

The College of Life Sciences, Northwest University¹; National Engineering Research Center for Miniaturized Detection Systems², Xi'an, China

Cytotoxicity of Fe₃O₄/Au composite nanoparticles loaded with doxorubicin combined with magnetic field

XU CHAO¹, FENG SHI¹, YING-YONG ZHAO¹, KE LI¹, MING-LI PENG², CHAO CHEN^{1,2}, YA-LI CUI^{1,2}

Received November 12, 2009, accepted November 17, 2009

Prof. Ya-Li Cui, The College of Life Sciences, Northwest University, No.229 Taibai North Road, Xi'an 710069, China
yalicui@nwu.edu.cn

Pharmazie 65: 500–504 (2010)

doi: 10.1691/ph.2010.9362

GoldMag (Fe₃O₄/Au) nanoparticles have the advantages of both magnetic response in an external magnetic field and the immobilization of molecules on their surface in a single step. The cytotoxicities of GoldMag nanoparticles and GoldMag nanoparticles loaded with doxorubicin (Dox-GoldMag) combined with an external magnetic field were tested *in vitro* on HepG2 malignant tumor cells. The results showed that cell viability remained above 92% when using GoldMag nanoparticles at a concentration as high as 2.0 mg/ml, suggesting the biocompatibility of the nanoparticles. The IC₅₀ (0.731 μg/ml) of the Dox-GoldMag group was higher than that (0.522 μg/ml) of the Dox group (P < 0.05). However, the Dox-GoldMag group combined with a magnetic field had an obviously increased inhibition rate for the HepG2 cell line and the IC₅₀ was lower than that of the Dox group (0.421 μg/ml). These results indicated that GoldMag nanoparticles loaded with doxorubicin combined with a permanent magnetic field are more cytotoxic and could be a potential targeted drug delivery system.

1. Introduction

Within the last two decades, the magnetically targeted-drug delivery system (MT-DDS) has been used in an attempt to increase the therapeutic efficacy and reduce the unpleasant side effects associated with chemotherapy for malignant tumors (Jon 2006; Sun et al. 2008). It involves binding an anticancer drug to biocompatible magnetic particles, injecting them into the blood stream and using an external magnetic field to pull them out of suspension in the target region (Alexiou et al. 2000, 2006; Mishima et al. 2006; Takeda et al. 2006). The drug can be released in the specific region, reducing its systemic distribution as well as allowing the possibility of administering lower but more accurately targeted doses of the drug treatment (Goodman et al. 2004; Jon 2006).

Incorporation of gold and magnetic nanoparticles in synthetic composite particles might be expected to offer the advantages of biocompatibility, surface functionality for coupling chemical or bio-medical agents and superparamagnetic properties. Therefore gold has become a favored material in the synthesis of magnetic composite nanoparticles. Particles with different structures such as gold coated magnetic nanoparticles (Park et al. 2007) and gold nanoparticles attached to functionalized magnetic nanoparticles (Caruntu et al. 2005) have been reported. Their applications in the biomedical area are currently active topics of research (Wang et al. 2008). We have prepared type composite nanoparticles of this kind using the iterative hydroxylamine seeding method (Cui et al. 2001) and named them GoldMag (Cui et al. 2005). Using GoldMag nanoparticles as a carrier, the kinetics of doxorubicin adsorption and drug release were investigated in this study. In addition, *in vitro* cytotoxicity assay was performed of GoldMag nanoparticles and GoldMag

nanoparticles loaded with doxorubicin combined with a permanent magnetic field to the human hepatocellular liver carcinoma cell line (HepG2).

2. Investigations, results and discussion

2.1. Kinetics of drug loading and release *in vitro*

Fig. 1 shows that the amount of drug adsorbed on the GoldMag nanoparticle surface increased rapidly within 30 min, and then there was a slow increase until the adsorbed drug reached saturation after 2 h. The maximum drug loading is about 10.2%. Dox becomes less positively charged as pH increases, so the kinetics of drug loading at different pH values were tested (Fig. 2). The drug loading of GoldMag nanoparticles was 4.63, 8.20 and 10.2% at pH 3.0, 4.5 and 7.4, respectively. The results indicate that electrostatic and covalent interactions of Au-N may be the main factors in the process of doxorubicin adsorption on GoldMag nanoparticle surfaces. The Zeta potential of GoldMag nanoparticles was –1.08, –9.17 and –21.3 mV at pH 3, 4.5 and 7.4, respectively. Therefore, electrostatic interaction is the dominant interaction at lower pH. However, the covalent interaction of Au-N between the elemental Au on the GoldMag surface and the amine group of doxorubicin plays a more important role in the immobilization process at higher pH.

In vitro drug release was carried on in PBS buffer and the results show (Fig. 3) that 18.1% of drug was released within the first 2 h of the experiment. Subsequently, there was a slow, steady and controlled release of drug conjugated on GoldMag nanoparticles up to 100 h. The maximum release of doxorubicin from the surface of GoldMag particles is about 93.4 %.

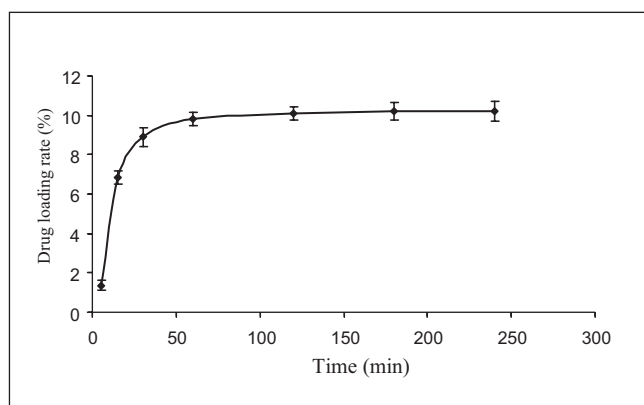


Fig. 1: Kinetics of doxorubicin adsorption by GoldMag nanoparticles

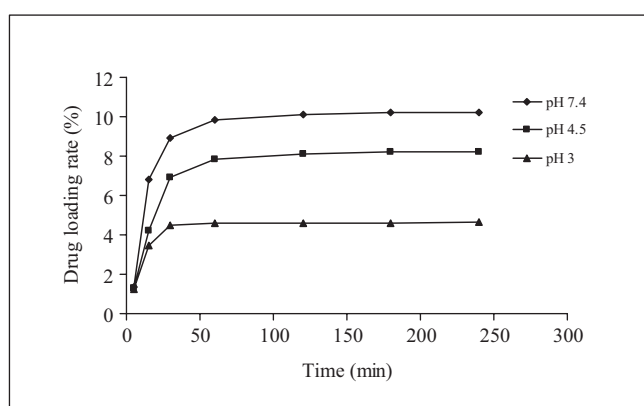


Fig. 2: Kinetics of doxorubicin adsorption by GoldMag nanoparticles at different pH values

2.2. Cytotoxicity of GoldMag nanoparticles

Low cytotoxicity is very important for nanoparticles to be used as a drug carrier. Viable cells have the ability to reduce MTT from a yellow water-soluble dye to a dark blue insoluble formazan product (Mosmann et al. 1983). Super paramagnetic iron oxide nanoparticles (SPION) have been found in tests to cause a significant reduction (80% of control) in cell viability at the concentration tested (0.05 mg/ml), and resulted in about 60% loss of cell viability at the concentration tested (2.0 mg/ml) (Gupta et al. 2005). Fig. 4 shows a dose-dependent reduction in MTT absorbance in cells treated with GoldMag nanoparticles (concentration range 0–2.0 mg/ml) for 72 h. The cells remained more than 92.1% viable relative to control at a concentration as high as 2.0 mg/ml. This indicated that GoldMag nanoparticles have low cytotoxicity's probably due to their Au component (Connor

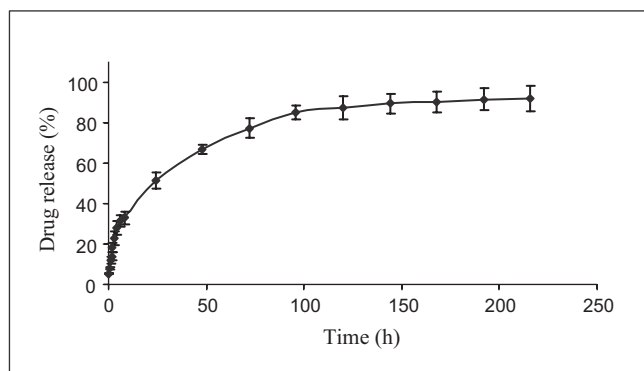


Fig. 3: Kinetics of doxorubicin release from Dox-GoldMag

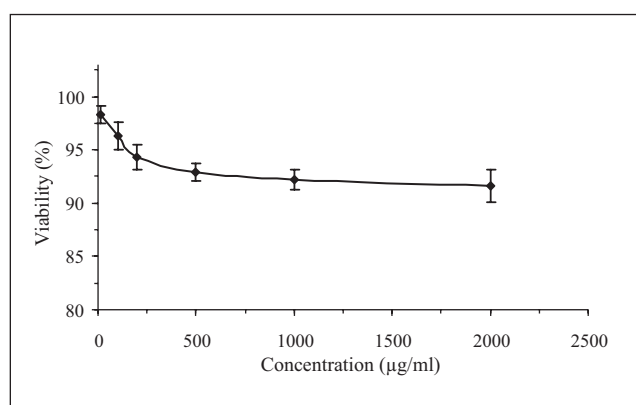


Fig. 4: Cytotoxicity profiles of GoldMag nanoparticles, incubated with HepG2 cell lines as determined by MTT assay. Percent viability of cells expressed relative to control cells (n = 6). Results represented as mean \pm SD

et al. 2005). The use of gold nanoparticles for drug delivery in mice has been described elsewhere and the result showed that gold colloid combined with TNF was less toxic and more effective in reducing tumor burden than native TNF (Paciotti et al. 2004). The results indicated that GoldMag nanoparticles have better biocompatibility and do not cause acute cytotoxicity to human cells.

Fig. 5(c) shows a multiple clusters of GoldMag nanoparticles confined to the cytosol (marked with arrows) and also some at the cell surface after incubation with medium containing GoldMag nanoparticles at 37 °C exposed to a magnetic field for 12 h. However, the cells (Fig. 5(b)) incubated with medium containing GoldMag nanoparticles without an external magnetic field showed little intracellular uptake of nanoparticles. These results demonstrate the effective uptake of GoldMag nanoparticles into cells to which an external magnetic field is applied.

2.3. Cytotoxicity of GoldMag nanoparticles loaded with doxorubicin combined with external magnetic field

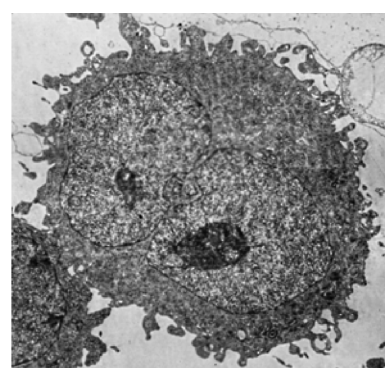
Gold nanoparticles provide an attractive system for diagnostic and therapeutic applications owing to their ready bioconjugation and good biocompatibility (Sokolov et al. 2003; El-Sayed et al. 2005; Loo et al. 2004; Schwartzberg et al. 2006a, b). They have already been studied for use in drug delivery (Paciotti et al. 2004; Mukherjee et al. 2005). However, it is hard to localize them because of their lack of super paramagnetic properties. Therefore, the influence of magnetic field on the degree of cell growth inhibition using GoldMag nanoparticles was studied by the following steps. For the HepG2 cell line, maximum cytotoxic activity of Dox, Dox-GolMag and Dox-GoldMag-M (M representing the magnetic field) were determined at a range of concentrations up to 4 µg/ml; the IC₅₀ values of Dox, Dox-GoldMag and Dox-GoldMag-M were also tested and the results are shown in the Table. They all exhibited significant dose-dependent inhibitory effects on HepG2 at from 0.04 to 0.8 µg/ml of Dox, Dox-GoldMag and Dox-GoldMag-M. Dox-GoldMag-M, Dox and Dox-GoldMag gave 97.2%, 92.2 and 79.8% inhibition respectively when the concentration of Dox was 4.0 µg/ml. The proliferation of HepG2 cells was more strongly inhibited when an external magnetic field was applied than with Dox and Dox-GoldMag without a magnetic field.

With the development of cancer therapy, it was discovered that magnetic fields can inhibit the proliferation of malignant cancer cells (Fitzsimmons et al. 1995). We investigated the inhibitory effect on malignant cancer using a magnetic field. The degree of cell growth inhibition was measured by MTT assay after 24,

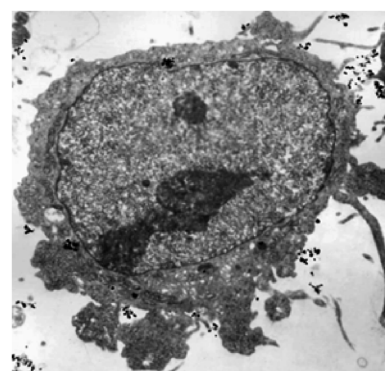
Table: Cytotoxic activity of Dox, Dox-GMNPs and Dox-GMNPs-M against HepG2 and IC₅₀ values (μg/ml)

	0.04	0.08	0.2	0.8	2	4	IC ₅₀
A	14.6 ± 0.6	19.4 ± 0.6	30.2 ± 0.4	68.7 ± 0.4	86.9 ± 0.2	92.2 ± 0.3	0.522 ± 0.040
B	13.8 ± 0.6	16.1 ± 0.3	24.9 ± 0.8	53.2 ± 0.4	72.3 ± 0.2	79.8 ± 0.9	0.731 ± 0.028
C	22.7 ± 0.4	24.8 ± 0.2	36.9 ± 0.4	75.9 ± 0.2	94.1 ± 0.3	97.2 ± 0.4	0.421 ± 0.020

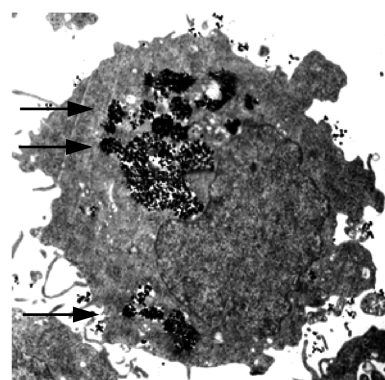
48 and 72 hours of treatment of HepG2 cells. Results showed that proliferation of the cells exposed to a magnetic field was inhibited compared to the cells without a magnetic field. The result is in accordance with a previous reference (Fitzsimmons et al. 1995). Using a 0.5T magnetic field, we also investigated the inhibition of cells subjected to DOX, GoldMag and Dox-GoldMag respectively. As shown in Fig. 6, the viability of the cells in the control group exposed to a 0.5T magnetic field fell to about 95.5% for 24 h and 86.6% for 48 h, and it continued



(a)



(b)



(c)

Fig. 5: Electron micrograph of HepG2 cells after uptake of GoldMag nanoparticles. (a) Control; (b) Image of cell incubated in medium containing GoldMag NPs without external magnetic field; (c) Image of cell incubated in medium containing GoldMag NPs without external magnetic field

to decrease, reaching a viability of 78.4 % after 72 h treatment. The viability of cells incubated with GoldMag nanoparticles also showed a similar trend compared with the control group. The data indicated that GoldMag particles do not significantly increase cytotoxic even when exposed to an external magnetic field. The viability of the cells in the Dox group was 56.9%, 43.3% and 31.6% when treated for 24, 48 and 72 h. However, if Dox was loaded on the GoldMag nanoparticles, the viability of the cells was 40.8%, 32.6% and 26.6% when treated for 24, 48 and 72 h respectively, a significant decrease when exposed to a magnetic field.

Chemotherapy combined with a permanent magnetic field has a more potent effect in killing cancer cells than chemotherapy alone (Hannan et al. 1994). Fig. 5 shows that there was an effective uptake of GoldMag nanoparticles into cells under an external magnetic field, and the results in Fig. 6 indicate that permanent magnetic fields can inhibit the proliferation of cells and suggest that Dox-GoldMag has more cytotoxicity than Dox when exposed to an external magnetic field. Due to the combination of the effect of magnetism and the cytotoxicity of doxorubicin, Dox-GoldMag nanoparticles combined with a magnetic field will probably be a potential magnetically targeted carrier in chemotherapy.

In this work, we investigated the kinetics of absorption and release of doxorubicin using GoldMag nanoparticles. The data showed that the maximum quantity of drug loaded on the particles is about 102.2 μg/mg. The GoldMag nanoparticles exhibit a slow, sustained and controlled release after 2 h. More than 85% of the drug is released up to 100 h, and the maximum release of doxorubicin from the GoldMag is about 93.4%. It is highly critical that the drug adsorbed on the particles can be released from the carrier. Also, the ideal drug delivery carrier is one from which the drug can be released in a sustained and controlled amount within a given time. It is clear that the GoldMag nanoparticles have this property. We validated that GoldMag nanoparticles loaded with doxorubicin combined with an external magnetic field have a more potent cytotoxic activity than the drug alone. However, GoldMag nanoparticles alone have little cytotoxic activity *in vitro*. The study also indicated that

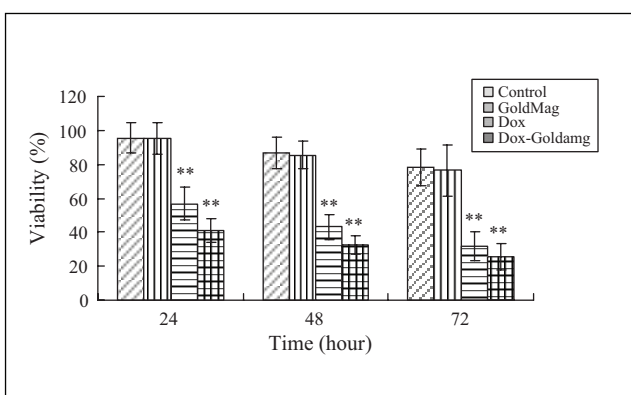


Fig. 6: Inhibition of cellular growth by magnetic field, when HepG2 cell lines exposed to 0.5 T for 24, 48 and 72 h, respectively. *P < 0.05; **P < 0.01 compared with controls using Student's unpaired t-test.

permanent magnetic fields can inhibit the proliferation of cells. However, the GoldMag particles have no significant cytotoxicity even with an applied magnetic field. The results also suggest that Dox-GoldMag has more cytotoxicity than doxorubicin with an applied magnetic field. These results indicate that GoldMag nanoparticles loaded with doxorubicin combined with permanent magnetically fields could be a potential magnetic targeted drug system.

3. Experimental

3.1. Reagents and materials

GoldMag nanoparticles (average diameter: 50 nm) were synthesized as described elsewhere in detail (Cui et al. 2005). Doxorubicin hydrochloride was obtained from Beijing HuaFeng United Technology Co., Ltd. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Amresco. Dimethylsulphoxide (DMSO) was purchased from Sigma. Human hepatocellular liver carcinoma cell line (HepG2) was obtained from the Fourth Military Medical University, China. Cell culture medium RPMI-1640 and fetal bovine serum (FBS) were products of Invitrogen Corporation. A 0.5 T permanent magnet (5 × 5 cm) was obtained from Northwestern Polytechnical University, China.

3.2. Preparation of doxorubicin loaded GoldMag particles and drug release in vitro

The capability of the particles to spontaneously adsorb the drug was detected by UV-visible spectrophotometry. The procedure was as following: 4 ml doxorubicin solution (1 mg/ml, dissolved in distilled water) was mixed with 16 ml GoldMag nanoparticles (1.25 mg/ml) and incubated in a shaker at room temperature for 15, 30, 60, 90, 120, 180 and 240 min, respectively. The adsorbent magnetic phase was magnetically separated with a permanent magnet, and the amount of drug left in the supernatant was calculated by measuring the absorption of the supernatant at 480 nm with a UV spectrophotometer (Agilent). Then the drug loading rate on the particle surface was calculated using the following formula:

Drug loading rate(%)

$$= \frac{\text{(Amount of drug on the particles/weight of particles)}}{\text{Amount of drug on the particles}} \times 100\% \quad (1)$$

Doxorubicin release kinetics were investigated as described elsewhere (Rana et al. 2007). 5 mg GoldMag nanoparticles loaded with doxorubicin (Dox-GoldMag) were incubated in 15 ml PBS buffer (pH = 7.4). The mixture was incubated at 37 °C under mild stirring. At specified time intervals the nanoparticles were magnetically separated, and the fluorescence intensity of the supernatant was measured at 555 nm with a fluorescence spectrophotometer (Hitachi F-4500 Fluorescence Spectrophotometer). The drug release rate from the Dox-GoldMag nanoparticles was calculated using the following formula:

Drug release rate(%)

$$= \frac{\text{(Amount of drug in the supernatant / Amount of drug on the particles)}}{\text{Amount of drug on the particles}} \times 100\% \quad (2)$$

3.3. Uptake and cytotoxicity of GoldMag nanoparticles in vitro

The uptake of magnetic nanoparticles into cells was investigated as described elsewhere (Becker et al. 2007). The HepG2 cells were seeded into glass culture dishes at 37 °C in a 5% CO₂ atmosphere. The cells adhered firmly to the bottom of the culture dish after 12 h, and the medium in the glass was then replaced with fresh medium containing nanoparticles at a concentration of 50 µg/ml and placed in an external magnetic field (0.5 T, permanent magnet). Cells incubated in medium containing nanoparticles at a concentration of 50 µg/ml but not exposed to an external magnetic field were also observed. After 12 h, the cells were thoroughly washed twice with PBS buffer, and then detached using 0.25% trypsin-0.02% EDTA solution. Finally, the cells were centrifuged, fixed with 3% (w/v) glutaraldehyde and processed for analysis by transmission electron microscopy.

An MTT assay was performed (Gupta et al. 2005) to determine the cytotoxicity of GoldMag nanoparticles. Cells were seeded at a density of 5 × 10³ cells/well in 96-well plates at 37 °C in a 5% CO₂ atmosphere. After 12 h of culture, the medium in the wells was replaced with fresh medium containing nanoparticles at a range of concentrations from 0 to 2.0 mg/ml. After 72 h, 20 µl of MTT dye solution (5 mg/ml in pH 7.4 phosphate buffer) was added to each well. And another 4 h of incubation at 37 °C, the medium was removed and formazan crystals dissolved in 150 µl DMSO added. The

absorbance of each well was read on an ELx800 Universal Microplate Reader (BIO-TEK Instruments) at 570 nm in 15 min. The cell viability (%) relative to control wells containing cell culture medium was calculated by the following formula:

$$\text{Relative cell viability(\%)} = \frac{[A]_{\text{Test sample}}}{[A]_{\text{Control}}} \times 100\% \quad (3)$$

where [A]_{Test sample} is the absorbance of the test sample and [A]_{Control} is the absorbance of the control.

3.4. Cytotoxicity of GoldMag nanoparticles loaded with doxorubicin combined with external magnetic field

The cytotoxic effect of GoldMag nanoparticles loaded with doxorubicin combined with an external magnetic field was also studied by the MTT method. The cultured HepG2 cell lines were divided into three groups: (a) doxorubicin group (Dox); (b) GoldMag nanoparticles loaded with doxorubicin group (Dox-GoldMag); (c) GoldMag nanoparticles loaded with doxorubicin combined with external magnetic field group (Dox-GoldMag-M). Each group included six final concentrations of doxorubicin: 0.04, 0.08, 0.2, 0.8, 2 and 4 µg/ml. Briefly, the cells were plated at a density of 5 × 10³ cells/well in 96-well plates at 37 °C in a 5% CO₂ atmosphere. After 12 h of culture, the medium in the wells was replaced with fresh medium. Then, 20 µl doxorubicin solution or a suspension of GoldMag nanoparticles loaded with doxorubicin was added at the series of concentrations mentioned above. In addition, a 0.5 T permanent magnet with was placed under the cell medium of group (c). The cells were incubated in a humidified atmosphere with 5% CO₂ for 72 hours. Then 20 µl MTT dye solution (5 mg/ml in pH 7.4 phosphate buffer) was added to each well and incubated at 37 °C for 4 hours. The medium was removed and formazan dissolved in 150 µl DMSO added and the absorbance at 570 nm was measured with an ELx800 Universal Microplate Reader (BIO-TEK Instruments). The growth inhibition rate was determined using the following formula:

Growth inhibition rate(%)

$$= \frac{([OD]_{\text{Control}} - [OD]_{\text{Sample}})}{[OD]_{\text{Control}}} \times 100\% \quad (4)$$

3.5. Inhibitory effect of magnetic fields on cells

The ability of a magnetic field to inhibit cellular growth was determined using the MTT assay. The cultured HepG2 cell lines were divided into control and three treatment groups: (a) cell culture medium group (Control); (b) doxorubicin group (Dox); (c) GoldMag nanoparticles group (GoldMag); (d) GoldMag nanoparticles loaded with doxorubicin group (Dox-GoldMag). After 12 h 20 µl cell culture media, 0.8 µg/ml doxorubicin solution, 7.84 µg/ml GoldMag nanoparticles suspension or GoldMag nanoparticle loaded with doxorubicin, respectively, were added to each well. Then the cell suspension was exposed to a 0.5T permanent magnetic field and incubated in a humidified atmosphere with 5% CO₂. After 24, 48 and 72 h, 20 µl MTT dye solution (5 mg/ml in pH 7.4 phosphate buffer) were added to each well and incubated at 37 °C for 4 hours. Then the medium was discarded. Finally, 150 µl DMSO was added to each well to dissolve the formazan crystals. The absorbance at 570 nm was determined using an ELx800 Universal Microplate Reader (Bio-Tek Instruments). The relative cell viability (%) was calculated.

Acknowledgment: This work was supported by the National High Technology Research and Development Program of China (2006AA020705).

References

- Alexiou C, Arnold W, Klein RJ, Parak FG, Hulin P, Bergemann C, Erhardt W, Wagenpfeil S, Lübke AS (2000) Locoregional cancer treatment with magnetic drug targeting. *Cancer Res* 60: 6641–6648.
- Alexiou C, Schmid RJ, Jurgons R, Kremer M, Wanner G, Bergemann C, Huenges E, Nawroth T, Arnold W, Parak FG (2006) Targeting cancer cells: magnetic nanoparticles as drug carriers. *Eur Biophys J* 35: 446–450.
- Becker C, Hodenius M, Blendinger G, Sechi A, Hieronymus T, Mueller-Schulte D, Schmitz-Rode T, Zenke M (2007) Uptake of magnetic nanoparticles into cells for cell tracking. *J Magn Magn Mater* 311: 234–237.
- Caruntu D, Cushing BL, Caruntu G, O'Connor CJ (2005) Attachment of gold nanograins onto colloidal magnetite nanocrystals. *Chem Mater* 17: 3398–3402.
- Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD (2005) Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 3: 325–327.

- Cui YL, Hu DD, Fang Y, Ma JB (2001) Preparation and mechanism of $\text{Fe}_3\text{O}_4/\text{Au}$ core/shell super-paramagnetic microsphere. *Science in China (Series B)* 44: 404–410.
- Cui YL, Hui WL, Su J, Wang YN, Chen C (2005) $\text{Fe}_3\text{O}_4/\text{Au}$ composite nanoparticles and their optical properties. *Science in China Series (Series B)* 48 (4): 273–278.
- Cui YL, Wang YN, Hui WL, Zhang ZF, Xin XF, Chen C (2005) The synthesis of GoldMag nano-particles and their application for antibody immobilization. *Biomed Microdevices* 7: 153–156.
- El-Sayed IH, Huang XH, El-Sayed MA (2005) Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. *Nano Lett* 5: 829–834.
- Fitzsimmons RJ, Ryaby JT, Mohan S, Magee FP, Baylink D (1995) Combined magnetic fields increase insulin-like growth factor-II in TE-85 human osteosarcoma bone cell cultures. *Endocrinology* 136: 3100–3106.
- Goodman CM, McCusker CD, Yilmaz T, Rotello VM (2004) Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjugate Chem* 15: 897–900.
- Gupta AK, Gupta M (2005) Cytotoxicity suppression and cellular uptake enhancement of surface modified magnetic nanoparticles. *Biomaterials* 26: 1565–1573.
- Hannan CJ, Liang Y, Allison JD, Pantazis CG, Searle JR (1994) Chemotherapy of human carcinoma xenografts during pulsed magnetic field exposure. *Anticancer Res* 14: 1521–1524.
- Jon D (2006) Magnetic nanoparticles for drug delivery. *Drug Del Res* 67: 55–60.
- Loo C, Lin A, Hirsch L, Lee MH, Barton J, Halas N, West J, Drezek R (2004) Nan shell-enabled photonics-based imaging and therapy of cancer technology. *Cancer Res Treat* 3: 33–40.
- Mishima F, Takeda S, Izumi Y, Nishijima S (2006) Three dimensional motion control system of ferromagnetic particles for magnetically targeted drug delivery systems. *IEEE Transact Applied Superconductiv* 16: 1539–1542.
- Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 95: 55–63.
- Mukherjee P, Bhattacharyal R, Bone N, Lee YK, Patra CR, Wang SF, Lu LC, Secreto C, Banerjee PC, Yaszemski MJ, Kay NE, Mukhopadhyay D (2007) Potential therapeutic application of gold nanoparticles in B-chronic lymphocytic leukemia (BCLL): enhancing apoptosis. *J Nanobiotechnol* 5: 4.
- Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, Tamarkin L (2004) Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. *Drug Deliv* 11: 169–183.
- Park HY, Schadt MJ, Wang L, Lim IS, Njoki PN, Kim SH, Jang MY, Luo J, Zhong CJ (2007) Fabrication of magnetic core-shell Fe Oxide-Au nanoparticles for interfacial bioactivity and bio-separation. *Langmuir* 23: 9050–9056.
- Rana S, Gallo A, Srivastava RS, Misra RD (2007) On the suitability of nanocrystalline ferrites as a magnetic carrier for drug delivery: functionalization, conjugation and drug release kinetics. *Acta Biomater* 3: 233–242.
- Schwartzberg AM, Olson TY, Talley CE, Zhang JZ (2006) Synthesis, characterization, and tunable optical properties of hollow gold nanospheres. *J Phys Chem* 110: 19935–19944.
- Schwartzberg AM, Oshiro TY, Zhang JZ, Huser T, Talley CE (2006) Improving nanoprobe using surface-enhanced Raman scattering from 30-nm hollow gold particles. *Anal Chem* 78: 4732–4736.
- Sokolov K, Follen M, Aaron J, Pavlova I, Malpica A, Lotan R, Richards-Kortum R (2003) Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Res* 63: 1999–2004.
- Sun C, Lee JS, Zhang M (2008) Magnetic nanoparticles in MR imaging and drug delivery. *Adv Drug Delivery Res* 60: 1252–1265.
- Takeda SI, Mishima F, Terazono B, Izumi Y, Nishijima S (2006) Development of magnetic force-assisted new gene transfer system using biopolymer-coated ferromagnetic nanoparticles. *IEEE Trans Appl Supercond* 16 (2): 1543–1546.
- Wang LY, Park HY, Lim SI, Schadt MJ, Mott D, Luo J, Wang X, Zhong CJ (2008) Core-shell nanomaterials: gold-coated magnetic oxide nanoparticles. *J Mater Chem* 18: 2629–2635.