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Effect of reduced graphene oxide-hybridized ZnO thin films on the photoinactivation of *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi

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ABSTRACT

The immobilization of photocatalyst nanoparticles on a solid substrate is an important aspect for improved post-treatment separation and photocatalyst reactor design. In this study, we report the simple preparation of reduced graphene oxide (rGO)-hybridized zinc oxide (ZnO) thin films using a one-step electrochemical deposition, and investigated the effect of rGO-hybridization on the photoinactivation efficiency of ZnO thin films towards *Staphylococcus aureus* (*S. aureus*) and *Salmonella enterica* serovar Typhi (*S.* Typhi) as target bacterial pathogens. Field-emission scanning electron microscopy (FESEM) revealed the formation of geometric, hexagonal flakes of ZnO on the ITO glass substrate, as well as the incorporation of rGO with ZnO in the rGO/ZnO thin film. Raman spectroscopy indicated the successful incorporation of rGO with ZnO during the electrodeposition process. Photoluminescence (PL) spectroscopy indicates that rGO hybridization with ZnO increases the amount of oxygen vacancies, evidenced by the shift of visible PL peak at 650 to 500 nm. The photoinactivation experiments showed that the thin films were able to reduce the bacterial cell density of *Staph. aureus* and *S.* Typhi from an initial concentration of approximately 10^8 to 10^3 CFU/mL within 15 min. The rGO/ZnO thin film increased the photoinactivation rate for *S. aureus* ($\log[N/N_o]$) from −5.1 (ZnO) to −5.9. In contrast, the application of rGO/ZnO thin film towards the photoinactivation of *S.* Typhi did not improve its photoinactivation rate, compared to the ZnO thin film. We may summarise that (1) rGO/ZnO was effective to accelerate the photoinactivation of *S. aureus* but showed no difference to improve the photoinactivation of *S.* Typhi, in comparison to the performance of ZnO thin films, and (2) the photoinactivation in the presence of ZnO and rGO/ZnO was by ROS damage to the extracellular wall.

1. Introduction

The presence of pathogenic bacteria in water poses a health risk to many communities around the world. Without access to clean water free of pathogenic bacteria, communities are susceptible to diseases such as gastroenteritis [1,2]. Climate change [3–7], industrial [8,9] and domestic [10,11] pollution threaten the continuous supply of clean water required to support a growing global population.

Solar purification methods such as the solar disinfection (SODIS) method were developed as an alternative treatment option for removing pathogenic bacteria in water [12,13]. The SODIS method is a cost-effective treatment with low carbon footprint, and does not pose a secondary contamination threat through the use and production of harmful chemical compounds [14], compared to conventional methods. Yet, the effectiveness of the SODIS method is limited by fluctuating weather conditions [15]. To overcome this issue, researchers turn their attention to disinfection processes which combine the use of photons and engineered nanostructures to accelerate the inactivation process.

Zinc oxide, ZnO is a photoactive semiconductor with high electron mobility, due to its wide bandgap and d^{10} electronic structure [16]. ZnO nanoparticles are easy to fabricate from zinc salt solutions using low-temperature methods such as hydrothermal [17], chemical bath deposition [18] and electrochemical deposition [19], yet with a high degree of control over its particle size and morphology. ZnO is effective as a photocatalyst due to its ability to catalyse the formation of reactive oxygen species (ROS). ROS refer to a group of chemical intermediates formed as a result of water photolysis e.g. hydrogen peroxide, superoxide and hydroxyl radicals. ZnO has demonstrated success in antibacterial applications [20,21].

The photocatalytic activity of ZnO has been improved through the incorporation of metal dopants e.g. Au [22,23] and Cu [24] in order to tune its band gap. To reduce the cost of improving photocatalytic ability, rGO has also been considered as a cost-effective co-catalyst to improve the photocatalytic ability of ZnO. Reduced graphene oxide (rGO) is a

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form of carbon which possesses a planar sp^2 hybridized structure. Similar in structure to graphene i.e. a single sheet of graphite, reduced graphene oxide is commonly obtained by subjecting graphite through a chemical exfoliation process [25], followed by a reduction step, either through thermal or chemical methods [26]. As a result of the chemical processes involved, residual oxygen functional groups remain present in or on the reduced graphene oxide surface [27]. Previous studies have shown that the combination of rGO and ZnO (rGO/ZnO hybrids) have resulted in improved performance for photovoltaic applications, and is attributed to the role of rGO as electron acceptor, in which photogenerated electrons are transferred between ZnO to rGO such that this reduces the recombination of photogenerated electron-hole pairs, which is a major issue that affects semiconductor solar conversion performance. Applications of rGO/ZnO hybrids include UV detector [28], anti-microbial packaging [29], synthesis catalyst [30], solar cells [31] and supercapacitor [32,33].

For environmental applications, rGO/ZnO hybrids have been extensively studied for photocatalytic degradation of inorganic pollutants [34–39] but less so for pathogenic bacteria [40], such as *S. aureus* and *S.* Typhi. Notably, most of the reports demonstrating the photocatalytic ability of rGO/ZnO hybrids were conducted using powdered photocatalysts, which raises practical concerns of post-treatment separation. Therefore, cost-effective methods to prepare immobilized photocatalysts that can be easily recycled, such as thin film photocatalysts, would benefit the design and development of efficient photocatalytic reactors [41].

In this paper, we report the simple preparation of rGO/ZnO hybrid thin films using a one-step electrochemical deposition method for the enhanced photocatalytic activity on a waterborne bacterial pathogen such as *Salmonella enterica* serovar Typhi (*S.* Typhi) and a common nosocomial pathogen, *Staphylococcus aureus* (*S. aureus*). The effect of rGO-hybridization to promote photoinactivation efficiency was investigated by applying the thin films in the photoinactivation of *S. aureus* and *S.* Typhi under simulated sunlight conditions. *S. aureus* and *S.* Typhi were selected as model bacterial strains as both cause diseases of public health concern. *S. aureus* is a Gram-positive, non-motile and spherical-shaped strain of bacteria which may cause disease such as food poisoning, cellulitis and toxic shock syndrome, due to direct infection or the production of toxins. *S.* Typhi is a Gram-negative bacteria strain, rod-shaped and possesses flagella which aid in cell motility. The virulence of *S.* Typhi causes disease syndrome such as typhoid. Many studies utilise *Escherichia coli* as microbial hygiene indicator according to international testing standards [42]. As different types of bacteria exhibit unique responses towards photoinactivation treatments, it becomes necessary to make a comparison on the effect of rGO-hybridization in promoting photoinactivation of pathogenic bacteria.

2. Experimental Method

2.1. Chemicals and Reagents

Zinc(II) nitrate hexahydrate was purchased from R & M chemicals whereas potassium chloride (KCl), tryptic soy agar and tryptic soy nutrient were purchased from Merck. The electrolyte solution for electrochemical deposition was prepared with deionised water. Graphene oxide (GO) was prepared using a modified Hummer's method.

S. aureus ATCC 25923, *S.* Typhi strain CR0063 were used as test microorganisms, representing Gram positive and Gram negative, respectively. Phosphate buffer solution was used in the photoinactivation experiments and serial dilution. Tryptone soy agar and tryptone soy broth were used in the growth and cultivation of bacteria.

2.2. Electrochemical Deposition of ZnO and rGO/ZnO Thin Films

ZnO and rGO-modified ZnO (rGO/ZnO) thin films were grown on a conductive, indium-doped tin oxide (ITO) glass substrate using a one-step electrochemical deposition method. A three-electrode electrochemical cell (Autolab PGSTAT302N, Metrohm) comprising (1) indium-doped tin oxide (ITO) conductive glass substrate, (2) platinum wire and (3) Ag/AgCl were used as working, counter and reference electrodes, respectively.

For the preparation of ZnO thin film, a fresh 50 mL solution containing 0.1 M Zinc(II) nitrate hexahydrate and 0.1 M KCl was used as the electrolyte. 0.2 g GO was dispersed into the above mentioned electrolyte solution for the preparation of the rGO/ZnO thin film, corresponding to a 4 mg/mL GO solution. The deposition of ZnO was performed at an applied potential of −1.2 V for 30 min. A water bath was used to maintain the electrolyte temperature at 60 °C throughout the deposition process. The *as*-prepared thin films were rinsed with deionised water and air-dried before annealed at 400 °C under argon gas flow for 3 h with heating rate of 1 °C min^{-1}. (See Fig. 1).

2.3. Materials Characterization of ZnO and rGO/ZnO Thin Films

The morphological features of the ZnO thin film were studied using field emission scanning electron microscopy (FE-SEM) (Zeiss SUPRA 35VP; Germany). Raman and photoluminescence spectroscopy was performed using Renishaw InVia Raman microscope (UK) between wavelength range of 100–3100 nm and with excitation source of 514 nm and 325 nm, respectively.

2.4. Evaluation of Thin Film Photoinactivation Efficiency

Prior to the photoinactivation experiment, *S. aureus* and *S.* Typhi were grown in Luria-Bertani broth to concentrations of 10^9 colony forming units per mL (CFU/mL), centrifuged, washed, and re-suspended in sterile phosphate buffer solution (PBS) at concentrations of approximately 10^6 CFU mL^{-1}.

Bacterial photoinactivation experiments were performed in polyethylene tubes containing 20 mL of the bacterial suspension and exposed to simulated sunlight (150 W xenon lamp). Treatment was performed without any thin film (light control experiment), and in the presence of ZnO and rGO/ZnO thin films. 1 mL aliquots were sampled at time intervals of 0, 1, 5, 10 and 15 min to determine the changes in bacterial cell density due to the photoinactivation treatment conditions (See Fig. 1) . The dark control experiment and ZnO treatment under dark conditions were performed by repeating the treatment in the absence of simulated sunlight, with and without using ZnO thin film, respectively.

The bacterial cell densities at each time interval were enumerated by standard plate count techniques. Briefly, aliquots were serially diluted by a series of ten-fold dilutions in PBS. The diluted solutions with appropriate dilution ratio were then plated on plate count agar plates to assay the colony forming ability using the easySpiral® Automatic Spiral® plater (Interscience, France). Plates were incubated at 37 °C for 24 h, and the colonies were counted using an automatic colony counter. All experiments were performed in triplicate, and the averages were obtained.

To determine the effects of ZnO and rGO/ZnO thin films on the cell surface of the bacteria cells as a result of each photoinactivation treatment, bacterial suspensions were collected at the end of the photoinactivation experiment (15 min) and prepared for FESEM imaging using the following method. Treated bacterial suspensions were centrifuged to remove excess PBS solution, followed by chemical fixation of the bacteria in 2% glutaraldehyde solution and washing in 5 mM Sodium cacodylate buffer at 4 °C. Next, the bacterial suspensions were post-fixed for overnight with 1% Osmium tetroxide at 4 °C and rinsed with distilled water twice for 15 min at room temperature. After fixation, the samples were dehydrated with a series of ethanol solution (20, 40, 60, 80, 100%) for 15 min each at room temperature, and dried with critical point dryer. After drying, the bacterial samples were