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Synthesis of oleic acid coated iron oxide nanoparticles and its role in anti-biofilm activity against clinical isolates of bacterial pathogens

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ABSTRACT

In the present study, iron oxide nanoparticles (IONPs) were synthesized using the solvothermal method. The surface of the IONPs was modified with oleic acid (OA) and its role in anti-biofilm activity against clinical isolates of bacterial pathogens was studied. The physiochemical properties of OA-IONPs were characterized using vibrating sample magnetometer, X-ray diffraction, Fourier transform infrared, dynamic light scattering and scanning electron microscopy. The biofilm inhibitory effect of OA-IONPs was tested against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*). The OA-IONPs showed a potent biofilm inhibitory effect in the case of Gram-positive bacteria and a less significant effect in Gram-negative bacteria. Further, enhanced biofilm inhibition was achieved against both the bacteria using external magnetic target and confirmed using fluorescence microscopy. In conclusion, OA-IONPs can be used as an effective surface modifier for biomaterials to prevent biofilm formation.

1. Introduction

Iron oxide nanoparticles (IONPs) are biocompatible and have a unique paramagnetic properties used for various biomedical applications including drug delivery [1], hyperthermia [2], and cancer therapy [3,4]. The magnetite and maghemite are two main forms which are paramagnetic in nature (*i.e.* shows magnetism only in the presence of an external magnetic field) [5]. This property is due to the presence of unpaired electrons that align in the direction of an external magnetic field. Iron oxide has an inverse spinel structure, with a space group number of 227 and has 8 atoms per unit cell. The Hermann Mauguin symbol is Fd3m and Hall symbol is F 4d 2 3 1d for iron oxide crystal. The crystal system is cubic and Bravais lattice is F.

Various methods were reported for the IONPs synthesis such as, chemical synthesis [6], sonochemical method [7], solvothermal method [8,9], reflux method [10], hydrothermal method [11,12] and co-precipitation method [13]. Among them, the most common method used for synthesis is co-precipitation, but this method produces non-uniform sized nanocrystals [14]. The main challenge is to synthesize nanoparticles that are uniform in size, shape, chemical composition, high yield production, and should be stable in biological environments. The solvothermal method has been reported to produce IONPs of uniform size [15].

Surface coating plays an important role to make stable aqueous IONPs suspensions. The surface of the IONPs requires a coating to prevent aggregation because of their magnetic property. The most common biocompatible coating materials are dextran, poly(acrylic acid), polysaccharides, hydrophilic synthetic polymers, poly(amino acid)s and oleic acid [16–20]. A polymer that does not stimulate the immune system and has a high affinity for the iron oxides can be considered as an ideal material for surface coating. Among the biocompatible coating material, oleic acid (OA) is most commonly used surfactant to stabilize magnetic nanoparticles [21]. The advantage of oleic acid is the strongest chemical bond between the carboxylic acid and the amorphous iron oxide nanoparticles [22,23]. OA modified IONPs are highly monodispersed, providing high biocompatibility, low toxicity, hydrophobic and long term stability [24]. OA has been reported to have antibacterial activity, especially toward Gram-positive bacteria, which inhibit the fatty acid synthesis in the bacteria [25].

Nowadays biomaterial implants are playing a crucial role in the modern medical field in enhancing the quality of life. Biomaterial-associated infection can occur from microbial contamination of implant surfaces during surgery, during the post-operative phase or, by haematogenous spreading of bacteria from infections elsewhere in the human system. It is one of the major causes for implantation failure and can develop several years after implantation. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common isolated pathogenic strains from infected biomaterial implant surfaces [26–28]. The bacteria causing biomaterial-associated infec-

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tion produces a matrix of exopolymeric substances which consist of polysaccharides, DNA, and proteins embedded within this matrix. Antibiotics and host immune system cells cannot penetrate this barrier, making these bacterial strains resistant to multiple drugs. Moreover, biocides or antibiotics are neutralized by reacting with constituents of the biofilm and it cannot penetrate the biofilm effectively. The biofilm resistance develops according to the different physiological microenvironments between the planktonic bacteria and bacteria embedded in biofilm [28,29].

In the present study, we investigated a novel alternative approach to inhibit the bacterial biofilm formation using OA-IONPs and kill the bacterial biofilm in the presence of an external magnetic target.

2. Materials and methods

2.1. Materials required

Oleic acid (≥99%), iron (III) chloride anhydrous (≥99.99%), sodium acetate anhydrous (≥99%), ethylene glycol, ethanol, sodium carbonate, acridine orange, propidium iodide, sodium chloride, Ethylenediaminetetraacetic acid (EDTA), citric acid and disodium hydrogen phosphate were purchased from Sigma-Aldrich. Luria broth (LB) and agar were purchased from Hi-media, India. Bacteria such as *P. aeruginosa* and *S. aureus* were obtained from SRM Medical College and Research Centre, Chennai, India. Milli-Q deionized water was used throughout the experiments.

2.2. Synthesis of OA-IONPs

The IONPs were synthesized using the solvothermal method. $FeCl_3 \cdot 6H_2O$ 2.70 g and anhydrous sodium acetate 7.20 g were mixed in 100 mL of ethylene glycol under vigorous stirring for 5 h. A yellow colored homogeneous solution was obtained. The solution was transferred to Teflon-lined stainless steel autoclave and heated to 200 °C for 8 h. After that, the solution was cooled to room temperature and centrifuged at 10,000 rpm for 10 min. A black colored pellet was obtained. The precipitate was washed 10 times with ethanol and acetone, and the obtained black nanoparticles were dried at room temperature. One mL OA was added to 0.6 g of IONPs on a Petri plate and kept overnight in a rocker at 10 rpm. Finally, the obtained OA-IONPs 5 mg was added to 1 mL of 0.1 M sodium carbonate and ultrasonication at 40% power for 10 min to obtain a stable colloidal dispersion.

2.3. Characterization of OA-IONPs

The magnetic property of OA-IONPs was examined using a vibrating sample magnetometer (Lakeshore VSM 7410, USA). The X-ray Diffraction analysis was performed using an X'PertPro A Analytical X-ray diffractometer instrument using Cu Kα radiation ($k = 1.54056$ Å) in the range of 30–80 (2θ values) at 40 keV. Fourier Transform Infrared (FTIR) (Agilent tech., USA) spectrum was recorded for pure OA, IONPs and OA-IONPs in the range of 600–4000 cm^{-1}. The particle size and zeta potential were measured using a Zetasizer Nano ZS (Malvern Instruments, UK). Measurements were carried out using an He–Ne laser of 633 nm at 25 °C. A morphological evidence of synthesized particles was studied using field emission electron microscopy (FE-SEM) equipped with EDS (Quanta FEG 200, USA) in an accelerating potential difference of 20 kV.

2.4. Determination of IC_{50} values of OA-IONPs

IC_{50} values were determined according to the modified method of Barbara et al. [30]. IC_{50} values of OA-IONPs were determined using different concentration (2– 256 μg/mL) of OA-IONPs incubated with *S. aureus* and *P. aeruginosa.* Bacterial cell suspension (*S. aureus* and *P. aeruginosa*) 10^6 CFU/mL was pipetted into 96 well plates in the presence of different concentration of OA-IONPs. The untreated bacterial

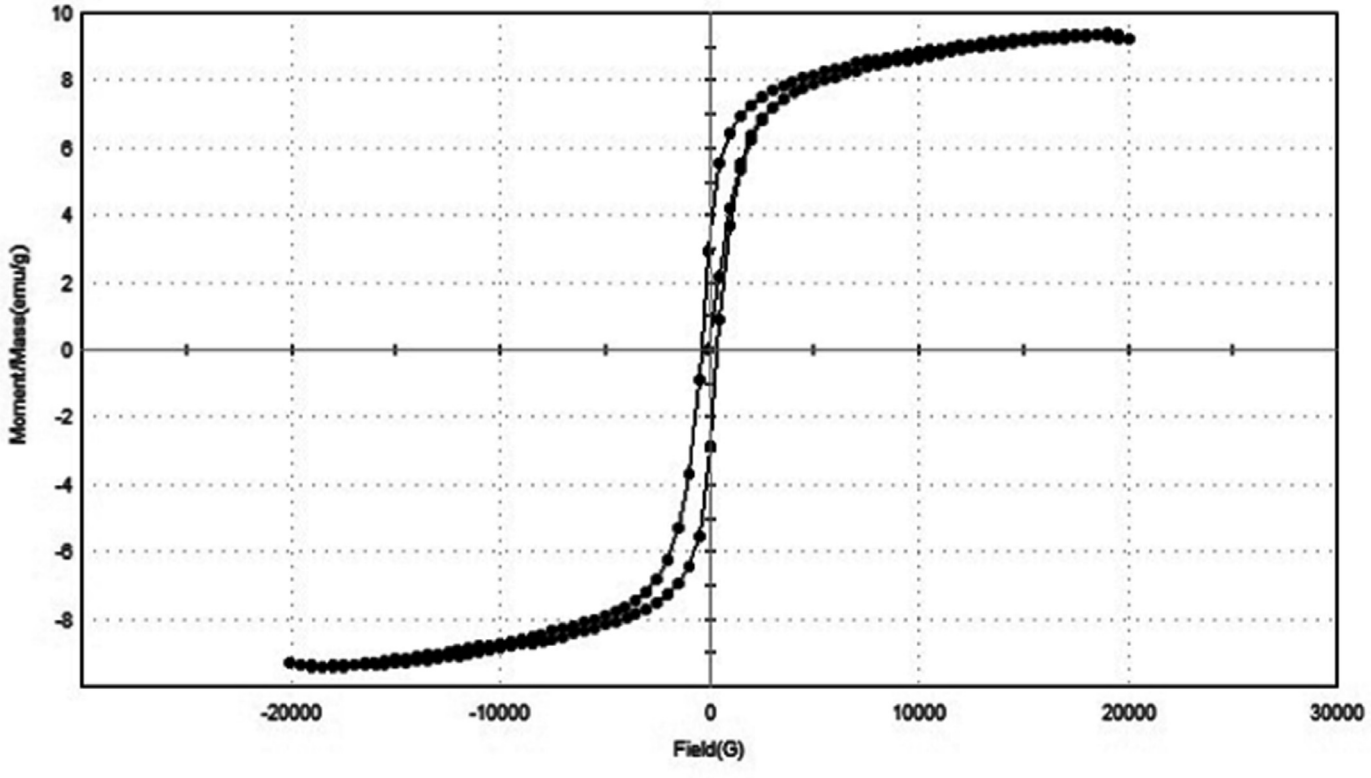

Fig. 1. Magnetic hysteresis loop of OA-IONPs measured at 300 K using vibrating sample magnetometer.