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Longitudinal assessment of dental erosion-abrasion by cross-polarization optical coherence tomography *in vitro*

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Abstract: This study tested a novel *in vitro* dental erosion-abrasion model and the performance of cross-polarization optical coherence tomography (CP-OCT) in longitudinally monitoring the simulated lesions. Thirty human enamel specimens were prepared and randomized to receive three dental erosion-abrasion (EA) protocols: severe (s-EA, lemon juice/pH:2.5/4.25%w/v citric acid), moderate (m-EA, grapefruit juice/pH:3.5/1.03%w/v citric acid) and no-EA (water, control). EA challenge was performed by exposing the specimens to acidic solutions 4x/day and to brushing 2x/day with 1:3 fluoridated toothpaste slurry, for 14 days. Enamel thickness measurements were obtained using CP-OCT at baseline (D0), 7 (D7) and 14 days (D14) and micro-computed tomography (micro-CT) at D14. Enamel surface loss was measured with both CP-OCT and optical profilometry at D0, D7 and D14. Data was analyzed with repeated-measures ANOVA and Pearson's correlation (r) ($\alpha = 0.05$). CP-OCT enamel thickness decreased over time in the s-EA group (D0 >D7 > D14, $p < 0.001$) and m-EA group (D0 > D14, $p = 0.019$) but did not change in the no-EA group ($p = 0.30$). Overall, CP-OCT and micro-CT results at D14 correlated moderately ($r = 0.73$). CP-OCT surface loss was highest for s-EA ($p < 0.001$) but did not differ between moderate and no-EA ($p = 0.25$). Enamel surface loss with profilometry increased with severity (no-EA>m-EA>s-EA, $p < 0.001$). D14 surface loss was higher than D7 for both methods except for the no-EA group with profilometry. CP-OCT and profilometry had moderate overall correlation ($r = 0.70$). Our results revealed that the currently proposed *in vitro* dental erosion-abrasion model is valid and could simulate lesions of different severities over time. CP-OCT was a suitable method for monitoring the EA lesions.

Keywords: Tooth Wear; Dental Enamel; Tomography, Optical Coherence.

Introduction

The irreversible nature of erosive tooth wear (ETW) substantiates the need for emphasis on the early detection, monitoring and use of specific preventive measures.[1] This is because in its advanced stages, the treatment of ETW could become more difficult and case prognosis could become poorer. However, the differential diagnosis of early ETW lesions and their

clinical monitoring can be difficult,[2] mostly due to the multitude of factors involved in their development and the lack of objective outcome measures.[1]

From a clinical standpoint, ETW is predominantly a surface phenomenon and its diagnostic procedure as well as progression assessment is traditionally done by visual examination, which is limited by its subjective nature.[3] The use of objective methods for the clinical assessment of ETW has been suggested,[4] and among them optical coherence tomography (OCT) stands out.[5,6] OCT allows for quick, safe, non-destructive, and repetitive measurements of enamel thickness;[7] and previous studies have shown promising results.[5,6,8,9] In order to improve some limitations on this method, such as surface reflection, variations of OCT were developed.[7] Polarization-sensitive OCT allows for higher contrast between sound and demineralized enamel, as well as for better dentin-enamel junction identification (DEJ).[7]

Along with the advances in ETW diagnosis and monitoring, there is still a need to improve research methods for the development and evaluation of anti-ETW agents. Previously, our group has proposed an *in situ* ETW model[10] that could reliably be used for such purpose. More recently, it was confirmed that cross-polarization optical coherence tomography (CP-OCT) was suitable for monitoring ETW lesions of different severities formed using the said *in situ* model.[11]

Nevertheless, while *in situ* studies are undoubtedly more clinically relevant than *in vitro* experiments, not all research laboratories are equipped to conduct such types of studies. Moreover, when evaluating experimental or novel anti-ETW agents, *in vitro* models would be more suitable in certain cases. The incorporation of mucin in artificial saliva has been found to produce *in vitro* lesions of similar surface loss with that of human saliva.[12] However, most previous *in vitro* models usually only involved short-term protocols.[6,8] It is then necessary to also develop longer-term *in vitro* models of dental erosion and abrasion that could better simulate clinical ETW and provide more reliable conditions for the evaluation of agents that may be used for preventive strategies.

In this study, we tested the performance of CP-OCT in assessing ETW lesions of different severities simulated using a novel *in vitro* dental erosion-abrasion (EA) model that mimicked the conditions of our previous *in situ* ETW model.[11] Objective outcome measures used were enamel thickness and enamel surface loss validated by micro-computed tomography (micro-CT) and optical profilometry, respectively.

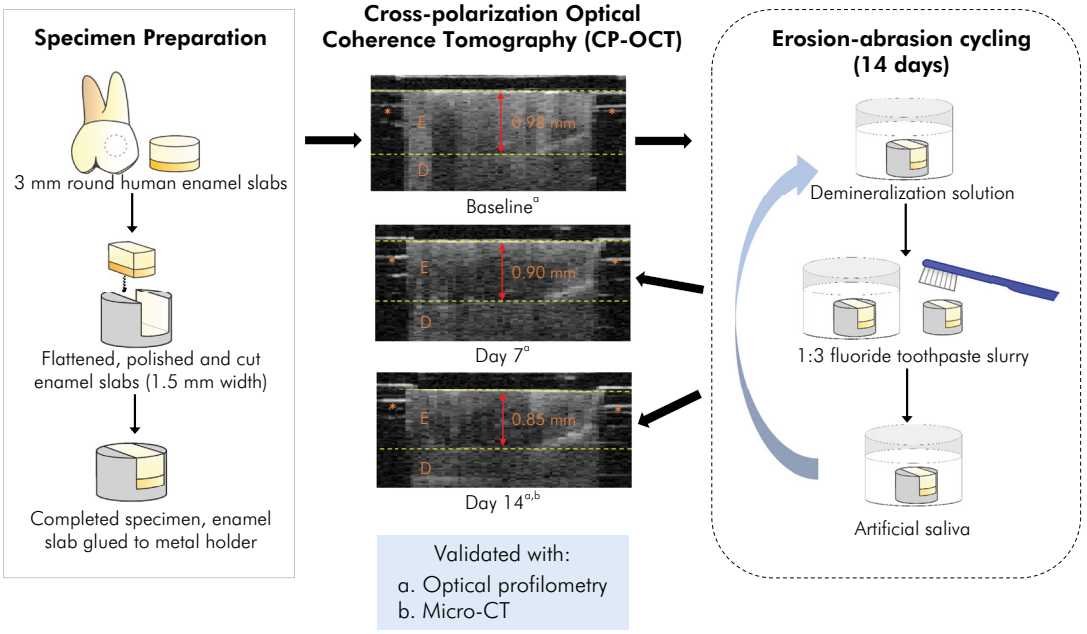
Methodology

Study design

This study followed a blind, randomized 3 × 3 factorial design wherein; EA lesion severity groups: 1) severe (s-EA, lemon juice/pH:2.5/4.25%w/v citric acid), 2) moderate (m-EA, grapefruit juice/pH: 3.5/1.03% w/v citric acid), and 3) no-EA (water, control) were assessed at time points: 1) baseline (D0), 2) Day 7 (D7), 3) Day 14 (D14). Human enamel specimens (n = 10) were prepared and submitted to the longitudinal development of EA lesions simulating different severities by exposure to the different test solutions. The specimens were measured with CP-OCT and profilometry for all time points (D0, D7, D14) and micro-CT at D14. Outcome measures were enamel thickness (CP-OCT and micro-CT) and enamel surface loss (CP-OCT and Profilometry). Figure presents a schematic summary of the study procedures and representative CP-OCT images of a specimen from the s-EA group over time with corresponding enamel thickness measured from the enamel surface to the DEJ.

Specimen preparation

Round-shaped human enamel slabs (of 3 mm diameter and 2 mm thickness) were cut from extracted permanent molars that were cleaned and kept in 0.1% thymol solution at 4°C. A total of thirty enamel specimens were prepared as previously described.[10] Briefly, the pulpal side of the cut enamel slabs were ground flat to a uniform thickness of 1.8 ± 0.2 mm. The top side (enamel) of the slab was then flattened and serially polished using 1,200- to 4,000-grit paper followed by 1-μm diamond polishing suspension to a final thickness of 1.5 ± 0.1 mm, with at least 1 mm of which was enamel. The sides of the slabs were cut with a precision cutting machine, so that their



E: enamel; D: dentin; *: reference metal specimen holder.

Figure. Schematic summary of the study procedures and CP-OCT analysis.

width was 1.5 mm. The enamel slabs were then cleaned under sonication and examined using a stereomicroscope. Slabs that were free from cracks, defects, or white spots were selected and glued into stainless steel holders using cyanoacrylate adhesive for medical use (Prism 4541; Loctite; Henkel, Rocky Hill, USA), with primer and accelerator (Prism 7701, 713; Loctite). The grinding and polishing procedures were repeated, so that the surfaces of the enamel and the metal holders were leveled, flattened, and highly polished. Baseline surface loss measurements were taken using optical profilometry (details below) and specimens that showed vertical differences of > 1 µm between enamel and metal surfaces were further rejected. Accepted specimens were randomly assigned to the different groups (n = 10).

Dental erosion-abrasion lesion simulation

A summary of the daily cycling protocol involving erosion-abrasion procedures can be seen in Table 1. Both pH and titratable acidities of the erosive solutions were measured in triplicate prior to the start of the experiment. Erosive challenge was done 4x/day for 14 days by immersion of the dental specimens for 5 minutes in 10 ml, per specimen, of their respective acidic solutions: pure lemon juice (Organic Pure Lemon Juice; Santa Cruz Natural Inc., Chico, USA) for the s-EA group; pure grapefruit juice (100% white grapefruit juice; Ocean Spray Cranberries, Lakeville-Middleboro, USA) for the m-EA group, and bottled water (Drinking Water, Kroger Co., Cincinnati, USA) for the negative control, no-EA group. The enamel specimens were manually brushed twice daily (after the first and last acid immersion) for 15 s using a soft-bristled manual toothbrush (Oral-B, Procter & Gamble, Cincinnati, USA) and 45 ml, per specimen, of 1:3 slurry of fluoridated toothpaste (Crest Cavity Protection (0.243% NaF/ 0.15%w/v F^-), Procter & Gamble, Cincinnati, USA) to simulate usual toothbrushing regimen. All the brushing procedures were performed by a single operator. The total exposure time of the specimens to the slurries was 2 min. After brushing, the specimens were rinsed with deionized water. When not subjected to the erosive and abrasive procedures, specimens were kept in a remineralization solution (1.45 mM Ca, 5.4 mM PO4, 0.1 M Tris buffer, 2.2 g/L porcine gastric mucin, pH 7.0)[12] under slight agitation at room temperature. The remineralization solution was renewed after every 24 h cycle.

Table 1. Daily erosion-abrasion cycling schedule.

Variable	Sequence	Procedures
1	Demineralization	5 min
	Manual brushing/slurry exposure	15 s (toothpaste slurry)
	Remineralization	60 min
2	Demineralization	5 min
	Remineralization	60 min
3	Demineralization	5 min
	Remineralization	60 min
4	Demineralization	5 min
	Manual brushing/slurry exposure	15 s (toothpaste slurry)
	Remineralization	60 min
5	Remineralization	Overnight storage to complete 24 h

Enamel thickness measurement

CP-OCT scanning and analysis

The specimens were kept moist in a container and were taken out and gently blotted dry prior to scanning to prevent dehydration. The specimens were then scanned and analyzed using a dental CP-OCT system (Santec Inner Vision IVS-300-S-L-C; Santec Corp, Komaki, Japan). Parameters similar to those previously used[6,8,13] were adopted. In brief, the CP-OCT system used a swept laser light with a center wavelength of 1,310 nm, a-scan rate of 30 kHz and imaging depth range (in air) of > 4 mm. Axial and lateral resolutions (in air) were ≤ 12 µm and 30 µm, respectively. The specimen to be scanned was positioned on an X-Y and Z translation stage and under the CP-OCT sensor, which was fixed in a positioning arm. The refractive index was set at 1.6 for enamel. 3D scans were acquired using the scanning probe which had a lateral scanning area of 5 × 5 mm and working distance of 1 mm. Central b-scans (2D image) in the X direction were selected from each 3D scan and saved for measurements. All saved 2D image files were then randomized for blind analyses in terms of both time and severity.

Enamel thickness measurements (from DEJ to surface of the specimen) on the 2D images were performed using Santec Inner Vision IVS-300 software (Santec Corp, Komaki, Japan) as previously described.[6] The measurement position was identified at the center of the enamel width with the aid of a screen ruler (A Ruler for Windows v3.3, Rob Latour). The distance in mm between the depths of the highest light intensity peaks at the enamel surface and DEJ areas was then calculated from the a-scan. CP-OCT scanning and enamel thickness measurements were performed at D0, D7 and D14.

Micro-CT scanning and analysis

The enamel specimens were removed from the metal specimen holders after D14 and were scanned with a micro-computed tomography scanner (SkyScan1172; Bruker microCT, Kontich, Belgium) to produce high-resolution 3D models. The specimens were then mounted on a rotary stage and scanned at 59 kV and 167µA with an X-ray beam perpendicular to the long axis of the specimen. A 0.5 mm aluminum filter was used for beam-hardening correction. Data were acquired and reconstructed applying previously used specifications.[5,6] Scan reconstruction was performed using NRecon v1.7.3.1 (Skyscan, Bruker microCT) and 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 2D scan position. The saved central X-Z images were then converted to bitmap (CT analyzer v1.17.7.2+, Skyscan, Bruker microCT) and were opened in Image J (ImageJ 1.52a, NIH) for enamel thickness measurements. Measurement positions were determined by locating the midline of the enamel width using the screen

ruler (A Ruler for Windows v3.3.3). Enamel thickness was measured as the distance between the enamel surface and DEJ in mm using the straight-line tool at the measuring position.

Enamel surface loss measurement

CP-OCT analysis

Enamel surface loss with CP-OCT was determined as the subtracted difference of enamel thickness measurements at D7 and D14 from baseline values.

Optical profilometry scanning and analysis

Optical profilometry scans were obtained at D0, D7, and D14. The specimens were fixed on acrylic blocks with wax and then scanned using an optical profilometer (Proscan 2000; Scantron, Taunton, UK) with an accuracy of 0.1%, a precision (SD) of ± 0.06 µm, and a detection limit of < 0.3 µm. The step size was set at 0.01 mm and the number of steps at 200 in the (x) axle; and 0.1 mm and 10, respectively, in the (y) axle. A surface area of 2 × 1 mm was scanned, comprising the reference metal and enamel surfaces. Using a dedicated software (Proscan 2000; Scantron), the depth of the treated enamel area was calculated in relation to the reference surfaces. A three-point height tool was used, which allowed the selection of a 1 × 1 mm area on the center of the enamel and two 1 × 0.5 mm areas on the adjacent metal surfaces. Measurements taken at D0, D7 and at the end of EA cycling, D14, were performed in a similar manner by an analyst blinded to the treatment regimens. Surface loss was calculated for each specimen based on the reference surfaces.

Statistical analysis

The effects of EA (s-EA, m-EA, no-EA) and time (D0, D7, D14) on CP-OCT enamel thickness and enamel surface loss and optical profilometry surface loss were analyzed using ANOVA models with repeated days within specimen. The effect of treatment on micro-CT enamel thickness at D14 was analyzed using one-way ANOVA. A natural logarithm transformation was used for the CP-OCT and optical profilometry surface loss analyses. Pearson's correlation coefficients (r) and scatterplots were used to examine the relationships between the measurements. A 5% significance level was used for all tests.

Results

A significant interaction was observed between EA severity and days for CP-OCT enamel thickness ($p < 0.001$). Results for enamel thickness assessed with CP-OCT and micro-CT and their correlations are shown in Table 2. For s-EA, enamel thickness decreased from D0 to D7 to D14 ($p<0.001$). For moderate EA, enamel thickness decreased from D0 to D14 ($p=0.019$), but D7 was not different from D0 ($p = 0.10$) or D14 ($p = 0.46$). There was no significant difference

Table 2. Mean (standard error) of enamel thickness (mm) with CP-OCT and μ-CT for each erosion-abrasion (EA) severity and their correlation coefficients (r).

EA Severity	Days	CP-OCT	Micro-CT	r	Overall r
None (no-EA)	0	0.89 (0.09)^{a}			
	7	0.89 (0.09)^{a}			
	14	0.88 (0.09)^{a}	0.90 (0.09)	0.98†	
Moderate (m-EA)	0	0.95 (0.09)^{a}			
	7	0.93 (0.09)^{ab}			0.73*
	14	0.92 (0.09)^{b}	0.95 (0.10)	0.8^{8}†	
Severe (s-EA)	0	1.05 (0.09)^{a}			
	7	0.97 (0.08)^{b}			
	14	0.92 (0.09)^{c}	0.94 (0.07)	0.30	

*Similar lowercase letters indicate no difference among the days (0, 7 and 14) within each EA severity ($p > 0.05$); there were no differences among EA severities within any of the study days; *with p-values < 0.001

among days for no-EA (p = 0.30). There were no differences in enamel thickness among the three EA groups (p = 0.76), regardless of treatment time. CP-OCT enamel thickness and micro-CT enamel thickness at D14 were moderately positively correlated overall (highly correlated for no-EA and m-EA but only weakly correlated for s-EA).

CP-OCT surface loss results revealed no significant interaction between EA severity and days (p = 0.10). CP-OCT surface loss was significantly higher for s-EA than m-EA (p < 0.001) and no-EA (p < 0.001) while m-EA and no-EA were not different from each other (p = 0.25). D7 had significantly less surface loss than D14 (p = 0.002). On the other hand, a significant interaction between EA severity and days was observed for optical profilometry surface loss measurements (p < 0.001). For D7 and D14, optical profilometry surface loss increased with EA severity (no-EA<m-EA<s-EA, p < 0.001). No significant optical profilometry surface loss difference was found between days for no-EA (p = 0.92), but for moderate and severe EA, D7 had significantly less surface loss than D14 (p < 0.001). CP-OCT surface loss and optical profilometry surface loss were moderately correlated overall (r = 0.70, p < 0.001) and when grouped according to days (D7: r = 0.66; D14: r = 0.72) but poorly to moderately correlated when grouped according to severity and severity per day (r = 0.08–0.56). Table 3 shows surface loss values for CP-OCT and profilometry for the different ETW severities and time points and their specific correlation coefficients. Correlations between surface loss and enamel thickness were generally negligible (between +/- 0.3).

Discussion

This study tested a novel model for the *in vitro* simulation of dental erosion-abrasion, adopted from our *in situ* ETW model.[10,11] Moreover, it tested the performance of CP-OCT in detecting and monitoring the progression of the simulated *in vitro* lesions longitudinally. Two objective outcomes were studied: enamel thickness and enamel surface loss, calculated as the change in enamel thickness over time. Previous studies have already reported that CP-OCT was useful in assessing these parameters both *in vitro*[6,8] and *in situ*.[11] Nevertheless, earlier *in vitro* studies have only used short-term protocols.[6,8]

The current *in vitro* model simulated ETW lesions similar to those in the more clinically relevant *in situ* model.[10-,1] Such a model would be useful for practical and more reliable laboratory development and evaluation of anti-ETW agents. It involved the use of mucin-containing artificial saliva, which has been reported to lead to similar surface loss results with that of human saliva in enamel erosion-abrasion models *in vitro*.[12] The addition of mucin has allowed the simulation of some functions of the pellicle. The organic layer it formed on the tooth surface has been reported to provide protection against enamel surface demineralization,[14] as well as interfered with remineralization[12] similar to that of human saliva.

However, even if the currently used conditions mimicked that of the *in situ* study,[11] the magnitude of enamel surface loss (measured by optical profilometry) observed *in vitro* was higher than *in situ* (*in vitro*

Table 3. Mean (standard error) surface loss with CP-OCT and OP (μm) after 7 and 14 days for each erosion-abrasion (EA) severity with corresponding correlation coefficients (r).

EA Severity	Days	CP-OCT	OP	r	Severity (r)	Days (r)	Overall (r)
None (no-EA)	7	-1.59 (7.85)^{aA}	2.29 (0.21)^{aA}	0.18	0.23	Day 7:	0.70†
	14	16.96(11.28)^{bA}	2.31 (0.25)^{aA}	0.28		0.6^{6}*	
Moderate (m-EA)	7	22.02 (10.84)^{aA}	12.58 (0.53)^{aB}	0.08	-0.07		
	14	31.65 (15.62)^{bA}	22.61 (0.94)^{bB}	-0.56		Day 14:	
Severe (s-EA)	7	81.03 (18.91)^{aB}	70.86 (0.89)^{aC}	0.23	0.52*	0.72*	
	14	137.67 (14.86)^{bB}	134.09 (2.53)^{bC}	0.28			

*Similar lowercase letters indicate no difference between the days (7 and14) within each EA severity (p > 0.05); similar uppercase letters indicate no difference among EA severities within the same study day (7 or 14); 8with p-values < 0.05.

vs. in situ, at D14: none: 2.31 vs. 0.62 µm; moderate: 22.61 vs. 18.80 µm; severe: 134.09 vs. 98.78 µm). This result was not surprising because even if mucin was present in the artificial saliva used, different biologic factors could not be simulated. As such, other salivary proteins from the acquired pellicle,[15] the saliva buffering and clearing capacities as well as the absence of oral mucosal surfaces for greater fluoride substantivity were not present and is thus a limitation of this *in vitro* study.

Our results for the CP-OCT enamel thickness showed a lack of EA treatment severity effect. This could be attributed to the high variation in enamel thickness among the specimens. Effect of time, on the other hand, was evident for both moderate and severe EA groups but not for the no-EA group, corroborating the results of our *in situ* study.[11] Enamel thickness decreased significantly after every time point with the s-EA group and between D0 and D14 for the m-EA group. There was no difference over time with the no-EA group. These findings suggest that while CP-OCT may not be able to differentiate EA severities cross-sectionally, it can be useful for the longitudinal monitoring the progression of EA lesions within the same tooth.

Overall, the enamel thickness measurements with CP-OCT at D14 correlated moderately with micro-CT. More specifically, a strong correlation was observed for the no-EA and m-EA groups, but a weak correlation existed for the s-EA group. This could be due to the difficulty in locating the actual DEJ in CP-OCT images, especially in several specimens in the s-EA group. Previous studies have similarly found loss of DEJ details in some of their scans.[8,16,17] It was reported that the degree of demineralization and surface micromorphology do not affect CP-OCT measurements.[8] However, differences likely existed in the lesion characteristics of the enamel specimens in the present study from those that had been previously examined.[6,8,11] Present study conditions could have effects on both de- and remineralization, which could respectively increase or decrease enamel surface reflectivity with OCT.[18] Enamel porosity also increases with demineralization and results to higher scattering coefficients.[19] These factors could then affect the OCT optical penetration depth and ultimately, the capability of CP-OCT in assessing enamel thickness and enamel surface loss. Likewise, the hydration state of enamel affects its optical properties.[20,21] Dehydration of demineralized specimens would then cause a greater increase in light scattering due to refractive index mismatch, consequently resulting in higher attenuation and a poorer resolution of the DEJ.[16] Thus, interpretation of results for the s-EA group must be taken with caution due to these limitations.

Similar to CP-OCT, micro-CT offers a non-destructive method that can take repeated measurements from the same position in the specimen.[6,8,22] However, micro-CT tomograms were done only at D14 after the enamel slab was detached from the metal specimen holder, due to the inherent limitations of using metal with a radiographic method. Metal holders were utilized in order to have reliable and stable reference surfaces over the extended period of erosion-abrasion challenges.

The enamel surface loss measured by optical profilometry increased with EA severity (no-EA<m-EA<s-EA), for both D7 and D14, similarly to our *in situ* study.[11] Likewise, no increase in surface loss over time was observed for the no-EA group in both studies, validating this dental erosion-abrasion *in vitro* model. These findings were generally similar to the trends observed with CP-OCT. A moderate overall correlation was observed between our CP-OCT and optical profilometry enamel surface loss results, which was also consistent with our *in situ* findings.[11] Nevertheless, specific correlations according to severity and severity per day were only poor to moderate. Differences in the sensitivity between CP-OCT and optical profilometry have been pointed out before as a possible reason.[8] The axial resolution of CP-OCT at ≤ 12 µm produces less accurate measurements compared to optical profilometry. The smaller range of measurements per group in comparison to the overall range across all severities as well as the lower number of specimens per group in this study compared to the previous *in situ* study[11] could have contributed to the poor individual group correlation but better overall correlation between the two methods.

Conclusions

Our results confirm the validity of this novel *in vitro* erosion-abrasion model, which can potentially be used for future testing of anti-erosion-abrasion agents in the laboratory. Even if the values obtained with CP-OCT were not directly interchangeable with micro-CT and optical profilometry, CP-OCT reflected similar trends for both enamel thickness and enamel surface loss parameters. These findings are consistent with our previous *in situ* study[11] and indicates that CP-OCT can be used to longitudinally monitor the erosion-abrasion lesions formed within the same tooth in this *in vitro* model. Caution however should be exercised when handling specimens of the severe EA group as their propensity for dehydration might negatively affect the CP-OCT applicability for this specific EA model.

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