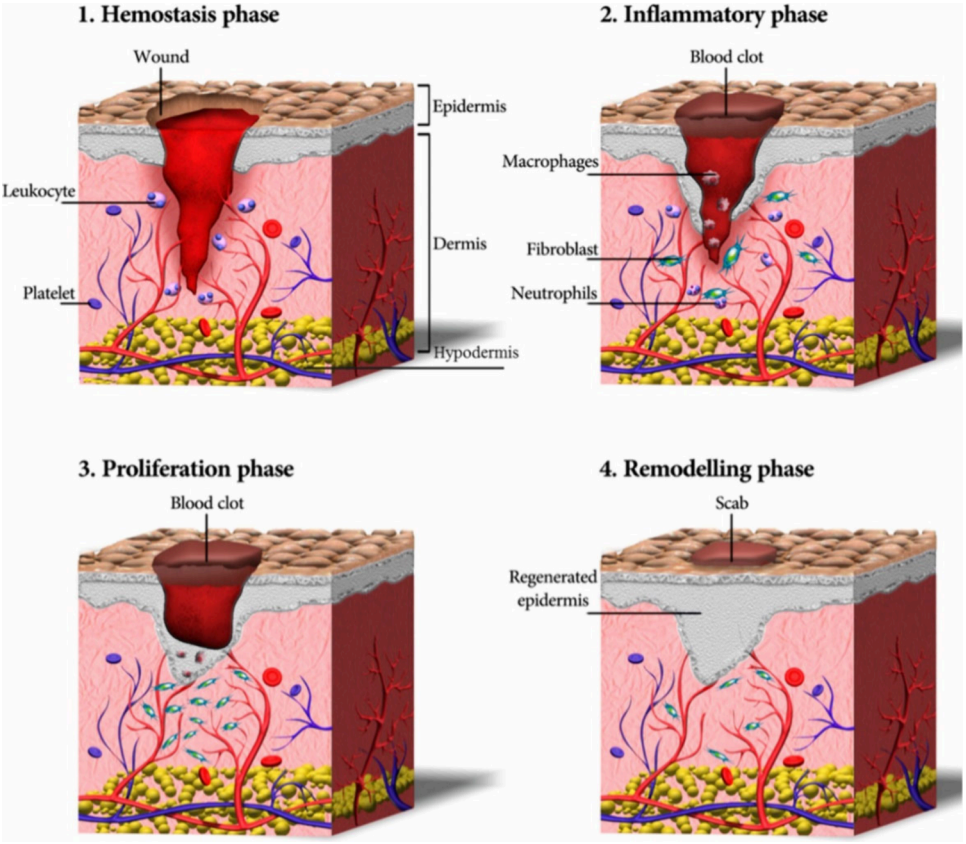

Figure 1. Different stages of wound healing: (1) hemostasis, (2) inflammation, (3) proliferation, (4) remodeling.

protein can be extracted from different varieties of silkworms which include mulberry and nonmulberry silkworms and spiders.[11] The properties of the SF protein extracted from different sources vary depending on the sequences of amino acid and crystalline structure.[12] Further, SF protein shows great promise as an efficient scaffold in the forms of gels, sponges, fibers, and films for faster recovery after an injury by facilitating cell adhesion, proliferation, differentiation, and re-epithelialization of the wounded area.[13] Even though it has been demonstrated that SF is a biocompatible material, it is still difficult to increase the efficacy of SF material by altering properties such as cell interaction and flexibility[14] to guarantee its use in skin wound healing. Natural polysaccharides such as chitosan, chitin, cellulose, alginate, HA, etc., are appropriate for fabricating biomaterials which have been explored by many researchers as they possess exceptional biocompatibility, moisture retention properties, nontoxic cell interactions that enable the proper healing of wounds.[15,16] For enhanced and specific properties, SF can be functionalized by blending it with natural polysaccharides, facilitating and ensuring faster wound healing in which the mentioned polysaccharides possess the desired properties that SF lacks.

Combining SF and polysaccharides can result in a material with enhanced biocompatibility, which is a desirable property for wound healing without posing a significant risk of infections. The intrinsic tensile strength of SF and the viscoelastic nature of polysaccharides can improve the mechanical properties of wound dressings. The processability of SF can be improved, and the pace of breakdown can be controlled by blending it with polysaccharides. Further, polysaccharides can retain adequate moisture at the wound bed, preventing it from drying out. Overall, the blending of SF and polysaccharides is a method to develop wound dressings with a wide array of synergistic and tailored properties by varying their ratios. For instance, to increase the hydrophilicity of SF and create porous composite scaffolds, HA, which has a larger number of hydrophilic groups, can be blended with it as stated in the work by Yang et al.[17] When combined with SF, sodium alginate (SA) also offers hydrophilicity and biodegradability. It is appropriate to blend carboxymethyl cellulose (CMC), a cellulose derivative with superabsorbent properties, with SF since it aids in absorbing wound exudates and shields the skin from further damage and infection. It is the fact that SF tackles the inadequate mechanical strength of CMC, which is reported in the work by Babaluei et al.[18] It may be feasible to utilize multi-functionalized SF dressings that incorporate various wound healing stimulants to protect the wound and promote wound closure by preventing scarring and keloid formation. Any bioactive molecules, including drugs, can be easily embedded into the SF-polysaccharide blends and released at the target place in a sustained fashion for wound healing with the aid of controlled drug delivery.

A preliminary literature assessment was conducted to identify the reviews that have previously been published in the relevant field. To the best of our knowledge, no review papers have been recently published in this fascinating field; hence, a comprehensive review highlighting the applications of blends of SF and polysaccharides that give directions for future research is needed. This review summarizes different blends of SF with certain natural polysaccharides that are being used in facilitating wound healing due to their synergistic effects with the underlying mechanisms. The review also discusses other

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treatment approaches used in conjunction with these blends and their applications in wound healing and highlights current limitations and future developments in this exciting area. By figuring out the mechanisms involved, applications, and challenges associated with SF-polysaccharide blends, researchers can create optimal wound dressings that can transform treatment strategies for the effective management of chronic wounds and facilitate tissue regeneration.

2. WOUND HEALING: DIFFERENT MECHANISMS INVOLVED

Skin wound healing is a complicated dynamic process occurring in four stages which are overlapping and backed by a wide range of cellular activities that need to be properly monitored and coordinated for the effective restoration of injured tissue. The skin is an excellent protective sheath against pathogenic microorganisms, while chronic wounds are the active sites for bacterial colonies to propagate by triggering the immune system. Chronic wounds that are difficult to heal can develop as a result of disruption in cellular activities associated with a wound occurring, especially due to underlying conditions such as aging and diabetes mellitus. These types of wounds are challenging, and they pose a substantial burden to society as it is prevalent. Therefore, better molecular and clinical knowledge of the mechanisms underlying wound repair is desperately needed.

There are four consecutive phases of wound healing, namely, hemostasis, inflammation, proliferation, and remodeling or maturation (shown in Figure 1). These stages have been shown to possess indispensable role in the healing of wounds. Impairments in any of these steps lead to complications in wound healing which need to be rectified with proper care.

2.1. Hemostasis. The initial phase involves the immediate reaction to damage and hemostasis, during which inflammatory cells and platelets gather at the wound site and adhere to the collagenous ECM. This process then triggers the release of clotting factors, including fibronectin, which enhances blood clotting and constriction. The activation of platelets takes place soon after an encounter with the vascular subendothelial matrix and becomes crucial when the injury takes place in the blood vessel. A well-renowned platelet receptor called glycoprotein VI seeks to connect with several proteins found in the ECM. An enzyme known as thrombin, which exists as prothrombin (an inactive enzyme found in the liver), exhibiting both anticoagulant and procoagulant properties, is the cause of platelet activation. The function of active thrombin is to convert fibrinogen to fibrin thereby enabling the clotting of the blood.[19] Consequently, this phase results in the wound site's bleeding being prevented.[20]

2.2. Inflammation. The second stage, which follows the damage immediately, is referred to as the inflammatory stage and is characterized by inflammation that lasts anywhere from 1 day to 6 days. After a fibrin clot forms, the complement system is activated, causing neutrophils to relocate to the wound site as a result of vasodilation. It was investigated that the blood monocytes and lymphocytes at the site of injury develop into tissue macrophages and endothelial cells, keratinocytes, and fibroblasts are activated for repairing the blood vessels that are damaged by the release of growth factors and cytokines.[21] At this point, macrophages and neutrophils will clear the wound bed of any tissue debris and external lesions, thus, preventing infection. Mast cells, whose key objective is to help draw neutrophils to the site of inflammation, are what cause wounds to produce histamine.[22] The wound becomes chronic when the phase of inflammation switches into a self-regenerating condition, and the body is unable to go through the regular sequence of certain events. At the inflammatory stage, bacterial infections are nearly always present and can be fatal, depending on the severity. Both Gram-positive bacteria such as *Staphylococcus aureus (S. aureus)*, *Streptococcus pyogenes (S. pyogenes)*, and Gram-negative bacteria which includes *Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa)* which are present at the wound area play a prominent role in the initial stage of infection. Furthermore, enzymes and a group of signaling proteins that regulate inflammation in our body called "cytokines" are secreted during this phase which eventually leads to the stimulation of both fibroblasts and myofibroblasts, and the moisture is maintained at the wound bed through exudate formation essential for faster healing of wounds.[23]

2.3. Proliferation. The third phase is the proliferative phase, which lasts for two or 3 days following the injury and ends until the wound closes. Macrophages, endothelial cells, keratinocytes, and fibroblasts are active during this phase and are in charge of matrix deposition, the process of angiogenesis and wound closure.[24] Growth factors, hydrogen peroxide, and cytokines promote keratinocyte activation during the proliferative phase of wound healing. Keratinocytes facilitate transitions in epithelial-mesenchymal cells and lead to the initiation of re-epithelialization in wounds as a result of this stimulation.[25] As a result of epithelialization, new immature granulation tissue is developed and starts to expand at the region of the wound to form a new ECM. Deposition of collagen and fibroblast proliferation are the key activities that occur at this phase. A large network of new blood vessels is formed throughout the granular tissue, and the process is known as angiogenesis. Angiogenesis is a key event in the process of wound healing, and the materials that are known to induce angiogenesis ensure faster as well as proper healing of wounds.

2.4. Maturation. The last step in the healing of wounds is remodeling, which is also known as the maturation phase in which maturation and tissue regeneration take place thereby causing an alteration in the composition of ECM. Fibroblast cells employ proteoglycans, hyaluronan, and fibronectin to relocate fibrin clots at this stage of wound healing. Following its removal, the wound site undergoes remodeling of the ECM, which produces matured collagen fibrils that aid in wound healing.[26] Proteoglycans aid in cell migration through the development of fully developed collagen fibrils that are cross-linked. This phase is also characterized by the replacement of collagen type III, which exists with collagen type I, causing the tensile strength of the regenerated tissue to rise for scar formation.[27,28]

2.5. Role of SF and Polysaccharides in Wound Healing. Various factors involved in wound healing have been proven to be modulated by SF, in both physiological and molecular aspects. Employing the nuclear factor-kappa B (NF-κB) signaling system, which regulates several cellular processes including cell attachment, proliferation, the removal of reactive oxygen species (ROS), and inflammation, SF is known to hasten the healing of wounds.[29] As a result, the NF-κB pathway is thought to be very significant in the healing of many types of wounds. The cells that were treated with SF were found to produce two important receptors, which are also mediators of NF-κB, i.e., Tumor necrosis factor receptor (TNFR) and toll-

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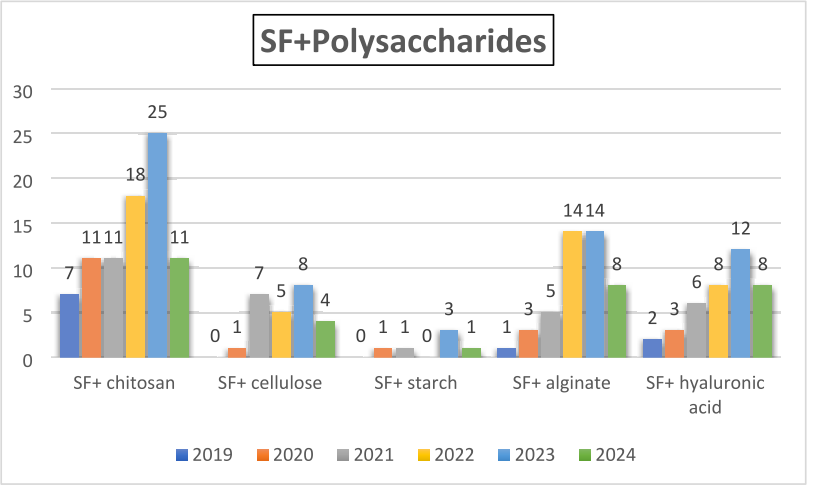

Figure 2. Number of research studies done in the past 5 years.

like receptors (TLR). As a result, various growth factors were also stimulated in the cells. A research study by Park et al.[30] revealed the significance of the NF-κB signaling pathway in wound healing through the release of several inflammatory factors. Some *in vitro* experiments have demonstrated that SF causes the stimuli to produce more integrin in the umbilical vein endothelial cells of human beings. Thus, it has proven the capability of SF in wound healing through cell motility enhancement as a result of increased integrin expression, which in turn led to the stimulation of three other pathways: mitogen-activated protein kinase, phosphoinositide 3-kinase, and c-Jun N-terminal kinase. Using a rat model, the findings from a work by Chou et al.[31] demonstrated the function of SF in the re-epithelialization of burn wounds as well as the development of granulation tissue.

Polysaccharides have been observed to possess many positive effects in the steps involved in wound healing. The structural components of polysaccharides serve as easily accessible reaction sites for the formation of hydrogels for different applications, including wound healing. Such hydrogels made of polysaccharides have excellent water retention capacity and act as a protective sheath against microorganisms, which aids their use in the healing of wounds. Alginate and chitosan are hydrophilic polysaccharides that can be employed to make wound-healing hydrogels, which further prevent the wound from desiccating out. The structure and topology of polysaccharides can be tailored to attain improved properties as per the requirements.[32] The inflammatory stage of wound healing can be modulated by polysaccharides, which are prominent in the proper healing of wounds. Some polysaccharides can induce fibroblast cell growth and migration, which are crucial for the development of new tissues. This may cause the formation of collagen, which is an essential protein for maintaining the structural environment of the skin. Several other polysaccharides like beta-glucans can actively participate in the process of wound healing by stimulating both immune and nonimmune cells facilitating the effective alleviation of wounds.[33] It can activate macrophages, thereby exhibiting an anti-inflammatory response at the wound site. Chitosan and its derivatives have been shown to have many positive effects on skin wounds, particularly favorable pharmacological activities, including hemostatic, antimicrobial, and skin-regenerating properties. This polymeric material has a remarkable role in the initial three stages of wound healing; hemostasis, inflammation, and proliferation. Initially in the hemostasis stage, they aid in ceasing bleeding by encouraging platelet and erythrocyte aggregation and preventing fibrin's disintegration. Consequently, they are effective in removing pathogens from the wound site while it is still inflamed; last but not least, they hasten the proliferation of skin by triggering the development of granulation tissue, often known as proliferation. The wound was then repaired, and the skin was remodeled to complete the healing process. HA supports cell adhesion, proliferation, and differentiation[34] which is contributed by its ability to interact with the receptors on the cell surface, a property employed for tissue regeneration. Another polysaccharide called heparin induces many crucial physiological processes of wound healing such as cell attachment, proliferation, inflammation, and angiogenesis, and inhibits coagulation[35,36] and has been widely explored as a drug of clinical importance. There are numerous works citing the ability of heparin to bind with the amino acid groups present in protein to regulate different biological phenomena, as in the case of binding of heparin with the amino acids of growth factors,[37] resulting in the formation of a complex, which ensures the stability of growth factors and enables the sustained release of growth factors. Bacterial cellulose facilitates faster wound healing by supporting cell adhesion, proliferation, and differentiation in tissue regeneration.[38]

The combination of SF and polysaccharides shows significant potential for creating sophisticated wound dressings that facilitate faster, safer, and more effective healing. A composite wound dressing can be fabricated by combining the cell attachment ability and mechanical strength of SF with the moisture retention capacity and other medicinal properties of polysaccharides. The presence of specific amino acid sequences of SF could contribute to the proliferation of fibroblasts cells and this property was discussed in a work proposed by Wang et al. where they reported the synergistic effect of SF, calcium alginate, a polysaccharide, and another polysaccharide extracted from Bletilla striata in the repair of wounds.[39] SF can be blended with natural polysaccharide polymers such as chitosan, alginate, HA, cellulose, and their derivatives to enhance the overall performance as well as to obtain desired properties like bioactivity, cell adhesion, and proliferation as well as the elasticity of the biomaterial.[40,41] Figure 2 reveals the

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number of research works conducted in the last 5 years. Increasing research interest can be seen in the particular area in the last year compared to the previous years.

3. FABRICATION TECHNIQUES OF WOUND DRESSINGS

There are various techniques for the fabrication of composite wound dressings named solvent casting,[42] electrospraying,[43] electrospinning,[44] and 3D printing[45] (as shown in Figure 3)

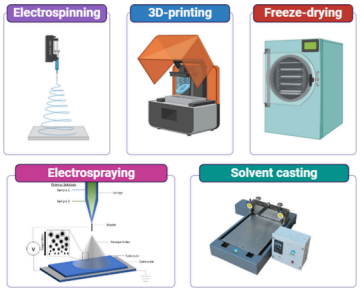

Figure 3. Different methods for fabrication of wound dressings.

and it is important to choose the appropriate method of fabrication as it is crucial to achieve the desired final product, which satisfies the requirements of the dressing. The requirements such as gas permeability and porosity of the material can be achieved through the proper fabrication of the dressing.

Solvent casting is the easiest approach and is generally used for fabricating wound healing films with no complications while casting.[46] Porosity cannot be guaranteed for the fabricated films, and in this case, an additional leaching process is needed, which is not appropriate as it causes the elimination of encapsulated drug molecules in the dressing. On the other hand, oxygen gas permeability and flexibility can be assured for the films. Arthe et al. fabricated a film composed of SF and paramylon using the solvent casting method for the treatment of chronic wounds. The fabricated films exhibited good thermal stability as well as excellent moisture absorption capacity.[47] Another scaffold made of SF and SA functionalized with allantoin was fabricated by Xie et al.[48] using the solvent casting method for repairing cutaneous wounds. The prepared scaffold was found to be transparent, ensuring good adhesion properties under wet conditions and moisture permeability.

Electrospinning is a versatile method for fabricating wound dressing, as it assures porosity and tunable surface area for the dressing to function at the wound site in a desired manner. A wide variety of biopolymers can be employed in electrospinning, which includes both natural and synthetic polymers for the preparation of dressings. In this technique, the blend of SF and polysaccharide is dissolved in an appropriate solvent, and then the optimally viscous solution is loaded into a syringe, in which its needle tip is subjected to high voltage. As a result, when the solution is ejected and solidified in the presence of the electric field, nanofibers are formed. The generated nanofibers are gathered on a rotating collector to obtain the fibrous mat.[49,50] The most fascinating feature of this technique is the successful encapsulation of drugs and biomolecules in a wound dressing. They can be easily loaded into the nanofibers generated to enhance therapeutic action by their prolonged release into the wound site. Peng et al. reported one work on the fabrication of electrospun nanofibers made up of SF, polylactic acid glycolic acid incorporating artemisinin for the healing of wounds. This biocompatible nanofiber could effectively load the drug employed, which is made possible through the electrospinning method of fabrication.[51] A modified method of spinning technique called free surface electrospinning was utilized by Yin et al.[52] to fabricate a nanofibrous scaffold of SF and hydroxypropyl methylcellulose for skin wound healing. This technique could restrict solvent evaporation during the process of spinning. The fabricated nanofibrous scaffolds with the combined properties of SF and polysaccharides show great promise in the field of skin tissue regeneration, as they provide an environment that is conducive to cell development and differentiation. For electrospinning to produce the desired final product, the blend ratio of SF and polysaccharide must be optimized, morphology must be maintained, and fiber diameter must be fixed. A composite scaffold of SF, alginate, and gelatin was prepared by Hajiabbas et al.[53] using the electrospinning technique with optimized blending ratios and working conditions. The scaffold fabricated was observed to resemble the natural ECM, and the structure of the composite material has a remarkable influence on the adhesion and growth of mesenchymal stem cells derived from the adipose tissue.

Electrospraying is also an approach for wound dressing fabrication which is very similar to the already discussed method "electrospinning". The difference between these two techniques is that instead of fiber ejection from the nozzle in the process of electrospinning; in electrospraying, droplets are ejected which are charged ones.[54]

Lyophilization often known as freeze-drying, is a simple process for creating porous scaffolds or sponges. In this manufacturing technique, a porous scaffold is fabricated by allowing the frozen solution of SF and polysaccharide to sublimate and remove the solvent through vacuum drying. Using this method, wound dressings that can adsorb excess exudates from infected wounds have been made successfully, with fewer challenges. Zhang et al. in their research work,[55] created a porous scaffold composed of SF and HA that resembled the natural ECM by using the freeze-drying process. The synergistic impact of the two polymers involved resulted in the manufactured scaffold exhibiting beneficial features for improved wound healing. Scanning electron microscopy (SEM) was used to validate the pore structure of the scaffold. One might adjust porosity by altering the temperature, it was observed that the porosity increased when the freezing temperature increased and the amount of HA decreased. Shen et al. in their study[56] used the previously stated freeze-drying approach to create a composite scaffold of SF and SA embedded with SF nanoparticles with improved surface roughness to enable cell adhesion and hemostasis for successful wound healing. Several physical and chemical characteristics were still retained within an acceptable range, even after the scaffold was fabricated.

3D printing is an advanced technique for the fabrication of wound dressing, and it is trending in various fields of medicine due to many attractive features of the composite fabricated. The scaffold can be fabricated with tunable porosity in the desired geometrical shape[57] to be fixed properly at the wounded region. Any drug molecule and bioactive compounds can be incorporated successfully into the matrix, and those encapsulated drugs are released in a sustained manner, which is remarkable in the area of wound healing using targeted drug delivery. Layer-by-layer deposition of bioactives and bio-

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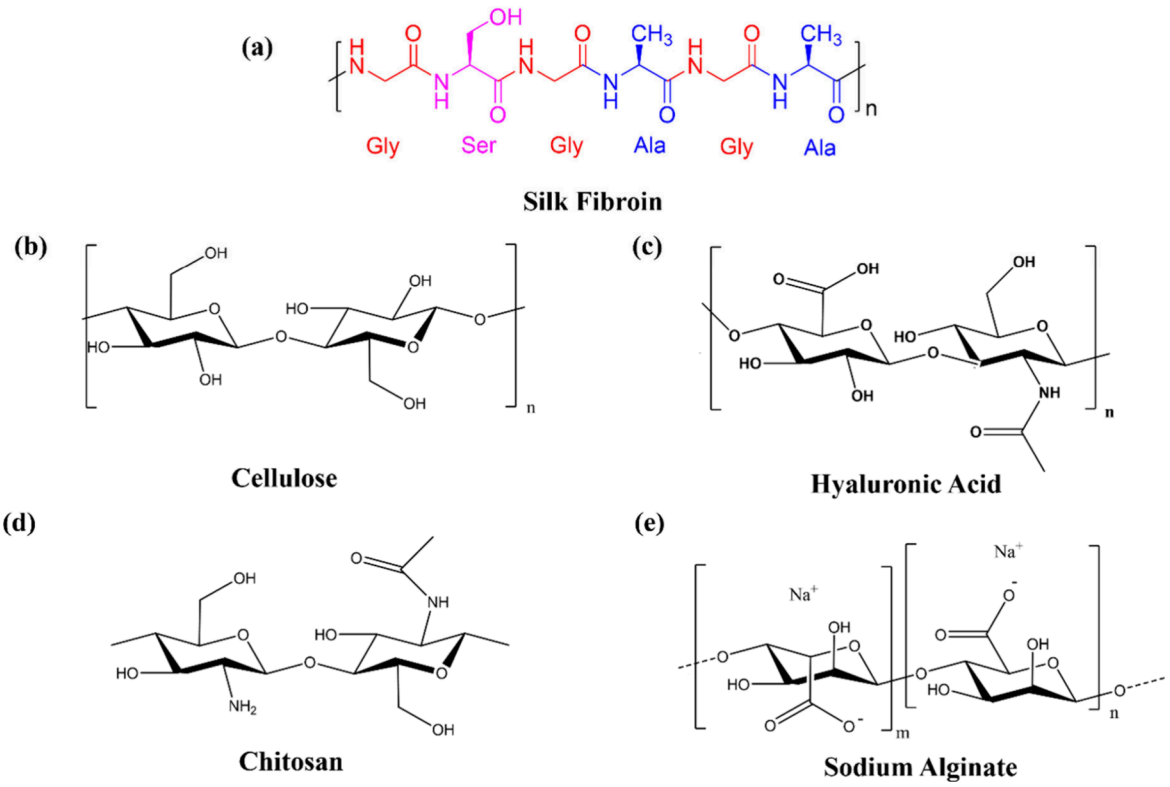

Figure 4. Structure of (a) SF, (b) cellulose, (c) HA, (d) chitosan, and (e) SA.

materials is an advantageous feature of this technique that eventually leads to faster healing of wounds through the synergic effect of all compositions of biomaterials present in the dressing. There are several 3D printing approaches available today: inkjet printing, extrusion printing, and photopolymerization printing; all of these mentioned methods have their advantages and limitations. Bioinks made of biomaterials can be employed in 3D printing strategy, and their effective optimization is required to ensure precision in printing. Choi et al. summarized a work on the effective use of SF as bio-ink for the 3D printing of skin and wound models. This printed hydrogel enabled cell viability and proliferation on different layers of the model. High-resolution printing was achieved in this reported work through the digital light processing (DLP) printer in which the biomaterial is assembled in a layer-by-layer manner.[58] The SF bio-inks are comparatively less viscous, and the material endures through a gelation phase before being printed using an extrusion method which is quite challenging.[59] To tackle this issue, SF can be combined with chitosan to enhance printability while maintaining the physical, chemical, and mechanical properties of SF.[60,61]

Depending on the fabrication techniques, the structural and functional characteristics of the wound dressings may be customized. Tunable properties are obtained by optimizing the fabrication parameters and blend ratios, irrespective of the technique employed for fabrication. Different properties of the wound dressing can be tailored and enhanced efficiently by adjusting and optimizing the ratio of different polymers comprised of the blend. Through optimization, a material with a variety of desired thermal, electrical, mechanical, and chemical properties can be produced. One can pick the manufacturing technique carefully to obtain a wound dressing that is cost-effective on a commercial scale. SF and polysaccharides have considerable potential in the field of wound healing, and their properties can be combined to fabricate advanced wound dressings by optimizing their blend ratios. To turn these intriguing possibilities into clinical applications, extensive research is needed.

4. PHYSICAL AND MECHANICAL PROPERTIES

The polymer system, which consists of polysaccharides and proteins, has been showing desirable features such as nontoxicity, good biodegradability, and excellent biocompatibility.[62] Glycine (Gly), Alanine (Ala), and Serine (Ser) are the amino acids that are present in the SF polymer[63] (Figure 4(a)), out of which Gly is hydrophobic as well as Ala and Ser are hydrophilic amino acids. Because of the presence of this amino acid sequence in SF, there is a balance between hydrophilicity and hydrophobicity. In the case of polysaccharides such as chitosan, HA, and cellulose, the existence of more hydroxyl groups that easily make hydrogen bonds in water makes them known to be more hydrophilic than SF. Hence, it is noteworthy that the blending of SF with polysaccharides may enhance the overall hydrophilicity of the material depending upon the specific polysaccharide involved, and also, the ratio of the individual polymers can be optimized to obtain tunable hydrophilicity.

The heavy chain of the fibroin protein has both crystalline and amorphous properties. The β sheet forming structures with a hydrophobic nature, which is present in the crystalline region imparts roughness and good mechanical strength to SF.[64,65] According to a study by Wang et al.,[66] SF nanofibers treated with ethanol may efficiently induce the β sheet structure, increasing the crystallinity of SF. This process was found to be made possible by the ability of ethanol to penetrate the SF peptide chain and form new hydrogen bonds

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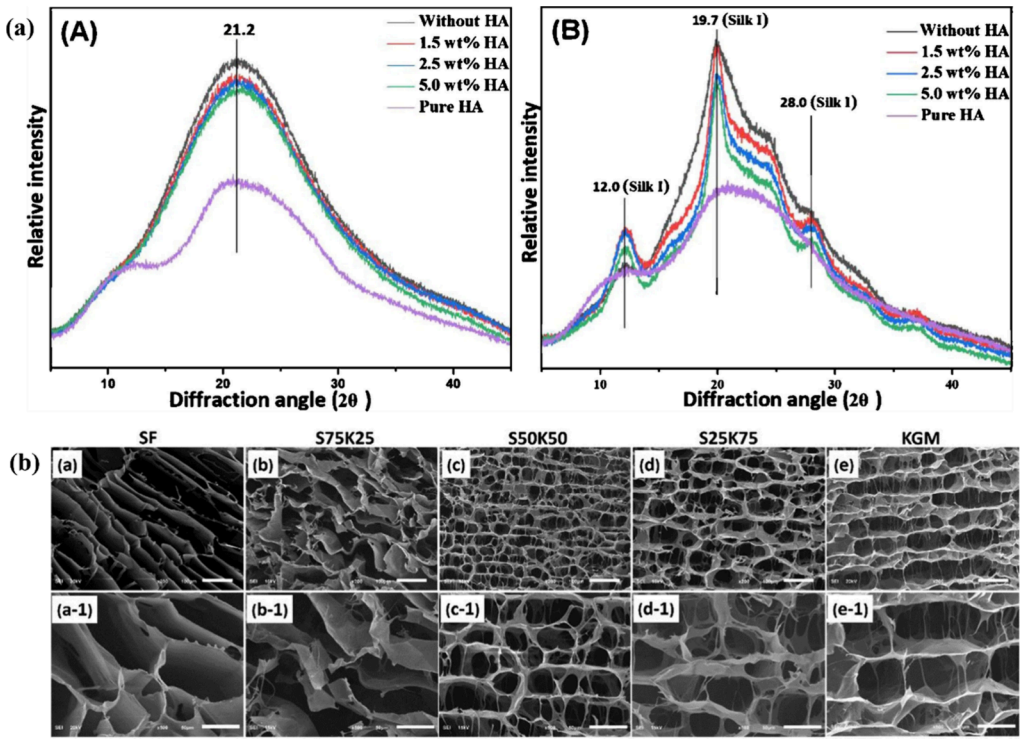

Figure 5. (a) XRD peak of SF/HA scaffold with different blend ratios (A) before the annealing process and (B) after the annealing process. Adapted with permission from ref 69. Copyright 2020 Elsevier. (b) Morphology of porous freeze-dried sponges containing different blend ratios of SF and the polysaccharide. Adapted with permission from ref 71. Copyright 2019 Elsevier.

by breaking existing ones. Further, the removal of ethanol caused bond recombination, which significantly increased the β sheet structure. As a result, it was observed that the ethanol-treated SF nanofibers had enhanced crystallinity and, thus, improved hydrophobicity. In a study conducted by Manchineella et al., a shift in vibrational frequency can be observed in the FT-IR spectrum of the fabricated SF film, indicating the β sheet structure of SF upon treatment with methanol.[67] SF is a material with inherent mechanical strength so that it can withstand severe forces up to a certain extent, and it can be enhanced further when a scaffold is prepared by the incorporation of some polysaccharide polymers depending on their source of extraction. Chitosan, alginate, HA, and cellulose are some polysaccharide polymers that are hydrophilic, biocompatible, and biodegradable (shown in Figure 4(b), (c), (d), (e)). These polysaccharides are readily functionalized and exhibit a range of mechanical properties based on their molecular weight and structure. The properties of the blends may vary according to the component ratios of the blend, the preparation techniques involved, and the choice of specific polysaccharides in the preparation.

Before fabrication, the blending of SF and polysaccharides results in synergistic properties whose physical and mechanical attributes may be customized by varying their ratios of blending. However, more tailored properties can be obtained with the selection of appropriate fabrication methods for use in wound healing. The physical and mechanical properties of SF-polysaccharide blends are influenced by the techniques used in the fabrication of wound dressings. Depending on the fabrication method selected, mechanical properties, microstructure, and porosity of the material can be determined. The SF-polysaccharide 3D porous structure can be fabricated using freeze-drying, electrospinning, or 3D printing techniques.[68]

Pore size can be regulated using the freeze-drying technique by controlling several variables, such as temperature, pH, and the quantity of organic solvent used. The XRD peak (shown in Figure 5(a)) showed structural changes before and after annealing in a study conducted by Guan et al. on the fabrication of SF/HA scaffolds utilizing the freeze-drying process. The XRD peak was observed to be broad upon the physical blending of SF and HA, but following the annealing process, the crystallinity of the material caused the β-sheet structure to be induced, which further decreased its solubility. The inclusion of SF with high crystallinity enhanced the overall stability of SF/HA scaffolds and enabled physical cross-linking during blending.[69] Nanosized structures can be created using the electrospinning technique, which is desirable for various processes including cell adhesion, proliferation, and differentiation. Scaffolds with high porosity and surface morphology can be fabricated using 3D printing, a computer-aided layer-by-layer manufacturing technique.[70] The porosity of the SF-polysaccharide composite sponge fabricated using the lyophilization technique in a work by Feng et al.[71] is evident from the morphology images shown in Figure 5(b). The proportion at which SF and polysaccharides are blended has a considerable effect on the material's mechanical properties. Several variables that are involved in the manufacturing process, including humidity, temperature, and type of solvent used, can also have an impact on the various physical and mechanical properties of the material. Further, cross-linking is a key strategy that improves the stability and tensile strength of the material. The physical and mechanical properties of SF-polysaccharide blends after fabrication are summarized in Table 1.

Dou et al.[72] fabricated an SF/SA scaffold functionalized with probiotics for scarless wound healing, and it was fascinating to observe that the mechanical strength of the SF scaffold was

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Table 1. Physical and Mechanical Properties of SF-Polysaccharide Blends after Fabrication Using Different Techniques

No.	Fabrication technique	Physical properties	Mechanical properties
1	Solvent casting	• Smooth surface • Less porous	• Modest tensile strength and elastic modulus
2	Electrospinning	• High surface area for functionalization • Nanofibrous structure • Controlled degradation	• High tensile strength and elastic modulus
3	3D-printing	• Complex 3D geometry • Optimal porosity • Controlled surface morphology	• Tailored properties depending on parameters involved in printing
4	Freeze-drying	• Highly porous • Customized surface morphology depending on processing parameters	• Improved tensile strength and elastic modulus
5	Electrospraying	• High porosity • Controlled fiber diameter	• Customized mechanical properties

increased by about 4.6-fold. The inherent brittleness of SF in dry conditions limits its utility in some applications.[73] When SF gets transformed into a membrane structure, it becomes relatively brittle and has little elasticity, making it easy to break during its usage, and this issue could be addressed by the addition of SA as in the above-mentioned work for application in wound healing. The material's capacity to precisely fit into growth factors for appropriate encapsulation is because of the porous nature.[74,75] It is possible to combine SF, which has intrinsic mechanical strength, with chitosan and HA, which have a lower tensile strength. Moisture absorbing ability, tensile strength, and swelling ratio as well as the porosity of the scaffold fabricated by Liu et al. using SF and chitosan were investigated and depicted in Figure 6. Many research works on SF highlight the unique features of the material such as biodegradability, biocompatibility, and less toxicity, and it was also found to be permeable to gas and water. The degradation property of SF is such that it undergoes slow degradation over time within the body. Alginate and HA are examples of water-soluble polysaccharide polymers that may be added to the SF polymer to manipulate its hydrophobicity.

The transparent scaffold which was fabricated after blending both SF and SA was found to exhibit considerably good cell adhesion properties, mechanical properties, and required permeability to oxygen.[77] Eivazzadeh-Keihan et al. developed an antibacterial, biologically active alginate hydrogel scaffold incorporated with poly(vinyl alcohol), SF, and magnesium hydroxide ($Mg(OH)_2$) in nano-dimensions with enhanced mechanical properties. The fabricated scaffold showed excellent porosity and cellular attachment with no toxicity observed.[78] Shefa et al. in their research work were able to successfully reduce the brittleness of SF through the effective cross-linking of the polymer with cellulose nanofiber for the treatment of wounds. With this view in mind, they could effectively fabricate cost-effective scaffolds with biocompatibility for the faster healing of wounds.[79] Eivazzadeh-Keihan et al. prepared a nanoparticle-loaded composite scaffold of SF and CMC as a wound dressing. The synergistic effect of carboxy methyl cellulose and SF causes the mechanical strength to increase, moisture retention capacity of the scaffold was found to be enhanced thereby supporting faster wound closure.[80] Babaluei et al. blended SF with CMC comprising functionalized graphene oxide to prepare injectable hydrogels for the treatment of burn wounds exhibiting antibacterial as well as antioxidant properties. The prepared hydrogel was observed to show enhanced biocompatibility, as expected due to the combination of both polymers. The hydrogel effectively promoted the closure of burn wounds as it facilitated the granulation tissue formation and collagen deposition, which are the key events in the healing of wounds.[81]

Moreover, by adjusting the composition, cross-linking, and processing parameters of the chosen manufacturing technique, scaffolds with customized physical and mechanical properties that facilitate quicker wound healing and patient satisfaction may be produced.

5. BIODEGRADABILITY AND BIOCOMPATIBILITY

In vitro and *in vivo* studies reveal that SF possesses certain specific properties to be used for tissue regeneration such as easy cell attachment, proliferation, and growth[82,83] without causing any toxicity to the skin tissue. SF mimics the environment of the skin to facilitate cell attachment, proliferation, and differentiation of fibroblast and keratinocyte cells and helps in the synthesis of collagen, which all are key events for the enhanced healing of wounds. The kind of polysaccharide, source, method of processing, and whether contaminants are present can all affect biocompatibility. Initial insights can be gained from *in vitro* experiments, but the complex environment of the body cannot be entirely mimicked. However, it is significant to prove biocompatibility through *in vivo* research. A crucial *in vitro* technique for analyzing the process of wound healing is a wound healing scratch assay also called a cell migration assay. The aforementioned technique enables the study of cell migration to the wound bed[84] in response to a different kind of stimuli, including chemokines, cytokines, and growth factors. It also allows for an assessment of the process of wound closure and the vital role that different cells play in it. Notably, this scratch test may be used to assess the impact of pharmacological agents on both cell migration and the healing process of wounds. Babu et al. in their work[85] investigated the wound healing activity of silver oxide nanoparticles incorporated SF spun with the aid of wound healing scratch assay. The images of cell migration to the wound bed within a specified period of 24 h revealing the wound healing activity are shown in Figure 7(a). It is also possible to quantify the cells that migrate into the wound scratch suitably as represented in Figure 7(b) to evaluate the process of new tissue development.[86] Cell adhesion tests, proliferation tests, cytotoxicity studies, and inflammatory response detection are often employed in both *in vitro* and *in vivo* investigations. Shefa et al. in their research work were able to successfully reduce the brittleness of SF through the effective cross-linking of the polymer with cellulose nanofiber for the treatment of wounds. With this view in mind, they could effectively fabricate cost-effective scaffolds with biocompatibility for the faster healing of wounds.[79] In a work proposed by Xia et al. where they have fabricated nanofibers of SF and chitosan, it was found that the nanofibers were biocompatible with the fibroblast cells which is a desirable property (cell viability results are depicted in Figure 7(c)).[87] Liu et al. created a wettable wound dressing, which is a combination of chitosan and SF encapsulated with silver nanoparticles for the treatment of infected wounds. The prepared wound dressing was identified as a biocompatible

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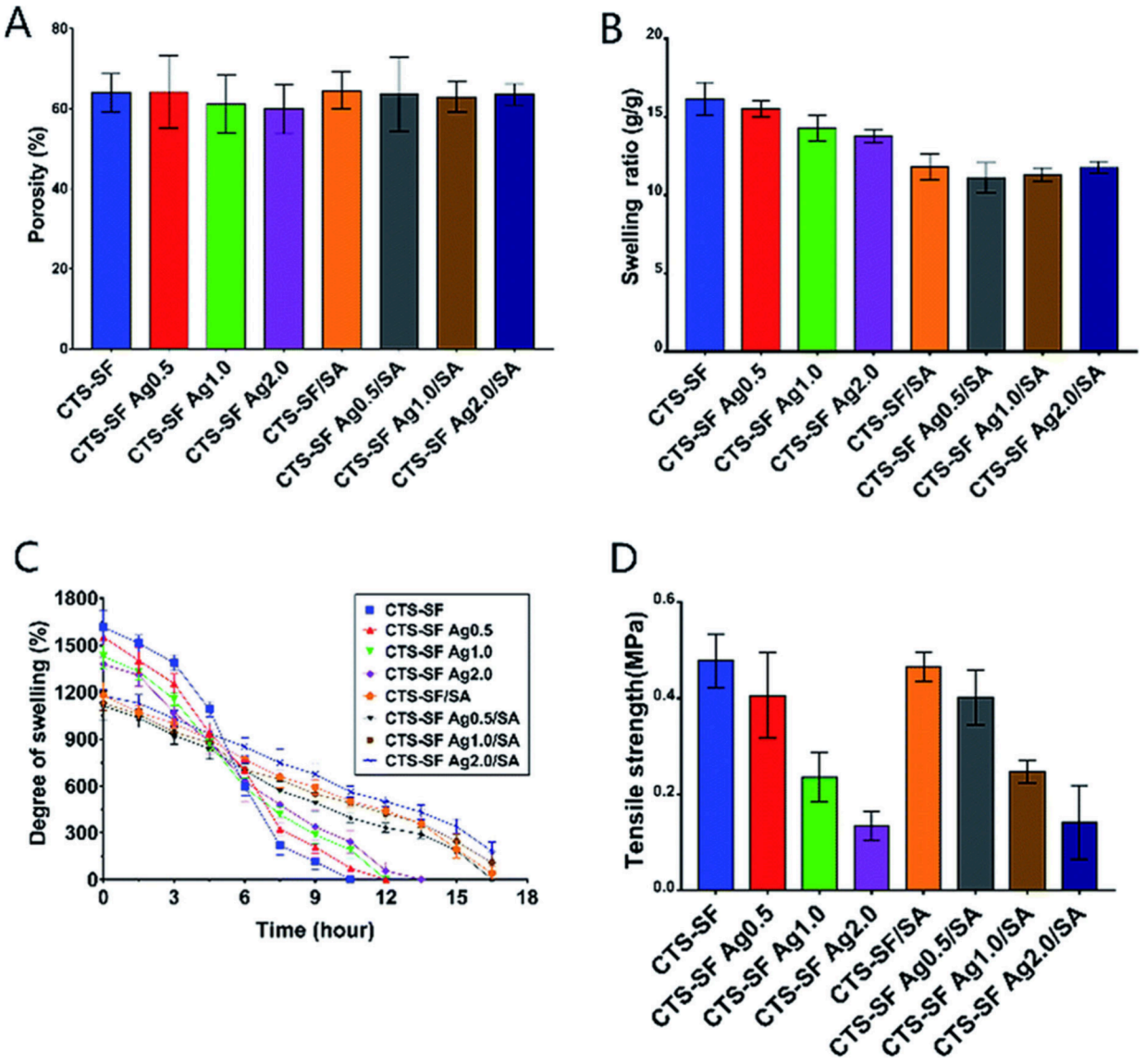

Figure 6. Different physicomechanical properties of the wound dressing: (a) Porosity of the dressing; (b) the ratio of swelling; (c) water retention capability; (d) tensile strength of the material. Adapted with permission from ref 76. Copyright 2017 Royal Society of Chemistry.

dressing from the MTT assay and the recovery from infected wounds was observed to be faster, on evaluating the results from *in vivo* experiments conducted on mice model.[76] A similar wettable wound dressing was fabricated by Qian et al. which comprised chitosan and SF encapsulated with a composite of silver nanoparticles and stem cell-derived exosomes for treating infected wounds. The respective wound dressing was fabricated to enhance the process called angiogenesis, which is the formation of new blood vessels. This wettable wound dressing showed moisture retention properties and exhibited excellent antimicrobial properties. It is evident from the *in vivo* animal studies that, recovery from infected wounds was made possible due to the ability of the dressing to promote collagen deposition and induce angiogenesis and nerve repair in the animal model.[88] It was investigated that the hydrogel composed of SF and thiolated HA prepared by Yu et al. could support the adhesion, proliferation, and differentiation of fibroblast cells, as well as human endothelial cells. *In vivo* animal studies showed promising results of wound healing as the composite could generate tissues in a 2-week duration of time.[89] Another less-discussed animal polysaccharide is heparin which induces many crucial physiological processes of wound healing such as cell attachment, proliferation, inflammation, and angiogenesis, and inhibits coagulation[35,36] and has been widely explored as a drug of clinical importance. Hama et al. investigated the effect of wound healing by an SF system modified with activated heparin, which was found to induce the process of re-epithelialization, which is a key event in the healing of wounds. The films were evaluated by using cell culture studies and analyzed for a better understanding of their cell adhesion and cell proliferation properties. Better cell adhesion and proliferation of fibroblast and endothelial cells were observed on the prepared films, which eventually led to faster wound healing. This work revealed the possibility of designing various materials through the successful alteration of their surface properties, cellular behavior, etc., by emphasizing on modification of the three-dimensional network structure of SF.[90]

Proteins and amino acids are the nontoxic byproducts of SF proteins upon degradation, which are readily adsorbed into the

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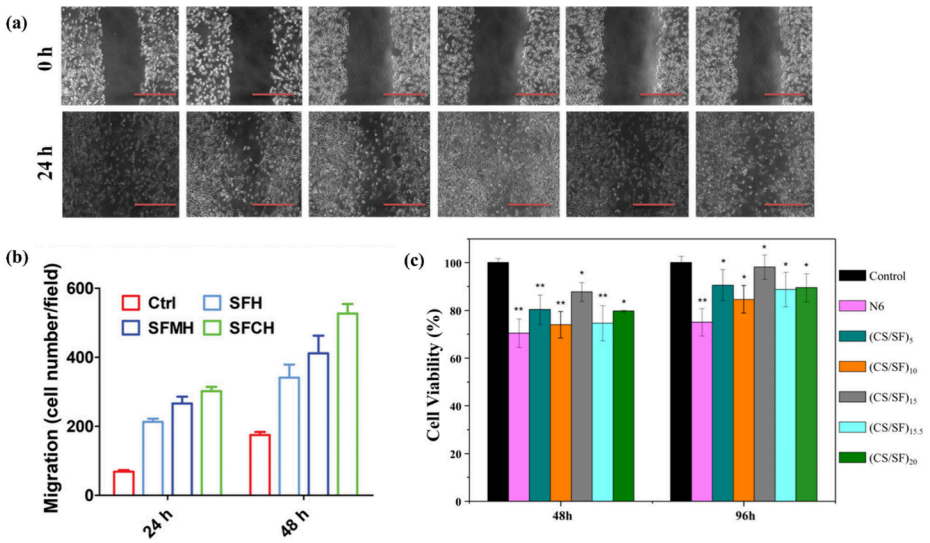

Figure 7. (a) A representation of *in vitro* wound healing activity assessed from the cell migration assay. Adapted with permission from ref 85. Copyright 2018 Elsevier. (b) Quantification of the cells that migrate into the scratch of the wound. Adapted with permission from ref 86. Copyright 2022 Wiley. (c) Results of cell viability of fibroblast cells on nylon6 and LBL deposited mats. Adapted with permission from ref 87. Copyright 2019 Elsevier.

body. There are several deciding factors for determining the rate of degradation of SF proteins. As the β sheet forming structures with a hydrophobic nature in the crystalline region imparts roughness and rigidity to SF, the material will degrade relatively slowly. "Degumming" which is a process for the separation of sericin proteins from the cocoon fragments by boiling them in an aqueous solution of sodium carbonate (Na_2CO_3)[8] also serves a role in the degradation of SF. Cross-linking methods for the enhancement of physical as well as chemical characteristics through the interaction between the molecules involved may also influence degradation.[91] Biodegradability may also be increased by several environmental factors like microbes, enzymes, etc. that hasten the degradation rate. Polysaccharides are also biodegradable polymers, and the polymers like chitosan, alginate, and HA with higher molecular weight tend to degrade slowly. Alginate which is anionic, breaks down into nontoxic units of mannuronic and guluronic acids, building blocks that are alternatively and repeatedly arranged in alginate which makes the polymer linear.[92] Wang et al. blended SF and SA for the fabrication of a porous composite with controlled degradation behavior using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) as the cross-linker. Blend that was cross-linked effectively was less vulnerable to degradation by enzymes due to the formation of strong bonds. The information gathered from this work about the fabricated porous scaffold is beneficial for future investigation and application for skin wound healing.[93] Chitin when undergoes N-deacetylation, produces chitosan which is an easily available semisynthetic[94] polysaccharide polymer available in nature.[95] More deacetylated chitosan undergoes faster degradation due to its high water solubility. Chitosan degrades into nontoxic units of glucosamine and N-acetyl glucosamine which are natural saccharides. Cellulose can be degraded into glucose molecules, which can be readily absorbed by the body, and degradation can be caused by microbes also. It is observed that the highly crystalline cellulose breaks down very slowly due to its rigid structure. Mehrabani et al. reported a work on the fabrication of 3D scaffolds made of SF and chitin with silver nanoparticles, which was found to be both biocompatible and biodegradable. *In vitro* biodegradation studies of the scaffold using an enzyme called lysozyme revealed that the degradation products of both SF and chitin showed no cytotoxicity to the selected cell lines and were observed to be controlled, which is a desirable feature for wound healing. Biocompatibility and cytotoxicity studies were also well agreed for wound healing applications.[96]

6. ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

SF with excellent properties to be used as a wound healing material lacks antibacterial activity in its natural state. Polysaccharides are known to possess broad-spectrum antibacterial activity, which is a desirable property for enhanced wound healing. They can retard or inhibit the growth of bacteria at the wound site by interfering with the metabolism or disrupting the cell wall of the microorganism. Hence, polysaccharides can be blended with SF to generate scaffolds that have antibacterial activity. Polysaccharides when combined with SF can modify the surface of the material by preventing the bacteria from attaching to the skin surface and inhibiting the biofilm formation, thereby reducing the chances of infection which further delays the wound healing process. It is interesting to notice the fact that the hydrogels made of polysaccharides have shown to possess high water retention ability which prevents the wound from drying out.[97] These hydrogels can act as carriers incorporating antimicrobial agents like drugs, essential oils, and medicinal plant extracts for their sustained release, ensuring the effective antibacterial activity of the SF material. Biomaterials with potential uses in wound healing are being developed by researchers through the utilization of the intrinsic features of polysaccharides, their surface modification properties, and their encapsulation abilities. The moisture content and the nutritious environment of the wound offer ideal habitats for the growth of microorganisms. When the immune system of the host is unable to eliminate all of the invasive pathogens, bacterial infections take place. Therefore, it is important to take into account the antibacterial capabilities of the wound dressings. Due to its outstanding antibacterial properties, chitosan is

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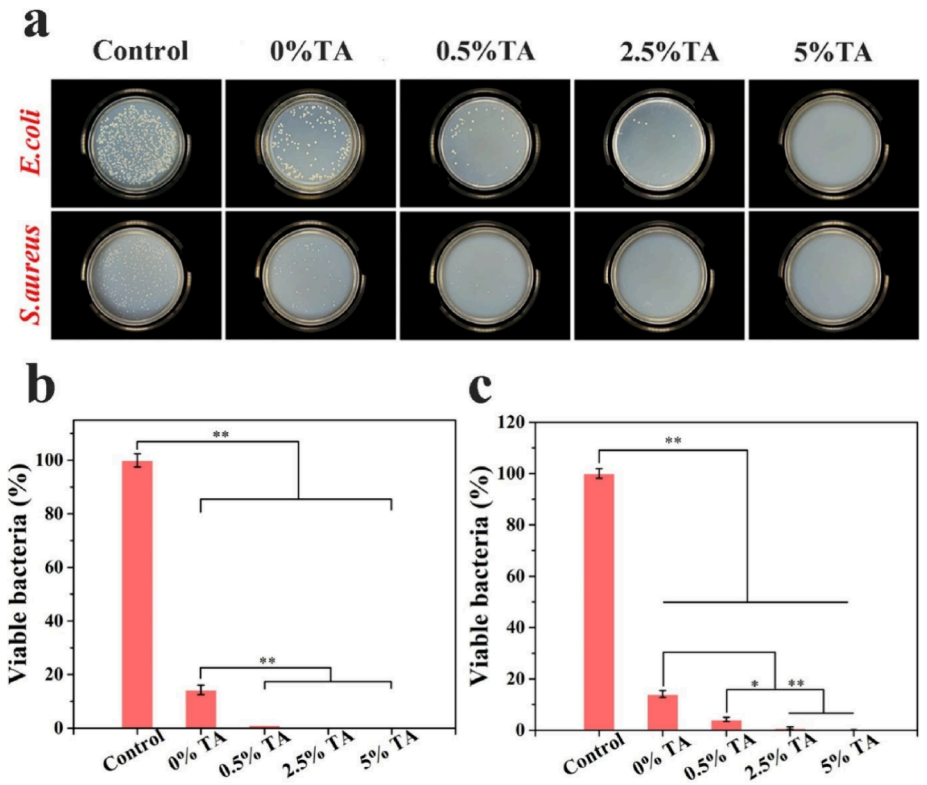

Figure 8. (a) Antibacterial activity of hydrogels against bacterial strains, (b) Rate of survival of *E. coli* bacteria on the treatment with hydrogels for 1 day, (c) Rate of survival of *S. aureus* on treatment with hydrogels for 1 day. Adapted with permission from ref 103. Copyright 2020 Elsevier.

extensively employed in wound care; however, its antibacterial processes are yet unknown. Presently, the recognized plausible mechanisms involve the destruction of bacterial cell walls and membranes, chelation of extremely small amounts of cationic metals, cellular interaction with the target molecules that are internally present, and the accumulation of bacteria.[98] Chitosan which is derived from shrimp shell is rich in lipids, proteins, and other chemical constituents[99] and it was envisaged that shrimp is antimicrobial active against various pathogens such as *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *and Enterobacter aerogenes*. Salama proposed work on the preparation of an antibacterial nanocomposite composed of SF and chitosan incorporated with zinc oxide (ZnO) nanoparticles to enhance its properties. The highly crystalline ZnO nanoparticles were found to have uniform size distribution, and they were evenly dispersed in the polymer matrix. The prepared composite with embedded ZnO nanoparticles was analyzed as an ideal candidate for various biomedical applications especially as an efficient biomaterial for skin wound healing as it possesses excellent antibacterial activity which prevents the growth of bacteria at the wound site.[100] Urzedo et al. prepared an antibacterial alginate hydrogel containing a nitric oxide donor and silver nanoparticles. The synthesized hydrogel was analyzed for its antibacterial activity against *Escherichia coli*, *Streptococcus mutans*, *Staphylococcus aureus* and it was found to exhibit considerable antibacterial activity against *Escherichia coli* variant of bacteria.[101] A nanofibrous membrane which is a blend of SF and cellulose acetate is shown to possess excellent antibacterial activity against *Escherichia coli*, hence identified as an ideal candidate for killing bacteria.[102] He et al. prepared a multifunctional hydrogel reinforced with tannic acid consisting of chitosan and SF in which both biopolymers were methacrylated for enhanced wound healing. Tannic acid was introduced as a cross-linker which increased the mechanical strength of the hydrogel, and several other properties such as antioxidant, antimicrobial, cell adhesion, and cytocompatibility on fibroblast cells were also enhanced upon the tannic acid introduction. The antibacterial efficacy of the prepared hydrogel with tannic acid toward *E. coli* and *S. aureus* as well as their survival to the hydrogels is depicted in Figure 8. The results from the *in vivo* studies were well satisfying as these hydrogels could successfully heal full-thickness wounds.[103]

Chemicals known as antioxidants have a special characteristic feature to transfer electrons to other molecules, like ROS, preventing them from gaining electrons from other molecules that are crucial to the body, such as DNA or proteins.[104] Antioxidants are known as free radical scavengers because they catalyze a convoluted series of events that transform ROS into oxygen and water molecules, which are more stable. Antioxidants can heal wounds by keeping an optimum level of ROS in the wound tissues.[105] Antioxidants are therefore becoming more and more popular in the area of wound healing, and several biomaterials involving polysaccharides have been created and evaluated in this regard. Polysaccharides are an interesting class with strong antioxidant activities. They provide a healthy way to support cellular health and fight against oxidative stress. Many structural variables of a polysaccharide, such as the type of sugar units present, kind of linkages, presence of charged groups, molecular weight, etc. determine the effectiveness of it as an antioxidant. Polysaccharides are scavengers, as free radicals are easily neutralized by the hydroxyl groups on their sugar units of polysaccharides, which can quickly donate hydrogen atoms to them. It is also noticeable that the antioxidant activity of polysaccharides depends on the extraction and purification methods involved.

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The α form of chitosan which is extracted from marine crustaceans and chitosan of insect origin are known to exhibit excellent antioxidant and antibacterial activities,[106] hence they are widely explored in the field of skin tissue regeneration. Also, excellent antioxidant properties can be observed in the chitosan material derived from crab that is enriched with protein and mineral contents, and researchers are showing huge interest in the fabrication of biomedical products that need to tackle free-radical-caused oxidative damage. Even though some studies highlight the free radical scavenging potential of SF, it is not a significant antioxidant on its own. Polysaccharides with antioxidant properties overcome the limitations of SF, forming a protective sheath against free radical damage that causes impairment in wound healing.

7. CLINICAL APPLICATIONS

Although research in the fascinating area of blends of SF-polysaccharides is still ongoing, it may be complicated to acquire information about the clinical trials employing these blends. The field of SF-polysaccharide blends is an emerging area of research. It may take some time before there are any documented human examples, as clinical studies are notoriously time-consuming. Currently, SF-polysaccharide blends are in the stage of preclinical trials where more attention is drawn to the characteristics of the material and several *in vitro* characterizations utilizing different cell lines. Extensive research is being conducted to determine how these blends can enhance the properties of the hydrogels, scaffolds, or bioactive delivery systems that are fabricated. There is no role for clinical studies until after the effectiveness and safety of SF-polysaccharide blends have been examined. *In vivo* animal studies are currently being conducted to demonstrate their efficacy for use in future clinical trials.

Peifen et al. fabricated a ternary scaffold for tissue regeneration by utilizing sulfated SF, chitosan, and also hydroxyapatite. The fabricated scaffold was found to be active against bacteria, biodegradable, and mechanically stable. Cell culture studies were performed, and the results showed low cytotoxicity and considerably good biocompatibility, and it was characterized using electron microscopes also. *In vivo* animal studies were conducted on neck wounds as depicted in Figure 9 and the results were promising, as the composite was able to cure the wounds properly. The ability to retain moisture was a well-appreciable property of the ternary composite as it is a desired property for a wound dressing for the effective treatment of wounds. The composite scaffold was able to facilitate enhanced wound healing because of its capability to support cell adhesion, proliferation, and differentiation and the system was identified as an ideal as well as novel candidate for the application of skin tissue regeneration.[107]

Several kinds of wounds occur. Acute wounds and chronic wounds are the two categorizations of wounds; acute wounds refer to the type of wounds that usually heal faster (surgical wounds and burn wounds) and chronic wounds do not heal within 3 months and require effective treatment to heal properly as they are vulnerable to infections and thus lead to complications in wound repair.[108] Chronic wounds that possess only a very low amount of extracellular matrix (ECM) are not associated with the well-orchestrated process to retain the structure and the normal functioning of the injured tissues within a short period, as it causes impairments to the inner layers of the skin. Chronic wounds are accompanied by the risk of infections and it is prevalent in

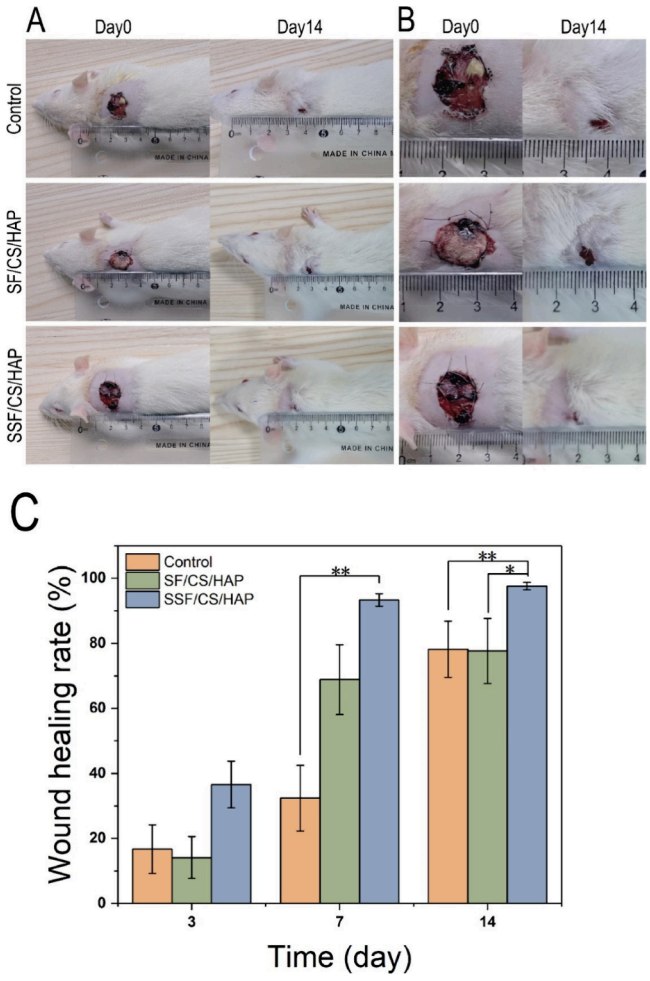

Figure 9. *In vivo* wound healing studies of the scaffold on rats. Adapted with permission from ref 107. Copyright 2023 Elsevier.

patients with underlying conditions such as diabetes, mechanical tension, and surgical excisions leading to chronic diabetic wounds, pressure ulcers, and surgical wound infections which are very challenging to take care of.[109] It is possible to modify SF according to the type of wound that needs to be treated. SF-polysaccharide blends with tailored properties can be fabricated as wound dressings to absorb exudates from certain types of wounds, thereby preventing any chances of infection. Some polysaccharides are known to exhibit anti-inflammatory properties that can aid in the treatment of chronic wounds, which are frequently accompanied by persistent inflammation. Diabetic ulcers are often accompanied by reduced blood circulation, and this issue can be fixed by certain polysaccharides, which can induce angiogenesis, a crucial process of developing blood vessels for enhanced wound healing. Diabetic wounds are also susceptible to bacterial infection; nevertheless, certain polysaccharides possess antibiofilm action, which stops bacteria from flourishing at the wound site. The risk factor associated with burn wounds is scar formation. SF-polysaccharide blends are found to guarantee proper scarless wound healing. Wang et al. fabricated a sponge scaffold of SF and chitosan cross-linked with rhubarb charcoal for the treatment of diabetic wounds, which was found to induce different stages of wound healing such as hemostasis, inflammation, and angiogenesis very rapidly. The scaffold was loaded with the Chinese traditional

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Table 2. Different Blends of SF and Certain Polysaccharide Polymers for Skin Wound Healing

No.	Composition of the blends	Wound type	Antimicrobial activity	Findings	ref
1	SF, modified chitosan	Full-thickness wounds	+	Nontoxic, cytocompatibility, good adhesion to the skin, supported cellular attachment and proliferation, stimulated angiogenesis, enhanced healing of wounds.	111
2	SF, chitosan, carbon quantum dot, α-tricalcium phosphate	Full-thickness wounds	+	Biocompatible, noncytotoxic, promoted rapid cell proliferation, enhanced re-epithelialization, and recovery from wounds within a time period of 12 days.	112
3	SF, chitosan, polydopamine	Infected diabetic wounds	+	Absorption of wound exudates, wound closure within 11 days, less cytotoxicity, an ideal candidate for diabetic wound healing.	113
4	SF, chitosan, Mg $(OH)_2$	Normal wounds	+	Biocompatible, hemocompatible, inhibits biofilm formation, viable to cells, little toxicity due to the presence of Mg $(OH)_2$.	114
5	SF, chitosan	Normal wounds	+	Capable of regulating any infections in the wounded area, promoted the sustained release of the loaded drug levofloxacin which caused the wound to heal rapidly.	115
6	SF, chitin, TiO_2	Normal wounds	+	Antifungal properties, cytocompatibility, desirable water uptake ability, biodegradability, supported cell adhesion, proliferation and differentiation.	116
7	SF, human fibrin and HA	Burn wounds	-	Enhanced the attachment, proliferation, and migration of cells, hemocompatible, nonimmunogenic scaffold, stimulated the process called angiogenesis thereby leading to the proper healing of burn wounds.	117
8	SF, chitosan, HA	Diabetic wounds	+	Biocompatible, enabled the controlled delivery of the drug loaded, reduced inflammation at the wound site, enhanced angiogenesis, accelerated the deposition of collagen, and effective cure for diabetic wounds.	118
9	SF, HA, ZnO	Burn wounds	+	Biocompatible, promoted better cell attachment, proliferation, and differentiation, facilitated the deposition of collagen, reduced the inflammation at the wound site, effective for burn wounds.	119
10	SF, SA	Full thickness wound	+	Re-epithelialization of tissues, granulation tissue development, facilitated the closure of wounds, nourished the wound by providing a moist environment, and enhanced angiogenesis.	120
11	SF, CMC, agarose (containing GO@PDA)	Full-thickness burn wounds	+	Enhanced deposition of collagen, development of granulation tissue, anti-inflammatory, stimulated neovascularization, enhanced healing of wounds.	121
12	SF, SA	Full thickness wounds	-	Stimulated protein synthesis in the ECM, induced the process called angiogenesis, quick re-epithelialization, reduction in inflammation, and facilitated wound closure.	122
13	SF, SA, probiotic	Infected wound healing	+	Stimulated angiogenesis, inhibited the bacterial colony, controlled the collagen ratio, and scarless healing of wounds.	123

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medicine "rhubarb charcoal", cross-linked, and fabricated via the freeze-drying method. The scaffold possesses a three-dimensional porous structure owing to the presence of SF as a main component. The well-known chitosan derivative, carboxymethyl chitosan, and the cross-linking agent rhubarb charcoal employed in this work showed excellent antibacterial activity and enhanced the process of wound healing. The scaffold was characterized using various techniques like wound healing scratch assay, hemostasis study, etc., which showed positive results, and it was evaluated for its antibacterial activity. The biocompatible scaffold prepared was found to exhibit good swelling properties and excellent antibacterial properties. The blend of SF and chitosan when cross-linked with rhubarb supported adhesion and proliferation of fibroblast cells upon conducting *in vivo* animal studies. Several key events that contribute toward faster healing of chronic wounds were promoted by the sponge scaffold fabricated. It is fascinating that the carbonized charcoal was released from the scaffold in a controlled manner and its effects include enhanced collagen deposition, faster re-epithelialization, and angiogenesis which have remarkable influence in the treatment of diabetic wounds.[110] The risk factor associated with burn wounds is the scar formation. SF-polysaccharide blends are found to guarantee the proper scarless wound healing. To target the requirements of a wound dressing for treating various types of wounds, the ratio of SF to polysaccharides can be varied, and the particular polysaccharide can be selected as per the requirements.

The blends of SF and some polysaccharides with their characteristic applications in the effective treatment of different types of wounds are summarized in Table 2.

The therapeutic value of SF-polysaccharide blends in wound healing applications is demonstrated by these studies. It is possible that more research is being done to improve formulations, evaluate efficacy in further studies, and eventually apply these discoveries to clinical practice.

8. FUTURE PERSPECTIVES AND CHALLENGES

Blends of SF-polysaccharides hold great promise for versatile applications soon, as there is growing interest in the development of biomaterials for different biomedical applications including wound healing. The blending of SF and polysaccharides can effectively result in the creation of biomaterials with enhanced and tailored properties desirable for improvement in the healing of wounds. Both SF and polysaccharides naturally decompose, minimizing their harmful effects on the environment, and the composition of the blends may be altered to provide wound therapy that is specific to a wound type. Blends of SF and polysaccharides hold great potential in the fabrication of next-generation wound dressings, providing a unique combination of features that can significantly treat a wide variety of wounds and facilitate wound healing, while extensive research in this fascinating area is still ongoing. Treatment approaches of different combinations of these blends in conjunction with some therapeutic agents are being explored by researchers in the hot field of tissue engineering. The need for some invasive treatment approaches in patients can be minimized through the effective use of these blends, as they have clinical potential in wound healing and tissue regeneration. As tissue engineering and regenerative medicine progress, we can expect even more tailored SF-polysaccharide blends with enhanced biocompatibility, bioavailability, biodegradability, stability, etc., which in turn further hastens wound healing. The problems associated with the delivery of certain therapeutic agents such as poor availability, low solubility, faster degradation rate, as well as less stability that limits their application can be addressed in the best way with the effective use of aforementioned blends as delivery systems.

However, the transformation of these blends to clinical applications is a bit challenging as they require several prerequisites, including lower manufacturing costs, easy fabrication, ease of handling, and storage. Even though biodegradability is a desirable quality for a wound dressing, as SF and polysaccharides are biodegradable, the pace of biodegradation needs to be regulated since it is vital to withstand the conditions at the wound site without hindering the regeneration of skin tissues. Further, to lower the possibility of cytotoxicity while treating serious injuries, extensive research is required to determine the long-term biocompatibility of SF and polysaccharide polymers to the surface of the skin. Despite favorable preclinical studies, more extensive studies and research in the form of organized clinical trials are required to ensure the safety, feasibility, and efficiency of these SF-polysaccharides in human beings. To ensure the biosafety of the blends, any reaction methods involved should be concise and the incorporation of less biocompatible therapeutic agents used in conjunction with these blends is undesirable. Moreover, SF-polysaccharide blends need to be specifically designed to address the complications of a sophisticated clinical system. The new combinations of SF and polysaccharides need to be explored for the effective incorporation of bioactive molecules for controlled delivery at the wound bed for enhanced wound healing and tissue regeneration. Further research is required to establish advanced fabrication techniques, surface modification of the polymers involved, easy functionalization, and optimization of the methods for the encapsulation of therapeutic agents for their controlled release at the wound site ensuring faster wound healing, all of which may support the easy transformation of the preclinical results to clinical trials.

9. CONCLUSION

The biopolymer blends of SF and polysaccharides are of potential interest in the field of skin tissue regeneration due to their favorable properties, (shown in Figure 10) such as biocompatibility, biodegradability, and tunable mechanical properties, and as discussed, this combinatorial approach has emerged as a significant strategy for the fabrication of wound dressings with tailored properties. The SF-polysaccharide blends that we already discussed have been shown to be beneficial in treating a variety of wounds, including chronic wounds associated with inflammation through their anti-inflammatory properties and they have also been shown to be able to keep the wounded region moist, which helps to prevent infections. In the case of diabetic wounds, where there is a risk of infection through bacterial adhesion, the antibiofilm activity of the blends was rather noticeable, and angiogenesis was also promoted in the wounds treated with SF-polysaccharide blends. These blends have also been shown to be able to reduce scar formation which is inherent in some types of wounds. In conclusion, Polysaccharides combined with SF constitute a pioneering approach to wound healing and these blends make a valuable contribution to the field of innovative biomaterial development and tissue regeneration. As there is an advancement in studies, these dressings have the potential

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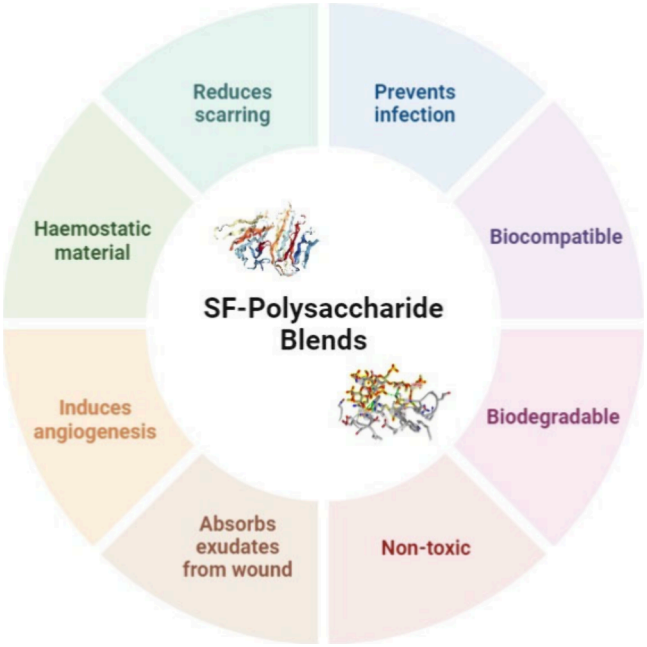

Figure 10. Various properties of SF-Polysaccharide blends.

to completely revolutionize wound care and greatly enhance patient satisfaction.

■ ASSOCIATED CONTENT

Data Availability Statement

No data was used for the research described in this article.

■ AUTHOR INFORMATION

Corresponding Author

D. Sangeetha − *Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore 632014 Tamil Nadu, India;* orcid.org/0000-0001-5834-9780; Email: dsangeetha@vit.ac.in

Author

Devipriya Vasudevan − *Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore 632014 Tamil Nadu, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c06518

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

SF:Silk Fibroin
ECM:Extra Cellular Matrix
HA:Hyaluronic acid
SA:Sodium Alginate
CMC:Carboxymethylcellulose
NF-κB:Nuclear factor-kappa B
ROS:Reactive oxygen species
Gly:Glycine
Ala:Alanine
Ser:Serine

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