Quantitative Swept-Source Optical Coherence Tomography of Early Enamel Erosion in vivo

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Keywords

Biophotonics · Optical coherence tomography · Salivary pellicle · Tooth wear

Abstract

Swept-source optical coherence tomography (SS-OCT) shows potential for the in vivo quantitative evaluation of micro-structural enamel surface phenomena occurring during early erosive demineralization. This randomized controlled single-blind cross-over clinical study aimed to evaluate the use of SS-OCT for detecting optical changes in the enamel of 30 healthy volunteers subjected to orange juice rinsing (erosive challenge) in comparison to mineral water rinsing (control), according to wiped and non-wiped enamel surface states. Participants were randomly allocated to 60 min of orange juice rinsing (pH 3.8) followed by 60 min of water rinsing (pH 6.7) and vice versa, with a 2-week wash-out period. In addition, the labial surfaces of the right or left maxillary incisors were wiped prior to SS-OCT imaging. An automated ImageJ algorithm was designed to analyse the back-scattered OCT signal intensity (D) after orange juice rinsing compared to after water rinsing. D was quantified as the OCT signal scattering from the 33 μ m sub-surface enamel, normalised by the total OCT signal intensity entering the enamel. The back-scattered OCT signal intensity increased by 3.1% (95% CI 1.1–5.1%) in the wiped incisors and by 3.5% (95%

CI 1.5–5.5%) in the unwiped incisors ($p < 0.0001$). Wiping reduced the back-scattered OCT signal intensity by 1.7% (95% CI –3.2 to –0.3%; $p = 0.02$) in comparison to the unwiped enamel surfaces for both rinsing solutions ($p = 0.2$). SS-OCT detected OCT signal changes in the superficial sub-surface enamel of maxillary central incisor teeth of healthy volunteers after orange juice rinsing.

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Detection of early erosive tooth wear is a problem of increasing clinical concern. Despite recent improvements in our understanding of the risk factors, including common dietary acids [Bartlett et al., 2013], currently, there are no clinically accepted techniques to measure early erosion in vivo. Optical coherence tomography (OCT) has significant potential for non-invasive, non-ionizing detection of the earliest signs of enamel erosion [Huysmans et al., 2011], such as changes in surface texture [Bartlett et al., 2008; Austin et al., 2015, 2016] or enamel mineral loss [Amaechi et al., 2003; Popescu et al., 2008; Chew et al., 2014] prior to irreversible tissue destruction. There have been significant in vitro developments for the detection of early erosion using OCT [Chew et al., 2014]; however, early erosive wear remains a very challenging clinical condition to detect in vivo. Therefore, further clinical research into early erosion detection is required, especially because

diagnosing erosion as early as possible has great therapeutic potential, so as to ensure that as much enamel as possible is preserved throughout the patient's lifetime.

Detection of enamel erosion using OCT signal changes is thought to rely on acid demineralization causing surface texture changes and increased sub-surface enamel porosity, and thereby quantitatively changing the back-scattered OCT signal in the immediate sub-surface layers [Huysmans et al., 2011]. The histology of the early erosion lesion is thought to involve partial demineralization confined to the enamel surface and immediate sub-surface, whereas, in contrast, the carious lesion involves the formation of a deeper sub-surface lesion of greater demineralization [Popescu et al., 2008; ten Cate et al., 2008]. This sub-surface demineralization significantly changes the refractive index of the demineralized tissue [Popescu et al., 2008; Meng et al., 2009; Hariri et al., 2013], which can then be accentuated by dehydration effects to enhance OCT detection [Nazari et al., 2013]. In vitro OCT erosion research has recently demonstrated that unpolished natural enamel surfaces demonstrated OCT optical changes after as little as 10 min of orange juice erosion; however, it was not clear how these changes should be optimally detected using the OCT A-scan profiles [Chew et al., 2014]. Previous research into optimization of dental OCT for enamel demineralization assessment has proposed that using near-infrared wavelengths (1,310 nm) is ideal to improve the axial imaging depth in enamel, and, moreover, that polarization-sensitive OCT resolves surface and immediate sub-surface demineralization by reducing surface artefact formation due to the strong Fresnel reflection [Ashtamker et al., 2011] occurring at the air-enamel interface, which may mask early demineralization [Jones et al., 2006]. However, there is no consensus on the optimal technical specifications for in vivo dental OCT. In addition, the impact of clinical variables influencing in vivo enamel OCT imaging is not clearly understood. Thus, quantitative detection of early enamel erosion using OCT in vivo is complicated by multiple interacting surface phenomena which are poorly defined, including the composition and function of the acquired enamel pellicle [Hannig et al., 2003; Carpenter et al., 2014; Moazzez et al., 2014], the degree of hydration of the dental hard tissue during imaging [Nazari et al., 2013; Chan et al., 2014], and the micro-textural changes of the curved enamel surface in vivo during erosion [Austin et al., 2015, 2016]. Therefore, carefully controlled clinical studies simulating early erosion are required to elucidate the optimal analytical techniques for imaging early erosive changes.

The primary aim of this study was to determine if swept-source OCT (SS-OCT) signal changes are detectable in the enamel of the maxillary incisors of healthy volunteers after orange juice rinsing (erosive challenge), in comparison with mineral water rinsing (control). The secondary aim was to determine if wiping the enamel surface with a damp cotton pledget immediately before imaging affected OCT measurements. The null hypotheses were: SS-OCT will not detect in vivo enamel OCT signal changes after rinsing with an erosive challenge in comparison to rinsing with a non-erosive challenge, and wiping of the enamel surface prior to imaging will make no difference to the enamel OCT signal properties for both rinsing solutions in comparison to not wiping the enamel surface.

Materials and Methods

A randomized, single-blind, controlled, cross-over clinical study was conducted on healthy volunteers. Ethical (Manchester University Research Ethics Committee 5 – project ref. No. 13136) and NHS R and D approvals were granted. The study was conducted in the National Institute of Health Research (NIHR) Biomedical Research Centre at Guy's and St Thomas' NHS Hospital Foundation Trust and King's College London by dentally qualified clinical researchers.

Figure 1 shows the study design and flow chart. Thirty participants were recruited from among King's College London students and faculty staff who responded to an e-mail advertisement inviting healthy volunteers to take part in research. The eligibility criteria included males or females aged ≥ 18 years, with sound unrestored maxillary incisors (12, 11, 21, and 22) with an absence of caries, erosion, dentine hypersensitivity, enamel cracks, opacities, hypomineralization, staining, orthodontic appliances, or imbrications affecting these teeth. Exclusion criteria included relevant medical conditions, also those involving any history of allergy to consumer products. Absence of erosion was defined as a cumulative BEWE (basic erosive wear examination) score of ≤ 9 , thus indicating no/low risk of erosive wear [Bartlett et al., 2008]. A cumulative BEWE score of >9 typically indicated the presence of moderately worn teeth in only 2 sextants (most commonly the mandibular first molars) displaying erosion lesions covering no more than 50% of the affected surface, whilst the remainder of the dentition displayed early erosion lesions, seen as initial change in surface texture and no dentine exposure. The age range of the participants was 19–28 years.

On entering the study, participants were provided with a 1,450-ppm F wash-out toothpaste (Colgate Cavity Protection, regular flavour, Colgate Palmolive Ltd., Piscataway, NJ, USA) and a soft-bristled toothbrush (Colgate Palmolive Ltd.), to be used for 2 min twice a day at least 2 weeks prior to the study (including 1 h before attending), and throughout the duration of the study including 2 weeks after completion.

Participants were randomly allocated to the order of rinsing (i.e., either orange juice rinsing in Arm 1 followed by water rinsing

Fig. 1. Flow diagram for each group showing the numbers of participants who were randomly assigned, received intended treatment, and analysed for the primary outcome. LHS, left-hand side; RHS, right-hand side.

Fig. 2. Clinical optical coherence tomography (OCT) imaging set-up: clinical OCT probe mounted in geometry-stabilizing unit for the in vivo imaging of four $1 \times 1 \times 1.5$ mm (x, y, z) volumes from the labial aspect of each of the maxillary incisors to acquire B-scan

stacks. Example of a resulting single B-scan (x, z) from which 250 individual A-scans were extracted for analysis using an algorithm that was custom designed to quantify the back-scattered OCT signal intensity from the superficial sub-surface enamel (D).

in Arm 2, or vice versa) using allocation concealment implemented by a study administrator based on simple randomization. Each participant was sub-randomized to enamel surface wiping on the right (teeth 11, 12) or left (teeth 21, 22) with a damp cotton wool roll, prior to SS-OCT imaging. Both Arms underwent 6 rinsing cycles, following previously published in vivo erosion models [West et al., 1998], with each cycle involving rinsing with 25 mL of the solution for 60 s, expectorating, and then repeating the rinse immediately afterwards until ten 60-s rinses had been completed in 10 min. The orange juice (Sainsbury's Orange Juice, Basics, Sainsbury's Ltd., London, UK) had a pH of 3.8 and titratable acidity 27 mL (measured as the volume of 0.1 M NaOH solution required to raise the pH to 7.00). The mineral water (Volvic™, natural still, Premier Waters Ltd., London) had a pH of 6.7. After a 2-week washout, the participants crossed over to the alternative rinse, according to the allocation schedule. All participants were followed up 2 weeks later to monitor for any adverse effects.

All SS-OCT imaging was carried out by a single operator, blinded to the rinsing solution, using a multi-beam, swept-source Fourier-Domain clinical OCT scanner (VivoSight Topical OCT system, Michelson Diagnostics Ltd., Maidstone, UK) with a Santec HSL-2000-11 wide-sweep laser (>25 mW peak power; centre wavelength $1,305 \pm 15$ nm). The optical resolution was <7.5 μ m and the A-line rate was 10 kHz with a frame rate of 20 fps (1-mm B-scan width/250 A-scans). As shown in Figure 2, the topical OCT probe was mounted in a custom-built unit, comprising an adjustable head and chin support with the OCT probe mounted on a platform [McGrady et al., 2012]. The geometry-stabilizing unit ensured that the subject remained static, and enabled the OCT probe to be reproducibly repositioned while the red laser spot guided the

imaging at a perpendicular axis to the tooth surface. Four $1 \times 1 \times 1.5$ mm volumes (x, y, z) of the labial aspect of each incisor tooth were scanned, so 16 B-stack volumes were captured per participant per cycle, with each volume consisting of 20 B-scans with x, z dimensions of 1×1.5 mm, each with 50- μ m y spacing. Immediately prior to imaging, a participant's lips were retracted using an oral soft-tissue retractor (Optragate, Ivolar Vivadent AG, Liechtenstein), and the labial aspect of the right or left pair of upper incisors (11 and 12, or 21 and 22) was gently wiped using a damp cotton wool roll as per the randomization schedule. All images were completed in 2 min 30 s for each participant in order to minimise the dehydration effects, with each tooth scanned in <10 s.

The primary outcome was expressed as the change in back-scattered OCT signal intensity (D), at the level of the incisor teeth pairs (i.e., 11 + 12, or 21 + 22). All analyses were carried out blinded, using an automated image analysis algorithm (ImageJ v1.45S; Wayne Rashband, NIH, Bethesda, MD, USA) designed to quantify changes in the enamel OCT signal after erosion, based on in vitro pilot data [Chew et al., 2014]. As shown in Figure 3, the algorithm analysed individual A-scan z profiles in order to quantify the OCT signal-scattering in the immediately sub-surface (<33 μ m) enamel. This was automatically calculated as the difference between the amount of OCT signal entering the tissue (Intensity I_{in}) and the amount of OCT signal scattered in the immediate sub-surface enamel, i.e., (Intensity I_{in}) – (Intensity I_{out}), which was then normalized by Intensity I_{in} , in order to provide a ratio between 0 and 1 (i.e., 1 = 100% back-scattered OCT signal intensity and 0.5 = 50% back-scattered OCT signal intensity). All analyses were carried out by a single operator who was blinded to all interventions.

Fig. 3. Principle of quantification of back-scattered OCT intensity (D) with reference to A-scan profiles from sound enamel and eroded enamel: entire A-scan profile from sound enamel showing the location of the air-enamel interface using peak intensity and subsequent decay in OCT signal across the entire 1,500- μm z depth into the enamel. D was quantified by first calculating the amount of OCT signal entering the tissue (Intensity In), by subtracting the signal intensity within the air prior to the signal entering the enamel from the peak intensity at the air/enamel interface. Intensity In thus represented the total light entering the enamel. The intensity of light reflected back out of the superficial enamel (Intensity Out)

was then calculated by identifying the first plateau as the OCT signal intensity decayed within the immediate sub-surface enamel (usually within the first 33 μm). Intensity Out was then subtracted from Intensity In, and this value was then normalised by the total intensity entering the enamel (Intensity In) to provide a ratio of the back-scattered OCT signal intensity (D). Representative A-scans show an example of the effect of a 24% increase in the back-scattered OCT signal intensity (D) after erosion: there is a distinct reduction in the peak intensity and an increase in the initial plateau <33 μm into the superficial enamel.

The precision of the measurement, at the level of the incisor teeth pairs, was estimated by calculating the reproducibility (SD) of 30 repeated measurements at the same sites of the same pair of teeth of the same participant. Using in vitro data, a sample size of 28 was estimated, based on the assumption that SS-OCT would detect a 5% change in back-scattered OCT signal intensity (D) after 30 min of orange juice rinsing (SD 5%) assuming a power of 95%, and $p < 0.01$ being regarded as statistically significant. The ImageJ macro was used to automatically analyse the image stacks prior to exporting the resulting data in a CSV format for the statistical software analysis. The Shapiro-Wilk test and histogram were used to determine whether the data demonstrated a normal

distribution, prior to statistical comparisons. Initially, a primary random-effects model to evaluate any possible unwanted interaction effects was fitted to test the various combinations of water versus orange juice rinsing, rinsing durations, wiping versus not wiping, and order of allocation. As the interaction effect was not significant, the final model included only the main effects of treatment and wiped status, in order to assess the overall effects of the rinsing solution according to the wiping status on the enamel SS-OCT optical properties. All statistical analyses were performed with SPSS v21 for Windows (SPSS, Chicago, IL, USA) and STATA v12.0, with $p < 0.05$ inferring statistical significance.

Fig. 4. Overall mean (SEM) back-scattered OCT signal intensity (D) of the labial enamel of maxillary incisor pairs ($n = 30$) after repeated rinsing with water or orange juice according to wiping status. **a** Wiped. **b** Not wiped.

Results

All 30 participants who met the eligibility criteria consented to take part in and completed the study, with no loss to follow-up. The reproducibility (SD) of the 30 repeated measurements was 0.003. Following automated quantification of all acquired SS-OCT images using the ImageJ-based algorithm, the randomisation code was unmasked and the resulting data were allocated to the appropriate group according to rinsing and wiping status. Data were normally distributed, so means and 95% CIs were quantified and parametric tests applied. The primary random-effects model included the interaction terms: Rinsing duration $\times$ Order of allocation, Rinsing duration $\times$ Wiping status, and Rinsing duration $\times$ Intervention. Since none of these interaction effects was statistically significant ($p > 0.05$), these terms were ignored for the definitive analysis that was carried out to analyse the overall effect in the back-scattered OCT signal intensity (D) of incisor pairs allocated to the rinsing solutions (orange juice/water) according to the status of the enamel surface (wiped or not wiped).

As shown in Figure 4, there were significant increases in the back-scattered OCT signal intensity (D) of the labial enamel of the incisor pairs after repeated rinsing with orange juice in comparison to water ($p < 0.0001$). The back-scattered OCT signal intensity increased by 3.1% (95% CI 1.1–5.1%) in the wiped incisors and by 3.5% (95% CI 1.5–5.5%) in the unwiped incisors. When the ef-

fect of the wiping was considered, there was a significant decrease in back-scattered OCT signal intensity of 1.7% (95% CI -3.2 to -0.3% ; $p = 0.02$), in comparison to the unwiped enamel surfaces. At follow-up, 6 (20%) participants reported experiencing mildly sensitive teeth immediately after the orange juice rinsing; however, this completely resolved in all cases within 5 days. Figure 5 shows representative OCT B-scans from the labial enamel of the same area of the same tooth after rinsing for 60 min with water (images on the left) and with orange juice (images on the right) for the unwiped and wiped tooth surfaces. All images display a high-resolution air/enamel interface with differing rates of decay of back-scattered OCT signal. For the tooth surfaces subjected to orange juice rinsing, the intensity of the back-scattered OCT signal in the sub-surface enamel appears slightly increased after erosion, which suggests that erosion has occurred.

Discussion

We investigated whether SS-OCT could detect changes in the OCT signal in healthy enamel subjected to rinsing with an erosive challenge (orange juice) versus rinsing with a non-erosive challenge (mineral water). There were statistically significant increases in the back-scattered OCT signal intensity after orange juice rinsing in comparison to water rinsing: 3.1% for wiped incisors and 3.5% for unwiped incisors ($p < 0.0001$). Previous studies have

Fig. 5. Representative OCT B-scans from the labial enamel of the incisors of a single participant before and after 60 min of rinsing, according to rinsing solution (water and orange juice) and wiping status (unwiped and wiped).

also found that demineralization results in an increased scattering of the near-infrared OCT signal in the enamel [Huynh et al., 2004; Hariri et al., 2013]; however, this is the first in vivo study to pick up these changes during early erosion. An ideal optical diagnostic method for enamel erosion should be able to detect the earliest pathological signs such as the quantitative optical changes induced by enamel mineral loss [Amaechi et al., 2003; Popescu et al., 2008; Chew et al., 2014] or changes in surface characteristics [Huysmans et al., 2011; Rakhmatul-

lina et al., 2011; Young and Tenuta, 2011; Austin et al., 2015], prior to irreversible bulk tissue loss [Wilder-Smith et al., 2009].

As shown in Figure 3, the algorithm we used was designed to detect changes in the intensity of the back-scattered OCT signal in the immediate sub-surface enamel (33 μm), and then to normalise this back-scattered OCT signal intensity as a ratio of the peak intensity at the air-enamel surface, in order to minimise the impact of optical artefacts which are known to complicate measurements

that are based purely on the enamel surface OCT signal [Huysmans et al., 2011; Chan et al., 2013].

Previous authors have postulated that enamel demineralization causes surface texture changes and increased sub-surface enamel porosity, which can be quantitatively measured as a change in the back-scattered OCT signal intensity of the OCT signal in the deep sub-surface layers [Huysmans et al., 2011]; however, concerns have been expressed about the negative impact of OCT artefact formation at rough surface interfaces, unless polarization-sensitive OCT is used [Jones et al., 2006]. This study has demonstrated that the immediate sub-surface depth of 33 μm was determined as optimal, in contrast to previous studies which excluded the superficial 30–40 μm and only used data from the profile of up to a 150- μm depth, due to concerns about surface artefacts and an inability to identify a more superficial plateauing of the decay in signal intensity [Chew et al., 2014]. For our study, a custom algorithm analysed all acquired A-scan profiles in order to exploit the improved depth of focus and image clarity from the 4 interferometer channels employed in the clinical SS-OCT system; this avoided the Fresnel reflection causing artificially initial steep decay in intensity of back-scattered OCT signal seen in previous *in vitro* systems [Chew et al., 2014]. As shown in Figure 5, the OCT signal algorithm was able to detect a more superficial plateau in the back-scattered OCT signal within the first 33 μm of the enamel sub-surface; this was discarded in previous studies [Chew et al., 2014]. So the swept-source OCT system and specialised image analysis software used in this study detected OCT signal changes in the immediate sub-surface enamel, which is contrary to previous concerns that only polarization-sensitive OCT would be able to quantify superficial enamel demineralization [Jones et al., 2006].

Clinical detection of early enamel erosion in natural incisors using OCT is more challenging than the *in vitro* detection of advanced erosion in flat polished enamel that was conducted in previous OCT research [Huysmans et al., 2011]. The relative translucence of enamel is significantly impacted by the inter-prismatic fluid content [Brodbeck et al., 1981] and SS-OCT is highly sensitive to changes in the refractive index of the enamel [Meng et al., 2009]. Therefore, for this study, it was paramount to standardise the relative hydration/dehydration of the teeth. On the surface of the enamel, the acquired enamel pellicle has a protective effect against erosion; however, controversy remains regarding the fundamental interactions between the acquired enamel pellicle and early dietary erosion *in vivo*. *In situ* studies have demonstrated that the pellicle has varying erosion-modifying properties that de-

pend on the exact structure and composition, especially with regard to thickness, and mineral and protein content [Hannig and Balz, 1999; Hannig and Joiner, 2006; Carpenter et al., 2014; Moazzez et al., 2014]. However, we have revealed more subtle superficial back-scattered OCT signal changes, not only from erosion but also from wiping the tooth with a damp cotton pledget, which resulted in significantly reduced back-scattered OCT signal intensity ($p = 0.02$) after both orange juice rinsing and water rinsing. For both wiping states, the enamel rinsed with orange juice showed consistent increases in sub-surface scattering compared to that rinsed with water ($p < 0.0001$), so the wiping did not enhance or detract from the erosion measurement. The damp cotton wool roll was used immediately prior to imaging to standardise the hydration state of the tooth surfaces, and thus ensure that no systematic drift occurred in the measurements due to changing hydration states as the cycles increased. Previous studies have shown that drying the enamel results in increased surface/sub-surface brightness [Nazari et al., 2013], so the reduction in sub-surface scattering after wiping, for both water- and orange juice-rinsed enamel, suggests that the wiped enamel surface was more hydrated than the unwiped surface. For the unwiped pellicle group, however, it is unlikely that dehydration confounded our measurements, as all OCT imaging took place rapidly with each scan. However, as dehydration may actually enhance discrimination between sound and demineralized enamel, the optimum clinical protocol for the quantification of *in vivo* demineralization has not yet been determined and this requires further research. Future *in vivo* SS-OCT studies should employ a robust standardisation of the relative levels of tooth hydration, in order to prevent confounding of possible changes in the enamel OCT signal from an erosive challenge.

In conclusion, *in vivo* SS-OCT was able to detect statistically significant increases in the enamel OCT signal after orange juice rinsing when compared to water rinsing ($p < 0.0001$), and the wiping of the enamel surface resulted in statistically significant reductions in the enamel OCT signal ($p = 0.02$). Whilst demineralization is known to increase the scattering anisotropy of dental enamel after artificial demineralization at 1,310 nm, the exact nature of these optical changes is not fully understood. Future studies are required, to elucidate, quantify, and characterize the exact nature of surface events occurring *in vivo* during enamel erosion, and how these events result in changes in the optical properties of the enamel. SS-OCT has potential for the non-invasive *in vivo* detection of early surface events occurring during dietary erosion.

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Author Contributions

R.S.A., F.F., R.C., J.G., I.A.P., and R.M. conceived and designed the experiments. R.S.A., M.H.T., and R.M. performed the clinical examination. R.S.A., J.G., M.H.T., and R.M. performed the experiments. R.S.A., F.F., M.A., R.C., and R.M. analysed the data. R.S.A., F.F., M.A., I.A.P., and R.M. wrote the paper.

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