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Synthesis of cationized nanofibrillated cellulose and its antimicrobial properties

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Abstract

Two types of cationized nanofibrillated cellulose (NFC) were prepared by redox initiated graft copolymerization and etherification with quaternary ammonium compounds (QAC). The QAC content and charge density of the products were measured. The NFC derivatives were tested for antimicrobial activity against Gram positive and negative bacteria, and yeast. Both NFC types exhibited broad spectrum antimicrobial activity. Etherification resulted in a higher degree of substitution and charge density, and the product also showed higher antimicrobial activity than the copolymerization product. Etherified NFC was more efficient against Gram negative than positive bacteria, whereas the polymer grafted NFC was equally active against both. This was attributed to the ability of the polymeric grafts to penetrate the thick cell wall of Gram positive bacteria, followed by the destabilization of the cellular membrane. Neither cationized NFC type showed cytotoxicity against human cells, providing means to manufacture safe, insoluble, and permanently antimicrobial materials via aqueous synthesis.

1. Introduction

Controlling microbe growth is often essential for the modern lifestyle, since man-made materials usually lack defenses against microbes. Prolific microbe growth creates a biofilm which protects the embedded microbes, making them more resistant to disinfection. These biofilms often excrete chemicals that are toxic or degrading to the underlying material. They also make the gene exchange between microbe cells more efficient, favoring the formation of bacterial strains with resistance to antibiotics [1,2]. For the purpose of microbial growth control, there are generally two methods. Disinfecting is used to sterilize an environment, but once exposed to a non-sterile environment it is soon contaminated by new microbes. The other option is to use materials that are inherently antimicrobial, eliminating the need for disinfection [3]. An example of
such material is silver that has been well-known for its antibacterial properties since antiquity. However, the antibacterial effect requires silver ions to dissolve into the medium [4]. The use of silver in food contact materials is severely limited by strict regulations that restrict the maximum allowed silver concentration below its biocidal level [4,5].

Quaternary ammonium compounds (QAC) are also known for their antibacterial and antifungal properties [6], offering an alternative to common disinfectants and silver. Polymers containing cationic ammonium groups have been found particularly effective as wide-spectrum biocides [7]. Moreover, microbes have not developed notable resistance to these compounds despite their wide use for over 40 years, nor have they shown improved resistance even after several generations of laboratory cultures [8]. Despite their strong bactericidal properties, cationic polymers have low human toxicity, since bacterial cells differ considerably from human cells in their structure and chemistry [9]. Moreover, biocidal polymers that are deactivated over a period of time due to cleavage of labile satellite groups by common enzymes have been developed to address concerns about possible long term effects of large scale use [10].

The antibacterial effect of cationic compounds is attributed to their ability to interact with the negatively charged phospholipids that are present in bacterial cell membranes, eventually disrupting the membrane. The interaction with mammalian cells is much weaker because their cell membrane is close to neutral charge [11]. The membrane disrupting ability has been explained by several models, including the “phospholipid sponge” effect where anionic phospholipids are gradually pulled away from the bacterial cell membrane by a nearby cationic surface. When a critical amount of phospholipids are removed, the membrane decomposes [12]. Another suggested mechanism involves the adsorption of a cationic polymer onto the cellular membrane. The adsorbed polymer binds to the anionic phospholipids scattered throughout the membrane, pulling them together into large anionic patches that destabilize the membrane [7,13]. It has also been proposed that the release of the membrane-stabilizing divalent cations upon polyelectrolyte adsorption is sufficient to disrupt normal membrane functionality [14,15]. A recent study of cationic polyionenes (polymers having cationic groups as part of their backbone) proposed that this class of polymers has antimicrobial mechanism not involving cellular membrane disruption and therefore also lower hemotoxicity [16].

Antimicrobial effect has been observed with modified natural polysaccharides, including N-alkylated chitosan [17] and cationized starch [18]. However, their use in packaging materials is limited by their solubility in water, which will likely cause them to migrate out of the material in humid conditions. This results in the loss of antimicrobial activity and also contamination of the product. An additional drawback with starch is its digestibility that facilitates the release of any grafted molecules inside the digestive tract. Cellulose on the other hand is also a readily available polysaccharide but unlike chitosan or starch it is insoluble in water [19] and, to a great extent, indigestible by humans [20]. Therefore, it is plausible that cellulose with covalently attached antimicrobial grafts does not dissolve or lose its activity when used as coating or packaging material, and also does not release the active compound when added directly to foodstuffs.

Nanofibrillated cellulose (NFC) is a plant-based nanomaterial, which is prepared by disintegrating native plant fibers into nanosized fibrils via mechanical homogenization. The fibrillation is accomplished by pumping a dilute pulp dispersion through a high pressure homogenizer where the fibrils are separated by strong shear forces. These nanofibers are targeted for several applications like transparent nanocomposites [21], novel packaging materials [22], and rheology modification in foodstuffs and cosmetics [23]. One of the drawbacks of NFC derives from its bio-based origin. Although mechanically strong, NFC is readily attacked by, for instance, cellulose consuming fungi. It also offers a large surface area for bacterial growth. Incorporating the antimicrobial properties of cationic compounds into the large specific surface area of nanofibrils could yield safe and efficient antimicrobial materials for food packaging or medical applications, for instance. The cationic NFC suspension also has a potential double functionality, being both an antimicrobial preservative/disinfectant, and, due to its high viscosity, also a thickening agent in cosmetics or foodstuffs.

We have previously reported successful graft copolymerization of NFC with several acrylates and methacrylates by a simple redox initiated reaction [24]. In this paper, the method was applied to graft a polymer containing cationic ammonium groups on NFC. However, cationization of NFC can also be accomplished by grafting cellulose fibers with small molecules instead of polymers [25,26]. Due to the lack of existing research about the effect of the graft length, we compared the feasibility of both methods and the antimicrobial potential of the products. The two NFC types were compared by their chemical composition, charge density, and broad spectrum antimicrobial properties. Health and safety concerns were also addressed by testing the products for human cell cytotoxicity.

2. Experimental

2.1. Materials

Nanofibrillated cellulose was provided by UPM Corporation (Helsinki, Finland) with the product name UPM Fibril Cellulose. The material was prepared by mechanical disintegration of bleached birch pulp, which was pre-treated with a Voith refiner and then fluidized by seven passes through an M7115 fluidizer (Microfluidics Corp, Newton, MA, USA). The solids content of the prepared water dispersion was 1.6 wt%. Bleached sulfite dissolving pulp (Dissolving Plus) was obtained from Domsjö Fabriker (Domsjö, Sweden). All other chemicals were purchased from Sigma–Aldrich (Germany) and were of analytical reagent grade.
2.2. Preparation of cationized NFC

Pre-cationized NFC (CNFC) was prepared by etherification of dissolving pulp with epoxypropyl trimethylammonium chloride (EPTMAC, Fig. 1) and subsequent homogenization as described elsewhere [26]. Grafted cationic NFC (NFC-PDMQ) was prepared by redox initiated graft copolymerization. NFC/water suspension containing 0.66 g of dry NFC was diluted to 0.7 wt % and mixed with 4.1 g (20 mmol) of [2-(Methacryloyloxy)ethyl]trimethylammonium chloride (DMQ, Fig. 1). Sodium chloride (1.2 g, 20 mmol) was added to reduce electrostatic repulsion between the charged monomer molecules. Nitrogen gas was bubbled through the mixture while it was heated to 35 °C for 35 min, cerium ammonium nitrate (0.55 g, 1 mmol) was added, and the mixture was stirred for 3 h. The product was centrifuged and redispersed in distilled water repeatedly, and the supernatant was analyzed with $^1$H NMR (Bruker Avance III 400 MHz, Switzerland). The washing was repeated until no more solute NMR signals were detected in the supernatant. For improved resolution, the water peak was suppressed by excitation sculpting (zgesgp pulse sequence) [27].

2.3. Microscopy

The size of the modified cellulose fibrils was studied by atomic force microscopy (AFM). The suspensions of NFC-PDMQ and CNFC were diluted to 1.5 g/L and 0.15 g/L, respectively, dispersed with a tip sonicator, centrifuged for 40 min at 10,000 rpm, and spin-coated on SiO$_2$ wafers which were then oven-dried at 60 °C for 30 min. To study the size of aggregates, similar samples were prepared without the sonication and centrifugation. The AFM images were taken by Bruker Nanoscope V using tapping mode in air, with 325 kHz resonance frequency and 40 N/m force constant. The samples containing aggregates were also imaged by scanning electron microscopy (SEM), using Zeiss Sigma VP at 4 kV acceleration voltage. The samples were coated with a thin layer of gold/palladium alloy by sputtering prior to the imaging.

2.4. Elemental analysis

Elemental composition of the products was determined with a Perkin–Elmer 2400 CHN analyzer (Waltham, MA) from powdered samples. Mass percentages of hydrogen, carbon, and nitrogen were measured. In order to convert the mass fractions into molar fractions, both cationized samples were assumed to contain one chlorine atom per each nitrogen atom as shown in Fig. 1. The rest of the mass was assumed to be oxygen. Since each cationic moiety has one nitrogen atom, their number per anhydroglucose unit (AGU) was then calculated by formula (1).

$$\text{DS} = \frac{N_{\text{AGU}}}{N_{\text{AGU}} = \frac{N_{\text{AGU}}}{(C-xN)/6}}$$

where

- $N$ is the molar fraction of nitrogen.
- $C$ is the molar fraction of carbon.
- $x$ is the number of carbon atoms in the grafted QAC.

2.5. Charge density measurement

Charge densities of unmodified NFC, NFC-PDMQ, and CNFC were determined by polyelectrolyte titration. For the dissolving pulp used to make CNFC, a value reported by Olszewska et al. [26] was used. Before titration, the samples were diluted with CO$_2$ free pH 9 buffer solution (1 mM NaHCO$_3$) to 0.1 g/dm$^3$. Different amounts of standard polyelectrolyte solution were added to the sample suspensions, using a cationic polyelectrolyte (hexamethrine bromide, PB) for unmodified NFC, and an
anionic polyelectrolyte (sodium polyethenesulfonate, PesNa) for cationized products. Zeta-potentials of resulting suspensions were measured using Malvern Zetasizer analyzer (Worcestershire, UK) equipped with a dip cell. Zeta-potential was plotted as a function of added charge, and the equivalent point was interpolated to give the charge density (Fig. S1). Theoretical charge densities were evaluated by the formula (2), and compared to the measured values.

$$\sigma_{\text{theor}} = \frac{w_{\text{QAC}}}{M_{\text{QAC}}} \cdot \sigma_{\text{cellulose}}$$

where $w_{\text{QAC}}$ is the weight fraction of the grafted cationic moiety. $M_{\text{QAC}}$ is the molecular weight of the cationic moiety. $\sigma_{\text{cellulose}}$ is the charge density of the unmodified cellulose.

### 2.6. Antimicrobial properties

Antimicrobial activity of cationic NFC was studied by suspension cultivation with three potential human pathogens: *Micrococcus luteus* (ML, Gram positive), *Escherichia coli* (EC, Gram negative) and *Candida oleophila* (CO, yeast). The microbes were incubated in a respective culture medium: nutrient agar (peptone 1%, meat extract 0.5%, agar 2%, pH = 7) for the bacterial strains and yeast extract peptone dextrose (1% yeast extract, 2% peptone, 2% glucose, 2% agar, pH = 7) for the yeast. Fresh cultures were pre-inoculated in Erlenmeyer flasks, and incubated overnight for bacteria and 24 h for yeasts at 30 °C in platform shakers (150 rpm) before antimicrobial assays.

The antimicrobial activities of unmodified NFC, NFC-PDMQ, and CNFC were evaluated by a method used by Li et al. [28]. Three different concentrations (500, 1000, and 2000 μg/mL) of each NFC variety were tested. Aliquots of 100 μL of the pre-cultivated strains were added into Erlenmeyer flasks containing 10 mL of culture medium and cultivated as described above. After cultivation, 1 mL aliquot from each batch was added into test tubes containing 9 mL of saline solution and vortexed to homogenize. 100 μL of microbial suspension was drawn from each tube and spread on a nutrient agar plate and incubated at 30 °C for 24 h, after which the colonies were counted. The inhibition rate was evaluated comparing the number of viable cells in each NFC suspension with a positive control without any NFC. The logarithmic reduction value ($\log_{10} N_0/N$) was calculated for all experiments showing inhibition higher than 90%.

### 2.7. Cytotoxicity tests

Renca renal adenocarcinoma cells (ATCC® CRL-2947) were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) at 37 °C and 5% CO₂. The original nanocellulose suspensions (1.08% NFC-PDMQ, 1.09% CNFC and 1.39% NFC) were diluted with DMEM to get final concentrations of 500, 1000, and 2000 μg/L. For the experiment, Renca cells were plated at a density of 100,000/6-well plate and incubated overnight. 1 mL of each of the diluted nanocellulose suspensions were pipetted onto a Transwell membrane (Corning, 0.4 μm pore) placed above cell monolayer and incubated for 24 h. Finally, nanocellulose was removed and cell death was assayed by SYTOX green (Thermo Fisher Scientific) nucleic acid stain that penetrates cells with compromised plasma membranes but does not cross the membranes of live cells.

### 3. Results and discussion

#### 3.1. Dispersion quality

Dispersion quality was studied by visual observation and microscopy. Both cationized NFC derivatives were stable dispersions, with no visible sedimentation observed over a period of several months. Individual nanofibrils were observed by AFM (Fig. 2), which is a convenient method for measuring their dimensions because it also measures the height profile of the sample. The fibril diameters were between 4 and 16 nm for NFC-PDMQ, and between 1 and 4 nm for CNFC. The fibril diameter of NFC-PDMQ was similar to the unmodified NFC [24], indicating that significant aggregation did not occur during the modification. The smaller fibril size reflected on the visual appearance of the CNFC dispersion, making it more translucent than NFC-PDMQ.

The aggregates were too large to fit in an AFM image and their sizes were evaluated with SEM instead. Surprisingly, CNFC samples contained some very large aggregates, up to 1 mm in size. Also large fibers of the same scale were present, indicating incomplete homogenization. However, most aggregates were in the order of 100 μm in diameter and highly irregular in shape. The aggregates of NFC-PDMQ were mostly below 50 μm in diameter, apparently consisting of piles of nanofibrils, rather than residual macrofibers (see Fig. 3).

#### 3.2. Chemical characterization

The nitrogen content of the cationized NFCS was measured via elemental analysis to determine the degree of substitution. The mass percentages of carbon, hydrogen, and nitrogen were converted into atomic percentages that were used to calculate...
the degree of substitution (DS) of the glucose units in the cellulose backbone (Table 1). The calculated DS of CNFC was nearly 50% higher than that of NFC-PDMQ. The difference in actual density of grafting was probably even higher, since the cationic groups of PDMQ were distributed into polymeric chains, whereas each EPTMAC group in CNFC was attached directly to the surface. The results indicated that EPTMAC functionalization was a more efficient cationization method in terms of obtaining higher DS.

Charge densities of unmodified NFC, NFC-PDMQ, and CNFC samples were determined using polyelectrolyte titration (Table 2). The equivalent point was interpolated from the steep linear section of the produced curve (Supplementary Figs. S1–S3). The charge density of CNFC raw material (28 meq/g) was obtained from an earlier publication [26].

The anionic charge of the unmodified NFC was considerably higher than that of the dissolving pulp used to make CNFC. The charge originated from the carboxylic groups in hemicelluloses which comprised 23% of the NFC [29]. The dissolving pulp on the other hand was highly purified cellulose with only 4.5% hemicellulose content, resulting in a low charge density. Due to this difference, and the higher DS, the charge density of CNFC was expected to be considerably higher than that of NFC-PDMQ. In fact, CNFC had nearly 70% higher charge density but only 15% more added charge, which factors in the different charge of the starting materials. It is possible that some of the hemicellulose was removed from NFC-PDMQ during washing, causing the added charge appear higher than it actually was. Although the charge densities of NFC-PDMQ and CNFC
were considerably lower than the predicted values, the ratio between the two was identical in both cationic NFC varieties (0.37 and 0.36, respectively), indicating similar accessibility of the charged groups regardless of the modification method.

### 3.3. Antimicrobial properties

Antimicrobial properties of unmodified and cationized NFC types were tested against three common, potentially pathogenic microorganisms of different types, namely Gram positive (\textit{M. luteus}) and negative (\textit{E. coli}) bacteria, and a yeast (\textit{C. oleophila}).

Unmodified NFC did not show significant inhibition to any of the tested microbial strains (Table 3). Minimal antifungal activity was detected, which may derive from the NFC network hindering the diffusion of nutrients to the yeast cells. This shows that practically all antimicrobial activity observed in the cationized derivatives was due to the introduced cationic groups. The pre-cationized CNFC had concentration dependent activity against both bacteria. The activity increased gradually with increasing concentration, reaching 93% inhibition (1.2 log) at 2000 \(\mu\text{g/mL}\) in the presence of the Gram negative strain. The effect was weaker against \textit{ML}, peaking at 79%. The CNFC exhibited highest activity against the yeast, killing 98% (1.7 log) of all yeast cells already at 500 \(\mu\text{g/mL}\) concentration and reaching 6 log reduction at 1000 and 2000 \(\mu\text{g/mL}\) concentrations. The NFC-PDMQ also showed moderate activity at 2000 \(\mu\text{g/mL}\) against both bacterial strains, however the growth inhibiting effect was limited to 43% against EC and 48% against ML at the highest concentration. At the two lower concentrations, NFC-PDMQ was 1.4–2.2 times more effective against ML than CNFC. There was also notable, concentration dependent activity against the tested yeast. The fungicidal activity was higher than the antibacterial activity. The microbe killing efficiencies of NFC-PDMQ and CNFC were comparable to earlier results with phosphonium-containing polycationic biocides, which were able to kill approximately 50–100% of the microbes at 2500 \(\mu\text{g/mL}\) concentration, depending on the species [30]. Interestingly, the materials synthesized in our study had similar level of activity, although

### Table 1

Mass percentages obtained by elemental analysis, molar percentages of carbon and nitrogen, and calculated values for DS and weight fraction of cationic substituents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>C mol%</th>
<th>N mol%</th>
<th>DS</th>
<th>w_{QAC}</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFC</td>
<td>42.80</td>
<td>5.82</td>
<td>0.13</td>
<td>28.41</td>
<td>0.07</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NFC-PDMQ</td>
<td>38.34</td>
<td>5.51</td>
<td>0.93</td>
<td>26.40</td>
<td>0.55</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>CNFC</td>
<td>42.65</td>
<td>7.11</td>
<td>1.30</td>
<td>26.03</td>
<td>0.68</td>
<td>0.19</td>
<td>0.18</td>
</tr>
</tbody>
</table>

\(a\) Mass percentage of the total sample mass.

\(b\) Degree of substitution.

\(c\) Weight fraction of the cationic substituent.

### Table 2

Measured and theoretical charge densities of unmodified and cationized NFC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured Charge density ((\mu\text{eq/g}))</th>
<th>Added Charge density ((\mu\text{eq/g}))</th>
<th>Theoretical Charge density ((\mu\text{eq/g}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFC</td>
<td>–136</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dissolving pulp</td>
<td>–28(^a)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NFC-PDMQ</td>
<td>239</td>
<td>375</td>
<td>641</td>
</tr>
<tr>
<td>CNFC</td>
<td>403</td>
<td>431</td>
<td>1131</td>
</tr>
</tbody>
</table>

\(^a\) Calculated by formula (2).

\(^b\) From [26].
the cationic compound comprised only 16–18% of their dry mass. The results suggest that the immobilization of the active compound on a high surface area carrier such as NFC allows its actual concentration to be lower without losing antimicrobial performance.

Although the limited scale of our study does not allow profound interpretations of the antimicrobial activities of our products, we can speculate on the possible reasons for the differences in their antimicrobial activities. First of all, the chemistry of the cationic groups did not explain the different antimicrobial properties since both CNFC and the grafted polymer in NFC-PDMQ contained trimethylammonium groups. Instead, the most apparent reason for the superior antimicrobial properties of CNFC was its charge density which was nearly 70% higher compared to NFC-PDMQ. In previous studies, charge density has been established as one of the key factors affecting antibacterial properties of cationic polymers [31].

The conformation of the cationic groups may also have been different. Since NFC has a negative charge, the cationic groups in a PDMQ polymer chain may have adhered to the fiber due to electrostatic interaction and chain flexibility, exposing only the neutral backbone to the surrounding medium. In contrast, the small cationic groups covering the surface of CNFC were unable to bend towards the fiber surface, which also had considerably lower negative charge than that of NFC. Moreover, the smaller diameter of CNFC fibrils also indicates larger active surface and better accessibility of the cationic groups. Due to these factors it was anticipated that the charge density of CNFC would be closer to the theoretical value. However, the measured charge density of both cationized NFC types was less than 40% of the charge density suggested by the chemical composition.

NFC-PDMQ showed similar activity against Gram positive and negative bacteria whereas CNFC was notably more efficient against the Gram negative bacteria. To understand this phenomenon, the cell structure of both bacterium types should be considered. The Gram positive bacteria have a single cell membrane that is surrounded by thick and porous cell wall that enables small molecules to diffuse through. In contrast, the Gram negative bacteria have a thin cell wall around the inner membrane, which is surrounded by another membrane. Typically, Gram negative bacteria have shown higher tolerance of cationic polymers which has been attributed to this additional membrane that acts as a diffusion barrier [32]. However, cationic groups attached on a cellulose nanofiber may be too bulky to properly penetrate the cell wall of Gram positive bacteria, hence making them less efficient against this type of bacteria. The main difference of the two cationized NFC types is that NFC-PDMQ has long polymeric grafts that may be able to diffuse through a thick cell wall despite being anchored onto a bulky carrier [33]. The consistent activity of NFC-PDMQ, regardless of the bacterial type, supported this hypothesis. The CNFC still had higher overall activity, probably due to its significantly higher charge density.

The strong antifungal activity of our samples shows that the membrane disrupting ability of cationized NFC also applies to some eukaryotic cells. Fungal cells, despite being considerably different from bacterial cells, have structural features in their outer lining similar to Gram positive bacteria. Both have a thick cell wall around a single negatively charged phospholipid membrane, although their compositions are different [34]. This negative charge most likely also made the yeast susceptible to the cationized NFC.

3.4. Cytotoxicity

Possible leaching of harmful chemicals from cationized NFC derivatives was studied using a Renca cell culture separated from the NFC by a porous membrane. The cytotoxicity of NFC proved difficult to study when it was applied directly on the cells at the desired concentrations. In this case, the nanofiber network would block the diffusion of oxygen and nutrients to the cells, causing massive cell death even when using unmodified NFC. Since our NFC derivatives were not intended for use as cell growing media, a porous membrane was used to keep NFC and cells separate, while allowing soluble chemicals to pass through. The observed degrees of cell death (Fig. 4) were approximately 1% in the control, and 1–2% with all NFC types.

![Fig. 4. Quantification of cell death results (percentage of dead cells of total cell number).](image-url)
showing very low cytotoxicity. A slightly positive dose response was observed with NFC and NFC-PDMQ. However, the statistical relevance of this trend was ambiguous because the differences between the samples and the control were within the margin of error. The results indicate that neither NFC derivative released notable amounts of chemicals toxic to human cells. Meanwhile, they were effectively antimicrobial at the same concentrations.

4. Conclusions

Two cationized NFC varieties were synthesized and tested for antimicrobial activity and cytotoxicity. Cationization was implemented via graft copolymerization of NFC, and through the etherification of dissolving pulp prior to nanofibril separation. The DS and charge density were significantly higher in the latter product, indicating better efficiency of the pre-cationization. On the other hand, the etherification required strong alkaline reaction medium, whereas the graft copolymerization was feasible in very mild conditions.

The pre-cationized NFC showed strong broad-spectrum antimicrobial effect at 2000 μg/mL concentration. The polymer grafted NFC had a more limited effect, exhibiting strong fungicidal activity and moderate antibacterial activity. The microbe killing efficiency was clearly concentration dependent with both materials. CNFC was notably more efficient against the Gram negative than Gram positive bacterium, while NFC-PDMQ exhibited consistent activity. A possible explanation was the thicker cell wall of gram positive bacteria, which may be easier for the long polymeric grafts on NFC-PDMQ to penetrate. Pre-cationized NFC showed significant fungicidal activity already at concentrations as low as 500 μg/mL, with log 6 inhibition being observed at 1000 μg/mL. Cytotoxicity tests with human cells showed no migration of toxic compounds from either NFC derivative, indicating them as safe candidates for novel, bio based, and permanently antimicrobial materials.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2015.12.008.

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