Anti-Cancer Drug Loaded Iron–Gold Core–Shell Nanoparticles (Fe@Au) for Magnetic Drug Targeting

Sibnath Kayal and Raju Vijayaraghavan Ramanujan*

School of Materials Science and Engineering, Nanyang Technological University, 639798, Singapore

Magnetic drug targeting, using core–shell magnetic carrier particles loaded with anti-cancer drugs, is an emerging and significant method of cancer treatment. Gold shell-iron core nanoparticles (Fe@Au) were synthesized by the reverse micelle method with aqueous reactants, surfactant, co-surfactant and oil phase. XRD, XPS, TEM and magnetic property measurements were utilized to characterize these core–shell nanoparticles. Magnetic measurements showed that the particles were superparamagnetic at room temperature and that the saturation magnetization decreased with increasing gold concentration. The anti-cancer drug doxorubicin (DOX) was loaded onto these Fe@Au nanoparticle carriers and the drug release profiles showed that up to 25% of adsorbed drug was released in 80 h. It was found that the amine (–NH₂) group of DOX binds to the gold shell. An in vitro apparatus simulating the human circulatory system was used to determine the retention of these nanoparticle carriers when exposed to an external magnetic field. A high percentage of magnetic carriers could be retained for physiologically relevant flow speeds of fluid. The present findings show that DOX loaded gold coated iron nanoparticles are promising for magnetically targeted drug delivery.

Keywords: Core–Shell, Reverse Micelle Method, Superparamagnetic Nanoparticles, Magnetic Drug Targeting.

1. INTRODUCTION

Magnetic nanoparticles have exciting biomedical applications such as magnetic separation,¹⁻⁶ magnetic resonance imaging (MRI) contrast agents,⁷⁻¹⁵ targeted drug delivery for cancer therapy,¹⁶⁻⁴⁰ as well as conventional applications such as magnetic seals, printing, recording.³¹⁻³³ The main goal in cancer therapy is to destroy cancer cells without damaging normal cells. Conventional methods of cancer therapy such as surgery, radiation and chemotherapy are either invasive or have undesirable side effects. magnetically directed drug delivery using magnetic nanoparticles, optionally combined with hyperthermia, is a very attractive technique to improve the performance of current methods of cancer treatment.³⁴⁻⁴⁹ The strategy is to concentrate the drug loaded magnetic nanoparticle carriers at the tumor site by using an external magnetic field. The drug can then be released from the carriers either via enzymatic activity or changes in physiological conditions such as pH, osmolality, or temperature,⁴⁰ to be taken up by tumor cells. If the magnetic particles are coated with a gold shell, this shell can provide advantages such as improved computed tomography (CT) contrast,⁵¹ it can be heated by near infrared (NIR) light irradiation; the resulting heat can destroy tumor cells without damaging healthy tissues, this modality of treatment is known as hyperthermia resulting from this photothermal effect.⁴²⁻⁴⁴

Nanoparticles can exhibit novel material properties that differ considerably from those of the bulk state.⁴⁵ Magnetic nanoparticles smaller than about 30 nm can exhibit superparamagnetism, in superparamagnetic nanoparticles the magnetic moment can reorient in times less than 1 ns due to thermal agitation.⁴⁶ As the particle size decreases, the reactivity of the particles also increases and the magnetic properties are influenced by surface effects.⁴⁷ Hence, a more robust structure for nanoparticles is a core/shell configuration where the magnetic core is coated by a shell layer. Such structures are useful for studying proximity effects and ideal for structure stabilization, as the shell can protect the core from oxidation. Additionally, the shell provides a platform for surface modification, functionalization, tuning magnetic properties and biocompatibility.⁴⁸

The synthesis of gold coated magnetic nanoparticles and the study of their structural, magnetic properties have been the focus of recent experimental studies.⁴⁹⁻⁶⁰ The synthesis of gold coated iron nanoparticles (Fe@Au) is of special interest since gold provides useful surface chemistry and
Anti-Cancer Drug Loaded Iron–Gold Core–Shell Nanoparticles (Fe@Au) for Magnetic Drug Targeting

Kayal and Ramanujan

Biological reactivity. Bare iron nanoparticles cannot be directly used for drug delivery since (a) free iron induces the formation of dangerous free radicals, (b) iron nanoparticles can aggregate resulting in the formation of thromboses and (c) free iron nanoparticles are easily oxidized.

Coating the iron nanoparticles with a stable noble metal like gold results in air-stable nanoparticles which are protected from oxidation. Gold is a favored coating material because of well known synthesis procedures as well as its chemical functionality. Surface derivatization with gold also helps to reduce particle agglomeration by steric or electronic repulsion and improves biocompatibility.

Considerable interest has been directed towards the functionalization of gold nanoparticles by the use of thiol chemistry, which facilitates the attachment of biologically relevant molecules using a variety of thiol linkers. Coating the iron nanoparticles with a stable noble metal like gold results in air-stable nanoparticles which are protected from oxidation.

2. EXPERIMENTAL PROCEDURES

2.1. Materials

All chemicals were used without further purification. Chemicals used for the synthesis of gold coated iron nanoparticles were cetyltrimethylammonium bromide (CTAB) (C15H31BrN), iron (II) sulphate heptahydrate (FeSO4·7H2O), hydrogen tetrachloroaurate (HAuCl4), sodium borohydride (NaBH4), octane (C8H18) and n-butanol (C4H10O). Deionised water was used for all the experiments. CTAB was obtained from Acros Organics, FeSO4·7H2O from Fischer Chemicals, NaBH4 from Fluka Chemical, other chemicals were obtained from Aldrich. Doxorubicin hydrochloride (Aldrich) was used for the drug loading and release studies.

2.2. Synthesis of Gold Coated Iron Nanoparticles (Fe@Au)

The synthesis reaction was carried out in an oxygen free glove box in two steps, in each step predetermined amount of aqueous reactants were mixed with CTAB, n-butanol and octane to form the reverse micelle solution. The procedure and the quantity of components used for the synthesis are schematically shown in Figure 1. In a typical experiment, in a beaker (A), 0.333 g FeSO4 was dissolved in 2.4 ml deionised water and 6 g CTAB, 5 g 1-butanol and 15 g octane were added. In another beaker (B), 0.9 g NaBH4 was dissolved in 2.4 ml water and mixed with 6 g CTAB, 5 g 1-butanol and 15 g octane. Micellar solutions containing FeSO4 (beaker A) and NaBH4 (beaker B) were mixed together under stirring, color change from pale green to black was observed. NaBH4 reduces FeSO4 resulting in the formation of iron core material. Once the iron nanoparticles formed, a micellar solution containing 0.0245 (M) HAuCl4 (aq). 3 g CTAB, 2.5 g 1-butanol and 15 g octane was added followed by the addition of a micellar solution of NaBH4 (aq). Au (III) was reduced to Au (0) by NaBH4 and gold forms a coating on the outer surface of the iron particles. The volume of HAuCl4 was varied to alter the gold coating thickness. The iron containing nanoparticles were separated by placing the final reaction solution in a magnetic field. The remaining surfactants and byproducts were removed by repeated washing with 1:1 methanol/water mixture. The particles were dried under vacuum and stored, the resulting powder was black. Following the above synthesis method, three sample sets were prepared by varying the concentration of gold.
0.333 g FeSO$_4$ in 2.4 ml deionised water
6 g CTAB
5 g 1-Butanol
15 g Octane

Mix

N$_2$

Fe nanoparticles

N$_2$

0.108 g NaBH$_4$ in 1.8 ml deionised water
3 g CTAB
2.5 g 1-Butanol
15 g Octane

Fe@Au nanoparticles

XM$_L$ (X = 0, 5, 10) of 0.0245 (M) HAuCl$_4$ (aq)
3 g CTAB
2.5 g 1-Butanol
15 g Octane

Fe nanoparticles

0.9 g NaBH$_4$ in 2.4 ml deionised water
6 g CTAB
5 g 1-Butanol
15 g Octane

Fig. 1. Schematic diagram showing a typical procedure for formation of Fe@Au nanoparticles by reverse micelle technique. FeSO$_4$ and HAuCl$_4$ are reduced by NaBH$_4$, CTAB is surfactant, 1-butanol is co-surfactant and octane is oil phase.

Table I. Summary of sample sets synthesized by reverse micelle technique.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>S-1</th>
<th>S-2</th>
<th>S-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmole of Au/mmole of Fe</td>
<td>0</td>
<td>0.102</td>
<td>0.204</td>
</tr>
</tbody>
</table>

(597x424)
4 cm\(^{-1}\) resolution. FTIR samples were prepared by mixing and grinding nanopowders with KBr reference followed by pelleting. UV-visible spectra were obtained with a UV-visible spectrophotometer (Shimadzu UV 1700) in the range of 400–700 nm equipped with quartz 1 cm optical length cuvettes (Hellma). The hydrodynamic diameter of magnetic nanoparticle suspension was measured based on dynamic light scattering principles with a Brookhaven 90 plus particle sizer. Magnetic properties were evaluated by a Lakeshore 7404 vibrating sample magnetometer (VSM).

### 2.4. Doxorubicin (DOX) Drug Loading and Release Study

DOX loading was carried out by dispersing 5 mg of gold coated iron (Fe@Au) nanoparticles (carriers) in 5 mL aqueous DOX solution (0.1 mg/mL) following the experimental procedure described by Kuznetsov et al.\(^{75}\) At fixed time intervals, the carriers were separated from the liquid by means of a permanent magnet and the optical density of residual DOX in the supernatant was measured at 498 nm by UV-visible spectrophotometry.\(^{76}\) After the measurements, carriers were redispersed for further DOX adsorption. Beyond a certain adsorption time, there were no further changes in the concentration of DOX since the loading capacity of the carriers had reached saturation. The drug loading was determined as the difference between the initial DOX concentration and the DOX concentration in the supernatant. The drug loaded magnetic carriers were then magnetically separated and dried. The release profile was obtained by dispersing the dried drug loaded magnetic nanoparticle carriers in 5 mL PBS buffer at 37 °C. As in the uptake experiments, the released concentration of DOX in the particle free liquid was determined at fixed time intervals at 498 nm by UV-visible spectrophotometry.

### 2.5. In Vitro Targeting

*In vitro* tests for targeting of gold coated iron (Fe@Au) magnetic carriers were conducted to test the retention of magnetic carriers under various flow speeds of fluid with different magnetic field gradients. An *in vitro* apparatus (Fig. 2) simulating the human circulatory system was used to test the retention of ferrofluid (suspension of Fe@Au in MilliQ water) under various flow speeds of fluid with various magnetic field gradients. PBS buffer (simulating blood) in beaker (A) could be pumped through the tube (I) of inner diameter of 1 mm into beaker (J) by a peristaltic pump (C). The flow speed could be changed by adjusting the peristaltic pump. The fluid containing the Fe@Au nanoparticles could be injected at the intake (D). A permanent magnet (F) was set around the target region (G) and the magnetic fields could be changed by altering the position of magnet. The retention was determined by measuring the percentage of Fe@Au magnetic nanoparticles at the target region after fluid flow.

### 3. RESULTS

Here we present structural, morphological and magnetic properties of Fe@Au nanoparticles. The particles were characterized by XRD, TEM, XPS and VSM. DOX loading and release studies and *in vitro* targeting of Fe@Au nanoparticles under physiologically relevant flow speeds of fluid are also presented.

#### 3.1. Structural Properties

Representative powder X-ray diffraction patterns of reverse micelle synthesized samples are presented in Figure 3. The X-ray diffraction pattern of S-1 shows peaks corresponding to the magnetite (Fe,O\(_4\)) phase. As there is no coating of gold in S-1, iron is oxidized to form iron oxide (Fe,O\(_3\)). For sample sets S-2 and S-3, peaks corresponding to gold are observed at \(2\theta = 38.1^\circ, 44.4^\circ, 64.6^\circ, 77.6^\circ\) and 81.8°. In sample sets S-2 and S-3, the peaks of \(\alpha\)-iron are overlapped with the peaks of gold at \(2\theta = 44.4^\circ, 64.6^\circ\) and 81.8°, consistent with previous reports.\(^{71,77}\) The Rietveld refinement of corresponding powder diffraction pattern was performed and it was found that 83% Fe, 17% Au and Fe, 49.4% Fe, 50.5% Au were present in S-2 and S-3, respectively. Figure 2 shows the selected area electron diffraction (SAED) pattern of S-3 obtained from transmission electron microscopy. The diffraction rings were indexed (Fig. 4) and it was observed that gold 200, 220, 222 peaks overlapped with iron 110, 200, 211 peaks. The EDX spectrum of S-3 (Fig. 5) generated with an electron nanoprobe (~20 nm in diameter) revealed both iron and gold peaks. No oxygen peak was found, suggesting that there was negligible oxidation of iron. The peaks corresponding to carbon and copper arise from the sample holder.

\[ R = \frac{1}{S} \text{compB}^\text{compB} \times 100 \]
nanoparticles (S-2 and S-3). The position of Fe 2p$_{1/2}$ line at 723.2 eV and that of Fe 2p$_{3/2}$ line at 710 eV (Fig. 6(a)) correspond to the bulk Fe (0) state$^{78}$ in both the gold coated samples. Analysis of XPS spectra reveals that the surface elemental atomic ratio of gold to iron increases from S-2 to S-3 with increasing gold concentration. The detection of Fe core with the Au shell in XPS spectra suggests that Au shell thickness is less than 5 nm.$^{49}$ We have calculated the thickness of gold coating which is presented in Section 3.3.
3.2. Particle Size

The size and morphology of individual particles can be observed in the TEM micrographs (Fig. 7). It is observed that the particles are equiaxed for both coated and uncoated samples. Uncoated particles have an average size of 10 nm and coated particles have an average size of 15 nm.

3.3. Magnetic Properties

Representative plots of magnetization versus field curve of the three sample sets measured at room temperature are shown in Figure 8. Interestingly, magnetic measurements reveal that the particles are superparamagnetic as no remanence is observed. In S-1, the magnetization is due to iron oxide, while in S-2 and S-3, magnetization is due to the iron core. It is observed that with increasing gold concentration, the saturation magnetization ($M_s$) of the gold coated iron nanoparticles decreases. The values are 30.5 emu/g, 18.2 emu/g and 11.9 emu/g for S-1, S-2 and S-3, respectively (Table II). Our magnetization results measured at room temperature agree well with previous reports.$^{48, 71, 74}$

We have calculated the magnetic particle size ($D$) by fitting the magnetization curves (Fig. 8) using the Langevin function:$^{79}$

$$M = M_s \left[ \coth \left( \frac{\mu H/K_B T}{D/6} \right) - \frac{K_B T}{\mu H} \right];$$

where, $\mu = M_s \pi D^3/6$ is the magnetic moment, $K_B$ is the Boltzmann constant, $T$ is the absolute temperature and $M_s$ is the saturation magnetization. From the data fitting, the size of uncoated particle is found to be 12.5 nm, that of S-2 and S-3 are 14.5 nm and 16 nm, respectively. Therefore, the gold coating is 2 nm and 3.5 nm in S-2 and S-3, respectively.

3.4. Stability of Fe@Au Nanoparticles Under Physiological Condition

The colloidal stability of Fe@Au nanoparticles in PBS (pH = 7.4) was monitored by dynamic light scattering (DLS). PBS represents the typical pH of physiological medium and is very common biological buffer. Figure 9 shows the average hydrodynamic diameter of uncoated and gold coated iron nanoparticles as a function of time. The flocculation of uncoated iron nanoparticles occurred within 1 h, whereas Fe@Au nanoparticles were relatively stable up to 4 h, after which aggregation started to produce large aggregates by 6 h. Here we observed the short term

<table>
<thead>
<tr>
<th>Sample set</th>
<th>$M_s$ (emu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1 (uncoated)</td>
<td>30.5</td>
</tr>
<tr>
<td>S-2 (coated with 0.102 mmole of Au)</td>
<td>18.2</td>
</tr>
<tr>
<td>S-3 (coated with 0.204 mmole of Au)</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Table II. Saturation magnetization ($M_s$) of nanoparticles.
stability of Fe@Au nanoparticles which are electrostatically stabilized. In order to get stability for several days, macromolecular stabilizers are used such as dextran, polyethylene glycol (PEG), Pluronics which prevent flocculation by steric stabilization.

3.5. Doxorubicin (DOX) Drug Loading and Release Studies

We studied DOX loading and release profile of gold coated iron (Fe@Au) nanoparticles. The starting ratio by weight of Fe@Au nanoparticles to DOX was 10. The drug loading of the Fe@Au nanoparticles can be qualitatively monitored by a change in the color of DOX, which changed from deep orange (Fig. 10(a)) to a much lighter color with increasing time as DOX was adsorbed to the Fe@Au nanoparticles (Fig. 10(c)). We also used uncoated iron nanoparticles for drug loading, but we observed no change in the color and concentration of DOX which indicates that there is no drug adsorption on the uncoated iron nanoparticles (Fig. 10(b)). The binding of DOX with gold coated iron (Fe@Au) nanoparticles was studied by FTIR spectroscopy. Figure 12 shows FTIR spectra of Fe@Au, pure DOX and DOX-attached Fe@Au nanoparticles. In case of Fe@Au nanoparticles, peaks at 3380 cm\(^{-1}\) (H–O stretching), 1626 cm\(^{-1}\) (H–O–H bending) are due to adsorbed water on the surface of nanoparticles and at 1392 cm\(^{-1}\) (C–C stretching) from the surfactant. The FTIR spectrum of pure DOX shows multiple peaks at 2932 cm\(^{-1}\) (C–H stretching), 1730 cm\(^{-1}\) (C=O stretching), 1618 cm\(^{-1}\) (N–H bending), 1414 cm\(^{-1}\) (C–C stretching), 1280 cm\(^{-1}\) (C–O–C stretching), 1070 cm\(^{-1}\) (C–O stretching), 997 cm\(^{-1}\) (C–O–C stretching) and peaks at 870 and

![Fig. 9. Average hydrodynamic diameter of S-1 (uncoated), S-2 (0.102 mmole gold coated iron nanoparticles) and S-3 (0.204 mmole gold coated iron nanoparticles) in PBS. Uncoated iron nanoparticles flocculate within 1 h, whereas Fe@Au nanoparticles are relatively stable up to 4 h.](image)

![Fig. 10. The color of (a) pure DOX solution, (b) DOX solution after 26 h mixing with uncoated iron nanoparticles and (c) DOX solution after 26 h mixing with gold coated iron (Fe@Au) nanoparticles. The change in color of DOX indicates that the DOX is attached onto Fe@Au nanoparticles only.](image)

![Fig. 11. DOX (a) loading and (b) release profile of gold coated iron (Fe@Au) nanoparticles. The DOX loading and release increases with increase in gold content in the magnetic carriers.](image)

Fig. 12. FTIR spectra of (a) Fe@Au, (b) pure DOX and (c) DOX-attached Fe@Au nanoparticles. Amine (–NH₂) group of DOX is involved in attachment to the Fe@Au nanoparticles.

Fig. 13. UV-vis spectra of (a) pure DOX, (b) DOX-attached gold and (c) gold colloid. Peak is broadened and red shifted at 535 nm upon addition of colloidal gold to DOX.

805 cm⁻¹ corresponding to amine (N–H) wag. Interestingly for pure DOX, peaks at 3450 cm⁻¹ due to N–H stretching vibrations for primary amine structure and at 3330 cm⁻¹ due to O–H stretching vibrations are observed. However, for DOX-attached Fe@Au nanoparticles, peaks due to N–H stretching vibrations and O–H stretching vibrations overlap and are broadened (∼3340 cm⁻¹). The sharp peaks at 870 and 805 cm⁻¹ observed in pure DOX due to amine (N–H) wag also diminish in the FTIR spectrum of DOX-attached Fe@Au nanoparticles. From this FTIR analysis, it can be interpreted that –NH₂ group of DOX is the active site for the attachment to the Fe@Au nanoparticles.

The interaction of DOX with gold was further investigated by measuring the optical properties of gold colloid, pure DOX solution and DOX-attached gold colloid (Fig. 13). The absorption band observed for gold colloid at 520 nm in the UV-visible spectrum is due to surface plasmon resonance (SPR) which is characteristic of gold nanoparticles. Pure DOX shows an absorption maximum at 485 nm and it has been observed that with the addition of colloidal gold to DOX, peak is broadened and red shifted at 535 nm due to interaction of DOX with the Au surface. This interaction is due to the attachment of DOX with gold arising from large electrostatic attraction of active groups (–NH₂) in DOX with gold nanoparticles.

The zeta potential of Fe@Au nanoparticles and DOX-attached Fe@Au nanoparticles were determined by Malvern Zetasizer. The average values of zeta potential of Fe@Au and DOX-attached Fe@Au nanoparticles were −28.5 mV and −23.6 mV, respectively. In DOX-attached Fe@Au nanoparticles, a fraction of negative charges was neutralized by complex formation, leading to change of zeta potential from −28.5 mV to −23.6 mV.

3.6. In Vitro Targeting

The retention of Fe@Au nanoparticles (S-2 and S-3) at distances 5, 10 and 15 mm away from the magnet surface with various flow rate of fluid is shown in Figure 14.

Fig. 14. Retention of (a) S-2 (0.1225 mmole gold coated iron nanoparticles) and (b) S-3 (0.245 mmole gold coated iron nanoparticles) at various flow rate of fluid with various magnetic field gradient. The retention is maximum at lower flow rate of fluid and higher magnetic field gradient.
This in vitro experimental study shows the capture of magnetic carriers within fluidic system simulating the flow regime encountered in diseased capillary blood vessels (1.5–10 mm s$^{-1}$). It is evident from Figure 14 that the retention of Fe@Au nanoparticle carriers is maximum at lower fluid flow rate and higher magnetic field gradient. The maximum retention of S-2 at flow rate of 1.5 mm s$^{-1}$ is about 70%, 65% and 58% (Fig. 14(a)) and that of S-3 is about 58%, 53% and 46% (Fig. 14(b)) under the magnetic field gradient of 25, 15 and 8.5 T m$^{-1}$, respectively. The difference in retention of S-2 and S-3 is due to higher saturation magnetization of S-2 compared to S-3. As expected, retention of magnetic carriers decreases with increasing flow rate of fluid and decreasing magnetic field gradient. This in vitro study reveals that the capture of magnetic carriers within the tumor is influenced by magnetic field gradient, flow rate of fluid and saturation magnetization of the magnetic carrier particles, the counterpart modeling studies will be reported elsewhere.

4. DISCUSSION

Our results show that Fe@Au nanoparticles are promising novel agents for DOX loading, release and targeting. We now discuss some specific results in the context of drug targeting.

4.1. Characterization of Fe@Au Nanoparticles

Due to its attractive combination of optical and magnetic properties, several groups have studied the structural, optical and magnetic properties of gold coated iron (Fe@Au) nanoparticles. Lin et al. synthesized Fe@Au nanoparticles of average size of 10 nm by the reverse micelle technique, the particles were superparamagnetic with $M_s$ of 17 emu/g at 300 K. They reported the absorption bands of gold colloid and Fe@Au colloid at 526 nm and 555 nm, respectively. Zhou et al. reported the reverse micelle synthesis of Fe–Au core–shell nanoparticles with 8 nm diameter and the saturation magnetization ($M_s$), remanent magnetization ($M_r$) and coercivity ($H_c$) were 48 emu/g, 13.67 emu/g and 400 Oe respectively at 2 K. Cho et al. also prepared Fe core/Au shell of average size of 18 nm by inverse micelle technique and measured the magnetic properties at temperature of 5 K and 300 K. They reported Fe/Au nanoparticles having coercivity ($H_c$) of 400 Oe, remanent magnetization ($M_r$) of 14 emu/g and saturation magnetization ($M_s$) of 43 emu/g at 5 K; whereas Fe/Au nanoparticles were superparamagnetic with $M_s$ of 17 emu/g at 300 K. The saturation magnetization ($M_s$) decreases with increasing temperature since thermal energy randomizes magnetic moment in different directions. Pana et al. reported a saturation magnetization of 4.4 emu/g for Fe–Au core–shell nanoparticles at room temperature, the nanoparticles had a broad size distribution with an average size of 25 nm.

Kinoshita et al. reported gold–iron composite particles of 5 nm, an absorption peak at 520 nm was observed in UV-Visible spectra due to surface plasmon resonance (SPR) of gold. Our results show that superparamagnetic Fe@Au nanoparticles with a narrow particle size distribution and an average size of 15 nm could be synthesized by reverse micelle technique. In the context of drug delivery, a narrow particle size range such as those obtained (Fig. 7) in the present work is useful since such particles offer equal probability of magnetic capture of drug loaded nanoparticles and are characterized by similar drug content. The uncoated particles are highly aggregated compared to gold coated particles. The nanoparticles have a general tendency to aggregate to reduce their surface energy regardless of the magnetic properties. When the particles are coated with surfactant, polymer and dispersed in a carrier fluid, this aggregation is reduced. The uncoated particles (S-1) were oxidized to Fe$_3$O$_4$ (magnetite) due to exposure to air (Fig. 3) as nanoparticles have very large surface area to volume ratio, promoting oxidation to more stable state. By coating with the noble metal gold, this oxidation of iron can be prevented. The gold coating is thin (2 nm and 3.5 nm in S-2 and S-3, respectively), hence Fe as well as Au peaks were observed in XPS spectra (Fig. 6). The positions of Fe and Au peaks (S-2 and S-3) in our XRD studies agree well with previous reports.

In this study, the observed saturation magnetization ($M_s$) of Fe@Au nanoparticles at room temperature is comparable to previous reports. However, the observed $M_s$ of Fe@Au nanoparticles is lower than that of bulk iron (220 emu/g) because of two effects:

(a) gold is a diamagnetic material, interparticle coupling between iron and gold decreases the magnetic properties of the coated nanoparticles and

(b) the $M_s$ generally decreases with a decrease in magnetic particle size.

The reduced magnetization can also be attributed to the spin disorder in the surface layer of magnetic nanoparticles, their high surface area to volume ratio magnifying this effect as the size decreases. Veranda et al. also reported a linear relationship between $M_s$ and particle size. In the context of drug delivery, the superparamagnetic nanoparticles we have obtained are useful because they do not retain magnetization before and after exposure to an external magnetic field, reducing the probability of particle aggregation due to magnetic dipole attraction.

4.2. DOX Loading and Release

Our results show that a significant amount of DOX could be loaded on to the Fe@Au nanoparticles, this loading can be increased by increasing the gold coating. This is sufficient to achieve DOX release in the range of $\mu$g with conservative concentration of magnetic nanoparticles.
Considering breast cancer, the required dosage of anticancer drug is calculated based on Mosteller equation\textsuperscript{94} to find the BSA (Body surface area). Mosteller equation is given by, $\text{BSA (m}^2) = \left[ \frac{\text{Height (cm)} \times \text{Weight (kg)}}{3600} \right]^{1/2}$. If a female patient of body weight of 55 kg and height of 165 cm, then her BSA is 1.59 $m^2$. Generally, the treatment of DOX requires a dose of 50 mg/$m^2$.\textsuperscript{95} Therefore, the required dosage of DOX is $(1.59 \text{ $m^2$} \times 50 \text{ mg/$m^2$}) = 79.5 \text{ mg}$. The total body water (TBW) of woman is calculated by Watson’s formula:\textsuperscript{96}

\[
\text{Female TBW} = (-2.097) + \left[ 0.1069 \times \text{height (cm)} \right] + \left[ 0.2466 \times \text{weight (kg)} \right]
\]

So the total body water is 29.1 L. Therefore, the DOX concentration should be 2.73 $\mu$g/mL. In the present work, we have used 5 mg DOX loaded Fe@Au carriers in 5 mL PBS buffer for the drug release. We achieve the concentration of DOX (2.73 $\mu$g/mL) in 2 h from S-2 and 1 h from S-3, respectively (Fig. 11).

The interaction of DOX to the Fe@Au nanoparticles is due to the attachment of $-\text{NH}_2$ group of DOX with the gold shell, as evidenced by the peak broadening of N–H stretching vibrations of DOX-conjugated Fe@Au nanoparticles in FTIR spectra (Fig. 12) and red shifting of absorption band of gold colloid with addition of DOX in UV-visible spectra (Fig. 13). Selvaraj et al. reported that $-\text{NH}$ group of 5-Fluorouracil drug was involved in binding the drug onto the gold nanoparticle surface\textsuperscript{97} and Aslam et al. showed that gold has a strong affinity towards the amino group.\textsuperscript{98} The drug release can be explained by the covalent conjugation model postulated by Ringsdorf,\textsuperscript{99} where the cleavage of Au-DOX coordinate linkers results in the release of attached drug. The DOX loading of Fe@Au nanoparticles is comparable to that of Arruebo et al.\textsuperscript{100} where zeolite-magnetite nanocomposite was loaded with DOX. Kuznetsov et al. reported that a maximum of 62 $\mu$g of DOX was loaded per mg of ferro-carbon adsorbent and approximately 25% of adsorbed DOX was released from iron–carbon adsorbent in 24 h.\textsuperscript{75}

### 4.3. In Vitro Targeting

To date, there is limited study on the pharmacokinetic and biodistribution of gold coated magnetic nanoparticles.\textsuperscript{100} In general, physicochemical properties of nanoparticles such as size, shape, morphology, charge, and surface chemistry affect their pharmacokinetics and biodistribution. The size of the nanoparticles should be small enough to avoid immediate uptake by phagocytic cells of the reticulo-endothelial system (RES) and big enough to avoid rapid renal clearance. Very small particles can easily pass through the capillary wall in the tumor but can easily be pushed out from the tumor by blood flow.\textsuperscript{101} As the magnetic force acting on the magnetic particles is proportional to the volume of the particles, fluidic drag force can overcome the magnetic force experienced by the smaller particles. Therefore, small particles may have good permeability but poor retention. On the other hand, larger particles have higher magnetization and experience higher magnetic forces which offer better in vivo manipulability in the bloodstream by an external magnetic field for guidance to the tumor. This comes at the cost of circulation time since larger particles are likely to be opsonized earlier. Various particle sizes have been successfully used in clinical trials and in vivo trials with animals, e.g., the average size of 100 nm for magnetic drug targeting,\textsuperscript{102} and a size range of 100–200 nm in animal trials.\textsuperscript{103,105}

In addition to size, surface charge plays a critical role in blood circulation time of nanoparticles.\textsuperscript{106,107} Positively charged coatings nonspecifically stick to cells\textsuperscript{108} whereas the negatively charged particle surface is easily taken up by liver due to sequestration by phagocytes.\textsuperscript{109} Therefore, it is generally agreed that nanoparticles with a neutral surface experience extended blood circulation times.\textsuperscript{109}

In previous studies, it was shown that a magnetic field of 0.8 T is sufficient to exceed linear blood flow in the intratumoral vasculature to localize 100% of magnetic carrier.\textsuperscript{110} Lubbe et al. studied the targeting of ferrofluid containing starch coated iron oxide nanoparticles (100 nm) loaded with mitoxantrone anti-cancer drug using a permanent magnet (0.5 T).\textsuperscript{111} Tumor bearing mice and rats were used for the experiment which showed that ferrofluid complex was well tolerated by the animals and tumor remission was achieved. Another major advantage is that the applied dose of drug could be reduced to 20% of the regular systemic dose.\textsuperscript{35} Clinical experiments in human patients using magnetic drug targeting were reported by Lubbe et al. who used anti-cancer drug epirubicin attached to starch coated iron oxide nanoparticles in form of ferrofluid (100 nm) to concentrate at the breast tumor by means of a permanent magnet (0.8 T) and demonstrated that the infusion of ferrofluid was well tolerated in most of the 14 patients studied without associated organ toxicity.\textsuperscript{34,102} Goodwin et al. used magnetic carriers to target at the liver and lungs in the swine model, a permanent magnet was used (0.025–0.1 T), the depth of targeting was 8–12 cm and particle size was 0.5–5 $\mu$m.\textsuperscript{112} However, disadvantages in using large particles ($\sim 5 \mu$m) are that they may clog the blood vessels prior to reaching the tumor and may not reach the brain tumors because particles are too large to cross the endothelial barrier.\textsuperscript{113} Therefore, use of a stronger magnetic field would be better choice to target nanocarrier particles to the tumor located deep inside the body. Preliminary investigations of the hydrodynamics of drug targeting suggest that a magnetic field of 0.2 T with field gradient of 8 T m$^{-1}$ is sufficient to target magnetic nanoparticle carriers in femoral arteries.\textsuperscript{114}

In the present work, we used ferrofluids containing Fe@Au nanoparticles for in vitro targeting. The magnetic fields measured at 5, 10 and 15 mm distance
from the magnet surface were 0.25, 0.15 and 0.098 T with field gradients of 25, 15 and 8.5 T m⁻¹, respectively. A significant percentage of Fe@Au nanoparticles were captured for physiologically relevant flow speed of fluid (1.5–10 mm s⁻¹) encountered in diseased capillary blood vessels under these magnetic fields. Our in vitro targeting results are comparable to that of Udrea et al, who showed the targeting of ferrofluid containing iron oxide nanoparticles under fluid flow rate of 1.5–9 mm s⁻¹ at a field gradient of 15 T m⁻¹ generated by a C-shape bipolar magnet.³⁵

In summary, magnetic carriers comprising of Fe@Au nanoparticles were synthesized, characterized and studied for anticancer drug loading, drug release and drug targeting applications. These studies show that Fe@Au nanoparticles are promising novel agents for magnetically targeted drug delivery.

5. CONCLUSIONS

• Superparamagnetic gold coated iron (Fe@Au) nanoparticles were synthesized by the reverse micelle method and characterized by XRD, XPS, TEM and VSM.
• Fe@Au nanoparticles show superparamagnetic character at room temperature, \( M_r \) of Fe@Au nanoparticles decreases with increasing gold concentration.
• Fe@Au nanoparticles show great promise as potential magnetic drug carriers through binding with anti-cancer drug DOX and a high percentage of carriers could be retained in the tumor when a suitable magnetic field is present.
• Fe@Au nanoparticles are promising candidates as magnetic drug carriers for tumor targeted drug delivery.

References and Notes

RESEARCH ARTICLE


Anti-Cancer Drug Loaded Iron–Gold Core–Shell Nanoparticles (Fe@Au) for Magnetic Drug Targeting

Kayal and Ramanujan

References


Received: 4 September 2009. Accepted: 12 October 2009.