Antibiofouling polymer coated gold nanoparticles as a dual modal contrast agent for X-ray and photoacoustic imaging

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Abstract. X-ray is one of the most useful diagnostic tools in hospitals in terms of frequency of use and cost, while photoacoustic (PA) imaging is a rapidly emerging non-invasive imaging technology that integrates the merits of high optical contrast with high ultrasound resolution. In this study, for the first time, we used gold nanoparticles (GNPs) as a dual modal contrast agent for X-ray and PA imaging. Soft gelatin phantoms with embedded tumor simulators of GPNPs in various concentrations are clearly shown in both X-ray and PA imaging. With GNPs as a dual modal contrast agent, X-ray can fast detect the position of tumor and provide morphological information, whereas PA imaging has important potential applications in the image guided therapy of superficial tumors such as breast cancer, melanoma and Merkel cell carcinoma.

1. Introduction
X-ray is one of the most useful diagnostic tools in hospitals in terms of frequency of use and cost. The X-ray imaging modality can obtain high resolution anatomical images based on X-ray’s attenuation when X-ray passes through tissue. It has been chosen as the morphological imaging modality for small animals because of the accuracy of the anatomical information [1]. PA imaging is an emerging non-ionizing, noninvasive imaging modality which can overcome the limitations of purely optical imaging methods that result from the intense optical scattering in soft tissue, and combines the spectroscopic capability of light with the spatial resolution of ultrasound may lead to a new medical imaging technique. PA imaging is interesting for some materials in the body having different optical absorption coefficients, which is a wavelength dependent tissue property. Thus, oxygenated or deoxygenated hemoglobin, water or melanin etc, can be distinguished based on their absorbed spectrum. These merits make PA imaging can be used to identify different functional activities of tissues that may be indistinguishable in other imaging modalities. PA imaging has been employed to image vasculature structure, detect breast tumors, track contrast agent, monitor glucose, measure
oxygen saturation, perform molecular and function imaging, count cell with photoacoustic flow cytometry [2-7].

Current contrast agents for X-ray are based on iodinated small molecules, because among nonmetal atoms, iodine has a high X-ray absorption coefficient [8-9]. Iodinated compounds, however, allow only very short imaging time due to rapid clearance by the kidney, which can also cause them to have renal toxicity [10-11]. Instead, PEG coated GNP can overcome these limitations [12-13]. Furthermore, based on high optical absorption coefficient, GNP has been used as a contrast agent for PA imaging [14-15].

In this study, for the first time, we use GNP as a dual-modal contrast agent for X-ray and PA imaging. Soft gelatin phantoms with embedded tumor simulators of GNP in various concentrations are clearly shown in both X-ray and PA imaging. X-ray can fast detect the position of tumor and provide morphological information, whereas PA imaging has important potential applications in the image guided therapy of superficial tumors such as breast cancer, melanoma and Merkel cell carcinoma.

2. Materials and imaging system

2.1 Gold nanoparticles

GNP were synthesized by citrate reduction of auric chloride (HAuCl₄) following the procedure of Frens [16]. The various factors controlling the size, size distribution and shape of the GNP in the citrate reduction method have been studied in detail [17-19]. In this paper, for the synthesis of 30 nm GNP, 100 mL of 0.01 wt % HAuCl₄ was added to a 250 mL round-bottom and the solution was brought to a boil. 2 mL of 1 wt % sodium citrate was added quickly, which resulted in a color change from blue to burgundy and then the solution was allowed to boil for an additional 15 min. After the solution was cooled to room temperature, 1 mg mPEG-SH was added and stirred for 12 h to covalently modify the surface of the GNP with PEG. The resulting PEG-coated GNP were collected by centrifugation at 16,800g for 30 min and washed twice with distilled water.

2.2 X-ray imaging system

A X-ray system (piXarray 100 digital specimen Radiography system, Bioptics, Inc, America) were used. This system incorporates a CCD camera with a phosphor as the X-ray detector that is irradiated with a low-power X-ray source, with image acquisition, system control, and image display and reconstruction performed on the standard desktop computer. Imaged area is 50 mm × 50 mm. The detector of the system consists of a scintillating screen fiber-optically coupled to two 1024 × 1024 CCD image arrays. The tube current of this system is 0.5 mA fixed, and the tube potential from 5 to 45 kVp adjustable. The resolution of this system is 10 lp/mm (50 microns) in contact mode, and 14 lp/mm (36 microns) in 1.4:1 magnification mode (50 micron focal spot).

2.3 Photoacoustic imaging system

For all experiments, a mechanical scanning PA system with a single acoustic transducer to collect the acoustic signals was utilized. An optical parametric oscillator (OPO) (Vibrant 532 I, Opotek, Carlsbad, Calif) was used for light illumination. The OPO operated at 532 nm with pulse duration of 10 ns and
pulse repetition rate of 15 Hz. The laser was expended by a concave lens and then homogenized by a piece of ground glass, so that the incident energy density was controlled below 20 mJ/cm² accordingly to the ANSI standard. A needle hydrophone (Precision Acoustics Ltd, Dorchester, UK; diameter, 1 mm; sensitivity, 850 nV/Pa; frequency response, 200 KHz to 15 MHz), which was pointed to the center of the samples and controlled by a precision stepper to scan circularly around the sample, and receive the PA signals. Total of 200 steps with a constant 1.8° interval were taken for covering a 2π receiving angle. The PA signals were recorded by the computer through the amplifier and a digital oscilloscope (TDS3032, Tektronix, USA); the sampling rate of the oscilloscope was 250 MSamples/s.

3. Results and discussion
The GNP s were prepared by the citrate reduction of the gold ions. Particle size and morphology were determined by TEM. Figure 1(a) shows the representative TEM picture of the 30 nm GNP s. UV–vis absorption spectra of gold nanoparticles showed that the absorption peak was 527 nm.

![Figure 1](image-url) (a) Representative TEM picture of 30 nm GNP s (scale bar 30 nm). (b) Optical absorption of 30 nm nanoparticles used in the experiments showing maxima to be 527 nm.

To verify the ability of the GNP s as the X-ray and PA imaging contrast, we constructed a phantom made of 5% gelatin in which 4 inclusions with different concentrations of nanoparticles were embedded (Fig. 2). Each cylinder was 3 mm in diameter, 5 mm in deep, where the nanoparticles concentrations were 10, 5, 2.5, and 1.25 mg/mL respectively. The X-ray imaging is demonstrated in Fig. 2(a), for a cylinder containing 4 inclusions of different GNP s concentrations. The inclusion with a GNP s concentration of 2.5 mg/mL can hardly be recognized from the background, leading us to conclude that this X-ray system has a detection sensitivity of the order of 5 mg/mL. In the corresponding PA image (Fig.2 b), the lowest concentration still supplies good contrast from the background which may owe to the high sensitivity of PA imaging. The X-ray signals are quantified as a function of concentration in Fig2. (c). As the concentration mounts, the X-ray signals increase linearly. Figure2 (d) shows a linear increase of the PA signals as function of concentration. These results strongly support the dual functionality of PEG coated gold nanoparticles for X-ray and PA imaging.
Figure 2. X-ray (a) and PA (b) image of PEG coated GNP s at various concentrations, 10, 5, 2.5, and 1.25 mg/mL, respectively. The quantification of the X-ray intensity (c) and PA signals (d) at the corresponding concentrations of GNP s.

To study the quality of GNP s in animal tissues, a 0.5-mm-diameter tube filled with 10 mg/mL GNP s as simulate vascular was embedded in chicken breast tissues. With a 5-mm thick layer of chicken breast tissue, the tube was clearly shown for X-ray imaging in Fig.3 (a) and also had a good contrast for PA imaging in Fig.3 (b), which may take advantage of PA imaging with high resolution.
Due to X-ray’s strong penetrating ability and high X-ray absorption coefficient of gold, with deeper chicken breast tissue, tube could still be visible using this X-ray system in our experiments. These results imply that X-ray imaging is potentially to fast detect the position of target and provide morphological information, while PA imaging has the potential to monitor the change of target.

Cell cytotoxicity assay was performed with a colorimetric tetrazolium salt-based assay, Cell Counting Kit-8 (CCK8). To determine the cytotoxicity of GNP\-s, tumor cells (103 per well) were cultured in a 96-well microplate for 24 h and then co-incubated with PEG coated GNP\-s of different concentrations for 12 h, rinsed with PBS, and incubated for another 72 h. OD450, with an absorbance value of 450 nm, was read with a 96-well plate reader (Infinite M200, Tecan, Switzerland) to determine the viability of the cells. The viability of cells was calculated as: cell viability (% of control) = ODTre/ODCon × 100% (where ODTre was the absorbance value at 450 nm of treated cells and ODCon was the absorbance value at 450 nm of control cells). Cell toxicity tests (see Fig. 4) show that PEG coated GNP\-s display satisfactory biosafety even at 1mg/mL, which is probably a much higher concentration than encountered in vivo.

**Figure 4.** Cell toxicity of GNP\-s measurement. Cell viability of tumor cells after 12 h of incubation with increasing amounts of the PEG coated GNP\-s. Cell viability was measured using Cell Counting Kit-8 (CCK8).

### 4. Conclusion

In conclusion, we successfully tested the feasibility of PEG coated GNP\-s as a dual-modal contrast agent for X-ray and PA imaging. We demonstrated that the nanoparticles have high X-ray intensity and PA signal due to elevated optical absorption. The combined imaging methods are complementary and together they provide the rich information needed for the reliable detection and diagnosis. This dual-modality imaging has the potential to become a useful tool in detecting early stage tumor and monitoring the progress of anti-angiogenic therapy. By treating the surface of GNP\-s to target specific molecules, the detection of tumor such as breast cancer and image guided therapy will be more accurately and the applications of these nanoparticles can be broaden to molecular imaging of primary and metastatic tumor.

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