A route to uniaxially oriented ribbons of bacterial cellulose nanocrystals based on isomalt spun sacrificial template

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KEYWORDS: Bacterial cellulose nanocrystals, Isomalt, Shear-induced orientation

SUMMARY: We have carried out orientation of bacterial cellulose nanocrystals (BCNC) by implementing a process based on mechanical shearing BCNC dispersed in a viscous temporary isomalt glass. After the orientation, the isomalt matrix was selectively solubilized to afford uniaxially highly oriented BCNC ribbons as demonstrated by SEM and X-Ray studies. The 2D WAXS determined Herman's order parameter reached 0.85.

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Decrease of oil-based resources prompts the use of biomass as a feedstock to produce renewable materials which are expected to offer improved functionalities such as biodegradability, enhanced biocompatibility or decreased environmental impact. Cellulose is the most abundant naturally occurring polymer. Cellulose is composed of β-1,4 glucan chains and occurs in the cell wall as microfibrils comprising highly ordered crystalline zones and less ordered "amorphous" zones. Harsh hydrolysis of cellulosic fibers allows the isolation of crystalline nanorods referred as cellulose nanocrystals (CNC) or whiskers. The first report of the production of CNC was published in the early 50's (Rånby 1951, Rånby 1952). Since that time, these biosourced nano-objects have been the subject of intense research yielding numerous reports that have tremendously increased in the two last decades. Due to their crystalline nature inducing high mechanical properties, CNC have been proposed as fillers in nanocomposite materials (Azizi et al. 2005). The first CNC reinforcing effect was reported by Favier using tunicin CNC dispersed in a rubbery latex matrix (Favier et al. 1995). Moreover triggered by the outstanding anisotropic properties of liquid crystalline polymers there is a strong momentum to orient unidimensional fillers and further improve the mechanical response of reinforced or filled composites. From literature, three main strategies can be identified to induce CNC orientation by applying external forces (Elena, et al., 2012). Two are related to the application of physical fields, i.e. electrical fields (Bordel et al. 2006, Habibi et al., 2008) leading to coaxial orientation or magnetic fields (Sugiyama et al. 1992, Cranston, Gray 2006) leading to orthogonal orientation. The third one uses shear gradients to achieve CNC alignment either directly in a concentrated colloidal suspension (Nishiyama et al. 1997) or in a spun gel subsequently stretched. In the last approach, mechanical shearing can be obtained either by wet spinning (Ureña-Benavides and Kitchens 2011, Ureña-Benavides et al. 2010) or under tensile loading (Osorio-Madrazo et al., 2011). Similarly, for structural study purposes, Nishiyama et al. (2010) have reported excellent orientation in stretched PVA/borax fibres.

In the present work, we propose a new and complementary method to obtain highly oriented flat ribbons of neutral bacterial cellulose nanocrystals (BCNC) (*Fig 1*), using direct melt spinning of a sacrificial soluble matrix composed of a low molecular weight sugar-alcohol blend (Isomalt ®) (*Fig 1a*). Isomalt® is a commercial sugar alcohol derived from sucrose and widely used in food industry. The melt obtained above 150°C conveniently converts to the glassy state around 60°C. Shaping in the rubbery state affords lamination or fiber spinning which will carry the orientation of eventual fillers. Subsequent selective solubilization of isomalt leads to the formation of highly oriented BCNC ribbons that were characterized by SEM and X-ray diffraction.

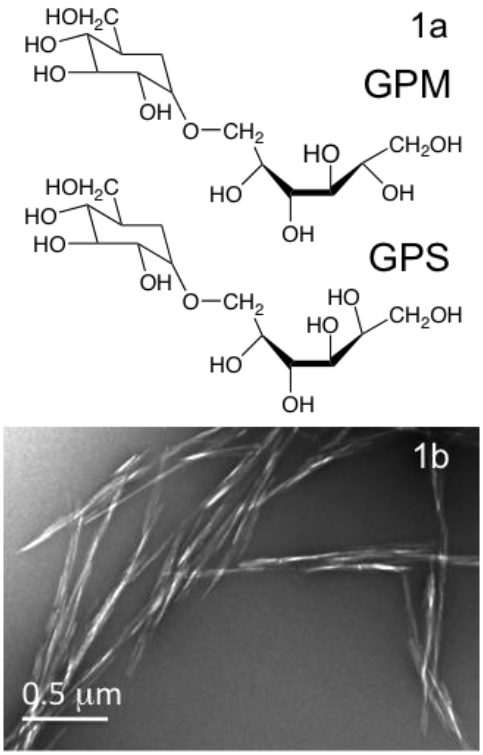

Fig 1 - (a) Chemical structures of isomalt isomers; D-glucopyranosyl -1-6-mannitol (GPM) and α-D-glucopyranosyl -1-6-sorbitol (GPS); and (b) TEM image of BCNC bundles.

Materials and Methods

Isomalt ® ST-F was a gift from BENEO-Palatinit GmbH, Mannheim, Germany.

BCNC preparation: Bacterial cellulose was extracted from commercial nata de coco. Heavily rinsed cubes suspended in water and ice chunks were ground at maximum speed in a Waring Blender for 3*1mn. The resulting paste was filtered, resuspended in 0.5 M NaOH and treated under constant stirring in a closed flask at 70°C for 2 h. Alkali was removed by rinsing with distilled water down to neutrality. Bleaching was conducted twice under reflux with 8.5 g/l $NaClO_2$ in sodium acetate buffer (pH 4.5) at 70 °C for 2 h. The bleached cellulose was rinsed with distilled water up to neutrality. Bacterial cellulose nanocrystals (BCNC) were produced by hydrolyzing purified bacterial cellulose with 2.5 M HCl at 70 °C under reflux for 2 h as described by Gilkes et al (1992). Acid was removed by successive centrifugation (10000 g) and redispersion in ultrapure water (18.2 MΩ, Synergy, Millipore, Molsheim, France) up to neutrality. The hydrolysis yield was calculated by determining the mass loss during hydrolysis (Yield 75%). Mechanical disintegration was obtained by 1 min sonication (Ultrasonic Processor XL 2020, MISONIX, Farmingdale, USA) at Power level 6 for 500 ml suspension.

Melt spinning and solvent extraction procedure: Isomalt 3g was dissolved in 10 ml water containing 2% freshly sonicated BCNC. The resulting suspension is concentrated by evaporation up to 155°C in a warming sleeve for several hours until bubbling ends. After reducing the temperature to 80°C threads are manually pulled from the viscous melt with a pair of tweezers adjusting the pulling speed to the diameter desired (about 1 m/s). Long threads are cut into multicentimeters segments and stored in a dessicator with silica gel to prevent recrystallization or deliquescence. To remove the sugar alcohol matrix, selected segments were laid onto pure Teflon membranes supported on a sintered glass disk (Savillex 450-47-2/0.45 µ) and rimmed with a silicone gasket. Isomalt was extracted under static conditions by soaking in Methanol/Water solutions (95/5) in slanted sealed tubes with membranes hanging at mid-height to take advantage of convections induced by concentration gradients. Methanol/water was replaced 3 times every 8-12h by gentle suction/injection. When extracting the films to open air the solvent evaporated rapidly and let viscous narrow bands appear. Once fully dried the initial threads were replaced by flat bands of aligned cellulose or composite films with clear edges, these were pealed off with a pair of microscopy tweezers and stored under dry conditions for further characterization.

Scanning Electron Microscopy (SEM): The dried films were metalized with platinum and visualized with a JEOL 6400F.

X Ray diffraction: A stack of 8 ribbons segments 5 mm long was inserted into a 1.5 mm Lindemann Glass Capillary Tube (Hilgenberg GmbH, Malsfeld , Germany) and diffraction pattern was obtained with a Bruker D8 Discover diffractometer (Madison, WI, U.S.A.) using Cu Kα1 radiation (λCuKα1 = 1.5405 Å) produced by a sealed tube at 40 kV and 40 mA, selected using a Göbel mirror parallel optics system, and collimated to produce a 500 µm beam diameter at 8.5 cm from detector in transmission mode. The degree of orientation was evaluated with Herman's order parameter as in ref (Nishiyama et al. 1997) calculated from the azimuthal intensity distribution of the (2,0,0) Ib reflection.

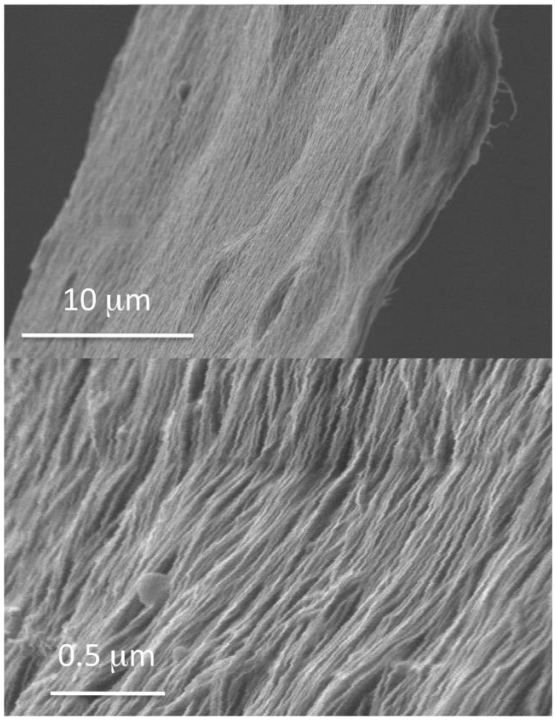

Fig 2 - Scanning electron micrographs of a longitudinal cross section of BCNC oriented ribbons.

Results and discussion

BCNC were obtained by harsh acid hydrolysis that released the crystalline part of the bacterial cellulose as highly crystalline rod shaped nanoparticles (*Fig 1*). BCNC were prepared by HCl hydrolysis that yields only a few surface charges as reported earlier (Winter et al. 2010). Hence, the BCNC used in the present work had almost no charges and exhibited low colloidal stability that appears clearly in TEM images. Indeed negatively stained BCNC display strong aggregation. Nearly all the nanocrystals participate in laterally associated bundles in good agreement with a previous work (Winter et al. 2010). Thus, prior to the addition of isomalt to proceed to the orientation, the dispersions were sonicated. Nevertheless, individual BCNC can be distinguish within the bundles. Analysis of the TEM images indicated an average length of 855 nm and width of 17 nm. A thickness of 7 nm was determined by atomic force microscopy (Kalashnikova et al. 2013).

The principle of the orientation procedure we develop relies on the use of a temporary carrier matrix that must fulfill at least three criteria. First, the matrix has to form a spinnable viscous dope to sustain shear stress upon stretching. Secondly it has to be removable after shaping by i.e. non-aqueous solvents to prevent the loss of orientation of BCNC by redispersion in water. Thirdly,

the matrix needs to be chemically compatible with cellulose and hemicelluloses so as to limit phase separation or unwanted reaggregation. All these criteria have led to select low molecular weight carbohydrates that can form molten dopes with viscosities similar to polymeric alternatives (i.e. PVA for instance) when the process temperature is properly selected. Low molecular weight carbohydrates can also be solubilized by alcohol/water mixture and present hydroxyl and aliphatic groups similar to those of glucose. Cotton candy confectionary offers a good example of spinnable sugar. However the saccharose based sugar glasses usually employed bear some limiting factors such as recrystallization and hydroscopicity, susceptibility to condensation or dehydration reactions at higher temperatures (caramel formation) due to the reducing end instability. They also usually present rather high glass transition. An alternative sugar alcohol blend (isomalt ®) offers competing advantages such as lower Tg (56-60°C) and non reducing character (Borde, Cesàro 2001), while preserving the appropriate glassy character which favors spinnability. Isomalt is a sugar alcohol appreciated in the food industry that is obtained from the enzymatic conversion of sucrose to isomaltulose followed by hydrogenation. Isomalt is a mixture of α-D-glucopyranosyl -1-6-mannitol and α-D-glucopyranosyl -1-6-sorbitol (*Fig 1a*). Compounding isomalt with CNC starts from dilute water solutions which are converted to a molten dope by evaporation up to high temperature (150°C) and subsequent spinning of the degazed melt around the glass transition temperature (70°C). Removal of the suspending matrix takes advantage of the differential solubility of low molecular weight saccharides in methanol or similar solvents. Loaded fibers are thus converted into flat ribbons preserving the high orientation of the filler particles possibly aided by self-assembly mechanisms.

The high degree of orientation of the ribbons is suggested by the SEM images of the longitudinal cross section of a metalized split ribbon (*Fig 2*) and X-Ray diffraction diagram (*Fig 3*). SEM images display a highly oriented pattern along the fiber axis. However, it is difficult to estimate from the images if the BCNC arise as individual nano-objects or as associated bundles. Further analysis of nanocrystals rebundling would require TEM analysis at higher resolution on thin sections. The averaged orientation is better illustrated by the X-Ray diffraction pattern (*Fig 3a*). The texture analysis from the azimuthal intensity distribution of the 200m main elongated spot allows to calculate the Herman's order parameter S (see Nishiyama et al. 1997 for details) which yields S=0.85. This figure may be compared to ramie highly aligned bast fiber (S= 0.9) and excellent nematically oriented dried gels of *Cladophora* sulfated whiskers (0.96) (Nishiyama et al. 1997). However, the lower value obtained in our work can also be attributed to that fact that in order to get enough signal, eight ribbons were manually and thus imperfectly stacked inducing possible edge disorder. Nevertheless, the degree of orientation is really high demonstrating the efficiency of the method.

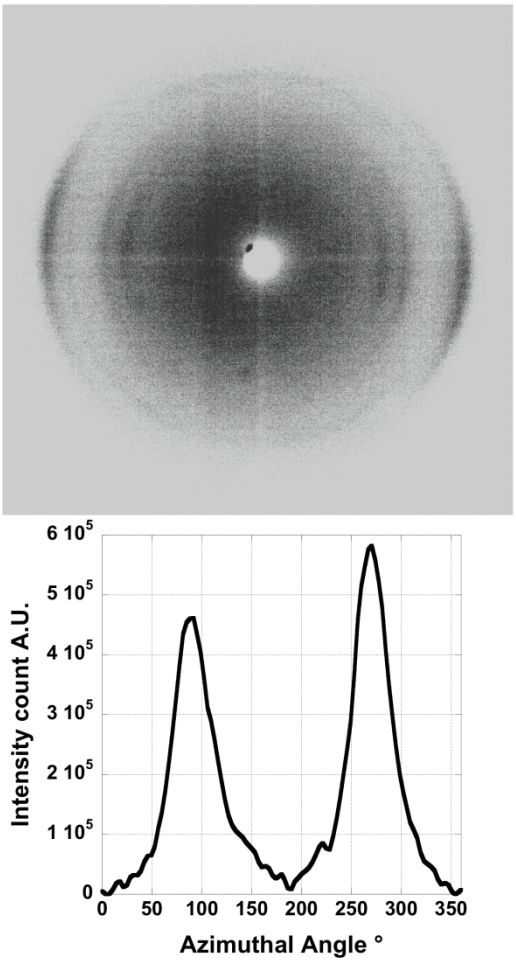

Fig 3 - (a) X-ray diffraction area scan and (b) azimuthal distribution of the main reflection spot (i.e. 200 of monoclinic Iβ allomorph 0.39nm inter-reticular distance) of a stack of oriented BCNC ribbons perpendicular to the incident beam.

Conclusions

We report preliminary work of a new method to obtain highly oriented BCNC ribbons thanks to the melt spinning of an embedding glass former : isomalt which is easily removed thanks to its differential solubility with respect to polysaccharides. Further studies would be necessary to increase the homogeneity of evenly thicker samples before addressing the evaluation of the mechanical properties of such oriented nano-cellulose based materials. As an extension this technique should be tested with long nanofibrils so as to mimic the effects of entanglement in the process of reconstituting plant mimetic cell wall assemblies.

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