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To Study the Properties of Biopolymers: Natural Silk Fibroin

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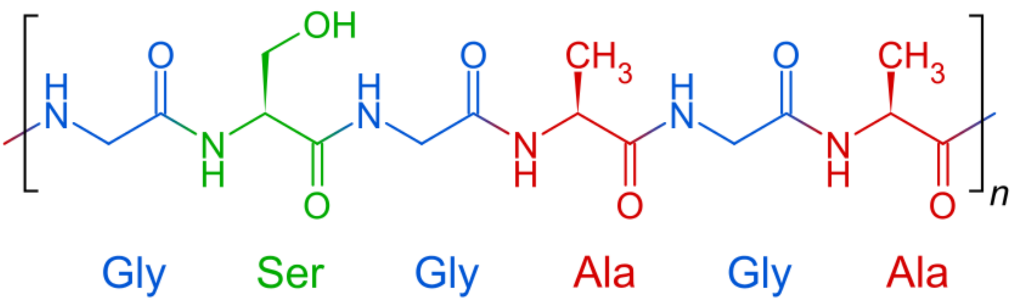
Abstract: All polymers are made of repetitive units called monomers. Biopolymers often have a well-defined structure, though this is not a defining characteristic. Fibroin is an insoluble protein present in silk produced by numerous insects, such as the larvae of Bombyx mori, and other moth genera such as Antheraea, Cricula, Samia. The silk worm produces fibroin with three chains, the light, heavy, and the glycoprotein.

Keywords: biopolymers, synthetic polymers, silk fibroin, Bombyx mori, Antheraea, Cricula, Samia, Gonometa, Silk fibroin solution.

Major defining difference between **biopolymers** and **synthetic** polymers can be found in their structures. All polymers are made of repetitive units called monomers. Biopolymers often have a well-defined structure, though this is not a defining characteristic (example: lignocellulose): The exact chemical composition and the sequence in which these units are arranged is called the primary structure, in the case of proteins. Many biopolymers spontaneously fold into characteristic compact shapes (see also "protein folding" as well as secondary structure and tertiary structure), which determine their biological functions and depend in a complicated way on their primary structures. Determination of structural content is the study of the structural properties of the biopolymers. In contrast, most *synthetic polymers'* have much simpler and more random (or stochastic) structures. This fact leads to a molecular mass distribution that is missing in biopolymers. In fact, as their synthesis is controlled by a template-directed process in most *in vivo* systems, all biopolymers of a type (say one specific protein) are all alike: they all contain the similar sequences and numbers of monomers and thus all have the same mass. This phenomenon is called monodispersity in contrast to the polydispersity encountered in synthetic polymers. As a result, biopolymers have a polydispersity index of 1.[1]

Silk fibroin primary structure , (Gly-Ser-Gly-Ala-Gly-Ala)$_n$

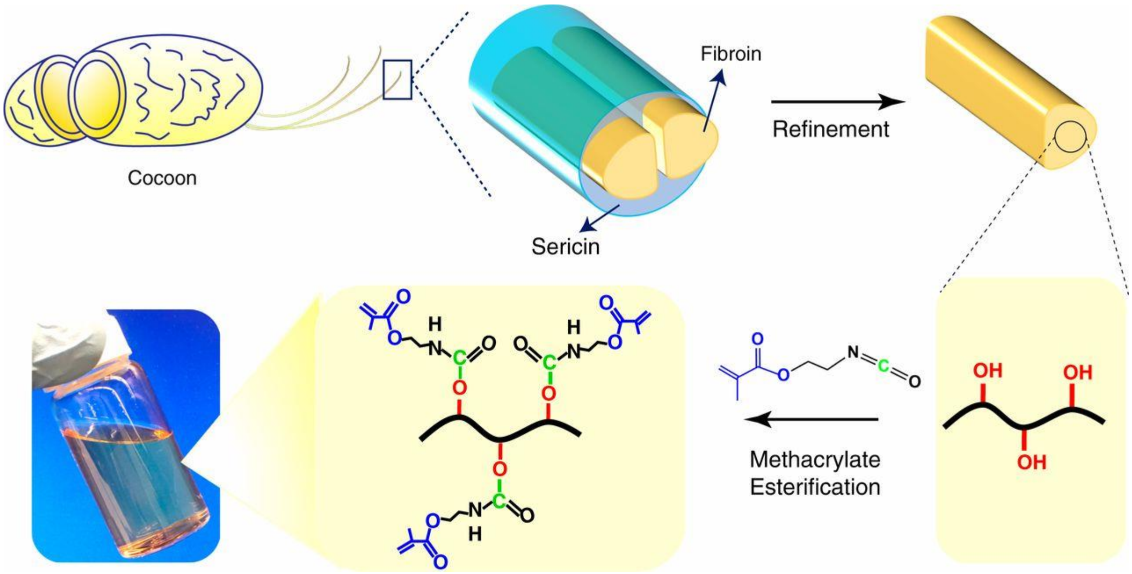


Fibroin is an insoluble protein present in silk produced by numerous insects, such as the larvae of *Bombyx mori*, and other moth genera such as *Antheraea*, *Cricula*, *Samia* and *Gonometa*. Silk in its raw state consists of two main proteins, sericin and fibroin, with a glue-like layer of sericin coating two singular filaments of fibroin called brins. [2][3] [4]

The silk worm produces fibroin with three chains, the light, heavy, and the glycoprotein P25. The heavy and light chains are linked by a disulphide bond, and P25 associates with disulphide-linked heavy and light chains by noncovalent interactions. P25 plays an important role in maintaining integrity of the complex.[4]

The heavy fibroin protein consists of layers of antiparallel beta sheets. Its primary structure mainly consists of the recurrent amino acid sequence (Gly-Ser-Gly-Ala-Gly-Ala)$_n$. The high glycine (and, to a lesser extent, alanine) content allows for tight packing of the sheets, which contributes to silk's rigid structure and tensile strength. A combination of stiffness and toughness make it a material with applications in several areas, including biomedicine and textile manufacture.

Fibroin is known to arrange itself in three structures, called silk I, II, and III. Silk I is the natural form of fibroin, as emitted from the *Bombyx mori* silk glands. Silk II refers to the arrangement of fibroin molecules in spun silk, which has greater strength and is often used in various commercial applications. Silk III is a newly discovered structure of fibroin.[5] Silk III is formed principally in solutions of fibroin at an interface (i.e. air-water interface, water-oil interface, etc.).



Silk Fibroin reveals some known applications like the preparation of scaffolds for bone and meniscus regeneration [6], small-diameter graft for vascular substitution [7]and transparent thin films for biophotonics.

From the silkworm plumage, not all silk fibers are obtained; 10-15% of the silkworm plumage fiber remains together with the plumage. This is a fiber waste of silk. In addition, if the silkworm butterfly comes out of the cocoon, such a cocoon becomes unsuitable for pulling thread and becomes a waste. There are options for the preparation of promising materials through the extraction of silk fibroin from such fiber waste.

Today, preparations from silk fiber are used in pharmacology, medicine, cosmetics and other fields. From silk fibroin, skin resurfacing materials are obtained. [8,9]

“HF” obtained as a result of the hydrolysis of silk fibroin fibers, has a high sorption capacity and is used in cosmetics and pharmacology. Applied in cosmetics, “HF” positively affects and restores the skin, nails and hair. Thanks to this, the aging process in the skin slows down. The chemical composition of HF is similar to the composition of proteins of the skin and nails, hair. [10]

In addition, SF as protein has amino acids that act as cell receptors and mediate important interactions between mammalian cells and extra cellular matrix (ECM) facilitating cell adhesion and growth and it presents antimicrobial activity. However, the regenerated SF has some disadvantages, such as brittleness, easy fragmentation, and difficulty in creating a uniform thickness.[11]

Thus, the aim of this study was to prepare porous scaffolds based Silk Fibroin by lyophilization process, in order to maintain their properties and complement each other as a composite, taking advantage of BC's surface modification with amino acids extracted from Silk Fibroin. Toward meeting these objectives, the resultant nanocomposites were characterized by physicochemical techniques and the cytocompatibility was assessed by the investigation of the cytotoxicity and genotoxicity of the developed material.

Silk fibroin solution

Silk fibroin (SF) solution was obtained from silk cocoons produced by *Bombyx mori* silk worms supplied by Bratac, Fiação de Seda S.A. (Bastos/SP, Brazil). The method was based on previous reports from literatures. Raw silk was degummed with 0.02 M Na_2CO_3 solution at 100 °C for 30 min and washed thoroughly with distilled water. Degummed silk was dissolved in a solution composed of $CaCl_2$, H_2O, and ethanol (1:8:2 molar ratio) in a proportion of 1 g of silk to 4 mL of the solvent. The resulting viscous solution was dialyzed against mili-Q water for 48 h in order to remove salts. A 3.7% (w/V) aqueous fibroin solution free of impurities was obtained after the centrifugation (twice) of the dialyzed solution at 20,000 rpm at 4 °C for 30 min. The final concentration of aqueous SF solution (3.7 wt.%) was determined by weighing the dried solids. The final solution was stored at 4 °C before use. The stock fibroin solution (3.7%) was used to prepare BC/fibroin composites.

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