



Synthesis and cytotoxicity study of magnesium ferrite-gold core-shell nanoparticles



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ABSTRACT

In this work, the core-magnesium ferrite (MgFe_2O_4) nanoparticles were prepared by hydrothermal technique. Completed gold (Au) shell coating on the surfaces of MgFe_2O_4 nanoparticles was obtained by varying core/shell ratios via a reduction method. Phase identification, morphological evolution, optical properties, magnetic properties and cytotoxicity to mammalian cells of these MgFe_2O_4 core coated with Au nanoparticles were examined by using a combination of X-ray diffraction, scanning electron microscopy, transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy, UV-visible spectroscopy (UV-vis), vibrating sample magnetometry and resazurin microplate assay techniques. In general, TEM images revealed different sizes of the core-shell nanoparticles generated from various core/shell ratios and confirmed the completed Au shell coating on MgFe_2O_4 core nanoparticles via suitable core/shell ratio with particle size less than 100 nm. The core-shell nanoparticle size and the quality of coating influence the optical properties of the products. The UV-vis spectra of complete coated MgFe_2O_4 -Au core-shell nanoparticles exhibit the absorption bands in the near-Infrared (NIR) region indicating high potential for therapeutic applications. Based on the magnetic property measurement, it was found that the obtained MgFe_2O_4 -Au core-shell nanoparticles still exhibit superparamagnetism with lower saturation magnetization value, compared with MgFe_2O_4 core. Both of MgFe_2O_4 and MgFe_2O_4 -Au core-shell also showed *in vitro* non-cytotoxicity to mouse areola fibroblast (L-929) cell line.

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1. Introduction

Recently, there have been enormous publications related to magnetic nanoparticle-based hyperthermia materials [1] due to heat generating ability of these filled nanoparticles in tumor cells when applying alternating current magnetic field from external coils [2]. Specific magnetic properties like superparamagnetism and defined size of magnetic nanoparticles (MNPs) are important properties for use in this biomedical application to create heating spot for destroying the tumor cells and/or to release the drugs either attached to the MNPs or embedded inside the matrix of thermosensitive polymer matrices at the target area [3]. In addition, superparamagnetism prevents the aggregation of MNPs [4]. The MNPs size also plays an important role in the penetration to target tissue (cancerous tissue) through small vascular pores between tumor endothelial cells of blood vessels [4] and in the uptake by phagocytes system when the MNPs are too large [5]. Due to these important criterions, the total size of MNPs should be smaller than

100 nm [5–7]. In addition, biocompatibility improvement of synthesized MNPs must be considered in order to reduce toxicity for healthy cells when the MNPs are injected into the patient body. Consequently, surface modification or coating of the synthesized MNPs by biocompatible materials should be considered. From our previous work [8], we successfully synthesized MgFe_2O_4 nanoparticles (MgFe_2O_4 NPs), one kind of the soft magnetic materials [9], by hydrothermal technique with superparamagnetism and the smallest size of about 65 nm. Finding out the optimal method to synthesize the biocompatible MgFe_2O_4 NPs followed by characterization for further utilization is therefore interesting. Moreover, core-shell systems or core-shell structures are challenging due to their multifunctionality originated from combination of different properties generated by core and shell (*i.e.* different material compositions can be accumulated into single particles) [3]. So far, there are many kinds of shell materials normally be used to coat MNPs, such as silica [10] and gold nanoparticles (AuNPs) [11]. Even though, both of them are biocompatible but silica could affect the saturation magnetization value of MNPs because of the diamagnetic contribution of the thick silica shell [12]. Thus, the AuNPs seem to be a better choice for coating on MNPs because AuNPs are inert and non-

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toxic [13], ease of synthesis [14], ready functionalization via amine/thiol linkages [15,16] and have photophysical properties that could stimulate drug release at remote area [17]. In general, tissue and blood are transparent in the NIR electromagnetic (EM) spectrum. Thus, some gold shells which can display absorbance properties at this EM region will be the possible candidates for the applications of therapeutics [18, 19]. In connection with this, many researchers have focused on synthesis of Fe₃O₄-Au core-shell NPs with appropriate properties for drug delivery and hyperthermia treatment applications [20–22]. However, to date, the potential of MgFe₂O₄-Au core-shell NPs for these applications is not available in literatures. Similar to other gold-coated magnetic nanoparticles [23,24], gold shells are considered as oxidation and corrosion protecting layers for MgFe₂O₄ core, exhibiting good biocompatibility and providing further functionalization on their surfaces. Where superparamagnetic MgFe₂O₄ provides magnetic inducibility facilitated for delivery and hyperthermia properties converted from incident alternating magnetic field, AuNPs also could absorb the incident energy from alternating magnetic field, NIR light, etc. and then convert the energy to heat. Thus, a combination of MgFe₂O₄ and Au in the form of core-shell structure is promising as high performance hyperthermia materials and/or drug release materials induced from thermosensitive encapsulating polymer degradation [3,20]. In addition, some related works had been only focused on using MgFe₂O₄ NPs as supporting materials, due to their capability of magnetic separation, for AuNPs decorating in case of catalytic application [25,26]. In continuation of our prior studies where we had successfully synthesized MgFe₂O₄ NPs as mentioned earlier, the aim of the present work therefore is to prepare MgFe₂O₄-Au NPs with core-shell structure and then investigate their physical, chemical and biological properties. A combination of X-ray diffraction, scanning electron microscopy, transmission electron microscopy, energy-dispersive X-ray spectroscopy, UV–visible spectroscopy, vibrating sample magnetometry and resazurin microplate assay techniques were used to reveal phase identification, morphological evolution, chemical compositions, optical properties, magnetic properties and *in vitro* cytotoxicity to mammalian cells (L-929) of the synthesized core-shell nanoparticles as a function of core/shell ratio.

2. Experimental

2.1. Synthesis of MgFe₂O₄ NPs

In this work, pure phase of the MgFe₂O₄ NPs with superparamagnetism and smallest average size (65 ± 8 nm), as reported earlier [8] was synthesized by using a hydrothermal method [27]. In brief, 1 mmol of Mg(NO₃)₂·6H₂O (Loba Chemie, 99% purity) and 2 mmol of Fe(NO₃)₃·9H₂O (Carlo Erba, 98% purity) were dissolved in 30 mL ethylene glycol (Carlo Erba, 98% purity). The 15 mmol of CH₃COONa (Loba Chemie, 99.5% purity) was added into the mixture solution and then transferred to a Teflon-lined stainless steel autoclave (HP series 5500 compact reactor). After heating inside the autoclave at 180 °C for 14 h [8], the precipitates were collected by magnetic separator, washed three times with deionized water, another three times with ethyl alcohol and dried at 70 °C for 12 h [28].

A combination of X-ray diffraction (XRD; Bruker D2 phaser diffractometer) technique, scanning electron microscopy (SEM; JEOL JSM-6335F), transmission electron microscopy (TEM; JEOL JEM-2010) and energy-dispersive X-ray (EDX) analyzer was used to reveal their phases, morphologies, selected area electron diffraction (SAED) patterns and chemical compositions of the obtained products. Finally, magnetization measurements were carried out at room temperature using an in-house developed vibrational sample magnetometer (VSM). Crystallinity degree by XRD amorphous subtraction method is calculated as the ratio between the area of the crystalline contribution and the total area (crystalline + amorphous) [29].

2.2. Synthesis of MgFe₂O₄-Au core-shell NPs

A schematic illustration of the synthesis is provided in Fig. 1. The details of synthesis are described as following sections.

2.2.1. Surface modification of MgFe₂O₄ NPs

0.05 g of synthesized MgFe₂O₄ NPs obtained from 2.1 was dispersed in 50 ml of absolute ethyl alcohol (J.T. Baker, 99.9% purity) in three-necked round bottom flask. After N₂ gas flow for 15 min, 0.5 ml of 3-Aminopropyltriethoxysilane, APTES (Sigma-Aldrich, 99% purity) was slowly added to the suspension. The mixture was refluxed at 70 °C for 1.5 h then cooled to room temperature. The precipitates were collected by centrifugation and washed at least 2 times with absolute ethyl alcohol. The final product was kept in 10 ml of absolute ethyl alcohol. The Fourier transform infrared spectroscopy (FTIR; Bruker TENSOR 27) was used to characterize the functionalization of APTES on the surface of MgFe₂O₄ NPs.

2.2.2. Attachment of AuNPs on surface modified MgFe₂O₄ NPs

Small colloidal AuNPs with average size ~2 nm were synthesized by using Duff and Baiker's method [30]. Briefly, 0.5 ml of 1 M NaOH (Ajax Finechem, 99% purity) and 1.0 ml of 0.067 M Tetrakis (hydroxymethyl) phosphonium chloride, THPC solution (Sigma-Aldrich, 80% in water) were added into 45 ml of deionized water. The solution was stirred for 5 min and then 2.00 ml of 1% HAuCl₄ (Sigma-Aldrich, 99.999% purity) was added and continuously stirred for 30 min providing aqueous AuNPs. To attach small colloidal AuNPs on MgFe₂O₄ NPs, 0.5 ml of surface modified MgFe₂O₄ NPs obtained from 2.2.1 was added into 30 ml of the as-synthesized aqueous AuNPs. The mixture was left overnight and then centrifuged for 10 min. The precipitates were washed once by centrifugation and redispersed into 25 ml with deionized water, defined as MgFe₂O₄-Au NPs. UV–visible spectroscopy (UV–vis; PerkinElmer LAMBDA 25) was employed to monitor the optical properties of the MgFe₂O₄-Au NPs. A combination of the SEM, TEM and EDX techniques was performed to reveal their morphologies and chemical compositions.

2.2.3. Growth of Au shell

The MgFe₂O₄-Au NPs cores were coated with the Au shell by following Pham *et al.* work [31]. The K-gold solution was used as gold source. The K-gold solution was prepared by dissolving 0.18 mmol of K₂CO₃ (Fisher Scientific, 99.87% purity) in 100 ml of deionized water. After stirring the solution for 10 min, 2.00 ml of 1% HAuCl₄ was added into the stirred solution. The solution mixture changed from yellow to colorless within 30 min. Various amounts of MgFe₂O₄-Au NPs core were then mixed with the 4 ml of K-gold solution by using homemade mechanical stirrer, following by adding 20 μl of formaldehyde (Ajax Finechem, 38% w/w). In order to optimize the preparation condition of shell coating, various amounts of MgFe₂O₄-AuNPs core *i.e.* 0.05, 0.2, 0.3, 0.4, 0.5, 1.0 and 2.0 ml were designed. The mixture color changes from yellow brown to purple within 15 min. The mixture was stirred for 30 min with final color in red to red brown. The precipitates were separated by magnetic separation and washed twice by deionized water. The final product was redispersed in deionized water to give black colloidal MgFe₂O₄-Au core-shell NPs. XRD technique was performed for phase identification. A combination of the SEM, TEM and EDX techniques was employed to identify their morphologies, SAED patterns and estimated chemical compositions of the products. Room temperature magnetization measurements were carried out using an in-house developed VSM. UV–vis technique was carried out to reveal the optical properties of the samples.

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