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● *Original Contribution*

BUBBLE-INDUCED COLOR DOPPLER FEEDBACK CORRELATES WITH HISTOTRIPSY-INDUCED DESTRUCTION OF STRUCTURAL COMPONENTS IN LIVER TISSUE

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Abstract—Bubble-induced color Doppler (BCD) is a histotripsy-therapy monitoring technique that uses Doppler ultrasound to track the motion of residual cavitation nuclei that persist after the collapse of the histotripsy bubble cloud. In this study, BCD is used to monitor tissue fractionation during histotripsy tissue therapy, and the BCD signal is correlated with the destruction of structural and non-structural components identified histologically to further understand how BCD monitors the extent of treatment. A 500-kHz, 112-element phased histotripsy array is used to generate approximately 6- × 6- × 7-mm lesions within *ex vivo* bovine liver tissue by scanning more than 219 locations with 30–1000 pulses per location. A 128-element L7-4 imaging probe is used to acquire BCD signals during all treatments. The BCD signal is then quantitatively analyzed using the time-to-peak rebound velocity (t_{prv}) metric. Using the Pearson correlation coefficient, the t_{prv} is compared with histologic analytics of lesions generated by various numbers of pulses using a significance level of 0.001. Histologic analytics in this study include viable cell count, reticulin-stained type III collagen area and trichrome-stained type I collagen area. It is found that the t_{prv} metric has a statistically significant correlation with the change in reticulin-stained type III collagen area with a Pearson correlation coefficient of −0.94 ($p < 0.001$), indicating that changes in BCD are more likely because of destruction of the structural components of tissue. (E-mail: macoskey@umich.edu) **© 2017 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Histotripsy, Therapy feedback, Color doppler.**

INTRODUCTION

Histotripsy is a therapeutic ultrasound technique that employs inertial cavitation to destroy mechanically and fractionate unwanted target tissue within the body non-invasively without damaging surrounding tissue (Lin et al. 2014; Xu et al. 2007). Histotripsy has been investigated for the treatment of deep vein thrombosis (Zhang et al. 2016, 2017), liver tumors (Vlaisavljevich et al. 2013), benign prostatic hyperplasia (Roberts 2005; Schade et al. 2012), congenital heart diseases (Xu et al. 2004), and transcranial brain applications (Kim et al. 2014).

Quantitative imaging feedback for indicating tissue fractionation is important for monitoring and guiding non-invasive ablation techniques such as histotripsy to ensure treatment accuracy and efficacy. Magnetic resonance–guided focused ultrasound (MRgFUS) is used to provide a 3-D temperature map as real-time feedback during high-intensity focused ultrasound (HIFU) thermal treatments (Allen et al. 2012; Hynynen et al. 1993; McDannold et al. 1998) and has been approved by the US Food and Drug Administration to treat several conditions including essential tremor (Lipsman et al. 2013), uterine fibroids (Roberts 2005) and prostate cancer (Gyöngy and Coussios 2010). However, magnetic resonance imaging (MRI)-guidance for histotripsy would require MRI-compatible histotripsy arrays, as well as procedural MRI scanners.

Ultrasound imaging has previously been used to guide HIFU therapy and for monitoring treatment and the

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development of HIFU-induced cavitation (Farny et al. 2006; Owens et al. 2011). For histotripsy, B-mode ultrasound imaging has also been used in real-time during treatment to monitor changes in the bubble cloud, which appears as a dynamically changing hyperechoic zone (Owens et al. 2011, 2012). When substantial tissue liquefaction has occurred, the tissue appears to be hypoechoic on B-mode imaging (Hall et al. 2007; Xu et al. 2009). However, B-mode ultrasound is sub-optimal for monitoring the treatment effect because of its low sensitivity, as the hypoechoic zone only occurs when substantial tissue fractionation is achieved (Wang et al. 2009). Additionally, as the intensity of the tissue speckle varies across various tissues both temporally and from patient to patient, it is unlikely that a universal-intensity threshold could be set to indicate complete tissue fractionation for various tissues and patients.

Ultrasound elastography, such as acoustic radiation force impulse (ARFI) imaging, has also been investigated to detect histotripsy lesion formation. As the histotripsy treatment progresses, the target tissue is fractionated until it turns into a liquid-appearing homogenate, indicating that the elasticity of the tissue decreases throughout treatment (Cain and Wang 2012; Miller et al. 2012; Wang et al. 2012a, 2012b, 2014). A reliable elasticity method would be especially useful for histotripsy in viscoelastic tissues such as the liver where the collagenous elements of the extracellular matrix are the primary structural component. These elastography methods have been experimentally successful in the detection of tissue fractionation with higher sensitivity than B-mode speckle intensity. However, as the tissue becomes more liquefied, the shear-wave propagation used for elastography-imaging techniques become increasingly restricted, resulting in decreased accuracy of elastography measurements later in histotripsy treatment (Wang et al. 2012a, 2012b). Additionally, reflections from the histotripsy-induced cavitation nuclei are known to contribute to and interfere with the radiation force used for ARFI imaging, which may introduce artifacts in the elastography methods (Wang et al. 2014).

Recently, a new modality called bubble-induced color Doppler (BCD) has been developed to monitor histotripsy-induced tissue fractionation in real-time (Macoskey et al. 2017; Miller et al. 2016; Zhang et al. 2015). This method uses Doppler ultrasound on a conventional clinical ultrasound machine to obtain velocity estimations of the residual cavitation nuclei that persist after cavitation during histotripsy therapy. Our previous work has shown that this motion is because of the disturbance caused by the rapid expansion and collapse of the bubble cloud, which has also been observed previously elsewhere (Khokhlova et al. 2011). This motion is only observed when the histotripsy bubble cloud is formed, and we have found that this residual motion can last for at least 20 ms after each histotripsy pulse, thus indicating that it is likely not because of acoustic radiation force. Furthermore, we have found that as the tissue becomes increasingly fractionated, the motion of residual nuclei lasts longer, and that the change of the BCD signal correlates somewhat with increased tissue destruction (Miller et al. 2016). However, in this previous study, only cellular destruction was quantified over a small sample of pulse numbers. Thus, a more-detailed physiologic investigation of the correlation of the BCD signal with tissue destruction is required. Interestingly, in Vlaisavljevich et al. (2014), it was shown that tissues with higher Young's moduli result in cavitation bubbles with shorter lifespans and smaller maximum radii (Vlaisavljevich et al. 2014). This suggests that as the tissue becomes more fractionated and the elasticity of the tissue decreases, the cavitation bubble cloud should last longer with a higher maximum radius. Therefore, because the BCD signal is highly dependent on cavitation dynamics, which are dependent on tissue mechanics and we know that the residual nuclei motion responsible for the BCD signal lasts increasingly longer throughout treatment, we hypothesize that the BCD signal is more closely correlated with the destruction of structural components of tissue that contribute to the overall mechanical properties of tissue rather than the cellular components.

In this study, we investigated how changes in the BCD signal throughout histotripsy treatment are quantitatively correlated to the levels of fractionation of specific structures within liver tissue induced by histotripsy. Histotripsy therapy was applied to *ex vivo* bovine liver tissue by a 112-element histotripsy array at varying levels of tissue fractionation (*i.e.,* number of histotripsy pulses), and BCD signals were acquired for all samples. Each tissue sample was histologically stained to quantify cellular destruction, type III collagen destruction and type I collagen destruction.

METHODS

Ex vivo *bovine liver preparation*

Freshly excised bovine liver acquired from a local abattoir was preserved in room-temperature PBS-buffered saline and was used within 24 h of harvest. Before treatment, the liver was sectioned into approximately 4-cm cube sections and was then placed in degassed PBS-buffered saline under vacuum in a desiccator for 5 h. Liver samples were then removed from the desiccator and were embedded in blocks of 1.5% agarose to maintain structural stability. The blocks were suspended in place *via* two carbon skewers. This was done so that no structural support material other than agar was present between the histotripsy array and the tissue, thus minimizing aberration and the chance for cavitation on the surface of the sample. Once the agarose was solidified after roughly 1 h, the tissue