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Silk: history, obtaining, structure and properties of an old material in the development of new technologies

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Abstract: For thousands of years, the silk produced in the Bombyx mori glands and used in the preparation of cocoons has been employed in the textile industry to produce fabric. These cocoons consist mainly of fibroin (SF – silk fibroin), a fibrous protein with unique mechanical properties and which is biocompatible, biodegradable, and inexpensive. SF can be extracted from the cocoons by processing in aqueous medium, and it can be employed to obtain materials for different applications, including biomaterials for body implants, scaffolds for tissue engineering, and materials for photonic devices such as sensors, waveguides, and lasers.

Keywords: Silk.Fibroin. Applications.

Introduction

Silk is a natural and semicrystalline polymer with high malleability, unique mechanical properties, and superior tenacity as compared to any currently employed synthetic fiber.[1,2] For thousands of years, since silk was discovered in China in the middle of 2600 BC, the textile industry has explored this material in its natural form.[3] Silk is produced in specific glands called sericigenic glands. Some invertebrates spin it into the fiber form during metamorphosis, but silk composition will depend on source.[4,5] The silk from silkworm (Bombyx mori) is the most produced and commercialized. It consists chiefly of fibroin and sericin proteins, and Bombyx mori uses it to manufacture the cocoons, to which fibroin and sericin acids provide resistance and protection, respectively.[2,6] Sericulture refers to the cultivation of silkworm. This culture was spread in Brazil in the beginning of the last century. It occurs mainly in the state of Paraná, in the so-called Vale da Seda paranaense, where silk threads are produced and exported to several countries.[7–9] However, in the 1940s, fibroin, a fibrous protein and a major component of the silk thread, started to arouse scientific interest. Structurally, this protein consists of two chains, one ~25-kDa chain and one ~325-kDa chain.[6] The largest chain comprises distinct regions known as silk I and silk II, which present different degrees of crystallinity.[10] Fibroin can be extracted from silk threads by degumming, a low-cost process that requires water.[2] The solution obtained after this process can be used to develop numerous materials such as films, fibers, and spheres, among others. Additionally, the solution can be submitted to several treatments, to give a series of products;[2] e.g., silk sutures,[6,11] which have been employed for decades. Moreover, the solution can be used to obtain biomaterials, including implants and scaffolds for tissue engineering[12,13] as well as photonic devices like sensors, waveguides, and lasers.[14] Silk-based materials have the advantage of being biocompatible,[2,12,13] biodegradable,[15] resorbable,[16] and mechanically robust[1], among other characteristics, which allows them to be widely applied and opens various possibilities for their use in countless areas. In addition, the cocoons can be obtained as waste from the textile industry, which reduces the cost of producing new materials.

History

The story that describes the discovery of silk by humanity is full of legends and divergences.[3] According to the account of the Chinese thinker and philosopher Confucius (571–479 BC), the most accepted to date, in 2640 BC silk was accidentally discovered by the Chinese Empress Hsi-Ling-Shi, wife of Emperor Huang Di (or Huang-Ti), also known as the "Yellow Emperor", who is thought to have ruled China from 2697 to 2597 BC, before the Xia dynasty. Legend has it that silk was discovered while the Empress was drinking a cup of tea under a mulberry tree—a cocoon fell into her cup and, upon contact with the hot water, it broke apart, revealing the fiber produced by silkworm.[3,17–19] Moved by curiosity, Hsi-Ling-Shi would have managed to transform that material into a piece of fabric and slowly started the weaving process, which is practically the same as the one used today. Although the textile industry was established at that time, it only peaked around 1500 BC, during the Shang dynasty. In the two following millennia, the Chinese were the exclusive

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manufacturers and exporters of silk: being aware of the commercial value of the fabric, the Chinese government banned mulberry seeds and silkworm eggs from being exported and threatened those who disrespected the ban with the death penalty.[3,20]

However, the ban was bypassed in 552 of the common era, when Justinian, the Roman Emperor, sent monks in disguise on a spy mission to China, to hide silkworm eggs in their luggage and take them to Constantinople inside bamboo sticks. As a result, the capital of the Roman empire at the time, present-day Istanbul, became the first European silk center, and this episode marked the arrival of sericulture in the Western World and the beginning of its dissemination to all continents in the following centuries. Due to its lightness, brightness, and beauty, silk, like precious stones and gold, has always been treated as a valuable object. The high commercial interest in it motivated the creation of the largest commercial route in the world, the so-called "Silk Road", leading to the foundation of great civilizations.[3,20] This path, which was the most important cultural and commercial link between the West and the East for hundreds of years, only received the name of "Silk Road" in the 19th century. Even though many products have been sold and traded along it, the one with the greatest prominence and attracting the most attention has always been the Chinese fabric, which led the German traveler and geologist Ferdinand Freiherr to name the path "Silk Road" (from the original "Die Siedenstrasse", in German).[3,21–24]

In Brazil, the history of silk fabric production began centuries later. In this country, the first mulberry trees were apparently grown in the middle of the 18th century in the state of Minas Gerais, as demanded by Queen Dona Maria I (also called "The Pious" and "The Crazy"), who ruled Portugal and Brazil at the time, until she was considered mentally incapable. However, although Brazil already had the basic subsidies for the cultivation of silkworm, silk fabric production only started in the Second Empire (1850). This is because Portugal had an agreement with England that forced the Portuguese colonies to import English fabric, thereby delaying the emergence of the Brazilian textile industry. In fact, this industry only developed after the Second World War, in the second half of the 20th century. Nevertheless, today Brazil is the fifth largest producer of silk fabric in the world. China still dominates the market, accounting for almost half of the silk fabric production on the planet. Even though Brazil remains behind China, India, Uzbekistan, and Thailand, the Brazilian silk fabric industry stands out for the quality of its yarns, produced mainly in the state of Paraná.[3,23,24]

Production and obtainment

Obtaining silk on a large scale is only possible thanks to sericulture, the part of zootechnics that deals with the study and development of silkworm (Kingdom: Animalia, Phylum: Arthropoda, Clade: Pancrustacea, Order: Lepidoptera, Genus: Bombyx, Species: Bombyx mori). [25,26] Sericulture is a thorough process that has been carried out pretty much the same way since silk was discovered approximately 4,500 years ago. It is the oldest known agroindustrial activity, and it is currently practiced by approximately 40 countries.[18] The so-called "Silk Farms" are in fact small family productions that operate without the need for advanced technologies given the delicacy of the cocoons produced therein. Therefore, production can be implemented in small areas and is promising for the social and economic development of rural areas. This activity keeps producers in the countryside, and it depends on some factors, especially climatic ones.[7] In Brazil, the only Western country that produces silk on a commercial scale, production began in the 19th century, in the state of São Paulo. Sericulture is integrated with the industry and employs approximately 8,000 families. These families receive the eggs – supplied by companies specialized in the production of yarns – to cultivate silkworm and to obtain the cocoons. These families also grow mulberries (Morus sp) to feed silkworm. Indeed, silkworm feeds on the leaves of these pants, which must be grown isolated and without pesticides, so as not to interfere in the quality of the silk threads. In Brazil, this activity is currently concentrated in the state of Paraná, as already mentioned. More specifically, it occurs in the so-called Vale da Seda paranaense, which comprises 29 cities.[7–9,18,27] This activity started there in 1950 and, 30 years later, this region became the largest national producer, with about 53% of all the Brazilian silk production. This percentage increased to 90% in the 1990s and has remained so until today. The largest share of silk production is exported to textile industries worldwide. To ensure the quality of the yarns produced nationally, local producers select and use only high-quality cocoons; the others (10 to 20% of all the production) are discarded.[7,8] Silk is composed of natural proteins[28] produced in specific glands called sericigenic glands. It is spun into fibers by invertebrates such as mites, moths, spiders, and silkworm;[5] its composition depends on the source. The best-known and best-characterized silks originate from silkworm (*Bombyx mori*) and spiders (*Nephila clavipes*),[5,29–31] which stand out for their strength and hardness.[32] However, spiders have predatory nature and low production,[6] so the silk produced by them is not commercialized very frequently. On the other hand, silk production by silkworm is expressive and occurs through sericulture.

Silkworm uses silk to produce the cocoons, where it lives until it undergoes metamorphosis and becomes a moth. Silkworm development comprises four stages – egg, larva, pupa, and moth – and lasts around 60 days. In the first step, around 300–400 eggs are deposited by the female moth at a time; the moth dies almost immediately after egg deposition. Fourteen days later, these eggs

hatch into larvae. The larval stage lasts approximately 27 days and comprises five stages, during which the larvae feed on mulberry leaves and begin to prepare the cocoon, a process that takes approximately four days and which is carried out by using a single wire measuring between 700 and 1500 m long. Once this is done, silkworm moves to the pupal phase, where it remains for 14 days, inside the cocoon. Finally, it turns into a moth, which lives for seven days.[33–35] Figure 1 illustrates these steps.

Figure 1 – Representation of the *Bombyx mori* life cycle.

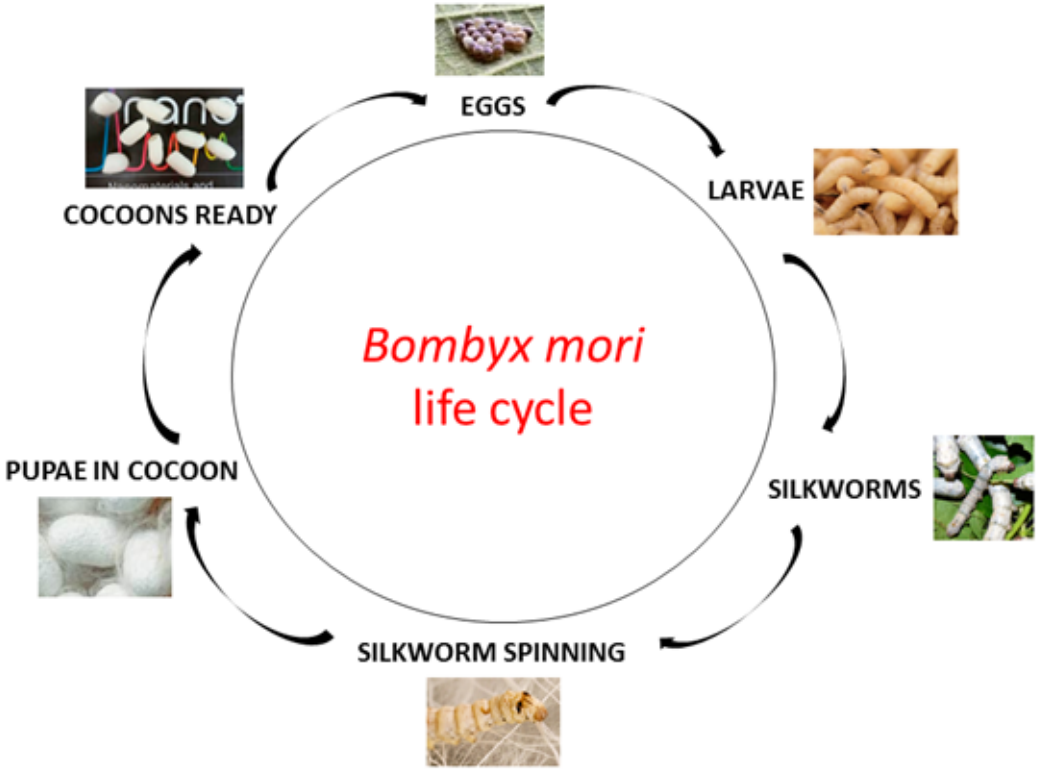
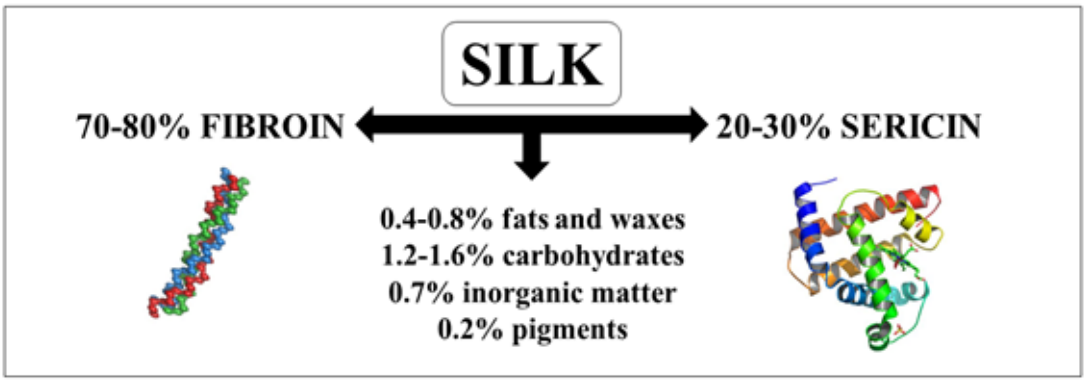


Figure 2 – Representative scheme of the composition of silk threads.



The textile industry is interested in the cocoons. Starting from the egg, the estimated time for obtaining them is approximately 45 days. These cocoons consist mainly of two proteins: fibroin (~70%), which belongs to the fibrous class and provides the cocoons with resistance, and sericin (~30%), which belongs to the globular class and provides the cocoons with protection.[2] There are also other materials[36], as shown in the scheme represented in Figure 2.

From a chemical standpoint, proteins are macromolecules built up from a set of 20 amino acids. The latter are smaller molecules bearing two functional groups – carboxyl and amino – in their structure.[28] In the case of the proteins constituting the main core of the silk thread, there is high percentage of glycine and alanine in fibroin and of sericin and threonine in sericin, as well as other subunits (in smaller proportions)[33]. Please see Table 1.

Table 1– Percentage of amino acids constituting the proteins present in the silk thread.[33]

AMINO ACID	% in fibroin	% in sericin
Glycine (Gly)	42.8	8.8
Alanine (Ala)	32.4	4.0
Serine (Ser)	14.7	30.1
Tyrosine (Tyr)	11.8	4.9
Valine (Val)	3.0	3.1
Aspartic acid (Asp)	1.9	16.8
Glutamic acid (Glu)	1.7	10.1
Threonine (Thr)	1.2	8.5
Phenylalanine (Phe)	1.2	0.6
Isoleucine (Ile)	0.9	0.6
Arginine (Arg)	0.9	4.2
Leucine (Leu)	0.7	0.7
Proline (Pro)	0.6	0.5
Lysine (Lys)	0.5	5.5
Tryptophan (Trp)	0.5	0.5
Histidine (Hys)	0.3	1.4
Methionine (Met)	0.2	0.1
Cysteine (Cys)	0.1	0.3

Fibroin, the major component in the silk thread, is a protein of the fibrous class. It plays a structural role and constitutes the nucleus of the silk filaments, providing the cocoons with resistance. On the other hand, sericin is a protein of the globular class, which functions as a "glue" that unites two fibroin filaments, to form a silk thread that protects the cocoon.[2,6,37]

Thus, silk is composed almost entirely of organic components.[33] Its production is sustainable given that this material can be processed in water.[2] Moreover, it is edible (so it can be used in the food industry),[38–40] it originates from natural sources, it is inexpensive,[6] and it has outstanding mechanical properties. Indeed, it is one of the most resistant natural fibers,[1,33,41] which allows it to be used in several other applications, inside or outside the textile industry. A considerable part of the cocoons produced during sericulture is discarded.[7,8,42,43] Therefore, in the last decades, hundreds of studies aiming at reusing these cocoons in the textile industry itself or in the production of "less noble" products have been conducted. An example of such studies is the Brazilian project "Casulo Feliz", created by the zootechnician Gustavo Augusto Serpa Rocha in 1988.[44] Alternatively, the discarded cocoons can be employed to develop cutting-edge technology based on isolation of the protein constituents of the cocoons.[1,2,12–14,45,46]

Fibroin, the major component of silk: extraction, structure, and properties

Although the history regarding the use of silk as sutures is centuries old,[6,11] it was in the mid-1940s that fibroin derived from cocoons aroused scientific interest. In 1947, the first academic article reporting the use of fibroin outside the textile industry was published.[47] Since then, the possibilities of using this protein have extended to several areas, such as tissue engineering.[4,48] Fibroin has also been employed in the development of various types of biomaterials[12,13] and photonic devices[14]. Today, it is one of the most applied natural fibers.

Compared to fibroin, sericin is still less often used due to biocompatibility and hypersensitivity issues.[6,49] However, many studies have investigated the application of sericin in other areas[50] including the development of compounds with antioxidant and bactericidal properties.[34,40,51] Sericin has also been employed in the prevention against UV rays,[52] in the composition of cosmetics,[53] and in the food industry.[40] This protein is commonly known as the "binding" protein. It involves and assembles the fibroin fibers, thereby providing the cocoon with a structure.[32] Because it is a globular protein consisting predominantly of the amino acids serine (Ser) and aspartic acid (Asp) (Table 1), sericin is highly hydrophilic and is highly soluble in hot water,[52] which allows it to be removed from silk during the "degumming" process – the procedure through which fibroin is extracted from the silk threads, to give a solution of this fibrous protein.[2]

While the textile industry disassembles the cocoon built from a single thread to produce fabrics, degumming is a "reverse engineering" process that starts from the cocoon, to return to the original components, proteins and water, present in the silkworm sericigenic glands and which are spun to produce the cocoons.[54,55] This protein splitting process comprises four steps: (I) separation of proteins and extraction of fibroin from the silk threads; (II) fiber dissolution; (III) dialysis to remove excess salt

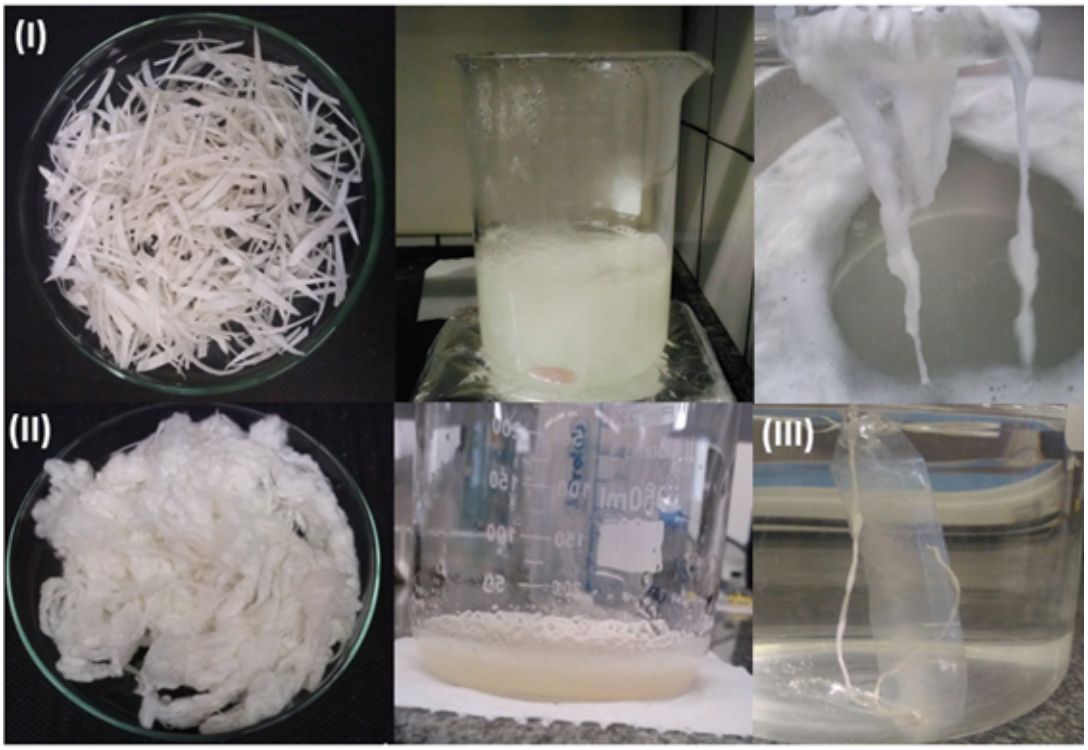
from the medium; and (IV) centrifugation to remove impurities from the cocoons itself. At the end of these steps (shown in Figure 3), an aqueous, viscous, and yellowish solution (~5% mass/volume) is obtained. This solution must be kept at 4 °C and remains stable for approximately 30 days.[2]

In step (I), the cocoons are heated to 100 °C in an alkaline solution of sodium carbonate (Na_2CO_3). A salting–in effect takes place – the low concentration of salts increases the solubility of the proteins because the saline ions interact with the ionic charges of the proteins, increasing the effective charge and the amount of water molecules attached to the protein ionosphere.[28] As already mentioned, the silk thread consist of two fibroin filaments linked together by a natural "glue", sericin. Sericin is a globular protein and is more soluble than fibroin. Thus, it is solubilized, becoming loose and remaining in solution, while the fibroin fibers remain rigid.[2]

These now dried loose fibroin fibers undergo dissolution (II), which can be carried out in the presence of various organic solvents and/or aqueous solutions[2] such as $CaCl_2$–EtOH–H_2O,[56,57] $Ca(NO_3)_2$–MeOH–H_2O,[58] LiBr–EtOH–H_2O,[41] LiSCN–H_2O,[59] NaSCN–H_2O,[60] and LiBr–H_2O.[2] The latter is the most commonly used system. In this case, LiBr acts as a chaotropic agent. At high concentration in aqueous solution, LiBr "removes" the water molecules surrounding the fibroin molecules, thus affecting the stability of the native conformation of these macromolecules, weakening the hydrophobic effect.[4,28]

After the fibroin fibers are completely dissolved, a yellow viscous gel is obtained. This gel is then dialyzed (III) against ultrapure water for 48 h. After this period, the solution is centrifuged (IV) at 3500 rpm, to remove impurities.[2]

Figure 3 – Photograph of the fibroin extraction process steps. (I) A sequence of three images showing cut cocoons, cocoons being heated in Na_2CO_3 solution, and loose fibroin fibers. (II) The first image shows dry fibroin fibers; the second image shows dissolved fibroin fibers after dissolution in LiBr solution. (III) Dialysis of the obtained solution.

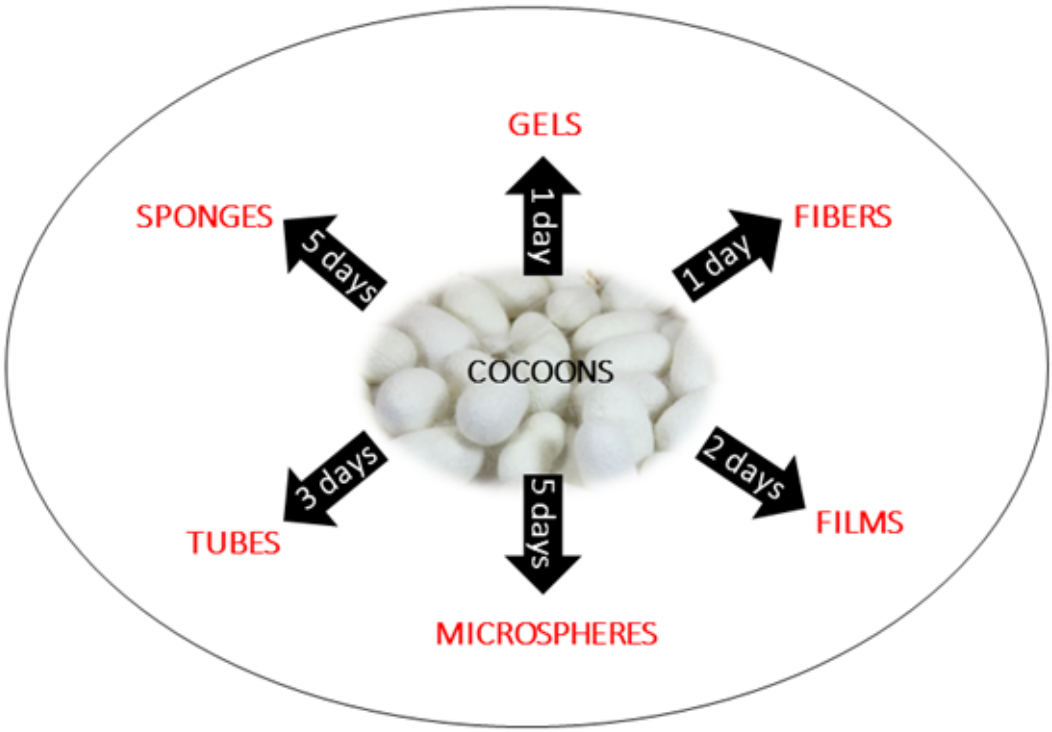


Structurally, fibroin consists of two chains: a larger one, with ~325 kDa ((H) –chain), and a smaller one, with ~25 kDa ((L) –chain)[6]. These chains are linked by a disulfide bond formed between two cysteine residues,[71] leading to an HL complex, which at the same time is also non–covalently linked to a P25 glycoprotein (~25 kDa). The whole structure of this protein[62–64] was first characterized by Marsh in collaboration with Corey and Pauling in 1955.[65] The high–molar–mass region underlying the tension properties of fibroin is a crystalline structure composed by repetitions of the amino acids that build up its primary structure (in that order Gly – Ala – Gly – Ala – Gly – Ser) and which may have the so–called silk I and silk II. Silk I is a hydrophilic, less crystalline region consisting of alpha–helix structures or random coil; silk II is hydrophobic and is more crystalline due to the presence of antiparallel beta–leaves.[10] Silk I can be converted to silk II by specific treatment with methanol. In addition to these two regions, a small and unstable structure, known as silk III, may occur at the air/water interface of the fibroin in solution, but its structure has been less explored.[66] The low–molar–mass chain accounting for chemical resistance and moisture retention is an amorphous, hydrophilic structure that does not present the repetition of the amino acids that build up the primary structure.[62–64,67–71] Fibroin comprises 5263 amino acid residues; Gly, Ser, and Ala are the main constituents of the primary structure, whereas other residual amino acids exist in smaller proportions,[72,73] as shown in Table 1.

Because fibroin belongs to the fibrous class and presents many hydrophobic amino acid residues in its structure, it is a water–insoluble protein.[33] As mentioned previously, this protein has unique characteristics that allow it to be used in several areas. Among these characteristics, its biocompatibility,[6,12,13,48] biodegradability,[15] resorbability,[16] high mechanical resistance,[1,74] relative stability to the environment it occupies,[6] flexibility,[75] ability to self–crystallize[62] and to self–organize,[2] structural functionality,[2,76,77] possible coordinative environment for ions or molecules of interest,[78,] permeability to vapors of interest,[80] transparency in all the visible spectrum, smooth surface, and variable refractive index[81–83] stand out.

After sericin is completely removed from the silk thread, the resulting fibroin solution can be used to prepare different materials, which is promising for several applications. Some designs are illustrated in Figure 4 and include gels, fibers, films, microspheres, tubes, and sponges. The time required to obtain these materials varies from one to five days,[2] depending on the method.

Figure 4 – Scheme of the numerous shapes that can be obtained from the fibroin solution. The number of days that are necessary to obtain each shape is counted only after the fibroin solution is prepared.[2]



The fibroin crystalline structure, and hence its mechanical properties, can be modified during the preparation of these materials, by altering the fibroin concentration in the solution, the solvent used during treatment (water or alcohols), and the treatment temperature[84] and by adding other components or creating/distributing pores.[2,4,6,12,14,76,85,86] The thickness and degradation time for materials such as films and tubes can be controlled by changing the fibroin concentration in the solution used during the preparation.[2,87] Through a simple drying process in ambient conditions, insoluble materials, like films, can be obtained – beta sheets (conversion of silk I to silk II) are formed as a result of water removal. However, the materials obtained by this method a simple drying process in ambient conditions, insoluble materials, like films, can be obtained – beta sheets (conversion of silk I to silk II) are formed as a result of water removal. However, the materials obtained by this method are brittle, but this can be improved through treatment with hot water or methanol.[4,76,88–90] Treatment with water vapor only affords more flexible materials that degrade within a shorter time than materials treated with methanol, a widely used method for preparing materials of different shapes, such as spheres and fibers.[2] In addition to the previously mentioned films and tubes, because methanol can induce fibroin crystallization, a larger number of beta-sheet structures arise.[76,89,90] In general, treatments with water vapor only give a smaller amount of beta sheets than treatment with alcohol, such as methanol. A small number of beta sheets results in less crystalline, more malleable, and softer materials, whilst an increase in the number of these structures affords more crystalline and rigid materials.[76]

Besides these treatments, the mechanical properties of fibroin can be improved by adding other components;[91–95] for example, fibroin blends with acrylic polymers,[60] polysaccharides,[96] collagen,[97] and sodium alginate,[98] among others, can be obtaimed.[99,100] Porous materials can also be achieved by using polyethylene oxide (PEO)[2], thus creating matrixes with defined porosity and modified surface properties.[101–103] Another factor that can influence the properties of the resulting materials is the age of the cocoons that are used to obtain the fibroin solution, as demonstrated by Ramirez and collaborators.[104]

Film is one of the simplest forms into which fibroin can be molded. To prepare a film, the typical feature of proteins must be considered: these macromolecules tend to self-organize as they spread over a surface. Therefore, they acquire the shape of the surface after the solvent present in the protein solution, in this case water, is evaporated under ambient conditions (dry casting).[2,4,105] In this way, non-patterned or patterned fibroin films can be achieved by changing only the substrate where the film is prepared. The choice of substrate will depend on the target application given that this film will "load" the characteristics of the substrate. Apart from this preparation method involving manual deposition, these materials can be obtained by other techniques, such as layer-by-layer (LbL) deposition,[74,86,106–108] which produces ultrafine fibroin films, as well as spin coating,[109,110] Langmuir-Blodgett (LB) process,[66,111,112] and others.[4]

Fibroin tubes can also be obtained by a simple method like dip coating, by immersing a template in a solution with high fibroin concentration, as described by Rockwood and collaborators.[2] After immersion, the system must be homogenized and placed in contact with methanol, which can induce formation of beta-sheet structures and stiffen the material.[4,76] After treatment, the templates with the solution are dried at room temperature. This method enables tube thickness to be controlled through control of the fibroin concentration in the solution and the number of layers deposited on the template;[2,115] furthermore, porous tubes can be created.[113] Another methodology involves spinning gel (gel spinning).[102]

Wenk and collaborators[114] obtained fibroin microspheres by a method that uses mild conditions and a vibrating mouthpiece. In this system, the fibroin solution is added with a syringe and ejected in the form of drops by action of the mouthpiece with controlled vibration and frequency. The drops are poured into a reservoir containing liquid nitrogen and solidify upon contact with it.

Spherical fibroin materials can also be prepared by other methods. For instance, fatty acid lipids (DOPC: 1,2-Dioleyol-sn-glycerol-3-phosphocholine) can be employed to encapsulate the aqueous fibroin solution. The fatty acid lipids can be in the pure form or in the presence of a molecule of interest, which act as a template. After vesicles are generated, the lipid can be removed, and the resulting spheres are resuspended. In the case of DOPC, which is soluble in methanol and can stiffen fibroin, methanol is used to remove the lipid.[2,115] Phase separation between the aqueous fibroin solution and another polymer, such as PVA (polyvinyl alcohol), is another useful method that is simple and dismisses the use of other solvents.[2,116]

Fibroin fibers are generally obtained by electrospinning, a process that affords materials with large surface area and reduced diameter.[117–122] This methodology is widely employed due to the simplicity of the experimental electrical force apparatus – it basically consists of three components, syringe, high voltage source, and collector – not to mention its high efficacy and low cost.[2,117]

Fibroin is also widely used to produce three-dimensional scaffolds with controlled morphology and porosity. These scaffolds can be obtained from gel, hydrogels, or sponges. Sponges can be achieved by adding and subsequently removing salts from the fibroin solution in aqueous medium; pore size can be controlled.[2,123] In addition to this aqueous method, sponges can also be prepared by using organic solvents, like the alcohol HFIP

(1,1,1,3,3,3–hexafluoro–2–propanol).[2,124]

Finally, hydrogels can be prepared by a series of methods involving decreasing the pH value of the medium,[125] sonication[126], or application of an electric current.[127] In all cases, the material is produced in a simple and fast way, and the choice of method will depend on the target application.[2] Nogueira et al.[128] demonstrated that these materials can be achieved by fibroin solution dialysis at different temperatures, without the need for further treatments, constituting another simple and effective route that affords promising structures for biological applications.

Applications

Because fibroin provides positive responses due to its biocompatibility,[2,12,13,48] among other properties, it has been widely explored for the development of diverse biomaterials in the medical field for decades;[12,13] moreover, it has promising use in the development of photonic devices.[14] Among the possible applications of this natural polymer, we can mention its use in drug delivery systems,[115,130–135] as scaffold in tissue engineering for repair of tissues (such as cornea[136–140] and vascular[102,113,141–144] and bone[145–154] tissues), and as dressing for healing skin lesions.[155–158] Other application areas include cancer diagnosis and treatment,[159] photodynamic therapy,[160] enzyme immobilization,[161–166] photonic devices,[16,167] lasers,[168–174] biosensors,[175–180] waveguides,[72,181,182] fuel cells,[183] energy storage devices,[184] and materials with antithrombogenic properties.[185,186]

Drug delivery

Controlled drug delivery using implantable biopolymers offers numerous advantages over conventional methods. Advantages include controlled and constant administration at the required therapeutic rate without peaks or valleys.[187,188] The release rates are determined by the vehicles themselves. The vehicles must be biocompatible and amenable to large–scale production, and they must work for days or even years.[187] In this scenario, carrier materials based on fibroin stand out for their biocompatibility, excellent mechanical properties, controlled degradation through crystallinity control (quantity of beta–sheet structures), ability to immobilize enzymes, and other therapeutic properties. Besides that, they can be processed in mild conditions and molded into different shapes.[129–135]

Through the most diverse forms, fibroin–based materials can be used to encapsulate/release a series of molecules and bioactive compounds. For example, Wang and colleagues prepared carrier microspheres in the presence of lipids and subsequently treated them with methanol and sodium chloride solution, to obtain materials with different surface characteristics, for different applications.[115]

Moraes and collaborators prepared fibroin hydrogels in the presence of diclofenac molecules for controlled delivery of this compound. They dissolved the drug in water or ethanol and later added it to fibroin, to obtain carrier hydrogels. In the case of the drug dissolved in water (SF–H_2O), hydrogel formation took three days, but this time decreased to 10 minutes for the drug dissolved in ethanol (SF–EtOH), confirming that ethanol accelerated the gelling kinetics. The authors studied the delivery profile of the drug in the two cases, under the same conditions. For SF–EtOH, drug release was faster at the beginning of the experiment and reached equilibrium after 10 h, whereas equilibrium was reached after 5 h in SF–H_2O. The data suggested that hydrogels containing diclofenac dissolved in ethanol presented more sustained controlled delivery because the SF–EtOH hydrogel emerged more rapidly and in the presence of well–organized beta–sheet structures, resembling cross–linking processes, thereby resulting in more controlled diclofenac release.[132]

Among the possible applications of carrier systems, tissue engineering to induce the growth of tissues like the cornea and vascular, bone, and skin tissues stand out.[133]

Through the most diverse forms, fibroin–based materials can be used to encapsulate/release a series of molecules and bioactive compounds. As an example, Wang and colleagues demonstrated the preparation of carrier microspheres in the presence of lipids and subsequent treatment with methanol and sodium chloride solution, where it was possible to obtain materials with different surface characteristics, widening the use for different purposes115.

Moraes and collaborators have shown the preparation of fibroin hydrogels in the presence of diclofenac molecules with potential use in controlled delivery of this compound. In this research, the drug was dissolved in water and in ethanol, and later added to fibroin to obtain carrier hydrogels. As for the drug dissolved in water (SF–H2O) the time required to obtain the hydrogel was 3 days, while in the case of dissolution in ethanol (SF–EtOH) the gelation time decreased to 10 minutes, confirming the effect of acceleration on the gelling kinetics by ethanol. The delivery profile of this medication in the two mentioned cases was studied under the same conditions as shown in Figure 7. In Figure 7 is also possible to notice that for SF–EtOH the drug release occurred more quickly at the beginning of the experiment and reached equilibrium after 10 h. On the other hand, in SF–H2O the equilibrium was reached after 5 h. According to the researchers, the data presented suggest that hydrogels containing diclofenac dissolved in ethanol present a more sustained controlled delivery compared to those obtained with the drug dissolved in water. This can be explained by the fact that the SF–EtOH hydrogel formation occurred more rapidly and in the presence of well–organized beta–sheet structures, similar to what occurs in cross–linking processes, thus,

resulting in a more controlled release of this medication[132]

Among the possible applications for carrier systems, the most studied and explored is in the area of tissue engineering in inducing the growth of the most diverse types of tissues such as the cornea, vascular, bone, and skin tissues[133].

Tissue Engineering

Tissue engineering, a sub–area of biomedical engineering, is an interdisciplinary area that gathers knowledge from several fields to develop systems that can act in the maintenance, restoration, or performance improvement of different organs and tissues. Therefore, this sub–area requires the use of biocompatible matrixes that can receive bioactive cells or molecules and later be implanted at the destination site, where they adapt to the microenvironment and mimic the damaged area. The resulting arrays are commonly called "scaffolds". Apart from the minimum biocompatibility requirement expected for these materials, their degradation time along with the regeneration time of the tissue in which they are inserted also determines their application. In the search for biomaterials that can act in the reconstruction of tissues, structural proteins such as collagen, elastin, and albumin stand out for being natural and easily found components.[2,4,6] In this context, silk fibroin has been increasingly explored due to its elasticity properties, mechanical resistance, controlled biodegradation, and biocompatibility.[4,189–196]

The most reported tissues for potential regenerative treatments using fibroin–based materials, whether pure or in the presence of other compounds,[153] are bone (36%),[145–154] cartilaginous (17%),[197–203] vascular (10%),[102,113,141–144] and cutaneous (5%) tissues,[155–158] as well as cornea (2%)[136–140], as described by Kasoju and Bora in 2012.[189]

Compared to other types of tissues, fibroin has been mostly employed in bone repair.[189] Bone tissue is made up mainly of hydroxyapatite and collagen, and it can be recovered by inducing osteoblast cell proliferation. Since the role of fibroin as a biomaterial was first reported in 1995,[204] many studies have been carried out, and bone regeneration using this material in the form of fibers obtained by electrospinning,[152] of hydrogels,[146–149] of porous membranes,[151] of films in the presence of hydroxyapatite,[145] as well as functionalized with other molecules[205,206] has already been successfully demonstrated.

Two decades ago, Sofia and collaborators[205] reported images of the systems they prepared. The images were obtained by calcein fluorescence and showed maximum calcification in the fibroin and RGD (peptide sequence) substrate after four weeks. These results supported the concept that fibroin can induce bone tissue growth, especially when it is functionalized with molecules of interest.

Skin dressing

Due to their biocompatibility, low immunogenicity, and nontoxic and non–allergenic nature, biopolymer dressings have been widely used in the treatment of skin lesions.[207] The mechanism through which these lesions are recovered occur in different parts of the skin and is complex: it involves various types of cells and requires a material with good adhesion.[208] Therefore, materials that can incorporate and carry molecules, like drugs, in their structure and which can be molded in different ways have gained prominence in this area.[207] Fibroin–based materials have been investigated as dressings for skin wound healing since the 1990s. These materials are effective substrates for proliferation of adherent cells and can be an alternative to replace collagen.[209] Indeed, studies have shown the effectiveness of materials based on pure fibroin[210,211] and of materials obtained through previous treatments and as blends in the presence of other compounds.[155–158]

Vasconcelos and collaborators prepared fibroin and elastin compounds via freeze–drying and proved that they effectively induced the recovery of wounds caused by skin burns. The combination of the self–organizing properties of fibroin with elastin resulted in a system that mimicked the extracellular matrix and was able to accelerate wound healing. According to the authors, treatment with a material composed of 50% fibroin and 50% elastin provided the fastest improvement.[158]

Enzyme immobilization

Silk fibroin started being used as a support for enzyme immobilization around 1977.[212] These systems can be easily obtained, not to mention that they increase enzymatic activity and stability.[161] Furthermore, silk fibroin can be submitted to different treatments, to optimize enzymatic stabilization through a low–cost material with interesting biological properties.[163,213,214] Enzymes can be immobilized on fibroin substrates by methods such as covalent bond formation,[215] adsorption,[12] and encapsulation,[164–166] among others.[162]

Many enzymes have been immobilized on a fibroin substrate; e.g., HRP (Horseradish peroxidase),[216] glucose oxidase,[217] lipase,[218] and alkaline phosphatase.[219] In a paper published in 2015, Tao *et. al.*[220] used a colorimetric reaction to demonstrate that the HRP activity was maintained after the enzyme was added to the fibroin solution. This led to a functional ink for 3D printing, which would have been impossible to achieve with the enzyme printed in isolation.

Systems comprised of enzymes of interest and fibroin can be used to develop materials for numerous applications in the medical[162] and industrial areas.[165] One example is their use as biosensors for diagnosis.[14,81]

Electronic and Photonic devices

In the field of electronics and photonics, biopolymer

films have been used to obtain flexible devices.[75] This field requires materials that can be applied in unconventional interfaces, such as curvy and soft interfaces, to which traditional devices are unable to adhere. Indeed, electrodes that can intimately and non-invasively integrate with these surfaces offer important opportunities for disease treatment and diagnosis.[16,75] In this regard, fibroin has been extensively studied and stands out against other materials thanks to its unique properties, which include being mechanically robust[1,14,74] and transparent throughout the visible spectrum, presenting smooth surface and variable refractive index,[81–83] and being susceptible to physical changes in its structure.[76,77] All these characteristics allow the preparation of passive or active devices[75] based on pure fibroin[16] or fibroin in the presence of other components,[167] to provide materials like supports for electrodes,[16,221] lasers,[168–174] biosensors,[175–180] waveguide,[83,181,182] fuel cells,[183] and energy storage devices.[184]

Kim and collaborators built an electrode consisting of a chip supported on an ultrathin and resorbable fibroin substrate. To this end, they obtained a pure, smooth, and thin film in which the chip was placed. They observed that tissue adhesion improved with decreasing device thickness. The chip could be reabsorbed by the body within a programmed time because fibroin can be undone in days, months, or even years depending on the treatment to which it is submitted.[16] This work introduced a concept that could be extended to other areas: the preparation of electronic devices with biodegradable components as an alternative to replace some types of plastics.

Silva et al.[172] took advantage of the ability of fibroin to copy the characteristics of the substrate to which it is added[2,14] to prepare a laser system based on the DFB (distributed feedback grating) mechanism by using a commercial DVD as substrate. They poured the fibroin solution on the surface of the DVD and let it dry. The resulting film displayed the diffraction pattern of the DVD. This pattern is what causes amplification in this type of laser system, thus being an example of an active device based on fibroin in the presence of nanoparticles and dye incorporated into the protein structure.[172]

A dielectric material with thickness (l) and refractive index (n_1) and that can support wave propagation is known as a waveguide. Its refractive index (n_1) must be different from the refractive index of the material on which the guide is supported (n_2). In a study published in 2015, researchers obtained biocompatible waveguides from fibroin fiber (n_1 = 1.54) encapsulated with fibroin hydrogel (n_2 = 1.34) and demonstrated that the materials can guide light in tissues by means of a robust system, thereby allowing great advances in the use of light in therapy or image acquisition.[83,181]

The fibroin protein structure is also of great interest in the development of photonic devices. Among the amino acids that make up such a structure are the aromatic amino acids tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe) (Table 1), which are commonly employed as fluorescent probes for interpretation of the protein structure (conformation, dynamics, and molecular interaction). In the case of fibroin, Trp stands out because its fluorescence is sensitive to the surrounding environment. This amino acid residue, with broadband emission between 300 and 400 nm, can sensitize lanthanide ions by energy transfer, populating the emitter levels more efficiently, as demonstrated in a study published in 2018.[78] When the emitting levels are more efficiently populated, the emission of these ions intensify, a widely explored phenomenon in photonics.

Understanding how the physicochemical properties of fibroin affect the spectroscopic properties of lanthanide ions can be a strategy for the development of a new generation of photonic devices, such as biocompatible and biodegradable sensors with high transparency in the visible spectral region. In a recently published study, thin, highly transparent films consisting of fibroin and a europium ion (Eu^{3+}) complex, produced by immersion under controlled conditions, were presented. The study showed the highly intense emission typical of Eu^{3+} complexes and the dependence of the intensity of the $^5D_0 \rightarrow {}^7F_2$ transition on the concentration of ammonia vapor, thus demonstrating the possibility of using this system as a photonic vapor sensor.[180]

Final considerations

Silk fibroin has been the focus of several studies on the development of the next technological advancements in the fields of medicine, photonic devices, energy conversion and storage, 3D printing, and electronic devices, for instance. This is because this protein has properties such as mechanical robustness, smooth surface, high transparency (> 95%) throughout the visible region of the spectrum, and high moldability. Therefore, this old and amazing material remains at the cutting-edge of knowledge. Its properties can be improved by further biochemical functionalization, which may provide it with greater versatility for the development of new devices through incorporation of different ions or molecules in the protein structure.

In this way, combining the mechanical and optical properties of fibroin with the multifunctionality of lanthanide ions could be an interesting strategy to develop new, distinguished photonic materials. However, the properties resulting from lanthanides in this protein host have not been fully exploited yet, which opens a wide field for future studies.

Sometimes, fibroin-derived materials do not outperform other inorganic materials. Nevertheless, progress in the synthesis of new composite materials from inorganic particles and fibroin can overcome some drawbacks of pristine fibroin, leading to new materials for solar cells, 3D print ink, and sensors and to new biomaterials

that can be developed on the basis of a green production strategy.

Finally, the silk cocoons that have been produced by the caterpillar *Bombyx mori* for millennia and which have been employed for fabric production had their history changed when their proteins were separated. Fibroin has now become the focus of several research groups and is key for the development of new materials.

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