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Resonant ultrasound spectroscopy measurements of the elastic constants of human dentin ☆

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Abstract

The technique of resonant ultrasound spectroscopy (RUS) was used to measure the second-order elastic constants of hydrated human dentin. Specimens were placed between two transducers, and the resonant frequencies of vibration were measured between 0.5 and 1.4 MHz. The elastic constants determined from the measured resonant frequencies in hydrated dentin exhibited slight hexagonal anisotropy, with the stiffest direction being perpendicular to the axis of the tubules ($E_{11} = 25.1$ GPa). This hexagonal anisotropy was small ($E_{33}/E_{11} = 0.92$), and almost disappeared when the specimens were dried. In addition, there was a pronounced anisotropy in the Poisson's ratio of wet dentin: $\nu_{21} = 0.45$; $\nu_{31} = 0.29$. With drying in air, this anisotropy vanished: $\nu_{21} = \nu_{31} = 0.29$. The isotropic Young's modulus of dried dentin was 28.1 GPa. RUS shows promise for determining the elastic constants in mineralized tissues.

Keywords: Dentin; Calcified tissues; Mechanical properties; Resonant ultrasound spectroscopy; Elastic constants

1. Introduction

Dentin, which is similar in composition to bone, is the most abundant mineralized tissue in the human tooth. It has a characteristic microstructure consisting of a hydrated intertubular matrix of type I collagen that is reinforced with a nanocrystalline, carbonated apatite mineral phase. This matrix is perforated by nearly cylindrical tubules that run from the dentin–enamel junction to the pulp chamber. The tubule lumens, which are roughly 1 μm in diameter, are surrounded or lined by a hypermineralized cylinder of peritubular dentin. This peritubular cuff is approximately 0.5–1.0 μm in thickness (Marshall et al., 1993, 1997). The mineralized collagen fibrils in the intertubular dentin matrix form a felt-like structure, lying preferentially in the plane perpendicular to the axis of the tubules (Jones and Boyde, 1984). This pronounced structural anisotropy is believed to produce a corresponding anisotropy in the mechanical properties (Palamara et al., 2000).

An accurate knowledge of the elastic properties of dentin is critical for developing new approaches to conservative and restorative dentistry. Yet, in spite of this importance, half a century of research has failed to yield consistent values for any of its elastic properties. There have been three-fold discrepancies in the reported magnitude of Young's modulus in the past few years (Huo et al., 2000; Kishen et al., 2000; Palamara et al., 2000). These discrepancies have often been attributed to the difficulties of making accurate mechanical tests on small specimens: uniform stress states within the gauge region of the specimens are probably never realized in practice. Sonic measurements of sound speed are also difficult to perform, and they are often criticized because of the high strain rates and their restriction to measurements of longitudinal or shear wave velocities along a single orientation (Waters, 1980). Reconstruction of the complete stiffness matrix usually relies on the assumption of isotropic symmetry (Gilmore et al., 1970).

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Resonant ultrasound spectroscopy (RUS) is a method for measuring single crystal elastic constants with great precision (Migliori et al., 1993). Since its development, its range of application has been extended to include geological structures and complex particulate and fiber-reinforced composites (Schwarz and Vuorinen, 2000). However, we are unaware of any attempts to apply this method to a mineralized tissue. The present study was intended to: (a) test the feasibility of performing RUS on dentin; (b) obtain more accurate values of the elastic constants of dentin; and (c) test the hypothesis that structural anisotropy of dentin produces a corresponding anisotropy in the elastic constants.

2. Materials and methods

Unlike the measurement of sound speed, RUS makes use of Hooke's law and Newton's second law to predict the resonant modes of mechanical vibration of a specimen of known shape (Maynard, 1996; Migliori et al., 1993)

$$C_{ijkl}\frac{\partial^2 u_k}{\partial x_j \partial x_l} = \rho\frac{\partial^2 u_i}{\partial t^2}. \quad (1)$$

In Eq. (1), C_{ijkl} are the elastic constants and ρ is the density of the specimen. The entire C_{ijkl} tensor can be determined by comparing the frequency spectrum produced by the resulting eigenvalue problem (Eq. (1)) with the measured resonant frequencies (vibrational eigenmodes) of the specimen.

Three unerupted human third molars were used in this study. Cubes, approximately 2 mm on an edge, were removed from the mid-coronal dentin with a slow speed diamond saw; tubules were oriented at right angles to the top and bottom surfaces of the cube. The specimens were dried, placed between right angle polishing blocks, and the opposing surfaces were polished flat and parallel. The edges of the specimens were polished to different lengths to avoid a cubic geometry and better separate the resonant peaks; a typical final dimension was $1.7 \times 1.1 \times 0.8\,\text{mm}^3$. The approximate 2:1 aspect ratio was chosen to increase the sensitivity to detecting anisotropy in low-Q specimens (Ulrich et al., 2002).

Three days prior to scanning, the specimens were rehydrated in deionized water and mounted on opposing corners between two transducers in the RUS system (Fig. 1). The transducers were thin foils of PVDF coated with 300 nm of gold. The resonant frequencies between 0.5 and 1.4 MHz were measured. An approximation of the stiffness tensor was used to generate initial values for the resonant frequencies using Eq. (1). The experimental and predicted frequency distributions then were compared, and the residuals, F, were determined from (Migliori et al., 1993):

$$F = \sum_i w_i (f_i^* - f)^2. \quad (2)$$

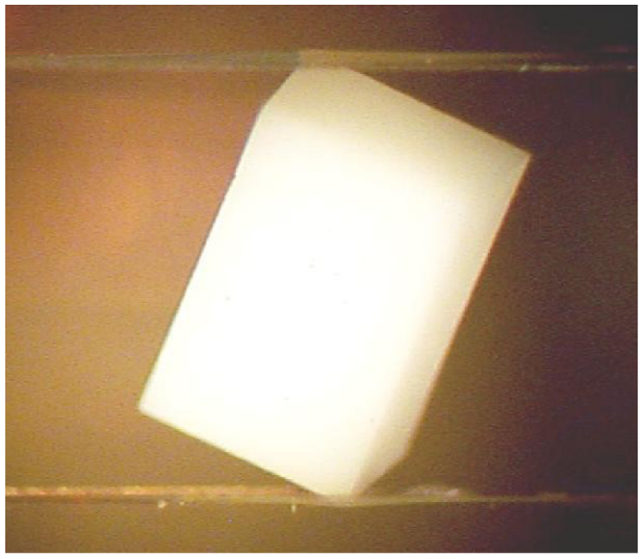

Fig. 1. A photograph showing a dentin specimen (approximately 2-mm in length) mounted between the transducers in the RUS assembly. From a measurement of the vibrational spectrum in this single orientation, it is possible to derive all of the second-order elastic constants of the specimen.

In Eq. (2), w_i was the weighting based on goodness of fit given to peak i, and f_i^* and f_i were the predicted and measured ith resonances, respectively. The elastic constants were adjusted from their initial values to minimize Eq. (2). A range of possible material symmetry groups, such as isotropic, cubic, and hexagonal (transverse isotropic), was modeled.

After performing the first RUS scans on the hydrated specimens, the air inside the transducer assembly was evacuated by pumping. The RUS scans were repeated every 15 min during pumping for 9 h in order to study the change in the elastic constants with drying.

3. Results

A typical RUS spectrum for a dentin specimen is shown in Fig. 2. In greater detail in the inset in Fig. 2 is a small region of the spectrum, near 1.1 MHz, showing the data fitted with a Lorentzian curve (superposed smooth curve) including an arbitrary phase factor. The elastic constants determined from peak fits to these spectra are listed in Table 1 for both the isotropic and hexagonal symmetry models. Deviations from isotropic symmetry were less than 10%. The angular deviation of the Young's modulus with respect to the axis of the dentinal tubules is charted in Fig. 3. The angular deviation was calculated from the RUS-measured compliance matrix using the well known formulas derived in Nye (1972). The maximum in the Young's modulus was in the direction perpendicular to the tubules, and in the plane of the mineralized collagen fibrils.

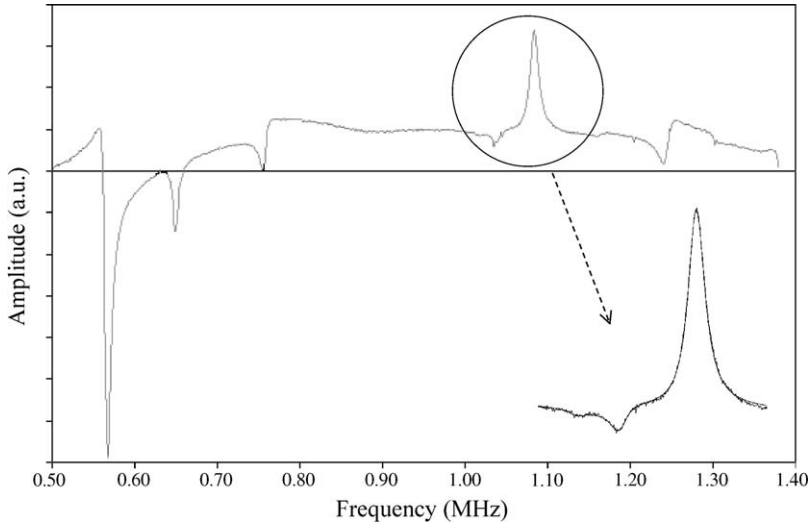

Fig. 2. A RUS scan of a dentin specimen showing the resonances between 0.5 and 1.4 MHz. The region of the spectrum near 1.1 MHz is shown in expanded detail below, along with the curve fit (smooth line). An hexagonal symmetry model provided excellent fits to all of the features of the spectrum.

Table 1
RUS results for dry and wet human dentin as determined for both isotropic and hexagonal symmetry models

Condition	Symmetry	C_{11}	C_{33}	C_{12}	C_{13}	C_{44}	ν	E	G
Dry	Isotropic	36.5	—	14.5	—	—	0.28	28.3	11.0
Dry	Hexagonal	36.7	36.5	14.7	15.1	11.1	a_d	b_d	c_d
Wet	Isotropic	66.11	—	49.01	—	—	0.43	24.4	8.6
Wet	Hexagonal	42.6	34.6	25.4	19.7	9.4	a_w	b_w	c_w
Gilmore et al. (1970)	Isotropic							19–29	7–11

Units are GPa with the exception of the Poisson's ratio (v), which is dimensionless. E is the Young's modulus and G is the shear modulus. The standard deviation was less than 5% in all cases. For hexagonal symmetry, the Poisson's ratio, and E and G depend on the direction. The (11) direction is perpendicular to the tubule axis, and the (33) direction is along the tubule axis. [a_d: $v_{21} = v_{31} = 0.29$; b_d: $E_{11} = 28.1$, $E_{33} = 27.6$; c_d: $G_{23} = 11.1$, $G_{12} = 11.0$]. [a_w: $v_{21} = 0.45$, $v_{31} = 0.29$; b_w: $E_{11} = 25.0$, $E_{33} = 23.2$; c_w: $G_{23} = 9.4$, $G_{12} = 8.6$]. For comparison purposes, we include the range of values from Gilmore et al. (1970) based on an isotropic fit to sound speed measurements in bovine dentin.

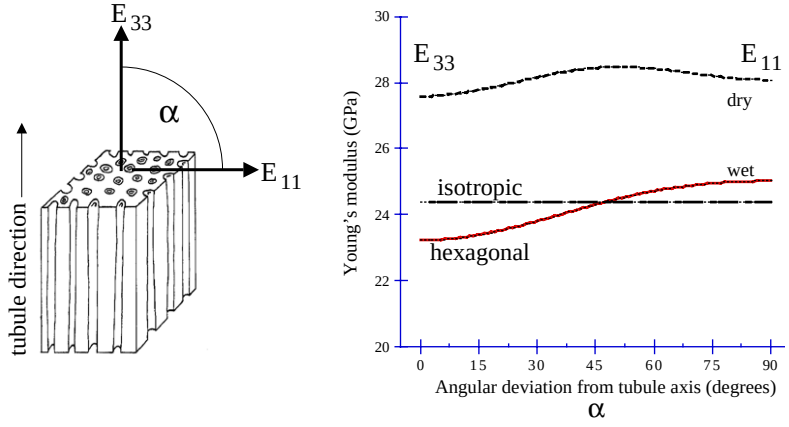

Fig. 3. The angular dependence of the Young's modulus for wet and dry dentin specimens measured in degrees from the tubule axis. The best fit to an isotropic model is also included for wet dentin. The anisotropy is about ±5% of the magnitude of the isotropic value. The magnitude of the Young's modulus in dry dentin is about 4 GPa greater than in wet dentin. Furthermore, much of the anisotropy seen in the wet dentin has been removed by drying.

There was considerable anisotropy in the Poisson's ratio in wet dentin: $v_{21} = 0.45$, $v_{31} = 0.29$. With drying in air, this anisotropy disappeared: $v_{21} = v_{31} = 0.29$. At the same time, the Young's modulus increased, as expected (Balooch et al., 1998), by about 4 GPa.

4. Discussion

The elastic properties of a material are completely determined by the elements of its elastic tensor, which relate stress to strain. While elastic constants may be determined with static measurements, a better method is to use an elastic vibration, as in a sound wave, where the elastic constants are related to various speeds of propagation. A complication is that the elastic tensor has off-diagonal elements, so that sound propagation in an arbitrary direction may be quite complicated. The conventional method for alleviating this complication is to use a sample with a face perpendicular to one of the principal axes of the elastic tensor; in this case, three relatively straightforward plane wave sound modes may be generated, and their speed of propagation may be used to determine three elastic constants. If there were more than three independent elastic constants, it would be necessary to re-orient the sample, re-attach transducers, and measure again.

Unfortunately, the plane wave method requires, in theory, a semi-infinite sample. The boundaries of a finite-size sample couple to modes other than the three plane wave modes, so that the sound speeds do not exactly represent three elastic constants. A new method of measuring elastic constants, RUS, turns the finite-size sample complication into an advantage. Rather than trying to force the propagation of a particular plane wave mode, the RUS method lets the sample vibrate as it wants, in the normal modes for that particular sample. Even for a simple shape, like the rectangular parallelepipeds used in this study, these normal modes may be quite complicated and result in a non-trivial spectrum of natural frequencies. But the RUS technique uses this to advantage as well. Because of the complicated nature of the normal modes, a set of values for a reasonable number of natural frequencies (typically a dozen or more) contains complete, indeed redundant, information that may be used to uniquely determine all of the elastic constants. In the past, analyzing a set of frequencies to determine the elastic constants required prohibitive computer resources. But with current desktop computers, the entire process including measuring the natural frequencies and determining all of the elastic constants may be accomplished in tens of minutes.

The major purpose of this study was to determine if dentin could be made to resonate. Indeed, the major conclusion of this study is confirmation that RUS is well suited for measuring the continuum elastic constants of dentin. Though surface acoustic microscopy (SAM) could, in principal, provide elastic properties in plane, SAM probes a length scale that is small with respect to the heterogeneities in the dentin microstructure. Continuum properties would then have to be inferred by micromechanics arguments.

Historically, the collagen and mineral phases have been the principal focus of biomechanical investigations of mineralized tissues (Currey, 1999; Mann and Weiner, 1999; Weiner et al., 1999). However, the presence of tubule lumens and their associated peritubular cuffs complicate dentin, and many investigators have focused on the tubules rather than the mineralized collagen fibrils as a potential source of elastic anisotropy. In a recent study, we used a micromechanics approach to rule out any significant contribution of the tubules, and inferred instead that the intertubular dentin matrix would control the symmetry of the continuum elastic constants (Kinney et al., 1999).

Our finding of a slight hexagonal anisotropy, with the stiffest direction perpendicular to the tubules, was consistent with the micromechanics model and the planar organization of the mineralized collagen fibrils. This hexagonal anisotropy appeared to vanish when the dentin was dried, perhaps explaining why previous studies with dry dentin did not detect anisotropic behavior in either the contact stiffness (Kinney et al., 1999) or microhardness (Wang and Weiner, 1998).

The magnitudes of the elastic constants determined with RUS are in good agreement with values determined from sound speed measurements in bovine dentin (Gilmore et al., 1970), and indentation measurements on human dentin. In the sound speed measurements, based on an isotropic symmetry model, the Young's modulus ranged from 19 to 29 GPa, and the shear modulus ranged from 7.2 to 10.8 GPa. Our RUS measurements lay midrange of these values for both isotropic and hexagonal symmetry models. In addition, when atomic force microscope (AFM) indentation data on wet human dentin are corrected for creep compliance, an isotropic reduced Young's modulus in the range of 25 GPa is obtained (Kinney et al., 2003). Thus, we are confident that the RUS technique provides a reliable determination of the elastic constants in dentin.

The present study only considered dentin from a single location in the crown. It is possible that other locations, such as root dentin where the collagen fibers are more highly oriented, might exhibit greater or lesser degrees of anisotropy. More study is necessary. RUS is a promising new technique for exploring the biomechanical consequences of age and disease-related changes in dentin.

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