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Three-dimensional profilometric assessment of early enamel erosion simulating gastric regurgitation

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ABSTRACT

Objectives: A priority research area in minimal intervention dentistry is the characterization of the early stages of dental erosion. The aim of this *in vitro* study was to assess the effect of short, repetitive erosive challenges to human enamel over 2 min at pH 1.5 and 3.0 under conditions simulating gastric regurgitation.

Methods: Enamel surfaces were subjected to erosive challenges at pH 1.5 (Group 1, $n = 10$) and pH 3.0 (Group 2, $n = 9$) for periods of 30 s (stage 1), 60 s (stage 2) and 120 s (stage 3). Quantitative changes were assessed longitudinally by measuring the 3D average surface roughness (Sa) values using 3D confocal microscopy. Qualitative micrographic assessment of surface changes was also conducted by using environmental scanning electron microscopy.

Results: Linear mixed model analysis showed significant effects of the pH values ($p < 0.001$) and the stages ($p < 0.001$) on the observed Sa values. Post hoc tests showed significant increases in the Sa values between baseline and other stages in both groups ($p < 0.01$). The mean Sa values also increased significantly from stage 1 to stage 2 in Group 1 ($p < 0.05$). Micrographic analysis displayed severely etched enamel rods in Group 1, but only subtle changes in Group 2.

Conclusions: The complexity of the enamel surface is influenced by both acid concentration (pH value) and duration of acid exposure during early stages of erosion. Erosion occurring under conditions simulating GORD can be detected in its initial stages, opening up the possibilities of early diagnosis and management of this condition.

Clinical significance: Erosive tooth wear occurs progressively and insidiously, often creating complex treatment challenges. This emphasizes the need for early diagnosis and management in accordance with minimal intervention philosophy. Our findings provide a foundation for further research that could lead to the development of highly-sensitive clinical diagnostic tools and preventive strategies.

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1. Introduction

Dental erosion is an issue of growing concern in developed countries,[1,2] and it is often only detected clinically after extensive tooth wear has occurred.[3] In order to prevent a lifetime of compromised dental health that may require complex and extensive rehabilitation, it is now generally recommended that, in accordance with the principles of minimal intervention dentistry, emphasis should be placed on early diagnosis and prevention before restorative management is considered.[4–7] Reported recent increases in the prevalence of erosion in children and young adults have been attributed to extrinsic erosion from increased consumption of acidic beverages,[8] with the relative effect of erosion due to intrinsic factors generally being considered minor.

Recent findings of an increasing prevalence of gastro-oesophageal reflux disease (GORD) in many countries (particularly in "silent refluxers"), and improved understanding of its oral and extra-oral manifestations (including Barrett's oesophagus and adenocarcinoma),[9–11] highlight the need for early management of intrinsic GORD-related erosion. However, GORD medications (for example, proton pump inhibitors) are less effective than originally believed,[12–14] and remineralizing agents provide little protection from strong acids.[15–18] Thus, there is a need for stronger collaboration between both dental and medical practitioners in the management of GORD and associated erosive tooth wear at an early stage.[5,19]

Current *in vivo* methods lack sensitivity and reliability to detect early erosion. In particular, they lack fixed intra-oral reference points for the assessment of longitudinal changes on the tooth surface.[20] Thus, our existing knowledge of the pathogenesis of erosion has come mainly from *in vitro* models using flat, polished samples. In addition, profilometric assessment on such samples has required around 5–10 min of demineralization prior to detection,[21–24] exceeding *in vivo* exposures to erosive agents that only tend to last from seconds to a few minutes.[25] As a result, detection of erosion within two minutes of acid exposure will be of clinical significance

By measuring changes in three-dimensional surface roughness (Sa) values, the aim of the present study was to assess both quantitatively and qualitatively the characteristics of early, repetitive erosive challenges to enamel surfaces up to a period of two minutes, simulating moderate and severe forms of gastric regurgitation. The null hypothesis was that erosion cannot be detected within the first two minutes of acid exposure irrespective of the pH value.

2. Materials and methods

2.1. Sample preparation

Twenty sound, maxillary human third molar teeth were selected from a pool of extracted teeth that had been stored in 0.2% thymol solution. The teeth had been extracted as part of dental treatment, and the protocol for the collection of extracted teeth was approved by the University of Adelaide Human Research Ethics Committee (H/27/90). The roots of these teeth were removed using a water-cooled high-speed sectioning machine, with the coronal halves being further sectioned into buccal and palatal portions using a water-cooled slow-speed sectioning machine. The palatal portions were polished flat to enable calculation of Sa values at high magnification. The portions were embedded with the uncut side down in a chemically cured transparent epoxy resin in SeriForm mounting cups (Struers, Ballerup, Denmark) ($n = 20$). The uncut enamel surfaces were polished flat progressively with 800, 1200 and 2400 grit silicon carbide discs under constant water supply on a rotating polishing machine, followed by polishing with 9 μm, 3 μm, 1 μm and 0.25 μm TegraSystem abrasion discs under a constant supply of lubricants (DP-Lubricant Blue and DP-Suspension P) (Struers, Ballerup, Denmark). The polishing removed an outer layer of 400 μm of enamel and exposed a flat area of around 15 mm^2. The dimensions of the epoxy cylinders were 1.2 cm (height) × 1.0 cm (diameter).

2.2. Study design

Early enamel erosion was investigated by subjecting the specimens to demineralization in hydrochloric acid solutions, simulating a severe form of gastric regurgitation in Group 1 (pH 1.5) and a moderate form of gastric regurgitation in Group 2 (pH 3.0). The titratable acidity of hydrochloric acid solutions at pH 1.5 and pH 3.0, calculated in three aliquots, were 0.0499 M and 0.0025 M, respectively. Their corresponding hydrogen ion concentrations, calculated using the formula '$pH^+ = -\log_{10}[H^+]$', were 0.0316 M and 0.0010 M. These two acidic conditions were chosen to represent different levels of acidity of the gastric refluxate in the oral cavity.[26] Enamel specimens were divided randomly into two groups, each comprising 10 specimens. One specimen from Group 1 was excluded because of technical (procedural) error, resulting in the final sample size of 9 in that group.

All specimens were stored in deionized water before commencement and between all stages of experimentation. They were subjected to repetitive erosive challenges for 2 min in three stages consisting of 30 s in stage 1, 30 s in stage 2 and 60 s in stage 3. Each erosion stage was conducted by placing 2 ml of hydrochloric acid solution on the enamel surface, followed by immediate coverage with a glass slide to ensure consistency in application of acid in the form a thin film. At the end of each stage, the specimens were washed with ultra-pure distilled water for 30 s and dried with nitrogen gas for 15 s.

2.3. Assessment of erosion

Quantitative changes on the enamel surfaces were assessed longitudinally by measuring the 3D average surface roughness (Sa) values before and after erosion using a 3D Laser Confocal Microscope (LEXT 4000, Olympus, Hamburg, Germany) at a magnification of 6376× (cut off wave length, $\lambda_c = 8$ μm using a Gaussian filter). This magnification was set automatically by the LEXT software when the objective lens of 100× was used at 3× zoom. This microscope has a resolution of 100 nm in the z-axis. Our preliminary investigation showed that locating reference points on flat epoxy surfaces around the tooth specimens did not yield accurate data to assess changes in erosion depth at this magnification, therefore the