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ORIGINAL PAPER

The sonochemical coating of cotton withstands 65 washing cycles at hospital washing standards and retains its antibacterial properties

Ilana Perelshtein · Yelena Ruderman · Nina Perkas · Jamie Beddow · Gagandeep Singh · Mircea Vinatoru · Eadaoin Joyce · Timothy J. Mason · María Blanes · Korina Mollá · Aharon Gedanken

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Abstract Hospital-acquired nosocomial infections are a major health, and consequently financial issue, in the world healthcare system. The problem of bacterial infections in general, and in hospitals in particular, has led to extensive scientific and industrial efforts to fabricate antibacterial textiles. A sonochemical coating machine was developed and built and its ability to coat antibacterial nanoparticles (NPs) onto 40–50 meter length of materials on a roll to roll basis at a speed of 22 cm/min. Cotton coated sonochemically with copper oxide nanoparticles (CuO NPs) was found to maintain its antibacterial properties even after 65 cycles of washings according to hospital protocols of hygienic washing (75 °C). This demonstrates the good quality and high stability of this sonochemically produced NPs coating on textiles. Durable antibacterial textiles such as these may be suitable for wide spread use in future hospital environments where hygiene control is of paramount importance.

Keywords Sonochemistry · Antibacterial nanoparticles · Copper oxide · Coating method

Introduction

Hospital-acquired nosocomial infections are a major health, and consequently financial, issue in the world healthcare system. The financial impact of these infections counteract medical advances and increase

I. Perelshtein · Y. Ruderman · N. Perkas · A. Gedanken (✉)
Kanbar Laboratory for Nanomaterials, Department of Chemistry, Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, 52900 Ramat-Gan, Israel
e-mail: gedanken@mail.biu.ac.il; Aharon.Gedanken@biu.ac.il

Y. Ruderman
e-mail: rulena@gmail.com

N. Perkas
e-mail: Nina.Perkas@biu.ac.il

J. Beddow · G. Singh · M. Vinatoru · E. Joyce · T. J. Mason
Faculty of Health and Life Sciences, Sonochemistry Centre, Coventry University, Coventry CV1 5FB, UK
e-mail: j.beddow@coventry.ac.uk

G. Singh
e-mail: Singhg28@coventry.ac.uk

M. Vinatoru
e-mail: m.vinatoru@coventry.ac.uk

E. Joyce
e-mail: e.joyce@coventry.ac.uk

T. J. Mason
e-mail: t.mason@coventry.ac.uk

M. Blanes · K. Mollá
Textile Research Institute, AITEX, Plaza Emilio Sala, 1, 03804 Alcoy, Spain
e-mail: mblanes@aitex.es

K. Mollá
e-mail: kmolla@aitex.es

the cost of medical care by increasing the length of hospital stay by at least 8 days on average per affected patient (http://www.healthfirsteurope.org/index.php?pid=82). This adds more than 30 million patient days in hospitals around the world each year (Branger et al. 2005).

The problem of bacterial infections in general, and in hospitals in particular, has led to extensive scientific and industrial efforts to fabricate antibacterial textiles. Since silver has been known as antibacterial agent for generations, the early stages of R & D have centered on its use. With the progress of nanotechnology Ag NPs were extensively studied for their antibacterial properties (Aymonier et al. 2002). Silver-containing products can already be found on the healthcare market. For example, Smith and Nephew produce a composite multi-layered product under the trade name ACTICOAT^{TM}. This wound care product is composed of three layers—a layer of polyethylene film, a middle layer of rayon/polyester blend non-woven fabric, and a third film layer. Nano-crystalline silver particles are deposited on the film layers to provide the antimicrobial effect. However, this technology has faults as the fabric tends to release large amounts of silver initially, often in the form of flakes that may enter the wound and cause irritation.

Another example of this is a highly porous, silver-impregnated charcoal cloth, sandwiched between two nylon non-woven layers marketed by Johnson & Johnson under the trademark ACTISORB.^{RTM}.

It is known that metallic copper and copper complexes have been used to disinfect liquids, solids, and human tissue for centuries (Dollwet and Sorenson 1985; Grace et al. 2009; Chattopadhyay and Patel 2010). However, as far as we know, there are not many publications on the production and application of the copper-textile composite with the exception of that of Gabbay and co-workers (Gabbay et al. 2006). They describe that the copper-impregnated fibers of cotton and polyester containing 10 %wt Cu demonstrated significant antifungal and antimicrobial properties. They performed the impregnation of copper oxide in the cotton fibers by a multi-phase soaking procedure, and in the polymer fibers, the copper oxide was inserted during the master batch preparation stage using the preliminary synthesized copper oxide powder.

The preferred coating technique in most scientific and industrial examples is the direct impregnation of textiles in the reactant solution. Other methods such as chemical vapour deposition (CVD) have also been used for depositing silver NPs on textiles (Foster et al. 2010). Recently there has been a shift from the use of silver to other NPs as a result of the FDA's attitude reducing the permitted amount of nanosilver to be used in medicine and health care products. The most promising alternatives for silver NPs are metal oxides, such as MgO, ZnO, CuO, and TiO_2.

Sonochemistry has been proven as an excellent technique for surface coating NPs on ceramic, polymeric, metallic, glass, textiles, and even paper (Kotlyar et al. 2007; Gedanken et al. 2011; Perkas et al. 2007). We have demonstrated that the metal oxides can be deposited on cotton, PE (polyester), Nylon and their mixtures as well as on nonwoven materials (Perelshtein et al. 2008).

For ZnO, a good level of antibacterial efficacy has been shown, and it has been demonstrated that there is no leaching of the NPs to the environment during washing (Perelshtein et al. 2009).

In our view the hospital of the future will replace conventional fabrics that are currently used (bed sheets, doctor aprons, pajamas, curtains, pillow covers) with antibacterial textiles. To be successfully incorporated into all of these applications the antibacterial properties of the coated textiles must be durable enough to withstand many washing cycles. The washing carried out in hospitals is generally aggressive and is conducted at high temperatures in order to maintain adequate levels of hygiene.

We have already published an article (Gedanken et al. 2011) describing a pilot machine that can sonochemically coat NPs, roll to roll, onto 40–50 m length of textiles at a speed of 22 cm/min. In this study we have coated CuO NPs on cotton. CuO was chosen because its $K_{sp} \sim 10^{-21}$ (at room temperature) is much smaller than that of ZnO (10^{-12}). The lower value of the Ksp leads to smaller amount of ions that are removed from the surface during the washing cycles.

Experimental

Materials

Bleached woven 100 % cotton fabric (145 g/m^2) was used in the current study. $Cu(CH_3COO)_2{\bullet}H_2O$ and aqueous solution of ammonia (28 % wt) were purchased from Aldrich and used without further purification.

The roll to roll coating of CuO on cotton fabric

Strips of cotton (10 meter long and 10 cm wide) were coated with CuO NPs using a roll to roll feed with a machine that has been described elsewhere (Abramov et al. 2009). In Fig. 1 the photo image of the pilot installation during the coating of the cotton by CuO is presented. The front panel was open to show the change in the color of the fabric after coating with CuO nanoparticles. In the inset one can observe that with the deposition of CuO nanoparticles the color of the textiles was changed from white to brown. The configuration of the sonochemical coating machine allows coating of both sides of substrate.

The methodology used for the CuO coating process has also been described previously (Gedanken et al. 2011). Briefly, 8.0 g of $Cu(CH_3COO)_2 \bullet H_2O$ was dissolved in 380 ml of DDW, and 3.6 liters of ethanol was added to the aqueous solution. The solution was heated to 55 °C using the sonotrodes, and 20 ml of ammonia (28 % wt.) was added dropwise until a pH of 8 was reached. At this stage the fabric was passed through the solution. The fabric speed was 22 cm/min. In order to keep the concentration of CuO constant along the coated fibers, 10 % of the initial concentration of the precursor was added to the reaction slurry every 10 min during the reaction. In this way new nanoparticles were formed, and a regular coating and a

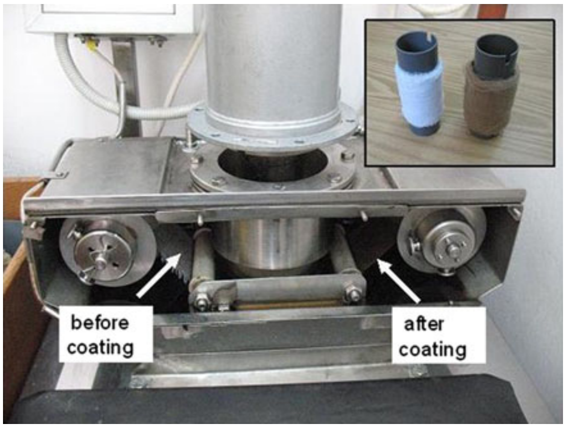

Fig. 1 Photo image of the pilot installation. In the inset of the figure the CuO coated and uncoated spools are shown

constant amount of CuO was immobilized on the cotton bandage. The XRD analysis of the coated bandage demonstrates that the copper oxide is crystalline in nature, and the diffraction peaks match very well with the PDF file 80–1916.

Antimicrobial test

The antibacterial performance of the textiles was measured using the absorption method from BS EN ISO 20743:2007 (BS EN 20743:2007). Samples of CuO coated fabric (test fabrics) and uncoated fabric (plain cotton control) were cut into pieces weighing 0.40 ± 0.05 g. Prior to testing these samples were sterilized in covered glass universals by autoclaving them for 15 min at 121 °C and 103 kPa. The suspensions of bacteria used for inoculating the fabrics were prepared as follows. Overnight cultures of bacteria were grown in nutrient broth (NB, Oxoid) at 37 °C and 110 rpm. Following this incubation, a 0.4 ml aliquot of the overnight culture was transferred to 20 ml of fresh nutrient broth and incubated for 3 h at 37 °C and 110 rpm. The 3 h culture was then serially diluted threefold in dilute nutrient broth (1 in 20 dilutions in water) and the concentration of bacteria in the suspension was adjusted to be between 1×10^5 and 3×10^5 colony forming units per ml (CFU/m). For each bacterial strain, 6 pieces of the control fabric and 6 pieces of the test fabric were inoculated with 0.2 ml of the dilute bacterial suspension. Each 0.2 ml of suspension was slowly added drop wise to each piece of fabric in order to allow it to be fully absorbed. Following the inoculation step, 3 of the control samples and 3 of the test samples were placed in an incubator at 37 °C in capped vials. The remaining 3 control and 3 test samples were immediately mixed with 20 ml of neutralizing medium (SCDLP). The resulting suspensions of bacteria were serially diluted in NB. 1 ml of each dilution was mixed with 17 ml of molten plate count agar (PCA, Oxoid) and allowed to set in a sterile Petri dish. All plating was carried out in duplicate. These agar plates were then incubated for 24 ± 4 h at 37 °C. After this incubation the number of colonies on the plates were counted and the inoculum cell density was calculated (CFU/ml). The remaining 3 control and 3 test pieces were incubated with the absorbed bacterial suspensions for between 18 and 24 h at 37 °C. After this incubation the fabrics were washed out with SCDLP and plated out as described above. The number of colonies on these plates was used to calculate the growth values on the control and test fabrics.

The antibacterial efficiency value (A) was calculated using the following formula:

$$A = F - G \quad (1)$$

where **F** = growth value on the control fabric sample ($\log_{10}$ CFU/ml post incubation—$\log_{10}$ CFU/ml prior to incubation). **G** = growth value on the treated fabric samples.

Antibacterial tests were carried out using the following bacterial strains: *Staphylococcus aureus* ATCC 6538, Methicillin-resistant *S. aureus (*MRSA) NCTC 10442, *Acinetobacter baumannii* NCTC 10303, *Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 8739.

Characterization

The coated cotton fabric was sent to a commercial company which carried out the washings according to standard hospital practices at 75 °C using an ECOS detergent (Free & Clear Liquid Laundry Detergent/Earth Friendly Products, USA). For textile washing the European standard EN ISO 6330 "Textiles—Domestic washing and drying procedure for textile testing" was applied. The CuO content on the textile was determined by inductive coupled plasma ICP (ULTIMA JY2501) and SEM (FEI) after a series of sequential of washing cycles.

The protocol for preparation of the coated samples for ICP analysis is as follows: The CuO coated cotton was placed in 10 ml 0.5 N HNO_3 and heated for 20 min. The copper oxide coating is dissolved by the acid. In the next stage 50 ml of H_2O is added and the solution is heated up for 20 additional minutes. Then, 5 ml of the solution is taken for the ICP analysis, for detection of the concentration of Cu^{+2} ions in the solution.

Results and discussion

Amount of CuO on cotton after washing cycles

The initial amount of CuO on fabrics was measured by ICP and was found to be $\sim$0.7 % (wt%). In Fig. 2 the amount of CuO retained on the washed fabric as a function of the number of washing cycles as determined by ICP is presented. The error of the ICP method is around 8 %. A main decrease in the amount of coating occurs after 15 cycles of washing. Additional cycles don't affect significantly on the amount of the well deposited coating. Although, the amount of CuO after 65 cycles was reduced by half, we have already demonstrated in our previous study (Perelshtein et al. 2009), that this is not due to the leaching of nanoparticles. According to our explanation, the reduction in the amount of CuO on the fabric is due to the dissolution of Cu^{+2} ions in the washing solution. The slight solubility of CuO can be explained by the very low K_{sp} of CuO ($\sim 10^{-21}$ at room temperature) that dictates a very low concentration of Cu^{+2} in the solution. The washing in industrial washing machines is carried out at a high temperature (75 °C) that has led to an increase in the solubility of the copper.

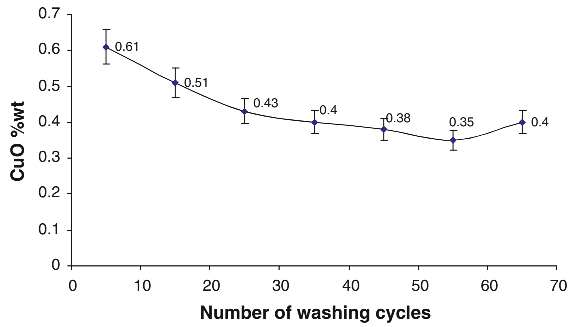

Fig. 2 Effect of number of washing cycles on the CuO content on the fabrics

Morphology of the coated textiles after washings

To substantiate the ICP measurements, and demonstrate that the CuO NPs are retained on the cotton fabric after washing SEM measurements were carried out. The SEM images of cotton after 65 cycles illustrated in Fig. 3, indicate that the fibers are coated with NPs. The inset in Fig. 3 shows the coated fabric under higher magnification. A homogeneous monodispersed coating is observed with particles ranging in size from 20 to 50 nm. The particle size of the CuO NPs after the deposition was observed to be 60–80 nm, a few larger aggregates were also observed. It appears that the washing cycles and consequent dissolution of Cu has led to the reduction of the particle size and the elimination of aggregates.

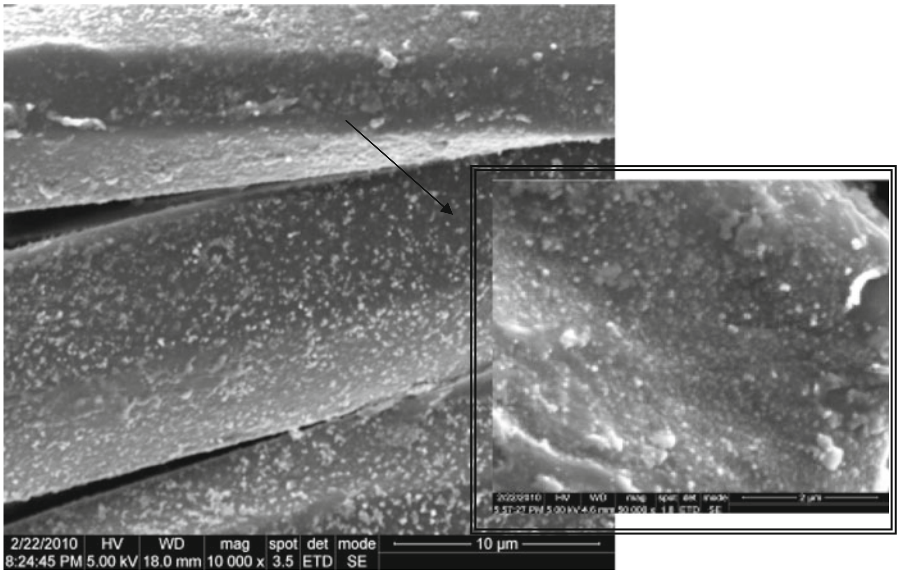

Fig. 3 SEM of CuO coated cotton following 65 wash cycles. The inset image was taken in a higher magnification (MAGX50 K)

We have already shown that the particle size has a major effect on the antibacterial properties (Applerot et al. 2009), and smaller NPs kill the bacteria more efficiently. It is therefore not surprising that the results obtained after washing were as good despite the loss of copper as measured by ICP.

One of the most efficient techniques available for the coating substrates in terms of stability is sonochemistry. This is due to the chemistry associated with acoustic cavitation collapse near to surfaces. This generates powerful shock waves and microjets that cause effective stirring and mixing of the liquid layer next to the surface (Suslick 1989).In our case, the ultrasonic waves promote the fast migration of the newly-formed metal oxide nanoparticles to the fabric surface. The coating is not based on the creation of chemical bonds between the surface and the metal oxides but rather due to the physical impregnation of the NPs. This physical phenomenon is a result of the conditions developed by ultrasound irradiation.

Antibacterial results

In Fig. 4 the antibacterial activity of the CuO coated cotton against 5 different bacterial species is shown. An excellent level of antibacterial activity was observed against each of the test bacterial species. In each case a greater than five log reduction in bacterial numbers on CuO coated cotton compared to plain cotton control samples was observed. In tests of ZnO coated cotton, antibacterial activity levels were less than one for *P. aeruginosa* and *A. baumannii*, between 3 and 4 for *S. aureus* and MRSA and greater than 5 *for E. coli*.

The antibacterial efficacy levels of the CuO coated cotton against *S. aureus* following 0, 5 and 65 washing cycles is shown in Fig. 5. The levels of activity observed were very high in all cases with a greater than 4 log reduction in bacterial numbers on CuO coated cotton compared to uncoated plain cotton. Although there was some decrease in antibacterial activity after 65 wash cycles the level was still well above the minimum acceptable antibacterial activity level of 2.

The antibacterial activity levels of the fabrics against the Gram-negative bacterium *E. coli*, as indicated in Fig. 6, were not as good as against *S. aureus* (which is a Gram-positive bacterium). The antibacterial activity decreased after each set of 20 wash cycles by nearly 60 %. After 25 washes the fabrics had a good level of antibacterial activity, but this decreased to a poor value (0.5) after 65 wash cycles. The two species of bacteria differ in that *E. coli* has a second outer lipid membrane. Once the antibacterial activity of the fabrics has been reduced by washing, this extra barrier layer may be enough to protect *E. coli* from the membrane damaging effects of the nanoparticles (Borkow and Gabbay 2005). The mechanism of the antimicrobial activity of metal

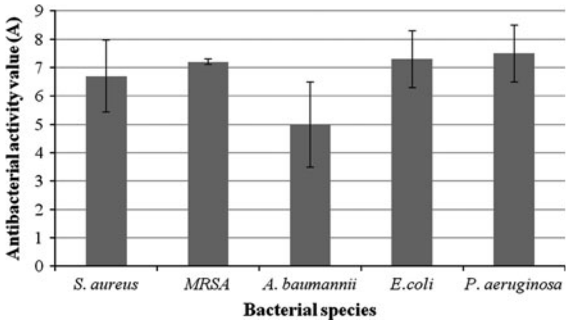

Fig. 4 Antibacterial activity values of CuO coated fabrics against bacterial species commonly associated with hospital aqcuired infections. The *error bars* show SD, $n = 3$

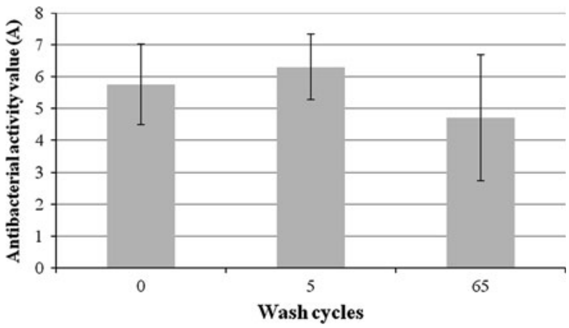

Fig. 5 Antibacterial activity values of washed CuO coated fabrics against *Staphylococcus aureus* (ATCC 6538). Testing was carried out according to the absorption method from BS EN ISO 20743:2007. The *error bars* show SD, $n = 3$

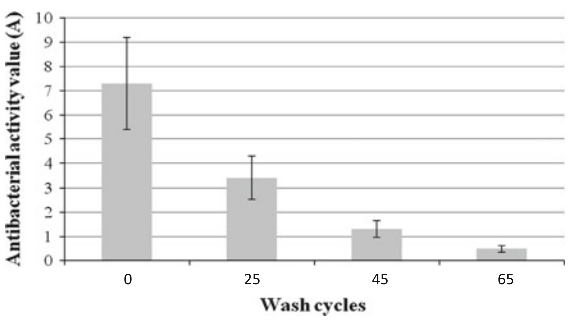

Fig. 6 Antibacterial activity values of washed CuO coated fabrics against *Escherichia coli* (ATCC 8739). The *error bars* show percentage error, $n = 3$

oxides is based not only on the amount of the coated material but also on the particles' size and the production of reactive oxygen species (ROS) that are generated from the metal oxides nanoparticles (BS EN 20743:2007). The amount of coating after 25 and 65 cycles is found to be almost the same, and sufficient for the inhibition of *S. aureus* after 65 washing cycles, but not of *E. coli*. The explanation for the reduced killing of *E.coli*, despite the presence of the same amount of NPs on the fabric, might be because the production of ROS species after repeating washings is decreased and not enough ROS are formed for the inhibition of bacterium having 2 lipid membrane structures.

The sonochemical coating process is an "in situ" process in which the NPs are formed and subsequently deposited on the surface. The strong adherence of the NPs to the fabric is thought to be due to a local melting of the fibers at the contact sites—essentially a sort of "welding". This melting is the result of the very high speeds at which the NPs are propelled under ultrasonic irradiation. The NPs are effectively embedded in the fabric and are resistant to removal by the washing cycles. The only loss appears to be due to the dissolution of Cu^{+2} ions which is very small even at 75 °C (see the above discussion).

Conclusions

The results presented here show that by application of a sonochemical coating technology it is possible to produce antibacterial fabrics that are durable enough to withstand 65 wash cycles under hospital washing conditions. This was demonstrated for CuO NPs deposited on cotton. Very good levels of antibacterial efficacy were observed for CuO coated cotton against a range of bacterial species. The antibacterial efficacy values post washing were found to vary with the species of bacteria used for testing. After washing the fabric proved to be much more effective against the Gram-positive bacterium *S. aureus* than the Gram-negative bacterium *E. coli*. The existence of CuO on the cotton, after the long washings cycles, was also probed by ICP and SEM studies. The durability of alternative metal oxide NPs on other fabric types is under investigation.

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