Magnetic Gold Nanoparticles: Synthesis, Characterization and Its Application in the Delivery of FITC Into KG-1 Cells

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In this article, we report a new method—a sonication method to disperse iron oxide nanoparticles into smaller nanoparticles and make gold ions absorb onto the surface or trapped in the micropores of the iron oxide nanoparticles using sonication action. By using quick reduction of ascorbic acid and post-HCl solution treatment, gold covered magnetic nanoparticles (mGNPs) with spherical morphology and uniform size were synthesized in a water solution. The size of the mGNPs was found to be 20–30 nm. Some ideal mGNPs possessed a core–shell structure. The mGNPs were non-cytotoxic and mGNP-fluorescein isothiocyanate (FITC) can enter KG-1 cells when driven by an external magnetic force, which was confirmed by confocal imaging. The confocal image also showed the FITC inside the KG-1 cells was near the nucleus. The fluorescein isothiocyanate (FITC) delivery efficiency is about 100% according to the flow cytometry results.

Keywords: Magnetic Gold Nanoparticles, Synthesis, Characterization, Fluorescein Isothiocyanate Delivery, KG-1 Cells.

1. INTRODUCTION

In recent years, researchers have paid more attention to synthesizing novel nanomaterials and applying them in various biomedical applications,¹,² such as making gold nanoparticles for drug and gene delivery into mammalian cell lines³ and microbial detection.⁴ For some cell lines such as KG-1 cells, it is difficult to ensure sufficient cellular uptake, and magnetic force can be used to help pull nanoparticles into these cells.³,⁶ Using magnetic nanoparticles as special biomolecule carriers is promising. For instance, magnetic nanoparticles were used in enzyme, protein, and DNA transport. They were also used in the targeted delivery of anti-cancer drugs through magnetically controlled force.⁷ However, due to the non-biocompatibility of magnetic iron oxide, its application is limited.

Gold nanoparticles, on the other hand, are biocompatible materials. Gold covered magnetic nanoparticles (mGNPs) are one of the most desired nanomaterials for biological applications, especially in the areas of drug and gene delivery, MRI imaging and CT imaging. However, synthesis of mGNPs is difficult, and only a few research papers have been published. The ideal structure for gold coated iron oxide nanomaterials should be a core–shell structure. Lin et al. prepared so-called Fe@Au nanoparticles via a reverse micelle mechanism. They made 1-dodecanethiol (C₁₂H₂₅SH) bound onto the gold surface to avoid aggregation.⁸ Zelenáková et al. also reported the synthesis and characteristics of gold coated magnetic nanoparticles via a reverse micelle method.⁹ Robinson et al. synthesized gold coated magnetic nanoparticles using toluene as solvent via a reverse micelle method.¹⁰ Gilles et al. reported gold-coated magnetic nanoparticles as a DNA sensor.¹¹ Fan et al. synthesized gold-coated magnetic nanoparticles using the normal method mentioned above, took advantage of a magnetic separation/mixing process and used gold-coated magnetic beads for immunoassay development.¹² Seino et al. reported the magnetic nanocarrier composed of gold and iron oxide using Gamma-ray.¹³

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In this paper, we exploited a sonication method to make magnetic iron oxide nanoparticles into smaller nanoparticles that were 15–20 nm in diameter. We also utilized this sonication method to make gold ions in a H\text{AuCl}_4 solution adsorb onto the surface or become trapped inside the micropores of the iron oxide nanoparticles. In this method, the smaller iron oxide nanoparticles are crystal seeds, and the metallic gold was produced on the surface or outside of iron oxide nanoparticles using ascorbic acid as reduction agent and aggregated together to form a shell on the surface of the iron oxide nanoparticles. The shape and size of gold coated iron oxide produced was uniform, round with sizes ranging from about 20–30 nm. Some have ideal core–shell structure.

Delivering biomolecules or drugs into the KG-1 cell line is difficult due to its small size and poor cellular uptake. Few researches have reported instances of KG-1 uptake. We took advantage of our mGNPs covalently linked with FITC (fluorescein isothiocyanate) through a polyethylene glycol (PEG) bridge and successfully delivered the FITC into the KG-1 cell line by driving the mGNPs using an external magnetic force, which was confirmed using confocal imaging. FACS (Flow Cytometry) results showed that the FITC delivery efficiency is about 100%. Due to the presence of gold, mGNPs can be covered by thiol PEG, and are non-cytotoxic.

2. MATERIALS AND METHODS

2.1. Chemicals

The sodium citrate trihydrate, chloroauric acid, ascorbic acid, fluorescein isothiocyanate (FITC), dimethylformamide (DMF) and sodium dodecyl sulfate (SDS) used in this study were from Sigma-Aldrich. Iscove’s Modified Dulbecco’s Medium (IMDM), Fetal Bovine Serum and Penicillin/streptomycin used are from GIBCO. Thiol polyethylene glycol (PEG) with amino functional group was purchased from NANOCS company with molecular weight 5000.

2.2. Cells

KG-1, acute human leukemia cell lines were purchased from the American Type Culture Collection (ATCC HTB22, Rockville, MD. USA).

2.3. Synthesis of Magnetic Gold Nanoparticles (mGNPs)

The following procedures outline the synthesis of mGNPs. (1) Synthesis of iron nanoparticles: 2.78 g of Iron(II) sulfate heptahydrate and 3.25 g of Iron(III) chloride hexahydrate were transferred into to a clean 125 ml conical flask containing 25 ml of MilliQ high purity de-ionized water. 0.85 ml of concentrated HCl was transferred into the flask. This solution was added dropwise into 250 ml of 1.0 N NaOH solution until a black solution was obtained. 400 \mu l of the black solution was diluted to 80 ml using MilliQ high purity de-ionized water, and was sonicated for 2 hours. (2) Synthesis of mGNPs: 1 ml of 25 mM chloroauric acid and 2 ml of 20% sodium dodecyl sulfate solution (SDS) were transferred to a clean 20 ml vial containing 16 ml of MilliQ high purity de-ionized water. 1 ml of iron nanoparticle solution prepared above and 300 \mu l of the above H\text{AuCl}_4 solution were transferred into a 20 ml vial. The vial was sonicated for 15 min. Meanwhile, a solution of ascorbic acid (AA) was prepared by dissolving 0.0400 g of AA powder in 20 ml of MilliQ water. 180 \mu l of AA solution was transferred into the vial and stirred for 30 min. 200 \mu l of 10% HCl solution was transferred into this vial and stirred for an additional 30 min. The nanoparticles were separated by an magnet into mGNPs and GNPs.

2.4. Synthesis of mGNP-FITC

(1) 0.0116 g HS-PEG-NH₂ (MW 5000) was dissolved into a 20 ml vial containing 10 ml of MilliQ water. 1 ml of the above mGNP solution was transferred into this vial and stirred for 5 min. This vial was kept at 4 °C overnight. (2) The solution was centrifuged at 10000 rpm for 30 min. The supernatant was discarded and the sediment was washed once using the same centrifuge conditions. The sediment was dissolved in 0.5 ml of MilliQ water (mGNP solution). Meanwhile, 100 mg FITC was dissolved into 0.5 ml of DMF, and then mixed with above mGNP solution. The mixture was stirred for 5 minutes before being kept at room temperature overnight. The mixture was dialyzed until no free FITC in solution remained.

2.5. Cell Culture and Magnetic-Field-Driven Cellular Uptake Experiment

KG-1 cells with a density of 5 \times 10^5 cells per plate were placed in poly-L-lysine (10 \mu g ml⁻¹)-coated 35 mm culture dishes and incubated for 45 min at 37 °C, 5% CO₂. The magnetic-field-driven delivery method was to place a culture dish containing 1 ml IMDM media with 18.8 nmol Au ml⁻¹ of mGNP-FITC or mGNP on the top of an Nd–Fe–B permanent magnet for 2–6 hrs, then the culture dish was put back in incubator overnight. The uptake experiment was terminated by washing the cells with PBS buffer.

2.6. MTS Experiment

(1) 30,000 cells were seeded per well in 96-well plates. The experiment was conducted in quadruplicate. (2) mGNP stock solution was diluted in growth medium to concentrations of 4.7, 9.4, 18.8, 37.5, and 75 nmol Au ml⁻¹.

(3) 200 µl of mGNP-FITC containing growth medium was added per well and the 96-well plates were put back into the incubator to continue culture for 24 and 48 hrs. (4) 20 µl of MTS solution was added (5 mg ml⁻¹ in 1× DPBS), then the cells were incubated for additional 3 hrs. (5) Absorbance at 490 nm was measured.

2.7. Flow Cytometry Measurement

KG-1 cells exposed to mGNP-FITC for different amounts of time on magnets were collected and centrifuged at 1200 rpm for 10 min. The collected cells were extensively washed using PBS and then fixed in 1% paraformaldehyde and resuspended in 400 µl of PBS. The mGNP-FITC delivery efficiency was evaluated with Flow Cytometry (FACscan, Becton-Dickinson, San Jose, CA, USA) at an excitation wavelength of 488 nm.

2.8. Atomic Force Microscope (AFM) Image

A small amount of sample solution was directly transferred dropwise onto a silicon wafer. The sample was covered and kept at room temperature until the solution was dry. AFM images were taken using a Veeco Multimode V SPM operating in tapping mode.

2.9. Fluorescent Microscopy

The fluorescent images were taken by using Fluorescent Microscopy of Leica CW 225 A with Nikon digital camera DXM1200.

2.10. Confocal Microscope Images

KG-1 cells were seeded at a density of 1 × 10⁵ cells cm⁻² on cover slips previously coated with poly-L-lysine (10 µg ml⁻¹) for 45 min at 37 °C, 5% CO₂. The cells were exposed to 18.8 nmol Au ml⁻¹ mGNP-FITC and mGNP (the control) on an Nd–Fe–B permanent magnet. Uptake was terminated by washing the cells twice with ice-cold PBS. After 4 hrs of incubation on an Nd–Fe–B permanent magnet, the cells was incubated in an incubator for an additional 12 hours, then fixed in 2% paraformaldehyde, stained and examined under a confocal laser scanning microscope (Quorum Wave FX-Sinning Disk) equipped with imaging software – Hamamatsu EMCCD (C9100-13).

Fig. 1. Synthesis of iron oxide nanoparticles. (A) Solution of magnetic iron oxide nanoparticles. (B) Solution of magnetic iron oxide nanoparticles beside a magnet. We can clearly see the nanoparticles were driven towards the magnet side. (C) AFM image of 15–20 nm magnetic iron oxide nanoparticles. (D) TEM-scan image of 15–20 nm magnetic iron oxide nanoparticles. (E) AFM analysis showing the vertical height (15 to 20 nm) of the nanoparticles in image C.
2.11. TEM Image

The TEM images were taken using Philips-FEI Morgagni 268 instrument, and operated at 80 kV. The sample solution was deposited on the copper support coating with carbon.

3. RESULTS AND DISCUSSION

3.1. Synthesis of mGNPs

The synthesis of mGNPs consists of two steps. The first step was to synthesize iron oxide nanoparticles with suitable size. Figure 1 shows the design process and characterization of the iron oxide nanoparticles, and we followed this typical method to synthesize iron oxide nanoparticles. The color of the iron oxide nanoparticle solution was black (Fig. 1(A)). When put a magnet beside the solution, the iron oxide nanoparticles quickly moved towards the magnet (Fig. 1(B)), which conformed the magnetism of iron oxide nanoparticles. The iron oxide nanoparticles were big enough to let us see that they migrated towards magnet. However, this size is too big for the creation of mGNPs so we have to make them become smaller. This problem can be solved by using sonication treatment. After sonication treatment, the black-colored solution of magnetic iron oxide nanoparticles became light yellow (in the middle of Fig. 1). According to AFM and TEM images (Figs. 1(C and D)), the size of iron oxide nanoparticles was about 15–20 nm. The morphology was uneven. AFM analysis of the vertical height of the particles also gave a similar result (Fig. 1(E)). The second step was to synthesize mGNPs. The synthesis of these mGNPs is shown in Figure 2. By sonicating the mixture of HAuCl₄, surfactant SDS (sodium dodecyl sulfate) and the 15–20 nm iron oxide nanoparticles, the gold ions were adsorbed on the surface or trapped inside the micropores of the iron oxide nanoparticles. During the sonication treatment, the size of iron nanoparticles became smaller due to weak acid solution (from HAuCl₄). After quick reduction, the gold ions became gold nanoparticles and aggregated together due to the instability of nanoparticles. Some aggregated gold nanoparticles formed a shell outside iron oxide nanoparticles; some aggregated together surrounding iron oxide nanoparticles (Fig. 1(A)). The solution was purple and the absorbance in the UV-Vis spectrum was at 556 nm, which indicates the nanoparticles were bigger (Fig. 1(B)). The size indicated using this UV-Vis spectrum method should reflect the mean size of the gold, iron oxide and their aggregation. After the purple solution was treated using a 5% HCl solution, the solution became red and the absorbance in UV-Vis spectrum was at 532 nm, which indicated the nanoparticles were about 20–30 nm (Fig. 1(D)) according to the normal UV spectrum character of gold nanoparticles. During this treatment, the most iron oxide outside the nanoparticles were dissolved and removed; only the iron oxide inside
the nanoparticles remained. Therefore, the cluster of iron oxide and gold nanoparticles was broken, the aggregation of nanoparticles was dispersed into smaller nanoparticles. Because the metallic gold was formed on the surface or inside the micropores of iron oxide nanoparticles, the iron oxide remaining must have been inside gold nanoparticles. The morphology and size of mGNPs became consistent (Fig. 2(C)). The ideal configuration would be for the metallic gold aggregated to form a shell around the surface of iron oxide nanoparticles. This structure was confirmed by the zoomed-in TEM images (Fig. 3(A)). The core–shell structure of the ideal mGNP can be clearly seen. There was a relative black shell and relative gray core. Because the contrasts of gold and iron are different in TEM image and the contrast of gold is larger, the black shell in this zoomed-in images should belong to gold and relative gray core should belong to iron oxide. Due to the spherical-like structure, there was a small amount of darker coloring in relative gray core produced by outside gold. The EDX analysis in Figure 3(B) showed that the nanoparticles in Figure 3(A) were composed of Fe and Au, which verified the core–shell structure. The schematic of the core–shell structure is shown in Scheme 1.

**Scheme 1.** Synthesis of mGNP-FITC and cell uptake for mGNP-FITC. (A) Synthetic process. (B) Cell uptake for mGNP-FITC.
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3.2. FITC Delivery into KG-1 Cell Line Using mGNPs

Scheme 1 (Fig. 4(A)) shows the linking process between mGNP and FITC molecules; and Figure 4(B) shows the cellular uptake experiment design of delivering FITC into KG-1 cells driven by an external magnetic force. By taking advantage of the gold covering the magnetic nanoparticles, we can bind PEG with mGNPs because thiol-PEG with amino functional groups can interact with gold through thiol functional groups. An FITC molecule can react with an amino functional group to form a covalent bond through an amide (Scheme 1(A)). Therefore, through PEG bridges, FITC molecules can link to the surface of mGNPs through covalent bonds which can avoid the FITC lost during uptake process. Due to the solubility of PEG, mGNP-FITC can dissolve in the culture medium of the KG-1 cell line to form a uniform solution. Therefore, after the cellular uptake experiment, most of the mGNP-FITC left on cell surfaces can be removed by completely washing the cells twice using PBS buffer, therefore, the fluorescent signals in FACS measurement should only come from the mGNP-FITC inside KG-1 cells. When the KG-1 cells with mGNP-FITC in culture medium were put on top of the magnet, the mGNP-FITC moved towards the bottom of culture dish and adsorbed on the surface of KG-1 cells. These mGNP-FITCs may have continued to move into cells due to the magnetic force and may have been engulfed by the cells themselves (Scheme 1(B)). The FACS results showed that standing for four hours on the magnet was enough for FITC delivery into cells driven by magnetic forces because no identifiable difference was observed for standing on the magnet for 4 and 6 hours (Figs. 4(A, B)), but for 2 hours, there was little left shift, which indicated more FITCs entered the cells after standing for over 4 hours. The FITC delivery efficiency was about 100% for standing for 2, 4 and 6 hours. Figure 4(C) showed the viability of KG-1 cell cultured in mGNP solution treated for 2 hours on the top of a magnet was similar to that of control cell, therefore, mGNPs is no cytotoxicity for both 24 and 48 hours among concentrations ranging from 4.7–75 nmol Au/ml using the MTS method.

In order to confirm the results of the FITC delivery into the KG-1 cell line, images from both fluorescent and confocal microscopy were taken (Fig. 5). Compared with the blue channel (checking cell nucleus), the image (Fig. 5(A)) in the green channel (fluorescent signal) of fluorescent microscopy showed that not all KG-1 cells took up the mGNP-FITCs even though the delivery efficiency from FACS was 100%. From the confocal image in Figure 5(B), we can clearly see that the green fluorescent signal surrounded the nucleus of the cells, there were some especially highlighted spots near the nucleus, which confirmed that mGNP-FITCs actually entered into KG-1 cells and migrated towards cell nucleus.

4. CONCLUSION

Sonication can disperse iron oxide nanoparticles into smaller nanoparticles and also make gold ions adsorb on the surface or become trapped in the micropores of the iron oxide nanoparticles. Through a quick reduction of ascorbic acid and post-HCl solution treatment, mGNPs with a...
uniform spherical morphology and sizes around 20–30 nm can be synthesized in a water solution. The ideal mGNPs have a core–shell structure. mGNPs were non-cytotoxic and mGNP-FITCs can enter into the KG-1 cell line, which was confirmed by the confocal images.

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References and Notes

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