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High-resolution vascular tissue characterization in mice using 55 MHz ultrasound hybrid imaging

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ABSTRACT

Ultrasound and Duplex ultrasonography in particular are routinely used to diagnose cardiovascular disease (CVD), which is the leading cause of morbidity and mortality worldwide. However, these techniques may not be able to characterize vascular tissue compositional changes due to CVD. This work describes an ultrasound-based hybrid imaging technique that can be used for vascular tissue characterization and the diagnosis of atherosclerosis. Ultrasound radiofrequency (RF) data were acquired and processed in time, frequency, and wavelet domains to extract six parameters including time integrated backscatter (T_{IB}), time variance (T_{var}), time entropy (T_E), frequency integrated backscatter (F_{IB}), wavelet root mean square value (W_{rms}), and wavelet integrated backscatter (W_{IB}). Each parameter was used to reconstruct an image co-registered to morphological B-scan. The combined set of hybrid images were used to characterize vascular tissue *in vitro* and *in vivo* using three mouse models including control (C57BL/6), and atherosclerotic apolipoprotein E-knockout (APOE-KO) and APOE/A_1 adenosine receptor double knockout (DKO) mice. The technique was tested using high-frequency ultrasound including single-element (center frequency = 55 MHz) and commercial array (center frequency = 40 MHz) systems providing superior spatial resolutions of 24 μm and 40 μm, respectively. Atherosclerotic vascular lesions in the APOE-KO mouse exhibited the highest values (contrast) of −10.11 ± 1.92 dB, −12.13 ± 2.13 dB, −7.54 ± 1.45 dB, −5.10 ± 1.06 dB, −5.25 ± 0.94 dB, and −10.23 ± 2.12 dB in T_{IB}, T_{var}, T_E, F_{IB}, W_{rms}, W_{IB} hybrid images ($n = 10$, $p < 0.05$), respectively. Control segments of normal vascular tissue showed the lowest values of −20.20 ± 2.71 dB, −22.54 ± 4.54 dB, −14.94 ± 2.05 dB, −9.64 ± 1.34 dB, −10.20 ± 1.27 dB, and −19.36 ± 3.24 dB in same hybrid images ($n = 6$, $p < 0.05$). Results from both histology and optical images showed good agreement with ultrasound findings within a maximum error of 3.6% in lesion estimation. This study demonstrated the feasibility of a high-resolution hybrid imaging technique to diagnose atherosclerosis and characterize plaque components in mouse. In the future, it can be easily implemented on commercial ultrasound systems and eventually translated into clinics as a screening tool for atherosclerosis and the assessment of vulnerable plaques.

1. Introduction

Atherosclerosis is considered one of the main causes of cardiovascular disease (CVD) that is the number one killer globally and the incidence of disease is increasing [1]. According to the American Heart Association Statistics Committee and Stroke Statistics Subcommittee, 1 in 3 people are estimated to have one or more types of CVDs [2]. Genetically modified mouse models provide a powerful tool for understanding the pathogenesis of human cardiovascular diseases like human atherosclerosis [3]. Among available models, the apolipoprotein E-knockout mouse (APOE-KO) that is particularly popular because of its tendency to develop atherosclerotic lesions of similar complexity as those found in humans [4]. In a previous report, our research group has demonstrated that by removing A_1 adenosine receptor (AR) gene from APOE-KO, the resulting development of atherosclerosis in APOE and A_1 AR double knockout mice (DKO) was significantly decreased [5]. Accordingly,

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availability of these mouse models in cardiovascular research has motivated the development of diagnostic imaging techniques to assess the cardiovascular function.

Several noninvasive imaging techniques have been used for the *in vivo* assessment of CVD including electron-beam computed tomography [6], magnetic resonance imaging [7], positron emission tomography [8], and optical coherent tomography [9]. Although commercial ultrasound scanners have been used to image human hearts, they might not be appropriate in small animal due to their limited spatial resolution (0.3–1 mm). Mice have extremely small arteries (~0.07–1 mm in diameter) and elevated heart rates (500–800 beats/min) which presents a great challenge for commercial ultrasound scanners. High-frequency (>20 MHz) ultrasound systems were developed in order to achieve a spatial resolution of 50 μm or smaller and can provide adequate imaging of mouse vasculature [10].

High-frequency (high-resolution) ultrasound systems have been developed during recent decades. In 1987, Sherar et al. [11] was the first to show the enormous potential of high-frequency ultrasound for tissue imaging. Further developments in high-frequency ultrasound were performed by Sun et al. [12,13] to image small animal hearts providing high-resolution ($\leqslant$50 μm). Most ultrasound studies on small animals are currently carried out using either custom high-frequency ultrasound systems or the Vevo commercial systems (Vevo 770 and Vevo 2100, VisualSonics Inc., Toronto, ON, Canada). However, the diagnosis, monitoring, or treatment of atherosclerosis may require an imaging system able to provide both quantitative morphological measurements and compositional characterization of the disease. Such quantitative tissue characterization techniques are not available in the aforementioned systems without the use of contrast agents.

Quantitative ultrasonic tissue characterization for the heart has been demonstrated by Miller's group in 1980's [14,15]. They described the potential of using integrated backscatter (IB) measurements to assess dog cardiac tissues using 3.5–5 MHz ultrasound transducers [14]. Bridal et al. reported the use of ultrasonic parameters such as IB and attenuation to characterize plaques in human arteries using high-frequency ultrasound (30–50 MHz), in an *in vitro* study [16]. Tissue characterization techniques were also used to diagnose and identify the composition of atherosclerosis in human arteries using 30 MHz and 40 MHz intravascular ultrasound imaging (IVUS) [17–19]. These techniques extract parameters from ultrasound backscatters to render virtual histology maps for quantitative assessment of atherosclerosis. IVUS elastography maps were also introduced to characterize atherosclerotic plaque components by measuring mechanical stiffness [20,21]. However, most of the aforementioned techniques are invasive and would not be ideal for monitoring disease longitudinally. Noninvasive cardiovascular ultrasound elastography was recently adopted to characterize atherosclerosis in human carotid artery. However, the resolution of the technique ($\geqslant$125 μm) is still a limiting factor in small animal models of atherosclerosis [20]. For *in vivo* small animal studies, however, high-resolution ($\leqslant$50 μm) tissue imaging and characterization shall be required to assess the cardiovascular system and diagnose CVD.

In this study, we demonstrated the feasibility of an ultrasound-based noninvasive tissue characterization algorithm using high resolution ($\leqslant$40 μm) hybrid imaging, which was applied to diagnose atherosclerotic plaques. The algorithm was applied to RF data collected from both a single-element transducer [22] and a commercial array systems (Vevo 2100) to characterize atherosclerosis in mice. In addition, we investigate the use of novel ultrasonic quantitative parameters extracted from the time and wavelet domains (time variance (T_{var}), time entropy (T_E), wavelet root mean square value (W_{rms}), and wavelet integrated backscatter (W_{IB})) to characterize atherosclerotic plaques and compare with existing parameters (time integrated backscatter (T_{IB}), frequency integrated backscatter (F_{IB})). The technique was tested *in vitro* and *in vivo* using control and atherosclerotic mouse models. This paper is organized as follows. Section 2 describes the ultrasound systems, tissue characterization algorithm, and *in vitro* and *in vivo* experiments. The results are shown and discussed in Sections 3 and 4, respectively. Finally, Section 5 provides the concluding remarks of this study.

2. Materials and methods

2.1. Custom ultrasound system

The high-frequency ultrasound imaging system used for *in vitro* testing was described in [22]. An ultrasound transducer (29–81 MHz) of 55 MHz center frequency and 9.7 mm focal length was used (Olympus NDT Inc., Waltham, MA, USA). Received RF signals were pre-amplified and filtered, then fed to a high-speed (400 MHz) 14-bits waveform digitizer (Signatec Inc., Newport Beach, CA, USA). The imaging system used a PC for control, synchronization, and further signal processing. The PC controlled precisely a two-axis positioning system using ±1 μm resolution (Danaher Corp., Washington, DC, USA). The positioning system was synchronized with the data acquisition to collect ultrasound RF signals continuously *on-the-fly* during the transducer movement down to 8.5 μm apart. A custom user-friendly computer graphical user interface (GUI) was designed using Microsoft Visual C++ (Microsoft Corp., Redmond, WA) for control and data acquisition. Data was then transferred to MATLAB 7.1 (MathWorks, Inc., Natick, MA, USA) for post-processing and image reconstruction. To obtain a high-resolution B-mode ultrasound images, several signal and image processing algorithms were applied to the high-frequency echo signals [22,23]. This ultrasound system provided access to ultrasound data at all processing stages including synthetic aperture focusing (SAF), envelop detection, and final B-mode processed images, which was essential for the tissue characterization technique described below. The SAF procedure was applied using the weighted synthetic aperture focusing to overcome the limited depth of field for a highly focused single-element [24,25]. The system spatial resolution was experimentally evaluated using a B-mode image for an 8 μm tungsten wire immersed in water. The experimental values of the axial and lateral resolutions were approximately measured to be 24 μm and 123 μm, respectively. An average signal to noise ratio (SNR) of 109 dB was estimated experimentally for the system using a planar reflector (glass plate).

2.2. Quantitative ultrasound tissue characterization

Fig. 1 described the main procedures of the tissue characterization technique. First, a region of interest (ROI) was segmented and extracted from the raw ultrasound data after SAF. ROI was then divided into 2D kernels of small size, such as 0.15 mm × 0.15 mm with approximately 90% overlapping. To reconstruct hybrid images, a vector $S_{i,j}$ was generated for each spatial location (i, j) within the ultrasound scan. This vector was reconstructed from the neighborhood pixels $P_{i,j}$ within the kernel $K_{i,j}$, and defined as:

$$S_{i,j} = \left[\left(P_i - \frac{n}{2}, j - \frac{m}{2}\right) \dots (P_{i,j}) \dots \left(P_i + \frac{n}{2}, j + \frac{m}{2}\right)\right], \quad (1)$$

where $n+1$ and $m+1$ are the kernel length and width in pixels, respectively. For each spatial location within the ROI, $S_{i,j}$ vector was reconstructed from ultrasound data. This vector was considered a 1D signal incorporating embedded features of the neighborhood characteristics, which can be used to extract different parameters in time-, frequency- and wavelet-domains. In the