The Applications of Nanoshells and Nanocapsules in Magnetic Resonance Molecular Imaging

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Introduction

Nanoparticle systems are promising new paradigms in pharmacotherapy and provide many applications in the areas of gene therapy, drug delivery [1], imaging [2], and novel drug discovery techniques [3]. The aim of nanodiagnostics is to identify disease at its earliest stage, particularly at the molecular level. Nanoparticle-based molecular imaging has set a unique platform for cellular tracking, targeted diagnostic studies, and image-monitored therapy. Magnetic resonance imaging (MRI) has been recognized as the most important development in medical diagnosis since the discovery of the X-ray. MRI measures the characteristics of the hydrogen nuclei of water and gives spatial distribution of the intensity of water protons. The signal intensity depends on the amount of water in the image area. Contrast agents accelerate the rate of relaxation of nearby water molecules, thereby greatly increasing the contrast between the specific tissue or organ of interest and surrounding tissue. The effective MR contrast agents must have a strong effect to accelerate spin-lattice relaxation (T_1), which produces bright or positive contrast images, or shorten spin-spin relaxation (T_2), which creates a dark or negative contrast effect. Currently, MR contrast agents can be categorized into T_1-positive agents of paramagnetic species and T_2-negative agents of superparamagnetic particles. Recently, some Gd-based nanoparticle contrast agents have also been reported [4]. The superparamagnetic particles (T_2-negative agents) are nano- or sub-micrometer sizes and can be classified as superparamagnetic iron oxide (SPIO), ultrasmall SPIO (USPIO), or monocrystalline iron oxide nanoparticles (MION) [5]. Superparamagnetic particles consist of many magnetic ions with significant, large, unpaired spins; they are superparamagnetic when the magnetic ions are mutually aligned. In this work, we present a new class of bimetallic MR contrast agent: Au_3Cu_1 hollow nanospheres (Scheme 1).

Materials and Methods

Preparing Au_3Cu_1 nanoshells and polyelectrolyte-coated Au_3Cu_1 nanocapsules Cu colloidal solution was added to $HAuCl_4$ (dehydrated) and was sonicated for 10 min to form Au_3Cu_1 hollow nanospheres. The colloidal color changed almost immediately (< 1 min) from wine-red to grayish blue after it had been added to the $HAuCl_4$. We added 5 mL of 2-propanol to dilute Au_3Cu_1 colloidal solution before coating polymers with the biocompatible polyelectrolytes polyethylenimine (PEI) and polyacrylic acid (PAA). We have used a layer-by-layer (LbL) method to prepare the PEI/PAA/PEI-coated Au_3Cu_1 nanocapsules.

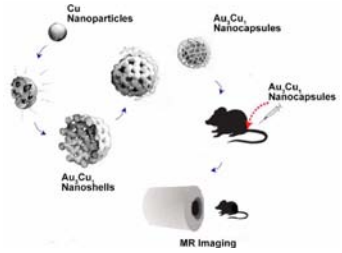
Evaluating the biocompatibility of Au_3Cu_1 nanocapsules and nanoshells coated with PEI/PAA/PEI polymers We used a WST-1 assay on a Vero cell line to measure mitochondrial dehydrogenase activity known to be associated with cell viability and two approaches were performed to evaluate the cytotoxicity of the newly synthesized Au_3Cu_1 nanocapsules and nanoshells *in vitro*. The quantification determining cell viability was done using optical absorbance (450/690 nm) and an ELISA plate reader.

***In vitro* MR imaging** The experiments were done using a spectroscope (3T MRI Biospec; Bruker, Ettlingen, Germany). A gradient system mounted on the table of the 3T magnet with an inner diameter of 6 cm and a maximal gradient strength of 1000 mT m^{-1} was used to yield high-resolution images. A quadrature coil with an inner diameter of 3.5 cm was used for RF transmission and reception. For *in vitro* MR images and both T_1 and T_2 measurements, all nanoshells and nanocapsules were dispersed in 5% agarose gel of various concentrations. Acquired images had a matrix size of 256 × 192, a field of view of 60 × 60 mm, and a slice thickness of 6 mm yielding an in-plane resolution of 234 mm after image smoothing. T_1 value measurements were done using a multi-slice multi-echo sequence with a repetition time (TR) of 6000 ms, an echo time (TE) of 8.7 ms, and 45 inversion recovery points (TI from 13.3 ms to 6000 ms). The field of view was 60 × 60 mm, the slice thickness was 6 mm, and the image matrix was 128 × 128. This allowed for simultaneous imaging of 26 vials with 0.3 mL of contrast agent for each vial. An average signal of 50 voxels was evaluated for all TI values. T_2 value measurements were performed with a spin echo sequence of TR/TE of 4000/10.1 ms, 60 echo points of 60, and a NEX of 5. The field of view was 60 × 60 mm, the slice thickness was 6 mm, and the imaging plane was 256 × 192.

Results

Biocompatible polyelectrolyte materials, polyethylenimine (PEI) and polyacrylic acid (PAA), are used on the particle surfaces forming polyelectrolyte multilayer nanocapsules. We used a layer-by-layer (LbL) method based on electrostatic attraction between oppositely charged polymer layers to first assemble a positively charged PEI and then a negatively charged PAA layer on Au_3Cu_1 nanoshells (shown in Fig. 1): 3-layer polyelectrolytes (PEI/PAA/PEI), with PEI as the outermost layer in the polyelectrolyte shells, were deposited, and the thickness of the polymer shell was 6.1 ± 1.2 nm.

We then evaluated *in vitro* spin-lattice relaxation time (T_1) weighted images and spin-spin relaxation time (T_2) weighted images in 0.5 % agarose gel for Au_3Cu_1 nanocapsules (Au_3Cu_1 coated with PEI/PAA/PEI) and Au_3Cu_1 nanoshells (not coated with polymers) with different concentrations: 0 (0.5 % agarose gel only), 0.125, 0.25, 1.25, 2.50, and 5.00 mg mL^{-1} (Fig. 2 and 3). Cell viability experiments were conducted on a vero cell line (monkey kidney cell line) using a WST-1 assay (Roche Diagnostics, Mannheim, Germany). Both Au_3Cu_1 nanocapsules and Au_3Cu_1 nanoshells were delivered over a range (0 ~ 200 μg mL^{-1}) of dosages (Fig. 4).



Scheme 1. The as-prepared Au_3Cu_1 nanocapsules used as the contrast agents in animal MR imaging.

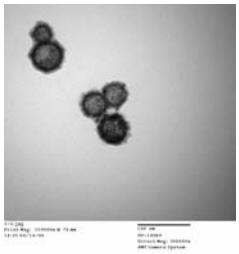


Figure 1 The TEM image of Au_3Cu_1 nanocapsules showing polyelectrolyte shells.

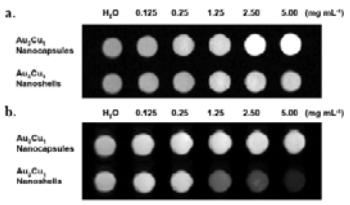


Figure 2 MR *in vitro* assays of Au_3Cu_1 nanocapsules and nanoshells. (a) Multi-slice multi-echo T_1-weighted images of Au_3Cu_1 nanocapsules and nanoshells in water containing 0.5 % agarose gel. (b) Fast spin echo T_2-weighted images of Au_3Cu_1 nanocapsules and nanoshells in water containing 0.5% agarose gel at 125.3 MHz (3T MR system).

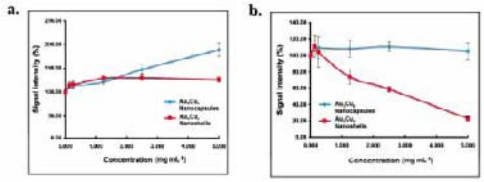


Figure 3 *In vitro* magnetic resonance imaging intensity plots of (a) T_1- and (b) T_2-weighted signals in H_2O/0.5 % agarose gel containing increasing concentrations of Au_3Cu_1 nanocapsules (blue) and nanoshells (red).

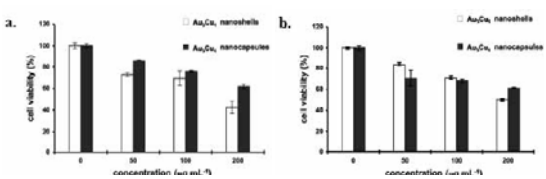


Figure 4 The biocompatibility of the Au_3Cu_1 nanoshells and Au_3Cu_1 nanocapsules. The biocompatibility was analyzed using a WST-1 assay. The nanoshells (white column) or nanocapsules (black column) were coated on the microplate with various dosages (50, 100, 200 μg mL^{-1}) first, followed by adding cells for (a) 6 h and (b) 24 h.

Conclusion

The anomalously high oxidation rate of copper, Au_3Cu_1 hollow nanostructures are believed to be the first bimetallic MR contrast agents. Au_3Cu_1 nanocapsules enhanced signal contrast not only in T_1-weighted imaging, but also in T_2-weighted imaging at lower doses, and the increase the brightness of T_2-weighted MR images has resulted in the potential development of this agent for MR angiography. We believed that the cooperativity originating from the form of the nanoparticles and the large surface area of the water because of its porous hollow morphology are primarily involved. Furthermore, the amine groups on the outermost PEI polymer shell of the nanocapsules, which further provides surface modification for the attachment of biological signals, suggest great potential for use in multifunctional composite capsules as functional carriers and imaging agents. Overall, these results might lead to the creation of other non-Gd- and iron oxide-base bimetallic contrast agents.

References

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