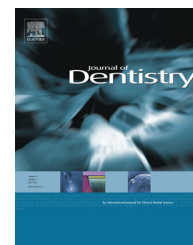


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Clinical enamel surface changes following an intra-oral acidic challenge

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ARTICLE INFO

Article history:

Received 17 February 2015

Received in revised form

27 March 2015

Accepted 1 April 2015

Keywords:

Enamel

Erosion

Clinical study

Acidic soft drink

Replica impression

ABSTRACT

Objectives: Investigation of early enamel erosion using replica impressions to compare changes in enamel surface topography in vivo prior to and over a 24 h period following acid challenge.

Method: A single treatment, blinded, enamel replica clinical study was undertaken in 20 healthy subjects. Replica tooth impressions were taken at baseline, following acid challenge and 2, 4, 7 and 24 h post challenge. Subjects consumed 500 ml of acidic soft drink over 30 min. Scanning electron microscopy of surface topography was characterised with a descriptive 5 point scale by four judges. Duplicate impressions were taken to assess reproducibility.

Results: 18 subjects had scorable sequences. Descriptive analyses showed erosive changes following acid consumption and reparative changes in the subsequent 24 h period. Comparing baseline replica to the 24 h replica, there were no significant differences ($p = 0.26$) in tooth surface characteristics. Comparing the replica taken immediately following acidic challenge with the subsequent replicas at 2, 4, 7 and 24 h, showed clear reduction of erosive effects on the enamel surface at 2 h ($p = 0.02$) and a highly significant reduction at 4, 7 and 24 h ($p < 0.001$).

Conclusion: This methodology demonstrated the ability to follow the progression and recovery of early erosive enamel lesions over 24 h being accurate and reproducible. This study suggests enamel repair commences within 2 h following a substantial acidic challenge and is completed 4–24 h later. After 24 h, the tooth surface appeared visibly indistinguishable from the original tooth surface, suggestive of a recovery process occurring.

Clinical significance: Healthy erosive lifestyles often culminate in tooth wear. The time taken for enamel remineralisation following acidic challenge is unknown however, this study suggests the repair process is relatively slow following a substantial acidic challenge, and at least 4–24 h should elapse prior to further acidic consumption to allow for recovery.

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<http://dx.doi.org/10.1016/j.jdent.2015.04.002>

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1. Introduction

Dental erosion is a condition that results in loss of hard tooth tissue due to dissolution by acids without bacterial involvement.¹ The very early stages of enamel erosion are thought to involve acids diffusing through the acquired pellicle, releasing minerals from the enamel and resulting in dissolution of the prism sheath followed by the prism core.² This produces the well known honeycomb appearance.³ It is generally agreed that at this stage, the condition can be reversed since a mineral scaffold exists for remineralisation by saliva.^{4,5} If the softened layer of enamel is not rehardened, and the acid impact continues, progressive softening and dissolution of the consecutive layers of enamel lead to permanent loss of volume, with a softened layer on top of the remaining tissue.⁶ This softened layer on the enamel surface is highly susceptible to wear from, for example, an abrasive toothbrush insult.⁷ In severe, long-term erosive wear, when there is frequent substantial erosive attack on the enamel surface, the volume of hard tissue permanently lost is clearly visible clinically, as a defect in the tooth structure. Erosive tooth wear is becoming more common, particularly amongst young adults, a recent European study of >3000 individuals aged 18–35 demonstrating 30% of individuals had a Basic Erosive Wear Examination (BEWE) score of 2 or 3,⁸ and 27.7% BEWE 1.

The dynamics of demineralisation and remineralisation of the enamel surface of a vital sound tooth following an erosive challenge, will depend on the interaction between the characteristics of the erosive challenge, the biological variability of the host's intra-oral environment and behaviour of the individual. The interplay between these factors modifying these processes.⁷ Early erosion and subsequent remineralisation/rehardening duration studies have been assessed mainly by *in vitro* methodologies, the majority of techniques unable to be used *in vivo*.⁹ The length of time needed for remineralisation to occur following an acid challenge *in vitro*, using profilometry and scanning electron microscopy (SEM), has been shown to be between 6 and 24 h.⁵ Surface microhardness changes are often used to detect early enamel erosion. An *in situ* study showed hardness changes after a few minutes of acid exposure to enamel surfaces,^{10,11} and a further *in situ* study using microhardness, Fushida *et al.*¹² found a third of the remineralisation occurred after 24 h. However, the microhardness technique requires flat polished surfaces, and lacks accuracy on curved natural tooth surfaces,¹³ although there are two documented *in vivo* studies.^{14,15}

Scanning electron microscopy has been the most common technique to examine the natural curved surface morphological changes of eroded enamel qualitatively especially *in vitro*.^{16,17} Recently, a new accurate replica impression technique has been developed for scanning electron microscopy to examine morphological changes of hard tissues.^{18,19} With this technique, Sauro *et al.*²⁰ proved *in vivo* the replica impression technique provided an accurate method of tracing enamel morphological alterations induced by acidic drinks.

To date, the time taken for a human tooth enamel reparative processes to occur in the oral environment following an erosive insult, can only be estimated, at best from *in situ* data.¹¹ *In vivo* data would provide invaluable

insight with regards to the enamel recovery time processes and improve knowledge for public oral health messages. The aim of this clinical study was to explore the use of a replica impression methodology to both acquire accurate scanning electron microscope images of vital tooth enamel surfaces and qualitatively assess the enamel morphological changes in the oral environment up to 24 h following an erosive challenge.

2. Materials and methods

2.1. Ethical considerations and inclusion/exclusion criteria

The study protocol was approved following review by a Governance Review Board. Written informed consent was obtained from all subjects prior to their screening visit. Sufficient subjects were screened to ensure that 20 participants fulfilled the entry criteria. Medical history and concomitant medications were recorded and inclusion/exclusion criteria applied. Participants needed to be aged at least 18 and in good health. Subjects were excluded if they were on medication which might result in decreased salivary flow rate, showed signs of xerostomia or susceptibility to acid regurgitation. Continued eligibility was then determined following an oral soft tissue (OST) examination and an evaluation of relevant dentition exclusions. Individuals displaying moderate to severe erosion were excluded, BEWE 2 and 3.²¹

2.1.1. Study design

This was a single site, blind with respect to study analysts (microscopist, image grader) single treatment clinical study carried out in a Dental Hospital. The study comprised of 6 clinical visits over 3 consecutive days.

The study was planned to recruit 20 participants. The degree of shift in score following acid challenge that is detectable with 80% power at the usual 2-sided 5% alpha level is 0.63 times the standard deviation representing the degree to which these changes in score vary between individuals.

Following screening two suitable upper incisor teeth were identified for study assessments. A prophylaxis scale was undertaken of the 2 identified teeth to remove surface deposits, the teeth were gently washed with the triple syringe and baseline impressions taken of the facial aspect using a silicone impression material (Aquasil® Dentsply™, Weybridge, Surrey). One tooth was subsequently identified for study following examination of the replicas under SEM.

The following day, participants drank 500 ml of a commonly available fruit based health drink diluted to the manufacturers instructions (PLJ Healthy Food Brands Ltd.) over a period of 30 min at their leisure and with a normal drinking pattern, no restrictions or instructions were given. The pH of the drink was 2.57 and neutralisable acidity was 7.28 (mls of 0.1 M NaOH). This was followed by impressions of the identified teeth immediately and 2, 4, 7 and 24 h following the acidic exposure. Two individuals were randomly chosen for duplicate impressions of each time point for comparison purposes. Impressions were disinfected per the study site protocol for cross infection purposes. Prior to taking the impressions, the nominated tooth surface was gently washed

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