

Abstract

# Synthesis and Antibacterial Efficiency of Chitosan–Copper Oxide Nanocomposites <sup>†</sup>

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Antimicrobial resistance is among the most serious global healthcare problems today. In Europe, a third of the estimated 8.9 million yearly hospital-acquired infections in 2016 and 2017 were caused by antibiotic-resistant bacteria [1]. One possible source for novel, efficient antimicrobials is nanotechnology. Some metallic nanoparticles (NPs) like silver, CuO, and ZnO are already commercially available for biomedical applications. NPs are often coated with different polymers to improve their characteristics. An intriguing biopolymer to combine with metal NPs is the antimicrobial, biocompatible, and immunomodulating chitosan, presently used in bandages and wound dressings. In this study, novel chitosan–CuO nanocomposites (NCs) were synthesized via the precipitation of copper acetate by sodium hydroxide in the presence of chitosan (50–190 kDa, Sigma Aldrich, Schnellendorf, Germany). Varying copper to chitosan weight ratios (1:0.3, 1:1, and 1:3) were used. The NCs were characterized by DLS, EDX, FTIR, SEM, XPS, and XRD. The minimum bactericidal concentrations (MBC) against clinically relevant gram-negative (*Escherichia coli* ATCC 25922, *E. coli* MG1655, *Pseudomonas aeruginosa* ATCC 27853) and gram-positive (*Staphylococcus aureus* ATCC 6538) bacteria were determined by the Spot test [2]. Flow cytometry and confocal laser scanning microscopy were used to reflect the interactions between NCs and bacteria. The zeta potential of the synthesized NCs was >40 mV. The NCs' hydrodynamic diameter and polydispersity index increased with higher chitosan content, varying within 90–180 nm and 0.24–0.33, respectively. Based on the XRD analysis, the CuO portion of the NCs had a crystalline structure. The NCs were similarly effective against gram-negative and -positive bacteria, displaying MBC values of 0.13–0.25 mg Cu/L after 24 h of exposure. Interestingly, after a 1-h period of exposure, the NCs were more toxic against gram-negative bacteria than Cu ions, suggesting that chitosan may facilitate the interaction of NCs and bacterial cells, enabling the immediate shedding of Cu ions alongside the bacteria. Furthermore, after a 1-h exposure, the NCs with a higher chitosan content were up to two times more biocidal against gram-negative bacteria than NCs with a lower chitosan concentration.



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