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In vitro demineralisation of the cervical region of human teeth

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ABSTRACT

Objective: The aim of this study was to investigate a possible role for demineralisation of the cervical region of human teeth in the development of non-carious cervical lesions (NCCLs).
Materials and methods: Freshly extracted human premolars were demineralised and prepared for nanoindentation and scanning electron microscope (SEM) observation. After 1 day or 2 days demineralisation in a solution of pH 4.5, specimens were embedded, cut and polished to 1 μm diamond paste. Nanoindentation was done at the cementum–enamel junction (CEJ) region with an interval of 30 μm, to develop mechanical properties maps. After the indentation, SEM with back-scatter detector was employed to observe the degree of demineralisation at the CEJ.
Results: After 1 day and 2 days demineralisation, the mechanical properties of enamel and dentine at the CEJ decreased by ~50% and ~90%, respectively. SEM images illustrate that artificial demineralisation generated typical demineralised zones in enamel near the CEJ. Moreover, 2 days demineralisation penetrated the sound enamel at the CEJ, and the dentine beneath was undermined.
Conclusion and significance: One day and 2 days demineralisation reduced the mechanical properties of teeth at the CEJ significantly. Demineralised enamel and dentine with low mechanical properties are prone to wear and abrasion. The findings of the investigation indicate that acid typical of that produced by dental plaque may compromise the mechanical properties of enamel and dentine at the CEJ to the extent that they would be susceptible to tooth brush abrasion, producing NCCLs.

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1. Introduction

Non-carious cervical lesions (NCCLs) have commonly been described as the loss of tooth substance at the cementum–enamel junction (CEJ) without the involvement of micro-organisms.[1–5] There is an increasing interest in NCCLs due to their high prevalence, which has been reported in up to 90% of 31–60 year old patients.[6] Although several factors have been considered in the development of the lesion, the true aetiology of NCCLs is not fully understood. In the past decades, the discussion on aetiological factors of NCCLs was mainly concentrated on abrasion[7] and stress[8] or abfraction,[9–11] which are all physically and biomechanically based theories. Few studies have considered chemical degradation of the surface as a factor in the development of NCCLs, possibly due to the characteristics of NCCLs and the contradicting clinical investigations. NCCLs are almost exclusively found on buccal surfaces and exhibit a site specific prevalence,[12] which is difficult to correlate to endogenous erosion. Although Bader et al. found that drinking fruit juices more than once a day and a low salivary buffering capacity were correlated with increased NCCLs (odds ratio: 6.11 and 5.73, respectively),[13] a similar study by Pegoraro et al. on 70 surveyed participants who

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drank acidic beverages and/or had regurgitation problems, did not support the conclusion.[10]

Clinically, accumulation of plaque and bacteria at the buccal cervical region of teeth is not uncommon, especially in patients with poor oral hygiene. Bacteria within plaque ferment carbohydrates producing acid, resulting in a pH of ~4, with some variation depending on site.[14] At this pH level, enamel undergoes demineralisation. Saliva counteracts the decrease in pH by clearing fermentable carbohydrates and buffering acids. However, it has been shown that saliva clearance is slowest in the vestibular region of the mandibular premolars,[14] which is also a region with high prevalence of NCCLs (12.7% in the mandibular left premolar region).[12] These findings suggest that demineralisation of the CEJ region due to plaque accumulation might be a plausible factor in the development of NCCLs. Therefore, this study aimed to verify the hypothesis that demineralisation of the CEJ region could be a contributing factor to the development of NCCLs.

2. Materials and methods

2.1. *Sample preparation*

Four freshly extracted human mandibular premolars were selected for this study. They were caries free, and without restorations or obvious cracks. Ethical approval for the use of these teeth was obtained from the Lower South Region Ethics Committee (Project Key: LRS/09/04/015). Any soft tissue remaining on the specimens was removed carefully with a scalpel blade. The surfaces of each sample were protected by nail varnish excluding a 5 mm × 5 mm window that included the CEJ on the buccal aspect. Prepared samples were randomly placed into two equal groups. Each group underwent a different period of demineralisation: 1 day and 2 days.

The demineralisation formula used in this study has been well documented.[15–17] It consisted of 2.2 mM $CaCl_2 \cdot 2H_2O$ (BDH Chemicals, AnalaR, Poole, England), 2.2 mM KH_2PO_4 (BDH Chemicals), and 50 mM Glacial acetic acid (BDH Chemicals) in de-ionised water. The pH of the solution was adjusted to 4.5 using 1 M KOH (BDH Chemicals) with a pH metre (pH 211, Hanna instruments, Australia). Prepared samples were completely immersed in 500 mL of the demineralizing solution for different time periods (1 day or 2 days) under constant low-speed magnetic stirring. For the 2 days group, the demineralizing solution was replaced after 24 h.

After the demineralisation process, all samples were embedded in cold-curing epoxy resin (Epofix, Struers, Copenhagen, Denmark). The embedded samples were sectioned buccal-lingually into halves with a high-speed cutting machine (Accutom-50, Struers) utilizing a diamond cut-off wheel (331CA, Struers). The samples were cut at a spindle speed of 3000 rpm under constant water irrigation. The cut surfaces of the samples were then polished (TegraPol-21, Struers) to a grade of 1 μm diamond polishing paste.

2.2. *Nanoindentation test*

Samples were indented using a nano-based indentation system (Ultra Micro-Indentation System, UMIS-200, CSIRO, Australia). Finished samples were mounted onto a magnetic metal base to ensure sufficient contact with the test base of the UMIS. The nanoindentation experiments were conducted at a load of 10 mN with a calibrated Berkovich indenter. The maximum load was held for 10 s to minimise the effect of creep on the unloading curve. Elastic modulus and hardness were calculated using the Oliver–Pharr analysis method.[18] Each prepared sample underwent different indentation arrays in the region of the demineralised enamel above or at the CEJ on the buccal surface. Indentation arrays were also performed on the CEJ region of the lingual side of the samples as the control. The sizes of the indentation arrays were dependent on the region of interest on each sample. An interval of 25 μm between each indentation was used. Images of the indented regions were captured by a light microscope fitted to the nanoindentation machine. The elastic modulus and hardness data were imported into OriginPro 8 (OriginLab Corp., Northampton, Mass., USA) to allow the data to be plotted as a z value, against the x and y position coordinates. The resulting contour plots are colour-coded to emphasise the change of elastic modulus and hardness over the indented region. For further comparison, data points at different regions close to the demineralised CEJ were measured and compared by a Student's t test with α value of 0.05.

2.3. *Scanning electron microscope (SEM) observation*

After nanoindentation tests, samples were carbon coated for SEM observation using a field emission SEM (JEOL 6700 FESEM, JEOL, USA) with a back-scatter detector.

3. Results

3.1. *Mechanical properties*

Fig. 1 clearly illustrates the influence of demineralisation on the mechanical properties of CEJ areas of the samples. In both 1 and 2 day samples, acid penetrated through the thin cementum near the CEJ into the dentine. One day treatment generated a demineralisation zone of ~30 μm at the surface of enamel near the CEJ (Fig. 2B), whilst 2 days treatment demineralised the enamel near the CEJ at its full thickness and the dentine was affected (Fig. 2C). However, different periods of demineralisation generated similar demineralised zones of ~60 μm in root dentine underneath cementum but with different degrees of demineralisation.

Fig. 3 illustrates that after 1 day of exposure to the acidic solution of pH 4.5, enamel and dentine at the CEJ region are significantly demineralised, with a reduction of mechanical properties of ~50%. Two days demineralisation reduced the mechanical properties of the affected area by ~90%. Statistical analysis indicated that both experimental groups had significant differences from the control values ($p < 0.05$, Student's t-test).

3.2. *SEM observation*

The demineralised regions are clearly displayed in back-scatter SEM (BS-SEM) images (Fig. 2). One day demineralisation generated a typical artificial demineralised zone of ~30 μm in enamel near the CEJ (black arrow in Fig. 2B) due to the

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