Thermal stability of biodegradable plasmonic nanoclusters in photoacoustic imaging

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Abstract: The photothermal stability of plasmonic nanoparticles is critically important to perform reliable photoacoustic imaging and photothermal therapy. Recently, biodegradable nanoclusters composed of sub-5 nm primary gold particles and a biodegradable polymer have been reported as clinically-translatable contrast agents for photoacoustic imaging. After cellular internalization, the nanoclusters degrade into 5 nm primary particles for efficient excretion from the body. In this paper, three different sizes of biodegradable nanoclusters were synthesized and the optical properties and photothermal stability of the nanoclusters were investigated and compared to that of gold nanorods. The results of our study indicate that 40 nm and 80 nm biodegradable nanoclusters demonstrate higher photothermal stability compared to gold nanorods. Furthermore, 40 nm nanoclusters produce higher photoacoustic signal than gold nanorods at a given concentration of gold. Therefore, the biodegradable plasmonic nanoclusters can be effectively used for photoacoustic imaging and photothermal therapy.

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1. Introduction

There is a tremendous interest in exploiting metal nanoparticles in various biomedical applications including imaging, biosensing, therapeutics, and drug delivery [1–7]. Metal nanoparticles are very appealing agents because their size range is similar to that of biological macromolecules and they can be designed to incorporate specific properties for manipulation or detection of biological systems [8–11]. Furthermore, nanoparticles have strong optical scattering and absorption properties in the visible and near-infrared (NIR) regions [12,13]. Due to these properties, strongly absorbing metal nanoparticles such as nanospheres, nanorods and nanoplates have been used for photoacoustic imaging and image-guided photothermal therapy [14–22]. However, long-term accumulation of these nanoparticles in the body is a major roadblock toward their clinical translation [23].

The metal particles often used in biomedical applications range from ca. 20 to 150 nm; therefore, these nanoparticles are not easily cleared from the body because of their large size. Particles smaller than ~6 nm can be rapidly cleared from the body by renal clearance [24]. However, these particles cannot be easily utilized for imaging and therapy because the signal from these particles is very low due to their low optical cross sections [25]. In addition, small particles have short blood residence times, which do not allow sufficient time for efficient delivery.

We recently introduced biodegradable nanoclusters, which consist of sub-5 nm primary gold nanoparticles stabilized by small amounts of biodegradable polymer [26]. The nanocluster assembly is controlled with by a weakly adsorbing biodegradable polymer through a combination of electrostatic, van der Waals, steric, and depletion forces. These nanoclusters were demonstrated to biodegrade into primary 5 nm gold spheres in both solution and cells [26]. The 5 nm spheres can undergo efficient clearance from the body through a renal mechanism. Therefore, the biodegradable nanoclusters can enable clearance of nanoparticles and expedite the translation of plasmonic gold nanoparticles to the clinic. Furthermore, we have demonstrated that biodegradable nanoclusters can be used as a contrast agent in photoacoustic imaging because they have enhanced absorption in the near-infrared (NIR) spectrum due to plasmon resonance coupling between the primary spherical nanoparticles [27].

During photoacoustic imaging, nanoparticles are exposed to short laser pulses with a peak laser power that is extremely high when compared to CW laser irradiation. Therefore, the thermal stability of photoacoustic contrast agents is important in producing a consistent and reliable photoacoustic signal. Previous studies have shown that gold nanorods, which are very promising photoacoustic contrast agents due to their high absorption cross-sections in the NIR region, are susceptible to melting and reshaping at the laser fluences used in photoacoustic imaging [15,22,28,29].

In this study, the stability of biodegradable nanoclusters of various sizes under nanosecond laser pulses was investigated. In addition, we analyzed the amplitude of the photoacoustic signal generated from nanoclusters of different sizes and compared them with the photoacoustic signal produced by the gold nanorods. Based on the thermal stability, optical absorption coefficient, and photoacoustic signal strength, we identified the optimal nanocluster size for photoacoustic imaging and photothermal therapy.

2. Materials and methods

4.1 Synthesis of different sizes of biodegradable nanoclusters

We designed and synthesized biodegradable nanoclusters with controlled sizes from 40 to 130 nm by varying the ratio of polymer stabilizer to primary gold particles, the concentration of gold nanoparticles, and the surface ligands on the primary gold particles. Forty nm
nanoclusters were formed using a method slightly modified from previous work [30]. Briefly, 1 ml of a citrate-capped gold nanoparticle dispersion was prepared at a concentration of 3 mg/ml Au. 42 µl of a 1% (w/v) lysine solution was then added to the dispersion and stirred for 15 min, in order to create a dispersion consisting of gold nanoparticles capped with a mixture of lysine and citrate ligands. This dispersion was then diluted with deionized water to 1 mg/ml Au, and 21 mg of the biodegradable polymer PLA(1k)-PEG(10k)-PLA(1k) was added to the 1 mg/ml dispersion, resulting in a polymer/Au ratio of 7/1. The polymer-gold nanoparticle dispersion was bath sonicated for 5 min, and the resulting mixture was placed under an air stream and evaporated to a film. 2 ml of deionized water was then added to the dried film, and the mixture was bath sonicated for ~15 min, resulting in a dispersion of nanoclusters.

The formations of 80 nm and 130 nm nanoclusters have been described previously [30]. For 80 nm clusters, citrate/lysine capped primary nanoparticles were used with a polymer/Au ratio of 16/1, and for 130 nm clusters, primary nanoparticles were capped by only citrate, and a polymer/Au ratio of 16/1 was used. In these cases, a 3 mg/ml Au dispersion was used before evaporation.

4.2 Characterization of the photothermal stability of the biodegradable nanoclusters

To test the stability of nanoclusters exposed to a nanosecond pulsed laser irradiation, 100 µL nanocluster suspensions of three different sizes (40, 80 and 130 nm) of nanoclusters were prepared by a seed-mediated growth method [31,32].
prepared and placed in a 96-well plate. The concentration of nanoparticles in each solution was adjusted by diluting the sample with nanopure water to achieve optical density (O.D.) of ~0.6 at 780 nm for each sample. The optical density was measured at room temperature using the microplate reader. Each well was then irradiated from the top with 300 laser pulses (7 ns pulse duration, 10 Hz repetition rate, 780 nm wavelength) generated using a tunable OPO laser system (Vibrant, OPOTEK, Inc.). The fluence of the laser beam was varied from 4 to 20 mJ/cm². Following laser irradiation, the O.D. of each sample was measured again, and the stability of nanoclusters under the nanosecond pulsed laser irradiation was assessed by comparison of absorbance spectra before and after the laser exposure.

The stability of the photoacoustic signal was explored by measuring the photoacoustic signal intensity of 40, 80, 130 nm nanoclusters and nanorods suspensions at 1.2 mg/mL of gold concentration exposed to 200 pulses with laser fluences ranging from 4 to 20 mJ/cm². A custom-built system to measure the photoacoustic signal from a small sample of nanoclusters in solution is presented in Fig. 2. The photoacoustic signals from the aqueous nanoparticle solutions were measured as a function of the number of pulses. An acrylic PMMA tube with inner diameter of 378 µm and outer diameter of 500 µm was positioned in a plastic water cuvette with an optical window for laser irradiation. Solutions of nanoclusters of different sizes but with the same overall mass of gold, measured by flame atomic absorption spectroscopy (FAAS, GBC Scientific Equipment Pty Ltd.), were injected into the tube and were kept stationary during the experiment. A 7.5 MHz single element ultrasound transducer (focal depth = 50.8 mm, aperture = 12.7 mm) was mounted on a one-dimensional translation stage and the focal point of the ultrasound transducer was located at the center of the tube containing nanocluster solution. A collimated laser beam from nanosecond pulsed laser was incident on the PMMA tube through the optical window in the water cuvette. The samples were irradiated with five different laser fluences: 4, 8, 12, 16, and 20 mJ/cm². For each laser pulse, the photoacoustic signal was collected by the ultrasound transducer and stored for off-line processing to determine the change of photoacoustic signal from each nanocluster solution. At each fluence, three independent measurements for each sample were performed.

4.3 Photoacoustic imaging

To investigate the importance of the thermal stability of the nanoparticles at high laser fluence in photoacoustic imaging, a tissue mimicking phantom was made of 6% polyvinyl alcohol (PVA) and 0.2% 15 µm silica by weight was constructed to simulate the ultrasound and optical properties of tissue. Four cylindrical compartments of 6 mm in diameter were created.
within the PVA phantom. All compartments were filled with 6% gelatin solution containing the 40, 80, 130 nm nanoclusters and the nanorods. In each inclusion, the concentration of nanoclusters and nanorods was standardized to 0.5 mg/mL of gold.

An ultrasound and photoacoustic imaging system (Vevo 2100, Visualsonics, Inc.) with an array ultrasound transducer operating at 20 MHz center frequency was used to obtain photoacoustic images of the tissue-mimicking phantom with embedded inclusions. At each position, the ultrasound array transducer was placed at the center of the inclusions. Nanosecond laser pulses at 780 nm were used to irradiate the samples and 4 photoacoustic signals were collected and averaged. The laser fluence was kept at 16 mJ/cm² which is below the safety limit set by American National Standards Institute (ANSI) of 20 mJ/cm² in the visible spectral region [33].

3. Results and discussions

The thermal stabilities of the 40, 80, 130 nm nanoclusters and the nanorods were measured using a UV-Vis-NIR spectrophotometer. Figure 3 shows the absorbance spectra of nanoparticles before and after laser irradiation with 300 pulses at various fluencies. Changes in the absorbance spectra indicate that the laser irradiation reaches the damage threshold fluence. Laser fluence above 8 mJ/cm² caused visible spectral changes in the 130 nm nanocluster solution. The NIR absorbance of the 130 nm nanoclusters dramatically decreased when the fluence was increased to 20 mJ/cm². Nanoclusters with 40 and 80 nm sizes showed minimal spectral changes after irradiation with fluences up to 20 mJ/cm². Similar to 130 nm nanoclusters, gold nanorods also exhibited reduction in the NIR optical absorbance above 8 mJ/cm² laser fluence. Further increase in the fluence induced a strong blue shift of the longitudinal peak of optical absorbance of nanorods in the 750-800 nm range. The reduction in the absorbance of the 130 nm nanoclusters is most likely associated with degradation of the clusters to their primary particles or the smaller clusters because of the corresponding increase in the absorbance at ca. 520 nm; this correlation between nanocluster sizes and spectral changes was described previously [30]. In the case of nanorods, the changes in the longitudinal plasmon absorption peak between 760 and 810 nm suggest the reshaping of the nanorods [15,28,29]. The results indicate that the 40 and 80 nm nanoclusters have excellent thermal stability under the nanosecond pulsed laser as compared to the larger 130 nm nanoclusters as well as gold nanorods.

The observed thermal stability of nanoclusters is closely related to their composition and binding forces between primary particles forming the clusters. The 40 and 80 nm clusters were formed using primary Au nanoparticles capped with a combination of citrate and lysine ligands and a Z-potential of \( -30.1 \pm 2.4 \) mV, while the primary nanoparticles in 130 nm clusters were capped only with citrate ligand resulting in a Z-potential of \( -44.0 \pm 4.9 \) mV. Based on the discernible particles in the periphery of TEM images (see Fig. 1(a)), the interparticle distances between the primary gold particles within the 40, 80, 130 nm clusters were estimated to be 1.8 \( \pm 0.7 \), 1.8 \( \pm 0.6 \), and 2.4 \( \pm 1.4 \) nm, respectively. The larger charge repulsion between the citrate capped primary particles in 130 nm clusters leads to a more weakly assembled nanocluster and a greater particle-to-particle spacing in comparison to the 40 and 80 nm clusters. Therefore, the increased thermal stability of the 40 and 80 nm clusters can be largely attributed to the relatively small interparticle distance incurred by lower electrostatic repulsion, which results in an overall more attractive interaction between the primary nanoparticles in the clusters.
Fig. 3. UV-Vis-NIR spectra of (a) 40 nm, (b) 80 nm, (c) 130 nm nanoclusters and (d) gold nanorods before and after laser irradiation with nanosecond laser pulses with various fluences.

The photoacoustic signal intensity was observed as a function of number of laser pulses (Fig. 4). While the standard deviation was measured in all experiments, for visualization purposes the error bars (plus/minus one standard deviation) are only shown in Fig. 4(d) corresponding to the worst-case condition. A consistent photoacoustic signal response from contrast agents in photoacoustic imaging is important because the image analysis is based on the assumption that the photoabsorbers remain the same in terms of the concentration and absorbance during the imaging. The photoacoustic signal was stable for all nanoparticles at 4 mJ/cm², which is below damage threshold measured by UV-Vis-NIR spectroscopy (see Fig. 3). The 40 and 80 nm nanoclusters produced similar photoacoustic signals, while the 130 nm nanoclusters only generated a very small photoacoustic signal which is just above the background signal measured from the sample without nanoparticles. The photoacoustic signals from the 40 and 80 nm nanocluster solutions were stable up to 12 mJ/cm²; however, a decay in the signal was observed for both nanoclusters exposed to fluences above 12 mJ/cm². At 20 mJ/cm², the 40 nm nanoclusters produced the highest photoacoustic signal among all the samples, which was 3.8 times higher than the signal from the nanorod solution. The photoacoustic signal from 40 nm nanoclusters increased 4.5 times when the laser fluence was raised from 4 to 20 mJ/cm². In general, the photoacoustic signal generated by photoabsorbers is linearly increases with the laser fluence. However, the photoacoustic signal from the 40 nm nanoclusters only increased 4.5 times while the fluence was increased 5 times. This can be attributed to photothermal damage of the nanoclusters which is shown as a small reduction in the absorbance at 20 mJ/cm² (Fig. 3(a)). Using the same conditions, the photoacoustic signal increase for the nanorods was only 1.1 times. This can be associated with melting and reshaping of the nanorods when exposed to elevated laser fluences. At equivalent gold mass concentrations, the measurements indicate that the 40 nm nanoclusters can produce a photoacoustic signal that is higher than the nanorods when the fluence is higher than 12 mJ/cm² because of their superior photothermal stability and photoacoustic signal enhancement due to clustering.
Using a tissue-mimicking phantom, the importance of the stability of nanoparticles in photoacoustic imaging was demonstrated. The photoacoustic images of the phantom with inclusions were obtained at 16 mJ/cm² laser fluence and 780 nm wavelength corresponding to the peak optical absorption wavelength of the nanorods. More than 50 pulses were used to irradiate each phantom before the photoacoustic images were collected. Inclusion with 130 nm nanoclusters produced the weakest photoacoustic signal among the samples (Fig. 5(c)). The photoacoustic signal from 40 nm nanoclusters showed the brightest signal at this laser fluence (Fig. 5(a)). Interestingly, both the 40 and the 80 nm nanoclusters exhibited stronger photoacoustic signal than that of nanorods. These results are in good agreement with the results presented in Fig. 5(f) where stability of nanoparticles was measured at 16 mJ/cm² laser fluence using the system described in Fig. 2.

In general, the photoacoustic signal intensity is proportional to the optical absorption coefficient of the sample. The absorbance of the 40 nm nanocluster solution measured by UV-Vis-NIR spectrometry at 780 nm ($\lambda_{\text{max}}$ of gold nanorods) was only half of that of the nanorod solution at the same amount of gold (Fig. 1(c)). However, both nanoparticles produce similar levels of photoacoustic signal at 4 mJ/cm², which is below the damage threshold for both types of nanoparticles as demonstrated in Fig. 4(a). The result clearly indicates that nanoclusters provide an enhanced photoacoustic signal. Several mechanisms including optical, thermal [34], and acoustic coupling effects can contribute to the enhancement of the photoacoustic signal from closely spaced primary nanoparticles. Indeed, a photoacoustic signal enhancement effect and a non-linearity with fluence have recently been reported for other forms of clusters [35,36]. Reasons for this effect could be local change in the temperature distribution and thermal conductivity. It has been reported that the effective thermal conductivity can be significantly enhanced due to the thermal transport along nanoparticles chains [37]. Others found that the thermal conductivity of gold spheres with a
polymer shell is higher than predicted, based on the bulk properties with the addition of an organic co-solvent to the aqueous medium [38], and it was demonstrated that the increased thermal interfacial conductivity enhances the photoacoustic signal [22,39]. Therefore, the enhancement of photoacoustic signal in biodegradable plasmonic nanoclusters may be attributed to the laser induced thermal coupling, transport effects in clusters, and/or an increased thermal transfer through gold interface induced by the clustering and the biodegradable polymer stabilizer.

4. Conclusion

In summary, we investigated the stability of biodegradable plasmonic nanoclusters of three different sizes in an aqueous solution under nanosecond laser pulses. Photoacoustic signals from the nanoparticles at various fluences were also studied and compared with that of gold nanorods. Finally, the photoacoustic signal amplification from clustering of primary gold nanoparticles was observed. The results indicate that 40 nm nanoclusters have superior photothermal stability for photoacoustic imaging and produce stronger photoacoustic signal as compared to nanorods at a given concentration of gold. Therefore, biodegradable plasmonic nanoclusters may serve as effective contrast agents for clinical photoacoustic imaging and photothermal therapy.

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