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Cross-Polarization Optical Coherence Tomographic Assessment of *In situ* Simulated Erosive Tooth Wear

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Abstract

This clinical study tested cross-polarization optical coherence tomography (CP-OCT) monitoring of erosive tooth wear (ETW). Twenty participants completed a 14-day/arm, 3-arm crossover study simulating different ETW severities. Participants received two enamel specimens (per arm) and were randomized to: severe (s-ETW, lemon juice/pH:2.5/4.25%w/v citric acid), moderate (m-ETW, grapefruit juice/pH:3.5/1.03%w/v citric acid), and non-ETW (water). Enamel thickness was measured with CP-OCT (day(D) 0, 7, 14) and micro-computed tomography (μ-CT; D14). Enamel surface loss was determined with CP-OCT and optical profilometry (OP; D7, D14). CP-OCT showed higher enamel surface loss for D14 than D7 for m-ETW (p=0.009) and s-ETW (p=0.040) and differentiated severity at D14 (s-ETW>non-ETW, p=0.027). OP was able to differentiate surface loss between days (D7<D14, p<0.001) for m-ETW and s-ETW, and ETW severity effect after 7 and 14 days (non-ETW<m-ETW<s-ETW, p<0.001). At D14, CP-OCT and μ-CT were positively correlated (r=0.87, ICC=0.62). CP-OCT showed potential as a tool for clinical ETW monitoring.

Graphical Abstract

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CONFLICT OF INTEREST
All authors report no conflicts of interest.

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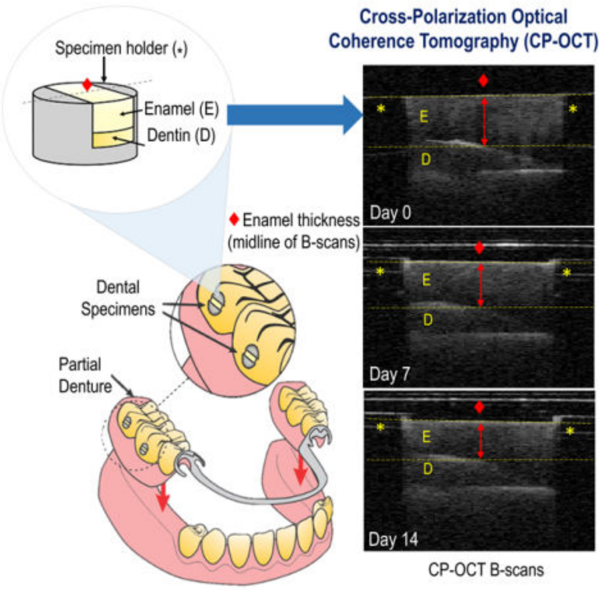

Keywords

Erosive Tooth Wear; Optical Coherence Tomography; Enamel; Dental Erosion

INTRODUCTION

Erosive tooth wear (ETW) is a multifactorial condition clinically manifested by the irreversible loss of tooth structure, affecting its form, function, and esthetics [1]. It is caused by enamel and dentin exposure to intrinsic (gastric) and/ or extrinsic (dietary) acids [2] that soften dental surfaces, leaving them susceptible to abrasive forces [1] from mastication and toothbrushing. ETW affects 30–50% of children and 20–45% of adults [3,4], hence, its management should focus on prevention, early detection and intervention. Currently, clinical assessment and monitoring of ETW lesions are performed through visual examination and scoring using subjective indices [5]. However, subclinical dental structure loss happens during ETW progression even before becoming visually detectable. Therefore, there is a need for objective methods to clinically monitor ETW [6].

Optical Coherence Tomography (OCT) is a promising non-invasive, non-destructive imaging tool used for various biomedical applications including dental caries [7,8] and dental materials [9]. It has also been used to clinically quantify ETW in patients with gastroesophageal reflux [10], evaluate early enamel erosion [11] and more recently, estimate occlusal tooth wear *ex vivo* [12]. The more functionally developed polarization sensitive OCT (PS-OCT) has been used to quantify enamel thickness and surface loss in ETW lesions *in vitro* [13–15]. Polarization influences the visibility of high scattering structures such as dentin, increases its contrast with enamel and enhances dentin-enamel junction (DEJ) resolution [16]. Nevertheless, the ability of PS-OCT, specifically the cross-polarization type (CP-OCT), to monitor ETW in clinically relevant conditions has yet to be investigated.

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This study aimed to validate the performance of CP-OCT under well-controlled and clinically relevant conditions, by using a 14-day *in situ* cross-over ETW experimental model [17] simulating different ETW severities. Enamel thickness and enamel surface loss were used as objective outcomes for CP-OCT and compared to reference methods micro-computed tomography (µ-CT) and optical profilometry (OP).

MATERIALS AND METHODS

Study Design

This study was a randomized, single-blind (analyst), three-treatment, three-arm crossover *in situ* ETW simulation utilizing a previously established model [17]. Subjects were randomized to receive all three ETW-severity protocols: severe (s-ETW, lemon juice/pH:2.5/4.25±0.007%w/v citric acid), moderate (m-ETW, grapefruit juice/pH:3.5/1.03±0.007%w/v citric acid) and non-ETW (water, control) (Figure 1). Each arm lasted 14 days during which subjects wore their mandibular partial denture containing two enamel specimens 24h per day, removing the denture only during extraoral erosive-abrasive challenges (4x/day). Objective outcome measurements using CP-OCT and OP were performed for each within arm time point (D0, D7, D14) while µ-CT was conducted after D14. Outcomes were enamel thickness (mm) and enamel surface loss (µm). Comparative outcome measures assessment across different ETW severities was performed to validate the use of CP-OCT for monitoring of ETW.

Ethics and Participants

The study protocol was reviewed and approved by the Institutional Review Board of the Indiana University-Purdue University Indianapolis (#1810761388). It was conducted at the Oral Health Research Institute (OHRI), Indiana University School of Dentistry following the laws and regulations of the Declaration of Helsinki. Written informed consent was signed by each subject prior to screening. Twenty healthy adults from the OHRI study database were enrolled meeting the inclusion criteria: 18–85 years of age, wearing a removable mandibular partial denture that could accommodate two specimens, stimulated and unstimulated salivary flow rates of ≥0.8ml/min and ≥0.2ml/min, respectively, and willingness to comply with all study procedures. Subjects were excluded if they exhibited untreated cavitated caries lesions, moderate to severe periodontal disease and/or severe dental wear; were unable to comply with study procedures; were pregnant or nursing; or were taking medications that could preclude the safe conduct of a pre-study dental prophylaxis.

Specimen Preparation

One hundred and twenty enamel slabs (3-mm round, 2-mm thick) were cut from extracted human permanent molars [17], had the dentin side ground flat and enamel side flattened and polished to a final thickness of 1.5mm ± 0.1mm. Sides of the slabs were cut to a final width of 1.5mm and cleaned under sonication. Specimens with defects, cracks or white spot lesions were excluded [17]. Qualified slabs were glued to a stainless-steel holder and the specimens were again ground and polished to make the enamel slab and reference metal holder surfaces flat, leveled, and well-polished. Specimens were sterilized using ethylene oxide [17]

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Clinical Phase

Dental prophylaxis and denture cleaning were performed prior to the first arm of this study. Subjects were assigned to one of three crossover randomization schedules (Figure 1). In each arm, two human enamel specimens were cemented (Revotek LC, GC America, Alsip, IL, USA) in the recesses made on the buccal surfaces of adjacent molars of the mandibular partial denture (Figure 2). Baseline CP-OCT scans were obtained. Specimen impressions using vinyl polysiloxane impression material (President Jet Regular Body, Coltene Whaledent, USA) were taken for D0 OP measurements. The specimens were brushed (Oral-B 40, Procter and Gamble, Cincinnati, OH, USA) for 10s under tap water prior to impression.

The subjects wore the partial denture 24h a day for the 14-d duration of each study arm, except during the extraoral procedures (Table 1). Erosive challenges were done four times a day: after breakfast, lunch, dinner and before bedtime using: bottled water (Drinking Water, Kroger Co., Cincinnati, OH, USA) for the non-ETW group; pure grapefruit juice, pH 3.5, 1.03±0.007% w/v citric acid (100% White Grapefruit Juice, Ocean Spray Cranberries, Lakeville-Middleboro, MA, USA) for the moderate ETW group; and pure lemon juice, pH 2.5, 4.25±0.007% w/v citric acid (Organic Pure Lemon Juice, Santa Cruz Natural Inc., Chico, CA, USA) for the severe ETW group; following the crossover randomization schedule. Subjects were provided weekly with sufficient test solutions and were instructed to keep them refrigerated. Toothbrushing was standardized using the provided toothbrush (Oral-B 40) and fluoride toothpaste (1,100ppm fluoride, Crest Cavity Protection, Procter and Gamble). When subjects returned at D7 and D14, they brushed their partial denture with water prior to CP-OCT scans and impressions. The specimens were removed from the partial denture at D14 and the enamel slabs were detached from the metal holders for μ-CT scanning.

Daily procedures and study diary completion training was given to the subjects during the first day of each arm. At the end of each arm, remaining products were returned and cross-checked with the study diary to verify compliance. One week washout period was observed between each arm, during which the denture recesses were filled with a temporary restorative material (Coe-Soft, GC America Inc., Alsip IL, USA). After study completion, the recesses of the partial dentures were restored (Tetric, EvoCeram Bulk Fill, Ivoclar Vivadent AG, Schaan, Liechtenstein).

Measurement of Enamel Thickness

CP-OCT Imaging and Analysis—Three-dimensional enamel scans were acquired using a portable dental CP-OCT system with a handheld probe (Santec Inner Vision IVS-300-S-L-C; Santec Corp, Komaki, Japan). The device employed a swept source laser light with a center wavelength of 1310 ± 30nm and high scan rate of 30kHz. The probe had a maximum lateral scanning area of 5×5mm and a working distance of 1mm. Axial imaging (in air) was >5.6 mm with a 3mm depth of focus. Axial and lateral resolutions in air were ≤12μm and 30μm, respectively.

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While cemented to the partial denture, each specimen was gently air dried for ~10s prior to scanning at D0, D7 and D14. A 3D tomogram was obtained for each specimen with its long axis parallel to the long axis of the probe using a dedicated imaging and analysis software (Inner Vision IVS-300, Santec Corp, Komaki, Japan). Refractive index was set to 1.6 for enamel. Subsequently, the central B-scan in the Y-direction was selected from each 3D scan. The saved files of the image data matrices generated from the central B-scans were renamed for blinded analyses. Randomized CP-OCT data were then postprocessed in MATLAB (R2019b, The MathWorks, Inc., Natick, MA) using a customized algorithm and a rotating kernel transformation (RKT) filter [18]. An image depth profile was generated from the enamel surface to the DEJ at the center of the image located using a screen ruler (A Ruler for Windows, version 3.4.2, Rob Latour). Enamel thickness was calculated as the millimeter-converted pixel depth difference between the abscissae of the enamel surface and DEJ signal peaks.

μ-CT Scanning—A micro-computed tomography (μ-CT) scanner (Skyscan 1172, Bruker MicroCT, Kontich, Belgium) was used to create high-resolution 3D models of the enamel specimens. Each enamel slab was subjected to X-ray conditions previously described [15]. Central X-Z images corresponding to central CP-OCT B-scans were located and saved. Subsequently, bitmap-converted images (CT analyzer v1.17.7.2+, Skyscan) were saved using specimen codes then analyzed in ImageJ (ImageJ 1.52a, NIH) for enamel thickness measurements taken as the distance between the enamel surface and DEJ along the specimen midline.

Determination of Surface Loss

CP-OCT Surface Loss Measurements—Surface loss measurements with CP-OCT were assessed after D7 and D14 and were determined for each specimen by subtracting D7 and D14 from D0 CP-OCT enamel thickness values.

Optical Profilometry Scanning and Analysis—Specimen impressions taken at D0, D7 and D14 were scanned by a blinded analyst using an optical profilometer (Proscan 2000; Scantron, Taunton, UK) [17]. Briefly, the profilometry system used had a detection limit of <0.3μm and precision of ± 0.06μm. A 4×4mm area was scanned with 0.02mm step size and 200 steps in both x and y directions. Impression scans were inverted to represent a positive specimen image and analyzed using a dedicated software (Proscan 2000; Scantron). A three-point height tool with auto-leveling function was applied for analysis. A 1×1mm central enamel area and two 1×0.5mm areas on adjacent reference metal surfaces were selected to measure relative enamel depth. Surface loss at D7 and D14 were calculated as the depth measurement difference for each specimen relative to D0.

Statistical Analyses

Effects of ETW severity and time on CP-OCT enamel thickness and surface loss and OP surface loss were analyzed using ANOVA models suitable for a 3-arm 3-treatment crossover design with repeated days within specimen. The models for CP-OCT enamel thickness and OP surface loss accounted for correlation within each subject due to the crossover design, accounted for correlation between specimens within the same treatment for each subject

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with different correlations allowed for each treatment, and allowed different variances and correlations between days within a specimen for each treatment. The model for CP-OCT surface loss was similar except it did not require each treatment to have a different set of variances and correlations between days within a specimen. Effects of treatment on μ-CT enamel thickness at D14 was analyzed using an ANOVA model suitable for a 3-arm 3-treatment crossover design; the model accounted for correlation within each subject due to the crossover design and for correlation between specimens within the same treatment for each subject with different correlations allowed for each treatment. Treatment sequence effect was examined and removed from the model when not significant. A natural logarithm transformation was used for the CP-OCT surface loss, while the ranks of the data were used for the OP surface loss. Intraclass correlation coefficients (ICCs) for agreement, Pearson correlation coefficients (r) for associations, and scatterplots were used to examine the relationships between the measurements. A 5% significance level was used for all tests.

RESULTS

Overall effects

Treatment sequence was not significant and therefore removed from the ANOVA models (p=0.49 for CP-OCT enamel thickness, p=0.84 for CP-OCT surface loss, p=0.66 for OP surface loss, and p=0.83 for μ-CT enamel thickness).

Enamel Thickness

A significant interaction was observed between ETW and days for CP-OCT enamel thickness (p=0.021). ETW severity did not affect CP-OCT enamel thickness (p=0.76 overall, p=0.83 D0, p=0.74 D7, p=0.45 D14) and μ-CT enamel thickness at D14 (p=0.43). Table 2 shows mean enamel thickness values for CP-OCT and μ-CT. For CP-OCT, no difference was observed between days for non-ETW (p=0.26). For m-ETW, however, D7 was significantly higher than D14 (p=0.005), while D0 and D7 were higher than D14 (p=0.030 and p=0.021, respectively) for s-ETW. CP-OCT and μ-CT were strongly positively correlated (r>0.8), but actual agreement between the measurements for the two methods was only moderate (ICC 0.51–0.85). Representative longitudinal CP-OCT B-scans, postprocessed images with RKT filter and their corresponding image depth profiles can be seen in Figure 3.

Surface Loss

Table 3 shows the mean surface loss for CP-OCT and OP after D7 and D14 for each ETW severity. Significant interaction was found between ETW severity and days for CP-OCT surface loss (p=0.041). ETW severity did not affect CP-OCT surface loss for D7 (p=0.21). However, for D14, non-ETW had less CP-OCT surface loss than s-ETW (p=0.027). In terms of days, no significant CP-OCT surface loss difference was found among all time points for non-ETW (p=0.68). For m-ETW and s-ETW, however, D7 had less CP-OCT surface loss than D14 (p=0.009 and p=0.040, respectively).

A significant interaction was similarly observed between ETW severity and days for OP surface loss (p=0.001). For D7 and D14, OP surface loss increased with ETW severity (non-ETW<m-ETW<s-ETW, p<0.001). Comparison between days revealed no difference

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in OP surface loss for non-ETW (p=0.34) or m-ETW (p=0.91). For s-ETW, however, D7 had significantly less surface loss than D14 (p<0.001). Moderate correlation was found between CP-OCT and OP surface loss (r=0.44 for m-ETW and 0.54 for S-ETW), but actual agreement between the measurements for the two methods was low (ICC 0.00–0.46).

DISCUSSION

Our findings showed that CP-OCT has the potential to monitor ETW lesion progression, by measuring changes in enamel thickness, corroborating our previous *in vitro* data [15,19]. However, determination of enamel thickness in OCT-generated images can be challenging, considering the difficulty in accurately locating the anatomical structures involved, especially the DEJ. The cross-polarization OCT system used contributed to the improvement of DEJ resolution, by minimizing image artifacts from enamel hydroxyapatite birefringence and improving enamel-dentin contrast [14,16]. CP-OCT also reduces intensified surface scattering and attenuation from subsurface demineralization and increased surface roughness in ETW lesions to still resolve the DEJ [13]. A previous study showed that surface micromorphology and demineralization severity did not interfere with CP-OCT monitoring of ETW lesions [19]. Despite these CP-OCT advantages, we observed that locating the DEJ, a scalloped structure, can in some cases still be difficult. To further improve our outcomes, we explored the application of an RKT filter that suppresses speckle noise by increasing signal-to-noise ratio and optical penetration [13,18]. The RKT filter was applied during the postprocessing of CP-OCT images; however, a separate analysis was also performed without the RKT filter (data not shown). While the RKT filter was able to enhance the DEJ detection, overall CP-OCT results were not markedly different in this particular study. In order to provide the most optimized data, we chose to present the CP-OCT data with the RKT filter.

The results for CP-OCT enamel thickness confirm the validity of the methods used to simulate different ETW severities in this study. Time-effect was observed for both m- and s-ETW groups but not in the non-ETW control group. Both m- and s-ETW groups showed a significant decrease in CP-OCT enamel thickness at least between D0 and D14. The effect of ETW severity was, however, not observed relative to time, which was probably due to inter-specimen variability. These results suggest the overall ability of CP-OCT to longitudinally monitor ETW through enamel thickness change within the same tooth [10].

Specimen scanning under μ-CT was only performed at D14. Ideally, μ-CT enamel thickness measurements should have been conducted for all time points for better comparison with CP-OCT. However, the use of a metal holder in this *in situ* model precluded μ-CT scanning at D0 and D7 and was therefore a limitation of this study. Nevertheless, CP-OCT enamel thickness measurements at D14 strongly correlated and were in moderate to strong agreement with the gold standard μ-CT across the ETW severities. This suggests that while CP-OCT and μ-CT measurement values cannot be used interchangeably, CP-OCT is reliable for the comparison and monitoring of enamel thickness longitudinally within a study.

Surface loss with CP-OCT showed a numerical increase towards the most severe ETW challenge. However, the large variation observed did not allow for a significant effect at D7.

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But at D14, mean surface loss for s-ETW was greater than non-ETW. This is in contrast with the CP-OCT enamel thickness results wherein no treatment effect was observed. This implies that CP-OCT enamel surface loss might be the more suitable outcome parameter when comparing ETW severities. Some calculated values were negative, which technically imply enamel ‘surface gain’. While saliva and fluoride toothpaste use could positively impact ETW lesions, true surface gain was not expected in the present model. The negative values were likely measurement errors, within the ≤12 μm axial resolution of the CP-OCT system.

Surface loss measured with CP-OCT correlated moderately overall with OP, and correlation coefficients increased with ETW severity. Actual agreement between the two methods was however low. OP is highly sensitive with a <0.3μm detection limit. The poor correlation between the two methods for surface loss measurements in the non-ETW group and the low agreement might have been magnified by the effect of the relatively higher detection error compared to the actual value being measured in CP-OCT compared to OP. Low agreement between the two methods was consistently observed previously [19]. Overall, these results indicate that CP-OCT was suitable for ETW severity differentiation (cross-sectional), and for studying longitudinal changes, when substantial surface loss is involved (approximately >80μm for cross-sectional; and >20μm, longitudinally).

This study used an earlier proposed longer-term ETW *in situ* model [17]. Lemon juice used for s-ETW simulation had lower pH and four-fold higher calculated titratable acidity at pH 7.0 [20] than grapefruit juice (m-ETW), hence had greater erosive potential than the latter [21]. Previous ETW models are mostly of *in vitro* or short-term *in situ* nature. The present model enabled a more clinically relevant but still controlled simulation of ETW lesion formation, while considering the influence of not only chemical but also biological factors such as saliva and the acquired pellicle, as well as the individual’s behavior in relation to erosion-abrasion challenges. The influence of the abovementioned factors resulted in greater variability among lesions formed *in situ* than *in vitro*. With regards to OCT, both saliva and pellicle could affect imaging through alteration in optical path length [13]. Hence, standardized cleaning of specimen surfaces, using toothbrush and water, were employed to minimize these effects prior to CP-OCT imaging. This step could be applied if OCT is to be used clinically. Clinical OCT imaging has elements that were not presently simulated and is thus a limitation of this study. Subsequent validation of direct clinical CP-OCT use for ETW monitoring is then warranted.

Within the limits of the currently adopted ETW model, CP-OCT showed limitations differentiating ETW severities. However, CP-OCT was able to monitor ETW progression within the studied time-frame, while also demonstrating a strong correlation and moderate agreement with μ-CT. This indicates CP-OCT’s potential as an objective tool for clinical longitudinal monitoring of ETW. Further investigations in this area should focus on methods to reduce CP-OCT variability.

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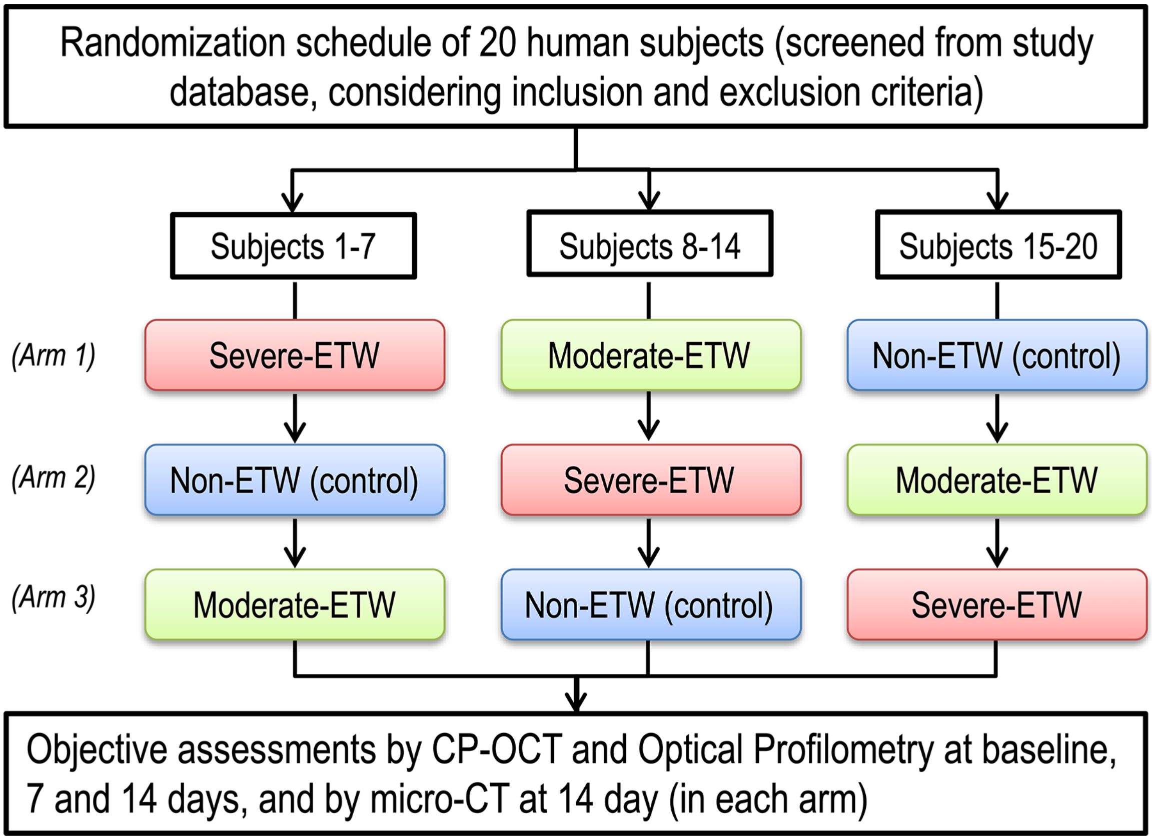

Figure 1.

Experimental design and randomization schedule for this 3-arm, 3-treatment crossover *in situ* ETW simulation study.

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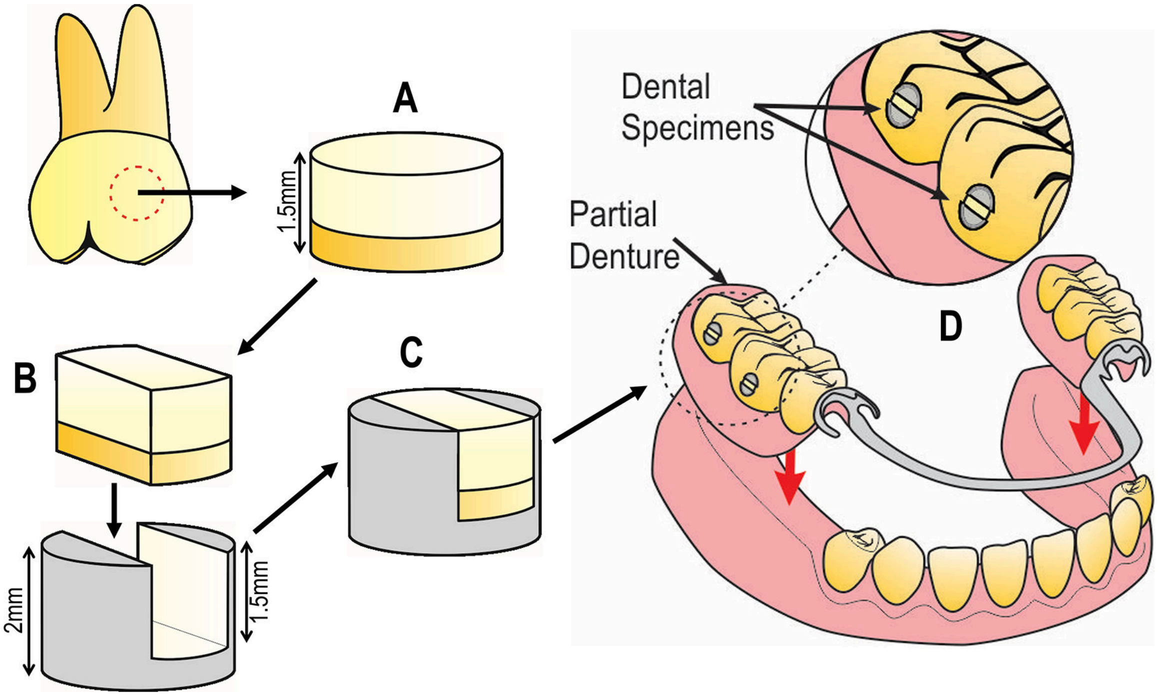

Figure 2.

Schematic representation of the specimen preparation and placement. (A) 3 mm round enamel slab cut from human molars. (B) Flattened, polished and cut enamel slabs to a width of 1.5 mm. (C) Completed specimen with the enamel slab glued to the stainless steel holder. (D) Two specimens attached to the recesses created on the molars of mandibular partial dentures.

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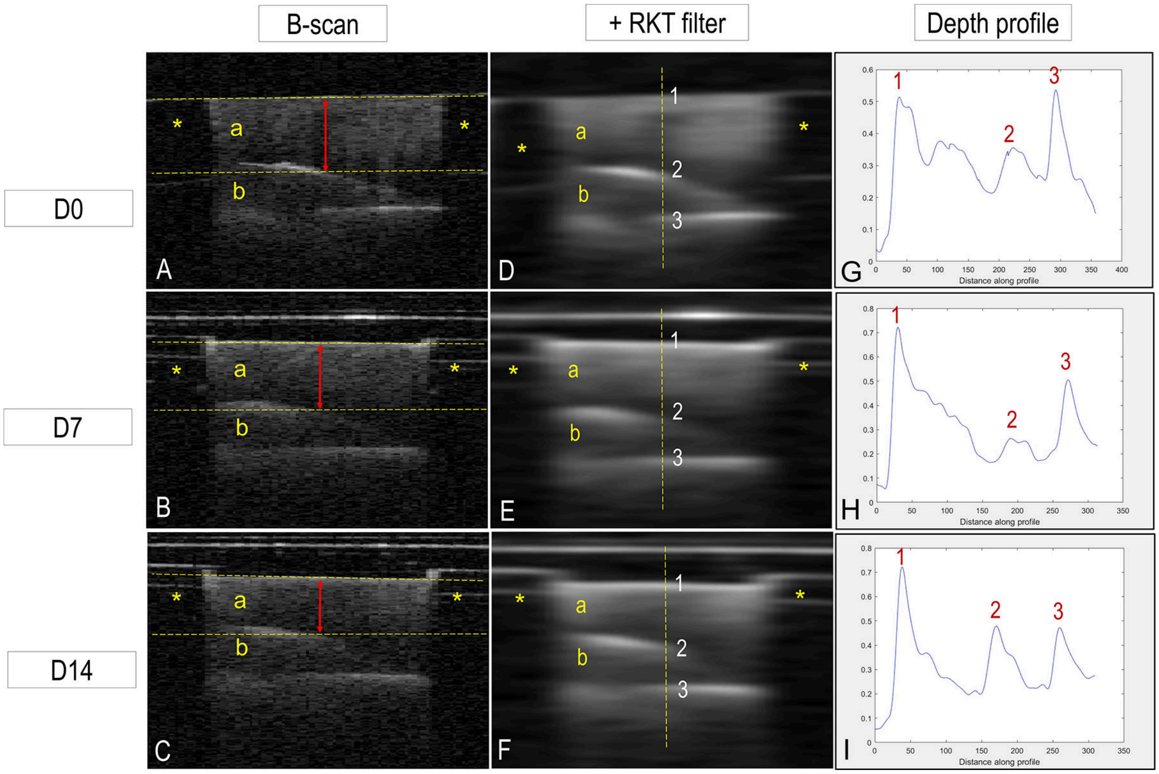

Figure 3.

Longitudinal CP-OCT B-scans (A-C); corresponding postprocessed images with applied RKT filter (D-F); and generated depth profiles (G-I) of a representative specimen from the severe ETW group scanned at D0 (A,D,G); D7 (B,E,H); and D14 (C,F,I).

*(A-F) a: enamel; b: dentin; *: reference metal specimen holder; (D-I)1: enamel, 2: dentin-enamel junction (DEJ), 3: base of the specimen. (A-C): Enamel thickness measured at the midline (double-ended red arrows) as the distance between the peaks of the enamel surface and DEJ (horizontal dashed yellow lines) (data not shown); (D-I) enamel thickness measured as the converted pixel depth difference from the abscissae of 1 and 2 in the depth profiles generated from the midline (vertical dashed yellow lines) of the filtered images.

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Table 1.

Summary of daily procedures to be performed by the subjects during each study arm.

Procedures	Breakfast	Lunch	Dinner	Bedtime
1. Clean denture with toothbrush and water	✓			✓
2. Immerse denture in 30 ml of test solution (5 mins)	✓	✓	✓	✓
3. Rinse denture with tap water (~10 s)	✓	✓	✓	✓
4. Place denture in mouth and brush with provided toothbrush and toothpaste	✓			✓
5. Swish toothpaste slurry in mouth for ~5 s	✓			✓
6. Spit, remove denture and brush specimens (~10s)	✓			✓
7. Rinse denture with tap water (~10s)	✓			✓
8. Brush natural teeth, rinse, and swish with tap water	✓			✓
9. Place denture back in mouth	✓	✓	✓	✓
10. Wait 30 minutes before eating or drinking	✓	✓	✓	✓

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Table 2.

Mean (standard error) of enamel thickness with CP-OCT and μ-CT for each ETW severity and their intraclass correlation coefficients (ICC) and Pearson correlation coefficients (r)

ETW Severity	Days	n	CP-OCT (SE) (mm)*	μ-CT (SE) (mm)	ICC, r†	ICC, r†
None	0		0.96 (0.03)^a	-	-	0.62, 0.87†
	7		0.96 (0.03)^a	-	-	
	14	40	0.96 (0.03)^a	0.94 (0.03)	0.85, 0.82†	
Moderate	0		0.93 (0.04)^{ab}	-	-	
	7		0.94 (0.04)^a	-	-	
	14	40	0.91 (0.04)^b	0.90 (0.05)	0.51, 0.88†	
Severe	0		0.97 (0.04)^a	-	-	
	7		0.92 (0.05)^a	-	-	
	14	39	0.90 (0.05)^b	0.87 (0.05)	0.64, 0.89†	

[*] Similar lowercase letters indicate no difference within ETW severity ($p>0.05$); there were no differences among ETW severities within any of the study days

[†] with p-values < 0.001

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Table 3.

Mean (standard error) surface loss with CP-OCT and OP (μm) after 7 and 14 days for each ETW severity with corresponding intraclass correlation coefficients (ICC) and Pearson correlation coefficients (r).

ETW Severity	Days	n	CP-OCT (SE) (μm)*	OP (SE) (μm)*	ICC, r†	ICC, r†	ICC, r†
None	7	39	−7.89 (5.33)^{aA}	0.85 (0.40)^{aA}	0.00, −0.18	0.05, −0.09	0.38, 0.36†
	14	39	−8.91 (6.59)^{aA}	0.62 (0.40)^{aA}	0.12, −0.03		
Moderate	7	38	−8.64 (9.67)^{aA}	5.75 (1.68)^{aB}	0.21, 0.12	0.39, 0.44†	
	14	39	21.99 (12.84)^{bAB}	18.80 (6.50)^{aB}	0.46, 0.65†		
Severe	7	39	50.37 (41.27)^{aA}	72.11 (40.84)^{aC}	0.25, 0.47†	0.24, 0.54†	
	14	38	70.05 (41.20)^{bB}	98.78 (40.84)^{bC}	0.22, 0.67†		

[*] Similar lowercase letters indicate no difference within ETW severity ($p > 0.05$); similar uppercase letters indicate no difference among ETW severities within the same study day

[†] with p-values < 0.001