First publ. in: Caries Research 44 (2010), 2, pp. 127-134

DOI: 10.1159/000302901

Effects of Carboxymethyl Cellulose-Based Saliva Substitutes with Varying Degrees of Saturation with Respect to Calcium Phosphates on Artificial Enamel Lesions

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Key Words

Brushite · Carboxymethyl cellulose · Enamel · Microradiography · Octacalcium phosphate · Saliva substitute

Abstract

The aim of the present study was to evaluate the effects of experimental saliva substitutes based on carboxymethyl cellulose (CMC) differing in degrees of saturation with respect to calcium phosphates on the mineral loss of enamel in vitro. Demineralized bovine specimens (subsurface lesions) were exposed to one of six experimental CMC-based solutions with theoretical degrees of saturation with respect to octacalcium phosphate (S_{OCP}) of S0, S0.5, S1, S2, S4, and S8 for 10 weeks. A previously studied saliva substitute (Glandosane) and two aqueous solutions (C0 and C1) served as controls. Mineral losses and lesion depths before and after storage were evaluated from microradiographs. Free and bound calcium as well as phosphate and fluoride concentrations were determined. According to these measurements, S_{OCP} of S2, S4, and S8 was 0.3, 1.1, and 3.4, respectively. Storage in Glandosane and both negative controls resulted in significant demineralization ($p < 0.05$). Only S2 significantly remineralized the specimens ($p < 0.05$). All other solutions showed neutral effects. No significant differences in mineralization between S0 and C0 as well as between S1 and C1 could be observed ($p > 0.05$). It can be concluded that a CMC-based solution actually unsaturated with respect to octacalcium phosphate (S2) shows most pronounced remineralization capability under the conditions chosen. This might be explained by a more favorable balance between calcium bound to CMC in an adsorbed layer at the enamel-liquid interface and heterogeneous nucleation of calcium phosphates within a solution compared to solutions either supersaturated or having lower levels of saturation.

The subjective feeling of a dry mouth as a sequel of radiation therapy in the head and neck area is accompanied by oral discomfort and impaired oral functions such as the rapid destruction of dentition [Vissink et al., 2003; Kielbassa et al., 2006]. In patients with diminished salivary flow, an increasing caries incidence can be observed unless strong preventive efforts are made [Guchelaar et al., 1997]. Besides the application of fluoride gels and motivation for regular tooth brushing, saliva substitutes based on carboxymethyl cellulose (CMC), mucin, or linseed are administered to support caries prevention and to

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improve oral complaints. Nevertheless, some of the marketed products seem to have demineralizing effects on dental hard tissues [Joyston-Bechal and Kidd, 1987; Meyer-Lueckel et al., 2002]. Moreover, the saliva substitutes 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 withdrawn from the German market, most probably due to pH instabilities and bacterial growth. Therefore, a stable saliva substitute with remineralizing properties is still needed. Although not being a natural lubricant, CMC still seems to be a good choice as a basis for a saliva substitute [Momm et al., 2005]. Nonetheless, a CMC-based saliva substitute often prescribed in Germany (Glandosane) showed considerable demineralizing effects in vitro [Joyston-Bechal and Kidd, 1987; Meyer-Lueckel et al., 2002].

An adequate degree of saturation with respect to relevant calcium phosphates is crucial for a saliva substitute to achieve remineralization [Chow, 2001]. The degree of saturation with octacalcium phosphate (S_{OCP}) and dicalcium phosphate dihydrate (S_{DCPD}) plays an important role in mineralization [Johnsson and Nancollas, 1992]. In a previous study, varying the calcium phosphate ratio did not influence de- and remineralizing properties of rather viscous (20 g/l CMC) experimental CMC-based saliva substitutes. The solution with the highest level of S_{OCP} being evaluated (S_{OCP} = 3) was the only one that induced significant remineralization after a relatively short study period of 2 weeks. Solutions with a pH of 6.5 were shown to be more stable with respect to pH compared to solutions having a lower pH (5.5) [Meyer-Lueckel et al., 2007]. It remained unclear from these results whether lower viscous solutions (10 g/l CMC) have similar effects, and if solutions having higher S_{OCP} might even induce more pronounced remineralization. For dentin, these lower viscous CMC-based solutions showed most promising results with S_{OCP} = 2, whereas solutions with an S_{OCP} = 4 and 8 revealed neutral effects after longer storage periods of up to 10 weeks [Tschoppe et al., 2008]. The role of brushite (dicalcium phosphate dihydrate) and amorphous calcium phosphate as major precursors in remineralization [Johnsson and Nancollas, 1992] has not been considered previously.

Therefore, the present study was undertaken to compare the effects of experimental CMC-based solutions differing in S_{OCP} and S_{DCPD} on the mineral loss of predemineralized bovine enamel in vitro. A CMC-based saliva substitute often prescribed in Germany (Glandosane) was used to control the study design. Moreover, calcium (Ca), phosphate (P_i), and fluoride (F) concentrations were measured to elucidate the mechanisms underlying these effects. It was hypothesized that with increasing S_{OCP} and S_{DCPD} of the solutions, a significantly reduced mineral loss/increased mineral gain might be observed.

Materials and Methods

Specimen Preparation

Thirty-two recently extracted permanent bovine central incisors were used. From each crown four slabs (3 × 4 mm^2) were prepared from the labial aspect under running tap water using a diamond-coated band saw (Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany). Subsequently, the 126 enamel specimens were embedded in epoxy resin (Technovit 4071; Heraeus Kulzer, Wehrheim, Germany) keeping the natural surface free from resin. Specimens were ground flat and hand-polished using up to 4,000 grit (silicon carbide; Struers, Copenhagen, Denmark), removing the outer parts of enamel (approximately 200 μm).

Subsequently, one quarter of each specimen's surface was covered with an acid-resistant nail varnish (Betrix, Frankfurt/Main, Germany) to serve as control of sound enamel. Lesions were prepared by immersion in a solution containing 6 mM methylhydroxydiphosphonate, 3 mM $CaCl_2 \cdot 2H_2O$, 3 mM KH_2PO_4, and 50 mM CH_3COOH (Merck, Darmstadt, Germany) at pH 4.95 in an incubator (37°C, BR 6000; Heraeus Kulzer) for 7 days. The pH value was controlled daily (pH-Meter CG 819; Schott Geräte, Hofheim, Germany) and slight elevations were corrected with lactic acid to maintain a constant pH value between 4.92 and 4.98 during the demineralization 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).

Calculation of the Degrees of Saturation and Preparation of the Solutions

CMC (Merck) was dissolved (10 g/l) as a base for experimental saliva substitutes using distilled water. For aqueous solutions the degrees of saturation with respect to calcium-containing compounds (DCPD, OCP, hydroxyapatite, and calcium fluoride, CaF_2) can be calculated [Shellis, 1988] (table 1). For both CMC as well as aqueous control solutions Ca:P_i ratio was defined at 1:1.6. According to theoretical S_{OCP} values of 0, 0.5, 1, 2, 4, and 8 at pH 6.5, the respective amounts of Ca ($CaCl_2$) and P_i (KH_2PO_4) were added to the CMC base (S0, S0.5, S1, S2, S4, and S8) as well as to distilled water (C0 and C1, table 2). Additionally, all solutions contained methyl (6.5 mM) and propyl hydroxyl benzoate (1 mM) as preservatives and sorbitol (20 g/l = 110 mM) as well as NaF (0.1 mM) (table 1). KCl (16 mM), NaCl (15.1 mM), and $MgCl_2$ (0.3 mM) were added to increase ionic strength (all chemicals from Merck, Darmstadt, Germany). Glandosane (pH 5.3) served as a means of controlling external validity of the present study.

In vitro Storage in Saliva Substitutes

After demineralization half of each demineralized surface was covered with nail varnish (control of demineralization). Subsequently, specimens were randomly divided into nine groups (n = 14) and stored in the respective solution at 37°C for 10 weeks (20 ml each, table 2). After 5 weeks exposed surfaces of each specimen were partly varnished (effect after 5 weeks). Every second day the solutions were replenished, the pH was checked, and slight changes were corrected with hydrochloric acid or potassium hydroxide to maintain a constant pH value during the remineralization period. Small amounts of 0–470 μl of hydrochloric acid were added to keep a constant pH of 6.5 during the study period of 10 weeks.

Calcium, Phosphate, and Fluoride Measurements

Calcium binding capacity and free calcium concentrations were measured with an automated titrator (Metrohm, Filderstadt, Germany) using a calcium electrode (Ca-ISE; No. 6.0508.110; Metrohm) and software (tiamo 1.1; Metrohm) [Gebauer et al., 2008]. Degassed distilled water was used for sample preparation and calibration of the Ca-ISE. All measurements were performed at pH 6.5. The pH value was kept constant by adding 0.01 M NaOH and 0.01 M HCl. The resulting volume dilution was taken into account.

For calcium binding measurements the various original solutions were diluted 1:333. A 0.025 M calcium chloride solution (≥99.5%; Sigma-Aldrich, Munich, Germany) was dosed at a rate of 0.01 ml/min to 25 ml of the sample solution while the electrode potential was recorded. The parallel shift of free Ca along the x axis indicates Ca binding. From the intercept of extrapolation of the linear Ca graph with the x axis, the adsorbed amount of Ca ions can be determined. The selectivity of the Ca-ISE is high with $\log K_{Ca\text{-}X} < -6.3$ for interfering cations X present in our solutions according to the electrode manual, maintaining a sensitive calcium measurement. The Ca-ISE was calibrated prior to each measurement by adding the diluted calcium chloride solution to 25 ml of degassed distilled water under the same conditions. Free calcium concentrations of the unfiltered solutions could be calculated from the initial Ca^{2+} potential of these curves.

Free calcium measurements of the ultrafiltered (membrane 5000 D) original solutions (dilution 1:10) were performed (single measurement) using the same procedure as above.

Calcium and phosphor concentrations of S1, S2, S4, and S8 were determined by optical emission spectroscopy (ICP-OES; Optima 2100 DV; Elmer Perkin, Fremont, Calif., USA). For each measurement 2.5 ml of the respective CMC solution (ultrafiltered using 5000 D membrane) was acidulated with 3 ml nitric acid (65%, Merck) and diluted in 50 ml of deionized water. The measurements were repeated once (three measurements each time).

Fluoride concentrations of all solutions were measured (Orion Autochemistry System 960; Fisher Scientific, Ulm, Germany) using an ion-specific electrode (type 96–09 BNC; Fisher Scientific). TISAB II (1:1; Fisher Scientific) was used to control ionic strength. The instrument was standardized with 0.1 M fluoride solution (Fisher Scientific). Three measurements of each solution were performed. Degrees of saturation with respect to calcium-containing compounds were recalculated taking into account these measurements (table 1).

To analyze the S8 precipitate, samples were centrifuged at 37,000 g. Wide-angle X-ray scattering of the sediments was performed using a diffractometer (PDS 120; Nonius, Solingen, Germany).

Transversal Microradiography

After in vitro exposure, samples were cut perpendicular to their demineralized/exposed surfaces. Specimens were mounted on microscopic slides and sections (200 μm) were cut (Exakt 300 CL). The slabs were ground (1,200, 2,400, and 4,000 grit, Exakt Mikroschleifsystem 400 CS; Exakt Apparatebau) from the opposite side to a uniform thickness of 100 μm. Parallelism of the samples was tested with a digital micrometer at a precision of 0.001 mm (Mitutoyo, Japan). Contact microradiographs of the enamel specimens were obtained with a nickel-filtered copper (CuKa) X-ray source (PW 1730; Philips, Kassel, Germany) operating at 20 kV and 20 mA. For all microradiographs an aluminum step wedge was used. The radiation source-to-film distance was 34 cm. Exposure time was 10 s, which is optimum for the type of section used as shown in preliminary experiments. A high-resolution film (high-speed holographic film, Kodak Professional, SO-253; Kodak, Stuttgart, Germany) was used and developed under standardized conditions according to the manufacturer's recommendations. Microradiographs were studied with a digital image-analyzing system (CCD video camera Modul XC77E; Sony, Japan) that was interfaced to a microscope (Axioplan; Zeiss, Oberkochen, Germany) and a computer. Mineral loss (vol% × μm) was calculated by integrating the difference between the mineral content (vol%) in sound and demineralized enamel over the depth of the lesion (μm). Lesion depth was defined as the distance from the surface (20 vol%) to the site in the lesion where the mineral content was more than 95% of the mineral content of sound enamel. Mineral volume percent of sound enamel was set at 88 vol% (TMR for Windows 2.0.27.2; Inspektor Research System BV, Amsterdam, The Netherlands).

Mineral loss and lesion depth of sound and demineralized areas as well as of those areas being exposed for 5 or 10 weeks to the various solutions were evaluated. Mineral loss (ΔZ_{Sound}) and lesion depth (LD_{Sound}) of sound areas were subtracted from the respective values of demineralized surfaces (ΔZ_{Demin}, LD_{Demin}) as well as from those of the surfaces that were exposed to the various solutions for 5 ($\Delta Z_{Effect5}$, $LD_{Effect5}$) or 10 weeks ($\Delta Z_{Effect10}$, $LD_{Effect10}$). These corrected values were then analyzed. Changes

Table 1. Theoretical and nominal degrees of saturation (DS) with respect to DCPD, OCP, hydroxyapatite (HA), and CaF_2 according to Shellis [1988]

Name		DS (as added)				DS (as measured)			
		DCPD	OCP	HA	CaF_2	DCPD	OCP	HA	CaF_2
Glandosane	G	0.2	0.3	0.9	0	–	–	–	–
CMC	S0	0	0	0	0	0	0	0	0
	S0.5[a]	0.2	0.5	2.2	0.28	0.1	0.2	0.6	0.13
	S1	0.5	1	4.5	0.36	0.1	0.3	1.0	0.15
	S2	1.1	2	9.1	0.47	0.3	0.6	2.5	0.21
	S4	*2.4*	*4*	*18.3*	*0.59*	*1.1*	*1.8*	*7.3*	*0.34*
	S8	*5.2*	*8*	*36.2*	*0.72*	*3.4*	*5.1*	*22.2*	*0.55*
Control	C0	0	0	0	0	0	0	0	0
	C1[a]	0.5	1	4.5	0.36	0.5	1	4.5	0.36

Solutions showing precipitations are italicized.

[a] Phosphate concentrations were deduced from measurements of S1, S2, S4, and S8.

Table 2. Ion concentrations of the various solutions

Name		pH	Added concentrations, mM			Measured concentrations, mM					
			Ca	P_i	F	free Ca[a] unfiltered	free Ca[b] filtered	free Ca[c] (ICP)	Ca binding[d]	phosphate (ICP)[e]	F[f]
Glandosane	G	5.3	1	2.6	0	–					
CMC	S0	6.5	0	0	0.1	0.01	0.01	–	9	–	0.09
	S0.5	6.5	0.34	0.54	0.1	0.03	0.02	–	9	–	0.1
	S1	6.5	0.8	1.25	0.1	0.10	0.08	0.05 ± 0.001	8	1.2 ± 0.003	0.1
	S2	6.5	1.8	2.9	0.1	0.17	0.07	0.16 ± 0.001	7	2.9 ± 0.02	0.1
	S4	6.5	4.5	7.2	0.1	0.57	0.55	0.78 ± 0.001	5	7.1 ± 0.02	0.07
	S8	6.5	12	19.5	0.1	5.00	3.49	4.58 ± 0.04	3	17.9 ± 0.1	0.07
Control	C0	6.5	0	0	0.1	0.01	–	–	<0.5	–	0.08
	C1	6.5	0.8	1.25	0.1	0.52	–	–	<0.5	–	0.09

Ion concentrations of Glandosane were derived from the manufacturer; pH was measured. To CMC (10 g/l) and control solutions calcium and phosphates were added as $CaCl_2$ and KH_2PO_4, respectively. Solutions additionally contained $MgCl_2$ (0.3 mM), NaCl (15.1 mM), KCl (16 mM) as well as methyl (6.5 mM) and propyl hydroxyl benzoate (1 mM) as preservatives. The CMC solutions contained sorbitol (20 g/l = 110 mM). For fluoride measurements, standard deviations were below 0.01 mM, for all other measurements standard deviation is only given in the table when applicable.

[a] Unfiltered original solutions (dilution 1:333). [b] Ultrafiltered (5000 D membrane) original solutions (dilution 1:10) measured by Ca-ISE. [c] Ultrafiltered (5000 D membrane) original solutions (1:20) measured by optical emission spectroscopy (ICP-OES). [d] Calcium binding capacity of filtered (membrane 5000 D) original solutions (1:333). [e] Ultrafiltered (5000 D membrane) original solutions (dilution 1:20) measured by ICP-OES. [f] Unfiltered original solution diluted in TISAB II (1:1).

in mineral loss ($\Delta\Delta Z_5 = \Delta Z_{Demin} - \Delta Z_{Effect5}$, $\Delta\Delta Z_{10} = \Delta Z_{Demin} - \Delta Z_{Effect10}$) as well as changes in lesion depth ($\Delta LD_5 = LD_{Demin} - LD_{Effect5}$, $\Delta LD_{10} = LD_{Demin} - LD_{Effect10}$) were calculated.

Statistical Analyses

Statistical analyses were performed using SPSS software (SPSS for Windows, version 11.5; Munich, Germany). Baseline values after demineralization were compared using ANOVA (Bonferroni correction). Differences in mineral loss and lesion depth before and after storage in the various solutions (adjusted paired t test: factor 9) as well as $\Delta\Delta Z$ and ΔLD values of subgroups with comparable S_{OCP} were analyzed (unadjusted independent t test) for both storage periods. All tests were performed at a 5% level of significance.

Results

All in vitro de- and remineralized specimens (n = 9–14) developed subsurface lesions consistently revealing a surface layer that was more mineralized than the body of the lesion with no signs of erosive loss. Seventeen specimens were lost during preparation. Calcium binding capacity of the CMC solutions was highest (9 mM) for S0 and lowest (3 mM) for S8. For aqueous control solutions calcium binding was below the sensitivity of the electrode (<0.5 mM). Free calcium concentrations of S0, S0.5, and C0 were below 0.02 mM. Values measured for C1 (0.52 mM), S1 (0.05 mM), S2 (0.16 mM), S4 (0.78 mM), and S8 (4.58 mM) were considerably lower than the respective added calcium concentrations, which resulted in a lower degree of saturation as intended (table 1). P_i concentrations of S1, S2, and S4 were similar to the concentrations added. Only S8 had 1.6 mM less P_i than previously added (19.5 mM). Measured fluoride concentrations were similar to the added ones (0.1 mM) for control solutions as well as S0, S0.5, S1, and S2. For S4 and S8 lower fluoride concentrations (0.07 mM) were measured (table 2). In S4 and S8, a fluffy precipitate was observed. The absence of sharp peaks in wide-angle X-ray scattering of the precipitate in S8 indicated absence of crystallinity and that the solid was most presumably amorphous calcium phosphate.

Mineral loss as well as lesion depth did not differ significantly between the various groups at baseline ($p > 0.05$; ANOVA). Borderline significance (t test) could be found for $\Delta\Delta Z_5$ ($p = 0.193$) and $\Delta\Delta Z_{10}$ ($p = 0.055$) for the

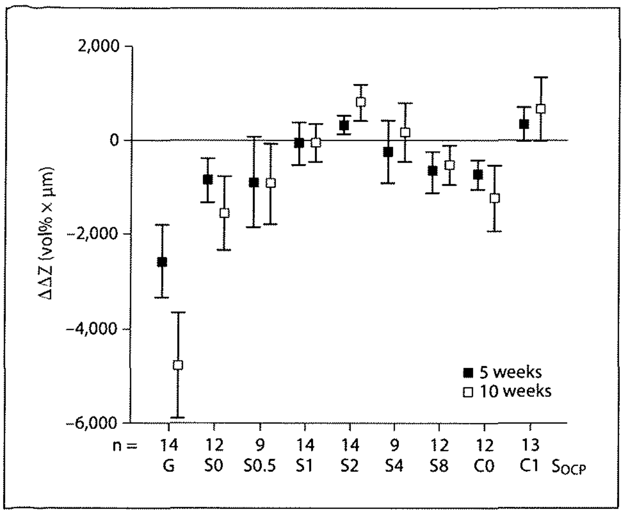

Fig. 1. Means and confidence intervals (95%) of the differences in mineral loss ($\Delta\Delta Z$; vol% $\times$ μm) of the various solutions (G = Glandosane; S0, S0.5, S1, S2, S4, and S8 = CMC-based solutions with S_{OCP} = 0, 0.5, 1, 2, 4, and 8, respectively; C0 and C1 = water-based control solutions with S_{OCP} = 0 and 1, respectively) after 5-week as well as 10-week storage.

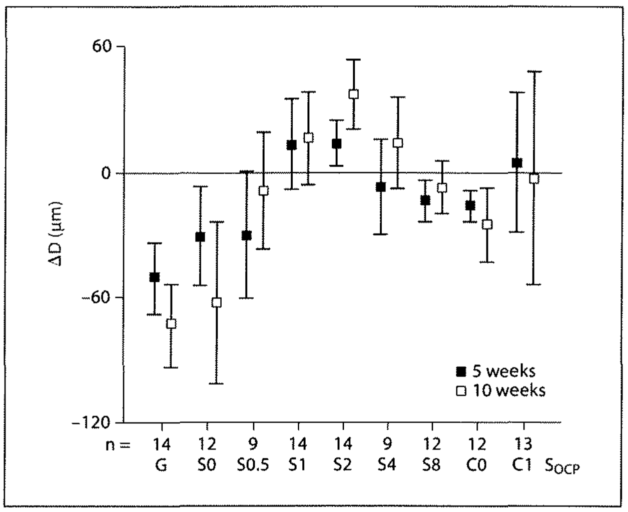

Fig. 2. Means and confidence intervals (95%) of the differences in lesion depth (ΔD; μm) of the various solutions (G = Glandosane; S0, S0.5, S1, S2, S4, and S8 = CMC-based solutions with S_{OCP} = 0, 0.5, 1, 2, 4, and 8, respectively; C0 and C1 = water-based control solutions with S_{OCP} = 0 and 1, respectively) after 5-week as well as 10-week storage.

comparison of S1 with C1 as well as for ΔLD_5 ($p = 0.182$) and ΔLD_{10} ($p = 0.066$) for the comparison of S0 with C0 (fig. 1, 2).

After 5- and 10-week exposure, significantly increased mineral loss as well as lesion depth could be observed for specimens stored in Glandosane compared with baseline ($p < 0.05$; adjusted t test). Both negative control solutions ($S_{OCP} = 0$) induced significantly increased $\Delta Z_{Effect5}$ and $\Delta Z_{Effect10}$ values. C0 and S0 also showed significantly increased $LD_{Effect5}$ and $LD_{Effect10}$, respectively, compared with baseline demineralization ($p < 0.05$). Specimens stored in CMC-based solutions with $S_{OCP} = 2$ for 10 weeks revealed significantly decreased levels of mineral loss and lesion depth. This was also the case after 5 weeks' storage for $\Delta Z_{Effect5}$ ($p < 0.05$, tables 3, 4).

Discussion

This study mainly shows that CMC-based and aqueous solutions theoretically saturated with respect to calcium phosphates at a similar level did not differ significantly in their capacities to either de- (C0 and S0) or remineralize (C1 and S1). Moreover, CMC solutions highly supersaturated with respect both to OCP and DCPD revealed a neutral (S4) or even a slightly demineralizing (S8) effect, whereas a solution that was unsaturated (S2) induced remineralization. Thus, the hypothesis that with increasing degrees of saturation of these solutions, an enhanced remineralization can be observed has to be rejected. The following mechanisms seemed to have played some role: (1) establishment of an adsorbed layer of CMC at the enamel-liquid interface; (2) interaction of calcium bound to CMC in the adsorbed layer with phosphate, and (3) interference of calcium diffusion in S4 and S8 due to heterogeneous nucleation.

In the present study using a remineralization model, CMC did not hamper remineralization significantly. Nonetheless, some nonsignificant but distinct differences between C1 and S1 were found in favor of C1 with respect to remineralization properties. An earlier study had shown that growth of hydroxyapatite crystals was inhibited even by low concentrations of CMC (0.005% w/v) in a supersaturated solution at neutral pH [Van der Reijden et al., 1997]. At lower pH (5.2) hydroxyapatite dissolution was inhibited as well. Under pH cycling conditions CMC inhibited both de- and remineralization, despite shorter contact times of the CMC solution to enamel in the pH cycling model compared to the present design. It was speculated that formation of an absorbed polymer layer

Table 3. Means and standard deviations (SD) of the mineral losses (ΔZ; vol% $\times$ μm) after in vitro demineralization (ΔZ_{Demin}) and storage for 5 ($\Delta Z_{Effect5}$) as well as 10 weeks ($\Delta Z_{Effect10}$) in the various solutions

Solution		ΔZ_{Demin}		$\Delta Z_{Effect5}$		Demin effect$_5$	$\Delta Z_{Effect10}$		Demin effect$_{10}$
		mean	SD	mean	SD	p	mean	SD	p
Glandosane	G	1,673	930	4,244	1,553	*0.008*	6,457	2,328	*0.008*
CMC	S0	1,605	1,181	2,400	1,410	*0.009*	3,077	1,265	*0.009*
	S0.5	1,884	948	2,593	1,450	0.270	3,229	1,428	0.326
	S1	1,796	1,010	1,868	871	1	1,867	920	1
	S2	1,714	622	1,396	552	*0.027*	913	453	*0.009*
	S4	2,065	627	2,507	1,053	0.612	1,720	691	1
	S8	1,765	918	2,334	1,216	0.099	2,336	1,065	0.171
Control	C0	1,474	580	2,283	891	*0.008*	2,737	1,440	*0.027*
	C1	1,611	703	1,333	867	0.882	1,015	795	0.540

Significant differences between the values after demineralization and storage in the various solutions within each group (n = 9–14) as analyzed by adjusted t tests are indicated by p values and italicized.

Table 4. Means and standard deviations (SD) of the lesion depths (LD, μm) after in vitro demineralization (LD_{Demin}) and storage for 5 ($LD_{Effect5}$) as well as 10 weeks ($LD_{Effect10}$) in the various solutions

Solution		LD_{Demin}		$LD_{Effect5}$		Demin effect$_5$	$LD_{Effect10}$		Demin effect$_{10}$
		mean	SD	mean	SD	p	mean	SD	p
Glandosane	G	82	35	133	32	*0.008*	156	51	*0.008*
CMC	S0	78	34	106	34	0.081	146	52	*0.030*
	S0.5	95	42	116	63	0.532	113	46	1
	S1	109	41	96	33	1	93	40	1
	S2	95	25	81	23	0.171	57	22	*0.008*
	S4	113	27	123	35	1	95	24	1
	S8	97	36	107	40	0.756	104	40	1
Control	C0	93	29	108	32	*0.008*	115	49	0.073
	C1	101	28	97	59	1	105	78	1

Significant differences between the values after demineralization and storage in the various solutions within each group (n = 9–14) as analyzed by adjusted t tests are indicated by p values and italicized.

on the hydroxyapatite or enamel surface may provide protection against acidic attacks [Van der Reijden et al., 1997]. A similar mechanism can be deduced from the present results with respect to the comparison of remineralization capabilities of aqueous and CMC-containing solutions.

Establishment of an adsorbed layer might also explain why we observed remineralization of a solution that was theoretically supersaturated, but actually unsaturated, with respect to DCDP and OCP. In an aqueous solution free calcium and phosphate ions are able to affect enamel directly. In CMC solutions diffusion of calcium and other ions is generally slower. Calcium is initially bound to CMC, which has been revealed for polycarboxylates [Gebauer et al., 2009], and could be observed for the present solutions as well. This layer functions as an 'ion sponge' that binds calcium to carboxylate, leading to calcium-enriched regions near the enamel interface. As can be inferred from the lower solubility of calcium phosphate compared to calcium carbonate, the calcium affinity of phosphate is higher than that of carbonate or carboxylate. Since phosphate is available in the solutions according to our measurements, calcium that was previously bound to CMC carboxylate groups was available for calcium phosphate formation in a competition reaction in analogy to polymethacrylate-bound calcium abstracted by phosphate [Antonietti et al., 1998] or polyacrylate-bound barium abstracted by sulfate [Wang and Cölfen, 2006].

S4 and S8 tended to precipitate spontaneously, presumably since these solutions were supersaturated with respect to DCPD and OCP [Johnsson and Nancollas, 1992]. Preliminary tests for colloidal species in solutions using dynamic light scattering revealed the absence of nanosized calcium/phosphates. In fact, amorphous calcium phosphate was determined as the main constituent of the precipitate. Hence, this might have influenced the mineralization process. Aqueous solutions with $S_{OCP} = 2$ or higher showed distinct precipitations as well. Thus, the solution with the highest degree of saturation, in which no precipitates were observed (C1), was chosen as a positive control.

Previous studies revealed that a solution with $S_{OCP} = 3$ induced the most pronounced remineralization in enamel. However, solutions with very high S_{OCP} of 16–58 [Meyer-Lueckel et al., 2007] demineralized the specimens because of instabilities of the solutions with respect to pH, which was not the case in the present study. Nonetheless, S4 and S8 showed less remineralization compared with S2. For S4 and S8 free calcium ions could be measured, but not for S2. According to our results, amorphous calcium phosphate precipitated in these solutions. This might have occurred not only in the solution, but also on top of the adsorbed CMC layer, since solutions were in contact with the enamel surface for rather long periods compared with the clinical situation. These precipitates can lead to local heterogeneous nucleation or growth on the amorphous precipitates, resulting in less calcium and phosphate available for remineralization in the adsorbed CMC layer at the enamel interface. Heterogeneous nucleation is always favored in terms of energy compared to homogeneous nucleation, since the surface energy which needs to be invested to generate the particle surface according to classical nucleation theory is lower as a result of the already existing surface [Mullin, 2001]. As mentioned, the clinical impact of this mechanism is uncertain, since similar absorbed polymer layers might not be established in vivo.

Despite not being a natural lubricant, CMC still seems to be a good clinical choice as a base for a saliva substitute. A recent prospective crossover study showed that most patients suffering from xerostomia preferred a CMC spray (Glandosane) compared to solutions based on cellulose gel, oil, or mucin, due to taste and handling [Momm et al., 2005]. Nonetheless, preference of saliva substitutes by various groups of patients has been discussed controversially [Vissink et al., 1983; Visch et al., 1986; Epstein and Stevenson-Moore, 1992; Furumoto et al., 1998; Regelink et al., 1998]. In particular Glandosane revealed high demineralizing potential in several in vitro studies [Joyston-Bechal and Kidd, 1987; Meyer-Lueckel et al., 2002; Tschoppe et al., 2008]. The rather low pH value (high concentrations of titratable acids), being more than 1 unit lower compared to experimental saliva substitutes, seems to be responsible for the demineralizing effect of Glandosane. Moreover, low levels of fluoride as added to the CMC solutions decrease the solubility of hydroxyapatite [ten Cate and Duijsters, 1983]. As a result, the fluoride-free product Glandosane demineralized the specimens to a greater extent than the CMC-based solution with the lowest S_{OCP}.

Several methodological issues should be considered when interpreting the results. Specimens were stored for a period of 10 weeks in the various solutions. This procedure simulated an extremely intensive contact that cannot be expected under clinical conditions. Patients suffering from hyposalivation reveal long demineralizing periods in their oral cavities alternating with short, but frequent periods when a saliva substitute is used. However, certain differences compared to the clinical situation need to be taken into account, first and foremost longer contact times of the solutions to enamel. Nonetheless, with the present model possible remineralizing effects on dental hard tissues could be monitored more efficiently compared with a pH cycling procedure, since recurrent demineralizing periods were not included [Featherstone, 1996]. Bovine enamel was used since the biological variation in bovine teeth is considered to be relatively small compared with human teeth. Consequently, a uniform reaction to the de-/remineralization challenge can be expected [Mellberg, 1992]. A 'physiological' $Ca:P_i$ ratio of 1:1.6 was chosen as previously [Tschoppe et al., 2008], since data do not clearly support a higher [Exterkate et al., 1993; Lynch et al., 2007] or a lower [Exterkate et al., 1993; Hara et al., 2008] ratio with respect to overall remineralization.

The pH value of the various solutions was adjusted to 6.5, similar to the one of human saliva. It was assumed that a lower pH would rise within a short oral period. However, irradiated patients are known to have lower pH values of saliva [Dreizen et al., 1976] that might affect those of saliva substitutes. Moreover, more pronounced remineralization has been reported for mucin- and CMC-based solutions with pH 6.5 rather than pH 5.5, which has been attributed to an easier adjustment (buffering) of the S_{OCP} at higher pH, resulting in more stable solutions [Meyer-Lueckel et al., 2006, 2007]. Therefore, a higher pH seems to be preferable for a saliva substitute from a cariological point of view.

It can be concluded that experimental CMC-based saliva substitutes that are theoretically slightly supersaturated but nominally unsaturated with respect to DCPD and OCP are capable of remineralizing dental hard tissues after longer storage periods in vitro. This might be due to a more favorable balance between calcium bound to CMC in an adsorbed layer at the enamel-liquid interface and heterogeneous nucleation of calcium phosphates on precipitates compared to solutions being either supersaturated or having lower levels of saturation.

Acknowledgments

This study was supported in part by the Deutsche Krebshilfe e.V. (grant 70-3117-Me-1). The authors wish to thank Mrs. Sara Nasiri and Mrs. Margarita Nitka for their contribution to the laboratory work and Margit Barth for her support while conducting the calcium measurements. We are grateful to Dr. R.P. Shellis for his constructive feedback.

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