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Effect of carboxymethylcellulose-based saliva substitutes on predemineralised dentin evaluated by microradiography

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ABSTRACT

Objective: This study investigated the effect of six lab-produced saliva substitutes based on carboxymethylcellulose (CMC) differing in octacalciumphosphate saturations (OCP-s) on mineralisation of bovine dentin in vitro.
Design: Dentin specimens were prepared ($n = 234$); prior to and after demineralisation (37 °C; pH 5.0; 7 d), one-third of each specimen surface was covered with nail varnish (control of sound dentin). Subsequently, specimens ($n = 13$) were exposed to either one of the six CMC-based solutions (OCP-s: 0, 0.5, 1, 2, 4, and 8) at pH 6.5 or to Glandosane for 5 and 10 weeks (37 °C). Two aqueous solutions (OCP-s: 0 and 1) served as controls. After storage, thin sections were prepared and mineral loss was calculated by transversal microradiography.
Results: After both storage periods specimens immersed in Glandosane revealed a significantly increased mineral loss compared to all other solutions ($p < 0.05$; Bonferroni post hoc test). Control solution with OCP-s = 1 induced a significant remineralisation ($p < 0.05$; adjusted paired t-test). Only after 5 weeks exposure to the CMC-based solution with an OCP-s = 2 a significant remineralisation compared to baseline ($p < 0.05$) as well as a significantly increased mineral gain of the surface area compared to higher saturated solutions ($p < 0.05$; Bonferroni post hoc test) could be observed.
Conclusions: CMC seems to hamper dentin remineralisation, although after 5 weeks a mineral gain could be induced with slightly supersaturated CMC-solutions with respect to OCP.

1. Introduction

Xerostomia often emerges after radiation therapy in the head and neck area accompanied by oral discomfort and impaired oral functions. Hyposalivation has been identified as being a co-factor for the rapid destruction of the dentition generally known as 'radiation caries'.[1] Without preventive measures (oral hygiene) and supportive therapy (i.e. fluoridation) the dentition can be destroyed within a few months.[2,3] As most patients suffering from these symptoms are elderly people recessions and subsequently exposed dentin surfaces are very common. Since dentin is not as acid resistant as enamel an earlier and more severe demineralisation can be expected.[3]

Providing moisture to the oral mucosa helps to relieve the symptoms of hyposalivation in these patients. For this purpose saliva substitutes have been developed.[4] Various thickening agents (e.g. carboxymethylcellulose [CMC], linseed, ptyalin, mucin) have been added to saliva substitutes to improve visco-elastic properties.[5,6] However, saliva medac (mucin-based; medac, Hamburg, Germany), ptyalin (ptyalin-based; TMP Tüshaus, Velen, Germany) as well as salinum (linseed-based; Sinclair, Surrey, United Kingdom) have been

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withdrawn from the German market recently because of pH instabilities and bacterial growth. Moreover, a prospective cross-over study in patients with xerostomia comparing four different polymers (gel, carmellose spray, oil, mucin spray) used in saliva substitutes showed that most patients preferred the carmellose spray (Glandosane; Cell Pharm, Hannover, Germany), due to its good taste and easy handling.[7] Therefore, CMC seems to be a suitable basis for saliva substitutes.

With regard to caries reduction it is important to note that saliva substitutes should be capable to prevent demineralisation and/or to have remineralising effects on dental hard tissues. So far, mainly fluoride gels and/or mouthwash solutions have been used for caries prevention in xerostomic patients.[8] However, in particular patients after radiotherapy of the head and neck often show poor compliance with these products.[9,10] Preferably, a remineralising saliva substitutes could cope with both outcomes (dental caries and xerostomia) in patients with hyposalivation.

Previous in vitro studies demonstrated that remineralisation of dentin could be achieved with a saliva substitute with an almost neutral pH value containing calcium, phosphates, and fluorides (Oralube; Orion, Balcatta, Australia).[11] However, this product is only available on the Oceanian (Australasian) market. Without the mentioned ions only a neutral effect (Saliva medac and Saliva Orthana; A.S. Pharma, Polegate, United Kingdom) or even a further demineralisation of dentin could be observed (Salinum, Glandosane, and biotene; Laclede, CA, United States).[3,11] Therefore, the addition of calcium, phosphates, and fluorides to saliva substitutes is essential for the protection of dental hard tissues.

Degrees of saturation for aqueous solutions with respect to various apatites can be calculated.[12] With regard to remineralising properties of artificial saliva the first detectable transient crystal during the formation of the stable hydroxyapatite (HA) has been reported as octacalciumphosphate (OCP).[13] Supersaturated saliva substitutes with respect to OCP should preferably be used, since calcium and phosphate may form complexes with the polymer ingredients (CMC, linseed, mucin). Regarding experimental linseed-based saliva substitutes remineralisation of dentin could not be achieved with solutions supersaturated with respect to OCP,[14] whereas remineralisation could be revealed for supersaturated mucin-based saliva substitutes.[15]

With experimental CMC-based saliva substitutes (20 g/l CMC, OCP-s = 3) remineralisation of enamel has been observed after storage for 2 weeks. Here, various ratios of calcium and phosphates had no significant effects on mineralisation.[16] However, it remained unclear from these results, whether lower viscous solutions (10 g/l CMC) that are mainly used as saliva substitutes show similar effects. As mentioned above the effects on dentin mineralisation have rarely been in focus. Moreover, the results might also be influenced by the exposure time that was quite short (2 weeks) in the previous study. Nonetheless, from a chemical point of view it has to be considered that in supersaturated saliva substitutes (OCP-s > 1) precipitations might occur within the solution altering de- and remineralisation.

Therefore, the present study was undertaken in order to compare the effects of various CMC-based solutions differing in OCP-s on the mineral loss of predemineralised bovine dentin in vitro. It was hypothesised that with increasing OCP-s an enhanced remineralisation can be observed. For comparison, a commercially available saliva substitute based on CMC (Glandosane) often prescribed in Western Europe was tested, as well.

2. Materials and methods

2.1. *Specimen preparation*

Fifty-nine recently extracted permanent bovine central incisors were used. Blocks of 4 mm thickness were prepared from the cervical region and quartered under cooling, running tap water using a diamond-coated band saw (Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany). The 234 dentin specimens were embedded in epoxy resin (Technovit 4071; Heraeus Kulzer, Wehrheim, Germany), while the natural surface was kept free from resin. Specimens were ground flat and hand-polished up to 4000 grit (silicon carbide; Struers, Copenhagen, Denmark), thereby removing the cementum and outer parts of the dentin layer (approximately 200 μm).

Subsequently, one-third of each specimen surface was covered with an acid-resistant nail varnish (Betrix, Frankfurt/Main, Germany) to serve as control of sound dentin. Following earlier studies[11,14,15] dentinal lesions were prepared by immersion in a solution of constant composition containing 0.0476 mM NaF, 2.2 mM $CaCl_2 \cdot 2H_2O$, 2.2 mM KH_2PO_4, 50 mM CH_3COOH, and 10 mM KOH (Merck, Darmstadt, Germany) at pH 5.0 in an incubator (37 °C) (BR 6000; Heraeus Kulzer) for 7 days. The composition of the demineralising solution was modified according to a previous study.[17] The pH value was controlled daily and slight elevations were corrected with lactic acid to maintain a constant pH value between 4.98 and 5.02 during the demineralisation period. Standard buffer solutions (Sigma–Aldrich, Steinheim, Germany) with nominal pH values of 4.0 and 7.0 with an accuracy of 0.01 units were used to calibrate the pH meter (pH-Meter GMH 3510; Greisinger, Regenstauf, Germany).

2.2. *Calculation of the degrees of saturation and preparation of the solutions*

For aqueous solutions the degree of saturation with respect to apatites [octacalciumphosphate (OCP), hydroxyapatite (HA), and calcium fluoride (CaF_2)] can be calculated, if the pH and the concentrations of certain ions are known.[12] The calcium to phosphate ratio of the solutions was defined at 1:1.6. Addition of the respective calcium and phosphate concentrations was determined by calculation of the OCP-s (0, 0.5, 1, 2, 4, 8) of the various CMC-based solutions as well as the two aqueous solutions (0, 1) at pH 6.5 (Table 1). Additionally, all solutions contained methyl- (6.5 mM) and propyl hydroxyl benzoate (1 mM), sorbitol (109.9 mM) as well as fluoride (0.1 mM), KCl (16 mM), NaCl (15.1 mM), and $MgCl_2$ (0.3 mM) (all chemicals from Merck, Darmstadt, Germany). A short-scale double-blind test with regard to the taste of the solutions was performed with 10 participants.