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Simultaneous sonochemical-enzymatic coating of medical textiles with antibacterial ZnO nanoparticles

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ABSTRACT

The antimicrobial finishing is a must for production of medical textiles, aiming at reducing the bioburden in clinical wards and consequently decreasing the risk of hospital-acquired infections. This work reports for the first time on a simultaneous sonochemical/enzymatic process for durable antibacterial coating of cotton with zinc oxide nanoparticles (ZnO NPs). The novel technology goes beyond the "stepwise" concept we proposed recently for enzymatic pre-activation of the fabrics and subsequent sonochemical nano-coating, and is designed to produce "ready-to-use" antibacterial medical textiles in a single step. A multilayer coating of uniformly dispersed NPs was obtained in the process. The enzymatic treatment provides better adhesion of the ZnO NPs and, as a consequence, enhanced coating stability during exploitation. The NPs-coated cotton fabrics inhibited the growth of the medically relevant *Staphylococcus aureus* and *Escherichia coli* respectively by 67% and 100%. The antibacterial efficiency of these textile materials resisted the intensive laundry regimes used in hospitals, though only 33% of the initially deposited NPs remained firmly fixed onto the fabrics after multiple washings.

1. Introduction

The use of antibacterial textiles is in the list of preventive measures to reduce the bioburden in clinical settings and consequently diminish the risk of hospital-acquired infections [1,2]. Such materials prevent the transmission of microorganisms, impairing the common transfer routes for pathogen spreading [3–5]. Consequently, the antibacterial treatment became an integrated step in the production of medical textiles, such as wound bandages, hospital bed sheets, surgical uniforms and patients' pajamas [6]. The choice of an antibacterial agent is the key to obtain effective bactericidal or bacteriostatic coatings and depends primarily on the required efficacy towards specific microorganisms. However, a major concern remains the release of the active agents during fabric exploitation, which compromises the durability of the antibacterial effect and the safety at use [5,7]. The routine adoption of antibacterial textiles in clinical practice inevitably calls for facile in terms of application and durable coatings, whereas the market demands attractive manufacturing cost [8].

Inorganic nanoparticles (NPs) are claimed to be more biocidal than many conventional antibiotics, which utilization at high concentrations can induce adverse effects and toxicity to human cells [9,10]. The use of antibacterial NPs is also among the most promising strategies to overcome the microbial drug resistance [11]. The mechanism of NPs antibacterial action involves generation of reactive oxygen species (ROS) followed by the disruption of the bacterial cell membrane [10,12–14]. However, the antibacterial potential of the inorganic NPs, except for the silver NPs, has not been exploited sufficiently for the development of medical textiles.

The production of durable antibacterial textiles embedded with inorganic NPs often requires time-consuming fabric pretreatments such as chemical or plasma activation, in addition to subsequent coating stabilization using different cross linking techniques [15–17]. Using enzymes as tools for activation of textile surfaces would avoid the use of harsh chemicals and allow to impart new functionalities to the fibrous substrates at mild processing conditions [18].

As an alternative to the existing finishing technologies, a facile one-step sonochemical route has been suggested for uniform deposition of inorganic NPs on the surface of solid substrates, including textiles [19,20]. The formation, growth and collapse of cavitation bubbles, formed upon sonication of liquids, determine the main features of the deposition process. Microjets formed after the bubbles' collapse drive at huge velocities the NPs encountered in their vicinity towards the solid surface where physico-chemical

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interactions occur during the collision. In a previous work, we combined the sonochemical deposition of NPs with an enzymatic pre-activation of cotton fabrics in order to create anchoring points for embedding the NPs on the textile surface, thereby improving the durability of the coating. In such way, antibacterial fabrics with durable antibacterial effect were obtained in a two-step process comprised of a cellulase pre-treatment followed by a sonochemical coating with ZnO NPs [21]. The particles were generated *in situ* from zinc acetate in water/ethanol alkaline solutions, and deposited on the fabrics using high intensity ultrasound. ZnO NPs were selected due to their general acceptance as biologically safe for humans and the lack of coloration [10,22]. The enzymatic pre-activation of the fabric surface resulted in deposition of smaller particles with enhanced antibacterial activity, improved adherence on the fibers and, consequently, durable antibacterial effect. Despite of these benefits, the industrial acceptance of such two-step process is hampered by the requirements for shorter processing time and simplicity of the operations. Therefore, the challenge was to achieve the above effects using a single step process.

Normally, heterogeneous enzyme catalysis for modification of solid substrates, such as textile fibers, is a time-consuming operation. In particular, the efficiency of hydrolytic enzymes, e.g. cellulases, depends on the mass transfer from the enzyme solution to the solid substrate. Thus, intensifying the mass transfer would shorten the time for fabric activation and lower the amount of the biocatalyst necessary for hydrolysis. Ultrasound (US) has been applied previously as a way to improve the performance of cellulases in de-sizing, scouring, bleaching, mercerization and dyeing of cotton [23–25].

The objective of this work is to combine biocatalysis and physicochemical processing in a single-step, industry-attractive technology for durable coating of medical textiles with antibacterial NPs. The coating consists in embedding of ZnO NPs onto cotton fabrics in a 30 min simultaneous sonochemical/enzymatic process. The US is employed to boost the rate of the enzymatic hydrolysis and create on the cotton surface a larger number of reducing sugar ends for better NPs adhesion and durability of the antibacterial effect. The activity of the fabrics will be evaluated against the clinically relevant bacteria *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) after multiple washing cycles with a non-ionic detergent at hospital laundry regimes (75 °C).

2. Experimental section

2.1. Materials, reagents and bacteria

Bleached 100% woven cotton fabric (144 g/m^2) was supplied by Davo SRL (Romania). Cellulase formulation Kappacell ETU 39 for biopolishing of cotton textiles (5.86 mg/mL protein, 1.2 mM glucose equivalents released per min, averaged over 60 min, pH optimum 5–8) was purchased by Kapp-Chemie GmbH & Co. KG (Germany). ZnO NPs (size < 100 nm) dispersion in water, potassium sodium tartrate tetrahydrate and sodium hydroxide and 3,5-dinitrosalicylic acid (DNSA) were purchased from Sigma–Aldrich (Spain). Gram-negative *E. coli* (ATCC 25922) and Gram-positive *S. aureus* (ATCC 25923) were used in the antibacterial activity assays. Plate count agar and all other reagents for bacterial studies were purchased from Sigma–Aldrich unless otherwise specified.

2.2. Cellulase activity measurements

The cellulase activity was measured using the filter paper (FPU) assay [26]. Reactions were carried out in 50 mL test tubes with Whatman No. 1 filter paper strip (1 × 6 cm, 50 mg) in 1.0 mL distilled water and 0.5 mL enzyme formulation. The mixtures were incubated at temperatures ranging from 20 to 65 °C for 1 h. Thereafter, a colorimetric reagent (DNSA) was added to quantify the amount of reducing sugars. The DNSA solution was prepared by dissolving 120 g sodium potassium tartrate in 80 mL of previously heated (60 °C) 0.2 M NaOH, prior to addition of 200 mL of 96 mM DNSA and volume completion to 400 mL with distilled water. The reaction mixtures were placed in a boiling water bath for 5 min, cooled to room temperature and diluted with 20 mL distilled water prior to measuring the absorbance at 540 nm with Infinite M200 (Tecan, Austria) multiplate reader. All experiments were performed in triplicate.

2.3. Enzyme stability in US field

One mL of cellulase product diluted to 50 mL with water was subjected to US irradiation (20 kHz, 21.5 W, 17.30 W/cm^2, 0.43 W/cm^3 and 35% of amplitude) for 30 min at temperatures ranging from 20 to 60 °C. After the treatment, enzyme aliquots (0.5 mL) were incubated for 1 h at 55 °C with Whatman No. 1 filter paper strip (1 × 6 cm) and the reducing sugars released were measured using the FPU assay as previously described. The residual enzyme activity was calculated as a percentage of the activity of the enzyme not exposed to US. The possible effect of the sonochemical treatment on the tertiary and secondary structure of cellulase was assessed by measuring the intrinsic fluorescence of the enzyme as a result of protein unfolding and denaturing. For the purpose of the assay, cellulase water solutions not exposed (control) and exposed to US treatment (30 min) were evaluated. The fluorescence was measured at room temperature (25 ± 1 °C) with Quanta Master 4 spectrofluorometer (PTI, USA) at 280 nm excitation wavelength (slit = 2 nm), 300–450 nm emission wavelength (slit = 2 nm) and 1200 nm/s of scanning speed.

2.4. Effect of the US parameters on the enzyme performance

To study the hydrolytic potential of the cellulase toward cellulose substrate under sonication, the US amplitude of vibration (and thus intensity) was varied to determine its effect on the yield of the enzyme catalyzed reaction expressed in reducing sugars concentration. For this aim, 1 mL of cellulase solution (diluted to 50 mL with water) was subjected to ultrasonic irradiation at different US amplitudes (range of 20–40%) for 30 min at 55 °C and in presence of 0.5 g of Whatman No. 1 filter paper as a substrate. Thereafter, the liberated reducing sugars were determined using the aforementioned method (FPU assay). Control treatments without enzyme were carried out in parallel. All results are reported as mean values ± standard deviation (n = 3).

2.5. Ultrasound-enzyme assisted coating of cotton with ZnO NPs

The sonochemical coating was carried out using an ultrasonic transducer Ti-horn (20 kHz, Sonics and Materials VC750, USA). By measuring the time-dependent increasing of the temperature in the ultrasonic glass jacked vessel, the US intensity (17.30 W/cm^2), density (0.43 W/cm^3) and power (21.5 W) used for the textile treatment were calorimetrically determined. The cotton samples (5 × 10 cm, approx. 0.7 g) were immersed in the ultrasonic pot containing 50 mL ZnO NPs aqueous solution (1 mM) and 2% of weight of fabric (owf) cellulase formulation and the coating of the cotton samples was carried out during 30 min at 55 ± 2 °C and amplitude of 35%. To maintain the fabric at the bottom of the US pot without using any additional accessories, the fabric sample was cut bigger than the diameter of the pot (4 cm). Thereafter, the sample was folded in a way that its diameter is slightly wider than the diameter of the pot, thereby once placed at the bottom of the pot the contact/friction of its edges with the walls prevents it from moving during