Development of Nanoparticle-Based Gold Contrast Agent for Photoacoustic Tomography

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ABSTRACT

Photoacoustic tomography (PAT) utilizes non-ionizing energy to obtain structural/functional information with high spatial resolution and sensitivity. Heterogeneous absorption of optical energy in biological tissues results in differentially expressed acoustic signals yielding spatial/temporal information. A critically underdeveloped area in PAT is the development of contrast agents. Gold-containing nanoparticles can be a good choice due to their high bio-compatibility, low toxicity, and high feasibility of surface modification/conjugation. Here we present a broad evaluation of gold nanosphere with diameters 2-60nm for PAT contrast agents at ~550nm. The acoustic signal enhances with increasing size. Encapsulation of gold nanospheres using liposomes shifted optical absorption to > 600nm with increased enhancement. The highest contrast enhancement was obtained for 5nm-Au and 30nm-Au nanosphere encapsulated in PDA. Future work focuses on modifying the liposome for in-vivo targeted delivery.

Keywords photoacoustic tomography, gold nanosphere, gold nanoparticle, contrast agent, molecular imaging

1 INTRODUCTION

Photoacoustic tomography (PAT) is an emerging imaging technique. The object of interest is heated with laser pulses. The laser power is absorbed by the object and converted to heat. An acoustic wave is generated based on the specific thermo-elasticity property of the object. This acoustic wave is detected using ultrasound transducers [1]. This technology combines the advantages from both optical and ultrasound imaging, while overcoming several of their disadvantages [2]. It utilizes non-ionizing energy to obtain both structural and functional information with high spatial resolution, high sensitivity, and low scattering. Several biological molecules have intrinsic PAT signal, such as hemoglobin and melanin. Applications have been reported using these endogenous contrast agents including tumor angiogenesis monitoring, functional brain mapping, blood oxygenation mapping and cancer detection [3-5].

In order to extend applications to molecular imaging, the development of exogenous contrast agents for this modality becomes critical. Contrast agents with proper size, shape, composition and functionality will be able to increase the signal intensity, penetration depth and specificity for this imaging modality. Typically, metal-containing particles provide excellent efficiency in light absorption [6]. One of the most promising candidates for *in vivo* applications is gold-containing nanoparticles. They have high *in vivo* bio-compatibility and low toxicity. In addition, these particles are amenable to surface modification and conjugation for increased contrast enhancement or targeting capability. To date, gold-based nanoparticles that have been explored and developed include nanoshells [7,8], nanorods [9-11], and nanocages [12,13] with feasibility of bio-conjugation.

In the present study we characterize a broad array of gold-containing nanosphere suitable for PAT contrast agents with different size and optical absorption properties. Potential application and future directions for *in vivo* PAT imaging is discussed.

2 MATERIALS AND METHODS

Gold nanospheres with diameter 2-60nm were purchase from Corpuscular, Inc. (Cold Spring, NY). Phosphatidylcholine (PC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). 10,12-pentacosadiynoic acid (PDA) was purchased from Alfa Aesar (Ward Hill, MA). PC and PDA-based liposomes were prepared based on procedures published previously [14]. Characterizations of the nanoparticles were performed using dynamic light scattering with multi angle particle sizer, scanning electron microscopy, UV-Vis absorption spectroscopy, and inductively coupled plasma atomic emission spectroscopy.

Four groups of nanoparticles were prepared. Group A: gold nanospheres with diameters ranging from 2-60nm. Group B: gold nanospheres encapsulated in PC-based liposomes. Group C: gold nanospheres encapsulated in PDA-based liposomes. Group D: control substances including pure vegetable oil, deionized water and dye. Vessel-mimicking phantom samples were prepared by injecting nanoparticles into the polyethylene tubes with I.D. of 0.045". Both tube ends were heat-sealed and secured using clamps to maintain a fixed distance to the acoustic transducer.

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Q–switched Nd:YAG laser or OPO tunable laser was used to generate pulse energy. The Nd:YAG laser features a fixed wavelength at 532nm with pulse duration of 10ns and frequency of 20Hz. OPO tunable laser features tunable wavelength 680-2500nm with 3-5ns pulse width and 20Hz frequency. Acoustic waves were detected using either a 2-D array of capacitive micromachined ultrasonic transducers (CMUTs) or piezoelectric ultrasound transducer. A thorough design and fabrication of the 2-D CMUT has been published elsewhere [15]. Piezoelectric ultrasound transducer had a center frequency of 25 MHz, focal depth of 8 mm. The acoustic wave was received and the output was amplified, digitized and recorded by a digital oscilloscope synchronized to the laser.

3 RESULTS

Figure 1 shows the normalized molar absorbance of the nanospheres. Group A: gold nanospheres with diameters of 2-60nm have maximum absorption wavelength around 520-540nm. Their molar absorbance increased with size. Group B: encapsulation of the gold nanosphere with PC-based liposomes. They have size average of ~85nm and shelf life of at least 2 months. Encapsulation of the nanospheres did not significantly alter their maximum absorption wavelength. The bandwidth of the absorption increased and the molar absorbance increased as compared with the gold nanospheres alone. PC-encapsulated 30nm Au had the highest molar absorbance. In contrast, encapsulation of the gold nanosphere with the PDA-based liposomes (Group C) significantly altered the optical absorption profile. These particles have an averaged size of 120nm and a shelf life of at least 5 months. PDA alone has a wide absorption band from 500-700nm. Thus when the gold nanosphere is encapsulated in PDA liposomes, the composite nanoparticle has a wide absorption band extended to 700nm due to the additive optical absorption from the two components. PDA-5nmAu and PDA-30nmAu had the highest molar absorbance.

Photoacoustic signals were generated by applying laser pulse to the nanoparticle samples at approximately the wavelength where the nanoparticle had maximum optical absorption. Setup of samples were described earlier. Four groups of samples were measured and the individual photoacoustic signals are presented in Figure 2. Gold nanospheres (Group A) showed moderate photoacoustic signals that peaked at 30nm. Encapsulating of the nanosphere with PC-based liposome did not significantly enhance the photoacoustic signal (Group B). In contrast, PDA-based encapsulation provided a significant increase in contrast enhancement by approximately 5 folds (Group C). Upon encapsulation, 5nm and 30nm gold nanosphere had the highest photoacoustic signal. Pure oil and deionized water were used as negative controls (Group D).

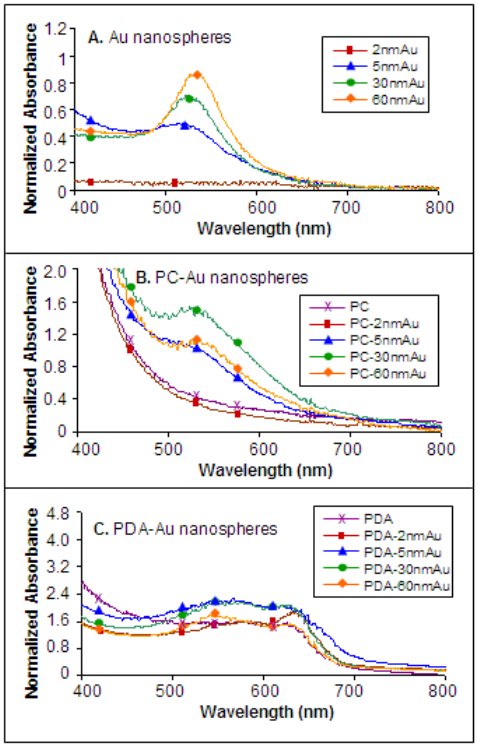

Figure 1. Optical absorption properties (A) Gold nanospheres, (B) PC-encapsulated gold nanospheres and (C) PDA-encapsulated gold nanospheres.

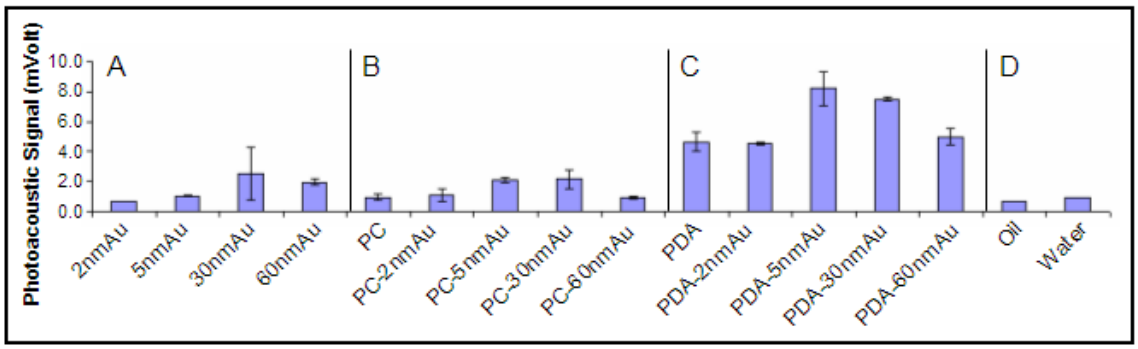

Figure 2. PA signal intensity of gold nanoparticles obtained using CMUT transducers with laser pulses at 532nm

Since PDA encapsulated gold nanosphere (Group C in Figure 2) showed a board optical absorption profile from 500-700nm (Figure 1C), we have further investigated the photoacoustic properties of this group of molecules using a tunable laser, allowing us to obtain photoacoustic signals at a higher wavelength that is more suitable for future clinical application. Signal intensities for PDA-encapsulated gold nanosphere from either 532nm laser pulses received by CMUT transducer, or 685nm laser pulses received by piezoelectric single transducer are comparable (Figure 3, dashed vs. solid bars).

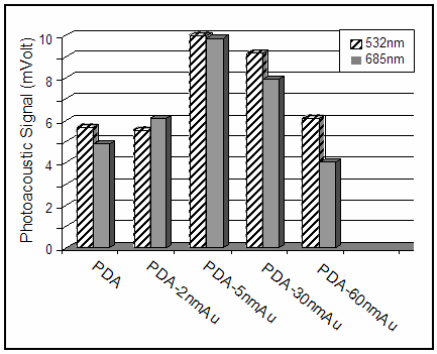

Figure 3. Comparison of the PA signal intensity of PDA-encapsulated gold nanosphere using CMUT at 532nm (dashed) and piezoelectric transducer at 685nm (solid).

Photoacoustic image of gold nanoparticles was obtained using piezoelectric transducer coupled to the OPO laser tuned at 685nm. Sample setup is shown in Figure 4A. Gold nanoparticles were sealed in tubes mimicking blood vessels. The tubes were aligned and secured on the holder and immersed in water for imaging. Images were acquired by slewing the transducer-laser unit across all the tubes in x direction (90mm) and a small area along the tube in the y direction (4mm) (Figure 4A, the dashed area). The laser was focused on the center of the tube for maximize signal coverage. Figure 4B presents the macro view of the photoacoustic contrast enhancement from the top across all the sample tubes at the same orientation as shown on Figure 4A. Note that the dimension on the x (90mm) and the y axis (4mm) is not to scale. The y axis is elongated for better visualization.

The orientation of the image slice relative to the sample tubing is shown on the top of Figure 4C. Dashed area represents the area where the images were acquired. The contrast enhancement was along the vessel phantom walls, as shown on the bottom of Figure 4C. Since acoustic signals are likely to arise at interfaces between different materials, it is reasonable to question whether the contrast signal is from the interfaces or from the contrast agent. If solely from the interfaces, the signal intensity should remain the same across all the tubes regardless of the type of contrast agents kept in the tubes. Figure 4D shows the images for all the samples (zoomed-in of Figure 4B). The differential signal intensities along the vessel-mimic tube walls indicate that the laser was the best absorbed and the contrast was further enhanced along the vessel-mimic tube wall. For PDA-encapsulated gold nanoparticles, the highest contrast enhancement was observed when the gold nanospheres are 5nm and 30nm in diameter. For gold nanosphere along, the contrast enhancement is fairly weak regardless of the particle size. Since gold nanospheres alone have a rather narrow optical absorption band in the region of 550nm (Figure 1A), at 685nm the gold nanospheres should not generate significant contrast enhancement. Thus the more intense signals observed for the PDA-encapsulated gold nanospheres suggests that this class of nanoparticles should be further evaluated and characterized for use as a photoacoustic contrast agent. In particular the PDA-5nm and PDA-30nmAu gives the best PAT contrast enhancement.

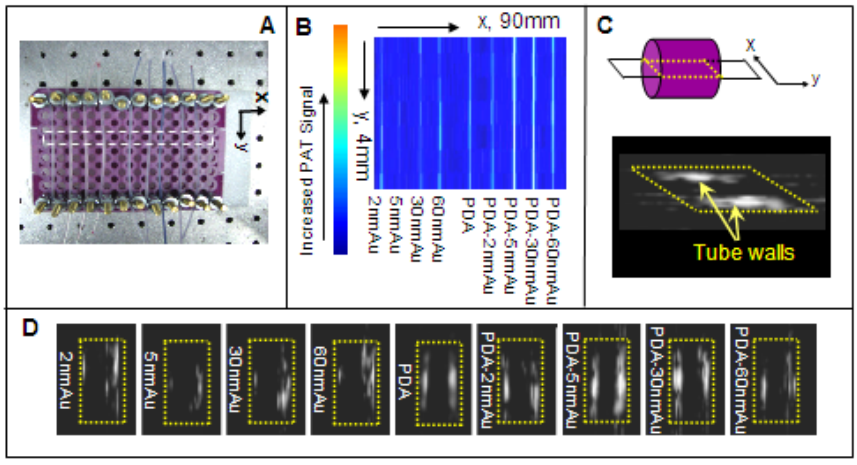

Figure 4. Photoacoustic images of gold nanoparticles PAT contrast agent using piezoelectric transducer with laser wavelength at 685nm. Images of gold nanospheres were also shown. (A) Sample setup with a scanned area of 90mm x 4mm marked with the dashed square. (B) Top view of the images in color scale acquired by scanning through the dashed area marked in (A). Note that for better visualization, the y axis has been elongated and not in scale with the x axis. (C) Top: schematic orientation of the plane, marked with a dashed box, where the image was acquired in relative to the sample tube. Bottom: representative photoacoustic image shows high contrast enhancement along the tube walls. (D) Serial photoacoustic images for PDA-encapsulated gold nanospheres and gold nanospheres alone with different sizes as labeled. Dashed boxes represent image slices corresponding to what on top of (C). Images are shown in the XY plane as indicated in (A) with a dynamic range of 40 dB.

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4 DISCUSSION AND SUMMARY

In the present study we have characterized different gold-based PAT contrast agents. We had hypothesized that optimum PAT contrast agents would have a metallic core the would act as a heat sink, coated with and external material that would have an enhanced expansion in response to the core heating and expansion of the core material. We demonstrated that although gold nanospheres alone generate PAT contrast enhancement, composite materials made from a metallic (gold core) in combination with and external coating produced and enhanced PAT signal. Encapsulation of gold nanospheres using polymerized liposomes altered the photoacoustic properties of these nanoparticles. Phosphotidylcholine (PC)-based liposomes slightly increased the contrast enhancement, while pentacosadiynoic acid (PDA)-based liposomes extended the optical absorption energy to around 700nm with significant increase of the contrast enhancement. For these liposome-encapsulated contrast agents, the PAT signal can be generated from both the liposome and the gold nanosphere, and the signal can be modulated by the interaction between the two components. Liposome encapsulation provides an environment where the liposome, depending on the composition, may generate additional photoacoustic wave at the same or different absorption energy region. PC-based liposomes do not have significant optical absorption above 500nm. Therefore the photoacoustic signals for PC-encapsulated gold nanospheres are mainly from the gold nanospheres only and remained similar upon encapsulation. Alternatively, PDA-based liposomes is chromatic with a wide bandwidth of optical absorption up to 700nm, thus generating additional photoacoustic signal when used with gold nanosphere. However, the increased PAT signal in PDA-based liposome became less significant when the size of the gold nanospheres increased. The highest contrast enhancement was obtained for the PDA-encapsulated 5nm and 30nm Au nanospheres. Since the size of the PDA-based liposome is ~120nm for optimal thermodynamic stability, the liposome accommodates fewer particles with increased gold nanoparticle size, resulting in decreased PAT signal.

Since PAT signals were measured using two different setups: Laser pulses at 532nm with CMUT 2-D array transducer, or laser pulse at 685nm with piezoelectric ultrasound transducer, the reliability and feasibility of either system is demonstrated, providing flexibility for future development in instrumentation. PDA-encapsulated 5nm and 30nm gold nanosphere generated the best contrast enhancement. When using vessel phantoms, the best enhancement occurred along the wall. This behavior suggests that the contrast enhancement may be further increased if the agents can be targeted to vessels, which holds potential application for monitoring and therapy in tumor angiogenesis.

In summary, we have shown the capability of preparing the optimal contrast agent for photoacoustic tomography by fine-tuning the size and the composition of the nanoparticles. We believe nanoparticles with a core that is an optimum heat sink, coated with material that readily expand upon heating and can provide functionalization and surface modification would be ideal PAT contrast agents. Here, among the contrast agents we tested, the small size (5nm or 30nm) gold nanosphere encapsulated in the PDA-based polymerized liposome produced the strongest PAT signal. It is advantageous to have liposome encapsulation since polymerized liposomes can readily be modified to carry other molecules such as fluorophores and targeting agents, which have been well-characterized with proven applications for molecular targeting and therapeutic delivery [16]. We have synthesized gold nanorods with optical absorption in the near-IR region (900nm). They can be encapsulated in the liposomes in a similar manner. Future work focuses on further development of the gold nanorods with optical absorption at near-IR region, and the modification of the liposome to make these agents suitable for in-vivo vascular targeted imaging.

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