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Title Page

Title of paper: *In vivo* reproducibility study of ultrasound for monitoring enamel thickness

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Abstract

Title: *In vivo* reproducibility study of ultrasound for monitoring enamel thickness

Objectives: This work assesses ultrasound's reproducibility for monitoring enamel thickness *in vivo*.

Study Design: This clinical reproducibility study recruited thirty healthy consented volunteers. The enamel thickness on an intact maxillary central incisor was evaluated at three sites on three separate visits, one week apart. Bland-Altman statistical test and intra-class coefficients (ICC) were used to assess reproducibility.

Results: Reproducibility results were highest for the cervical site (Bias (mm) = -0.01, 95 % limits of agreement = -0.05, 0.04) followed by mid-buccal (Bias = 0.01, 95 % limits of agreement = -0.04, 0.06) and incisal site (Bias = 0, 95 % limits of agreement = -0.25, 0.25). ICC was highest for the cervical site (0.96) followed by mid-buccal (0.71).

Conclusions: Ultrasound is a sufficiently reproducible and reliable technique for monitoring change in enamel thickness, as in erosive tooth surface loss (TSL). The preferred sites for ultrasonic measurements are cervical and mid-buccal.

1 Introduction

Erosive tooth surface loss (TSL) is defined as the loss of tooth tissue due to a non-bacterial acidic source and is of great concern to both patients and dentists. Patient's appearance and dental function are compromised as the enamel gradually wears away under cyclic attacks of acids which are exacerbated by abrasion and attrition. Global interest is increasing towards finding a non-destructive clinical tool that directly monitor enamel thickness in sub-millimetre scales without the need for impressions, expensive equipment or laboratory assessments.¹⁻³

The current clinical techniques available for monitoring erosive TSL include: sequential study casts,⁴ silicone putty index, clinical photographs and erosion indices, which are subjective and unreliable.⁵ Laboratory-based techniques such as profilometric assessment⁶ have drawbacks, such as the need for fixed reference points, impressions, study casts and expensive laboratory equipment (for a detailed review see ^{7,8}). Ultrasound is a non-invasive, non-destructive imaging method that has previously been shown to measure enamel thickness with a reproducibility of 0.08 mm *in vitro*.⁹ It should be noted that only incisors were used in this study and it might be expected that these teeth would be advantageous because they are relatively planar. The simplest ultrasound technique is the pulse-echo technique, where the enamel thickness is calculated by measuring the time of flight (TOF) between the enamel surface echo and the echo from dentino-enamel junction (DEJ) using the mean value reported in the literature for the speed of sound (SOS) in enamel (6000 ms⁻¹).

For any technique to be useful in monitoring TSL, its reproducibility has to be better than the change which is to be detected.⁵ There is little quantitative data on the amount of TSL in affected patients. Generally, the range of the reported values for erosive TSL varied from 17.6-108.2 $\mu\text{m}/6$ months¹⁰ and 250 $\mu\text{m}/\text{year}$ ¹. However, these erosive TSL rates were from

patients with Gastro-Esophageal Reflux Disease (GERD) and caution is required before generalising to different populations.⁷ The technique must also be simple to perform, acceptable for patients and cost effective⁵ in the dental surgery. Monitoring erosion *in vivo* requires fixed reference points from which serial measurements can be taken. Fortunately, the DEJ does not move or change with erosion, facilitating the use of ultrasound compared to more complex methods where, for example, metal discs were cemented on the teeth to serve as fixed reference points on impressions undergoing profilometry assessments in the laboratory.¹⁰

It is important to distinguish between absolute accuracy and reproducibility of the enamel thickness measurement. If monitoring erosive TSL were to require accurate and precise measurement of enamel thickness on each occasion, the variation in the SOS in enamel between patients will be a limiting factor. However the challenge in the case of erosive TSL measurements is not to make the individual measurement very accurate (knowing the exact remaining thickness of enamel) but rather to enable detection of change between visits so that the rate of erosive TSL can be determined. The key performance parameter in such cases is not accuracy (i.e. agreement with a gold standard) but rather reproducibility. The smallest amount of detectable change in enamel thickness will be greater than the reproducibility of individual measurements. The aim of this study was to assess the reproducibility of enamel thickness measurements *in vivo* using an ultrasonic system.

2 Materials and Methods

2.1 Ethics

Ethical approval for this work was granted by the Dental Research Ethics Committee (020712/KS/46) and Leeds Research and Development Directorate (DT12/10538). Good

Clinical Practice standards were followed in line with: Helsinki declaration. All volunteers consented in writing.

2.2 Recruitment

Thirty volunteers were required for this study, an initial dental examination to determine their suitability was carried out. This included a trial scan performed (by KS) on each maxillary central incisor to confirm that the tooth was echogenic. If echogenic the right tooth was chosen in preference otherwise the left was used.

2.3 Inclusion Criteria

Consenting healthy adults over 18 years (females and males) with at least one maxillary central incisor that was intact with no obvious cracks in its crown. At least one maxillary central incisor had to be caries-free and with no obvious periodontal disease and echogenic.

2.4 Exclusion Criteria

Volunteers with the following were excluded: abnormal, replaced, hypoplastic enamel on maxillary central incisors; teeth that had orthodontic appliances, removable appliances, fixed crown or bridgework, hypersensitivity and periodontal disease.

2.5 Measurements

This work was carried out at the Leeds Dental Translational and Clinical Research Unit using a consistent set up. Prior to making measurements each site on the tooth was cleaned using a cotton wool roll. A pulse-echo technique was used¹⁰ with 15 MHz hand-held focussed transducer (VR 260, Olympus Inc., Waltham, USA). This was fitted as standard with a Perspex tip, delay line, Ø2 mm and a separate tip was used for each volunteer. The tip was placed in good contact with the tooth using a drop of water as a couplant (Fig.1).



Fig 1. Ultrasound transducer position when taking measurements

Measurements were taken from three sites: cervical (site 1), mid-buccal (site 2) and incisal (site 3) (Fig. 2). Three measurements were taken for each site. Measurements were repeated on subsequent second and third visits, which were a week apart and the data was analysed after all measurements had been collected to prevent bias. A single operator (KS) was used to make all measurements.

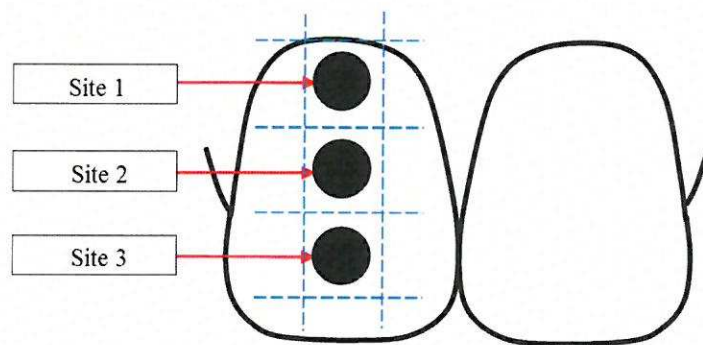


Fig 2. Schematic of maxillary central incisor and the measurements' sites. The dashed lines represent the 'visual' marking of the scan sites

The transducer was excited by a pulser/receiver (PR-5742, Olympus[®] Inc., MA, USA) and the ultrasound waveform was captured on a digital oscilloscope (LT-3542, Teledyne LeCroy[®], NY, USA) and digitized to 2500 data points. A 1000 such pulses were averaged by the oscilloscope and displayed. The operator was only allowed to view the echo signature on the display and was blinded from any additional information. Some angle adjustment was

required in order to maximise the echo signature when the transducer was normal to the tooth surface. This information was exported to an American Standard Code for Information Interchange (ASCII) data file.

2.6 Data Analysis

A total of 810 ASCII data files were analysed using OriginPro[®] version 8.6 (OriginLabs[®], Northampton, MA, USA). Each file was plotted and a waveform was produced. A “find peaks” function in the program was used to locate the peaks. The first peak corresponded to the transducer-enamel interface and the second peak corresponded to the DEJ. When there were multiple peaks for an echo, the first echo was used. When a waveform was uninterpretable, its measurement was omitted. The time difference from the first to the second peak was used to calculate the enamel thickness using the range formula,¹² based on a mean SOS of 6000 ms⁻¹.¹¹⁻¹³

$$(d = \frac{ct}{2})$$

where

d: thickness, *c*: speed of sound, *t*: time of flight.

Bland-Altman¹⁴ statistical test was used to examine agreement between visits. This was analysed in three pairs, weeks 1-2, 2-3, 1-3. In order to achieve a holistic evaluation of the reproducibility, the intra-class correlation coefficient (ICC) was used to assess same visit agreement¹⁵. Both were carried out using IBM[®] SPSS[®] version 20 (IBM SPSS[®], IBM[®] Corp., NY, USA).

3 Results

3.1 Recruitment

A total of 30 volunteers were recruited to the study with all meeting the inclusion criteria; with the mean age 35 (20-63) years and the female to male balance 27:3.

3.2 Data

Table 1 shows the mean values obtained from the study. The range of mean values for sites 1,2 and 3 were (0.54-1.15, 0.54-1.11, 0.58-1.36) respectively.

Table 1. Enamel thickness measurement with ultrasound on three sites for each tooth (n = 30)
(mean of week 1, 2 and 3)

Volunteer	Age (years)	Site 1 Mean Thickness (mm)	SD (mm)	Site 2 Mean Thickness (mm)	SD (mm)	Site 3 Mean Thickness (mm)	SD (mm)
1	27	1.16	0.07	1.11	0.40	1.20	0.21
2	28	0.85	0.45	0.79	0.05	1.15	0.16
3	34	0.56	0.01	0.61	0.08	0.71	0.25
4	26	0.58	0.01	0.59	0.01	0.58	0.02
5	63	0.56	0.02	0.56	0.01	0.87	0.03
6	38	0.54	0.01	0.56	0.01	1.36	0.18
7	55	0.57	0.02	0.55	0.01	1.33	0.13
8	30	0.56	0.03	0.56	0.03	0.91	0.06
9	21	0.60	0.02	0.57	0.02	0.86	0.03
10	36	0.54	0.03	0.54	0.02	0.86	0.13
11	51	0.56	0.03	0.54	0.02	0.97	0.19
12	27	0.57	0.00	0.59	0.02	1.26	0.12
13	20	0.61	0.02	0.57	0.03	1.17	0.11
14	40	0.55	0.01	0.56	0.02	1.05	0.03
15	35	0.56	0.00	0.57	0.01	0.92	0.14
16	35	0.57	0.01	0.60	0.06	0.90	0.02
17	42	0.57	0.02	0.57	0.04	0.94	0.09
18	27	0.55	0.01	0.57	0.01	1.09	0.09
19	32	0.57	0.01	0.57	0.04	1.19	0.09
20	22	0.57	0.00	0.56	0.01	1.02	0.17
21	27	0.56	0.00	0.56	0.01	1.01	0.23
22	41	0.57	0.00	0.56	0.01	1.05	0.06
23	41	0.56	0.02	0.57	0.01	1.09	0.06
24	42	0.58	0.01	0.57	0.00	0.94	0.09
25	38	0.58	0.01	0.56	0.02	1.08	0.04
26	41	0.56	0.02	0.56	0.03	0.98	0.13
27	29	0.57	0.01	0.55	0.01	0.93	0.10
28	36	0.58	0.05	0.58	0.02	0.88	0.03
29	25	0.55	0.02	0.57	0.01	0.91	0.04
30	49	0.56	0.01	0.57	0.01	0.97	0.09

3.3 Reproducibility

The intra-examiner reproducibility is shown in Table 2.

Table 2. Intra-examiner reproducibility of the ultrasound thickness measurements (mm)

	Site 1	Site 2	Site 3
Week 1 and Week 2			
Mean difference w2-w1	-0.01	0.01	-0.04
Standard deviation of difference	0.02	0.04	0.20
Lower limit of agreement	-0.05	-0.07	-0.43
Upper limit of agreement	0.04	0.09	0.34
Week 1 and Week 3			
Mean difference w3-w1	0.00	0.01	-0.05
Standard deviation of difference	0.04	0.03	0.18
Lower limit of agreement	-0.07	-0.04	-0.39
Upper limit of agreement	0.06	0.06	0.30
Week 2 and Week 3			
Mean difference w3-w2	0.00	0.00	0.00
Standard deviation of difference	0.03	0.05	0.13
Lower limit of agreement	-0.06	-0.10	-0.25
Upper limit of agreement	0.06	0.10	0.25

The first set of analyses examined the agreement between the measurements from week 1 and week 2 using the Bland-Altman method. The results showed that in all analyses there was a relatively small mean difference between the repeat measurements, i.e. one set of scores was not consistently higher than the other. This suggests no consistent trend of higher or lower values at week 2 compared to week 1.

The 95 % limits of agreement of site 1 were -0.30 and 0.24 with all measurements included. However this result was confounded by one tooth which had a large difference. Excluding this tooth reduces the limits of agreement to -0.05 and 0.04 (Fig. 3).

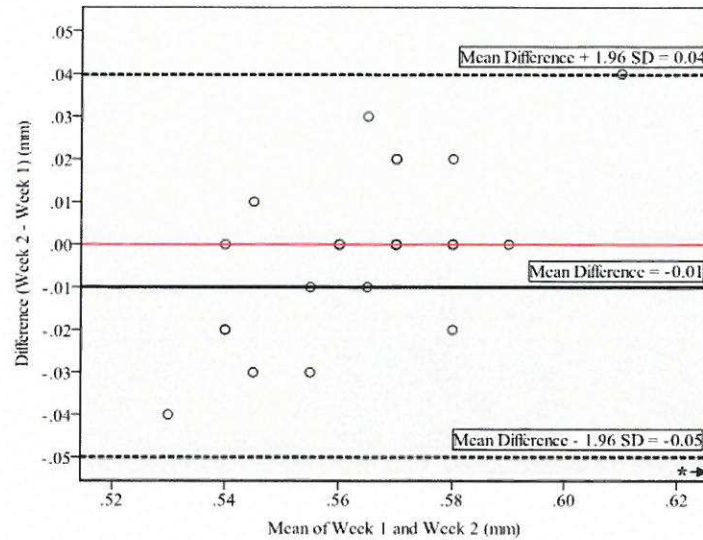


Fig 3. Bland-Altman plot comparing week 1 and 2 on site 1 (cervical) ($n = 29$). The bias line at -0.01 mm shows very good agreement. The 95 % limits of agreement span zero at -0.05 and 0.04 mm (omitting one outlier). The asterisk represents a data point removed for graph clarification (data point location was $x = 1.02$, $y = -0.05$).

The results for site 2 showed a similar pattern. The interval was narrower with the exclusion of data from one tooth, although the interval was wider than that observed for site 1 (Fig. 4).

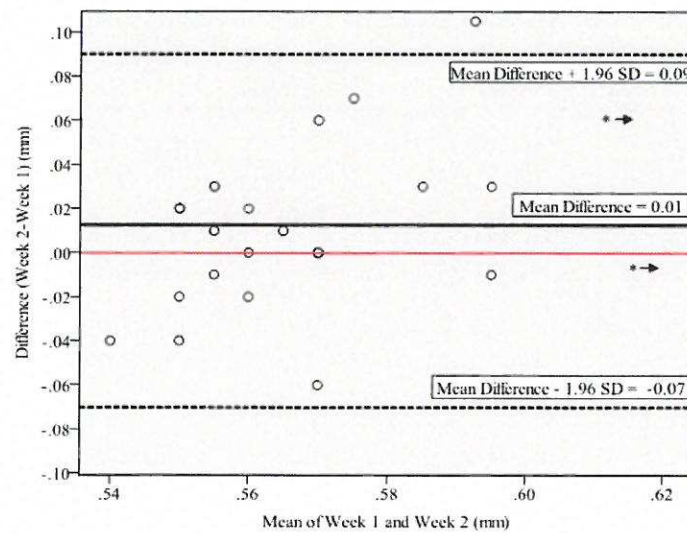


Fig 4. Bland-Altman plot comparing week 1 and 2 on site 2 (mid-buccal) ($n = 29$). The bias line at 0.01 mm shows very good agreement. The 95 % limits of agreement span zero at -0.07 and 0.09 mm (omitting one outlier). The asterisks represent data points removed for graph clarification (top to bottom their locations are $x = 0.63$, $y = 0.14$ and $x = 0.77$, $y = -0.04$).

The site 3 results show the widest limits of agreement ranging from -0.43 to $+0.34$ (Fig. 5).

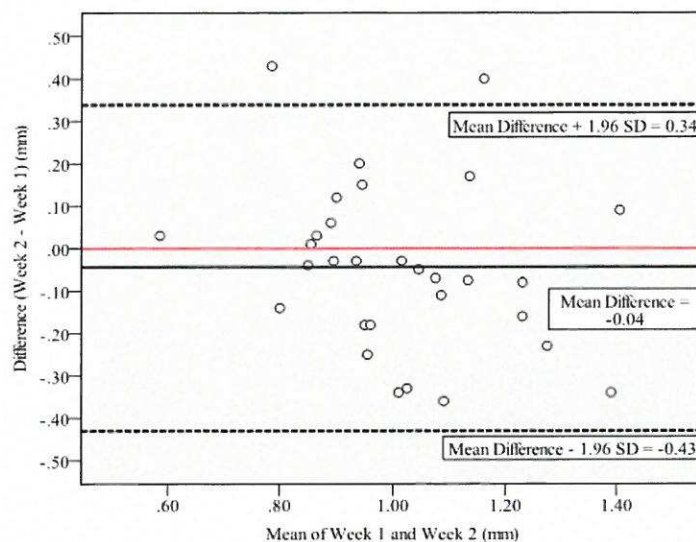


Fig 5. Bland-Altman plot comparing week 1 and 2 on site 3 (incisal) ($n = 30$). The bias line at -0.04 mm shows very good agreement. The 95 % limits of agreement span zero at -0.43 and 0.34 mm

The results comparing weeks 1 and 3 showed a pattern similar to that found for weeks 1 and 2. There were relatively wide limits for sites 1 and 2 when all data values were used, but these limits were much reduced after omitting one outlying value. Site 3 again demonstrates the poorest agreement, with the widest Bland-Altman limits (Figs. 6-8).

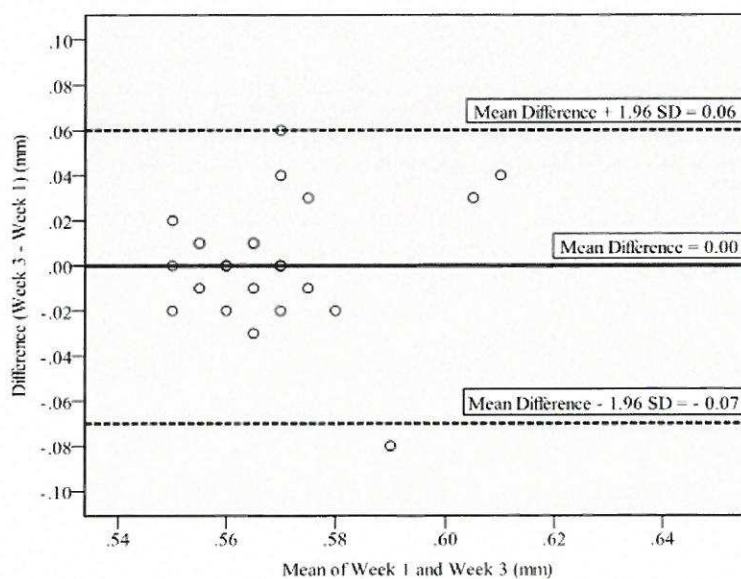


Fig 6. Bland-Altman plot comparing week 1 and 3 on site 1 (cervical) ($n = 29$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at -0.07 and 0.06 mm (omitting one outlier)

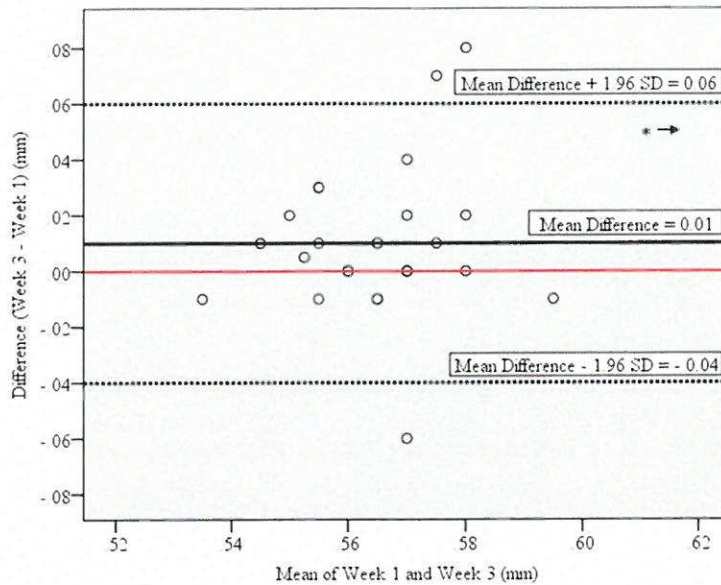


Fig 7. Bland-Altman plot comparing week 1 and 3 on site 2 (mid-buccal) ($n = 29$). The bias line at 0.01 mm shows very good agreement. The 95 % limits of agreement span zero at - 0.04 and 0.06 mm (omitting one outlier). The asterisk represents a data point removed for graph clarification (data point location was $x = 0.82$, $y = 0.05$).

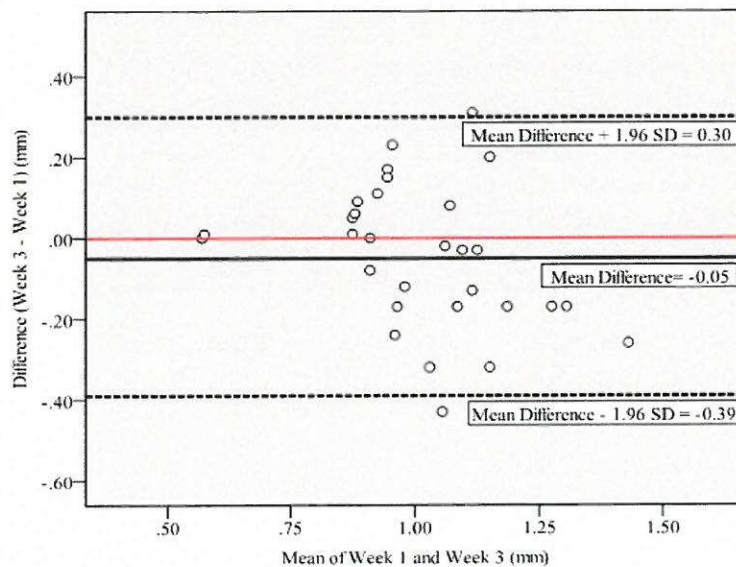


Fig 8. Bland-Altman plot comparing week 1 and 3 on site 3 (incisal) ($n = 30$). The bias line at - 0.05 mm shows very good agreement. The 95 % limits of agreement span zero at - 0.39 and 0.30 mm

The differences between measurements on weeks 2 and 3 are summarised in Table 2. No outlying values were found in these sets. Overall the results showed relatively narrow

intervals for sites 1 and 2, with the interval for site 1 the narrowest, indicating the best agreement (Table 2). The Bland-Altman intervals for site 3 are much wider than those for sites 1 and 2, indicating the poorest agreement for this site (Figs. 9-11).

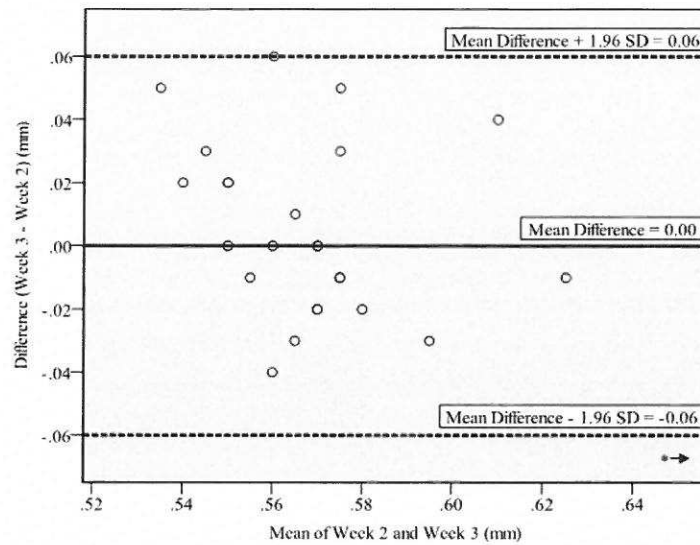


Fig 9. Bland-Altman plot comparing week 2 and 3 on site 1 (cervical) ($n = 30$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at - 0.06 and 0.06 mm. The asterisk represents a data point removed for graph clarification (data point location was $x = 1.13$, $y = -0.08$).

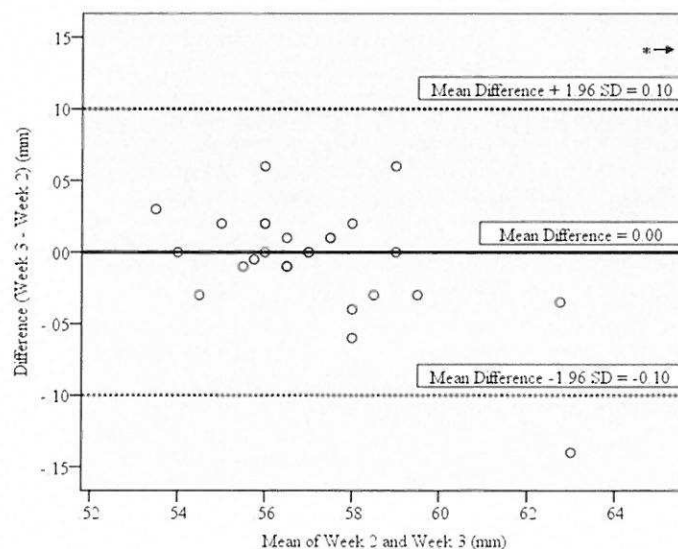


Fig 10. Bland-Altman plot comparing week 2 and 3 on site 2 (mid-buccal) ($n = 30$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at - 0.10 and 0.10 mm. The asterisk represents a data point removed for graph clarification (data point location was $x = 1.34$, $y = 0.17$).

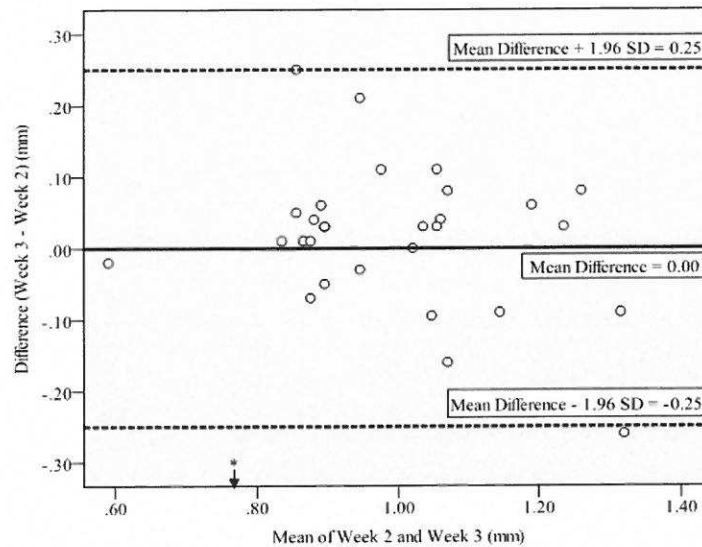


Fig 11. Bland-Altman plot comparing week 2 and 3 on site 3 (incisal) ($n = 30$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at - 0.25 and 0.25 mm. The asterisk represents a data point removed for graph clarification (data point location was $x = 0.79$, $y = -0.43$).

3.4 Intra-Class Correlation

When results from all teeth were included in the analysis, the ICC values were around 0.6. This implies that only around 60% of the total variability in enamel thicknesses was between different teeth with the remaining 40% between repeat measurements of the same teeth.

However, the results for sites 1 and 2 seemed to be heavily influenced by one outlying value. Therefore, the analyses were repeated omitting this tooth. The revised results showed an ICC of 0.96 for site 1 and 0.71 for site 2. This increases the total variability to 96 and 71 % respectively between teeth, with only 4 and 29 % within teeth (i.e. between repeat measurements of the same teeth). This shows that the agreement at this site was not particularly high (Table 3).

Table 3. Summary of the ICC values across the three sites.

Site	ICC (*)	ICC (**)
Site 1	0.63	0.96
Site 2	0.61	0.71
Site 3	0.62	-

(*) Analysis using data from all teeth

(**) Omitting 1 outlier

4 Discussion

The results in this study demonstrated that ultrasound's use to 'measure' enamel thickness was highly reliable and reproducible. The aim of this study was to measure the reproducibility of the technique *in vivo* rather than the true enamel thickness. For the purposes of time dependent erosive TSL assessment, it is the difference in serial measurements which is critical and this hinges on reproducibility.

4.1 Reproducibility of Ultrasound Measurements

The applicability of ultrasound for longitudinal monitoring of erosive TSL hinges on the reproducibility of the measurement *in vivo*. Overall, the cervical and mid-buccal sites scored highest in terms of reproducibility of enamel thickness measurements with 95 % limits of agreement of -0.05 to 0.04 and -0.04 to 0.06 mm respectively. This was similar to the *in vitro* intra-examiner reproducibility results of Huysmans and Thijssen ⁹ where the 95 % limits of agreement for cervical, mid-buccal, incisal and palatal sites were -0.06 to 0.06 mm for the first observer and -0.08 to 0.06 mm for the second observer.

In this work, the incisal site had the lowest reproducibility with a 95 % limits of agreement ranging from -0.43 to 0.34 mm. This wide range of agreement could be due to the fact that the DEJ was least parallel to the enamel surface and consequently a weak echo was produced from that site, which was difficult to detect. It is important to consider why there was an

outlying value in the data set. This outlying value was from the second volunteer (Table 1). The reasons as to why this might have occurred are twofold: First, the transducer might not have been at a right angle to the enamel surface and as such the reflected echo took a longer TOF resulting in larger apparent enamel thickness¹⁶; second, a small echo from the ADJ of that tooth may lead to inaccurate location of the echo peak on the waveform during data analysis might have contributed to this larger thickness value.

Angle adjustments and orientation of the hand held transducer was required before an interpretable echo was seen. This does not undermine its use clinically as the operator of such a device would be able to make angle adjustments easily, guided by the instantaneous height of the received echoes. In the first instance this would be a manually optimised system but there is clear potential for a more automated system in which the device would monitor the received signal and feedback to the operator either visually or by sound when the optimal angle had been attained.

The results demonstrated that the site of the measurement affected the reproducibility. The incisal site was not of adequate reproducibility so it could not be of value in the TSL monitoring process using this technique. This would not be a problem because the cervical or mid-buccal site could be utilised for monitoring the change on the eroded tooth. In an *in vitro* study researchers found that the cervical site (site 1 in this work) had the lowest reproducibility because enamel was thin in that area and because of difficulty in aligning the ultrasound transducer at the site; they added that if this measurement were performed *in vivo*, the reproducibility would be higher as teeth are stationary in the jawbone⁹. However, this was not the case in the work described here, as demonstrated by the reproducibility results for the cervical site in in this work (Table 2). It would be interesting to know why the cervical site examined in this work resulted in reproducible measurements, contrary to the previously

reported results of Huysmans and Thijssen⁹. A reason for this could be that the measurements were made further away from the cervical region where enamel thickness changes rapidly with position. A second reason could be that the tooth was stationary and thus the position of the measurements was defined more⁹. The preferable site for making ultrasonic measurement was the cervical site followed by the mid-buccal site. This renders the technique helpful in monitoring erosive TSL in patients with frequent intake of acidic fruit juice and carbonated beverages, which have been linked to extreme erosive TSL on the labial surfaces. Unusual drinking habits, such as swishing, sucking or retaining drinks in the mouth also aggravate the erosive process¹⁷ as it has been shown that the drinking method significantly affects the pH of the tooth surface¹⁸. This technique could also be potentially useful in monitoring younger patients with suspected anorexia or patients with GERD.

It is important to remember the location of the ultrasonic scan site when taking serial ultrasonic measurements on a tooth. Previous *in vitro* work¹⁹ has demonstrated that using ink markers to guide in ultrasound measurements did not have significant effect on the reproducibility of the measurements, but this had to be tested *in vivo* to see if the same was achievable. We have found that reproducible measurements can be obtained without the need for positioning stents or marker points on the teeth. This was achieved by visually dividing the maxillary central incisor into 3 sites and orienting the transducer perpendicular to and in the middle of the site under investigation (Fig. 2). This negates the need for positioning stents or ink markers which might take additional time and maybe inconvenient for the patient.

The results indicate that ultrasound is a reproducible and reliable technique for monitoring enamel thickness *in vivo* with a reproducibility of 0.05 mm, which is similar to the value of 0.08 mm reported by Huysmans and Thijssen⁹ in their *in vitro* work. Of course this assumes that the SOS in enamel is consistently equal to 6000 ms⁻¹. It is well known that the actual

enamel SOS value varies between individuals and between teeth for any one individual. However it is not necessary to know the true SOS to detect a change in thickness by serial measurement as long as the SOS in the tooth in question does not itself change during that period. The requirement in this case is that the percentage change in thickness is greater than the percentage reproducibility. West and co-workers²⁰ performed a study on ten participants to investigate the effect of consuming proprietary orange juice on enamel thickness. The participants had to wear an intra-oral appliance with human enamel specimens attached palatally for seven hours each day. They found that the consumption of proprietary orange juice 4 times a day for 15 days led to enamel loss of $2.69 \pm 0.49 \mu\text{m}$ *in situ* and $24.06 \pm 1.62 \mu\text{m}$ *in vitro*. Although the study was conducted over a short period of time, it demonstrated the erosive potential of acidic drinks. However, the volunteers were not allowed to brush their teeth while the appliance was worn. It is reasonable to assume that the enamel loss would be higher if tooth brushing had also taken place²¹. The TSL value quoted above equates to a loss of $65 \pm 11.76 \mu\text{m}/12$ months. This is from orange juice only and it is expected that this value would be higher in real oral conditions, especially labially because of the direct contact with the erosive agent when consumed. This level of TSL renders the ultrasonic system described in our work capable of monitoring erosive TSL every 12-18 months and more frequently than this in GERD patients.

Directly measuring the progressive loss of enamel due to erosive TSL with this level of precision and reliability is something that would be of great value to the dental and research community. This could be commenced as soon as the permanent maxillary central incisor erupts in children and adolescents, so that preventive measures could be used if the condition progresses into adulthood. It could aid in flagging up cases requiring conservative restorative intervention, such as dental composites, to protect the remaining enamel layer. This could be

part of a dental examination where preventative measures are applied and oral hygiene instructions are reinforced, although the frequency of dental visits is dependent on the level of oral health of each patient.

It is known that incisors are prone to acid attack from frequent acidic intake and erosive TSL in patients with frequent consumption of acidic fruit juice and carbonated beverages is an increasing concern in young patients. Unusual drinking habits, such as swishing, sucking or retaining drinks in the mouth also aggravate the erosive process¹⁷ as it has been shown that the drinking method significantly affects the pH of the tooth surface¹⁸. Patients with suspected anorexia or patients with GERD could also be monitored using this technology. The level of erosive TSL occurring in patients as a result of the habits mentioned above cannot be monitored reliably in the dental surgery with available methods (indices, study casts, photographs), whereas ultrasound has shown excellent precision (0.05 mm), making it a promising alternative for reliably monitoring erosive TSL in the dental surgery on native teeth.

The accuracy of our ultrasonic system was established in our previous *in vitro* work (unpublished data) on human premolar teeth and the difference between the enamel thickness obtained with histology (true value) and the values obtained with the hand-held transducer was 0.01 ± 0.09 mm. It is pertinent to note that what could be measured with this technique is the progressive loss of the enamel layer and not the early stages of enamel demineralisation (chemical process). On the other hand even if demineralisation occurs, it is confined to a thin layer of approximately 2-12 μm thickness^{22,23}, and hence its effect on the overall SOS within the enamel is negligible.

This study is of great clinical significance in that it demonstrates for the first time *in vivo* that

ultrasound is a reproducible, reliable and direct method with sufficient precision (0.05 mm) that can be used to quantify and serially monitor erosive TSL. Future work should focus on comparing this ultrasonic system with an established technique such as profilometry assessment of sequential study casts. Also, optimising the technique by using higher frequencies and signal processing which could increase the resolution to detect finer changes (< 0.05 mm). Finally, refining the equipment to render them more user friendly because the equipment as used in this study is not suitable as a clinical device and would need further development.

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7 Figure Captions

Fig 1. Ultrasound transducer position when taking measurements

Fig 2. Schematic of maxillary central incisor and the measurements' sites. The dashed lines represent the 'visual' marking of the scan sites

Fig 3. Bland-Altman plot comparing week 1 and 2 on site 1 (cervical) ($n = 29$). The bias line at -0.01 mm shows very good agreement. The 95 % limits of agreement span zero at -0.05 and 0.04 mm (omitting one outlier). The asterisk represents a data point removed for graph clarification (data point location was $x = 1.02, y = -0.05$).

Fig 4. Bland-Altman plot comparing week 1 and 2 on site 2 (mid-buccal) ($n = 29$). The bias line at 0.01 mm shows very good agreement. The 95 % limits of agreement span zero at -0.07 and 0.09 mm (omitting one outlier). The asterisks represent data points removed for graph clarification (top to bottom their locations are $x = 0.63, y = 0.14$ and $x = 0.77, y = -0.04$).

Fig 5. Bland-Altman plot comparing week 1 and 2 on site 3 (incisal) ($n = 30$). The bias line at -0.04 mm shows very good agreement. The 95 % limits of agreement span zero at -0.43 and 0.34 mm

Fig 6. Bland-Altman plot comparing week 1 and 3 on site 1(cervical) ($n = 29$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at -0.07 and 0.06 mm (omitting one outlier)

Fig 7. Bland-Altman plot comparing week 1 and 3 on site 2 (mid-buccal) ($n = 29$). The bias line at 0.01 mm shows very good agreement. The 95 % limits of agreement span zero at -0.04 and 0.06 mm (omitting one outlier). The asterisk represents a data point removed for graph clarification (data point location was $x = 0.82, y = 0.05$).

Fig 8. Bland-Altman plot comparing week 1 and 3 on site 3 (incisal) ($n = 30$). The bias line at -0.05 mm shows very good agreement. The 95 % limits of agreement span zero at -0.39 and 0.30 mm

Fig 9. Bland-Altman plot comparing week 2 and 3 on site 1 (cervical) ($n = 30$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at -0.06 and 0.06 mm. The asterisk represents a data point removed for graph clarification (data point location was $x = 1.13, y = -0.08$).

Fig 10. Bland-Altman plot comparing week 2 and 3 on site 2 (mid-buccal) ($n = 30$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at -0.10 and 0.10 mm. The asterisk represents a data point removed for graph clarification (data point location was $x = 1.34, y = 0.17$).

Fig 11. Bland-Altman plot comparing week 2 and 3 on site 3 (incisal) ($n = 30$). The bias line at 0.00 mm shows very good agreement. The 95 % limits of agreement span zero at -0.25 and 0.25 mm. The asterisk represents a data point removed for graph clarification (data point location was $x = 0.79, y = -0.43$).