Ultrasonic Monitoring of the Dentin Demineralization Dynamics

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***Abstract*—Caries, one of the most important oral diseases, is associated to a progressively demineralization process. The aim of the present work is the study of the dynamics of the artificial demineralization process of human dentin by means of a non destructive ultrasonic pulse-echo technique. Human dentin samples were sliced and artificially demineralized with phosphoric acid at 10%. The demineralization process was monitored using a 15-Mhz pulsed-echo transducer. The reduction of the stiffness of the dentin, and suggests that the acid demineralization, lasting about one hour, develops at a non-constant penetration rate. A model to study the diffusion of the acid into the dentine tissue is proposed to evaluate demineralization lesions produced by natural caries in-vivo.**

***Index Terms*—Dentin demineralization, Ultrasound, NDT, Tubules, Pulse-echo technique, Phosphoric acid.**

I. Introduction

Cariosity affects between 60% and 90% of children and practically 100% of adults. According to the World Health Organization (WHO), caries is considered a highly prevalent disease. It is the pathological process that most frequently affects teeth and is considered the leading cause of dental consultations in all age groups. [1], [2]. This is a multifactorial disease where dentin loses its minerals due to the acids produced by bacterial metabolism [3], [4].

Dentin is composed of 70% inorganic material (hydroxyapatite), 20% organic material (mineralized collagen fibers) and 10% water. [5]. The demineralization process can be divided into two parts: the dissolution of the biomaterials by acids and the degradation of the dentin matrix by protease [6]. Natural demineralization processes are complex, with variations in colour, size, depth and severity. For this reason, it is difficult to obtain samples with the same characteristics for in vitro studies [6]. Consequently, standardized artificial demineralization protocols using acids have been proposed in order to reproduce real lesions [6].

Artificial standardized demineralization lesions offer many benefits compared to the use of natural lesions for research because these protocols make it possible to reproduce lesions with different sizes based on demineralization kinetics [7]. Mainly two acids are used, ethylenediaminetetra-acetic acid (EDTA) or phosphoric acid (PA), these acids allow to selectively demineralize extrafibrillar mineralies while keeping collagen degradation to a minimum [8]. The effect of the acid opens the dentin tubules demineralizing the surface, increasing the porosity of the dentin. The concentration of the dental tubules and their diameter are greater as they get closer to the root of the tooth [9].

In the present work we study the demineralization of the human dentin procedure under controlled laboratory conditions using a non-destructive ultrasonic technique deepening on what was presented in [10].

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II. Materials and Methods

A. Sample Preparation

A group of 3 healthy human molars without caries or cavities extracted for periodontal or orthodontic reasons were obtained with patient consent. The molars were stored in demineralised water. The occlusal surfaces were polished using

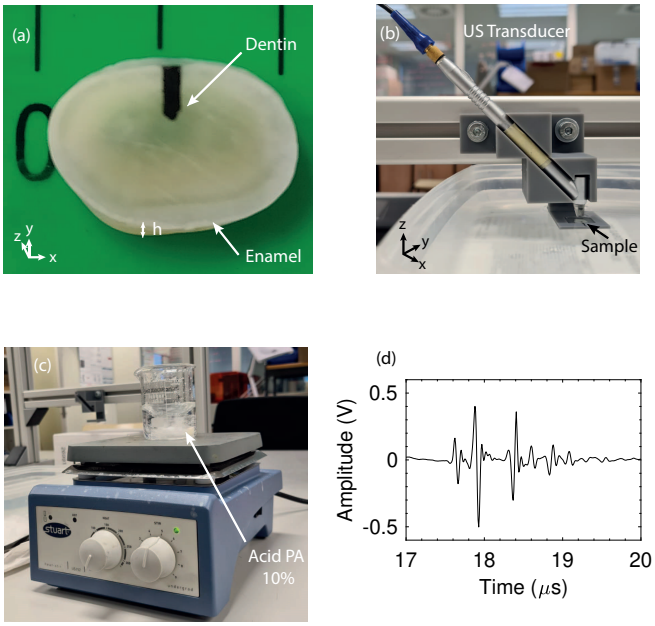


Fig. 1. Experimental setup. (a) Photograph of a sample after preparation, the line indicates the position in the measuring system. (b),(c) Photograph of the experimental set-up. (d) Example of an RF-signal obtained for a partially demineralized sample showing several echoes.

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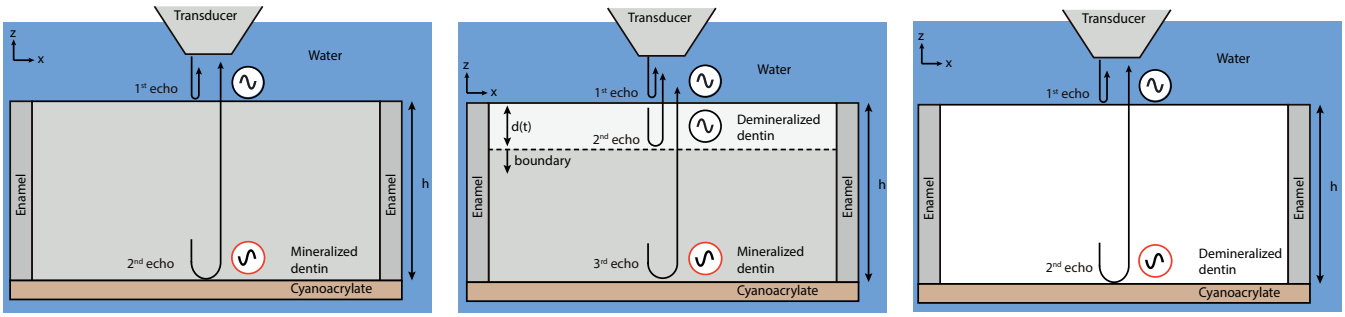


Fig. 2. Models describing the different scenarios along the demineralization process when the sample is previously sealed on one side with cyanoacrylate. At each interface the echoes and its corresponding phase have been drawn. The red circles highlight changes in phase. (a) Initially, the echoes correspond to the acoustic impedance change between the mineralized dentin and the experimental outer layouts (water and cyanoacrylate, respectively). (b) The acid penetration generates a layered medium. (c) When the sample is fully demineralized, the inner boundaries vanish.

the same procedure as in [10]. All samples were immersed in distilled water. The debris was removed using an ultrasonic bath. After that, the samples were stored in distilled water.

B. Demineralization and Measurement Protocol

The 3 samples were located with phosphoric acid (Panreac Química, Barcelona, Spain) at 10% concentration in a Pyrex recipient, during the demineralization process, the samples were stirred with a magnetic shaker, see Fig. 1(c). Samples 1 and 2 had been previously sealed on one side with cyanoacrylate to prevent demineralization and to characterize acid penetration on one side only. The demineralization in sample 3 was applied on the two sides simulteneously.

First of all, an initial ultrasonic measurement is done to characterize the actual speed of sound for the mineralized sample under study. The iterative measurement protocol consists of: (1). The inclusion of the sample in acid with the magnetic stirrer for the stipulated time, (2). The sample is removed from the acid solution and introduced in distilled water, (3). The sample is dried and weighed with a digital scale (TL-series, Homgeek In corporation, UK, ± 0,001 grams), (4). The sample is introduced in distilled water for one minute to eliminate bubbles and measured, (5). The position is slightly changed and another measurement is done. The measurement flow chart can be observed in Fig. 3.

Table I summarizes the particular information related to the measurement protocol applied on each sample. Sample 1 is measured every 5 min for 55 min and samples 2 and 3 every 2 min for 48 and 42 min, respectively. h is the thickness, and W_i and W_f are the initial and final weight respectively.

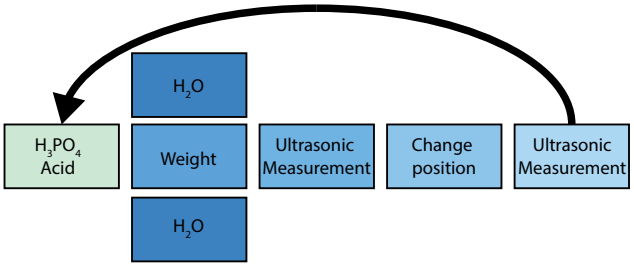


Fig. 3. Measurement protocol established along the demineralization process.

TABLE I
SAMPLE CHARACTERISTICS AND EXPERIMENTAL PARAMETERS.

	Sample 1	Sample 2	Sample 3
h (mm)	1.2	1.05	1.3
W_i (g)	0.240	0.218	0.190
W_f (g)	0.081	0.124	0.091
Cyanocrylate	Yes	Yes	No
Measurement Period (min)	5	2	2
Total Measurement Time (min)	55	48	42

C. Ultrasonic Measurement

Figure 1(b) shows a picture of the experimental setup. The specifications ultrasonic system and the 15-MHz transducer are explained in [10]. A 3D-printed holding support was designed to perpendicularly place the transducer and the sample facing each other with a water delay line to record the signals and ensure repeatability. On the backing side, the sample is in contact with a rigid surface to enhance the ultrasonic reflection.

An example of the ultrasonic signal recorded for a in part demineralized sample is shown in Figure 1(d). A total of 200 measurements were made by changing the tooth position (100 acquisitions by position). The approximate duration of each ultrasonic measurement was 3 minutes. After each measurement, the dentin sample has been introduced into the acid solution. All experiments were performed at a controlled room temperature of 23 $\pm$ 0.2° C. For samples 1 and 2, ultrasonic measurements were taken on the single unsealed side. For sample 3, ultrasonic measurements were performed on both sides.

D. Detection algorithm

RF signals were processed using MATLAB (Mathworks, USA) to estimate the time delay of the echoes. A peak detection algorithm was used taking into account the changes in impedance between layers. According to the reflection factor given by:

$$R = \frac{Z_2 - Z_1}{Z_1 + Z_2}, \tag{1}$$

where R is the reflection coefficient, Z_1 is the impedance of the first medium and z_2 is the impedance of the second

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medium. A phase change exists when the impedance of the first medium is higher than the impedance of the second medium. Figure 2 models the three possible scenarios in a single-faced demineralization experiment where the echoes with each corresponding phase have been pointed out.

The demineralized distance of the acid penetration effect is calculated geometrically as:

$$h_{demin} = \frac{2 \cdot h - c_{min} \cdot \Delta t}{2}, \tag{2}$$

where h is the total sample height, c_{min} is the propagation velocity in the mineralized dentin layer and Δt is the time difference between the third and the second echoes. Note that the speed of sound in the demineralized dentin has not been used in the expression since it is a priori unknown.

III. Results

A. Weight

In order to monitor the loss of minerals, samples were weighed at each iteration. Figure 5 shows how the weight decreases as the samples demineralized. Depending on the characteristics of each sample this variation may change: the more enamel the more weight is lost and faster. Note that for sample 3, as cyanoacrylate has not been used, the piece is demineralized faster and as a consequence the weight decreases faster. In about 60 minutes, all the pieces have lost half of their weight.

B. Ultrasonic experiment

Figure 6 show RF-signals acquired during the ultrasonic monitoring of the demineralization process of two of the samples with (a) and without (b) cyanoacrylate, respectively. From the results obtained we propose a model where we consider that dentin is a homogeneous medium for the frequencies used in our experiments. We deduce this from the lack of scatterers of the first and last signal where the echoes correspond to the change of acoustic impedance between the transducer and the dentin surface (first echo) and between the dentin bottom

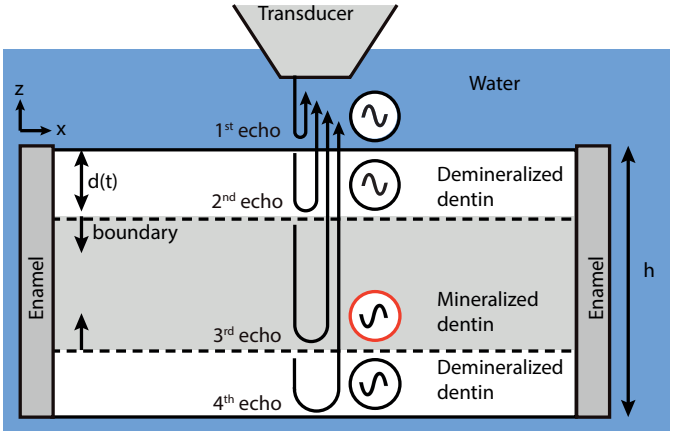


Fig. 4. Model describing the resulting layered medium when the acid penetrates by both sides simultaneously.

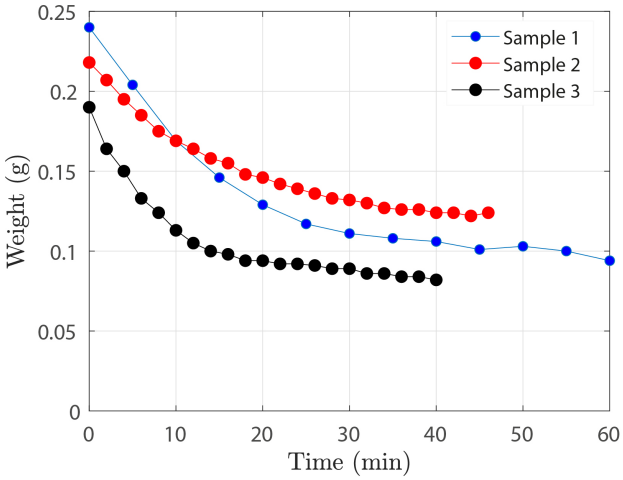


Fig. 5. Evolution of the weights of each of the samples as the time of the experiment progresses. Samples 1 (blue) and 2 (red) were demineralized on one side only, and sample 3 (black) on both sides simultaneously.

and the dentin clamping surface (last echo). From the dentin dimensions we calculate the longitudinal propagation velocity of healthy dentin (3415 $\pm$ 30) m/s and demineralized dentin (1710 $\pm$ 70) m/s.

On the other hand, during the demineralization process, well-defined echoes are appreciated, after the demineralization process begins (signals for $t > 0$ min), two clear echoes appear in the center of the signals, due to the appearance of new boundaries within the dentin. As the demineralization process increases, the arrival time of these echoes changes progressively due to the action of the acid on the dentin. A simple model to explain how the pulse-echo signals change is presented in Figs. 2(a-c). The hypothesis is that the action of the acid during the demineralization process produces a layered medium due to the penetration of the acid in the direction of the center of the sample until it demineralizes it completely.

For the sample without cyanoacrylate, as the acid takes effect from the tooth surfaces, the mechanical properties of the samples show a layered structure with well-defined boundaries that produce echoes. Finally, when the sample is completely demineralized, the inner boundaries disappear.

C. Acid Penetration Characterization

If we analyze the penetration of the acid (Fig. 7), different peculiarities are detected. Samples 1 and 2 follow a very similar trend. After 20 min of demineralization, the acid had penetrated 0.15 mm. This common trend also coincides with face 2 of sample 3. However, the opposite face (face 1 of sample 3) shows a faster penetration ratio. This might be explained by looking at the microstructure of the dentin which shows internal tubules [9]. The density of tubules increases closer to the root of the tooth. Therefore, the dynamics of the demineralization may change according to the height of the tooth and the exposed face.

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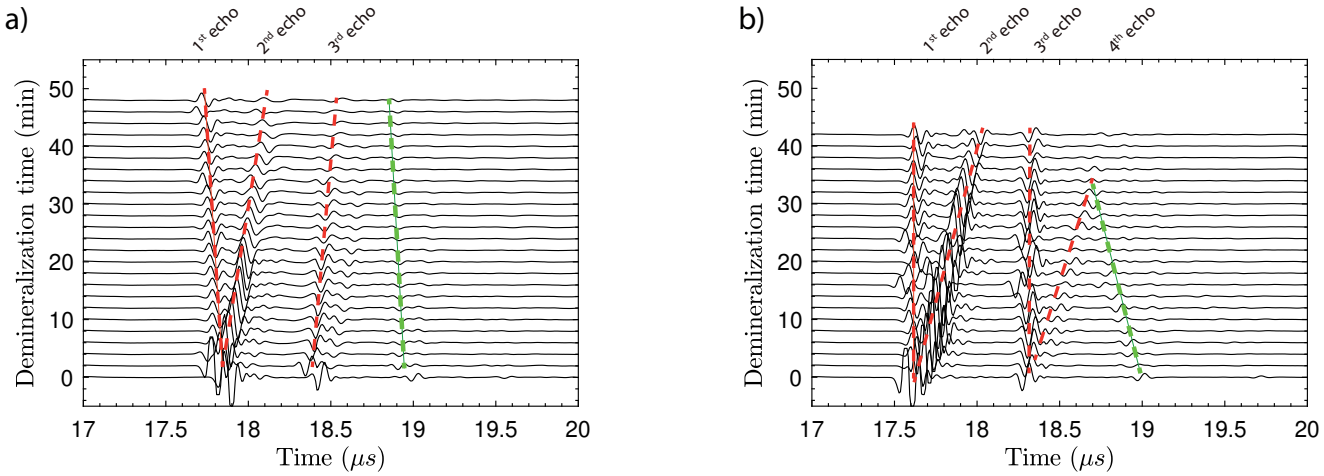


Fig. 6. RF-signals corresponding to the ultrasonic monitoring of the demineralization process of two different samples: a) with cyanoacrylate and b) without cyanoacrylate. The dashed lines were drawn manually as an eye-guide. Sample 1 was demineralized for 48 minutes and measured each 2 minutes (24 signals) and sample 3 was demineralized each for 42 minutes and measure each 2 minutes (21 measurements).

IV. Conclusions

This work has demonstrated the ability of ultrasound to monitor a process of artificial demineralization of dentin with acid. An optimal set-up as well as the appropriate pulse-echo signal processing allow us to demonstrate that the acid-penetration process provides a clear boundary between two layers with different mechanical properties. The present ultrasonic technique, which incorporates a delay line by reducing the contact area between the emitter and the sample is suitable for future in vivo studies. In addition, it has been observed that the acid penetrates faster on one of the faces. So that, it is necessary to take into account the density and dimension of the tubules during the whole dentin demineralization process. In future works, different chemical acids and/or different concentrations must be analysed in order to characterize the differences in dynamics related to the acid penetration, characterizing the face through which the acid penetrates, analyzing the effect of the density and size of the dentin tubules.

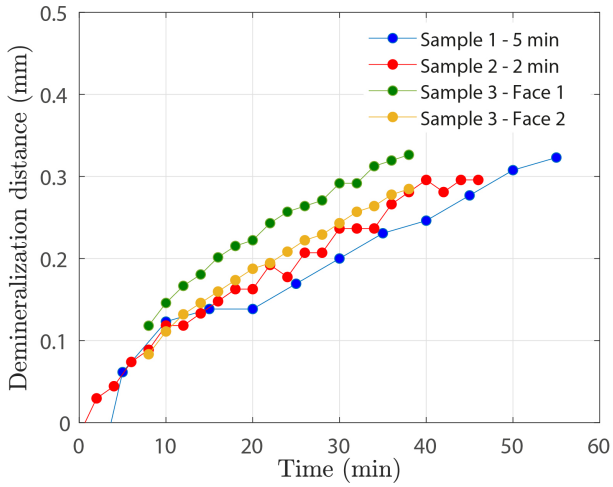


Fig. 7. Acid penetration of the different samples tested, sample 1 and 2 was measured with cyanoacrylate and the sample 3 are measured from both sides of the sample.

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