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In vitro longitudinal evaluation of enamel wear by cross-polarization optical coherence tomography

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ABSTRACT

Objectives. Enamel thickness determination by Cross-Polarization Optical Coherence Tomography (CP-OCT) is a promising approach for quantitative monitoring of tooth wear progression. This study evaluated the ability of CP-OCT to quantify the thickness of natural enamel before, during and after tooth wear simulation.

Materials and Methods. Natural, unpolished human dental enamel slabs were submitted to five wear stages (Wear 1: to level the surfaces; Wear 2 to Wear 5: 0.05 ± 0.02 mm reduction each) simulated by an automatic grinding/polishing machine. Enamel thickness was evaluated with CP-OCT and a gold-standard method (micro-CT) at baseline and after every wear stage. Data were analyzed using ANOVA with pairwise comparisons for wear stages' impact on the thickness and wear depth measurements. The inter-method agreement was analyzed using intra-class correlation coefficients, the difference between means, and Bland-Altman plots.

Results. Enamel thickness measurements (mean ± standard error, in mm) with natural (1.40 ± 0.05) and worn surfaces (1.08 ± 0.02) by CP-OCT did not differ significantly from those measured by micro-CT (natural = 1.39 ± 0.05; worn = 1.09 ± 0.02; p-values = 0.30 and 0.39, respectively). CP-OCT and micro-CT showed excellent agreement on natural (ICC = 0.98) and worn surfaces (ICC = 0.98) enamel thickness measurements. Among and between

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wear stages, there were significant differences in enamel thickness and wear depth measurements for both methods (p -value <0.0001 for all). Both methods yielded similar measurements' mean (0.14 ± 0.01 ; p -value = 0.87) and were in good agreement ($ICC = 0.77$) for wear depth estimation.

Significance. CP-OCT allows accurate measurement of enamel thickness on natural tooth surfaces. Enamel thickness measurement by CP-OCT allows quantitative monitoring of enamel thickness changes and wear depth following progressive wear.

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1. Introduction

Tooth wear is the irreversible loss of dental hard tissue of either physiological or pathological nature. The rate of pathological enamel loss varies considerably among individuals and depends on the nature of the underlying related wear mechanisms, whether abrasion, erosion, attrition or abfraction, combined or not [1], and the degree of enamel wear resistance [2]. Therefore, tooth wear should be clinically monitored and controlled, preferably using objective methods.

Among the available potential methods, optical coherence tomography (OCT) offers remarkable advantages, as it allows a quantitative analysis of enamel thickness to be performed at chairside. Additionally, OCT creates cross-sectional tomograms as well as 3D reconstructed enamel images non-invasively without X-ray ionizing radiation [3–5]. Those tomographic images enable dentin-enamel junction (DEJ) visualization, which can be used as a landmark, ruling out the need for intact enamel surface or artificially created reference areas or points for tooth wear measurement. This approach was first proposed and tested by Wilder-Smith et al. [6] to evaluate enamel erosive tooth wear by endogenous acids, although further validation was deemed necessary. The performance of this method was also tested on eroded enamel surfaces [7,8]. However, these studies considered the simulation of severe dental erosion and use of surface-flat enamel. Moreover, the capability of this approach to detect lower enamel wear rates has not been explored.

The objectives of this study were to a) evaluate the accuracy of enamel thickness measurements performed by CP-OCT for enamel with natural, unpolished surfaces compared to measurements by micro-CT, and b) to evaluate the capability of CP-OCT to quantify the progression of enamel wear from enamel thickness and wear depth measurements, compared to micro-CT measurements.

2. Methods

2.1. Study design

Human enamel specimens were submitted to five wear stages created by use of an automatic grinding and polishing machine and guided by digital micrometer specimen thickness measurements. Enamel thickness was evaluated by a trained analyst with CP-OCT and the gold-standard method,

micro-CT, at baseline (natural tooth surface), and after every wear stage. The capability of CP-OCT to measure the thickness of natural-surface and worn-surface enamel and to differentiate the wear depths was assessed and compared to the gold-standard method.

2.2. Natural-surface enamel specimen preparation

Extracted intact human molars previously collected and stored in a tooth bank (IRB approval #NSO 911-07) were used to obtain the study's specimens. All the teeth were deidentified. Ten enamel slabs ($4 \text{ mm} \times 4 \text{ mm} \times 2 \text{ mm}$) were cut from smooth surfaces of the molars using a low speed saw (IsoMet, Buehler, Lake Bluff, IL). Then, they were mounted on an acrylic polishing block with the dentin side facing upward and stabilized with sticky wax. The specimens' dentin surface was flattened with #500 grit SiC paper (MDFuga, Struers) on an automated grinding and polishing machine (Rotoforce-4, Struers Inc.) under running deionized (DI) water. An orientation notch for remounting and scanning was prepared on the center of the outer dentin edge using a round carbide bur in a low-speed handpiece. Specimens were then removed from the block, cleaned of wax and each stored individually in a closed labeled vial in humid conditions to prevent specimen dehydration.

2.3. Wear stages

The specimens' enamel surface was reduced in five stages: Wear 1 (to flatten the enamel surface); Wear 2, 3, 4 and 5 (target wear depth: $0.05 \pm 0.02 \text{ mm}$ for each stage). At each wear stage, the specimens were re-mounted on an acrylic polishing block with the enamel surface facing upward and stabilized with sticky wax. On the automated grinding and polishing machine, the enamel surface was reduced with #1200 SiC paper then polished with #2400 and #4000 grit SiC papers and finally with $1\text{-}\mu\text{m}$ diamond suspension on a polishing cloth. The grinding time was guided by target wear depth; thus, specimens' thicknesses were measured with a digital micrometer indicator (Brown & Sharpe Digit-Dial Plus Indicator #599-1033) before, during and after the grinding procedure. Each sample thickness measurement with the digital micrometer was an average of two measurements. After grinding/polishing, the specimens were rinsed with DI water for 3 min, cleaned in an ultrasonic bath with a detergent solution for 3 min then rinsed with DI water for 3 min. Specimens were then removed

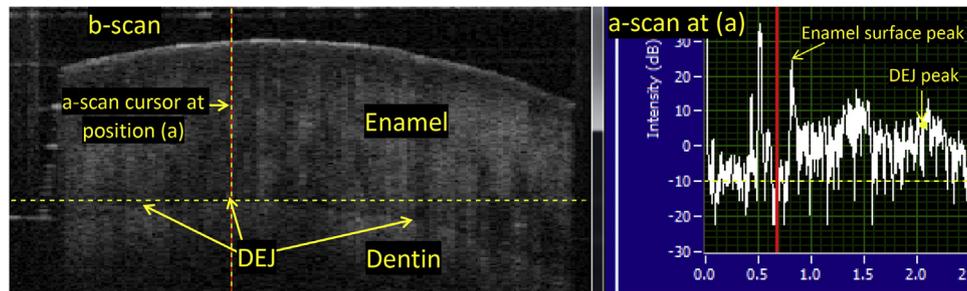


Fig. 1 – CP-OCT b-scan (left) and a-scan (right) analysis for enamel thickness measurements.

from the mounting block, cleaned and stored as described in subsection 2.2.

2.4. Enamel thickness and wear depth evaluation with CP-OCT

A cross-polarization OCT (CP-OCT) dental imaging device (Santec Inner Vision IVS-300-S-L-C; Santec Corp, Komaki, Japan) was used in this study to obtain and analyze cross-sectional images of the enamel. The device uses a swept source laser light at a center wavelength of 1310 ± 30 nm, with a scan rate of 30 kHz. The device's maximum lateral scanning area was $5 \text{ mm} \times 5 \text{ mm}$ with axial and lateral resolutions (in air) of $12 \mu\text{m}$ and $30 \mu\text{m}$, respectively. The range of axial imaging (in air) was $>5.6 \text{ mm}$ and the depth of focus was 3 mm.

Three specific positions on the specimen were assigned to obtain the enamel thickness measurement by CP-OCT at every measurement time point (for natural surface testing and for worn surface testing from Wear 1 to Wear 5). Consistent identification of the three sites was achieved by obtaining a 3D CP-OCT scan of the whole specimen, followed by determination of the center and thirds (by the aid of screen ruler) of the middle Y plane CP-OCT slice. Therefore, there was no need for a specimen and scanning probe positioning jig to determine the measurement positions precisely before and after wear stages.

The specimen to be scanned was gently air dried for 10 s then positioned under the sensor of the CP-OCT probe with the notch oriented toward the probe handle. In the scanning and analysis software (Inner Vision IVS-300, Santec Corp, Komaki, Japan), a 3D scan ($5 \text{ mm} \times 5 \text{ mm} \times 5.6 \text{ mm}$) was obtained for each entire specimen at refractive index = 1.6 [9]. After scanning, the specimen was kept in the storage vial. For randomized and blinded analysis, specimen codes were generated and used to rename the 3D scans. From each 3D scan, the central b-scan in the Y direction was selected and saved to be evaluated for enamel thickness measurements. On the b-scan, three measurement positions were located at the half (b) and thirds (a and c) of the enamel width using a screen ruler (A ruler for windows v3.3.3) (Fig. 1). Enamel thickness distance in mm (from DEJ to surface of the specimen) was measured at each of the three positions by measuring the distance between the depth of light intensity change at the enamel surface and the DEJ from each a-scan (Fig. 1).

CP-OCT scans were obtained and analyzed for enamel thickness measurement before wear (natural-surface enamel) and after every wear stage. The wear depth measurement by CP-OCT was estimated by subtracting the enamel thickness measurement after each wear stage from Wear 1 measurement.

2.5. Enamel thickness and wear depth evaluation with micro-CT

An X-ray micro-computed tomography (micro-CT) scanner (Skyscan1172, Bruker microCT) was used as a gold-standard method to obtain cross-sectional images of the enamel. The specimens were scanned at 59 kV and $167 \mu\text{A}$ with 0.5 mm aluminum filter using medium binning mode and an image pixel size set at $5.88 \mu\text{m}$ [9].

The scans were reconstructed on the reconstruction software (NRecon v1.7.3.1, Skyscan, Bruker microCT) using smoothing at 2, post alignment at -5, ring artifact correction at 20, beam hardening correction at 41, and the minimum and maximum attenuation coefficient values at 0 and 0.329744, respectively.

The reconstructed scans were viewed on the associated software (DataViewer v1.5.6.2, Skyscan, Bruker microCT) to obtain the central X-Z image that corresponds to the CP-OCT b-scan position. To locate the central X-Z image, the reconstructed scan was viewed on the X-Y plane and oriented until the notch was facing the lower border of the viewer. Then, the screen ruler was used to locate half of the enamel width on the Y-axis. At the located point, the X-Z image was viewed and saved for enamel thickness measurement.

Then, the saved X-Z image ($8.817 \text{ mm} \times 3.527 \text{ mm}$) for each specimen was converted into a bitmap with a scale bar using the analysis software (CT analyzer v1.17.7.2+, Skyscan, Bruker microCT). Specimen codes were generated and used to rename the images for blinded and randomized analysis.

The coded images were opened in Image J (ImageJ 1.52a, NIH) for enamel thickness measurement. On the image, three measurement positions were located at the half (b) and thirds (a and c) of the enamel width using the screen ruler (A ruler for windows v3.3.3) (Fig. 2). Enamel thickness distance in mm was measured at each of the three positions by measuring the distance between the enamel surface and DEJ using the straight line tool (Fig. 2).

Micro-CT images were obtained and analyzed for enamel thickness measurement before wear (natural-surface enamel)

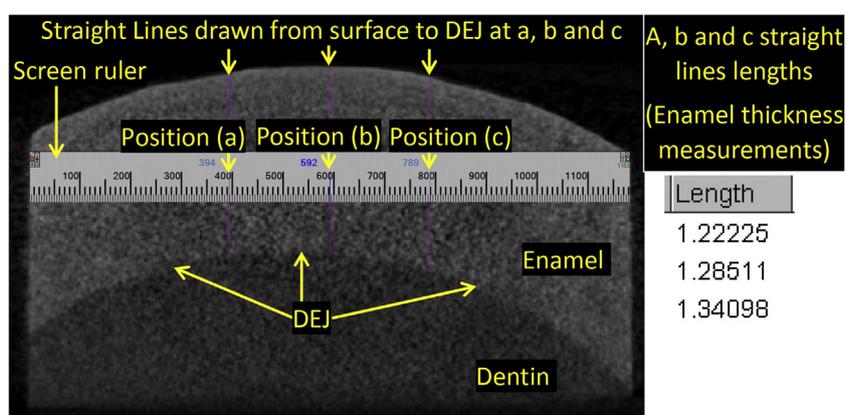


Fig. 2 – Micro-CT image analysis for enamel thickness measurements.

and after every wear stage. The wear depth measurement by micro-CT was estimated by subtracting the enamel thickness measurement after each wear stage from Wear 1 measurement.

2.6. Statistical analysis

Agreement between CP-OCT and micro-CT for thickness measurement of natural-surface enamel was evaluated using an intra-class correlation coefficient and the significance of the difference between the methods' means. Agreements between the methods for thickness measurement of worn enamel and wear depth estimation were evaluated using intra-class correlation coefficients, the significance of the difference between the methods' means, and Bland-Altman plots.

The effects of wear stages on enamel thickness and wear depth measurements by micro-CT and CP-OCT were tested using ANOVA followed by pairwise comparisons. The ANOVA included a random effect to account for correlations among the three enamel positions within each specimen, and a repeated effect to account for the correlations among the wear stages. A 5% significance level was used for all the tests. Statistical analysis was performed with SAS 9.4 (SAS Institute Inc., Cary, N.C., USA).

3. Results

3.1. Thickness measurement of natural surface enamel by CP-OCT and micro-CT

The mean of enamel thickness measurements for natural surface enamel by CP-OCT (1.40 mm) did not significantly differ from the mean measurements by micro-CT (1.39 mm; p -value = 0.30). There was excellent agreement between CP-OCT and micro-CT for thickness measurement of natural-surface enamel (ICC 0.98, Table 1).

3.2. Thickness measurement of worn enamel

Following enamel wear, the mean of enamel thickness measurements by CP-OCT (1.08 mm) did not significantly differ from the mean measurements by micro-CT (1.09 mm; p -

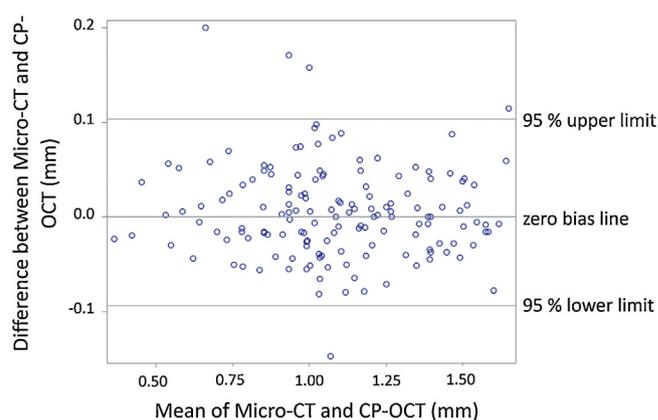


Fig. 3 – Bland-Altman plot representing the mean (in X-axis) and the difference (in Y-axis) between micro-CT and CP-OCT enamel thickness measurements pairs.

value = 0.39). There was an excellent agreement between CP-OCT and micro-CT for thickness measurement of worn enamel (ICC 0.99, Table 1). A Bland-Altman plot (Fig. 3) did not show a specific pattern of disagreement between the two methods and shows an agreement limit of approx. ± 0.10 mm.

Among wear stages, there were significant differences in enamel thickness measurements between all wear stages for both methods, the micro-CT and CP-OCT (p -value < 0.0001 for all; Table 2).

3.3. Wear depth estimation

The estimated wear depth measurements at each wear stage compared to wear 1 are presented in Table 2. There were significant differences in wear depths between all wear stages for both methods, the micro-CT and CP-OCT (p -value < 0.0001 for all). The mean of wear depth measurements by CP-OCT (0.14 mm) did not significantly differ from the mean measurements by micro-CT (0.14 mm; p -value = 0.87; Table 3). There was a good agreement between CP-OCT and micro-CT for wear depth estimation (ICC 0.77, Table 3). A Bland-Altman plot (Fig. 4) did not show a specific pattern of disagreement between the two methods for wear depth estimation and shows agreement limits of approx. ± 0.12 mm.

Table 1 – Enamel thickness (mean \pm SE and range, in mm) by CP-OCT and micro-CT obtained at baseline (natural surface) and following enamel wear.

Enamel Thickness	CP-OCT		Micro-CT		CP-OCT vs. micro-CT	
	Mean \pm SE	Range	Mean \pm SE	Range	p-value	ICC
Natural surface	1.40 \pm 0.05	0.81–1.81	1.39 \pm 0.05	0.81–1.83	0.30	0.98
Worn surface	1.08 \pm 0.02	0.38–1.64	1.09 \pm 0.02	0.35–1.71	0.39	0.99

ICC, intra-class correlation coefficient; SE, standard error.

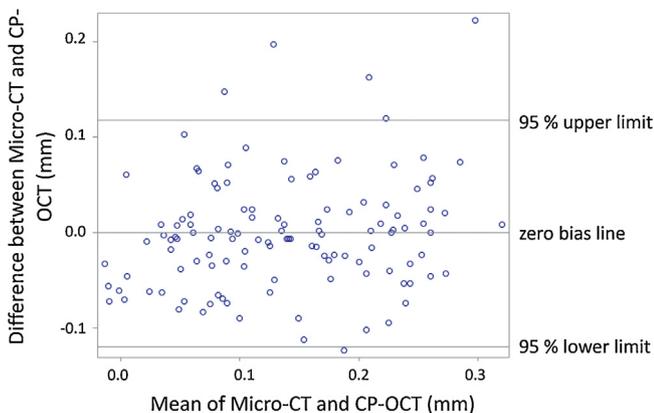
Table 2 – Comparison of enamel thickness measurements and wear depth measurements in mm among wear stages.

Wear stage	CP-OCT				Micro-CT			
	Enamel Thickness		Wear depth*		Enamel Thickness		Wear depth*	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Wear 1	1.19 \pm 0.28 ^a	0.56–1.64	—	—	1.20 \pm 0.26 ^a	0.71–1.67	—	—
Wear 2	1.15 \pm 0.28 ^b	0.55–1.59	0.04 \pm 0.03 ^a	–0.03–0.09	1.16 \pm 0.28 ^b	0.60–1.71	0.04 \pm 0.05 ^a	–0.05–0.16
Wear 3	1.09 \pm 0.27 ^c	0.53–1.58	0.10 \pm 0.03 ^b	0.03–0.16	1.09 \pm 0.27 ^c	0.53–1.57	0.10 \pm 0.05 ^b	0.02–0.23
Wear 4	1.03 \pm 0.29 ^d	0.43–1.55	0.17 \pm 0.04 ^c	0.06–0.25	1.03 \pm 0.28 ^d	0.47–1.52	0.16 \pm 0.06 ^c	0.06–0.29
Wear 5	0.95 \pm 0.27 ^e	0.38–1.42	0.24 \pm 0.03 ^d	0.19–0.32	0.96 \pm 0.28 ^e	0.35–1.51	0.24 \pm 0.05 ^d	0.16–0.41

The different uppercase letters within mean \pm SD column indicate significant difference (p-value < 0.0001); * Enamel thickness subtracted from Wear 1.

Table 3 – Mean \pm SE and range of wear depth measurements (estimated following Wear 2, 3, 4 and 5) in mm by CP-OCT and micro-CT with a p-value of inter-method measurements difference and intra-class correlation coefficient value.

Wear depth	CP-OCT		Micro-CT		CP-OCT vs. micro-CT	
	Mean \pm SE	Range	Mean \pm SE	Range	p-value	ICC
Wear 2 to Wear 5	0.14 \pm 0.01	–0.03–0.32	0.14 \pm 0.01	–0.05–0.41	0.87	0.77

**Fig. 4 – Bland-Altman plot representing the mean (in X-axis) and the difference (in Y-axis) between micro-CT and CP-OCT wear depth measurements pairs.**

4. Discussion

In the present in vitro study, we evaluated the effect of natural and worn enamel surfaces on enamel thickness measurements by CP-OCT, and assessed the capability of CP-OCT to monitor simulated incremental enamel wear.

As the DEJ landmark was identified in all CP-OCT scans, we were able to obtain measurements in all specimens with natural surface enamel. As the tooth specimens had variable

enamel thicknesses, CP-OCT also allowed us to identify a wide range of enamel thicknesses, ranging from 0.81 mm to as high as 1.81 mm.

CP-OCT measurements of enamel thickness with natural unpolished surface agreed very well with the measurements by micro-CT. Both methods yielded nearly similar measurement means and they exhibited an excellent ICC value. Our findings also support the fact that the optical penetration of cross-polarization images of polarization-sensitive OCT device is unaffected by surface topography of the tooth, which is in agreement with Jones et al. [10]. Our results indicate that natural enamel surface did not impact the enamel thickness measurement by CP-OCT and support the use of CP-OCT for an accurate baseline evaluation of enamel thickness.

OCT along with ultrasonography are in vivo non-invasive imaging technologies that work by the same principle of utilizing non-ionizing radiation (light and sound, respectively) and by analyzing the back-reflected waves from the examined tissues to construct structural images. Their ability to determine enamel thickness has shown to be encouraging. Ultrasound measurements of enamel thickness showed an excellent agreement with histology (ICC of 0.97, Bland-Altman plot limits of agreement from –0.17 to 0.21 mm) [11]. However, the curvature of the natural enamel surface and the DEJ, whether on occlusal or smooth surfaces of teeth, pose a challenge in ultrasound probe positioning and imaging [11–15]. On the other hand, our study shows that the natural curvature in the smooth surfaces of molars did not affect the measurements by CP-OCT.

CP-OCT also allowed enamel thickness measurement after wear simulation. We were able to measure the thickness in all worn specimens, ranging from 0.38 to 1.64 mm. These findings are consistent with the findings obtained for sound flattened enamel [9]. There was an excellent agreement between the thickness measurements of worn enamel by CP-OCT and the measurements by the gold-standard method. Based on the inter-method agreement analysis, the performance of CP-OCT in the current study for enamel thickness measurement seems to be superior to the co-polarization OCT imaging reported in a previous study [9]. The difference in mean measurements was significant between co-polarization OCT and micro-CT (0.06 ± 0.02 mm; ICC of 0.95) [9], whereas the difference was not significant between standard method and CP-OCT in the current study (0.01 ± 0.01 mm, ICC of 0.95).

CP-OCT was able to monitor enamel thickness along the five stages, as the measurements were decreasing with wear progression. Also, CP-OCT was able to differentiate the thickness at each wear stage from the other stages as exhibited by significant differences in measurements among wear stages, which was comparable to the micro-CT findings (Table 2).

The magnitude of incremental wear we created in this study allowed us to evaluate the ability of the CP-OCT to detect the small wear changes. The CP-OCT wear depth measurements were comparable to the measurements by micro-CT, which ranged from 0.04 ± 0.03 mm to 0.24 ± 0.03 mm. This similarity in measurements between the two methods was also evidenced by the good inter-method agreement (ICC of 0.77). The significant differences we found in CP-OCT wear depth measurements among and between the wear stages indicate that CP-OCT allowed differentiation of small changes in wear depth between each wear stage. Despite the accurate measurements by CP-OCT, the method presented low measurement precision for wear depth measurements compared to micro-CT, as depicted from the limits of agreement (approximately ± 0.12 mm) in the Bland-Altman plot (Fig. 4).

There is limited information on the ability of OCT to quantify enamel thickness changes by wear smaller than 0.1 mm. Kim et al. [16] used OCT as a gold-standard to evaluate QLF's ability to measure occlusal enamel thickness measured every 100 μ m. Oguro et al. [17] used OCT to measure enamel thickness of labial surfaces of incisors following serial enamel trimming by approximately 100 μ m for evaluation of enamel thickness effect on the tooth color. Because OCT performance was not the main focus in these studies, there was no information on the challenges encountered or the limitations observed with enamel thickness measurement by OCT.

In the current study, we demonstrated that using the DEJ on an OCT image as a reference is a suitable approach for quantifying and monitoring enamel thickness changes. This method involves scanning the area directly on the tooth, thus it eliminates the problem of dimensional changes with impressions in an indirect tooth wear analysis [18]. The CP-OCT results in our study compared well with micro-CT, the most appropriate non-destructive method available to date for enamel thickness measurement. We chose micro-CT as a gold standard method in this study because it provides tomographic images similar to OCT and it allows the output 2D images to be viewed on any plane from a 3D image (b-scans). In addition, micro-CT has been used for enamel thickness measurement in

previous *in vitro* studies focusing on clinical and anthropological applications [9,19–21]. Also, this method is non-destructive and allows repeated measurements from the same position in the specimen. It is noteworthy that the wear depth measurements utilizing CP-OCT exhibited lower variability than those with micro-CT, which could be interpreted as a superior performance of CP-OCT for wear depth estimation. A possible source of discrepancy in measurements within each of the methods (micro-CT and CP-OCT) might be related to errors in the localization of the measurement position and the DEJ landmark, as noted in our previous work [8]. Despite possible limitations, micro-CT was the most appropriate gold standard method available for this study. Histological analysis could also be considered for the cross-sectional measurement of enamel thickness as done in Algarni et al. (2016); however, it would not allow repeated measures in the same specimens as done in this study.

This study evaluated the performance of CP-OCT on smooth surfaces of molars; thus, the results can be projected clinically to the accessible smooth surfaces of teeth only. It is still necessary to verify that results would be similar on more complicated topographies, such as the occlusal and incisal tooth surfaces. Besides, it is still not known whether the change in the probe angulation, which may be necessary for scanning lingual surfaces of teeth, would affect the quality of the scans and measurements. Since we have tested the performance CP-OCT under a controlled specimen hydration condition, the degree of teeth hydration is another aspect that need to be evaluated to ensure the usefulness of the method in the variable hydration environment intraorally. It was evident from the previous studies that enamel surface roughening with the silicon carbide grinding papers [8] and pumice prophylaxis [22] did not affect the CP-OCT enamel thickness measurement. Further testing involving enamel abrasive wear combined with other intra-oral factors, such as dental erosion and dental pellicle, on the clinical monitoring of tooth wear by CP-OCT would allow the better understanding of this method's capabilities and limitations.

The performance of the tested method indicates that it can be a viable option to clinically monitor enamel wear longitudinally for patients at high risk of tooth wear.

Declarations of interest

None.

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