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Sonochemical Coating of Textiles with Hybrid ZnO/Chitosan Antimicrobial Nanoparticles

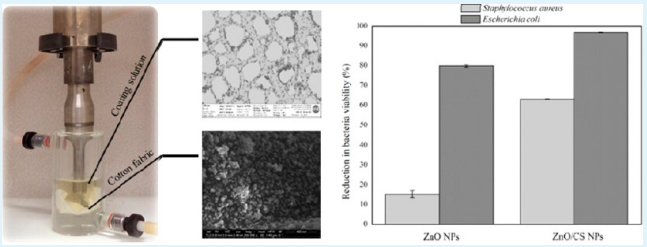
Petya Petkova,[†] Antonio Francesko,[†] Margarida M. Fernandes,[†] Ernest Mendoza,[‡] Ilana Perelshtein,[§] Aharon Gedanken,[§] and Tzanko Tzanov*[,†]

[†]Grup de Biotecnologia Molecular i Industrial, Department of Chemical Engineering, Universitat Politècnica de Catalunya, Rambla Sant Nebridi 22, 08222, Terrassa, Barcelona, Spain

[‡]Grup de Nanomaterials Aplicats, Centre de Recerca en Nanoenginyeria, Universitat Politècnica de Catalunya, c/Pascual I Vila 15, 08028 Barcelona, Barcelona, Spain

[§]Department of Chemistry, Kanbar Laboratory for Nanomaterials, Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat-Gan 52900, Israel

ABSTRACT: Textiles are good substrates for growth of microorganisms especially under moisture and temperature conditions found in hospitals. Microbial shedding from the body occurs continuously at contact of the patient with textile materials used in medical practices, contributing to the occurrence of hospital acquired infections. Thus, the use of efficient antimicrobial textiles is necessary to prevent the transfer of pathogens and the infection incidence. In this work, hybrid antimicrobial coatings were generated on cotton fabrics by means of a one-step simultaneous sonochemical deposition of ZnO nanoparticles (NPs) and chitosan. The process was further optimized in terms of reagents concentration and processing time in order to improve the antibacterial properties of the fabric and ensure their biocompatibility. The highest antibacterial activity of the fabrics against two medically relevant bacterial species was achieved in a 30 min sonochemical coating process using 2 mM ZnO NPs suspension. When chitosan was simultaneously deposited with the same amount of ZnO, the obtained hybrid NPs coating displayed higher by 48 and 17% antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, respectively. The presence of biopolymer also improved the durability of the antimicrobial effect of the coatings by 21% for *Staphylococcus aureus* and 40% for *Escherichia coli*, evaluated after applying multiple washing cycles at hospital laundering regimes. Finally, 87% biocompatibility improvement supported by fibroblast viability was observed for the hybrid ZnO/chitosan coating compared to the steady decrease of cells viability over one week in contact with the fabrics coated with ZnO alone.



KEYWORDS: *zinc oxide, chitosan, sonochemistry, hybrid nanoparticles, antibacterial coatings, medical textiles*

INTRODUCTION

Metal oxide nanoparticles (NPs) are largely claimed as efficient antibacterial agents suitable for biomedical applications.[1] However, studies to evaluate the biological toxicity of nanometals still continue, since hazard issues should be considered when using metal oxide antimicrobials as a therapy in humans.[2−4] Optimizing the metal concentrations to nonhazardous levels without affecting their functional properties would be a way to diminish the cytotoxic effect of these systems. This target is achievable by combining metal NPs with antimicrobial polymers that enhance their stability and efficacy. Such composites have already been used as drug delivery devices and antibacterial coatings.[5] In particular, complexing metal NPs with chitosan (CS), a biopolymer with intrinsic antimicrobial properties,[6] resulted in enhanced antibacterial performance,[7] homogeneous NPs distribution on solid surfaces, and improved cytotoxicity profiles due to the low metal dissolution rates from such complexes.[7,8] Thus, a hybrid material comprising metal/CS NPs is expected to exhibit low toxicity coupled to high antimicrobial efficiency even in low particles concentration.

The development of stable hybrid NPs coatings on textiles, however, almost exclusively involves the use of precursors, chemical pretreatment, or additional processing steps, such as thermal curing, which renders the production technology rather complicated, time-consuming, and cost-inefficient.[9,10] For example, in a multistep process, CS was covalently attached to previously oxidized cellulose, followed by the incorporation of ZnO microparticles into the CS layer by 'equilibration-cum-hydrothermal' approach.[11] Another time-consuming process was employed to impart both antimicrobial and UV protection properties to cotton by padding with ZnO/CS NPs suspension followed by squeezing, high-energy drying, and curing.[12] In an attempt to overcome the aforementioned manufacturing

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hurdles, namely the need of pre- or post-treatments and consequently long processing times, the sonochemistry appeared as an efficient, simple, and fast technique for coating of different solid surfaces with NPs.[13,14] The application of high intensity ultrasound (US) prevents the NPs aggregation, whereas the obtained coatings are extremely stable and uniformly deposited on the substrates.[15] In our previous work, cotton/polyester antimicrobial textiles were obtained in a one-hour sonochemical process to simultaneously synthesize and immobilize ZnO/CS composite NPs from water/ethanol solution at basic pH.[16]

A one-step sonochemical technique is again employed here for coating of textiles with hybrid ZnO/CS NPs. This study, however, goes beyond the already reported results, improving the processing of the antimicrobial textiles by (i) shortening the time for sonochemical coating, (ii) optimizing the ratio between the individual coating components toward decreasing the metal concentration without deteriorating the antibacterial properties, and (iii) assessing their cytotoxicity toward human cells. Such development is in the scope of a general strategy to decrease the occurrence of nosocomial infections in clinical settings through sustainable manufacturing of efficient antimicrobial hospital textiles. The sonochemical process is carried out in aqueous solutions, in line with the current focus on development of environmentally friendly manufacturing technologies. The durability of the antimicrobial effect is also evaluated after multiple high-temperature washing cycles used in hospitals against two medically relevant bacterial species – the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli*.

EXPERIMENTAL SECTION

Materials, Reagents, and Bacteria. Bleached woven 100% cotton fabric (144 g/m^2, warp/weft density 25/21 threads/cm) was provided by Davo, Romania. Low molecular weight chitosan (15 kDa, 87% DDA) supplied by Kitozyme (Belgium) was dissolved prior to use in 1% acetic acid. Water dispersion of ZnO NPs (size <100 nm) was purchased from Sigma-Aldrich (Spain). Gram-positive *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and Gram-negative *Escherichia coli* (*E. coli*, ATCC 25922) were used in the antimicrobial assays. Plate count agar and all other reagents for cell culture studies were purchased from Sigma-Aldrich unless otherwise specified.

Ultrasound-Assisted Coating of Cotton. The sonochemical coating process was carried out using an ultrasonic transducer (Ti-horn, 20 kHz, 750 W, Sonics and Materials VC750, USA). The power (21.5 W) and intensity (0.43 W·cm^{-3}) were determined calorimetrically by measuring the time-dependent temperature increase in the ultrasonic vessel. The coating of the cotton samples (0.70 ± 0.05 g, approximately 5 × 10 cm) was performed during 30 min at 20 ± 2 °C and 35% amplitude, in a glass jacked vessel containing 50 mL of 0.2, 2, or 20 mM ZnO NPs aqueous solution and 0.3% (w/v) chitosan. After sonication, the samples were thoroughly washed with distilled water to remove the loosely fixed particles and chitosan. Control samples were prepared by deposition of ZnO NPs alone.

Quantification of Deposited ZnO. The amount of ZnO embedded on the fabrics was determined after extraction with 0.5 M nitric acid. The concentration of solubilized Zn^{2+} ions was probed by inductive coupled plasma (ICP) analysis using ULTIMA JY2501 (France).

Characterization of the Coatings and Nanoparticles. The surface morphology of the coatings and size of the deposited NPs were studied with environmental scanning electron microscope (ESEM) model Quanta 200 FEG from FEI (USA). Additionally, the presence of zinc and chitosan on the coated fabric was detected by energy dispersive X-ray spectroscopy (EDS). ZnO/CS mass and molar ratios were determined by X-ray photoelectron spectroscopy (XPS) on a SPECS system equipped with an Al anode XR50 source operating at 150 mW and a Phoibos 150 MCD-9 detector (Germany). The morphology of the ZnO NPs in the remaining after the sonochemical process suspensions was investigated by a Ziess Neon FIb microscope (Carl Zeiss, Germany) in scanning transmission electron microscopy (STEM) mode operating at 30 kV acceleration voltage. The samples for observation were drop-casted on a TEM holey carbon grid. Additionally, STEM images were taken to evaluate the leaching of the NPs from the fabrics. Prior to STEM analysis the coated fabrics were incubated for 1 h in 0.3 mM KH_2PO_4 solution at 37 °C and 230 rpm.

Antibacterial Tests. Antimicrobial activity of the coated samples was assessed according to the standard shake flask method (ASTM-E2149-01) recommended for permanently immobilized active agents on fabrics. The method provides quantitative data for measuring the reduction rate in a number of colonies formed, converted to the average colony forming units per milliliter of buffer solution in the flask (CFU·mL^{-1}). For preparation of *E. coli* and *S. aureus* suspensions, a single colony from the corresponding stock bacterial cultures was used. The culture was then inoculated overnight in 20 mL sterile nutrient broth (NB, Sharlab, Spain) in a 100 mL Erlenmeyer flask and incubated at 37 °C and 110 rpm. The inoculated bacterial culture was diluted with sterile buffer (0.3 mM KH_2PO_4) until solution absorbance of 0.28 ± 0.01 at 475 nm was reached, which corresponds to 1.5 ÷ 3.0 × 10^8 CFU·mL^{-1}. Thereafter, the cotton fabrics (0.35 g) were incubated with 5 mL of bacterial suspension at 37 °C and 230 rpm. For determination of the inoculum cell density the suspensions were withdrawn before introducing the textile sample and after 15, 30, and 60 min in contact with the fabrics. These suspensions were serially diluted in sterile buffer solution, plated on a plate count agar, and further incubated at 37 °C for 24 h to determine the number of surviving bacteria. Antimicrobial activity is reported in terms of percentage of bacteria reduction calculated as the ratio between the number of surviving bacteria before and after the contact with the coated textiles using the following formula:

$$\text{Bacteria reduction (\%)} = ((A - B)/A) \times 100$$

where A and B are the average number of bacteria before and after the contact with the coated textiles, respectively.

The durability of the antibacterial effect was evaluated after performing 10 washing cycles in a laboratory dyeing machine (Ahiba Nuance, Datacolor) at 75 °C, 30 rpm, for 15 min with 0.1 g·L^{-1} nonionic surfactant Cotemol NI (Colorcenter, Spain), in liquor to good ratio 30:1.

Cell Culture. The BJ-5ta cells (human foreskin fibroblasts, ATCC-CRL-4001) were maintained in 4 parts Dulbecco's Modified Eagle's Medium (DMEM, ATCC) containing 4 mM of L-glutamine (ATCC), 4500 mg·L^{-1} glucose, 1500 mg·L^{-1} sodium bicarbonate and 1 mM sodium pyruvate, and 1 part of Medium 199 supplemented with 10% (v/v) of fetal bovine serum (FBS) and 10 g·mL^{-1} Hygromycin B, at 37 °C in a humidified atmosphere with 5% CO_2. The culture medium was replaced every 2 days. At preconfluence, cells were harvested using trypsin-EDTA (ATCC-30-2101, 0.25% (w/v) trypsin/0.53 mM EDTA solution in Hank's BSS without calcium or magnesium) and seeded at a density of 4.5 × 104 cells/well in a 96-well tissue culture-treated polystyrene plate (Nunc, Spain).

Cytotoxicity Evaluation by Indirect Contact. Coated fabric samples (25 mg) were first sterilized under UV light for 1 h. The samples were then placed in contact with 3 mL of complete growth medium (DMEM) in a CO_2 incubator at 37 °C for 1 and 7 days. At the end of these periods, the samples were removed and the growth media withdrawn. Medium without the contact with the cotton was used as a negative control, whereas a 30% dimethyl sulfoxide (DMSO) prepared in fresh culture medium was used as a toxicity positive control.

The previously seeded cells were put into contact with the withdrawn culture media and incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h. The cells were further examined for signs of toxicity using an Alamar Blue assay kit (AlamarBlue, Invitrogen). Resazurin, the active blue ingredient of the kit, is a nontoxic, cell permeable compound that, once in a viable cell, is

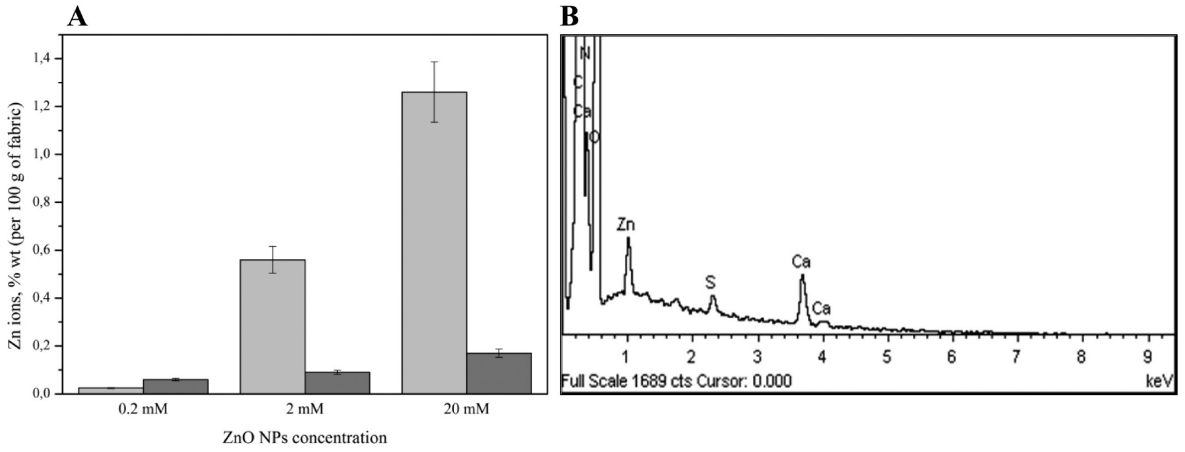

Figure 1. (A) Amount of zinc sonochemically deposited on cotton fabrics from solutions of ZnO NPs, in the absence (light gray bars) and presence (dark gray bars) of 0.3% (w/v) CS, and (B) EDS spectrum of cotton coated with 2 mM ZnO in the presence of CS.

reduced to red colored resorufin. AlamarBlue was diluted in culture medium and added to each well after aspirating the culture medium containing the samples. After 4 h incubation at 37 °C the absorbance at 570 nm was measured, using 600 nm as a reference wavelength, in a microplate reader Infinite M200, Tecan (Austria). The quantity of resorufin formed is directly proportional to the number of viable cells. The error bars for each data are the standard deviation of three independent measurements. No cell viability was detected after the contact with the positive control regardless of the incubation time (data not reported).

RESULTS AND DISCUSSION

The properties of hybrid polymer−metal nanocomposites are influenced by the components interactions which affect the composite shape, size distribution, and stability. Unlike other natural compounds, chitosan strongly complexes with metal ions due to its free amino groups.[17,18] Two models are proposed for hybrid chitosan and metal ions structures: "pendant model" where an ion is bound to only one amino group of CS[19] and "bridge model" where an ion is bound to several nitrogen atoms and hydroxyl groups of one or bridging more CS chains.[20] This ability of chitosan was explored in our previous study to sonochemically synthesize ZnO/CS composite NPs and simultaneously deposit them on cotton fabrics. Here, the study is extended to the optimization of the sonochemical process in terms of metal oxide concentration and reaction time in order to obtain efficient and durable antimicrobial textiles that do not cause toxic effects to human cells.

Characterization of the Coated Fabrics. ICP measurements were performed for determination of zinc on the cotton treated with different ZnO NPs concentrations in the presence and absence of CS. As expected, increased ZnO NPs concentration in the coating process led to a higher amount of zinc deposited on the fabric (Figure 1A). On the contrary, considerably less amount of zinc was detected on the fabrics treated in the presence of CS. Moreover, the EDS analysis of the hybrid coatings detected the presence of N and Zn on the fabric, thereby confirming the deposition of both chitosan and ZnO (Figure 1B).

XPS analysis was conducted to determine the atomic concentrations of Zn and N and to calculate the molar and mass ratios of ZnO/CS on the fiber surface (Table 1). As expected the ZnO/CS molar and mass ratios were highest for the sample treated with 20 mM ZnO NPs while the ratios for

Table 1. N and Z Atomic Concentrations from XPS Analysis[a]

hybrid coating	N atomic concn, %	Zn atomic concn, %	mass ratio ZnO/CS	molar ratio ZnO/CS
0.2 mM ZnO/CS	82.945	17.063	0.090	16.711
2 mM ZnO/CS	83.724	16.281	0.086	15.798
20 mM ZnO/CS	70.263	29.741	0.187	34.388

[a]Mass and molar ratios of ZnO/CS in the hybrid coatings deposited on the fibers obtained by XPS analysis.

the samples obtained with 0.2 and 2 mM ZnO NPs were found to be quite similar. The energy spectrum of the photoelectrons, produced by the X-rays photoelectronic effect, allows the determination of the sample composition (up to 10 nm sampling depth). The position of the peaks (binding energies of electron orbitals) in the spectrum and their relative areas are used to quantitatively identify the composition of the sample surface (Figure 2A). Figure 2B shows high energy resolution carbon C 1s spectrum obtained for the hybrid coating. Three peaks are assigned to the different chemical bonds of carbon atoms, namely C−O (286.21 eV), C═O/O−C−O (287.29 eV), and C−H/C−C (284.80 eV). The N 1s spectrum peaks (Figure 2C) with binding energies of 399.35 and 401.90 eV were assigned to the chitosan amino and protonated amino groups, respectively. Zn 2p 3/2 spectrum is presented with the peak with binding energy at 1021.97 eV (Figure 2D).[16]

The ESEM surface analysis of the sample coated only with ZnO showed a dense layer of NPs on the fibers (Figure 3A, B). A comparatively lower amount of NPs with bigger average size was found on the fabric treated with the ZnO/CS system (Figure 3C, D), in good agreement with the ICP data. It is hard to distinguish between the individual and hybrid ZnO/CS particles, because chitosan itself is able to form nanospheres under sonochemical irradiation that are similar in shape to the ZnO NPs.[16] Indeed, the pure CS coating (Figure 3E, F) appeared morphologically quite similar to the hybrid coating. Nevertheless, the NPs within the hybrid coating were bigger in size than the pure ZnO or CS particles, indicating that these probably were comprised of both components. It was reported that the mean diameter of CS nanoparticles increased when metal ions were loaded to the polymer.[21] On the other hand,

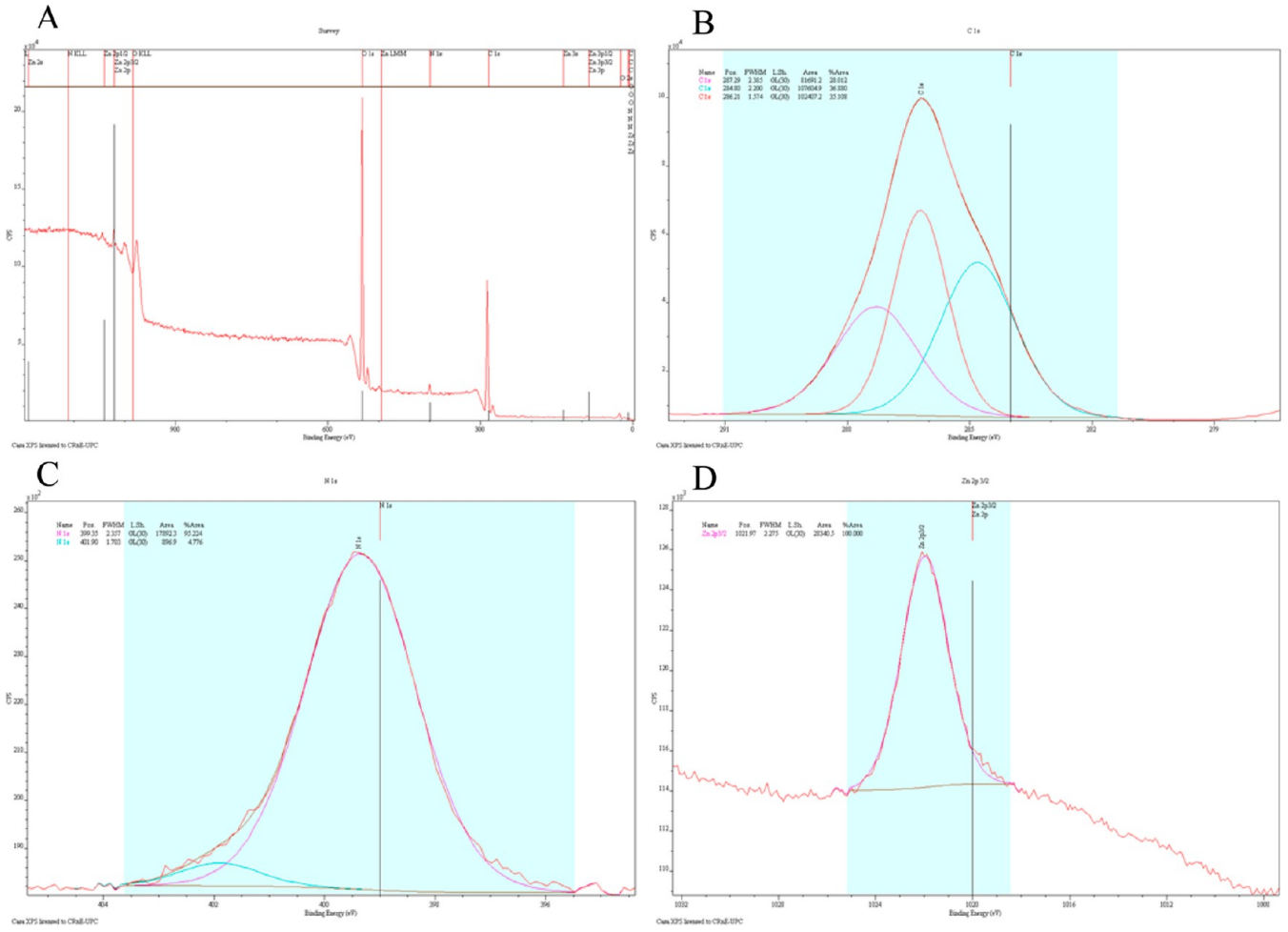

Figure 2. XPS spectra of fabric cotton sonicated in the presence of 2 mM ZnO NPs and CS.

the effect of CS on the metal NPs is resumed in its action as a controller of the nucleation or stabilizer.[22,23]

To explain the differences in the amounts of ZnO deposited on the fibers, the remaining after the sonochemical process solutions were evaluated for the presence of ZnO NPs using STEM. Given the lower amount of Zn detected on the fabrics treated in the presence of chitosan, it was expected that a higher amount of metal oxide particles would be found in the corresponding remaining solution compared to the one remaining after coating in the absence of chitosan. Surprisingly, the ZnO NPs density was also lower in the solution left after the hybrid coating (Figure 4A). The lower amount of NPs both on the fabric and in the remaining solution could be explained only if the effect of pH on the solubility of both ZnO and CS is considered. ZnO dissolution occurs over a wide pH range,[24] whereas the oxide is stable at pH 7.2 due to a minimal interference of the dissolution products. On the other hand, due to intermolecular and intramolecular hydrogen bonding, chitosan can be dissolved only in aqueous solutions of organic or mineral acids below pH 7. At these conditions the stability of ZnO rapidly decreases due to reaction with acidic substances. It is thus expected that the addition of CS dissolved in CH_3COOH to the ZnO aqueous suspension will decrease the pH of the system and affect the ZnO stability. After CS addition the pH of the ZnO NPs suspension dropped from 8.2 to 6.9. Zn^{2+} complexation could also lower the pH similarly to already reported metal ion systems containing CS.[25−27] The complex reaction could be described according to the Lewis acid−base theory, where the acid (Zn^{2+}) is an acceptor of a pair of electrons, provided by the base (CS).[25] Thus, the physicochemical properties of the individual composite components most probably dictate the formation of the Zn^{2+}/CS complex within the hybrid coatings. On the other hand, no NPs leaching was detected from the coated fabrics, revealing a high stability of both ZnO and the hybrid coatings (Figure 4B).

Antibacterial Activity. Both ZnO and CS are largely reported as efficient antibacterial agents. The most widely accepted mechanism of ZnO antibacterial action involves the oxide dissolution to Zn^{2+} further associated with oxidative stress in bacteria cells and generation of reactive oxygen species (ROS).[28,29] ROS cause inhibition of cell enzymes, lysosomal and mitochondrial damage, and consequently cell death.[30] The antimicrobial effect of CS depends on its molecular weight and varies among different microorganisms.[31,32] Among the proposed mechanisms of CS antimicrobial action are as follows: (i) binding to the cell DNA to inhibit the protein synthesis and (ii) interaction with negatively charged microbial membranes to alter the cell permeability.[33,34] Recently, hybrid complexes of CS and metal ions showed several-fold enhancement of their antibacterial activity as compared to the individual components.[25,35] In addition, the antibacterial activity was directly proportional to the amount of metal ions in these complexes.

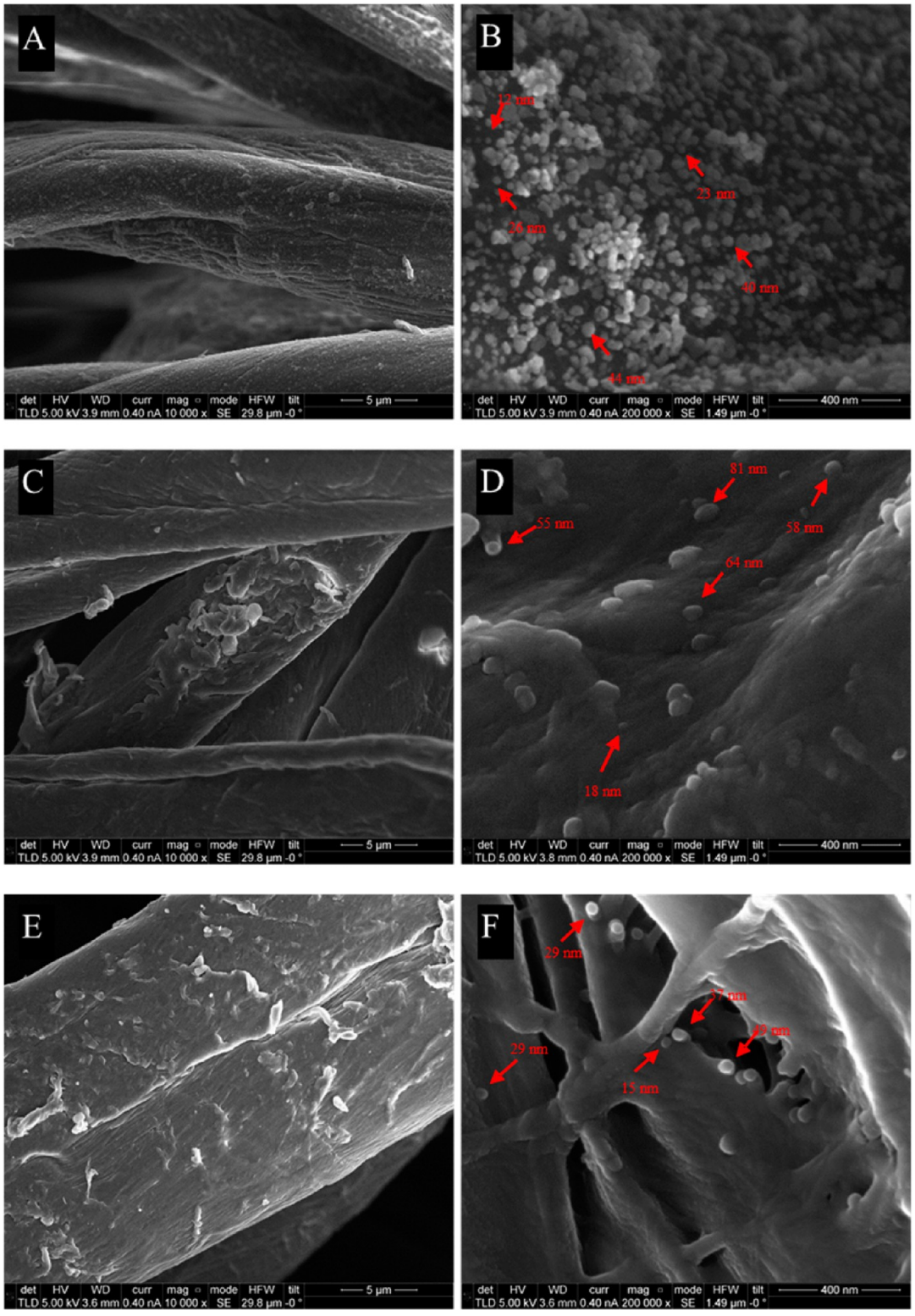

Figure 3. SEM micrographs of cotton fabrics coated with ZnO (A, B), ZnO/CS (C, D), and CS (E, F). Composition of the coating solution: 2 mM ZnO NPs and 0.3% (w/v) CS. The images on the left were taken with 10 000×, whereas the right-side images were taken with 200 000× magnification.

In the next step, the antibacterial efficiency of the sonochemically generated on cotton fabrics hybrid Zn^{2+}/CS was evaluated against two medically relevant bacterial species and further compared to the effect of pure ZnO coatings. Although to a different extent, the Zn^{2+}/CS coatings reduced the viabilities of both *S. aureus* and *E. coli* (Figure 5). As expected, the presence of chitosan enhanced the antibacterial effect of the coatings against both strains regardless of zinc concentration in the complex. However, the fabrics coated with the lowest NPs concentration (0.2 mM) in the presence of CS brought about only 30% reduction of bacteria viabilities. Increasing the amount of zinc in the coatings resulted in

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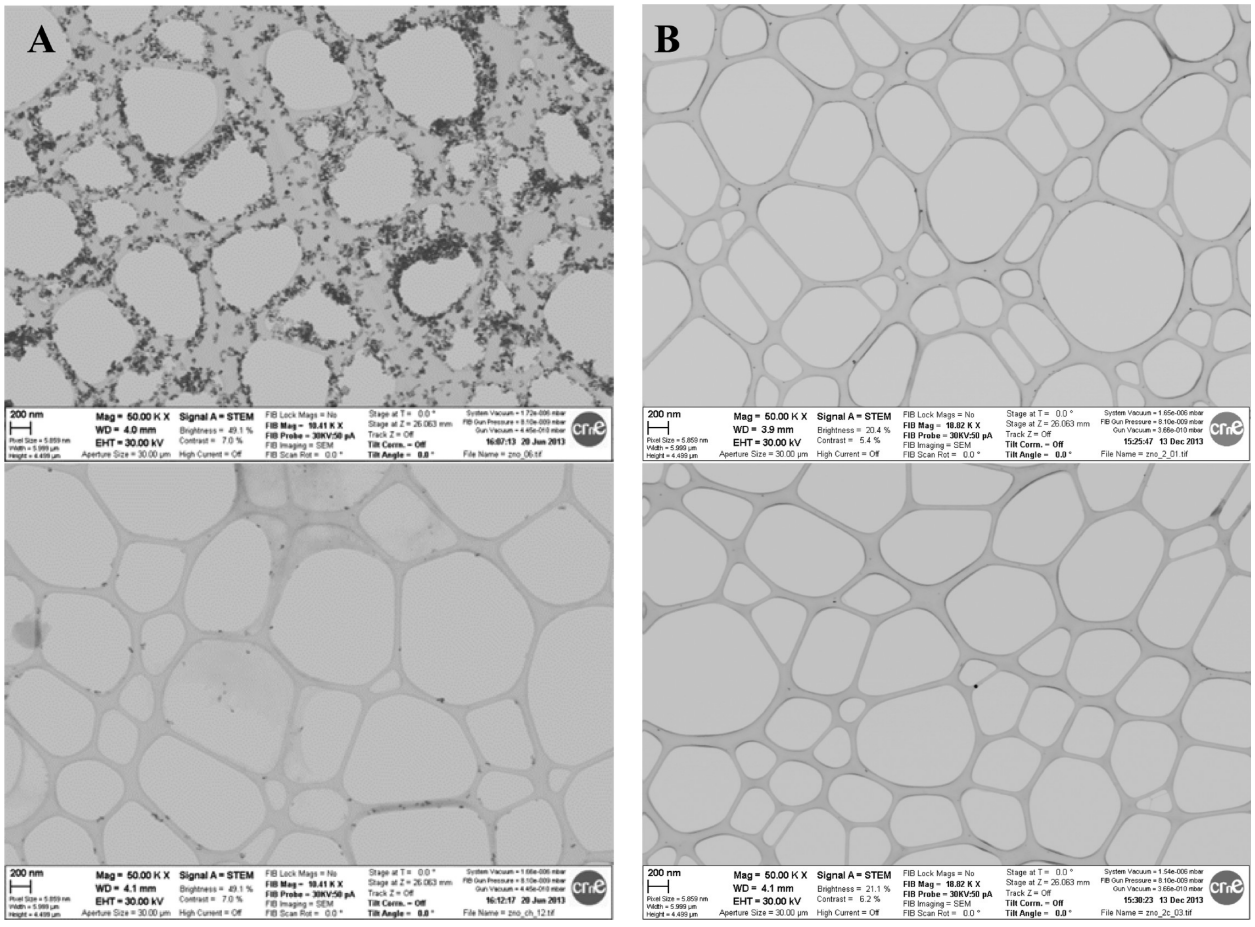

Figure 4. STEM images taken to evaluate: (A) the NPs presence in the remaining solutions after the sonochemical coating of cotton fabrics with 2 mM ZnO in the absence (top image) and presence (bottom image) of 0.3% (w/v) CS; and (B) the NPs leaching into a solution incubated with the fabrics coated with ZnO (top image) and ZnO/CS (bottom image).

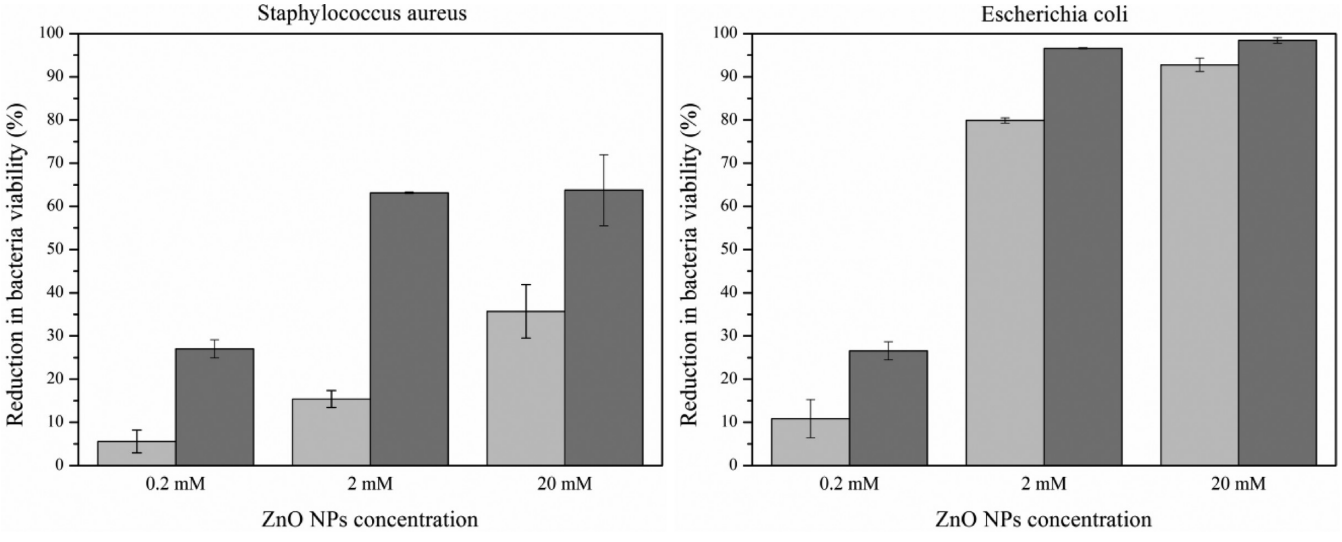

Figure 5. Antibacterial activity of ZnO (light gray bars) and Zn^{2+}/CS (dark gray bars) coated cotton fabrics toward *S. aureus* and *E. coli* after 15 min of contact.

enhanced antibacterial effect. The coatings with 2 and 20 mM ZnO showed comparable antibacterial efficiency for both bacteria. Thus, all further experiments were carried out with the fabrics treated with 2 mM NPs, as this concentration was considered appropriate to produce efficient antimicrobial textiles.

In Table 2 the antibacterial activity of fabrics coated with 0.3% (w/v) CS, 2 mM ZnO, and the corresponding hybrid is shown as a function of the incubation time. The hybrid coating

Table 2. Antibacterial Activity of the Coated Fabrics against *S. aureus* and *E. coli* after Different Incubation Time

	Staphylococcus aureus reduction in viability, %		
coating	15 min	30 min	60 min
CS	26.84 ± 4.07	33.00 ± 1.94	30.99 ± 1.81
2 mM ZnO NPs	15.39 ± 1.93	41.50 ± 2.12	60.77 ± 0.16
2 mM ZnO NPs/CS	63.15 ± 0.23	81.62 ± 3.58	98.48 ± 0.22
	Escherichia coli reduction in viability, %		
coating	15 min	30 min	60 min
CS	25.18 ± 1.02	72.66 ± 1.02	78.06 ± 0.51
2 mM ZnO NPs	79.88 ± 0.60	89.81 ± 1.31	99.86 ± 0.02
2 mM ZnO NPs/CS	96.60 ± 0.12	96.72 ± 0.18	99.88 ± 0.04

led to 98% reduction of bacteria growth for *S. aureus* after 60 min, whereas ZnO and CS alone reduced the bacteria growth by 61 and 31%, respectively. It is worthy to mention that the CS coating reached its maximum activity against *S. aureus* already after 30 min, while the longer incubation time resulted in improved antimicrobial effect for the coatings containing ZnO. In the case of *E. coli* a progressive improvement in antibacterial effect was observed for both CS and pure ZnO coatings. The different efficiency of the CS-containing coatings against *E. coli* (78% after 60 min incubation) in comparison to *S. aureus* (31%) could be due to the low M_w of the biopolymer (15 kDa) used in the study. Low M_w chitosans are reported to be more active against Gram-negative than against Gram-positive bacteria.[32] On the other hand, the hybrid coating reduced the bacterial viability by more than 96% even after 15 min of incubation. Nearly 100% reduction was observed for both fabrics treated with ZnO and the hybrid coating after 60 min incubation.

Durability of Antimicrobial Effect. Despite the advances in antimicrobial finishing, the hospital textiles still become contaminated at use.[36] Frequent laundering is therefore necessary in order to prevent the transfer of pathogens. In order to evaluate the durability of the antimicrobial coatings, the fabrics were subjected to 10 washing cycles at 75 °C, and their antibacterial performance was assessed afterward against the selected bacterial strains. The results are expressed in percentage of remaining biocide activity compared to non-washed fabrics (Figure 6A). The ZnO coating retained about 50% of its initial activity against both strains, whereas the hybrid coating preserved about 70% and 85% of the efficacy against *S. aureus* and *E. coli*, respectively (illustrated at Figure 6B). It is the presence of CS that improved the washing stability of the hybrid coating, as the individual CS coating maintained between 85 and 90% of its initial activity. The sonochemical deposition of polymers onto solid surfaces usually results in their stable embedding on the substrate.[37] Under ultrasound irradiation of liquids, the microjets and shock waves produced after cavitation collapse are able to drive the biopolymers at such high velocities toward a solid surface that fusion and strong adherence by physical or chemical interactions with the fabric occurs during collision.[38] Conversely, sonochemically deposited metal oxides are not particularly stable, and additional pretreatment of the solid substrate is required to improve the coating stability.[39]

Cell Viability. Although zinc and copper oxides are safer alternatives to the more toxic silver, the studies continue to highlight their biological toxicity using soil and aquatic organisms,[40] and mammalian cell lines.[41,42] The toxic action of metal and metal oxide NPs is mainly a consequence of the release of cytotoxic metal ions from such systems.[28,43] Since ZnO NPs partially dissolve in water, their dissolution in aqueous systems is expected to involve both ionic and particulate species. Solubilized Zn^{2+} proved to contribute substantially to the cytotoxicity of ZnO NPs.[4,40] Another assumption claims that ZnO produces toxic species associated with its photocatalytic property. ROS generated under environmental UV radiation increase significantly the toxicity of these systems to human cells.[28] Some studies even report the generation of ROS in the absence of photochemical energy.[44,45] It is therefore crucial to evaluate the potential toxicity to humans of the systems comprising ZnO.

After optimizing the ZnO concentration in the antimicrobials-coated textiles, the potential cytotoxicity of these systems

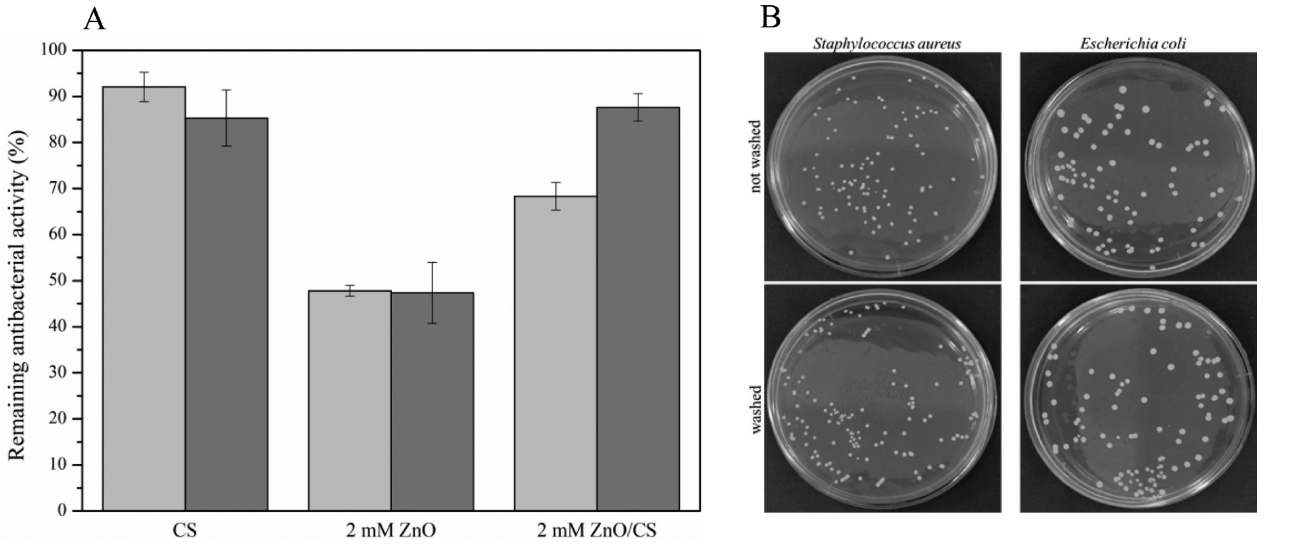

Figure 6. (A) Percentage of remaining antibacterial activity of the coatings after 10 washing cycles at 75 °C against *S. aureus* (light gray bars) and *E. coli* (dark gray bars), after 15 min of contact. (B) Antibacterial activities of the Zn^{2+}/CS fabrics before and after washing.

was evaluated for medical application requiring contact with human skin (Figure 7). An indirect contact method was used to

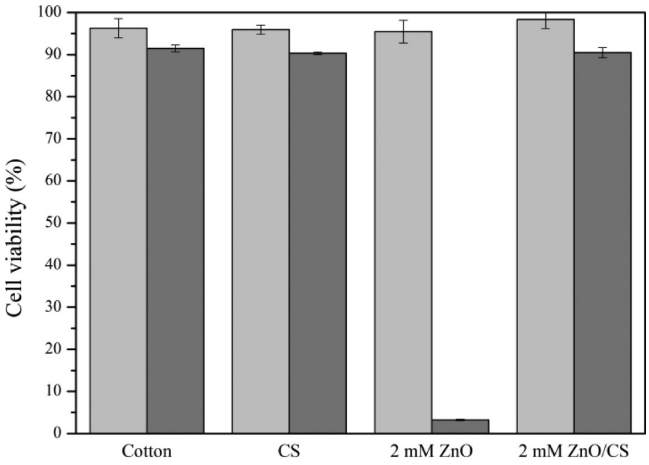

Figure 7. Human skin fibroblasts viability after 24 h (light gray bars) and 1 week (dark gray bars) contact with the coated cotton fabrics.

determine the potential toxicity that CS, ZnO, or hybrid nanocoatings might induce to fibroblasts cell culture. During the first 24 h the cultured cells were metabolically active with no difference in cell viability observed among the experimental groups. However, after one week of contact with the ZnO coated fabric cell viability dropped to less than 5%. In contrast, the CS and the hybrid coating did not induce considerable cell toxicity even after one week. As in the case of bacteria killing, ZnO NPs induce oxidative stress in human cells through the generation of free radicals and ROS.[46] The reason for the lower cytotoxicity value in the case of the hybrid coating could be the lower amount of ZnO impregnated on the fabric compared to the fabric treated with ZnO alone (Figure 1A). It could be also hypothesized that the CS inhibits the generation of ROS below the threshold of oxidative stress due to its antioxidant capacity.[47]

CONCLUSIONS

Hybrid antimicrobial coatings were generated on cotton fabrics via a one-step sonochemical deposition of ZnO NPs in the presence of chitosan. The process was optimized in terms of ZnO concentration and ultrasound irradiation time toward obtaining highly efficient and noncytotoxic antibacterial textiles for use in hospitals. The synergy between ZnO NPs and chitosan resulted in enhanced antibacterial efficiency against *S. aureus* and *E. coli* even at low ZnO concentration, compared to the individual ZnO and chitosan coatings. The antimicrobial effect of the treated fabrics was resistant to multiple washing cycles at 75 °C according to the laundry regimes used in hospitals. Moreover, the presence of chitosan substantially improved the biocompatibility of the ZnO coatings avoiding the risk of adverse effects on human health. The sonochemically generated antimicrobial textiles showed potential, in terms of easiness of the process coupled to enhanced antimicrobial effect and high washing stability, for uses in a hospital environment to prevent the spread of nosocomial infection.

AUTHOR INFORMATION

Corresponding Author

*Phone: +34 93 739 85 70. Fax +34 93 739 82 25. E-mail: tzanko.tzanov@upc.edu. Corresponding author address: Universitat Politècnica de Catalunya, Grup de Biotecnologia Molecular i Industrial, Edifici Gaia, TR14, Rambla Sant Nebridi, 22, 08222 Terrassa, Barcelona, Spain.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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