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The Silk, Versatile Material for Biological, Optical, and Electronic Fields: Review

By Luigi Bibbo, Karim Khan & Ayesha Khan Tareen

DIIES University

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The Silk, Versatile Material for Biological, Optical, and Electronic Fields: Review

Luigi Bibbo α, Karim Khan σ & Ayesha Khan Tareen ρ

Abstract- Silk, seen as a material, is a fiber made from silkworm cocoons and spiders. They have standard structural components and hierarchical structures. Different manufacturing techniques allow obtaining silk in films, fibers, hydrogels, microspheres, and sponges. We can tune the properties through the structure of secondary proteins. The paper explores the application in biomedical, optics, and electronic fields by analyzing the technological trend.

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I. Introduction

Silk is one of the oldest fibers used above all in the textile field. The continuous research developed on its properties, the chemical modifications of silk fibers, and the functionalization, lead it to be a versatile material used in many contexts from the biomedical field (tissue engineering and regenerative medicine), pharmaceutical, cosmetic, up to optical and electronic.

Previous silk reviews have focused on a single specific sector such as biomedical or optical, or electronic. At the same time, after researching the literature to analyze more recently updated testimonials, the one we propose embraces a wide range of more applications in the same single review. Attractive in different fields. It reports both recent progress in manufacturing processes and innovative applications. The objective of the manuscript is to investigate both of these aspects, from the biological to the industrial one, considering the different process methodologies applied to create a variety of structural forms. The approach is to analyze the nature of the fiber and its potential applications. It starts with the structure and properties and continues with the innovative preparation technologies and functionalization to modify its mechanical properties or give the silk the desired functionality. Then the latest emerging applications are reported. In some sections, representing the experiences carried out, we can see how the silk, among other things, can effectively contribute to the achievement of the objectives of sustainable engineering.

Particular attention is paid to silkworms for their availability in large quantities from sericulture. The arachnid species is difficult to raise due to their cannibal tendencies, but their silk has the best characteristics. The biotechnological production of proteins (spindrois) makes it possible to overcome this obstacle. The availability of recombinantly produced spider silk protein is essential for experimentation and their use in applications. Their application was mainly in the biomedical field. Silk extracted is combined with other biomaterials to form biopolymer compounds. The review analyzes applications that use worm and spider silk, although for the latter to a limited extent due to the fewer testimonies in literature. If not clearly expressed, we refer to both types.

II. Structural Aspects

Historically the silk used in the textile field is obtained by Bombyx mori silkworm (Fig.1a).

In nature, there are different types of silks produced by other animals, such as the Trichoptera, mites, and spiders [1]. It is precisely the silk of spiders that becomes the subject of study by exceptional properties (Fig.1b). There are 48,000 species, divided into 120 families. There are several different types of silk. One of the most interesting is the dragline which has features superior to other silks. The silk fibers are suitable for implants. Both spiders and silkworms use protein spun in the salivary glands. While the former weaves silk to create cobwebs to capture prey, the silkworm uses silk to produce cocoons for the metamorphosis cycle. Silk fibers show up resistant to traction, and some are distinguished by having a high degree of elasticity. Silks, with these properties, exhibit significantly greater hardness than those of synthetic fibers. Silkworms produce silk with a consistent thickness, while spiders produce silk with varying thicknesses, high resistance under stress, exceptional elasticity, resistance to high temperatures, have piezoelectric properties but at the molecular level haven't sericin. Mechanically the silk of the silkworm is much weaker and less extensible than that of the spider. We can modify the characteristics of the silk produced by the worm according to the spinning conditions.

Author α: DIIES University "MEDITERRANEA" Reggio Calabria, Italy.
e-mail: luigi.bibbo@unirc.it
Author σ ρ: School of Electrical Engineering & Intelligentization, Dongguan University of Technology, Dongguan 523808 China.

Global Journal of Researches in Engineering (F) Volume XXI Issue III Version I Year 2021

Tab. 1 shows the features of two different kinds of silk.

Bombyx mori Spider

Fig. 1: Bombyx mori, Spider

Tab. 1: Features of silkworm silk and spider silk

Feature	Spider silk	Silkworm silk
Glands	Multiple glands near	Secreted via mouth
External coating	Glycoprotein	Sericin
Protein	Spidroin	Fibroin
Glycine	37%	46%
Alanine	21%	29%
Serine	4.5%	12%
Beta Sheet	30%	40-50%

The silk spun are different but have a typical structure: they have a protein chain and a hierarchical structure [2]. Fibroin is the core protein of fiber, and it is composed of amino acids arranged in β-sheets. The protein chain is an alternation of hydrophobic and hydrophilic domains. Fig. 2 shows the primary structure.

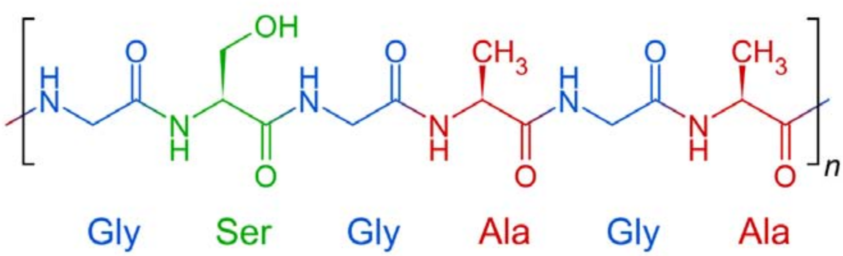

Fig. 2: Primary structure-Gly-Ser-Gly-Ala-Gly-Ala amino acid sequence

Fibroin is a fibrous protein-containing serine, alanine, glycine (Fig. 3), and tyrosine.

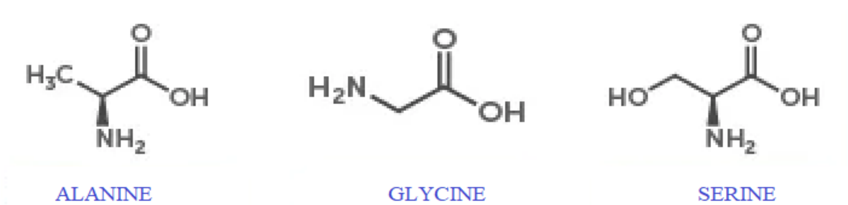

Fig. 3: Alanine, Glycine, Serine

Silkworm cocoon is sericin and fibroin, in proportion to 25% sericin and 75 % of fibroin. The fibroin is linked by glycoprotein sericin [3], with a small amount of waxy and dye material. Sericin is composed of serine and glycine. Fig. 4 shows the cross-section view, while Fig. 5 highlights its structure.

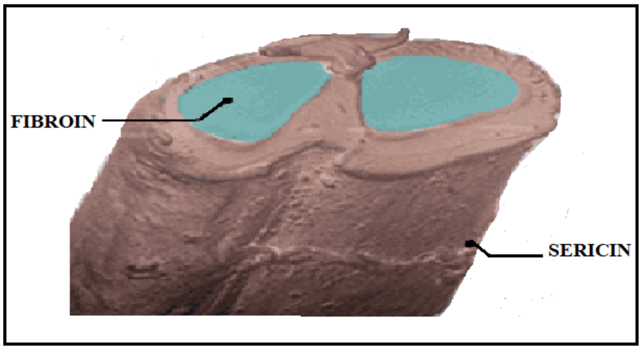

Fig. 4: Cross-section of fiber structure of Bombyx mori

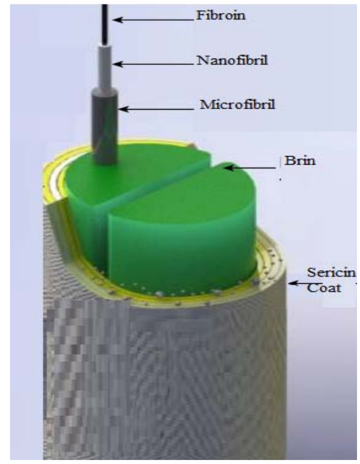

Fig. 5: Hierarchical structure

III. Properties

Silk is the most potent natural fiber, made up of thin films (β-sheets) with a controllable thickness from 100nm to 100μm. Lightweight, strength and toughness, elasticity, good resistance to failure in compression, and significant crystallinity are the characteristics that have greatly enhanced silkworm silk in the textile sector. In addition to these physical properties, silk as a natural biopolymer also shows biocompatibility, programmed biodegradability, and non-immunogenicity. Thanks to the evolution of materials manufacturing techniques, it is now possible to obtain polymeric materials based on silk protein for specific purposes. We modified the silk with organic composites to fabricate an interface between a bulk biopolymer and a conductive substrate of a polymeric optical device [4]. SF materials for their exceptional characteristics and structure also represent active optics, photonics, electronics, and optoelectronics applications. Silk materials with suitable morphologies and architectures are the right components for wearable sensors [5].

The efforts made in the production processes focused on the preparation of biotechnological methods for the recombination of silk fibers.

a) *Biologically properties*

The hierarchical structure of the hydrophobic parts and the blocks in silk show an extraordinary capacity for self-assembly, allowing devices with particular biological characteristics for innovative biotechnological applications. In particular, fibroin-based biomaterials show specific flexibility properties, rigidity, hydrophobicity, and hydrophilicity. We can control time and degradation under certain conditions. These characteristics stem from the conformation taken from the regenerated fibers during the dissolution process. The presence of amino acid sequence allows making chemical changes. For the functionalization of silk fibers, a series of reagents such as amines, alcohol, phenols, carboxylic groups were tested [6].

i. *Biocompatibility and cell interaction in vitro and in vivo*

We can obtain SF in various formats. For biocompatibility, after sericin removal, different studies demonstrated that the protein could shape structures to favor the attack and growth of cells.

Surgeons apply silk sutures in neurosurgery and cardiac surgery. In many cases, we have to remove the silk suture for an inflammatory reaction.

Previous studies believed that sericin might be the cause of the inflammatory state. Recent studies supported by information from in vitro, in vivo, and clinical trials show that silk sericin does not cause allergic reactions and is safe for medical applications. It can be considered an antioxidant, anti-tyrosinase, and tumor inhibitor for several biological properties in vitro and in vivo. However, sericin in the presence of fibroin should be used with caution because generating biological responses can be dangerous to human cells [7].

Overall, we can compare the degummed and sterilized silk products to polylactic acid (PLA) and collagen [8].

Many studies in vitro show that silk fibroin in contact with human cells has no interaction with a component of the inflammatory system, and fibroblast and other cells proliferate in silk fibroin scaffold. The use of water vapor induces the transition to β-sheets structure that makes cell proliferation easier [9]. Many studies involving subcutaneous implants showed no inflammation and their tolerance in the integration with the living tissue.

ii. *Biodegradability*

Biodegradability is a critical aspect of the biomedical field. Degradation refers to both in vitro and in vivo. The US Pharmacopeia defines bioabsorbable materials that degrade 60 days after implantation in vivo. Though natural silk fibers are considered not degradable by this definition, they degrade over a long period (months, years). Regenerated silk materials

Global Journal of Researches in Engineering (F) Volume XXI Issue III Version I Year 2021

(films, gels, etc.) degrade over a short time (days, weeks).

Many factors can influence the degradation process, such as implantable site, patient's medical conditions, type, and shape [10].

Some in vivo studies showed that changing the method and variables to processing silk scaffolds can modify the degradation process [11].

Cell culture highlighted that the silk fibroin scaffold degrades slowly with loss of mass after four weeks. Bacterial and enzymatic degradation influence its biodegradability [12]. Methanol degradation may reduce the rate of degradation. Implanted scaffolds degrade as tissues regenerate, and the time depends on the type of tissue affected. We can tune degradation by controlling dissolution, hydrolysis conditions, and lyophilization [13, 14].

We also found that it can control the degradation of a silk fibroin scaffold through the contents of the sheet structure β [15]. The possibility to control silk material properties is an essential advantage over other biopolymers like collagen, chitosan, and alginate, favoring the silk over other biomaterials in tissue reconstruction. The control over the physical form and the insertion, through different post-processing techniques, such as alpha -helices and beta-sheets, favor the process of biodegradability.

For bone tissue engineering, the ability to maintain an intact scaffold for a long time favors the transport of nutrients and waste products. Meanwhile, we have bone growth and vascular network formation.

IV. Processability and Modulation of Silk Properties

Silk is composed of water, fibroin, and sericin. We produce it in a neutral PH, in an aqueous solution, and at ambient temperature.

The first step of the manufacturing process is the elimination of cocoons from the fibroin solution. In the next step, we remove the sericin by boiling the silk cocoons in solution with degumming agents. The obtained silk fibers are then dissolved with appropriate solvents in an aqueous solution of purified fibroin by removing salts by dialysis. We can activate the solution of water and fibroin by inserting different organic dopants (enzymes, proteins) and inorganic (metal nanoparticles, quantum dots). We then deposit it on a particular substrate. The crystallization process occurs through the self-assembly of the proteins stored in the air. Thus, we can create different films or absorbable substrates for electronic or photon devices.

We report the steps of the manufacturing process in the following flowchart (Fig. 6). The degumming represents the first step to the removal of sericin for biomedical applications.

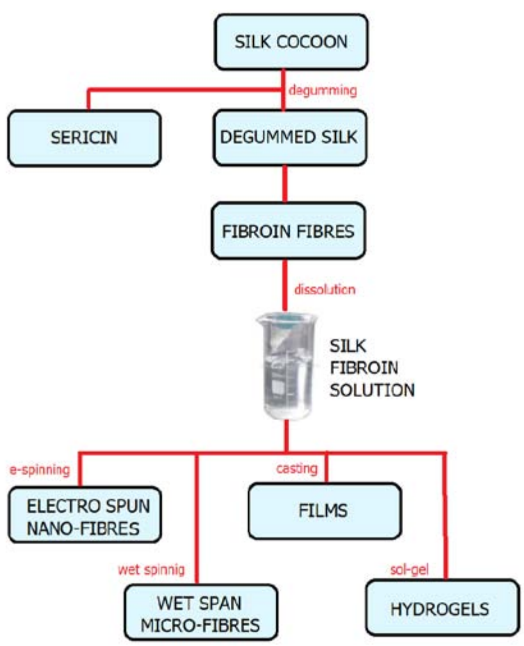

Fig. 6: Stages of the silk manufacturing process

Different degumming techniques obtain fibroins. New methods based on microwaves, ultrasounds, and CO2 supercritical fluid have been introduced [16]. Chemical dissolution of sericin is obtained partly by hydrolysis and partly by dispersion, independent of the method.

Soapy degumming is a commonly used technique that determines a chemical bond between the alkalis, produced by the hydrolysis of soap, with sericin making soda salts. The sericin is separated from the soap and dissolves in the water [2]. Mixtures of soaps and alkalis improve the quality of the silk. The degumming phase is associated with the bleaching phase obtained using hydrogen peroxide in alkaline conditions, acting on the PH through ammonia [17].

Silkworm degummed with citric acid treatment at 30% gave better results than treatment with soap and alkali, obtaining the removal of the sericin at almost 100 %; the total weight loss resulted of 25.4% in the silk fibers. Degumming with citric acid improves tensile strength and surface morphology [18].

The use of chemical components causes environmental pollution; therefore, it is preferable to replace them with natural enzymes extracted from papaya peel or pineapple peel that help improve the degumming process. Enzymes act with a method of proteolytic degradation of sericin without affecting fibroin. In India, the researchers tested unripened papaya as an enzyme. The proteolytic enzymes promote the hydrolysis process of the peptide bonds produced by amino acids. The experimentation required careful tuning of some elements such as degumming time, PH, process temperature, the percentage of the enzyme, and simultaneous selection of mordanting and dyeing [19].

Traditional degumming processes are expensive and harmful to the environment. They require a large quantity of hot water to eliminate contaminants such as oil wax and natural pigments, producing high temperatures and dispersing many chemicals into the environment. The new process has become more ecological and less expensive. One of the most valuable techniques in removing sericin is supercritical fluid CO2, requiring less water and energy. This process involves a pre-treatment with an organic acid which can be citric acid or tartaric acid. It involves soaking the silk in purified water or deionized water for many hours and then transferring the silk from the bath to containers of CO2 with a glycol-type non-ionic surfactant. With containers heated to temperatures around 100 ° C, keeping CO2 levels between 150 and 400 atm. After the removal of the CO2 emissions, we obtain the raw silk without sericin. [20].

Sericin contains water-soluble proteins such as serine and aspartic acid [21]. The degumming process is based precisely on the difference in solubility between silk sericin (SS) and silk fibroin (SF), so using alkaline hot water, SF fibers remaining insoluble are separated.

As an alternative to techniques based on hot solutions, the microwave technique penetrating inside the particles heats them simultaneously.

Irradiation, compared to other methods, takes less time to achieve the same degree of degumming. The process involves verifying the effects produced on the properties of silk through the scanning electron microscope [22]. The survey examined the weight loss, strength, and elongation of the samples found 10 % of weight loss and 8 %. It also occurred that the addition of Marseille soap improves the efficiency of the process. Adding baking soda to Marseille soap, the performance improves further. It turned out that the increase in degumming time, on the one hand, improved both weight loss and elongation of the silk while the strength worsened. The removal of the sericin allowed to obtain these results.

The good results obtained in the wet processing of fabrics have led to extending ultrasonic irradiation in the field of silk degumming. Traditional degumming is expensive and polluting. A group of researchers applied ultrasonic irradiation to a conventional heating bath. They then carried out a comparative analysis using degumming agents: citric acid, sodium carbonate, and papain [23]. Tests carried out on sericin degumming rate, fiber morphology, characteristics of their structure, and their tensile properties have shown that ultrasonic irradiation compared to the conventional heated bath is more effective, especially degumming at a temperature of 60°.

Furthermore, having found that the frequency variation in the wet processing of fabrics influences the efficiency of the process, they also analyzed their effect in the degumming of silk. They obtained the best results with ultrasound at a lower frequency. Regarding degumming agents, they found that with papain, compared to citric acid and sodium bicarbonate, we get a more significant elimination of sericin. They obtained a degumming rate of 22% at a temperature of 60° C and 40 kHz. However, the presence of papain causes excessive loss of the whiteness of the silk. Tests with infrared spectrography and X-ray diffraction showed minor changes in the characteristics of the structure of the silk fibers. The ultrasonic frequency has negligible effects on fiber structure and tensile properties.

The process based on the use of ultrasound represents a green alternative to the conventional method.

After the degumming step follows the dissolution of silk fibroin to make regenerated silk fibers in different forms [24], it is necessary to use solvents that require other dissolution times and temperatures depending on their solubility power [25].

In the process of dissolution, there is always a search for suitable and cheap solvents. An efficient practice washes the one developed by some researchers [26] who used a system with a methanol solvent instead of water and a low concentration of CaCl2, which is a protein denaturant.

They obtained a change in the solvation scabbard of ions in the solvents with the volatilization of methanol.

The process continues up to a stable concentrated SF solution. The remaining solvent

Global Journal of Researches in Engineering (F) Volume XXI Issue III Version I Year 2021

interacts with fibroin chains to complete the solvation sheath. They found a concentration of CaCl2 of 26% at the end of the process, and silk fibroin almost totally dissolved. The residual calcium interacted with SF molecules to form nanofibril molecules. Other techniques allow obtaining different structures, starting from fibroin solution for producing films, fiber, and sponges.

Silk fibroin offers a great opportunity in the biomedical field thanks to its particular anti-inflammatory and biocompatible characteristics when it's implanted in the human body [27].

The possibility to tune the silk properties offers advantages compared to other commonly used polymers. The researchers can tune the properties thanks to the secondary structures: α-helix and β-sheets produced with different processes. These processes may act on the degree of hydrophobicity, degradation, mechanical stress, porosity, oxygen permeability, and thermal stability [28].

Different treatments use solvents and water vapor. The technique with methanol produces mainly secondary structures as β-sheets, although there are other structures. The transition to the β-sheet forms depends on the exposure time and the level of concentration of the solvent (Fig.7). It ensures better conditions for tissue engineering, such as mechanical properties and resistance to degradation.

The method water vapor acts as a lubricant for the movement of the chains of protein [29]. With the increase of secondary structures, there is an improvement in degradation and hydrogel bond.

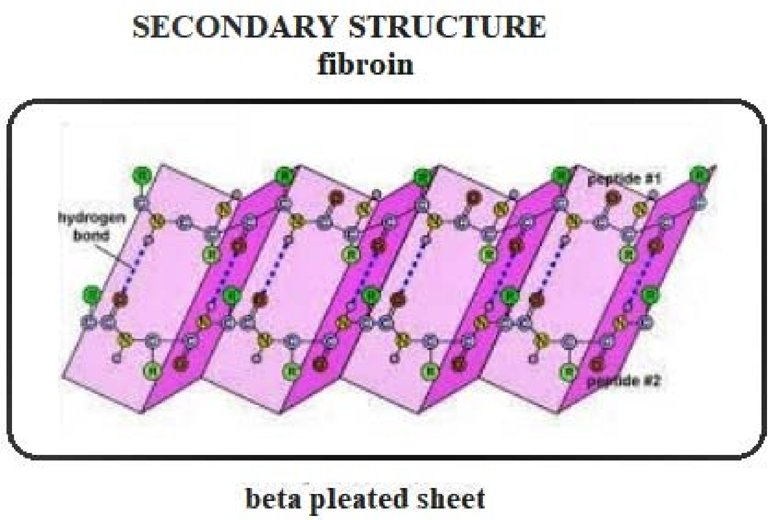

Fig. 7: Secondary structure of fibroin

V. Silk Biomaterials

SF finds application in the biomedical field for its particular characteristics of biocompatibility and biodegradability and its high tensile strength; it can be obtained through different processes or starting from silk fibers or silk fibroin solutions as regenerated fibers. Its combination with other materials favors the creation of various mechanical properties and physical characteristics. The milling process makes it possible to obtain silk in the form of a submicron particle [30].

Dissolution is the phase of the reprocessing of silk fibroin (SF) that allows the production of films, hydrogel, porous scaffolds, and electrospun fibers. Dissolving SF produces molecular chains and different properties, depending on the single solvent system, for each specific application [31].

Treatment with methanol is reported in several experiences, highlighting the formation of β-sheet, although they are also present in α-helix and random coils regions. The concentration of the solvent and its exposure time strongly influenced this transition [32].

a) *Native silk fiber*

Native silk fibers, derived from the Bombyx mori silkworm, represent the raw material used to produce regenerated silk solutions. Native silk fibers are composed of fibroin, which provides high tensile strength. They are applied initially in the medical field as sutures. Moreover, silk fibers also have the property of permeating water vapor and oxygen and promoting skin fibroblasts and collagen formation. Subsequently, they have found wide applications in the biomedical field [33].

The filaments are suitable for constructing a porous nonwoven silk material for cell seeding, the fabrication of the mesh structure, and scaffolds for ligament repair [34].

Methylcellulose (MC) is also valid in tissue reconstruction for its resistance and anti-toxicity properties. Recently, however, composites in silk fiber and methylcellulose (SF/MC) have been made to engineer bone. The results obtained showed improved osseointegration between bone and composite and provided a reasonable degradation rate, bioactivity, biocompatibility, and mechanical properties. The solvent casting technique allows obtaining a porous composite [35].

b) Regenerated Silk

Silk solution allows you to produce several different regenerated structures (Fig. 8). After the phase of degumming, we can dissolve the silk fiber with a salt solution followed by dialysis to obtain an aqueous silk solution; the silk solution is formed [36, 37].

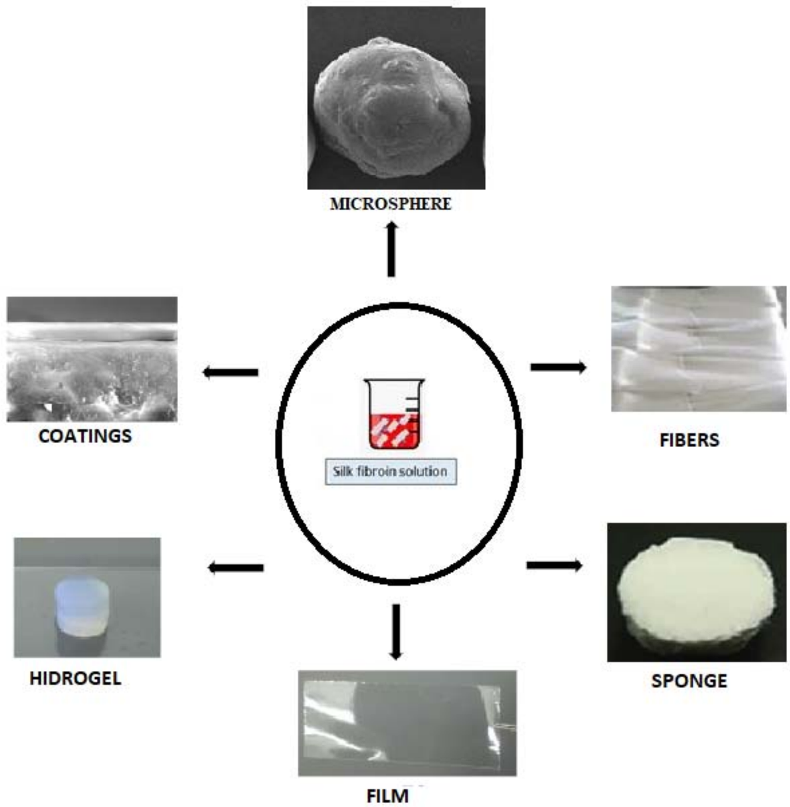

Fig. 8: Derivatives of regenerated silk

i. *Silk film*

For corneal engineering, centrifugal casting produces fit silk fibroin films [38]. They show lower roughness than those made by dry casting and are also better for elasticity and transparency, revealing a good proliferation of human corneal keratocytes. Another suitable technique is the spin coating and layer-by-layer deposition [39]. At the end of the synthesis process, silk fibroin solution has approximately 7.5% concentration wt./vol. The spin-coating solution with glass substrates produced ten samples with a different number of layers: drying at room temperature for some and heating at 60°C for others. Then the optical transmittances observed over the visible-to-near-infrared region showed the values of 95% in the samples at room temperature and 98% in those at 60°C. The number of layers and the heating time don't affect the results.

Each specific biomedical application has modulated biodegradability and its mechanical-optical properties. Studies have shown that the film can support various cell-like epithelium, endothelium, and fibroblasts for tissue engineering [40].

Recently some researchers have designed a technique for the production of patterned silk films to improve cell proliferation [41].

The technique of water annealing after casting allows producing water-insoluble films by forming secondary structures α-helix and β-sheets [42].

Recent work pointed out the validity of the silk fibroin matrix for storing the antibiotic tetracycline and rubella vaccine for six months at 60°C [43].

The silk films constitute the substrate to fabricate electronic components that can be readily integrated and applied by medical care and wearable device. Recently by patterned "casting" strategy, conductive Ag nanowires have been introduced into the silk film for manufacturing conductive devices. The silk fibroin film with patterned Ag nanowires can operate as an interdigital capacitive sensor [44].

ii. *Hydrogel*

The transition to hydrogel occurs under specific requirements [45]. Their preparation takes place without the need for any chemical crosslinking agent.

An increase in temperature or protein concentration and a decrease in ph can control the process. In these conditions, with the change from random coil formation to β-sheets formation, the solution will gel. Subsequently, further aggregation can create gels with a three-dimensional network structure [46].

The low PH and high temperature make the solution unstable. The concentration of the solution shapes the mechanical properties and pore size of the hydrogel.

Currently, the hydrogel products are classified into physical or chemical gels depending on the cross linking method used. The formation of hydrogels occurs through physical interactions such as hydrogen bonding, hydrophobic interaction, electrostatic interaction, and ion interaction in the physical cross linking process. The applied methods are self-assembly, ultrasonication, cutting, electric field, choice of appropriate values of temperature and pH, use of organic solvents and surfactants, etc. The chemical crosslinking method favors the creation of a spatial network structure through covalent bonds between the molecular chains of silk. Chemical hydrogels are physically more stable and more resistant to traction than physical ones. The methods used are essentially photopolymerization, irradiation, use of chemical and enzymatic agents [47].

The possibility of controlling the structural and functional characteristics of silk hydrogels and integrating new biological features have made it possible to obtain a new generation of hydrogels suitable for tissue engineering and drug delivery [48].

Researchers applied also ultrasound energy for the manufacture of silk fibroin hydrogel. Ultrasound causes structural changes and can control the gelation rate through sonication parameters, such as power output, ultrasound time, and silk fibroin concentration [49]. This method is faster and more effective. Adhesive hydrogels can repair hard tissues such as teeth and bones and soft tissues such as the liver and kidney [50].

The Hydrogels are also helpful in maxillofacial and dermal filling applications [51].

iii. *3D Porous Scaffold*

Different experiences have reported the use of silk scaffolds for bone repair and regeneration [52].

The porous 3-D sponges have a high surface area that creates a suitable environment where cells grow at different times [53]. They also model the growth of hydroxyapatite facilitating the osseointegration process [54]. Silk 3-D porous scaffolds require other manufacturing techniques [55]. The silk fibroin concentration represents a decisive element for final porosity formation and mechanical properties. Some work shows that the porosity could be dialed from 80% to 90% by ranging the fibroin concentration between 8% and 16%, respectively [56]. The implanted scaffolds degrade as tissues regenerate, and the degradation rate depends on the type of tissue involved. The researchers can determine the degradation rate through controlling dissolution, hydrolyzing conditions, and freeze-drying [57]. Different techniques allow 3-D scaffolds for bone and cartilage repair with particular reference to porosity and pore size [58].

Researchers had a new experience by inserting the structure of the sponge into a collagen gel. The collagen gel/sponge composite scaffold exhibited better mechanical compression characteristics than the simple collagen gel. It also presented favorable conditions for the proliferation of human mesenchymal stem cells (hMSCs) [59].

iv. *Electrospun fiber*

The scaffolds are a temporary structure to promote tissue regeneration.

Some works show the use of electrospinning to manufacture scaffolds for various tissue engineering applications [60]. The electrospun fibers have a large surface area and porous morphology that favor cell adhesion, proliferation, and differentiation. The authors have further functionalized on the surface by incorporating biomolecules, such as DNA, growth factors [61]. Thus, they can control the proliferation, differentiation, and integration of cells seeded on the scaffold. With this technique, in which the action of an elevated electric field can stretch a polymer jet, it is possible to produce filaments of 2-5nm. The high fineness of the electrospun fibers allows the production of materials with a very high surface/volume ratio and a high porosity. An electrospinning plant mainly consists of an extruder that pushes the molten or solution polymer inside a capillary and a collector placed in front to it (Fig.9). Electrostatic repulsion between the charges at the surface of the solution droplet produces the fiber. The high voltage power supply is applied to the tip of the capillary tube. As the electric field strength increases, the hemispherical surface of the fluid at the end of the capillary tube elongates to form a cone called "Taylor cone," with further expansion, the charged fluid jet is expelled, and stretches becoming very long and thin. Following the evaporation of the solvent, the filled fibers solidify and their collection is done on the collector [62].

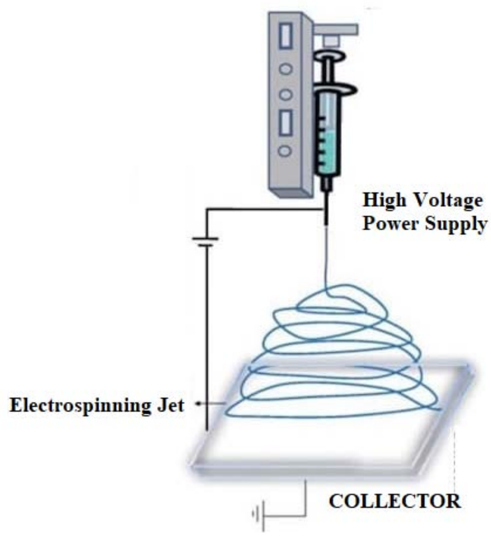

Fig. 9: Scheme of the electrospinning process

We can use two processes: solution electrospinning (employs a system polymer-solvent binary), melt electrospinning (uses melted polymer). Then we characterize the scaffold's properties by acting on various process parameters, such as flow rate, voltage, air gape distance, and solution concentration.

Human platelet lysate (hPL), a set of growth factors and cytokines, can significantly help regenerative medicine. Still, the rapid degradation at room temperature and the difficulty of handling hPL gels have limited its application. Recent studies have shown the possibility of enclosing hPL in an electrospun matrix of silk fibroin to allow its wide use [63]. Fibroin is suitable for preserving hPL activity at temperatures up to 60 ° C. The porosity, conferred by electrospun fibers, favors cell proliferation and makes simple the absorption of exudate.

Electrospun nanofibers have also attracted tremendous attention in manufacturing bone tissue [64], concerning the following conditions: stem cells that differentiate into bone cells, scaffolds that can simulate the extracellular matrix (ECM), and growth factors for a cell. A characteristic that the scaffolds must possess is the high porosity to favor the adhesion of osteogenic cells. Experimental data confirm that patterns obtain the best results with a large surface area, high porosity, and pores of adequate size. Additionally, most tissue regeneration applications require fully biodegradable or absorbable scaffolds. The rate of degradation must be in tune with the growth of the tissues to have a good interaction between fibers and ECM. The electrospun silk fiber osteogenic agents are incorporated into the electrospun silk fibers, thus making the SF a suitable material for reconstructing bone tissue. With the addition of polyethylene oxide (PEO), an increase in viscosity is obtained [65].

These materials have proved useful to produce tubular scaffolds as a small-diameter vascular graft [66]. The methanol promotes the transition from random coil structure to β-sheets.

v. *Microspheres*

Microspheres are materials with spherical shapes and diameters of micro-nanometer dimensions widely used in the biomedical field. The evolution of nanotechnologies made their manufacture possible. Its manufacturing process is complex and requires rigorous control; there are different fabrication methods [67] Recently, researchers developed a method to meet the requirements of crystalline β-sheet structure and size in silk nanospheres [68]. The crystalline content of the β sheet structures is strictly connected with the drug delivery capacity and with the biodegradability rate. The process requires adding polyethylene glycol so that preparation does not require other toxic chemicals and solvents. The salt added to the solution makes the microspheres more homogeneous.

We can produce microspheres by adding lipid vesicles for the controlled application for drug delivery to a targeted area of the body [69]. The removal of lipids occurs subsequently using methanol or sodium chloride, getting microspheres with β-sheets structure. The transition rate from random coil structures to β-sheets depends on methanol concentration. The microspheres treated with NaCl have a smoother surface compared to the methanol treatment. Both types have a mixture of multilamellar and unilamellar structures. For the process is used a lipid (e.g., 1, 2-dioleoyl-sn-glycerol-3-phosphocholine) film to emulsify the solution [70]. Later, with freezing/thawing cycles and lyophilization, water is removed. With centrifugation, the lipid is removed, getting SF microspheres.

The application of polyvinyl alcohol (PVA) is another method for preparing silk microspheres [71]. This methodology foresees a fibroin solution with PVA. The foreseen steps are drying of the solution in the form of films and subsequent dissolution of the films, then removal of the residual PVA by centrifugation. By acting on the concentration of the silk or PVA solution, we can change the size of the spheres. Before mixing the silk solution with the PVA, we must proceed with encapsulating the drugs. The mass ratio between silk and drug is in the measure of 100:1.

VI. Applications

Silk is a textile fiber, also a biopolymer. Its extraordinary biological and functional properties have found wide application from the biomedical field to microelectronics (Fig.10).

Application in the biomedical field

One of the first silk applications was in the medical field due to its essential properties of:

- Biocompatibility, is not rejected by the immune system, does not favor the onset of thrombi;
- Biodegradability, the possibility of regulating its degradation rate in the absence of inflammatory reactions, and its
- Easy sterilization makes it preferred to other materials of a synthetic nature;
- Realization of scaffolds represents a structure suitable for in vivo tissue repair by promoting cell adhesion and growth.

Application in the pharmacological and therapeutic field

Another silk application is the controlled release of drugs to pre-established organs and the monitoring of definite pathologies. We create different forms to encapsulate pharmacological molecules, bioactive macromolecules such as enzymes, or other types of cells.

Application in the field of microelectronics

A further silk application is the realization of biosensors for monitoring physiological parameters such as heart rate or body temperature and implantable silk bio-electrodes in the human body to replace invasive needles.

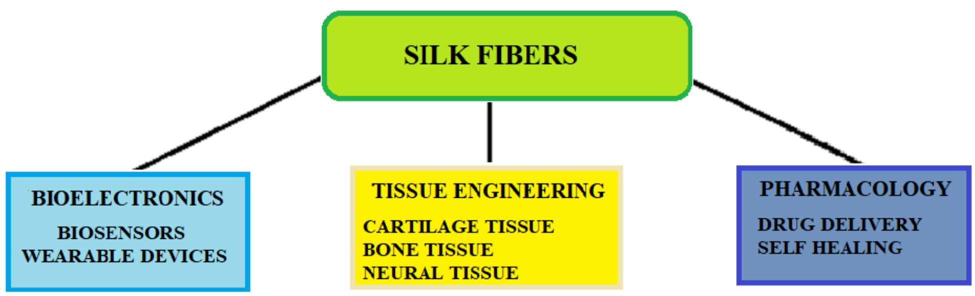

Fig. 10: Silk applications

VII. Biomedical Applications

The recognized natural silk has extraordinary properties that make it suitable to regenerate or improve the functions of damaged tissues and organs. They are biocompatibility, biodegradability, anti-inflammatory, the capacity of promoting the attachment, proliferation, and differentiation of many different cells type. For these properties, it is also an essential structure for the adhesion of growth factors and to incorporate drugs [72]. In addition to rebuilding damaged areas, silk matrices also provide bioactive molecules, genes, and cells.

The path for the preparation of tissue for reconstructive medicine consists of several steps (fig. 11).

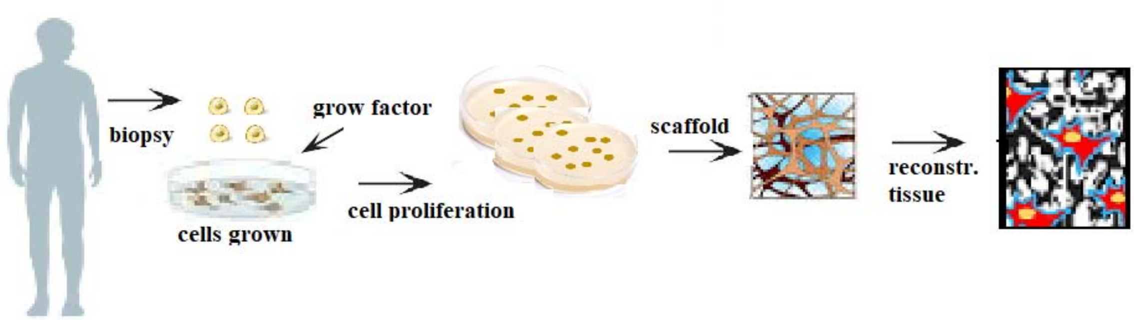

Fig. 11: Engineered tissue preparation steps

Cells are biopsied from patients and grown in 2D culture to which we add growth factors. Subsequently, these cells, once expanded, are seeded on scaffolds for the engineering of specific tissues. We can transplant the reconstructed tissue into the patient.

a) Wound healing

The skin protects the human body from dehydration, infectious agents, and, more generally, from environmental conditions. Any damage produced by burns or wounds on the skin reduces its protective effect, so tissue engineering interventions restore skin loss. Studies conducted in vitro and in vivo highlighted that the silkworm or spider SF-based biomaterials favor cellular adhesion and fibroblast proliferation on skin wounds and improve plasmatic imbibition capabilities to promote wound healing [73]. Thanks to its properties, this biomaterial is applied alone or scaffolds for nanofibrous mats, hydrogels, sponges, or films tailored to tissue engineering. SF films showed to have more cure potential than conventional hydrocolloids. Wounds treated with silk films have better collagen regeneration, easier re-epithelialization, and heal earlier. The nonwoven SF is biocompatible and allows the growth of any type of human cell [74]. The histological finding revealed that dermal fibroblast proliferates on fibroin coating and scaffold without an inflammatory response. Oral keratinocytes can proliferate on woven fibroin meshes. Researchers developed chitosan-based (CS) hydrogels loaded with silk proteins (SF) and l-proline (LP), an amino acid necessary for collagen synthesis, via physical crosslinking to speed up the healing process. Studies showed that hydrogels incorporated into LP, compared to other composites, reduce the healing time [75]. The nanofibrous membranes have a high surface-to-volume ratio.

Furthermore, interconnected pores promote cell penetration and nutrient exchange and favor hemostasis and absorption of exudate from the wound. To increase wound healing were produced, electrospinning, nanofibrous asymmetric membranes. These had a top layer which is composed of SF and poly (caprolactone) (PCL) and a bottom layer compound of SF with hyaluronic acid (HA). The combination of SF with PCL lets it get epidermis-like properties such as hydrophobic character, waterproof ability, and mechanical resistance. The combination of SF/HA allows dermis-like properties such as absorption of the exudate from the wound, cell adhesion, and proliferation [76].

b) Drug Delivery

We can use fibroin nanoparticles for the controlled delivery of drugs for specific clinical needs (Fig.12) adjusting the encapsulation capacity and release rate through the crystallinity and concentration of the silk fibroin. They are also used to deliver proteins and peptides [77]. Recently, they are applied to target cancer cells. The technique used allows to target the diseased cells and save the healthy ones, thus reducing the toxic effects and improving the effectiveness of the therapy [78]. Some researchers applied a therapy based on lyophilization of SF with emodin-loaded liposomes and methanol to treat breast cancer [79]. Also, curcumin in silk nanoparticles has proven to be an effective method for treating breast cancer [80]. Another innovative experience is that carried out for the release of anticonvulsive adenosine with silk encapsulation of adenosine dust reservoirs. The thickness, crystallinity, and morphology of silk were analyzed to study the relationships between the silk coating of the adenosine reservoirs and the release time. The thickness of the tank coating and the number of layers applied to affect adenosine release; with their increase, the average rate of release decreases by increasing its duration [81].

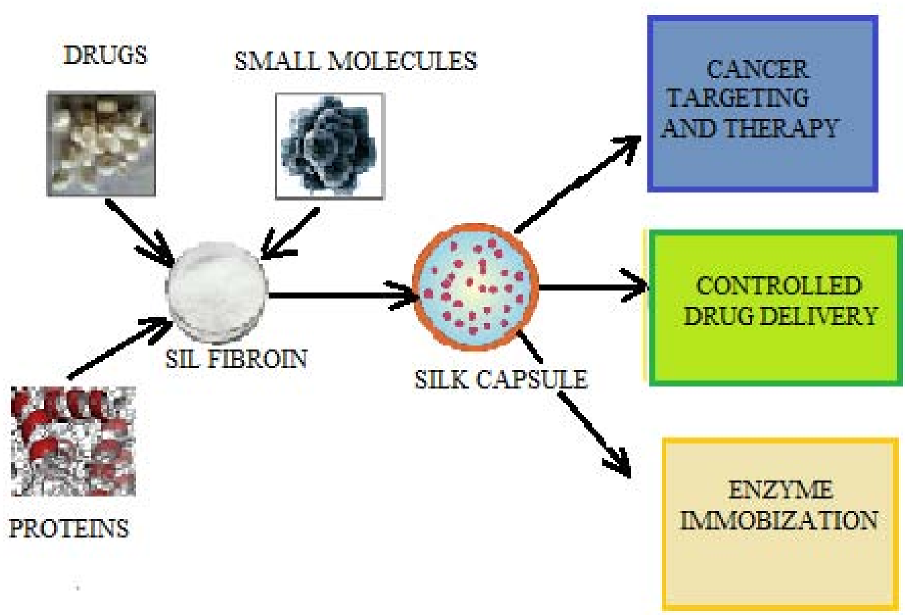

Fig. 12: Drug delivery

Global Journal of Researches in Engineering (F) Volume XXI Issue III Version I Year 2021

c) Bone Tissue Engineering

The application of silk proteins in bone regeneration allows modelling the growth of hydroxyapatite by improving the osseointegration process [82].

The healing time in fractures or bone defects is closely related to the extent and extension of the damage.

There are two different repair techniques; one is called autograft and the other allograft. The first refers to the transplant of tissue within the same body. The second refers to how the tissue comes from a foreign body. The first research for human tissue reconstruction by silk took place at Tufts University of Boston (USA) [83], where they applied different forms, such as film, electrospun scaffold, and 3-D porous scaffold. They preferred the use of silk, as a basic structure, over other different materials available, for its property to improve the osseointegration process [84]. Then with the addition of rBMP2 (recombinant human bone morphogenetic protein-2), a rapid diversification of osteoblasts is also obtained [85]. Researchers have made compounds by incorporating silk into various biopolymers. Compounds of silk and hydroxyapatite (HA) have also been effective in bone repair as HA is biocompatible and has good osteoconductivity and osseointegration [86, 87]. It has been noted that combining SF with HA, a good compound biopolymer suitable for bone engineering, is obtained [88].

Researchers experimented with solutions of poly (ethylene oxide) PEO mixed in fibroin and analyzed how fibroin on PEO matrices influences the healing process of the breast implant [89]. The level of silk concentration affects the conformation of the PEO scaffolds structure and the process of cell adhesion and proliferation of HaCaT (human keratinocytes cells). The first cells surrounded the wound but subsequently the following factors influenced their increase: growth factors, degree of cell adhesion, and their differentiation. Therefore, it is possible to have composites without defects by acting on the silk concentration during the electrospinning process. Silk hydrogels with Arg-Gly-Asp (RGD) peptide gelling adhesive and bone marrow-derived mesenchymal stem cell encapsulation are suitable for bone engineering beyond improving cell adhesion and favoring osteogenic differentiation. [90].

Xenografting refers to the transplantation of human tissues or cells into animal models and is widely used in research to evaluate the effectiveness of the graft and its physiological interactions [91]. The graft must satisfy specific characteristics to ensure the formation of new and healthy tissues. In the first place, a porosity of such dimensions allows the vascularization and construction of new bone. Secondly, a surface will enable vascular growth, attachment of bone cells, migration, and proliferation. The third is adequate mechanical resistance to compression and elasticity. The last requirement is sufficient dimensional stability. Xenograft models find use in cancer research and therapy. In addition to applications in regenerative medicine, xenograft models of human bone are also used as experimental models of skeletal diseases, allowing for studying disease states in vivo, which would otherwise be impossible on human subjects.

Stem cell-based (BTE) bone tissue engineering represents a clinically meaningful solution for bone repair. We present a pure 3D silk fibroin (SF) scaffold used and fabricated by the freeze-drying method in the study. Human adipose-derived mesenchymal stem cells (hASCs) were seeded into the scaffold to facilitate bone regeneration [92]. The researchers evaluated the efficacy of xenograft SF-hASCs scaffold on the ability of bone regeneration in critical vital defects in the rat.

Previously, the culture of HASCs in organic and inorganic sources highlighted their potential for osteogenic differentiation. This coupling produced rapid vascularization of the implanted area and improved bone formation. The researchers after generating two 5 mm (diameter) cranial bone defects in the skull of the model of 30 mice model, with the aid of a dental bur, inserted the xenografts The defects were partly filled with the SF-hASCs scaffold and partly with the SF scaffold only. The implant was successful without any bleeding or infection complications, confirming the perfect biocompatibility of silk fibroin. After a period of six and twelve weeks, the researchers performed the micro-CT analysis to verify the growth of new mineralized bone. The results confirmed the newly formed bone is densely localized at the edges of the defect, especially in the xenograft SF-hASCs compared to the SF. They also observed that the degradation rate of the scaffolds was compatible with the regeneration of bone tissues. The results obtained revealed that the SF scaffold incorporated with hASCs had superior biocompatibility and osteogenic capacity to promote bone regeneration.

The presence of hASC in the SF scaffold favored the transformation of osteoblasts into osteocytes producing an effective bone remodeling process and an improved bone extracellular matrix in the defect area. These results confirmed that the union of stem cells and SF may be an excellent functional bio-scaffold for forming new and healthy tissues.

Alloplastic bone repair materials are a valid alternative to autograft. Alloplastic is synthetic bone substitutes easily accessible and that do not require a patient donor. The alloplastic materials must possess specific requirements such as biocompatibility with host tissues, non-inflammatory and non-antigenicity. Furthermore, they must reproduce the porosity of the cancellous bone. The pores of the bone substitute must be of sufficient size so that there can be migration of cells and passage of blood vessels through them. The pores must be connected, not isolated.

Additionally, they must stimulate bone induction, resorbable and replaceable by bone, stable in varying temperature and humidity.

The researchers analyzed many materials to make these synthetic grafts, including metals, ceramic materials, polymers, and their composites. Choosing the most suitable material is essential as it must have the mechanical properties suitable to support the expected loads depending on the type of application.

Metals such as titanium, nickel-titanium, and magnesium alloys are biocompatible, resistant, workable but have a higher elastic modulus than bone which can cause stress-shielding. (This phenomenon consists in a loss of density due to the reduced load on the natural bone compared to the metal fixation device, no stimulus for continuous remodeling necessary to maintain bone mass).

Because of their fragility, ceramic materials are challenging to use as a scaffold for synthetic bone tissue.

Polymers are excellent candidates for bone grafts due to their physical properties and chemical, have excellent biocompatibility. Still, their poor properties of mechanical strength make them inappropriate to manufacture bone scaffolds that they must instead support excessive loads [93].

In recent years, researchers have developed manufacturing studies on alloplastic grafts based on organic and inorganic materials that can mimic the structure and function of natural biomaterials. They obtained satisfying results with the combination of SF membranes with hydroxyapatite (HA). Hydroxyapatite (HA) is a hydroxylated calcium phosphate salt with a high degree of hardness and a significant component of the inorganic substance found in bones and teeth. For its characteristics of bioactivity and resorption and facilitating the binding of the graft to living tissues, it allows obtaining the slow and gradual degradation of the implanted material simultaneously with the growth of new tissues.

SF / HA scaffolds are preferred over other HA-based bio-materials as they reduce the risks during bone implantation due to their excellent bioactivity, proliferation activity, and osseointegration. Their porous structures ensure better transport of blood and body fluids for metabolism and bone growth. These properties make them very similar to natural bones. The researchers apply several techniques to prepare SF / HA scaffolds, such as freeze-drying, electrospinning, gelation, and cold drying.

Researchers at Tufts University in Boston, led by Kaplan, were the first to study the efficacy of SF / HA old scaffolds in bone regeneration. They found that hydroxyapatite is a substance with exceptional biocompatibility and bioactivity and is substituted, after grafting, with the bone growing through the osteoinduction process. Regeneration occurs via two methods, osteoconduction of the surrounding bone in the defect area and nucleation. They noted that reconstruction in the presence of the hybrid composite was faster than regeneration by the surrounding bone and that ossification was constant in all areas, including the center of the bone defect [94].

Researchers investigated bone regeneration using silk hydroxyapatite hybrid composite in a rat alveolar defect model [95]. For testing, they were used thirty-six male Sprague-Dawley rats of 9 to 10 weeks of age and weighing 240–250g, divided into three groups of 12. The first group was sutured without a scaffold bone graft, the second group was sutured with a silk scaffold graft, while the third group was grafted with a hybrid scaffold of silk and hydroxyapatite. In rats, a 7 x 4 x 1.5 mm alveolar defect was created by incision using a power drill in the mucous membrane between the hard palate of the right upper jaw and the alveolar bone. They produced scaffold by mixing an aqueous silk fibroin solution, previously refined, with granular hydroxyapatite at a 10: 1 ratio and sterilized by irradiating with gamma rays after freeze-drying three days. The pretreated scaffold was cut to the same size as the bone defect and grafted into the created cavity. The mucosa was then sutured using black silk. Inspections were carried out every four weeks from the fourth to the twelfth to verify the state of growth of the new tissues. They used different types of analysis, visual analysis, tissue analysis, and CT of the bone defect; in the twelfth, they used the Western Blot technique to verify the degree of bone generation. The authors found that mature osteoids appear at the eighth week and only at the twelfth week, observed through the bone defect tissue analysis, forming bone cells. Moreover, they observed that in the samples in which the hybrid scaffolds were present, the generation was faster, and there was constant ossification in all parts of the defect, including the center. The new bone is generated via two processes, osteoinduction, and osteoconduction, from the boundary and the center of the bone defect.

d) Ligament/tendon

Ligament/tendon injuries are common in sports. The repair frames must have high mechanical strength, ligament formation capacity, and biodegradability. Silk can be aggregated with other compounds, making it suitable for the repair of ligaments and tendons. Experiences have shown that silk scaffolds are valid for repairing the anterior cruciate ligaments (ACL), Achilles' tendon, and other ligaments [96]. Researchers found that silk scaffolds and, in particular, knitted silk scaffolds and silk sponges facilitate the growth of mesenchymal stem cells. The silk fibers deprived of sericin can be wrapped in yarns for the production of ligament matrices. These matrices have the same structure as collagen fibers present in human ligaments. It also features the same mechanical stimulation as the native ligament. The native Bombyx mori SF lacks the peptide sequence RGD (arginine-glycine-aspartic acid) responsible for cell adhesion to the ECM [97]. It is necessary to resort to either the surface coating or the chemical coupling with the RGD or specific growth factors to favor the adhesion.

Another study experimented with a composite formed by an electrospun scaffold of SF and collagen with the addition of BMP-13 (bone morphogenetic protein-13). Subsequently were crosslinked with methanol and ethanol, the treatment with methanol ensured a higher mechanical resistance. There was a good adhesion and proliferation of stem-derived adipose cells (ASC) on these structures, demonstrating that BMP-13 is a factor that improves cell infiltration. The scaffold obtained is a regenerating matrix with improved tensile strength [98].

TEND (Tissue Engineered Device), obtained by mixing type 1 collagen and PDLLA (poly DL-lactide) solubilized in DMSO (dimethyl sulfoxide), represents an effective solution. The morphological dimensioning of this structure allows improving cell adhesion, ensuring tissue integration and mechanical resistance [99].

e) Cartilage

Hyaline cartilage is the cartilage most present in the body and covers the surfaces of the joints, favoring the transmission of mechanical loads with a low coefficient of friction. The hyaline cartilage matrix is rich in collagen fibers type II and proteoglycans; it is an avascular and aneural tissue. It can be damaged if subjected to significant mechanical strain. The damage is produced either by trauma or by deterioration due to age. Surgery on cartilage essentially takes place in the orthopedic sector to restore joint function or repair the loss in an auricle, trachea, nose, or eyelid. Silk scaffolds are also valid in the regeneration of cartilage tissues, but they must be appropriately structured to allow the growth and differentiation of chondrocytes. The scaffolds can be used for in vitro cultures or transplanted directly into the organism as a colonization medium for stem cells [100].

Pioneers in the combined use of silk scaffolds and human chondrocytes were researchers from Tufts University in Boston (USA) [101]. They experimented with the combination of SF with PLLA (polylactide) scaffolds. The results obtained from in vitro experiments found that the adhesion, growth, and proliferation of chondrocytes are significantly better than unmodified PLLA scaffolds [102]. They also compared porous silk fibroin scaffold with collagen scaffold regarding adhesion and proliferation of human articular chondrocytes and mesenchymal stem cells. In vitro cultures porous silk scaffolds showed the best results; the GAG (glycosaminoglycan) content was also higher. Based on previous experiences on the ability of silk fibroin scaffolds to behave in the same way as ECM (extracellular matrix) towards chondrocytes [103], some researchers have fabricated curcumin and silk scaffolds with saline leaching method [104]. The scaffolds obtained were porous and rough, thus favoring cell adhesion [105]. Curcumin is a yellow polyphenolic pigment found in turmeric spice. It is known to have antioxidant, anti-carcinogenic, antiangiogenic, and anti-inflammatory effects [106]. These last two properties lengthen the cellular lifespan. The tests carried out showed that increasing the concentration of curcumin improves the device's mechanical strength and increases cell proliferation and ECM formation.

The reconstruction of the auricular cartilage uses composites with PVA [107]. Polyvinyl alcohol (PVA) hydrogels form hydrophilic 3D polymer networks. They ensure an excellent elasticity that makes them preferable in reconstructing tissues and, in particular of cartilage. In particular, they possess some specific properties of ear cartilage, such as cell immobilization, solidity, flexibility, and resistance to pressure and traction [108]. Its mechanical strength is very similar to that of human cartilage [109]. We tried to create composites with different percentages of silk and PVA, also using various techniques such as salt leaching and freezing-thawing. But from the results obtained for the growth of chondrocytes and mechanical characteristics, the best were those with 50% PVA and 50% S.F. They also did not exhibit any rejection or inflammation reactions.

f) Vascular Tissue

SF produces highly biocompatible tubular vascular graft architectures by preventing fibrous tissue responses, thus providing an adequate solution for vascular dysfunctions. In coronary or peripheral vascular bypass surgery, it is necessary to integrate the current procedures to replace vascular tubular tracts taken from one's own body or with artificial lots. Sometimes auto-grafts are not possible for several critical factors related to the presence of already carried out self-grafts or advanced auto sclerosis of the arteries. Even small-caliber wholesalers made from polyethylene compounds involve several complications that discourage their use. For its characteristic properties of biodegradability or biocompatibility, silk fibroin is a suitable solution for small-caliber blood vessels. Silk electrospun tubular scaffolds are used for vascular tissue engineering as they are porous and resistant mechanically [110]. The porosity is ideal for the endothelization of vascular grafts.

Different techniques for the production of tubular scaffolding range from filament winding to spinning gel weaving and electrospinning. The research aims to construct multilayer tubular scaffolds to get as close as possible to the functional characteristics of the artery [111-112]. Crosslinking agents are also used to make them more porous and elastic and to set degradation. A tubular scaffold SF ES-TEX-ES (Silk Graft) provided good results to treat small-caliber blood vessels [113]. The structure has a three-layer hybrid architecture, formed by E.S. (electrospun) layers, one inside and outside, and in the middle TEX (textile) layer. The hybrid architecture was engineered for the pre-surgical manipulation of the device and to improve the aggregation between the tissues and the mechanical resistance. It is the TEX layer that gives the device a high mechanical resistance. It is obtained, first electrospinning, during the manufacturing process, coating the TEX surface with the ionic liquid to achieve complete adhesion between the three layers, ensuring the high mechanical resistance. After a series of in vitro tests, the researchers implanted artifacts in large animals for in vivo tests. Experiments allowed to study the type of interaction with fibroblasts and endothelial cells; cytokines and chemokines were analyzed to verify the proliferative and anti-inflammatory capacities. The researchers also performed a blood component test. The results obtained were all encouraging for subsequent in vivo experimental studies.

Micro-nanoscale topographies influence the process of cell adhesion, proliferation, and migration.

Experiences made, designing fibroin silk with different structures [114] confirmed their suitability for the manufacture of micro blood vessels. Small-diameter graft structures of S.F. electrospinning and coated with silk sponges showed good mechanical resistance and water permeation [115]. The results obtained found that the coating with silk sponges increases endurance and elastic modulus of the device while the permeability decreases, enhancing the fibroin silk sponges.

When the fibroin is coated with gelatin, the properties to promote endothelial cell attachment and the development of a micro vessel-like structure improve [116]. Studies showed adequate morphological properties combined with good stability in an aqueous environment and good mechanical properties.

Experiments proved that the culture of endothelial and osteoblasts in silk fibroin is essential in forming micro vessel-like structures. These materials have good anticoagulant activity and platelet response. The ability to promote vascularization while withstanding vascular pulsating pressure makes silk fibroin a suitable graft for blood vessel repair.

VIII. Optical and Photonic Applications

Thanks to its physical and structural properties, technological evolution in production, and functionalization techniques, it was possible to apply silk in technical fields, including optics and photonics. By modulating their self-assembly through the regeneration process of the silk fibers, it is possible to obtain materials in different formats also in the optical sector. Among other things, the possibility of getting structures on a micro-nanometric scale associated with its biocompatibility and biodegradable properties has made it possible to create implantable and sustainable optical devices, contributing to the reduction of environmental pollution [117].

Furthermore, taking into account the variability of its structure in correlation to surrounding factors, it was possible to manufacture tunable optical devices in the field of environmental sensors [118]. The regenerated silk fibers show many attractive characteristics, such as making them suitable for realizing devices in the optical and photonic fields with the other property of creating optically transparent and multifunctional supports.

One of the sectors in which silk has found application, using its high refractive index, is that of the manufacture of optical fibers and waveguides with low optical losses [119]. The guides were made either by integrating regenerated silk with other optical platforms or combining different silk formats with distinct refractive indices or using natural silks spun directly from silkworms or spiders with higher optical losses.

It is possible to manufacture plasmonic devices by integrating metallic plasmonic nanostructures with silk matrices [120]. These hybrid structures generally consist of periodic or aperiodic arrays of nanocylinders, nanospheres, and nanoantennae. Some designs applied matrices of silk and metals such as gold and silver.

They can function as biosensors using the refractive index shift in correspondence with strong plasmonic resonances [121].

Other integrated compounds are the metamaterials with silk proteins. Metamaterials are artificially structured compounds whose properties arise from their structural composition rather than from materials. Silk represents the substrate of the device in which plasmonic and metamaterials patterns are incorporated. It constitutes the platform of optoelectronics devices that sensors or implantable devices can use. [122]. The variation of the dielectric constant of silk in the presence of humidity monitored the level [123].

Researchers resort to silk to obtain structural color materials that have different optical characteristics than pigment colors. The interaction of light with periodic nanostructures makes structural colors. We can achieve structural color functions through nanofabrication techniques associated with the intrinsic properties of silk. Many structurally colored materials are iridescent, such as opal; the color changes with the viewing angle and orientation. The experience made on the possibility of obtaining controllable structural color through nanostructured periodic lattices in silk protein films is helpful [124]. The periodic lattices of nanopores give particular scattering features to the films by realizing a

Global Journal of Researches in Engineering (F) Volume XXI Issue III Version I Year 2021

coloration when they are hit by white light. The lattices exhibit a different coloration as a function of the variation of occupied space. Furthermore, by varying the refractive index, a shift in the spectral response occurs. This phenomenon makes it possible for photonic gratings as substrates for optical sensors.

Researchers developed numerous experiences in recent years; some considered innovative solutions are listed below.

a) Sensors

Due to their excellent features, silk fibers also find application as optical fibers. Dragline spider silk acts as a sensor to detect humidity, and more generally, as a chemical substance detector (ethanol, ammonia, etc.), representing a better alternative to glass fibers that usually require coatings [125]. The physical principle underlying this experience consists of the optical fiber's reaction in contact with the targeted chemical species. Under the action of these substances, the fiber will change throughout its volume. Consequently, the parameters of the light (intensity, phase, spectrum) that propagate along it will also vary. From the analysis of the light spectrum, it is possible to trace the presence of the substance. The light is kept wholly confined to the fiber, and the effect accumulates over its entire length. We produce Dragline silk extracted from spider glands and spinning with a constant diameter and smooth surface as a chemical sensor for its sensitivity to different chemical substances. Their presence affects the birefringence of the silk fibers [126]. Due to humidity, the phenomenon of infiltration of water molecules occurs, which attacking the coil structures of silk fibers breaks the hydrogen bonds that hold the protein threads together. This phenomenon will cause the silk to relax, causing it to contract. The sample designed to measure the relative humidity of the environment is placed on a support and blocked at the two end parts.

As the fiber is held back from resting, it tends to swell, producing a change in its geometry. The researchers tested the sample using a set-up consisting of a laser to produce polarized light injected into the silk fiber positioned inside an air chamber and an analyzer polarization located downstream of the air chamber to analyze the state of polarization (SOP). The hermetic closure of the air chamber ensures that the pressure and temperature are kept constant so as not to affect the test results. The points of change of the SOP of the outgoing light are reported as positions on the Poincarè sphere. By increasing the humidity level inside the chamber from 40% up to a maximum of 65%, a corresponding variation is noted on the sphere's rotation angle between the initial and final positions. The remained almost constant when the experiment was performed, keeping the humidity rate stable. This result confirms the high sensitivity of the sensor.

Another experience is the realization of a capacitive type sensor for respiratory monitoring based on the detection of the dielectric constant of silk fibroin film [127]. The variation of the capacity produced by the introduction of steam provides information on the respiratory state. The knowledge of the respiratory state is a determining factor for knowing the state of health of the subject concerning the activity performed such as sleep or physical exercise or for the detection of abnormal cardiovascular situations such as the presence of flu, pneumonia, and asthma [128]. The changes in the respiratory state are related to the changes in humidity produced during the inhalation and breathing phases. The changes in resistance or capacity of the humidity sensors provide the measure of respiratory changes. The researchers used sensors consisting of interdigital electrodes of silver nanowires (IDE Ag NW) associated with an S.F. sensing film. The sensor can distinguish the type of breathing, whether regular, deep, and fast, by detecting the frequency value for a range up to 4 Hz. The sensor is placed under the nose because there are local changes in humidity in the mouth and nose area. The results obtained showed that, with the penetration of vapor, through the variation of the film's dielectric constant, it is possible to detect human respiration.

b) Filter

Researchers developed an eco-friendly physical dispersion process with partial diffusion to exfoliate silk fiber into mesostructures like nanorods, nanofibrils, nanoparticles. The resultant mesh silk dispersion can be treated to produce mesostructures of different sizes. Compared to those produced by silk fiber solutions, these materials have maintained the same microarchitectures but are also enriched with structures and properties deriving from mesh sizes effects such as ultra-high specific surface area and a system like extracellular matrix. These characteristics make it possible to use them in electronic and environmental applications, making them better than other commonly used materials. They were applied as a filter for water treatment, recycling organic solvent, like sensors, and nano fertilizers [129].

Another application is a thin silk paper created as a filter by adding two wt% silk microfibr il suspensions for the water treatment. At the end of the air drying, silk paper in A4 format was obtained, which was softer and foldable in different shapes compared to the traditional cellulose-based paper. SEM showed a uniform mesoporous structure. This mesostructure was used directly as a microfilter of water for bacteria and other micro-sized contaminants. To remove organic solvents, it was necessary to reduce the pore diameter of the silk paper using nanofibrils. SEM images confirmed the filtering effectiveness of these silk papers. The membranes used for water treatment were produced from Bombyx mori silk nanofibrils [130]. Organic solvents dissolve them; Antheraea pernyi silk nanofibrils were chosen because most solvents tolerate them. Therefore A. pernyi silk nanofibrils were preferred as filters for organic solvent.

Scientists used silk paper as a conductive device by printing an electronic pattern such as a circuit board and sensors loops. It can be chosen as electronic prototyping. They are suitable for modulating electrical signals but being flexible can measure the deformation of objects subjected to stress. Devices subjected to several bending cycles show no deformation when returning to the initial realignment position.

The excessive use of pesticides and fertilizers in agriculture has created significant environmental problems such as soil foul-up from antibiotics and acidification and pollution of the aquatic environment due to excess nitrates and phosphates. As a result, nano fertilizers have developed in recent years and controlled release pesticides. The goal is to create products absorbed by the cultures without interaction with the external environment [131]. For this purpose, researchers tested whether the use of A. pernyi silk nanoparticles of 11 $\pm$ 4 nm can provide fertilizers. Bulbs of Narcissus pseudonarcissus (N. pseudonarcissus) incubated in a culture medium were grown, using homogeneous dispersion of silk and rhodamine B (RhB) nanoparticles to deliver small molecules for drugs and fertilizers. The bulbs continued to grow during the eight days of incubation, showing that both RhB and silk nanoparticles were not harmful. They studied cultures with a fluorescence microscope, and synchrotron Fourier transform infrared microspectroscopy (S-FTIR); the provided images validated that the silk nanoparticles facilitated the release of nutrients. Furthermore, they found that adjusting the rate of release of the nano-fertilizers can reduce the quantities.

c) Waveguide

Silk is suitable for manufacturing implantable optical fibers and waveguides thanks to its high refractive index. We can also integrate it with other optical platforms for the realization of light guiding. Non-regenerated Bombyx mori silk fibers are designed as waveguides, especially when embedded in living tissue, thanks to their biocompatibility and bio absorbability. The images obtained from the samples produced [132] showed configuration defects such as helical torsion and symmetry breaking in the fiber center. These factors are both determined at the pre-processing stage [133]. Their effects are limited through multiple rinsing operations but not entirely removed by pre-processing. To evaluate their applicability as waveguides, we can characterize them optically using two methods: the cutback method commonly used to determine losses in waveguides as is usually done in telecommunications for optical fibers.

The technique uses the Beer-Lambert law of attenuation, which considers that the propagation losses along the fiber length are prolonged. The other method used is Image-based Analysis, according to which the performance of the fibers is evaluated directly from the microscope images. This method is based on the scattered light at a certain point of the fiber that is proportional to the radiation of the wave that propagates within it. Therefore, the measure of wave attenuation inside can be deduced from scattered light as a function of position along with it. The researchers experimented that when a large amount of light is highlighted in a certain point of the fiber, it means that point is the localized scattering center of the light wave that travels in it. From that point on, the optical power gradually decreases along with it. The optical wave continues to propagate with a lower intensity. The loss coefficient obtained depends on many factors, such as the effects produced by the localized scatterers, the structural characteristics of fiber and waveguide. From the images acquired by a non-linear microscope, the second and third harmonic presence was noted in the signal, with the third harmonic present along with the entire fiber. In contrast, the second was more relevant in the center where the center-symmetry breaks due to twisting of the fiber around this point.

A further experience gained in the field of optoelectronics was the design of waveguides with the use of direct femtosecond laser writing (fs-DLW). This innovative approach represents a suitable platform for biophotonic applications [134]. A femtosecond laser is widely applied to fabricate optical devices presenting physical effects and interactions with various materials different from traditional processes [135]. The laser beam develops intense energy capable of producing structural changes at the micrometric level. It also has the advantage of reducing thermal effects. The SEM images found that the magnitude of the impulse determines the groove width without damaging the surrounding areas. The analysis of AFM images showed that the tracing of the profiles is obtained by transferring material from the central location towards the edges. This effect is due to the way the laser beam operates according to the Gaussian profile. RAMAN spectroscopy made it possible to highlight structural changes in densification zones. The spectra analysis showed that there are no significant structural changes in the irradiated fibers. The images obtained confirmed that the light propagates along with the areas where there is the densification of the material.

The same behavior occurs for both linear and curved guides. From research emerged that propagation losses were (5.8 $\pm$ 1.0) dB / cm and (6.8 $\pm$ 1.0) dB / cm at 632.8 nm for linear and curved waveguides. The propagation losses in these guides are more significant than those obtained by direct ink writing. This greater loss is probably attributable to surface roughness in the thickening areas of the material. Although the losses are high, they are comparable with those found in unprocessed silk fibers (10.5±4) dB/cm, in the range of 800–850 nm [136]. The results obtained through simulation and experimentally showed an almost symmetrical mode in which the light confines to the waveguide edges. The radiation produces deformation on the edges of the irradiated area by varying the refractive index, which is lower in the central region than the edges with a value equal to $\Delta n \sim 1 \times 10$-3. A He-Ne laser with 632.8 nm light produced the characterization of the waveguides. We obtain the optimal conditions for the manufacture of the waveguides by varying the pulse energy in the range of 16-34 nJ and the scan speed in the field of 50-400 μs, obtaining the best results for 26 nJ and 100 μs.

d) Lenses

The silk fibroin protein can be a biocompatible alternative to conventional emitting diode polymers [137]. The blue light-emitting diodes allow enormous energy savings by implementing a total replacement of traditional light sources [138]. Recently, light-emitting diodes are also present in automotive, home, office, and display applications. The increase in the use of LEDs will consequently also lead to a more excellent production of electronic waste upon their disposal, which, although in part is recycled, the remainder still damages the environment [139]. In a typical LED chip, among the various components, there is also a lens manufactured with non-biodegradable silicone-based polymers and epoxy resins. Therefore, finding solutions on eco-compatible materials to realize LEDs represents a significant step toward improving environmental protection. Since there are currently applications of silk hydrogels in electronic devices, their feasibility for manufacturing light-emitting diode lenses has been tested. The tests carried out found that the concentration rate of the silk and the type of crosslink used characterized the optical properties. By decreasing the concentration of proteins, the transmittance increases, while the average transmittance of the hydrogel decreases slightly in the visible spectrum compared to the phase of the silk solution. The refractive index was also evaluated, its value is slightly higher than that of the silk solution, resulting in 1.35 at 448 nm.

The distribution of the intensity of the spatial radiation was tested using silk hydrogel lenses in the shape of a dome and crater. The comparison of the emission profiles showed that the dome hydrogel lens focused in the center similar to that of the LED without lens, while the crater lens had two maximum values at 20 ° and -20 ° of the viewing angle. Then the researchers tested the stability of the silk hydrogel lenses. To increase their durability, they synthesized polyester-urethane biocompatible reference material with which coated the lens; stability is increased approximately three times and significantly by using edible paraffin.

This type of lens can be considered an environmentally friendly electronic device.

IX. Electronic Applications

Silk-based devices have characteristics that make them suitable for applications in the electronics field. Silk as planar structure replaced inorganic oxide layers such as PMMA or SiO2. It is present in organic transistors as gate electrodes, source and drain electrodes, or also as a substrate on ITO gate layers and as a p-type and n-type organic semiconductor (Fig.13).

Silk is a material whose properties are suitable for flexible electronics. We must generate silk fibroins in films (RFS) that exhibit the desired optical, mechanical, and chemical properties. The creation of fibroin structures around carbon nanotubes (CNTs) has made it possible to create flexible and transparent films that are more sustainable and degradable, although similar to synthetic polymers.

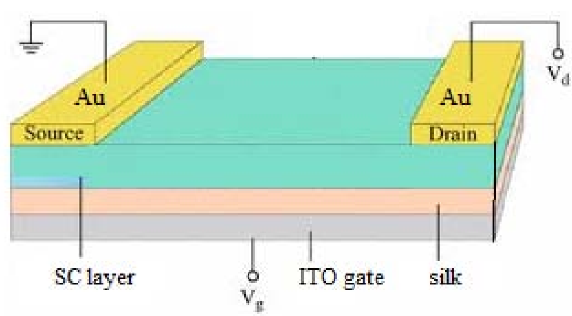

Fig. 13: Organic silk transistor

a) Semiconductor

Researchers used regenerated Bombyx mori silk matrices integrated with zinc oxide and copper oxide nanoparticles to produce n-type and p-type semiconductors, respectively [140]. To optimize the electrical conductivity of the silk nanocomposite films and metal oxide produced by the solvent casting method, samples with different metal oxide concentration values and film thicknesses were tested [141]. The tests carried out showed that the nanocomposites films with lower thickness and the higher concentration of metal oxide exhibited the best conductivity. The presence of metal oxide nanoparticles in the matrix influences the mobility of the vector (electron-hole) in the matrix by modifying the conductivity of the films. The SF/ZnO nanocomposite film had the highest conductivity between the two semiconductors. The results showed that the conductivity of the semiconductor depends on the type of carrier concentration, the entity of the metal oxide nanoparticles, and the content of the β sheet.

To study the semiconductor behavior of nanocomposite films, the researchers used the Hall effect, which is a physical phenomenon. A potential difference is produced in a transverse direction in a conductor or semiconductor when it is crossed by an electric current in a longitudinal direction and is subjected to a perpendicular magnetic field. Through the Hall effect, we can consider the semiconductor type p if its value is positive or type n if its value is negative. The incorporation of zinc oxide nanoparticles makes the device an n-type semiconductor, while copper oxide makes it p-type. From the analysis of the optical properties of the nanocomposite films, the researchers identified an increase in transmittance and attributed it to the presence of metal oxide nanoparticles that produce structural changes in the device, and a decrease in the refractive index. Furthermore, by the structural analysis, a homogeneous surface is observed due to the difference in the crystal structures of the β sheet of the regenerated silk.

b) Electro-tendon

The researchers made robotic humanoid hands replace human limbs with a transmission system based on a fiber that simulates the human tendon to strengthen the joints (Fig.14). Currently, the materials available for their manufacture are characterized by low toughness and conditioned by significant friction [142]. Furthermore, these tendons must be conducive to the transmission of signals to the tendons. Available materials such as nylon, silicon rubber, etc., do not simultaneously possess all those properties that need to manufacture robotic tendons: elasticity, conductivity, and toughness. Some researchers engineered an electro tendon using spider silk characterized by the toughness of 420 MJ/m3 and conductivity of 1,077 S/cm [143]. Electro tendon has been equipped with carbon nanotubes to provide further toughness and PEDOT: PSS electrically enhance it. Electro-tendon subjected to tests for more than 40,000 flexion-elongation cycles, it turned out tough and durable. It did not show variations in conductivity; mounted on a robot enabled the finger to handle different objects. This tendon represents the most suitable solution for solving the need for a robotic finger to have the same fiber for both the transmission of activation and detection signals.

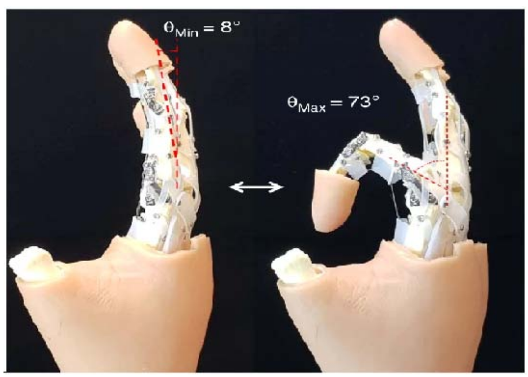

Fig. 14: Electro-tendon from Liang Pan et al. Nature Communication 2020

c) Conductive composites

Conductive silk-based biomaterials are suitable for the fabrication of systems for healthcare and tracking human motion. The transformation of the silk fiber into conductive biomaterial requires the filling of conductive fillers such as carbon nanotubes (CNTs.) or graphene. In the experiment reported, the researchers used bio-based carbons as a conductive filler to make SF biomaterial conductive [144]. They obtained this material called hydro char [145] from the aqueous thermochemical process (HTP) that transforms the biomass into a carbonaceous solid (biocarbon-HC). Then they converted it, by physical activation, into conductive biobased carbon. By acting on the HTP parameters, it is possible to adjust their nanostructure and their chemical functionality. The bio-based carbon can be rapidly doped with polar functionalities, adding oxygen and nitrogen, promoting dispersion in silk fiber suspensions. They studied the effects produced by the variation of the dopant elements. Bio-based carbons thus obtained dissolve in formic acid with CaCl2 silk fibers to produce thin films. The films are flexible and stretchable at room temperature and humidity of 50%. The tests carried out showed that the mechanical properties improve due to the presence of carbon nano materials. The resistivity of these films is lower than those produced without resorting to the thermo chemical process. These materials show good cytocompatibility due to the absence of toxic solvents. From the molecular dynamic simulations, a good conductivity has emerged without causing significant changes in its secondary structure or the bonds with hydrogen. The results obtained highlight the possibility of getting bio-based carbons with different morphologies and properties starting from biomass with other biochemical parameters such as temperature, water biomass ratio, and reaction time.

The fields of application are different from biomedicine to electronics.

d) Flexible electronics

One of the most recent applications in which silk has found wide use is flexible electronics with the construction of wearable and implantable devices [146]. Soft and extensible electronic devices are crucial in wearable and implantable applications to easily conform to the skin's surface. Many flexible materials such as polydimethylsiloxane (PDMS), polyimide, or silicone rubber that show remarkable mechanical characteristics of foldability and elasticity are suitable for the manufacture of flexible electronic devices. Still, none possess the characteristics of biocompatibility and biodegradability essential for implantable devices. Therefore, research has focused on developing flexible natural materials such as cellulose, melanin, pectin, chitosan, and silk fibroin. The latter is preferred not only for its specific properties but also for its simplicity and favorable cost. In recent years, manufacturing oriented on devices in which SF, in the form of silk films, silk hydrogels, and silk fibers, is the fundamental component of wearable electronics. Researchers prefer silkworm because, despite having characteristics of less resistance and extensibility than that of spider, can be produced more efficiently on a large scale. The wide variety of shapes associated with its mechanical, optical, and biological properties have made silk a fundamental or functional component in the manufacture of wearable devices. There are different experiences in flexible electronics in which silk allowed the construction of specific devices with predetermined functions such as substrates for optical devices [147], transistor dielectrics [148], and active layers of memristors [149]. Carbon nanotube coated silk due to its Young's modulus found application in strain sensor.

Flexible electrodes have favorable characteristics for the manufacture of wearable and implantable devices. Compared to traditional electronic systems, possessing the same mechanical characteristics as human organs, they can ensure accurate signals even during movement [150]. The silk film is preferred as it is more suitable for the preparation of functional substrates. However, its use is limited in cases where good elasticity is required, such as producing electronic devices conformable to the epidermis.

In the experience proposed, the authors overcame this problem by manufacturing a plasticized silk film [151]. Silk proteins represent the base layer on which to manufacture soft and elastic electrodes on the skin. The plasticization was achieved by adding CaCl2 and with subsequent environmental hydration. This process made it possible to obtain silk with a softness-like skin. The electrodes are manufactured by metalizing a thin silk film and creating a rough surface after hydration. The electrodes obtained, by modifying both Young's modulus and the elasticity by tuning them respectively to 0.1-0.2 MPa and> 100%, were highly conductive and extensible, making them suitable for recording high-quality electrophysiological signals. The softness of the electrodes allows perfect adhesion to the skin with a low interfacial impedance, ensuring the high-quality recording of EMG signals on the skin. The water with Ca2 ions determines a lower Young's modulus and better elasticity from the instrumental results and those of the dynamic molecular simulations.

The electrodes must also be transparent as well as flexible, biocompatible, and biodegradable. In recent years, efforts have focused on fabricating different types of transparent materials for SnIn2O3 doped electrodes. But ITO film on flexible substrates with polyethylene (PET) and polyimide is unsuitable for wearable devices, making it not biocompatible with the human body. Compared to ITO film, the researchers found that the use of silver nanowires embedded in a silk fiber substrate fully satisfies the requirements of flexible electronics.

In recent research, the authors created a highly extensible, transparent, flexible SF-based film with a low modulus of elasticity, perfectly adherent to the skin. The film integrated with silver nanofibers (AgNFs) / SF synthesized through a water-free procedure showed low resistance (10-5Ω/Sq), high transmittance, good extensibility. After numerous bending cycles, it is offered excellent stability. We can identify it as an electrode for the manufacture of intelligent touch sensors [152]. The sensor was sensitive to pressure and deformation, showing permeability to water and air and not producing inflammation; it was implanted directly on human skin. The experiment was done by gluing it on the arm at the elbow and on the larynx. Positioning on the larynx enabled the trachea and esophagus vibrations to be detected in real-time, thereby monitoring swallowing, drinking, and pronunciation. Abnormal pressure values are related to the onset of diseases of the laryngeal organs. Positioning on the arm allows monitoring finger/arm movement and muscle contraction. The functional element of the pressure-strain sensor is given by the sandwich structure in which the authors placed an Eco-flex layer acting as a dielectric between the two AgNF / SF electrodes (Fig.15).

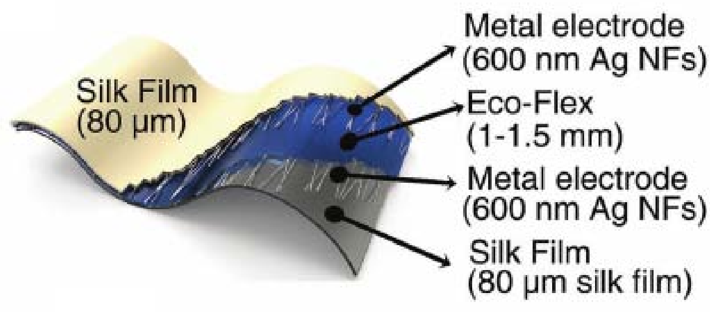

Fig. 15: Structure of the pressure-strain sensor from C. Hou et al. Small 2019

The results obtained in the absence of inflammation confirm the feasibility of this technology in the manufacture of wearable electrodes.

The electronic skin can simulate the human skin and implement tactile sensation made by thin, soft, and elastic devices, suitably functionalized to realize these functions. Research is developing new solutions to improve flexibility and elasticity and make the various electronic skin adaptable to human skin. Silk films are an ideal material to make electronic skins.

Below is an experience based on SF (SFCM) composite membrane with polyurethane that acts as a substrate of electronic skin for human thermoregulation [153]. The SFCM showed characteristics of high transmittance (>90%), excellent stretch-ability (>200%), heat-resistant ability (up to 160 °C). The use of inkjet print allowed to manufacture flexible circuit on substrate: two networks on each of the faces, one based on Ag nanofibers (NFs) and one on Pt NFs introduce functions of heaters and temperature sensors. This integrated protein-based electronic skin (PBES) with Ag NFs/SFCM/Pt NFs sandwich structure can realize temperature control mounted to the human body. Electric circuits were printed to transfer temperature and heating signals. The Ag NFs network as the heater highlight high thermal resistance, heating cyclicity, tensile stability, while the Pt NFs net-work highlights temperature sensitivity (0.205% °C-1), reliability, and rapid response (<2 s).

Some researchers [154] used engineered silk protein hydrogel for artificial electronic skin. They showed that devices based on silk protein hydrogels glued to the skin are suitable for collecting piezoelectric (PZ, based on mechanical stress) and triboelectric (TB, based on electrostatic induction) energy. They obtained poor results compared to conventional inorganic-based PZ devices, such as ZnO, CuO, SnO2, et al. incorporated in polymers as polyvinylidene fluoride (PVDF), polyvinylchloride (PVC), and polydimethy lsiloxane (PDMS). For the manufacture of artificial skin PZ with silk hydrogel, among the materials with good PZ performance, ZnO was chosen, which in addition to being biocompatible and economical, is also physically / chemically stable. [155]. As an artificial PZ energy-generating skin (EG-skin), the structure, adhesive to human skin, incorporates ZnO nanotubes (ZnONR), capable of collecting biomechanical energy and detecting movements. The presence of ZnONR improves the piezoelectricity eight times compared to native silk hydrogels. The PZ response can be further enhanced when the EG-skin is encapsulated within two thin silk membranes. Tests carried out on biodegradability, and adhesive properties on the skin showed that these EG-skin devices constitute a perfect interface between the artificially generated skin and natural tissues. To verify the energy generation capacity of the EG-skin, the device was placed on the forearm to measure the muscular effort when we apply a force to the hand: the action induces a power of 6.2 μW / cm2. The device was also positioned on the elbow to collect the energy produced by the flexion-release of the elbow: the power had 0.2 μW / cm2. In light of the results obtained, the hybrid skin made of engineered silk protein hydrogel can be a versatile platform for wearable and implantable devices.

Due to its biodegradability and programmable water solubility properties, researchers used SF as a component of implantable devices both with different shapes (silk film, silk sponges, and silk hydrogels) and for various applications (wireless thermal therapy, drug release, bone repair). Programmable water solubility is achieved through a post-treatment process to change the crystal structure. The possibility of controlling some of its specific properties made SF a suitable biomaterial for biological transfer, allowing for a perfect integration between biological tissue and electronic devices. The use of SF to manufacture implantable and degradable biosensors allowed us to overcome the problems connected with mechanically flexible and implantable sensors. For their removal, it is necessary to proceed with an extraction surgery.

The experience made by some researchers, in realizing an organic biosensor, shows a structure in which only conductive silk ink is used plus a flexible silk substrate without any metallic component[156]. The structure consists of an integrated three-electrode configuration. The organic electrode as a working electrode, Ag / AgCl as the standard reference electrode, and Pt as the counter electrode are manufactured by an organic conducting ink. The conductive silk sericin ink is micro-patterned on a layer of fibroin with a photolithographic process. The designers realized the electrical connections using a conductive polymeric wire coated with silk fibroin. This entirely organic device can allow the biomonitoring of analytes in the human body or the environment for a controlled period followed by a degradation phase [157]. The researchers tested the device to verify performance as a biosensor; they chose ascorbic acid (AA) as a target. It is essential for the human organism as it is decisive in collagen formation to develop bones, muscles, and blood vessels. The rapid response time of the sensor was on the order of few seconds, showing a high sensitivity. They also extended the test to the proteolytic degradation phase. The researchers found operating stability during the first four days, followed by an improvement in sensitivity, presumably due to the biodegradation of the silk matrix, which exposed PEDOT: PSS led to an improvement in the transfer of electrons from the electrolyte to conductive polymers. From 4th to 7th, sensitivity remained unchanged; to 10th connections deteriorated, and it was no longer possible to detect its behavior. These results suggest replacing transitional devices to monitor phenomena where a short observation period is required. They verified the performance of the biosensor against biofouling. In vivo monitoring of metabolites can lead to contamination of biosensors with a consequent reduction in performance [158]. Nonspecific adsorption of proteins or cells can lead to biosensor occlusion. The presence of a membrane layer of organic material can reduce the biofouling phenomenon. The biosensor having an organic structure has shown a natural resistance to biofouling. Therefore, the fully organic silk biosensor with core-sketch PEDOT: PSS silk fibroin conductive wires can permit real-time, continuous monitoring of health parameters without compatibility problems, with perfect adherence to the epidermis. In addition to AA, we can investigate a wide variety of target metabolites. It is possible to immobilize enzymes and antibodies in the conducting ink.

For the performance shown, the device can be a transient biosensor for monitoring metabolites in cases where a long period of operation is not required.

Energy harvesting technology is a cutting-edge topic for application in wearable technology and the energy stored connected with motion tracking. The availability of intelligent clothing has been beneficial for monitoring human health conditions by preventing harmful events and favoring health improvement conditions. These clothes are possible thanks to the advent of wearable, elastic devices and the possibility of transforming the mechanical energy associated with human activities into electrical energy [159]. We can compare the triboelectric nanogenerators (TENGs) with conventional power supply systems such as electromagnetic generators, thermoelectric generators, and solar cells. They have the advantages of possessing excellent sustainability characteristics, diversified material selection, and efficient low-frequency energy harvesting. The operation mode of TENGs exploits the phenomenon that when two materials come into contact with each other, there is a transfer of charges between them due to their difference in polarity; consequently, a current is generated. The integration of polymeric fibers/tissues with nanomaterial allows manufacturing fabrics to collect energy with TENG technology [160]. The mechanical robustness and structural stability of these devices are not able to provide high functional performance. SF is selected in the manufacture of TENGs because it has the necessary prerequisites.

Moreover, SF has a solid ability to lose electrons; therefore, pairing it with those materials that quickly gain electrons can ensure high performance. In the application we propose [161], the authors with 3D printing made a TENG device with a coaxial composite fiber of carbon nanotubes and SF, where CNT acted as a conductive core and SF acted a dielectric sheath. The dielectric material, another component of the triboelectric pair, was polyethylene terephthalate (PET). The tests showed that this configuration resulted in a maximum power density of 18 mW/m2. This structure has the advantage of having a high integration of functional and electrode materials and simple fabrication for the 3D printing technology used.

The following table highlights the field of applications of silk.

Tab. 2: Summary of silk applications

Application	Component	Properties
Wound healing	Scaffold, hydrogels, sponges, layers	Biocompatibility, biodegradability, reduced healing time
Drug Delivery	Nanoparticles, encapsulation	Biocompatibility, biodegradability
Bone Tissue Engineering	Film, scaffold, electrospun scaffold, 3D porous scaffold	Biocompatibility, osteoconductivity, osteointegration.
Ligament/ tendon	Scaffold, sponges	Biocompatibility,biodegradability, good adhesion and cell proliferation
Cartilage	Porous scaffolds	Biocompatibility, good adhesion, growth and chondrocytes
Vascular Tissue	Electrospun tubular scaffold, graft structure of SF electrospun coated with sponges.	Biocompatibility, good aggregation between tissue and mechanical resistance, water permeation.
Sensors	Functional layer	Biocompatibility, flexibility, good sensitivity to chemical substances
Filters	Substrate, functional layer	Flexibility, conductive pattern for circuit board
Waveguide	Substrate	Biocompatibility, biodegrability, complete transparency, high refractive index
Lenses	Functional layer, hydrogel	Transparency, crosslink, stability
Semiconductor	Substrate	Conductivity, structure β sheet
Electro tendon	Fiber composite SWCNT	Elasticity, conductivity, toughness
Conductive composite	Silk fibroin composite CNT or graphene	Biocompatibility, conductivity, flexibility, stretchability
Wearable electronics	Substrate	Biocompatibility, biodegrability, elasticity, softness
Implantable electronics	Substrate, encapsulation	Biocompatibility, biodegradability, flexibility, low surface resistance, high transmittance
Energy harvesters	Functional layer	Biocompatibility, flexibility, stretchability, piezoelectricity

X. Conclusion

For future developments, silk, with its specific properties, is the solution to meet the need to have multifunctional, non-polluting, and economical devices.

It can be subject to different processes modifying its original structure with different shapes and chemical and mechanical properties for tissue engineering application. Recent work shows the possibility of using silk for peripheral nerve regeneration and intervertebral discs. The particular characteristics of the material, from the structure to the process, the flexibility of modification of the properties, and the availability of functionalization techniques, have made silk ideal as a technological platform. It is suitable for creating sustainable, implantable, and bioabsorbable optical and electronic systems to replace traditional materials.

The Sustainable Development Goals (SDGs) require a drastic reduction of electronic waste. Current technology in electronic and photonic devices applies processes that use chemicals that generate equally hazardous waste once their lifespan has expired.

Therefore, we must orient technological efforts towards applying materials of biological origin to cope with the depletion of oil and the reduction of toxic by-products.

The silk can provide a substantial contribution to solving the problem.

Due to its exceptional biodegradability and biocompatibility properties, it can represent a promising material for biomedical applications. For the future, we can think of entirely organic electronic devices, implantable for a controlled period, in which silk fibroin and sericin can allow the creation of structural and functional elements with the same characteristics as conventional systems which have the disadvantages of creating possible damage to living cells and are not biodegradable.

It usefully satisfies the objectives proposed by the Green Economy.

Furthermore, future developments will be oriented towards the realization of new generation multifunctional devices. The biomedical need for intelligent materials has led to a structural change in the design of biomaterials. Having devices with multiform and multifunctional integration represents a promising application opportunity towards "intelligent" optics. The silk can be the ideal solution for the realization of dynamic biopolymer systems. Controlling the ability of structural proteins to modify their conformation and response behavior to stimuli leads to the creation of innovative solutions for micro-nanopatterning of dynamic and multifunctional biocompatible interfaces.

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