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Sono-photoacoustic imaging of gold nanoemulsions: Part I. Exposure thresholds

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ABSTRACT

Integrating high contrast bubbles from ultrasound imaging with plasmonic absorbers from photoacoustic imaging is investigated. Nanoemulsion beads coated with gold nanopsheres (NEB-GNS) are excited with simultaneous light (transient heat at the GNS's) and ultrasound (rarefactional pressure) resulting in a phase transition achievable under different scenarios, enhancing laser-induced acoustic signals and enabling specific detection of nanoprobes at lower concentration. An automated platform allowed dual parameter scans of both pressure and laser fluence while recording broadband acoustic signals. Two types of NEB-GNS and individual GNS were investigated and showed the great potential of this technique to enhance photoacoustic/acoustic signals. The NEB-GNS size distribution influences vaporization thresholds which can be reached at both permissible ultrasound and light exposures at deep penetration and at low concentrations of targets. This technique, called sono-photoacoustics, has great potential for targeted molecular imaging and therapy using compact nanoprobes with potentially high-penetrability into tissue.

1. Introduction

Contrast-enhanced imaging with exogenous contrast agents is a rapidly developing technique for both photoacoustic (PA) and ultrasound (US) systems. Microbubbles have been used in US harmonic imaging [1] of vasculature, and for drug delivery [2] or cavitation-based treatments [3]. Their large size (typically 1–10 μm), however, inhibits high penetrability into tissue through leaky vasculature (300–500 nm endothelial gaps) and nanoscale pores, and through active mechanisms such as cellular uptake. Making stable nanobubbles at the dimensions required for enhanced transport is currently a challenge [4].

To solve this problem, phase-change contrast agents, which change state from liquid to gas if exposed to thermal and/or acoustic energy, were introduced. Liquid perfluorocarbon nanodroplets with a low-boiling point and a physical dimension appropriate for enhanced penetrability into tissue have been developed [5–8]. The phase-transition of these nanodroplets into a microbubble (vaporization) produces high contrast for US imaging. Compared to other US contrast agents, nanoemulsions can have a long circulation in the body (up to 2 h [8]). Because of increased surface tension, however, US exposure parameters must be relatively high, usually involving high repetition rates [7] or long excitations (~100 ms) [6]. This means that heat can be involved in the vaporization, increasing the possibility to damage surrounding tissues or limiting the repetition rate of the imaging modality. This mechanism is also not efficient using low-frequency ultrasound because of low acoustic absorption.

To achieve molecular imaging at significant depths within tissue, nanoscale, laser-activated PA contrast agents have been introduced [9–13]. Plasmonic absorption enhances the efficiency of compact nanoprobes (10–200 nm) in PA generation. However, a significant concentration is usually required to get single shot measurements for real-time in vivo imaging (e.g. ~10 nM) [9–13]. Recent studies have shown that combining nanodroplets with efficient optical absorbers in a single contrast agent can enhance the PA signal from the absorbers [12]. Here we build on this work to demonstrate a highly sensitive, and potentially specific, approach for molecular imaging and therapy using a nanoscale contrast agent integrating

perfluorocarbon nanodroplets with plasmonic absorbers. In particular, we explore the non-linear acoustic response of these agents to simultaneous light and sound excitations at exposure levels well within safety limits for routine diagnostic imaging.

In our previous work, we introduced a nanoemulsion of perfluorohexane droplets (100–200 nm) surrounded by amphiphilic gold nanospheres (12 nm in diameter). It was used as a non-linear PA contrast agent by inducing a phase transition of the oil through plasmonic absorption of light by the gold nanoparticles [14]. This approach can produce highly sensitive PA images with high background suppression (i.e., also highly specific contrast), but it requires a relatively high optical fluence to observe non-linear contrast (at 750 nm, threshold at 4 mJ/cm^2, 10 dB contrast around 6 mJ/cm^2), limiting its use for deep imaging applications.

An approach to greatly reduce the optical fluence required for a non-linear response from these contrast agents is suggested by recent work showing that laser generation of water vapor bubbles around plasmonic absorbers can be enhanced by adding ultrasound pressure fields [15–17]. This technique, named "PA cavitation", enables high contrast imaging at lower exposure thresholds and concentrations. Building on this work, we investigate here the PA cavitation exposure thresholds needed to trigger vaporization of a nanoemulsion bead coated with gold nanospheres (NEB-GNS). This approach leverages short-lived (a few μs) bubbles made possible by the high boiling point (BP) of the perflurocarbon used in this nanoemulsion. In contrast to another approach using long lifetime bubbles [12], a short-lifetime strategy using reversible phase transitions is preferred for molecular imaging applications requiring extended exposures. Using PA cavitation, the detection sensitivity for imaging can be enhanced by two physical effects: higher thermal expansion will enhance the PA response and the emitted ultrasound field will instantly scatter on the generated bubbles.

In the remainder of this paper, we investigate the benefit of clustering small gold nanospheres around high-BP perfluorocarbon droplets for combined PA/US applications. After introducing the physical characteristics of the NEB-GNS agent, vaporization thresholds are quantified and compared to single gold nanospheres. The potential use of the vaporization signals for imaging is then studied and discussed. To specifically retrieve the vaporization signal in tissues loaded with NEB-GNS, photoacoustic and backscattered ultrasound signals must be canceled by a procedure described in a future publication called "sono-photoacoustic" imaging.

2. Materials and methods

2.1. Nanoemulsion samples

NEB-GNSs were synthetized using the procedure described in previous reports [18,19]. Colloidal GNSs (diameter 12 nm) were synthetized using a citrate reduction method [20]. The particles were functionalized using PEG-thiol and butane-thiol (Sigma–Aldrich, St. Louis, MO, USA), with dosages of 0.8 chains/nm^2 Au and 700 molecules/nm^2 Au respectively. Attraction between the hydrophobic butane-thiol ligands resulted in clustered GNS dispersions. A solution of 1 vol% perfluorohexane (Sigma–Aldrich, St. Louis, MO, USA) and 0.012 vol% Au clusters in water was sonicated (102C, Branson, Danbury, CT, USA, pulsed regime – 1 s on, 4 s off) for 13 s in a cold water bath.

The absorbance was measured with a spectrophotometer (UV 1601, Shimatzu, Kyoto, Japan). The absorbance spectrum (cf Fig. 1a, blue curve) exhibits a red shift of the resonance from 520 nm to 547 nm compared to the original gold nanopsheres (black curve) and a broad tail enhancing absorption in the optical window ([600–1100] nm wavelengths). The size was measured by DLS as a broad dispersion between 30 nm and 1.2 μm with a peak at 132.1 nm and a mean size of 303 nm (cf Fig. 1b). Additional complexes were identified between 2 and 6 μm, but DLS may not be quantitative in this range as it is heavily affected by sedimentation or flotation [21]. In our case, the effective density of perfluorohexane beads (1.7 g/cm^3) can be increased by the GNS (density 19.3) resulting in sedimentation speeds depending on size.

To confirm the presence and quantify the micron-size particle number, the size distribution and concentration of the emulsion were obtained prior to and after all experiments using a Coulter Multisizer III (Beckman Coulter, Miami, FL). A 20 μm aperture was used, which can size particles with diameters from 0.56 to 12 μm and considers any count below 0.56 μm as the noise level. The sample was diluted ~1250× on a 0.2 μm filtered ISOTON II electrolyte (Beckman Coulter, Miami, FL). A 50 μL sample was used each time, and all measurements were repeated 6 times using a volumetric count mode. Individual particles were sized and binned in 300 evenly spaced bins with 0.039 μm width. All data are reported as a histogram with count vs. diameter, with the count (bin height) showing the number of particles in each bin interval. The reported concentration is computed for all ranges in question and accounts for the dilution factor and sampling volume used above.

A near Gaussian distribution of NEB-GNS was identified between 1 and 3 μm (cf inset of Fig. 1b). The sample was then split in two volumes. Assuming an ideal covering of the bead by gold nanospheres (80% surface density), the weight of each bead size was calculated. Then, a basic model of sedimentation (Stokes force = gravity) was used to calculate the sedimentation speeds of different particles: e.g., in 24 h a 425 nm particle sinks 22 mm. In contrast, a 132 nm bead should sink only 6 mm in the same period. A sample of 10 ml was left to settle in a scintillation vial. As 22 mm was the height of our solution in the vial, we assume that all
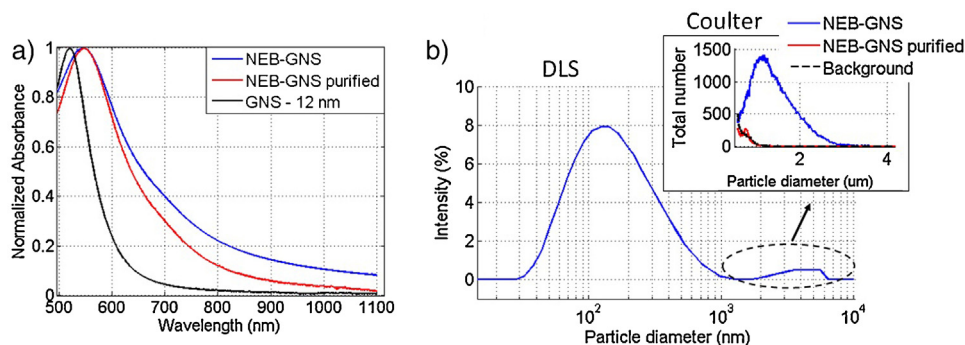
Fig. 1. (a) Absorbance spectra of the nanoemulsion before (blue) and after purification (red), and of gold nanospheres in suspension (black). (b) Sizing of the nanoemulsion by DLS (intensity). Inset: Sizing in the micron range using a Coulter counter: nanoemulsion before (blue) and after purification (red); background signal is shown in black.