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● *Original Contribution*

IN VITRO ENAMEL THICKNESS MEASUREMENTS WITH ULTRASOUND

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Abstract—In the work described here, agreement between ultrasound and histologic measurements of enamel thickness *in vitro* was investigated. Fifteen extracted human premolars were sectioned coronally to produce 30 sections. The enamel thickness of each specimen was measured with a 15-MHz hand-held ultrasound probe and verified with histology. The speed of sound in enamel was established. Bland–Altman analysis, intra-class correlation coefficient and Wilcoxon sign rank test were used to assess agreement. The mean speed of sound in enamel was 6191 ± 199 m s^{-1}. Bland–Altman limits of agreement were −0.16 to 0.18 mm when the speed of sound for each specimen was used, and −0.17 to 0.21 mm when the mean speed of sound was used. Intra-class correlation coefficient agreement was 0.97, and the Wilcoxon sign rank test yielded a *p*-value of 0.55. Using the speed of sound for each specimen results in more accurate measurement of enamel thickness. Ultrasound measurements were in good agreement with histology, which highlights its potential for monitoring the progressive loss of enamel thickness in erosive tooth surface loss. (E-mail: drsindi@gmail.com) © **2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Erosion, Tooth wear, Tooth surface loss, Non-destructive, Enamel thickness measurement.**

INTRODUCTION

Ultrasound is a non-invasive, non-destructive imaging tool that has been used in medicine since the 1940s. It was first used as an imaging tool in dentistry by Baum and co-workers (1963), who employed an ultrasound device, originally designed for ophthalmology, to scan teeth in B-mode. However, the images produced were not of sufficient clarity to render the ultrasound device usable in the dental surgery. Later, Lees and Barber (1968) attempted to use ultrasound to examine teeth, with more encouraging results. Recently, Huysmans and Thijssen (2000) reported the use of ultrasound to measure enamel thickness in a sample of nine extracted human incisors. Tagtekin et al. (2005) investigated ultrasound for monitoring occlusal enamel on worn molars *in vitro* and concluded that ultrasound was a promising tool for that task. Indeed, several studies have compared ultrasound measurements with histology, the gold standard in the field, but with mixed results (Harput et al. 2011; Louwerse et al. 2004; Slak et al. 2011; Tagtekin et al. 2005). One factor that may explain the variation between these studies is the assumed speed of sound (SOS) in enamel. This value is used to derive enamel thickness. The variation in SOS within the enamel tissue of teeth is well established and ranges between 4500 and 6500 m s^{-1}. Table 1 summarizes the various reports. The variations in SOS occur both within single teeth and between different patients, and it is likely that much of the variation is due to the orientation of the enamel rods with respect to the incident ultrasound beam (John 2005). Sound travels faster in enamel rods that are parallel to the ultrasound beam, and the opposite holds true.

The reliability of the measurement itself is also influenced by the orientation of the ultrasound transducer with respect to the enamel surface. Ideally, the measurements would be carried out at normal incidence. Dwyer-Joyce and co-workers (2010) investigated the incidence angle after which no echo was seen from the amelodentinal junction (ADJ) and found that in human molar teeth, this angle was 10°. In a preliminary study investigating the echo amplitude from the external surface of synthetic incisors, we found that 50% of the echo amplitude

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Table 1. Speed of sound (SOS) in enamel as reported in several published studies (m s^{-1}).

Study	SOS (m s^{-1})	Tooth type
Huysmans and Thijssen (2000)	6500	Human incisors
Ng et al. (1989)	6450	Human incisors and molars
Barber et al. (1969), Blodgett (2002)	6250	Human incisors
Hamano et al. (2003)	6244	Human molars
Ghorayeb and Valle (2002)	6200	Human molars
Bozkurt et al. (2005)	6132	Human premolars
Slak et al. (2011)	6100	Human incisors
Lees and Barber (1971)	6000	Human molars
Maev et al. (2002)	5900	Human molars
Hedrick et al. (1995)	5800	Incisors and molars
Reich et al. (1967)	5700	—
Kossoff and Sharpe (1966)	4500	Human incisors and molars

plummeted when the incidence angle was ≥25° (Sindi 2013). This angle discrepancy between the two studies might be due to the non-planar nature of molar teeth compared with incisors. Of course, if the transducer is normal to the enamel surface when taking a measurement, a consistent orientation of the beam relative to the enamel rods will be ensured.

For absolute measurement of enamel thickness, knowledge of the SOS is essential, and hence, it might be assumed that the SOS uncertainties preclude the use of ultrasound in routine clinical applications of enamel thickness assessment. On the other hand, if the enamel SOS does not change in a particular tooth over time, then changes in enamel thickness can be monitored without knowledge of the SOS. This is the case in erosive tooth surface loss, a multifactorial disease that is increasing in prevalence (Lussi and Jaeggi 2008). It is defined as the loss of hard dental tissues by acids of non-bacterial origin (intrinsic, extrinsic or both) and causes enamel demineralization. Erosive tooth surface loss causes poor aesthetics, deterioration of dental function and hypersensitivity, and diagnosis is made by obtaining a medical and dental history with thorough investigation of dietary intake. Early detection and monitoring of erosion are crucial to prevent its progression and avoid the aforementioned complications.

To date, no *in vivo* dental tool is available that can aid dentists in diagnosing and monitoring the progression (or stabilization) of the erosive process reproducibly and quantitatively (Amaechi and Higham 2005). The currently used methods for monitoring erosive tooth surface loss are sequential study casts (Wickens 1999), silicone putty index (Shaw and Smith 1999), photographs and erosive tooth surface loss indices (Bartlett et al. 2008; Eccles 1979; Larsen et al. 2000; Linkosalo and Markkanen 1985; O'Brien 1994; O'Sullivan and Curzon 2000), which are subjective and are not reproducible and do not measure enamel at a submillimeter level. Laboratory-based methods, such as profilometry (Bartlett 2003), are costly and cannot be used in the dental surgery. Profilometry also requires an impression of the teeth from which replicas are made, but it has been found that impressions can lead to inaccurate measurements (Rodriguez and Bartlett 2011).

One important question that arises is the extent to which it is possible to take a single assumed value of the SOS and use it to obtain a useful measure of enamel thickness. Hence the aim of this work was to assess the agreement between enamel thickness measurements by ultrasound and histology using the same SOS value for each tooth (selected as being the mean of our sample) and compare it with the agreement obtained when using individualized SOS values. This is important clinically because it would open the possibility of a routine clinical tool using a standard value.

METHODS

Tooth selection and storage

Fifteen extracted human premolar teeth were randomly chosen from the Skeletal Tissue Bank, University of Leeds, after obtaining ethical approval (130109/DS/19) from the Dental Research and Ethics Committee, University of Leeds, according to the Human Tissues Act 2004 (UK). The teeth were kept hydrated in 0.1% thymol (Sigma Aldrich, St. Louis, MO, USA) solution and stored in the laboratory refrigerator at 5°C.

Sectioning of the premolar teeth and storage media

The crowns of all premolars were inspected for near-planar areas (buccally, palatally, mesially and distally) so that the cut sections could include these acoustically preferential regions. All 15 premolars were sectioned coronally using a cutting machine employing a 250-μm water-cooled diamond cutoff wheel (Accutom, Struers, Denmark). Two disk-shaped specimens with a thickness of 2.50 ± 0.02 mm were obtained from each premolar's crown (an "occlusal" specimen and a "cervical" specimen) (Fig. 1a), which resulted in a total of 30 specimens. Specimen thickness was determined with a digital micrometer (293-766-30, Mitutoyo, Kawasaki, Japan). The specimens were stored in labeled vials filled with Hanks' balanced salt solution (HBSS) (Thermoscientific, Hyclone Laboratories, Radnor, PA, USA) in a refrigerator at 5°C for subsequent ultrasound measurements.

Marking specimens

Each specimen was marked with a permanent marker (twin tip, Sharpie, Newell Rubbermaid, Kalamazoo, MI, USA) at two locations on the enamel surface ('V' and 'T' in Fig. 1b). For each specimen, the V-marked