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An in-situ pilot study to investigate the native clinical resistance of enamel to erosion

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ABSTRACT

Objectives: To investigate the differences in susceptibility of the surface of native and polished enamel to dietary erosion using an in-situ model.
Methods: Thirty healthy volunteers (n = 10 per group) wore mandibular appliances containing 2 native and 2 polished enamel samples for 30 min after which, the samples were exposed to either an ex-vivo or in-vivo immersion in orange juice for 5, 10 or 15 min and the cycle repeated twice with an hour's interval between them. Samples were scanned with a non-contacting laser profilometer and surface roughness was extracted from the data, together with step height and microhardness change on the polished enamel samples.
Results: All volunteers completed the study. For native enamel there were no statistical difference between baseline roughness values versus post erosion. Polished enamel significantly increased mean (SD) Sa roughness from baseline for each group resulting in roughness change of 0.04 (0.03), 0.06 (0.04), 0.04 (0.03), 0.06 (0.03), 0.08 (0.05) and 0.09 (0.05) μm respectively. With statistical differences between roughness change 45 min in-vivo versus 45 min ex-vivo ($p < 0.05$). Microhardness significantly decreased for each polished group, with statistical differences in hardness change between 30 min in-vivo versus 30 min ex-vivo ($p < 0.05$), 45 min in-vivo versus 30 min ex-vivo ($p < 0.01$), 45 min in-vivo versus 45 min ex-vivo ($p < 0.01$).
Conclusions: The native resistance to erosion provided clinically is a combination of the ultrastructure of outer enamel, protection from the salivary pellicle and the overall effects of the oral environment.
ClinicalTrials.gov identifier: NCT03178968.
Clinical significance: This study demonstrates that outer enamel is innately more resistant to erosion which is clinically relevant as once there has been structural breakdown at this level the effects of erosive wear will be accelerated.

1. Introduction

The first sign of erosive tooth wear is described as early surface texture loss. There have been recent developments this surface change using surface roughness parameters [1]. Surface roughness measurements have been specifically advocated for erosion studies investigating early erosion changes without tissue loss [2]. The origins of surface roughness measurements come from nanometrology and are based upon the principle that every surface is combined of three component forms; (profile), waviness and roughness (which combined are texture). The form is described as the underlying shape, whereas waviness and roughness are deviations from the shape. In particular roughness is the minute wavelengths that give an indication of the nature of a substance [3,4]. When quantifying changes in enamel following erosive wear using surface roughness measurements it is the activity at the level of an enamel prism that is being measured, which has been postulated to be relevant for quantifying initial erosion [2]. Different parameters are used to quantify surface roughness including amplitude. Amplitude parameters calculate roughness as height deviations from the form. The parameter used in this study was Sa roughness, which is a 3 dimensional measure which represents the mean roughness of a studied surface [5].

Most erosion studies use enamel samples that have been polished flat, a process which removes the outer layer of enamel and alters the overall form and creates a surface that is easier and more reproducible to measure [6,7]. The effects of erosion have been recently investigated in vitro using both polished and native enamel samples (where the outer surface has been left intact) [8–10]. We reported data from in-vitro studies using surface roughness changes on polished enamel and native enamel over different locations, and identified that five discrete

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measurements from the centre of native enamel were representative of the overall surface of the sample [9]. We also identified that whilst polished enamel becomes rougher, native enamel becomes smoother after 45 min immersion in orange juice [8,9]. However, polished enamel exhibited statistically significant changes in surface roughness after only 15 min immersion in orange juice whereas it took 45 min for native enamel to show quantifiable change. This suggests that as well as behaving differently to polished enamel, native enamel is innately resistant to erosion.

The native enamel surface provides a more clinically relevant substrate, however, to be truly clinically representative the effects of the total native defences to erosion must be investigated. In vitro studies can overestimate erosive changes by approximately 10 fold [11]. Currently there are no validated methods to measure surface roughness in-vivo. Therefore, the optimum method to simulate the clinical environment is an in-situ study with in-vivo erosion, which was used in this study. The aim of this study was to investigate the differences in susceptibility of native and polished enamel to dietary erosion using an in-situ model and compare the influence of native biological defences to erosion. The null hypothesis was: there is no change to the enamel surface following immersion in orange juice.

2. Methods

2.1. Experimental design

This was a single-blind, randomised intervention study involving 30 healthy volunteers (10 volunteers per study group) who met the inclusion/exclusion criteria, to measure surface changes of native enamel and polished enamel in-situ following an orange juice acid challenge. Ethical approval for the study was granted by the Stanmore Health Research Authority REC ref 15/LO/0417, and the study was conducted using the guidelines for Good Clinical Practice. The inclusion criteria stipulated mild erosive tooth wear maximum score of 2 in each sextant and cumulative score no more than 8, aged 18 years and over, willing to participate, not enrolled in any other research, possessing more than 20 anterior and posterior teeth, no active carious lesions and a maximum BPE score of 2 in one sextant (no periodontal disease). The exclusion criteria stipulated pregnancy or breast feeding, medical history likely to impact on attendance or mobility, insulin dependent diabetes, saliva diagnoses (xerostomia), lower orthodontic appliances, dentine hypersensitivity, defective restoration of the occlusal or incisal surfaces of upper anterior teeth and first molars and any condition that precluded consumption of 300 mL of orange juice a day for 5 consecutive days. Following recruitment into the study, lower impressions were recorded in alginate, using stock trays, to fabricate custom-made lower soft orthodontic appliances. The appliances were designed to accommodate four enamel samples (one polished and one native on each side) and positioned buccally in the premolar/molar region. The volunteers underwent a 5 day wash out period during which they used a non-fluoridated toothpaste (Kingfisher, Norwich, UK) and standard manual toothbrush. They were also asked to refrain from eating or drinking for two hours prior to the start of the study appointment.

Extracted human molars without visible signs of caries or tooth wear were stored in sodium hypochlorite for a minimum of three days [9]. The roots were removed and the crowns sectioned using a circular diamond saw (XL 12205, Benetec Ltd., London, UK) to produce 120 (4 × 4 mm) buccal enamel sections. These enamel sections from the buccal surfaces of teeth were randomly allocated to produce 60 native and 60 polished (flattened) samples. Both groups were embedded in bisacryl composite (Protemp4 3M ESPE, Germany) using custom made mould trays ensuring the outer curved surfaces remained untouched; and were cleaned using a soft toothbrush and non-fluoridated toothpaste (Kingfisher, Norwich, UK) and wiped with ethanol. The polished samples, were fully embedded in bisacryl composite, placed in a water-cooled rotating polishing machine (LaboForce 100, Struers, ApS, Ballerup, Denmark) and polished flat using a series of Silica Carbide Grits (Versocit, Struers A/S, Copenhagen, Denmark) to produce 60 optically flat samples with a flatness tolerance within 0.4 µm [12]. Following preparation all the samples (native and polished) were ultrasonicated in deionised water for 15 min and immersed in sodium hypochlorite prior to baseline measurements being recorded. For the polished samples, PVC tape was applied over the enamel to create a window of exposed enamel (1 mm) with a reference area of enamel either side (each 1 mm) and used for step height measurement.

Three erosion times were investigated at 15, 30, or 45 min, which were achieved using a 3-cycle model. The volunteers were randomly allocated into these groups using statistical software (GraphPad). Ex-vivo and in-vivo erosion was carried for each participant. For the ex-vivo erosion one native and one polished sample were removed from the splint and immersed in 20 mL of orange juice and agitated at 62 rpm (Stuart Scientific, Mini Orbital Shaker S05, Bibby) for either 5, 10, or 15 min. Following which they were reinserted into the splint and worn during the rest periods. During the ex-vivo erosion the participant rinsed with orange juice for the same time duration. The process for in-vivo and ex-vivo was repeated twice with an hour's rest between giving 3 cycles of erosion thereby totally 15, 30 or 45 min erosion, a flow chart of the study schedule is shown in Fig. 1. After the 3rd and final erosion

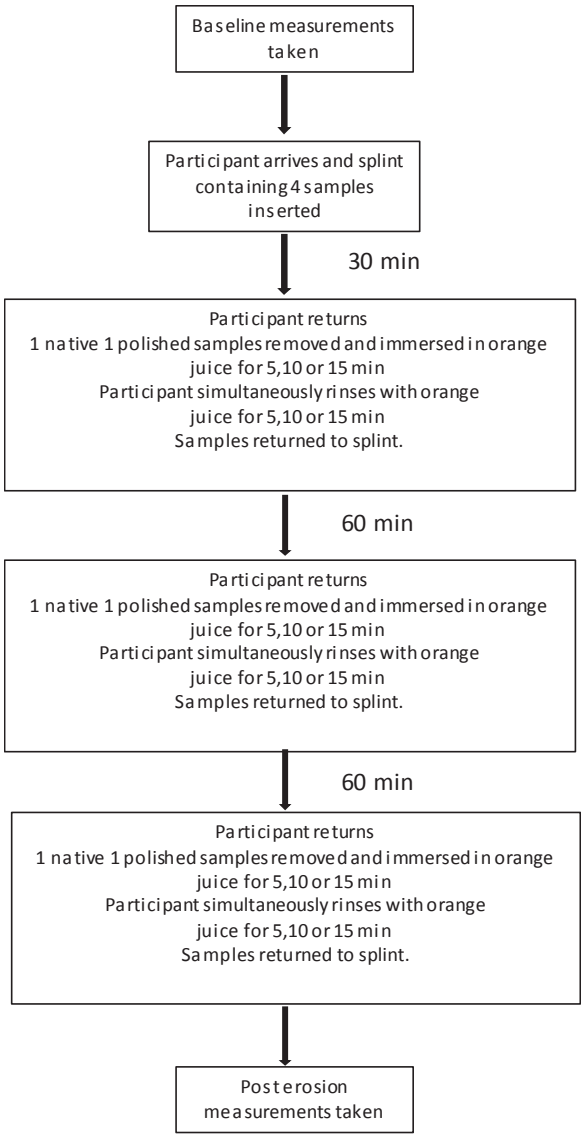

Fig. 1. Flow chart of study visit.