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Synthesis and *in vitro* cellular interactions of superparamagnetic iron nanoparticles with a crystalline gold shell

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ABSTRACT

Fe@Au core–shell nanoparticles (NPs) exhibit multiple functionalities enabling their effective use in applications such as medical imaging and drug delivery. In this work, a novel synthetic method was developed and optimized for the synthesis of highly stable, monodisperse Fe@Au NPs of average diameter ~24 nm exhibiting magneto-plasmonic characteristics. Fe@Au NPs were characterized by a wide range of experimental techniques, including scanning (transmission) electron microscopy (S(T)EM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), energy dispersive X-ray spectroscopy (EDX), dynamic light scattering (DLS) and UV–vis spectroscopy. The formed particles comprise an amorphous iron core with a crystalline Au shell of tunable thickness, and retain the superparamagnetic properties at room temperature after formation of a crystalline Au shell. After surface modification, PEGylated Fe@Au NPs were used for *in vitro* studies on olfactory ensheathing cells (OECs) and human neural stem cells (hNSCs). No adverse effects of the Fe@Au particles were observed post-labeling, both cell types retaining normal morphology, viability, proliferation, and motility. It can be concluded that no appreciable toxic effects on both cell types, coupled with multifunctionality and chemical stability make them ideal candidates for therapeutic as well as diagnostic applications.

1. Introduction

Synthesis of magnetic nanoparticles (MNPs) of tunable sizes has received a great scientific attention owing to their applications in targeted drug delivery, magnetic resonance imaging (MRI) and treatment of cancer by hyperthermia [1–8]. A common property exploited in life sciences and biomedicine is superparamagnetism allowing the alignment of all spins under an external magnetic field [9]. Despite their tremendous use in biomedical applications, MNPs often tend to aggregate due to strong interparticle dipolar interactions in high ionic strength environment of biological solutions [10]. This causes the enlargement of nanoparticle size, influencing their magnetic properties and limiting their practical use. While MNPs with higher magnetic moment and higher anisotropy would be ideal to improve their performance and dosage, they are often hampered by a low degree of chemical stability, increased toxicity coupled with low plasma half life [11]. Therefore, a major challenge is to increase solution stability and reduce NP toxicity without compromising their magnetic properties.

To overcome these limitations, several procedures have been reported whereby the MNPs are modified with a thin layer of polymers/organic molecules, metal oxide or a metal [12–14]. Among these, a thin protective shell of gold (Au) around the MNPs provides high stability due to its chemical inertness, low cytotoxicity, simple bioconjugation through well-understood surface chemistry such as Au–S [15], and high catalytic activity while supported on metal or metal oxide supports [16]. Upon further functionalization with molecules such as PEG (poly (ethylene glycol)), NP aggregation can also be prevented, besides exploiting cloaking properties of PEG [17]. Au NPs display tunable and environmentally sensitive localized surface plasmon resonance (LSPR) within the visible range, which makes them suitable candidates for biosensors, and

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good contrast agents for computed tomography (CT) [18,19] as well as photo-acoustic imaging [20]. Thus, core@shell NPs formed by unifying a nanoscale magnetic core within a thin metallic shell can act as a dual contrast agent for MRI and CT. To date, various approaches have been developed for the synthesis of MNPs@Au, such as hydroxyl amine seeding, reverse micelle templating, attachment of Au NPs onto amino-silane modified iron oxide NPs, laser ablation, sonochemical reaction, γ-ray radiation, etc. [8,21–24].

Despite a small number of successful approaches for the synthesis of MNPs@Au and the exploration of their applications in different areas, many issues related to the precise control of Au shell thickness, and the detailed characterization of resultant MNPs@Au need to be addressed. In addition, some limitations associated with earlier methods also include time-consuming purification steps, intricate sequence of chemical reactions, broad size distribution, and poor magnetic responses due to uncontrolled or uneven coating of Au shell around MNPs [25]. The control over size of the resultant MNPs@Au is very important which not only has a pronounced effect on toxicity and retention but also on mode of administration. In some cases, successive gold coating steps are needed to ensure sufficiently stable Fe@Au NPs [26]. Remaining solvents or surfactants may also result in opsonization *in vivo* and other potential side effects, and thus biocompatibility becomes a serious concern [27].

Here, we report a new synthetic procedure for the formation of Fe@Au NPs with a crystalline Au shell on amorphous Fe NPs, their solution and magnetic properties, as well as their interaction with two cell lines. Our approach provides control over Au shell thickness *via* tuning the concentration of Au salt in the solution. First, Fe NPs were produced *via* thermal decomposition of iron pentacarbonyl ($Fe(CO)_5$) in the presence of oleylamine (OAm) [28]. Later, these NPs were transferred to an aqueous phase, and a shell of Au was grown over presynthesized Fe NPs seeds *via* reduction of Au salt in the presence of sodium citrate. Scanning (transmission) electron microscopy (S(T)EM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), energy dispersive X-ray spectroscopy (EDX), dynamic light scattering (DLS), and ultraviolet–visible spectroscopy (UV–vis) were used to characterize the Fe@Au NPs. These NPs were subsequently functionalized with O-[2-(3-Mercaptopropionylamino)ethyl]-O′-methylpolyethylene glycol (PEG-SH) molecules which have been used at various concentrations for *in vitro* labeling of two different cell types which are promising candidates for regenerative therapy of the central nervous system: [29,30] olfactory ensheathing cells (OECs) and human neural stem cells (hNSCs). These two cell types differed in terms of uptake and localization of the Fe@Au NPs post-labeling, while no cytotoxic effects were observed irrespective of label concentration or length of co-incubation with the NPs. The magnetic properties of Fe, Fe@Au, and PEGylated Fe@Au NPs were measured by Quantum Design MPMS system indicating supermagnetic behavior. Thus, Fe@Au NPs were found to be have low cytotoxicity, aptly suited for a wide array of applications including bioimaging, drug delivery and other biodiagnostic and/or biomedical applications.

2. Materials and methods

Iron pentacarbonyl ($Fe(CO)_5$, 99.99%), octadecene (ODE, 90%), oleylamine (OAm, 70%), chloroauric acid (99.999%), sodium citrate, O-[2-(3-Mercaptopropionylamino)ethyl]-O′-methylpolyethylene glycol (PEG-SH) of molecular weight 5000 Da were purchased from Sigma–Aldrich.

2.1. Synthesis of Fe NPs

Fe NPs were synthesized *via* thermal decomposition of $Fe(CO)_5$ in ODE in the presence of OAm. The reaction scheme modified from the one reported by Sun et al. [31] is detailed herein. In essence, a mixture of ODE (50 mL) and OAm (740 μL) was degassed under Ar atmosphere and vigorous stirring at 120 °C for 30 min. The temperature was raised to 180 °C and 1.8 mL of $Fe(CO)_5$ was injected into the hot reaction mixture and the reaction was continued for 20 min. After cooling down to room temperature, the supernatant was decanted and the magnetic bar coated with Fe NPs was washed with 20 mL hexane and 40 mL acetone. Fe NPs were magnetically separated, and the product was washed two times with 20 mL acetone. Subsequently, these Fe NPs were dried in a stream of nitrogen.

2.2. Synthesis of Fe@Au NPs

The schematic protocol for Fe@Au NPs synthesis is illustrated in Fig. 1. 5 mg of the as synthesized Fe NPs were dissolved in 10 mL of 10 mM sodium citrate solution using sonication at 80 °C for half an hour. Citrate stabilized Fe seed solution (brown solution) was added to a 50 mL reaction flask and the resultant solution was maintained to 70 °C under mild stirring. 10 mL of 1.5 mM chloroauric acid (the concentration of the gold precursor was optimized by performing experiments at concentrations both below and above this experimental value) was added dropwise and allowed to react for 20 min under vigorous stirring. The solution turned purplish red around 8 min after reaction. Thereafter, the solution was cooled down to room temperature, and Fe@Au NPs were magnetically separated to remove free Au NPs. A video of the synthesis protocol showing the color change during the course of the reaction and influence of the magnet on the final product can be found in the supporting information.

2.3. PEG coating of Fe@Au NPs

Two milligrams of PEG-SH was mixed with 5 mg of the as synthesized Fe@Au NPs dissolved in 5 mL of MQ water and stirred for 1 h to covalently modify the surface of the NPs [32]. The resulting PEG coated Fe@Au NPs were collected by centrifugation at 10,000 rpm for 20 min and washed twice with MQ water. These

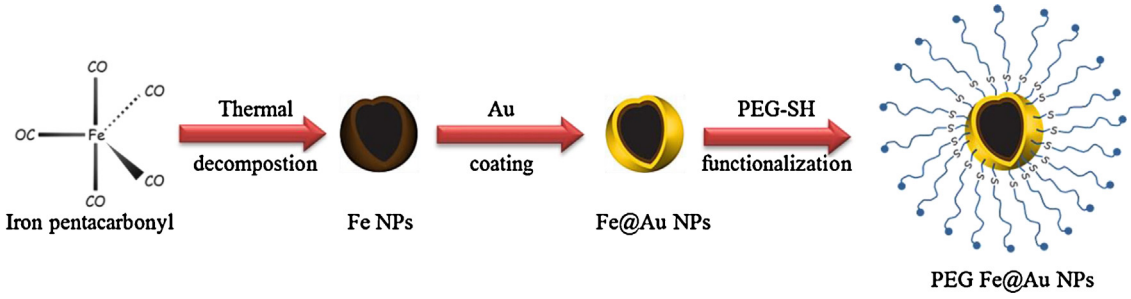

Fig. 1. Simplified schematic showing sequence of synthesis of PEG modified Fe@Au NPs.