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Synthesis, characterization, and antimicrobial activity of chitosan–zinc oxide/polyaniline composites

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ABSTRACT

Organic–inorganic materials of chitosan–zinc oxide/polyaniline (CS–ZnO/PANI) composite were prepared via precipitation with a polymerization method and characterized by FT-IR, XRD, EDXS and TEM analysis, thereby providing evidence of composite formation. The size of the prepared CS–ZnO/PANI composite was found to be 100–200 nm, thereby rendering the morphology suitable for biomedical applications. Antibacterial activities of chitosan–ZnO (CS–ZnO), polyaniline (PANI) and CS–ZnO/PANI composites were determined against Gram-positive bacterium, *Staphylococcus aureus* (*S. aureus*), and Gram-negative bacterium, *Pseudomonas aeruginosa* (*P. aeruginosa*) and were tested in-vitro at 5–50 μg/mL. Results showed that CS–ZnO/PANI composite had broad-spectrum antibacterial activity that was greatly enhanced in comparison with CS–ZnO. In addition, CS–ZnO/PANI composite has tested fungal strains of *Candida albicans* (*C. albicans*) and relatively higher activities were observed than the known antibiotics. Finally, the antimicrobial activity of CS–ZnO/PANI composite against established biofilms was also examined and resulted in more than 95% inhibition in biofilm formation.

1. Introduction

Bacterial and fungal contamination is currently of great concern in the health care and food industries; hence development of antimicrobial agents has been gaining increased attention in recent years. Due to the spread of antibiotic resistant bacteria and fungi, interest in alternative antimicrobial agents, such as polymeric and inorganic materials, has been rising [1–3]. Based on this concept, chitosan (CS) is a nontoxic biopolymer derived from chitin, which is found in the crustacean's shells, insect's cuticle and cell wall of fungi [4]. It is a bioadhesive that readily binds to negatively charged surfaces and has excellent antimicrobial and antifungal activities.

Three mechanisms have been proposed as an explanation to CS antimicrobial properties. In the first one, positively charged CS and negatively charged bacterial cell would alter the bacterial cell permeability, resulting in the leakage of intracellular components and cell death. Secondly, CS acts as chelating agent, creating compounds from traces of metals essential to the cell, while the third mechanism establishes that chitosan of low molecular weight is capable of entering the cell's nucleus itself, interacting with the DNA, interfering with the messenger RNA synthesis, affecting the synthesis of proteins and inhibiting the action of various enzymes [5]. On the other hand, polyaniline (PANI) is a highly functional synthetic polymer which is unique among the family of π-conjugated polymers because of its ease of synthesis, good environmental stability and simple doping/dedoping chemistry.

PANI has found immense applications in diverse areas such as artificial muscles, controlled drug release and the stimulation of nerve regeneration [6]. It was reported that

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PANI-coated conductive cotton fabrics have potent antibacterial and antifungal activities [7]. In particular the antibacterial activity of conducting polymer PANI is associated with the ability to act as an electron acceptor or donor. It is explained through the electrostatic adherence between polymer molecules and bacteria, which carry charges of different signs; thus the walls of the bacteria break down and the intracellular fluid leaks out, causing death [8].

However, the use of individual CS and PANI is limited because of its insolubility in water and most organic solvents, thereby making it difficult to process. Additionally, it showed relatively low antifungal activity compared with commercial fungicide. Hence, numerous researchers tried to improve both solubility and antimicrobial activity of CS and PANI via chemical modification [9,10]. For example, Chen et al. reported that CS incorporated PANI gives biocompatible chitosan–polyaniline (CS–PANI) matrix, which increases the flexibility and solubility in common solvents for biomedical applications [11]. More recently, introduction of asparagines into CS significantly improved the bactericidal activity and minimum inhibitory concentration, which could be attributed to the higher number of amino groups present in the polymer chain [12].

Compared to organic materials, inorganic materials such as metaloxide nanoparticles possess superior durability, greater selectivity and heat resistance, can produce increased levels of reactive oxygen species (ROS), mostly hydroxyl radicals, H_2O_2 and singlet oxygen, which results in the enhanced cell damage. Among the various types of nanomaterials that have been developed, highly ionic nanoparticulate ZnO nanoparticles are unique with unusual crystal structures, antibacterial and antifungal agents at lower concentrations [13]. Besides, zinc is a mineral element essential to human health and used in the form of ZnO in the daily supplement for zinc. Recently several studies have reported that CS functionalized ZnO nanoparticles are effective at inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* [14,15,21].

In addition to their direct bactericidal activity, nanoparticles are also known to disrupt the biofilm formation [16]. Biofilms are formed because of the attachment of bacteria to the solid surfaces of in vivo medical devices such as contact lens, artificial joints, and synthetic valves resulting in the conglomeration of bacterial cells that are highly resistant to antimicrobial therapy. Currently biofilm preventive strategies are essentially coating medical surfaces with antimicrobial agents. Recent reports reveal that MgF_2 nanoparticles inhibit the biofilm formation of *Pseudomonas aeruginos* and *S. aureus* [17].

The aim of this paper is to report a simple and reliable method to prepare CS–ZnO/PANI composite with improved capabilities inhibiting the growth of bacterial and fungal pathogens. The present study divulges the anti-bacterial and anti-fungal activity of synthesized CS–ZnO/PANI composite against two pathogenic bacteria – Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* – and fungal pathogens (*Candida albicans*). In addition, the antimicrobial activity of CS–ZnO/PANI composite against established biofilms was examined. To our knowledge, this is the first report on the antibiotic effect of CS–ZnO/PANI composite on *S. aureus*, *P. aeruginosa* and *C. albicans* and its effect on biofilm formation.

2. Experimental

2.1. Materials

Chitosan (90% deacetylation; M/s South India Sea Foods, Kochi, Kerala, India) and aniline (Merck, India) were purified by vacuum distillation in the presence of zinc dust (SD Fine-Chem Limited, India, atomic weight 65–37 g/mol) prior to use, Ammonium persulfate (APS, Merck) and zinc (II) chloride hexa hydrated ($Zncl_2 \cdot 6H_2O$) (Sigma-Aldrich). The microorganisms of bacterial and fungal strains and reagents for culture media were provided by the Department of Animal Health and Management, Alagappa University, Karaikudi, India. All other reagents used in the study were of analytical grade.

2.2. Preparation of CS–ZnO/PANI composite

Preparation of CS–ZnO/PANI composite has been reported previously [18]. The typical procedure is as follows. 0.12 g of CS dissolved in 50 mL of CH_3COOH (4%) was added with vigorous stirring for 1 h at ambient temperature. Then 2.5% (w/v) $ZnCl_2$ was added in 50 mL of distilled water and stirred continuously for 1 h at 80 °C. After cooling to room temperature, 2 M NaOH was added into white precipitate and left to react during 24 h. The supernatant solution was discarded and the precipitate was rinsed with double distilled water several times; it was labeled beaker (A). Then separately aniline hydrochloride (0.9 mL aniline in 1 M HCl) was polymerized with 0.25 M APS in an ice bath (0–5 °C) for 1 h; it was labeled beaker (B). Finally the solution (B) was slowly poured into beaker (A) after stirring for 30 min, and allowed to react for 24 h to complete the chemical reaction, followed by washing more times with distilled water, filtered and dried at 80 °C, which is designed as CS–ZnO/PANI. In a similar manner, the preparation of CS–ZnO without the addition of PANI which was designed as CS–ZnO was carried out and PANI was also prepared without the addition of CS–ZnO, which is designed as PANI.

2.3. Characterization

The Fourier transform infrared (FTIR) spectra were recorded on a PerkinElmer 2000 spectrophotometer which was in the range of 4000–450 cm^{-1} at a 4 cm^{-1} resolution with KBr pellets at room temperature. The powder X-ray diffraction (XRD) pattern measurement (Rigaku D/Max 2550 VB/PC) was employed to study the crystal structures of the composites. The elemental analysis of the composites was observed via (HRSEM-FEI Quanta FEG 200) at an accelerating voltage of 10.0 kV. The morphology of the samples was characterized by high resolution transmission electron microscopy (HRTEM-CM200 model).

2.4. *in vitro* anti-microbial activity of synthesized compounds against human pathogens

The antimicrobial activity of the synthesized materials was checked with the Gram-positive and Gram-negative pathogens through an agar well diffusion method. The well