Impact of surface micromorphology and demineralization severity on enamel loss measurements by cross-polarization optical coherence tomography

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ABSTRACT

Objectives: Optical Coherence tomography (OCT) is a promising clinical imaging technology for quantitative and objective assessment of dental erosion. We aimed to determine the influence of enamel surface roughness and demineralization severity (by erosive challenge) on dental surface loss measurements by cross-polarization OCT (CP-OCT).

Materials and methods: Human enamel specimens were prepared with three surface roughness levels (very rough, rough and polished; n = 10 each). They were evaluated using CP-OCT and optical profilometry (gold standard) at baseline, and after 1, 2, 4, 6, 8, 16 and 24 h of erosion demineralization. The effects of roughness and demineralization on enamel loss were analyzed using ANOVA (alpha = 0.05). Intraclass correlation coefficients (ICCs) and Bland-Altman plots were used to evaluate inter-method agreement and intra-examiner repeatability.

Results: CP-OCT surface loss measurements did not significantly differ with the changes in enamel surface roughness (p = 0.27). Among demineralization severities, CP-OCT surface loss measurements at 1, 2, 4 and 8 h did not differ among each other, but they showed significantly lower enamel loss than 16 and 24 h; 6 and 16 h were significantly lower than 24 h (p < 0.05). Overall, CP-OCT and optical profilometry measurements did not differ (p = 0.73); however, ICC was relatively low (ICC = 0.34). Enamel loss estimation by CP-OCT presented an error of approximately ± 150 μm compared to profilometry. Intra-examiner repeatability with CP-OCT was excellent (ICC = 0.98).

Conclusions: Enamel roughness did not affect CP-OCT measurements. The estimated error of CP-OCT measurements limited the appropriate assessment of enamel erosion surface loss, in the magnitude simulated in this study.

Clinical relevance: Enamel thickness measurement by CP-OCT presents potential as an objective method for monitoring dental erosion lesions; however, its use may be limited for the assessment of the initial stages of enamel surface loss by erosion.

1. Introduction

Dental erosion is an increasing oral health problem in modern societies [1]. It is characterized by loss of dental hard tissues due to exposure to extrinsic (e.g. dietary) or intrinsic (e.g. gastric) acids [2,3]. Appropriate clinical diagnosis and monitoring of dental erosion lesions would benefit from a method that yields objective and quantitative outcome measures. Of existing clinically available methods, optical coherence tomography (OCT) imaging technology holds promise. It is a non-destructive, sensitive [4] and safe [5] clinical imaging tool. OCT technology can be used for various applications in dentistry [6–9]. Since 1991, OCT was considered to have great potential for monitoring

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demineralization and remineralization in vivo [10–12]. In the pioneering work of Wilder-Smith et al. [13], an early OCT system was able to quantify enamel surface loss related to dental erosion in patients suffering from gastroesophageal reflux disease by measuring changes in enamel thickness [13].

Despite promising results, concerns have been raised with the use of OCT for this specific application, including the loss of DEJ resolution in images and unreliable measurements after extended periods of erosive challenge [14]. It is well known that increases in surface scattering and demineralization can greatly attenuate the penetration of the OCT signal interfering with the ability to resolve the underlying DEJ [10,15]. During erosion, increased surface roughness and subsurface demineralization can both occur. It is unclear from prior studies to what degree increases in surface roughness contribute to the attenuation of the OCT signal. As the enamel surface micromorphology of erosive lesions may vary from rough with loss of luster in active lesions to smooth with some wear marks in inactive stages [16], it is important to understand how changes on eroded enamel surfaces affect CP-OCT performance.

The objective of this study was to test whether enamel surface micromorphology and erosive severity (demineralization time) impact the ability of CP-OCT to quantify enamel surface loss. Optical profilometry was used as a gold standard method. A secondary objective was to test the repeatability of CP-OCT measurements.

2. Methods

2.1. Study design

In this in vitro study, we investigated the effects of two experimental factors on longitudinal measurement of enamel surface loss with CP-OCT: enamel surface roughness (very rough, rough, and polished) and severity of erosive demineralization challenge (1, 2, 4, 6, 8, 16, and 24 h). Human enamel specimens were prepared and distributed into three groups (n = 10 each) according to their surface roughness. They were submitted to seven different levels of erosive challenge, and evaluated using CP-OCT and optical profilometry (gold-standard method) at baseline, and after each demineralization period. The primary outcome measure was enamel surface loss (in μm), calculated both by subtracting the enamel thickness (for CP-OCT) and enamel surface profile (for profilometry) at each demineralization point from baseline. For the secondary objective of testing CP-OCT measurement repeatability, enamel thickness measurements (in μm) of original CP-OCT scans were compared with the measurements of the repeated scans from baseline, and after 4 and 24 h of demineralization. The experimental procedures including sample preparation and demineralization, profilometry and CP-OCT scanning and scan analysis were performed by one trained analyst (MAA).

2.2. Specimen preparation

Enamel slabs (4 × 4 × 2 mm) were prepared from coronal smooth surfaces of intact human molars using a low speed saw (IsoMet, Buehler, Lake Bluff, IL), flattened on the pulpal side and randomized into the surface morphology groups (very rough, rough and polished). Specimens were mounted on polishing disks with the enamel surface facing upward and stabilized with sticky wax. Next, the enamel surface was ground or polished with an automated grinding and polishing machine (Rotoforce-4, Struers Inc.) according to the roughness level for each group. The Specimens in the "very rough" group were ground flat with 500-grit SiC grinding paper (MDFuga, Struers). The specimens in the "rough" group were sequentially ground flat with 500-, 1200-, 2400-grit SiC papers. The specimens in the "polished" group were sequentially ground with 500-, 1200-, 2400-, 4000-grit grinding papers and then polished with 1-μm diamond suspension (DP-Suspension P, Struers). Specimens were then rinsed with deionized (DI) water,

sonicated for 3 min in detergent solution and rinsed again with DI water for 3 min. The specimens' enamel surfaces were visually inspected to ensure no dentin was exposed. Specimens with areas of exposed dentin were excluded. Surface roughness was measured using an optical profilometer (see "Surface roughness measurement" section below). Ten specimens from each group were chosen and removed from the polishing disks. Then, they were individually mounted on acrylic blocks and stabilized with cyanoacrylate adhesive. The occlusal surface of the specimen, which has the thickest enamel layer, was identified and the mounting block was labeled on the same side. Specimens were stored in a closed container in moist conditions to prevent specimen dehydration.

2.3. Surface roughness measurement

Surface roughness was measured by optical profilometry using an S5/03 sensor (Proscan 2000, Scantron, Venture Way, Tauton, UK) in a central area (2 × 2 mm) of the specimen and expressed in Ra values. The step size was set at 0.01 mm and the number of steps at 200 in the (X) axis; and at 0.1 mm and 20, respectively, in the (Y) axis. The scans were analyzed using the dedicated software (Proscan Application software v. 2.0.17) by applying auto leveling and surface filter function, which was set at 1. Then, Ra values in (X) and (Y) axes were recorded and averaged.

2.4. CP-OCT scanning and measurement

A commercial dental CP-OCT device (Santec Inner Vision IVS-300-S-L-C; Santec Corp, Komaki, Japan) was used to acquire the 3D scans. It uses a swept laser light source to generate the scans at a center wavelength of 1310 nm. The a-scan rate was 30 kHz and the range of imaging depth (in air) was > 4 mm. The system's axial and lateral resolution (in air) was ≤ 12 μm and 30 μm, respectively. The scanning probe had a lateral scanning area of 5 × 5 mm and its working distance was 1 mm.

The specimen to be scanned was individually removed from the storage container, gently blot-dried at the surface with absorbent paper (KimWipes, Kimberly-Clark Corp.). Then, it was positioned on an X-Y and Z translation stage and under the CP-OCT sensor with the labeled surface oriented toward the probe handle, which was fixed in a positioning arm. A 3D scan (5 × 5 × 5.63 mm) was performed for each specimen. The refractive index was set at 1.6. From the 3D scan, the central b-scan in X direction was selected and saved for measurements (position of the b-scan on the specimen is indicated in Fig. 1). The positioning and scanning were performed within 2 min to ensure that adequate moisture was maintained in all specimens during scanning.

After obtaining the b-scans, the enamel thickness measurement (from DEJ to surface of the specimen) was performed in a blinded manner and it was based on the methods described by Chan et al. [14] and Algarni et al. [17]. Briefly, the b-scan was opened in Santec Inner Vision IVS-300 software (Santec Corp, Komaki, Japan) then the measurement position (b) was identified at the center of the enamel width with the aid of a screen ruler (JR Screen Ruler, Spadix Software; www.spadixbd.com) (Fig. 2). The DEJ measurement landmark along (b) position was annotated to help in identifying its depth on the b-scan (Fig. 2). Then, the a-scan at (b) position was viewed by dragging the a-scan cursor line to position (b) (Fig. 2) and clicking on "peak detect" function. On a-scan, the depths of the highest light intensity peaks at the enamel surface and DEJ areas were identified and confirmed by the depths of the measurement landmarks on b-scan (Fig. 3). The distance between the depths of the two peaks on a-scan was calculated by clicking on "add layer" function. The distance in mm was recorded then converted to μm to represent the enamel thickness measurement at position (b). CP-OCT scanning and enamel thickness measurements were performed at baseline and following each demineralization period. To calculate surface loss, enamel thickness measurements at every demineralization time point were subtracted from the baseline
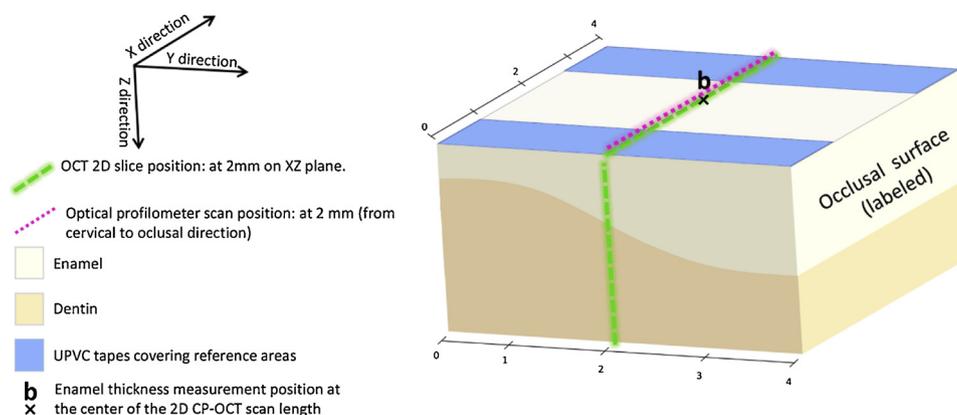
Fig. 1. Schematic illustration showing specimen dimensions, position of the label, test and reference areas, position of CP-OCT b-scan, CP-OCT measurement position (b), and position profilometric scan.
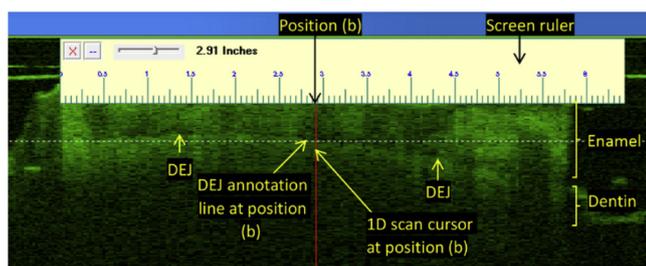

Fig. 2. A screenshot showing a sample b-scan on the CP-OCT viewer and the JR screen ruler for locating the enamel measurement position (b) on the x- axis.
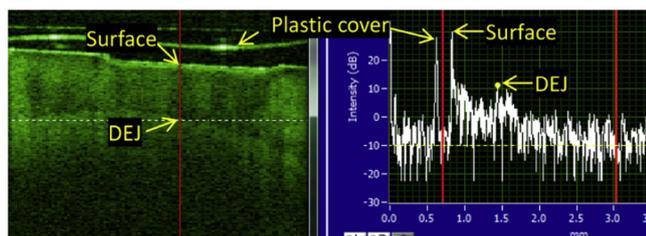

Fig. 3. Enamel thickness determination using CP-OCT b-scan (left) and a-scan (right) along position (b) in the Z-plane. The position of the highest intensity peaks at the enamel surface and DEJ (indicated with labelled arrows) were identified. The distance between the two peaks was calculated by the "add layer" function, and distance in mm was recorded, which represents the enamel thickness measurement. The first high intensity peak above the enamel surface was ignored as it represents the probe's plastic cover placed for infection control purposes.

measurements.

2.5. Optical profilometric scanning and enamel surface loss measurement

Following enamel thickness measurements using CP-OCT, the specimens were scanned with an optical profilometer with a S5/03 sensor (Proscan 2000, Scantron, Venture Way, Tauton, UK). A central line (0.01 × 4 mm) was scanned, which overlaps the area of CP-OCT b-scan Y direction (Fig. 1). The length of the scan covered both the treated and reference areas. Fig. 1 shows the profilometric scanning line (dotted with pink lines). The step size was set at 0.01 mm and the number of steps at 400 in the (X) axis; and at 1 and 3 mm, respectively, in the (Y) axis. Surface profile analysis was performed with the use of a dedicated software (Proscan Application software v. 2.0.17) by applying a three-point height tool with an auto leveling function. Profilometric scanning and surface profile measurements were performed at baseline and following each erosive challenge. Then, the surface loss was calculated by

subtracting enamel profile measurements at each demineralization time point from the baseline enamel profile measurements.

2.6. Dental erosion simulation

After CP-OCT/Profilometric scanning and before every erosive challenge, UPVC tapes were placed on two sides of the specimens to protect the reference areas from demineralization, leaving a testing area of 2 × 4 mm exposed in the center of the specimen surface (Fig. 1). After each erosive challenge, the tapes were removed to allow the areas to be rescanned with CP-OCT and optical profilometry.

After generating CP-OCT and profilometric scans and test surface delimitation, the specimens were exposed to the demineralizing solution (0.3% citric acid, natural pH 2.40–2.41), at room temperature and with no agitation. A volume of 30 ml of demineralization solution was used per specimen. After immersion for the designated time, specimens were removed from the solution and rinsed thoroughly with DI water for 10 s. Specimens were then stored in humid conditions, in a sealed container. Measurements were performed after 1, 2, 4, 6, 8, 16 and 24 h of acid exposure, respectively.

2.7. Repeatability of CP-OCT measurements

For intra-examiner repeatability evaluation, a second set of 3D scans was performed for specimens at baseline, after 4 and 24 h of demineralization challenges. Enamel thickness measurements were repeated on the second 3D scans at least seven days after analyzing the original scans. The repeated measurements were then compared with the measurements of the original scans to evaluate the intra-examiner repeatability.

2.8. Statistical analysis

The effects of enamel surface roughness and demineralization time on surface loss (CP-OCT and optical profilometry) were analyzed using ANOVA. The ANOVA included fixed effects for roughness, demineralization time, and their interaction. Pairwise comparisons using ANOVA with a repeated effect were conducted.

Intraclass correlation coefficients (ICCs) and Bland-Altman plots were used to evaluate the intra-examiner repeatability of the CP-OCT and the agreement between the CP-OCT and optical profilometry surface loss measurements. Statistical analysis was performed with SAS 9.4 (SAS Institute Inc., Cary, N.C., USA). A 5% significance level was used for all tests.

Table 1
Mean ± standard-deviation of enamel surface loss profilometry measurements (μm) for each roughness group and after each demineralization time.

	Demineralization time (h)								
	1	2	4	6	8	16	24	n	
Very rough	−5 ± 2	−9 ± 2	−20 ± 3	−29 ± 4	−39 ± 5	−70 ± 5	−105 ± 8	70	A
Rough[a]	−5 ± 2	−9 ± 2	−19 ± 7	−29 ± 7	−39 ± 7	−72 ± 9	−106 ± 8	35	A
Polished	−5 ± 1	−10 ± 1	−21 ± 2	−32 ± 2	−42 ± 4	−74 ± 6	−106 ± 6	70	A
n	25	25	25	25	25	25	25		
	a	b	c	d	e	f	g		

Similar uppercase letters in the last column represent no statistical difference among roughness groups means indicated by the ANOVA (p > 0.05).
Different lowercase letters in the last row represent statistical difference among demineralization times means indicated by the ANOVA (p < 0.05).
[a] Five enamel specimens from rough group were excluded from testing and analysis because their surface did not show signs of demineralization, possibly due to surface contamination with wax.

3. Results

3.1. Baseline enamel surface roughness characteristics

The mean ± standard-deviation of enamel surface roughness at baseline, expressed in Ra parameter in μm, for very rough, rough and polished groups were 0.49 ± 0.07; 0.28 ± 0.04 and 0.10 ± 0.02, respectively.

3.2. Enamel surface loss with optical profilometry

There was no significant interaction effect (roughness × demineralization time) in enamel surface loss measurements (p = 0.75). Among roughness levels, there were no significant differences in enamel surface loss measurements (p = 0.43). However, among demineralization times, there were significant differences in measurements, with all of them differing from each other (p < 0.0001; Table 1).

3.3. Enamel surface loss with CP-OCT

There was no significant interaction effect (roughness × demineralization time) in enamel surface loss measurements (p = 0.49). Among roughness levels, there were no significant differences in measurements (p = 0.27), and as with profilometry results, there were significant differences in measurements among demineralization times. Mean CP-OCT measurements after 1, 2, 4, and 8 h of demineralization were significantly lower than 16 and 24 h (P < 0.05); and 6 and 16 h were significantly lower than 24 h (P < 0.05). Table 2 shows the mean enamel surface loss measurements of CP-OCT for each roughness group and after each demineralization time, presented in μm.

3.4. Agreement between CP-OCT and Profilometry for enamel surface loss

Agreement between the two methods as evaluated by the intraclass correlation coefficient (ICC) and the mean difference is presented in Table 3. There was no significant difference between the methods' measurements means (p = 0.73). However, the ICC was poor (ICC = 0.34). The Bland-Altman Plot (Fig. 4) shows that the measurement error by CP-OCT was approximately ± 150 μm compared to optical profilometry.

3.5. Intra-examiner repeatability for CP-OCT

Intra-examiner repeatability as evaluated by the difference between means, intraclass correlation coefficient and Bland-Altman plot can be found in Table 4 and Fig. 5. There was no significant difference between the original and repeated measurements' means (p = 0.14). Additionally, the ICC was excellent (ICC = 0.98). The Bland-Altman Plot (Fig. 5) shows that the limits of agreement were at approximately ± 150 μm.

4. Discussion

In the present study, we tested whether enamel surface characteristics of roughness and demineralization by erosion simulation would affect CP-OCT measurements. Overall, our data showed that different levels of enamel surface roughness did not affect CP-OCT measurements.

Regarding the demineralization time effect, no significant differences were found among time points up to 8 h by CP-OCT. A possible explanation might be that the magnitude of early enamel surface loss remained below the resolution limit of the CP-OCT system. According to the manufacturer, the CP-OCT system's axial (depth or Z plane) resolution in air is ≤ 12 μm. Changes in the water content and variation in the enamel composition with depth may cause small changes in the

Table 2
Mean ± standard-deviation of enamel surface loss CP−OCT measurements (μm) for each roughness group and after each demineralization time.

	Demineralization time (h)								
	1	2	4	6	8	16	24	n[b]	
Very rough	−16 ± 40	9 ± 83	−75 ± 69	−79 ± 128	−21 ± 67	−71 ± 17	−112 ± 27	54	A
Rough[a]	2 ± 7	−10 ± 13	−4 ± 37	−36 ± 36	−36 ± 20	−71 ± 12	−111 ± 28	25	A
Polished	14 ± 89	−1 ± 43	13 ± 69	0 ± 72	−17 ± 41	−75 ± 25	−113 ± 53	51	A
n[b]	17	19	19	21	20	16	18		
	a	a	a	ab	a	b	c		

Similar uppercase letters in the last column represent no statistical difference among roughness groups means indicated by the ANOVA (p > 0.05).
Different lowercase letters in the last row represent statistical difference among demineralization times means indicated by the ANOVA (p < 0.05).
[a] Five enamel specimens from rough group were excluded from testing and analysis because their surface did not show signs of demineralization, possibly due to surface contamination with wax.
[b] Lower n values by CP-OCT compared to profilometry because DEJ was not clear in some CP-OCT scans.

Table 3
Agreement between Profilometry and CP-OCT enamel surface loss measurements according to the mean difference and intraclass correlation coefficient.

	Mean (SE) Profilometry (μm)	Mean (SE) CP-OCT (μm)	P-Value	SD within-sample (μm)	ICC
Profilometry vs. CP-OCT	−40 (4)	−39 (0.5)	0.73	44	0.34

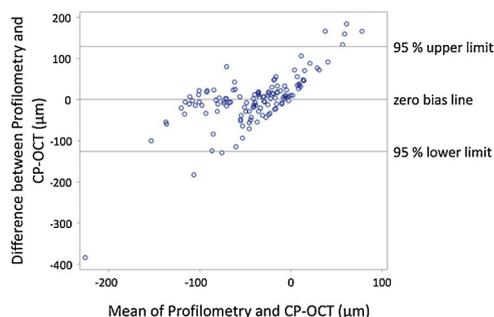SE-standard error; SD-standard deviation; and ICC-intraclass correlation coefficient.

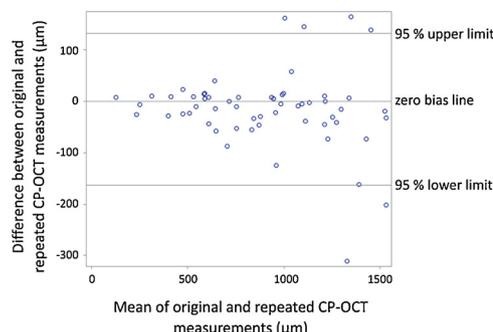**Fig. 4.** Bland-Altman Plot for inter-method agreement.

Fig. 5. Bland-Altman Plot for showing the differences between pairs of CP-OCT enamel thickness measurements (μm) obtained from different scans and analyzed on different time points.

refractive index that can influence the path length [18].

Another possible explanation is the inherent measurement errors that were higher than the amount of enamel loss simulated at the early time points, as evidenced by the high standard deviation of the means, corroborating the findings of Chew et al. [19]. A possible source of error might be a slight shift in locating the measurement position in the 3D scan or b-scan or both, leading to measuring a different enamel position with a relatively longer or shorter enamel thickness. This limitation was also encountered when comparing and analyzing the before and after OCT images in a previous study [20], causing a larger error than the magnitude of the examined shallow lesions. Furthermore, potential errors could be related to difficulties in selecting the DEJ peak on a-scans for enamel thickness measurements, which are difficult to avoid due to the irregular nature of the DEJ [18]. Additionally, the adoption of a plastic cover (infection control purposes) of the CP-OCT handheld probe may have caused uneven light refraction and attenuation patterns [21].

Despite the use of an advanced CP-OCT system under well-controlled laboratory conditions, we could not reliably detect surface loss of 7.2 μm, as reported clinically [13]. In that study and according to the authors, the process of the enamel thickness measurement and re-positioning of the imaging probe using a stent was relatively un-problematic and they did not report any of the problems noted in the present or other studies [3,14].

On the other hand, after 16 and 24 h demineralization our results showed that the differences in surface loss by CP-OCT were significant and the results were more aligned with profilometry mean enamel surface loss of 70 μm or more. It can be speculated that the loss of enamel thickness at the advanced demineralization timepoints reduced the light signal attenuation [22] and thus, improved the resolving capabilities of the OCT device, compared to the thicker enamel at early demineralization challenges. Chan et al. (2013) found that enamel thickness increased at 48 h of demineralization, when it was expected to decrease [14]. Our results differ from their observation, in fact the mean enamel thickness loss in our study at the maximum demineralization duration tested (24 h) showed a decreasing trend from

previous timepoints and it is more consistent with profilometry data.

We were able to measure enamel thickness in most of the scans after every demineralization timepoint (1, 2, 4, 6, 8, 16 and 24 h; Table 2). However, we were unable to measure enamel thickness in some scans due to the loss of DEJ details. Similar observations were also reported by Chan et al. [14] and Aden et al. [23], who hypothesized that the difficulty in visualizing the DEJ in scans was in part due to specimens drying out during scanning [14]. Demineralized dehydrated specimens caused increased light scattering because of a mismatch in refractive index, which in turn interfered with the light propagation deeper to the DEJ area [14]. The loss of DEJ details in our study did not seem to be associated with the degree of demineralization. It is possible that this finding relates to the specimens' moisture condition during CP-OCT scanning [24]. Chung et al. demonstrated the dramatic influence of internal hydration on the transparency of enamel at 1300-nm and have reported that it is extremely important that teeth are kept well hydrated for optical coherence tomography studies [25]. In corroboration with earlier findings, Hariri et al. [26] and Nazari et al. [27] have found a significant difference between OCT signals of enamel in wet and in dry conditions, with the difference being more pronounced in demineralized enamel, increasing with demineralization time. While there is still no consensus on the optimal hydration condition for specimens during OCT scanning [24,28], we controlled it during scanning to some extent by blotting the excess water and standardizing the time of the scanning procedure.

For CP-OCT agreement with optical profilometry for surface loss measurements, although the difference between the methods' measurements means was non-significant, the ICC value was low. Also, most of the differences presented in the Bland-Altman plot were larger than the magnitude of enamel surface loss measured by the gold-standard method. This indicates that the agreement of CP-OCT with optical profilometry was not satisfactory for measurement of enamel erosive surface losses created in this study. This could potentially be caused by

Table 4
Intra-examiner repeatability analysis of CP-OCT original and repeated enamel thickness measurements (μm) for specimens at baseline, after 4 and 24 h of demineralization, according to the mean difference and intraclass correlation coefficient.

	Mean (SE) original (μm)	Mean (SE) repeated (μm)	P-Value	SD within-sample (μm)	ICC
Original vs. repeated measurement	958 (44)	943 (44)	0.14	53	0.98

SE-standard error; SD-standard deviation; and ICC-intraclass correlation coefficient.

the lower resolution of CP-OCT compared to profilometry (CP-OCT lateral and axial resolution = 30 μm and ≤ 12 μm, respectively in air; Profilometry S5/03 sensor resolution = 10 nm [0.01 μm]). Despite the low agreement with profilometry, the CP-OCT scanning and measurement approach used was repeatable. This may indicate that the measurement discrepancies were primarily due to changes of enamel optical properties associated with erosion simulation and the inherent disadvantages of the CP-OCT imaging and analysis approach used.

Both OCT and optical profilometry are non-contact, non-invasive technologies. They both use a light to acquire information from a specimen and are able to generate a two- or three-dimensional output [29]. Profilometry also is the most commonly applied quantitative method to measure enamel loss in in-vitro and in-situ studies, owing to its high sensitivity and accuracy [29]; therefore, we employed it in this study as a gold standard method. Optical Profilometry was also used in the current study to characterize the enamel surface roughness changes using a commonly used roughness parameter in dental research, the arithmetic average (Ra) [30]. Measuring the surface roughness provides an information about the range of enamel surface roughness changes created in this study.

We utilized 3D CP-OCT scanner to acquire b- and a- scans for analysis. With using the 3D OCT scanning system, the repositioning is potentially easier by having the outer borders of the specimen as landmarks for acquiring 3D repeated scans. Clinically, the gingival margin can be used as a position reference for repeated scans [31]. Enamel thickness measurements were performed on raw b-scans and a-scans without rendering and analyzed using the associated software. This approach allows clear understanding of the device capabilities without analysis bias [32].

Dental erosion lesions are characterized by surface loss and a relatively small amount of subsurface demineralization that would most likely be few microns deep [33–36]. The extent of the subsurface portion of the lesion remains relatively constant as the dental erosion lesion progresses [23]. Thus, enamel loss estimated from the changes in the enamel thickness measurements from the anatomical surface is more indicative outcome measure for monitoring changes during erosion progression.

In the proposed CP-OCT approach, the DEJ was considered as a reference because it has an advantage over the surface reference points that are subjectively determined. An earlier study reported the difficulties with in-vivo longitudinal measurement of maximum depth and horizontal width of NCCL lesions with using reference points on the enamel surface [37]. This was explained by the subjectivity in placing the reference points and by the changes in reference points positions due to abrasive loss at the tooth surface [37].

There are some important limitations that need to be considered. Firstly, we performed the testing using surface-flat specimens, which does not reproduce the natural tooth curvature. It was necessary to flatten the surface to rule out any confounding factors related to variability in tooth curvature and to allow the surface to be analyzed accurately with optical profilometry. Secondly, the levels of surface roughness were not formed to simulate the roughness levels that occur in enamel erosion lesions inside the oral cavity. We arbitrarily created the degrees of enamel surface roughness to test the performance of the device on wide range of surface micromorphologies. Also, the continuous demineralization challenge induced in this study was planned to test the capability of the device to evaluate enamel surface loss and thus, to establish the device's usefulness for quantifying enamel thickness loss following dental erosion. Thirdly, the lower sample size in the 'rough' group. Five enamel specimens (50%) were excluded due to potential experimental error. It was observed that they had no measurable (by profilometry) demineralization, which was not as expected. We suspect that there was inadvertent enamel surface contamination with wax in those specimens, therefore justifying their exclusion from the study. In order not to interfere with the original randomization schedule and laboratory procedures initially planned, the samples were

not replaced. Finally, we did not consider the presence of the acquired salivary pellicle. The pellicle can be visualized by OCT [38] and possibly affect OCT signal intensity [28]. It can also modulate the enamel surface roughness effect on the reflection intensity measurement because it fills the irregularities of the roughened enamel surface [39]. The magnitude of the pellicle effects on the outcomes of this study is unknown and should be further investigated. Within the above-mentioned limitations, this work provides valuable information regarding the capabilities and drawbacks of CP-OCT for enamel erosive surface loss measurement estimated from the remaining enamel thickness. The approach in this study seems to have little clinical applications for early stages of enamel erosive surface loss, owing to the unreliable surface loss measurements. On the other hand, the measurement approach showed to be practical and repeatable. Based on the significant demineralization effects on CP-OCT measurement, future studies will be aimed to elucidate the ability of CP-OCT to identify advanced erosive tooth wear levels and to clarify the effects of depth of demineralized enamel on the enamel thickness measurement by CP-OCT.

In conclusion, our results suggest that surface roughness changes do not affect CP-OCT measurements of enamel erosive surface loss. However, the estimated error observed for CP-OCT measurements limited the appropriate assessment of enamel erosive surface loss, in the magnitude simulated in this study.

Declarations of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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