Ultrasmall dopamine-coated nanogolds: preparation, characteristics, and CT imaging

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ABSTRACT
Water-dispersible ultrasmall nanogolds (WDU AuNPs) and their dopamine-coated nanogolds (WDU AuNPs@DPAs) were prepared by a reduction method with sodium borohydride as a reducing agent and a stabilised agent of 2-mercaptosuccinic acid in aqueous solution. The effects of these nanoparticles on computed tomography (CT) imaging were evaluated. The size distributions and Zeta potential of the nanoparticles were measured with a Malvern size analyser, and nanoparticle morphology was observed by transmission electron microscopy. These characteristics were confirmed by Fourier transform spectroscopy and ultraviolet/visible spectra. It was found that WDU AuNPs@DPAs were 5.4 nm in size with clear core–shell structure. The 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide assay results showed that the WDU AuNPs and WDU AuNPs@DPAs were hypotoxic to different cells. The WDU AuNPs@DPAs showed a much longer circulation time and a larger CT attenuation coefficient than iohexol and could be excreted by the kidney and bladder. These nanoparticles showed considerable potential for future application in CT imaging.

KEYWORDS
AuNPs; dopamine; CT imaging

1. Introduction

X-ray computed tomography (CT), which allows 3D visual reconstruction and segmentation of organs and tissues, is currently among the most efficient noninvasive imaging and diagnostic tools. CT contrast agents that contain high atomic number elements, such as iodine,[1] gold,[2] ytterbium,[3] and bismuth,[4,5] allow the easy identification of soft tissues by CT. Commercial CT contrast agents mostly comprise iodinate molecules, such as diatrizoic acid and Omnipaque (iohexol). These small molecules are cleared by the kidney rapidly and could induce serious adverse effects on the excretion pathways in vivo[6] because of their viscosity and the osmotic pressure due to the high doses using in clinic. Nanogolds (AuNPs) are new and promising CT contrast agents that allow 2.7-fold higher CT absorption value per unit weight than iodinate compounds.[7] AuNPs have become popular in CT imaging because of their excellent characteristics, such as hypotoxicity in vivo,[8] low dosage, long circulation time, and controllable shape, size, and surface. AuNPs’ surfaces have easily modificable characteristics with different functional groups. The shape, size, and surface characteristic of the AuNPs are important factors that could affect cellular uptake[9,10] in vivo; for
example, AuNPs smaller than 1.4 nm will induce cell toxicity [11] and AuNPs at sizes smaller than 8 nm may be excreted by the kidney.[12] Moreover, according to the report of Sun et al., AuNPs of different sizes provide various X-ray attenuation coefficients. Small AuNPs lead to large X-ray attenuations.[13] Therefore, AuNPs of sizes in the range 1.4 to 8 nm are the best choice for improving CT imaging abilities. Moreover, AuNPs of such sizes are safe for the organism in the long run.

To synthesise 1.4 to 8 nm AuNPs, the most common route is to use borohydride to reduce gold salts in the presence of a capping agent, which contains mercapto group; such methods could produce ultrasmall AuNPs in nonaqueous solutions.[14] However, convenient and efficient methods that can synthesise stable ultrasmall AuNPs in water besides dendrimer-entrapped AuNPs,[15] polymer-stabilised AuNPs,[16] are lacking. The high cost of dendrimer limits the mass production of dendrimer-entrapped AuNPs, whereas polymer-stabilised AuNPs usually fail to produce monodispersed samples. In 2003, Negishi et al. used meso-2, 3-dimercaptosuccinic acid (DMSA) both as reducing and stabilising agent to prepare subnanometer-sized gold clusters in aqueous solution.[17] The two $-COOH$ groups of the molecule resulted in strong reducibility of the DMSA, whereas the two $-SH$ groups resulted in super hydrophilicity. The intensely hydrophilic DMSA reduced the gold salts to gold cluster rapidly, and capped the surface of the gold cluster via the strong binding of S and Au$^0$. Such effects made it difficult for the newly synthesised gold cluster to grow in size thus gold clusters smaller than 1 nm could be obtained. Furthermore, in 2007, Niu et al. reportedly used 2-mercaptosuccinic acid (MSA) as a reducing agent to synthesise 30 to 150 nm quasi-spherical nanogolds by a one-step, seed-mediated growth method.[18] It can be found that hydrophilic molecules (DMSA and MSA), which contain the mercapto group, were used as a stabilising and reducing agent to prepare AuNPs in water. However, controlling the nucleation and growth of AuNPs by changing the ratio of reducing and stabilising agent and moderating the sizes of AuNPs optionally were impossible. Thus, 2 to 8 nm ultrasmall AuNPs could not be prepared by the abovementioned methods.

We assumed that MSA can be used as the stabilising agent, and another strong reducing agent was required to make the reduction and stabilisation procedures separate. By adjusting the proportion of gold salts, reducing agents, and MSA in the preparation process, we could control the rate of the nucleation and growth of AuNPs. Thus, differently sized AuNPs can be obtained.

However, bare AuNPs will aggregate under the complicated physiological conditions in vivo, making them unable to produce in large scale and unsuitable for CT imaging. Thus, little studies about ultra-small AuNPs in vivo have been found. A biocompatibility coating might overcome this difficult for further application. Dopamine, a mussel-inspired adhesive molecule and the analogue of 3,4-dihydroxy-L phenylalanine (L-DOPA) found in adhesive protein,[19] has recently become popular as a novel coating material because of its excellent biocompatibility, biodegradability, and antifouling characteristics.[20] Dopamine can be used to easily coat nearly all materials with controllable thickness and permanent stability under mild polymerisation conditions. Dopamine coating on the surface of the AuNPs can improve their stability.

In this study, we developed a novel protocol for the preparation of 4 to 5 nm AuNPs in the aqueous phase (Scheme 1). MSA and sodium borohydride (NaBH$_4$) were used as stabilising and reducing agents, respectively. The procedures were conducted under ice bath conditions to limit the reducing ability of MSA. We mixed the gold salts and MSA uniformly, and NaBH$_4$ was added rapidly as the main reducing agent in this reaction. The hydrophilic MSA could stabilise the newly developed AuNPs in aqueous solution, and water-dispersed ultrasmall nanogolds (WDU AuNPs) with sizes ranging from 4 to 5 nm were prepared with an appropriate proportion of MSA, gold salts, and NaBH$_4$. In addition, dopamine was used to encapsulate the WDU AuNPs, and this process improved WDU AuNPs stability,[21] concentration ability, hypotoxicity, and antifouling characters in vivo. The dopamine-coated AuNPs (WDU AuNPs@DPAs) have ideal characteristics for CT imaging, indicating that these water-dispersed multifunctional ultrasmall nanogolds have considerable potential application in CT imaging.
2. Experimental

2.1. Chemicals

MSA, hydrogen tetrachloraurate trihydrate (HAuCl₄•3H₂O), KBr, and iohexol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). NaBH₄, sodium carbonate (Na₂CO₃), dopamine hydrochloride (DPA), Tris hydroxy methyl aminomethane (Tris), were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were obtained from Sigma (St. Louis, USA). All reagents were of analytical grade and used without further purification. Double distilled water was used throughout this study.

HeLa, PC-3, and IMR-90 cell lines were obtained from the School of Life Science and Technology, Xi’an Jiaotong University, China. PRMI-1640 and high glucose medium, penicillin, streptomycin, and foetal bovine serum (FBS) were obtained from ThermoFisher Scientific Inc. (MA, USA).

Female Balb/c mice (weighing 20.0 to 25.0 g) were purchased from the School of Medicine, Xi’an Jiaotong University, China. The mice were fed and maintained under standard housing conditions. The animal experiments were approved by the Ethics Committees of Xi’an Jiaotong University.

2.2. Preparation of WDU AuNPs

Briefly, HAuCl₄•3H₂O at 0.5 ml of 1% (wt), MSA at suitable concentrations, and 0.5 ml Na₂CO₃ at 0.1 mol L⁻¹ were mixed uniformly in a 50 ml volumetric flask. Double distilled water was used to measure the volume. Subsequently, the mixture was transferred into an acid-cleaned conical flask. After a few minutes in ice water bath, 2.5 ml of 0.5 mg ml⁻¹ NaBH₄ solution was added into the flask. The reaction was conducted under strenuous stirring for 15 min. The water-dispersible ultrasmall nanogolds (WDU AuNPs) with sizes of 4 to 5 nm were obtained.

2.3. Preparation of WDU AuNPs@DPAs

The dopamine shell was made thin using the following process. Tris (9.9 ml) at 100 mmol L⁻¹ and dopamine solution (0.1 ml) at 1 mg ml⁻¹ were mixed uniformly in a clean conical flask. WDU AuNPs (10 ml) were added, and the solution was stirred slowly for 3 to 4 h at room temperature. The obtained water-dispersed ultrasmall nanogolds coated with dopamine shell (WDU AuNPs@DPAs) were concentrated by centrifuging at 11,000 rpm for 30 min. The procedure was repeated thrice.
2.4. Measurements and characterisations

The composition and morphology of WDU AuNPs and WDU AuNPs@DPAs were analysed by transmission electron microscopy (TEM) (JEOL JEM-100SX) and high-resolution TEM (JEOL JEM-2100F) (Tokyo, Japan). For TEM, samples were dropped in aqueous solution on a carbon-coated ultra-thin copper grid and measured using a 75 kV electron source. High-resolution TEM was performed using a 200 kV electron source.

The Zeta potential and the hydraulic radius data were obtained by Malvern Zetasizer Nano ZS90 (Malvern, UK). A sample (0.8 ml) was added into the suitable cell, and Zeta potential and hydraulic radius were measured at room temperature.

Ultraviolet/Visible (UV/Vis) measurements were performed with T90 UV/Vis Spectrophotometer (PG INSTRUMENTS, Ltd., China). To evaluate the stability of WDU AuNPs and WDU AuNPs@DPAs, we mixed the two aqueous solutions at the same concentrations with phosphate buffer saline (PBS) and FBS, respectively. Their size changes could be directly determined by the UV/Vis spectra and their colours. Therefore, we observed the colour changes that suggest the stability of these nanoparticles in high ionic strength of PBS. The Fourier transform infrared spectroscopy (FTIR) spectra of the samples were recorded with a Bruker Optics Tensor 27 spectrometer (Ettlingen, Germany). The samples were dried in a vacuum freeze drier for 8 h prior to analysis. KBr was utilised and mixed with the sample powders to produce tablets for measurements.

2.5. Cell culture and cytotoxicity assay

PC-3, HeLa, and IMR-90 cells lines were cultured in PRMI-1640 and high glucose media, which were supplemented with 50 μg ml⁻¹ of penicillin, 40 μg ml⁻¹ of streptomycin sulphate, and 10% FBS in a humidified incubator (Sanyo, Japan) at 37 °C and 5% CO₂. Cells were harvested and re-suspended in fresh medium before plating.

Cytotoxicity was measured by the MTT assay. PC-3, HeLa, and IMR-90 cells were seeded into a 96-well plate and subsequently incubated at 37 °C and 5% CO₂ for 24 h. After incubation with different concentrations of WDU AuNPs and WDU AuNPs@DPAs for another 24 h, the medium was discarded, and cells were washed with PBS thrice prior to the addition of a new medium containing MTT at final concentration of 0.5 mg ml⁻¹ and incubation at 37 °C for 4 h. The medium was discarded, and the formazan was dissolved with DMSO at 200 μl per well. The absorbance was recorded using a Thermo Scientific Multiskan GO Microplate Spectrophotometer (New York, USA) at 495 nm. The experiments were repeated five times, and the data were analysed by GraphPad Prism 5.

2.6. CT imaging

To evaluate CT contrast efficacy of WDU AuNPs@DPAs and to compare the effects of WDU AuNPs@DPAs with that of commercial CT contrast agent iohexol, we dispersed samples in water at the same concentration and measured the CT values by scanning. Citric acid-stabilised AuNPs (CA AuNPs), served as the control group, were obtained following the method reported by Kim et al.[22]

For in vivo CT, 200 μl of WDU AuNPs@DPAs (200 mg Au Kg⁻¹ body weight) and iohexol were intravenously injected into mice. After injection, mice were anesthetised with 100 μl of 2 wt% of phenobarbital by intraperitoneal injection. The CT values at 30 min and 1, 2, 4, 24, and 72 h were obtained. The CT values of WDU AuNPs@DPAs and iohexol in vivo at different times and in various organs were obtained and quantitatively calculated. CT image was acquired using CT series 5000 Apogee tube (Oxford instrument, USA). Imaging parameters were as follows: tube voltage, 50 kVp; tube current, 1 mA; spot size, 35 μm; resolution ratio, 1944 × 3072.

To study the distribution of the WDU AuNPs@DPAs in vivo, the mice organs were made as pathological sections following standard methods and evaluated using a microscope (Nikon, Japan).
3. Results and discussion

3.1. Preparation of WDU AuNPs

Various studies on the reagent preparation method of AuNPs for CT, such as the dendrimer-entrapped AuNPs [15] and the polymer-stabilised AuNPs,[16] have been reported. However, few convenient and efficient WDU AuNPs preparation methods have been found. Negishi et al. synthesised subnanogold clusters with DMSA as the reducing and stabilising agent.[17] The relatively strong reducing ability of the two $-\text{SH}$ groups in the DMSA molecule was demonstrated, and the monolayer protection of the DMSA on the surface of the newly reduced Au core was observed. In this study, we intended to use MSA as the stabilising agent and NaBH$_4$ as the reducing agent. Thus, separation and reduction were separate processes in the synthesis of ultrasmall AuNPs. The processes were performed under ice bath conditions to limit the reducing ability of MSA. NaBH$_4$ was the main reducing agent in this reaction (Scheme 1). By adjusting the ratio of stabilising and reducing agents to control the reduction rate of the gold salts and control the nucleation and growth of AuNPs, water-dispersed ultrasmall nanogolds (WDU AuNPs) of approximately 4 to 5 nm in size were produced.

To verify our assumption, we initially used different stabilising agents that contained the $-\text{SH}$ group, such as cysteine (Cys) and sodium dimercaptosulfonate (DMPS), to synthesise the AuNPs. Figure S1(a) (Supplemental data) shows the UV/Vis absorption peak of the Cys- and DMPS-stabilised AuNPs, whereas Figure S1(b) and S1(c) (Supplemental data) showed the diameter distribution of Cys-stabilised AuNPs and the size of DMPS-stabilised AuNPs, as measured by Malvern size analyser, respectively. The two solutions showed no typical absorption peaks from 400 to 700 nm, and the diameters of the AuNPs were 36 and 182 nm, respectively. Cys and DMPS failed to synthesise the ultrasmall AuNPs, even if both compounds contained the mercaptan group. We used MSA as the stabilising agent at different proportions of gold salt and reducing agent to synthesise AuNPs. Figure S2(a)–S2(c) (Supplemental data) showed the sizes of the produced AuNPs, as measured by Malvern size analyser. The typical UV/Vis absorptions of AuNPs were 516, 516, and 517 nm, whereas the diameters were at 4, 4.9, and 5.9 nm, respectively. Using MSA as the stabilising agent could lead to the rapid synthesis of differentially sized ultrasmall AuNPs. Synthesis was completed by changing the ratio of the MSA and gold salts in water. We finally determined the optimal proportion of the MSA and gold salts and reducing agent. We used 200 equivalent units of gold salts and 50 equivalent units of reducing agent (equivalent to MSA) to synthesise ultrasmall AuNPs (approximately 4 to 5 nm in size).

3.2. Characterisation of WDU AuNPs and WDU AuNPs@DPAs

TEM images revealed that WDU AuNPs have a relatively uniform spherical shape with a mean diameter of $4.5 \pm 1.07$ nm and a narrow size distribution (Figure 1(a)). In comparison, the WDU AuNPs@DPAs were slightly larger than bare AuNPs. WDU AuNPs@DPAs had a uniform mean diameter of $5.4 \pm 1.27$ nm and narrow size distribution (Figure 1(b)). High-resolution TEM images revealed the clear core–shell structure of the WDU AuNPs@DPAs and the crystalline nanostructure of the WDU AuNPs (Figure 1(a) and 1(b) inset), indicating the successful coating of dopamine. The thickness of the dopamine shell was less than 1 nm, which indicates the presence of an ultrathin dopamine shell capping on the surface of the WDU AuNPs.

The UV/Vis spectrum of newly synthesised WDU AuNPs is presented in Figure 2(a). The maximal absorption peak of the WDU AuNPs was at 517 nm thus the WDU AuNPs appeared in watermelon red colour. The WDU AuNPs@DPAs sample showed a maximal absorption peak at 524 nm. The slight red-shift of the UV/Vis peak to WDU AuNPs@DPAs was due to the increase of refractive index around WDU AuNPs after dopamine coating. The Zeta potentials of WDU AuNPs and WDU AuNPs@DPAs were $-20.6$ and $-17.2$ mV, respectively. The deprotonation of phenolic hydroxyl group on the dopamine shells contributed to the negative charge on the surface of
WDU AuNPs@DPAs, indicating that the ultrathin dopamine shell had little effect on the surface charge of the AuNPs. In the inserted pictures in Figure 2(a), 1 and 2 showed the appearance of WDU AuNPs and WDU AuNPs@DPAs, respectively. The different compositions and absorption peaks of the two solutions were observed, whereas 3 and 4 showed the colour of WDU AuNPs and WDU AuNPs@DPAs mixed with PBS at the same concentrations, respectively. Bare WDU AuNPs in PBS transformed in colour from watermelon red to purple or blue in seconds, whereas WDU

Figure 1. Characterisation of WDU AuNPs and WDU AuNPs@DPAs. (a) The TEM imaging and the size distribution histogram of WDU AuNPs, and the inset picture is the TEM imaging measured by a high-resolution TEM; (b) the TEM imaging and the size distribution histogram of WDU AuNPs@DPAs, and the inset picture is the TEM imaging measured by a high resolution TEM to show the core–shell structures.

Figure 2. (Colour online) (a) The UV/Vis absorption spectra of WDU AuNPs and WDU AuNPs@DPAs. The inset pictures show the appearances of (1) WDU AuNPs, (2) WDU AuNPs@DPAs, and (3,4) their isomotic solutions in PBS with the Au atom concentration at 0.01 mg ml⁻¹; (b) The FTIR spectra of WDU AuNPs@DPAs, dopamine (DPA), poly-dopamine (PDA).
AuNPs@DPAs retained their red colour in PBS, which was a high ionic strength solution. AuNP sizes were significantly different relative to their absorption peaks; purple or blue colour indicated large particles, whereas red colour indicated small particles.[23] The two solutions in PBS showed different colours, indicating that bare WDU AuNPs in PBS were not as stable as WDU AuNPs@DPAs. This phenomenon was confirmed by the results of Malvern size analysis. It is the reason the previous works about the AuNPs used in CT imaging were need of a polymer or dendrimer coating. The WDU AuNPs@DPAs were stable in PBS, blood serum, and complete medium for more than one month (as shown in Figure S2(d) and S2(e), Supplemental data). WDU AuNPs were not suitable for CT imaging in vivo, whereas WDU AuNPs@DPAs showed excellent stability and potential use in CT applications.

Figure 2(b) showed the FTIR spectra of WDU AuNPs@DPAs, dopamine and poly-dopamine, respectively. The peaks at approximately 3400 cm$^{-1}$ in each spectrogram indicated the stretching vibrations of $\equiv$C0NH. The phenolic hydroxyl group characteristic peaks appeared at approximately 3147 cm$^{-1}$. The typical functional group of C$\equiv$O in the polydopamine showed high absorption at 1300 cm$^{-1}$, indicating that the parts of dopamine characteristic peaks did not change after the coating process. The FTIR spectra provided additional evidence of successful dopamine coating on the surface of WDU AuNPs.

### 3.3. Cytotoxicity of WDU AuNPs and WDU AuNPs@DPAs

It is reported that AuNPs were taken up by human cells but acute cytotoxicity was not observed.[8] According to the report by Shukla et al. in 2005, AuNPs are not cytotoxic and can reduce the production of reactive oxygen and nitrite, indicating that these compounds are suitable candidates for nanomedicine and nanobiotechnology applications [24] and CA AuNPs were safe for HepG2 cells [22]. However, different perspectives have been proposed. In 2007, Pan et al. reported that the cytotoxicity of AuNPs was size dependent. Distinct cell death was induced by 1.4 nm AuNPs within 12 h by necrosis.[11] MTT assay was used to assess the cytotoxicity of the WDU AuNPs and WDU AuNPs@DPAs. Different concentrations (0.045 to 450 $\mu$g ml$^{-1}$) of the WDU AuNPs@DPAs solution were chosen for this assay. Figure 3 shows that the two nanoparticles are safe for HeLa, PC-3, and IMR-90 cells and WDU AuNPs@DPAs showed lower cytotoxicity than bare WDU AuNPs in general. Under certain concentrations, the differences were statistically significant. WDU AuNPs@DPAs showed low cytotoxicity and can be applied in vivo.

### 3.4. CT imaging

Many tissues are easily visualised with high reliability by CT. The ability of the attenuation of X-ray is measured by Hounsfield units (HU). For soft tissues comprising carbohydrates, the CT values are in the range of 50 to 100 HU, which make the tissues difficult to visualise while bones are defined at 1000 HU. CT contrast agents containing high atomic elements to attenuate X-ray are used to make
the soft tissues easy to distinguish. The most common agent used in clinical practice is Omnipaque (iohexol), which is a nonionic small molecule with a short circulation time and shows renal toxicity when used in large dosages. AuNPs are new promising CT agents that have already been used for cancer and blood imaging because of their high X-ray absorption and hypotoxicity. Typical CT values of the iohexol, CA AuNPs, and WDU AuNPs@DPAs at different concentrations are shown in Figure 4(d). At the same concentrations of the abovementioned solutions, the CT value of iohexol was approximately 360 HU, whereas that of CA AuNPs and WDU AuNPs@DPAs was larger than 420 HU, indicating that AuNPs provided much higher CT values than iohexol at the same concentration. For in vivo CT imaging, we measured the CT attenuation value by intravenous injection of the newly synthesised WDU AuNPs@DPAs, CA AuNPs, and iohexol in mice to assess X-ray absorption ability of the tested compounds. The metabolic pathways and the bio-distribution of the nanoparticles in vivo were studied by measuring the CT value at different time points from pre-injection to 3 d after injection.

Figure 5 showed the CT of the different organs (heart, liver, kidney, and bladder) before and after intravenous injection of the WDU AuNPs@DPAs, iohexol, and CA AuNPs at different times. The remaining CT results are shown in Figure S3 and S4 (Supplemental data). After 1 d of WDU AuNPs@DPAs injection, most of the sample accumulated in the bladder, which made the bladder much brighter than other organs and the bladder itself at previous times. WDU AuNPs@DPAs in vivo would be excreted by kidney and bladder as well as iohexol while CA AuNPs were enriched in the liver and spleen. At 30 min after injection of the iohexol, the bladder was much brighter than the other organs and showed a CT value of 943 HU, indicating that iohexol had shorter circulation time in vivo than the WDU AuNPs@DPAs. By quantitative calculation of the CT values shown in Figure 4(a)–(c), the CT values of the liver were approximately 416 HU with WDU AuNPs@DPAs treatment and no significant differences

![Figure 4](image-url)
were observed at different time points. Thus, the WDU AuNPs@DPAs were not concentrated in the liver. WDU AuNPs@DPAs had the same excretion pathway \textit{in vivo} as the small iodinate molecule iohexol because both are excreted by the kidney and bladder. Figure S4 (Supplemental data) showed the CT results of iohexol solution at the commonly used concentration of 250 mg Kg$^{-1}$ body weight, iohexol would be rapidly cleared by the kidney in 10 min. This finding revealed the extraordinary short circulation time of iohexol. In 2007, Kim et al. reportedly used polyethylene glycol (PEG)-coated, AuNPs of approximately 30 nm in size as CT contrast agents.\cite{22} PEG-coated AuNPs showed a relatively long blood circulation time (longer than 4 h) and accumulated in the phagocyte cell-rich organs, such as the spleen and liver. Thus, PEG-coated AuNPs were not excreted by the kidney and bladder. Moreover, the CT values of the organs where the agent was concentrated increased by 40 HU for the liver and by 70 HU at 233 mg Au Kg$^{-1}$ body weight. WDU AuNPs@DPAs showed much better characteristics in this study than in the previous one. The WDU AuNPs@DPAs at a low dosage of 200 mg Au Kg$^{-1}$ body weight was satisfactory for CT imaging. WDU AuNPs@DPAs showed satisfactory imaging effects and long circulation time. WDU AuNPs@DPAs could be excreted by the kidney and concentrated in the bladder in 24 h, indicating the potential for its application to targeted imaging in the future. In contrast, PEG-coated AuNPs would accumulate in liver and spleen which would do harm to the organism in the long run. The pathological sections shown in Figure 6 provided additional evidence that WDU AuNPs@DPAs were not concentrated in liver. In the liver of the mice injected with CA AuNPs, there were much black dots while the liver from the WDU AuNPs@DPAs was similar to the normal liver, indicating WDU AuNPs@DPAs were not concentrated in the liver.

WDU AuNPs@DPAs can be an effective CT contrast agent and will provide a much higher CT values than either iohexol or other lager AuNPs at the same concentrations because of their ultrasmall sizes and biocompatibility coating. WDU AuNPs@DPAs had the longer circulation time than iohexol. WDU AuNPs@DPAs can be cleared by kidney in 24 h, which means WDU AuNPs@DPAs do not result in long-term damage to the organism. Safety is an important characteristic of a contrast agent for targeted CT. Their ultrasmall size and biocompatibility with antifouling dopamine coating make WDU AuNPs@DPAs ideal for CT imaging.
4. Conclusion

We developed a novel strategy to prepare WDU AuNPs and WDU AuNPs@DPAs. Both nanoparticles show the characteristics of biocompatibility, biodegradability, stability in aqueous solution, and lower toxicity to cells. WDU AuNPs@DPAs showed better efficiency in larger CT attenuation coefficient than iohexol. WDU AuNPs@DPAs can be excreted by the kidney and bladder in vivo. Thus, WDU AuNPs@DPAs have the potential use in CT imaging.

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Disclosure statement

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