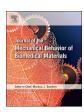


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Mechanical characteristics of nanocellulose-PEG bionanocomposite wound dressings in wet conditions



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ABSTRACT

Wood nanocellulose has been proposed for wound dressing applications partly based on its capability to form translucent films with good liquid absorption capabilities. Such properties are adequate for non-healing and chronic wounds where adequate management of exudates is a requirement. In addition, the translucency will allow to follow the wound development without the necessity to remove the dressing from the wound. Understanding the mechanical properties of nanocellulose films and dressings are also most important for tailoring optimizing wound dressing structures with adequate strength, conformability, porosity and exudate management. Mechanical properties are usually assessed in standard conditions (50% relative humidity, RH), which is not relevant in a wound management situation. In this study we have assessed the mechanical properties of three nanocellulose grades varying in the degree of nanofibrillation. The effect of nanofibrillation and of polyethylene glycol (PEG) addition, on the tensile strength, elongation and elastic modulus were assessed after 24 h in water and in phosphate-buffered saline (PBS). The results reveal the behavior of the nanocellulose dressings after wetting and shed light into the development of mechanical properties in environments, which are relevant from a wound management point of view.

1. Introduction

Nanocellulose is biodegradable by nature and renewable material, with a range of advantageous characteristics. Cellulose nanofibrils (CNF) is a type of nanocellulose that can be produced from a series of natural resources including wood pulp fibres, annual crops and agricultural residues (Iwamoto et al., 2008; Syverud et al., 2011; Alila et al., 2013; Siró and Plackett, 2010; Lee et al., 2014; Paschoala et al., 2015; Boufi and Chaker, 2016). Depending on the production process, nanocellulose can be highly nanofibrillated, containing a low fraction of residual fibres, and nanofibrils with diameters less than 20 nm and lengths in the micrometer scale. 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO)-mediated oxidation is one of the most applied pre-treatments for production of CNF. This chemical pre-treatment is regiospecific and oxidates the C6-position of the glucose molecule into carboxyl groups. The regiospecific oxidation creates repulsive forces between the nanofibrils and facilitates the swelling and the disintegration of the fibre wall structure into structurally homogeneous and individualized nanofibrils (Saito et al., 2006; Chinga-Carrasco et al., 2011; Fukuzumi et al., 2013).

TEMPO CNF can form films, which are translucent, strong and with a great capacity to absorb liquid (Fukuzumi et al., 2009; Nordli et al., 2016; Liu et al., 2016). Such characteristics have motivated extensive research regarding the application of nanocellulose as a functional biomedical material, e.g. as scaffolds for tissue regeneration (Lou et al., 2014; Ninan et al., 2013; Syverud et al., 2015) or as dressings for wound healing (Powell et al., 2016; Jack et al., 2016; Hakkarainen et al., 2016). Various studies have confirmed that different types of nanocelluloses are not cytotoxic (Vartiainen et al., 2011; Pitkänen et al., 2014; Čolić et al., 2015). Specifically, TEMPO CNF from P. radiata pulp fibres are not cytotoxic against 3T3 cell lines (Alexandrescu et al., 2013) and human skin cells (Nordli et al., 2016; Tehrani et al., 2016). In addition, we have most recently developed an ultrapure TEMPO CNF from P. radiata kraft pulp fibres with low endotoxin levels (less than 50 endotoxin units/gram cellulose) which complies with FDA requirements for biomedical devices (Nordli et al.,

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2016).

Well-fibrillated and homogeneous TEMPO CNF form films, which are strong, but brittle. The strength and modulus of some films have been quantified to be in the range of 200-300 MPa and 10-12 GPa, respectively (Henriksson et al., 2008; Fukuzumi et al., 2013; Josefsson et al., 2014). CNF can also serve as a building block for soft materials with high compliance and ductility (Esposito et al., 1996; Gatenholm and Klemm, 2010). Additionally, the brittleness of CNF films can be counteracted with plasticizers, e.g. glycerol, sorbitol and polyethylene glycol (PEG) (Minelli et al., 2010; Hansen et al., 2012; Tehrani, 2016). CNF films are strong in dry state, but considerably lose strength when the films are exposed to moisture (Tehrani et al., 2016). Furthermore, CNF films have been proposed as biomedical device for management of chronic wounds having high amounts of exudates management (Nordli et al., 2016). Hence, it is thus important to assess the effect of liquid absorption on the strength of the films and shed more light on how they could potentially behave in a wound management situation.

Although much attention has been given to well-consolidated CNF films with high stiffness and strength, it is important to assess in detail the mechanical properties (tensile strength, strain to failure, and elastic modulus) of CNF dressings in wet conditions, considering that wound management will involve the use of nanocellulose dressings to keep a moist environment in the wound bed. The purpose of this study is thus to perform a detailed mechanical assessment of nanocellulose dressings in contact with liquid (water and phosphate-buffered saline; PBS), considering also the influence of the added plasticizer and the degree of fibrillation on important physical, structural and liquid absorption properties.

2. Materials and methods

2.1. Nanocellulose production

Pinus radiata bleached kraft pulp fibers were used for CNF production. The carbohydrate composition of the pulp fibres have previously been quantified to be 87% cellulose, 12.2% hemicellulose and 0.8% lignin (Chinga-Carrasco et al., 2012). The pulp fibres were pretreated with TEMPO-mediated oxidation (Saito et al., 2006), using 3.8 mmol hypochlorite (NaClO) per gram cellulose. The CNF were produced through homogenization using a Rannie 15 type 12.56X homogenizer. The material (1% solids content) was homogenized with a pressure drop of 1000 bars and collected after 1 and 2 passes through the homogenizer. The TEMPO nanocellulose after 2 passes was diluted to 0.5% solids content and homogenized again for a third time. The carboxyl and aldehyde content was reported by Rees et al. (2014).

2.2. Characterization

Films (grammage 20 g/m²) were made of the TEMPO CNF without and with addition of 40 wt% polyethylene glycol (PEG 400 mw) in the composition (see Table 1). The films were made by casting a 0.2% suspension in petri dishes. The suspensions were allowed to dry at room temperature (~20 °C) for approx. 5–7 days. Previously, we have verified that addition of 40% PEG to the CNF film composition has various advantages, without compromising the cytotoxicity (Tehrani

Table 1
CNF series, without and with PEG in the composition.

	#Passes	PEG (%)
CNF01	1	
CNF01_PEG	1	40%
CNF02	2	_
CNF02_PEG	2	40%
CNF03	3	_
CNF03_PEG	3	40%

et al., 2016).

Ten topographical images were acquired from each sample by laser profilometry (LP). The topside was assessed. The size of the images was 1 mm \times 1 mm, with a lateral resolution of 1 μ m. The roughness of the films was quantified according to Chinga-Carrasco et al. (2014), suppressing the lateral wavelengths larger than 160 μ m.

A multimode atomic force microscope (AFM; with Nanoscope V controller, Digital Instruments) was used for microscopic analysis. All images were recorded at room temperature in air, in ScanAsyst mode (peak force tapping mode). The AFM tips of spring constant value $\sim\!0.4~\text{N/m}$ were purchased from Bruker AFM probes. The image size was $2\!\times\!2~\mu\text{m}$, with a resolution of 1.95 nm/pixel. The wavelengths larger than 30 nm were suppressed by fast Fourier-transform bandpass filtering to better visualize the nanofibrillar structure.

Film samples (20×20 mm) from each series (Table 1) were immersed in distilled water and PBS for 24 h and then freeze-dried at -80 °C, as an attempt to preserve the swelled structure. The water and PBS series (6 series×2 treatments) were embedded in epoxy resin and prepared for SEM cross-sectional analysis. Ten SEM cross-sectional images were acquired in a Hitachi scanning electron microscope, SEM, SU3500, in backscatter electron imaging (BEI) mode for thickness quantification (Chinga-Carrasco et al., 2011). For exemplification purposes, cross-sections of the films before and after 24 h in water were prepared by ion milling (using an IM4000 system), where the milling time was 5 h at 2.5 kV. Images of the ion-milled samples were acquired in the SEM (SU3500), in low vacuum mode.

2.3. Liquid absorption

The absorption of distilled water and PBS was quantified during a period of 24 h, and measurements were undertaken at time points 0, 15, 30, 60, 120, 240, 480 and 1440 min. The liquid absorption was calculated as $(w_l-w_d)/w_d$, where w_l is the weight of the film immersed in the liquid (water or PBS) and w_d is the weight of the film in its dry form.

2.4. Mechanical assessment

Strip-shaped samples were cut from each series of films, with the width of 15 ± 1 mm and the length of 112 mm. The films conditioned in distilled water and PBS were assessed with a 10 N load cell in order to obtain a better resolution in stress for the softened material. Tensile tests were performed at a speed of 20 mm/min, and each test took around 3-4 min (including the sample fixation), assuring that the samples practically remained in their conditioned state during testing. For each series of films, more than five samples were tested.

3. Results and discussion

3.1. Structural assessment

In this work, we have produced three batches of CNF, based on the same pretreatment but varying on the number of homogenization passes. As expected, the higher the number of passes through the homogenizer, the more fibrillated the material becomes. The degree of fibrillation is effectively quantified by measuring the surface roughness of CNF films. Provided that the films are produced with the same grammage and are dried under the same conditions, the smoother the CNF films are the higher the degree of fibrillation (Chinga-Carrasco et al., 2014). This is confirmed in this study, considering the samples CNF01, CNF02 and CNF03, without PEG in the composition (Figs. 1 and 2). In addition, PEG has been proposed as a plasticizer for nanocellulose films. The PEG addition increases the roughness of the top side of the films (Figs. 1 and 2), which confirms our previous findings (Tehrani et al., 2016).

The AFM analysis of the samples CNF01, CNF02 and CNF03

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