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Effect of variable aminoalkyl chains on chemical grafting of cellulose nanofiber and their antimicrobial activity

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ABSTRACT

Recent focus on the preparation of antimicrobial surfaces using cellulose nanofibers (CNF) has gained considerable attention. In this work, functionalization of CNF films in 100% aqueous solution with three different aminosilanes, including 3-aminopropyl trimethoxysilane (APMS), 2-aminoethyl 3-aminopropyl trimethoxysilane (DAMS)and 3-2-(2-aminoethylamino) ethylamino propyl-trimethoxysilane (TAMS) is reported for the fabrication of contact active antimicrobial materials. Grafted CNF films were comprehensively characterized by FTIR, TGA, contact angle, elemental analysis, solid-state ^{29}Si NMR, FEG-SEM and SEM-EDX. It was found that all the silanes were grafted on the surface of nanofibers without any change in the morphology or fibril structure through different grafting efficiency, depending on the aminoalkyl chain length. The effect of variable aminoalkyl length and initial grafting concentration was analyzed against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) by qualitative and quantitative standards. The most promising results were obtained with 3-2-(2-aminoethylamino) ethylamino propyl-trimethoxysilane at very low concentration which completely restrict bacterial growth after 24 h with Gram-positive bacteria. This study, for the first time, established the relationship between the aminoalkyl chain length and its corresponding antibacterial activity.

1. Introduction

CNF, first produced in 1980′s [1], have attracted considerable attention for the design of novel, efficient and biobased materials for several applications, such as barriers, nanocomposites, paper and controlled drug release. This is due to their exceptional features, such as high aspect ratio, web like nanoporus network of films, high surface ratio, flexibility and apparent surface functionalization [2]. Physical incorporation and chemical surface grafting of bioactive agents are the two main methods for the fabrication of antimicrobial surfaces from CNF. These bioactive agents or biocides play an apparent role in the preservation of different products, e.g., food, cosmetics or medicines.

Indeed, various antimicrobial compounds, such as metal nanoparticles, enzymes, polysaccharides, drugs and plant extracts have been introduced into CNF [3–6] with success but limited actions due to accessibility and release. However, some of these compounds are toxic to the environment or human health and also uncontrolled rate of diffusion constitutes a major problem.

As a result, in last decade, the preparations of contact active antimicrobial surfaces have gained attention due to advantages, such as chemical stability, low volatility and release, resulting in multiple times utilization [7–9].

For this reason, contact active antimicrobial CNF films were prepared recently by chemical immobilization of quaternary ammonium [10–12], silanes [13], isocyanates and anhydrides [14], as well as penicillin [15]. For grafting on CNF, the use of solvent for the preparation is also a major concern related to food and medical applications.

The motivation of this study was derived from the intrinsic bactericidal activity of chitosan, which is associated with the presence of free amino groups along the polymeric chain. Indeed, very recently biomimetic approach demonstrated that APMS is a good candidate to mimic antibacterial activity of chitosan [13]. Our investigation confirms such result but also provides an intensive insight with the effect of free amino groups and increased alkyl chain. It also provides an aqueous-based green process to consumers and industries for large scale application.

Amino silanes were primarily studied as a coupling agent for the absorption/complexing of elements [16,17], in heterogeneous catalysis [18–20] or very recently in controlled drug delivery [21,22] but not sufficiently explored for antimicrobial activity. Herein, the impact of variable aminoalkyl chains on the inhibition of bacterial growth was compared. To the best of our knowledge, this is the only study that

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compares the ability of amino silane on the basis of its structure. In this study, the main application is the preparation of antimicrobial surfaces; however, the obtained results could help establishing the best amino silane for other applications as well.

In this work, amino silanes were selected for chemical grafting on CNF films due to their distinct properties, such as high solubility in water, fast kinetics at natural alkaline pH and their well-known potential for the surface modification of hydroxyl bearing surfaces [23–25]. As shown in Fig. 1, three different commonly used aminosilanes: 3-aminopropyl trimethoxysilane (APMS), 2-aminoethyl 3-aminopropyl trimethoxysilane (DAMS) and 3-2-(2-aminoethylamino) ethylamino propyl-trimethoxysilane (TAMS), were preferred among other choices due to the variable aminoalkyl chain.

Indeed, previous investigation has emphasized the importance of the nature and position of the nitrogen bearing function in the structure of the silane [26]. This previous study proved that aminosilanes with the longest aminoalkyl chain i.e. TAMS have the highest stability and available for grafting in the form of silanol groups for longer period of time. Present study took a one step forward by applying the previously established information and used to study its effect on the grafting with CNF.

2. Material and methods

2.1. Materials

CNF suspension was kindly supplied by Centre technique du Papier (CTP), France at 2 wt.% consistency, prepared as reported in previous literature [27]. This suspension was produced from Domjso softwood dissolving pulp using enzymatic pre-treatment (during 2 h with endoglucanase) and mechanical fibrillation in a homogenizer, Ariete NS3075, GEA NiroSoavi, Italy (one pass at 1000 bar and four passes at 1450 bar). Acetone, were obtained from Chimie Plus, France. Aminopropyl trimethoxysilane (APMS), 2-aminoethyl 3-aminopropyl trimethoxysilane (DAMS) and 3-2-(2-aminoethylamino) ethylamino propyl-trimethoxysilane (TAMS) were purchased from Sigma-Aldrich, France. Nutrient broth, nutrient agar, petri plates and sodium thiosulphate were obtained from Roth, France. Lecithin, Tween 80 and Histidine were purchased from Merck. *Bacillus subtilis* spores were provided by Humeau, France and other bacteria *Staphylococcus aureus ATCC 6538* and *Escherichia coli ATCC 8739* were purchased from Thermo scientific, USA and Microbiologics, Germany respectively in lyophilized form. All bacteria were revived before use. For all experiment, only deionized water was used.

2.2. CNF films

CNF films were prepared using a 159-mm-diameter handsheet former (the apparatus is particularly described in T 205 sp.—95 standard). CNF suspensions (2 g of oven-dry weight for each film) were filtered on nitrocellulose membranes with a pore size of 65 μm under a pressure of 600 mbar to obtain a wet cake (for ca. 2 min). Drying was performed using pressure dryers (Karl Frank GMBH) at 80 °C for 15 min and ensuing CNF films were stored in conditioned room (50% of relative humidity and 25 °C) at least for 24 h.

2.3. Grafting of CNF films

The chemical grafting of cellulose took place in 3 facile steps. The first steps consisting of dissolving aminosilanes in deionized water at 25 °C, pH 10. Then, absorption of silanes in CNF films by dipping for ca. 10 s. The second step including functionalization of CNF by thermally treated at 110 °C for 2 h to promote the covalent bonding between the silanol groups and the hydroxyl groups of CNF. In final grafting step, CNF films were washed thoroughly by soxhlet extraction with solvent (acetone) for 12 h and grafted films were dried in a desiccator at room temperature.

Five samples were prepared with different amino silanes by changing their quantity in the grafting solution, as following: CNF-N1 (0.28 mol/L of APMS), CNF-N2 (0.28 mol/L of DAMS), CNF-N3 (0.28 mol/L of TAMS), CNF-N2N1 (0.14 mol of DAMS) and CNF-N3N1 (0.10 mol of TAMS). CNF-N2N1 and CNF-N3N1 were prepared to attain a similar molar quantity of nitrogen, analyzing their impact on antibacterial activity.

First, similar molar concentration of each amino silane (APMS, DAMS and TAMS) was grafted on the surface of CNF films to correlate the efficiency of the grating with respect to the type of silane. Second, grafting concentration was limited with respect to the amino groups to analyze the impact of the amino alkyl chain on the antibacterial activity. As detailed in experimental section, three steps grafting process was used with first step of impregnation of respective silane in the CNF film for 10 s. Then, thermal treatment for 2 h at 110 °C was performed in order to form a covalent bond between silanol groups and cellulose

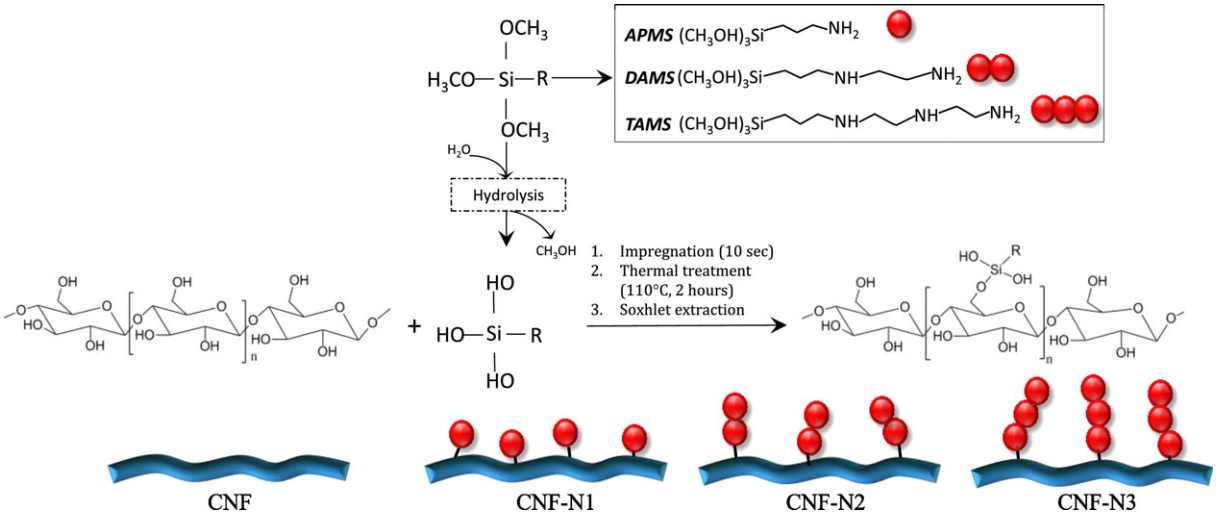

Fig. 1. Schematic illustration of the mechanism for aqueous-based silylation reaction with amino silanes on the surface of CNF films.