Title page

Title: Quantitative swept-source optical coherence tomography of early enamel erosion in vivo

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Short Title
Optical coherence tomography for detection of enamel erosion in vivo

Keywords
Biophotonics, Optical Coherence Tomography, Tooth wear, Salivary pellicle

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There are no conflicts of interest.
Abstract:
Swept-source optical coherence tomography (SS-OCT) has potential for in vivo quantitative evaluation of microstructural enamel surface phenomena occurring during early erosive demineralisation. This randomized controlled single-blind crossover clinical study aimed to evaluate the use of SS-OCT for detection of 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 minutes orange juice rinsing (pH 3.8) followed by 60 minutes water rinsing (pH 6.7) and vice versa, with a two-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 backscattered OCT signal intensity (D) after orange juice rinsing compared to after water rinsing. D was quantified as the OCT signal scattering from the ~33 µm subsurface enamel, normalised by the total OCT signal intensity entering the enamel. The backscattered OCT signal intensity increased 3.1% (95% CI 1.1% to 5.1%) in the wiped incisors and 3.5% (95% CI 1.5% to 5.5%) in the unwiped incisors (P<0.0001). Wiping reduced the backscattered 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). Swept-source Optical Coherence Tomography detected OCT signal changes in the superficial subsurface enamel of maxillary central incisor teeth of healthy volunteers after orange juice rinsing.
**Introduction**

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] to date 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 [Austin et al., 2015 ; Austin et al., 2015; Bartlett et al., 2008] or enamel mineral loss [Amaechi et al., 2003; Chew et al., 2014; Popescu et al., 2008], prior to irreversible tissue destruction. There have been significant in vitro developments for 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, in order 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 subsurface enamel porosity thus quantitatively changing the backscattered OCT signal in the immediate subsurface 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 subsurface lesion of greater demineralization [Popescu et al., 2008; Ten Cate et al., 2008]. This subsurface demineralization significantly changes the refractive index of the demineralized tissue [Hariri et al., 2013; Meng et al., 2009; Popescu et al., 2008], 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 minutes 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 have proposed that using near-infrared wavelengths (1310 nm) are ideal to improve axial imaging depth in enamel and moreover that polarization-sensitive OCT resolves surface and immediate subsurface 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 2016]. However there is no consensus on the optimal technical specifications for in vivo dental OCT and additionally, the impact of clinical variables influencing in vivo enamel OCT imaging are 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 [Carpenter et al., 2014; Hannig et al., 2003; Moazzez et al., 2014], the degree of hydration of the dental hard tissue during imaging [Chan et al., 2014; Nazari et al., 2013] and the micro-textural changes of the curved enamel surface in vivo during erosion [Austin et al., 2015 ; Austin et al., 2015].

Therefore, there 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 crossover clinical study was conducted in healthy volunteers. Ethical (Manchester University Research Ethics Committee 5 - Project Ref 13136) and NHS R&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. 30 participants were recruited from King’s College London students and faculty staff who responded to an email advertisement inviting healthy volunteers to take part in research. The eligibility criteria included males or females aged 18 or more with sound unrestored maxillary incisors (12, 11, 21, 22) with an absence of caries, erosion, dentine hypersensitivity, enamel cracks, opacities, hypomineralisation, staining, orthodontic appliances or imbrications affecting 12, 11, 21, 22. Further exclusion criteria included relevant medical conditions, including any history of allergy to consumer products. Absence of erosion was defined as a cumulative Basic Erosive Wear Examination score ≤9 thus indicating no or low risk of erosive wear [Bartlett et al., 2008]. A cumulative BEWE score less than 9 typically indicated that participants had 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. Participant’s age range was 19 to 28 years old.

On entering the study, participants were provided with a 1450 ppm F wash-out toothpaste (Colgate Cavity Protection Regular Flavour, Colgate Palmolive Ltd, Piscataway, USA) and a soft-bristled toothbrush (Colgate Palmolive Ltd) to be used two minutes twice a day at least 2 weeks prior to the study (including 1 hour before attending), 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 in Arm 2 or vice versa) using allocation concealment implemented by a study administrator based on simple randomization. Each participant was sub-randomized to right-hand side (tooth 11, 12) or left-hand side (tooth 21, 22) enamel surface wiping with a damp cotton wool roll, prior to SS-OCT imaging. During both Arms, participants underwent six 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 seconds, expectorating and then repeating the rinse immediately afterwards until ten 60 second rinses were completed in 10 minutes. The orange juice (Sainsbury's Orange Juice, Basics, Sainsbury’s Ltd, London, UK) had pH 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 pH 6.7. After a two 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 events.
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 Limited, Maidstone, UK) operating using a Santec HSL-2000-11 wide sweep laser with a >25 mW peak power and centre wavelength 1305 ± 15 nm. The optical resolution was <7.5 µm 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 custom-built, 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 whilst the red laser spot guiding the imaging at a perpendicular axis to the tooth surface. Four 1 mm x 1 mm x 1.5 mm volumes (x, y, z) of the labial aspect of each incisor tooth were scanned, thus sixteen B-stack volumes were captured per participant per cycle, with each volume consisting of 20 B-scans with x, z dimensions of 1 mm x 1.5 mm, each with 50 µm y spacing. Immediately prior to imaging, participant’s lips were retracted using an oral soft tissue retractor (Optragate, Ivolcar Vivadent AG, Liechtenstein) and the labial aspect of the right or left pair of upper incisors (12 and 11 or 21 and 22) was gently wiped using a damp cotton wool roll as per the randomization schedule.

All images were completed in 2½ minutes for each participant in order to minimise dehydration effects, with each tooth scanned in less than 10 seconds.

The primary outcome was expressed as the change in backscattered OCT signal intensity (D), at the level of the incisor teeth pairs (i.e. 12+11 or 21+22). All analyses were carried out blinded using an automated image analysis algorithm (Image J version 1.45S; Wayne Rashband, NIH, Bethesda, Maryland), 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 µm) enamel. This was automatically calculated as the difference between the amount of OCT signal entering the tissue (Intensity In) and the amount of OCT signal scattered in the immediate subsurface enamel (Intensity In minus Intensity Out), which was then normalized by Intensity In in order to provide a ratio between 0 and 1 (i.e. 1 = 100% backscattered OCT signal intensity and 0.5 = 50% backscattered OCT signal intensity). All analyses were carried out by a single operator who was blind to all interventions.

The precision of measurement, at the level of the incisor teeth pairs, was estimated by calculating the reproducibility (SD) of thirty repeated measurements of 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 backscattered OCT signal intensity (D) after 30 minutes orange juice rinsing with a standard deviation of 5% assuming a power of 95% and p<0.01 regarded as statistically significant. The ImageJ macro was used to automatically analyse the image stacks prior to exporting the resulting data as .CSV format for analysis using statistical software. A Shapiro-Wilk’s test and histogram were used to determine whether 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 vs. orange juice rinsing, rinsing durations, wiping vs. 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 to assess the overall effects of the rinsing solution according to the wiping status on the enamel SS-OCT optical properties. All statistical analyses with the Statistical
package for Social Sciences (SPSS Ver.21 for Windows, SPSS, Chicago, Illinois) and Stata version 12.0 with \textit{P}<0.05 inferring statistical significance.

**Results**

All 30 participants who met the eligibility criteria, consented to take part in the study 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 resulting data were allocated to the appropriate group according to rinsing and wiping status. Data were normally distributed, therefore means and 95% confidence intervals (95% CI) were quantified and parametric tests applied. The primary random effects model included the interaction terms ‘Rinsing duration’ X ‘Order of allocation’, ‘Rinsing duration’ X ‘wiping status’ and ‘Rinsing duration’ X ‘Intervention’. Since none of these interaction effects were statistically significant (\textit{p}>0.05), these terms were ignored for the definitive analysis which was carried out to analyze the overall effect in the backscattered OCT signal intensity (D) of incisor pairs allocated to the rinsing solutions (orange juice / water) according to the enamel surface status (wiped or not wiped).

As shown in Figure 4, there were significant increases in the backscattered OCT signal intensity (D) of the labial enamel of the incisor pairs after repeated rinsing with orange juice in comparison to water (\textit{P}<0.0001). The backscattered OCT signal intensity increased 3.1% (95% CI 1.1% to 5.1%) in the wiped incisors and 3.5% (95% CI 1.5% to 5.5%) in the unwiped incisors. When the effect of the wiping was considered, there was a significant decrease in backscattered OCT signal intensity of 1.7% (95% CI -3.2% to -0.3%) (\textit{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 minutes with water (left hand side images) and with orange juice (right side images) for unwiped and wiped tooth surfaces. All images display high-resolution air/enamel interface with differing rates of decay of backscattered OCT signal. For the tooth surfaces subjected to orange juice rinsing, the intensity of the backscattered OCT signal in the subsurface enamel surface appears slightly increased after erosion, which suggests erosion has occurred.

**Discussion**

The present study investigated whether SS-OCT could detect changes in the OCT signal in healthy enamel subjected to rinsing with an erosive challenge (orange juice), in comparison to rinsing with a non-erosive challenge (mineral water). There were statistically significant increases in the backscattered OCT signal intensity after orange juice rinsing in comparison to water rinsing of 3.1% for wiped incisors and 3.5% for unwiped incisors (\textit{P}<0.0001). Previous studies have also found that demineralization results in increased scattering of the near-infrared OCT signal in the enamel [Hariri et al., 2013; Huynh et al 2004], 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 quantitative optical changes induced by enamel mineral loss [Amaechi et al., 2003; Chew et al., 2014; Popescu et al., 2008] or changes in surface characteristics [Austin et al., 2015; Huysmans et al., 2011; Rakhmatullina et al., 2011; Rakhmatullina et al., 2011].
et al., 2011; Young and Tenuta, 2011], prior to irreversible bulk tissue loss [Wilder-Smith et al., 2009]. As shown in Figure 3, the algorithm in the present study was designed to detect changes in the intensity of the backscattered OCT signal in the immediate subsurface enamel (33 µm) and then normalise this backscattered OCT signal intensity as a ratio of the peak intensity at the air-enamel surface, in order minimise impact of optical artefacts which are known to complicate measurements purely based on enamel surface OCT signal [Chan et al., 2013; Huysmans et al., 2011]. Previous authors have postulated that enamel demineralization causing surface texture changes and increased subsurface enamel porosity which can be quantitatively measured as a change in the backscattered OCT signal intensity of the OCT signal in the deep subsurface 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., 2016]. This present study has demonstrated that the immediate subsurface depth of 33 µm was determined as optimal in contrast to previous studies, which have excluded the superficial 30 – 40 µm and only used data from the profile up to 150 µm depth, due to concerns about surface artefacts and inability to identify a more superficial plateauing of the decay in signal intensity [Chew et al., 2014]. For this present study, a custom algorithm analysed all acquired A-scan profiles in order to exploit the improved depth of focus and image clarity from the four interferometer channels employed in this clinical SS-OCT system which avoided the Fresnel reflection causing artificially initial steep decay in intensity of backscattered OCT signal seen in previous in vitro systems [Chew et al., 2014]. Therefore, as shown in Figure 5, the OCT signal algorithm was able to detect a more superficial plateau in the backscattered OCT signal within the first 33 µm of the enamel subsurface, which has been discarded in previous studies [Chew et al., 2014]. Therefore, the swept-source OCT system and specialised image analysis software used in this present study detected OCT signal changes in the immediate subsurface enamel, which is contrary to previous concerns that only polarization-sensitive OCT would be able to quantify superficial enamel demineralisation [Jones et al., 2016].

Clinical detection of early enamel erosion in natural incisors using OCT is more challenging than in vitro detection of advanced erosion in flat polished enamel, as conducted in previous OCT research [Huysmans et al., 2011]. The relative translucency of enamel is significantly impacted by the interprismatic fluid content [Brodbelt et al., 1981] and as SS-OCT is highly sensitive to changes in refractive index of the enamel [Meng et al., 2009]. Therefore, for this present study clinical study, it was paramount to standardise the relative hydration/dehydration of the teeth. At the enamel surface, the acquired enamel pellicle has a protective effect against erosion; however there remains controversy 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 depending on the exact structure and composition, especially with regard to thickness, mineral and protein content [Carpenter et al., 2014; Hannig and Balz, 1999; Hannig and Joiner, 2006; Moazzez et al., 2014]. However, this present study has revealed more subtle superficial backscattered OCT signal changes, not only from erosion but also from the tooth wiping with a damp cotton pledge, which resulted in significantly reduced back scattered OCT signal intensity (p=0.02) after both orange juice rising and water rinsing. However, for both wiping states, the orange juice rinsed enamel showed consistent increases in subsurface scattering compared to water rinsing (P<0.0001) therefore the wiping did not enhance or detract from the erosion measurement. The damp cotton wool role was used immediately prior to imaging, in order 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/subsurface brightness [Nazari et al., 2013], therefore the reduction in subsurface scattering after wiping, for both water and orange juiced 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 the measurement in this present study, 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 quantification of in vivo demineralisation, has not been determined and requires further research. Future in vivo SS-OCT studies employ 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 swept-source Optical Coherence Tomography was able to detect statistically significant increases in the enamel OCT signal after orange juice rinsing in comparison to water rinsing (P<0.0001) and the wiping of the enamel surface resulted in statistically reductions in the enamel OCT signal (P=0.02). Whilst demineralization is known to increase scattering anisotropy of dental enamel after artificial demineralization at 1310-nm, the exact nature of these optical changes are not fully understood. Future studies are required to elucidate, quantify and characterize the exact nature of the surface events occurring in vivo during enamel erosion and how these events results in changes in the optical properties of the enamel. SS-OCT has potential for non-invasive in vivo detection of early surface events occurring during dietary erosion.

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Conceived and designed the experiments: RA, FF, RC, JG, IP, RM. Performed the clinical examination: RA, MHT, RM. Performed the experiments: RA, JG, MHT, RM. Analyzed the data: RA, FF, MA, RC, RM. Wrote the paper: RA, FF, MA, IP, RM
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**Figures**
Figure 1 Participant flow diagram for each group showing the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome.
Figure 2 Clinical Optical Coherence Tomography imaging set up: Clinical OCT Probe mounted in geometry stabilizing unit for in vivo imaging of four 1 mm × 1 mm × 1.5 mm (x, y, z) volumes from of the labial aspect of each of the maxillary incisors in order to acquire B-scan stacks. Example of resulting single B-scan (x,z) from which 250 individual A-Scans were extracted for analysis using a custom designed algorithm, designed to quantify the back scattered OCT signal intensity from the superficial subsurface enamel (D).
Figure 3 Principle of quantification of backscattered OCT intensity (D) with reference to A-Scan profiles from sound and eroded enamel. Entire A-Scan profile from sound enamel showing location of air-enamel interface using peak intensity and subsequent decay in OCT signal across entire 1500 μm Z depth into enamel. D was quantified by first calculating the amount of OCT signal entering the tissue (Intensity IN) by subtracting from the peak intensity, at the air/enamel interface, the signal intensity within the air prior to the signal entering the enamel thus, 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 subsurface 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 backscattered OCT signal intensity (D). Representative A-Scans show an example of the effect of a 24% increase in the backscattered 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.

Figure 4 Overall mean (SEM) backscattered 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 and B: not wiped) with P values.
Figure 5 Representative OCT B-scans from the labial enamel the incisors of a single participant before and after 60 minutes rinsing, according to the rinsing solutions (water and orange juice) and the wiping status (unwiped and wiped).