Silk Fibroin-Based Therapeutics

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Silk fibroin, the fibrous structural-protein component in silk, has emerged as a promising treatment for these impaired processes by promoting functional tissue regeneration. Silk fibroin's dynamic properties allow for customizable nanoarchitectures, which can be tailored for effectively treating several wound healing impairments. Different forms of silk fibroin include nanoparticles, biosensors, tissue scaffolds, wound dressings, and novel drug-delivery systems. Silk fibroin can be combined with other biomaterials, such as chitosan or microRNA-bound cerium oxide nanoparticles (CNP), to have a synergistic effect on improving impaired wound healing.

Keywords: silk fibroin ; nanosilk ; diabetes ; wound healing ; biomaterials ; polymers

1. Introduction

Skin wounds are a natural part of life; therefore, organisms are generally well prepared to repair the resulting damaged tissue. When a wound occurs under normal, healthy circumstances, an extraordinarily complex and delicate process is initiated to reverse the damage [1]. The wound repair process consists of four overlapping phases: initial hemostasis, the inflammatory phase, the proliferation phase, and the maturation phase [2]. Each phase employs numerous cells, cytokines, and growth factors to facilitate the structural repair and closure necessary to restore tissue to its undamaged state. Tissue regeneration can quickly become deranged if one or more of these components is dysregulated. Dysregulation can occur for many reasons; chronic conditions such as diabetes mellitus, cancer, malnutrition, and even sequelae of COVID-19 can each lead to the development of chronic wounds [3]. In 2014 alone, it was estimated that Medicare patients spent up to 96.8 billion USD on wound care [4]. It is estimated that more than 29 million people in the United States alone—nearly 1-in-10—have been diagnosed with diabetes mellitus, with rates continuing to rise [5]. Worldwide, it is estimated that 552 million people will have diabetes by 2030 [6]. The increasing prevalence of individuals with impaired wound healing and the substantial associated costs illustrate the need for cost-effective treatment options.

The ideal wound healing treatment is composed of highly biocompatible, bioactive materials that aid in wound closure, degrade or metabolize at an appropriate rate, protect the wound from microbial infection, can be removed without damaging the underlying tissue, and are easily attainable. However, traditional wound healing treatments, such as skin autografts, can be costly, invasive, inefficient, and even harmful to the wound [7]. There are currently no widely-used wound treatments that reach these ideal standards. Biomaterials such as collagen, elastin, and gelatin have all been studied as wound treatments due to their presence in nature. Ultimately, many of these biomaterials are hindered by processing limitations, structural degradation, and by their immunogenicity in wounds.

Silk fibroin (SF) has emerged as a dynamic biomaterial that meets the aforementioned criteria for an ideal wound treatment. SF is derived from cocoons of *Bombyx mori* silk worms and also from the webs spun by spiders, mites, and other insects [8][9]. Silk contains two proteins: SF and sericin. SF is a fibrous structural protein and is the component typically isolated for therapeutic applications. Sericin creates a gum-like structure of glycoproteins surrounding the unprocessed SF and can exhibit immunogenicity; therefore, it is usually removed [9]. In order to separate these two elements, sericin is removed via a degumming process, and the remaining SF is regenerated via electrospinning, a fairly simple and inexpensive process. SF's dynamic properties as a biomaterial can be applied to many wound treatment approaches, such as molecular scaffolds, topical applications, and novel therapeutic delivery systems [10]. This natural bioactive polymer is effective, readily available, and cost effective, making it an ideal candidate for widespread usage. While SF has been studied as a regenerative therapeutic for a plethora of tissues, including bone, cornea, nerve, and cartilage, this review focuses on the use of SF as it relates to cutaneous wound healing and tissue engineering.

2. Physicochemical Properties of Silk Fibroin

SF has been widely researched as a wound therapeutic due to its dynamic properties and biocompatibility. Both the physical and chemical properties of this natural biopolymer lend to the benefits of using SF as a wound treatment.

However, there are some disadvantages to SF, which are discussed as well.

Three components make up SF: a heavy chain, a light chain, and a glycoprotein, which are 350 kDa, 25 kDa, and 30 kDa, respectively [11]. The heavy chains consist of hydrophobic domains, while the light chains are hydrophilic. These two chains form both secondary structures commonly seen in SF: silk I and silk II [12]. Silk I forms α-helices, while silk II forms β-sheets. In particular, the β-sheets form hydrogen bonds and, alongside glycine and alanine bonds, lend the biomaterial its renowned mechanical strength. Genetic manipulation of SF can lead to tunable properties which affect how the biomaterial behaves [13]. These tunable properties include permeability, composition, and sequence.

Ultimately, the physicochemical properties of SF combine to create an ideal biomaterial for wound treatment. High water retention keeps the wound hydrated, while antimicrobial properties prevent infection [7]. Improved cytocompatibility and efficient carbon dioxide and oxygen gas exchange allow cells to more efficiently proliferate within the wound. Little to no immunogenicity keeps inflammation low, reducing the risk of adverse reactions [7]. Additionally, it can work synergistically when used in conjunction with other biomaterials such as chitosan or microRNA-conjugated cerium oxide nanoparticles (CNP) [14].

However, SF also has its disadvantages (**Table 1**). The cross-linking of the β-sheets that confer mechanical strength can be vulnerable to enzymatic degradation. Matrix metalloproteinases (MMPs) have been shown to degrade SF in solution. Proteinase K has high affinity for the β-sheet component of SF, and collagenase degrades the amorphous regions [15]. Given that chronic wounds typically over-express various proteases, it is important to consider degradability when using SF as a therapeutic [16]. Some research suggests that tightly conformed β-sheets have significant resistance to enzymatic degradation [17]. Additionally, SF hydrogels have been shown to have poor mechanical strength and can undergo swelling in the wound [11]. Mechanical strength is a central feature of SF as a wound treatment, and the loss of this greatly reduces effectivity. Fortunately, there are solutions to this. Combining SF hydrogels with various polymers can greatly improve SF-hydrogel properties [11][18].

Table 1. Advantages and disadvantages of SF.

SF Biomaterial	Advantages	Disadvantages	Applications	References
tissue scaffolds	**ECM mimic dynamic properties biomechanical strength**	**invasive treatment**	**tissue repair strengthens skin**	**[7][19][20]**
solutions	**topical application biomechanical strength**	**decreased solubility**	**drug delivery strengthens skin wound repair**	**[9][14]**
biosensors	**biomarker detection**	**minimal therapeutic value**	**wound monitoring**	**[21][22]**
nanoparticles	**customizable size short-term drug release**	**degrades over time unsuitable for long-term release**	**drug delivery**	**[23][24][25]**
hydrogels	**efficient drug delivery**	**swelling decreased mechanical strength**	**wound healing drug delivery**	**[11][26][27]**

3. SF Nanoparticles

Over the last decade, the effects of nanoparticles on dermal wounds have been extensively studied. Nanoparticles are 100 nanometers (nm) or smaller, and often have unusual and unique chemical and physical properties independent of their macro forms. In the wound environment, nanoparticles can aid the healing process by improving angiogenesis, decreasing inflammation, conferring antimicrobial effects, regulating gene expression, and altering the ECM [28]. Nanoparticles also enable targeted delivery of therapeutics, metals, exogenous RNA, and other organic and inorganic wound treatments; they are most often used in the form of polymeric nanoparticles, nanotubes, micelles, liposomes, nanometals, drug conjugates, and protein carriers.

Traditional medication-delivery systems, such as oral ingestion and intravenous or intramuscular injections, often limit the delivery of novel therapeutics. These systems are frequently limited by high degradation, low bioavailability, and poor targeting of diseased tissues [29]. Ideal drug delivery systems should be non-toxic, biocompatible, and allow for controlled dosing and targeted release. Nanoparticles have become a primary focus in the development of drug delivery systems. Various synthetic and natural nanoparticles have been used to successfully modulate the localization, timing, and uptake of growth factors, proteins, and drugs in tissues. While numerous synthetic biomaterials have shown success as slow-release drug delivery systems, they are not ideal for carrying all novel therapeutics due to instability and toxic synthesis

processes [30][31]. Organic solvents, surfactants, and cross-linking agents are often used to make synthetic polymers, which can lead to adverse reactions in vivo. Nanoparticles from natural polymers, like SF, stand out as ideal drug delivery systems because of their modifiable nanostructures, biocompatibility, and customizable degradation. However, natural polymers' variability in structure and drug release could potentially limit their applications in select cases [10].

The behavior of a given nanoparticle is determined by both its structural and chemical properties. Particle size is the most influential property as it determines targeting ability, cell uptake, and drug release. While specific rates vary by material, smaller nanoparticles have been shown to have higher cell uptake than their larger microparticle counterparts [32]. Larger particles are associated with slower drug release as the encapsulated drug is further from the nanoparticle's surface; conversely, smaller particles exhibit faster release as the drug is closer to the nanoparticle's surface [33]. Additionally, smaller particles diffuse through tissue more easily than larger particles, leading to wider drug distribution. Shape is also an important factor in how a nanoparticle behaves. One study demonstrated that spherical nanoparticles had much more efficient uptake than rod-shaped nanoparticles of the same material [34]. Chemical properties such as hydrophobicity and particle charge can determine the target cell's fate. Hydrophobic particles are more likely to attract phagocytes, leading to targeted cell death [35].

SF is presented here as a nanoparticle for controlled delivery of bioactive therapeutics as it is highly dynamic and can be manipulated for various biomedical applications through well-characterized, non-toxic processing methods [10][36][37][38][39]. The molecular and physical properties of SF allow for highly customizable particle size, ranging from about 10 nm to over 100 nm [24]. SF nanoparticles have also been made into different shapes as well as combined with other biomaterials, depending on their intended applications [25]. SF is an FDA-approved therapeutic biomaterial and has been widely used in sutures, wound dressings, and tissue scaffolds because of its biocompatibility and non-cytotoxicity [40]. It has been shown to efficiently deliver growth factors, proteins, and other novel therapeutics to wounds and other tissues.

The use of SF nanoparticles as a delivery system for novel therapeutics has grown in popularity due to the non-toxic processes used to prepare the drug delivery systems; SF nanoparticles can be prepared without organic solvents or other cytotoxic chemicals through a variety of processes including milling, electrospraying, freezing, and desolvation [41]. The nanoparticles can then be loaded with drugs and other therapeutics by the simple process of adsorption, which is enabled by SF's porosity [25][42][43][44]. For subsequent drug delivery, nanoparticles can be engineered for different release behaviors. Importantly, SF nanoparticles are small enough to easily penetrate tissues, thus increasing the efficiency of drug uptake [24]. After drug release, the high biocompatibility and low immunogenicity of SF nanoparticles leads to either natural degradation or passive clearance, without adverse effects [10].

Topical delivery of SF nanoparticles conjugated to therapeutic agents can effectively improve wound healing. Aerosolized SF nanoparticles were used to topically deliver *Avicennia marina* extract and neomycin into full-thickness rat wounds to successfully enhance healing [45]. *Avicennia marina* is a wooded plant, the extract of which has been found to have anti-inflammatory, antioxidant, and antimicrobial effects [46]. The extract has also been shown to stimulate the proliferation of fibroblasts and induce epithelization [45]. Neomycin is a common antibiotic used to prevent infection in a variety of tissues, including cutaneous wounds [47]. On the first day after treatment, SF nanoparticles released 49% and 68% of the neomycin and *Avicennia marina* extract, respectively. This was followed by a gradual release of the remaining treatments over the next 24 days. The initial release could be beneficial for immediately killing any bacteria in the wound bed and stimulating the proliferation of fibroblasts, while the subsequent prolonged release could continue to promote a healthy wound bed throughout the healing process. Wounds treated with SF-loaded nanoparticles healed within 15 days, while negative control wounds remained open in the same timeframe [45]. The success of topical SF nanoparticles as a drug delivery mechanism suggests that the nanoparticles could be used dynamically with various drug combinations to treat a number of common wound ailments.

Dual-drug delivery involves multiple drugs being delivered and released through one system. These systems aim to selectively deliver drugs to target tissues for a synergistic effect while also controlling their release [48][49]. Hydrogels are a popular wound treatment and drug delivery system because they can carry multiple therapeutics within their polymeric networks and can enact their release with the appropriate stimulus, such as heat or pH changes [41]. However, the variable network structure of the biomaterials used to synthesize hydrogels can lead to difficulty modulating drug release in certain situations [50]. SF nanoparticles provide a promising solution for this as they can be loaded with bioactive materials and cross-linked into the polymeric network of both synthetic and natural hydrogels for regulated release of novel therapeutics such as genes, proteins, and growth factors [51].

Bacterial infection is a leading cause of chronic wound development and can be life threatening if left untreated [3]. Drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) have become increasingly difficult to treat

with traditional therapeutics [52]. Chronic wounds are well documented to have reduced epidermal growth factor (EGF) expression, slowing tissue regeneration [53]; however, EGF therapeutics have historically been unsuccessful due to their instability in the harsh conditions of a wound bed. Novel SF-nanoparticle-based drug delivery has recently been shown to enhance healing in chronic wounds and kill pathogenic bacteria through the controlled local release of antibiotics and growth factors [54]. Alginate-SF nanoparticles loaded with vancomycin are cross-linked to poly(*N*-isopropylacrylamide) (PNIPAM) hydrogels containing EGF. Alginate-SF nanoparticles release vancomycin, a powerful antibiotic against MRSA, at a pH-controlled rate in the presence of the alkaline conditions of chronic wounds [55]. EGF is steadily released and stabilized by the PNIPAM hydrogel. After treatment, both EGF and vancomycin were released into the wound for the entire 20-day study duration. Importantly, the bioactivity of the therapeutic agents was maintained at 80%, demonstrating the stabilizing ability of the SF-based delivery system. Treated wounds showed improved wound healing at 21 days—91% closure in treated wounds compared with 42% closure in controls—higher growth factor expression, and reduced bacterial infection, which demonstrated the potential of SF nanoparticles as a means of novel drug delivery in wounds.

While a wide range of therapeutics has been delivered in vitro and to pathogenic tissue by SF nanoparticles, research into SF nanoparticles as a wound healing treatment is scarce. However, these therapeutic applications can still provide insight into the underlying release mechanisms and interactions of SF nanoparticle-based drug delivery and how they could be applied in cutaneous wounds. For example, an in vitro study examined the prospect of sustained growth-factor release by SF nanoparticles as a novel therapeutic [56]. Vascular endothelial growth factor (VEGF) conjugated to SF nanoparticles was successfully released into cells over a three-week period. The nanoparticles delivered stable VEGF at a controlled, rapid pace for the first five days before release slowed, suggesting this delivery system could be used to supplement growth-factor-deficient wounds before slowing release to maintain normal levels. However, the in vivo stability of protein therapeutics is much more difficult to achieve, implying that more research needs to be conducted.

Model drugs can also be used to form an understanding of how an SF nanoparticle interacts with different drugs and therapeutics. For example, a study was conducted on the controlled release of model drugs by SF-nanoparticle-SF-hydrogel delivery systems [57]. Fluorescent dyes were conjugated to SF nanoparticles, allowing the characterization of release behavior in the presence of mesenchymal stem cells. Three dyes were loaded onto SF nanoparticles: Rhodamine B (RhB) and Texas Red (TR), which are both hydrophilic, and fluorescein isothiocyanate (FITC), which is hydrophobic. The release behavior, encapsulation efficiencies, cumulative release, and conjugate structure were observed; hydrophobicity and size ultimately determined how each dye behaved as smaller RhB and TR followed a nearly identical pattern in contrast with the much larger FITC [57]. RhB and TR were quickly released from within the nanoparticles, while the hydrophobic interactions between FITC and SF were reported to slow proteolytic degradation and, consequently, FITC release. The difference in degradation rates alone suggests that the combination of the hydrophilic and hydrophobic biomaterials loaded onto SF nanoparticles can provide dual-drug delivery with independent release behaviors.

While the behavior of therapeutic nanoparticles is clearly multifactorial and sound conclusions cannot be made from in vitro therapeutic behavior, new research avenues could be elucidated. SF nanoparticles should be examined as a novel, in vivo drug-delivery system in wounds.

4. SF Hydrogels

Hydrogels are three-dimensional networks of cross-linked polymers [58]. They also require a high proportion of water. When applied to wounds, hydrogels can facilitate drug delivery through their networks of polymers. Hydrogels can also create an exudate for wounds to absorb, keeping them moist for proper wound healing conditions. While hydrogels have been made from many different types of polymers, natural polymers have the most appeal for treating chronic wounds due to their biocompatibility and low toxicity. Of the natural polymers that have been used, SF has emerged as a promising candidate due to its dynamic structure and controlled biodegradability.

SF hydrogels are easy to manufacture. The pH of the SF solution is simply lowered with an acidic solution, and gelation is induced. As a drug carrier, this can be limiting as a lower pH may not be suitable for all drugs [10]. Other methods, such as sonication, have been applied to the gelation of SF solutions [59]. These methods are more complex and less practical for the quick and widespread production of treatments; however, they do provide an alternative to pH-restricted drug delivery. Additionally, SF hydrogels do not possess the same mechanical strength of their SF counterparts [18]. For these reasons, SF hydrogels are somewhat limited in application. Nevertheless, they have still been used to significantly improve wound healing.

Due to the limitations of SF hydrogels, other biomaterials are often employed to help increase functionality. One such study combined tannic acid, chitosan, and SF into a hydrogel [60]. The addition of tannic acid increased mechanical

strength by up to five-fold by cross-linking into the existing SF polymeric network. Tannic acid also conferred antimicrobial properties to the hydrogel. When applied to full-thickness murine wounds, wound closure was achieved significantly faster than in controls. The authors speculated the addition of tannic acid may have improved free-radical-scavenging capacity.

Despite the apparent limitations of SF hydrogels, researchers have found innovative applications for the hydrogels in wound treatment. Another study found that SF hydrogels can be made to undergo gelation at the local treatment site [61]. The use of silk fibroin from both *B. mori* and *Antheraea assama* led to the self-assembly of β-sheets and subsequent cross-linking. Wound healing was improved when compared with collagen controls.

While SF hydrogels may not be the optimal wound treatment, they have still been shown to improve healing overall. Unfortunately, hydrogels are hindered by limitations in drug delivery and manufacturing. This suggests that more focus should be put into other areas of SF therapeutics as they relate to wound healing. However, it is not unreasonable to research drugs that are stable in a low-pH environment for use in SF hydrogels.

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