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Comparing the properties of *Bombyx mori* silk cocoons against sericin-fibroin regummed biocomposite sheets

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ABSTRACT

This paper considers the utility of sericin, a degumming waste product, in the regumming of *Bombyx mori* silk fibroin fibres to form sericin-fibroin biocomposites. Regummed biocomposites have a chemical character that is somewhat closer to fibroin than sericin, though sericin presence is confirmed through FT-IR spectroscopy. Using direct measurements we further find the weight fractions of sericin in the regummed biocomposites and the native cocoons differ by only 5%. Mechanically, *B. mori* cocoons exhibit brittle stress-strain characteristics, failing at strengths of $\overline{X} = 16.6$ MPa and at strains of $\overline{X} = 13\%$. Contrarily, aligning fibroin fibres to a unidirectional axis in the regummed biocomposites causes them to exhibit characteristics of strain hardening, which is itself a typical characteristic of silk fibre pulled in tension. Though they are half as strong ($\overline{X} = 7.2$ MPa), regummed biocomposites are able to absorb five times more mechanical energy ($\overline{X} = 5.6$ MJm^{-3}) than the *B. mori* cocoons ($\overline{X} = 1.1$ MJm^{-3}) and are furthermore able to elongate to more than ten times ($\overline{X} = 180\%$) that of the native cocoons prior to failure. Our research shows that degummed *B. mori* cocoons can be regummed into sheets that have potential for use as load bearing engineering biocomposites.

1. Introduction

Bombyx mori caterpillars are responsible for a major share in the global production of silks [1]. Fibroin, the core fibrous protein component of *B. mori* cocoons, has been a ubiquitous biopolymer used primarily in the textiles industries. Nevertheless, there is growing impetus towards the application of silk in composites [2,3], sometimes as the matrix material and other times as the reinforcement. A number of researchers have considered fibroin as a polymer matrix for nanotube reinforcements [4–6]. Single walled nanotube (SWNT) reinforcements [5] are able to increase stiffness but due to complexities associated with the alignment of SWNT along fibre lengths, other properties such as strength are compromised. Contrarily, multi-walled nanotubes (MWNTs) are able to align more readily, which improves the strength and stiffness of the fibres, but reduces elongation [6]. This results from the development of significant hydrogen bond networks between the fibroin fibres and the MWNT reinforcements, which concurrently improves resistance to washing and sonication techniques [7]. Jin and co-workers [8] applied fibroin fibres within a polyethylene matrix to yield high strength composites with respectable elongation. To ensure that the fibroin fibres would carry sufficient levels of strength-inducing $\beta-$sheets [9], Jin and co-workers manufactured the composites in a humidified environment. $\beta-$sheets control essentially, the mechanical character of silks. They improve strength by creating nanoconfined networks of hydrogen bonds [10] and influence the development of semi-crystalline matter at their interfaces, which in turn, moderates stress transfer between the crystalline and amorphous regions of silk [11].

A fundamental benefit of silk is that it is a renewable, sustainable and biodegradable (RSB) material. As such there is growing interest in combining fibroin fibres to other biodegradable polymers to form composites. In combination with cellulose, silk has been shown to develop considerable hydrogen bonding [12], which if sufficiently close-range may also instigate molecular anchoring [13], further raising bond strength. When compared against pure fibroin fibres, cellulose-fibroin composites exhibit superior mechanical performance, as do fibroin-polylactic acid composites [14]. The contrary is true for fibroin-collagen films, which have reduced mechanical performance as compared to pure fibroin fibres [15] even though they also develop vast networks of hydrogen bonds.

Unlike fibroin, sericin is a proteinous waste product from the degumming of silk cocoons, which is a necessary means of isolating and purifying silk fibroin. Since sericin makes up 20–30% by weight of each cocoon, there is a considerable volume of waste that arises through the process of degumming [16]. Several methods of degumming are utilised industrially including; heating in water, heating in soap water, enzymatic degumming and acid treatments [17]. Nevertheless, degumming has been reported to detrimentally alter the properties of silk proteins, the extent of which is a function of the duration and type of degumming treatment [18]. Water and soap treatments, enzyme treatments and acid treatments have all been shown to cause undesirable

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effects [19] such as loss in tensile strength, damage of the protein molecules and bond breakage. All treatments (excluding enzymatic treatment) increase the elongation properties of fibroin fibres, whilst concurrently reducing its elastic modulus [20].

The utilisation of sericin waste in biomaterials for mechanical benefit has been considered, [21], though for the most part it has been blended into polymer melts to improve the properties of the melt. Some researchers hypothecate the use of sericin in pharmaceuticals [22]. Nevertheless, there is still a significant quantity of sericin that is left unused, much of which is left as unrecovered waste [16]. The successful recovery and utility of waste sericin holds potential for both environmental and economic benefit. Sericin is the natural bioglue for fibroin, yet regumming of fibroin fibres by sericin has never been considered or undertaken as a process by which means native cocoons can be converted into engineering biocomposites. Its utilisation as a bioglue seems a natural move towards cleaner biocomposites production and waste management. The objective of the research herein, is to manufacture biocomposite sheets of fibroin fibres and waste sericin *via* degumming and subsequent regumming processes. We subsequently aim to compare the mechanical properties of regummed biocomposite sheets against those of natural silk cocoons.

2. Materials and Methods

2.1. Degumming process

B. mori cocoons were purchased from the Kabondo Silk Factory and Marketplace, Kisumu, Kenya. The cocoons were cut in half to remove dried larvae after which they were heated in batches of 30 g in 1500 ml deionised water at 95 °C for 4 h. The temperature was checked every 30 min using a thermometer. This procedure results in the separation of sericin waste from fibroin fibres, the sericin remaining in solution. The solution of sericin was then separated from the fibroin fibres by filtration through 30 nm pore diameter Whatman paper after which the waste sericin solution was stored in a refrigerator at approximately 7 °C prior to biocomposite fabrication. To ensure that all the sericin was removed from the *B. mori* fibroin fibres [23,24], the cocoons were then heated again in deionised water at 95 °C for one more hour, however this time in the presence of a dishwasher tablet (Kiilto Green-Easy Tabs) containing polycarboxylates, non-ionic tensides, enzymes and oxygen based whiteners. Following this, the fully degummed fibroin fibres were washed with deionised water several times to remove all traces of these chemicals.

2.2. Manufacture of sericin-fibroin biocomposite sheets

Degummed silk cocoons were stretched out while still wet to straighten and separate them using a wooden board. These were left to dry at room temperature overnight. Fibroin fibres were then cut into equal lengths and aligned as unidirectional fibres in a containment unit after which sericin was poured over the fibres to essentially, regum the fibres. The mixture was dried at 25 °C and at a relative humidity (RH) of 55% for several days until solidified. The dried manufactured biocomposite sheets were then used to determine the fractions of sericin and fibroin in the biocomposite sheet since sericin can migrate away from the fibroin fibres during the process of drying. This migration occurs at the edges and free surfaces of the material where the liquid surface area to volume ratio is high, exacerbating the rate of dynamic motion and pulling more of the free floating sericin molecules to the free surfaces. Here, the sericin shows high affinity to the containment unit and we notice this results in sericin migration. To determine the weight fractions of each, offcuts from the biocomposite sheets were dried, weighed, heated in deionised water for at 95 °C for 4 h for sericin removal, dried again and then re-weighed as fibroin only.

2.3. FT-IR spectroscopy

Fourier Transform Infrared (FT-IR) spectroscopy was performed in ATR mode to characterise the chemistry and secondary structures of pure *B. mori* silk cocoon, pure sericin, pure fibroin and the regummed biocomposite sheets. This method was also used to qualitatively differentiate the biostructures and chemistry of the biocomposite sheets against the native *B. mori* cocoons.

2.4. Mechanical Testing

Biocomposite sheets were cut into dog bone shapes with the fibre axis extending parallel to the gauge length of the dog bone. The samples were 4 mm wide and had a 40 mm gauge length. These were conditioned in a humidity chamber overnight at 25 °C/55% RH prior to testing. An Instron 8872 was used to perform tensile tests in the fibre axis direction at a testing speed of 10 mm/min. Native, unprocessed *B. mori* cocoons were cut into rectangular sections, and tested in a direction parallel to the equatorial axis of the cocoon at the same testing speed. Rectangular sections were used because it was difficult to accurately cut the preferential dog bone shapes from the cocoons. The samples were 10 mm wide and 40 mm long. In total, 22 tests were conducted for each of the biocomposites and 10 tests were conducted for the native cocoons. Though samples were of similar gauge length and width, thickness measurements were taken and averaged for each sample prior to testing for the accurate conversion of load to stress.

2.5. Scanning electron microscopy (SEM)

To elucidate the microstructural arrangements in both native *B. mori* cocoons and in the regummed biocomposites, electron microscopy was conducted using a Jeol-JSM-6335-F Field Emission Scanning Electron Microscope. SEM was also used to characterise the fracture profiles of native cocoons and regummed biocomposites. Samples were sputter coated with 30–40 nm of platinum prior to SEM.

2.6. Molecular dynamics modelling

Molecular dynamics simulations were conducted in Ascalaph Designer to determine characteristics of adhesion and binding sites of sericin to fibroin fibre surfaces under the conditions of high humidity. The sericin molecule was built using the following amino acid sequence reported in [25]: SSTGSSSNTDSNSNSVGSSTSGGSSTYGYSSNSRDGSV. This was then folded to its energetic steady state in a vacuum using an AMBER94 force field, implicit water (Sheffield) conditions defining solvation energy electrostatics [26] at a time step of 2.5 fs. The AMBER94 force field was chosen since it is commonly used for protein simulations with a focus on inter and intramolecular interactions [27]. This force field includes Coulombs law to define electrostatic values, Fourier series to estimate torsion terms and potentials to calculate angle and VDW terms [28]. The sericin after degumming is in solution and will naturally fold in aqueous conditions, prior to its use as a regumming agent for fibroin fibres.

To develop a fibroin fibre surface upon which the sericin molecule could attach, individual fibroin molecules were packed into a periodic box and simulated to steady state. The fibroin molecules were built based on the most frequent motifs that are reported to recur in fibroin [29]. The following four chain sequences of fibroin that were modelled follow.

1. SAGSGAGAGYGAGVGAGYGAGYGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGAG
2. SAGSGAGAGYGAGAGAGYGAGYGAGVGAGYGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAG
3. SAGSGAGAGYGAGAGSGAGAGSGAGAGSGAGSGAGAGSGAGAGSGAGAGSGAGAGAGSGAGA