In vivo imaging of gold nanorod contrast agents using photothermal optical coherence tomography

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ABSTRACT

Photothermal optical coherence tomography (PT-OCT) has the potential to increase the molecular specificity of OCT for in vivo pre-clinical studies of cancer, in order to better understand drug uptake and treatment response. However, the use of PT-OCT to image contrast agents in vivo has yet to be demonstrated. Here, we characterize PT-OCT imaging of gold nanorod (GNR) contrast agents, and we further apply these techniques for in vivo imaging. The PT-OCT signal was characterized and compared to a numerical model of the bio-heat equation with respect to varying photothermal chop frequency, photothermal laser power, OCT image reflectivity, and concentration of GNRs. PT-OCT images were taken of GNR+ and GNR- solid agarose phantoms in capillary tubes, and 400 pM GNR matrigel injections into a mouse ear. Experimental PT-OCT data varied as predicted with closed form models of the bio-heat equation. Increasing the concentration of GNRs caused a linear increase in the PT-OCT signal, with GNR sensitivity as low as 7.5 pM compared to a scattering control (p<0.01). PT-OCT images in capillary tubes and the live mouse ear demonstrated an appreciable increase in signal in the presence of GNRs compared to controls. The demonstrated in vivo PT-OCT capabilities using GNR contrast agents is sufficient to image molecular expression, based on published molecular imaging studies employing GNR contrast agents in vivo. Therefore, this work demonstrates an important transition of PT-OCT to in vivo imaging, and marks the next step towards its use for in vivo molecular imaging.

Keywords: Optical coherence tomography, nanoparticles, photothermal effect

1. INTRODUCTION

Cell-to-cell heterogeneities in molecular expression play a vital role in pathogenesis and therapeutic resistance in many diseases, including cancer1-2. Therefore, significant efforts have focused on three-dimensional longitudinal imaging of molecular expression in pre-clinical models, with the goal of designing more effective therapies. Optical coherence tomography (OCT) fills a unique niche between microscopy and ultrasonic imaging modalities, with cellular-level resolution and imaging depths that exceed traditional and multi-photon microscopy. As a result, OCT provides an attractive platform to study disease formation and to test experimental drugs in pre-clinical models. However, OCT lacks inherent molecular specificity because the scattering cross-section does not vary widely between molecular species. Pump probe3, spectroscopic4, magnetomotive5, and photothermal optical coherence tomography (PT-OCT)6-11 all seek to increase the molecular specificity of OCT. PT-OCT leverages the photothermal heating effect, in which photon absorption triggers local heating, and thus changes in optical path length via thermoelastic expansion and thermorefractive index changes. PT-OCT has gained much attention in recent years, with in vitro and ex vivo imaging of targeted and non-targeted nanoparticles6-10. PT-OCT has also been used to characterize blood oxygen saturation in vivo with multiple second long A-scan acquisitions (non-imaging mode)11. However, in vivo PT-OCT has yet to be demonstrated with the use of contrast agents. Therefore, the goal of this study is to demonstrate the ability of PT-OCT to differentiate contrast agent from background in vivo. Gold nanorods (GNRs) were used in these studies because they are attractive contrast agents for in vivo molecular imaging due to their finely tunable near infrared (NIR) absorption peaks, well documented fabrication and molecular functionalization, safety, and desirable size scale for infiltrating tissues. The PT-OCT signal was characterized and compared to the bio-heat equation with respect to varying photothermal chop frequency, photothermal laser power, OCT reflectivity, and concentration of GNRs. PT-OCT contrast
enhancement with GNR injections was also confirmed with in vitro and in vivo imaging. This work provides the next step towards using PT-OCT for high resolution molecular imaging in vivo at depths greater than traditional microscopy.

2. METHODS

2.1 Gold nanorod samples

GNRs were fabricated in house using well established seed-mediated growth methods using the surfactant hexadecyltrimethyl ammonium bromide (CTAB). CTAB surface molecules were then displaced with covalently linked 5000 molecular weight polyethylene glycol (PEG), a common surface modification to increase stability in salt solutions and biocompatibility. The GNR extinction peak was found to be 725 nm using spectrophotometry both before and after the exchange for PEG surface molecules (Fig. 1a), with minimal shifting over time at room temperature. Average GNR size was approximately 45.2±5.7 nm long by 13.2±1.8 nm wide (n=20), assessed by transmission electron microscopy (Fig. 1b).

![Figure 1: GNR characterization. (a) Spectrophotometry extinction spectra of GNR samples after PEG exchange. (b) TEM images of GNR samples.](image)

A number of liquid GNR suspensions ranging in concentration from 0 to ~600 pM were prepared for analysis. For PT-OCT signal characterization, 5 μl of liquid sample in 1X PBS were placed on a microscope slide and covered with a cover slip. Solid agarose phantoms in capillary tubes were also prepared for imaging. 4% low gelling temperature agarose was dissolved into water using a hot plate. An 800 pM GNR sample was then mixed 1:1 with dissolved 4% agarose, suctioned into a capillary tube using capillary action, and allowed to cool while covered in parafilm. The resulting solid agarose phantom consisted of two capillary tubes, one containing 2% agarose/400 pM GNRs, and one negative control with only 2% agarose. Finally, for in vivo injection studies, an 800 pM GNR sample, which was cooled on ice, was mixed with Matrigel, to create a 400 pM GNR sample suspended in Matrigel.

2.2 Imaging instrumentation

A spectral domain OCT imaging system was altered for PT-OCT imaging by adding a photothermal laser, in this case a wavelength tunable titanium:sapphire laser (Fig. 2). Light from a 860 nm center wavelength, 51 nm bandwidth (6.4 μm axial resolution in air) superluminescent diode (SLD) is sent through a circulator and 50/50 fiber splitter, where the light is joined by the photothermal beam and split between a reference and sample arm (~16.5 μm spot size on the sample). The returning light is captured by a 2048 pixel CCD, at a 10 kHz line rate. For each PT-OCT A-scan, 1000 repeated temporal scans (M-mode) are acquired for analysis. During acquisition, the photothermal beam, tuned to 725 nm (the GNR resonance peak), is amplitude modulated via mechanical interference from a chopper, set to a 200 Hz square wave with a 50% duty cycle. The oscillations in photothermal heating are processed via the phase information in the repeated OCT scans to create PT-OCT images. Unless otherwise noted, the photothermal beam power is 10 mW. For a majority of the experiments, common path imaging geometry was used to increase phase stability, with the reflection of a coverslip on top of the sample serving as the reflection sample. However, for imaging of agarose phantoms, as well as in vivo imaging, the reference arm remained intact.
2.3 Data processing

PT-OCT data was processed offline with a custom Matlab script. M-mode data from each A-scan was resampled to k-space, dispersion corrected, and DC subtracted. Magnitude and phase information as a function of depth and time were then calculated using a Chirp-Z transform along the wavenumber axis. Whereas traditional OCT images are visualized with the magnitude information from the Chirp-Z transform, the PT-OCT data is calculated using the phase information. PT-OCT images the oscillations in optical path length due to amplitude modulated photothermal heating. OCT phase information contains sub-resolution changes in optical path length, and therefore the phase information is operated upon to investigate the PT-OCT signal. The derivative of the phase data along the temporal axis is calculated. The derivative of the phase information is Fourier transformed along the temporal axis in 20 separate overlapping and windowed segments, and averaged to reduce noise in the signal. The PT-OCT signal for the A-scan at each point in depth is defined as the magnitude of the Fourier transformed data at the chopping frequency of the amplitude modulated photothermal beam, minus the mean of the surrounding frequency components (baseline removal). PT-OCT images are then median filtered in both dimensions to minimize noise.

2.4 PT-OCT characterization and imaging

The PT-OCT signal was characterized using liquid GNR phantoms placed on a microscope slide. PT-OCT signal as a function of GNR concentration was assessed to determine signal linearity and imaging sensitivity to GNR contrast. The PT-OCT signal with respect to varying chopping frequency, photothermal laser power, and OCT image reflectivity were also assessed. Normalized experimental PT-OCT signals were directly compared to computer simulations of previously developed closed form solutions to the bio-heat equation, which simulates photothermal heating with the assumption of radial heat conduction dominance. Control and 400 pM GNR solid agarose phantoms in capillary tubes were imaged. Finally, matrigel injections into the ears of a 10 week old nude female mouse (Foxn1
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) were imaged with OCT, PT-OCT, and Doppler OCT while the mouse was under isoflurane anesthesia, to demonstrate the ability of PT-OCT to identify GNR contrasts in a live animal (conducted with institutional approval at Vanderbilt University). For in vivo imaging, a lower resolution lens (~40 μm spot size) was used, and photothermal laser power was increased to 40 mW to account for the increased spot size.

3. RESULTS

3.1 PT-OCT signal characterization

Outputs from computer simulations of photothermal heating using the closed form solution of the bio-heat equation agree with previous results. The computational model agrees well with all experimental results. Increasing the laser power on the sample linearly increases the PT-OCT signal (Fig. 3a), and increasing the chopping frequency causes a logarithmic falloff in PT-OCT signal (Fig. 3b). Assessing the effect of the OCT image reflectivity on the PT-OCT signal...
is also essential due to the speckle and spatial heterogeneity in an OCT image. The photothermal heating is independent of OCT image reflectivity, and is strictly a function of the thermodynamics of the photothermal laser parameters and imaging specimen; therefore the PT-OCT signal should be independent of the OCT magnitude signal. Indeed, decreasing the reflectivity in the OCT image had no effect on the mean PT-OCT signal (Fig. 3c). However, due to the decreased phase stability of weak OCT reflections, the noise floor of the PT-OCT signal increased with decreased OCT image reflectivity, thus increasing the variability of the PT-OCT signal (error bars, Fig. 3c). Finally, increasing the concentration of GNRs linearly increased the PT-OCT signal, and PT-OCT in common path imaging geometry was able to differentiate GNR samples as low as 7.5 pM from a scattering control (p<0.01, Fig. 3d).

**Figure 3**: PT-OCT signal characterization. (a) Linear increase in PT-OCT signal (modeled and experimental) with increased laser power. (b) Logarithmic decrease in PT-OCT signal (modeled and experimental) with increased chopping frequency. (c) Constant PT-OCT signal with varying OCT image reflectivity. (d) Linear increase in PT-OCT signal with increasing GNR concentration. PT-OCT signal is significantly greater in 7.5 pM sample compared to scattering control (red, p<0.01).

### 3.2 Phantom and in vivo PT-OCT imaging

Images of capillary tubes and the in vivo mouse ear demonstrated the ability of PT-OCT to differentiate GNR contrast from background signal. Capillary tube images demonstrated increased PT-OCT signal between GNR+ and GNR- capillary tubes (Fig. 4b), while OCT magnitude images had no appreciable differences between GNR+ and GNR- tubes (Fig. 4a).
The background PT-OCT signal in vivo is negligible in control ears with matrigel-only injections (green channel, Fig. 5a), but injections of matrigel-suspended GNRs into the ears of experimental mice demonstrate appreciable increases in PT-OCT signal (green channel, Fig. 5b). The photothermal signal due to GNRs is spatially distinct from blood vessels imaged with Doppler OCT (red and blue channels, Fig 5b), which is noteworthy because blood is the primary source of endogenous absorption and background PT-OCT signal in vivo in nude mice (that have no melanin) at 725nm.

4. CONCLUSIONS

To our knowledge, this work provides the first demonstration of in vivo PT-OCT imaging of contrast agents. PT-OCT was sensitive to relevant concentrations (400pM) of GNRs for in vivo molecular imaging, providing sufficient sensitivity to image the uptake of GNRs in mouse xenograft tumors with systemic (tail-vein) delivery. This represents an important translational step towards in vivo molecular imaging with PT-OCT in pre-clinical models.
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