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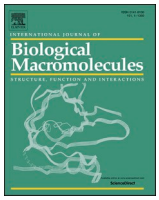

Review

Bioactive silk fibroin hydrogels: Unraveling the potential for biomedical engineering

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ABSTRACT

Silk fibroin (SF) has received special attention from the scientific community due to its noteworthy properties. Its unique chemical structure results in an uncommon combination of macroscopically useful properties, yielding a strong, fine and flexible material which, in addition, presents good biodegradability and better biocompatibility. Therefore, silk fibroin in various formats, appears as an ideal candidate for supporting biomedical applications. In this review, we will focus on the hydrogels obtained from silk fibroin or in combination with it, paying special attention to the synthesis procedures, characterization methodologies and biomedical applications. Tissue engineering and drug-delivery systems are, undoubtedly, the two main areas where silk fibroin hydrogels find their place.

1. Introduction

1.1. The intriguing world of silk fibroin hydrogels

Silk fibroin hydrogels, an area of burgeoning scientific interest, captivate researchers and innovators alike with their remarkable properties and diverse applications. These hydrogels, derived from silk fibroin, a protein abundant in silk produced by silkworms and spiders, are poised at the intersection of biology, materials science, and medicine [1,2]. Their unique blend of biocompatibility, mechanical strength, and water-absorption capacity make them a true marvel in the world of biomaterials [3].

Imagine a material that effortlessly mimics the intricate balance of strength and flexibility found in natural silk fibers, all while providing a hospitable environment for cell growth and tissue regeneration. Silk fibroin hydrogels achieve just that. Their biocompatible nature makes them ideal candidates for applications in regenerative medicine, tissue engineering, and drug delivery systems. These hydrogels not only support cell adhesion and proliferation but also promote controlled drug release, offering novel avenues for targeted therapies [4].

Beyond their biomedical applications, silk fibroin hydrogels find utility in a plethora of fields. These hydrogels have been harnessed for use in wound dressings, bioadhesives, and even wearable electronics [4–6]. Their ability to retain water and adapt to various shapes makes them suitable for creating responsive materials and sensors, further expanding their potential in cutting-edge technologies [7].

In this review, we embark on a multi-faceted exploration of silk fibroin hydrogels. We will journey through their synthesis methods, delve into the intricacies of their structural properties, and unravel the dynamic range of biomedical applications they enable. From regenerating damaged tissues to crafting next-generation biomaterials, silk fibroin hydrogels beckon us to unlock their full potential.

By the end of this review, you will not only grasp the captivating world of silk fibroin hydrogels but also appreciate the boundless opportunities they offer in revolutionizing the realms of biotechnology, materials science, and healthcare while contributing to a more sustainable and eco-friendly future [1].

1.2. Biodegradability of biopolymers and silk fibroin

In the preceding section, we explored the captivating world of silk fibroin hydrogels, highlighting their unique properties and applications. Now, let us delve deeper into the broader context of biopolymers and the pivotal role silk fibroin plays within this domain. Biopolymers, the building blocks of living organisms, stand as pivotal players in today's scientific and industrial arenas. These remarkable molecules, synthesized within the complex metabolic processes of organisms, have garnered immense importance due to their biodegradable nature and

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their crucial role in various biological functions [5,8].

Biodegradable biopolymers serve as a testament to nature's ingenuity. They are synthesized through enzymatic catalysis and polymerization reactions, embodying the essence of sustainability. Biopolymers are commonly characterized by their susceptibility to biological degradation (biodegradation) which, according to IUPAC, leads to a decrease in their molar mass and a transformation into environmentally acceptable substances with desirable properties [9].

Biopolymers are intrinsically linked with biodegradation, an extracellular process catalyzed by enzymes, resulting in the breakdown of these organic macromolecules [5]. The diverse range of biodegradation mechanisms hinges on the environmental conditions in which they operate. Ensuring that these biopolymers degrade at their intended usage sites is of paramount importance, as this enhances their environmental sustainability.

Biodegradation pathways encompass the actions of microorganisms and enzymes. Microorganisms such as fungi and bacteria possess the remarkable ability to biodegrade both natural and synthetic polymers. In parallel, enzymes orchestrate the biodegradation of biopolymers through physical and chemical processes, involving biological oxidations and hydrolysis [8].

Silk fibroin emerges as a prominent biopolymer within this fascinating realm. Derived from the silken threads of silkworms, silk fibroin represents a marvel of nature's engineering. It aligns seamlessly with the concept of biodegradability, as these silk fibers can undergo enzymatic degradation. Moreover, silk fibroin's unique properties make it a versatile material for various applications, from textiles to biomaterials. Its biocompatibility and biodegradability render it a key player in the pursuit of sustainable materials [1,10].

1.3. Silk: A fascinating protein

As we continue our journey into the realm of biopolymers, our spotlight turns to one of nature's most captivating creations: silk. Silk, a proteinaceous fiber produced by certain arthropods, has fascinated humanity for millennia with its remarkable properties and structural complexity [1,2]. Its unique blend of strength, flexibility, and fineness makes it a valuable natural resource.

The primary sources of silk production are silkworms, with the silkworm species *Bombyx mori* being the most prominent contributor [2,11]., The choice of silkworms as the preferred source of silk is no accident; it is rooted in their remarkable metamorphosis process and the exquisite properties of the silk they produce [11].

Silkworms, the larvae of the *Bombyx mori* moth, embark on a truly remarkable journey in their transformation from egg to cocoon. During this process, silkworms secrete a proteinaceous substance known as silk fibroin to form protective cocoons. These cocoons are composed primarily of silk fibroin, accounting for 75 % of the cocoon's structure, with the remaining 25 % being sericin, a protein that acts as an adhesive, maintaining the structural integrity of the fibers. Remarkably, sericin can be readily removed by subjecting the cocoons to an alkaline solution, a process known as degumming [8,10–12].

The structural complexity of silk is a testament to nature's artistry. Silk consists of two chains, a heavy (H) chain, approximately 390 KDa in size, and a light (L) chain, approximately 26 KDa. These chains maintain a 1:1 ratio and are linked by disulfide bonds [13]. Additionally, glycoprotein 25, also known as P25 (25 KDa), is non-covalently linked to the H-L complex, resulting in a 6:6:1 ratio for the H chain, L chain, and P25 [14].

The H chain, rich in glycine (43 %) and containing X-Gly sequences, where X can be alanine, serine, tyrosine, or valine, continuously repeats the sequence of Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ser, along with Gly-Ala/Ser/Tyr motifs, leading to the formation of twelve crystalline domains. The abundance of glycine in silk prompts the formation of stable antiparallel beta-sheets, a key factor contributing to silk's rigidity and tensile strength. These beta-sheets are held together by hydrogen bonds, imparting properties like self-healing and self-assembly to silk [11,15].

On the other hand, the L chain, more hydrophilic and elastic compared to the H chain, adds to the structural diversity of silk. Together, these structural intricacies result in two distinct silk structures: Silk I, a metastable crystalline zigzag structure, and Silk II, composed of antiparallel beta-sheets belonging to the monoclinic system. Silk II predominantly defines silk's properties of stiffness and strength, while Silk I can be converted to Silk II under specific conditions, expanding the range of silk's applications [16–18]. Likewise, although less common, it is possible to find a third silk structure, Silk III, which normally appears in regenerated SF solutions and at the water/air interface [11].

The unique properties and structural complexity of silk from silkworms make it an exceptional biopolymer of interest, not only for the textile industry but also for a myriad of applications in biomaterials and beyond.

1.4. Silk fibroin hydrogels: Bridging the world of biopolymers and hydrogels

As we continue our journey through the intricate realm of biopolymers and explore the remarkable silk protein, our focus now shifts to a convergence of nature's ingenuity and modern materials science: silk fibroin hydrogels. These hydrogels serve as a testament to the versatility and sustainable nature of biopolymers within the hydrogel matrix.

Hydrogels, as the name suggests, are three-dimensional structures composed of natural or synthetic polymers with the remarkable ability to retain water within their network [19]. These materials have gained recognition for their exceptional stability and modifiability, making them valuable in fields such as drug delivery, tissue engineering, biosensors, and self-healing materials. Two primary types of hydrogels exist: synthetic hydrogels, characterized by hydrophobic properties, strong chemical bonds, low degradation rates, and high mechanical strength, and natural hydrogels, known for their biocompatibility and biodegradability. Silk fibroin hydrogels, a subject of our focus in the subsequent sections, belong to the latter category [3,20].

In this article, we will delve deeper into the intriguing world of silk fibroin hydrogels, exploring their preparation methods, unique characteristics, and their pivotal role in diverse applications (see Fig. 1). As we embark on this journey, we invite you to unravel the fascinating possibilities that silk fibroin hydrogels offer, and their contribution to the development of sustainable and effective materials.

2. Synthesis of silk fibroin hydrogels

The synthesis of silk fibroin hydrogels is a fundamental process in creating these versatile materials. Various approaches are employed in achieving this synthesis, with polymer modification through crosslinking being one of the most common methods. Crosslinking can be carried out in several ways, including chemical, physical, and enzymatic approaches [3]:

- *Chemical Crosslinking*: This approach involves the inter or intramolecular bonding of two or more molecules through covalent bonds. The resulting chemical bonds are irreversible, meaning they are permanent. Crosslinkers, compounds designed for this purpose, are used to facilitate this union. Chemical crosslinking is employed, for example, in studying protein-protein complexes [21,22].

- *Physical Crosslinking*: In contrast, physical crosslinking entails the bonding of molecules through non-covalent interactions, such as hydrogen bonding, hydrophobic interactions, electrostatic forces, ionic interactions, and chain entanglement. Unlike chemical crosslinking, physical crosslinking does not require crosslinking agents,

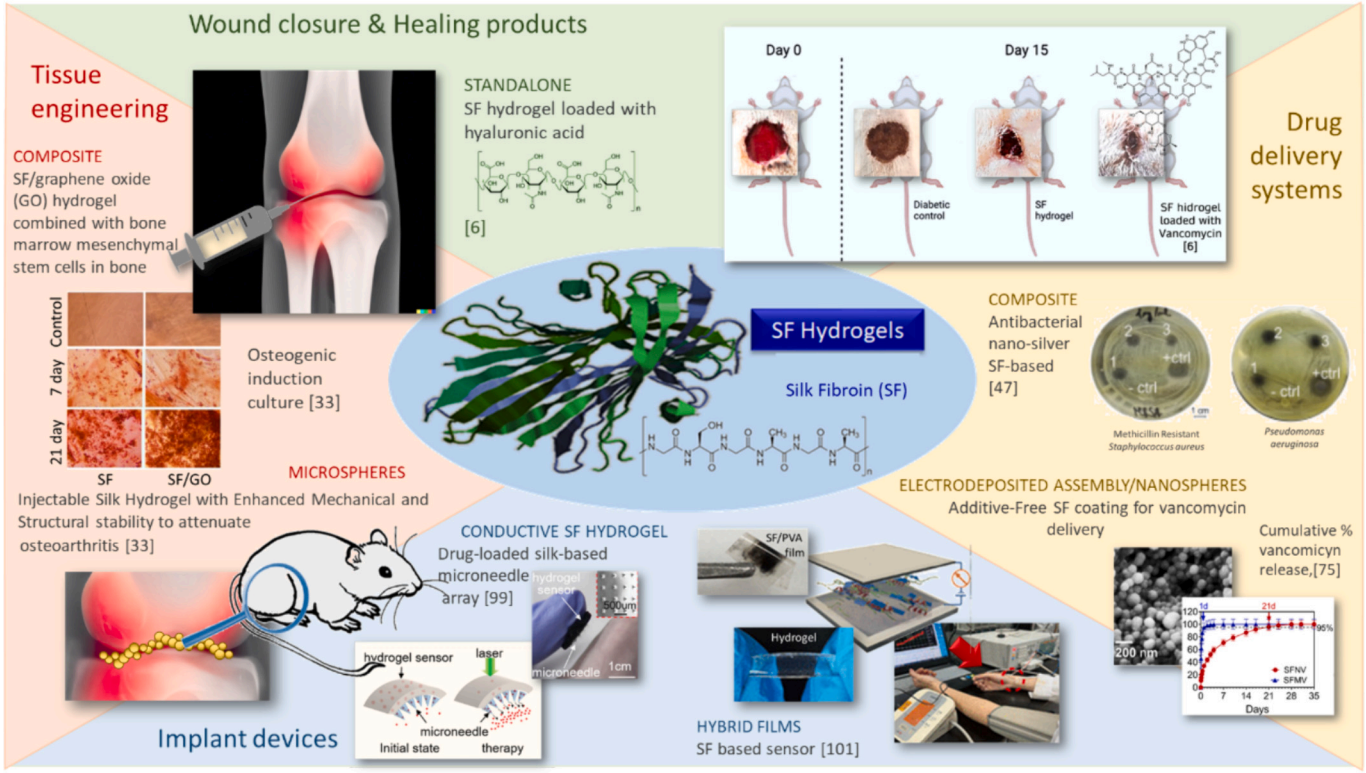

Fig. 1. Biomedical applications of silk fibroin hydrogels categorized into four key perspectives: drug delivery systems, wound closure & healing products, tissue engineering and implant devices. (Figure created adapting elements from figures included in articles [6,33,47,102,127 and 129]).

and the interactions are reversible. By adjusting physical conditions, it is possible to revert to the original state [19,21].

- *Enzymatic Crosslinking*: In this approach, the catalytic action of enzymes is harnessed to activate specific groups in the fibroin chain, promoting crosslinking. Commonly used enzymes include horseradish peroxidase (HRP) or mushroom tyrosinase, among others [20].

Although the physical crosslinking of SF is straightforward and reversible, either using fibroin alone or in combination with other macromolecules such as hyaluronic acid [23] (Fig. 2a) or xanthan gum [24], and presents as a suitable scaffold for the loading and release of bioactive agents, it often results in hydrogels with limited mechanical properties. Chemical crosslinking yields more stable hydrogels and allows for the regulation of properties such as porosity and strength. In general, SF hydrogels obtained through chemical crosslinking provide robust structures effective for tissue regeneration, improving joint injury treatment, or acting as biolubricants [25]. For example, hyaluronic acid has been used for chemical crosslinking through the lysine residues of SF

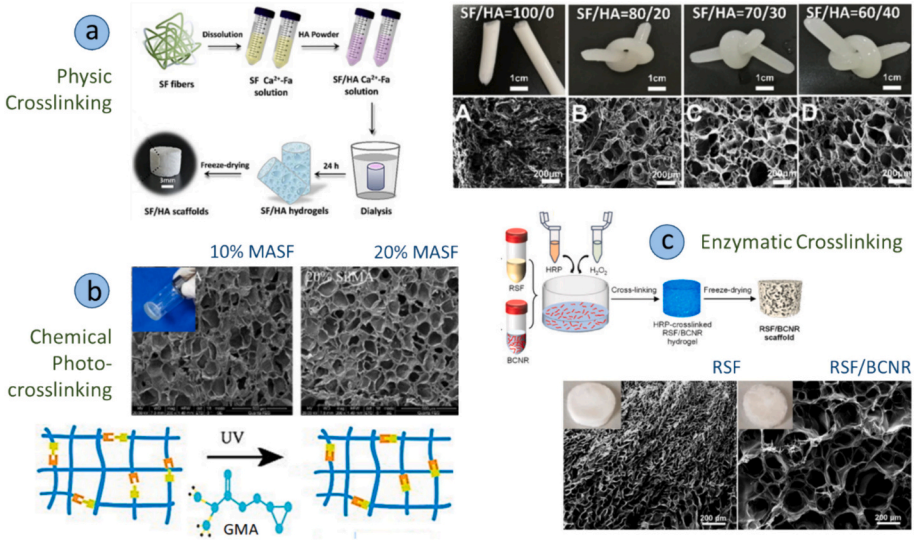

Fig. 2. SEM images and synthesis scheme of c) SF/Hyaluronic acid hydrogels derived from $CaCl_2$-formic acid (Ca^{2+}-Fa) system. (adapted from ref. [23]), b) hydrogel of glycidyl methacrylate (GMA)-modified silk fibroin (MASF) obtained by photo-crosslinking (adapted from ref. [29]) and c) scaffold of regenerated silk (RSF) with and without bacterial cellulose nanofiber ribbon (BCNR), created by freeze-drying after enzymatic crosslinking (adapted from ref. [35]).

by Schiff base formation [26], maleoyl functionalized chitosan through the reaction between the SF methacrylate groups and the maleoyl groups [27] and polyethylene glycol diacrylate [28] or methacrylated silk fibroin (Fig. 2b) have been employed to obtain hydrogels via photopolymerization [29–31]. These materials also prove effective for loading active ingredients that contribute to regenerative activity. However, it requires the use of crosslinking agents that may be cytotoxic. For this reason, especially in biomedical applications, careful consideration of the reagents used is crucial, and enzymatic crosslinking is often favored due to its safety, mild working conditions and the absence of the need for crosslinking agents [3,20]. Consequently, new porous scaffolds of silk fibroin [32], SF/graphene oxide [33], or SF/carboxymethyl chitosan [34] have been developed using horseradish peroxidase (HRP)-mediated crosslinking (Fig. 2c), exhibiting high biocompatibility.

Certainly, let's focus on discussing the various methodologies used in the synthesis of SF hydrogels. From recent studies conducted from 2018 to the present, a diversity of approaches in the synthesis of these hydrogels has been observed. One common method is vortex-induced gelation, as demonstrated in 2023 by Singh et al., who used a 1:1 ratio of SF solution and water to achieve drug release applications [6]. Another strategy involves combining SF with other biomaterials, such as the work by Gu et al. in 2022, who used regenerated silk fibroin (RSF) in combination with bacterial cellulose nanofibers (BCNR) for cartilage reconstruction and regeneration [35] (Fig. 2c). Enzymatic gelation techniques have also been employed, as seen in Dorishetty's 2021 work mixing SF with soy protein isolate (SPI) for bone tissue regeneration, highlighting the safety of this approach in biomedical applications [36]. The synthesis of SF hydrogels has been explored in different contexts, including the incorporation of nanoparticles, as demonstrated by Wu et al. in 2019 by combining SF with iron oxide nanocubes (IONCs) for cancer therapy applications [37]. These diverse methodologies illustrate the flexibility and adaptability of SF hydrogels in a wide range of biomedical and tissue engineering applications. In Table 1, a comprehensive and concise overview of the various methodologies used in the synthesis of SF hydrogels is provided.

3. General aspects of bioactivity of SF hydrogels

Hydrogels are considered bioactive due to its ability to interact with biological systems in ways that promote specific cellular and tissue responses are. The bioactivity of hydrogels appears as a consequence of different characteristics such as biocompatibility, cell adhesion and interaction or controlled release of bioactive molecules, among others [63]. Some of these characteristics can be improved with chemical modifications of the SF-hydrogel, whose multifunctionalization enhances the cell adhesion, long-lasting antimicrobial properties among others [64]. SF-hydrogels often include peptides that mimic the natural extracellular matrix, allowing cells to adhere and grow on the surface of the hydrogel. This is especially important in tissue engineering and regenerative medicine [65,66]. However, natural products are not the only materials used for improving bioactive properties of SF-hydrogels, but also graphene or polymeric materials. As an example, scaffolds made out of graphene oxide/SF show a better adhesion of mouse mesenchymal stem cell compared to the neat SF material [67], as well as methacrylate has been studied for creating a nanocomposite with SF-hydrogel for bone tissue engineering [68]. In the same line it is interesting to mention the work by Wang et al., who developed a bioactive composite hydrogel by integrating LAPONITE®, an osteo-inductive inorganic component, into methacrylated silk fibroin. The resulting hydrogel is intended for bone tissue engineering, offering improved mechanical strength and bioactivity to facilitate bone regeneration [69].

Bioactive hydrogels can be designed to respond to specific physiological conditions like pH, temperature, or the presence of a specific enzyme. This ability to respond allows them to adapt to the biological environment and to release their cargo or to change their properties in a controlled manner [70]. As an example, Pham et al. obtained SF-hydrogel nanoparticles functionalized with Eudragit® as a pH-responsive drug delivery system showing high versatility for biomedical applications [71].

Thus, the bioactivity of hydrogels can also be characterized by the bioactive components and the controlled removal of these components. Bioactive hydrogels can incorporate and release therapeutic agents, such as drugs or cytokines, in a controlled manner. This targeted release can enhance tissue regeneration and repair at the site of injury or disease [66,70].

Many bioactive hydrogels are also designed to be biodegradable, so they can decompose in the body over time. This degradation process can be coordinated with tissue regeneration, ensuring that the hydrogel provides the necessary support and then gradually dissipates as new tissue is created [72,73].

Mechanical properties are also important hydrogel characteristics which affect the bioactivity of these materials, as they have to fit with the host tissue. Elasticity and resistance can be tailored to those of the target tissue, thus ensuring that the hydrogel provides adequate mechanical support and integrates seamlessly with surrounding tissue [65,74].

Although numerous experimental results have demonstrated the bioactivity of silk fibroin hydrogels, the specific molecular mechanisms through which SF regulates these effects remain not fully understood, highlighting the need for further investigation into its molecular properties.

Some in vivo studies have provided additional insights into SF's molecular mechanisms. Enzymatic hydrolysates of SF have been shown to protect against memory and learning impairments by increasing levels of acetylcholine, brain-derived neurotrophic factor, and phosphorylated cAMP, response element-binding protein through the activation of p-PI3K/p-AKT/mTOR/PSD95, ERK, and CaMKII pathways in hippocampal tissue. These processes also reduced inflammatory cytokines IL-1β, TNF-α, and IL-6, suggesting SF's neuroprotective and anti-inflammatory roles [75]. SF has also been observed to enhance the expression of proteins involved in the NF-kB signaling pathway, crucial for wound healing and cellular functions such as proliferation and oxidative stress management [76,77]. Additionally, SF promotes the expression of type III collagen and MMP-12 in burn wound models and human fibroblasts, and positively regulates cell migration and differentiation pathways like MEK1, PI3K, and JNK in human keratinocyte and mammary gland cells [78,79]. Furthermore, SF scaffolds have shown superior performance in bone regeneration, increasing biomarkers of osteoblast differentiation compared to traditional ceramic scaffolds [80]. These studies underscore the complexity of SF's molecular interactions and the necessity for ongoing research to fully elucidate its role in wound healing and regenerative medicine.

4. Characterization of hydrogels

Characterization is a mandatory process after the synthesis and preparation of new hydrogels. Different tools can be used depending on the parameter which is intended to evaluate. Morphological characterization tools cover electron microscopy and X-Ray Diffraction, whereas chemical characterization used to resort to UV–Vis or FTIR spectroscopy as well as thermogravimetric assays. Furthermore, mechanical testing is also important for certain hydrogel applications.

4.1. Transmission electron microscopy (TEM)

In TEM, the electron beam is transmitted over a thin sample, so that it interacts with it as the electron beam moves through it. TEM provides us with structural information, as well as the composition and properties of the samples analyzed, being able to see down to sizes of less than a micron. However, although the images obtained have very good resolution, they are bidimensional. Sample preparation uses to be difficult,

Table 1
"Summary of silk fibroin hydrogel synthesis methods from recent studies (2018-2024)".

1st Author	Ref	Biomaterial used	Type	Synthesis used	Application
Singh V.	[6]	Silk fibroin (SF)	Raw hydrogel	Vortex-induced gelation. 1:1 SF solution:H_2O	Release of drug for diabetic wound healing.
Yan S.	[23]	SF and HA	Composite hydrogel	On the solution of SF it was added HA powder, both with different weight ratios. The final concentration of SF/HA is 10 %, and it was dialyzed, frozen at −80 °C and lyophilized, to obtain scaffolds	Tissue repair. And to be used as a biodevice.
Byram P.K.	[24]	SF and xanthan gum (XG)	Raw hydrogel	Heated XG was added to a solution of SF in different ratios, and then it was sonicated. After, it was incubated at −20 °C overnight and lyophilized. To have a sterile and strong hydrogel, it was treated with EtOH for several hours.	Tissue regeneration and repair.
Wang T.	[25]	SF and 1,4-butanediol diglycidyl ether (BDDE)	Microspheres and bulk hydrogels	Bulk SF/BDDE hydrogels: 10 % SF solution was mixed with NaOH, and it was added BBDE. After, it was incubated 90 min at 60 °C. SF/BDDE hydrogel microspheres were obtained by using an emulsion system.	To palliate the pain caused by osteoarthritis.
Giang Phan V.H.	[26]	Hyaluronic acid (HA) and SF	Raw hydrogel	Mixing 2–4%SF and 0.5 % HA at 50 °C 3 min. Then use the ultrasonic bath until gelation starts.	Cartilage regeneration.
Shao J.	[27]	SF and chitosan (CS)	Nanocomposite hydrogel	6 % CS and 0.1 % SF were mixed with an UV photo-initiator (0.05 %). It was put in a mold with the recombinant human transforming growth factor (TGF-β1) and irritated with U*V* light.	Regeneration of articular cartilage tissue.
Ciocci M.	[28]	SF	Raw hydrogel	SF and 10 % PEG were mixed with 0.1 % Irgacure™ 2959 to achieve the photopolymerization of the hydrogel. Then it was exposed under 365 nm (UV light) for 5 min.	Used as a stem cell-carrier system. Tissue repair.
Zhou L.	[29]	Methacrylate (MA) and SF	Raw hydrogel	From SF solution it was added GMA and stirred at 60 °C 300 rpm for 4 h.	Regeneration of spinal cord.
Jiang W.	[30]	SF and MA	Raw hydrogel, nano and microspheres	MA was added to SF solution and stirred at 300 rpm for 3 h at 60 °C. After it was dialyzed and lyophilized	Osteochondral tissue engineering.
Zhou Y.	[31]	SF and CS	Raw hydrogel	First, it was synthetized methacrylated SF (MSF) NPs and maleilated CS (MCS) NPs to obtain in a second step the hydrogels, by putting in contact both NPs with a photoinitiator for it to happen the photocrosslinking.	Cartilage repair.
Ribeiro V.P.	[32]	SF	Scaffold	SF solution was mixed with HRP and H_2O_2, and then it was placed in a mold and it was added NaCl. All of it was put in the incubator at 37 °C. To eliminate the NaCl undissolved it was filtered 3 days. Finally, it was frozen at −80 °C, and lyophilized.	Cartilage regeneration.
Wang L.	[33]	SF and graphene oxide (GO)	Composite hydrogel	GO and SF solutions were mixed using concentrations of 0.05/0.1/0.2 % and 0.4 %, respectively. To this is added HRP and H_2O_2.	Bone tissue engineering.
Li T.	[34]	SF and carboxymethyl chitosan (CMCS)	Composite hydrogel	SF/CMCS composite hydrogel was prepared in two steps. The first one consists of a chemical crosslinking with HRP and H_2O_2 and then, it was done a physical crosslinking with ethanol.	Scaffold cartilage.
Gu M.	[35]	Regenerated silk fibroin (RSF) combined with bacterial cellulose nanofiber (BCNR)	Scaffold and scaffold composite	RSF scaffold: horseradish peroxide (HRP): H_2O_2: RSF (6 %), 1:1:20. The mixture was 40 min at 37 °C, then frozen at −80 °C, and lyophilized. RSF/BCNR composite: HRP:H_2O_2:RSF/BCNR (4/5/6 % of RSF mixed with 2 % BCNR) 1:1:20. Preparation equal at the RSF scaffold.	Cartilage reconstruction and regeneration.
Dorishetty P.	[36]	Soy protein isolate (SPI) and SF	3D Hydrogels	SF and SPI were mixed with different weight ratios, and it was employed Ru and ammonium phosphate to mixed both materials.	Bone tissue regeneration.
Wu J.	[37]	SF, CS and bioactives glass nanoparticle (Cu-BG NPs)	Raw hydrogels	Cu-BG NPs was added to SF/CS solution, and cooled at 4 °C. After it was added 50 % of glycerophosphate (GP), to obtain Cu-BG/CS/SF/GP. To start the gelation it was put into an ice bath a few minutes and then in a water bath at 37 °C.	Bone repair and healing.
Narayana S.	[38]	Chitosan (CS) and SF	Raw hydrogel	Stirred CS:SF 1:0.6.	Wound healing and coagulation.
Yan K.	[39]	Hydroxypropyl cellulose methacrylate (HPCMA) and SF	Scaffold	Mixing rations of 5/10/15 % of SF and 5/10/15 % of HPCMA. The gelation was recorded.	Repaired articular cartilage defects.
Shen K.	[40]	SF	Raw hydrogel	SF solution (4 %) was sonicated 30s and kept at 37 °C until gelation.	Regeneration of the cartilage.
Kim D.K.	[41]	SF	3D SF scaffolds	SF solution was kept at −70 °C 24 h, and lyophilized, in order to obtain SF scaffolds. Those were crosslinked with methanol and after were washed with water.	Recovery and regeneration of bone defects.

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Table 1 (*continued*)

1st Author	Ref	Biomaterial used	Type	Synthesis used	Application
Li Q.	[42]	SF and gelatin	Hydrogel scaffold	It was used a printer to do the hydrogels. Different pressures and speed were used. When done that it was left in H_2O_2 30 min and then transferred to an MeOH solution and washed with water to obtain the final hydrogel.	Cartilage regeneration.
Dong Z.	[43]	SF and acidic calcium phosphate (ACP)	Composite hydrogel	Different ratios of ACP and SF were tested, 2/2, 3/2, 4/2, 5/2, and 6/2. The mixture was transferred into molds and incubated at 37 °C for 3 days.	Orthopedics such as void fillers, and drug vehicle.
Wang L.	[44]	SF and angiogenic peptides	Raw hydrogel	SV (0.7 %) and SF (1 %) solution were self-assembled by working at pH of 7.4 and by using Aluminum phosphide	Epidermal repair and vascular regeneration.
Ziadlou R.	[45]	SF and HA	Raw hydrogel	HA and SF were mixed until reaching 20 mg/mL of SF. The hydrogel was formed at 37 °C by adding HRP and H_2O_2.	Cartilage regeneration.
Qian K.Y.	[46]	SF	Raw hydrogel	SF solution was mixed in different ratios with Iron oxide nanocubes (IONCs) and then sonicated. After, it was placed in a syringe and incubated at 37 °C till formation of the hydrogel.	Cancer therapy.
Raho R.	[47]	RSF, silver nanoparticles (AgNPs) and carboxymethylcellulose-Na (CMC-Na)	Composite hydrogel	1.65 % RSF and 3 % CMC-Na were centrifuged at 1500 rpm for 5 min and sonicated other 15 min and melt in a PS boat. $AgNO_3$ was mixed in different ratios with RSF. After it was exposed under the UV radiation, then centrifuged, sonicated and melt in PS boat. Both were dried at 45 °C overnight and cured at 120 °C. Then it was washed several times with distilled water.	Wound healing and tissue repair.
Li D.	[48]	SF and RSF	3D scaffold	SF was turned into nonwoven fabric, and it was cut into circular columns. Then 4 % SF solution was put into in a mold that already has nonwoven fabric, and incubated at 4 °C, then at −80 °C overnight, lyophilized and crosslinked using EtOH.	Regeneration of tissues.
Han C.	[49]	SF	Raw hydrogel	4 % SF solution was sonicated several times and incubated at 37 °C for 1 h.	Preventing ageing-induced vascular dysfunction.
Roohaniesfahani I.	[50]	SF and MSM-10, which is a ceramic powder	Composite hydrogel	4 % SF solution was mixed with MSM-10 with different weight ratios. The mixture was sonicated to start the gelation.	Bone and tissue regeneration.
Amirikia M.	[51]	SF	Scaffolds	SF solutions with different concentrations from 4 to 8.4 % were cooled at 4 °C for 30 min and frozen at −80 °C overnight.	Bone repair and bone tissue regeneration.
Buitrago J.O.	[52]	SF and collagen	Raw hydrogel	SF solution and collagen solution were mixed using different weight ratios. Once mixed, it is placed on molds and incubated at 37 °C to complete gelation.	They can be employed as elastic tissue equivalents and can be applied for all types of tissue.
Li T.	[53]	SF and pullulan (PL)	Raw hydrogel	To get the SF/CMPL-TA hydrogel, the CMPL-TA (Carboxymethyl PL- Tyramine) must first be obtained, and the latter is mixed with the SF solution, HRP and H_2O_2.	It can be used as a cell carrier and be employed in tissue engineering.
Yan S.	[54]	SF and HA	Composite Hydrogel	SF and HA were mixed at different percentages and to produce the hydrogel it was used as crosslinkers EDC and NHS, that were added to the mixture by stirring, and then it was put in the oven at different temperatures.	Tissue regeneration.
Eivazzadeh-Keihan R.	[55]	CS, SF, GO and Fe_3O_4	Nanobiocomposite scaffold hydrogel	To prepare CS hydrogel/SF/GO/Fe_3O_4 nanobiocomposite, CS hydrogel:SF (1:1) was mixed with GO to form CS hydrogel/SF/GO. Finally, it was added $FeCl_2$ and $FeCl_3$ and ammonia.	Hyperthermia
Chaala M.	[56]	SF and HA	Hydrogel	HA was dissolved in a 5 mg/mL SF solution with SF/HA mass ratios of 100/0, 80/20, 60/40, 50/50, and 40/60. The solutions were sonicated to form hydrogels, which were then lyophilized.	Biomedical
Wang Y.	[57]	SF	Hydrogel membrane	Samples were cross-linked with riboflavin, HRP for 30 min. The solution was submerged in ethanol (50 %, 75 %, 90 %) for 6 or 24 h, washed, and incubated at 37 °C for 10 days to obtain pure SF hydrogel. Stored at 4 °C.	Bone regeneration
Ghorbani M.	[58]	SF and alginate	Scaffold hydrogel	Oxidized alginate was mixed with fibroin in various ratios and reacted for 3 h. Sodium alginate, CaCO3, and GDL were added to this mixture in specific ratios and concentrations. The solutions were poured onto a plate and crosslinked at room temperature.	Bone tissue engineering
Llang R.	[59]	SF	Scaffold hydrogel	A comprehensive approach to create SF-derived bone scaffolds with mechanical properties that mimic natural bone.	Bone regeneration

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Table 1 (*continued*)

1st Author	Ref	Biomaterial used	Type	Synthesis used	Application
Eivazzadeh-Keihan R.	[60]	Trgacanth gum (TG), SF and Fe_3O_4	Nanobiocomposite hydrogel	SF and TG hydrogel solutions were mixed, then combined with a Fe_3O_4 an N_2 atmosphere, and adjusted to pH 12. After stirring for 2 h at 70 °C, the TG hydrogel/SF/Fe_3O_4 nanobiocomposite was collected, washed, and dried at 60 °C.	Hyperthermia
Viola M.	[61]	SF and methacryloyl (MA) of SF	Hydrogel	SF was dissolved in LiBr at 60 °C, then GMA was added and stirred. SFMA was purified by dialysis, centrifuged, and freeze-dried. SFMA hydrogel was prepared in PBS with UV cross-linking.	Tissue engineering
Yao J.	[62]	CS methylacryloyl (CHMA) and SF methylacryloyl (SFMA)	Composite hydrogel	CHMA and SFMA were dissolved in water, mixed in specific ratios with 0.025 % LAP, and exposed to UV light to form hydrogels.	Hemostatic and wound repair

as the supporting grid easily bends and breaks. For a proper TEM analysis, hydrogels need to be stained with 2 % phosphotungstic acid for a certain time, which normally depends on the sample nature itself [32,81,82]. It enables the examination of microstructural disparities and, particularly in the field of silk fibroin and as illustrated in the article by Yan et al., the determination of unbound silk fibroin content. Consequently, it offers insights into the degree of amorphousness within the structure [82].

4.2. Scanning electron microscopy (SEM)

- In SEM, the electron beam is scanned over the entire sample causing electron emission or reflection from the most superficial layers of the sample. SEM gives us structural information, i.e. on the composition, structure and concentration of the sample, giving us 3D images, but it has a lower resolution than TEM. The preparation of the sample in SEM, in general requires mounting on a support, either a tape or conductive adhesive and, generally, a coating step with a conductive metal, for example gold, to obtain conductive samples.

In the case of hydrogel samples, it is common to employ freeze-dried samples, as this step facilitates the gold coating process. However, there are also some examples in the literature where these hydrogels can be characterized without a prior lyophilization step [81,83]. Furthermore, one of the most interesting features of SEM is its ability to characterize the size and morphology of the pores within the hydrogel, revealing the relationship between silk fibroin concentration and pore size, which plays a crucial role in drug encapsulation [83].

4.3. Fourier-transform infrared spectroscopy (FTIR)

The principles of FTIR spectroscopy are well-established, primarily involving the absorption of infrared radiation as a result of molecular vibrational mode changes. Consequently, FTIR furnishes valuable insights into the chemical bonds present within the molecular structure, yielding essential information regarding the sample's structural composition, bonding characteristics, and chemical properties under examination [84].

This ability of FTIR allows its use to verify the type of structure which SF presents. For example, the normal structure of fibroin presents random coils which absorb at 1650 cm^{-1} and 1534 cm^{-1}, but structures with alpha-helixes shift these absorptions to 1658 cm^{-1} and 1526 cm^{-1}. Likewise, the presence of beta-sheet type structure is identified by a peak shoulder at 1628 cm^{-1}. Following these wavenumbers, changes in the structure can be detected [83]. Besides, nonlinear least-square convergence criterion can be used to quantify β-sheet crystallinity from ATR-FTIR spectra [84].

4.4. X-ray diffraction analysis (XRD)

Another tool for morphological characterization is X-ray diffraction (XRD), which gives information about the crystal structure and chemical composition of the sample. XRD of proteins is a fascinating and complex world but, in the specific case of the polymeric structure of SF, XRD provides information about the degree of orientation, as well as its opacity/transparency [85].

In addition, both Silk I and Silk II conformations exhibit diffraction peaks at characteristic angles. For example, Silk I show peaks at 2θ 19.1° and 22.5° while Silk II conformation present peaks at 2θ at 9.6° and 20.1°, thus allowing a morphologic characterization [86]. Furthermore, XRD spectra allows also estimating the crystallinity using Hermans' method [87], which is a common practice in silk fibroin hydrogels [86].

4.5. Thermal analysis

There are two main types of thermal analyses: thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Briefly, a TGA instrument tracks changes in a sample's mass as it undergoes heating or cooling, while DSC quantifies the amount of energy a sample absorbs or releases during these temperature changes. With this information, quantitative and qualitative data can be obtained, allowing to determine both the purity and elemental composition of the sample as well as its thermal stability [24,88,89].

For example, TGA of SF scaffolds gives information about their degradation. In the work by Byram et al. this process is studied, occurring in two steps: an initial water loss at 100 °C and the chain length and peptide bonds breaking process, happening at 270 °C–380 °C [24].

On the other hand, DSC data complements the TGA results. It provides insight into whether the process is endothermic or exothermic, which is determined by the mass loss as temperature changes. For instance, when analyzing SF hydrogels, TGA assists in identifying the material's structural characteristics, including amorphousness, the presence of beta-sheet structures, or oriented fibers.

Thermal analysis of silk fibroin hydrogels may occur through different stages, depending on the composition of the degumming process SF has suffered [24,86]. It is common to have an initial weight loss around 100 °C (up to 150 °C for adsorbed water) corresponding to the loss of free water; then, a second weight loss appears between 270 °C and 380 °C arising from the breakdown of side chain groups and from the fragmentation of the peptide chain [24,86]. Finally, if the temperature rises over 450 °C, a third weight loss peak appears from the main chain disintegration [86].

4.6. Mechanical testing methods

These experiments are designed to provide information about the elasticity, durability, and the force that materials can withstand, among other specifications. For doing that, the materials are subjected to compression, tension, relaxation and creep tests [20].

These kinds of studies become particularly important when it comes to assessing changes in properties after a particular treatment, as is the case of Yan et al. [39]. These authors conducted a comparison of the

mechanical properties between SF scaffolds and those that were modified with hydroxypropyl cellulose methacrylate (HPCMA). Their findings revealed that the modified scaffolds did not undergo deformation when subjected to a constant force, in contrast to the unmodified scaffold. Moreover, the modified version demonstrated resilience during multiple bending processes, consistently returning to its original state in each instance.

4.7. UV–Vis spectrophotometry

UV–Vis spectrophotometry is one of the most fundamental techniques found in nearly every chemistry laboratory. By providing information about the energy of electronic states, it is particularly useful for identifying organic or inorganic species which present any chromophore groups [90]. In the case of SF, UV–Vis spectrophotometry is valuable for characterization, as SF contains aromatic amino acid residues with a chromophore aromatic ring. The main absorption peak, located around 280 nm, is attributed to the presence of tyrosine, phenylalanine, and tryptophan in the molecular chains, existing in quantities lower than 5 %. Phenomena such as the photoyellowing of silk, a consequence of UV irradiation, can be studied by monitoring a new band situated between 300 and 310 nm. This band appears due to the formation of photoproducts resulting from the creation of new cross-links and/or 3,4-dihydroxyphenylalanine, a product of the photooxidation between phenylalanine and tyrosine [91,92]. Additionally, UV–Vis radiation is an effective tool for photopolymerizing SF, resulting in crosslinked hydrogels with remarkable properties, such as an impressive modulus values [93]. This photopolymerization usually requires the presence of reducing agents, and habitually implies the formation of tyrosine radicals [93]. Furthermore, this technique can be employed to investigate drug release, enabling the quantification of released substances, thereby providing insights into the amount retained.

4.8. Rheological measurements

Rheometry quantifies the extent of deformation experienced by a material or liquid when subjected to an applied force. Rheology, the scientific study of material deformation, is founded upon the interplay between stress, strain, and shear behavior. Rheometric measurements are usually conducted under two different approaches: either at fixed temperature (isothermal) or in a temperature ramp. One of the main advantages of rheological measurements is their capability of giving insight into the structure-properties relationship, according to their viscoelastic behavior. The rheological tests, which are habitually performed on hydrogels, cover [94]:

- Flow curves: Shedding light into the variation of viscosity on the applied shear rate, which may result very interesting in applications such as drug delivery, and tissue engineering.
- Strain sweep tests: Consisting in using increasing oscillatory strain at a constant frequency. They give information about the storage (G') and loss (G'') moduli, related to the Newtonian behavior or the linear viscoelastic region of the material.
- Time sweeps tests: Commonly used to determining structural changes over a certain period of time.
- Temperature sweep tests: Give information about the structure of the hydrogel when subjected to a certain range of temperatures.
- Frequency sweep tests: They are helpful to establish the relationship between the testing frequency and the storage (G') and loss (G'') moduli of the hydrogel. This is related to the viscoelastic properties.
- Creep Compliance, Creep Recovery tests: Basically, these tests consist of applying a stress to the hydrogel for a time. Then, no further stress is applied, and the recovery of the sample is recorded over a certain fixed time. So, information about the tolerance of the material to the deformation is obtained.
- Stress relaxation tests: In a certain way, these tests are the opposite of the creep compliance ones. In this case, the hydrogel is subjected to a constant strain and measures the stress exerted by the sample.

Rheological studies may result very interesting for certain hydrogel applications. Thus, Zhang et al. designed a rheological study in three steps, which mimicked the injection process, allowing thus knowing the extent of structural destruction and recovery in the procedure [95].

5. Applications

After conducting a thorough literature review, the application of silk fibroin hydrogels can be categorized into four key perspectives (Fig. 1): drug delivery systems, wound closure & healing products, tissue engineering and implant devices. Among these applications, tissue engineering comprises half of the scientific publications, with drug delivery being the second most significant application for these materials. Fig. 3 depicts the distribution of publications related to silk fibroin hydrogels across various applications.

For practical applications, the activity of SF hydrogels should be assessed in terms of their intrinsic structural activity as well as the activity of the loaded molecule (when acting as carrier systems). Hydrogels' activity is closely related to mechanical properties although it is also crucial to consider other properties such as biocompatibility or biodegradability. For example, structural properties were deeply studied by G. Egan et al. [96]. In their research, they found that the secondary structure of SF hydrogels (measured by FTIR) has a direct relationship with their biological activity. SF hydrogels, synthesized by electro-gelation or sonication, exhibit different structures. The beta sheet secondary structure is higher when hydrogels are formed by sonication, resulting in a less amorphous structure and less fibroin elution compared to those formed by electro-gelation. They also examined how the leached silk influenced cellular behavior by exposing fibroblasts to soluble silk. They found that the phosphorylation of receptor tyrosine kinases is boosted by leached SF, and chemokinesis is stimulated as well. In addition, it had little effect on fibroblasts cytotoxicity, being a safe material for biomedical applications. These features are crucial for both wound healing and tissue engineering.

5.1. Tissue engineering

An insightful definition of tissue engineering can be discovered in Professor Berthiaume's contribution to the Encyclopedia of Physical Science and Technology [97]: "*Tissue engineering is the construction of bioartificial tissues* in vitro *as well as the* in vivo *alteration of cell growth and function via implantation of suitable cells isolated from donor tissue and biocompatible scaffold materials*". This field emerged in response to the significant challenges frequently encountered in transplantation. The fundamental concept revolves around the creation of new, functional living tissue using living cells, often in conjunction with an external biocompatible scaffold material designed to facilitate tissue development. Hence, tissue engineering generally tackles two key aspects. On one hand, fostering cell growth and/or differentiation becomes paramount to generate a substantial cell mass capable of serving as a natural tissue replacement. On the other hand, the scaffold material must possess a set of attributes, including biocompatibility, durability, and suitability for supporting cells. And this is precisely why silk fibroin hydrogels demonstrate significant potential in this field.

Among the potential applications of silk fibroin (SF) hydrogels in tissue engineering, bone and cartilage regeneration are the most prominent, as illustrated in Fig. 1, with skin regeneration following in third place. However, distinguishing between skin tissue regeneration and skin wound dressing, which has already been discussed, is challenging due to their close relationship. The role of SF in skin regeneration (see Fig. 4) has been associated with the modulation of protein expression responsible for the proliferation and remodeling phases

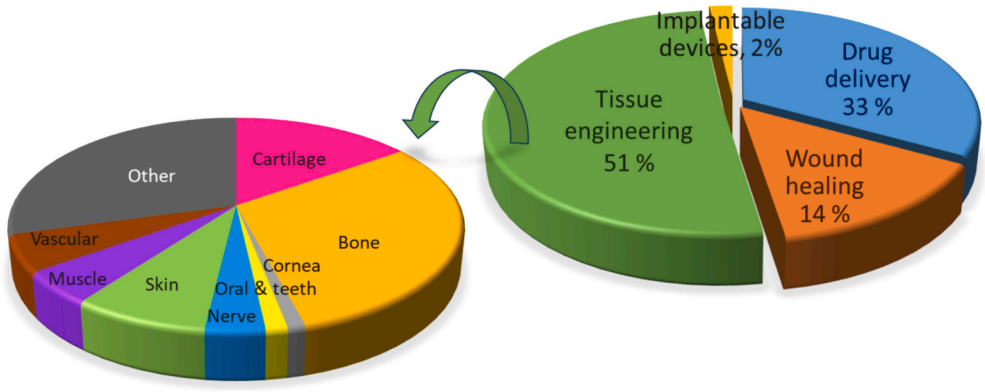

Fig. 3. Ratio of publications on general applications of silk fibroin hydrogels and their specific focus on tissue engineering (Source: WoS under the keywords tissue engineering with silk fibroin hydrogels).

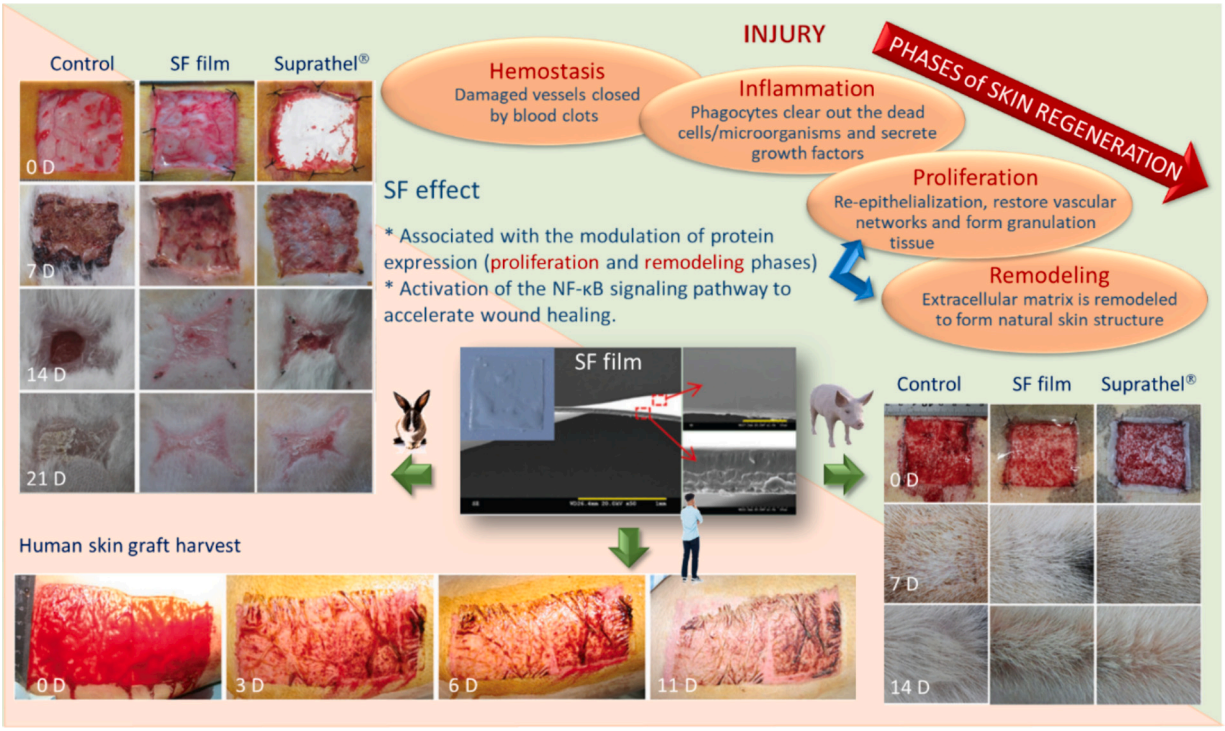

Fig. 4. Scheme of the skin regeneration process post-injury, highlighting the specific stages where silk fibroin (SF) plays a role, and comparative images showcasing the efficacy of SF films versus a commercial product in promoting skin regeneration. (Figure created adapting imagines from figures included in article [100]).

through the activation of the NF-κB signaling pathway, leading to accelerated wound healing [98,99]. Fig. 4 includes a schematic of the skin regeneration process after an injury, highlighting the stages where SF is involved, along with images from another study comparing regeneration using SF films and a commercial product [100].

The use of silk fibroin hydrogels in bone regeneration has proved to be promising, despite its weak osteogenic properties. This uses to be overcome by adding bioactive substances such as growth factors, showing a good repairing performance and inducing the differentiation of mesenchymal stem cells into osteoblasts in vitro [101,102].

Silk Fibroin hydrogels have also been described to accelerate at least one week the collagen deposition in second degree burns when compared to a classical medical gauze. Similarly, the presence of immune cells after one week is also higher in the SF-hydrogel treated burn compared to the medical gauze one [103].

5.2. Drug-delivery

Regarding drug delivery, hydrogels are a common option as they have shown a higher encapsulation efficiency compared to micelles or liposomes [7]. In addition, hydrogels offer two supplementary benefits: a higher protection degree to the encapsulated substance, thus improving its stability, and the possibility of encapsulating macromolecules and other bioactive substances, which are difficult to encapsulate in other carriers [104]. Particularly, silk fibroin-based hydrogels not only allow the controlled release in a specific area maintaining the drug concentration in a more-or-less constant rate, but they are biocompatible and biodegradable too. So, the presence of proteases and similar substances in the body leads to the breakdown of the material and its disappearance, contributing to an increasing use of these systems in the treatment of various diseases, such as joint injuries. This phenomenon can also be harnessed to create drug delivery systems with precise targeting within the body. For instance, Navamajiti et al. leveraged the

sensitivity of silk fibroin to enzymes found in the small intestine, such as chymotrypsin, while remaining resistant to the actions of gastric pepsin. They employed this property to synthesize a silk fibroin-polymethacrylate copolymer coating for oral dosage forms, whose intended drug release target was the intestine [105]. Alternatively, other researchers put their focus not on the stimuli-responsive capabilities, but on getting a superior control over the drug release. Thus, Cheng et al. prepared an electrodeposited assembly of SF coating from drug-loaded SF nanospheres which showed up to 21 times enhancement on the antibiotic release without therapeutical loss nor cytotoxic activity [106]. In the context of employing SF hydrogels for encapsulating macromolecules that prove challenging to encapsulate within alternative structures, we find an illustrative case in the research conducted by Zhou et al. They developed a photocrosslinked hydrogel based on silk fibroin and methacrylate for encapsulating basic fibroblast growth factor (bFGF), a peptide promotor of the recovery of nerve function after spinal cord injury. The resulting material showed a sustained drug release, improving mitochondrial function and the regeneration of axons from injured neurons while keeping a good biocompatibility [29].

It's worth noting the potential of silk fibroin hydrogels in gene therapy and gene delivery for cancer treatment as well. Ghandehari's research group, in the USA, has extensively worked on silk-elastin-like-based hydrogels (SELP) for gene transfection and delivery [107]. These materials facilitated the regulated discharge of adenoviral vectors from SELP hydrogels within a tumor environment for a duration of 28 days and showed enhanced duration of transgene expression and efficacy. Moreover, the incorporation of a matrix-metalloprotease responsive sequence within or next to the silk units affected the degradation kinetics of the hydrogel and, when present in the middle of elastin units, the degradation of those sequences allowed for vascular infiltration, aiding in the healing process. The same authors developed a silk-elastinlike copolymer hydrogel loaded with a sulfated glycosaminoglycan ether, GM-0111, a powerful anti-inflammatory useful for fighting inflammation derived from radiation-induced proctitis and cystitis. This hydrogel enabled controlled release over a period of 12 h [108].

It is, then, clear, that the special protection that silk fibroin hydrogels provide to the encapsulated drugs, as well as the slow release and the SF-hydrogel degradation through proteases, maximizes positive effects and reduces adverse effects compared to drugs that would be used in the traditional way. To illustrate, the drugs used as treatment for articular pain are non-steroidal anti-inflammatory drugs (NSAIDs) or painkillers, which have several side effects and if there is a large exposure to them, they can cause severe damage such as kidney problems or ulcers [53,109,110].

It is also interesting to mention the effect that lyophilization may exert on some hydrogels regarding their delivery properties. Guziewicz et al. described the possibility of a sustained local release of monoclonal antibodies using a lyophilized SF hydrogel material. Although lyophilized SF hydrogels at 3.2 % *w*/w did not show appreciable differences in antibody release compared to the non-lyophilized material, higher concentrations of 6.2 % w/w resulted in lyophilized hydrogels whose sustained antibody release lasted for 38 days, compared to 10 days for the non-lyophilized version [111].

5.3. Wound closure & healing products

When an injury occurs in the body, typically due to mechanical or thermal causes, but also from physical or chemical sources, it triggers a physiological response mediated by a cascade of events involving numerous agents, ultimately aimed at restoring damaged tissues. This process, known as wound healing, comprises several stages, including inflammation, a stage at which the process often halts when interrupted by factors like infections, burns, or other pathological disorders. In such cases, the use of specific dressings is beneficial, and silk fibroin-based hydrogels become particularly relevant [112].

Traditional wound healing systems often exhibit several common drawbacks, such as poor adhesion and limited degradability. Nevertheless, SF hydrogels excel in overcoming these challenges due to their robustness, antiseptic properties, biodegradability, and biocompatibility, among other advantages [6,104]. While silk sericin has shown some inherent antibacterial activity in certain studies [113], the antibacterial efficacy of silk fibroin-based materials typically relies on physical loading or chemical functionalization with various antibacterial agents, as well as bioinspired surface modifications [114]. Additionally, silk fibroin has been observed to closely resemble the skin's topology [115], potentially influencing the dermal wound healing process by activating the nuclear factor-kappa B (NF-κB) signaling pathway [76].

Nevertheless, wound closure and healing are intricately linked to drug delivery. In many cases, the SF hydrogel not only serves as a scaffold for wound healing but also typically carries drugs, thus serving a dual purpose: facilitating cellular interactions with damaged tissue cells and releasing antibiotics, anti-inflammatories, or other medications to the affected area [116,117]. Approximately 38 % of the articles gathered in the WoS database under the topic *hydrogel silk fibroin wound healing* involve the use of antibacterial agents as adjuncts in the healing process using hydrogels. In this context it is interesting to mention that the antibacterial activity is not only achieved through classic antibiotics but also gallium (III) ions in the case of *Pseudomonas aeruginosa*-infected wounds [84].

Furthermore, as mentioned earlier, hydrogels offer the opportunity to encapsulate other types of macromolecules and larger bio-structures that would be challenging to encapsulate otherwise. Hence, silk fibroin hydrogels have been employed for the encapsulation of exosomes derived from human umbilical cord mesenchymal stem cells (UMSC-Exo), which were subsequently evaluated for their potential in wound healing applications. The researchers concluded that, in addition to the improvement in wound repair facilitated by the freeze-dried composite hydrogel itself, the incorporation of UMSC-Exo within its structure further enhanced its wound healing properties [118].

Indeed, the remarkable versatility of hydrogels in accommodating various components enables the creation of tailored wound dressings for specific applications. In this context, while not exclusively limited to silk fibroin, the research conducted by Liang et al. presents a noteworthy collection of hydrogel-based wound dressings (HWD). It is important to highlight that, in the review work, the results obtained by R. Yu et al. are cited, referring to a supramolecular hydrogel obtained by photopolymerization from SF, acryloyl-β-cyclodextrin, and 2-hydroxyethyl acrylate, which demonstrates superior performance as a wound dressing compared to commercial alternatives. Furthermore, as a drug carrier, it has a high loading capacity due to the presence of abundant cyclodextrins. In in vivo trials, curcumin loading results in a reduced inflammatory response and positive regulation of vascular endothelial growth factor (VEGF) [119].

Furthermore, SF-hydrogels are also used to accelerate the closing of a wound, as it can be seen in the work of Pan et al., in which they use it to seal and reconstruct meniscus tears, providing a stable and durable repair [120], or in the work by Bhar et al., who employed a hydroscaffold composed of omentum extracellular matrix (ECM) and SF for promoting wound healing by two vias: vascularization (enhances the angiogenesis) and tissue remodeling (reconstructs the damage tissue) [121].

However, while silk fibroin serves as a promising foundation for wound dressings, it also presents certain challenges, with one of the primary concerns being its allergenic potential. Several studies have indicated that native silk fibers can trigger type I allergies, such as asthma, and lead to elevated IgE levels [122].

5.4. Implantable devices

Implantable devices generally suggest the idea of the material being either permanently or for long-term inserted or incorporated into the

body. Bearing this in mind, it becomes evident that many tissue engineering applications fall also within this category due to their long-term interaction with biological tissues. However, within the broad scope of implantable devices, we aim to highlight specific applications of silk fibroin (SF) hydrogels, particularly focusing on their use in drug delivery systems and implantable sensing devices.

Implanted SF hydrogels have been explored as in-site drug delivery systems seeking to help control tumors after surgery [123] or to prevent and treat infection [124]. The general considerations for these systems have been thoroughly discussed in another section of this review. However, we believe that implantable sensing devices made from SF hydrogels deserve special attention within this application category due to their unique capabilities and potential applications.

The majority of implantable and wearable silk fibroin hydrogel sensing materials exhibit sensitivity to various forms of mechanical excitation, including pressure, shear, strain, and torsion. These materials find wide-ranging applications in physiological contexts where force is applied to the sensing element. While there are some articles that explore the use of SF hydrogels in sensing non-mechanical stimuli, such as magnetic fields [125] or humidity [126], these studies are not primarily focused on implantable or wearable systems. Therefore, when it comes to discussing implantable mechanical-sensitive SF hydrogel sensing materials, we may remark the work by Zhang et al., who introduced a captivating innovation in the realm of flexible, stretchable, wearable, implantable, and degradable mechanical sensors, leveraging conductive SF-hydrogels [127]. Essentially, they harnessed carbon-nanotube-doped silk fibroin hydrogels as the sensing materials, with their electrical resistance dynamically responding to material deformation. These sensors exhibited promise in applications like sign language translation and real-time monitoring of physiological signals and sporting movements. Furthermore, when integrated with phenobarbital-loaded microneedle arrays and a light-induced degradation system, they demonstrated remarkable efficacy as a real-time monitoring and in situ treatment device for epilepsy in a rodent model.

In a similar vein, Qiu et al. put forth an ultra-thin, transparent, and highly flexible multifunctional sensor based on silk hydrogel with a self-patterned microstructure, showcasing impressive performance in temperature and humidity sensing. This device is useful in monitoring several aspects such as facial expression, joint movements or temperature fluctuations. Of particular significance is its ability to monitor breath and throat signals, making it especially noteworthy for tracking individuals with upper respiratory tract infections [128]. Similar approaches based on conductive SF polyvinyl alcohol hybrid hydrogel film were used to monitor pulse pressure, finger joint movement, and vocal cord vibrations [129].

In the same line polypropylene-glycol plasticized SF solution can be used as human skin sensing material. As described by Xiao et al., the resistance of such hydrogel suffers a reversible periodic change with the hydrogel stretching condition, and it was used to monitor a finger's movement [130]. Graphene oxide has also been successfully used as modifier of SF-hydrogels for developing mechanical sensors for human motion detection and gesture recognition [131].

Regarding wearable sensors, it is also worth mentioning the work by Wu et al. They developed a wearable sensor system based on 3D-printed SF hydrogels to monitor temperature, strain, and bioelectrical signals. Notably, their system's flexibility and biocompatibility suggest that it has the potential for long-term use [132].

5.5. In the world of nano

While it may not be considered an application, the integration of nanomaterials into the field of hydrogels is an increasingly intriguing aspect. Both the development of nanostructured silk fibroin hydrogels and the incorporation of nanomaterials into suprananometric hydrogel structures open up a highly fascinating range of possibilities. Incorporation of nanoparticles within the SF hydrogel structure definitively enhances some of the material properties such as mechanical, biological, thermal, luminescent, antibacterial or magnetic ones, and we suggest some comprehensive reviews to delve into this fascinating world, such as those by Xu et al. [133], Marin et al. [134] or Khan et al. [135].

Silk fibroin nanostructures (see Fig. 4) such as nanofibers might be obtained through electrospinning of an SF solution, although this process needs either the addition of poly(ethylene oxide) to increase the solution's viscosity, or dissolve the SF in a mixture of acidic acid, formic acid and hexafluoro-2-propanol, what might reduce biological applications because of the inherent toxicity of those chemicals. These nanofibers may be prepared in presence of silver salts for developing wound dressing applications, or may be incorporated with pre-synthesized or in situ prepared silver nanoparticles as antimicrobial agents.

On the other hand, silk fibroin – nanoparticle composites may be obtained also in different ways [133] (Fig. 5): i) the direct mixing of NPs into the SF solution prior to obtaining the film, nanofiber, scaffold or hydrogels which would retain the NPs adsorbed through physical interaction; ii) alternatively, SF is brought into contact with a precursor of the NPs, acting as a template for the in situ nucleation and growth of NPs; iii) a third alternative is feeding the silkworm with NPs-sprayed mulberry leaves, extracting afterwards the SF from the cocoons, which would naturally content the NPs distributed inside the SF fibers.

As an example, a practical application of a methacrylonylated SF-hydrogel and zinc oxide nanoparticles in combination with fisetin was described by Gu et al. to accelerate infected diabetic wounds through biofilm application [136]. In this case, the fisetin loaded hydrogel as well as de ZnO loaded hydrogel showed an increased biofilm inhibition compared to the control or the unloaded hydrogel. Likewise, the double-loaded hydrogel with ZnO and fisetin resulted in the most inhibited biofilm, thus illustrating the potential of the material in wound healing.

6. Conclusions and perspectives

As can be observed, silk fibroin, coupled with its ability to form hydrogels, has emerged as a revolutionary biomaterial. SF has demonstrated to be biocompatible, biodegradable, with a minimal immunogenicity and tunable biomechanical behavior, presenting an extraordinary mechanical strength and with a high water absorption capacity. These unique characteristics make it applicable in various fields, especially in biomedical sciences, where also it shows significant potential to transform healthcare technologies. However, there is still much work to be done in terms of synthesis. In most cases, silk fibroin is mixed with other biomaterials to obtain hydrogels, involving intricate choices among chemical, physical, and enzymatic methods, each offering distinct advantages and cutting-edge biomedical applications, ranging from drug delivery to tissue regeneration and cancer therapy. Researchers are continuously exploring and refining these methods, enhancing not only their versatility and efficacy but also their transformative impact on healthcare technologies.

One of the key areas where silk fibroin hydrogels excel is in drug delivery systems. Their unique properties, including high encapsulation efficiency and controlled release capabilities, have enabled targeted drug delivery strategies. Notably, these hydrogels have been harnessed in oral dosage forms, precisely releasing drugs in specific areas like the intestine. Moreover, they have demonstrated efficacy in gene therapy, facilitating enhanced transfection efficiency and prolonged therapeutic effects, especially in cancer treatments.

In the domain of wound closure and healing, silk fibroin hydrogels have redefined conventional approaches. Serving both as scaffolds for tissue regeneration and carriers for drugs, they offer comprehensive solutions. These hydrogels, enriched with antibacterial agents, nanoparticles, and exosomes, enhance wound healing properties significantly. Addressing potential allergenic reactions is a focal point for future research, ensuring their safe and widespread application.

Tissue engineering represents another frontier where silk fibroin hydrogels have made substantial contributions. Particularly in bone and

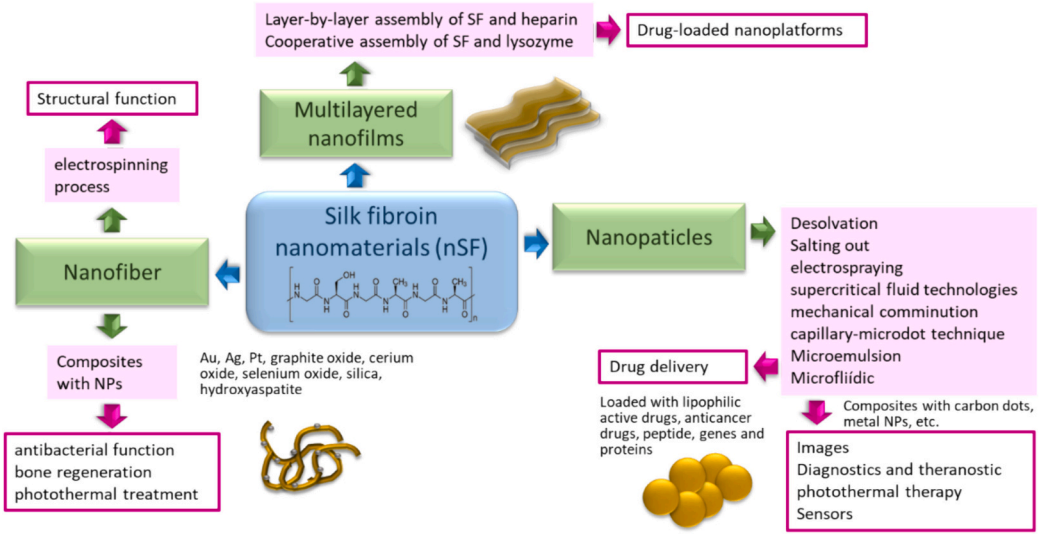

Fig. 5. Scheme of synthesis methods and application of silk fibroin nanomaterials.

cartilage regeneration, they serve as robust platforms. While their inherent osteogenic properties might be limited, these hydrogels, when combined with bioactive substances, promote cell differentiation and tissue repair. Balancing cell mass creation with scaffold biocompatibility, silk fibroin hydrogels have shown promise in advancing tissue engineering methodologies.

Implantable devices, especially sensors, underscore another dimension of their capabilities. Integration with conductive materials and microneedle arrays has facilitated real-time monitoring and in situ treatment. These devices have applications not just in healthcare but also in sports monitoring, highlighting their adaptability and potential impact on wearable technology.

Furthermore, the integration of nanomaterials into silk fibroin hydrogels has unlocked intriguing possibilities. Enhancing mechanical, biological, thermal, and magnetic properties, the inclusion of nanoparticles broadens their scope. Researchers are actively exploring innovative combinations, paving the way for refined applications and groundbreaking advancements.

In essence, silk fibroin hydrogels stand at the intersection of innovation and biomedical applications. Their seamless integration into drug delivery, wound healing, tissue engineering, and implantable devices marks a transformative journey. As interdisciplinary research continues to evolve, these hydrogels are poised to revolutionize the landscape of biomedical technologies, promising a future where targeted therapies and advanced medical solutions are the norm.

Nevertheless, while extensive experimental evidence has established the bioactivity of silk fibroin hydrogels, the precise molecular mechanisms underlying their regulatory effects remain incompletely elucidated. This underscores the necessity for deeper investigation into the molecular properties of silk fibroin, which is crucial for fully harnessing its potential and optimizing its applications in biomedical fields.

Declaration of generative AI in scientific writing

During the preparation of this work the authors used ChatGPT 3.5 in order to revise and improve the English quality of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Alfonso Fernández-González: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Clara de Lorenzo González:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sandra Rodríguez-Varillas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rosana Badía-Laíño:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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