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Dual-modality self-heating and antibacterial polymer-coated nanoparticles for magnetic hyperthermia

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ABSTRACT

Multifunctional nanoparticles for magnetic hyperthermia which simultaneously display antibacterial properties promise to decrease bacterial infections co-localized with cancers. Current methods synthesize such particles by multi-step procedures, and systematic comparisons of antibacterial properties between coatings, as well as measurements of specific absorption rate (SAR) during magnetic hyperthermia are lacking. Here we report the novel simple method for synthesis of magnetic nanoparticles with shells of oleic acid (OA), polyethyleneimine (PEI) and polyethyleneimine-methyl cellulose (PEI-mC). We compare their antibacterial properties against single gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria as well as biofilms. Magnetite nanoparticles (MNPs) with PEI-methyl cellulose were found to be most effective against both *S. aureus* and *E. coli* with concentration for 10% growth inhibition (EC10) of <150 mg/l. All the particles have high SAR and are effective for heat-generation in alternating magnetic fields.

1. Introduction

Magnetite nanoparticles (MNPs) have attracted broad interest for hyperthermic cancer treatment because they are non-toxic, biocompatible, and can be remotely heated by alternating magnetic fields [1–2]. The magnetic induction heating behavior also provides a benefit for biomedical applications, such as targeted drug delivery, and magnetic separation [3–6]. MNP can transform the energy of an alternating magnetic field into heat through two kinds of relaxation: Neel relaxation and the Brownian relaxation. Neel relaxation is caused by the reorientation of the magnetization, which is caused by the reorientation of the magnetization vector inside the magnetic core against an energy barrier [7]. Brownian relaxation is due to the rotational diffusion of the whole particle in the carrier liquid [8–11].

The size of nanoparticles influences the number of their magnetic domains, where small nanoparticles are composed of a single domain and the larger ones are composed of multiple domains minimizing the magnetostatic energy. Conversion of dissipated magnetic energy into thermal energy in magnetic nanoparticles shows promising applications in hyperthermic cancer treatment. Artificially induced hyperthermia, by locally raising the temperature of an affected region of the body to 42–46 °C, has the power to kill cancer cells without affecting the nearby healthy tissue [12–13].

The most common methods used for the synthesis of magnetite nanoparticles are co-precipitation process, thermal decomposition, and hydrothermal synthesis [14–18]. Co-precipitation process is considered one of the easier procedures for preparation of magnetic nanoparticles by precipitation of magnetic nanoparticles in solution media. The size and the morphology of the prepared magnetic nanoparticles strongly depend on the type of iron salts used, the reaction temperature, pH and speed of stirring [14]. Heat dissipation, morphological and magnetic properties are important for hyperthermia response.

Magnetic nanoparticles tend to aggregate due to the presence of high surface energy of nanoparticles and their strong van der Waals interactions. In situ formation of an organic or polymer layer on their surface is one of the efficient methods for decreasing nanoparticle aggregation, as well as providing functional groups on the surface and allowing further functionalization with drugs and therapeutic agents. The co-precipitation method used here is an easy method to produce such coatings [15–16]. Several types of organic layer capped superparamagnetic iron oxide nanoparticles have been prepared with cinnamaldehyde, citric acid, starch and oleic acid [17–21]. It was reported that poly(N-isopropylacrylamide) (PNIPAm), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polyethylene glycol (PEG), polyethyleneimine (PEI) and chitosan have also been used to stabilize magnetic nanoparticles [22–25].

It is highly desired to have new hyperthermic agents that are also antibacterial, which would decrease bacterial infections co-localized with cancers [26–28]. Gold nanorods capped with toluidine blue O (TBO) have been used as dual-function agents for photodynamic

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inactivation and hyperthermia against methicillin resistant *Staphylococcus aureus* [26]. Recent studies have focused on dual-action nanoparticles, Fe_3O_4–ZnO nanocomposite were prepared for inactivation of bacterial pathogens under magnetic hyperthermia [27]. In another report a rapid drug release system was designed with a near-infrared (NIR) light-activated molecular switch for dual-mode photothermal/antibiotic treatments of subcutaneous abscesses [28]. A graphene-based photothermal agent was used for rapid and effective killing of bacteria [29].

The aim of the present work, is the fabrication of magnetic nanoparticles coated with a functional layer having dual-action self-heating and antibacterial effects. Magnetic fields have full penetration in the human body, and by directly heating the particles themselves, no damage is incurred on the surrounding tissues. The multi-functional particles could also be conveniently guided from a convenient injection site to the site of internal infection without additional incisions and risks for re-infection.

2. Materials and methods

2.1. Materials

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ammonium hydroxide (26% NH_3 in H_2O), oleic acid, methylcellulose (M.W. 88,000), poly(ethyleneimine) solution ~50% in H_2O (M.W. 600,000–1,000,000) were all commercial products from Sigma–Aldrich, Germany.

2.2. Preparation of magnetite coated with oleic acid (OA-MNP), polyethyleneimine (PEI-MNP) and polyethyleneimine-methyl cellulose (PEI-mC-MNP)

1.9 g $FeCl_2 \cdot 4H_2O$ and 5.4 g $FeCl_3 \cdot 6H_2O$ (molar ratio of 1:2) are dissolved in 100 ml distilled water with mechanical stirrer for 1 h. This sample is named as *mixture 1*. Magnetite coated with oleic acid (OA-MNP) was prepared by addition 6 ml of ammonium hydroxide to *mixture 1* at 70 °C for 30 min. Particles are transferred into a mixture of dichloromethane 50 ml and oleic acid 1 ml to form OA-MNPs. The product was washed with water (to remove impurities, such as ammonium salt). Magnetite coating with polyethyleneimine (PEI-MNP) and polyethyleneimine-methyl cellulose (PEI-mC-MNP) was carried out by addition of (4 g PEI in 50 ml distilled water) and (5 g PEI and 5 g methyl cellulose in 50 ml distilled water) to *mixture 1*, respectively. PEI-MNP and PEI-mC-MNP were prepared by addition 6 ml of ammonium hydroxide to two solutions at 70 °C for 1 h. The product was washed several times with distilled water and after that, OA-MNP, PEI-MNP and PEI-mC-MNP were dried in a rotary evaporator at 40 °C (25 mbar, 80 rpm), until forming the fine powder.

2.3. Characterization

Fourier Transform Infrared (FTIR) was performed by Bruker Tensor 27 Infrared Spectrometer. Thermogravimetric Analysis (TGA) was measured by TA Instruments Q500, while zeta potential measurements were performed using a Zetasizer Nano analyzer (Malvern Instruments, USA) at pH = 7. Dynamic Light Scattering (DLS) analysis was employed to measure the hydrodynamic diameters of magnetic nanoparticle aggregates in DI water using a Zetasizer Nano DLS unit. Microscopy images were obtained through scanning electron microscopy (SEM) using a Zeiss ULTRA Plus field-emission SEM equipped with a Schottky cathode. The images were analyzed using Smart SEM software v5.05 (Zeiss, Germany) for imaging operated at 1.5 kV. Magnetic properties were measured by vibrating sample magnetometer (VSM), heating properties of the samples were measured by Cheltenham induction heating limited with constant frequency (142 kHz) and power (1, 0.7, 0.5, 0.3, 0.1 kW) and the temperature was measured by infrared thermometer.

2.4. Bacterial strains

Bacterial strains of Gram-negative *Escherichia coli* CCM 3954 and Gram-positive *S. aureus* CCM 3953 were obtained from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic. The bacterial inocula were always prepared fresh from a single colony growing overnight in a soya nutrient broth (Sigma Aldrich) at 37 °C. The culture was diluted to achieve optical density OD = 0.01–0.02 at 600 nm (OD_{600}). The growth rate was monitored by using a UV–Vis spectrophotometer (Hach Lange DR6000, Germany) to measure the increased scattering.

2.5. Bacterial growth rate exposed to MNPs

The freshly prepared bacterial cultures were transferred to 30 ml of soya broth and kept in 200 ml conical flasks. The MNPs stock suspensions (10 g/l) were added to the bacterial culture at a range of final concentrations, i.e. 50, 500 and 1000 mg/l. A 250 μl was then transferred to each well of 96-well plate. Negative controls: bacterial cells only in growth media and magnetic nanoparticles only in growth media were run in parallel. Each sample was prepared in duplicates. The plate was incubated at 37 °C and optical density of the samples was measured at 600 nm (OD600) every 2 h by using a Multi-Mode Microplate Reader (Synergy™ HT, Biotek). The bacterial growth rate (h^{-1}) was defined by a linear regression of cell density measurement (OD600) versus incubation time (hours).

As an earlier publication, the effect of nanoparticle concentration on bacterial growth rate (μ) was calculated for each nanoparticle type, based on Eq. (1):

$$(I\%) = (\mu_C - \mu_T)/\mu_C \times 100 \quad (1)$$

[30] where I is inhibition, μ_C is the mean value of growth rate of the control, and μ_T value is the growth rate of the culture affected by the nanoparticles. The EC10 value (effective concentration for 10% inhibition) was obtained by plotting I% versus concentration of nanoparticle tested.

2.6. Cell viability

In parallel with the determination of bacterial growth rate, bacterial viability test was carried out in the same MNP concentrations as described above. Each bacteria-MNP sample was prepared in replicates and cultures without nanoparticles were cultivated in growth media as controls. Bacterial samples, including MNP were centrifuged at 5000 rpm for 5 min after 24 h incubation at 37 °C. The cell pellet was washed and resuspended in physiological solution (0.85% of NaCl). After that, the cells were stained (Live/Dead BacLight kit L7007, Life Technologies) for 15 min in dark to distinguish viable and non-viable cells. Viable cells showed green fluorescence and non-viable cells were of red fluorescence. The viable and non-viable cells were observed in fluorescence microscope (AxioImager, Zeiss, Germany) with excitation 470 nm, emission 490–700 nm and counted by Zeiss AxioVision software. A range of 200 *E. coli* cells or *S. aureus* clusters (colonies) was inspected to get the ratio of dead cells versus live cells.

2.7. Determination of S. aureus biofilm

The biofilm assay with crystal violet staining was previously described [31]. *S. aureus* culture exposed to MNP as described for the growth rate test was further incubated for 24 h in 96-well plate. The growth media solution was then removed followed by drying the 96-well plate on air for 10 min. The formed biofilms were fixed with methanol and stained with 0.1% of crystal violet aqueous solution for 15 min. The staining solution