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Utility of biodegradable plasmonic nanoclusters in photoacoustic imaging

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Abstract

Plasmonic metal nanoparticles are used in photoacoustic imaging as contrast agents because of their resonant optical absorption properties in the visible and near-IR regions. However, the nanoparticles could accumulate and result in long-term toxicity *in vivo*, because they are generally not biodegradable. Recently, biodegradable plasmonic gold nanoclusters, consisting of sub-5 nm primary gold nanoparticles and biodegradable polymer stabilizer, were introduced. In this Letter, we demonstrate the feasibility of biodegradable nanoclusters as a photoacoustic contrast agent. We performed photoacoustic and ultrasound imaging of a tissue-mimicking phantom with inclusions containing nanoclusters at various concentrations. The results indicate that the biodegradable gold nanoclusters can be used as effective contrast agents in photoacoustic imaging.

In recent years, there has been growing interest in metallic plasmonic nanoparticles for various biomedical applications, including imaging, sensing, and therapeutics, because the nanoparticles have strong scattering and absorption in the visible and near-IR (NIR) regions [1]. Specifically, strongly absorbing gold nanoparticles have been shown as contrast agents in photoacoustic imaging [2–5]. These nanoparticles extend penetration depth and enhance sensitivity of photoacoustic imaging *in vivo*. In addition, molecular and cellular photoacoustic imaging can be achieved by using targeted gold nanoparticles [3–6].

However, because of accumulation of nanoparticles and potential long-term toxicity, the safety of the nano-particles in a biological environment could be a major hurdle for utilizing plasmonic nanoparticles *in vivo* [7]. The size of commonly used nanoparticles in biomedical imaging applications ranges between 20 and 150 nm; these nanoparticles may not be easily removed from the body. A previous study indicates that particles smaller than ~6 nm in diameter are rapidly cleared from the body [8]. However, the nanoparticles within this size range do not have sufficient blood residence time for imaging and therapy because they are removed too rapidly from the body. In addition, smaller nanoparticles produce smaller signals in comparison with larger nanoparticles [9].

Recently, nontoxic biodegradable plasmonic nanoclusters consisting of sub-5 nm primary gold particles stabilized by a weakly adsorbed biodegradable polymer have been reported [10,11]. The small spacing between primary gold nanoparticles within the clusters results in strong NIR absorbance [12]. The nanoclusters, stable at pH 7, degrade into primary gold nanoparticles at around pH 5 (the environment inside of the endosomes) within about seven days [10] (Fig. 1). Once biodegraded, the 5 nm primary gold nanoparticles can be excreted from the body [13].

In this study, we demonstrated the feasibility of biodegradable plasmonic nanoclusters as contrast agents in photoacoustic imaging. The sub-100 nm clusters, composed of primary 4 nm diameter gold nanoparticles, were synthesized using a previously published protocol [10,11]. The primary colloidal gold nanospheres have an absorbance peak at 520 nm [Fig. 2(a), solid curve]. However, the solution of 100 nm nanoclusters [see transmission electron microscopy (TEM) image in Fig. 2(b)] at pH 7 had a broad absorbance in the NIR region, where soft tissue and blood have low absorption [Fig. 2(a), dashed-dotted curve]. In contrast, after incubation at pH 5 for one week, the absorbance spectrum of the nanoclusters shifted toward the original spectrum of the colloidal gold nanospheres. The difference between two spectra is due to a small number (about 7%) of clusters remaining in the dispersion [10]. The result indicates that, over time, the biodegradable nanoclusters degrade to the primary gold nanospheres.

Photoacoustic imaging experiments were performed using tissue-mimicking phantoms. To simulate the ultrasound and optical scattering properties of tissue, an 8% gelatin solution with 0.1% (by weight) of 15 μm silica microparticles was poured into a mold. Once the gelatin solution was cooled, it formed the base layer of the phantom. To make an inclusion, a single drop of aliquot of the nanocluster suspension at a given concentration mixed with gelatin and silica particles was placed at the center of the base layer. The inclusion was then cooled and covered with the 8% gelatin solution mixed with 0.1% of 15 μm silica particles (same as the base layer). Four phantoms with inclusions were prepared. The concentrations of the nanoclusters in each inclusion were 550, 220, 110, and 55 $\mu\text{g/mL}$, respectively.

The combined ultrasound and photoacoustic imaging system (Fig. 3) consisted of a pulsed optical parametric oscillator laser system (750 nm wavelength, 7 ns pulse duration, 10 Hz pulse repetition frequency), an ultrasound transducer (25 MHz center frequency, focal depth = 12:7 mm, f number = 2), an ultrasound pulser/receiver, a three-dimensional motion axis, and a data acquisition unit. The phantoms were irradiated with laser pulses of four different energies: 2, 5, 10.5, and 16:5 mJ/cm^2 . The laser light was delivered by an optical fiber bundle consisting of seven fibers 600 μm in diameter. During the experiment, the tissue-mimicking phantom was placed into a water cuvette. The focal point of the ultrasound transducer was positioned at the inclusion's depth and aligned with the laser beam from the optical fiber bundle. A stepper motor mechanically scanned the phantom over the imaging region with a 50 μm step in the lateral direction. At each step, the phantom was irradiated by the laser light, and the ultrasound transducer was used to collect both photoacoustic transients and ultrasound pulse-echo signals. The recorded ultrasound and photoacoustic signals were Hilbert transformed, bandpass filtered, and spatially interpolated to form spatially coregistered two-dimensional cross-sectional ultrasound (US) and photoacoustic (PA) images of the phantoms.

The US, PA, and combined US/PA images of the phantom with inclusion containing a 550 $\mu\text{g/mL}$ concentration of nanoclusters are presented in Fig. 4(a). Each image covers a 4:7 mm $\times$ 4:4 mm field of view. The US image cannot identify the inclusion within the background —this is expected, since the nanoclusters do not significantly increase the ultrasound backscattering. However, the PA image clearly differentiates the inclusion, because the

nanoclusters have high optical absorption compared to the background. The PA and US images can be overlaid to show the position of the inclusion within the background.

The quantitative analysis of the photoacoustic signal amplitude with respect to the different laser fluence rate measured from the regions of interest (ROI) containing the nanoclusters' inclusion is shown in Fig. 4(b). A 2.33 mm × 0.67 mm ROI was selected for each PA image and divided into subareas measuring 0.33 mm × 0.33 mm. The mean and the standard deviation of the photoacoustic signal in the subareas were plotted as a function of the fluence rate of the laser. As the laser fluence increases, the amplitude of photoacoustic signal from the nanoclusters increases linearly (the R^2 value for the linear curve fitting is 0.9853).

The changes in the photoacoustic signal amplitude with the concentration of nanoclusters are quantified in Fig. 4(c). The solid lines represent the linear fit of the photoacoustic signal measured for 5 and 10.5 mJ/cm² laser fluence rates with R^2 equal to 0.9895 and 0.9901, respectively. As expected, the amplitude of the photoacoustic signal from the inclusions increases with the concentration of nanoclusters. These results suggest that nanoclusters remain structurally stable under relatively large laser fluences.

The individual nanospheres have a peak absorbance at around 520 nm wavelength, and therefore isolated spherical nanoparticles cannot be easily detected in photoacoustic imaging in vivo because of a spectral overlap with strong absorption of blood and tissue scattering. However, aggregation of targeted spherical nanoparticles mediated by biological molecules, such as growth factor receptors or actin, can lead to strong NIR absorption; this approach has been explored for optical [12,14,15] and photoacoustic [3–6] molecular imaging.

Nanorods are also promising photoacoustic contrast agents because of their high absorption cross section and tunability in the NIR region. However, nanorods are not thermodynamically stable structures and change their shape and therefore absorption properties at 8 mJ/cm² and higher laser fluence [2]. In comparison, the nanoclusters have strong NIR absorption and are expected to have reasonable thermodynamic stability, thus providing an alternative contrast agent for photoacoustic imaging.

In conclusion, we explored the usability of biodegradable nanoclusters (consisting of small primary gold nano-spheres and a polymeric stabilizer) as a photoacoustic imaging contrast agent. It was demonstrated that an inclusion with biodegradable nanoclusters, characterized by enhanced absorption within a broad NIR spectrum due to plasmon resonance coupling between closely spaced primary nanoparticles [10], can be imaged in an optically scattering tissue-mimicking environment using NIR photoacoustic imaging. We also demonstrated that the photoacoustic signal intensity is linearly proportional to the laser fluence and concentration of the nanoclusters, suggesting their structural and thermodynamic stability. Overall, the results of our study suggest that biodegradable plasmonic nanoclusters are promising contrast agents for photoacoustic imaging and other biomedical applications, such as photothermal therapy.

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Fig. 1.
(Color online) Diagram of degradation of gold nanoclusters at different pH levels.

Fig. 2.
(Color online) (a) Normalized absorbance spectra and (b) TEM image of biodegradable nanoclusters.

Fig. 3.
(Color online) Block diagram of combined ultrasound and photoacoustic (US/PA) imaging system and the gelatin-based tissue-mimicking phantom with biodegradable nanoclusters inclusion.

Fig 4. (Color online) (a) US, PA, and combined US/PA images of the phantom with inclusion containing nanoclusters. The concentration of the nanoclusters was $550 \mu\text{g}/\text{mL}$. (b) Photoacoustic signal amplitude with respect to fluence rate and (c) dependence of PA signal on nanocluster concentration at 5 and 10.5 mJ/cm^2 . The solid lines represent the linear regression fit of the data.