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A critical assessment of the in-vitro measurement of cortical bone stiffness with ultrasound

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ABSTRACT

Elasticity assessment based on bulk wave velocity (BWV) measurements is the most popular technique to characterize the anisotropic stiffness tensor in cortical bone. Typically, a cuboid bone specimen is cut with its sides along the different anatomical directions. Then, the velocity of shear and longitudinal waves propagating along different directions are assessed, from which stiffness coefficients are calculated. Despite the importance of obtaining accurate elasticity values for bone research, there is no generally accepted protocol to measure BWV and the precision of the technique has been seldom investigated. The purpose of this work is to critically assess the method to measure BWV on cuboid specimens in terms of ultrasound frequency, specimen size and signal processing technique. In this study, we measured polycarbonate specimens of different dimensions and 55 human bone specimens with different transducers using frequencies ranging from 2.25 to 10 MHz and 1–5 MHz for longitudinal and shear waves, respectively. We compared four signal processing methods to detect the wave arrival time. The main results are that, (1) the measurement of shear waves is more complex than that of longitudinal wave, being less precise and more sensitive to sample size; (2) the estimated stiffness depends on the signal processing technique used (up to 10% variation for shear coefficients of bone); and (3) bone stiffness assessed from BWV using the first arrival of the signal to determine the time-of-flight is not different from stiffness assessed using resonant ultrasound spectroscopy (RUS). These results evidence that the measurement method can have an effect on the stiffness values estimates and hence, a well-defined protocol is needed to accurately measure bone stiffness coefficients based on BWV.

1. Introduction

Measurement of bone elastic properties and the understanding of their variations are a key in elucidating the mechanical effects of skeletal pathologies such as osteoporosis. Also, elastic properties are necessary inputs of finite element models of the skeleton for the accurate computation of stresses and strains [30,8].

According to the Christoffel equation, the coefficients of the stiffness tensor of an anisotropic elastic solid can be deduced from the phase velocity of shear and longitudinal ultrasound (US) bulk waves propagating along different material directions. Most of the knowledge on the stiffness of cortical bone, which is an orthotropic or transverse isotropic material [1,25,23,26] has been obtained through the measurement of ultrasonic bulk wave velocity (BWV) ex vivo along different anatomical directions using cuboid specimens. Since the method was introduced to measure bone in the 1960s [16], and until recently, this technique has been applied to assess bone elasticity and relate it to age [19], anatomical location [23,27] or others bone properties such as microstructure [9] and extravascular bone tissue properties [3].

However, in bone studies such as those cited above, the phase velocity is not measured but rather a signal velocity that is used in place of phase velocity to determine the stiffness coefficients from Christoffel equation. The interpretation of this signal velocity is complicated by the modifications of the pulse shape during propagation due to frequency-dependent attenuation, dispersion and possible interference effects related to reflections within the specimen [12,13]. In practice the signal velocity is obtained by determining the first deviation from zero [22], one zero-crossing [12] or a thresholding [21]. In general, these different markers yield different ultrasound velocity values [12].

The possible biases of the method of measurement of BWV for the estimation of cortical bone stiffness have not been thoroughly discussed to the best of our knowledge. It appears that there is no generally accepted protocol to measure BWV in cortical bone in terms of US frequency (frequencies in the range 1–20 MHz have been used), signal processing technique and specimen size

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(specimens of characteristic size between 0.5 and 10 mm have been used) [16,13,1,19]. Furthermore, the signal processing method used to estimate the velocity is rarely specified in the studies.

The aim of this study is to elucidate the effects of the signal processing to measure BWV on cuboid specimens of cortical bone, discuss the choice of transducer frequency and the possible influence of specimen size. Based on BWV measurements of polycarbonate specimens of different sizes and bone specimens, and comparing with stiffness estimated by resonant ultrasound spectroscopy (RUS), we propose guidelines to standardize the BWV measurement of cortical bone stiffness. Section 2 presents the equations used to assess the stiffness tensor from BWV measurements. The methodology is described in Section 3, which contains four subsections. The samples included in this study are described in Section 3.1. Section 3.2 presents the ultrasonic velocity measurements and the RUS method is outlined in Section 3.3. Statistics are presented in Section 3.4. Finally, results are illustrated and discussed in Sections 4 and 5, respectively and concluding remarks end the paper in Section 6.

2. Theory

The stiffness tensor of an orthotropic material has nine independent coefficients. The tensor writes in matrix form using Voigt notation as,

$$C_{ij} = \begin{pmatrix} C_{11} & C_{12} & C_{13} & 0 & 0 & 0 \\ C_{12} & C_{22} & C_{23} & 0 & 0 & 0 \\ C_{13} & C_{23} & C_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & C_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & C_{55} & 0 \\ 0 & 0 & 0 & 0 & 0 & C_{66} \end{pmatrix} \quad (1)$$

According to the Christoffel equation [2], the 6 diagonal terms can be determined by propagating longitudinal and shear waves along the principal material axes (1,2,3),

$$\begin{aligned} &C_{ii} = \rho v_{ii}^2 \quad (i = 1,2,3) \\ &C_{44} = \rho v_{23}^2 = \rho v_{32}^2 \\ &C_{55} = \rho v_{13}^2 = \rho v_{31}^2 \\ &C_{66} = \rho v_{12}^2 = \rho v_{21}^2 \end{aligned} \quad (2)$$

where ρ is the mass density, v_{ii} is the velocity of a longitudinal wave propagating in direction i, and v_{ij} is the velocity of a shear wave propagating in direction i with particle motion in direction j. The off-diagonal terms of the stiffness tensor can be obtained by measuring BWV in directions at a 45 angle from the material axes. However this has rarely been done to measure cortical bone due to the limited size of the samples.

If the material is isotropic, then, its stiffness tensor can be characterized by two constants: $C_{11} = C_{22} = C_{33}$ and $C_{44} = C_{55} = C_{66}$.

3. Method

3.1. Specimens

Eleven polycarbonate (PC) specimens were prepared to investigate the effect of specimen size on BWV. PC was chosen because its acoustic properties (BWV, intrinsic attenuation) are close to those of cortical bone [24,14]. The density of the PC was $\rho = 1.187$ g/cm^3. Specimens of square cross-section of variable side length w ($w \in [4, 111]$ mm) were cut from the same PC plate of thickness 4.85 mm. The direction of ultrasound propagation was along the plate thickness. Note that thickness and lateral dimensions are larger than the US wavelength (see Table 1).

A total of 55 bone specimens were harvested from the left femora of 29 human cadavers. The femurs were provided by the Départment Universitaire d'Anatomie Rockefeller (Lyon, France) through the French program on voluntary corpse donation to science. The tissue donors or their legal guardians provided informed written consent to give their tissue for investigations, in accord with legal clauses stated in the French Code of Public Health. Among the 29 donors, 16 were females and 13 were males (77.83 ± 11.39 years old, mean ± SD). The fresh material was frozen and stored at −20 °C.

The specimens were slowly thawed and then, for each femur, approximately a 10 mm thick cross sectional slab was cut perpendicular to the bone axis at the mid-diaphysis. Then, using a water-cooled low-speed diamond wire saw (Model 3241, Well, Lyon, France), two rectangular parallelepiped shaped specimens were prepared in the lateral and medial anatomical quadrants of each cross section. The nominal specimen size was $3 \times 4 \times 5$ mm^3 in radial (axis 1), circumferential (axis 2) and axial (axis 3) directions, respectively, defined by the anatomic shape of the femoral diaphysis. All specimens were kept hydrated during specimen preparation and between measurements.

3.2. Ultrasonic velocity measurements

BWV was measured with different pairs of matched contact transducers (Table 1), differing both in central frequency and diameter ϕ. Transducers were excited with a pulser with 200 MHz (−3 dB) US bandwidth (Panametrics 5900PR). The received signal was digitized and stored using an acquisition card (Agilent Acquiris DP240) and post-processed with a custom MatLab program (The Mathworks Inc., Natick, MA). For PC specimens, all pairs of transducers were used while for cortical bone, only two pairs were used, namely V105 and V152, for longitudinal and shear waves, respectively. Typical wavelengths in PC associated to each transducer are given in Table 1. For bone, the choice of the transducers was driven by the dimensions of the specimens and by the scale of interest to measure the bone elasticity. That is, at the chosen frequency (2.25 MHz and 1 MHz, for longitudinal and shear waves, respectively), the resulting wavelength ($\sim$ 1.7 mm), which defines the probing scale, guaranteed to retrieve the bone mesoscopic elasticity (i.e. at a scale much larger than the vascular pores) [11].

The BWV (longitudinal or shear) v was calculated as $v = d/\Delta t$, where d is the propagation distance in the specimen, and Δt is a time delay. The latter was calculated as a difference between the time-of-flight (TOF) of the signal propagating through the specimen and that of a reference signal. The propagation distance, d, i.e., the specimen's dimension between the transducers, was measured by a digital calliper (precision ±0.01 mm). In order to minimize the uncertainty of this measurement, four measurements were averaged. Precisely, the measurement were taken after repositioning the calliper on different location of the opposite surfaces to account for possible parallelism defects.

Four different signal processing techniques were used to define the TOF (Fig. 1): (1) the first arrival of the wave defined by a threshold of 5% of the maximum amplitude of the signal; (2) the maximum amplitude of the envelope defined by the Hilbert transform; (3) the first zero-crossing of the signal; and (4) the phase velocity at the central frequency of the transducer [7].

For longitudinal waves, Δt was obtained as the difference between the arrival time of the US pulse (determined using one of the proposed signal processing techniques) with the specimen inserted in between the transducers and the arrival time of a refer-