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***In vivo* intravascular ultrasound-guided photoacoustic imaging of lipid in plaques using an animal model of atherosclerosis**

Bo Wang^{*}, Andrei Karpouk^{*}, Doug Yeager^{*}, James Amirian[†], Silvio Litovsky[‡], Richard Smalling[†], and Stanislav Emelianov^{*}

^{*}Department of Biomedical Engineering, University of Texas at Austin, Austin, TX 78731

[†]Division of Cardiology, University of Texas Health Science Center, Houston, TX 77030

[‡]Department of Pathology, University of Alabama Birmingham, Birmingham, AL 35249

Abstract

We present a preliminary study demonstrating the capability of ultrasound-guided intravascular photoacoustic (IVPA) imaging to visualize the depth-resolved distribution of lipid deposits in atherosclerotic plaques *in vivo*. Based on the characteristic optical absorption of lipid in the near infrared wavelength range, IVPA imaging at a single, 1720 nm, wavelength was used to provide a spatially resolved direct measurement of lipid content in atherosclerotic arteries. By overlaying an IVPA image with a spatially co-registered intravascular ultrasound (IVUS) image, the combined IVPA/IVUS image was used to visualize the distribution of lipid within the vessel wall.

Ultrasound-guided IVPA imaging was performed *in vivo* in the abdominal aorta of a Watanabe heritable hyperlipidemic (WHHL) rabbit. Subsequently, the excised rabbit aorta filled with a solution of red blood cells (RBC) was then imaged *ex vivo*, and the histology was obtained in the section adjacent to the imaged cross-section. To demonstrate the potential of future clinical application of IVPA/IVUS imaging, a sample of diseased human right coronary artery (RCA) was also imaged. Both *in vivo* and *ex vivo* IVPA images clearly showed the distribution of lipid in the atherosclerotic vessels. *In vivo* IVPA imaging was able to identify diffuse, lipid-rich plaques in the WHHL rabbit model of atherosclerosis. Furthermore, IVPA imaging at a single wavelength was able to identify the lipid core within the human RCA *ex vivo*. Our results demonstrate that ultrasound-guided IVPA imaging can identify lipid in atherosclerotic plaques *in vivo*. Importantly, the IVPA/IVUS images were obtained in presence of luminal blood and no saline flush or balloon occlusion was required. Overall, our studies suggest that ultrasound-guided IVPA imaging can potentially be used for depth-resolved visualization of lipid deposits within the anatomical context of the vessel wall and lumen. Therefore, IVUS/ IVPA imaging may become an important tool for the detection of rupture-prone plaques.

Keywords

intravascular photoacoustic; intravascular ultrasound; plaque characterization; lipid

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Address of correspondence: Stanislav Emelianov, Department of Biomedical Engineering, The University of Texas at Austin, 1 University Station C0800, Austin, TX, 78712-0238, Phone: (512) 471-1733, Fax: (512) 471-0616, emelian@mail.utexas.edu.

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INTRODUCTION

The location and size of the necrotic lipid core is critical for analyzing the stability of atherosclerotic plaques (Falk 2006). Identification of vulnerable plaques depends on the distribution of lipid (Virmani 2011). Unfortunately, current invasive imaging modalities cannot reliably delineate spatially resolved lipid distribution (Vancraeynest et al. 2011). Intravascular optical coherence tomography (OCT) can detect lipid, but it lacks the imaging depth to assess the area of the lipid-rich plaques and requires temporal removal of luminal blood during imaging. Intravascular magnetic resonance imaging (MRI) has a better imaging depth, but it requires an occluding balloon to stabilize the catheter. Intravascular near-infrared spectroscopy (NIRS) can be performed in presence of blood, but the signals are not depth resolved. Here we present a catheter-based imaging modality – ultrasound-guided intravascular photoacoustic imaging – to detect the depth-resolved distribution of lipid in atherosclerotic plaques *in vivo*.

Intravascular photoacoustic (IVPA) imaging is analogous to intravascular ultrasound (IVUS) imaging. In IVPA imaging, instead of sending acoustic waves into the tissue, a low energy, short laser pulse is emitted into the vessel wall. Upon absorption of the laser energy, thermal expansion of the tissue generates photoacoustic (PA) waves that are received by an ultrasound transducer. Among other factors, the amplitude of the PA signal is proportional to the optical absorption coefficient of the tissue (Oraevsky et al. 1997). Similar to IVUS imaging, the location of the absorber can be resolved based on the time of arrival of the PA signal. IVPA imaging is performed together with traditional IVUS imaging, which can delineate the anatomy of the vessel wall. Overall, the resolution of IVPA imaging is similar to IVUS imaging. For example, the axial resolution for IVPA imaging performed using a 40 MHz single element IVUS imaging catheter is tens of micrometers (Sethuraman et al. 2007). By scanning at multiple wavelengths, spectroscopic IVPA has been previously used to detect both endogenous and exogenous optical absorption contrast in arterial samples (Sethuraman et al. 2008; Allen and Beard 2009; Wang et al. 2009; Wang et al. 2010; Jansen et al. 2011).

Due to the abundance of C–H bonds in fatty acids, lipid absorbs strongly at around 1720 nm wavelength, around the first overtone of the C–H bond (Wang et al. 2002; Anderson et al. 2006). Therefore, IVPA imaging performed at 1720 nm wavelength can map the distribution of lipid deposits deep inside the plaques because imaging in this near infrared (NIR) wavelength range increases the imaging depth. Indeed, using excised samples of healthy and atherosclerotic rabbit aortas, 1720-nm IVPA imaging of lipid was successfully demonstrated *ex vivo* in the presence of luminal blood (Wang et al. 2012). In the current study, using an integrated IVPA/IVUS imaging catheter, we investigate the possibility of *in vivo* detection of lipid within the plaque in an atherosclerotic animal model using ultrasound-guided IVPA imaging.

METHODS

Animal model

A Watanabe heritable hyperlipidemic (WHHL) rabbit was used as an animal model of atherosclerosis. WHHL rabbits have a high level of low density lipoproteins (LDL) in their blood due to their genetic deficiency of LDL receptors, and can spontaneously develop atherosclerosis in the aorta and coronary arteries. Atherosclerotic plaques from WHHL rabbits are morphologically similar to human plaques (Masashi and Takashi 2009). In this study, an 18 month old WHHL rabbit under normal diet was used for *in vivo* IVPA imaging. The rabbit weighted 3.4 kg at the time of the experiment.

Human specimen

A human right coronary artery (RCA) autopsy sample was acquired from the Department of Pathology at the University of Alabama at Birmingham. The sample was formalin fixed and used for *ex vivo* ultrasound-guided IVPA imaging in a solution of human red blood cells (RBCs) acquired from the Blood Center of Central Texas. RBCs were diluted with saline to the concentration of RBCs in human whole blood.

IVPA/IVUS imaging system and catheter

The combined IVPA and IVUS imaging system consisted IVUS imaging system interfaced with a pulsed laser source. The overall system was triggered by a tunable optical parametric oscillator (OPO) laser system (OPOTEK, Inc. for *in vivo* imaging, and Spectra-Physics, Inc. for *ex vivo* imaging) operating at a repetition rate of 10 Hz. Laser pulses with 3–5 ns duration were coupled with an integrated IVPA/IVUS imaging catheter. After each laser pulse, the data acquisition (DAQ) card (GaGe, Inc.) started to sample the radio frequency (RF) data at a 200 MHz sampling rate. Then, after a user defined delay of 9 μ s, conventional IVUS imaging was performed using the integrated catheter (Fig. 1). Each scanning A-line consisted of spatially registered IVPA and IVUS signals from the vessel wall.

The integrated IVPA/IVUS imaging catheter was built with a single element 40 MHz IVUS imaging catheter (Boston Scientific, Inc.) and a light delivery system based on a side-fire fiber with a 600 μ m core. By aligning the optical and acoustic beams, simultaneous IVPA/IVUS imaging could be performed in the overlapping region of the two beams (Karpouk et al. 2010; Karpouk et al. 2012). Laser energy output from the integrated catheter was approximately 0.9 mJ/pulse. In this study, the total diameter of the distal end of the integrated catheter was around 2.2 mm (Fig. 1B). Cross-sectional scans of the vessel wall were realized by rotating the catheter using a stepper motor with a gear system. Each co-registered IVPA and IVUS image consisted of 256 scanning A-lines. No averaging was applied to IVPA or IVUS signals/images.

Image acquisition

Before *in vivo* imaging, 0.5 ml heparin (100 units/ml) was administrated to the anesthetized rabbit. The descending aorta of the WHHL rabbit was exposed via abdominal laparotomy. After isolating the descending aorta, a modified short 8F introducer was placed into the aorta distal to the renal arteries and advanced a short distance (~2.5 cm). The artery distal to the introducer was tied off with a 4-0 silk ligature and secured with a 4-0 silk suture. Then, an integrated IVPA/IVUS imaging catheter was advanced into the introducer to perform ultrasound-guided IVPA imaging of the abdominal aorta. After *in vivo* imaging, the rabbit aorta was procured and imaged *ex vivo* in a cuvette filled with human RBCs solution within 24 hours after the animal was sacrificed. The human RBCs solution was used to mimic whole blood during *ex vivo* imaging of rabbit aorta sample and human coronary artery sample. The experimental protocol was approved by the Animal Welfare Committee of the University of Texas Houston Health Science Center.

Histopathology

After *ex vivo* imaging of both artery samples, the imaged cross-sections were marked with a suture while the samples were still immersed within the blood cuvette. For the rabbit aorta, the marked cross-section was cut open using a scalpel blade and embedded in the tissue freezing medium. The frozen tissue section was then sliced using a cryostat for Oil red O stain and hematoxylin-eosin (H&E) stain. The formalin fixed human sample was paraffin embedded and sliced with a microtome for H&E and CD68 stain, an immunohistochemistry marker for macrophages.

RESULTS

In vivo IVPA imaging

A representative *in vivo* IVPA image (Fig. 2A) of the WHHL rabbit aorta shows the depth resolved distribution of lipid deposits inside the vessel wall. The spatially co-registered IVUS image (Fig. 2B) of the same cross-section clearly delineates the anatomy of the vessel wall. By combining the two images, the IVUS/IVPA image (Fig. 2C) shows that lipid deposits are located in the intimal layer of the aorta. IVPA signal generated from periaortic fat can also be detected (Fig. 2C, 5 o'clock). The amplitude of backscattered IVUS signal from luminal blood was relatively strong compared to typical IVUS images (Fig. 2B). The stronger signal may be a result of reduced blood flow caused by tying off the distal end of the aorta. The background noise in the images was due to ambient electromagnetic noise from the surgical unit in the room where the *in vivo* experiment was performed. No significant motion artifacts caused by cardiac cycle were observed in Fig. 2 because the introducer was secured to the aorta and minimized the tissue motion.

Ex vivo imaging of rabbit aorta

The excised rabbit aorta was immersed in human RBCs solution at the same concentration of RBCs in human whole blood for ultrasound-guided IVPA imaging. The inner boundary of the aorta can barely be distinguished because of strong acoustic scattering from the stationary RBCs solution in the vessel lumen (Fig. 3B). The combined IVPA/IVUS image demonstrates the relative location of the IVPA signal source in the vessel wall (Fig. 3C). Then, the RBCs solution was diluted to approximately 10% of RBCs concentration in human whole blood. When imaged in the diluted RBCs solution, deeper imaging depth is achieved due to reduced light attenuation in luminal blood (Fig. 3E). In addition, because of lower acoustic scattering from luminal blood, the vessel wall can be more reliably identified in the IVUS image (Fig. 3F). The combined IVPA/IVUS image shows that lipid-rich plaques are present in the thickened intimal layer (Fig. 3G). H&E and Oil red O stain of the imaged cross-section confirmed that the vessel contained diffuse, lipid-rich plaques in the intimal layer of aorta (Figs. 3D and 3H). Due to limited imaging depth of approximately 2.5 mm, lipid at 12 o'clock region in Fig. 3C and Fig. 3G were not visualized.

Ex vivo imaging of lipid core in human RCA

To demonstrate that ultrasound-guided IVPA can also detect lipid in human plaques, a human RCA sample was imaged in the RBCs solution at a concentration of human whole blood. Strong lipid signals are present from 3–6 o'clock in the IVPA image (Fig. 4A). The imaged artery has a thickened intimal layer (Fig. 4B). The hypo-echoic region from 4–6 o'clock next to the vessel lumen in the IVUS image (Fig. 4B) was caused by insufficient blood perfusion. The combined IVPA/IVUS image reveals the presence of lipid deposits located inside the intimal layer (Fig. 4C). Lipid from periaortic fat also generated photoacoustic signal (Fig. 4C, 11–5 o'clock). H&E stain shows the vessel has mostly fibrous intima, and a lipid core containing early calcification (Figs. 4D and 4E). CD68 stain for macrophages further confirmed inflammation in the lipid core (Fig. 4F).

DISCUSSION

Combination of IVPA imaging with IVUS imaging aids both imaging technologies – the distribution of lipid within atherosclerotic plaques (IVPA image) can be visualized within the context of the anatomical structure of the vessel (IVUS image). Furthermore, IVPA imaging shares similar data acquisition and signal processing procedures with IVUS imaging, which allows the integration of IVPA with IVUS imaging systems with minimal hardware changes. Finally, IVUS is already used in cardiac catheterization laboratories to

guide interventions, and therefore, combined IVUS/IVPA imaging can be readily introduced to clinical practice.

IVPA imaging depth inside the vessel wall is affected by the construction of the catheter and the distance between the lumen and the catheter. Optical attenuation by the blood cells attenuates the light energy reaching to the vessel wall. Imaging in the NIR wavelength range and the one-way transmission of light allows IVPA to have a superior imaging depth in the presence of luminal blood compared to pure optical based imaging modalities, such as intravascular NIRS (Wang et al. 2002). Because the amplitude of PA signals relates to the local laser fluence but not the coherence of the laser beam inside the biological tissue, removal of luminal blood, employed in intravascular OCT and intravascular MRI (Vancraeynest et al. 2011), is not needed. Using the current IVUS/IVPA integrated catheter, an imaging depth of approximately 2.5 mm was achieved in the presence of luminal blood (Fig. 2). Increasing the area of the overlapped region between the optical and acoustic beam can further improve the imaging depth. For example, deeper imaging can be achieved by adjusting the angle of the redirected laser beam relative to the acoustic beam of the imaging catheter (Karpouk et al. 2010). Further miniaturization of the imaging catheter will enable increased overlap between the acoustic and optical beams and, as a result, allow for improved imaging depth.

The size and flexibility of the current integrated IVPA/IVUS imaging catheter are not suitable to image coronary arteries. An integrated IVPA/IVUS catheter built with a 400 μm core side-fire fiber to a total diameter of 1.25 mm has been reported for *ex vivo* spectroscopic IVPA imaging in saline (Jansen et al. 2011). Because of the low laser energy required for lipid imaging at 1720 nm wavelength, an optical fiber with a smaller diameter can be used for the integrated catheter. In addition, the optical fiber should be incorporated into the driving cable of the catheter such that an integrated IVPA/IVUS imaging catheter will have a size similar to the clinically used IVUS imaging catheters.

The preliminary study presented here demonstrates that IVPA imaging is a promising technique for *in vivo* depth resolved visualization and quantification of lipid in plaques. The *in vivo* experiments were performed using a WHHL rabbit model of atherosclerosis and the results were confirmed using *ex vivo* imaging of both atherosclerotic rabbit aorta and human coronary artery samples. Prior to imaging, the human sample used in this study was fixed in formalin at zero transmural pressure. Although the preservation procedure has a possibility to change the structure of the plaque and therefore affect the IVUS and IVPA images, we observed excellent correlation between the ultrasound-guided IVPA imaging and the histopathology results. In the future, fresh human vessel samples with more diverse pathology will be imaged, and a quantitative study will be performed to demonstrate the sensitivity and specificity of this technique.

Unlike previously proposed spectroscopic IVPA imaging of lipid around 1210 nm wavelength (Wang et al. 2010), where background PA signals were suppressed through spectroscopic (e.g., multi-wavelength) analysis, in this study we used a single wavelength of 1720 nm for lipid imaging. Because the PA signal from lipid was higher compared to other types of tissues, no multi-wavelength scan was required to further differentiate lipid-rich and background tissues. As a result, single-wavelength IVPA imaging reduces the cost of the system and increases the achievable imaging speed. In the current ultrasound-guided IVPA imaging experiments, a combined IVUS and IVPA image was acquired in about 30 seconds. The imaging speed was severely limited by the laser pulse repetition frequency of 10 Hz determined by the OPO laser system. For real-time imaging (30 frames/s), a laser with high, a few kHz, pulse repetition frequency is needed. Such high pulse repetition frequency may be achieved by several laser systems, e.g. a diode laser, an OPO or dye laser driven by Q-

switched diode-pumped solid-state laser, etc. Currently, Nd:YAG lasers operating at 1064 nm wavelength with 10 kHz frequency and several millijoules output energy are available. Therefore, with further development, real-time ultrasound-guided-IVPA imaging is possible.

CONCLUSIONS

A catheter-based ultrasound-guided IVPA imaging system was successfully tested for detection of lipid presence *in vivo* in an atherosclerotic rabbit aorta and *ex vivo* in a human RCA sample. The lipid-rich plaques and lipid core inside a diseased artery can be spatially resolved in the presence of luminal blood. This combined imaging method might become a valuable asset for interventional cardiologists to guide percutaneous coronary intervention in the near future.

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Fig. 1. Combined IVPA/IVUS imaging system used in *in vivo* and *ex vivo* studies. (A) Block diagram of the combined IVPA/IVUS imaging system. (B) X-ray image of the WHHL rabbit with an integrated IVPA/IVUS imaging catheter advanced into the abdominal aorta. (C) Photograph of the distal end of the integrated IVPA/IVUS imaging catheter. The catheter consists of a single element IVUS imaging catheter and side-fire optical fiber.

Fig. 2.

In vivo IVPA/IVUS imaging of lipid in the abdominal rabbit aorta. (A) IVPA image shows the spatially resolved distribution of lipid deposits inside the vessel. (B) IVUS image shows the anatomy of the vessel wall. IVPA and IVUS images are displayed using 20 dB and 35 dB display dynamic ranges, respectively. (C) Location of lipid deposits in the vessel wall can be visualized in combined IVPA/IVUS image. Lipid deposits are mainly located in the intimal layer of the artery.

Fig. 3.

Ex vivo combined IVPA/IVUS imaging of lipid in the atherosclerotic rabbit aorta. (A–C) IVPA, IVUS and combined IVPA/IVUS images of the rabbit aorta acquired *ex vivo* in the human RBCs solution. The vessel wall can be barely visualized in the IVUS image because of the strong acoustic scattering from stationary luminal blood. (E–G) IVPA, IVUS and combined IVPA/IVUS images of the same cross-section of the vessel containing diluted RBCs solution. IVPA imaging depth is increased due to the reduced attenuation of light in the luminal blood. IVPA and IVUS images are displayed using 25 dB and 35 dB display dynamic ranges, respectively. (D,E) H&E and Oil red O stains confirmed lipid deposits in the intimal layer of the aorta.

Fig. 4.

Ex vivo combined IVPA/IVUS imaging of lipid core inside a human RCA. (A) IVPA image of the formalin fixed human RCA. Strong IVPA signals from lipid deposits can be observed at the 3–6 o'clock position. (B) IVUS image showed the luminal blood and vessel wall. (C) Combined IVPA/IVUS image shows that the lipid region is deep inside the intimal layer of the vessel. (D,E) 2X and 4X H&E stain of the imaged cross-section showed lipid core (pointed by an arrow in E) in the thickened intimal layer of the vessel wall. (F) CD68 stain confirmed that the lipid core (pointed by an arrow) is rich in macrophages. An intimal artifact occurred during section of the specimen (3 o'clock in D–F).