Photoacoustics 3 (2015) 11–19

Contents lists available at ScienceDirect

Photoacoustics

journal homepage: www.elsevier.com/locate/pacs

Research Article

Sono-photoacoustic imaging of gold nanoemulsions: Part II. Real time imaging

Bastien Arnal [a,*], Chen-Wei Wei [a], Camilo Perez [a,c], Thu-Mai Nguyen [a], Michael Lombardo [b], Ivan Pelivanov [a,d], Lilo D. Pozzo [b], Matthew O'Donnell [a]

[a] University of Washington, Department of Bioengineering, 616 NE Northlake Place, Seattle, WA 98105, United States
[b] University of Washington, Department of Chemical Engineering, Box 351750, Seattle, WA 98195-1750, United States
[c] University of Washington, Applied Physics Laboratory, Center for Industrial and Medical Ultrasound, 1013 NE 40th Street, Seattle, WA 98105-6698, United States
[d] International Laser Center, Moscow State University, Moscow, Russian Federation

ARTICLE INFO

Article history:
Received 30 October 2014
Received in revised form 31 December 2014
Accepted 11 January 2015

Keywords:
Photoacoustic cavitation
Vaporization
Gold nanoparticles
Nanoemulsion
Non-linear photoacoustics
Background suppression

ABSTRACT

Photoacoustic (PA) imaging using exogenous agents can be limited by degraded specificity due to strong background signals. This paper introduces a technique called sono-photoacoustics (SPA) applied to perfluorohexane nanodroplets coated with gold nanospheres. Pulsed laser and ultrasound (US) excitations are applied simultaneously to the contrast agent to induce a phase-transition ultimately creating a transient microbubble. The US field present during the phase transition combined with the large thermal expansion of the bubble leads to 20–30 dB signal enhancement. Aqueous solutions and phantoms with very low concentrations of this agent were probed using pulsed laser radiation at diagnostic exposures and a conventional US array used both for excitation and imaging. Contrast specificity of the agent was demonstrated with a coherent differential scheme to suppress US and linear PA background signals. SPA shows great potential for molecular imaging with ultrasensitive detection of targeted gold coated nanoemulsions and cavitation-assisted theranostic approaches.

1. Introduction

Photoacoustics (PA) can leverage the optical spectra of endogenous molecular absorbers in the body to provide image contrast. For example, hemoglobin is a fairly efficient optical absorber that can be used to image the vasculature in tissue [1]. Moreover, the oxygen saturation of hemoglobin can be measured using PA spectroscopy [2] to provide functional information [3]. However, the efficiency of PA signal generation makes it very difficult to acquire single shot images for deep targets, and it is difficult to distinguish endogenous PA sources because of a number of instrumental issues. For example, there are significant artifacts in reconstructed images unless a full 3-D tomography setup is used with unblocked access to all acoustic sources and wideband transducers at sufficient density to capture all spatial frequencies [4–6]. More practical setups for medical applications using ultrasound (US) arrays are further limited because of the finite bandwidth of these devices. Large optical absorbers (typically a few wavelengths at the central frequency) cannot be imaged correctly because the emitted low frequency content is out of the bandwidth of regular transducers, which results in high-pass filtering of the PA image. Generally, it amplifies heterogeneities and can create speckle images, typical for US images. This makes it nearly impossible to distinguish small resolved absorbers from large derivative ones. Finally, endogenous signal contrast provides limited specificity in differentiating the molecular source of the PA signal, especially at depth where wavelength dependent scattering limits the effectiveness of multi-spectral techniques [7,8]. With recently developed fast wavelength-tuning lasers, it has been shown that multi-spectral imaging can be done in real-time, providing a high level of specificity for contrast agents [9]. However, accurate reconstruction usually requires a tomographic approach using a large number of transducers for an environment where optical scattering does not greatly change the illumination pattern for different wavelengths.

To overcome some of the practical limitations of endogenous contrast, nanoparticle-based agents providing exogenous contrast and molecular targeting have been proposed [10]. For example,

* Corresponding author. Tel.: +1 2062218330.
E-mail address: bastien.arnal@gmail.com (B. Arnal).

http://dx.doi.org/10.1016/j.pacs.2015.01.001

plasmonic absorbers targeted to specific molecular biomarkers can help identify, and potentially quantify, tumor cells [11–15]. Their very small size (10–200 nm) means that they have a better probability to penetrate into any tissue from primary vessels (with typical endothelial gaps of 100 nm). However, since the PA image relies on the conversion of optical absorption into thermal expansion around the absorber to generate an US wave, a low agent concentration cannot be distinguished from surrounding endogenous absorbers. For many applications, a background suppression strategy must be used to isolate signals of interest [12,16–18], greatly improving the contrast specificity of the targeted agent.

Different kinds of non-linearities can be used to enhance both the sensitivity and contrast specificity of PA imaging [19–29]. Here we focus on a highly non-linear nanoagent that can be distinguished from surrounding linear absorbers. This non-linearity is directly related to a phase transition greatly enhancing thermal expansion, thus PA efficiency. In our previous work [23], we introduced vaporization-enhanced PA imaging of nanoemulsion beads containing perfluorohexane and coated with clusters of gold nanospheres (NEB-GNS). The amplitude of background PA signals increasing linearly with laser fluence can be easily distinguished from the much more rapidly growing nonlinear response of NEB-GNS. The advantage of this agent is a lower boiling point (~60 °C) compared to that of the tissue background, avoiding thermal damage of surrounding tissue. However, a clear distinction between linear and non-linear behavior was shown for relatively high laser fluences that limit the application of this technique to depths of about 1 cm in turbid tissue.

The combination of light and ultrasound was previously introduced under the term "photoacoustic cavitation" to facilitate vapor bubble formation around plasmonic nanoparticles much more efficiently than using pulsed laser radiation or US excitation (applying negative pressure) alone [25–27]. In a companion paper to this submission, we presented results showing that simultaneous US and light stimulation can facilitate vaporization of nanoemulsion beads [28]. After a reversible phase transition of the perfluorocarbon core, a vaporization signal can be retrieved along with a scattered US signal from the resulting microbubbles. The phase transition threshold of NEB-GNS was shown to be much lower than the one observed with dispersed GNS in water (10-fold decrease in laser fluence at 1 MPa peak negative pressure for 1.2 MHz US insonification), and the combination of light and sound induced the phase transition within MPE limits for both US and laser light: the US mechanical index (MI) was below 1.9 with a laser fluence corresponding to centimeter-scale penetration into tissue within the limit of 25 mJ/cm^2 on the skin for the 750 nm optical wavelength used in those studies.

In that previous paper, we used a 1.2 MHz focused, single-element transducer to excite the emulsion and a home-made ultra wide-band PVDF transducer to detect generated US signals. This is not a useable tool for potential clinical applications. A phase transition in the nanoemulsion can be reached at pressures of a few MPa and laser fluences of a few mJ/cm^2. Therefore, real-time imaging using a conventional US array both for excitation of the emulsion and for reception of generated US signals is investigated here. The goal is to develop an imaging protocol combining ultrasound and laser pulses to vaporize the nanoemulsion and produce highly sensitive and specific images of this agent. To demonstrate its potential utility even at very low concentrations, we present a specific imaging sequence we call sono-photoacoustic imaging (SPA) that can easily detect NEB-GNS at pM concentrations. Moreover, we show that the SPA signal can be retrieved even with a band-limited array thanks to heterogeneities in the PA efficiency profile and acoustic properties in the bubble distribution. A tomographic reconstruction is no longer needed to eliminate artifacts from out-of-plane sources since vaporization locations are within the imaging plane. Some alternative strategies, including spatial control of cavitation, are also proposed.

2. Material and methods

2.1. Nanoemulsion samples

NEB-GNSs were synthesized using the procedure described in previous reports [28–31]. Colloidal GNSs (diameter 12 nm) 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. A solution of 1 vol.% perfluorohexane (Sigma–Aldrich) and 0.012 vol.% Au clusters in water was sonicated (102C, Branson, Danbury, CT, USA, pulse regime – 1 s on, 4 s off) for 13 s in a cold water bath.

The resulting size distribution of the beads was measured by dynamic light scattering (Malvern Zetasizer Nano ZS, 633 nm wavelength, Malvern, UK). The intensity distribution was centered at a bead diameter of 129 nm with full width at half-maximum of 190 nm. This primary DLS peak ends around 500 nm. As was the case with the samples used in our companion publication, a secondary peak was present between 3 and 6 μm.

We then used another technique to purify the sample from large droplets by one-pass extrusion through a 400 nm membrane (model no. 110605, Whatman, Clifton, NJ, USA). The sample was first diluted 35×. To confirm and quantify the absence of large droplets, the size distribution and concentration of the emulsion were obtained prior to 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 analyzed 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 solution of molecular dye (IR-783, Sigma–Aldrich) and a solution of single GNS (12 nm diameter) were used as control samples. Absorbance spectra for all samples were measured with a spectrophotometer (UV 1601, Shimatzu, Kyoto, Japan). Normalized spectra for the two nanoemulsion samples and the two controls are presented in Fig. 1b. As shown in previous work [23,24,28–31], NEB-GNS (blue curve) and filtered NEB-GNS (black-dashed curve) have a broader tail due to plasmonic coupling between gold nanospheres. The NEB-GNS and filtered NEB-GNS were diluted to a low absorption coefficient so that there was minimal thermal coupling or interaction between resultant microbubbles from neighboring particles, ensuring that the solution response is dominated by the sum of individual nanoparticle responses to light/US excitation [32]. Consequently, all nanoemulsion samples, and the dye control sample, were diluted to an absorbance of 0.05 cm^{-1} at the operating wavelength of 750 nm (see Fig. 1a), corresponding to a solution with mean distance between each nanoemulsion bead of 7 μm and independent particle behavior in terms of thermal coupling and bubble growth dynamics. There is no thermal coupling when the light is absorbed because the distance is too large for individual thermal fields to interact, the bubbles are likely to grow and condense back without colliding, stabilizing the droplet distribution. The amount of GNS in the resulting NEB-GNS solution was then estimated. A pure GNS