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Self-assembled chitin nanofibers and applications

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ABSTRACT

Self-assembled natural biomaterials offer a variety of ready-made nanostructures available for basic science research and technological applications. Most natural structural materials are made of self-assembled nanofibers with diameters in the nanometer range. Among these materials, chitin is the second most abundant polysaccharide after cellulose and is part of the exoskeleton or arthropods and mollusk shells. Chitin has several desirable properties as a biomaterial including mechanical strength, chemical and thermal stability, and biocompatibility. However, chitin insolubility in most organic solvents has somewhat limited its use. In this research highlight, we describe recent developments in producing biogenic chitin nanofibers using self-assembly from a solution of squid pen β-chitin in hexafluoroisopropanol. With this solution based assembly, we have demonstrated chitin-silk composite self-assembly, chitin nanofiber fabrication across length-scales, and manufacturing of chitin nanofiber substrates for tissue engineering.

Contents

1. Introduction

The basic concept that biogenic materials and natural structures finely tuned by natural selection are recyclable and functional to ecosystems promotes research on biogenic materials and biomimetic structures. Beside this rational motivation, a curiosity driven researcher easily yields to the spell of elegant tiny structures of foraminifer and diatom shells or to the iridescent colors of butterfly wings. Butterfly wings are photonic crystals mainly made of chitin [1], which is the most abundant natural polymer after cellulose. Chitin is a structural component of the exoskeleton of arthropods and the cell walls of fungi and yeast [2–4]. Chitin was first described by the French botanist and chemist Henri Braconnot in 1811 [5]. Chitin is a polysaccharide whose repeating structural unit is β(1 → 4) linked 2-acetamido-2-deoxy-β-D-glucopyranose – or *N*-acetyl-D-glucosamine (GlcNAc) – that forms long linear chains (Fig. 1). Chitin is found in nature as semi-crystalline nanofibers with hydrophobic interaction between glucosamine rings and hydrogen bonding along the linear chains. This strong bonding between the chains also results in chitin being insoluble in water and common organic solvents. Partial N-deacetylation of chitin produces chitosan, which is the most commonly used derivative of chitin. The degree of acetylation (DA), i.e. the proportion of acetylated units (m) with respect to the total number of acetylated and deacetylated units (m + n), characterizes chitosan chemical properties. Chitosan has a typical DA of less than 0.35 and it is a copolymer composed of 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose – or D-glucosamine (GlcN) – (Fig. 1). The presence of amino groups renders chitosan soluble in acidic solutions with pH < 6.5 upon protonation of the primary amines. Highly deacetylated chitosan (low DA) does not self-assemble into nanofibers from solution; the nanofiber self-assembly process is driven by the intramolecular hydrogen bonding of the acetyl groups [6]. It is important to note that chitin and chitosan are not distinct chemical entities since they share same repeating units. Chitin and chitosan are analogs of cellulose, which is also a

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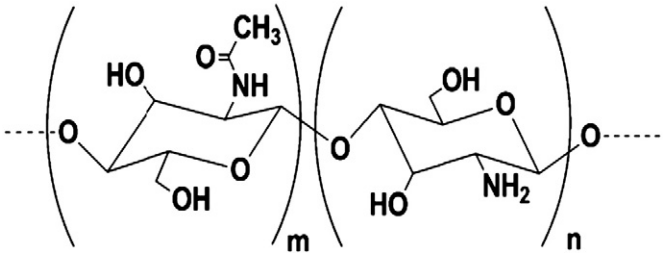

Fig. 1. Chitin and chitosan chemical structure. Deacetylation replaces the acetyl group in chitin (m block) with an amine (n block) resulting in a more hydrophilic and positively charged polymer. When the ratio between acetyl groups and amines is lower than 1:1 (m < n), the polymer is typically referred to as chitosan.

polysaccharide with a repeating unit of β(1 → 4) linked-D-glucose. As cellulose is the organic matrix of skeletal structures in plants, chitin is the organic matrix of skeletal structures in invertebrate. Their analog in mammals is collagen [7]. All these compounds generate fibrillar structures possibly from a converging natural chemical evolution. As in the case of cellulose, chitin exists in different polymorphic forms, which can be reduced to two forms: α and β. In both forms, hydrogen bonds between hydroxyls join longitudinally the linear polysaccharide chains to form sheets. In α-chitin the polysaccharide chains are antiparallel, and in β-chitin the polysaccharide chains are parallel [8,9].

The interest for chitin as a material began in the 1920s, when market pressure for low cost fibers promoted research of artificial silks. Chitin was an artificial silk candidate and many attempts were made to solubilize it. Artificial silk was never produced from chitin. The discovery of nylon in the late 1930s, followed by the success of synthetic polymer fibers, slowed down further research in this direction [10]. In the 1970s several interesting properties of chitin and chitosan were discovered. In particular, their biocompatibility and wound-healing properties made them attractive for a variety of biomedical applications including wound dressing and sutures [10], tissue engineering scaffolds [11–13], and biocompatible devices [14,15]. For these applications, often chitin and chitosan are used in the nanofiber form obtained from electrospinning [16]. While electrospinning produces large diameter nanofibers, smaller nanofibers as present in nature are difficult to produce. Self-assembly of small diameter chitin nanofibers for biomedical applications was a well-known challenge because of chitin intractability and water insolubility [17]. The latter impedes the use of the water-based self-assembly generative route, which is common in the synthesis of other biogenic nanofibers [17]. Therefore, conventional approaches to the production of chitin nanofibers either rely upon top-down procedures that break down the starting bulk material in harsh conditions, or involve electrospinning of depolymerized chitin solutions [10,18–21]. Most of these protocols use highly basic or highly acidic environments with strong mechanical forces and usually result in partially deacetylated or depolymerized nanofibers often 10–100 times larger than the biogenic counterpart. Several recent papers and excellent reviews exist on producing chitin nanofibers from electrospinning and processing of chitin [22–27]. In this research highlight, however, we focus on an alternate method for the production of chitin nanofibers that involves the self-assembly of ultrafine nanofibers from solution of squid pen β-chitin. This simple approach is amenable to several applications including biocomposites, microfabrication, and substrates for tissue engineering.

2. Chitin nanofiber self-assembly

One of us (MR) has developed a novel method for the in vitro self-assembly of biogenic chitin nanofibers [28]. This method exploits the lower degree of hydrogen bonding of β-chitin, with respect to α-chitin. β-Chitin is obtained from squid pen, while the more commonly available α-chitin is usually obtained from shrimp shells. Appropriate amounts of β-chitin were dissolved either in hexafluoro-2-propanol (HFIP) or LiCl/N,N dimethylacetamide (DMAC). These solvents caused hydrogen bond disruption. For chitin dissolved in HFIP the self-assembly process was initiated by solvent evaporation. Drying solutions of appropriate concentrations led to micron long nanofibers of α-chitin with a small diameter (2.8 ± 0.7 nm) (Fig. 2 a–e). The reported error is the measure standard deviation and it indicates a relatively large diameter distribution, which is not surprising taking into account the manifold variables affecting the self-assembling process of a naturally derived polymer [28]. Biogenic nanofibers with similar length and diameter are found in the tissues of living organisms such as shrimp shells and arthropod cuticle [29]. AFM images of these nanofibers (Fig. 2 c) did not show any surface corrugation, suggesting that these materials are composed of a single self-assembled filament. Chitin nanofiber structures were assembled from HFIP solutions having a range of concentrations from 0.5% w/v to 0.005% w/v. Different solution concentrations did not affect the nanofiber dimensions, but only the nanofiber density. For chitin LiCl/DMAC solutions, simple drying was not a practical approach to produce nanofibers due to low volatility of the solvent. Instead, fibers were precipitated out upon the addition of ample amounts of water, which reached 10–25 times the original volume. Nanofibers prepared in this fashion generally had a larger diameter (10.2 ± 2.9 nm) (Fig. 2 f–j) than those prepared from HFIP (Fig. 2 a–e). However, both types of nanofibers had similar lengths (Fig. 2 a–i). In LiCl/DMAC-prepared fibers, a complex structure composed of several smaller subunits was clearly discernible (Fig. 2 f and h). It is thus conceivable that fiber self-assembly for the two methods might have proceeded along different routes. X-ray diffraction measurements showed that the nanofibers produced by both methods were made of highly crystalline α-chitin. The crystallization of α-chitin was favored with respect to β-chitin because of the higher number of hydrogen bonds and higher thermodynamic stability of α-chitin [30]. Furthermore, FTIR measurements showed that deacetylation to water-soluble chitosan or other chemical degradation of the chitin starting material did not occur. This is quite important to confer robustness to chitin structures prepared with these methods [28].

3. Chitin nanofiber silk self-assembly

Many structural natural materials based on chitin such as the arthropod cuticle, crustacean exoskeleton, and mollusk shells are composites [31,32]. In these composites the organic phase, which is often biomineralized, is made of chitin nanofibers embedded in a silk-like protein matrix [29,33]. In an attempt to recreate the microstructure of these materials, we exploited the self-assembly properties into nanofibers of the chitin/HFIP solution to form a chitin-silk biocomposite [34]. Silk was added to solutions of squid pen β-chitin in HFIP, which were dried on a polydimethylsiloxane (PDMS) mold to yield homogeneous films (Fig. 3). These films were made of ultrafine (~3 nm) chitin nanofibers embedded in the silk fibroin matrix. The chitin nanofibers in the co-assembled composite shared the same entangled structure with the chitin nanofibers self-assembled from a chitin only-HFIP solution (Fig. 2 a) [28]. The chitin nanofiber content of the biocomposite was easily tunable by varying the solution chitin/silk ratio in CS31, CS11, and CS13 (CSXY = chitin/silk wt/wt ratio) (Fig. 3 b, c, and d). This was a desirable feature that afforded a simple strategy to fine-tune the biocomposite properties. In contrast, the surface structure of the silk film dried from the HFIP solution was smooth and did not contain nanofibers, confirming that addition of chitin was essential to create the biocomposite nanostructure (Fig. 3 e). The chitin-silk films were transparent. The chitin-silk biocomposite assembled from solution was easily manipulated with soft-lithography strategies that were developed for chitin [35] and silk [36] to manufacture optical elements such as diffraction gratings (Fig. 3 f).

4. Self-assembled chitin nanofiber fabrication

To enable the application of self-assembled chitin nanofibers in biocompatible structural devices, we merged the chitin/HFIP self-assembly

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