Hollow MnO$_x$P$_y$ and Pt/MnO$_x$P$_y$ yolk/shell nanoparticles as a T$_1$ MRI contrast agent

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**Abstract**

Hollow MnO$_x$P$_y$ and Pt/MnO$_x$P$_y$ yolk/shell nanoparticles were fabricated via the nanoscale acid-etching process from the solid MnO and Pt/MnO core/shell nanoparticles, respectively. In the synthesis, alkylphosphonic acid impurity in trioctylphosphine oxide was a key component, resulting in amorphous hollow metal phosphate nanoparticles. Hollow nanoparticles containing Mn$^{2+}$ ions showed positively enhanced T$_1$ relaxation, in which the $r_1$ values of the hollow MnO$_x$P$_y$ and Pt/MnO$_x$P$_y$ yolk/shell were greater than that of the original MnO nanocrystals. The increased surface area of the hollow nanoparticles enhanced interaction between Mn$^{2+}$ ions of the nanoparticle surface and water molecules. Cytotoxicity experiment revealed that Mn ions released from hollow walls of the MnO$_x$P$_y$ nanoparticles were responsible for the cytotoxicity, while Pt ions from the Pt/MnO$_x$P$_y$ yolk/shell were not released in the cells. These nanoparticles provide potential insights into an anticancer drug, enabling simultaneous T$_1$ magnetic resonance imaging (MRI) and therapy.

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1. Introduction

Hollow nanomaterials have attracted tremendous attention from researchers in various disciplines, because their high surface to volume ratio and the large pore volume are highly desirable for many technological applications including drug delivery vehicle [1–5]. Since magnetic nanoparticles are widely used as a contrast agent for magnetic resonance imaging (MRI), various magnetic hollow nanomaterials have been designed for the simultaneous MRI and drug delivery by loading drug molecules in their cavity [6–9]. For instance, the Xu group exhibited the anticancer effect of FePt/Co core/shell nanoparticles with hollow voids that were synthesized from FePt/Co core/shell nanoparticles via the nanoscale Kirkendall effect [10,11]. After cellular uptake, Pt atoms were released from the core and diffused through the outer shell. In their report, Pt atoms had the high cytotoxicity comparable to cisplatin. Similarly, they also designed multifunctional FePt/Fe$_3$O$_4$ yolk/shell nanoparticles as a T$_2$ MRI contrast and anticancer agent [12].

Basically, there are two different relaxation pathways in the MRI system [13,14]. One is longitudinal or T$_1$ relaxation, involving the decreased net magnetization recovering to the initial state. The other is transverse or T$_2$ relaxation, inducing magnetization on the perpendicular plane disappearing by the dephasing of the spins. Depending on their relaxation processes, contrast agents are classified as T$_1$ and T$_2$ agents. In principle, superparamagnetic iron oxide nanoparticles with strong magnetization cause microscopic field inhomogeneity and activate the dephasing of protons, resulting in short T$_2$ and T$_2$ relaxation times, and T$_2$ and T$_2$-weighted MRIs. In spite of their disadvantages such as dark domains with low signal intensity or susceptibility artifacts, many imaging systems have been concentrated on iron oxide nanoparticle-based T$_2$ MRI contrast agents, due to their a long circulation time and targeting capability. On the contrary, paramagnetic metal ions with abundant unpaired electrons such as Gd$^{3+}$ and Mn$^{2+}$ lead T$_1$ relaxation process, enhancing positive contrasts. Compared to T$_2$ agents, the T$_1$ contrast agent discriminates clearly from other pathogenic and biological conditions, and maximizes anatomic imaging with high spatial resolution by their bright signal [14]. Recently developed MnO nanoparticle-based T$_1$ MRI contrast agent provided clear T$_1$-weighted brain imaging which was comparable to fine anatomical brain structures, and allowed targeted imaging by conjugation of MnO nanoparticles with a tumor specific antibody [15–17].

Through the nanoscale acid-etching process, MnO nanoparticles
suffered transformation to hollow nanostructures with a void inside [18]. Because multifunctional nanomedical systems have been much focused on iron oxide nanoparticle-based T2 MRI contrast agents, development of new T1 MRI contrast agent using hollow nanoparticles of MnO or Gd₂O₃ are demanded for simultaneous MRI imaging and therapy with therapeutic molecules [19–23]. Herein we report on the fabrication of hollow MnO₉₆₆₃₉₃₉₄ of Pt/MnO₉₆₆₃₉₃₉₄ and Pt/MnO₉₆₆₃₉₃₉₄ via nanoscale acid-etching from solid MnO and Pt/MnO core/shell nanoparticles, respectively. By a 1.5-T clinical MR scanner, r₁ relaxation properties of hollow MnO₉₆₆₃₉₃₉₄ and Pt/MnO₉₆₆₃₉₃₉₄ yolk/shell nanoparticles as MRI contrast agents are compared. Cytotoxicity of the hollow nanoparticles is also evaluated by incubation of U87MG cancer cells with dye-labeled hollow nanoparticles.

2. Materials and methods

2.1. Synthesis of MnO nanoparticles

Preparation of Mn(II)-oleate complex and subsequent synthesis of MnO nanoparticles via thermal decomposition are performed according to the reported method [24]. For the typical synthesis of MnO nanoparticles, 1.24 g (2 mmol) of Mn-oleate dissolved in 10 g of 1-hexadecene (Aldrich 90%) was degassed at 70 °C for 1 h under vacuum to remove water and oxygen with vigorous stirring. The solution was heated to the reflux temperature (~288 °C) with a heating rate of 2 °C min⁻¹ and kept at this temperature for 1 h under an argon atmosphere. The color of the solution was changed from pink to deep green upon thermal decomposition. After cooling, the nanoparticle solids were separated with 80 ml of acetone by centrifugation and dispersed in 20 ml of hexane.

2.2. Synthesis of Pt/MnO core/shell nanoparticles

For the preparation of Pt nanoparticles, 0.08 g of Pt(acac)₂ (Strem Chemicals Inc., 0.2 mmol), 0.1 g of 1,2-hexadecanediol (Aldrich 90%), 0.06 ml of oleic acid (Aldrich 90%), and 7 ml of oleylamine were mixed and stirred vigorously. After reaction at 250 °C for 30 min, Pt nanoparticles with a diameter of 5–6 nm were separated by centrifugation and then re-dispersed in n-hexane. For Pt/MnO core/shell nanoparticles, 3 ml of Pt colloidal dispersion (ca. 20 mg Pt) was added to the solution of 0.34 g of Mn(OAc)₂ (2 mmol, Aldrich, 98%), 1.7 ml of oleic acid (5.3 mmol, Aldrich, 90%), and 18.5 ml of trioctylamine (TOPO). The solution was stirred for 10 min, and sonication was followed until the solution became clear. The mixture solution was degassed at 70 °C for 1 h under vacuum to remove hexane and residual moisture. The resulting solution was heated rapidly to 300 °C and kept at this temperature for 30 min. The solution was then cooled to room temperature and 40 ml of acetone was added to precipitate the nanoparticles. The Pt/MnO core/shell nanoparticles with a particle diameter of 40–50 nm were retrieved by centrifugation.

2.3. Synthesis of hollow MnO₉₆₆₃₉₃₉₄ and Pt/MnO₉₆₆₃₉₃₉₄ yolk/shell nanoparticles

For the preparation of hollow nanoparticles, technical grade trioctylphosphine oxide was used for nanoscale acid-etching process [18]. 10 ml dispersion of MnO or Pt/MnO core/shell nanoparticles in hexane ([Mn] = 0.5 M) was added to 15 g trioctylphosphine oxide (TOPO, Aldrich, 90%), in which small amount of alkylphosphonic acids were contained as an impurity. The solution was degassed under vacuum for 1 h. Subsequently, under argon atmosphere, the nanoparticle solution was heated up to 300 °C and maintained at that temperature for 2 h. During the reaction, the mixture solution became transparent. The resulting hollow MnO₉₆₆₃₉₃₉₄, P₆, and Pt/MnO₉₆₃₉₃₉₄ yolk/shell nanoparticles were re-dispersed in chloroform after centrifugation.

2.4. Preparation of water-dispersible nanoparticles

Multiparticulates dispersed in chloroform were encapsulated by PEG-phospholipids shell. 1 ml of the MnO or hollow MnO₉₆₆₃₉₃₉₄ nanoparticles dispersed in chloroform (10 mg/ml) was mixed with 2 ml of 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (mPEG-2000 DSPE, Avanti Polar Lipids, Inc.) in chloroform (10 mg/ml). After evaporation solvent, the mixture was incubated at 60 °C under vacuum for 1 h. The addition of 10 ml water resulted in transparent suspension. After filtration using 0.2 μm cellulose acetate syringe filter, excess mPEG-2000 PE was removed by ultracentrifugation. To conjugate fluorescences dyes, nanoparticles were encapsulated with a mixture of mPEG-2000 DSPE and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (NH₂-Peg2000 DSPE, Avanti Polar Lipids, Inc.). After ultracentrifugation, the nanoparticles with amine groups were reacted with 1 mg of rhodamine B isothiocyanate (RITC) for 6 h in PBS. Excess RITC was removed using a desalting column (PD-10, GE Healthcare Life Sciences).

2.5. Measurement of MR relaxation properties of MnO, MnO₆₆₃₉₃₉₄, and Pt/MnO₆₆₃₉₃₉₄ nanoparticles

The values of T₁ and T₂ relaxation times of the water-dispersed nanoparticles were measured with a 1.5 T clinical MRI scanner (GE Signa Excite) for various nanoparticle concentrations. An IR-FSE sequence, with 30 multiple values of Tls (TR/TE/Tl = 4400 ms/8.4 ms/50–4000 ms) for the T₁, measurement and a conventional CPMG sequence with 12 multiple TEs (TR/TE = 5000 ms/16–200 ms) for the T₂ measurement were employed with a head coil on a 1.5 T MRI scanner.

2.6. Cytotoxicity against U87MG cells

Cells were grown as monolayer cultures in 100 mm dish and subcultured 3 times for a week at 37 °C in atmosphere containing 5% CO₂ in air and 100% relative humidity. For in vitro cytotoxicity assay, cells at a logarithmic growth phase were detached and plated (0.2 ml per well) in 96-well flat-bottom microplates at a density of 1000 cells per well, which were then left for 1–2 days at 37 °C to resume exponential growth. After washing the cells with PBS, 0.1 ml of culture medium with various concentrations (3.8, 7.5, 15, 30, and 60 μg Mn/ml) of nanoparticles was added to the wells in quintuplicate. For control wells, the same volume of culture medium was included in each experiment. Following 24 h of continuous exposure to nanoparticles under 5% CO₂ atmosphere at 37 °C, cell survival was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) cell proliferation assay. Briefly, cells were incubated in media containing 0.1 mg/ml of MTT for 1 h. Thereafter, the media was removed, and the precipitated violet crystals were dissolved in 200 μl of DMSO. The absorbance was measured at 560 nm using a VersaMax™ microplate reader (Molecular Devices).

2.7. Cellular uptake and MR imaging of labeled cells

To prepare in vitro MR phantom, U87MG cells were seeded onto culture dish at a density of 1 × 10⁶ per plate in 10 ml of media and grown overnight. Subsequently, nanoparticles of various concentrations (0–8 μg Mn/ml) were added. After incubation for 24 h, the cells were washed twice with PBS to remove free nanoparticles,
and then detached by addition of 2 ml of trypsin/EDTA. After centrifugation at 1500 rpm for 5 min, cells were dispersed in 2 ml culture media and transferred to 1.5-ml microtubes. Cells pellets were prepared by centrifugation at 2000 rpm for 5 min. The measurement parameters are as follows: flip angle = 73, ETL = 1, TR = 400 ms, TE = 12 ms, field of view FOV = 100 × 100 mm², matrix = 256 × 256, slice thickness/gap = 1 mm/2 mm, NEX = 2.0.

To study cellular uptake of nanoparticles, the cells were cultured in a 4-well chamber slide (Nalgene Nunc), and incubated with nanoparticles for 24 h. Subsequently, the cells were washed with PBS, fixed with 4% paraformaldehyde, and stained with 4,6-diamidino-2-phenylindole (DAPI, 1 μg/ml in PBS, Roche). The fluorescence images were acquired with confocal laser scanning microscopy (LSM 510, Carl Zeiss).

2.8. Characterization of materials

Pt/MnO core/shell, hollow MnO-Py and Pt/MnO-Py yolk/shell nanoparticles were characterized by low- and high-resolution transmission electron microscopy (TEM, and HRTEM), and X-ray diffraction (XRD). The TEM images were obtained on JEM 2010 microscope operated at 200 kV. Powder XRD was performed using a Rigaku D/Max-3C diffractometer (Cu Kα radiation, λ = 0.15418 nm). The phase identification was performed using JCPDS-ICDD 2000 (JCPDS- International Centre for Diffraction Data). Hydrodynamic diameter of nanoparticles dispersed in water was measured with a particle size analyzer (ELS-Z2, Otsuka). We performed elemental analysis by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using ICPS-7500 spectrometer (Shimadzu).

3. Results and discussion

3.1. Preparation of hollow MnO-Py and Pt/MnO-Py yolk/shell nanoparticles for the MRI agent

Fig. 1a shows MnO nanoparticles with an average diameter of 20 nm synthesized via thermal decomposition of Mn(II)-oleate. After the acid etching using technical grade TOPO containing small amount of alkylphosphonic acid as an etchant, the MnO nanoparticles were transformed to hollow MnO-Py nanoparticles (Fig. 1b) [18]. Pt/MnO core/shell nanoparticles were fabricated by encapsulating 6 nm-sized Pt nanoparticles with MnO shells (Fig. 1c–d). Similarly, Pt/MnO-Py yolk/shell nanoparticles were generated from Pt/MnO core/shell nanoparticles, leaving voids in the MnO-Py walls (Fig. 1d–e). Fig. 1e shows that both wall thickness and pore size of the resulting hollow Pt/MnO-Py yolk/shell nanoparticles were ca. 15 nm, and their original particle size of 40–50 nm were maintained after the etching process. X-ray diffraction (XRD) pattern revealed that high crystalline MnO and Pt/MnO core/shell nanoparticles were transformed to amorphous hollow nanostructures containing manganese phosphate walls (MnO-Py) (Fig. 2).

Since the as-synthesized hollow nanoparticles are hydrophobic, they were encapsulated with polyethylene glycol-phospholipid (PEG-phospholipid) to impart hydrophilicity for biomedical applications [15]. Dynamic light scattering (DLS) data shows that the hydrodynamic diameter of encapsulated hollow nanoparticles wa ca. 78.3 nm (Fig. 3). The increase in hydrodynamic diameter compared with the size measured by TEM can be attributed to a hydrophobic lipid bilayer and a water-permeable PEG layer. In addition, no aggregate of nanoparticles was observed in TEM images.

T₁-weighted MR images were acquired using a 1.5-T clinical MR scanner to investigate their applicability for MRI contrast agents. Fig. 4a-c clearly show signal enhancement of the hollow nanoparticles in T₁-weighted MR images. We also obtained MR images of MnO nanoparticles of the diameter of 20 nm for the comparison (Fig. 4a). The r₁ value of the hollow Pt/MnO-Py yolk/shell nanoparticles (3.55 mM⁻¹ s⁻¹) was much higher than that of MnO nanoparticles (0.27 mM⁻¹ s⁻¹, Table 1). As a T₁ contrast agent, the magnetization of paramagnetic materials, such as manganese chloride, is directly dependent on the number of ions, and they have no magnetization in the absence of an external magnetic field. In our previous report, as the size of MnO nanoparticles became smaller, the signal was brighter in the T₁-weighted MR images [15]. When compared the r₁ values

![Fig. 1](image_url)

**Fig. 1.** Preparation of hollow MnO-Py and Pt/MnO-Py yolk/shell nanoparticles through the nanoscale acid-etching and their TEM images: (a) solid MnO and (b) corresponding hollow MnO-Py nanoparticles. (c) Pt, (d) Pt/MnO core/shell, and (e) hollow Pt/MnO-Py yolk/shell nanoparticles.
between MnO and hollow MnO$_x$P$_y$ nanoparticles ($r_1 = 2.35$ mM$^{-1}$ s$^{-1}$) with the same diameters (20 nm), $T_1$ contrasting effect was greatly enhanced in hollow nanoparticles, because the hollow MnO$_x$P$_y$ nanoparticles have much abundant Mn ions on the surface than those of solid MnO. Therefore, we conclude that the increased surface area of the hollow nanoparticles results in an enhanced interaction between nanoparticles and water molecules to generate a brighter contrast.
or Pt/MnO shells were almost released. and Pt/MnO yolk/shell nanoparticles have much higher T1 relaxivity derived from the hollow MnO nanoparticles. However, our experimental results revealed that the cytotoxicity of the Pt yolk. It was proven that Mn ions released from hollow walls of the Pt/MnO yolk/shell nanoparticles were responsible for the cytotoxicity. This nanomaterial is expected to be useful as a bifunctional nanomedicine as anticancer drug and T1 MRI contrast agent.

### 3.2. Cytotoxicity of hollow nanoparticles

In order to confirm cytotoxicity, we incubated U87MG cancer cells with MnO or hollow MnO nanoparticles or Pt/MnO yolk/shell nanoparticles labeled with rhodamine-B-isothiocyanate (RITC). The red fluorescence from the cytosol in confocal laser scanning microscopy (CLSM) image (Fig. 4d-f) clearly demonstrated the cellular uptake of the nanoparticles. The cellular uptake was also confirmed by the T1-weighted MR images in the Fig. 4c. As the concentration of the nanoparticles was increased, the MR signal of cells also enhanced. In Fig. 5, cytotoxicity of the hollow Pt/MnO yolk/shell nanoparticles was evaluated and compared with that of the hollow MnO nanoparticles and solid MnO nanoparticles by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.

As discussed previously, the Xu group demonstrated that Pt ions released from the core of the hollow yolk/shell nanoparticles bind with deoxyribonucleic acid (DNA) and interrupt replication and transcription, which leads to apoptosis of cancer cells [10–12]. However, our experimental results revealed that the cytotoxicity derived from the hollow MnO nanoparticles was comparable to that of hollow Pt/MnO nanoparticles, while solid MnO nanoparticles did not exhibit any notable toxicity as the Mn concentrations increased (Fig. 5). It is speculated that Mn ions that released continuously from fragile walls of the etched hollow nanoparticles caused cytotoxicity in the cells. Furthermore, it seemed that the core Pt nanoparticles did not contribute to the cytotoxicity in this experiment. TEM images taken after the assay show that Pt nanoparticles were still existed without dissolution, where as the MnO nanoparticles were almost released.

### 4. Conclusions

We fabricated hollow MnO and Pt/MnO yolk/shell nanoparticles via the nanoscale acid-etching process from MnO and Pt/MnO core/shell nanoparticles, respectively. Hollow MnO and Pt/MnO yolk/shell nanoparticles have much higher T1 contrasting effect than solid MnO nanoparticles due to their high surface area. Cytotoxicity experiment revealed that Pt ions from the core Pt nanoparticles were not released in the cells, because of the relative inertness of the Pt yolk. It was proven that Mn ions released from hollow walls of the Pt/MnO yolk/shell nanoparticles were responsible for the cytotoxicity. This nanomaterial is expected to be useful as a bifunctional nanomedicine as anticancer drug and T1 MRI contrast agent.

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### References


### Table 1

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<tr>
<th>Type of nanoparticle</th>
<th>( r_1 [\text{mM}^{-1} \text{s}^{-1}] )</th>
<th>( r_2 [\text{mM}^{-1} \text{s}^{-1}] )</th>
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<tr>
<td>Solid MnO</td>
<td>0.27</td>
<td>1.47</td>
</tr>
<tr>
<td>Hollow MnO(_x)Py</td>
<td>2.35</td>
<td>12.54</td>
</tr>
<tr>
<td>Hollow Pt/MnO(_x)Py</td>
<td>3.55</td>
<td>9.99</td>
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Fig. 5. In vitro cytotoxicity of solid MnO, hollow MnO, and hollow Pt/MnO yolk/shell nanoparticles against U87MG glioma cells after 24 h incubation.