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Natural Protein Films from Textile Wastes for Wound Healing and Wound Dressing Applications

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Abstract: In recent years, several studies have focused their attention on the preparation of sustainable biocompatible and biodegradable films of potential interest in wound healing and wound dressing applications. In this regard, natural macromolecules—such as proteins—represent an attractive alternative to synthetic polymers thanks to their numerous advantages, such as biocompatibility, biodegradability, and low immunogenicity. Among the most interesting proteins, keratin extracted from waste wool and fibroin extracted from *Bombyx mori* cocoons, possess all of the abovementioned features required for biomedical applications. In the present review, we therefore aim to give an overview of the keratin and fibroin based films designed for wound healing and wound dressing applications and devices.

Keywords: silk fibroin films; keratin films; wound healing; wound dressing

1. Introduction

Wound management is a complex field that requires continuous effort in scientific research to develop more effective therapies, especially in cases where conventional treatments are insufficient. Wound dressings have evolved from traditional materials (e.g., plasters, gauze, cotton, and bandages), which primarily protect the wound surface from infection and contamination and require manual intervention to remove, to more innovative and modern dressings that not only cover the wound but also accelerate the healing process. An ideal wound dressing should balance fluid absorption and release, effectively absorbing exudate while maintaining the necessary hydration for healing process and preventing the dressing from adhering to the lesion. The materials used for wound dressings should be biocompatible, biodegradable, and provide thermal insulation and mechanical stability. Moreover, they should allow gaseous exchange between the injured tissue and the environment, facilitating oxygen penetration. This is critical for cell proliferation, angiogenesis, protein synthesis, and for inhibiting the growth of anaerobic bacteria.[1–3]

A critical factor of wound healing is preventing the growth and colonization of harmful microorganisms, such as *E. coli*, *B. subtilis*, and *P. aeruginosa*, or fighting those already present. In healthy individuals, the immune system plays a key role in preventing infection by sending macrophages to the wound site, where they engulf pathogens through phagocytosis. However, if the immune system fails to eradicate these pathogens, infections can develop. Therefore, it is essential to create multifunctional wound dressings that support the body's natural healing processes and address issues such as severe inflammation, scarring, and wound infection. Consequently, the structure of the requested dressing should provide an efficient platform for drug delivery, controlling the release of active molecules. A variety of materials, both synthetic and natural, are available for wound healing dressings (e.g., sponges, hydrogels, hydrocolloids, films, membranes), and the appropriate selection of materials significantly influences the effectiveness of the dressing. The material choice should be guided by a thorough understanding of wound dressing properties and an assessment of the material's ability to adapt to the changing healing environment, taking into account factors such as wound depth, exudate volume, chronicity, and infection presence.

Tissue engineering primarily focuses on utilizing materials that can emulate and temporarily replace the native extracellular matrix (ECM), the most abundant component of the dermal layer. These materials must mimic the physicochemical properties of the ECM, providing inherent biological activity, cell adhesion, mechanical support, and a suitable structure for cell attachment, all without causing harm to the surrounding tissue.[4] Since the native extracellular matrix contains natural proteins and polysaccharides, biopolymers with similar characteristics—offering resistance and biological compatibility—have been proposed as promising biomaterials. These natural polymers can stimulate native tissue through specific interactions with cells, unlike synthetic polymers, which, despite their advantages in terms of reproducibility, are often biologically inert.[5,6] Hence, identifying biopolymers with wound healing capabilities and understanding their impact on fibroblast behavior represents a crucial opportunity to advance wound healing.

In this respect, recent research has focused on recovering protein fibers from textile industry waste, such as wool keratin (Ker) and silk fibroin (SF), as these materials offer valuable raw materials for developing innovative biomedical devices. These textile-derived biopolymers possess desirable properties, including strength, flexibility, biocompatibility, and biodegradability, making them ideal for supporting and promoting the wound healing process.[7,8] Reusing these materials has both economic and environmental benefits, as the technological processing of textile fibers generates large amounts of waste that can be reused, contributing to a greener and more sustainable society.[9]

SF is a natural protein which can be extracted from the fibers of silkworms *Bombyx mori* [10] and possesses several desirable features for biomedical use, including excellent structural properties, mechanical strength, controlled biodegradability, and non-inflammatory activity.[11–13] The FDA has approved its use for sutures, tissue regeneration, coating devices, and drug delivery systems.[14]

Ker, is the principal structural protein of wool, nails, hair, horns, and feathers.[15]

Its structure is rich in cysteine, which provides mechanical, thermal, and chemical stability, making keratin highly stable in water—a key advantage for biomedical applications. Additionally, its primary structure contains two motifs—"Arg-Gly-Asp" (RGD) and "Leu-Asp-Val" (LDV)—which facilitate cell adhesion by forming bonds with surface ligands on cells.[16]

Interestingly, both of these polymers are characterized by high molecular weights (45–60 kDa for keratin extracted from wool, and around 350 kDa for fibroin from *Bombyx mori* cocoons) that make these proteins particularly suitable for processing into several types of structural materials, such as sponges, electrospun fibres, nanoparticles and films.[17–20]

Among the various formats, films are particularly interesting as wound dressing materials due to the advantages they offer in terms of physical properties, including flexibility and adhesiveness, which enable easy conformation to hard-to-cover body areas.[21] More often these films are thin, transparent, and allow clinicians to monitor wound healing progress without removing the dressing. This minimizes trauma during dressing changes, reduces exposure to bacteria, and lowers the risk of infection.

This dressings category is frequently used in the medical sector for treating dry superficial wounds, minor burns, lacerations, and certain low-exudate ulcers, as they have a reduced capacity to absorb biological fluids due to their occlusive nature and their poor swelling properties. Hence, this limits their application for treating severe wounds with high exudates.[2,22] Films offer several advantages during different stages of wound healing. They provide a barrier to external contamination and to prevent blood loss and support the function of immune cells, cytokines, and growth factors. Moreover, films possess small pores that only allow the transmission of small molecules, such as oxygen, which are beneficial to prevent the invasion of microorganisms into the wound site and can be used for a direct drug delivery at the wound site, consequently exerting a therapeutic effect.[1] In this regard, fabricating thin adhesive films loaded with active ingredients and nano systems could enhance their efficacy.[23] This review aims to provide an overview of the preparation and application of keratin- and fibroin-based films, which are among the most commonly used scaffolds in medical applications for wound healing.

2. Methods for Films Preparation

The preparation methods used for polymeric films have a direct impact on the properties of the final product. These techniques are customized to suit the specific polymers and their intended applications. The most commonly employed preparation methods include solvent casting, salt leaching, spin coating and microfluidic spinning (Figure 1). Each of these offers its own unique set of advantages and disadvantages in terms of film properties, scalability, cost-effectiveness, and suitability for different polymer systems and biomedical applications (Table 1).
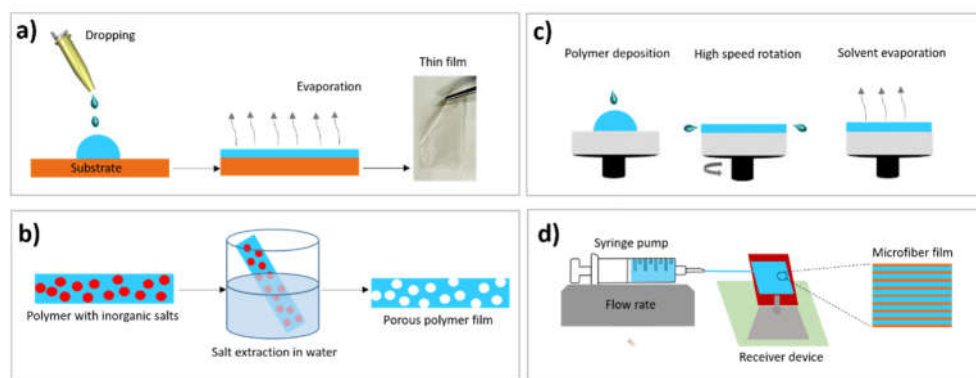

Figure 1. Schematic representation of the methods used for films preparation: a) solvent casting; b) salt leaching; c) spin coating; d) microfluidic spinning.

2.1. Solvent Casting

Among the various film manufacturing techniques, solvent casting is preferred for its cost-effectiveness, simplicity, practicality, and ability to generate robust films with suitable mechanical properties and homogeneity.[24]

This method involves the dissolution of biopolymers, in a suitable solvent and plasticizer (e.g. glycerin) if necessary, resulting in a suspension of different components with proper viscosity. The suspension is then cast onto a flat surface and dried without any external force except the temperature, allowing the solvent to evaporate and resulting in the formation of a polymeric film. After the drying process, the films are carefully removed and visually inspected for their integrity, color, and opacity and packed to keep their stability (Figure 1a).

The rheological properties of the polymeric mixture must be carefully monitored during the casting and drying steps, as they significantly impact critical parameters such as the drying rate, morphology, and content uniformity of the resulting film.[24] This process has some limitations as the prepared films may become brittle upon storage and the percent elongation may decrease due to the evaporation or loss of residual solvent within the film over time. Additionally, scaling up the production of films from a laboratory to a commercial level presents significant challenges. Achieving consistent film formation can be difficult, and variables such as heating, mixing speed, and environmental conditions may introduce inconsistencies in film quality across different production batches.[24] Furthermore, ensuring sterility throughout all manufacturing steps is also a challenge.[1]

2.2. Salt Leaching

The relatively smooth surface and low permeability of polymeric films can restrict their effectiveness in applications that would benefit from the increased permeability and improved nutrient transport provided by porous scaffolds, however, at the same time it is important to ensure that the porosity and pores do not compromise the mechanical properties of the films. To overcome this limitation, various methods have been used for the preparation of porous polymeric structures and porous films, such as for example the salt leaching technique.

This technique is based on the insolubility of inorganic salts in organic solvents that can dissolve biodegradable polymers. The salts are added to the polymer solution before casting, serving as

porogens. After casting, the polymer-salt composite is washed with deionized water to remove the salt crystals, and the resulting leached dressings are then dried to create porous wound dressings (Figure 1b). The porosity and pore sizes of the obtained porous structures can be varied by changing the salt-to-polymer ratios and the sizes of the leachable particles, respectively.[25] A large variety of inorganic salts, including potassium carbonate, sodium carbonate, sodium chloride, and ammonium bicarbonate, are commonly used as porogen agents.[26]

However, this method has some limitations and cannot be applied to water-soluble materials for scaffold formation, as these materials may be removed alongside the leaching of the salt.[27] Additionally, the process of incorporating porogens can be problematic, due to the lengthy processing time and the possibility of residual porogens that could affect certain applications, particularly in medical and food-related contexts. In this context, an alternative was found in a study conducted by Jian Liu J et al.[28] In this study, micro/nano-sized pores were introduced into SF films through the use of SF nanoparticles generated by autoclaving and collected through ultracentrifugation. Specifically, methanol treatment was used to induce a β-sheet structure in the film while the embedded particles were extracted by water.

2.3. Spin Coating

Spin coating is currently the leading technique used to produce uniform thin films of organic materials, with thicknesses in the micrometer and nanometer range. The typical process involves depositing a small amount of a homogeneous polymeric dispersion onto a rotating substrate, which is accelerated to the desired speed and the fluid is spread due to centrifugal force in uniform thin layers (Figure 1c). During the acceleration phase, the rotational motion causes a vigorous expulsion of fluid from the substrate, continuing until the fluid becomes thin enough to co-rotate with it.[29]

Once the final speed is reached and the substrate spins at a constant rate, the fluid gradually thins out, initiating the drying phase. The rotation enhances evaporation, increasing the polymer concentration and the viscosity of the fluid, resulting in highly uniform films with a smooth surface.

The spinning speed and the viscosity of the dispersion can be adjusted to control the final thickness, allowing for a versatile process, even though it may not be highly efficient.

In the study conducted by Kamol Wasapinyokul et al.,[30] SF films with different numbers of layers were fabricated by the spin-coating method. All samples exhibited high optical transmittance regardless of the number of layers and length of heating time.

2.4. Microfluidic Spinning

Among the various approaches, microfluidic spinning is a promising technology that enables precise manipulation of fluids within microscale channels. It is particularly suitable for volatile compounds that are sensitive to high voltage and temperature, operating based on the principles of microscale fluid dynamics.

This technique utilizes specially designed microfluidic devices with appropriate micro-channels. By injecting the solution at a specific flow rate, core and sheath flows create a coaxial flow, allowing the polymer dispersion to be solidified into microfibers using methods such as UV light exposure, ionic or chemical crosslinking, and solvent exchange (Figure 1d).[31] This method allows for the production of fibers in various shapes and sizes, which can be guided through a forward and reverse step process to create films. These films are then immobilized for a specific period at a controlled temperature to form the final product.

Table 2. Manufacturing methods for the preparation of films.

Methods	Advantages	Disadvantages
Solvent casting	Cost-effectiveness Feasibility Suitable mechanical properties Good homogeneity	Brittleness upon storage Difficult to scale up

Salt leaching	Easy and viable technique Tunable porosity and pore sizes	Not for water-soluble materials Not complete removal of salts
Spin coating	Thickness control via spin speed Thickness uniformity	Lack of material efficiency
Microfluidic spinning	Suitability for volatile compounds Reproducibility Capability of producing patterns	Costly technique Need to determine process parameters

3. Fibroin and Keratin Films for Wound Healing and Wound Dressing Applications

SF and Ker solutions exhibit excellent film-forming abilities under all-aqueous processing conditions, that have high water and oxygen permeability and are particularly appealing as wound dressings, skin or corneal replacement graft.[32] The water stability, biodegradability, mechanical resistance and cell response of the films can be controlled by modifying the fabrication conditions;[33] as well as adding plasticizer (such as dextrose and glycerol) and/or cross linking agents.[34]

The high demand for accelerated skin healing during medical treatment of skin wounds is not fully met by the therapeutic effects of a single polymeric-based material. Incorporating additional functions, such as promoting cell growth and antimicrobial properties, into the scaffold would significantly simplify the clinical process and shorten the time required to achieve better therapeutic outcomes for skin wound healing.

Based on a literature search, we have compiled a list of research articles that have explored the clinical potential of SF and Ker films loaded with different active compounds to produce or increase a specific effect on the wound. These articles have been classified into groups based on their therapeutic effects.

3.1. Films with Antimicrobial Function

Bacterial infection is one of the primary issues associated with impaired wound healing and, although SF and Ker based dressings have significant positive effects on wound repair and healing, they do not possess inherent antibacterial properties to address this problem, and could provide a good base layer for bacterial growth. Therefore, given fibroin and keratin versatility and ability to be combined with other substances,[35,36] incorporating antibacterial functions into biopolymer scaffolds is a promising strategy to improve their clinical use in treating open wounds and to decrease the risk of infections at the site. It is worth noting that, unlike SF, Ker already displays a mild antimicrobial effect, primarily due to the interaction between the positively charged amino groups in Ker and the negatively charged bacterial cell wall, which results in the inhibition of bacterial growth.[37,38] This is demonstrated, for example, in a study conducted by Sahil Goyal et al.[39] The addition of Ker extracted from wool conferred antibacterial properties against *S. aureus* and *K. pneumoniae* to polymer films containing alginate and pectin, which showed no clear growth inhibition zone in the absence of the keratin.

Over the past decade, researchers have investigated various approaches to create antimicrobial SF and Ker based biomaterials. In this contest, a practical method is to develop infection-resistant biomaterials by integrating specific antibiotics into the protein matrix. This can be achieved by effectively blending antibiotics into aqueous or organic protein solutions before casting, resulting in antibiotic-loaded protein films. Aparna Yerra et al.[40] evaluated the addition of 11 different antibiotics to a SF solution, which was then used to produce thin films tested against specific pathogenic strains. The resulting antibiotic-based SF films demonstrated antimicrobial effectiveness, successfully carrying and delivering the drugs to the attached microbial cells releasing them through passive diffusion. The localized drug delivery from biocomposite films offers a promising alternative to systemic antibiotic treatment, especially given the increasing concerns about microbial drug resistance that are important in clinical settings. In fact, this approach can provide a more targeted delivery, optimize pharmacokinetics, and reduce the required dosing frequency of drugs.

In response to the demand for effective alternatives, numerous studies have investigated the incorporation of various nanoparticles, microparticles, and natural compounds (including inorganic

substances, honey, plant extracts, and natural polymers) as potential antimicrobial agents in wound dressings to improve their antimicrobial properties.

So far, researches demonstrated that integrating small amounts of biocompatible nano-sized components such as metal and metal oxide nanoparticles, within SF scaffolds can not only promote various cellular and molecular mechanisms that support the wound environment, but also hinder bacterial adhesion and biofilm formation, thereby facilitating a more expeditious healing process.[4,41] 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 eliminate invading bacteria by generating reactive oxygen species (ROS), since high ROS levels can overwhelm the bacteria's antioxidant defence mechanisms, causing oxidative damage to essential cellular components such as enzymes, proteins, DNA, and lipids. Besides ROS generation, inorganic nanomaterials employ additional bactericidal mechanisms, including disrupting the cell wall, damaging chromosomes and DNA, and interfering with the metabolic activities of microorganisms.[41,42] Priyanka P. Patil et al.[43,44] developed nanocomposite films made of SF-polyvinyl alcohol embedded with ZnO NPs for use as a dressing material. *In vitro* evaluation showed that these films exhibited antimicrobial activity against both model Gram-positive and Gram-negative bacteria. In addition, the zone of inhibition (ZOI) increased with higher ZnO nanoparticle content in the composite films (Figure 2). Silver nanoparticles (AgNPs) are also a type nanoparticle that has attracted considerable attention due to their excellent electrical conductivity, remarkable chemical stability, and powerful catalytic and antibacterial properties. Smita Patil et al.[45] reported the development of antibacterial SF films containing *in situ* synthesized AgNPs with antimicrobial properties and activity against both sessile and planktonic *S. aureus* as well as against biofilm formation of antibiotic resistant *E.coli*. In addition, also AuNPs functionalized with 4,6-diamino-2-pyrimidinethiol (DAPT) were combined with fibroin to prepare films that demonstrated excellent antibacterial capabilities for wound healing applications.[46]
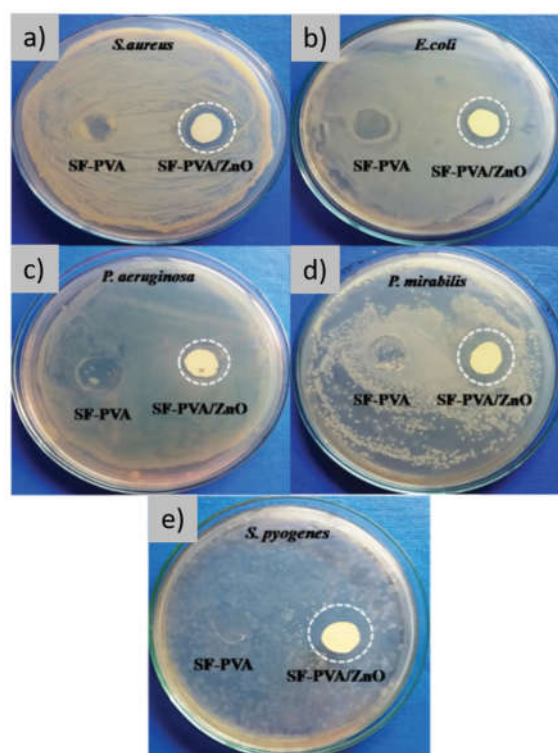

Figure 2. Antimicrobial activity of SF–PVA and SF–PVA/ZnO composite films against a) *S. aureus*, b) *E. coli*, c) *P. aeruginosa*, d) *P. mirabilis* and e) *S. pyogenes* microorganisms. Reprinted and adapted with permission from [43]. Copyright from Royal Society of Chemistry.

Particularly relevant is the use of a natural compound such as honey as an antimicrobial agent. Honey is a concentrated, viscous solution of floral sugars, proteins, enzymes, and amino acids derived from the bee crops. Nowadays, there is a more comprehensive scientific understanding of how honey's beneficial properties facilitate wound healing and regeneration. Specifically, the well-documented antibacterial property can vary across different types of honeys and it is influenced by various factors working either singularly or synergistically.[47] In most honeys it can be attributed to the action of the endogenous enzyme glucose oxidase from the bee's crop. This enzyme slowly breaks down glucose into gluconic acid, lowering the pH of honey and producing hydrogen peroxide, which sterilizes the wound and stimulates vascular endothelial growth factor production.[48–50] In the study conducted by Monika Rajput and colleagues[51] self-standing patterned and flat SF membranes containing honey were fabricated. It was observed that honey altered the physical properties of the membranes, specifically, its hydrophilic nature enhanced their wettability and water absorption capacity, resulting in increased swelling. This high degree of swelling indicates the membranes' potential to absorb nutrients from the surrounding media, which can nourish the cells and promote favourable cell adhesion and proliferation. Indeed, biocompatibility assay suggested that incorporation of honey into SF membranes increased the adherence, proliferation and viability of cells at higher honey concentrations and also enhances cell–cell and cell–matrix interaction. Furthermore, honey serves as a nutritional factor, as it is gradually released from the membranes into the surrounding environment, providing nourishment to the cells. Another research study conducted by N. Sukumar et al.[52] focused on combining SF with honey and with the epidermal growth factor (rhEGF) in order to promote the process of healing of diabetic wounds. The authors demonstrated once more that honey exhibits an extraordinary ability to promote the wound-healing process and great antimicrobial activity against *E. coli* and *Staphylococcus epidermidis* (S. epidermidis).

An alternative approach to achieve films with antibacterial properties is combining different antimicrobial natural polymers. For example, in the work of P. Ganesan et al.,[8] honey was combined with SF, wool Ker, and chitosan (CS) to produce, using a casting technique, curative films able of acting as drug-releasing agents on the wound surface and protecting the wound from secondary bacterial infection. Indeed, all the different dressings demonstrated good antimicrobial activity against both gram-positive and gram-negative bacteria such as S*taphylococcus aureus* and *Escherichia coli*. In the work of Meghann Rosewald et al.,[53] chitosan was used alongside cellulose to produce Ker composites. These materials have demonstrated the ability to maintain antibacterial and anti-inflammatory properties provided by the Ker and CS components. Furthermore, the composites have exhibited enhanced mechanical properties, including a significant increase in tensile strength, which allows them to be utilized in a broader range of practical and general applications. Additionally, Chieu D. Tran et al.[54] evaluated whether composites made of Ker, cellulose, and/or CS could encapsulate and control the release of a broad-spectrum antibiotic as ciprofloxacin (CPX). All three biopolymers were able of encapsulating the drug and subsequently releasing it at different rates, depending on the composition of the single, two, or three component systems. Particularly, it was found that the drug release rate can be controlled and adjusted at any desired rate by carefully selecting the appropriate concentration of Ker in the composite materials. This is due to keratin's structurally denser nature, in contrast with the more porous structure of cellulose and chitosan, which leads to a much faster drug release rate from those materials. By combining all three components into a composite, it is possible to integrate their respective properties and create a high-performance dressing that can effectively heal wounds, kill bacteria, and deliver drugs for the treatment of chronic ulcerous wounds in diabetic patients.

Polymeric scaffolds can be functionalized also by incorporating various phenolic compounds derived from diverse plant extracts, which have been reported to be used in the development of bactericidal protein based biomaterials.[55]

Additionally, antimicrobial peptides (AMPs), which are produced by bacteria, insects, plants, invertebrates, and vertebrates, are gaining attention as alternatives to traditional antibiotics. To decrease their cytotoxicity and improve their antimicrobial stability, AMPs are often immobilized on the surfaces of specific materials. SF and Ker are considered some of the most promising candidates

for surface functionalization due to their rich variety of active groups, such as carboxyl, hydroxyl, and amine. For example R. Si et al.,[56] developed a novel wound dressing by immobilizing the marine-derived antimicrobial peptide actinomycin X2 onto SF fibers and subsequently developing a versatile SF film (ASF) with intrinsic antibacterial and angiogenic properties. *In vivo* experiments on bacterially infected wound healing showed that the antimicrobial films (AMFs) prevented wound inflammation, facilitated repair, and enhanced the wound microenvironment, demonstrating that ASF film could be a promising candidate for skin wound healing (Figure 3).
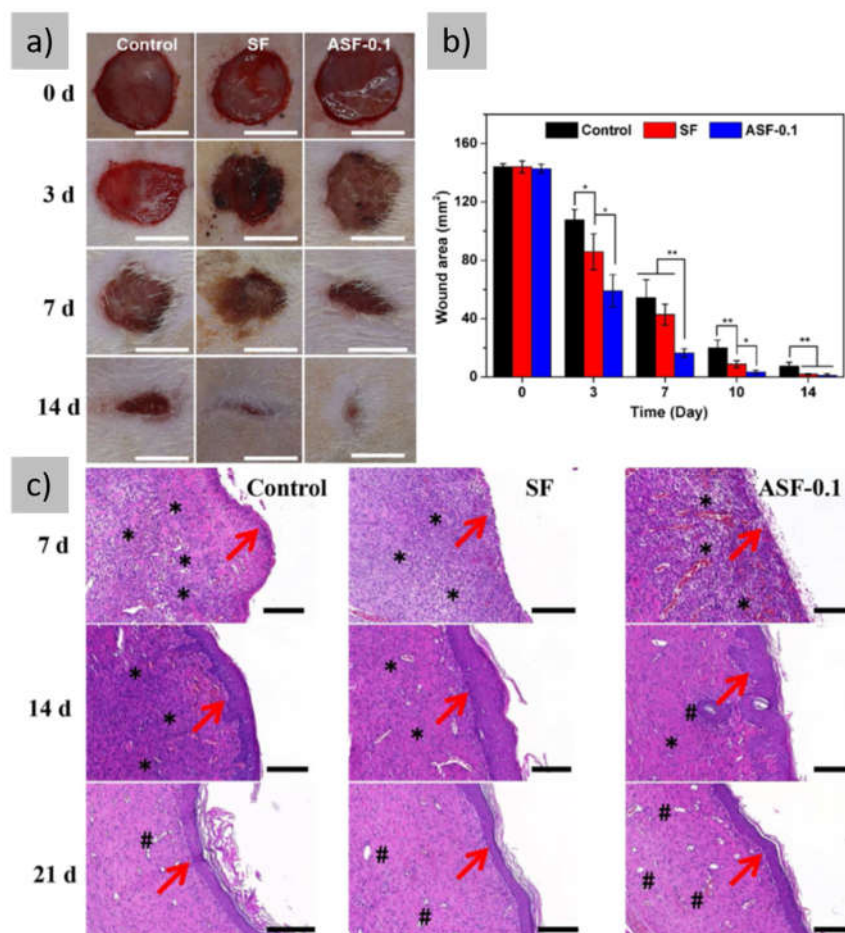

Figure 3. Wound healing ability of different films in a rat full-thickness wound model. a) Representative photographs of the wound healing process over 2 weeks (scale: 10 mm). b) Quantification of wound areas at days 0, 3, 7, 10, and 14 after operation. Note: $*p < 0.05$, $**p < 0.01$. c) Representative HE (hematoxylin and eosin)-stained photographs of the wound tissue regeneration process at 7, 14, and 21 days after surgery (scale: 200 mm). Note: /, new epithelium; *, inflammatory cells; #, hair follicle cells. Reprinted and adapted with permission from [56]. Copyright Elsevier Clearance Center 2024.

Another promising alternative to conventional antibiotic-based therapy is antimicrobial photodynamic therapy (APDT). This approach combines the use of visible light, photosensitizers, and oxygen to generate singlet oxygen and other reactive oxygen species, which can effectively kill bacteria. Aluigi et al.[57] selected methylene blue, a highly effective antimicrobial photosensitizer, to dope Ker films and create new biodegradable, biocompatible materials for tissue engineering and wound healing. It was demonstrated that these materials can exert antimicrobial photodynamic activity when exposed to visible light (Figure 4).
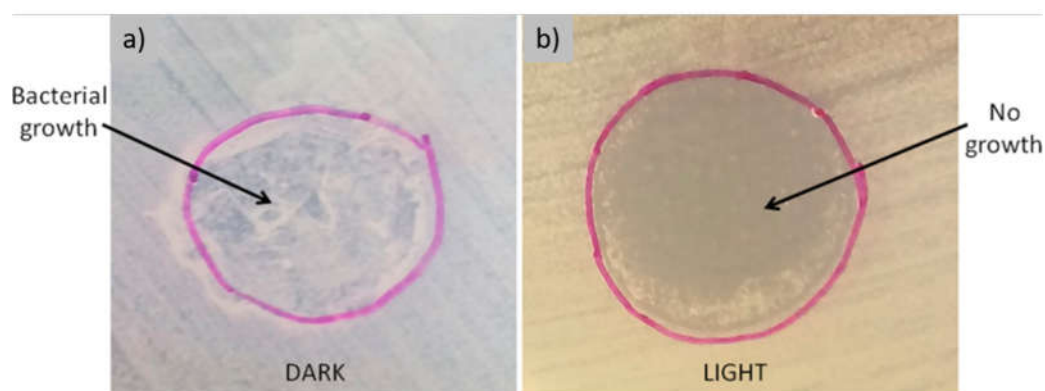
Figure 4. Antimicrobial "patch assay". KFMB400 film placed upon LB agar inoculated with *S. aureus* was incubated in the dark a) or irradiated b) for 75 min. After film removal bacteria growth 24 h at 37 °C. Reprinted and adapted with permission from[57]. Copyright Clearance Center American Chemical Society 2024.

3.2. Films with Antioxidant Function

Over the past few decades, by combining knowledge from biomaterials and skin tissue regeneration, researchers have developed intelligent wound dressings with antioxidant properties that regulate reactive oxygen species (ROS) and facilitate skin tissue recovery. These biomaterials have emerged as innovative solutions for medical applications, particularly in treating skin injuries. While physiological levels of ROS are beneficial for timely wound healing, excessive production can negatively impact the healing process. Under non-physiological conditions, the persistent overproduction of ROS, which is not adequately balanced by the body's antioxidant defenses, results in uncontrolled oxidative stress and has been linked to impaired healing in chronic, non-healing wounds.[58,59]

Antioxidant compounds can help prevent or inhibit oxidation by stabilizing, deactivating, or scavenging free radicals that can damage cells. Therefore, integrating antioxidants into polymeric biomaterials, which typically have limited radical-scavenging abilities, offers a promising strategy to inhibit molecular oxidation and restore normal physiological levels of reactive oxygen species. Furthermore, this approach aims to improve the efficiency of these antioxidants, which often suffer from low bioavailability and bioactivity when directly administered onto the wound.[60] These compounds are present in various plants and may accelerate wound healing, as demonstrated in a study conducted by Tingting Luo et al.[61] In this study, three natural antioxidants, vitamin C (VC), epigallocatechin gallate (EGCG), and curcumin, were physically incorporated into three types of silk films. The strong interaction between SF and the antioxidants not only preserved the structural integrity of the films but also stabilized the antioxidants, maintaining their activity for up to 14 days at 37 and 45 °C, compared to controls with free antioxidants in solution.

In this scenario, numerous studies have investigated the feasibility of developing various topical formulations loaded with curcumin, to overcome the poor bioavailability observed when this compound is directly applied to wound sites.[62] The wound healing potential of this natural polyphenolic molecule is primarily attributed to its antioxidant and anti-inflammatory properties. This is due to its ability to position itself within the cell membrane and act as a potent scavenger of various reactive oxygen species, including hydroxyl radicals and nitrogen dioxide radicals.[63,64] Moreover, curcumin exhibits strong antimicrobial properties,[65] both on its own and in synergy with antibiotics, thereby protecting wound tissue from bacterial infections. Curcumin also promotes cell proliferation, aiding in the repair and reconstruction of damaged tissue.[66] Thus, given the proven ability of this compound to effectively accelerate the wound healing process by targeting various stages of the natural wound healing cascade, developing a curcumin-based wound dressing represents a promising strategy to optimize the targeted delivery and therapeutic benefits of this compound for skin wounds.[62,67] In this contest, Zhang and co-workers[32] prepared sustained-release film by blending curcumin with SF using the solution casting method (Figure 5). The results

of this study indicate that the curcumin-loaded SF film is highly suitable for wound healing applications. It not only creates a moist environment for wound areas but also demonstrates effective sustained-release performance and impressive antibacterial activity against *S. aureus*, thereby protecting the wound from microbial invasion. Furthermore, in the study of Chunmei Li et al.[68] it is reported that curcumin, when physically bound to SF films, significantly enhanced the proliferation and differentiation of human mesenchymal stem cells when compared to free curcumin (in monomer or aggregate form) in cell culture medium.
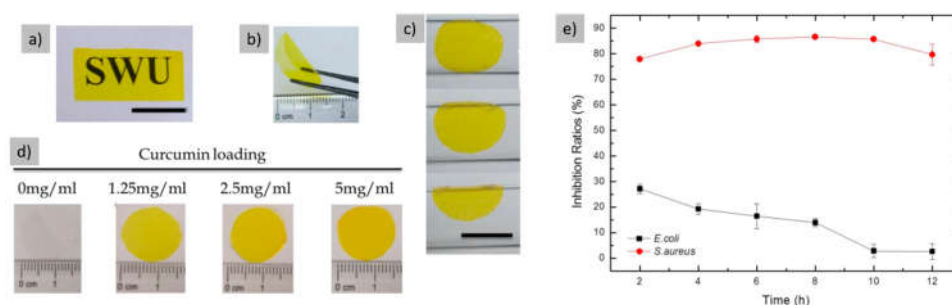

Figure 5. a) Transparency of curcumin-loaded SF film (scale bar 10 mm), b) free-standing curcumin-loaded SF film in the dry state, c) hydrated films follow surface contours (scale bar: 10 mm),   d) SF films can be loaded with a range of curcumin concentrations, e) Inhibition ratio kinetic curves of SF/Gly/GA/Cur composite film against *S. aureus* and *E. coli*. Reprinted and adapted with permission from [32].

Recently, our research[69] has demonstrated that bioactive and eco-sustainable films made by combining SF with antioxidant compounds extracted from pomegranate waste have a high capacity to reduce oxidative stress in cells. The transparent and flexible films have shown biocompatibility with the main skin cells (keratinocytes and fibroblasts) and were able to release bioactive compounds in a controlled manner, based on Fickian diffusion.

An important cause of ROS generation is the photochemical interaction of UVA radiation with biological materials, specifically with intracellular chromophores. To address this issue, Binbing Chen et al.[70] demonstrated that incorporating wool Ker into silk sericin films led to a microstructural transformation and a reinforcement of the molecular network, which conferred the films an improved UV absorption and excellent anti-UV light property. It must also be considered that the exposure to oxidizing agents like UVA or certain pathological conditions can severely disrupt intracellular iron homeostasis, which plays a key role in both oxidative stress and photo-induced skin damage.[71,72] In this regard, Anastasia Anceschi et al.[73] developed novel bioactive films capable of binding iron in chronic wounds. These films were created by casting an aqueous solution of wool-derived keratosis (the soluble fraction of keratin), and their stability and water insolubility were increased through a thermal cross-link treatment. The utility of these dressings stems not only from their iron-binding ability, but also from their capacity to swell and remove exudate from the wounds.

3.3. Films with Growth Factors

Growth factors are essential signaling molecules that play a key role in wound healing by enhancing wound closure, promoting the formation of granulation tissue, and facilitating the development of new blood vessels. Although the body naturally produces various growth factors, certain pathological conditions, like diabetes, which is marked by impaired microcirculation, hinder the effective delivery of these bioactive compounds needed for chronic wound healing. This results in the development of chronic non-healing wounds where re-epithelialization fails to occur. Consequently, it is generally believed that providing a continuous supply of growth factors to the localized wound area through a slow-release drug delivery system would be advantageous for chronic wound therapy and for the clinical applications of tissue engineering.[74] However, the delivery of these compounds is challenging, as many exhibit a relatively short half-life *in vivo*, presumably due to enzymatic degradation within the wound bed. For this reason, to maintain an

effective concentration of these growth factors, a formulation combining them with a suitable protein-based drug delivery system is recommended. In particular, SF has demonstrated excellent biocompatibility as a delivery platform, and functionalization of different SF forms with growth factors provides a synergistic approach to improve wound healing. This combination takes advantage of both the structural support provided by the SF matrices and their capability to extend the half-life of the growth factors complexed with them, as well as their biological activity. This approach can accelerate the healing process by promoting tissue regeneration and reducing healing time.

A study conducted by Eun Seok Gil et al., reports on the incorporation of epidermal growth factor and silver sulfadiazine, a widely used topical antimicrobial agent for preventing and treating wound infections, into silk biomaterials to assess their impact on wound healing.[75] Three types of silk biomaterials (porous films, electrospun mats, and non-porous silk films) were developed using two different drug functionalization techniques to assess the influence of silk material architecture and drug functionalization on wound healing. The study showed that silk biomaterials, likely due to their natural biocompatibility, ability to incorporate growth factors, controlled release properties, and potential to form porous structures, were able to improve the wound healing process. Additionally, Meng-Jin Lin et al. investigated the potential benefits of locally administering and sustaining the release of insulin-like growth factor-1 (IGF-1) from a SF film for patients with chronic, hard-to-heal wounds.[76] In fact IGF-1 is a well-known stimulator of keratinocyte growth and migration, which are crucial characteristics for the re-epithelialization of wounds. SF films demonstrated an excellent ability to deliver IGF-1, moreover, the cell growth-promoting activity of IGF-1 was maintained even after it is complexed with the SF films, which also significantly prolong its half-life (Figure 6).

In addition to the direct incorporation, research has recently moved toward other methods that provide significant supply of silk material containing growth factors, using genetic engineering technology, as investigated in the study of Sheng-Lan Wang et al.[77] Specifically, advancements in transgenic technology have enabled silkworms to express human growth factors (FGF, TGF-β1, PDGF, VEGF, and EGF) within their silk glands, allowing them to synthesize genetically engineered cocoons that can be processed into a range of functionalized silk-based biomaterials.

On the other hand, literature data suggest that keratin-based products have a stimulatory beneficial effect on their own, accelerating wound healing particularly in terms of re-epithelialization. This is achieved by enhancing keratinocytes migration and the production of basement membrane proteins, such as types IV and VII collagens.[78]
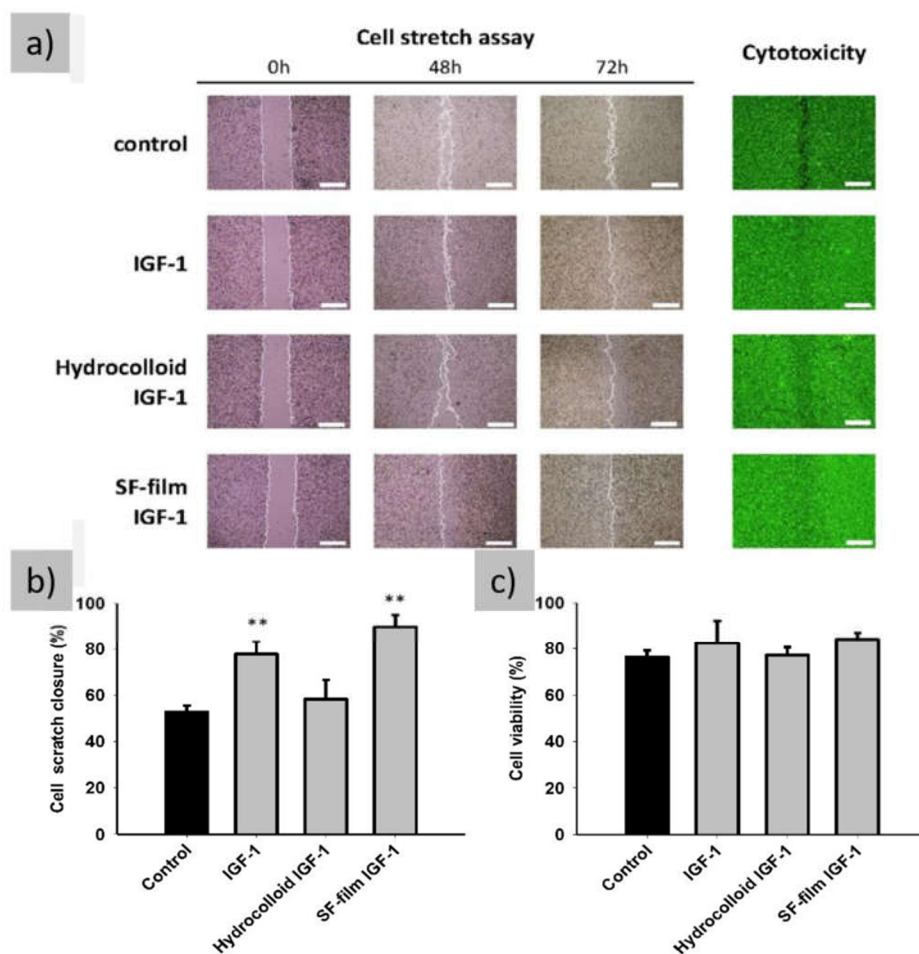
Figure 6. Effects of IGF-1, hydrocolloid IGF-1, and SF film IGF-1 on BALB/3T3 fibroblast scratch closure, cytotoxicity, and viability. a) Scratch closure and cytotoxicity of BALB/3T3 monolayer scratch closure in the presence of hyperglycemic medium at different times. Cells were stained with LIVE/DEAD stain and examined under a fluorescence microscope. b) Quantification of cell scratch closure after different treatment at 48 h in hyperglycemic medium. c) Cell viability in the presence after different treatment at 72 h. Significant differences between the control (black bar) and treatment groups were determined by Dunnett's multiple comparison post hoc test. ** $p < 0.01$; n = 3; mean ± SEM. (Scale bars = 500 µm). Reprinted and adapted with permission from [76].

3.4. Films with Anti-Inflammatory

While inflammatory responses by immune cells are essential for protecting against infections, excessive inflammation can be harmful. Macrophages, in particular, play a critical role in tissue injury and repair, significantly affecting the dynamics of wound healing. Their polarization into either an inflammatory (M1) or anti-inflammatory (M2) phenotype is crucial for pathogen clearance and promoting wound repair. However, chronic activation of either macrophage type has been linked to various diseases, highlighting the need for localized treatments to address chronic macrophage activation.

In this context, the design of wound dressings is particularly important. For example Doudou Hu et al. demonstrated that the physical properties of medical materials, especially their surface topography, play a crucial role in modulating intracellular signaling in macrophages.[79] In fact, SF films with varying and tunable surface roughness, produced by slow-drying casting and salting out techniques, were able to induce differential macrophage polarization (Figure 7). This established a direct connection between the specific surface morphology of the SF films and the macrophage response, subsequently influencing tissue regeneration. Mechanically, the rough surface of SF film enabled a positively-curved membrane of macrophages, promoting the internalization and

degradation of integrin αV and thus inhibiting the integrin-NF-κB signaling pathway. While SF film with low roughness activates the integrin-NF-κB signaling pathway. Another approach to control the polarization of macrophage and the biological response, optimizing regenerative needs while reducing inflammatory pathways, was shown in a study of Andrew R.D. Reeves et al.[80] The authors used silk protein to fabricate biopolymer films that released either IFN-γ or IL-4 to modulate macrophage polarization. By varying the duration of the films' exposure to water vapor, they were able to tune the solubility of the SF films and regulate their β-sheet content, enabling a short-term release of the respective cytokine. The released IFN-γ or IL-4 induced polarization of THP-1-derived macrophages into the M1 or M2 phenotypes, respectively.
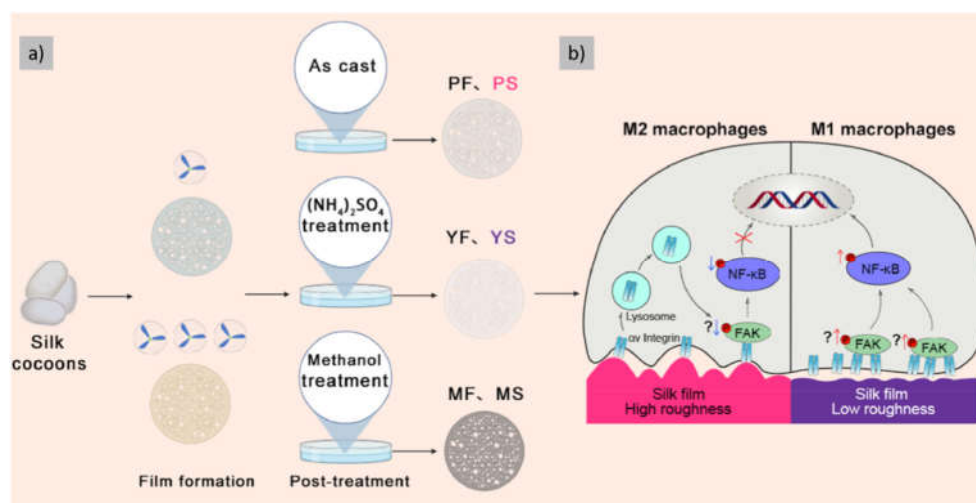

Figure 7. a) Preparation and screening of SF films with different surface roughness and b) the potential mechanism of roughness on macrophage polarization. Reprinted and adapted with permission from [79]. Copyright Elsevier Clearance Center 2024.

Furthermore, to modulate the inflammatory process, SF films can be designed as a delivery system for neurotensin (NT), a neuropeptide that acts as an inflammatory modulator in wound healing but is easily metabolized in biological environments.[81] Incorporating neurotensin into a SF film could be beneficial, as the film can protect the neuropeptide from degradation and sustain its release, prolonging its therapeutic effects. Notably, NT has been shown to effectively reduce the inflammatory status of wound and promote fibroblast migration to the wound site, ultimately facilitating the expression of extracellular matrix (ECM) proteins essential for skin repair.[82]The study by Camila Nunes Lemos et al.[83] developed SF films that successfully protected the peptide from rapid degradation, allowing it to have the desired effect. Additionally, the application of iontophoresis in the reported films promoted a rapid release of NT, ensuring its immediate availability for action and providing a high concentration of the drug in the wound environment at the beginning of the treatment.

Another approach to address this issue is to formulate drug-loaded films capable of managing inflammation and pain. For instance, our research [84] described the development of transparent and free standing organic-inorganic hybrid films for drug delivery purpose. Specifically, the researchers created a matrix of Ker extracted from wool filled with ZnAl hydrotalcites (HTlc) nanoparticles that had intercalated diclofenac, member of the non-steroidal anti-inflammatory (NSAID) group of drug (Figure 8). Previous studies have also shown that diclofenac-incorporated formulations have antimicrobial activity, to some extent, against various bacterial strains.[85–87] The composite films were characterized and evaluated *in vitro* as drug delivery systems, since the incorporation of the anionic drug into the lamellar structure of the hydrotalcites enabled its protection and controlled release from the polymer matrix.
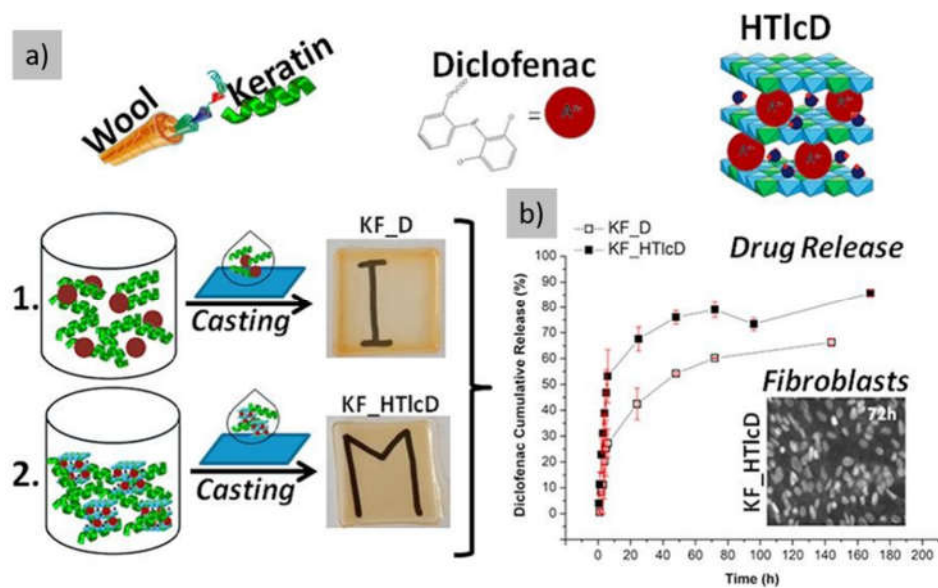
Figure 8. a) Preparation of Ker films loaded with HTlc and diclofenac (KF_D and KF_HTlcD respectively) and b) comparison of *in vitro* release profiles of KF_D and KF_HTlcD. Reprinted and adapted with permission from [84]. Copyright Elsevier Clearance Center 2024.

The work of Li Cui et al.[88] also involved the preparation of Ker films loaded with diclofenac. In order to maintain the mechanical strength and to improve the chemical stability of the materials, Ker was cross-linked with transglutaminase (TGase). In fact, this treatment led to the formation of new covalent bonds between the keratin proteins, resulting in increased tensile strength of the films, along with decreased elongation at break and reduced solubility in various solvents. Furthermore, the enzyme treatment resulted in a lower drug release rate from the films.

Films with antimicrobial function

	Mechanism of action	REF
Antibiotics	Antimicrobial activity	[40]
Metal and metal oxide nanoparticles /inorganic nanomaterials	Avoid bacterial adhesion and biofilm formation through ROS generation	[41–46,89–91]
Honey	Sterilization Foster endothelial growth	[47–52,92–95]
Chitosan	Antimicrobial and drug release agent	[8,53,54,96]
Phenolic compounds from plant extracts	Inhibit microbial proliferation	[55]
Antimicrobial peptides	Antibacterial Angiogenic	[56,97]
Photodynamic therapy	Antimicrobial through ROS generation	[57]

Films with antioxidant function

	Mechanism of action	REF
Vitamin C (VC), epigallocatechin gallate (EGCG), and curcumin	Preservative Stabilizing	[61]

Curcumin	Preservative Stabilizing ROS scavenging Enhancing cellular proliferation and differentiation	[22,62–68,98]
Pomegranate extract	Drug release agent Reducing oxidative stress in cells	[69]
Chromophore	UV absorption	[70]
Keratoses	Iron-binding ability	[73]

Growth factors

	Mechanism of action	REF
EGF	Increase keratinocyte proliferation and migration	[75]
IGF-1	Activation of the IGF1R pathway	[76,99,100]
Transgenic technology	Silkworm cocoons genetically engineered to produce human growth factors	[77]
Keratin-based products	Enhancing keratinocyte migration and collagen production	[78]

Films with anti-inflammatory function

	Mechanism of action	REF
Modulation of films' surface topography	Inducing differential macrophage polarization	[79]
Cytokines IFN-γ and IL-4	Modulating macrophage polarization	[80]
Neurotensin	Reducing the inflammatory status Promoting fibroblast migration	[82,83]
ZnAl HTIc-DIK	COX-1 and COX-2 inhibition Antimicrobial activity	[84–87]
DIK	COX-1 and COX-2 inhibition Antimicrobial activity	[88]

4. Conclusions

In this review, we focused on natural protein films as potential platforms for wound healing and wound dressing applications. Specifically, we examined two proteins, keratin and fibroin, extracted from textile wastes. Both fibroin and keratin offer exceptional properties, including mechanical

strength, biodegradability, biocompatibility, cell adhesion capabilities, and adaptability for processing into various formats, such as sponges, electrospun nanofibers, nanoparticles, and films. Furthermore, these natural proteins can be functionalized with a wide range of compounds with antimicrobial, antioxidant, and anti-inflammatory properties. Here, we first presented the main fabrication methods for SF and keratin films (e.g., solvent casting, spin coating, salt leaching, and microfluidic spinning) and then reviewed studies on their various functionalized films. In conclusion, the results obtained to date are very promising and support the potential use of natural biopolymers for developing innovative wound healing systems and devices.

References

1. Borbolla-Jiménez, F. V.; Peña-Corona, S.I.; Farah, S.J.; Jiménez-Valdés, M.T.; Pineda-Pérez, E.; Romero-Montero, A.; Del Prado-Audelo, M.L.; Bernal-Chávez, S.A.; Magaña, J.J.; Leyva-Gómez, G. Films for Wound Healing Fabricated Using a Solvent Casting Technique. *Pharmaceutics* 2023, *15*.

2. Sheokand, B.; Vats, M.; Kumar, A.; Srivastava, C.M.; Bahadur, I.; Pathak, S.R. Natural Polymers Used in the Dressing Materials for Wound Healing: Past, Present and Future. *Journal of Polymer Science* 2023, *61*, 1389–1414.

3. Çalamak, S.; Erdoğdu, C.; Özalp, M.; Ulubayram, K. Silk Fibroin Based Antibacterial Bionanotextiles as Wound Dressing Materials. *Materials Science and Engineering C* **2014**, *43*, 11–20, doi:10.1016/j.msec.2014.07.001.

4. Radulescu, D.M.; Andronescu, E.; Vasile, O.R.; Ficai, A.; Vasile, B.S. Silk Fibroin-Based Scaffolds for Wound Healing Applications with Metal Oxide Nanoparticles. *J Drug Deliv Sci Technol* 2024, *96*.

5. Pollini, M.; Paladini, F. Bioinspired Materials for Wound Healing Application: The Potential of Silk Fibroin. *Materials* 2020, *13*.

6. Lujerdean, C.; Baci, G.M.; Cucu, A.A.; Dezmirean, D.S. The Contribution of Silk Fibroin in Biomedical Engineering. *Insects* 2022, *13*.

7. Gupta, B.; Agarwal, R.; Alam, M.S. *Textile-Based Smart Wound Dressings*; 2010; Vol. 35;.

8. Ganesan, P. Natural and Bio Polymer Curative Films for Wound Dressing Medical Applications. *Wound Medicine* **2017**, *18*, 33–40, doi:10.1016/j.wndm.2017.07.002.

9. El-Sayed, H.; Taleb, M.A.; Mowafi, S. Potential Applications of Textile Wastes and By-Products in Preparation of Textile Auxiliaries. *Egypt J Chem* 2021, *64*, 4433–4447.

10. Rockwood, D.N.; Preda, R.C.; Yücel, T.; Wang, X.; Lovett, M.L.; Kaplan, D.L. Materials Fabrication from Bombyx Mori Silk Fibroin. *Nat Protoc* **2011**, *6*, 1612–1631, doi:10.1038/nprot.2011.379.

11. Numata, K.; Kaplan, D.L. Silk-Based Delivery Systems of Bioactive Molecules. *Adv Drug Deliv Rev* 2010, *62*, 1497–1508.

12. Sun, W.; Gregory, D.A.; Tomeh, M.A.; Zhao, X. Molecular Sciences Silk Fibroin as a Functional Biomaterial for Tissue Engineering. *J. Mol. Sci* **2021**, *22*, 1499, doi:10.3390/ijms2.

13. Nguyen, T.P.; Nguyen, Q.V.; Nguyen, V.H.; Le, T.H.; Huynh, V.Q.N.; Vo, D.V.N.; Trinh, Q.T.; Kim, S.Y.; Van Le, Q. Silk Fibroin-Based Biomaterials for Biomedical Applications: A Review. *Polymers (Basel)* 2019, *11*.

14. Vidya, M.; Rajagopal, S. Silk Fibroin: A Promising Tool for Wound Healing and Skin Regeneration. *Int J Polym Sci* 2021, *2021*.

15. Chilakamarry, C.R.; Mahmood, S.; Saffe, S.N.B.M.; Arifin, M.A. Bin; Gupta, A.; Sikkandar, M.Y.; Begum, S.S.; Narasaiah, B. Extraction and Application of Keratin from Natural Resources: A Review. *3 Biotech* 2021, *11*.

16. Xu, H.; Cai, S.; Xu, L.; Yang, Y. Water-Stable Three-Dimensional Ultrafine Fibrous Scaffolds from Keratin for Cartilage Tissue Engineering. *Langmuir* **2014**, *30*, 8461–8470, doi:10.1021/la500768b.

17. Giannelli, M.; Barbalinardo, M.; Riminucci, A.; Belvedere, K.; Boccalon, E.; Sotgiu, G.; Corticelli, F.; Ruani, G.; Zamboni, R.; Aluigi, A.; et al. Magnetic Keratin/Hydrotalcites Sponges as Potential Scaffolds for Tissue Regeneration. *Appl Clay Sci* **2021**, *207*, doi:10.1016/j.clay.2021.106090.

18. Posati, T.; Ferroni, C.; Aluigi, A.; Guerrini, A.; Rossi, F.; Tatini, F.; Ratto, F.; Marras, E.; Gariboldi, M.B.; Sagnella, A.; et al. Mild and Effective Polymerization of Dopamine on Keratin Films for Innovative Photoactivable and Biocompatible Coated Materials. *Macromol Mater Eng* **2018**, *303*, doi:10.1002/mame.201700653.

19. Aluigi, A.; Sotgiu, G.; Ferroni, C.; Duchi, S.; Lucarelli, E.; Martini, C.; Posati, T.; Guerrini, A.; Ballestri, M.; Corticelli, F.; et al. Chlorin E6 Keratin Nanoparticles for Photodynamic Anticancer Therapy. *RSC Adv* **2016**, *6*, 33910–33918, doi:10.1039/c6ra04208b.

20. dos Santos, F.V.; Siqueira, R.L.; de Morais Ramos, L.; Yoshioka, S.A.; Branciforti, M.C.; Correa, D.S. Silk Fibroin-Derived Electrospun Materials for Biomedical Applications: A Review. *Int J Biol Macromol* 2024, *254*.

21. Savencu, I.; Iurian, S.; Porfire, A.; Bogdan, C.; Tomuță, I. Review of Advances in Polymeric Wound Dressing Films. *React Funct Polym* 2021, *168*.

22. Alven, S.; Nqoro, X.; Aderibigbe, B.A. Polymer-Based Materials Loaded with Curcumin for Wound Healing Applications. *Polymers (Basel)* 2020, *12*, 1–25.

23. Hodge, J.G.; Zamierowski, D.S.; Robinson, J.L.; Mellott, A.J. Evaluating Polymeric Biomaterials to Improve next Generation Wound Dressing Design. *Biomater Res* 2022, *26*.

24. Karki, S.; Kim, H.; Na, S.J.; Shin, D.; Jo, K.; Lee, J. Thin Films as an Emerging Platform for Drug Delivery. *Asian J Pharm Sci* 2016, *11*, 559–574.

25. Hou, Q.; Grijpma, D.W.; Feijen, J. Porous Polymeric Structures for Tissue Engineering Prepared by a Coagulation, Compression Moulding and Salt Leaching Technique. *Biomaterials* **2003**, *24*, 1937–1947, doi:10.1016/S0142-9612(02)00562-8.

26. Draczynski, Z.; Kolesinska, B.; Latanska, I.; Sujka, W. Preparation Method of Porous Dressing Materials Based on Butyric-Acetic Chitin Co-Polyesters. *Materials* **2018**, *11*, doi:10.3390/ma11122359.

27. Aramwit, P.; Ratanavaraporn, J.; Ekgasit, S.; Tongsakul, D.; Bang, N. A Green Salt-Leaching Technique to Produce Sericin/PVA/Glycerin Scaffolds with Distinguished Characteristics for Wound-Dressing Applications. *J Biomed Mater Res B Appl Biomater* **2015**, *103*, 915–924, doi:10.1002/jbm.b.33264.

28. Liu, J.; Huang, R.; Li, G.; Kaplan, D.L.; Zheng, Z.; Wang, X. Generation of Nano-Pores in Silk Fibroin Films Using Silk Nanoparticles for Full-Thickness Wound Healing. *Biomacromolecules* **2021**, *22*, 546–556, doi:10.1021/acs.biomac.0c01411.

29. Sahu, N.; Panigrahi, S. Fundamental Understanding and Modeling of Spin Coating Process : A Review; 2009; Vol. 83;.

30. Wasapinyokul, K.; Kaewpirom, S.; Boonsang, S.; Chuwongin, S. Highly-Transparent Multi-Layered Spin-Coated Silk Fibroin Film.; SPIE-Intl Soc Optical Eng, October 24 2017; p. 31.

31. Cheng, J.; Jun, Y.; Qin, J.; Lee, S.H. Electrospinning versus Microfluidic Spinning of Functional Fibers for Biomedical Applications. *Biomaterials* 2017, *114*, 121–143.

32. Zhang, X.; Chen, Z.; Bao, H.; Liang, J.; Xu, S.; Cheng, G.; Zhu, Y. Fabrication and Characterization of Silk Fibroin/Curcumin Sustained-Release Film. *Materials* **2019**, *12*, doi:10.3390/ma12203340.

33. Sagnella, A.; Pistone, A.; Bonetti, S.; Donnadio, A.; Saracino, E.; Nocchetti, M.; Dionigi, C.; Ruani, G.; Muccini, M.; Posati, T.; et al. Effect of Different Fabrication Methods on the Chemo-Physical Properties of Silk Fibroin Films and on Their Interaction with Neural Cells. *RSC Adv* **2016**, *6*, 9304–9314, doi:10.1039/c5ra20684g.

34. Brown, J.E.; Davidowski, S.K.; Xu, D.; Cebe, P.; Onofrei, D.; Holland, G.P.; Kaplan, D.L. Thermal and Structural Properties of Silk Biomaterials Plasticized by Glycerol. *Biomacromolecules* **2016**, *17*, 3911–3921, doi:10.1021/acs.biomac.6b01260.

35. Belda Marín, C.; Egles, C.; Humblot, V.; Lalatonne, Y.; Motte, L.; Landoulsi, J.; Guénin, E. Gold, Silver, and Iron Oxide Nanoparticle Incorporation into Silk Hydrogels for Biomedical Applications: Elaboration, Structure, and Properties. *ACS Biomater Sci Eng* **2021**, *7*, 2358–2371, doi:10.1021/acsbiomaterials.1c00441.

36. Ghalei, S.; Handa, H. A Review on Antibacterial Silk Fibroin-Based Biomaterials: Current State and Prospects. *Mater Today Chem* **2022**, *23*.

37. Konop, M.; Rybka, M.; Drapała, A. Keratin Biomaterials in Skin Wound Healing, an Old Player in Modern Medicine: A Mini Review. *Pharmaceutics* **2021**, *13*.

38. Khajavi, R.; Rahimi, M.K.; Abbasipour, M.; Brendjchi, A.H. Antibacterial Nanofibrous Scaffolds with Lowered Cytotoxicity Using Keratin Extracted from Quail Feathers. *J Bioact Compat Polym* **2016**, *31*, 60–71, doi:10.1177/0883911515598793.

39. Goyal, S.; Dotter, M.; Diestelhorst, E.; Storck, J.L.; Ehrmann, A.; Mahltig, B. Extraction of Keratin from Wool and Its Use as Biopolymer in Film Formation and in Electrospinning for Composite Material Processing. *J Eng Fiber Fabr* **2022**, *17*, doi:10.1177/15589250221090499.

40. Yerra, A.; Mamatha, D.M. Antibiotic-Based Silk Fibroin Films for Burn Wound Healing. *Polym Adv Technol* **2021**, *32*, 861–871, doi:10.1002/pat.5137.

41. Kushwaha, A.; Goswami, L.; Kim, B.S. Nanomaterial-Based Therapy for Wound Healing. *Nanomaterials* **2022**, *12*.

42. Dizaj, S.M.; Lotfipour, F.; Barzegar-Jalali, M.; Zarrintan, M.H.; Adibkia, K. Antimicrobial Activity of the Metals and Metal Oxide Nanoparticles. *Materials Science and Engineering C* **2014**, *44*, 278–284.

43. Patil, P.P.; Meshram, J. V.; Bohara, R.A.; Nanaware, S.G.; Pawar, S.H. ZnO Nanoparticle-Embedded Silk Fibroin-Polyvinyl Alcohol Composite Film: A Potential Dressing Material for Infected Wounds. *New Journal of Chemistry* **2018**, *42*, 14620–14629, doi:10.1039/c8nj01675e.

44. Patil, P.P.; Bohara, R.A.; Meshram, J. V.; Nanaware, S.G.; Pawar, S.H. Hybrid Chitosan-ZnO Nanoparticles Coated with a Sonochemical Technique on Silk Fibroin-PVA Composite Film: A Synergistic Antibacterial Activity. *Int J Biol Macromol* **2019**, *122*, 1305–1312, doi:10.1016/j.ijbiomac.2018.09.090.

45. Patil, S.; Singh, N. Antibacterial Silk Fibroin Scaffolds with Green Synthesized Silver Nanoparticles for Osteoblast Proliferation and Human Mesenchymal Stem Cell Differentiation. *Colloids Surf B Biointerfaces* **2019**, *176*, 150–155, doi:10.1016/j.colsurfb.2018.12.067.

46. Zhu, G.; Sun, Z.; Hui, P.; Chen, W.; Jiang, X. Composite Film with Antibacterial Gold Nanoparticles and Silk Fibroin for Treating Multidrug-Resistant E. Coli-Infected Wounds. *ACS Biomater Sci Eng* **2021**, *7*, 1827–1835, doi:10.1021/acsbiomaterials.0c01271.

47. Almasaudi, S. The Antibacterial Activities of Honey. *Saudi J Biol Sci* **2021**, *28*, 2188–2196.

48. Bizerra, F.C.; Da Silva, P.I.; Hayashi, M.A.F. Exploring the Antibacterial Properties of Honey and Its Potential. *Front Microbiol* **2012**, *3*.

49. Minden-Birkenmaier, B.A.; Bowlin, G.L. Honey-Based Templates in Wound Healing and Tissue Engineering. *Bioengineering* **2018**, *5*.

50. Tashkandi, H. Honey in Wound Healing: An Updated Review. *Open Life Sci* **2021**, *16*, 1091–1100.

51. Rajput, M.; Bhandaru, N.; Barui, A.; Chaudhary, A.; Paul, R.R.; Mukherjee, R.; Chatterjee, J. Nano-Patterned Honey Incorporated Silk Fibroin Membranes for Improving Cellular Compatibility. *RSC Adv* **2014**, *4*, 44674–44688, doi:10.1039/c4ra05799f.

52. Nachiappan, S. Silk Based Scaffolds in Combination with Honey and RhEGF for Diabetic Wound Healing;

53. Rosewald, M.; Hou, F.Y.S.; Mututuvari, T. m; Harkins, A.; Tran, C. d (Invited) Cellulose-Chitosan-Keratin Composite Materials: Synthesis, Immunological and Antibacterial Properties. *ECS Trans* **2014**, *64*, 499–505, doi:10.1149/06404.0499ecst.

54. Tran, C.D.; Mututuvari, T.M. Cellulose, Chitosan, and Keratin Composite Materials. Controlled Drug Release. *Langmuir* **2015**, *31*, 1516–1526, doi:10.1021/la5034367.

55. Antibacterial Properties of Silk Fibroin-Chitosan Blend Films Loaded with Plant Extract.

56. Si, R.; Chen, W.; Chen, J.; Yang, Y.; Zhou, W.; Zhang, Q.; Chen, C.; Han, B. Green Chemistry Fabrication of Durable Antimicrobial Peptide-Immobilized Silk Fibroin Films for Accelerated Full-Thickness Wound Healing. *Mater Today Chem* **2023**, *29*, doi:10.1016/j.mtchem.2023.101468.

57. Aluigi, A.; Sotgiu, G.; Torreggiani, A.; Guerrini, A.; Orlandi, V.T.; Corticelli, F.; Varchi, G. Methylene Blue Doped Films of Wool Keratin with Antimicrobial Photodynamic Activity. *ACS Appl Mater Interfaces* **2015**, *7*, 17416–17424, doi:10.1021/acsami.5b04699.

58. Sanchez, M.C.; Lancel, S.; Boulanger, E.; Neviere, R. Targeting Oxidative Stress and Mitochondrial Dysfunction in the Treatment of Impaired Wound Healing: A Systematic Review. *Antioxidants* 2018, *7*.

59. Dunnill, C.; Patton, T.; Brennan, J.; Barrett, J.; Dryden, M.; Cooke, J.; Leaper, D.; Georgopoulos, N.T. Reactive Oxygen Species (ROS) and Wound Healing: The Functional Role of ROS and Emerging ROS-Modulating Technologies for Augmentation of the Healing Process. *Int Wound J* **2017**, *14*, 89–96, doi:10.1111/iwj.12557.

60. Fadilah, N.I.M.; Phang, S.J.; Kamaruzaman, N.; Salleh, A.; Zawani, M.; Sanyal, A.; Maarof, M.; Fauzi, M.B. Antioxidant Biomaterials in Cutaneous Wound Healing and Tissue Regeneration: A Critical Review. *Antioxidants* 2023, *12*.

61. Luo, T.; Yang, L.; Wu, J.; Zheng, Z.; Li, G.; Wang, X.; Kaplan, D.L. Stabilization of Natural Antioxidants by Silk Biomaterials. *ACS Appl Mater Interfaces* **2016**, *8*, 13573–13582, doi:10.1021/acsami.6b01636.

62. Mohanty, C.; Sahoo, S.K. Curcumin and Its Topical Formulations for Wound Healing Applications. *Drug Discov Today* 2017, *22*, 1582–1592.

63. Liang, G.; Yang, S.; Zhou, H.; Shao, L.; Huang, K.; Xiao, J.; Huang, Z.; Li, X. Synthesis, Crystal Structure and Anti-Inflammatory Properties of Curcumin Analogues. *Eur J Med Chem* **2009**, *44*, 915–919, doi:10.1016/j.ejmech.2008.01.031.

64. Ak, T.; Gülçin, I. Antioxidant and Radical Scavenging Properties of Curcumin. *Chem Biol Interact* **2008**, *174*, 27–37, doi:10.1016/j.cbi.2008.05.003.

65. Mun, S.H.; Joung, D.K.; Kim, Y.S.; Kang, O.H.; Kim, S.B.; Seo, Y.S.; Kim, Y.C.; Lee, D.S.; Shin, D.W.; Kweon, K.T.; et al. Synergistic Antibacterial Effect of Curcumin against Methicillin-Resistant Staphylococcus Aureus. *Phytomedicine* **2013**, *20*, 714–718, doi:10.1016/j.phymed.2013.02.006.

66. Joe, B.; Vijaykumar, M.; Lokesh, B.R. Biological Properties of Curcumin-Cellular and Molecular Mechanisms of Action. *Crit Rev Food Sci Nutr* 2004, *44*, 97–111.

67. Akbik, D.; Ghadiri, M.; Chrzanowski, W.; Rohanizadeh, R. Curcumin as a Wound Healing Agent. *Life Sci* 2014, *116*, 1–7.

68. Li, C.; Luo, T.; Zheng, Z.; Murphy, A.R.; Wang, X.; Kaplan, D.L. Curcumin-Functionalized Silk Materials for Enhancing Adipogenic Differentiation of Bone Marrow-Derived Human Mesenchymal Stem Cells. *Acta Biomater* **2015**, *11*, 222–232, doi:10.1016/j.actbio.2014.08.009.

69. Barbalinardo, M.; Giannelli, M.; Forcini, L.; Luppi, B.; Donnadio, A.; Navacchia, M.L.; Ruani, G.; Sotgiu, G.; Aluigi, A.; Zamboni, R.; et al. Eco-Sustainable Silk Fibroin/Pomegranate Peel Extract Film as an Innovative Green Material for Skin Repair. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms23126805.

70. Chen, B.; Xing, Y.; Yu, W.; Liu, H. Wool Keratin and Silk Sericin Composite Films Reinforced by Molecular Network Reconstruction. *J Mater Sci* **2018**, *53*, 5418–5428, doi:10.1007/s10853-017-1909-5.

71. Wright, J.A.; Richards, T.; Srai, S.K.S. The Role of Iron in the Skin and Cutaneous Wound Healing. *Front Pharmacol* 2014, *5 JUL*.

72. Aroun, A.; Zhong, J.L.; Tyrrell, R.M.; Pourzand, C. Iron, Oxidative Stress and the Example of Solar Ultraviolet A Radiation. *Photochemical and Photobiological Sciences* 2012, *11*, 118–134.

73. Anceschi, A.; Patrucco, A.; Bhavsar, P.; Zoccola, M.; Tessari, M.; Erbazzi, L.; Zamboni, P. Keratose Self-Cross-Linked Wound Dressing for Iron Sequestration in Chronic Wounds. *ACS Omega* **2023**, *8*, 30118–30128, doi:10.1021/acsomega.3c02525.

74. Zhang, Y.; Atala, A. Regenerative Medicine of the Bladder. In *Principles of Regenerative Medicine*; Elsevier, 2018; pp. 1263–1279 ISBN 9780128098806.

75. Gil, E.S.; Panilaitis, B.; Bellas, E.; Kaplan, D.L. Functionalized Silk Biomaterials for Wound Healing. *Adv Healthc Mater* **2013**, *2*, 206–217, doi:10.1002/adhm.201200192.

76. Lin, M.J.; Lu, M.C.; Chang, H.Y. Sustained Release of Insulin-like Growth Factor-1 from Bombyx Mori L. Silk Fibroin Delivery for Diabetic Wound Therapy. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22126267.

77. Wang, S.L.; Li, X.W.; Xu, W.; Yu, Q.Y.; Fang, S.M. Advances of Regenerated and Functionalized Silk Biomaterials and Application in Skin Wound Healing. *Int J Biol Macromol* 2024, *254*.

78. Tang, L.; Sierra, J.O.; Kelly, R.; Kirsner, R.S.; Li, J. Wool-Derived Keratin Stimulates Human Keratinocyte Migration and Types IV and VII Collagen Expression. *Exp Dermatol* 2012, *21*, 458–460.

79. Hu, D.; Li, T.; Bian, H.; Liu, H.; Wang, P.; Wang, Y.; Sun, J. Silk Films with Distinct Surface Topography Modulate Plasma Membrane Curvature to Polarize Macrophages. *Mater Today Bio* **2024**, *28*, doi:10.1016/j.mtbio.2024.101193.

80. Reeves, A.R.D.; Spiller, K.L.; Freytes, D.O.; Vunjak-Novakovic, G.; Kaplan, D.L. Controlled Release of Cytokines Using Silk-Biomaterials for Macrophage Polarization. *Biomaterials* **2015**, *73*, 272–283, doi:10.1016/j.biomaterials.2015.09.027.

81. Moura, L.I.F.; Dias, A.M.A.; Suesca, E.; Casadiegos, S.; Leal, E.C.; Fontanilla, M.R.; Carvalho, L.; de Sousa, H.C.; Carvalho, E. Neurotensin-Loaded Collagen Dressings Reduce Inflammation and Improve Wound Healing in Diabetic Mice. *Biochim Biophys Acta Mol Basis Dis* **2014**, *1842*, 32–43, doi:10.1016/j.bbadis.2013.10.009.

82. Da Silva, L.P.; Neves, B.M.; Moura, L.; Cruz, M.T.; Carvalho, E. Neurotensin Decreases the Proinflammatory Status of Human Skin Fibroblasts and Increases Epidermal Growth Factor Expression. *Int J Inflam* **2014**, *2014*, doi:10.1155/2014/248240.

83. Lemos, C.N.; Cubayachi, C.; Dias, K.; Mendonça, J.N.; Lopes, N.P.; Furtado, N.A.J.C.; Lopez, R.F.V. Iontophoresis-Stimulated Silk Fibroin Films as a Peptide Delivery System for Wound Healing. *European Journal of Pharmaceutics and Biopharmaceutics* **2018**, *128*, 147–155, doi:10.1016/j.ejpb.2018.04.019.

84. Posati, T.; Giuri, D.; Nocchetti, M.; Sagnella, A.; Gariboldi, M.; Ferroni, C.; Sotgiu, G.; Varchi, G.; Zamboni, R.; Aluigi, A. Keratin-Hydrotalcites Hybrid Films for Drug Delivery Applications. *Eur Polym J* **2018**, *105*, 177–185, doi:10.1016/j.eurpolymj.2018.05.030.

85. Goh, C.F.; Lane, M.E. Formulation of Diclofenac for Dermal Delivery. *Int J Pharm* **2014**, *473*, 607–616, doi:10.1016/j.ijpharm.2014.07.052.

86. Salem-Milani, A.; Balaei-Gajan, E.; Rahimi, S.; Moosavi, Z.; Abdollahi, A.; Zakeri-Milani, P.; Bolourian, M.; Salem Milani, A. *Antibacterial Effect of Diclofenac Sodium on Enterococcus Faecalis*; 2013; Vol. 10;.

87. Dutta, N.K.; Dastidar, S.G.; Asok Kumar, ; Kaushiki Mazumdar, ; Ray, R.; Chakrabarty, A.N. ANTIMYCOBACTERIAL ACTIVITY OF THE ANTIINFLAMMATORY AGENT DICLOFENAC SODIUM, AND ITS SYNERGISM WITH STREPTOMYCIN. *Brazilian Journal of Microbiology* **2004**, *35*, 316–323.

88. Cui, L.; Gong, J.; Fan, X.; Wang, P.; Wang, Q.; Qiu, Y. Transglutaminase-Modified Wool Keratin Film and Its Potential Application in Tissue Engineering. *Eng Life Sci* **2013**, *13*, 149–155, doi:10.1002/elsc.201100206.

89. Paladini, F.; Pollini, M. Antimicrobial Silver Nanoparticles for Wound Healing Application: Progress and Future Trends. *Materials* 2019, *12*.

90. Cadinoiu, A.N.; Rata, D.M.; Daraba, O.M.; Ichim, D.L.; Popescu, I.; Solcan, C.; Solcan, G. Silver Nanoparticles Biocomposite Films with Antimicrobial Activity: In Vitro and In Vivo Tests. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms231810671.

91. Panáček, A.; Kvítek, L.; Smékalová, M.; Večeřová, R.; Kolář, M.; Röderová, M.; Dyčka, F.; Šebela, M.; Prucek, R.; Tomanec, O.; et al. Bacterial Resistance to Silver Nanoparticles and How to Overcome It. *Nat Nanotechnol* **2018**, *13*, 65–71, doi:10.1038/s41565-017-0013-y.

92. Mandal, M.D.; Mandal, S. Honey: Its Medicinal Property and Antibacterial Activity. *Asian Pac J Trop Biomed* **2011**, *1*, 154–160, doi:10.1016/S2221-1691(11)60016-6.

93. Scepankova, H.; Combarros-Fuertes, P.; Fresno, J.M.; Tornadijo, M.E.; Dias, M.S.; Pinto, C.A.; Saraiva, J.A.; Estevinho, L.M. Role of Honey in Advanced Wound Care. *Molecules* 2021, *26*.

94. Ranzato, E.; Martinotti, S.; Burlando, B. Epithelial Mesenchymal Transition Traits in Honey-Driven Keratinocyte Wound Healing: Comparison among Different Honeys. *Wound Repair and Regeneration* **2012**, *20*, 778–785, doi:10.1111/j.1524-475X.2012.00825.x.

95. Yang, X.; Fan, L.; Ma, L.; Wang, Y.; Lin, S.; Yu, F.; Pan, X.; Luo, G.; Zhang, D.; Wang, H. Green Electrospun Manuka Honey/Silk Fibroin Fibrous Matrices as Potential Wound Dressing. *Mater Des* **2017**, *119*, 76–84, doi:10.1016/j.matdes.2017.01.023.

96. Guang, S.; An, Y.; Ke, F.; Zhao, D.; Shen, Y.; Xu, H. Chitosan/Silk Fibroin Composite Scaffolds for Wound Dressing. *J Appl Polym Sci* **2015**, *132*, doi:10.1002/app.42503.

97. Zhou, W.; Xie, Z.; Si, R.; Chen, Z.; Javeed, A.; Li, J.; Wu, Y.; Han, B. Actinomycin-X2-Immobilized Silk Fibroin Film with Enhanced Antimicrobial and Wound Healing Activities. *Int J Mol Sci* **2023**, *24*, doi:10.3390/ijms24076269.

98. Mohanty, C.; Sahoo, S.K. Curcumin and Its Topical Formulations for Wound Healing Applications. *Drug Discov Today* 2017, *22*, 1582–1592.

99. Garoufalia, Z.; Papadopetraki, A.; Karatza, E.; Vardakostas, D.; Philippou, A.; Kouraklis, G.; Mantas, D. Insulin-like Growth Factor-I and Wound Healing, a Potential Answer to Non-Healing Wounds: A Systematic Review of the Literature and Future Perspectives. *Biomed Rep* **2021**, *15*, doi:10.3892/br.2021.1442.

100. Lin, M.J.; Lu, M.C.; Chan, Y.C.; Huang, Y.F.; Chang, H.Y. An Insulin-like Growth Factor-1 Conjugated Bombyx Mori Silk Fibroin Film for Diabeticwound Healing: Fabrication, Physicochemical Property Characterization, and Dosage Optimization in Vitro and in Vivo. *Pharmaceutics* **2021**, *13*, doi:10.3390/pharmaceutics13091459.