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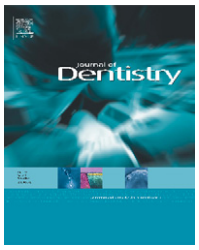

Effect of various calcium/phosphates ratios of carboxymethylcellulose-based saliva substitutes on mineral loss of bovine enamel in vitro

Hendrik Meyer-Lueckel [a], *Andreas J. Chatzidakis* [b,*], *Andrej M. Kielbassa* [a]

[a] *Department of Operative Dentistry and Periodontology, University School of Dental Medicine, Campus Benjamin Franklin, Charité - Universitätsmedizin Berlin, Germany*

[b] *Department of Prosthetic Dentistry, University School for Dental Medicine, Campus Benjamin Franklin, Charité - Universitätsmedizin Berlin, Germany*

ARTICLE INFO

Article history:
Received 18 June 2007
Received in revised form
13 August 2007
Accepted 13 August 2007

Keywords:
Demineralisation
Bovine enamel
Microradiography
Calcium
Phosphate
Carboxymethylcellulose
Saliva substitute

ABSTRACT

Objectives: The present study evaluated the effects of various calcium and phosphate concentrations and ratios of carboxymethylcellulose (CMC)-based solutions on the mineral loss of predemineralised bovine enamel in vitro.

Methods: Bovine enamel specimens were prepared, polished and partly covered with nail varnish, thus serving as control of sound enamel. After demineralisation (37 °C; pH 5.0; 14 days) the specimens were exposed to CMC-based solutions (20 g/l) with various saturations with respect to apatites containing 0.1 mM NaF, $CaCl_2$ (0–32 mM) and KH_2PO_4 (0–52 mM) at two different pH values (5.5 or 6.5). A fluoride-free solution served as control, and four commercially available products were tested as well. The differences in mineral loss ($\Delta\Delta Z$) between the values prior to (ΔZ_{Demin}) and after storage (ΔZ_{Effect}) in the various solutions were evaluated from microradiographs of thin sections (100 μm).

Results: The general linear model revealed a significant dependency for $\Delta\Delta Z$ on 'calcium' ($p < 0.001$), 'phosphate' ($p = 0.023$), 'fluoride' ($p = 0.002$) and 'pH' ($p < 0.001$). With increasing calcium and phosphate concentrations an increase in $\Delta\Delta Z$ could be observed up to the solution containing the third highest saturation with respect to octacalciumphosphate (3.2), showing a significant remineralisation ($p < 0.05$; *t*-test). The commercially available products as well as the control groups revealed significantly reduced $\Delta\Delta Z$ values compared to this group ($p < 0.01$; Bonferroni).

Conclusions: A saturation with respect to octacalciumphosphate of 3.2 and a pH of 6.5 enables CMC-based solutions to remineralise bovine enamel in vitro.

1. Introduction

Symptomatic dryness (xerostomia) is associated with objective evidence of reduced salivary secretion in about half the cases.[1] Dry mouth most often follows salivary gland disturbance of external origin or due to systemic diseases. Xerostomia may be accompanied by increased caries prevalence, candidosis, cheilitis, dysgeusia or dysphagia. Prominent causes of salivary gland hypofunction are drugs, organic diseases, psychogenic factors and at least to some extent, decreased mastication.

* *Corresponding author at*: Department of Prosthetic Dentistry, Charité Centrum 3 of Dental Medicine, Campus Benjamin Franklin, Charité - Universitätsmedizin Berlin, Assmannshauser Strasse 4-6, 14197 Berlin, Germany. Tel.: +49 30 8445 6220/6244; fax: +49 30 8445 6238.
E-mail address: andreas.chatzidakis@charite.de (A.J. Chatzidakis).

0300-5712/$ – see front matter
doi:10.1016/j.jdent.2007.08.006

Moreover, extensive mouth dryness is usually observed after irradiation of the head and neck region.[2–4] Inhibited remineralisation as well as the compromised self-cleaning, nocturnal discomfort, disturbed intake of food and difficulties in swallowing are responsible for the rapid destruction of dentition generally described as 'radiation decay'.[5]

For patients without residual salivary function, the only option for temporary symptomatic relief can be offered by moistening agents and saliva substitutes[6] which are mostly based on carboxymethylcellulose (CMC), mucin or linseed. From a dental point of view, it should be reasonable to expect that saliva substitutes will not damage sound enamel and dentin. Moreover, artificial salivas should have a remineralising effect on dental hard tissues.[7]

Previous studies showed a demineralising potential of linseed-based saliva substitutes on bovine dentin[8], whereas mucin-based solutions revealed a neutral behaviour on enamel[9] and dentin[5,8,10] solubility, and were able to remineralise dental hard tissues in case of an adequate composition of calcium and phosphates as well as fluoride.[8,9,11]

Commercially available saliva substitutes based on CMC (i.e. Glandosane) showed a high demineralising potential in vitro,[11,12] whereas other products induced no further demineralisation, but were unable to remineralise dentin[13] or revealed only slight remineralising capacities on enamel.[11] Therefore, the aim of the present investigation was to determine the effects of self-made CMC-based solutions differing in calcium and phosphate concentrations as well as ratios on the mineral loss of predemineralised bovine enamel at two different pH values in vitro. The solutions contained 20 g/l CMC, which is twice the amount usually added to the commercially available or formerly tested saliva substitutes and CMC-based lubricants, respectively. In addition, four commercially available products, bioXtra mouth rinse based on hydroxyethylcellulose (Butler, Kriftel Germany), Aldiamed mouthgel based on glycerin (Biomedica, Rodgau, Germany), Aldiamed mouth rinse based on hydroxyethylcellulose (Biomedica) and Paroex containing glycerin (Butler) that are recommended for xerostomia patients were tested in comparison to the self-made CMC-based solutions.

2. Materials and methods

2.1. Sample preparation

The crowns of 60 freshly extracted bovine incisors that were stored in Ringer's solution (Delta Select, Pfullingen, Germany) at 4 °C were separated clearly below the cemento-enamel junction using a diamond-coated band saw under continuous water cooling (Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany). From each crown, four slabs (3 mm × 4 mm × 2 mm) were prepared from the labial aspects. Subsequently, these 240 specimens were embedded in epoxy resin (Technovit 4071; Kulzer, Wehrheim, Germany) with maximal caution to keep the experimental site free from epoxy resin. The epoxy resin was allowed to cure for 5 min before the samples were stored in Ringer's solution until further preparation. Specimens were ground flat and hand-polished up to 4000 grits (silicon carbide; Struers, Copenhagen, Denmark), thereby removing about 200 μm of the outer enamel layer. One-third of each specimen's surface was partly covered with acid resistant nail varnish (Betrix, Frankfurt/Main, Germany) to serve as control. Prior to the in vitro demineralisation in a modified Buskes' solution containing 50 mM lactic acid, 6 μM methylhydroxydiphosphonate, 3 mM $CaCl_2 \cdot 2H_2O$, 3 mM KH_2PO_4 and vestiges of thymol.[14] The samples were immersed in 10 l of the demineralising solution in an incubator (Memmert, Schwalbach, Germany; 37 °C) at pH 5.0 for 14 days. The pH value was measured daily (pH-Meter CG 819; Schott Geräte, Hofheim, Germany), and slight elevations were corrected with lactic acid or potassium hydroxide to maintain a constant pH value between 5.0 and 5.1 during the whole demineralisation period.

2.2. In vitro exposure

After 14 days of demineralisation half of the demineralised surfaces were again partially covered with nail varnish. The 240 specimens were divided into 24 groups ($n = 10$) and exposed to various CMC-based solutions differing in NaF (0–0.1 mM), $CaCl_2$ (0–32 mM) and KH_2PO_4 (0–52 mM) concentrations at two pH values (5.5 or 6.5) and two different calcium/phosphates ratios (1/1.6 or 1/6.4) for 14 days (Table 1). All the solutions additionally contained KCl (16.3 mM), NaCl (14.6 mM), $MgCl_2$ (0.26 mM) and methyl- (6.5 mM) as well as propylhydroxybenzoate (1 mM) as preservatives. All chemicals were of analytical grade (Merck, Darmstadt, Germany). Four commercially available products recommended for patients suffering from xerostomia, bioXtra mouth rinse, Aldiamed mouthgel, Aldiamed mouth rinse and Paroex were used as well.

Two groups of the self-made CMC-based solutions, either at pH 5.5 or 6.5, but without any calcium, phosphates and fluorides served as control. Again, the pH values were checked daily (pH-Meter CG 819; Schott Geräte, Hofheim, Germany), and slight elevations were corrected with lactic acid or potash lye to maintain a constant pH value during the remineralisation period.

2.3. Microradiographic assessment

Subsequently, the specimens were cut perpendicular to their surfaces with a diamond-coated band saw (Exakt 300cl). Half of each specimen was mounted on a Plexiglas microscope slide (diaplus, Oststeinbeck, Germany), and sections of approximately 500 μm thickness were cut. Slabs were ground (Exakt Mikroschleifsystem, Exakt Apparatebau) to a uniform thickness of 100 μm, verifying the thickness of the paralleled surfaces by a digital micrometer (Mitutoyo, Tokyo, Japan). For microradiographic evaluation, specimens were fixed on sample holders (Plano, Wetzlar, Germany). Contact microradiographs were obtained and evaluated as described previously.[15]

2.4. Calcium analysis

Calcium concentrations of the various experimental solutions were measured using a calcium electrode (type Orion 97–20 ionplus; Thermo Electron Corporation, Beverly, MA, USA). To enable the electrode to measure, the solutions were diluted in