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Antibacterial activity and biodegradability assessment of chemically grafted nanofibrillated cellulose

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ABSTRACT

Nanofibrillated cellulose (NFC) and their derivatives were prepared using three chemical surface modification strategies. All grafting was characterized by FTIR and contact angle measurements in order to evaluate the efficiency of grafting. Antibacterial activities of neat and grafted samples were investigated against two kinds of bacteria (i.e. Gram+ (*Staphylococcus aureus*) and Gram− (*Klebsiella pneumoniae*)). All the grafted samples displayed promising results with at least bacteriostatic effect or bactericidal properties. They also strongly enhanced the photo-catalytic antimicrobial effect of TiO_2. This study proves that it is better to use grafted NFC either alone or for functionalization with TiO_2 if anti-bacterial properties are desired. The cellulose backbone is known to be easily biodegradable in different biodegradation conditions and environments. The chemical surface modifications applied on NFC in the present work did not negatively influence this valuable property of cellulose but help for monitoring this property, which could be very useful for paper, packaging and composites.

1. Introduction

Due to the renewable nature, abundance, biocompatibility, biodegradability and high specific strength of cellulose fibers, a growing interest has been devoted to the ensued nanofibrillated cellulose (NFC). NFCs were produced and isolated first in 1983 by Turbak et al. [1] using high pressure homogenizer process. Presently, nanofibrillated cellulose can be obtained, from cellulose fibers by different mechanical and/or chemical pretreatment methods [2–5], in the form of long flexible filament in aqueous suspensions displaying a range in diameter of 5 to 50 nm diameter and several micrometers in length. Their specific surface areas combined with remarkable strength and flexibility make them a good candidate in different applications. Very recent reviews are available in the literature dealing with their properties and their potential applications in composite, paper and packaging for example [3,6–9].

However, these advantages turn to constitute their drawbacks. The high viscosity at very low concentration (i.e. 2–5%wt.) and their ability to form films or aggregates once dried limit their use in some applications. Chemical surface modification [10–14] on hydroxyl groups of NFC has been developed in the last decade by scientists to overcome these limitations. However such treatments can be costly and only added value applications should be envisaged with such materials. Therefore, it could be interesting to add new functions like antimicrobial as already performed on chitosan substrates [15,16].

NFC has been explored for numerous innovative applications, including composites, emulsions and also viscosity modifier. However, NFC based materials for antimicrobial activity have not yet been fully explored. Only very few scientific papers (only 5 papers up to our knowledge) exist [14,17–20] and usually in this scientific papers, silver nanoparticles have been tested after adsorption onto NFC (contrary to our chemical grafting strategy). However, the release of inorganic nanoparticles might be an issue when health and safety are concerned and the future availability of these non-renewable materials might be an issue for the development of this solution. In addition a growing interest in antimicrobial development brought by metal oxide nanoparticles like MgO, ZnO or TiO_2 particles has been recently published [21–23]. In this case, the development of bacterial resistance is less favored because these nanomaterials attack a broad range of bacteria. Among them, TiO_2 nanoparticles have attracted particular interest and were successfully applied with cellulosic fibers [24–26]. The first results about NFC–TiO_2 have been presented this year [20]. Based on photo-catalytic activation, TiO_2 nanoparticles have lethal effects on the cells; they can damage DNA, cell membranes, cell proteins and may lead to cell death.

In spite of these studies, almost nobody (to our knowledge) tested the chemically modified NFC in this context. Indeed, even if some

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surfactant adsorption has already been tested for anti-microbial properties [14], only one paper [17] deals with chemical grafting of quaternary ammonium molecule via silane reaction.

No other grafted chemical functions have been tested that are well-known to have bactericidal (like carbamates, for instance) [27]. That is why this study deals with the effect of different chemical modification of NFC on the anti-bacterial properties with a view in using these "active" particles directly at the surface or into the material bulk for paper, packaging or composite applications. In such a case NFC should not only be active but should also keep (or help to control) their biodegradable character.

Indeed, cellulose is a well-known biodegradable polymer, in fact pure cellulose is usually recommended as the positive reference in the biodegradation tests [28]. However, in some applications like external application (e.g. Mulch), a lower kinetic of biodegradability is expected. Moreover, it has been recently shown that addition of nanocellulose in composite can increase their degradation kinetics [29]. It is not always the expectation in nano-composite. So this information is a key property for monitoring material end-of-life. To the best of our knowledge, biodegradability of modified NFC was never reported in literature.

The research described in this study involved neat and grafted NFC (by carbanilation and esterification) in order to check their antibacterial and biodegradation properties. The antibacterial activity has been evaluated against Gram positive (*Staphylococcus aureus*) and Gram negative (*Klebsiella pneumoniae*) bacteria and the mechanism of antibacterial action has been discussed. Moreover, neat and grafted NFCs were functionalized or not with TiO_2 nanoparticles to enhance their antibacterial properties and to check the influence of chemical grafting. To the best of our knowledge, this is the first time that a paper dealing with both bactericidal effect and biodegradation behavior of neat and chemically modified NFC is reported.

2. Experimental

2.1. Materials

The wood pulp was kindly delivered by Domsjö (Sweden) and corresponded to a mix between Spruce and Pine (60% and 40%, respectively). This material is a dissolving pulp referred to sodium based sulfite mill extraction and used for the production of NFC. A second wood pulp was used in this study for the chemical modification using Eucalyptus as a raw material for the production of NFC. Endoglucanase used for the pretreatment of cellulosic fibers was purchased in Novozyme (Denmark).

The coupling agent (n-octadecyl isocyanate — 98%) and the catalyst (dibutyltin dilaurate — 96%), as well as the other reagents, acetic (99%), butyric (99%), iso-butyric (99%) and hexanoic anhydrides (99%), the ionic liquid [bmim][PF6] (98% HPLC grade), all the solvents used (i.e. ethanol—98%, acetone—98%, toluene—98% and dichloromethane—98%) and the surfactant TetradecylTrimethylAmmonium Bromide (TTAB), with a purity of 99%, were purchased from Sigma-Aldrich (France). AlkylKetene Dimer (AKD) was kindly supplied by Hercules©. It corresponds to a pure liquid AKD without a stabilizer (Prequel 9000). Distilled water was used for all experiments.

Titanium dioxide nanoparticles were supplied by Colorobbia (Italy) and dispersions were synthesized via hydrolysis and condensation of alkoxide-based precursors in water.

All microbial strains used were provided by DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures). *S. aureus* ATCC 6538 (DSM 799) and *K. pneumoniae* ATCC 4352 (DSM 789) were maintained frozen (−80 °C) and transferred monthly on PCA (Plate Count Agar) made of 5 g/L tryptone; 2.5 g/L yeast extract; 1 g/L glucose and 9 g/L neutralized bacteriological agar.

2.2. Neat and modified NFC production

Nanofibrillated cellulose suspension was produced from wood pulp depending on the chemical grafting strategy. Eucalyptus was used for carbonylated NFC and Domsjö pulp for all the other treatments and neat NFC. Authors asses the hypothesis that pulp source has no influence for this study. A suspension of bleached cellulose fibers (2.0%wt.) was enzymatically pretreated with endoglucanase (Cellulase) during 1 h at 50 °C to facilitate the size reduction of the fibers. Then, the slurry was fibrillated using a Masuko Grinder© (Japan). Size reduction of the fibers into nanofibrillated cellulose was obtained after 10 passes between the rotating and the static stones at 1500 rpm. Solid content of the NFC suspension was around 2.6% (w/w).

Six grades of modified NFC (3 g of each) were produced following previously described procedures. The first grade (NFC_C_{18}NCO) is obtained by carbanilation of NFC in toluene [30]. The three next samples obtained with acetic, butyric, and hexanoic anhydrides, i.e. NFC_AA, NFC_BA, and NFC_HA, respectively, were produced following a procedure in ionic liquid detailed very recently [11]. The last surface chemical modification of NFC was performed using a nanoemulsion of Alkyl Ketone Dimer (NFC_AKD) [31].

The adsorption of titanium dioxide was performed with the 30 mL NFC (600 mg dry NFC) + "x" mL TiO_2 to obtain different NFC:TiO_2 ratios. After mixing, centrifugation steps (3 times, 15 min at 10,000 rpm) with water were done to eliminate excess TiO_2 not "adsorbed" to NFC. Final residue was suspended in about 6–7 mL of water giving suspensions at 5–7% dry weight. The reference sample with NFC followed similar treatment: 30 mL NFC (600 mg dry NFC) + centrifugation (3 times, 15 min at 10,000 rpm) with water. A control test was performed to evaluate the amount of TiO_2 retained by NFC: in fact some control preparations were obtained by filtration procedures. Instead of centrifugation, the NFC/TiO_2 suspension was filtered on glass fiber filters, to eliminate excess TiO_2 not attached to NFC (10 mL suspension was washed with 30 mL of water). The final NFC/TiO_2 nanocomposite was tested for antibacterial properties and similar activities were obtained in respect to the preparations obtained by centrifugation.

2.3. NFC characterizations

Microscopy characterization were carried out using a scanning electron microscope equipped with a field emission gun (FE-SEM), model Zeiss Ultra column 55 Gemini, which was used to observe neat and modified NFC. The accelerating voltage (EHT) was 3 kV for a working distance of 6.4 mm. The sample tested was deposited onto a substrate covered with carbon tape and then coated with a 2 nm layer of Au/Pd (gold/palladium) to ensure the conductivity of samples. The coating was obtained by sputtering Au/Pd atoms under a voltage of 6 kV and 300 μA during 20 s.

Infrared spectra were recorded on film for neat NFC and dried powder for modified NFC, using a Perkin-Elmer SP100 spectrometer. For each sample, the Diamond crystal of an attenuate total reflectance (ATR) apparatus was used. The torque applied was kept constant to ensure the same pressure on each sample. Triplicates were performed for each sample and the best representative spectrum was kept for consideration. All spectra were recorded between 4000 and 600 cm^{-1}, with a resolution of 4 cm^{-1} and 8 scans.

Contact angle measurements were carried out by depositing different water droplets at the surface of the studied substrates and by recording the formed angles using an OCA dataphysics system equipped with a CCD camera. The contact angle and the drop volume (5 μL) acquisition were realized during the first after deposition seconds until equilibrium has been achieved and taking 4 images/s. For unmodified NFC and modified NFC, the measurement was performed on dried films. All measurements were performed at least 5 times for each sample and averaged.