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The Effects of Desiccation on Skull Bone Sound Speed in Porcine Models

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***Abstract*—Pre- and postdesiccation sound speeds through *ex vivo* porcine skull specimens were determined by time-of-flight measurements with propagated broadband pulses centered at 0.97 MHz (Øs12.7 mm, −6-dB bandwidth = 58%). The measured longitudinal sound speed in the 13 porcine samples (predesiccation average sound speed = 1727 ± 57 ms^{-1}) changed by a statistically significant +2.3% after deionized water reconstitution (paired t-test, $\alpha = 0.05$, $p = 0.0332$).**

I. Introduction

Historically, experimental studies to characterize the acoustic transmission properties of the human skull bone have been vigorously performed in order to improve the efficacy of transcranial ultrasound as a diagnostic or therapeutic agent [1], [2]. The bulk sound velocity through layers of cranial bone tissue is a parameter that is often measured. This is one of the most crucial parameters because spatial variations in sound speed are largely responsible for transmission phase distortion and, in the case of focused ultrasound, focal intensity aberrations [3]. Ideally, ultrasound transmission measurements would be performed with specimens *in vivo*, but most often it is logistically more feasible to use excised tissues. Formaldehyde-preserved skull tissue has been shown to maintain the acoustic properties of fresh samples [1], but oftentimes, the *ex vivo* bone specimens are allowed to dehydrate, then they are reconstituted by submersion in formaldehyde solution, saline, or deionized water prior to experimentation. This study investigated the effects on sound speed measurements of 13 reconstituted *ex vivo* porcine calvaria after full desiccation.

II. Materials and Methods

Thirteen calvarium specimens excised from 13 separate *ex vivo* pigs—each animal was sacrificed less than 15 minutes before harvest—were temporarily stored at room temperature in phosphate-buffered saline (PBS), then transferred to 10% buffered formalin. For transmission measurements, each specimen was immersed in a rubber-lined tank filled with degassed deionized water at room temperature (monitored and recorded by a thermocouple) and placed in the path of ultrasound propagation between a broadband planar piezocomposite transducer and a needle hydrophone (Fig. 1). Normal angles of incidence between the ultrasound propagation vector and the outer surface of the skull specimens were obtained by geometrically matching skull surfaces at close proximity to the surface of the transducer, then translating the skull into the measurement setup position. The source transducer, which had a center frequency of 0.97 MHz, a circular 12.7-mm aperture, and a −6-dB bandwidth of 58% (Panametrics, Waltham, MA), propagated ultrasound pulses through the thickness dimension of the specimens in which the transmitted signals were received by a polyvinylidene fluoride (PVDF) needle hydrophone with a 0.2-mm diameter aperture (Precision Acoustics, Dorchester, UK). The skull specimens were positioned approximately half-way (±5 mm) between the source transducer and the receiving hydrophone. Data analysis was independent of the absolute distance between the source and receiver, so 20 cm was arbitrarily chosen (Fig. 1) to accommodate the geometries of the setup. Broadband pulses were generated by an ultrasound pulser-receiver (Panametrics, Waltham, MA), and the transmitted signals were received by the hydrophone and recorded by a digital oscilloscope (Tektronix, Beaverton, OR).

To calculate skull bone sound speed, ultrasound propagation measurements were taken with and without the skull specimens in place. The propagation times for the first pressure peak in the received signals were recorded, and the bulk longitudinal sound speed in bone was calculated according to

$$c_B = \frac{h}{t_w + \Delta t}, \quad (1)$$

where h is the measured thickness of the bone, Δt is the difference in the propagation time for the two cases, and t_w is the calculated ultrasound time-of-flight through a layer of water of thickness h. The speeds of sound in water used for this calculation were determined with measured water temperature values and a previously published numerical formulation [4]. Bone thicknesses were measured with digital calipers (Mitutoyo, Kawaski, Japan, model CD-6) at multiple locations corresponding to the beamwidth footprint of the transducer. The measurements then were averaged, and the error in measurement was calculated and propagated through the determination of c_B.

To compare the sound speeds of fresh versus previously-desiccated skull bone samples, each specimen was placed in an open container under a continuously venting fume hood for at least 1 week after predesiccation measurements were

Manuscript received October 11, 2006; accepted April 28, 2007. Formation of this work was funded in part by NIH grants R01EB003268, R21EB004353, U41RR019703, and 8 T32EB02177-09.

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Digital Object Identifier 10.1109/TUFFC.2007.443

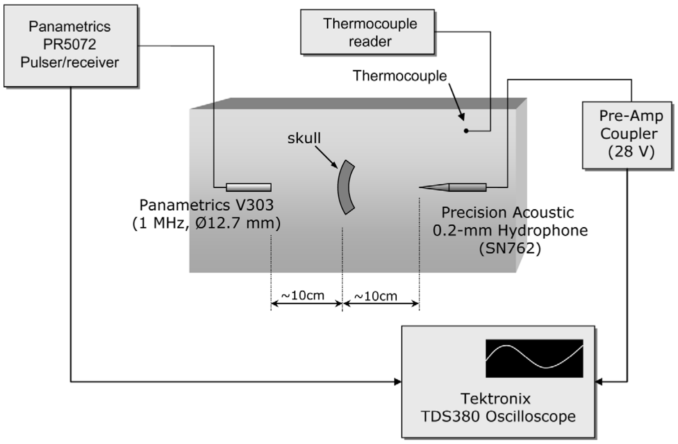

Fig. 1. Experimental setup for measuring sound speed through the thickness dimension of *ex vivo* skull bone specimens.

performed. After desiccation, the bone specimens were reconstituted by immersion in deionized water and degassed in an evacuated vacuum jar (Nalge, Rochester, NY) (Gast, Benton Harbor, MI) at room temperature for approximately 4 hours. By this method, bone marrow that may have been lost due to desiccation is replaced by water, which can be assumed to acoustically behave like bone marrow at the intended experimental parameters [5]. This assumption notwithstanding, the effects of bone marrow on transcranial ultrasound propagation is not readily observable due to the largely cortical structure of the skull bone, as compared to the porous morphology of cancellous bone, which is dominated by trabecular structures. The measurements then were repeated and tabulated for comparison. The combination of specimen geometric parameters (e.g., surface area, curvature, smoothness, etc.) and transducer beamwidth allowed one measurement per specimen.

III. Results

The average values for the measured speeds of longitudinal mode sound propagation through 13 *ex vivo* pig skulls for the predesiccation and the postdesiccation cases are presented in Figs. 2 and 3 and Table I. A representative example of the transmitted signal for the pre- and postdesiccation scenarios is shown in Fig. 4. The transmission of ultrasound at near 90° incidence to the outer surface of the skulls ensures a longitudinal mode of propagation with a negligible contribution of shear components [6]. Over the 13 pig skull specimens, the average sound speed was measured to be 1727 ± 57 ms^{-1} before desiccation. After reconstitution, the average of the measured sound speeds increased by 2.3% for a value of 1766 ± 61 ms^{-1}. This increase in sound speed was deemed by a paired t-test to be statistically significant ($\alpha = 0.05$, $p = 0.0332$). The least significant change (LSC) for the measured sound speeds for the predesiccation case, calculated as the measurement precision multiplied by 2.77 (95% confidence), was 9.1%. For the postdesiccation case, the LSC was 9.6%.

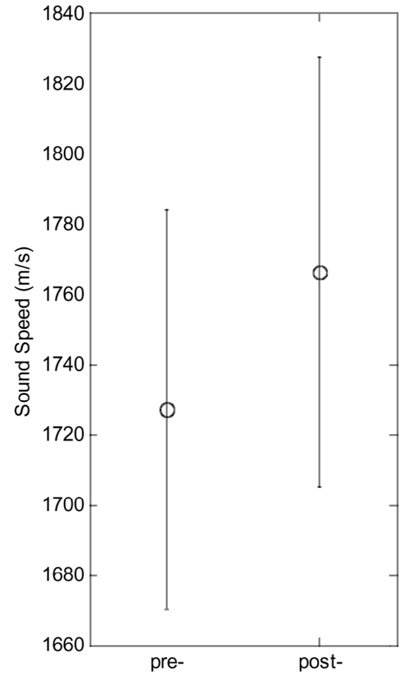

Fig. 2. Averaged results of sound speed measurements for bulk longitudinal ultrasound propagation in pre- and postdesiccated porcine skull bone.

IV. Discussion

The purpose of this study was to examine the effects of complete desiccation on the bulk ultrasound sound speed of skull bone. Freshly excised calvaria are oftentimes logistically difficult to obtain for *ex vivo* experimentation, especially those of human skulls. So, a crucial study [1] which demonstrated that formalin fixation has a negligible effect on sound speed in human skull bone has made possible extensive studies in transcranial ultrasound. Yet, post mortem skull samples available for research often are extensively exposed to air in which they desiccate and must be reconstituted by immersion degassing before ultrasound experiments can be performed. Whether significant structural rearrangement of the osseous structure takes place through the desiccation and reconstitution cycle remains an interesting question. But, most immediately, a comparison of the bulk ultrasound transmission properties both before and after desiccation would give a first-order indication of the significance of any change, if any does take place.

V. Conclusions

The statistical analysis of the results obtained from measuring the sound speed in pre- and postdesiccated pig skull samples pointed toward a significant, yet small, increase of 2.3%. An examination of the individual sets of measurements with the pig skull specimens and their accompanying error analyses (Fig. 2) reveals that the overall

TABLE I
Measured Thicknesses and Bulk Longitudinal Sound Speeds for Pre- and Postdesiccated Porcine Skull Bone.

	n	Predesiccation sound speed, c (ms^{-1})	Postdesiccation sound speed, c (ms^{-1})	Δc (m/s)	% change
Porcine skull	13	1727 ± 57	1766 ± 61	39 ± 58	+2.3

n	Predesiccation thickness (mm)	Postdesiccation thickness (mm)	n	Predesiccation thickness (mm)	Postdesiccation thickness (mm)
1	18.0 ± 0.6	18.3 ± 0.7	8	6.1 ± 0.2	5.2 ± 0.1
2	6.4 ± 0.7	5.9 ± 0.3	9	8.3 ± 0.9	8.4 ± 0.8
3	6.5 ± 0.3	7.4 ± 0.2	10	7.8 ± 0.7	7.9 ± 0.9
4	7.1 ± 0.3	7.2 ± 0.5	11	11.0 ± 0.3	11.3 ± 0.6
5	11.4 ± 0.8	10.8 ± 1.2	12	7.0 ± 0.8	6.8 ± 0.4
6	8.3 ± 0.5	7.7 ± 0.3	13	10.3 ± 0.3	10.9 ± 0.8
7	9.8 ± 0.7	8.6 ± 0.7			

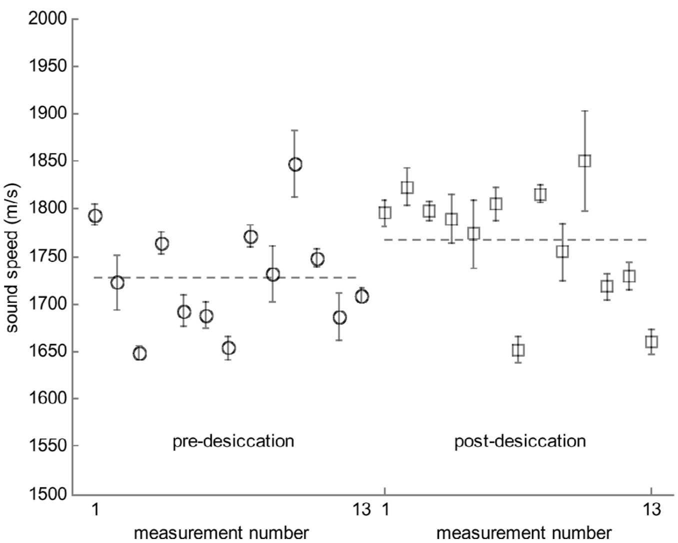

Fig. 3. Sound speed measurements for bulk longitudinal ultrasound propagation in pre- and postdesiccated porcine skull bone. The dashed lines represent the average value for each scenario.

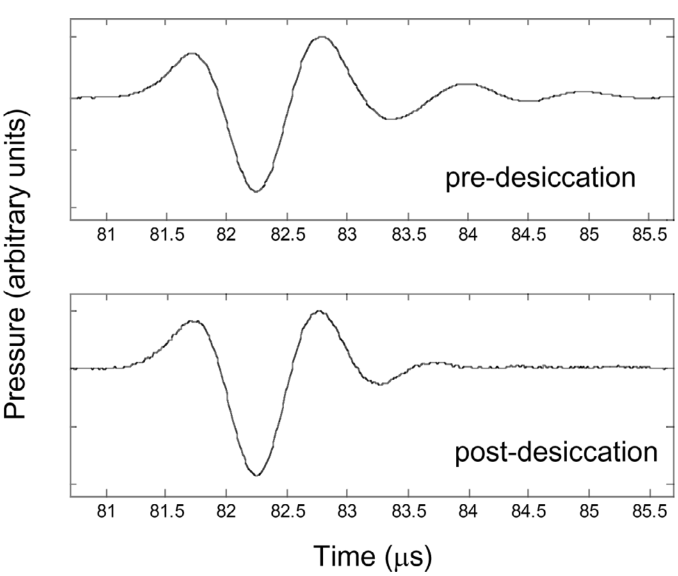

Fig. 4. A representative series of transmitted signals for pre- and postdesiccation *ex vivo* porcine skull specimens.

average increase in sound speed (2.3%) lies within the margins of error for the individual measurements.

Because this study was performed without frequency isolation, future studies could benefit from detailed measurements of desiccation effects on ultrasound transmission with respect to sonication frequency. Future studies also could include the characterization of changes in transmission attenuation and shear mode properties.

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