Photoacoustic viscoelasticity imaging for the detection of acute hepatitis: a feasibility study

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Received: 21 June 2018 / Accepted: 19 October 2018 / Published online: 13 March 2020

Abstract Biomechanical assessments are essential for the understanding of physiological states and the characterization of certain tissue pathologies such as liver cirrhosis. In this work, we showed by the photoacoustic viscoelasticity (PAVE) imaging that obvious mechanical change was also observed in the development of the acute hepatitis owing to the hepatocyte enlargement and intracellular fluid increment, indicating that the PAVE technique can be developed as a supplementary method for detecting acute hepatitis in future. The feasibility of the PAVE imaging is validated by a group of agar phantoms. Furthermore, acute hepatitis pathological animal models were established and imaged *ex vivo* and *in situ* by the PAVE technique to demonstrate its capability for the mechanical characterization of acute hepatitis, and the imaging results were consistent with pathological results. The feasibility study of detecting acute hepatitis by the PAVE technique proved that this method has potential to be developed as a clinical biomechanical imaging method to supplement current clinical strategy for liver disease detection.

Keywords Acute hepatitis, Viscoelasticity detection, Photoacoustic imaging

INTRODUCTION

Hepatitis infection is an important public health problem worldwide because hepatitis is one of the major causes of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Alter *et al.* 1999; Darby *et al.* 1997; Detre *et al.* 1996; Gao *et al.* 2015; Seeff *et al.* 1992; Tong *et al.* 1995). It is reported that more than two billion people worldwide are estimated to have evidence of current or past infection with hepatitis (Gao *et al.* 2013; Vedio *et al.* 2013), and usually some of them are asymptomatic. Liver function test (LFT) and liver biopsy (LB) are routine examination to diagnosis hepatitis. However, there are still problems in LFT such as low sensitivity of the enzyme detection in excess alcohol users (Yano *et al.* 2001). Even though LB is still considered as the gold standard in the evaluation of liver disease, it is invasive,

painful, and costly, which is not an ideal method for repeated assessment of liver disease progression such as acute hepatitis detection (Ferraioli *et al.* 2012). In the development of the acute hepatitis, hepatocyte enlargement and intracellular fluid increment usually lead to tissue edema, which potentially induces the changes of liver viscoelasticity. Therefore, the method that can detect the mechanical properties of acute hepatitis can be an alternative way to supplement current clinical strategy.

Photoacoustic (PA) imaging is a hybrid imaging modality that combines the advantages of high optical contrast and high ultrasonic resolution (Chen *et al.* 2012; Ermilov *et al.* 2009; Han *et al.* 2013; Hu *et al.* 2009; Lee *et al.* 2018; Song *et al.* 2014; Wang and Hu 2012; Yang *et al.* 2007a, b; Zeng *et al.* 2004; Zhang *et al.* 2014; Zhong *et al.* 2013). When excited by incident laser, tissues absorb photons and convert them into PA waves through thermal expansion (Gao *et al.* 2014; Liu

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and Yuan 2016; Yuan *et al.* 2012). As mechanical waves, the produced PA signal inherently carries mechanical information of the excited tissue (Gao *et al.* 2011, 2017; Hsieh *et al.* 2012; Singh and Jiang 2014; Yang *et al.* 2007a, b; Yuan *et al.* 2010), enabling PA imaging to be a potential strategy for tissue biomechanical characterization. Our group has proposed the PA viscoelasticity (PAVE) imaging for noninvasively characterizing tissue biomechanics through phase-resolved viscoelasticity ratio (VER) measurement (Gao *et al.* 2011; Shan *et al.* 2018), and has applied it to atherosclerosis characterization, tumor detection (Lv *et al.* 2018; Wang *et al.* 2004; Zhao *et al.* 2014, 2016), and liver fibrosis detection (Wang *et al.* 2018). In this study, the feasibility of this method for acute hepatitis detection is provided. Animal liver models are established and imaged *ex vivo* and *in situ*, demonstrating the method to be a potential technique supplement to clinical acute hepatitis assessment.

PRINCIPLE OF THE PAVE IMAGING

Figure 1A shows the principle of the laser-induced PA signal. When the tissue is excited by a sine-modulated laser beam with laser energy density of $I = I_0 \sin(\omega t)$, the tissue absorbs incident photons and causes a temperature variation with a form of $T = T_0 \sin(\omega t)$. The

sine-modulated temperature field will induce thermal expansion which then induces sinusoidal PA wave generation with the same modulation frequency of the incident laser. In the PA signal production process, the cyclical heating induces thermal stress in the local region, and then the strain is generated. Because of the viscoelastic damping effect of biological tissues, there will be a phase lag between the incident laser and the produced PA signal, as shown in Fig. 1B. According to the Kelvin–Voigt model, the phase delay corresponds to the tissues viscosity–elasticity ratio can be written as (Gao *et al.* 2011; Shi *et al.* 2017):

$$\delta = \arctan \eta\omega/E, \quad (1)$$

where δ is the PA phase delay, ω is the laser modulation frequency, E is the Young's modulus, and η is the tissue viscosity, respectively. In this work, we validated the feasibility of the PAVE technique for acute hepatitis detection. The pathological and mechanical changes of acute hepatitis are shown in Fig. 1C. When the liver tissue is inflamed, inflammatory cells grow and infiltrate normal liver cells. This induces liver cell enlargement and increases intracellular fluid and edema, and the biomechanical properties such as the viscoelasticity ratio of the liver tissue may suffer obvious changes. Therefore, the PAVE imaging can be used for the detection of acute hepatitis.

Fig. 1 **A** Principle of the PA imaging. **B** Principle of the PAVE imaging. δ : the tissue viscoelasticity-induced phase delay; η : tissue viscosity; E : Young's modulus. **C** The pathological and mechanical changes of acute hepatitis. The direction of the arrow represents the increase of the viscoelastic ratio in the liver tissue

THE CONSTRUCTION OF THE PAVE IMAGING SYSTEM

The systematic diagram of the PAVE imaging is shown in Fig. 2A. A high repetition frequency quasi-continuous laser (DS20HE-1064D/R, Photonics Industries) with a wavelength of 1064 nm and repetition frequency of 500 kHz is used as the excitation source. After laser collimation, the laser beam is focused by a microscope

objective (NA = 0.1) to illuminate the tissue sample which is fixed on a two-dimensional stage and immersed in a water tank. The produced PA signal is then detected by an ultrasound transducer with a flat spectrum from 200 kHz to 15 MHz (HPM05/3, Precision Acoustics Ltd.). The detected signal is then transferred to an amplifier (ZFL-500, Minicircuits) for signal amplification and then acquired by a lock-in amplifier

Fig. 2 **A** The schematic diagram of the PAVE imaging. MO: miniature objective; UT: ultrasonic transducer. **B** The photo of the PAVE imaging schematic. **C** The PAVE imaging of hair. **D** The resolution of the imaging system. ESF: edge spread function; LSF: line spread function

(SR830, Stanford Research Systems) to obtain the viscoelasticity-induced PA phase delay. By point-to-point scanning, the PAVE image then can be obtained through a MATLAB program. The two-dimensional stage is controlled by the computer with a custom program written by LabVIEW (National Instruments, USA). The photo of the PAVE imaging set-up is shown in Fig. 2B. In order to prove that the proposed PAVE imaging inherits the high-resolution imaging capability of conventional PA techniques, two carbon tubes were imaged by the PAVE imaging system, and the result is shown in Fig. 2C. The resolution experiment as shown in Fig. 2D indicates that the lateral resolution of the PAVE imaging system is about $6.4\ \mu\text{m}$, which is capable of providing micro-mechanical information of target sample with high resolution.

THE CAPABILITY OF THE PAVE IMAGING SYSTEM

To demonstrate the capability of the PAVE imaging in biomechanical characterization, tissue-mimic agar phantoms were prepared and tested. Four agar phantoms with different agar concentrations and India ink concentrations with 1% agar and 5% ink, 2% agar and 5% ink, 3% agar and 2% ink, 4% agar and 2% ink are shown in Fig. 3A. It is reported that the mechanical properties of agar phantoms are highly correlated with the agar concentrations (Hall *et al.* 1997; Singh and Jiang 2014). The conventional PA imaging of the four agar phantoms shown in Fig. 3B reflects sample's absorption that corresponds to the ink concentration. As shown in Fig. 3C, the PAVE image of the four agar phantoms has different phase delays which reflect the

Fig. 3 **A** The photo of agar sample. **B** The PA imaging of agar sample. **C** The PAVE imaging of agar sample. **D** The PA phase delay of agar phantoms obtained by rheometer and the PAVE imaging. **E** The photo of normal tissue (liver, fat). **F** The PAVE imaging of normal tissue. **G** The statistical results of PA phase delay for the normal tissue

agar concentration, and the four agar phantoms can be clearly distinguished. It is shown in Figs. 3C and D that the phase delay decreases with the agar concentration, and results obtained by the PAVE imaging agree with the measurement results by a rheometer. To further validate the capacity of the PAVE imaging in bio-applications, *ex vivo* normal fat and liver tissue (shown in Fig. 3E) were imaged by the PAVE imaging system, and the result is shown in Fig. 3F. Owing to the fact that more fibrin and less fat content are existed in liver tissue, the phase delay of the liver tissue is relatively small compared with that of the fat tissue. Figure 3G shows the phase delay along the dotted line in Fig. 3F. These phantom and *ex vivo* tissue imaging results demonstrate that the PAVE imaging is an effective method for biomechanical characterization.

The modeling process of acute hepatitis Sprague Dawley (SD) rat models with different pathological states is shown in Fig. 4A. 2 mL (10 mL/kg) carbon

tetrachloride (CCl_4) olive oil solution with 0.1% concentration was used and intraperitoneally injected to modeling the acute hepatitis (Campo *et al.* 2008). The mechanism of hepatic injury-induced acute hepatitis by CCl_4 injection was reported mainly owing to the free radical during its catabolism (LeSage *et al.* 1999). The generation of trichloromethyl radicals and trichloromethyl peroxy radicals due to CCl_4 injection can attack the phospholipid molecules on the cell membrane of the liver, which induces cell membrane injury. Meanwhile, the radicals can also combine with membrane lipid and protein molecular to destruct the protein metabolism. By this way, the injection of the mixture of CCl_4 and olive oil will finally induce the death of the liver cells and acute hepatitis. In our experiments, six rats were injected with the mixture of CCl_4 and olive oil, and were divided into three groups, and two of them were dissected soon after CCl_4 injection to provide as liver tissue control group. The other two groups of rat

Fig. 4 **A** The modeling process of acute hepatitis in rats. **B** The photograph of hepatitis with different pathological stages. **C** The corresponding PAVE imaging results. **D** The hematoxylin-eosin (H&E) staining of liver tissues. **E** The Masson staining of liver tissues. **F** The statistical results of viscosity and the ALT results

were dissected after 24 and 48 h after CCl_4 injection to provide acute hepatitis tissue with different pathological states (Campo *et al.* 2008). After 24-h injection of CCl_4 solution, the concentration of alanine aminotransferase (ALT) in liver cells reached highest value, indicating that the liver suffers the most acute hepatitis. The photos of liver tissue with acute hepatitis are shown in Fig. 4B. Results in Fig. 4C show that the viscoelastic phase delay significantly increases compared with normal liver. After 48-h injection, the concentration of ALT decreased due to immune self-healing, and the liver viscosity began to reduce. The viscosity images agree well with the histological results shown in Fig. 4D. Since the acute hepatitis model is a rapid-form process, the fibrin has been barely deposited in the liver, and thus the liver elasticity has little obvious changes, as demonstrated by the histological results in Fig. 4E. The statistical results of the liver phase delay and the variation of ALT in Fig. 4F indicate that the PAVE imaging can be used as a potential method for assessment of acute hepatitis.

Compared with existed clinical mechanical techniques, our method is capable of providing comprehensive tissue biomechanics with cellular resolution, and can be easily implemented into conventional PA microscopy, endoscopy, and laparoscope systems. To further demonstrate its capability for liver biomechanical characterization,

in situ liver tissues were imaged by the PAVE imaging. As shown in Fig. 5A and B, mouse models with normal and 24 h acute hepatitis livers were executed to expose the liver tissue for PAVE imaging. The obtained PAVE images corresponding to the dotted line frame in Fig. 5A and B are shown in Fig. 5C and D. It is shown in the PAVE images that the PA phase delay of the acute hepatitis liver is much larger than that of the normal liver, which agrees well with the statistical results in Fig. 5G and H. The corresponding ultrasound elastography (USE) in Fig. 5E and F shows that the elasticity of the liver has little changes during the acute hepatitis. This is because the acute hepatitis is a rapid process of mold formation, and thus there is no obvious deposition of fibrous components in the liver tissue. The comparison of the PA phase delay and the USE demonstrates that the PAVE imaging is an effective strategy for the assessment of acute hepatitis.

DISCUSSION

In this work, by using the phase-sensitive image contrast, the PAVE imaging is able to differentiate subtle changes in the biomechanical characteristics of the acute hepatitis, which can allow effective detection of acute hepatitis and help to improve the detection accuracy in clinic. Nevertheless, there are still some

Fig. 5 **A** The photo of normal liver. **B** The photo of acute hepatitis liver. **C** The PAVE image of normal liver. **D** The PAVE image of acute hepatitis liver tissue. **E** The USE of normal liver. **F** The USE of acute hepatitis liver. **G** The H&E staining of normal liver. **H** The H&E staining of acute hepatitis liver. **I** The comparison of the PA phase delay and the US elasticity results of liver tissue

limitations existed in the PAVE imaging. First, as we used a lock-in amplifier to obtain the PA phase delay, the imaging speed was restricted by the time constant (30 ms) of the lock-in amplifier. By reducing the time constant, higher imaging speed then can be obtained with a sacrifice of the signal-to-noise ratio. Second, due to distance-dependence phase deviation for the PA phase detection, ultrasonic localization will be necessary to calibrate the phase errors resulting from the sample surface roughness, which is essential to realize *in vivo* experiments. In this work, a 2.5-mm-sized broadband transducer with a flat spectrum from 200 kHz to 15 MHz was used to detect the PA signal. By using a narrowband transducer, the signal-to-noise ratio (SNR) in PAVE imaging can be further improved.

CONCLUSION

In summary, we present a method for the detection of the acute hepatitis by the PAVE imaging. The feasibility of the PAVE imaging was validated by a group of agar phantoms. Acute hepatitis animal models were established and imaged *ex vivo* to demonstrate the capability of the PAVE imaging for effective detection of the acute hepatitis. *In situ* animal models were imaged to further demonstrate the capacity of the proposed method in future biomedical applications. Overall, the experiment results show that the PAVE imaging has potential to be developed as a clinical biomechanical imaging method to supplement current clinical strategy for liver disease detection.

Acknowledgements This research was supported by the National Natural Science Foundation of China (61627827, 61331001, 91539127), the Science and Technology Planning Project of Guangdong Province, China (2015B020233016, 2014B020215003, 2014A020215031), the Postdoctoral Innovative Talent Support Program of China (BX201700084), and the Distinguished Young Teacher Project in Higher Education of Guangdong, China (YQ2015049). We thank the Third Affiliated Hospital of Sun Yat-Sen University for providing the results of shear wave elastography.

Compliance with Ethical Standards

Conflict of interest Qian Wang and Yujiao Shi declare that they have no conflict of interest.

Human and animal rights and informed consent All institutional and national guidelines for the care and use of laboratory animals were followed.

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