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Straining flow spinning: Simplified model of a bioinspired process to mass produce regenerated silk fibers controllably

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ABSTRACT

This paper describes a method of molecular self-assembly by using two interacting streams for the production of bioinspired and biocompatible fibers. A first stream of an aqueous solution (dope) of polymer molecules (fibroins) is extruded from a capillary. The end of the capillary is surrounded by a focusing fluid which is miscible with the dope solution. The interaction between the dope solution and the surrounding focusing fluid, which can be an environmentally friendly solvent like ethanol or a water-based solution, leads to hydrodynamic stretching of the dope and allows molecular diffusion processes between the fluids to be established. Polymer molecules within the solution at stretched regions of the jet interact and self-assemble, forming a fiber which could be wound onto a mandrel. This work provides an exhaustive description of the geometrical and hydrodynamic conditions required to achieve the optimum interaction which triggers the formation of fibers. A simplified theoretical model that accounts for the influence of the main parameters of the process on the fibers is also given and experimentally validated.

1. Introduction

In 1664, Robert Hooke wrote “I have often thought that probably there might be a way of making an artificial glutinous substance much resembling, if not fully as good, nay better than, the excrement or whatever substance it may be, out of which the silkworm wire-draws his clew”. Nature has been, and currently remains, a continuous source of inspiration for creating new products and processes. In this regard, one of the most solidly established areas in the new field of biomimetics deals with the production of fibers inspired in their natural counterparts and, in particular, in silk fibers. Silks are defined as fibers spun by arthropods from a protein solution stored in specialized glands. Although a large number of lineages can spin silk fibers, silk production is essentially associated with spiders and some Lepidoptera (butterflies) larvae [1].

The critical biological functions performed by silks have led to materials with a unique combination of properties. In effect, spider silk shows a combination of tensile strength and strain at breaking that yields the highest work to fracture of any material, either natural or artificial, in large excess of the values measured for high-performance fibers, such as Kevlar [2]. However, requiring a large amount of work in order to fracture a silk thread is not the only desirable characteristic of silk fibers that can be transferred to artificial materials. It has also been found that the mechanical properties of spider silk can be tailored predictably and reproducibly

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by a simple method known as wet-stretching [3]. In addition, a factor of major practical importance regarding the application of these fibers is the extreme biocompatibility of silk proteins [4].

The outstanding properties of silks are the result of a subtle interplay among composition, microstructure and processing. Silk proteins – fibroins – are characterized by sequences that have been conserved for millions of years [5]. In particular, the motif -GA- is a characteristic of the silk spun by silkworms (*Bombyx mori*) [6]. This -GA- motif leads to the formation of nanocrystals by piling up β-pleated sheets [7], with the β-nanocrystals being responsible for maintaining the structural integrity of the material. In addition to their nanocrystalline phase, silks also present an amorphous phase [8], which is far less characterized than the crystalline phase, although it is thought to control the mechanical behavior of the fibers [9].

In addition to composition and microstructure, processing plays a critical role in the properties of silks. Although the details of silk spinning are not known in full detail, there is general consensus on their essential features. In this regard, the basic event in the transition from protein solution to solid material is the formation of the β-nanocrystallites [10], which critically depends on the presence of the crystallite-forming motifs in the sequence of the proteins and is believed to occur in two consecutive steps as described below.

The first step involves the organization of the proteins in the gland lumen. There are two models, though not necessarily incompatible, which describe this organization: the liquid crystal model [11] and the micellar model [12]. In both models, the protein molecules acquire a given order in the solution (liquid crystalline order or, alternatively, formation of micellar structures) which decreases the viscosity of the fluid, provides it with a non-Newtonian character and prepares the proteins for the subsequent conformational changes that lead to solidification. A decrease in the pH of the solution at this step is thought to play a leading role in this conformational change [13].

The conformational changes which the proteins undergo in the first step make them susceptible to solidifying in a second step, which consists of imposing shear stresses on the fluid solution that induce relative displacements among the proteins [10]. It was also found that the large variability exhibited by natural spider silk fibers was controlled by the stresses exerted on the fiber during the final part of the spinning process [14,15]. The requirement of a stress-induced extensive protein reorganization to complete the spinning process of silk fibers appears to be a singular feature of these fibers that distinguishes their processing from other spinning routes. Initial estimates of the stresses that lead to fiber formation yielded values of 40 MPa, as calculated from rheological data [16]. Alternative measurements on the silking stresses exerted on fibers not subjected to additional stretching subsequently set a new upper limit of 20 MPa [17], although the actual value might be well below this stress level [18] since the silking stresses might be affected by processes different from the fiber formation, such as friction of the fiber with the gland walls.

The singular properties of silk fibers naturally led to an increased interest in the production of artificial fibers that would share the main features of the natural materials. Initial attempts were restricted to systems in which natural proteins were dissolved and then spun, yielding so-called regenerated fibers [19]. The advent of genetic engineering techniques and the possibility of synthesizing recombinant proteins inspired in natural fibroins extended this initial methodology to include so-called bioinspired fibers [20,21], obtained by spinning solutions of genetically engineered proteins. Initial production of regenerated and/or bioinspired fibers was mostly based on either conventional wet spinning or electrospinning methods. Both approaches are essentially based on the *chemistry* of the solutions involved and on the sequences of the silk or silk-bioinspired proteins. Thus, both techniques are based on the following: (1) use of a polar solvent that prevents uncontrolled formation of hydrogen bonds between silk proteins; (2) removal of the solvent from the dope, by using an appropriate miscible fluid (wet spinning) or by being evaporated to the atmosphere (electrospinning) [22]. Stresses and reorganization of the proteins are supposed to play a role in the formation of regenerated silk fibers, but no attempt was undertaken to measure or to control it. Subsequently, more sophisticated methods that relied on the principles of microfluidics [23–26] were developed to try to improve the performance of the process. In particular, a microfluidic approach was used to spin both regenerated [27] and genetically engineered bioinspired fibers [28], with significant results being obtained by combining microfluidics with a dry conventional spinning step [29].

From the above, it is clear that shortcomings are encountered when spinning regenerated or bioinspired fibroin fibers by using conventional wet spinning or, electrospinning techniques, or microfluidic approaches. Comparison of the natural spinning system in both silkworms and spiders with the artificial techniques reveals the convenience of the following: (1) increasing the interaction time between the dope and the coagulating bath in order to allow for the required conformational changes of the proteins to occur; (2) using a procedure that ensures (and possibly) controls geometrically- and stress-induced protein reorganization undergone by the dope proteins during spinning. In addition, with regard to physicochemical conditions, most artificial silk fibers have been produced by using toxic solvents and/or coagulants, or by placing them under high-temperature processing conditions. Consequently, the spinning of artificial silk fibers under mild conditions (aqueous dopes, low-toxicity coagulants and ambient temperature) is still a challenge to be faced.

This work describes the concurrent result of some decades of intense research by our group on the following: (1) the physics of micro-jetting, either by electrospray and electrospinning [30–32], by flow focusing [33–35] or by microfluidics [36]; and (2) the physics of natural and regenerated silk fibers [37–40] that led to the development of a new spinning process, named straining flow spinning (SFS). The new spinning process is inspired in flow focusing technology [33,41,42] and seeks to mimic certain characteristics of natural spinning like the dehydration of the dope and ion exchange during the spinning process between the dope and the surrounding media. As shown below, SFS is a versatile technique that offers a broad parametric space which allows, for instance, properties of the fibers in terms of their mechanical behavior to be optimized [43].

This work both includes a detailed study on the influence of the geometrical and hydrodynamic parameters on the outcome of SFS and complements previous work on the effects of modifying the chemical parameters of the process [44]. A simplified model that accounts for basic magnitudes of the technique, such as the diameter of the fibers and the definition of the draw ratio (DR), is