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Fabrication and investigation of a biocompatible microfilament with high mechanical performance based on regenerated bacterial cellulose and bacterial cellulose

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ABSTRACT

A high-strength regenerated bacterial cellulose (RBC)/bacterial cellulose (BC) microfilament of potential use as a biomaterial was successfully prepared via a wet spinning process. The BC not only consists of a 3-D network composed of nanofibers with a diameter of several hundred nanometers but also has a secondary structure consisting of highly oriented nanofibrils with a diameter ranging from a few nanometers to tens of nanometers which explains the reason for the high mechanical strength of BC. Furthermore, a strategy of partially dissolving BC was used and this greatly enhanced the mechanical performance of spun filament and a method called post-treatment was utilized to remove residual solvents from the RBC/BC filaments. A comparison of structure, properties, as well as cytocompatibility between BC nanofibers and RBC/BC microfilaments was achieved using morphology, mechanical properties, X-ray Diffraction (XRD) and an enzymatic hydrolysis assay. The RBC/BC microfilament has a uniform groove structure with a diameter of 50–60 μm and XRD indicated that the crystal form was transformed from cellulose Iα to cellulose III_I and the degree of crystallinity of RBC/BC (33.22%) was much lower than the original BC (60.29%). The enzymatic hydrolysis assay proved that the RBC/BC material was more easily degraded than BC. ICP detection indicated that the residual amount of lithium was 0.07 mg/g (*w*/w) and GC–MS analysis showed the residual amount of DMAc to be 8.51 μg/g (*w*/w) demonstrating that the post-treatment process is necessary and effective for removal of residual materials from the RBC/BC microfilaments. Also, a cell viability assay demonstrated that after post-treatment the RBC/BC filaments had good cytocompatibility.

1. Introduction

Bacterial cellulose (BC) is a biomass, synthesized by bacteria and has the same molecular structure as plant cellulose, and both are composed of polysaccharides with linear chains of β-1,4-D glucopyranose residues. The manufacturing process of BC is economical and environmentally friendly and BC exhibits outstanding properties and is one of the most abundant renewable organic materials on earth [1]. Due to its advantages in structure, performance, ease of production and application, BC is superior to plant cellulose [2] and has shown good promise in the field of biomaterials [3–7]. However, natural BC lacks structural diversity, so it is necessary to modify it in order to enlarge its field of potential applications.

BC can be converted into cellulose derivatives (ethers and esters) and regenerated bacterial cellulose (RBC) materials (membranes, fibers, food casings, sponges, etc.) [8]. In the current work, a wet spinning regeneration process was investigated since it has superior performance with regard to material, equipment and parameters compared to wet filming (casting film using BC solution) and there are a few reports of the preparation of RBC filaments from BC solution in the literature. Previous research on RBC mainly focused on the preparation of RBC film not fibers/filaments [9]. In industry, it is necessary for some materials to be processed in the form of fibers/filaments since the yarns or filaments made from wet spinning fibers can be further converted into non-woven, woven and knitted fabrics or sutures that can be used in medical or other areas [10,11]. As previously described [12,13], wet spinning technology is suitable for the preparation of such materials where the melting temperature is higher than its degradation temperature and BC fits into this category. To date, there have been few theoretical or experimental studies on RBC filaments prepared by wet spinning so consequently research on this issue is considered to be of considerable importance and timely.

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The use of an appropriate solvent which will dissolve BC into a spinning solution with a certain viscosity and rheological behavior is the premise of wet spinning. Recent reports have mainly focused on finding a good solvent, such as NaOH/urea solution [9], *N*-methylmorpholine-N-oxide monohydrate (NMMO) [14,15], ionic liquids [16,17], *N*,*N*-dimethylacetamide (DMAc)/lithium chloride (LiCl) [18,19] or $ZnCl_2$ [20] for the BC. Among these, the use of DMAc/LiCl in the dissolving process is the most common due to ease of operation, low cost and being less energy demanding. Thus, this paper describes the preparation, via a wet spinning process, and properties of an RBC/BC microfilament for potential use as a biomedical material. The main objective of this study was to use BC as the raw material, LiCl/DMAc as the solvent system and DMAc/water as the coagulation bath in order to improve the wet spun filaments by controlling the swelling and dissolution time of BC to enhance the mechanical strength (Fig. 1). BC has an ultra-fine fibrous 3-D network structure with high purity, high mechanical strength, wet capability and good biocompatibility [21]. In the current work, the nature of the BC was partially conserved during the dissolving process ensuring that its excellent properties, especially the high mechanical strength, was maintained and enhanced in the resulting RBC/BC microfilament. The rheological morphological, mechanical properties, X-ray Diffraction (XRD) as well as the enzymatic hydrolysis performances were all studied in order to determine the differences between the BC fiber membrane and RBC/BC filaments with respect to structure and properties.

An ideal biomaterial must provide a variety of shapes and sizes, be biocompatible, absorbable and capable of being replaced by new tissue formation, in the case of applications in tissue engineering. Currently, natural polymers are important alternatives as scaffolds for tissue repair [22] and over the past decade, types of materials based BC have been designed for a diversity of biomedical applications. Previous work in this area concentrated on BC or BC composites rather than RBC and no relevant study on the issue of residual solvent/ions produced during the process of BC regeneration has been reported. Residual solvents (RS) and residual ions (RI) may represent a potential risk for human health due to their toxicity and their undesirable side effects [23] and they should be removed through some form of effective post-treatment. Hence, for the first time, an attempt was made to detect RS in RBC filaments using gas chromatography–mass spectrometry (GC–MS) and Inductively Coupled Plasma Emission Spectrometry (ICP-MS) was utilized to measure the amount of RI. A cytocompatibility assay test was performed to examine the differences of cell (L929 cells) viability between the RBC/BC filaments with and without post-treatment. Moreover, in this work, not only is the preparation of a RBC/BC microfilament, with high mechanical performance, described but also data is given to provide for its better future application through a comprehensive analysis of the properties of the BC before and after regeneration.

2. Materials and methods

2.1. Materials

Bacterial cellulose was provided by Hainan Yida Co., Ltd. (Guangdong, China). Cellulase from Aspergillus niger (product #C0057, 10,000 U/g, slightly brown powder) was purchased from Okyo Chemical Industry (TCI). The following chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd.: dimethylacetamide (DMAc, ≥99%), lithium chloride (LiCl, ≥97%), sulfuric acid (≥98%), Avicel citric acid (≥99.5%), sodium citrate dihydrate (meets USP testing specifications), 3,5-dinitrosalicylic acid (DNS, ≥98%), ethyl alcohol (EtOH, ≥99%), acetone (≥99%).

2.2. Dissolution and regeneration of BC

The preparation of spinning dope was carried out as follows: firstly, wet BC membrane was cut into small pieces and crushed by a high speed homogenizer at 15,000 rpm for 30 min to form a BC slurry which was freeze-dried at −40 °C for 2 days. Secondly, a DMAc/LiCl (7%–8% *w*/w) mixed solvent was prepared and the dried BC slurry (3.0 g) was suspended in DMAc/LiCl (147.0 g). This superior dispersion system was stirred with mechanical agitation (50–60 r/min) for a certain time (6, 8, 10, 12, 14, 16, 18 h) at room temperature until visually all the BC dissolved and then a RBC/BC spinning solution (2%, *w*/w) was prepared as described in the supplementary documents (Fig. S1).

The method of recasting RBC film has been reported in the literature [15,19] and was utilized in this work. The solution prepared above was poured into a culture dish with a diameter of 60 mm and then coagulated in a DMAc/water solution (500 mL; 30:70 *v*/*v*) for 5 min. After freeze-drying at −40 °C for 2 days, a RBC film with a thickness of 0.1 mm was produced.

RBC/BC microfilaments were spun on a custom-made wet-spinning device (Fig. S2), which has been described previously, using the spinning process parameters shown in Table S1. A nitrogen pressure of 0–0.3 MPa was used to extrude the aqueous solutions (2% *w*/w) at 1.0 mL/min through a commercial spinneret plate with a single 0.25 mm diameter orifice. The spinning dope was extruded directly into an aqueous DMAc/water (50% *v*/*v*) coagulant solution kept below 15 °C. The total length of the coagulation bath, the second bath and the third bath was 60 cm and after passing though these baths and over the three rollers, the resulting microfilaments were wound onto spools and dried at room temperature for 2 h. The appearance of prepared RBC/BC filaments in the dry state and wet state are also shown in Fig. S2. Further, it is necessary to use a strategy called post-treatment process whereby the filament samples are soaked in Millipore hot water (≥60 °C) for 30 min, then centrifuged at 2500 rpm at 20 °C for 5 min. This process was repeated once more in order to remove as much residual solvent and ions as possible.

2.3. Optical microscope (OM) analysis

The optical imaging was performed using a custom-modified BX-51 Olympus optical microscope equipped with a color digital CCD camera (Lumenera Infinity 2 − 1C). Various diluted samples (50 μL) of the RBC/BC solution were spread on a glass slide and then observed by OM.

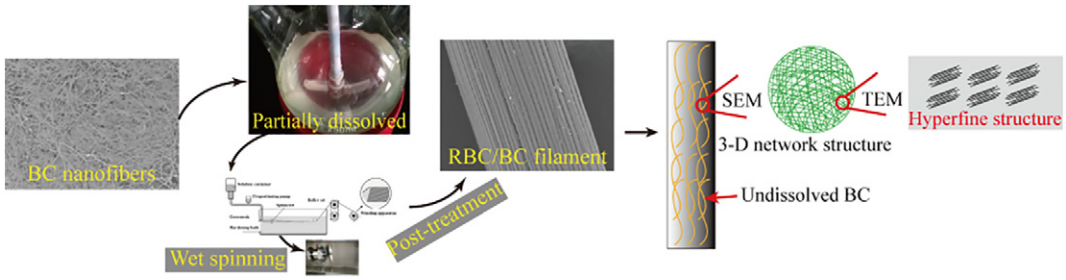

Fig. 1. The preparation strategy of strength enhanced RBC/BC microfilaments: Morphology transition from original BC nanofibers to RBC/BC microfilament.