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Quantum dots conjugated zinc oxide nanosheets: Impeder of microbial growth and biofilm

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ABSTRACT

The grieving problem of the 21st century has been the antimicrobial resistance in pathogenic microorganisms to conventional antibiotics. Therefore, developments of novel antibacterial materials which effectively inhibit or kill such resistant microorganisms have become the need of the hour. In the present study, we communicate the synthesis of quantum dots conjugated zinc oxide nanostructures (ZnO/CdTe) as an impeder of microbial growth and biofilm. The as-synthesized nanostructures were characterized by X-ray diffraction, ultraviolet–visible spectroscopy, photoluminescence spectroscopy, field emission scanning electron microscopy and high resolution transmission electron microscopy. The growth impedance property of ZnO and ZnO/CdTe on Gram positive organism, *Bacillus subtilis* NCIM 2063 and Gram negative, *Escherichia coli* NCIM 2931 and biofilm impedance activity in *Pseudomonas aeruginosa* O1 was found to occur due to photocatalytical action on the cell biofilm surfaces. The impedance in microbial growth and biofilm formation was further supported by ruptured appearances of cells and dettrered biofilm under field emission scanning electron and confocal laser scanning microscope. The ZnO/CdTe nanostructures array synthesized by hydrothermal method has an advantage of low growth temperature, and opportunity to fabricate inexpensive material for nano-biotechnological applications.

1. Introduction

Many basic properties of materials (optical, electrical, mechanical, physical, chemical, magnetic, etc.) can be expressed as a function of their size, composition, and structural order. Therefore, recently many serious attempts have been tried to synthesize nanoscale materials with functional properties [1], particularly, to impede/eradicate microbial growth in solutions or surfaces [1–7]. The microbial growth in solutions or surfaces are difficult to impede/eradicate due to the ability of some microorganisms to form biofilm. Biofilms are conglomerations of bacterial cells and it remains with extrapolymeric substance (EPS), the latter protects their constituent cells from various antimicrobial therapies thereby acting as a reservoir of microbial infections [8]. In fact, in the medical sector, a recent study shows that up to 60% of all human infections are caused by biofilm. Not only medical sector, the biofilm also has affected industrial sector. In industry, biofilm has been shown to contaminate installations in food industries by colonization of the interior of the pipes and resistance of vessels by initiation of 'biofouling' on the vessel hulls. It has been estimated that the yearly economic loss caused by 'biofouling' in the marine industry were about $ 6.5 billion [9,10].

Although, various nanocomposites have been reported to impede/eradicate microbial growth in solution or surfaces, the semiconductor metal oxide nanoparticles outsmart over all existing nanocomposites due to excellent photon absorption property and efficient transport of photogenerated-charge carriers [11]. One of the most important semiconductor metal oxide nanoparticles is zinc oxide (ZnO) with wide band gap energy of 3.37 eV and a relatively large exciton binding energy (60 meV). ZnO nanomaterials have received increasing attention as an excellent candidate for photocatalytical impedance/eradication of microbial growth because of their stability under harsh processing conditions [12,13]. Interestingly, despite conflicting reports on the cytotoxicity of

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ZnO nanoparticles [14–16], many studies have proven that ZnO nanoparticles have selective toxicity to bacteria [17–27]. But, fast recombination of the photogenerated electron–hole pairs in ZnO makes it less efficient. Therefore, in order to improve the photocatalytic efficiencies in ZnO, suppression of the recombination of photogenerated electron–hole pairs were undertaken, such as SnO_2/ZnO, ZnO/In_2O_3, Pt/ZnO, ZnO/ZnS, Bi_2S_3/ZnO, ZnO/CdS [28], CdS/CdSe [29], ZnO/CdS [30], CeF_3/ZnO [31], ZnO/CdTe and ZnO/CdS [32], ZnO/CdS/Cu(In,Ga)Se_2 [33], ZnO/CuO [34], SnO_2–ZnO [35], CdS/ZnO [36], Ag/ZnO–C [37], Pt–ZnO [38], CdS/CdSe [39]. Quantum dots (QDs) are favoured as sensitizers of ZnO because it possesses excellent properties such as (i) high emission quantum yield, sharp emission spectra, broad absorption spectra, (ii) possesses excellent chemical and photostability (iii) tunability of emission frequencies, particle size and their convenience of multiple charge carrier generation from a single high-energy photon. Therefore, in the present work we have attempted to synthesize CdTe QDs sensitized ZnO nanostructures and studied their application to impede/eradicate bacterial growth biofilm, which is hitherto unattempted.

2. Experimental

2.1. Materials

Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$, 98%), Potassium hydroxide (KOH, 99%), Mercaptopropionic acid (MPA), Tellurium powder (Te), Cadmium chloride ($CdCl_2$), Sodium borohydride ($NaBH_4$), are all analytical grade and used without further purifications.

2.2. Synthesis of ZnO nanostructure

For the synthesis of ZnO nanoparticles, 1 mmol of Zn $(NO_3)_2{\cdot}6H_2O$ and 5 mmol of KOH were dissolved in 100 mL aqueous solution. The mixture was immediately transferred into a Teflon-lined stainless steel autoclave. The hydrothermal synthesis was carried out at 85 and 120 °C for 2 h duration. The product obtained was washed with distilled water and dried in oven at 70 °C.

2.3. Synthesis of ZnO/CdTe nanostructures

The ZnO nanoparticles (100 mg) were firstly dispersed in 100 mL aqueous solution of $CdCl_2$ and sodium hydrogen telluride (NaHTe) with MPA as a stabilizer. The molar ratio of Cd^{2+}:Te^{2-}:MPA was set at 1.0:0.2:2.4. $CdCl_2$ (15 mM) and MPA (36 mM) were mixed under vigorous stirring in an N_2 saturated deionized water and the pH was adjusted to 9.0 with 1 M NaOH. Freshly prepared oxygen-free NaHTe was injected into it and the mixture was immediately transferred into a Teflon-lined stainless steel autoclave (200 mL). The hydrothermal synthesis was carried out 120 °C for 2 h duration. The resultant product (powder) was collected by centrifuge, washed with distilled water and dried at 70 °C.

2.4. Characterizations

ZnO and ZnO/CdTe nanostructures were characterized by X-ray powder diffraction (XRD) (Philips X'Pert PRO), field emission scanning electron microscopy (FESEM), (Hitachi S-4800) and high resolution transmission electron microscopy (HRTEM) (FEI Tecnai 300). The optical properties were studied by ultraviolet–visible (UV–vis) spectroscopy (JASCO V-670 spectrophotometer) and photoluminescence (PL) spectroscopy measurements were performed on a Perkin-Elmer LS 55 spectrophotometer.

2.5. Bacterial growth impedance

Bacterial growth impedance/eradication by ZnO and CdTe conjugated ZnO nanostructures were performed in *Bacillus subtilis* NCIM 2063 and *Escherichia coli* NCIM 2931 by colony count method, under visible light (80 W bulb). In brief, the ZnO nanoparticles and its conjugates (ZnO/CdTe) in the concentration range of 0 to 20 μg/mL each were added to Muller Hinton broth, and each separately inoculated with *B. subtilis* and *E. coli* (final cell density of 1×10^6 colony forming units per millilitre (CFU/mL) and incubated at 37 °C for 15 h. After 15 h, viable numbers of cells were recorded as CFU/mL in comparison to control (without nanomaterials). The data were recorded as survival rates (CFU/mL), based on 100% survival for the untreated control. The extent of damage caused by (ZnO/CdTe) to bacterial cells, cell surface structures were seen under FESEM. In short, the cells of *B. subtilils* and *E. coli* were grown to mid log phase (appro. 1×10^7 cells/mL) and treated with a respective MIC concentration of ZnO and ZnO/CdTe nanostructures for 3 h at 37 °C and 150 rpm. The cells were collected by centrifugation at 10,000 × *g* for 15 min at 4 °C. The pellets were then washed 3 times with 0.1 M phosphate buffer of pH 7.4, and fixed in 2.5% glutaraldehyde at 4 °C for 4 h. After rinsing twice with buffer, the pellets were dehydrated in ethanol serials (10,100%, 15 min per step), and then dried in air. Finally, the images were seen under FESEM.

2.6. Biofilm inhibition

Biofilm impedance/eradication ability of ZnO and ZnO/CdTe nanostructures was studied in *Pseudomonas aeruginosa* O1 at 70–100 lx intensity of visible light. It was performed by crystal violet retention assay as crystal violet has an affinity towards polysaccharides of biofilm. In short, The *P. aeruginosa* O1 was grown overnight in Luria Bertaini (LB) medium at 37 °C with agitation. After growth, the culture was diluted with LB medium (OD_{600} 0.02), and 50 μL of the diluted culture was added to 950 μL of LB medium and allowed to form biofilm. After the formation of biofilm on polystyrene plastic surfaces, the planktonic cells (cells which are suspended in the medium) were replaced with fresh medium supplemented with 0–125 μg/mL ZnO and ZnO/CdTe, separately, and incubated statically for 18 h at 37 °C. After incubation, planktonic bacteria were discarded, and the biofilms were washed three times with phosphate buffered saline. Washed biofilms were fixed with 1 mL of methanol. After 15 min, the methanol was discarded, and the plates were dried at room temperature. Crystal violet (0.1% in water) was then added to each well (1 mL/well), and the plates were incubated for 15 min at room temperature. Crystal violet was then discarded, and stained biofilms were washed three times with 1 mL of water. Acetic acid (33% in water) was added to the stained biofilms (2 mL) in order to solubilize the crystal violet, and the absorbance of the solution was read at 590 nm with a spectroscopy (Schimadzu, Japan). To further confirm the impedance of biofilm, FESEM and confocal laser scanning microscope (CLSM) of biofilm was performed. For FESEM analysis, the biofilm were dehydrated in a series of ethanol solutions (10–100%, each for 15 min), dried, mounted on aluminium stubs with conductive carbon cement, and finally coated with a gold film. For CLSM, after initial gentle washing, the biofilm was covered with 50 μL of 10 μg/mL of dye, concanavalin A alexaflour 488, and incubated for 30 min at 4 °C. The biofilm was finally washed with 0.1 M cocdylate buffer of (pH 7.4), and observed under fluorescence microscopy with an excitation of wavelength of 488 nm and emission at 519 nm.

The impedance/eradication of bacterial communities inside the biofilm was performed by enumerating viable number of cells inside biofilm. In short, the biofilm formed in the presence of ZnO and ZnO/CdTe nanostructures were washed in 0.8% sodium chloride solution, sufficiently diluted in same solution to decrease the