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In vitro examination of human teeth using ultrasound and X-ray diffraction

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The aim of this paper was to find whether an ultrasound velocity along a whole tooth reflects variety of its morphology and properties and to compare the results of ultrasonic measurements with X-ray diffraction data from a large area of tooth. 100 kHz pulses were transmitted in longitudinal direction of extracted teeth. A significant variation of velocity in teeth from different donors was stated (from 3200 m/s to 4200 m/s). The velocity was influenced both by the age of donors and the type of a tooth. For a given individual, the greatest difference was revealed between incisors and canine teeth. Considering X-ray diffraction results, a difference in size of crystallites between teeth was found. In an enamel, the size of crystallites ranged from 20 nm to 50 nm, and in dentin – from 5.5 nm to 39 nm. The size of crystallites in dentin was positively correlated with the ultrasound velocity.

Key words: human teeth, X-ray diffraction, ultrasonic measurements

1. Introduction

Enamel and dentin, two main dental tissues, differ greatly in both morphology and properties [1], [2]. Enamel, the hard tissue that surrounds tooth crown, contains ca. 96% (by weight) of mineral in the form of impure hydroxyapatite (HAP). HAP consists of hexagonal crystals or flattened hexagonal crystals whose symmetry line ranges between 30 nm and 70 nm, and the length is up to 1000 nm. These dimensions vary greatly with the distance from the enamel surface. 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 apatite mineral phase. The mineral content in dentin approaches 60–70% and consists of crystallites whose size is approximately of the order of magnitude less compared to enamel crystallites. 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 of tooth axis as well as the

size and texture of crystallites depend on their location in the tooth, kind of the tooth and maturity of the tissue [1]–[5].

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

The aim of this work was to find whether an ultrasound velocity measured along a whole 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.

2. 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 examined. Teeth with any signs of caries were excluded. The length of teeth varied from 20 mm to 23.5 mm and their diameter from 5 mm to 8 mm. The bottom and the top ends of each tooth were polished slightly to obtain flat surfaces that enable their good contact with ultrasonic transducers. An ultrasound transmission method was applied using a Tester CT-1 (Unipan – Ultrasonic, Poland), (figure 1). The pulses of 100 kHz ultrasonic waves were sent from one transducer, transmitted through the whole tooth along its longitudinal axis and detected by a receiving transducer. The time between sending and receiving the signal was measured by the tester with an accuracy of 0.1 μs. The mean velocity of the wave front along the tooth was calculated.

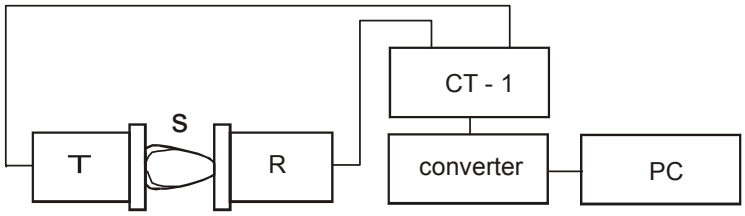

Fig. 1. Experimental set-up for ultrasonic measurements: T, R – ultrasonic transducers: tramsmitter and receiver, CT-1 – ultrasonic tester, PC – computer, s – sample

After ultrasonic measurements three incisors and four canine teeth from 30-year old and 39-year old donors were chosen for X-ray diffraction. The teeth were cut

along the longitudinal axis in buccolingual direction. Three one-millimeter-wide longitudinal sections were marked in the buccal halve of the intersection (figure 2). 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 by means of 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 $2\theta = 10^{o}$ to $2\theta = 60^{o}$ with a 0.02^{o} step size. A counting rate was 20 seconds per one step.

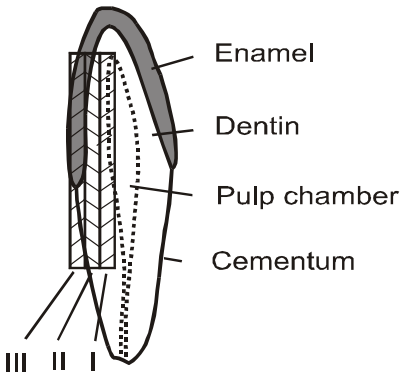

Fig. 2. A scheme of a tooth intersection;
I, II, III – sections used in X-ray diffraction analysis

The X-ray diffraction patterns of the reflections (002) and (310) were used to evaluate the crystallite size in the long dimension and the mean size in the plane of cross section [2]. The size D was calculated from the width at the half-maximum intensity $\beta_{1/2}$ 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.

3. Results

3.1. Ultrasound

Velocity of ultrasonic pulses in teeth examined in this experiment ranged from 3200 m/s to 4200 m/s. The velocity was influenced both by age of donors and a type of the tooth (figure 3).

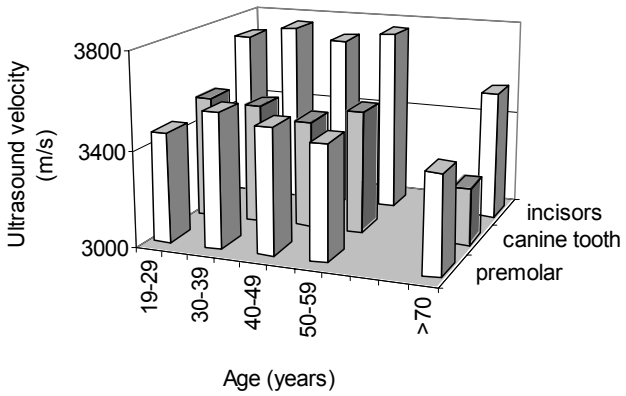

Fig. 3. Velocity of ultrasound in human teeth in different age groups

In figure 3, each bar represents a mean value from a group of 3–12 samples. Error bars are not shown for the sake of transparency but standard deviation (*SD*) did not exceed 120 m/s in any group. An average velocity assumed the highest value in incisors of adult persons (V = 3782 m/s; SD = 118 m/s), and the lowest in canine teeth of elderly people (V = 3406 m/s; SD = 89 m/s). Ultrasound velocity in premolar decreased gradually from the fifth decade of life, while in canine teeth and incisors this velocity was stable in adult persons 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 premolars 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.

3.2. X-ray diffraction

An example of diffraction patterns obtained from dentin and enamel of an incisor of a thirty-year old person is presented in figure 4. X-ray diffraction patterns from sections I and II display broad reflections and weak 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 section III including mainly enamel with larger crystallites and negligible organic fraction, peaks are much more distinct and sharper.

Length and width of crystallites in sections I–III of all teeth examined were calculated from diffraction peaks. Mean length h and mean dimension d in perpendicular plane in particular sections and averaged over all sections are given in table 1. From the table few conclusions can be formulated:

- the size of HAP plates increases from the tooth axis to the surface,

- the size of crystallites along the *c*-axis of HAP lattice (002) is significantly bigger than a mean size in *a*-plane, perpendicular to *c*-axis (310),
- crystallites in dentine seem to be more elongated than in enamel.

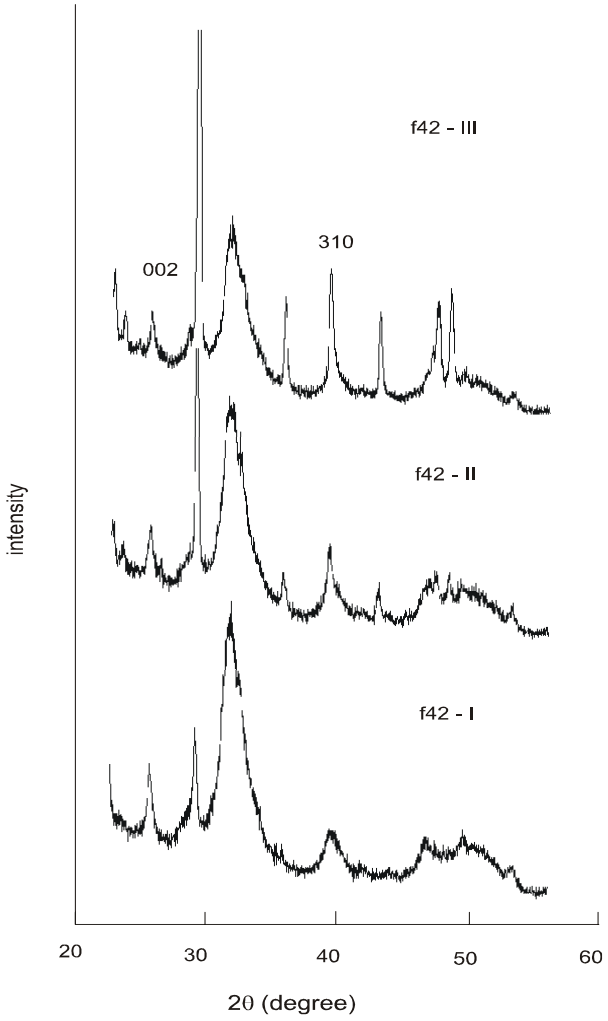

Fig. 4. An example of a set of diffraction patterns obtained from an incisor of a 30-year old woman: I – inner layer of dentin, II – a layer of dentin close to enamel, III – enamel

3.3. Ultrasound versus X-ray diffraction

The next step of the analysis of the data obtained in this experiment was an attempt to find whether velocity of ultrasonic pulses passing through teeth was influenced by crystallite size in dentin and in enamel. To obtain the samples that cover a broader

range of ultrasound velocity both incisors and canine teeth were used (see figure 3). Premolars were excluded from that analysis as the difference in the orientation of structural elements in these teeth and incisors is much greater compared to that observed in premolars and canine teeth [1].

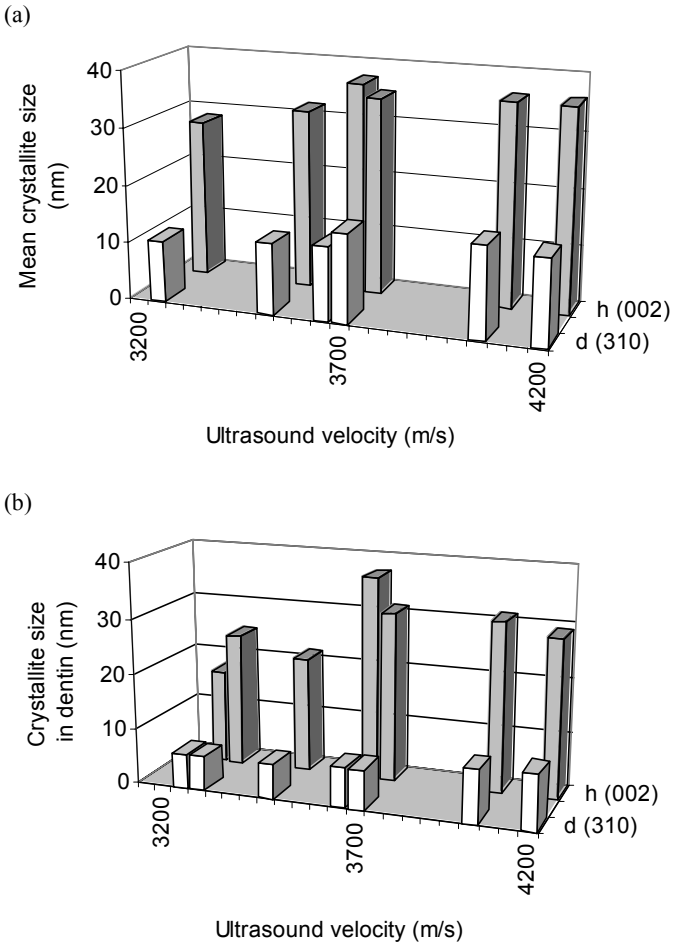

Fig. 5. Average size of crystallites versus velocity of ultrasound along tooth axis: $h_{(002)}$ – size in the direction of *c*-axis of HAP lattice, $d_{(310)}$ – average size in *a*–*b* plane of HAP lattice; (a) in dentin and enamel (sections I–III), (b) in dentin (sections I–II)

In figure 5, the mean size of crystallites in two main crystallographic directions of HAP crystals in each of teeth studied is plotted against the 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.

Table 1. Crystal size in teeth; mean values (*SD*). Sections I and II contain dentin, section III contains enamel and dentin–enamel junction

Section	I	II	III	I–III
h (002) (nm)	23.9 (13.0)	30.5 (7.6)	45.4 (5.2)	33.6 (3.4)
d (310) (nm)	6.6 (1.2)	8.7 (3.0)	26.0 (4.4)	13.8 (2.1)

Table 2. The Pearsons coefficient of correlation between ultrasound velocity in tooth and crystallite size *h* and *d*; crystallite size averaged over dentin and enemel (sections I–III) and crystallite size in dentin (sections I–II)

$h_{(002)}$ I–III	$d_{(310)}$ I–III	$h_{(002)}$ I–II	$d_{(310)}$ I–II
0.787	0.831	0.601	0.968

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

4. Discussion

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

The size of crystallites in dentin measured in our experiment is similar with that reported by others [1], [3]. The change in the shape of crystallites depending on the location in a tooth was also reported [3]. The size of crystallites in enamel estimated in this study is not as large as that usually reported which proves that enamel is not uniform as its structure is depth-dependent and that 1 mm wide X-ray beam in section III includes also dentin–enamel junction and cementum, where crystallites are smaller [1].

From the X-ray diffraction data presented here, it is impossible to establish the orientation of crystallites. One can suppose that there are differences in HAP orientation between teeth being examined because no systematic relation of the width to the intensity of a peak has been found.

On the basis of the results obtained we conclude that the velocity of ultrasound in teeth is positively correlated with the size of crystallites. Obviously, the correlation is not direct because there is a great discrepancy between the wavelength of ultrasonic pulses and the size of crystallites. However, the size of crystallites strongly determines orientation and density of tooth structures, and this organization significantly influences propagation of ultrasound.

Diffraction data presented in this study were obtained based on young donors only. The decreased velocity of ultrasound in advanced age (figure 3) should be attributed rather to deterioration of tissue and their mechanical properties than to smaller crystallite size, all the more because the crystallite size in calcified tissues has been shown to increase with tissue maturation [2].

We suppose that velocity of ultrasound is influenced also by the preferred orientation of crystallites in tooth. In teeth with similar size of crystallites, the higher velocity was measured in incisors, where crystallites are more uniformly organized in respect of the tooth axis compared to canine teeth [1]. However, our study is only preliminary and establishing a more univocal and quantitative relationship needs much larger number of data.

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