

Research Article

Preparation of AgI/Silica/Poly(Ethylene Glycol) Nanoparticle Colloid Solution and X-Ray Imaging Using It

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This work performed X-ray imaging of mouse by using aqueous colloid solution of AgI nanoparticles coated with silica (AgI/SiO₂) and then surface-modified with poly(ethylene glycol) (PEG) (AgI/SiO₂/PEG). A colloid solution of AgI nanoparticles was prepared by mixing silver perchlorate and potassium iodide in water. The AgI nanoparticles were surface-modified with 3-mercaptopropyltrimethoxysilane and then were silica-coated by a sol-gel reaction between tetraethylorthosilicate and H₂O catalyzed with NaOH in ethanol. The AgI/SiO₂ particle surface was modified with PEG by using methoxy PEG silane (CH₃O(CH₂CH₂O)_nCH₂CONHC₃H₆Si(OC₂H₅)₃). The AgI/SiO₂/PEG colloid solution revealed a computed tomography value as high as 1343.6 HU at an iodine concentration of 0.1 M, which was higher than a commercial X-ray contrast agent with the same iodine concentration. Tissues of mouse could be imaged by injecting the concentrated colloid solution into them.

1. Introduction

X-ray imaging is one of the quite useful techniques for medical diagnosis. Chemicals composed of iodine absorb strongly X-ray. This property has been utilized for making X-ray images clearer, or taking high contrast images. Various iodine compounds have been thus far proposed as X-ray contrast agents [1–5], and several iodine compounds are commercially available. The iodine compounds, however, cannot be used for patients, in which adverse events as allergic reactions may be provoked by the iodine compounds [6–8]. In iodine compound nanoparticles coated with shell of materials inert for living bodies, that is, core-shell particles composed of core of iodine compound nanoparticles and shell of materials inert for living bodies, the shell prevents the iodine compound particles from contacting with living bodies. As a result, toxicity of iodine compounds will decrease.

Several researchers have extensively performed coating of nanoparticles with silica, which is inert for living bodies [9–15]. Their coating methods are based on a sol-gel process. Our research group has studied silica coating of various nanoparticles with the sol-gel process [16–26]. Our group has also proposed a method for silica coating of nanoparticles of silver iodide (AgI) that is one of the iodine compounds [27–35].

The present work performed synthesis of colloid solution of silica-coated AgI nanoparticles (AgI/SiO₂) by our proposed method. Furthermore, the AgI/SiO₂ nanoparticles were surface-modified with poly(ethylene glycol) (PEG) (PEGylation), which is expected to improve its imaging ability in living bodies. X-ray imaging ability of the colloid solution of the PEGylated AgI/SiO₂ (AgI/SiO₂/PEG) nanoparticles was investigated using a mouse, into which the AgI/SiO₂/PEG nanoparticle colloid solution was injected.

2. Experimental

2.1. Chemicals. AgI nanoparticles were prepared from AgClO_4 (Kanto Chemical, 99%) and KI (Kanto Chemical, 99.5%). A silane coupling agent used to increase affinity between AgI particle surface and silica shell was 3-mercaptopropyltrimethoxysilane (MPS) (Sigma-Aldrich, 97%). A silica source, a catalyst for a sol-gel reaction of TEOS, and a solvent in silica coating were tetraethylorthosilicate (TEOS) (Kanto Chemical, 95%), NaOH aqueous solution (Kanto Chemical, 1 mol/L), and ethanol (Kanto Chemical, 99.5%), respectively. Used for PEGylation of AgI/SiO₂ particle surface was methoxy PEG silane ($\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CONHC}_3\text{H}_6\text{Si}(\text{OC}_2\text{H}_5)_3$) (M-PEG-SLN) (JenKem Technology, Mw: 5000). Iopamiron 300 (Bracco Eisai, iodine concentration: 2.36 M), a commercial X-ray contrast agent, was used for a comparison to the AgI/SiO₂ particles in measurements of computed tomography (CT) values. All chemicals were used as received. Water that was ion-exchanged and distilled with Yamato WG-250 was used in all the preparation.

2.2. Preparation. An AgI/SiO₂/PEG particle colloid solution was prepared by a process composed of a method for producing AgI/SiO₂ nanoparticle colloid solution, which was reported in our previous work [32] and a method for PEGylation of the AgI/SiO₂ particle surface. First, the AgI particle colloid solution was prepared by adding a KI aqueous solution to AgClO_4 aqueous solution under vigorous stirring at 20°C. Concentrations of AgClO_4 and KI in the colloid solution were 3.9×10^{-4} and 7.8×10^{-4} M, respectively; an AgI concentration in the colloid solution was 3.9×10^{-4} M. Color of the solution turned yellow immediately after the addition, which implied formation of AgI nanoparticles. Second, the AgI nanoparticles were silica-coated by performing a sol-gel method in the presence of the AgI nanoparticles, as follows. At 15 min after the preparation of AgI nanoparticle colloid solution, an MPS aqueous solution was added to the colloid solution. At 15 min after the addition, ethanol, TEOS, and NaOH aqueous solution were successively added to the colloid solution. The reaction time was 24 h. Initial concentrations of AgI, MPS, NaOH, H₂O, and TEOS in the final colloid solution were 1.0×10^{-4} , 4.5×10^{-6} , 1.2×10^{-3} , 15, and 4.0×10^{-3} M, respectively. TEM observation performed in our previous work [32] revealed that these preparation conditions resulted in production of silica-coated particles with an average particle size of 53.2 ± 9.3 nm, which contained the AgI nanoparticles with an average particle size of 13.5 ± 4.2 nm. Third, PEGylation of AgI/SiO₂ particle surface was performed by using the M-PEG-SLN, or PEG with a silicone alkoxide group, since the M-PEG-SLN is expected to be introduced on the AgI/SiO₂ particle surface through a reaction between the silanol groups on the particle surface and alkoxide groups of the M-PEG-SLN. The AgI/SiO₂ particle colloid solution was preconcentrated by a salting-out technique. The AgI/SiO₂ particles were sedimented adjusting NaCl concentration in the colloid solution to 10 g/L with addition of a saturated NaCl aqueous

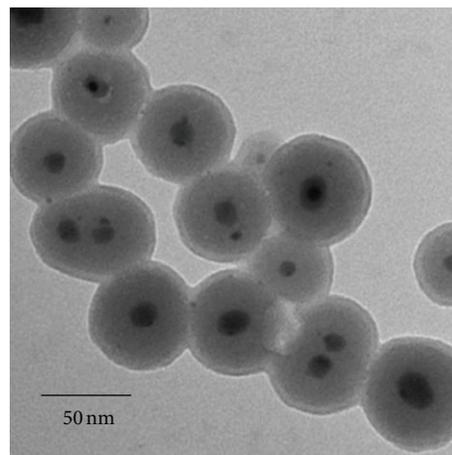


FIGURE 1: TEM image of AgI/SiO₂/PEG particles.

solution. Then, the supernatant of the colloid solution was removed by decantation. After the preconcentration by the salting out, the AgI/SiO₂ particles were washed by repeating a process composed of centrifuge, removal of supernatant with decantation, addition of the water, and shaking with a vortex mixer three times. The particle colloid solution was concentrated up to an AgI concentration above 0.1 M by decreasing the amount of added water in the washing process. For PEGylation of particle surface, or producing AgI/SiO₂/PEG particles, an M-PEG-SLN aqueous solution was added to the concentrated particle colloid solution, in which concentrations of AgI and M-PEG-SLN were 0.1 and 1.0×10^{-3} M, respectively.

2.3. Characterization. The particles were observed by a JEOL JEM-2000FX II transmittance electron microscope (TEM) operating at 200 kV. Samples for TEM were prepared by dropping and evaporating the nanoparticle suspensions on a collodion-coated copper grid. X-ray images and CT values of samples such as Iopamiron 300, the AgI/SiO₂/PEG particle colloid solutions, and an ICR mouse were obtained with an Aloka La theta LCT-200 CT system, according to our previous work [32]. The samples were put into a tube with a diameter of 3.7 cm and a length of 29.5 cm, and then their images were taken as if the samples were cut into round slices. The CT values were estimated on the basis of CT values of -1000 for air and 0 for water. The ICR mouse used was 5-6 weeks old. The mouse was put under anesthesia, and the colloid solutions (0.1 mL) were injected into the mouse from its tail veins.

3. Results and Discussion

3.1. AgI/SiO₂/PEG Particles. Figure 1 shows a TEM image of the AgI/SiO₂/PEG particles. The core-shell structure was reserved even after the PEGylation. The AgI/SiO₂/PEG particle size and the AgI core size were 55.7 ± 8.6 and 16.1 ± 3.9 nm, respectively, which were almost the same as those

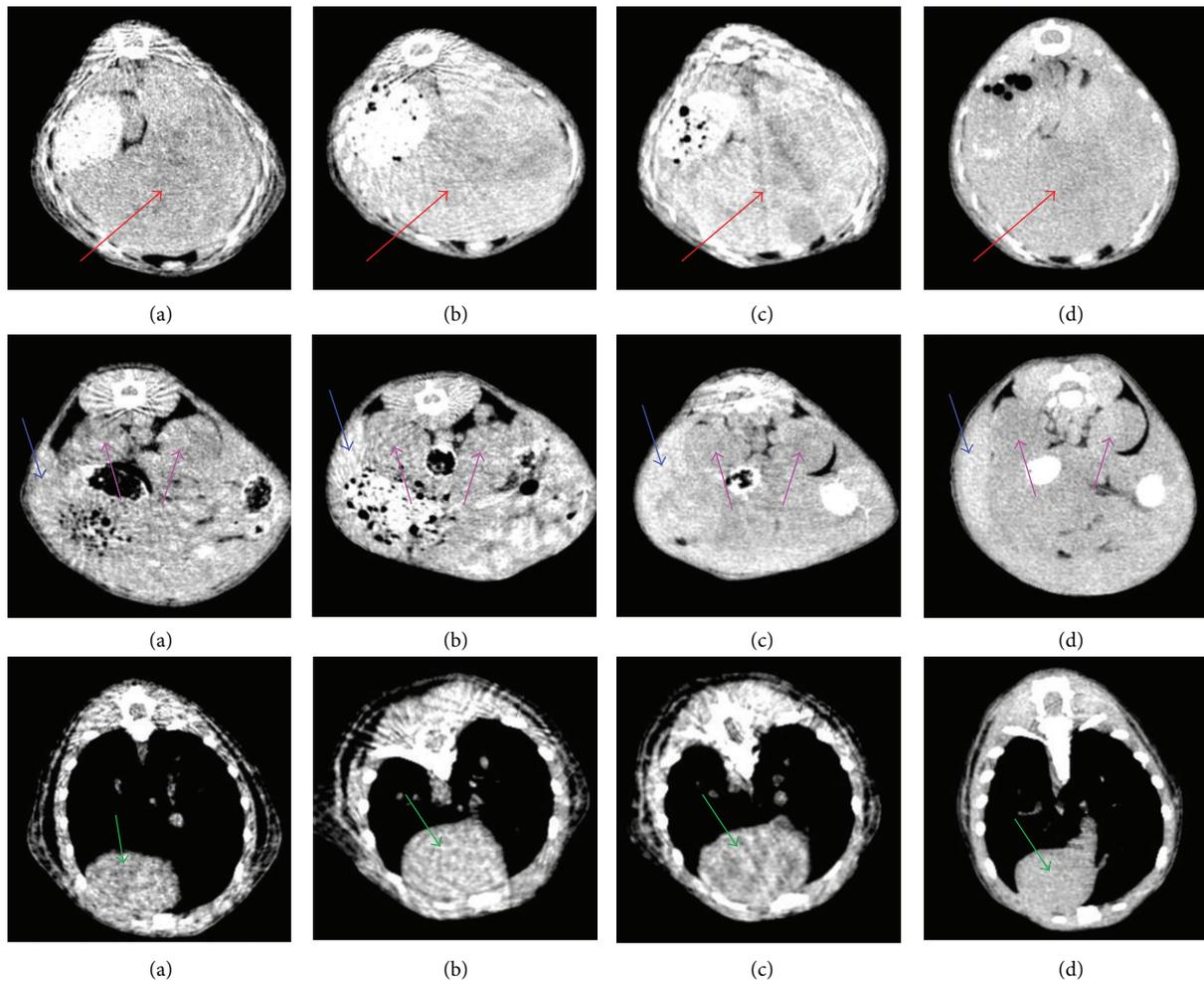


FIGURE 2: CT images of liver (red arrows), spleen (blue arrows), kidney (pink arrows), and heart (green arrows) of mouse after injection of AgI/SiO₂/PEG particle colloid solution. The images were taken (a) prior to injection, at (b) 5, (c) 1440, and (d) 2880 min after injection.

prior to the PEGylation. This indicated that the AgI/SiO₂ particle structure was preserved even after the PEGylation.

A CT value of the AgI/SiO₂/PEG particle colloid solution with the iodine concentration of 0.1M was 1343.6 HU, in which the AgI concentration in the particle colloid solution (0.1M) was regarded as the iodine concentration. Accordingly, the CT value with respect to the iodine concentration was 1.34×10^5 HU/M. For Iopamiron 300, our previous work gave that a CT value of Iopamiron 300 was 333.8 HU at an iodine concentration adjusted to 0.073 M [25]; the CT value with respect to the iodine concentration was 4.57×10^3 HU/M. These measurements indicated that the CT value of the AgI/SiO₂/PEG particle colloid solution was approximately three times larger than that of Iopamiron 300; the AgI/SiO₂/PEG particle colloid solution could function as an X-ray contrast agent sensitive compared to Iopamiron 300.

Two possible mechanisms on the large CT value for the AgI/SiO₂/PEG particle colloid solution were considered as follows. The first possible mechanism was based on adsorption of excess I⁻. For stabilizing the AgI particles colloiddally thorough adsorption of I⁻ ions on AgI particle surface [36], KI was excessively added to the AgClO₄ solution for the

preparation of AgI particle colloid solution. Some of the excess I⁻ were probably left on the AgI nanoparticles through the adsorption, which made an actual iodine concentration larger than the AgI concentration of 0.1M. The second possible mechanism was based on X-ray absorption ability of silver. The AgI nanoparticles are composed of both silver and iodine. Silver also absorbs X-ray strongly because of its large atomic number. Consequently, it was concluded that the two possible mechanisms explained that the AgI/SiO₂/PEG particle colloid solution revealed a CT value larger than expected.

3.2. X-Ray Imaging of Mouse. Figure 2 shows X-ray images of the mouse prior to and after the injection of the AgI/SiO₂/PEG particle colloid solution. Prior to the injection, tissues such as liver and spleen in the images were hard to be recognized because of small differences in contrast between them and other parts. Just after the injection (5 min), these tissues were imaged, since contrasts of these tissues became lighter than prior to the injection. Kidney and heart were easy to be recognized even prior to the injection, judging from their expected shapes and locations in body. Just after the injection, these tissues were imaged, though the images

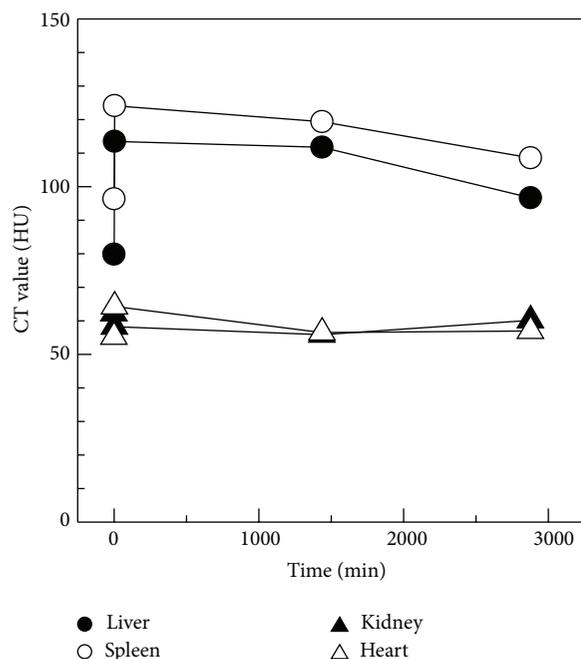


FIGURE 3: CT values of liver (●), spleen (○), kidney (▲), and heart (△) of mouse after injection of AgI/SiO₂/PEG particle colloid solution.

were not so clear as those of the liver and the spleen. This observation indicated that the AgI/SiO₂/PEG particles flew in blood tubes, reached the tissues, and then were probably recognized as alien substances and were intensively trapped in them. Over 5 min, the contrasts of liver and spleen tended to become darker with an increase in time.

Figure 3 shows dependencies of CT values of various tissues on time after the injection. The CT values of liver and spleen jumped from 79.9 to 113.5 and from 96.3 to 124.1 HU after the injection, respectively. Though the CT values of kidney and heart also jumped after the injection, their jumps were slight compared to those of liver and spleen. This supported the intensive trapping in liver and spleen. After the jumps, the values in liver and spleen decreased gradually with the increase in time. These decreases indicated that the AgI/SiO₂/PEG particles were metabolized and then were released from the liver and the spleen.

4. Conclusions

The colloid solution of AgI/SiO₂/PEG particle with the average size of 55.7 ± 8.6 nm and the AgI core size of 16.1 ± 3.9 nm was produced through PEGylation using M-PEG-SLN for the AgI/SiO₂ particles that were fabricated in our previous work. Its CT value was as high as 1343.6 HU at the iodine concentration of 0.1M, which was three times larger than that for the commercial X-ray contrast agent with the same iodine concentration. This indicated that the AgI/SiO₂/PEG particle colloid solution was sensitive for absorption of X-ray. The injection of the colloid solution into a mouse made the X-ray images of its tissues be taken clearly. From the aforementioned experimental results, it was found that the

AgI/SiO₂/PEG particle colloid solutions could work as an X-ray contrast agent. Further studies are in progress toward practical use.

Conflict of Interests

The Iopamiron 300, the MPS, the M-PEG-SLN, and the other chemicals were supplied by Bracco Eisai, Sigma-Aldrich, JenKem Technology, and Kanto Chemical, respectively, with no financial support from the four companies.

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