Nonleaching Antimicrobial Films Prepared from Surface-Modified Microfibrillated Cellulose

Martin Andresen,*,† Per Stenstad,‡ Trond Møretrø,§ Solveig Langsrud,§ Kristin Syverud,|| Leena-Sisko Johansson,⊥ and Per Stenius†,⊥

Ugelstad Laboratory, Department of Chemical Engineering, Norwegian University of Science and Technology, N-7491 Trondheim, Norway, SINTEF Materials and Chemistry, N-7465 Trondheim, Norway, Matforsk AS, Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway, The Norwegian Paper and Fibre Research Institute, Høgskoleringen 6b, N-7491 Trondheim, Norway, and Laboratory of Forest Products Chemistry, Helsinki University of Technology, Post Office Box 6300, 02015 TKK, Finland

Received March 16, 2007; Revised Manuscript Received April 26, 2007

We have prepared potentially permanent antimicrobial films based on surface-modified microfibrillated cellulose (MFC). MFC, obtained by disintegration of bleached softwood sulfite pulp in a homogenizer, was grafted with the quaternary ammonium compound octadecyldimethyl(3-trimethoxysilylpropyl)ammonium chloride (ODDMAC) by a simple adsorption-curing process. Films prepared from the ODDMAC-modified MFC were characterized by Fourier transform infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS) and tested for antibacterial activity against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The films showed substantial antibacterial capacity even at very low concentrations of antimicrobial agent immobilized on the surface. A zone of inhibition test demonstrated that no ODDMAC diffused into the surroundings, verifying that the films were indeed of the nonleaching type.

Introduction

The development of materials with antimicrobial surface properties has gained much attention during the past decade. Traditionally, such materials have been prepared by incorporating a leachable antiseptic into a polymeric surface matrix. The antimicrobial is gradually released and kills the microorganism by diffusing into and disrupting the cell.[1] Examples of typical leaching antimicrobial materials include compositions containing antibiotics, halogens, quaternary ammonium compounds, or heavy metals like silver or mercury.[2−5] There are, however, several disadvantages associated with this approach. Apart from the fact that a toxic substance is released into the environment, the antimicrobial activity of the material is only temporary, as all these systems eventually exhaust. Furthermore, the gradually decreasing level of the released compound may lead to subinhibitory concentrations of antimicrobial in the surroundings, which may provide conditions for development of bacterial resistance. In order to overcome these problems, the ideal strategy would be one where the antimicrobial agent is covalently immobilized onto the material surface rather than gradually released from it.[6] Several strategies for the preparation of such nonleaching materials have been reported; however, they often involve rather harsh reaction conditions and a number of chemical reaction steps.[7−12]

Microfibrillated cellulose materials (MFC) are known to form very thin, highly homogeneous, translucent films, with tensile strengths far superior to those of regular print-grade paper.[13,14] As a result, MFC have been proposed to have applications in paper coatings or other coating compositions.[15−17] Recently, we reported on the preparation of highly hydrophobic films based on surface-modified microfibrillated cellulose.[18] In the present study, we describe a simple method for the preparation of microfibrillated cellulose with a covalently bound antimicrobial agent as a step toward more permanent antimicrobial activity. The alkoxysilane octadecyldimethyl(3-trimethoxysilylpropyl)-ammonium chloride (ODDMAC), known for its antimicrobial properties and utilized by the textile industry for decades,[19−23] was grafted onto the surface of the MFC through an adsorption-curing process. By combining the unique properties of the MFC films, such as very high surface area-to-volume ratio and small pore sizes, with the antimicrobial properties of the ODDMAC molecule, we have prepared a fully biocompatible nanofiber material that could be very effective for wound healing applications, air filtration uses, or as an antimicrobial separation filter for submicrometer particles.[24−27] Furthermore, the grafting protocol described herein could easily be extended to other natural polymer fibers bearing surface hydroxyls, including textile fibers or fibers for use in packaging applications. Films prepared from the surface-modified MFC were characterized by Fourier transform infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS), and their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was evaluated.

Experimental Section

Reagents. Octadecyldimethyl(3-trimethoxysilylpropyl)ammonium chloride (ODDMAC) was purchased from Sigma−Aldrich as a 42% (w/w) solution in methanol. According to the manufacturer, the solution also contained 8% (w/w) (3-chloropropyl)trimethoxysilane. Chlorodimethylisopropylsilane was purchased from Aldrich and had a purity of 97%. The reagents were used without further purification.

* Author to whom correspondence should be addressed: tel + 47 73 55 05 37; fax + 47 73 59 40 80; e-mail martinan@chemeng.ntnu.no.
† Norwegian University of Science and Technology.
‡ SINTEF Materials and Chemistry.
§ Norwegian Food Research Institute.
|| The Norwegian Paper and Fibre Research Institute.
⊥ Helsinki University of Technology.

10.1021/bm070304e CCC: $37.00
Published on Web 06/02/2007

Microfibrillated Cellulose. Microfibrillated cellulose was obtained by disintegration of fully bleached spruce sulfite cellulose (Borregaard VHV−S) in a homogenizer. To avoid plugging of the homogenizing equipment, the fibers were cut to reduce the fiber length. Cutting the fibers into shorter lengths is also believed to increase the rate of homogenization by exposing an increased fiber cross-section area to fibrillation.[28] The pulp was dispersed in water and formed into thick sheets. The wet pulp sheets were then cut by repeated shredding in an ordinary office shredder, which resulted in an average fiber length of approximately 1 mm. The resulting pulp (4 kg of dry pulp) was diluted to ∼2% consistency and subjected to 20 passes through a Gaulin M12 homogenizer (from APV), with a flow rate of 11 L/min and a pressure drop of 600 bar at each pass. The temperature was controlled at 45−65 °C.

Prehydrolysis of ODDMAC. Before the reaction with MFC, the ODDMAC was prehydrolyzed in a mixture of methanol and water (90:10 w/w) at a concentration of 10% (w/w) for 18 h at room temperature.

Surface Modification of MFC with ODDMAC. MFC in aqueous suspension was solvent-exchanged by centrifugation and redispersion in a mixture of methanol and water (90:10 w/w). The required amount of prehydrolyzed ODDMAC (5−100 mol % with respect to cellulose repeating glucose units) was added as a 10% (w/w) solution in methanol/water (90:10 w/w), and the resulting suspension was diluted with methanol/water (90:10 w/w) to a final MFC concentration of 0.5% (w/w) with respect to the solvent. The reaction suspension was stirred at room temperature for 2 h. After the reaction, the suspension was centrifuged (3500 rpm, 30 min) and washed with the solvent, in order to remove any nonadsorbed silane. The surface-modified MFC was redispersed in methanol/water (90:10 w/w), to a concentration of ∼0.2% (w/w). Films were prepared by casting 5 mL of the suspension into a circular glass mold (diameter 4 cm), placed on S&S filter paper lying on top of a perforated metal plate. After the solvent was drained off, the films were dried for 1 h at 80 °C, followed by curing for 2 h at 100 °C.

Surface Hydrophobization of MFC with Chlorodimethylisopropylsilane. The MFC was hydrophobized as described previously.[18] MFC in aqueous suspension (∼1.2% w/w) was solvent-exchanged to acetone and then to dry toluene by seven successive centrifugation and redispersion operations. Chlorodimethylisopropylsilane (3 mol equiv with respect to cellulose repeating glucose units) was added, together with imidazole (amount equimolar to the chlorosilane) for trapping the HCl released during the reaction. The reaction mixture was stirred under argon atmosphere at room temperature for 16 h. A mixture of methanol and tetrahydrofuran (THF) (20:80 v/v) was then added, in order to terminate the grafting reaction and to dissolve the imidazolium chloride formed. The final suspension was centrifuged and the resulting pellet was washed twice with THF for removal of any disilyl ether byproduct. The pellet was resuspended in THF, and films were prepared by casting the suspension into a glass mold placed on S&S filter paper. After the solvent was drained off, the films were dried for 2 h at 80 °C. The hydrophobized MFC used in this work had a degree of surface substitution (DSS) of 1.1, as determined by XPS (for details, see ref 18).

Scanning Electron Microscopy. An unmodified MFC film was coated with platinum by sputtering and inspected with a Zeiss Gemini Supra 55VP field-emission scanning electron microscope (FESEM). The image was acquired with a lateral secondary electron (SE) detector at approximately 8 mm working distance. The accelerating voltage was 5 kV and the magnification was 50000×.

FT-IR Spectroscopy. Infrared spectra of the MFC samples were obtained on a Bio-Rad Excalibur FTX 3000 spectrometer. The samples were analyzed as KBr disks, with 1% (w/w) MFC in KBr.

X-ray Photoelectron Spectroscopy. XPS measurements were performed on a Kratos AXIS 165 electron spectrometer at the Laboratory of Forest Products Chemistry, Helsinki University of Technology, Finland. A small piece was cut from each MFC film and secured to the sample holder with metal clips only, to minimize contamination. Prior to the experiment, specimens were evacuated in a pretreatment chamber overnight in order to stabilize the water content. Survey scans were recorded with monochromated Al Kα irradiation, 1 eV step, and 80 eV analyzer pass energy. High-resolution spectra were recorded with monochromated Al Kα irradiation, 0.1 eV step, and 20 eV analyzer pass energy. Nitrogen data were determined by trace analysis in the N 1s range, 1 or 0.5 eV step, and 80 eV analyzer pass energy. The trace N 1s spectra were recorded with increased acquisition times (32 and 128 times that for normal survey scans) to enhance the detection limit of nitrogen. Data recorded from an in situ reference sample with each sample batch showed that the ultrahigh vacuum conditions remained satisfactory. The analysis area was less than 1 mm^2. Each sample was measured at three locations, in order to average over the heterogeneity of the sample and also in order to enhance the reliability. The standard deviations were less than 1.5% for the surface concentrations of oxygen and carbon, less than 0.8% for silicon, and less than 0.1% for nitrogen. The standard deviation of the oxygen to carbon ratio was 0.04. No degradation of the specimens during the XPS analyses was detected.

Determination of Antibacterial Activity. The antibacterial effect of the surface-modified MFC films was assessed by use of a modified version of the Japanese Industrial Standard Z 2801: Antimicrobial products—Test for antimicrobial activity and efficacy.[29] The effect of the material was tested against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 11229), and *Pseudomonas aeruginosa* (ATCC 15442). Bacteria were inoculated in brain heart infusion (BHI) broth and incubated at 37 °C overnight. An aliquot (25 μL) of the overnight culture, diluted 100 times in 1:500 strength BHI broth, was added onto the surface of a 7.5 cm^2 circular piece of the film to be tested. The inoculated samples were covered with a polyethylene film. The MFC films were incubated at 37 °C for 24 h in Petri dishes with lid in plastic bags. To prevent the samples from drying, a small piece of cotton wetted in water was present in each Petri dish. Two samples were tested in parallel for each strain. Upon sampling, the two parallels were combined in a Stomacher plastic bag and 10 mL of Dey−Engley neutralizing broth was added. After 1 min of mixing in the Stomacher, the resulting suspension was serially diluted in peptone water (0.85% NaCl and 0.1% peptone, pH 7.2) and plated onto BHI−agar plates. The plates were incubated at 37 °C overnight before determination of the number of colonies. The test was performed on all ODDMAC-modified MFC films, a film prepared from unmodified MFC (reference), and a film prepared from hydrophobized MFC (hydrophobic reference). The reference films were also sampled directly after addition of the bacteria (0 h). The experiment was performed in triplicate, on different days. The mean number of surviving cells and standard deviations were calculated from log-transformed data. Minitab (release 14.2, Minitab Inc., www.minitab.com) was used to calculate statistical significance of differences between means (two-sample *t*-test). The killing efficiency was defined as the percent reduction in number of surviving bacteria on the ODDMAC-modified films after cultivation relative to an untreated, hydrophobic reference. It was calculated according to

$$\text{killing efficiency (\%)} = \frac{B - A}{B} \times 100 \qquad (1)$$

where A and B are the mean number of surviving cells for the ODDMAC-modified MFC film and the hydrophobized reference, respectively.

Zone of Inhibition Test. Possible leakage of ODDMAC from the modified MFC films into the surroundings was examined by a zone of inhibition test. An overnight culture (300 μL) of *S. aureus* grown in BHI was mixed with 7 mL of BHI soft agar (0.7% w/v) and poured on top of a BHI agar (1.5% w/v) plate. An MFC film prepared from MFC modified with 100 mol % ODDMAC as described above, an unmodified MFC film, and an unmodified MFC film that had been soaked in diluted ODDMAC solution and dried in vacuo were placed on top of the soft

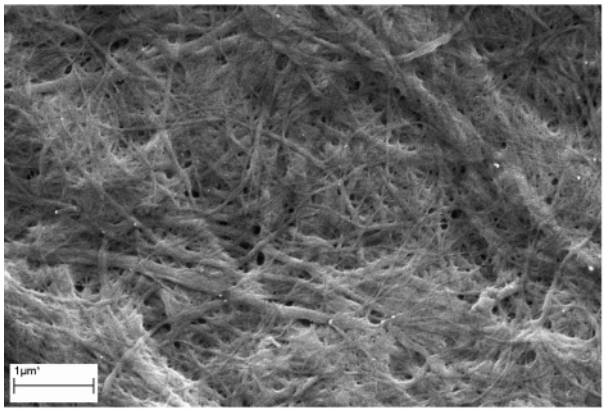

Figure 1. FESEM picture of film prepared from microfibrillated fully bleached sulfite pulp.

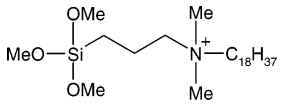

Figure 2. Structure of octadecyldimethyl(3-trimethoxysilylpropyl)-ammonium chloride (ODDMAC).

agar. The agar plate was incubated at 37 °C for 24 h before examination for clear zones around the test materials, where bacterial growth was inhibited.

Results and Discussion

Microfibrillated cellulose is usually produced by disintegration of pulp via a homogenization process, where high shear forces reduce the fibers to their fibrillar submorphologies.[15,28] The resulting material is greatly expanded in surface area, being composed of microfibrils, larger fibrils, and occasional fiber fragments. Upon drying, the MFC form a strong, nanoporous film network through interfibrillar hydrogen bonding. The structure of a MFC film is shown in the field-emission scanning electron micrograph in Figure 1. As can be seen in the FESEM picture, the diameters of the fibrils range from 20−30 nm for the smallest microfibrils up to several hundred nanometers for the larger fibril bundles. Thus, the sample is very polydisperse, but there is no doubt that the disintegration has resulted in a nanomaterial with very large specific surface area.

Surface Modification of MFC. In order to achieve MFC with antimicrobial properties, the quaternary ammonium compound octadecyldimethyl(3-trimethoxysilylpropyl)ammonium chloride (ODDMAC) was grafted onto the surface of the fibrils. The ODDMAC molecule consists of an alkoxysilane group, which functions as an anchor to the substrate surface, and a quaternary amine group with a long aliphatic tail, which enables it to act as a biocide (see Figure 2). The general mechanism for the surface modification of MFC with a methoxysilane is outlined in Figure 3.[30] In the presence of water, the methoxy groups of the silane are hydrolyzed to yield the corresponding silanol, which adsorbs onto the OH-rich MFC surface via hydrogen bonding. In addition, the hydrolyzed molecules can undergo self-condensation reactions and form siloxane bonds, resulting in oligomeric species (illustrated in Figure 3 by a dimer structure).[30,31] The growth of oligomeric structures from self-condensation is believed to enhance the adsorption, since each adsorbed oligomer would imply the presence of several silane molecules, capable of forming multiple hydrogen bonds to the cellulose surface.[31] Subsequent thermal treatment will lead to chemical condensation, resulting in covalent bonding to the surface.

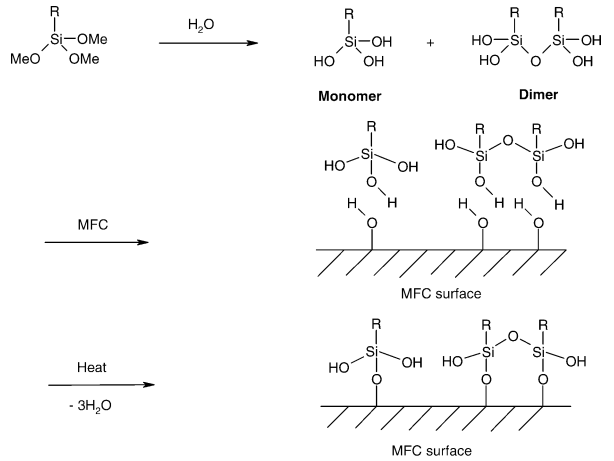

Figure 3. General mechanism for the prehydrolysis and adsorption/curing of alkoxysilanes onto a MFC surface.

The amount of ODDMAC added in the surface modification of MFC varied from 5 to 100 mol % with respect to cellulose repeating glucose units. All other reaction parameters were kept constant.

Infrared Spectroscopy. The reaction of ODDMAC with the MFC surface was verified qualitatively by FT-IR. Figure 4 compares the FT-IR spectra of native MFC, MFC modified with 50 mol % ODDMAC, and MFC modified with 100 mol % ODDMAC. The surface-modified samples show two distinct bands around 1260 and 800 cm^{-1}, which can be assigned to Si−C deformation and stretching, respectively. Furthermore, with increasing concentration of alkoxysilane, a sharp peak near 2965 cm^{-1} appears, corresponding to the asymmetric -CH_3 stretch, indicating the introduction of alkyl chains in the MFC surface.

Surface Characterization. We have shown previously that XPS is an excellent tool for quantitative characterization of surface-modified MFC.[18] Here, we have employed XPS to monitor the changes in chemical composition of the MFC surface with increasing ODDMAC concentration in the reaction. The atomic composition data derived from the XPS survey scans and N 1s trace analysis are summarized in Table 1. The nitrogen concentrations in the samples were too low to be measured by normal survey scan analysis, which has an N detection limit of 1 at. %. This was also the case for Cl, which in principle should be present in the surface in the same amounts as nitrogen (one Cl^- ion per ODDMAC molecule) and has a comparable detection limit. Nitrogen data were therefore determined by trace analysis in the N 1s range with increased acquisition times (this was not done for Cl).

As can be seen from Table 1, the surface constituents of untreated MFC are oxygen and carbon, with some small silicon and nitrogen contamination. The origin of the contamination is not clear, but it is most likely due to contaminants in the wood raw material or the storage atmosphere.[32] The O/C ratio was found to be 0.76, which is very close to the value obtained from an S&S ash-free pure cellulose filter paper sample (0.74)[18] but lower than the theoretical value for cellulose (0.82). This suggests that there are traces of noncellulosic compounds in the surface, most likely traces of wood extractives.[33] With increasing concentration of ODDMAC in the reaction, the O/C ratio decreased, indicative of a relative increase in carbon at the surface resulting from the alkyl chains of the grafted alkoxysilane groups. Furthermore, the amounts of silicon and nitrogen in the surface both increased with increasing concentration of alkoxysilane, consistent with an increasing degree of

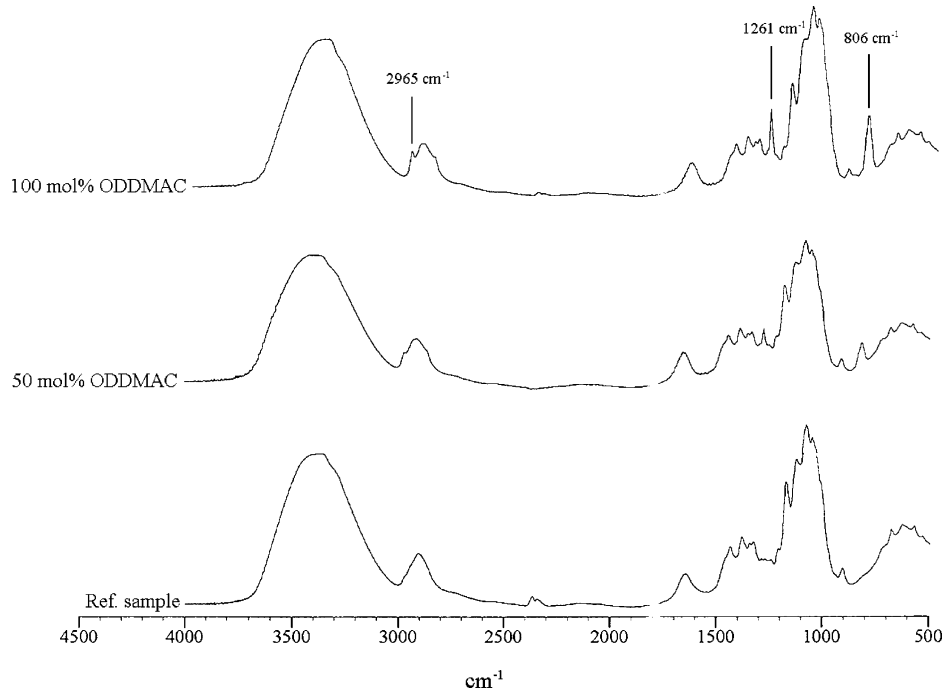

Figure 4. FT-IR spectra of unmodified MFC, MFC modified with 50 mol % ODDMAC, and MFC modified with 100 mol % ODDMAC. For explanation, see text.

Table 1. Atomic Concentrations from XPS Survey Scans and N 1s Trace Analysis[a]

sample	O 1s (%)	C 1s (%)	Si 2p (%)	N 1s (%)	O/C
MFC	43.1	56.7	0.2[b]	0.03	0.76
MFC-mod-5%	40.6	57.9	1.4	0.06	0.70
MFC-mod-10%	40.2	57.2	2.5	0.11	0.70
MFC-mod-50%	37.5	58.3	3.9	0.16	0.64
MFC-mod-100%	36.5	58.5	4.6	0.14	0.62

[a] Analysis is confined to the topmost 10 nm of the surface. Sample names refer to the mol % of ODDMAC with respect to cellulose repeating glucose units employed in each reaction [b] Below detection limit.

grafting to the MFC surface. However, the amount of nitrogen detected was considerably lower than that of silicon. This was unexpected, since the ODDMAC molecule contains one Si atom and one N atom, which means that the amounts of these two elements in the surface after grafting are expected to be the same, as there is virtually no N or Si in the native MFC. The ODDMAC used in these experiments was supplied as 42 wt % solution in methanol. However, according to the supplier, the solution also contained 8 wt % (3-chloropropyl)trimethoxysilane, which is one of the precursors in the synthesis of ODDMAC (Figure 5). The XPS data strongly indicate that a competitive reaction took place during the grafting of the ODDMAC, namely, between the (3-chloropropyl)trimethoxysilane and the MFC hydroxyls. Grafting of this alkoxysilane to the MFC would increase the concentration of Si in the surface, but it would not affect the N concentration. The concentration of (3-chloropropyl)trimethoxysilane in the solution was initially considered to be too low to cause this side reaction. However, it appears that the (3-chloropropyl)trimethoxysilane may react more rapidly with the OH groups than ODDMAC. This may be attributed to the size difference between the two molecules: the ODDMAC has a long aliphatic tail that provides steric hindrance, which presumably makes it less reactive toward the cellulose fibrils than the smaller (3-chloropropyl)trimethoxysilane. That steric factors influence the adsorption properties of alkoxysilanes has also been reported elsewhere.[34]

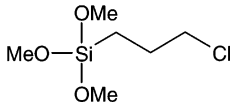

Figure 5. Structure of (3-chloropropyl)trimethoxysilane.

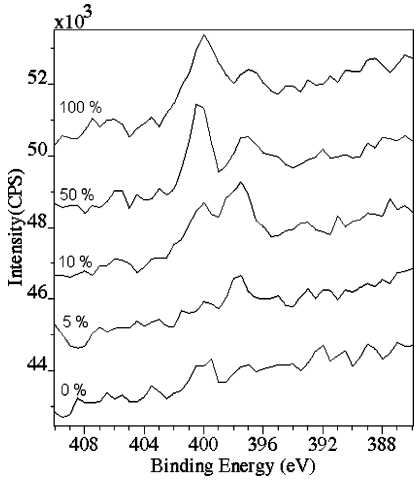

Figure 6. Trace region spectra of the N 1s peak near 400 eV in unmodified MFC and MFC modified with 5−100 mol % ODDMAC. For clarity, the spectrum for each concentration has been shifted upward by ~200 cps.

Although it seems that (3-chloropropyl)trimethoxysilane reacted to a larger extent than ODDMAC, the XPS data clearly indicate that a certain amount of ODDMAC was grafted onto the MFC surface. As there was no other source of nitrogen in the reaction, the increasing surface concentration of N observed when the mol % ODDMAC was increased must be attributed to an increasing amount of ODDMAC grafted.

This is also demonstrated in Figure 6, which shows the trace nitrogen spectra of unmodified MFC and MFC modified with 5−100 mol % ODDMAC. As can be seen from the spectra, there was clearly an increase in the intensity of the N 1s peak

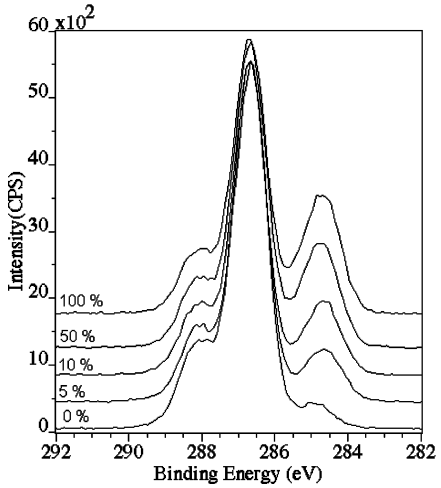

Figure 7. Superimposed high-resolution XPS spectra of the C 1s peak in unmodified MFC and MFC modified with 5−100 mol % ODDMAC. The peak near 285 eV corresponds to C1 carbon, the peak near 287 eV is C2 carbon, the peak near 288 eV corresponds to C3 carbon, and the C4 peak lies near 289−290 eV (not resolved in this figure). For clarity, the spectrum for each concentration has been shifted upward by ~200 cps.

Table 2. Relative Peak Areas from Deconvoluted C 1s Spectra

sample	C 1 (%)	C 2 (%)	C 3 (%)	C 4 (%)
MFC	6	76	18	0.7
MFC-mod-5%	14	70	16	0.9
MFC-mod-10%	16	68	16	0.8
MFC-mod-50%	24	62	14	0.6
MFC-mod-100%	29	58	13	0.5

near 400 eV with increasing mol % ODDMAC employed in the grafting reaction.

As will be shown later, the amount of ODDMAC grafted was sufficient to render the surface of the MFC antimicrobial. We assume that the increase in the concentration of nitrogen can be attributed solely to an increasing amount of ODDMAC bonded to the surface of the fibrils. Hence, we will use the amount of nitrogen as a relative measure of the amount of antimicrobial grafted onto the MFC.

Additional information on the carbon chemistry at the MFC surface can be obtained from the XPS high-resolution carbon data, which is derived from the deconvoluted C 1s spectra (Figure 7). As the carbon 1s binding energy depends on the number of bonds between the carbon and oxygen, the carbon signal can be resolved into four different peaks: C1, originating from carbons bonded only to other carbon atoms or to hydrogen (aliphatic carbon), and the C2, C3, and C4 peaks that originate from carbon atoms with one, two (or one double), and three oxygen bonds, respectively. The relative peak areas from the deconvoluted C 1s spectra of the native and ODDMAC-modified MFC samples are summarized in Table 2. Pure cellulose should only contain C2 and C3 carbon, characteristic of the C−O and O−C−O bonds (and very small amounts of C4 carbon). The presence of a small amount of aliphatic carbon in the unmodified MFC sample (5.8%) can probably be attributed, as already mentioned, to noncellulosic compounds inherent to the material or to airborne carbonaceous surface impurities. Aliphatic carbon is the most common surface contaminant of air-exposed surfaces, and in practice, the lowest values obtained for paper samples are about 2%.[35] The even smaller amount of C4 carbon in the native MFC most likely originates from traces of carboxylic groups in the fibril surface. With increasing ODDMAC concentration in the reaction, the relative amount of C1 carbon was found to increase, while the amounts of C2, C3, and C4 carbon decreased. This is clearly demonstrated in Figure 7, where the intensity of the C1 peak near 285 eV is seen to increase with increasing concentration of ODDMAC. This is consistent with an increasing degree of surface substitution, as the alkyl chains of the ODDMAC and (3-chloropropyl)-trimethoxysilane strongly contribute to the intensity of the C1 peak in the high-resolution carbon spectrum. A consequence of the increased density of aliphatic carbon in the MFC would be a hydrophobization of the surface, the hydrophobicity increasing with increased degree of grafting.

Table 3. Measured Surface Content of Nitrogen (from XPS) and Calculated Killing Efficiency toward *E. coli* and *S. aureus* for ODDMAC-Modified MFC Films

		killing efficiency (%)	
sample	% N	*E. coli*	*S. aureus*
MFC-mod-100%	0.14	>99	>99
MFC-mod-50%	0.16	>99	>99
MFC-mod-10%	0.11	98.5	>99
MFC-mod-5%	0.06	96.0	0

Evaluation of Bactericidal Activity. The bactericidal activity of the films prepared from the ODDMAC-modified MFC was tested against *E. coli*, *S. aureus*, and *P. aeruginosa*. Figure 8 shows the number of surviving cells for the different bacteria on the ODDMAC-modified films and the native MFC reference film after incubation for 24 h. Because the ODDMAC-treated MFC was partly hydrophobized as a result of the alkoxysilane grafting, we also included a MFC reference film hydrophobized through silylation with chlorodimethylisopropylsilane, with a low surface energy similar to that of the ODDMAC-treated films but no antibacterial activity. The hydrophobic reference was incubated with *E. coli* and *S. aureus*. Incorporation of the quaternary ammonium compound ODDMAC on the MFC surface resulted in significant reduction ($p < 0.05$) in viable *S. aureus* and *E. coli* on the films (Figure 8 and Table 3). The bactericidal efficiency was found to be smaller toward *P. aeruginosa* than toward *E. coli* and *S. aureus*. This was not unexpected, as it is well-known that *Pseudomonas* generally has a much higher level of resistance toward antimicrobial agents based on quaternary ammonium compounds than *Staphylococcus* spp. or *E. coli*.[36,37] The antimicrobial effect is also slightly lower against *S. aureus* than *E. coli*. In general, the bactericidal efficiency increased with increasing concentration of ODDMAC for all bacteria tested, with the samples modified with 50 and 100 mol % ODDMAC showing the lowest amount of surviving cells. This is in agreement with the XPS data presented in Table 1, which demonstrate that these samples also contained the largest concentration of nitrogen in the surface. The fact that there was little difference in the surface concentration of antimicrobial agent for the MFC-mod-50% and MFC-mod-100% samples (the N concentration in the 50% sample was actually slightly higher than in the 100% sample) might indicate that the adsorption of ODDMAC reached a plateau when 50 mol % was employed in the reaction. As can be seen from Table 3 and Figure 8, the films killed more than 99% of the bacteria when the surface concentration of ODDMAC nitrogen was 0.14% or higher. Below 0.10% N on the surface, the killing efficiency decreased, and all *S. aureus* survived on the film treated with 5 mol % ODDMAC.

Surprisingly, incubation for 24 h resulted in significant reduction in the number of bacteria isolated from the native MFC reference film (ref.24 vs ref.0). This phenomenon was

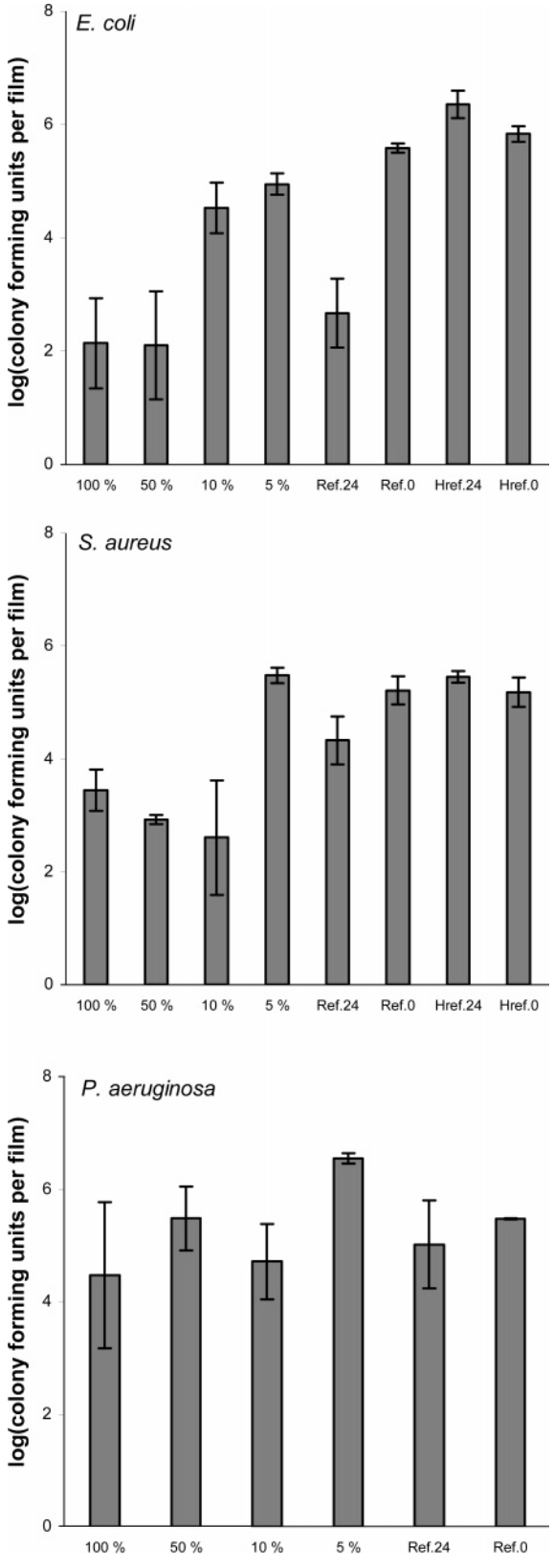

Figure 8. Number of surviving bacteria (colony-forming units) on films prepared from MFC modified with 5−100 mol % ODDMAC, after incubation with *E. coli*, *S. aureus*, and *P. aeruginosa* for 24 h. Included also are the number of surviving bacteria measured on an unmodified reference film incubated for 0 and 24 h (Ref.0 and Ref.24, respectively), and the number of *E. coli* and *S. aureus* on a hydrophobized reference film incubated for 0 and 24 h (Href.0 and Href.24, respectively). The error bars are the standard errors for triplicated experiments. Note that the scale on the *y*-axis is logarithmic.

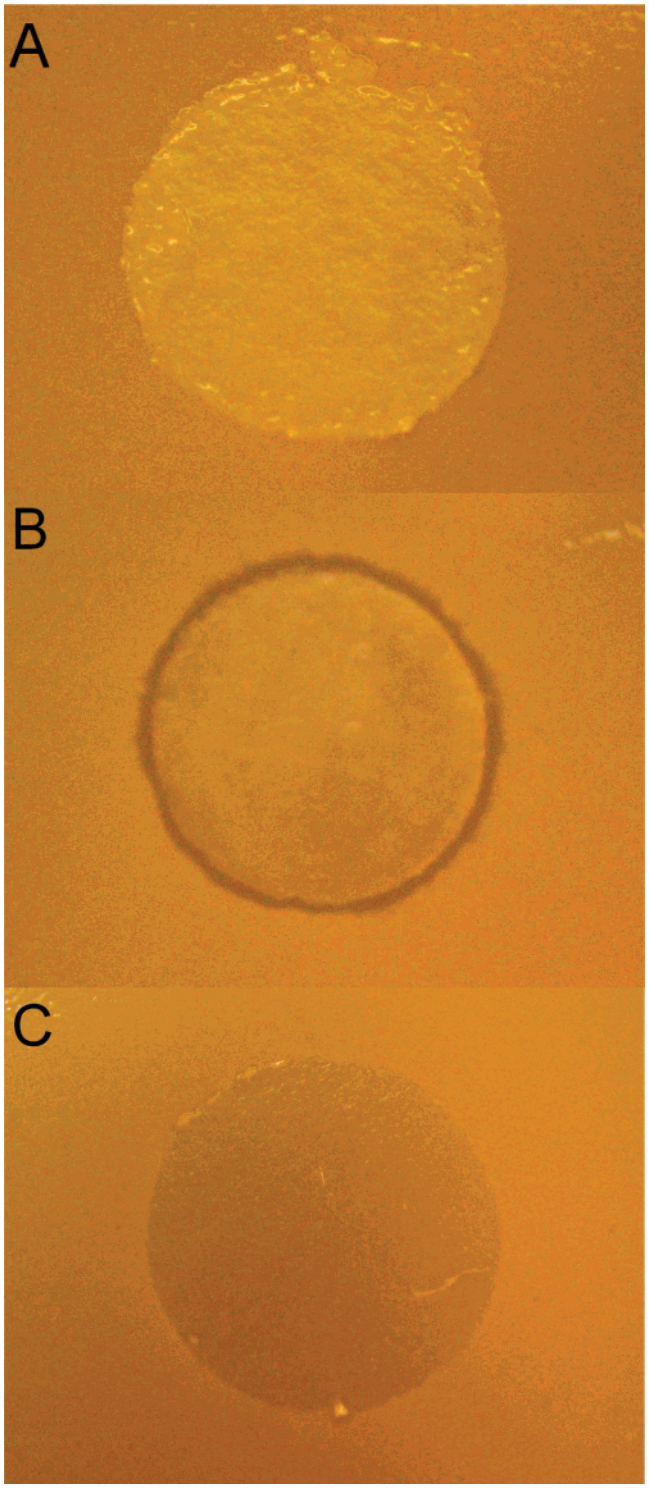

Figure 9. Pictures comparing the results of a zone of inhibition test for (A) an unmodified MFC film, (B) an unmodified MFC film soaked in diluted ODDMAC solution and dried (no prehydrolysis or curing), and (C) a film prepared from MFC treated with 100 mol % prehydrolyzed ODDMAC and cured for 2 h at 100 °C. The dark zone around film B indicates that growth of bacteria has been inhibited (films are 1 cm in diameter).

not observed for the hydrophobized reference. It is unlikely that native MFC, which is basically pure cellulose, should display any antimicrobial effect. We believe that the reduction could be related to its hydrophilic properties. In contrast to the ODDMAC-modified films and the hydrophobized control films, it was observed that the bacterial suspension absorbed rapidly into the MFC reference films, and possibly the detaching

procedure used in the evaluation of antimicrobial efficiency was less efficient for the latter. The hydrophobized reference has therefore been used in all calculations of lethal effect.

In order to determine whether any antimicrobial agent leached from the modified MFC films, a zone of inhibition test was performed. This test demonstrates whether the antimicrobial agent is able to leak from the film and kill the microorganisms in the surrounding agar. Figure 9 compares the results of the test for an unmodified MFC film (A), an unmodified MFC film that was soaked in diluted ODDMAC solution and dried (B), and a film prepared from MFC treated with 100 mol % ODDMAC according to the prehydrolysis−adsorption−curing procedure described in Figure 3C. As expected, the untreated MFC sample showed no zone of inhibition. For the MFC film that had been soaked in diluted, unhydrolyzed ODDMAC solution, a small zone of inhibition was observed. As the alkoxysilane had not been prehydrolyzed or cured onto the MFC surface, the antimicrobial was only weakly adsorbed and therefore capable of leaching into the agar, killing the surrounding bacteria. The film prepared from the MFC modified with 100 mol % ODDMAC showed no zone of inhibition, indicating that the antimicrobial was indeed covalently bound to the surface of the fibrils and thus unable to diffuse into the surroundings.

Conclusions

Nonleaching antimicrobial films from microfibrillated cellulose covalently grafted with octadecyldimethyl(3-trimethoxysilylpropyl)ammonium chloride could be successfully prepared. The surface-modified MFC films showed antibacterial activity against both Gram-positive and Gram-negative bacteria, even at very low concentrations of antimicrobial agent in the surface, killing more than 99% of *E. coli* and *S. aureus* when the atomic concentration of ODDMAC nitrogen in the film surface was 0.14% or higher. We believe that these antimicrobial MFC films could be used as, for instance, coatings in food packaging or in medical applications, such as wound healing. Moreover, due to the large surface-to-volume ratio of the MFC films and the fact that not only the film surface but also each microfibril and fibril are surface-modified with ODDMAC, these nanoporous films could prove to be highly efficient as antimicrobial separation filters.

Acknowledgment. We thank the NANOMAT research programme of the Norwegian Research Council for a grant to fund M.A. Södra Cell, Borregaard, Akzo Nobel, and Domsjö Fabriker are also acknowledged for financial support. We are grateful to Per Olav Johannesen at The Norwegian Paper and Fibre Research Institute for taking the SEM picture and to Dr. Joseph M. Campbell for performing the XPS experiments. Birgitta Baardsen and Anne Kristine Olsen at Matforsk are acknowledged for skilful assistance during the antibacterial testing of the MFC films.

References and Notes

(1) Denyer, S. P. *Int. Biodeterior. Biodegrad.* **1995**, *36*, 227.
(2) Lindstedt, M.; Allenmark, S.; Thompson, R. A.; Edebo, L. *Antimicrob. Agents Chemother.* **1990**, *34*, 1949.
(3) Klueh, U.; Wagner, V.; Kelly, S.; Johnson, A.; Bryers, J. D. *Biomed. Mater. Res., Appl. Biomater.* **2000**, *53*, 621.
(4) Pai, M. P.; Pendland, S. L.; Danziger, L. H. *Ann. Pharmacother.* **2001**, *35*, 1255.
(5) Worley, S. D.; Li, F.; Wu, R.; Kim, J.; Wei, C.-I.; Williams, J. F.; Owens, J. R.; Wander, J. D.; Bargmeyer, A. M.; Shirtliff, M. E. *Surf. Coat. Int. B* **2003**, *86*, 273.
(6) Lewis, K.; Klibanow, A. M. *Trends Biotechnol.* **2005**, *23*, 343.
(7) Tiller, J. C.; Liao, C.-J.; Lewis, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5981.
(8) Lin, J.; Qui, S.; Lewis, K.; Klibanov, A. M. *Biotechnol. Prog.* **2002**, *18*, 1082.
(9) Abel, T.; Cohen, J. I.; Engel, R.; Filishtinskaya, M.; Melkonian, A.; Melkonian, K. *Carbohydr. Res.* **2002**, *337*, *2495*.
(10) Cen, L.; Neoh, K. G.; Kang, E. T. *Langmuir* **2003**, *19*, 10295.
(11) Lin, J.; Murthy, S. K.; Olsen, B. D.; Gleason, K. K.; Klibanov, A. M. *Biotechnol. Lett.* **2003**, *25*, 1661.
(12) Lee, S. B.; Koepsel, R. R.; Morley, S. W.; Matyjaszewski, K.; Sun, Y.; Russel, A. J. *Biomacromolecules* **2004**, *5*, 877.
(13) Taniguchi, T.; Okamura, K. *Polym. Int.* **1998**, *47*, 291.
(14) Iguchi, M.; Yamanaka, S.; Budhiono, A. *J. Mater. Sci.* **2000**, *35*, 261.
(15) Turbak A. F.; Snyder F. W.; Sandberg, K. R. *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1983**, *37*, 815.
(16) Cash, M. J.; Chan, A. N.; Conner, H. T.; Cowan, P. J.; Gelman, R. A.; Lusvardi, K. M.; Thompson, S. A.; Tise, F. P. U.S. Patent 6,-602,994, 1999.
(17) Matsuda, Y.; Hirose, M.; Ueno, K. U.S. Patent 6,183,596, 2001.
(18) Andresen, M.; Johansson, L.-S.; Tanem, B. S.; Stenius, P. *Cellulose* **2006**, *13*, 665.
(19) Isquith, A. J.; Abbot, E. A.; Walters, P. A. *Appl. Microbiol.* **1972**, *24*, 859.
(20) Gettings, R. L.; White, W. C. *Polym. Mater. Sci. Eng.* **1987**, *57*, 181.
(21) Murray, P. R.; Niles, A. C.; Heeren, R. L. *J. Clin. Microbiol.* **1988**, *26*, 1884.
(22) Gottenbos, B.; van der Mei, H. C.; Klatter, F.; Nieuwenhuis, P.; Busscher, H. J. *Biomaterials* **2002**, *23*, 1417.
(23) Oosterhof, J. J. H.; Buijssen, K. J. D. A.; Busscher, H. J.; van der Laan, B. F. A. M.; van der Mei, H. C. *Appl. Environ. Microbiol.* **2006**, *72*, 3673.
(24) Ignatova, M.; Starbova, K.; Markova, N.; Manolova, N.; Rashkov, I. 2006. *Carbohydr. Res.* **2006**, *341*, 2098.
(25) Thomas, H.; Heine, E.; Wollseifen, R.; Cimpeanu, C.; Möller, M. *Int. Nonwovens J.* **2005**, *14*, 12.
(26) Son, W. K.; Youk, J. H.; Lee, T. S.; Park, W. H. *Macromol. Rapid Commun.* **2004**, *25*, 1632.
(27) Son, W. K.; Youk, J. H.; Park, W. H. *Carbohydr. Polym.* **2006**, *65*, 430.
(28) Herrick F. W.; Casebier R. L.; Hamilton J. K.; Sandberg, K. R. *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1983**, *37*, 797.
(29) JIS Z 2801:2000, *Antimicrobial products*—Test for antimicrobial activity and efficacy; Japanese Standard Association: Tokyo, 2000.
(30) Daniels, M. W.; Francis, L. F. *J. Colloid Interface Sci.* **1998**, *205*, 191.
(31) Salon, M.-C. B.; Abdelmouleh, A.; Boufi, S.; Belgacem, M. N.; Gandini, A. *J. Colloid Interface Sci.* **2005**, *289*, 249.
(32) Matuana, L. M.; Balantinecz, J. J.; Sodhi, R. N. S.; Park, C. B. *Wood Sci. Technol.* **2001**, *35*, 191.
(33) Johansson, L.-S.; Campbell, J. M.; Fardim, P.; Hultén, A. H.; Boisvert, J.-P.; Erntsson, M. *Surf. Sci.* **2005**, *584*, 126.
(34) Abdelmouleh, M.; Boufi, S.; ben Salah, A.; Belgacem, M. N.; Gandini, A. *Langmuir* **2002**, *18*, 3202.
(35) Johansson L.-S.; Campbell J. M.; Koljonen, K.; Stenius, P. *Appl. Surf. Sci.* **1999**, *144*−*145*, 92.
(36) Russell, A. D.; Gould, G. W. *J. Appl. Bacteriol.* **1988**, *65* (Suppl.), 167S.
(37) Langsrud, S.; Sidhu, M. S.; Heir, E.; Holck, A. L. *Int. Biodeterior. Biodegrad.* **2003**, *51*, 283.

BM070304E