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ORIGINAL ARTICLE

Enamel erosion depths measured on impressions by a white light interferometer

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Abstract

Objective. To investigate if measurements of the step heights on an impression of eroded enamel surfaces may give reliable values of the etching depths measured directly on the enamel. ***Materials and methods.*** Twelve human enamel specimens, with one circular amalgam reference surface in each, were mounted on an epoxy block and ground flat. Baseline images were taken by a White Light Interferometer (WLI) followed by 12 min etch by 0.01 M HCl, pH 2.2. The mean etch depth on each specimen was calculated from three repeated measurements, both on the specimen surfaces and on the silicone polymer impressions of the surfaces. Paired samples *t*-test was used when comparing the precision of measurements on enamel vs on impression. ***Results.*** The mean lesion depths after 12 min etch measured on the 12 enamel surfaces and impressions were 9.9 μm and 10.6 μm, respectively. The direct and indirect measurements correlated well ($r^2 = 0.95$), with 7% higher mean etch depth measured on the impressions ($p < 0.05$). The reliability of three repeated measurements on enamel and impressions expressed by Intra Class Correlation (ICC) were 0.98 and 0.99, respectively. The average relative precisions of three repeated measurements on the 12 tooth and impression surfaces were $(0.5 \pm 0.1)\%$ and $(0.1 \pm 0.02)\%$, respectively ($p < 0.05$). The accuracies were 0.4% and 7%, respectively. ***Conclusion.*** WLI gave reliable but slightly higher etch depth measurements on impressions compared to enamel surfaces.

Key Words: *enamel erosion, impression, interferometer*

Introduction

Most studies regarding prevention and treatment of dental erosive wear have been performed *in vitro* and *in situ*. Particular focus has been on various fluoride compounds and their working-mechanism on enamel and dentine that are exposed to acid attacks. The erosion inhibiting effect of these fluoride agents is influenced by factors like abrasion, pellicle and saliva. Therefore, it is important to study the effect of repeated applications and acid attacks over a period of time in a natural oral environment. The possibility to follow-up the progression and/or the effect of treatment of dental erosions in patients with intrinsic risk factors like bulimia and GORD (Gastro-Oesophageal Reflux Disease) or subjected to external risk factors like soft drinks is also desirable. At present, instruments that measure loss of enamel or dentin cannot be used directly in the mouth. So far, *in situ* studies [1–4], *in vivo* studies using teeth planned for extraction [5,6] and an *in vivo* approach where teeth were exposed to acid by a peristaltic pump intraorally [7–9] are study designs more clinically relevant than laboratory studies. Another possibility for periodic assessments of erosive loss on the same surfaces after different interventions *in vivo* is by using a replica technique where measurements are performed on casts or impressions.

Assessment of tooth substance loss on casts or directly on impressions has been reported in several studies [10–18]. However, the authors have pointed out some limitations involved in the replica procedures. The impression and casting procedures are complex and time-consuming and may introduce errors related to both contraction of the impression material and inaccurate replicas. Difficulties related to type of reference area have also been reported. The measurement techniques used are influenced by

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different factors, for example that optical profilometers, mechanical profilometers and laser scanners perform best on ground surfaces and profilometers with a red laser light source cannot scan impressions of the same colour.

The present study design may have the advantages that the white light interferometer (WLI) can measure directly on the impression material, the reference area is easy to identify correctly on the impression and WLI does not favor any color of the impression material since the light consists of the whole spectrum of wavelengths. In a study by Holme et al. [12] it was shown in a pilot experiment that the difference in average step height measured by a white light interferometer (WLI) on a polished enamel specimen vs on a negative replica (impression) was very small. The authors suggested that it could be possible to measure the etch rate or progression of erosion on human enamel *in vivo* using this technique. The WLI has proved to be an accurate and precise instrument for measuring dental erosions [12] also in comparison with other instruments [19].

The aim of this study was to determine if measurements by WLI of the step height on an impression (negative replica) of eroded enamel could give reliable values of the etching depths measured directly on enamel.

Materials and methods

Enamel specimens

Twelve freshly extracted human permanent molars were collected and kept in containers with Thymol crystals in 100% humidity to prevent bacterial growth. Ethical approval for the study was given by the Regional Committee for Medical Research Ethics, Norway, and The Norwegian Institute of Public Health's Biobank Register. The enamel was polished with pumice in water, wiped free of debris and rinsed in tap water. One circular amalgam filling (~ 1 mm in diameter) was made in each tooth ~ 2 mm above the enamel–cement junction and used as a reference surface during the analysis. One specimen including the amalgam filling was prepared from each tooth ($n = 12$) using a high speed turbine. The 12 specimens were mounted together in one epoxy resin block (Epofix®, Struers, Denmark) parallel to and level with the epoxy surface. The specimen surfaces were then ground flat with abrasive paper to grit 4000, rinsed with water to remove any traces from the polishing procedure and wiped free of debris with a cotton pellet soaked in 20% ethanol.

Analytical methods

White light interferometry (WLI). Prior to acid exposure, the specimens were analyzed by a white light

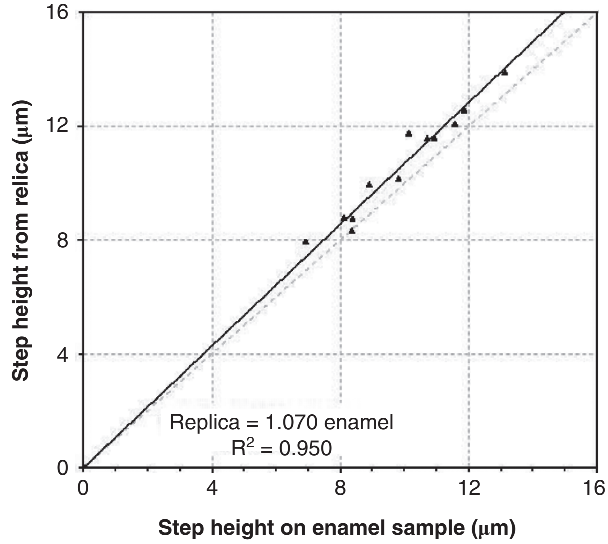

Figure 1. Comparison of mean etch depths (µm) for the 12 enamel specimens after 12 min etch, measured by WLI on the enamel surfaces and their respective impressions. Each point is based on the average of three measurements. Standard errors of the means all lay within the triangular markers.

interferometer (WYKO® NT-2000, Veeco, Tucson, Arizona, USA), which is a computerized optical interference microscope operated in the Vertical Scanning Interferometry mode (VSI). The topographic images made by the WLI consisted of ~ 1/3 amalgam and 2/3 enamel. A baseline image was made of every specimen before etch and a new image was made after 12 min acid etch both on enamel and impression. The baseline images were subtracted from the image taken after acid exposure for every separate specimen, thus creating a 'difference image' which shows the changes in topography. The amalgam surface is not influenced by acid and is easily identified on the WLI

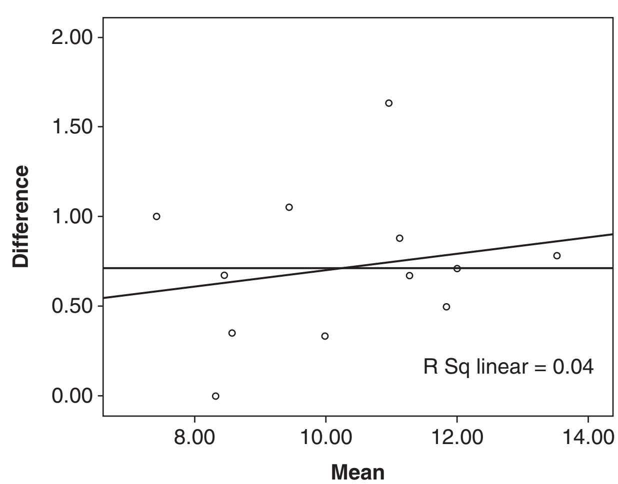

Figure 2. Mean etch depths (µm) of measurements on the 12 enamel- and impression surfaces (x-axis) and the differences in mean etch depths (µm) measured on the 12 enamel surfaces vs on the respective impression surfaces (y-axis). The reference line on the y-axis represents the mean difference in etch depth.

Table I. Mean etch depths (SD) for 12 specimens measured on the enamel surfaces and the respective impressions. Reliability analyses (ICC) of repeated measurements on enamel and impressions. Relative precision (SD/mean etch depth) averaged for 12 specimens and the accuracies for the measurements are also given.

	Mean etch depth (SD)	ICC	Average relative precision (%)	Accuracy (%)
Enamel surface	9.9 µm^{a} (1.0)	0.98	0.46^{c} ± 0.09	0.4
Replica/impression	10.6 µm^{b} (1.0)	0.99	0.11^{d} ± 0.02	7.0

a, *b* Values are significantly different $p < 0.05$.
c, *d* Values are significantly different $p < 0.05$.

images so exactly the same area can be measured at baseline and during the experiment within a few pixels exactness. A computer program calculated the depth distribution of pixels in amalgam and enamel separately to get goodness of fit value and the lateral variability of etch rate, respectively. The technique has been described in detail in a previous study [12,19,20]. A 10× magnification objective was used and gave a pixel size of 11.4 µm^2 with x-size 3.13 µm and y-size 3.65 µm of the rectangular pixels. The image resolution was 368 × 240 pixels (1.0 mm^2).

Variations in room temperature, dust level and vibration were kept at a minimum during the analyses. The WLI was run by a certified operator. The instrument was calibrated at the beginning and end of every measurement series by checking against a 10.07 µm glass step standard with an absolute uncertainty at the height of 0.04 µm. The average accuracy (difference from the glass standard value of 10.07 µm) after the six calibration runs was 0.007 ± 0.002 µm (given as the mean value ± one standard error of the mean). Therefore, the overall accuracy of the WLI measurements for ideal stepped surfaces was in this case limited by the step uncertainty of 0.04 µm, corresponding to 0.4%. The precision of the glass step measurements was 0.008 ± 0.001 µm, which for the 10.07 µm step becomes 0.08%. Etch depth measurements by WLI on enamel has been shown to be accurate and precise within 0.4% and 0.5%, respectively [19]. The precision was not as good on enamel as on glass, mainly because the polished enamel was still 15-times rougher than the very smooth glass step surfaces. The rough enamel thus gave more noise in the image data.

Acid exposure. The epoxy block with the 12 specimens was exposed to 15 ml 0.01 M hydrochloric acid (HCl) (pH 2.2) for 12 min under gentle agitation.

Replica/impression. After 0.01 M HCl (pH 2.2) acid etch for 12 min, an impression was made of the epoxy block with all 12 specimens by Microset® 101TH (Microset Products Ltd., Warwickshire, UK) (a two-component polymer) which is able to reproduce features down to 0.1 µm [21]. It was applied with a mixing pistol similar to the type used to apply dental impression materials. The lesion depths both on the enamel specimens and on their respective impressions were measured by WLI by three repeated measurements after acid etch and directly after impression making. By making a topographic image of the impression and inverting the data, a 3D image similar to that of the specimen was obtained. The inverted impression images were analyzed in the same way as the images of the enamel surfaces in order to measure the step height.

Statistics. Statistical procedures were performed with SPSS 16.0 (Statistical Package for Social Sciences Inc., Chicago, IL). A Bland Altman plot of mean etch depth for repeated measurements on enamel and impressions vs the difference in etch depth measured on enamel and impressions was made with mean deviation from zero (95% CI) of the differences, as well as the ordinary least square regression of the scatter plot. p-values related to this difference and to the slope were calculated. The precision of the measurements from each method after 12 min acid exposure was calculated by reliability analysis and expressed by single measures Intra Class Correlation (ICC), a two-way mixed effects model that measures the reliability of repeated measurements by one instrument.The correlation of measurements by WLI on enamel surfaces vs on impressions after 12 min etch was calculated by regression analysis. The paired samples t-test was used with a 5% significance level when comparing the precision of repeated measurements on enamel vs on impressions.

Results

The mean lesion depths measured after 12 min etch by 0.01 M HCl on the 12 enamel surfaces and on impressions of the same enamel surfaces were 9.9 µm and 10.6 µm, respectively, which means 7% higher values measures on the impressions compared to the actual enamel surfaces (Table 1). Despite the systematic over-estimation, there was generally a good linear correlation between the impression and enamel data, with a few exceptions that resemble statistical scatter (Figure 1). The ICC values expressed a good reliability of the repeated measurements on both enamel and on the impression with coefficients of 0.981 and

0.994, respectively. The average relative precision of three repeated measurements for all 12 samples on enamel was 0.5 ± 0.1% and significantly smaller on replica 0.10 ± 0.02%. The Bland Altman plot (Figure 2) showed that measurements on impressions were systematically higher than on enamel, as indicated by 0.71 μm mean difference (95% CI: 0.45–0.98) from zero ($p < 0.001$). The over-estimation of the etch depths did not depend on the size of the mean etch depth (in the range from 7–14 μm) and was within 0.0–1.0 μm except for one outlier. The SD of the mean difference was 0.42. From the least square regressions of the plot the intercept with the y-axis was found to be 0.247 ($p = 0.742$) and the slope was 0.045 ($p = 0.533$).

Discussion

An advantage with the impression technique in clinical studies is that it will make it possible to measure tooth wear on the same area at different points in time. This is relevant for studies of the activity, prevention and treatment of dental erosions, abrasion and attrition. The two-component impression material that was used in the present study has a smooth, black surface that gave little scattering and absorption of light and offered a high number of pixels for the imaging in the white light interferometer (WLI). In contrast to this observation, studies using optical profilometers with red laser of 785 nm wavelength have shown that the results are affected by the colour and transparency of the investigated surface. If an impression material absorbs color in the same wavelength as the light source, the surface may not be correctly scanned by the profilometer [22]. It has also been found that the homogeneity of the color of a polyvinyl siloxane impression material influenced the accuracy of the laser scanning process and that the blue color was most favorable in combination with a 785 nm red laser [15]. Silicone impression materials can reproduce features down to 0.1 μm [21]. However, in clinical studies it is equally important that the smaller lesions can be detected by the measuring instrument and analyzed in the software. Previous and present studies indicate that this is the case with WLI both on enamel and negative replicas [2,12,19,20,23]. In the study by Holme et al. [12], assessment of topographic images based on impressions vs enamel, gave etch depths with an agreement of 0.1 μm. In the present study the mean etch depth was 0.7 μm higher measured on impressions than measured on enamel (Table 1). An explanation for this may be that the measurements are performed on different types of surfaces and that the black impression surface offered more registered points in the WLI than on the enamel surface. As can be seen in Figure 1, the mean etch depth measured on enamel vs on impression correlated well, but was consistently higher when measured on impressions.

The present results also show that WLI measurements both on enamel and impression can be repeated precisely as expressed by the calculated precision and ICC. These results were confirmed by the Bland Altman plot (Figure 2), where the least square regression of the plot showed that neither the slope nor the intercept were significantly different from zero, thus indicating no proportional or relative biases in comparison to the true value (measurements on enamel). The Bland Altman plot further indicates that measurements on these impressions in the range from 7–14 μm, are over-estimated by 7%, independent of the mean etch depth of the two methods (x-axis).

In order to obtain accurate and precise successive measurements there are several factors that should be taken into consideration, since they may influence the outcome of the analysis. One factor is the reference area which must be unaffected by wear and easy to identify. Various types of reference devices such as metal discs [10,11,18], metal stars [17] and metal rings [15] have been used for *in-vitro* and *in-situ* studies. The purpose of these reference devices is both to ensure identification of the measurement area, but also to level the measurement plane for analyses in the optical profilometers. In the present study, an amalgam filling was used as reference surface since it has been tested and shown to be unaffected by the acid etch [2,12,19,20,23]. Printouts of the baseline topographic maps (images) of each enamel specimen (which included 1/3 amalgam and 2/3 enamel) enabled us to identify exactly the same area for re-measurements after etch in the white light interferometer within a few pixels exactness.

The accuracy and precision of the replica technique reported in other studies have varied. Azzopardi et al. [10] used metal discs as reference surfaces on the tooth specimens and erosive loss was measured directly on Panasil impressions with a non- contact laser profilometer. The technique was proven to be accurate and reproducible. However, the progression of erosive wear was extensive in that study, with lesion depths > 120 μm, and it did not demonstrate if the technique is able to detect small lesions with a good reliability. In a study by Mitchell et al. [14], repeated measurements of erosive wear were performed on painted electro-conductive replicas made from silicone impressions. Erosion depths down to 50 μm could be presented with a precision of 15 μm, but the authors reported difficulties regarding the placement of control markers for identification of the analytic area. Schlueter et al. [17] reported that measurements by a mechanical stylus on epoxy models of ground enamel differed from −2.8% to +7.7% from the 'true value' measured directly on ground enamel, depending on the epoxy material. The authors considered one casting material unsuitable for further evaluation since the step height was over-estimated by 7%, but the high SD was even more troublesome. It may have been caused by the glass-like properties of the material which made it difficult to adjust in the microscope. In the present study the over-estimation of the etch depths by 7% when measured on impressions was in the same range, but here the standard deviation was low and the precision of the repeated measurements on the enamel vs on the impression was 0.05 μm and 0.01 μm, respectively. The latter may be explained by registration of a higher number of pixels for analyses on the impression surfaces than on the enamel surface due to a less reflective and rougher enamel surface. In addition, the amalgam reference and enamel reflects light differently than an impression of both, which also could lead to a few bad pixels/noise in the data. Stenhagen et al. [19] evaluated measurements of etch depths on enamel surfaces by a white light interferometer in comparison with four other techniques and etch depth measurements on enamel (with 0.07 μm accuracy and 0.05 μm precision) correlated well with those of both an optical and a mechanical profilometer. Measurements performed directly on impressions instead of making casts, are less time consuming and minimize the uncertainty that is easily introduced by multiple procedure steps. However, the instrument of choice should be of non-contact character in order to avoid scratches on the impression surface, which excludes the accurate and precise mechanical stylus profilometer.

The present study is the first attempt at a systematic comparison of measurements by WLI of etch depths on impressions vs directly on the tooth samples. To qualify this technique for clinical use, there is a need for further studies, preferably *in situ* or *in vivo* in order to evaluate the accuracy and stability of impression materials and techniques for taking repeated accurate impression. However, the composition and mixing procedure of the industrial silicon material that was used in the present study is based on the same material science as for dental impression materials. It is also important to test other reference devices that would be clinically acceptable and applicable for the process of the analyses by the interferometer. Amalgam is forbidden in Scandinavia and its use as a reference surface *in vivo* implies invasion of tooth structure and would not be acceptable for ethical reasons in clinical studies today. Possible reference devices could be orthodontic brackets or gold tooth jewellery which are easily accepted by patients and could be retained for years in the mouth. In addition it is important to measure tooth wear with high accuracy and precision on natural tooth surfaces in order to imitate the clinical conditions, since it has been shown that erosive loss is more extensive on ground enamel surfaces than on natural enamel surfaces under the same experimental conditions [24]. When WLI is used for analyses of tooth substance loss, the curvature of the tooth surface is not a problem since a topography image of the original surface (baseline) is subtracted from a topography image of exactly the same area after an experimental/observation period. This implies that extraction of depth change information from the topographic images is performed on a 'difference image'. By the use of specially designed software the original curvature is therefore removed by the subtraction and the surface loss may be calculated in addition to information about local changes in the topography. Measurements by WLI have previously been successfully performed on natural enamel surfaces [2,23], but need further investigation regarding a broader range of etch depths in order to evaluate the applicability of the method for clinical studies. The etch depths measured in the present study (7–14 μm) are over a limited range, but are representative for etch depths that may occur after several erosive challenges in a clinical situation.

If the replica technique works satisfactorily under these conditions, it will make clinical studies easier both with regard to monitoring the progression of tooth substance loss and the evaluation of the effect of preventive measures against dental erosion and tooth wear in general.

In conclusion, the white light interferometer (WLI) gave reliable repeated measurements of etch depths in the range from 7–14 μm on impressions of eroded ground enamel surfaces. However, the present study was the first step for the validation of a method that could make dental *in vivo* studies possible and there is a need for further adjustment of the method before it could be adopted in the clinic.

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