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Antibacterial activity of nanocomposites of silver and bacterial or vegetable cellulosic fibers

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Abstract

Cellulose/Ag nanocomposites were prepared using two distinct methodologies and two cellulose substrates: vegetable and bacterial cellulose. These nanocomposites were characterized in terms of their morphology and chemical composition. Detailed studies on the antibacterial activity of these materials were carried out for *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Silver nanoparticles present in the cellulosic fibers in concentrations as low as 5.0×10^{-4} wt.% make these nanocomposites effective antibacterial materials. We anticipate that the versatile use of these cellulose-based nanocomposites can bring a promising strategy to produce a wide range of interesting materials where antibacterial properties are crucial.

Keywords: Nanocomposites; Vegetable cellulose; Bacterial cellulose; Silver nanoparticles; Antibacterial activity

1. Introduction

Cellulose is one of the oldest and most abundant natural, renewable, biodegradable and biocompatible polymers. Cellulose and its derivatives have been used in a variety of applications in several areas, such as the textile industry, the paper industry and in the medical field, where they are used as tissue engineering materials, due to their good biocompatibility, mechanical properties similar to those of hard and soft tissue and easy fabrication into a variety of shapes with adjustable interconnecting porosity [1]. Recently, a growing interest has been devoted to bacterial cellulose (BC) [2]. Although identical in chemical composition, the mechanical properties and microstructure of bacterial cellulose differ from those of vegetable cellulose (VC). BC shows high tensile strength and modulus; for instance, the BC microfibrils have a density of 1600 kg m^{-3}, Young's modulus of 138 GPa and tensile strength of at least 2 GPa, which are almost equal to those of aramid fibers [3]. Compared to vegetable cellulose, BC possesses higher water-holding capacity, higher crystallinity, higher tensile strength and a finer web-like network. BC has been widely used in processed foods and, to a less extent, in acoustic diaphragms for audio speakers and headphones and for making unusually strong paper [4]. BC has also medical applications such as wound dressings and artificial skins, artificial blood vessels and (bio)membranes [5–7].

Silver, both as a metal and in ionic form, exhibits strong cytotoxicity towards a broad range of microorganisms, and its use as an antibacterial agent is well known [2,8]. It has been reported that the mode of antibacterial action of silver nanoparticles is similar to that of silver ion. However, the effective biocidal concentration of silver nanoparticles is at a nanomolar level in contrast to a micromolar level of silver ions [9].

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Silver has an oligodynamic effect, that is, silver is capable of causing a bacteriostatic (growth inhibition) or a bactericidal (antibacterial) impact [10,11]. Research indicates that silver is also effective in purification systems for disinfecting water or air [12,13].

In the above context, the association of Ag nanoparticles to biopolymers like vegetable or bacterial celluloses represents an interesting approach to developing new nanocomposites that might find a variety of new applications.

In this work, we wish to report a comparative study on the antibacterial effects of Ag containing cellulose nanocomposites prepared by a variety of simply chemical strategies. A detailed characterization of these nanocomposites and the determination of their antibacterial activity using *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae* are presented in this article.

We anticipate that this might have a beneficial consequence for future applications of cellulose fibers with antibacterial properties.

2. Materials and methods

2.1. Materials

Silver nitrate ($AgNO_3$, Vaz Pereira), sodium borohydride ($NaBH_4$) (95%, Riedel-de Haën), poly(diallyldimethylammonium chloride) (PDDA, 20 wt.% in water, MW 100,000–200,000) and poly(sodium 4-styrenesulfonate) (PSS, MW 70,000) were purchased from Aldrich and were used as received. Wood cellulose fibers (*Eucalyptus globulus*, ECF bleached kraft pulp, average length 0.9 mm, average width 20 μm) composed essentially of cellulose (~85%) and glucuronoxylan (~15%) were supplied by Portucel (Portugal). The VC fibers were disintegrated and washed with distilled water before use. Pure BC was produced by *Acetobacter xylinus*, in the form of a wet 3D network of ribbon-like nanofibril structures (50–100 nm width).

2.1.1. Stock cultures and culture media

All microbial strains cited in the paper were provided by DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures). *B. subtilis* ATCC 6633 (DSM 347), *K. pneumoniae* ATCC 4352 (DSM 789), *S. aureus* ATCC 6538 (DSM 799) were maintained frozen (−80 °C) and transferred monthly on TSA (Tryptone Soya Agar) made of 15 g l^{-1} tryptone; 5 g l^{-1} soya peptone; 5 g l^{-1} NaCl and 15 g l^{-1} neutralized bacteriological agar.

2.2. Instrumentation

Scanning electron microscopy (SEM) images were obtained using an FEG-SEM Hitachi S4100 microscope operating at 25 kV. Samples were deposited on a glass plate and coated with carbon for SEM analysis. The optical spectra were recorded using a Jasco V-560 UV–vis spectrophotometer; for the solid samples the spectra were recorded in the diffuse reflectance mode using MgO as the reference. Zeta potential measurements and dynamic light scattering analyses (DLS) were performed using a Zeta Sizer Nano Series (Malvern). In order to quantify the Ag retained in the fibers, these were treated with a mixture of HCl, HNO_3 and HF in a microwave oven. Silver was analyzed in the resultant solutions by ICP (inductively coupled plasma) using a Jobin Yvon 70 Plus equipment.

2.3. In situ synthesis of cellulose/silver nanocomposites

2.3.1. Reduction of Ag^+ with borohydride

Silver nanoparticles were grown at the fiber surfaces by direct reduction of $AgNO_3$ with an excess of aqueous $NaBH_4$. Typically, a solution of $AgNO_3$ 1 mM (80 ml) was added drop-wise to an ice-cold $NaBH_4$ 2 mM solution (240 ml) containing the cellulose, under vigorous stirring. After a few minutes, the suspension turned yellow and then was stirred over 2 h. The fibers were separated and thoroughly washed with distilled water. The resulting nanocomposites of bacterial cellulose with silver (BC/Ag) were lyophilized and the nanocomposites of vegetable cellulose with silver (VC/Ag) were dried at 50 °C overnight.

To prepare nanocomposites with different Ag content, the $AgNO_3$ concentration was varied between 0.033 and 2 mM, keeping molar relation to $NaBH_4$.

2.3.2. Reduction of Ag^+ under UV radiation

The cellulose substrates were immersed in the aqueous $AgNO_3$ solution mentioned above and then were exposed to a UV lamp ($\lambda = 254$ nm; 8 W) for different times (from 5 to 60 min). At the end, the fibers were collected and washed in order to remove vestigial $AgNO_3$ and finally dried as described above.

2.4. Post-deposition of pre-synthesized Ag nanoparticles

First, silver colloids were prepared from the borohydride method as described above but in the absence of the cellulose substrates. Several diluted solutions with different concentrations were then prepared from the initial colloid.

2.4.1. Layer by layer (LbL) assembly of silver nanoparticles at vegetable cellulose surface

Aqueous solutions of 1% of PDDA and PSS have been prepared in NaCl (0.5 M). In a first step, the cellulosic substrates were treated with the polyelectrolytes by alternate dipping in PDDA, PSS, and again in the PDDA solutions. The substrates were then rinsed with deionized water to remove the excess of polyelectrolytes and were then immersed in the inorganic colloids for 10 min. After, the resulting nanocomposites were washed thoroughly in water

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