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● *Original Contribution*

ACOUSTIC PROPERTIES OF SMALL ANIMAL SOFT TISSUE IN THE FREQUENCY RANGE 12–32 MHZ

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Abstract—Quality assurance phantoms are made of tissue-mimicking materials (TMMs) the acoustic properties of which mimic those of soft tissue. However, the acoustic properties of many soft tissue types have not been measured at ultrasonic frequencies >9 MHz. With the increasing use of high-frequency ultrasound for both clinical and pre-clinical applications, it is of increasing interest to ensure that TMMs accurately reflect the acoustic properties of soft tissue at these higher frequencies. In this study, the acoustic properties of *ex vivo* brain, liver and kidney samples from 50 mice were assessed in the frequency range 12–32 MHz. Measurements were performed within 6 min of euthanasia in a phosphate-buffered saline solution maintained at 37.2 ± 0.2 °C. The measured mean values for the speed of sound for all organs were found to be higher than the International Electrotechnical Commission guideline recommended value for TMMs. The attenuation coefficients measured for brain, liver and kidney samples were compared with the results of previous studies at lower frequencies. Only the measured kidney attenuation coefficient was found to be in good agreement with the International Electrotechnical Commission guideline. The information provided in this study can be used as a baseline on which to manufacture a TMM suitable for high-frequency applications. (E-mail: adela.rabell@ed.ac.uk) **© 2017 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Ultrasound, High frequency, Mice, Brain, Liver, Kidney, Speed of sound, Attenuation.**

INTRODUCTION

The purpose of tissue-mimicking materials (TMMs) is to mimic the acoustic properties of soft tissue. Currently, the International Electrotechnical Commission (IEC 2001) guideline recommends standard values for the speed of sound (SoS) (1540 ± 15 m/s) and an attenuation coefficient for TMM of 0.5 ± 0.05 dB/cm at frequencies up to 10 MHz. Also, the International Commission on Radiation Units and Measurements (ICRU 1998) reports that for non-fatty tissues, the attenuation at 1 MHz should be 0.6 dB/cm. With the increasing use of high-frequency ultrasound in both clinical (2–15 MHz) and pre-clinical (>15 MHz) (Banchhor et al. 2016; Cook et al. 2011; Machet et al. 2009; Moran 1995; Rhee 2007; Schmitt et al. 2010; Sundholm et al. 2015; Xu et al. 2012) imaging applications, there is a need to extend the frequency range of these recommended acoustic values. Furthermore, the development of phantoms that incorporate TMMs that realistically mimic the acoustic properties of small animal soft tissue will enable a reduction in the use of small animals to optimize ultrasound imaging techniques.

The acoustic properties of brain, liver and kidney, among other organs, have previously been measured in small animals (Foster et al. 2000; Frizzel and Gindorf 1981; Goss et al. 1979; Gray et al. 2013; Szabo 2014; Tervola et al. 1985), humans (Bamber and Hill 1979; Bamber 1981; Kremkau et al. 1981; Ludwig 1950; Parker 1983; Rajagopalan et al. 1979; Sehgal et al. 1986) chickens (Martínez-Valdez et al. 2015) and mammals (Bamber et al. 1977; Ghoshal et al. 2011; Goss et al. 1979; López-Haro et al. 2010; Martial and Cachard 2007). These studies measured the acoustic properties up to 9 MHz at either room temperature (22 °C–26 °C) or human body temperature (37 °C). Wirtzfeld et al. (2015) measured the extracellular matrix (ECM) attenuation coefficient of murine liver and kidney across the frequency range 15–35 MHz using

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a decellularized method and found that the ECM of the organ contributes to the ultrasonic properties. Additionally, Frizzel and Gindorf (1981), O'Brien (1988) and Tervola et al. (1985) performed very high frequency acoustical measurements up to 100 MHz using a scanning laser acoustic microscope (SLAM). Measurements performed at 100 MHz were undertaken at room temperature (20 °C–26 °C).

It has been reported that the SoS and attenuation coefficient of soft tissue increase with increasing temperature (Bamber and Hill 1979; Ghoshal et al. 2011; López-Haro et al. 2010; Rajagopalan et al. 1979; Suomi et al. 2016). However, there is no further increase in the SoS in soft tissue above 50 °C (Duck 2012). Furthermore, it is well known that *ex vivo* soft tissue samples deteriorate with time after excision as gas bubbles form within the tissue, affecting its acoustic properties (Bamber 1981; Duck 2012). To prevent this, soft tissue should be excised and measured as soon as possible after euthanasia or stored at 4 °C (Bamber et al. 1977; Bamber and Nassiri 1985; Foster and Hunt 1979).

The acoustic properties of soft tissue have also been measured *in vitro* or by embedding the organ sample in an ultrasound-compatible acoustic material such as TMM (Bamber and Hill 1979; Bamber et al. 1977; Goss et al. 1979; Martínez-Valdez et al. 2015; Muleki-Seya et al. 2016; Sundholm et al. 2015; Suomi et al. 2016), but very few experiments have been undertaken using *ex vivo* tissue (Kumagai et al. 2014) or *in vivo* tissue (Kagadis et al. 2010; Zderic et al. 2004).

To address the current limited data on the acoustic properties of soft tissue, the study described here sought to measure the acoustic properties of *ex vivo* mouse brain, liver and kidney immersed in phosphate-buffered saline (PBS, Sigma-Aldrich, Saint Louis, MO, USA) at 37 °C, over the frequency range 12–32 MHz.

METHODS

Soft tissue sample preparation

We analyzed 20 brains, 20 livers and 20 kidneys from 50 recently euthanized healthy male C57BL/6 mice, a common inbred laboratory mouse strain. The mice were euthanized by cervical dislocation under the auspices of the Animals (Scientific Procedures) Act 1986 (Schedule 1) approved by the University of Edinburgh Animal Welfare and Ethical Review Board (AWERB). Within 6 min of euthanasia, the organs were extracted and sliced in either the coronal or transverse plane, and their acoustic properties were measured. Excised mouse tissues were sliced using a 1-mm adult rat brain acrylic slicer matrix (Zivic Instruments, Pittsburgh, PA, USA).

Twenty brains were excised and sliced in the frontal plane at the superior colliculus, which included the cerebral cortex (Fig. 1a). For brain tissue, the sample thickness was 3 mm, as thinner samples tended to disintegrate during handling. Acoustical measurements were made in the center of each sample, within the grey matter. Twenty murine left lateral liver lobes were excised and sliced in the coronal plane, to a thickness of 2 mm (Fig. 1b). Twenty kidneys from 10 mice were excised and sliced (2 mm) as follows: the right kidney was sliced in the coronal plane (Fig. 1c), and the left kidney was sliced in the transverse plane (Fig. 1d). Acoustical measurements were undertaken in the center of each sliced kidney sample in an endeavor to ensure location within the *medulla* of the kidney. Only one tissue sample was collected from each organ. The lateral (radial) dimensions of the samples were 0.5 cm for brain, 1 cm for liver and 0.5 cm for both dissection planes in the kidney.

Experimental setup using the high-frequency Vevo 770 ultrasound scanner

A temperature-controlled water-filled reservoir (Grant Instruments, Cambridge, UK) with dimensions of $15 \times 33 \times 19$ cm was used to heat phosphate-buffered saline (PBS; Sigma-Aldrich, Saint Louis, MO, USA) to 37.2 ± 0.2 °C. A smaller glass container ($10 \times 8 \times 7.5$ cm and 0.6 cm thick) was placed inside the water reservoir. A 1-cm layer of acoustic absorber (Aptflex F28, Precision Acoustics, Dorset, UK) was fixed at the bottom of the glass container. A 2.5-cm-diameter, and 5-mm-thick cylindrical acoustic reflector made from polymethylpentene (TPX; Boedeker Plastics, Shiner, TX, USA) was glued to the absorber. A 1-mm-thick, 2.5-mm-inner-diameter, 2.5-cm-outer-diameter circular washer made of the acoustic absorber was attached to the top surface of the TPX reflector, as illustrated in Figure 2. The circular washer acted as a tissue holder and ensured there was a space between the soft tissue sample and the TPX reflector. The aim of this separation was to allow the echoes from the tissue and from the TPX reflector to be differentiated during later analysis.

Acquisition and analysis of the acoustic data

Radiofrequency (RF) data from 60 soft tissue samples were acquired using a single-element, high-frequency probe RMV707 B attached to the Vevo 770 ultrasound scanner (Visualsonics, Toronto, ON, Canada). The RMV707 B probe has a center frequency of 30 MHz, focal depth of 12.7 mm and 3-dB bandwidth from 12 to 32 MHz (Rabell Montiel et al. 2017). The acoustic properties of the soft tissues were measured while immersed in PBS at 37.2 ± 0.2 °C. The TPX reflector was located at the focal point of the probe (Fig. 2). Data were collected at 10% of maximum acoustic output power (peak negative pressure: 1.05 MPa), which gave a satisfactory signal-to-noise ratio while avoiding significant non-linear propagation effects (Sun et al. 2012). By use of a broadband