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Nano-bio control of bacteria: A novel mechanism for antibacterial activities of magnetic nanoparticles as a temporary nanomagnets

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ABSTRACT

The aim of this study is to investigate the role of interaction pattern between the Fe_3O_4 exposed by magnetic field as a temporary nanomagnets (TNMs) against bacterial interfaces, affecting antimicrobial trends of TNMs. The magnetite nano particles were prepared by the coprecipitation of Fe^{3+} and Fe^{2+} ions. The Fe_3O_4 nanoparticles without any modification is called MNPs and Fe_3O_4 nanoparticles treated in the magnetic field is called TNMs. Functionalization by azo compounds using 3-Amino propyl triethoxysilane as a linker resulted in azo-compound @ MNPs. Azo-compound @ TNMs is a temporary nanomagnets treated by external magnetic field and during all tests it has magnetic property. The structural and magnetic properties of magnetic nanomaterials are identified by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and vibrating sample magnetometer (VSM) instruments. FT-IR and XRD were also used for the identification of these structures. All of the antibacterial tests were performed using MNPs and TNMs against two Gram positive and two Gram negative bacterial species. It is determined that the exposing of magnetic fields to magnetic nanoparticles can change the biological activity and increase the bacteriostaticity of these nano composites in bacterial medium. The TNMs may serve as a useful model system to apply electromagnetic interactions of Nano particles in biological system. Since TNMs are very unstable and oxidized in aqueous solution, surface modification of TNMs is crucial for biomedical applications. Functionalizations of TNMs by azo compounds protect them from red-ox reactions and improve their stability making nanoparticles better antibacterial nanocomposite.

1. Introduction

In the past decade, magnetite was developed for basic scientific interest and exceedingly for applications in nano catalyst [1,2], magnetic storage media [3], nanoscavengers [4], biosensors [5], medical applications [6,7] and biological probes [8]. Research interest has been in the chemical aspects of nanoparticles, such as synthesis and biomedical applications aiming at developing very successful antibacterial agents for biochemical and biological applications [9–11] with emphasis on the chemical properties of the nanoparticles to improve their efficiencies [12–14].

As magnetic nanoparticles are generally considered to be biologically and chemically inert [15]. The iron is one of the functional requirement for bacterial growth [16] and surprisingly external magnetic field is the enhancing factor for growth of bacteria [17,18] and must be typically coated with other metals, chemical compounds, enzymes or antibodies to increase their functionality [19–21]. Molecules with structures or properties which can be manipulated by external actuators have attracted considerable interest in different fields of science [22]. Molecules with carrier magnetic fields are a novel method for new version of antibacterial agents [4,23]. Advantageously the physicochemical properties of nano particles exposed by magnetic field, using temporary nanomagnets (TNMs) was used to control the bacterial activities. Furthermore, azo compounds are such an important class of chemicals with application in coordination chemistry [24,25], electro-optical devices [26–28], biological and chemical reactors in various fields [29–34]. In this article azo-azomethines synthons are effectively used to provide improved nanoparticles properties for use as modified and antibacterial system.

2. Experimental section

2.1. Materials

All chemicals were prepared from Merck, Germany. The azo compounds (1–7) were synthesized by condensation reaction of primary aromatic amines and various derivation of salicylaldehyde in aqueous medium [33,35,36]. Magnetite nano particle were prepared by common co-precipitation method [37]. Bacterial species were as *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 9027).

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Melting points were measured with Electrothermal 9300 apparatus. IR spectra in the range of 4000–400 cm^{-1} were obtained on Shimadzu 8400 S spectrometer with samples investigated as KBr discs. The structure of all synthesized compounds were confirmed by ^{1}H and ^{13}C NMR spectra, in DMSO d_6 as solvent on a Bruker AV 400 MHz spectrometer. Utilized X-ray diffractometer is Philips PW 1840 (Germany), CU-Kα radiation (λ = 1.5418 Å) at voltage of 40 KV and current of 30 mA with a scan rate of 5°/min and step size of 0.02° over 2θ range of 10° to 90°. The Field Emission Scanning Electron Microscope (FE-SEM, MIRA3 LMU. TESCAN Company, Czech Republic) was employed to investigate the shape of bacteria and morphological structure of MNPs at an accelerating voltage of 15 KV upon gold coating for 3 min. Tunneling electron microscope (TEM, Zeiss - EM10C – 80 KV) is also used to complete proves. Growth curve of target bacteria was obtained by ELISA reader (Bio-Rad, USA).

2.2. Preparation of azo-compounds functionalized magnetic nano particles

Preparation of azo-compounds functionalized magnetic nano particles (azo-compound @ MNPs) are performed by common method [38]. 1 g of Fe_3O_4 was dispersed in 50 ml of toluene, followed by addition of 4 ml of 3-amino propyl trimethoxysilane (3- APTES), and stirred 12 h at 80 °C, then the resultant mixture was collected by external magnetic field and washed with hot deionized water to remove the residuals. Next, the azo compound were (1 g) dissolved in 50 ml mixture of ethanol and deionized water (1:1) followed by addition of 3-APTES modified MNPs (1 g) and addition of 0.3 ml acetic acid as a catalyst. The mixture was then heated at 75 °C for 1–2 h and then stirred at room temperature for 4–6 h. The resultant precipitate was washed separately by deionized water and ethanol (2 × 50), in every step the product was magnetically separated and the eluent was discarded.

2.3. Preparation of MNPs and TNMs

The Fe_3O_4 nanoparticles without any modification is called MNPs and Fe_3O_4 nanoparticles treated in the magnetic field of 1000 ± 200 G at 2.54 cm is called TNMs. TNMs is a temporary nanomagnets prepared by external magnetic field and during all tests it has magnetic property (made by three Neodymium Block Magnets, NdFeB, Grade N42, 5.08 × 2.54 × 0.317 cm thick, model: BY0X02, K&J Magnetics, Inc.).

2.4. Antibacterial activity

2.4.1. Growth curve of target bacteria

Growth curve of target bacteria was obtained in the presence of MNPs, TNMs, azo compound @ MNPs and azo compound @ TNMs by known method [39]. 20 mg/ml concentration of each compound was prepared in Mueller- Hinton broth and the tubes were separately inoculated with 0.5 McFarland suspension of *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis*. Then the cultures were incubated at 37 °C and bacterial growth was monitored every 2 h measuring culture turbidity at 655 nm by using microplate ELISA reader.

2.4.2. Viable bacterial count

Colony Forming Unit (CFU) per ml of bacterial culture, i.e., $^{CFU}/_{ml}$ was used to determine the viable bacterial count following treatment of bacterial species with MNPs and TNMs [40]. For this purpose, a 20 mg/ml concentration of compounds were prepared in Mueller–Hinton broth and each of target bacteria was inoculated to different culture tubes. The tubes were incubated at 37 °C for 24 h. Then 1 in 10^{-5} dilution was prepared from treated cultures and spread on a Nutrient agar. Following overnight incubation at 37 °C, the number of colonies formed on the surface of agar plates were counted and the results were presented as $^{CFU}/_{ml}$ and used for quantitative comparisons. The effect of TNMs is directly related to the inhibition of the bacterial growth which was confirmed by bacterial colony count (CFU) measurements for *E.coli* as a sample.

2.4.3. MIC and MBC determination

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured for antimicrobial agents. According to CLSI guidelines [41], two fold serial dilutions from 32 to 1 mg/ml of compound were prepared in 1 ml sterile Mueller–Hinton broth and all tubes were inoculated with 0.5 McFarland suspension of each target bacterium. The cultures were incubated at 37 °C for 24 h. The least concentration that inhibited bacterial growth and turbidity was regarded as MIC. All growth negative cultures were cultured on Mueller- Hinton agar and incubated as previously described. The least concentration that was able to inhibit bacterial growth and colony formation was regarded as MBC.

3. Results and discussion

The magnetite nano particles were prepared by the coprecipitation of Fe^{3+} and Fe^{2+} ions. The Fe_3O_4 nanoparticles without any modification is called MNPs and Fe_3O_4 nanoparticles treated in the magnetic field is called TNMs. Reduction at 80 °C and functionalization by azo compounds (1–7) using 3-Amino propyl triethoxysilane as a linker resulted in azo-compound @ MNPs. Azo-compound @ TNMs is a temporary nanomagnets prepared by external magnetic field and during all tests it has magnetic property (Scheme 1). All of the antibacterial tests were performed using MNPs and TNMs against two Gram positive and two Gram negative bacterial species.

Growth curve of target bacteria was obtained in the presence of MNPs and TNMs (Fig. 1). As shown, significant growth inhibition occurred in TNMs treated bacterial cultures with comparison to MNPs treated ones in specified duration. The design principles of TNMs are influenced by their intrinsic characteristics namely magnetic nature and surface chemistry. Such attributes allow the TNMs to electrostatically adhere to the negative charge of microbial cell surface disrupting its initial surface through reaction with the functional groups of cell-surface bilayer membrane [42]. Another factor is the difference between cavities in the cell wall of target bacteria which affect the penetration potential of nano particles. In recent reports it has been noted that iron oxide nanoparticles treated with magnetic field produce local heating and in situ temperature increase [43]. Membrane fluidity may be increased as a result of temperature increase. Apart from these, there may be non-bonded interactions and non-covalent linkages important in the large natural macromolecules [44]. Hence existing iron atoms and external magnetic field simultaneously, showed different impacts on the bacteria.

Membrane depolarization of *E. coli* was checked (Fig. 2) using Fe-SEM and the interaction between damaged bacteria and fresh bacteria was confirmed using Energy-dispersive X-ray spectroscope (EDX). Unlike control, the EDX spectra of TNMs treated bacterial surface showed the traces of Fe, confirming the interaction of TNMs with bacteria causing disruption and depolarization of the membrane (Fig. S-8). For this part, the bacteria may have phase transformations, free energy releases, restructuring and dissolution at the nanomaterial surface and make colloidal phases ahead of dynamic bio-physicochemical interactions.

In magnetic nanoparticle, the surface of Fe_3O_4 act as Lewis acid and can coordinate with electron donors such as H_2O. Since hydroxyl groups are amphoteric the surface of magnetite can be negative or positive charge depending on the pH of the solution. Around the isoelectric point at pH 6.8 [point of zero charge (PZC)], the surface charge density (Σ) is too small and the particles are not stable in water and will flocculate [45,46]. Under basic conditions, the oxidation of Fe_3O_4 involves the oxidation-reduction of the nanoparticle's surface, whereas, under anaerobic or acidic medium, surface of Fe^{2+} ions are desorbed as hexa-aqua complexes [47]. On the other hand, magnetite can be transformed into maghemite (γ-Fe_2O_3) in the presence of oxygen [47]. Hence the