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Biocompatibility of Near-IR Sensitive Au-based Nanoparticles As the Potential Drug Delivery Carriers

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Abstract. We successfully synthesized near infrared (NIR) sensitive Au(shell)-Au_2S(core) nanoparticles, where Au_2S dielectric core was encapsulated by a thin gold shell. The cytotoxicity *in vitro* and biodistribution *in vivo* of Au-Au_2S nanoparticles was studied by using NIH3T3 cells and KM mice, respectively. The quantitative analysis of Au in each tissue of mice was done by using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Au-Au_2S nanoparticles (< 300 μg/ml) showed good biocompatibility. Au-Au_2S nanoparticles were preferentially taken up by the liver and spleen, and ultimately eliminated mostly in the feces.

Introduction

A major goal of nanotechnology is to develop materials with the functional properties. One approach has been to construct materials from nanoscale hybrids. A number of reports on the synthesis of Au-based nanoscale hybrid systems can be found in literature [1-5], because gold has unique applications in many areas such as drug delivery [1], immunoassay labeling [2], nonlinear optical switching, and Raman spectroscopy enhancement [3]. We have recently synthesized a new kind of NIR sensitive nanoparticles composed of Au and Au_2S, where Au_2S dielectric core was encapsulated by a thin gold shell [4, 5]. The surface that the Au-Au_2S nanoparticles present to the environment is a contiguous layer of gold. Gold is essentially a bioinert material and has been found to be useful in fields ranging from dental surgery to arthritis treatments [6], Hence, the gold shell surrounding Au-Au_2S nanoparticles should also take advantage of the inherent biocompatibility of gold, not requiring further surface functionalization or protective layer growth. The most unique feature of Au-Au_2S nanoparticles is that their plasmon resonance (wavelength of optimal optical extinction) can be designed to fall in the near infrared (NIR) region [7]. The near-infrared (NIR) light in controlled doses has been used for medical applications, because it is non-destructive property to human tissues. In the previous report, Ren *et al.* absorbed an anti-tumor drug, *cis*-platin, onto the surface of Au–Au_2S nanoparticles successfully, which exhibited the possible clinic application of these potential drug delivery carriers [8]. In this paper, the biocompatibility *in vitro* and biodistribution *in vivo* of Au-Au_2S nanoparticles were studied by using NIH3T3 cells and Kun Ming (KM) mice, respectively.

Materials and methods

Preparation and characterization of Au-Au_2S nanoparticles. Following the reference [8], the growth of Au–Au_2S nanoparticles was initiated when aqueous solutions of $HAuCl_4$ (provided by Acrobs Organics) and Na_2S (obtained from Sigma) were mixed. Briefly, 20 ml of 1 mM Na_2S was mixed with 20 ml of 2 mM $HAuCl_4$, and stored at 25ºC for 1 day. Sample for UV–Vis study were placed in quartz crystal cuvettes, and the absorption spectra were acquired at room temperature using a UV-2550 spectrophotometer (Shimadzu). Samples for TEM were prepared by placing a drop of solution onto carbon-coated copper grids and left to dry prior to investigation using a TEM microscope (100kV, Hitachi H600).

Cytotoxicity assay. We used the colorimetric methyl-thiazol-tetrazolium (MTT) assay to study cell survival after incubation with NIH3T3 cell. Briefly, Cells were seeded at 1×10^4 cells/well in a 96-well plate and incubated at 37ºC in 5% CO_2 for 24h. The medium was then removed and replaced with fresh medium containing Au-Au_2S nanoparticles at various concentrations ranging from 0 to 600 μg/ml (100 μl/well). Cells treated with medium only as negative control group. Incubations were made for 24 and 72 h before removal of media containing Au-Au_2S nanoparticles. 20 μl MTT (5 mg/ml in PBS) was added to each well and then the plate was further incubated for 4 h under normal growing conditions. All remaining supernatant were removed and 150 μl DMSO was added to each well. Plates were then incubated for 30 min at 37°C to ensure all crystals were dissolved. Finally, the absorbance at 490 nm for each well was measured on a microreader (Bio-tek ELX800). Cell survival at each dose was calculated relative to the negative control group. Every sample was repeated 4 times, and the data were analyzed statistically by using *t*-test.

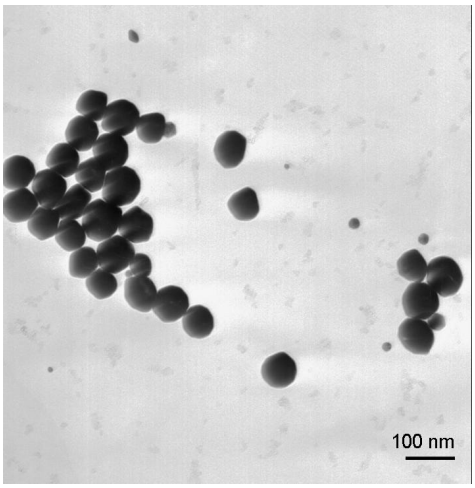

Fig. 1 TEM image of Au-Au_2S nanoparticles

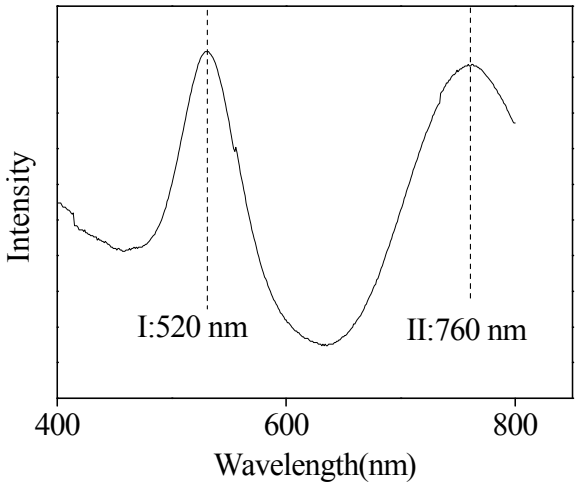

Fig. 2 UV– vis spectrum of Au–Au_2S nanopaiticles

Biodistribution in vivo. KM male mice (provided by Anti-Cancer Research Center, Xiamen University), aged 7 weeks and weighing 18-21 g, were used in the experiments. After filtration through 0.22 μm filter membrane, 200 μl nanoparticles solution (1 mg/ml) was injected into a KM mouse via the tail vein. All animal experiments were performed in compliance with the local ethics committee. At 30 min, 1h, 12h, 24h, and 7d, blood, muscle (left thigh), bone (left femur with marrow), heart, liver, spleen, lung, kidney, brain of KM mice were collected. Except the blood, every sample was washed thoroughly with deionized water and dried for 4 hours at 120ºC [9]. In order to prepare ICP solution, the samples were digested by a microwave accelerated reaction system (CEM MARS 240/50), following the preset protocols. The digested solutions were then

qualitative analyzed for Au by using a Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer SCIEX ELAN DRC-e). 10 ppb Au aqueous solution (purchased from National Research Center for CRM'S) was used as the standard control. Every sample was repeated 5 times.

Results and discussion

Characterization of Au-Au_2S nanoparticles. Fig. 1 illustrated a typical TEM image of Au-Au_2S nanoparticles. The spherical particles were about 55 nm in diameter. Fig. 2 displayed a UV–Vis spectrum of Au–Au_2S nanoparticles which consisted of two absorption bands. The band I at 520 nm was assigned to the surface-plasmon resonance of the Au nanoparticles, whereas the band II at 760 nm was due to Au-coated Au_2S nanoparticles [8].

Cytotoxicity of Au-Au_2S nanoparticles. Initial studies were performed to examine the cytotoxicity of the Au-Au_2S nanoparticles in NIH3T3 cells. As shown in Fig. 3, the cellular viability depended on the concentration, namely increasing Au-Au_2S nanoparticles resulted in a decrease of cell viability. The concentration inhibited cell growth by 50% (IC_{50}) was reached 300 μg/ml. As the concentration < 300 μg/ml, no toxicity was found even up to 3 days culture, indicating the expose time with Au-Au_2S nanoparticles has little effect on the cytotoxicity. Thus, it may be concluded that the Au-Au_2S nanoparticles with concentration < 300 μg/ml are biocompatible and can be used as drug carrier without causing cytotoxicity.

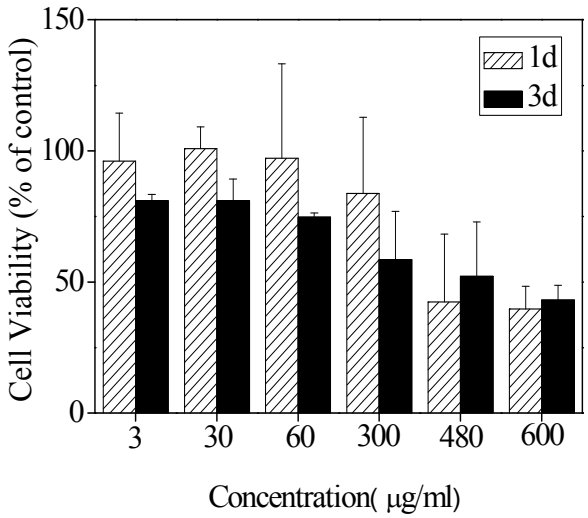

Fig. 3 Cytotoxicity of Au-Au_2S nanoparticles

Biodistribution of Au-Au_2S nanoparticles. Since the male mice exhibit more severe toxic symptoms for nanoparticles than the females [10], we chose male KM mice in our experiments. The concentration of Au in each tissue was determined by ICP-MS. No gold was found in blood, bone, muscle, heart and brain. Most of Au deposited in the liver, spleen, and little in kidney and lung, which decreased slightly with increasing times. This was consistent with Yoshifumi's observation that nanoscale substances may be easily taken up by the reticuloendotheliar system (RES) [11].

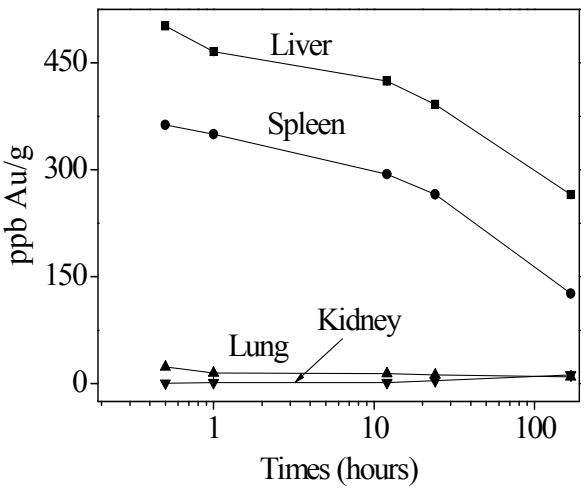

Fig. 4 Biodistribution of Au-Au_2S nanoparticles in mice up to 7 days of injection

We calculated the amount of remained Au-Au_2S in the mice as shown in Fig. 5. By increasing the time from 30 min to 7 days, the remained Au-Au_2S decreased from 14.1% to 7.4%. We also found some gold in the urine of mice after injection up to 7 days, indicating those particles may be expelled through from the body in feces. This was in accordance to the references [12-13]. In order to check if there were pathological

changes in each organ due to Au-Au_2S nanoparticles' deposition, we examined the histological sections of tissues. No pathological changes were found in each organ such as liver, spleen, lung, and kidney and so on, which showed similar to the control group (pictures not showed here). Hence, Au-Au_2S nanoparticles may possess good bio-safety.

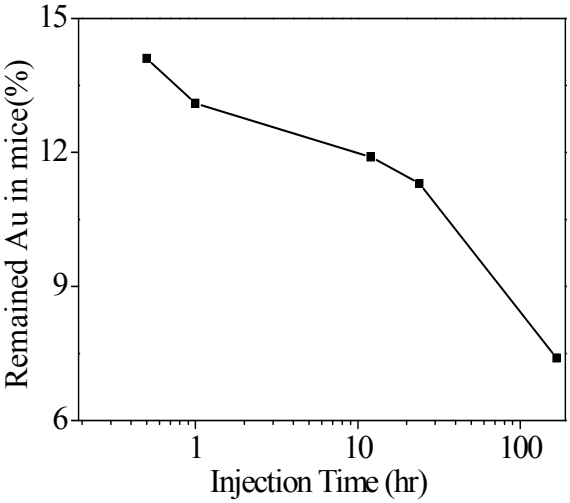

Fig. 5 The amount of the remained Au-Au_2S nanoparticles in the mice after injection for 7 days

Conclusions

We successfully synthesized near infrared (NIR) sensitive Au(shell)-Au_2S(core) nanoparticles, where Au_2S dielectric core was encapsulated by a thin gold shell. Au-Au_2S nanoparticles were preferentially taken up by the liver and spleen and ultimately eliminated mostly in the feces. The biodistribution examinations revealed that liver, spleen, kidney and lung are the target organs for Au-Au_2S nanoparticles. Considering the cytotoxicity assays and pathological examinations, they appear to be potential drug delivery vectors in future.

Acknowledgements

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