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Translucent and ductile nanocellulose-PEG bionanocomposites—A novel substrate with potential to be functionalized by printing for wound dressing applications

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ABSTRACT

There is potential that nanocellulose structures can act as a substrate for biomedical applications in which printing can expand its use as a functionalized biomaterial. Nanocellulose has a variety of advantages, which make the material suitable for use in biomedical devices that include wound dressings. The material does not promote bacterial growth, allows for production of translucent films and provides a moist wound-healing environment. However it is intrinsically brittle so research is needed to develop its flexibility and strength through the addition of plasticisers. In this work, we explore the effect of Polyethylene Glycol (PEG 400) as a plasticizer on nanocellulose film formation and performance. The nanocellulose used was prepared with TEMPO mediated oxidation. We also demonstrated different methods such as laser profilometry and atomic force microscopy to observe the topography and morphology of the films. FTIR, UV–vis spectroscopy was used to look at the characteristics of the nanocellulose films. In addition, the mechanical strength of the films with and without plasticizers was assessed. This led to the formulation of films that included PEG400 at 10–40% by weight. These demonstrated properties that are suitable for wound dressings. Additionally, the PEG modification yielded films that showed a surface morphology adequate for surface modification by printing. Importantly, a cytotoxicity test was performed using Human Dermal Fibroblasts and Human Epidermal Keratinocytes. The results showed no effect on the metabolic activity when fibroblasts were incubated in the presence of films containing 10 and 25% PEG. A reduction was measured in the presence of PEG at 40%. However, no significant cell death was detected in any of the cell-types. Hence, the nanocellulose-PEG films are not considered to be cytotoxic against human skin cells at the concentrations applied in this study.

1. Introduction

Nanocellulose has been the subject of extensive research during the last years. This is due to the wide range of potential applications that have been attributed to the material, including strength enhancement in paper and bionanocomposites, as an oxygen barrier for food packaging, and viscosity enhancers, to name a few. Additionally, biomedical applications of nanocellulose have attracted considerable attention. Presently, most of the biomedical applications of nanocellulose have been based on bacterial cellulose (see e.g. Jorfi and Foster, 2015). Considering that nanocellulose can be effectively produced from wood and in large quantities, makes the material promising, provided that the material is biocompatible with the human body. Recently, nanocellulose from wood has been proposed as a main component in scaffolds (Bhattacharya et al., 2012; Syverud et al., 2015), with potential as an artificial ligament (Mathew et al., 2013), as a component of bioinks for 3D bioprinting (Rees et al., 2014; Markstedt et al., 2015) and in wound dressings with potential to impair bacterial growth (Powell et al., 2016).

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Nanocellulose from wood has previously been shown to be compatible with different cell lines (Vartiainen et al., 2011; Alexandrescu et al., 2013; Hua et al., 2014). However, a concentration dependent reduction in metabolic activity and cell proliferation has been seen in the fibroblast cell line L929 and human peripheral blood mononuclear cells (PBMNCs) (Čolić et al., 2015). Recently, ultrapure cellulose nanofibrils from wood have been produced for the first time for biomedical applications, having low levels of lipopolysaccharides (Nordli et al., 2015), which reduces the potential immunogenicity of the material. This has demonstrated that ultrapure nanocellulose can be produced for applications within biomedicine, e.g. films for wound dressings.

Films made of cellulose nanofibrils are commonly brittle, which may limit its application. Ductile films are desirable for e.g. surface modification by printing. Various additives have been proposed for increasing the ductility of nanocellulose films, including cationic hydroxyethylcellulose (CHEC) and glycerol (Spoljaric et al., 2015) and quaternary alkylammoniums (QAs) by casting on 2,2,6,6-tetramethylpiperidine-1-oxyl-oxidized cellulose nanofibrils (TOCNs) (Shimizu et al., 2014). In general, there are different classifications of plasticizers used that include hydrophilic, hydrophobic and oligomeric-polymeric types. Polyethylene glycol (PEG) belongs to the oligomeric and polymeric group (Snejdrova and Dittrich, 2012). In addition, polar and polarizable groups can be used to improve tensile strength, but flexibility may be improved only moderately because of cohesion at points along the chain (Wypych, 2004). The border between hydrophilic and hydrophobic plasticizers is not sharp, due to their solubility in water and hence their ability to retain it. Water can act as a plasticizer (Snejdrova and Dittrich, 2012), but in reviewing the literature it was found that the ability to control this indirectly through the addition of PEG to cellulose nanofibrils (CNF) has not been investigated to date. Thus the impact of this addition on both flexibility and strength will be investigated in this work. Ductile films, with adequate strength, elongation and surface smoothness, can pave the way for surface modification by printing in a roll-to-roll process.

There are several techniques that can be used for printing on flexible substrates, including contact (flexographic, gravure, offset, screen) and non-contact (inkjet and aerosol) printing. Inkjet printing is suitable for depositing various functional materials (Delaney et al., 2009; Tobjörk and Österbacka, 2011; Tekin et al., 2008; Singh et al., 2010; Di Risio and Yan, 2012; Chinga-Carrasco et al., 2012a,b; Orelma et al., 2012), because of the low ink consumption, low cost and simplicity of changing digital print patterns. However, the current key disadvantage of inkjet printing is processing speed for large area coverage, the level of stress that is applied to the fluid during the jetting process and the narrow rheology window for process operation. The screen printing process also enables the deposition of active material and polymers on flexible substrates (Tehrani et al., 2015). Characteristically it is used to deposit thick films, which requires inks with high viscosity. Flexography as a contact process has also been developed for high-speed, roll-to-roll printing of fine features (Deganello et al., 2011, 2012) and has been demonstrated for the deposition of antibodies (Phillips et al., 2012).

The purpose of this study was to investigate the effect of polyethylene glycol (PEG) on the morphology and physical properties of nanocellulose films, with the intention of improving their ductility. It was found that a nanocellulose-PEG formulation facilitated the formation of flexible and smooth films, suitable for printing purposes. Cytotoxic aspects of the nanocellulose-PEG biocomposite films were assessed using primary Human Epidermal Keratinocytes and Human Dermal Fibroblasts.

2. Materials and methods

2.1. Production of cellulose nanofibrils

A nanocellulose material has been applied in this study, derived from *Pinus radiata* bleached kraft pulp fibres. According to Chinga-Carrasco et al. (2012a,b) the relative carbohydrate composition of the same *P. radiata* pulp fibres is 87% cellulose, 12.2% hemicellulose and 0.8% lignin. The pulp fibres were pretreated with TEMPO mediated oxidation (using 3.8 mmol NaClO per gram cellulose), according to Saito et al. (2006). The oxidized pulp fibres (1% consistency) were homogenized using a Rannie 15 type 12.56X homogenizer with a pressure drop of 600 bars. The nanocellulose was collected after 3 passes through the homogenizer.

2.2. Characterisation

Films were made using the TEMPO nanocellulose (20 g/m^2) having increasing PEG fractions (0, 10, 25, 40%). The film series were thus composed of 20 g/m^2 nanocellulose with additional PEG, i.e. 0, 2, 5 and 8 g/m^2 respectively. The films were made by casting a 0.2% suspension in polystyrene petri dishes and the suspensions were allowed to dry at room temperature (~20 °C). Following drying, the substrates were characterized by using laser profilometry (LP) and atomic force microscopy (AFM). Ten topographical images were acquired from each sample by LP and measurements were made on both sides. The size of the images was 1 mm × 1 mm, with a resolution of 1 μm/pixel. The roughness of the films was quantified at various wavelengths (for details see Chinga-Carrasco et al., 2014). Additionally, AFM imaging was performed with a Multimode AFM (with Nanoscope V controller), Digital Instruments. The images (2 μm × 2 μm) were acquired in ScanAsyst mode at room temperature. The thickness of the films, without and with 40% PEG, was estimated with X-ray microtomography, according to Miettinen et al. (2015). Cross-sections of the 40% PEG and a thin paper sample were prepared with a Hitachi ion-milling equipment (IM4000). The milling time was 10 h at 2.5 kV. The samples were covered with a thin layer of gold and cross-sectional images were acquired with a Hitachi scanning electron microscope (SEM, SU3500), in secondary electron imaging mode.

A UV–vis spectrophotometer (Cary 300 Conc., Varian) was used to quantify the Ultraviolet–visible (UV–vis) transmittance at a wavelength of 600 nm. A PerkinElmer Spectrum 100 and Spotlight 400 Fourier transform infrared spectroscopy (FTIR) microscope was used to characterise the film. At certain resonant frequencies characteristic of the specific sample, the Mid-IR radiation will be absorbed by a molecule's functional groups resulting in a series of peaks forming a spectrum, which can then be used to identify the sample surface chemistry.

Mechanical properties (maximum force and strain at break) were measured with a Zwick material tester (T1-FRxxMOD.A1K). Five samples of 15 × 20 mm were cut from each film series. The samples were tested with a speed and load cell of 20 mm/min and 2.5 kN, respectively. Three to four measurements were undertaken from each series.

2.3. Swelling and drying

Films were cut into squares of approximately 2 cm × 2 cm. Samples were soaked in either phosphate buffered saline (PBS) or MQ-water. PBS is a buffer solution with an osmolarity and ion concentration that is similar to fluids in the human body, and thus relevant for testing the films as wound dressing. At given timepoints (0.5, 2, 4, 8, and 24 h) the samples were lifted up with a pair of tweezers and weighed. After 24 h soaking the samples were left