EXPERIMENTAL INVESTIGATION OF HUMAN TEETH *IN VITRO* USING ULTRASOUND AND X-RAY DIFFRACTION

H. Gawda, L. Sekowski, H. Trebacz,
Department of Biophysics, Medical University of Lublin, Lublin, POLAND
trebacz@panaceum.am.lublin.pl

Abstract

The aim of this work was to find if ultrasound velocity along a complete tooth reflects variety of teeth morphology and properties and to compare the results of ultrasonic measurements with X-ray diffraction data from a large area of tooth. 100kHz pulses were transmitted in longitudinal direction of extracted teeth. A significant variation of velocity in teeth from different donors was stated (3200m/s to 4200m/s). Velocity correlated with age of donors. For a given individual, the greatest difference was between incisors and canine teeth. Considering X-ray diffraction data, a significant difference in size of crystallites between teeth was stated. In enamel the size of crystallites was 20nm - 50nm and in dentin 5.5nm - 39nm. Crystallites size was positively correlated with velocity of ultrasound. The velocity was also influenced by the preferred orientation of crystallites in tooth; the highest velocity was found in teeth with crystallites directed along tooth axis.

Introduction

The mechanical properties of tooth tissues are of considerable interest in the field of dental research. Understanding the physical properties of teeth and their constituents, both enamel and dentine is of particular importance to clinical tooth preparation and for selection of filling materials. Scientific interest is focused also on application of acoustic methods for dental diagnosis as one of the most attractive practical purposes. Before any clinical application, it is necessary to accumulate a detailed knowledge on various aspects of acoustic properties of tooth tissues and their relationships with histology and microstructure of tooth.

Enamel and dentin, two main dental tissues differ greatly in morphology and properties [1,2]. Enamel, the hard tissue that surrounds tooth crown, contains around 96% (by weight) of mineral in the form of impure hydroxyapatite (HAP). HAP crystals are hexagons or flattened hexagons that in enamel are around 40 - 70 nm across and up to 1000 nm in length. The crystals are packed into prisms with crystallographic c-axes preferentially parallel to the prism axis. Dentin, the mineralized hard tissue that surrounds the pulp in teeth is a composite of type I collagen and an apatitic mineral phase. The mineral content in dentin is about 60-70%. The c-axis of crystallites in dentin, as in other mature hard tissues, is distributed along extended collagen fibers. Orientation of prisms in enamel and collagen fibers in dentine in respect to tooth axis as well as size and texture of crystallites depends on location in the tooth, kind of the tooth and maturity of the tissue [1-5].

The aim of this work was to find if ultrasound velocity measured along a complete tooth reflects variety of teeth morphology and properties and to compare the results of ultrasonic measurements with X-ray diffraction data from a relatively large area of tooth enamel and dentin.

Material and Methods

Ultrasonic measurements were performed on 112 human teeth from donors ranging in age from 19 to 82 years. Incisors, canine and premolar teeth extracted from mandible were used. Teeth with any signs of caries were excluded. The length of teeth was 20 mm to 23.5 mm and the diameter was 5 to 8 mm. The bottom and the top ends of each tooth were polished slightly to obtain flat surfaces that enable a good contact with ultrasonic transducers. An ultrasound pulse of 100 kHz frequency was transmitted through the whole tooth, along its longitudinal axis. The time of fly between sending and receiving the signal was measured using standard equipment. The mean velocity of the wave front along the tooth was calculated.

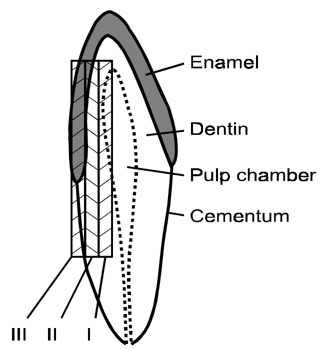

Figure 1. A scheme of a tooth intersection;
I, II, III – section used in X-ray diffraction analysis.

After ultrasonic measurements seven teeth from two donors of similar age but with different velocity of ultrasound were chosen for X-ray diffraction. The teeth were cut along the longitudinal axis in buccal-lingual direction. Three one-millimeter-wide longitudinal sections were marked in the buccal halve of the intersection. (Fig. 1). Sections I and II included mainly dentin while section III contained enamel, dentin-enamel junction and cementum. X-ray diffraction patterns of each section were obtained using a scanning method using DRON-3 apparatus (Russia) with Cu lamp. A Ni filter and a linear amplifier with high impulse analyzer were applied, in order to obtain sufficiently monochromatic radiation. Each sample was scanned from 2θ =10° to 2θ =60° with a 0.02° step size. A counting speed was 20 seconds per one step.

The X-ray diffraction patterns of the reflections (002) and (310) were used to evaluate the crystallite sizes. D values, which are related to the crystal size in the long dimension (h) and the mean dimension in the cross-section (d) of the HAP crystals, were calculated from the width at half-maximum intensity using the Scherrer equation

$$D= K\lambda/\beta_{1/2} \cos\theta$$

Where λ is the x-ray wavelength, θ is the diffraction angle and K is a constant depending on type of crystal; for apatites chosen as 0.9.

Results

Ultrasound

Velocity of ultrasonic pulses in teeth examined in this experiment ranged from 3200m/s to 4200m/s. The velocity was influenced both by age of donors and a type of the tooth (Fig. 2).

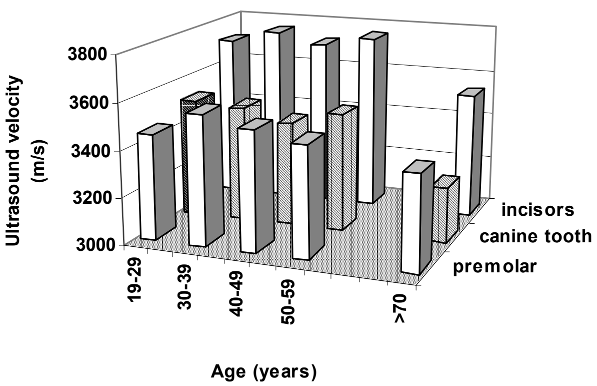

Figure 2. Velocity of ultrasound in human teeth in different age groups.

In the Figure 2 each bar represents a mean value from a group of 3 to 12 samples. Error bars are not shown for transparency but standard deviation (S.D.) did not exceed 120m/s in any group. The highest velocity was found in incisors of adult persons (V = 3750 m/s; S.D. = 75m/s) and the lowest in canine teeth of elderly people (V = 3406m/s; S.D. = 89 m/s). Ultrasound velocity in premolar decreased gradually from the fifth decade of life, while in canine teeth and incisors velocity was stable in adult person and significantly decreased in elderly. For a given individual, the greatest difference was found between incisors and canine teeth. The ultrasound velocities in canine teeth and premolar were similar with the exception of the elderly group. Velocity in canine teeth in this age group was significantly lower than in all other groups.

X-ray diffraction

An example of diffraction patterns obtained from dentin and enamel of an incisor of thirty years old person is presented in Figure 3.

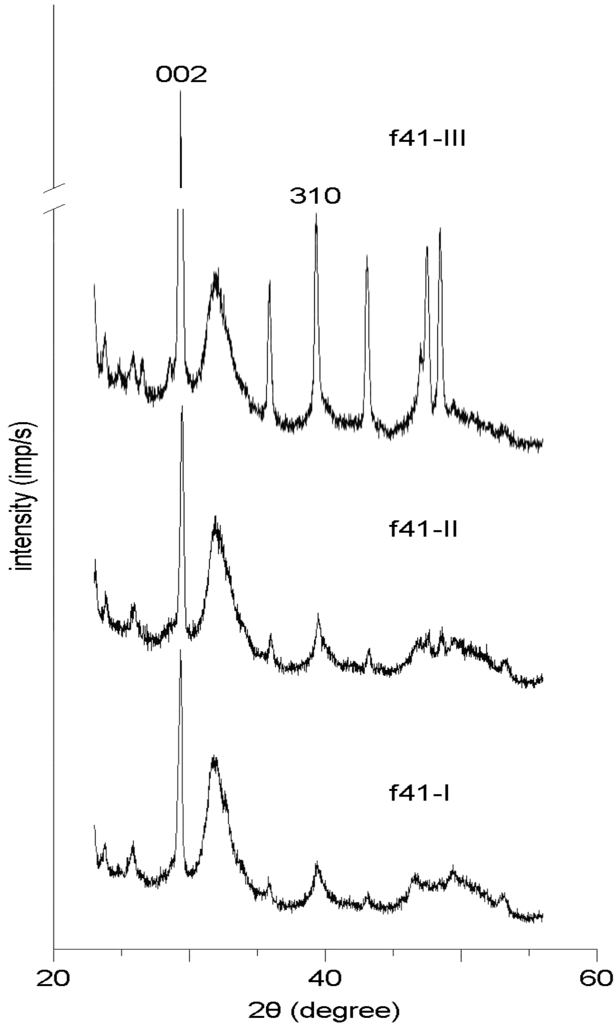

Figure 3. An examples of a set of diffraction patterns obtained from an incisor of a 30 years old women; I – inner layer of dentin, II – a layer of dentin closer to enamel, III – enamel.

X-ray diffraction patterns from sections I and II displays broad reflections and week counting statistics because small size of dentine apatite crystals implies a large surface to mass ratio, and therefore a high proportion of structurally distorted or destroyed lattice units [2]. The second reason of peaks broadening is a significant organic fraction in dentin. In the section III including mainly enamel with larger crystallites and negligible organic fraction peaks are much more distinct and sharper.

The values of crystallite length and width calculated from diffraction peaks from different sections are listed in Table 1. Both dimensions are averaged over all teeth examined. From the Table 1 few conclusions can be formulated:

- size of HAP plates raises from the tooth axis to the surface
- size of crystallites along c-axis of HAP lattice (002) is significantly larger than the mean size in a-plane (310)
- crystallites in dentine seem to be more elongated than in enamel

Table 1: Crystal size of tooth apatites; sections I and II contain dentin and section III contains enamel and dentin-enamel junction; mean values (S.D.)

section	I	II	III
h (002) (nm)	23.9 (13.0)	30.5 (7.6)	45.4 (5.2)
d (310) (nm)	6.6 (1.2)	8.7 (3.0)	26.0 (4.4)

Ultrasound versus x-ray diffraction

The next step of the analysis of data obtained in this experiment was an attempt to find if velocity of ultrasonic pulses passing through teeth was influenced by crystallite size in dentin and in enamel. To obtain samples that cover a broader range of ultrasound velocity both incisors and canine teeth were used. Premolars were excluded from that analysis as orientation of structural elements in these teeth much greatly differs from incisors than canine teeth [1].

In Figures 4 and 5 the mean size of crystallites in two main crystallographic direction of HAP crystals in each of teeth studied is plotted against velocity of ultrasonic pulses along that tooth. As the differences in crystallite size between both sections of dentin (I and II) were not significant (Table 1), they were analyzed together.

Results of Pearsons analysis (Table 2) shows that despite a small number of samples studied correlation between velocity and crystallite size is significant. In (310) crystallographic direction correlation is high both in dentin and averaged over dentin and enamel. In the direction (002) the correlation is significant only after averaging crystallite.

Table 2: Pearsons coefficient of correlation between ultrasound velocity in a tooth and average crystallite size in dentin and enamel (Mean) and crystallite size in dentin (Dentin).

Mean h (002)	Mean d (310)	Dentin h (002)	Dentin d (310)
0.787	0.831	0.601	0.968

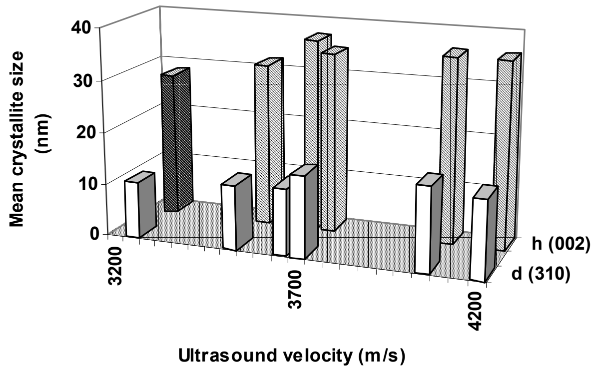

Figure 4. Average size of crystallite in dentin and enamel versus velocity of ultrasound along tooth axis; h(002) - size in the direction of c-axis of HAP lattice, d(310). - size in the direction of a-axis of HAP lattice.

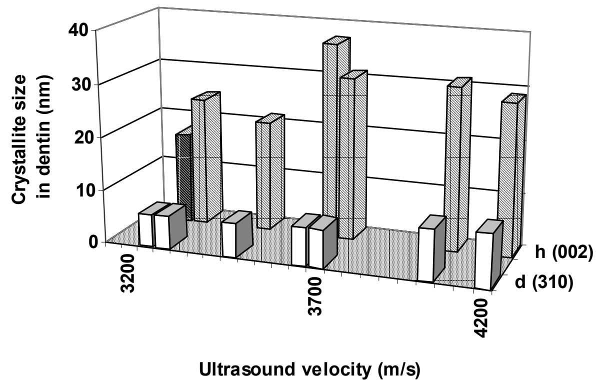

Figure 5. Average size of crystallites in dentin versus velocity of ultrasound along tooth axis;; h(002) - size in the direction of c-axis of HAP lattice, d(310). - size in the direction of a-axis of HAP lattice.

Discussion

Results of ultrasonic measurements indicating large differences between teeth reflects the correlation between teeth physical properties and their composition and structure reported from investigations on teeth and dental pieces using mechanical testing, other ultrasonic measurements and theoretical considerations [4-12]. The mechanical properties of dentin were reported to be largely determined by the intertubular dentin matrix - a complex composite of type I collagen fibers and apatite crystals of different orientation [3,9]. Elasticity and hardness were found to be a function of the microstructural texture. From ultrasonic measurements elastic properties of enamel were found to be depth dependent [4]. From FEM consideration stiffness of bone tissue is dependent on chemical composition and crystal orientation [12]. Anisotropy in mechanical properties of bone was explained by considering axial distribution and orientation of mineral [5]. Differences between enamel and dentin were also proved in ultrasonic and acoustic microscope measurements [4,8,10]

The results obtained in our experiment considering to crystallite size in dentin are consistent with data obtained by others [1,3]. The change of shape of crystallites depending on the location in a tooth was also reported [3]. Size of crystallites in enamel obtained in this study is not as large as usually reported. That result reflects the fact that enamel is not uniform in depth and that 1mm wide X-ray beam in section III included also dentin-enamel junction and cementum, where crystallites are smaller [1].

Considering that crystallite size in calcified tissues have been shown to increase with tissue maturation [2] the decreased velocity of ultrasound in advanced age found in our study should be attributed rather to deterioration of tissue than to smaller crystallite size.

From the X-ray diffraction data presented here it is impossible to establish orientation of crystallites. One can suppose that there are differences between HAP orientation between teeth included in the study because no systematic dependence between with and intensity of a peak was found.

On the basis of obtained results we conclude that velocity of ultrasound in teeth is positively correlated with crystallites size. The velocity is also supposed to be influenced by the preferred orientation of crystallites in tooth. In teeth with similar crystallites size, the higher velocity was found in incisors, where crystallites are more uniformly organized in respect to the tooth axis then in canine teeth [1].Our study is only preliminary and establishing the more univocal and quantitative relationship needs much larger number of data.

References

[1] F.H. Jones, “Teeth and bones: applications of surface science to dental materials and related biomaterials, “ Surface Science Reports, vol. 42, pp.75-205, 2001.

[2] R.G. Handschin, W.B. Stern, “X-ray diffraction studies on the lattice perfection of human bone apatite,” Bone, vol. 16(4, Suppl.), pp.355S-363S, 1995.

[3] J.H. Kinney, J.A. Pople, G.W. Marshall, “Collagen orientation and crystallite size in human dentin: a small angle x-ray scattering study,” Calcif. Tissue Int, vol. 69, pp.31-37, 2001.

[4] S. Lees, F.R. Rollins, “Anisotropy in hard dental tissues,” J. Biomechanics, vol.5, pp.557-566, 1972.

[5] N. Sasaki, N. Matsushima, T. Ikawa, H. Yamamura, A. Fukuda, "Orientation of bone mineral and its role in the anisotropic mechananical properties of bone - transverse anisotropy," J Biomechanics, vol.22(2), pp.157-164, 1989.

[6] B. Al-Navas, K.A. Grotz, E. Rose, H. Duschner, P. Kann, W. Wagner, "Using ultrasound transmission velocity to analyse the mechanical properties of teeth after in vitro, in situ, and in vivo irradiation," Clin Oral Invest, vol.4, pp.168-172, 2000.

[7] M. Finke, J.A. Hughes, D.D. Parker, K.D. Jandt, “Mechanical properties of in situ dmineralised human enamel measured by AFM nanoindentation,” Surface Science, vol. 491, pp.456-467, 2001.

[8] T.N. Gardner, J.C. Eliott, Z.Sklar, G.A. Briggs, "Acoustic microscope study of the elastic properties of flourapatite and hydroxyapatite, tooth enamel and bone," J Biomechanics, vol.11, pp. 1265-1277, 1992.

[9] W.J.Landis, "The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix," Bone, vol. 16, pp.533-544, 1995.

[10] R.G. Maev, L.A. Denisowa, E.Y. Maeva, A.A. Denissov, “New data on histology and physico-mechanical properties of human tooth tissue obtained with acoustic microscopy,” Ultrasound in Med. & Biol., vol. 28(1), pp. 131-136, 2002.

[11] F. Pavolo, E.B. Hermida, "Measurement of the elastic modulus of dental pieces," Journal of Alloys and Compounds, vol. 310, pp. 392-395, 2000.

[12] I.R. Spears, “A three dimensional finite element model of prismatic enamel: a re-appraisal of the data on the Young’s modulus of enamel,” J. Dent. Res., vol. 76(10), pp.1690-1697, 1997.