International Biodeterioration & Biodegradation 123 (2017) 182–190

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Antimicrobial activity of graphene oxide-metal hybrids

K.A. Whitehead [a, *], M. Vaidya [a], C.M. Liauw [a], D.A.C. Brownson [b], P. Ramalingam [b, c], J. Kamieniak [b], S.J. Rowley-Neale [b], L.A. Tetlow [a], J.S.T. Wilson-Nieuwenhuis [a], D. Brown [a], A.J. McBain [d], J. Kulandaivel [c], C.E. Banks [a, b]

[a] School of Healthcare Science, Manchester Metropolitan University, Chester Street, Manchester, M1 5GD, UK
[b] Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, Chester Street, M1 5GD, UK
[c] Centre for Nanoscience and Nanotechnology, School of Physics, Bharathidasan University, Tamil Nadu, 620024, India
[d] Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, M13 9PT, UK

ARTICLE INFO

Article history:
Received 16 November 2016
Received in revised form
5 May 2017
Accepted 28 June 2017
Available online 10 July 2017

Keywords:
Antimicrobials
Graphene oxide
Biocide
ESKAPE
Nano/Micro particles
Pathogens

ABSTRACT

With resistant bacteria on the increase, there is a need for new combinations of antimicrobials/biocidal agents to help control the transmission of such microorganisms. Particulate forms of graphite, graphene oxide (GO) and metal-hybrid compounds (silver-graphene oxide (AgGO) and zinc oxide graphene oxide (ZnOGO)) were fabricated and characterised. X-Ray diffraction and Diffuse Reflectance Infrared Fourier Transform Spectroscopy demonstrated the composition of the compounds. Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy determined the compounds were heterogeneous and irregular in shape and size and that the level of silver in the AgGO sample was 57.9 wt% and the ZnOGO contained 72.65 wt % zinc. The compounds were tested for their antimicrobial activity against four prominent bacteria; *Escherichia coli, Staphylococcus aureus, Enterococcus faecium* and *Klebsiella pneumoniae*. AgGO was the most effective antimicrobial (Minimum inhibitory concentration *E. coli/Enterococcus faecium* 0.125 mg mL^{-1}; *S. aureus/K. pneumoniae* 0.25 mg mL^{-1}). The addition of Ag enhanced the activity of GO against the bacteria tested, including the generally recalcitrant *K. pneumoniae* and *Enterococcus faecium*. These findings demonstrated that GO-metal hybrids have the potential to be utilised as novel antimicrobials or biocides in liquid formulations, biomaterials or coatings for use in the treatment of wounds where medically relevant bacteria are becoming increasingly resistant.

1. Introduction

Concerns about bacterial resistance from community-acquired and food-borne pathogens has been growing for a number of years at both national and international levels. Several Gram-positive and Gram-negative bacteria including *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecium* and *Staphylococcus aureus* are currently considered as emergent global pathogens, which pose a huge global health problem (Boucher et al., 2009).

Metals have been used for decades to treat various infectious diseases, and their antimicrobial efficacies are now being re-evaluated owing to the emergence of resilient pathogens. A particular interest has emerged particularly in the use of these compounds for topical/therapeutic use as well as for disinfection to prevent the adhesion and transmission of bacterial species. Silver is one of the most widely investigated metals for antimicrobial applications, and is being used in a number of medical purposes including catheters, biomaterials and wound dressings. Zinc oxide (ZnO) is used in such applications as food packaging (Tayel et al., 2011), textiles (Velmurugan et al., 2016), as antimicrobials (Deokar et al., 2016), and in wound dressings (Chaturvedi et al., 2016). Nanoparticles are interesting in that they can be synthesized with a high surface area to volume ratio and with unusual morphologies that contain sharp edges and corners. Graphite and the graphene derivatives have traditionally been used in electrochemistry, from applications in energy technologies, such as batteries and fuel cells and they have also been used in an array of functional composites (Unwin et al., 2016). Work has recently suggested that the graphene family of compounds also possess antimicrobial properties (Liu et al., 2011; Wang et al., 2012). By

* Corresponding author.
E-mail address: k.a.whitehead@mmu.ac.uk (K.A. Whitehead).

http://dx.doi.org/10.1016/j.ibiod.2017.06.020
0964-8305/

combining the antimicrobial activity of metals together with the physical effect of GO on the bacterial cell walls, it may be hypothesised that the antimicrobial activity of graphene products may be increased.

A number of disinfectants and antiseptics have been reported to be showing signs of becoming less effective so there is a need for the development of novel microbicides due to the current limitations (Russel and Chopra, 1990; Jennings et al., 2015). Transmission and infection problems due to bacterial adhesion to surfaces can be mitigated in part by the development of alternative antimicrobial sources/biocides. The aim of this work was to determine if metal-GO hybrid compounds demonstrated increased antimicrobial efficacy compared to graphite and GO, against a range of bacteria. The development of such alternative antimicrobial actives may prove beneficial for use in such formulations such as biocidal, disinfecting or topical antimicrobials or cleaning agents or for incorporation into biomaterial coatings.

2. Materials and methods

2.1. Synthesis of compounds and characterisation

For the synthesis of the compounds, all chemicals (analytical grade or higher) were used as received from Sigma-Aldrich (UK) without any further purification and all solutions were prepared with deionised water of resistivity not less than 18.2 MΩ cm. Synthetic graphite powder was commercially obtained from Gwent Group (Pontypool, UK).

Graphene oxide (GO) was synthesized by the Hummers method *via* the oxidation of synthetic graphite (Hummers Jr. and Offeman, 1958). Graphite flakes (5 g) and $NaNO_3$ (2.5 g) were combined in 115 mL of H_2SO_4 (conc.) and stirred for 30 min. Whilst kept in an ice bath (<5 °C), $KMnO_4$ (15.0 g) was gradually added to the suspension and the rate of addition was controlled to keep the reaction temperature below 15 °C. The mixture was heated to 35 °C for a 30 min period and underwent continuous stirring producing a brown paste. A further dilution was made by adding 250 mL of water to the mixture and the temperature was increased to 70 °C for 15 min. The resultant mixture was diluted by adding H_2O until a final volume of 1 L was obtained. Finally, the solution was treated with 15 mL of H_2O_2 (30% w/w) to terminate the reaction, at which stage the solution became yellow in appearance. For purification, the mixture was filtrated and the obtained solid was washed thoroughly with Milli Q water several times in order to avoid sulphate contamination. After purification, the powder was dried at 60 °C during 48 h.

In the preparation of the AgGO, a sonochemical reduction method was utilised (Anandan and Muthukumaran, 2015). Following preparation of the GO, 0.5 g was added to 150 mL of ethylene glycol and sonicated for 30 min. In a separate vesicle, 1.0 g of silver nitrate was added to 20 mL of ethylene glycol and sonicated for 30 min. The silver nitrate dispersion was added drop-wise to the GO solution whilst undergoing sonication for 30 min to produce a homogeneous mixture. Finally, 50 mL of 0.1 M $NaBH_4$ was added to the resultant AgGO mixture and a further 30 min of sonication was performed. The product was purified with repeated steps of H_2O and ethanol washing, after which the solution was dried at 50 °C.

The ZnOGO was fabricated by dissolving 5.0 g GO in 200 mL of N, N,-dimethylformamide (DMF), along with 20 mL of 1 M zinc acetate dihydrate (pH of 6.5). The homogeneous solution was heated to 60 °C and was stirred continuously for 120 min, after which the solution was heated to 250 °C. Following solvent evaporation, partial ZnO/ZnOHGO was produced. The resulting dried product was collected and ground in an agate mortar prior to being annealed at 450 °C for 120 min within atmospheric conditions to obtain the final ZnOGO product (Liu et al., 2012).

2.1.1. Preparation of compounds for testing

For the analysis of the fabricated compounds, 20 mg of each test compound was added to 20 mL of sterile distilled water. The samples were vortexed for 10 s and immediately 10 μL of prepared sample was pipetted onto a 10 mm × 10 mm polished silicon wafer (Montco Silicon Technologies, USA) and air dried for 30 min. The samples were stored at room temperature, in desiccators until use.

2.1.2. X-Ray Diffraction (XRD)

In order to identify the crystal phase of the compounds, X-Ray Diffraction (XRD) was performed using a PANalytical X'pert powder diffraction platform. Nickel filtered copper K_α radiation (l = 1.54 Å) was used, with an anode voltage of 40 kV an anode current of 30 mA. A reflection transmission spinner stage (15 rpm) was implemented to hold the powder samples. The XRD parameters were step size: 0.13; sample: powder; slit (antiscatter) size: 1/4°. The 2q range was set between 10° and 100°, in correspondence with literature ranges associated with the characterised samples (Li et al., 2007; Zhou et al., 2007; Kumar et al., 2013; Chowdhuri et al., 2015; Liu et al., 2016). Additionally, to ensure well-defined peaks, an exposure of 50 s per 2q step was implemented.

2.1.3. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFTS) was carried out using a Spectra-Tech DRIFTS cell fitted in a Thermo − Nicolet Nexus FTIR spectrophotometer. The instrument was thoroughly purged (30 L/min) with CO_2 and water-free air, produced using a Balaston purge gas generator. All samples were diluted to ca. 5 % wt. in finely ground KBr (Sigma, UK). The samples were used as received, with no further grinding. The sample was folded into the pre-ground KBr using a micro-spatula. The micro-sampling cup was over-filled slightly and the cup dropped from a height of 1 cm onto the bench in order to shake off the excess mixture whilst at the same time, produce a slightly domed and naturally randomised, surface of KBr diluted sample. The same batch of ground KBr was used as the background. The background and sample spectra were made up of 164 scans with resolution set to 4 cm^{-1}. As the sample was diluted with KBr there were no specular reflection components so a blocker was not used. Spectra were plotted in absorbance (Liauw, 2003).

2.1.4. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX)

In order to determine the shape, size and atomic elemental weight of the compounds, the samples were fixed to stubs using carbon tabs (Agar, UK). Scanning Electron Microscopy (Carl Zeiss Ltd.) was carried out using a Supra 40VP SEM with SmartSEM software. Energy Dispersive X-Ray (EDAX Inc.) was carried out using an Apollo 40 SDD system with Genesis software.

2.2. Microbiology and antibacterial testing

2.2.1. Stock cultures of bacteria

In preparation for the antimicrobial assays, stock cultures of *S. aureus* NCTC 4137, *K. pneumoniae* NCTC 9633 or *E. coli* NCTC 10418 were inoculated onto nutrient agar (NA) or nutrient broth (NB) and incubated at 37 °C for 24 h. Stock cultures of *Enterococcus faecium* NCTC 7171 were cultured onto Columbia blood agar with horse blood in a 5%, Brain heart infusion agar (BHIA) (Oxoid, UK) or brain heart infusion broth (BIHB) and incubated in 5% CO_2 for 24 h at 37 °C. All medias were obtained from Oxoid (UK).