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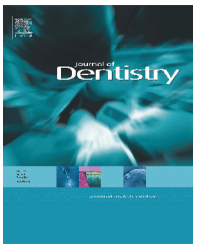

Introduction of an interproximal mineralisation model to measure remineralisation caused by novel formulations containing calcium silicate, sodium phosphate salts and fluoride

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KEYWORDS

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ABSTRACT

Objectives: To introduce a new interproximal mineralisation model and to investigate the effectiveness of novel toothpaste and dual phase gel formulations to remineralise acid softened enamel in a simulated interproximal environment.

Methods: Specimens were positioned opposite each other with an approximately 100 μm space between enamel surfaces to simulate an interproximal environment. Target specimens were demineralised in 1% (w/v) citric acid, pH3.75. Specimens were daily immersed in artificial saliva (AS) for 1h, treated with formulations, re-immersed in AS for 6h, re-treated and re-immersed in AS for a further 1h. Study 1 evaluated prototype calcium silicate/phosphate fluoride toothpaste formulations. Study 2 evaluated novel calcium silicate/phosphate fluoride toothpaste and dual phase gel formulations. Both studies contained fluoridated and non-fluoridated controls. The surface microhardness of each target enamel block was measured following demineralisation and following days three, seven and fourteen for study one and after days one, three and seven for study two.

Results: This new mineralisation model was able to show increased remineralisation from calcium silicate/phosphate fluoride prototype formulations over fluoridated formulations alone, after three and seven days of treatment. Using this new model, the combined application of novel calcium silicate/phosphate fluoride toothpaste and novel calcium silicate/phosphate fluoride dual phase gel showed the greatest amount of remineralisation, which was significantly greater than sodium fluoride and non-fluoride controls.

Conclusions: Employing a new interproximal mineralisation model successfully determined the remineralisation potential of novel calcium silicate/phosphate fluoride oral healthcare formulations.

Clinical significance: Modifying a mineralisation model to include specimens positioned in an interproximal environment allows us to better understand the remineralisation potential of oral healthcare products. It is important to minimise mineral loss at interproximal sites as the enamel within these areas is thinner than the rest of the crown.

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1. Introduction

Enamel mineral loss leads to the degradation of the surface and subsurface structures of teeth. Frequently, this is caused by the action of acid on the enamel giving rise to demineralisation and is present in both the caries and erosion processes[1]. Dental caries is a multifactorial disease caused by the simultaneous interplay of dietary sugars, dental plaque, the host and time[2]. In brief, dietary carbohydrates are metabolised by plaque bacteria to generate a range of organic acids, causing a fall of pH within the plaque and giving conditions where demineralisation may occur[3]. Dental erosion occurs when enamel is subjected to an acidic solution, usually in the form of dietary acids commonly found in soft drinks but also as intrinsic acids following stomach regurgitation[4]. In the early stages of erosion, the enamel surface is demineralised resulting in the disruption of the mineral structure and softening of the enamel. Saliva, which contains calcium and phosphate that is supersaturated with respect to hydroxyapatite, can help remineralise acid softened enamel[5]. However, in the later stages of erosion gross tissue loss can be substantial, especially following abrasion, to an extent where the natural repair processes will be insufficient to replace the lost tissue[6]. Erosion can be clinically diagnosed at these developed stages by the loss or smoothing of dental features and a rounding of tooth cusps[7]. At the cervical margin, non-carious cervical lesions arising from the loss of tooth tissue are often observed[8]. These types of lesions are thought to be caused by misalignment of occlusal forces causing stress to the enamel at the cervicular region and hence become more susceptible to demineralisation[9]. The enamel at the cervicular and interproximal areas of the tooth are thin compared to the rest of the crown[10] so if tooth tissue in this area is softened and removed following exposure to acid, the dentine will be exposed much sooner than other parts of the crown[11]. With the increased consumption of acidic drinks[12], more research is justified to investigate the promotion of repair of thin enamel in the cervicular and interproximal areas.

In order to investigate interproximal de- and re-mineralisation processes it is necessary to have a model which can accurately re-create the interdental crevice environment to simulate the unique chemistry that can take place in the confines of these spaces. To date, a number of *in vitro* interproximal models for caries have been developed, but no *in vitro* interproximal erosion models exist. Interproximal models that are used to examine caries generally use typodonts, or whole real or artificial teeth, mounted such that they have interproximal contact[13-15]. Artificial fissures have also been created to recreate the interproximal areas for specifically investigating interproximal caries[16].

Treatments designed to remineralise early stages of enamel mineral loss are needed in order to reduce the processes and prevalence of erosion. Fluoride is well known for helping to provide some protection against demineralisation and promoting remineralisation[17]. An alternative approach is to increase the amount of calcium or phosphate available in the oral environment where elevated concentrations of calcium or phosphate can provide some protection from demineralisation during acid exposure and enable remineralisation to occur at a lower pH[18]. New toothpaste formulations containing calcium silicate and sodium phosphate salts (mono sodium phosphate and trisodium phosphate) combined with 1450 ppm fluoride as sodium monofluorophosphate (SMFP) has been developed to increase the potential of enamel remineralisation. This toothpaste is proposed to deliver calcium silicate to the enamel surface and form new hydroxyapatite, leading to the remineralisation of the enamel surface[19, 20]. An adjunct oral health care product comprising a dual phase gel has also been developed containing SMFP, calcium silicate and sodium phosphate salts in one phase with sodium fluoride in the second phase. This adjunct is proposed for short term application along with daily use of the toothpaste.

The aims of the current studies are to introduce a new interproximal mineralisation model and to investigate the effectiveness of novel calcium silicate/phosphate fluoridated toothpaste and dual phase gel formulations against fluoridated and non fluoridated controls. The null hypothesis to be tested is that calcium silicate/phosphate fluoride formulations will not provide any significant remineralisation benefits versus control formulations to enamel in a simulated interproximal environment.

2. Materials and methods

2.1 Preparation of enamel specimens

Bovine enamel specimens were prepared by trepanning enamel cores from sound bovine incisors. The enamel cores were embedded in 25 mm resin discs (Stycast 1266: Hitek Electronic Material Ltd., South Humberside, UK). The enamel was exposed using P60 grit paper and then serially polished with graded grit paper P800 and P2500 and finished with 1 μm diamond polish until the surface was highly polished. Strips 1 cm wide were cut so that the polished enamel core was located slightly off centre. The strips were rinsed in deionised water and then sonicated for 10 minutes in fresh deionised water. Strips were allocated to either be used as facing (control) specimens or as target specimens. Baseline microhardness readings were taken of all specimens to confirm that the enamel was sound (acceptance criterion of >VHN 300). Target enamel specimens were demineralised in a pre-warmed 1% (w/v) citric acid (Sigma-Aldrich, Gillingham, Dorset, UK) solution adjusted to pH 3.75 so that they sat approximately 2 cm below the surface of the solution and incubated for 10 min at 37°C. Specimens were then rinsed in deionised water and indented once more. Target and facing enamel specimens were allocated to treatment groups based on their hardness at the start of the study (facing control specimens) or after their initial acid exposure (target specimens). Specimens were grouped such that each treatment group had a similar range of hardness values for both the target and facing groups. Within each group the target enamel and facing enamel were paired such that there was a similar difference in hardness for each pair in the group. The blocks remained in the pairing for the whole study. Specimens were mounted so that paired specimens were opposite one another and double sided sticky tape was placed between the resin on the lower side of the strip such that the distance between the paired specimens was approximately 100 μm. All target specimens were set on one side of the sticky tape and all facing specimens were set on the other side of the tape as shown in Figure 1. The 15 pairs of specimens were taped together for ease of handling.