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Effects of enamel matrix derivative on remineralisation of initial enamel carious lesions *in vitro*

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ABSTRACT

Objective: The purpose of this study was to investigate the effect of enamel matrix derivative on remineralisation of initial enamel carious lesions *in vitro*.

Design: Initial enamel carious lesions were created in bovine enamel blocks *in vitro*. The lesions were subjected to a pH-cycling regime of 24 days. Each daily cycle included 4 × 3-min applications with one of four treatments: 1 g/L NaF aqueous solution (positive control), 6% propylene glycol alginate (PGA) or distilled and deionised water (DDW) (both negative controls), and a gel of enamel matrix derivative and PGA (EMDgel). Samples were subjected to surface microhardness (SMH) testing, polarised light microscopy (PLM) and transverse microradiography (TMR) to measure SMH, mineral loss, lesion depth and mineral content of the surface layer and lesion body before and after pH-cycling.

Results: NaF samples showed the highest SMH recovery of all the groups ($P < 0.05$). EMDgel samples showed significantly higher SMH recovery than did PGA ones ($P < 0.05$). NaF samples showed significantly less mineral loss and shallower lesions than all other groups ($P < 0.05$). The DDW and EMDgel samples showed significantly less mineral loss and shallower lesions than PGA samples. Mineral deposition predominated much more at the surface layer in the EMDgel group than in the PGA group ($P < 0.05$).

Conclusions: EMD, the active ingredient of EMDgel, may play an essential role in promoting remineralisation of initial enamel carious lesions. However, EMDgel as a whole did not cause detectable remineralisation of such lesions *in vitro*.

1. Introduction

Caries is the result of numerous de- and remineralisation events, rather than a single demineralisation process.[1] Both processes are dynamic and involve the flow of calcium and phosphate in and out of tooth enamel. The processes should be balanced in order to prevent the progression of caries.[2] Remineralisation is an important natural repair process that is crucial for preventing the progression of caries lesions.

Fluoride is a classic anti-caries agent. The decline in dental caries experienced in most industrialised countries over the last 25 years can be attributed largely to the widespread use of

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Abbreviations: PGA, propylene glycol alginate; EMD, enamel matrix derivative; EMPs, enamel matrix proteins; DDW, distilled and deionised water; SMH, surface microhardness; KHN, Knoop hardness number; SMHR, surface microhardness recovery; PLM, polarised light microscopy; TMR, transverse microradiography.

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fluoride.[3] However, overexposure to fluoride may lead to fluorosis.[4] Although fluoride presents no problems to healthy individuals when used properly, it is recommended that certain groups limit their exposure, such as groups living in areas where the drinking water has a high fluoride content.[5,6] Therefore, effective non-fluoride anti-caries agents are still needed.

Enamel matrix proteins (EMPs) are secreted by Hertwig epithelial root sheath during the development of dental germ. These proteins are secreted into the enamel extracellular matrix, where they nucleate and regulate the growth of hydroxyapatite crystals in order to form the mineralised enamel covering the crowns of teeth.[7] The biomineralisation activity of EMPs has been well documented.[8] The hydroxyapatite crystals of mature enamel are unusually large, uniform and arranged regularly within the tissue, implying that their development is a highly controlled process. EMPs are presumed to play an important role in the modulation of mineral deposition and growth during tooth morphogenesis.[8] Initially crystals are small and rich in magnesium and carbonate, resulting in relatively poor crystallinity. During crystal development, the concentrations of amelogenin and albumin in the matrix increase.[9] Enamel maturation is characterised by massive increases in crystal width and thickness. This maturation is controlled by a series of events that are carefully orchestrated temporally and spatially, requiring the coordinated degradation and removal of the endogenous enamel matrix.[10]

Enamel matrix derivative (EMD), a commercially available derivative of EMPs, has been used widely in dentistry. It is often used in periodontics to stimulate regeneration of periodontal tissues and induce a regenerative process that mimics odontogenesis.[11,12] Recent experiments have confirmed that EMD may have other applications, such as in dental implantation,[13,14] dental replantation,[15] and dental pulp capping.[16]

In a previous study, our group found that enamel organic matrix interacts significantly with the Chinese medicine (*Galla chinensis*) while inhibiting enamel demineralisation,[17] and it was hypothesised that functional peptides mimicking EMP function and structure might promote remineralisation of enamel caries.[18] However, whether EMPs can be used to prevent and treat caries has not been investigated, and we are unaware of studies that explore the potential of EMPs to promote the remineralisation of initial enamel carious lesions.

The purpose of the present study was to investigate the effects of EMD on the remineralisation of initial enamel carious lesions during pH-cycling *in vitro*, and to assess whether EMPs may be a suitable anti-caries agent.

2. Materials and methods

2.1. EMD preparation

EMDgel (Emdogain-Straumann, Biora, Sweden), containing 30 mg/mL EMD in propylene glycol alginate (PGA), was stored at 4 °C and delivered from syringes loaded with 0.7 mL. During each treatment, one drop of EMDgel[19] was placed on the surface of the carious lesion and daubed into a very thin layer that covered the lesion surface.

2.2. Preparation of enamel samples

Bovine permanent incisors free of lesions, cracks and fluoric mottle were selected for this study. The crowns were separated from the roots and cut into sections approximately 5 mm × 5 mm × 2 mm using a diamond-coated band saw with continuous water cooling (Struers Minitom, Struers, Copenhagen, Denmark). All enamel blocks were embedded in polymethylmethacrylate and painted with two layers of acid-resistant nail varnish, leaving a 4 mm × 4 mm window exposed on the labial enamel surface. The labial enamel surfaces were then ground flat with water-cooled carborundum discs (800, 1000, 1200 and 2400 grit, waterproof silicon carbide paper; Struers) and hand-polished with diamond paste (15 μm Diamond Paste, Struers). In this way, approximately 150 μm of the outermost enamel layer was removed, creating a flat, uncontaminated surface.

2.3. Surface microhardness prior to lesion formation

Surface microhardness (SMH) of the prepared enamel blocks was measured prior to lesion formation (SMH_0) using a microhardness tester (Duramin-1/-2, Struers) and a Knoop indenter at a load of 50 g for 15 s. Five indentations spaced 100 mm from each other were made at the centre of the enamel surface. A total of 160 enamel blocks with SMH_0 between 310.7 and 380.3 Knoop hardness number (KHN) were selected for further study.

2.4. Carious lesion formation

Early artificial caries lesions were produced in the 160 selected enamel blocks as described previously.[20] Blocks were placed in demineralisation solution containing 50 mM acetic acid (pH 4.5), 2.2 mM $Ca(NO_3)_2$, 2.2 mM KH_2PO_4, 5.0 mM NaN_3 and 0.5 ppm NaF. The demineralisation test was performed at 37 °C with continuous low-speed magnetic stirring (100 rpm) for 3 days. After the SMH of the samples (SMH_1) was measured using the same method as described in Section 2.3, half of the exposed window on each enamel sample was sealed with film and two layers of acid-resistant nail varnish, leaving only a 4 mm × 2 mm exposed window.

2.5. Measurement of SMH at baseline

The consistency of the mineral content of exposed enamel windows was checked at baseline (prior to pH-cycling) by measuring SMH_1. A total of 40 enamel blocks with SMH_1 between 49.56 and 89.32 KHN were selected for pH-cycling.

2.6. pH-cycling

The 40 samples with 4 mm × 2 mm exposed windows on the enamel surfaces were divided into four treatment groups: 1 g/L NaF aqueous solution (positive control), 6% propylene glycol alginate (PGA) or distilled and deionised water (DDW) (both negative controls), and EMDgel. A standard regime[21] of pH-cycling was carried out using remineralisation solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, 1.0 mM NaN_3 and 20 mM HEPES, pH 7.0) and demineralisation solution