Characterizing the Effects of Indirect Histotripsy Treatment on Tendon Structure and Function

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INTRODUCTION: Focused ultrasound (FUS) therapies are an emerging non-invasive approach for ablating various tumors, including osteosarcoma (OS) and soft tissue sarcomas (STS), which pose significant clinical challenges in both human and veterinary medicine[1]. While current standards of care (e.g. limb amputation and thermal ablation methods) are used to ablate and/or provide pain relief, there are risks and limitations associated with them, including potential deleterious effects on surrounding tissues[2, 3]. Histotripsy is a non-invasive, non-thermal, and non-ionizing focused ultrasound ablation methods that has been proposed as a novel limb-salvage technique[2, 3]. In order to establish the clinical safety of histotripsy for the treatment of these musculoskeletal tumors, it is important to ensure that there is no damage to supporting structures, particularly tumor-adjacent tendons that are vital to joint movement. Therefore, the objective of this study was to evaluate the effects of indirect histotripsy treatment on tendon structure and function via biomechanical testing, ultrasound (US) image texture analysis, and histological analysis. It was hypothesized that tendons treated indirectly at 4000 ppp will exhibit significant differences in 1st and 2nd order image texture parameters, inferior biomechanical properties, and inferior histologic grades compared to untreated tendons and those treated indirectly at 1000 ppp.

METHODS: A total of 36 canine extensor tendons were collected from medium to large breed male (n=19) and female (n=17) dogs and randomly assigned to one of the 3 treatment conditions: control (untreated), histotripsy using 1000 ppp (pulses per point, a clinical dose), and 4000 ppp (supraclinical dose). In this *ex vivo* model, treated tendons were fixed in 7.5% porcine gelatin and treated indirectly in the middle region of the tendon (i.e. focal point is below the tendon) at the appropriate dose with a hydrophone-calibrated 500 kHz 32-element tranducer[2-4] (**Figure 1**). For n=8 tendons/group, grayscale and shear wave elastography (SWE) US images were acquired before and after histotripsy treatments for each dose, followed by load-to-failure mechanical testing at 20 mm/min. Image standardization of the grayscale images was performed using a histogram specification technique to account for variability of gain[5]. Pixelwise US texture analysis for the three regions (distal, middle, and proximal) was performed to calculate 1st (mean, median, variance, skewness, kurtosis, and entropy) and 2nd order (contrast, homogeneity, and energy) statistics. Samples for histological analysis (n=3 tendons/group) were treated with histotripsy within 48 hours of collection (treatment groups) or placed in formalin for further processing (control group, n = 6 tendons). Cell-matrix features were evaluated from Trichrome and H&E stained sections using a custom histologic grading scale.

RESULTS: Biomechanics: In comparing tensile properties across the three experimental groups, only one of the six quantified material properties exhibited significant differences, with this change attributable to the supraclinical dose. Strain at maximum stress was significantly increased in tendons treated at 4000 ppp ($p = 0.0361$) relative to untreated tendons. No significant differences among groups were observed for the remaining material properties (maximum stress, yield stress, yield strain, elastic modulus, and post-yield strain). Collectively, these results indicate that the low and high doses do not compromise tissue stiffness (modulus) or yield behavior. However, failure mode analysis revealed a significantly greater proportion of failure localized to the treatment region at 4000 ppp, indicating weakening within the treatment region that is not evident when examining bulk mechanical properties **(Figure 2)**. Ultrasound Texture: Two of the nine grayscale parameters exhibited a significant difference at the lower dose, with treated tendons showing increased kurtosis at 1000 ppp and increased variance at 4000 ppp in the treated region (**Table 1**). At 4000 ppp, three parameters (median, variance, and skewness) exhibited either borderline or statistically significant differences relative to the pre-treatment state in the treatment region. 2nd order texture parameters, which were quantified for the full region of interest (ROI), showed no change post-treatment. Mean shear wave speed (SWS) significantly increased following 1000 ppp but was unaltered by 4000 ppp treatment. However, SWS of the untreated group was also significantly higher than pre-treatment 1000 ppp samples. Histology: Histological evaluation with H&E and Trichrome straining revealed no differences between untreated and treated tendons. All samples received a score of 0, indicating no histologically detectable changes due to off-target effects of histotripsy. Statistical Correlations: Skewness was positively correlated with yield strain ($R = 0.64$, $p < 0.001$). Correlations between the remaining texture parameters and mechanical properties were moderate to weak ($R < 0.5$).

DISCUSSION: The results of this study partially support our hypothesis. While significant changes and trends in ultrasound texture and biomechanical properties revealed subtle dose-dependent, localized changes in the histotripsy treatment region, these alterations did not compromise overall tendon structure and function. Strain at maximum stress (i.e., failure strain) was significantly elevated relative to the untreated group, indicating that tendons subjected to supraclinical doses can tolerate greater elongation prior to complete tissue failure. Higher texture variance in the high-dose treatment group indicates greater heterogeneity in pixel intensity, while higher (positive) skew suggests more hypoechoic pixels, which in this *ex vivo* model may indicate tissue microstructural damage. A limitation of this study was the heterogeneity of tendon size attributable to the medium to large breed dogs available to the investigative team. This variability potentially confounds the interpretation of our SWS results, which differed between untreated tendons and pre-treated tendons (low dose group).

SIGNIFICANCE/CLINICAL RELEVANCE: This work establishes histotripsy as a safe and effective limb-salvage treatment for musculoskeletal tumors that preserves tendon function. The data from this study can be used to refine histotripsy treatment protocols for tumor treatments and establish a safety profile for tendons. From a clinical perspective, this data can inform treatment planning, such as cases where higher histotripsy doses are required, so that the appropriate approaches, including delayed return to activity or rehabilitation, can be implemented to allow for tendon recovery and reduce the risk of reinjury. Finally, this study establishes an experimental platform for understanding how specific histotripsy parameters influence tendon tissue, which will facilitate the design of non-invasive, ablative treatments, such as direct histotripsy (e.g., debridement)[6].

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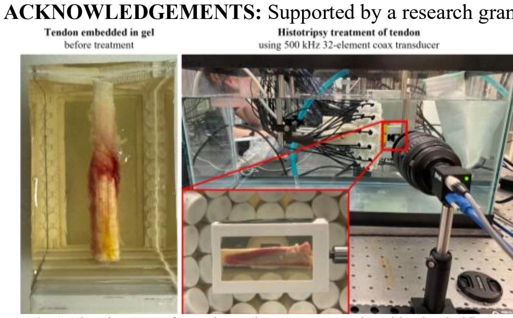

Fig. 1: Histotripsy set-up for ex vivo tendon treatments, starting with gel embedding. Samples are placed in a tank of degassed water, where a motorized positioning system was used to align the embedded tendon sample to the focus by positioning the marked focal point below the tendon in the gel to simulate an indirect treatment.

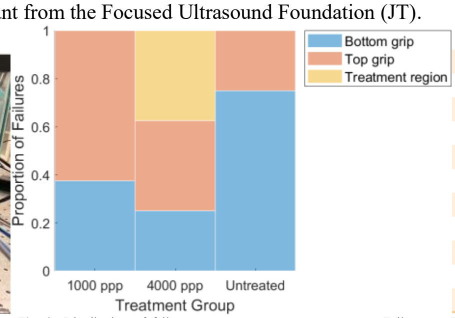

Fig. 2: Distribution of failure patterns across treatment groups. Failure pattern differed significantly ($p = 0.045$) among groups, with tendons in the supra-clinical dose more likely to rupture within the treatment region. This occurred in 3 of the 8 tested samples, whereas failure within this corresponding tendon region was not observed for any samples within the clinical dose or non-treated groups.

	Pre- vs. Post- Treatment			
Parameters	1000 ppp	p-value	4000 ppp	p-value
Mean	NC	0.58	⇩	0.0950
Median	NC	0.4125	⇩	0.0552
Variance	NC	0.1190	⬆	0.0037*
Skewness	NC	0.9356	⇧	0.0583
Kurtosis	⬆	< 0.0001*	NC	0.7899
Entropy	NC	p > 0.9	NC	p > 0.9
Contrast	NC	0.5856	NC	0.1969
Energy	NC	0.8279	NC	p > 0.9
Homogeneity	NC	0.6629	NC	0.5687
Shear Wave Speed	⬆	0.0178*	NC	p > 0.9

Table 1: Post-treatment changes in 1st (for treated region) and 2nd (for full ROI) order texture parameters as well as mean SWS. NC indicates no change, filled arrows indicate a significant difference, and open arrows indicate trends toward significance.