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Full Length Article

Comparison of the newer preventive therapies on remineralisation of enamel *in vitro*

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ABSTRACT

Objectives: To investigate in vitro the effect of MI Paste, MI Paste Plus and 2800 ppm fluoride (F) toothpaste (TP) on the remineralisation of enamel subsurface lesions and to compare these to the effect of 1450 ppm and 0 ppm F toothpastes.

Methods: Enamel subsurface lesions were created in bovine enamel slabs (n = 120) which were assigned randomly to five groups; (1) 0 ppm F TP, (2) 1450 ppm F TP, (3) 2800 ppm F TP, (4) 1450 ppm F TP + MI Paste (Tooth Mousse-TM,10% w/v CPP-ACP) and (5) 1450 ppm F TP + MI Paste Plus (Tooth Mousse Plus,10% w/v CPP-ACP, 900 ppm F as 0.2% w/w sodium fluoride). The enamel slabs were subjected to a pH cycling regimen for 21 days. Quantitative Light-induced Fluorescence (QLF) images were taken and analysed. Data analysis was carried out using one way ANOVA.

Results: In all groups, both ΔF (percentage fluorescence loss) and ΔQ (ΔF times the area) values improved significantly within the same group after the treatment. In addition, the mean difference in ΔF of the non-fluoride control group was significantly lower than all other groups but not for the 2800 ppm F^- group. Whereas the mean difference in ΔQ of the non-fluoride control of group was significantly lower when compared with all other groups ($p < 0.05$).

Conclusions: Both MI Paste and MI Paste Plus when used in conjunction with 1450 ppm F did not show a significant increase in efficacy for the remineralisation of bovine enamel subsurface lesions in the model used in this study.

Clinical significance: Newer preventive agents such as MI paste and MI paste plus are advocated as promoting remineralisation when used in addition to routine oral care. This in vitro study shows that they may have a limited value in promoting remineralisation over and above that of 1450 ppm F toothpaste used twice a day.

1. Introduction

Dental caries remains a significant public health problem in most developed countries [1,2]. Restoration of carious teeth has significant cost implications [3]. Remineralisation of at least early lesions would provide a significant benefit to the patient in that it would avoid invasive dental treatment and also reduce costs for management of disease.

In the last decade a number of products and therapies, both for professional application and home use have been introduced into the market. These are supposed to be used in addition to normal twice a day tooth brushing with a fluoride toothpaste. In particular products based on milk proteins, such as casein phosphopeptide have been reported to enhance remineralisation of early carious lesions [4].

The anti-cariogenic properties of milk and milk products have been demonstrated in human and animal models [5,6] and the chemical effect of the phosphoprotein casein and calcium phosphate components were proposed as the main mechanism for this action [7,8]. Casein phosphopeptide (CPP) has the ability to stabilise calcium and phosphate in high concentrations at the tooth surface thereby inhibiting demineralisation and enhancing remineralisation.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been shown to have anti-cariogenic activity in laboratory, animal, human in situ and clinical experiments. The ability of CPP-ACP to enhance the remineralisation of enamel subsurface lesions has been revealed in several studies [9–13].

The role of CPP-ACP involves the localisation of ACP on the tooth surface; this in turn leads to buffering of the free calcium and phosphate ion activities, which help to maintain a state of super-saturation with respect to enamel by suppressing demineralisation and enhancing

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remineralisation. The presence of CPP-ACP might permit a rapid return to resting calcium concentration and allow earlier remineralisation of enamel substrate [14].

The synergistic effect of CPP-ACP and fluoride in caries prevention has been reported in a number of studies [15,16]. This effect has been attributed to the formation of CPP-stabilised amorphous calcium fluoride phosphate (CPP-ACFP) [17], which results in an increase in the concentrations of fluoride ions together with bio-available calcium and phosphate ions and their localisation at the tooth surface by the CPP.

Most of the studies on the efficacy of CPP-ACP have investigated its effect when used alone for early lesion remineralisation [18,19]. On the other hand, there is a lack of evidence on its effect when added to the regular oral hygiene practice that involves brushing the teeth with fluoridated toothpaste.

Therefore, the aims of the current study were to investigate the effect of the commercially available topical crèmes containing CPP-ACP and CPP-ACFP on the remineralisation of enamel subsurface lesions in vitro when used supplementary to fluoridated toothpaste 1450 ppm F, and also, to compare their effect in vitro on the remineralisation resulting from the use of a higher concentration (2800 ppm) fluoride toothpaste alone.

Null hypothesis. There is no difference in the remineralisation of enamel subsurface lesions with the following regimes: 1450 ppm F TP + MI Paste, 1450 ppm F TP + MI Paste Plus, 0 ppm F TP, 1450 ppm F TP, and 2800 ppm F TP.

2. Materials and methods

This was an in vitro study using bovine enamel and pH cycling regime.

2.1. Power calculation

Statistical advice was sought and the sample size was calculated by using data from a previous PhD thesis 'Investigations into the effect of casein phosphopeptide-amorphous calcium phosphate on enamel demineralisation and remineralisation [20]. A total of 22 enamel slabs per group were needed. This calculation was based on the assumption that the standard deviation of the response variable was 2.0, power 90%, 0.05 significance level and a true difference between treatments would be adjusted to 3 units. This was based on the calculations by *MGH* Biostatistics Centre software [21].

2.2. Preparation of the Enamel Slabs and Sub-Surface Lesions

All enamel slabs used in the present study were obtained from bovine incisors.

Approval for collection of bovine teeth was sought and obtained from the Food Standards Agency. The teeth were obtained from an abattoir and stored immediately in 0.1% thymol (Sigma Aldrich) solution in the fridge. Before sectioning, the teeth were cleaned using a spoon excavator and a toothbrush to remove any soft tissue remnants. To detect any defects, caries or cracks, all teeth were screened by transillumination and transmitted light using low-power microscopy (Leitz, Wetzlar®, Germany).

Each tooth was mounted using "green stick" impression compound (Kerr, UK) on plates. The crowns were sectioned using a water cooled, diamond wire saw, cutting machine (Well@Walter EBNER, CH-2400 Le Loche). The buccal and palatal surfaces of each crown were separated, and each buccal section was cut into two slabs that were approximately 6 × 5 x 3 mm in size.

Each enamel slab was mounted on a plastic rod using "sticky wax" to hold the slab in the demineralising gel. The rod was secured to the lid of a "Sterilin" type universal tube so that when the top was screwed onto the tube, the tooth was suspended in the centre of the tube free space. Two coats of an acid resistant, coloured nail varnish (Max Factor, "Glossfinity") were then applied on the enamel slabs, except for a small window of approximately 2 × 3 mm on the centre of each slab that was left exposed. An interval of 24 h was left between the two applications to allow the nail varnish to dry completely.

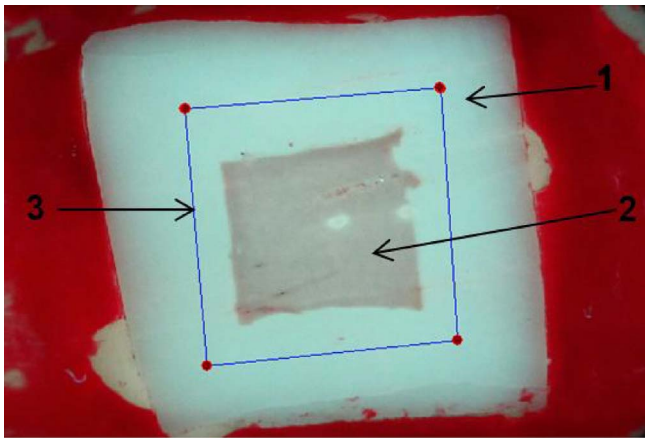

Fig. 1. QLF image taken with the blue light showing the demineralised lesion with an intact surface in the centre of the enamel slab as well as a patch drawn around the lesion with the border in sound enamel. (1: sound enamel, 2: demineralised enamel, 3: patch drawn around the lesion to include sound enamel for reference).

Once the enamel slabs were prepared, they were kept in deionised water in plastic containers at room temperature to prevent dehydration.

Acidified hydroxyethyl cellulose gel was prepared by adding 0.1 M sodium hydroxide (BDH Analar Grade) to 0.1 M lactic acid (Sigma Aldrich D/L GPR 87% Lactic acid) to give a pH value of 4.5 and then 6% w/v hydroxyethyl cellulose (Sigma Aldrich) was added to the solution and stirred for one hour. The mixture was left to settle for 24 h. Each enamel slab was immersed in 15 ml of acid gel for 10 days to produce an artificial enamel subsurface lesion (Fig. 1). The enamel slabs were removed from the acid gel and washed with distilled water, the nail varnish was then removed using methanol (HPLC Gradient grade, method development, Fisher Scientific) to prepare the enamel slabs for the baseline QLF measurements.

2.3. The ΔF range of the artificial lesions

After performing the QLF baseline analysis for all enamel slabs, the range of ΔF values were found to vary between −7.66 and −31.98. The enamel slabs within the ΔF range of −14.12 to −26.65 with an average of −20.75, were selected for use in the study to enable detection of the differences in ΔF post-treatment. A sufficient number of lesions were made, in order to discard the outliers with a ΔF less than −14.00 or with ΔF more than −27.00).

2.4. Study materials

- Non-fluoride toothpaste (the Boots Company PLC, Nottingham, England).
- Fluoride toothpaste 1450 ppm F (0.32% w/w sodium fluoride) (Colgate cool stripe. Colgate–Palmolive (UK) Ltd, Guildford, England).
- High fluoride toothpaste 2800 ppm F (0.619 w/w sodium fluoride) (Duraphat®. Colgate–Palmolive (UK) Ltd, Guildford, England).
- MI Paste 10% w/v CPP-ACP (GC MI Paste™, GC Corp, Tokyo, Japan).
- MI Paste Plus 10% w/v CPP-ACP, 900 ppm F (0.2% w/w sodium fluoride) (GC MI Paste Plus™, GC Corp, Tokyo, Japan).