COMPOSITE OF GRAPHENE OXIDE WITH ZINC OXIDE NANOPARTICLES AS AN ANTIMICROBIAL AGENT AGAINST PATHOGENIC MICROORGANISMS

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Abstract

The synthesis process of composites based on graphene oxide is described and tested as a carrier of zinc oxide nanoparticles and subsequently tested as an antimicrobial agent for some bacterial strains (*Staphylococcus aureus* (*S. aureus*), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E. coli*). Scanning electron microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX), and Fourier transform infrared spectroscopy (FT-IR) confirmed the formation of this composite. The composite was tested by colony-forming capability test. Graphene oxide as well as zinc oxide exhibit antibacterial properties, but the composite consisting of graphene oxide and zinc oxide shows stronger antimicrobial effect against pathogenic microorganisms. Stronger antimicrobial effect was found in *S.aureus* and MRSA as a G+ bacteria and *E.coli* as a G- one, due to different composition of cell wall.

Keywords: Graphene oxide, antibacterial, antimicrobial, *Escherichia coli*, *Staphylococcus aureus*, MRSA

1. INTRODUCTION

General increase in the number of resistant microorganisms is a significant global problem, although specific patterns vary considerably across countries [1]. The resistant strains of bacteria are rather present in hospital facilities, where genetic mutations in bacteria occur due to the long-term administration of antibiotics. This phenomenon leads to the expansion of various defensive mechanisms able to inactivate the applied chemical substance [2]. Organic compounds used for disinfection aren't suitable due to toxicity to the human body [3]. Nanoparticles (NPs) especially have demonstrated broad-spectrum antibacterial properties against both Gram+ and Gram− bacteria [4]. Nano-sized ZnO can interact with bacterial surface and/or with the bacterial core, by entering the cell, and subsequently exhibiting bactericidal mechanisms [5]. The high antibacterial efficiency of graphene oxide (GO) is caused by damage of cell membranes *via* generation of reactive oxygen species and exceptionally sharp edges of graphene oxide [6-8]. The advantages of graphene oxide, enabling it to kill or inhibit bacteria, in comparison to other antibacterial materials can be summarized in these three specific characteristics: (i) the antibacterial mechanism of graphene oxide is influenced by both, physical destruction and chemical oxidation, which decrease bacterial resistance, (ii) graphene oxide has mild cytotoxicity to mammalian cells in low dose, and (iii) in comparison to other carbon nanomaterials, its large scale production potential, easy processing, and low cost of production, guarantee graphene oxide as a good antibacterial agent [6, 9, 10]. GO is continuously demonstrating its excellent membrane characteristics and offer huge potential for practice applications. One of the important applications of graphene based membranes is bacterial inactivation and removal [11]. The modifications of graphene with antibacterial ZnO nanoparticles could render this phenomenon more effective.

2. MATERIAL AND METHODS

2.1. Preparation of graphene oxide

GO was prepared by chemical oxidation of 5.0 g graphite flakes (Sigma-Aldrich, and 100 mesh, ≥75% min) in a mixture of concentrated H_2SO_4 (670 mL, Sigma-Aldrich) and 30.0 g $KMnO_4$ (Sigma-Aldrich) according to the

simplified Hummer's method [12, 13]. The reaction mixture was stirred vigorously. After 4 days, the oxidation of graphite was terminated by addition of H_2O_2 solution (250 mL, 30 wt% in H_2O, Sigma-Aldrich). Formed graphene oxide was washed 3 times with 1 M HCl (37 wt% in H_2O, Sigma-Aldrich) and several times with Milli-Q water (total volume used 10 L) until constant pH value (3-4) was achieved.

2.2. Preparation of composite of grapheme oxide with zinc oxide nanoparticles

1 mL of 1% polyoxyethylene (40) stearate (PES) was mixed with 600 µL GO and zinc oxide nanoparticles (Sigma-Aldrich). The obtained solution was rotated for 24 hours.

2.3. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) spectra were collected using a Nicolet iS10 FT-IR spectrometer with a diamond ATR attachment (Thermo Electron Inc., San Jose, USA). IR spectra were recorded from 4000 to 650 cm^{-1} at a resolution of 2 cm^{-1}. Each spectrum was acquired by adding together 128 interferograms. Spectra were taken at 22 °C. The OMNIC™ software was used for IR spectra recording, and the JDXview v0.2 software (Norbert Haider, Department of Drug Synthesis, University of Vienna, Austria) was used for further spectra evaluation.

2.4. Scanning Electron Microscopy

The structures of the GO with ZnO-NPs composites were characterized by scanning electron microscopy (SEM). For documentation of the nanoparticles structure, a MIRA3 LMU (Tescan, Brno, Czech Republic) was used. This model is equipped with a high brightness Schottky field emitter for low noise imaging at fast scanning rates. The SEM was fitted with In-Beam SE detector. For automated acquisition of selected areas a TESCAN proprietary software tool called Image Snapper (Tescan, Brno, Czech Republic) was used. The software enabled automatic acquisition of selected areas with defined resolution. An accelerating voltage of 15 kV gave satisfactory results regarding maximum throughput.

2.5. Cultivation of bacteria strains

Staphylococcus aureus (NCTC 8511), *Escherichia coli* (NCTC 13216) and MRSA (ST239) were obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University (Brno, Czech Republic). Cultivation media (Mueller Hinton, Oxoid) were inoculated with bacterial culture and were cultivated for 24 h on a shaker at 120 rpm[14] and 37 °C.

2.6. Colony-Forming Capability Test

Bacterial cultures (*E.coli*, *S.aureus*, MRSA) were diluted with MH medium to an absorbance of 0.1 measured using a spectrophotometer Ultrospec 10 (Biochrom, Cambridge, United Kingdom) at a wavelength of 600 nm. After that cultures were diluted by decimal dilution (to 10^{-7} cells per millilitre) and incubated at 37 °C for 2 h. The pH value of composite was adjusted to 7. After being exposed to different concentrations of composites of GO with ZnO-NPs at 37 °C for 2 h. 100 µL of the cell suspension was spread onto MH agar plates. The number of the colonies was counted after agar plates were incubated at 37 °C in the dark overnight. The survival percentage was used to evaluate the antimicrobial effect of composites of GO with ZnO-NPs and it was defined as the following formula:

$$\text{Survival\%} = \frac{\text{Colony numbers of treated bacteria}}{\text{Colony numbers of control bacteria}} \times 100\%$$

3. RESULTS AND DISCUSSION

3.1. Characterization of composite

The SEM micrographs (**Figure 1**) confirmed the preservation of the original structure of the large area, which remained maintained in comparison with starting material. This method also enabled determining the degree of exfoliation, which is crucial for nanoparticle character. The SEM micrographs also allowed the rating of ZnO NPs adhesion to GO-based carrier.

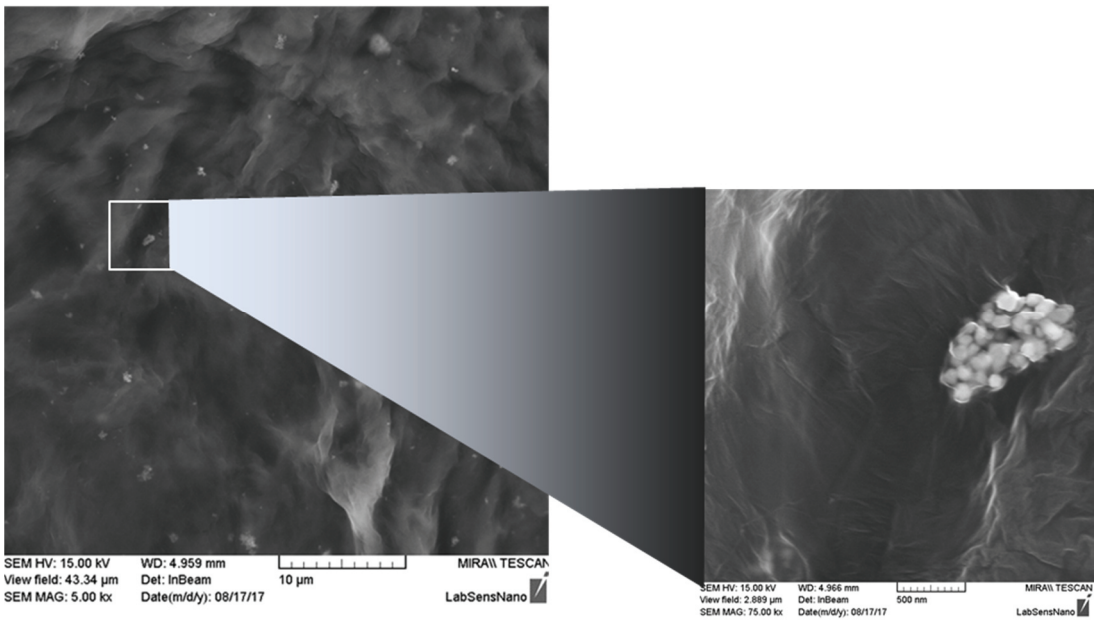

Figure 1 Characterization of composite of GO with ZnO nanoparticles by using SEM

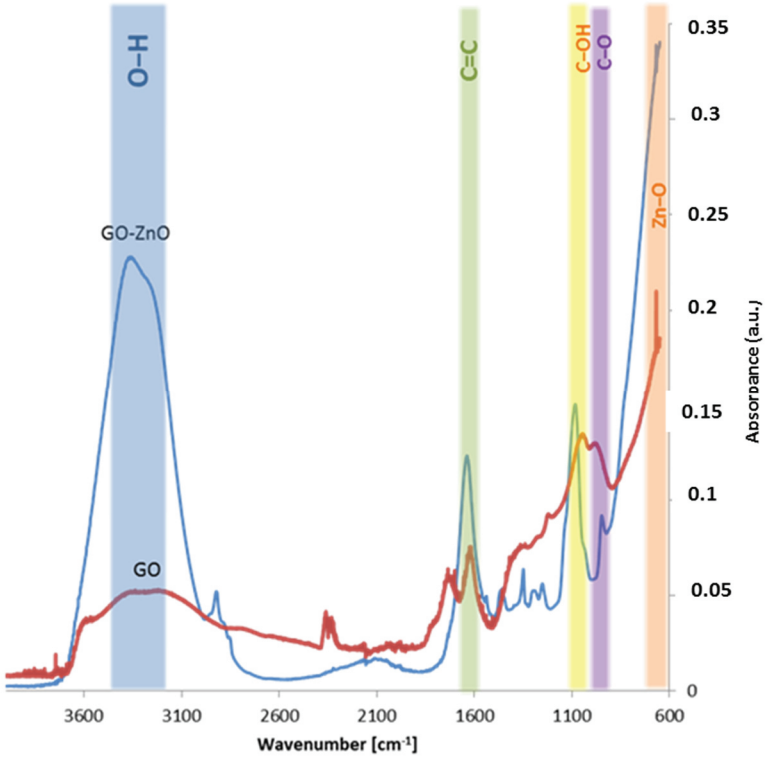

Figure 2 Characterization of composite of GO with ZnO nanoparticles by using FT-IR

The characterization of composite of GO with ZnO NPs was further supported with FT-IR spectroscopy (**Figure 2**). The peak at 3350 cm^{-1} was from the O-H groups due to water remaining in GO. The C=C groups peaks were observed at 1629 cm^{-1}. The peak at 1071 cm^{-1} was attributed to C-OH vibrations of the graphitic domains. The peak at 940 cm^{-1} are assigned to the stretching of C-O. It has been shown that the composite of GO with ZnO NPs shared identical peaks with graphene oxide, but a new broad peak was observed at 650 cm^{-1} and was attributed to Zn-O vibrations. These vibrations indicate that ZnO particles were anchored to the graphene oxide sheets [15]. Complete water removal is impossible because GO absorbs water from the air [16-18].

3.2. The composites influence on pathogenic microorganisms

Antibacterial activity of GO and GO with ZnO NPs was determined using colony-forming capability test and expressed in terms of the colonies after incubation. GO and composites of GO with ZnO NPs were tested based on their antimicrobial effects on *S. aureus*, MRSA and *E. coli* strains. Effect of GO and composites of GO with ZnO NPs on bacterial strains is shown in **Figure 3** Stronger antimicrobial effect was found at G+ bacteria. It could by caused by the fact that G− bacteria have an outer membrane situated above a thin layer of peptidoglycan. The cell walls of gram-negative bacteria follow a more general structural format than that of G+ bacteria, which is strictly adhered [19]. Each experiment was repeated three times. The student t-test was used to evaluate statistically significant differences ($p < 0.05$) between experimental group and control group. The null hypothesis that the frequency was not different between different samples was rejected at a value less than or equal to 0.05.

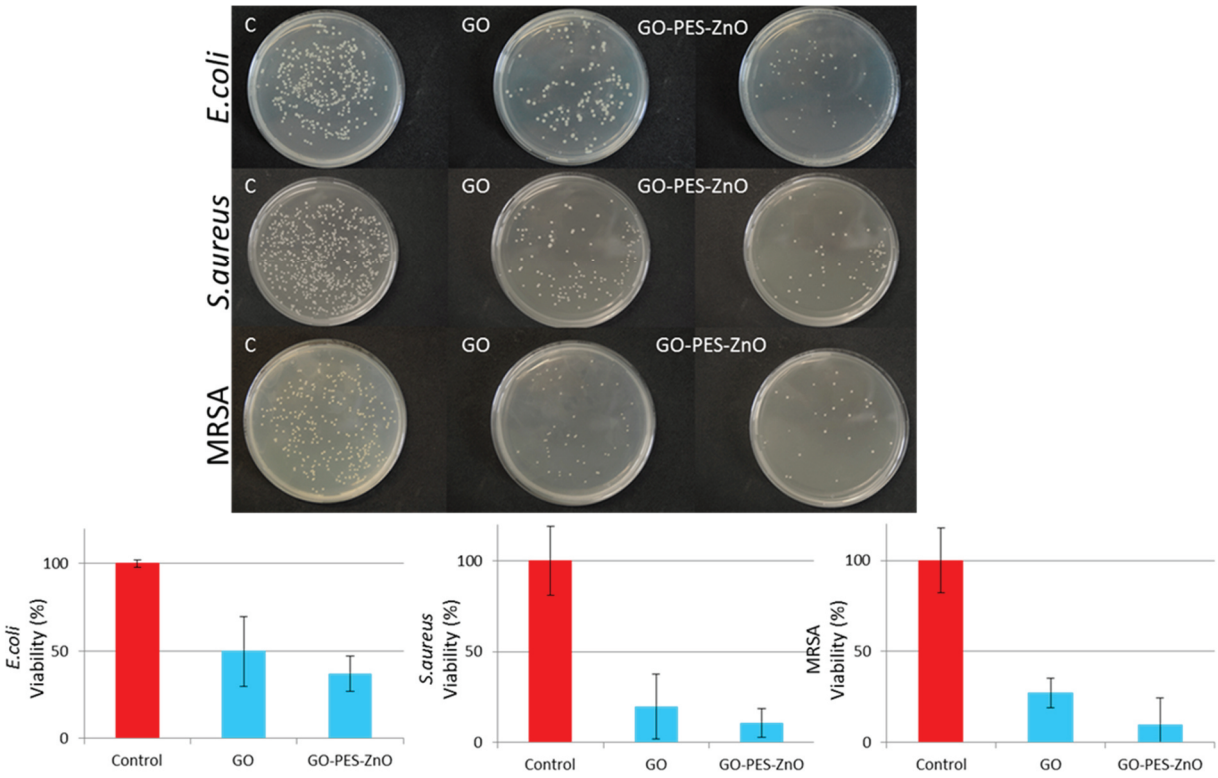

Figure 3 Colony-Forming Capability Test

4. CONCLUSION

In this study, the composites of GO with zinc oxide NPs were synthetized and characterized by different methods and their effect on bacterial strains was tested. The composite showed inhibitory effect on three selected bacterial strains (*S. aureus; E. coli*; MRSA) and the inhibitory effect were stronger than in case of GO

itself. It can be said that combination of GO with metal NPs seems like a promising way to fight drug-resistant bacteria.

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