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Polyaniline/$CoFe_2O_4$ nanocomposite inhibits the growth of *Candida albicans* 077 by ROS production

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ABSTRACT

In recent years, polyaniline/$CoFe_2O_4$ nanocomposites have gained attention because of their wide utilization in optoelectronics and biomedical studies. However, very limited research has been carried out on the anticandidal activity of polyaniline/$CoFe_2O_4$ nanocomposite against *Candida* spp. Thus, the study was designed to investigate the anticandidal potential of PANI/$CoFe_2O_4$ nanocomposite against *Candida albicans* 077. PANI/$CoFe_2O_4$ nanocomposite (denoted as "cfPNCs") was synthesized by polymerization of aniline in the presence of $CoFe_2O_4$ nanoparticles. The structural and thermal properties of the synthesized PANI/$CoFe_2O_4$ nanocomposite were investigated. It was noteworthy that PANI/$CoFe_2O_4$ nanocomposite showed promising anticandidal activity in a dose-dependent manner. Results also showed that the protection of histidine (a ROS quencher) against ROS clearly suggested the implication of ROS in anticandidal activity of PANI/$CoFe_2O_4$ nanocomposite. It is encouraging to conclude that PANI/$CoFe_2O_4$ nanocomposite bears the potential of their applications in biomedicine, especially nanotherapy for diseases caused by *C. albicans*.

1. Introduction

Polyaniline (PANI) is a conducting polymer that has received immense attention in recent years owing to its physical, chemical and biological properties [1]. Therefore, the PANI-containing polymeric nanocomposites (PNCs) are widely being used for the fabrication and development of bioelectronics, optoelectronics, and sensor-based devices [2,3]. Among them, particularly, the PNCs of metal oxides such as cobalt iron oxide ($CoFe_2O_4$) nanoparticles (NPs) have been studied extensively to understand their unique physicochemical properties, not only for scientific research, but also for technological applications [4]. $CoFe_2O_4$ belongs to the family of spinel-type ferrites and is one of the most important ferrites, with high coercivity, moderate magnetization, high magnetocrystalline anisotropy, chemical stability, and mechanical hardness [5]. These $CoFe_2O_4$-based PNCs frequently exhibit unexpected hybrid properties derived from both components and have great potential applications in the areas of electronics, photonics, catalysis, etc. [6].

Magnetic NPs of iron oxides and their hybrid forms represent the most suitable candidates for the preparation of magnetic nanocomposites owing to their application-convenient magnetic (e.g., superparamagnetism) and biochemical (e.g., non-toxicity, biocompatibility, biodegradability) properties and low price. These oxides that can

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play the role of nano-antimicrobial agents are believed to be non-toxic, and some of them even contain mineral elements essential to the human body [7]. Moreover, biomedical applications of polyaniline/$CoFe_2O_4$ nanocomposite have also been reported in recent years owing to its suitable magnetic behaviour at room temperature for MRI contrast enhancement and hyperthermia treatments [8]. Although conventional antibacterial agents have several disadvantages, i.e., toxicity to the microbiota and sensitivity towards the temperatures and pressures [9], therefore, the interest in metal-oxide-based nano-antimicrobial agents are rising over the years [10,11]. These metal-oxide-based nano-antimicrobial agents exhibited strong antimicrobial activity at low concentrations, with high stability in extreme conditions [12,13]. The suggested mechanism for the antimicrobial activity of metal-oxide-based NPs is based mainly on the production of reactive oxygen species (ROS) from the catalysis of water [14]. Although many controversies have been raised on the mechanistic aspects of the antimicrobial activity of the metal oxide NPs, there is copious evidence that metal-oxide-based NPs increase oxidative stress through the generation of reactive oxygen species (ROS) that include predominantly hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen ($^1O_2$). These ROS mainly contribute to the antimicrobial activity of metal-oxide-based NPs [14,15]. Other antimicrobial mechanisms involving metal-oxide-based NPs, like cytoplasmic membrane disruption and electrostatic binding of metal oxide NPs to the cell surface of the microbial pathogen, have been reported [16–18]. However, recently the production of ROS governed by the electronic band gap property of metal-oxide-based NPs has been considered as triggering the actual mechanism [14]. The energy band gap property of metal-oxide-based NPs are influenced by various physical and chemical parameters [19]. Therefore, a fundamental understanding of the energy band gap property of metal-oxide-based NPs becomes crucial to the tailoring of novel nano-antimicrobial agents in an economic way.

Recent preliminary studies on $CoFe_2O_4$ NPs have demonstrated that they may be appropriate antimicrobial agents to control and manage human microbial pathogens [20,21]. Ferrite NPs exhibit an intrinsic peroxidase-like activity by generating ROS via the Fenton reaction [22]. $CoFe_2O_4$ NPs interacts with the microbial cell surface and elevates the oxidative stress that disrupts the integrity of the cell wall/membrane and damage the biological macromolecules [23]. Although NPs coated with polymers exhibited stronger antimicrobial activity in comparison to the bare [24], extensive work has been done in recent years to develop promising nanocomposite antibacterial agents based on synthetic materials or biopolymers. These polymers possess an intrinsic antibacterial activity through (*i*) coating or adsorption of an antibacterial agent onto the polymer surface; (*ii*) grafting/fabrication of an antibacterial agent in the polymer via ionic or covalent bonding, or (*iii*) direct incorporation of an antibacterial agent into the polymer during in situ synthesis [25,26]. Among them, conducting polymer polyaniline (PANI) has inherently a broad spectrum of antibacterial and antifungal properties. Thus, the PANI has been the subject of considerable attention due to its potential in biomedical applications [27,28]. These findings motivated us to use PANI/$CoFe_2O_4$ composite as an anticandidal agent.

Over the past years, researches have been carried out on the assessment of the antimicrobial activity of NPs and nanomaterials. However, research focused on the antimicrobial properties of NPs and nanomaterials were confined to the prokaryotic pathogens in comparison to the eukaryotic pathogens. Therefore, in this study, we have chosen *Candida albicans*, an eukaryotic human pathogen, for assessment of the anticandidal properties of PANI/$CoFe_2O_4$ nanocomposite. *C. albicans* is amongst the most common fungal causative agent in superficial and deep-seated candidiasis, depending on the patient's immune system status [29]. The brutality of antifungal drugs in pharmacotherapy has led to the development of wide-spread multi-drug resistance (MDR) in *C. albicans* [30]. The failure of antifungal drugs to control infection makes it crucial to find alternatives to currently available drugs. Unfortunately, most of these antifungal drugs chemical in nature and bulk form that are being investigated are too reactive and are unsuitable for the treatment of *C. albicans* infections in humans [31]. With the continuous development of MDR in *C. albicans*, the search for new medical treatments beyond conventional antifungal drugs has become a key aim of public health research [32]. Possible innovative strategies encompass the inhibition of *C. albicans* growth with the use of NPs and nanomaterials [33]. There is thus a need for the identification of a novel class of PNCs that can be able to inhibit efficiently *C. albicans* growth. Identification of such metal-oxide-based or hybrid nano-antimicrobial agents could present us with new opportunities for the development of novel non-antibiotic drugs for treating *C. albicans* diseases [30,32,33]. The anticandidal activity of PANI/$CoFe_2O_4$ against *C. albicans* has not been studied yet. Therefore, in this study, we have synthesized a stable and surface-protected PANI/$CoFe_2O_4$ nanocomposite (denoted as "cfPNCs") using a conducting polymer and investigated its anticandidal activity against *C. albicans* 077.

2. Materials and methods

2.1. Materials

All reagents were of analytical grade. Cobalt nitrate, iron nitrate, ammonium peroxydisulfate, citric acid, *N*-methyl-2-pyrrolidone (NMP) and hydrochloric acid were purchased from SRL, India. The nutrient media, Sabouraud's dextrose (SD) broth and agar for the *C. albicans* 077 were obtained from the HiMedia Laboratories, Mumbai, India. Histidine and 2,7-dichlorofluorescin diacetate (DCFH-DA) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used were of the highest purity available from commercial sources.

2.2. Nanocomposite materials synthesis

2.2.1. $CoFe_2O_4$ NPs synthesis

$CoFe_2O_4$ NPs were synthesized by the gel-combustion citrate–nitrate method reported previously [34]. The salts