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ORIGINAL PAPER

Electrostatic assembly of Ag nanoparticles onto nanofibrillated cellulose for antibacterial paper products

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Abstract Nanofibrillated cellulose offers new technological solutions for the development of paper products. Here, composites of nanofibrillated cellulose (NFC) and Ag nanoparticles (NP) were prepared for the first time via the electrostatic assembly of Ag NP (aqueous colloids) onto NFC. Distinct polyelectrolytes have been investigated as macromolecular linkers in order to evaluate their effects on the building-up of Ag modified NFC and also on the final properties of the NFC/Ag composite materials. The NFC/Ag nanocomposites were first investigated for their antibacterial properties towards *S. aureus* and *K. pneumoniae* microorganisms as compared to NFC modified by polyelectrolytes linkers without Ag. Subsequently, the antibacterial NFC/Ag nanocomposites were used as fillers in starch based coating formulations for *Eucalyptus globulus*-based paper sheets. The potential of this approach to produce antimicrobial paper products will be discussed on the basis of complementary optical, air barrier and mechanical data.

Keywords Nanofibrillated cellulose · Silver colloids · Nanocomposites · Paper coating · Antibacterial activity

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Introduction

Due to the renewable nature, biocompatibility, biodegradability and high specific strength of vegetable cellulose fibers, a growing interest has been devoted to its use in new composite materials in the last decades (Gandini 2011; Belgacem and Gandini 2008; Klemm et al. 2005; Amash and Zugenmaier 2000; Karlsson et al. 2000; Smiechowicz et al. 2011). In addition to conventional vegetal cellulose fibers, other forms of cellulose have attracted the attention of the scientific community in the last few years, namely, nanofibrillated cellulose (NFC) and microbial cellulose, due to the nanosized dimensions of these fibers that might impart improved, and in many cases new properties to the ensuing (nano)composite materials. Nanofibrillated cellulose can be obtained from cellulose fibers by distinct methods, in the form of aqueous suspensions of nanoscale fibers with high aspect ratio (5–30 nm diameter and lengths in the micrometer range) and specific surface areas combined with remarkable

strength and flexibility (Paakko et al. 2007; Henriksson et al. 2007; Saito et al. 2007; Siró and Plackett 2010). The excellent mechanical properties of NFC make it a good candidate for reinforcement materials in nanocomposites (Zimmermann et al. 2004, 2010; Yano and Nakagaito 2005; Eichhorn et al. 2010; Hubbe et al. 2008; Klemm et al. 2011). Nanosized cellulose based composites have potential for applications in several areas, such as transparent materials (Tome et al. 2011; Fernandes et al. 2010), biomedical applications (Maneerung et al. 2008) or gas barrier films (Hubbe et al. 2008). Although the preparation of polymer coated Ag nanoclusters in NFC has been recently reported (Diez et al. 2011), the production of innovative NFC based materials has not yet been fully explored, especially in what concerns the context of antimicrobial paper research. Therefore, the use of NFC/silver nanocomposites for the preparation of antibacterial paper is reported here for the first time.

With the increasing awareness of infectious diseases caused by different microorganisms and the development of antibiotic resistance, the search for new and efficient antibacterial materials is imperative. Among inorganic antibacterial agents, silver has been extensively studied and its use to fight infections dates back to ancient times (Rai et al. 2009). Silver, both in metallic or in ionic form, exhibits strong inhibitory and bactericidal activities towards a broad spectrum of microorganisms. In recent years there has been a renewed interest on silver as antimicrobial agent, namely due to unprecedented progress observed on nanomaterials science in the last decades that prompted innovative strategies on the use of colloidal Ag in antimicrobial products (Marini et al. 2007; Pinto et al. 2009). As compared to the bulk form, Ag NP have higher surface area per volume and therefore surface mediated phenomena are expected to be more effective (Maneerung et al. 2008). Although the antibacterial mechanism of Ag NP is still not completely understood, some mechanisms have been suggested that highlight the relevance of the metal surface (Sondi and Salopek-Sondi 2004; Morones et al. 2005; Potara et al. 2011).

In the last years, reports on the production of antibacterial nanocomposites of vegetal or bacterial cellulose and silver nanoparticles have been published (Pinto et al. 2009; Maneerung et al. 2008; Silva and Unali 2011; El-Shishtawy et al. 2011), but to the best of our knowledge, the preparation of nanocomposites of NFC and Ag NP using polyelectrolytes as macromolecular linkers has not been reported. Cellulose-silver nanocomposites can be used as functional materials on textiles and polymers, leading to innovative products with antibacterial properties. For example, the use of such nanocomposites in paper coating formulations is an interesting approach to produce antibacterial papers with improved mechanical, surface and barrier properties that might find interest in packaging and air filters. Recently, reports concerning the production of antibacterial paper coated with Ag nanoparticles have appeared in the literature, namely by using the direct deposition or in situ synthesis of Ag nanoparticles (Dankovich and Gray 2011; Tankhiwale and Bajpai 2009; Gottesman et al. 2011). However these approaches might present limitations such as tendency for particle agglomeration and poor adherence of the nanoparticles onto the cellulose fibers (Dankovich and Gray 2011). Therefore the fabrication of antibacterial paper was investigated here using an alternative strategy. In this context, we report the preparation of antibacterial NFC/Ag nanocomposites by the electrostatic assembly of Ag NP onto NFC using polyelectrolytes, and their use in paper coating formulations to produce papers with antibacterial properties.

Materials and methods

Materials

Nanofibrillated cellulose suspension (2 % solid content) was kindly supplied by Centre Technique du Papier (France) and was obtained by submitting a softwood pulp to an enzymatic pre-treatment followed by mechanical treatment using a high-pressure homogenizer. The aqueous silver colloid (1 % w/w, average diameter 40 nm ± 12.4 nm) was supplied by Colorobbia (Italy) and was obtained by reducing Ag(I) at 85 °C, using an aqueous solution of glucose as a soft reducing agent, in the presence of soda and polyvinylpyrrolidone. Water diluted suspensions of Ag NP with metal contents between 0.8×10^{-5} and 5.0×10^{-3}% (w/v) were prepared from the initial Ag colloid and were used in the preparation of the nanocomposites. The polyelectrolytes poly(diallyldimethylammonium chloride) (PDDA, 20 % in water, MW 100,000–200,000), poly(sodium 4-styrenesulfonate) (PSS, MW 70,000), poly(allylamine hydrochloride) (PAH, MW 15,000) and branched

polyethylenimine (PEI, MW 25,000) were purchased from Aldrich and used as received. Native starch was provided by Grupo Portucel-Soporcel, Figueira da Foz.

Non-commercial A3-size papers sheets (100 % *E. globulus* bleached kraft pulp with a grammage of 76.4 g/m^2 and an average thickness of 100 μm, produced by AKD-based sizing system and filled with precipitated calcium carbonate) without any surface treatment, gently ceded by the Grupo Portucel-Soporcel, Figueira da Foz, Portugal, were used as paper substrates (control sheets CS) for the coating essays.

Stock cultures and culture media

All microbial strains used were provided by DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures). *Staphylococcus aureus* ATCC 6538 (DSM 799) and *Klebsiella 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.

Preparation of NFC/Ag nanocomposites

Solutions (0.1 % w/v) of cationic (PDDA, PAH and PEI) and anionic (PSS) polyelectrolytes were prepared in 0.5 M aqueous NaCl. In a first step, NFC (5.8 g of the 2 % suspension) was surface modified with the polyelectrolytes by alternate mixing with a cationic polyelectrolyte (70 mL), anionic polyelectrolyte (70 mL) and again with the cationic polyelectrolyte (70 mL) solutions. After each mixing step with a given polyelectrolyte (20 min), NFC fibers were separated by filtration and washed twice with distilled water to remove the excess of polyelectrolyte. Finally, the surface modified NFC was mixed with the Ag colloid (70 mL) during 20 min and the resulting nanocomposite was filtered and washed with distilled water. For nanocomposites submitted to more than one deposition cycle, the materials were mixed with cationic polyelectrolyte followed by mixing with Ag NP and this procedure was repeated until the required number of deposition cycles. The resulting NFC/Ag nanocomposites were lyophilized, with exception of those samples evaluated for their antimicrobial activity as aqueous suspensions. Table 1 lists the samples reported in this work.

Paper coating experiments

Among the several NFC/Ag nanocomposites prepared with one deposition cycle, the Ag/PDDA6 sample presented the highest Ag content per mass of composite and as such was selected for paper coating experiments. Two different starch-based coating

Table 1 List of all NFC/Ag nanocomposites prepared

Sample code	Number of deposition cycles	Polyelectrolyte	Colloid concentration (% w/w)	Ag content (% w/w)
Ag/PDDA1	1	PDDA/PSS/PDDA	5.0×10^{-4}	1.1×10^{-1}
Ag/PDDA2	2	PDDA/PSS/PDDA	5.0×10^{-4}	2.9×10^{-1}
Ag/PDDA3	3	PDDA/PSS/PDDA	5.0×10^{-4}	4.1×10^{-1}
Ag/PDDA4	4	PDDA/PSS/PDDA	5.0×10^{-4}	6.1×10^{-1}
Ag/PDDA5	5	PDDA/PSS/PDDA	5.0×10^{-4}	7.7×10^{-1}
Ag/PDDA6	1	PDDA/PSS/PDDA	5.0×10^{-3}	5.3×10^{-1}
Ag/PDDA7	1	PDDA/PSS/PDDA	2.5×10^{-4}	3.5×10^{-2}
Ag/PDDA8	1	PDDA/PSS/PDDA	1.2×10^{-4}	1.8×10^{-2}
Ag/PDDA9	1	PDDA/PSS/PDDA	0.8×10^{-4}	1.1×10^{-2}
Ag/PDDA10	1	PDDA/PSS/PDDA	0.8×10^{-5}	5.3×10^{-3}
Ag/PEI11	1	PEI/PSS/PEI	5.0×10^{-4}	2.8×10^{-1}
Ag/PEI12	1	PEI/PSS/PEI	0.8×10^{-4}	1.9×10^{-2}
Ag/PAH13	1	PAH/PSS/PAH	0.8×10^{-4}	2.1×10^{-3}

aqueous formulations with a total solid content of 6 % and distinct NFC/Ag contents (11 and 29 % of Ag/PDDA6 with respect to starch content) were prepared. Paper sheets were then coated using a size press machine (MathisLAB reverse roll coater type RRC-BW 350 mm). The coating speed was fixed at 20 m/min and the distance between the cylinders at zero micrometer (adjusting precision 0.001 mm). Two different coating levels were applied, with one or two layers on one side of the paper sheets.

The coated-papers obtained were dried after each single layer deposition for 120 s at 100 °C in the dryer section of the size press. Five replicates were prepared for each coating formulations. An A4 sample was then cut out from the inner region of each original A3 sheet in order to eliminate the inevitable irregularities associated with its coated borders. Before their characterization, all coated papers were conditioned at 23 ± 1 °C and 50 ± 5 % RH during 3 days following the TAPPI T402 om-93 standard. The coating weights (pick-up) were obtained by subtracting the weight of the paper sheets after and before the coating procedure.

Assessment of antibacterial activity of NFC/Ag nanocomposites and coated papers

The adopted testing method for the assessment of antibacterial activity was based on the AATCC 100 standard test method under static conditions (AATCC Test Method 100, Quantitative assessment of antibacterial activity). The antibacterial tests were carried out on NFC based nanocomposites (water suspensions) as well as on paper coated derivatives. In the cases of the water suspensions of NFC nanocomposites, a suspension of known concentration of bacteria was dispersed in the nanocomposite sample, while in the cases of paper samples, the paper specimens were impregnated by the bacteria suspension. After bacteria inoculation the samples were set at conditions for optimal microbe growth.

The inoculated samples were subjected to 24 h incubation at 37 °C and at the end of the incubation period the bacteria were extracted from the samples under investigation by using a neutralizing solution. The number of living cells (CFU = colony forming units) in the extracted suspension was evaluated by count plate agar method. Specifically the following testing conditions were adopted:

- *Tested Microorganisms*: Gram positive bacteria: *Staphylococcus aureus* ATCC 6538. Gram negative: *Klebsiella pneumoniae* ATCC 4352.
- *Tested material*: About 400 mg of NFC composite suspension (corresponding to 4 mg NFC dry weight) or about 60 mg of treated paper sheet (size paper specimens: 3×3 cm). The samples were subjected to sterilization by autoclave before testing.
- *Quantity of inoculum*: 100 μL of nutrient broth (NB) or a solution of 12.5 % diluted NB in physiological saline, the initial number of bacteria amounted to about 1×10^5 CFU/100 μL (1×10^6 CFU/ml).
- *Bacteria extraction*: At the end of antibacterial tests the surviving bacteria were extracted by using 50 ml of neutralizing solution: Azolectin 3 g/L, Polysorbate 80 30 g/L, sodium thiosulphate 5 g/L, L-Hystidine 1 g/L, KH_2PO_4 0.68 g/L, (pH a 7.2 ± 0.2).
- *Control Samples*: NFC suspension non treated with polyelectrolytes and not subjected to Ag addition or non coated paper.

The antibacterial activity of the sample, as bacteria log reduction, was calculated as follows:

$$\log \text{ reduction} = \log \text{ CFU } T_{24} \text{ blank (control)} - \log \text{ CFU } T_{24} \text{ sample}$$

where CFU T_{24} is the colony forming number corresponding to the bacteria living cells at time 24 h.

Instrumentation and materials characterization

Scanning electron microscopy (SEM) images were obtained using a HR-FESEM SU-70 Hitachi instrument operating at 4 or 15 kV. Samples were placed on carbon tape and coated with carbon before SEM analysis. The optical spectra of the solid nanocomposites were recorded using a Jasco V-560 UV/VIS spectrophotometer in the diffuse reflectance mode using MgO as reference. Zeta potential measurements were performed using a Zeta Sizer Nano Series (Malvern) equipment. The Ag content in the composite materials was evaluated by Inductively Coupled Plasma (ICP) using a Jobin–Yvon 70 Plus instrument. Before ICP analysis the samples were submitted to acid digestion with an acidic mixture of HCl, HNO_3 and HF in a microwave oven.

The Brightness of the papers was measured using an Elrepho 2000 data color unit according TAPPI T 452 m-98. The air permeability was measured according to ISO 5636/3:1992 using an L&W Bendtsen tester (lorentzen & Wettre, model 114). The tensile index was determined using an Alwetron TH1 tensile tester (model 65-F, AB Lorentzen & Wettre). The preconditioned sheets were cut into 15 mm × 180 mm strips and tested according to ISO 1924/2. The initial clamp distance was 100 mm, and the strain rate was 20 mm/s. The burst index was determined in accordance with ISO 2758 with a Burst-O-Matic (model 04BOM, Lorentzen & Wettre).

Results and discussion

Preparation and characterization of NFC/Ag nanocomposites

The NFC/Ag nanocomposites were produced by electrostatic assembly of Ag NP onto NFC via polyelectrolytes linkers. It is well known that cellulose fibers are negatively charged in aqueous medium over a wide range of pH, due to the presence of ionisable moieties such as carboxylic acid groups, resulting from chemical processing, or from the minor presence of polysaccharides such as glucuronoxylans. Typically, the NFC aqueous suspensions exhibited a zeta (ζ) potential of -16.3 mV and the Ag colloid a ζ potential -18 mV, both at pH 6.0. As both materials surfaces were negatively charged the direct attachment of Ag NP onto NFC was not favored and this was confirmed here. In fact, the simple mixture of colloidal Ag NP with NFC led to agglomerates loosely attached to the cellulose fibers. Therefore the deposition of the AgNP was mediated by the assembly of oppositely charged polyelectrolytes. Cellulose nanofibers were firstly treated with a cationic polyelectrolyte, followed by an anionic polyelectrolyte and again with the cationic polyelectrolyte. As previously reported for a variety of cellulose based hybrid nanostructures (Pinto et al. 2007, 2009; Goncalves et al. 2009), this preliminary modification promotes charge homogeneity of the nanofibers surfaces hence favoring the subsequent deposition of inorganic NP.

Table 1 lists the nanocomposite samples investigated here along with the respective Ag content as determined by ICP. These nanocomposites have been prepared by varying the number of deposition cycles (n), chemical nature of the polyelectrolytes and Ag content in the starting colloid. SEM analysis of the NFC/Ag nanocomposite samples confirmed the presence of Ag NP covering the NFC fibers, as illustrated in Fig. 1 for the samples Ag/PDDA1 and Ag/PDDA5. A typical UV–VIS spectrum of a NFC/Ag nanocomposite is shown in Fig. 2 whose maximum peaked at 435 nm is characteristic of the Ag NP surface plasmon resonance. The increment of the number of deposition cycles led to an increase of the Ag content (% w/w) in the nanocomposites as inferred by visual inspection of the color of the samples but also confirmed by ICP (Table 1). Representative SEM images (Fig. 1) seem also to indicate higher amounts of AgNP at the NFC surface for the composite Ag/PDDA5 as compared to Ag/PDDA1.

Among the polyelectrolytes investigated in this work, the most efficient deposition of Ag NP onto NFC has been accomplished by using the cationic polyelectrolytes PDDA and PEI, and the anionic polyelectrolyte PSS (Table 1). The use of PAH as cationic polyelectrolyte led to a deposition of Ag NP about 20 and 10 % of the minimal values achieved with PDDA and PEI respectively (Table 1). This might be due to charge effects because for solutions with a pH near the neutrality, such as the Ag colloid used here (pH = 6.5), a high fraction of the PAH polyelectrolyte is in the non-ionized form (pK_a value of 8.5) as compared to PEI (pKa $\sim$ 9.7) and PDDA (side groups are ionized at a wide pH range) (Mak et al. 2008). Finally, and as expected, we note that the Ag content in the final nanocomposites increased with increasing amounts of Ag in the starting colloid (Table 1).

Paper coating with NFC/Ag nanocomposites

Non-commercial A3-size papers sheets (control sheets CS) were coated with two starch-based coatings with different amounts of Ag/NFC nanocomposite, specifically 11 and 29 % (with respect to the starch content) of Ag/PDDA6. The AgPDDA6 sample has been selected as filler due to its higher Ag content after one single deposition cycle. Blank samples were also produced using a coating formulation containing only starch (6 %).

The coating weights (pick-up) of the paper samples and their Ag content, as determined by ICP, are presented in Table 2. As expected, the coating weights increased with the number of layers. The silver contents are below the detection level of the analytical

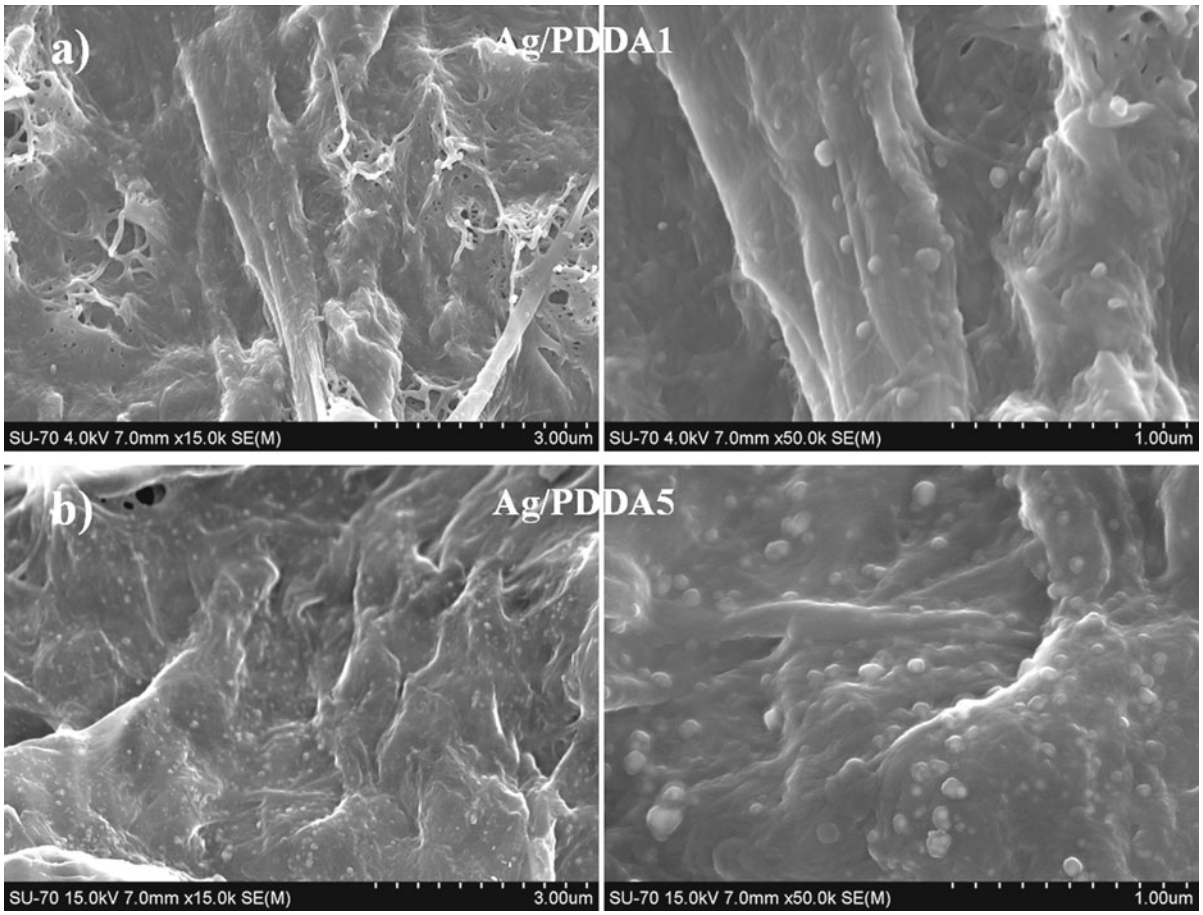

Fig. 1 SEM images of NFC/Ag nanocomposites obtained using different number of deposition cycles: **a** Ag/PDDA1 (n = 1, 15 k× and 50 k×); **b** Ag/PDDA5 (n = 5, 15 k× and 50 k×)

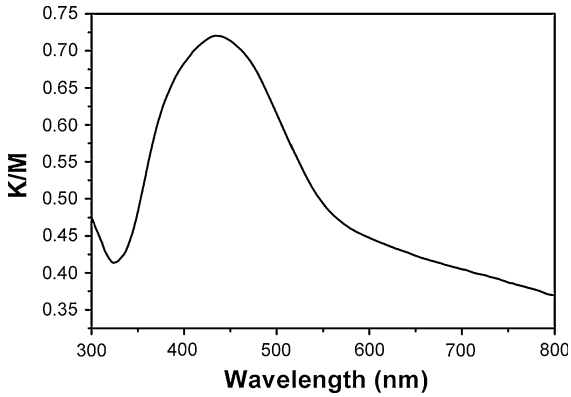

Fig. 2 UV-visible diffuse reflectance spectrum of Ag/PDDA1 nanocomposite (Kebelka-Munk units)

technique used (ICP), in the case of NFC-Ag/starch 1, NFC-Ag/starch 2 and NFC-Ag/starch 3; in fact Ag was only detected by ICP in sample NFC-Ag/starch 4.

The morphology of CS and coated papers was investigated by SEM analysis (Fig. 3). The micrographs of the CS and paper coated with starch clearly show the presence of the characteristic features of a paper: fibers and inorganic fillers (calcium carbonate). The papers coated with NFC-Ag/starch formulations show large areas were the fibers and fillers are covered with the coating components. The NFC fibers and Ag NP are clearly observed at high magnification of the paper coatings.

Antibacterial activity of cellulosic composites

The antibacterial activity of NFC/Ag suspensions and coated papers was tested towards gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumoniae*) bacteria. NFC modified with polyelectrolytes, i.e. in the absence of Ag NP, have also

Table 2 Coating weights and Ag content of coated papers

Paper sample	% of AgPDDA6 in respect to starch	Number of layers	Coating weight (g/m^2)	Ag content (% w/w)
Starch 1	–	1	2.1 ± 0.2	–
Starch 2	–	2	3.0 ± 0.3	–
NFC-Ag/starch 1	11	1	1.8 ± 0.3	ND
NFC-Ag/starch 2	11	2	2.4 ± 0.2	ND
NFC-Ag/starch 3	29	1	1.9 ± 0.2	ND
NFC-Ag/starch 4	29	2	2.6 ± 0.4	4.5×10^{-4}

ND not detected, below the ICP detection level

been used for comparative purposes. All the tests were performed in the presence of nutrients (100 or 12.5 % diluted nutrient broth) in the buffer testing media (bacteria growing conditions). In these conditions, the antimicrobial activity has been evaluated for (1) *bactericidal effect*: reduction (killing) of the number of bacteria initially inoculated (at least 1 log reduction respect to the inoculated bacteria, CFU at time 0) and (2) *bacteriostatic effect*: inhibition of bacteria growth, at least 1 log reduction respect to growth in the control sample at time 24 h.

Modified NFC suspensions

Antibacterial tests carried out on NFC modified by the sole addition of the different polyelectrolytes revealed distinct behavior depending on the nutrient conditions. In Fig. 4 the results obtained at low (12.5 %) and high (100 %) nutrients concentration are reported in respect to *S. aureus* and *K. pneumoniae*. These results show that NFC treated with different polyelectrolytes display strong antibacterial activity (complete killing) at low nutrients concentration. However, these samples have no antibacterial activity at high nutrients concentration. In fact, the polyelectrolytes modified NFC samples support the same bacteria growth as the untreated NFC (control sample). At low nutrient concentration and in contact with chemicals the bacteria cells might be more sensible to stress action. In particular, cationic polyelectrolytes, as well as other molecules with a net positive charge, are capable of killing microorganisms (Rabea et al. 2003; Melo et al. 2010; Friedrich et al. 2000). The mechanism of antibacterial action of cationic polyelectrolytes is not completely understood but it has been suggested that these polymers can interact electrostatically with anionic groups at the bacterial cell walls causing an increase of membrane permeability and subsequent leakage of cellular proteins which ultimately leads to cell death (Rabea et al. 2003). In the context of this research, the observed antibacterial action of the polyelectrolytes is an interesting finding because in principle the antimicrobial activity of the final NFC/Ag composites can be adjusted using favorable ratios for the amounts of polyelectrolyte and Ag NP.

Figures 5 and 6 show the results of the antibacterial tests for Ag based nanocomposites prepared by polyelectrolytes (PDDA/PSS/PDDA) assembly. As expected, at low nutrient concentration (Fig. 5) the Ag based samples showed strong antibacterial activity (complete killing), as previously observed for the polyelectrolytes modified NFC (Fig. 4). However, at high nutrient concentration (Fig. 6), corresponding to conditions in which the polyelectrolyte did not show antibacterial effect, the NFC/Ag composites still exhibit antibacterial activity due to the presence of Ag NP. As shown in Fig. 6, the antibacterial effect depends on the Ag content in the nanocomposite. Bacteriostatic and partial bactericidal activity could be detected respect to *S. aureus* for Ag concentrations higher than 0.1 % (w/w), while similar effects were observed in respect to *K. pneumoniae* for Ag content as low as 0.018 %. As previously observed for other Ag based composites (Pinto et al. 2009; Yuan et al. 2010), these results suggest that the NFC/Ag composites are more active against *K. pneumoniae* microorganisms. It is known that Gram positive bacteria (e.g., *S. aureus*) have a stronger defense system as compared to Gram negative bacteria (e.g., *K. pneumoniae*) (Rai et al. 2009; Gottesman et al. 2011). While Gram positive bacteria have a thick peptidoglycan cell wall, Gram negative bacteria membrane is mostly made of tightly packed lipopolysaccharides (LPS) that offer a less effective protection against silver ions penetration in the cytoplasm.

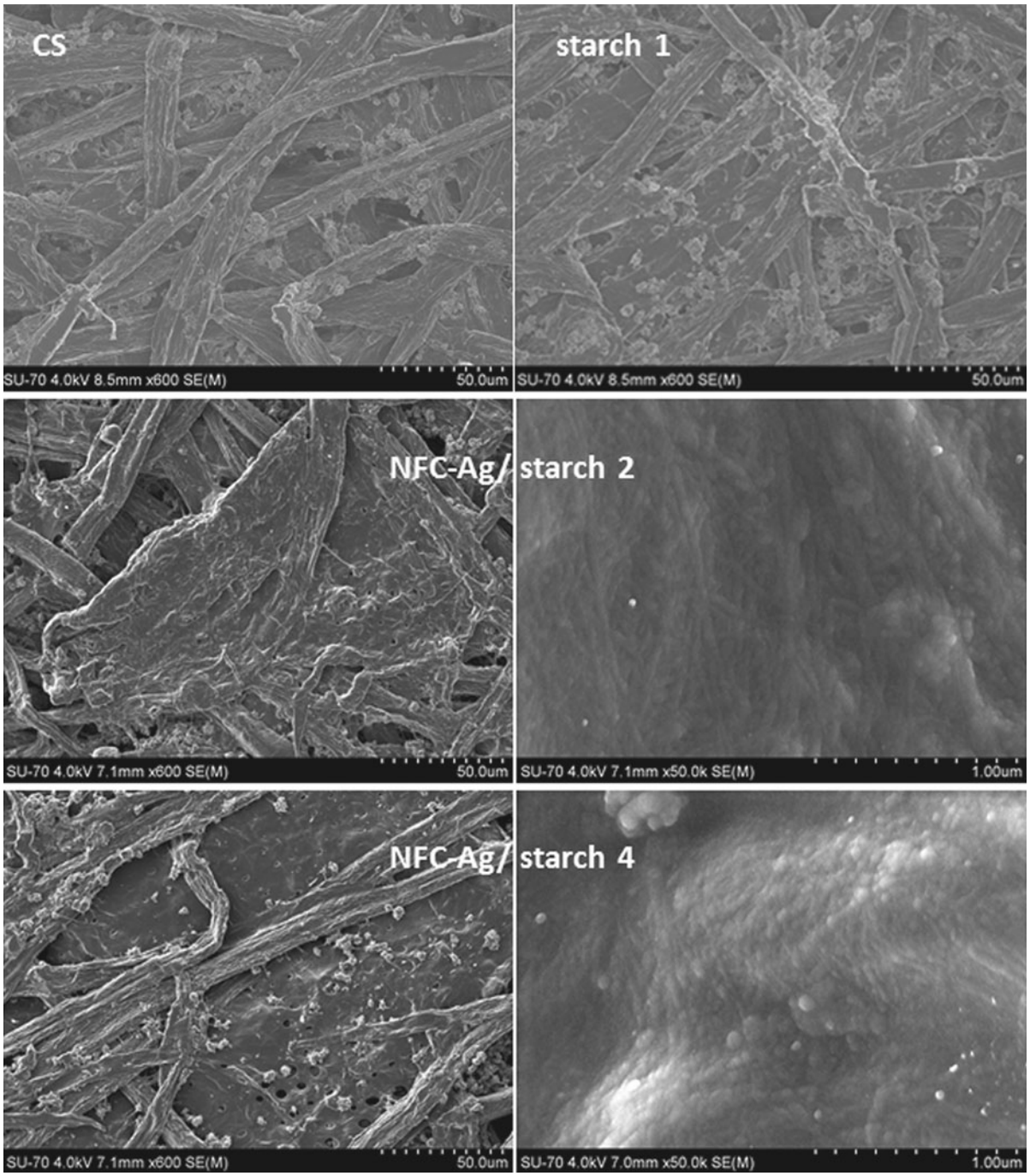

Fig. 3 SEM images of paper samples: CS (600×), starch 1 (600×), NFC-Ag/starch 2 (600× and 50 k×) and NFC-Ag/starch 4 (600× and 50 k×)

NFC/Ag coated paper

The paper samples were tested for antibacterial activity toward *S. aureus* microorganism. As can be observed in (Fig. 7), no significant antibacterial effect was detected for samples coated with the formulations that contain 11 % AgPDDA6 relative to starch (NFC-Ag/starch 1 and NFC-Ag/starch 2), probably

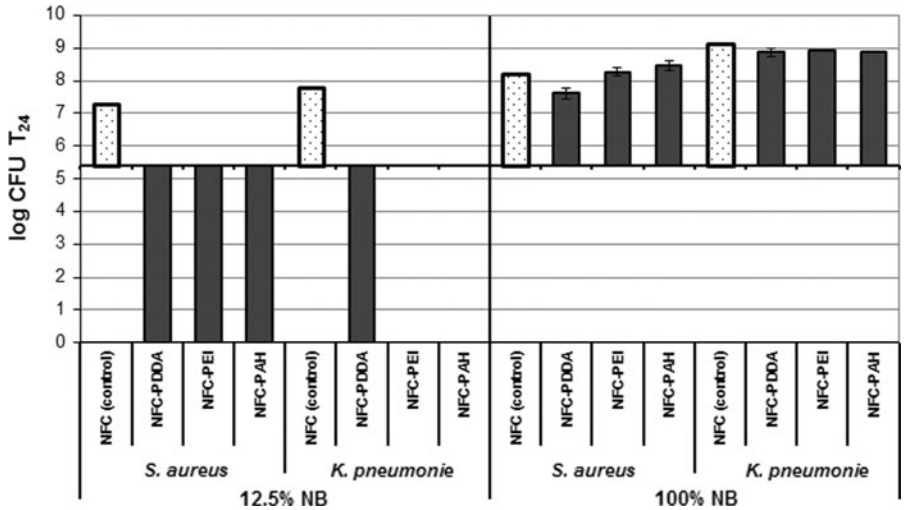

Fig. 4 Antibacterial activity of NFC treated with polyelectrolytes, toward *S.aureus* and *K.pneumoniae* at low and high nutrient concentration. Analysed samples: NFC-PDDA (NFC treated by PDDA/PSS/PDDA); NFC-PEI (NFC treated by PEI/PSS/PEI); NFC-PAH (NFC treated by PAH/PSS/PAH). Horizontal dark line refers to the initial inoculum (log CFU at time 0)

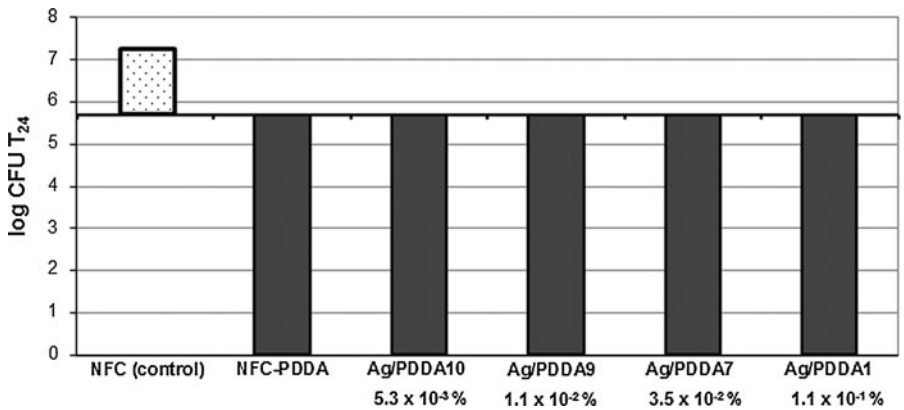

Fig. 5 Antibacterial activity at low nutrients concentration, toward *S.aureus*, of NFC/Ag nanocomposites. The bacterial load after 24 h contact time has been reported for untreated NFC (control), NFC only treated by polyelectrolytes (NFC-PDDA: NFC treated by PDDA/PSS/PDDA) and Ag nanocomposite samples. In the figure the Ag concentration in the different nanocomposites is reported as % w/w

because of the low Ag content in these samples (Table 2). However, the samples coated with formulations that contain 29 % AgPDDA6 relative to starch (NFC-Ag/starch 3 and NFC-Ag/starch 4) show significant antibacterial effects. NFC-Ag/starch 3, that possesses one layer of coating, inhibits bacteria growth (bacteriostatic effect) despite of its low Ag content. With NFC-Ag/starch 4 both bacteriostatic and bactericidal effects (partial killing of the inoculated bacteria, about 2 log reduction respect T_0 inoculum) are observed for an Ag content as low as 4.5×10^{-4} (% w/w). These results show that antimicrobial paper was produced.

Optical, barrier and mechanical properties, of the antibacterial NFC-Ag/starch coated paper

The NFC-Ag/starch coated paper sheets that presented antibacterial activity were further characterized in terms of their optical (brightness), barrier (air permeability) and mechanical (tensile strength and Bursting strength) properties (Table 3). The papers coated with the unfilled starch formulation showed a small decrease of their brightness. However, the papers coated with NFC-Ag/starch presented a dramatic brightness reduction. This behavior is obviously related with the fact that the NFC/Ag nanocomposite

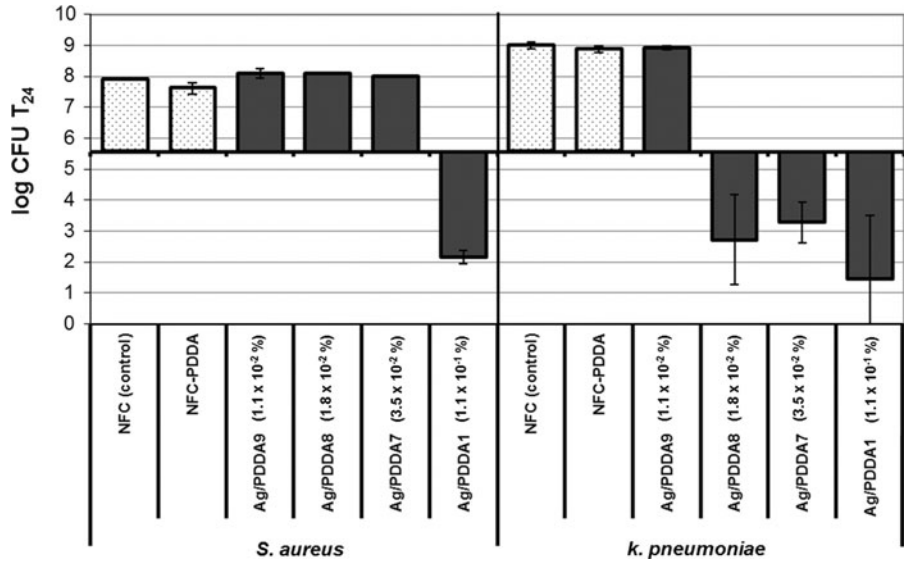

Fig. 6 Antibacterial activity at high nutrients concentration, toward *S.aureus* and *K.pneumoniae* of NFC/Ag nanocomposites. The bacterial load after 24 h contact time has been reported for untreated NFC (control), NFC only treated by polyelectrolytes (NFC-PDDA: NFC treated by PDDA/PSS/PDDA) and Ag nanocomposite samples. In the figure the Ag concentration in the different nanocomposites is reported as % w/w

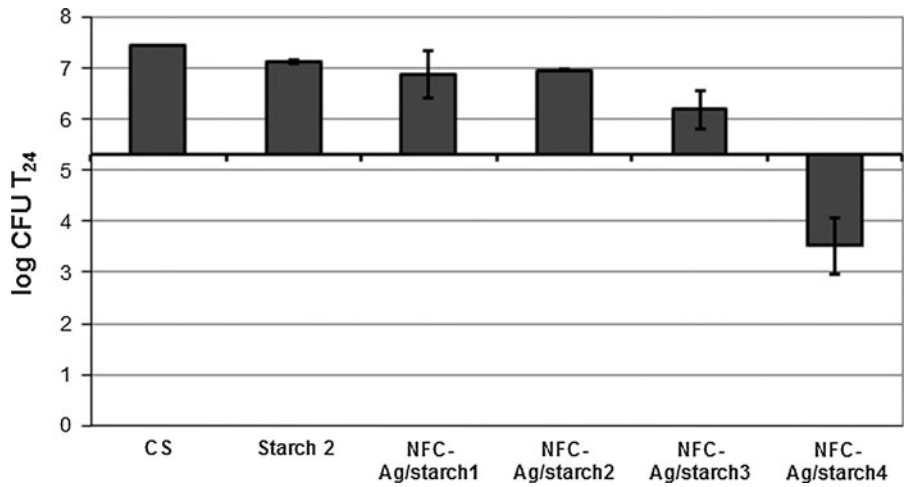

Fig. 7 Antibacterial activity at high nutrients concentration toward *S.aureus* of control sample, starch2, and NFC/Ag coated papers

Table 3 Optical, barrier and mechanical properties of control sheet and papers coated with starch and NFC-Ag/starch formulations

Paper sample	Brightness (%)	Air permeability (nm/Pa. s)	Burst index (KPa m^2/g)	Tensile index (N.m./g)	
				MD	CD
CS	95.21 ± 0.07	11.49 ± 0.4	2.62 ± 0.30	83.7 ± 3.81	23.7 ± 1.2
Starch 1	94.78 ± 0.10	10.82 ± 0.5	2.74 ± 0.20	77.5 ± 2.70	23.2 ± 0.9
Starch 2	94.44 ± 0.12	10.76 ± 0.5	2.86 ± 0.14	81.1 ± 2.46	25.2 ± 0.9
NFC-Ag/starch 3	65.82 ± 2.59	9.54 ± 0.5	2.77 ± 0.21	83.4 ± 2.41	22.9 ± 1.5
NFC-Ag/starch 4	56.25 ± 1.17	8.18 ± 0.7	3.18 ± 0.14	82.2 ± 1.96	25.5 ± 1.5

MD machine direction, *CD* cross-machine direction

used in the formulations displayed a typically intense brown color. This is a drawback for applications such as for printing papers, but might be less relevant for example for air sanitization filters or for packaging applications in which the color of the paper is not so relevant. For the applications where brightness is not

critical, the observed reduction in air permeability promoted by the coating with NFC/Ag and starch together with the maintenance of the mechanical properties of the paper sheets (Table 3) and with the reported antibacterial properties can indeed open interesting possibilities for the application of this materials for example for food packaging and preservation.

Conclusions

The electrostatic assembly of Ag NP onto nanofibrillated cellulose mediated by polyelectrolyte linkage has been demonstrated for a series of NFC/Ag nanocomposites. This approach was successfully employed to impart antibacterial properties to NFC whose relevance for the paper making industry has increased considerably in the last years. The antibacterial activity observed for NFC/Ag materials against *S. aureus* and *K. pneumoniae* strains was due to both the polyelectrolytes and Ag NP, however the presence of the latter was crucial in order to observe antimicrobial activity for high nutrient conditions. This preparative method may also find applications in the development of paper-based products whose antibacterial properties can be adjusted by varying the amount and characteristics of NFC/Ag composites used as nanofillers.

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