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Measuring enamel erosion: A comparative study of contact profilometry, non-contact profilometry and confocal laser scanning microscopy

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ABSTRACT

Objectives. To compare three instruments for their ability to quantify enamel loss after acid erosion.

Methods. 6 randomized parallel groups of bovine enamel samples were subjected to citric acid (higher acidity) or orange juice (lower acidity) erosion and remineralisation in a cycling model. Two protected shoulders were created on each of the samples using tape, to serve as reference for analysis. The time of exposure to each acid was varied, along with presence or absence of agitation. After treatment, samples were measured on 3 instruments capable of measuring step height: a contact profilometer (CP); a non-contact profilometer (NCP); and a confocal laser scanning microscope (CLSM) by three different examiners. Additionally, 3D (volume) step height was also measured using the CLSM.

Results. Increasing acid concentration and exposure time resulted in greater erosion, as did agitation of samples while in acid solution. All instruments/methods identified the same statistically significant ($p < 0.05$) pair-wise differences between the treatments groups. Further, all four methods exhibited strong agreement (Intra-class correlation ≥ 0.96) in erosion level and were highly correlated, with correlations of 0.99 or higher in all cases.

Significance. All instruments/methods used in this study produced very similar conclusions with regard to ranking of enamel loss, with data showing very high agreement between instruments. All instruments were found to be equally suited to the measurement of enamel erosion.

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1. Introduction

Research in the area of tooth wear has been growing over the last decade or so. There are a number of reports detailing this as a growing problem among Western populations [1]. Tooth wear is widely considered to consist of 3 main aspects, two of which are physical wear (abrasion, attrition) the third which is chemical dissolution (erosion) [2]. It is challenging to separate the two components in clinical models, so in vitro models are often used to help understand each component separately, although combined models which include both aspects are also important [3].

The methods used for measuring damage caused by tooth wear are many and varied, and discussed at length by Attin [4]. Quantitative assessment of tooth wear has most often been reported using surface profilometry. This has the advantage of being reasonably straightforward to conduct, and simple to understand, as it allows a step measurement (in microns) of enamel lost after exposure to acid, compared to a protected/undamaged (control) portion of the sample. However, surprisingly little research has been reported comparing the different types of instruments for surface profile measurement [5,6]. A notable comparison the authors could find was conducted by Heurich (2010) [5], where two contact profilometers (CP) were compared to a confocal laser scanning microscope (CLSM) and an Atomic Force Microscope (AFM). All instruments performed well and the same experimental conclusion would have been reached with each instrument.

Two main types of profilometer are available, contact and non-contact. Contact profilometers use a stylus moved across the surface to record the surface profile. While relatively simple, this traditional method has the potential risk of affecting the reading or even damaging the sample as a consequence of the contact [5]. Non-contact profilometers generally use some type of laser to scan the surface to create the profile. In addition, non-contact profilometers usually generate a surface plane rather than just simple line profiles, which allows volumetric loss analysis [7]. However, while removing the risk of surface damage due to contact, the type of laser scanning system needs careful selection, as reflective and/or translucent surfaces (such as enamel) can cause inconsistencies when profiled. A recent alternative/variation on the non-contact profilometer is the confocal laser scanning microscope (CLSM). This combines the laser scan with capture of a traditional visible light microscope image, producing a detailed 3D image of the surface. Traditionally these instruments did not provide quantitative data (i.e. scans/images calibrated to the μm level), but more modern instruments are now able to do this [5,8]. CLSM is used extensively elsewhere in science and engineering research [9,10].

The null hypothesis for the study was that differing instruments measuring the same samples provided data with poor agreement. The approach was to utilize a laboratory model for erosion to compare the data output from surface profiling instruments with differing analytical protocols and located at different institutions in the UK.

2. Materials and methods

2.1. Study design and procedure

2.1.1. Summary outline

Each of 48 bovine enamel samples (Therametric Technologies Inc., Noblesville, IN, USA) [11] was randomly assigned into one of 6 parallel groups (A1–A6), 8 samples per group. Samples were visually examined under a magnifier prior to inclusion in the study to check for significant defects and discarded as appropriate. A cycling model was employed to induce erosive damage on the samples. Each cycle commenced with 30 min immersion in remineralizing solution (remin solution described by Eisenburger et al. [12]), at mouth temperature with gentle agitation (Nickel Electro Clifton NE528D Shaking Waterbath with horizontal linear agitation at 24 cycles/min.), followed by deionized water rinse. Samples were then immersed in 100 mL of acid, either citric acid or commercial orange juice, at room temperature (RT, 20 ± 1 °C), followed by a final rinse in deionized (DI) water. The cycle was repeated 24 times. The 0.05 mol/L citric acid, (CA), was freshly made in the laboratory on a daily basis from deionized (DI) water and monohydrate citric acid and had a pH 2.26, with a titratable acidity of 18.0 [13]. The orange juice (Waitrose Essentials Orange Juice, referred to as OJ) had a pH of 3.8 and a titratable acidity of 10.5. The central portion of each sample was exposed to acid demineralization, the remainder of the sample masked to prevent acid contact. Groups A1, A2, A3 and A5 were treated with OJ, while groups A4 and A6 were treated with CA. Groups A1–A4 had a total acid exposure time of 180 min (7.5 min/cycle), while the other two groups had a total acid exposure time of 300 min (12.5 min/cycle). Further, while in acid, group A2 was gently agitated to induce increased erosion (Stuart See-Saw Rocker SSL4, set at 24 cycles/min). Group A3 samples were suspended upside-down in the acid (enamel surface facing down). See Table 1 for a summary. All acid exposure was done at room temperature (20 ± 1 °C).

Table 1 – Study design groups.

Group	Acid type[a]	Agitation?	Suspended?	Acid immersion time/cycle (min)	Total acid exposure (min)
A1	OJ	None	No	7.5	180
A2	OJ	Rocker	No	7.5	180
A3	OJ	None	Yes	7.5	180
A4	CA	None	No	7.5	180
A5	OJ	None	No	12.5	300
A6	CA	None	No	12.5	300

[a] OJ = Orange Juice, CA = 0.05 M citric acid.