Mechanical evaluation of lipid accumulation in atherosclerotic tissues by photoacoustic viscoelasticity imaging

**YUE ZHAO, CONGGUI CHEN, SIHUA YANG, AND DA XING**

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou 510631, China

*Corresponding author: xingda@scnu.edu.cn

Received 6 June 2016; revised 1 September 2016; accepted 2 September 2016; posted 6 September 2016 (Doc. ID 267699); published 27 September 2016

Photoacoustic viscoelasticity imaging (PAVEI) is a technique that directly provides the morphology of biological tissues with correlative mechanical information. In this Letter, we demonstrate the use of PAVEI to successfully characterize early-stage atherosclerotic plaques. Lipid, as the main material in early plaque lesions, embedded in gelatin was imaged to test the feasibility of PAVEI. Atherosclerosis of rabbits was studied *ex vivo*, and the rabbit arteries were imaged to show the intrinsic contrast of PAVEI. Our results demonstrate that PAVEI can provide valuable viscoelasticity information for early detection of atherosclerotic plaques, which yields new insights into biomechanical diagnosis of cardiovascular diseases. © 2016 Optical Society of America

**OCIS codes:** (120.5050) Phase measurement; (170.3880) Medical and biological imaging; (170.5120) Photoacoustic imaging; (170.6935) Tissue characterization. http://dx.doi.org/10.1364/OL.41.004522

Observations from animal and human models of atherosclerosis suggest that plaque development is initiated by lipid accumulation in the arterial wall extracellular matrix, leading to activation of inflammation and intimal fibrosis [1,2]. Atherosclerotic plaques with large lipid necrotic cores and thin fibrous caps are more susceptible to rupture, which is responsible for the development of the majority of acute cardiovascular events [3]. A plaque’s stability is related to its morphology and histological composition. Hence, accurate identification of plaque morphology and components may allow the detection of vulnerable plaques before they rupture.

Currently, as a hybrid imaging modality, photoacoustic (PA) imaging combines high optical contrast with the high spatial resolution of ultrasound and provides the structural and compositional information of biological tissues [4–10]. Intravascular PA tomography is an emerging application of PA imaging that allowed localization and quantification of lipid content in atherosclerotic plaques in coronary syndromes [11–15]. However, the risk of plaque rupture is directly linked to the mechanical properties of the plaques [16]. Noninvasive measurement of the mechanical properties of the arterial walls is useful for diagnosing atherosclerosis, because there are significant differences between normal arterial walls and those affected by atherosclerosis [17,18]. A comprehensive understanding of the mechanical properties of the plaques will not only advance our understanding of the pathology of atherosclerotic disease but also provide critical information for medical or surgical treatment and for evaluating the efficacy of therapeutic interventions. Reliable techniques that are capable of characterizing the mechanical properties of plaque components may bear clinically relevant diagnostic values.

Viscoelasticity is one of the crucial physical parameters for characterizing the mechanical properties of biological tissues [19]. In this Letter, we describe a photoacoustic viscoelasticity imaging (PAVEI) technique for precisely evaluating the viscosity–elasticity ratio of atherosclerotic plaques. When a biological tissue is irradiated with a high-frequency quasi-continuous laser, the optical absorption converts to cyclical heat and causes thermoelastic stress that generates a strain in the form of the force-produced PA wave. The PA wave is out of phase with the stress in a viscoelastic medium. The phase delay is directly related to the viscosity–elasticity ratio of the medium. By recording the phase delay of the PA signal, the viscoelasticity image of the sample can then be reconstructed [20]. In atherosclerotic plaques, due to the relatively high viscosity of lipid, the phase delay of a PA signal will be higher than that in normal regions. Therefore, PAVEI can provide viscosity–elasticity ratio information for the early detection of atherosclerotic plaques, which will yield new insights into biomechanical diagnosis of cardiovascular diseases.

When a high-frequency quasi-continuous laser is used to radiate absorptive isotropic viscoelastic structures, the light intensity is given by

$$I = \frac{1}{2\overline{I_0}} (1 + \cos \omega t),$$  \hspace{1cm} (1)

where $\overline{I_0}$ is the time-averaged light intensity and $\omega$ is the modulation frequency. Light absorption by the absorber results in a...
sinusoidal temperature variation, which then causes thermal expansion and shrinkage as well as PA wave generation based on the thermoelastic mechanism. Because of the cyclical changes of the light intensity, a PA wave whose dominant frequency is equal to the modulated frequency is excited periodically. In the above process, the cyclical heating in the local region induces thermal stress, and strain is generated due to the stress in the form of the force-produced PA waves. Because of the damping effect due to the viscoelasticity of biological tissues, it could be found that the strain also alternates periodically, but it would be out of phase with the stress. Considering the generation of a PA signal, the rheological Kelvin–Voigt model is chosen to represent biological tissue with viscoelasticity; we know the relationship between the phase delay $\delta$ and the viscosity–elasticity ratio $\eta/E$ as \[ \delta = \arctan \frac{\eta \omega}{E}, \] \tag{2}

where $E$ is Young's modulus, $\omega$ is the modulation frequency, and $\eta$ is the coefficient of viscosity. From Eq. (2), we can obtain the viscoelasticity of the tissues.

Figure 1 shows the schematic diagram of the experimental setup. A high-frequency quasi-continuous laser operating at a wavelength of 1024 nm was used as the excitation source, with a frequency of 50 KHz. The transmitted laser was focused by a $4 \times$ microscope objective ($\text{NA} = 0.1$) to produce a high resolution and illuminate the target. The transverse resolution of the system is approximately 65 $\mu$m, tested by PAVEI of a blade edge. The center frequency of the customized ultrasonic transducer was also 50 KHz. A lock-in amplifier (SR830, Stanford Research Systems, Sunnyvale, CA, USA) was used as a phase-sensitive detector to detect the preamplified PA signals. The lock-in detector implemented the orthogonal vector arithmetic, which took time 300 ms because of the time constant (30 ms) of the lock-in detector and an average of 10 times, to output the averaged phase delay between the PA signal and the reference signal of the laser. The two-dimension photoacoustic viscoelastic images were obtained by mechanically scanning the specimen over the desired region. In the images, each pixel's gray level represents the averaged phase delay detected by the lock-in detector, which describes the integrated viscoelasticity of the tissue within the illuminated volume of the laser.

New Zealand white rabbits (Guangdong Province Medicine Laboratory Animal Center, China) with an average weight of 2.4 kg were used as animal models. To induce atherosclerosis, rabbits were fed a high-fat/high-cholesterol diet (97% normal chow, 2% lard, and 1% cholesterol) for 0 to 5 months. The rabbit aortas were dissected from the coronary artery to the femoral artery and then subdivided into sections. Each section was cut open longitudinally, exposing the luminal surface. Digital photos of the luminal surface were acquired, and regions of interest were identified prior to measurement. After the photoacoustic experiments and subsequent formalin fixation, the marked arterial segments were dissected. Sections of atherosclerotic plaques were sliced near the center of the marked segment. For each segment, cross sections were stained for lipid with oil red O stain, and stained for collagen with Masson's trichrome stain.

To verify the ability of PAVEI to identify plaque lipids, plaque-mimicking phantoms were studied, as shown in Fig. 2. Lipid, as the main material in early plaque lesions, was mixed with the gelatin in different concentrations to simulate atherosclerotic plaques. Figure 2(a) shows the relation between the lipid content and phase delay. Four circular targets are clearly shown in the PAVEI images acquired from the gelatin phantoms in Fig. 2(b). As predicted theoretically, the observed phase delay of the PA signals increased significantly with the increasing density of the lipid.

In Fig. 3 as the laser beam was scanned across the plaque, the phase delay varied significantly depending on tissue type: the phase delay was low ($\sim 30$ deg) in the normal regions and higher ($\sim 50$ deg) in the lipid-rich regions. The atherosclerotic plaque is clearly differentiated from the normal arterial wall because of the high viscoelasticity of lipid. The oil red O stain results of the two sections clearly showed the surface composition changes of the artery. The correspondence between the histological sections and the PAVEI results showed that the lipid produces the contrast in the PAVEI.

A representative example was used to demonstrate the ability of a PAVEI system to retrieve characteristics from an atherosclerotic plaque, as shown in Fig. 4. Figure 4(a) was acquired from the atherosclerotic plaques found in a 4-month-old rabbit. The phase delay, which was used to characterize plaque composition, changed with the compositional change at the lumen.

![Fig. 1. Schematic setup of the photoacoustic viscoelasticity imaging system.](image1)

![Fig. 2. (a) Relation between the lipid concentration and phase delay; (b) PAVEI of phantoms containing various concentrations of lipid.](image2)
surface of the plaque, as in Fig. 4(b). The histological results show the surface composition changes of the plaque [Fig. 4(c)]; the lipid components obviously increased, and the collagen decreased significantly. For a semi-quantitative analysis of the lipid content of the plaque, we calculated the integrated optical density (IOD) per stained area (pixels) (IOD/area) in the histological results using the Image-Pro Plus program (IPP; Media Cybernetics, Inc., Bethesda, Maryland, USA) [22]. The average phase delay values within three region of interest were compared against histological results that are independent from the measurement. The plaque type in region I was determined to be healthy from histology (0.024 ± 0.003 IOD/area); from PAVEI, and an average phase delay of 31.46 ± 4.48 deg was observed. Compared with region I, which exhibited a relatively shorter average phase delay, the area shown in region II had more lipid (0.102 ± 0.005 IOD/area), as indicated by histology, and showed an averaged phase delay of 60.40 ± 11.20 deg. In comparison, a different trend in the advanced fatty region (region III) was observed, where the average phase delay increased to 66.14 ± 12.65 deg with an increase in lipid (0.112 ± 0.006 IOD/area). In this manner, the lipid composition of the plaque surface (i.e., the vessel lumen) was characterized by PAVEI.

Figure 5(a) shows the region of the arterial lumen in a healthy rabbit and also the atherosclerotic lesions found in 3- and 5-month-old rabbits. In Fig. 5(b), PAVEI images from the surface of the advanced arterial plaques show abundant lipid-rich structures differing in appearance from the luminal elastic lamina that dominate the images of healthy vessels. The average phase delay (30.52 ± 3.24 deg) obtained from images of the lumen surface in the healthy region is significantly lower than that for the atherosclerotic regions. Meanwhile, the average phase delay (56.40 ± 9.79 deg) from the plaque found in the 3-month-old rabbit is much lower than that (70.35 ± 10.27 deg) found in the 5-month-old rabbit. Thus, the phase delay from regions of plaque increased in a linear fashion with the age of the rabbits, representing the overall burden of plaque in the aorta. The lipid accumulation is clearly shown in Fig. 5(c). A dense knot of lipid, as observed in the histological results, led to an overall increase in phase delay compared to the surrounding area. The lipid content increased from a basal average IOD/area of 0.018 ± 0.002 in a healthy artery to 0.108 ± 0.005 IOD/area in the fatty artery after 3 months of a high-fat/high-cholesterol diet. After 5 months of this diet, plaque had accumulated over large areas of the lumen surface of the aorta, and the 0.147 ± 0.008 IOD/area value of the lipid plaque showed a significant difference compared with other groups. The increase in the average viscosity–elasticity ratio was predominantly due to the increase in the amount of accumulated lipid and the decrease of collagen content [Fig. 5(d)], which plays an important role in the development of vulnerable plaque.

In this study, a PAVEI technique that measured the mechanical properties of atherosclerotic plaques was described, which provided an index of viscoelasticity for diagnosis of atherosclerosis. Through PAVEI analysis of atherosclerosis plaques and validation with the histological results, we found that there is a positive correlation between the lipid content and the phase delay measured by PAVEI. Lipid plays an important role in the development of plaques and directly relates to the mechanical

![Fig. 4.](image) (a) Photo of the atherosclerotic arterial wall. The red dashed rectangle indicates the detection area. (b) PAVEI of the region of interest. The lipid-rich plaque with clearly demarcated borders in the color map is corroborated by the accompanying gross pathology photograph. (c) The oil red O and Masson’s trichrome stain images of the area in the dashed rectangle. Bars = 300 μm.

![Fig. 5.](image) (a) Photos and (b) representative PAVEI images acquired from the luminal surface of the rabbit arteries, with healthy lumen (top row), early atherosclerotic plaque (middle row), and atherosclerotic plaque with higher lipid content (bottom row); (c) the oil red O and (d) Masson’s trichrome stain images of the area in the dashed rectangle. Bars = 300 μm.
properties of the vessel. This is consistent with weakening of the plaques, as it has been suggested that plaques containing more lipids are more likely to break [23]. Rupture of the plaque occurs mostly at the thinnest part of the plaque’s fibrous cap. In the future, the relation between the thickness of a plaque’s fibrous cap and phase delay must be investigated. Moreover, additional specific analysis of the lipid and collagen content of the atherosclerotic lesions and detection of a larger number of specimens could further improve the reliability of the PAVEI for the quantification of plaque burden in the rabbit models.

Although this PAVEI system was well applied to the ex vivo detection of lipid accumulation in atherosclerosis, to extrapolate the results to a clinical situation, a prospective study is needed for in vivo validation. However, the in vivo application of intravascular viscoelasticity imaging is still limited by the long acquisition time and the large diameter of the transducer. In the future, a small-diameter focused transducer will be employed to highly improve the imaging quality and accelerate the imaging speed of the system, which is an attractive prospect for the in vivo intravascular viscoelasticity evaluation of plaque. Meanwhile, in this work, the atherosclerotic specimens used in the ex vivo study were stored at 4°C in PBS before imaging, and it is possible that minor degradation of the specimens may have occurred. Hence, it is possible that the absolute measurement of phase delay may vary slightly under in vivo conditions. However, in the present study, because the difference in phase delay between the lipid and surrounding tissue was highly statistically significant, we anticipate that these relative differences would be maintained under in vivo conditions. Given that human atherosclerosis development goes through stages involving the same key biomechanical parameter that can be sensitively detected by PAVEI, this work suggests that a similar strategy could be used to realize the identification of human atherosclerosis in acute coronary syndrome.

It has been demonstrated that two-dimensional maps can be reconstructed to evaluate the spatial variation in plaque viscoelasticity by PAVEI; however, using only this single index to evaluate the risk of plaque rupture is not comprehensive. Imaging of the three-dimensional structure of plaques and the distribution of lipid can provide more comprehensive information for the evaluation of plaque vulnerability. In PAVEI, the phase delay values were computed over all the PA signals and the reference signals in the lock-in detector. As a result, the effects of viscoelasticity were integrated over the illuminated volume in the atherosclerotic plaque, and depth-resolved spatial information was lost. To overcome this problem, an electrically tunable lens will be used in the future to realize a fast variable-focus PAVEI with a large range of imaging depth [24], with which it may be possible to obtain three-dimensional volumetric maps of plaque viscoelasticity distributions. Although an electrically tunable lens may be a usable method for axial scanning and depth viscoelasticity imaging, the focal depth is still limited by the aperture angle of the lens. Conventional PA imaging has the potential to provide both the structure and the composition information of plaque with sufficient imaging depth. Therefore, through the combination of PAVEI and PA imaging, we can realize the integration of en face mechanical evaluation and in-depth structural information of lipid plaques, which would provide insight into the functional and compositional characterization of vulnerable plaques.

In summary, we have introduced PAVEI as a method for the mechanical evaluation of atherosclerotic plaques. The sensitivity and reliable contrast of PAVEI was demonstrated via gelatin phantoms. Furthermore, PAVEI was successfully applied to determine the viscoelasticity of atherosclerotic tissues and differentiate lipid plaque from the artery. Applying the PAVEI method to ex vivo atherosclerotic tissues indicated the potential of PAVEI to distinguish different plaque morphologies.

Funding. National Natural Science Foundation of China (NSFC) (91539127; 61331001; 61361160414); National High Technology Research and Development Program of China (2015AA020901); Science and Technology Planning Project of Guangdong Province, China (2015B020230316, 2014B0215003); Science and Technology Planning Project of Guangzhou, China (201508020112).

REFERENCES

5. V. Nitzschke, Nat. Methods 7, 603 (2010).