DENTAL MATERIALS XXX (2018) XXX–XXX

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

In vitro evaluation of the early erosive lesion in polished and natural human enamel

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ARTICLE INFO

Article history:
Received 20 December 2017
Received in revised form
12 March 2018
Accepted 7 June 2018
Available online xxx

Keywords:
Early erosion
Erosion
Surface topography
Surface analysis
Optical coherence tomography
Surface profilometry

ABSTRACT

Objective. This study evaluated the capability of profilometry, microhardness, Optical Coherence Tomography (OCT) and Tandem Scanning Confocal Microscopy (TSM) in characterising the early erosive lesion in polished and natural human enamel *in vitro*.

Methods. Polished (n = 60) and natural (n = 60) human enamel surfaces, were immersed and agitated in 0.3% citric acid erosion at 0 s, 10 s, 30 s, 60 s, 120 s, and 300 s (n = 10). Changes in the surface were measured with 3D-step height change (μm), surface roughness (μm), surface microhardness (KHN), and images were assessed qualitatively with OCT and TSM.

Results. Mean (SD) 3D-step height change (μm) was measurable for polished enamel at: 60 s (0.24 ± 0.1), 120 s (1.16 ± 0.71), 300 s (2.01 ± 0.47; $p < 0.05$); a step height change was not detectable on acid challenged natural enamel surfaces. Mean (SD) surface roughness (μm) of polished enamel was detected at 10 s (0.270 ± 0.013; $p < 0.05$) and all erosion periods; and in natural enamel detected after 120 s (0.830 ± 0.125) and 300 s (0.800 ± 0.140; $p < 0.005$). Polished enamel Mean (SD) microhardness (KHN) statistically significantly decreased at all time points ($p < 0.001$); this was unmeasurable for natural enamel. Qualitative image analysis of both surface types indicated erosive change at the surface level, with progression after increasing erosion time.

Significance. The early erosive lesion in polished enamel could be characterised quantitatively surface roughness and microhardness and qualitatively using OCT and TSM; whilst in natural enamel only surface roughness could be utilised. Further investigation of early erosion in natural enamel is required to develop new more clinically relevant models.

1. Introduction

Erosive tooth wear is a common oral condition which if not treated can compromise the longevity of teeth and a person's quality of life [1]. Understanding what happens in the early stages of this condition, particularly to the integrity of the enamel surface, should enable a better understanding of prevention [2]. The time acidic foods or drinks are exposed in the mouth during eating and or drinking is not precisely known, due to individual variation, but it's likely to be only for a few seconds. But how long it takes for acidic challenge to change the enamel surface is unknown. It is apparent that frequency of consumption of acidic foods and drinks cre-

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https://doi.org/10.1016/j.dental.2018.06.018
0109-5641/

ates a greater risk of developing erosion but they are cleared quickly from the mouth [3]. Current understanding of erosive tooth wear suggests that following a short duration (less than 5 min) acid exposure the enamel surface may soften to a depth of between 0.2 μm and 5 μm [4,5]. This softening is believed to reflect partial loss of surface minerals leading to increasing surface roughness and decreased surface microhardness [5–8]. But these times are derived from laboratory investigations which are partly influenced by the model, presence of artificial or natural saliva, the biofilm, but also by the sensitivity of the measuring technique. Clinically, we appreciate that irreversible loss of tooth structure occurs if acid exposure is prolonged, and increases if further attritive or abrasive factors are introduced [4,9–12]. The definition of what is 'short duration' acid-erosion, in the literature, has not been specified to one particular time period or to the number of acid erosion cycles used, but rather by the duration of acid exposure utilised; typically lasting for several seconds or minutes, and which do not exceed 5 min duration [4,11,13–15].

The physical properties of the early erosive lesion are ill-defined due to the difficulty in detecting quantifiable changes to the enamel structure within short acid erosion time periods [16]. This is primarily a consequence of the sensitivity of most methods to measure any change. Studies have evaluated the formation of early erosive lesions with a range of acid immersion periods and/or number of immersion cycles and varies from 5 s [17] up to 2 h [7] for 1 acid cycle [6,7,11,18–24] to greater than 5 cycles of acid erosion with varying immersion times [17,23,25–27]. Most early enamel erosion studies have been conducted on polished enamel surfaces to ensure consistency of sample preparation, and allow reproducible and accurate measurement of effects occurring from acid exposure [16,18,28,29]. Laboratory simulation of the early erosive lesion on natural, unpolished enamel has been challenging due to its topography and morphology [30].

Previous erosion studies have utilised surface profilometry, to measure step height loss [17,22,27,31] and surface microhardness to determine surface and sub surface softening [6,18,27,29]. Moreover, the equipment and acquisition parameters utilised, varied between studies, and thus it is difficult to generalise the overall detection capabilities of specific measurement techniques. However, these techniques have produced varying degrees of success when detecting the earliest changes following acid erosion. To complicate matters further different measuring systems produce different data, although it appears that the comparative changes are consistent between them [31,32]. A recent study concluded that chromatic non-contacting optical profilometry had measurement uncertainty of 0.49 μm [33] suggesting enamel loss from early enamel erosion may not be reliably detected below 0.4 microns. Whilst another study reported areal textural changes after 30 s exposure to citric acid, utilising non-contacting optical microscopy [27]. Surface microhardness has been shown to be sensitive in detecting early changes due to acid erosion after 30 s exposure to citric acid [27], and this technique may be useful for times less than this on polished or natural enamel surfaces. Scanning electron microscopy (SEM), has been used to evaluate morphological changes following exposure to acid, however this technique alters the sample surface to allow imaging [21,24]. Tandem scanning microscopy (TSM), involves no sample preparation and high image acquisition, has previously been evaluated for dentine occlusion studies [34,35] but its use in evaluating enamel erosion has yet to be considered. Optical coherence tomography (OCT) has been used to determine surface [16,36] and subsurface [16,37,38] changes that occur to enamel after erosion but to different degrees of success. A recent study determined that alterations in enamel surface reflectivity could be utilised to study the early erosive lesion with change detectable after 1 min acid exposure in polished bovine enamel surfaces [36]. However, longer erosive time periods of 60 min [38] up to 6 h [37] were required in order for subsurface changes in natural enamel to be detectable.

The aim of the study was to determine the minimum time that acid exposure causes change on the enamel surface can be measured by profilometry, surface roughness, surface microhardness, OCT, and TSM. Our null hypothesis was that the formation of an early erosive lesion is independent of time

2. Methods

Enamel slabs (n = 120) were sectioned from the mid-buccal surfaces of previously extracted caries-free human molar teeth (REC ref 12/LO/1836), using a water-cooled 300 μm diamond wafering blade (XL 12205, Benetec Ltd., London, UK). Each slab was mounted, unpolished enamel surface down, in self-cured bisacryl material (Protemp^{TM}4, 3M ESPE, Seefeld, Germany) using a custom-made silicone mould. Sixty slabs were polished using successively finer silicon-carbide discs (Versocit, Struers A/S, Copenhagen, Denmark) of grit 500, 1200, 2000, and 4000 for 25 s, 30 s, and 60 s respectively, using a water-cooled rotating polishing machine at 150 rpm and 10 N constant pressure (LaboPol-30, Struers ApS, Ballerup, Denmark) removing approximately 300 μm of enamel and achieving flatness tolerance ±0.2 μm. The newly polished surfaces were then ultrasonicated (GP-70, Nusonics, Lakewood, USA) in 100 ml deionised water (pH 5.8) for 15 min to remove smear layer and air-dried for 24 h at room temperature. PVC adhesive tape was placed on the polished enamel slab/bis-acryl embedding material surface such that each the polished enamel surface had a 1 mm × 3 mm window of exposed enamel, protected by two zones of reference tape either side; allowing for comparison of eroded and protected enamel regions after erosion and after tape removal [27].

Natural surfaces were not cleaned using the same method as for the polished surfaces because this did not result in a sufficiently cleaned enamel surface upon which to conduct the acid erosion challenge. As a result, to ensure they were free from surface debris and organic contamination, all natural enamel surfaces (n = 60) underwent a standardised cleaning regime consisting of: a 10-min immersion in 4.7% sodium hypochlorite solution (Coventry Chemicals Ltd, Coventry, UK), followed by 30-min ultrasonic cleaning in deionised water and air dried for 1 h followed by a 2-min clean with ethanol and cotton wipes, and final air dry for 1 h. They were examined under TSM before and after cleaning to ensure surface cleanliness which was determined as the visible lack of surface material after cleaning and clear visualisation of enamel surface topography.

Please cite this article in press as: Mylonas P, et al. *In vitro* evaluation of the early erosive lesion in polished and natural human enamel. Dent Mater (2018), https://doi.org/10.1016/j.dental.2018.06.018