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The effects of lesion baseline characteristics and different Sr:Ca ratios in plaque fluid-like solutions on caries lesion de- and remineralization

Frank Lippert*
Department of Preventive and Community Dentistry, Oral Health Research Institute, Indiana University School of Dentistry, 415 Lansing Street, Indianapolis, IN 46202, USA

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ABSTRACT

This study investigated the effects of lesion baseline characteristics and different strontium (Sr) to calcium (Ca) ratios in plaque fluid-like solutions (PF) on lesion de- and remineralization. Caries lesions were formed in enamel using three protocols: methylcellulose acid gel (MeC) and partially saturated lactic acid solutions containing carboxymethylcellulose (CMC) or not (SOLN). Lesions were exposed to PF with four distinct Sr:Ca molar ratios (0:1/3:1:3), but otherwise identical composition and total Sr+Ca molarity, for seven days. Lesions were characterized using transverse microradiography (TMR) at baseline and post-treatment. At baseline, MeC and CMC had similar integrated mineral loss values, whereas SOLN lesions were more demineralized. All lesions showed significant differences in their mineral distributions, with CMC and SOLN having lower R values (integrated mineral loss to lesion depth ratio) than MeC. Post-PF exposure, no interaction was found between lesion type and Sr:Ca ratio. Within lesion type, MeC demineralized, whereas CMC and SOLN exhibited some remineralization, with the differences between MeC and the other lesion types being of statistical significance. Within Sr:Ca ratio, the 1:3 ratio exhibited some remineralization whereas other groups tended to demineralize. Only the difference between groups SrCa1/3 and SrCa0 was of statistical significance. In summary, both lesion baseline characteristics and Sr:Ca ratio were shown to effect lesion de- and remineralization. Under the conditions of the study, high-R lesions are more prone to demineralize under PF-like conditions than low-R lesions. In addition, partial Sr substitution for Ca in PF was shown to enhance lesion remineralization.

1. Introduction

The baseline characteristics of caries lesions have been reported to greatly affect the lesion's subsequent behaviour to de- and remineralization challenges, with the greatest impacts being attributed to baseline mineral loss (ΔZ_{base}) and R value, which is ratio of ΔZ_{base} to lesion depth. Several studies[1–4] reported an increasing predisposition of lesions to net remineralization with increasing ΔZ_{base}. Unlike ΔZ_{base} effects, which are comparatively well understood, the effect of R value has been studied to far lesser extent as the majority of investigators concentrate only on one (preferred) lesion type. Nonetheless, Lynch et al.[5] found that high-R lesions are more responsive to remineralizing treatments than low-R lesions, presumably due to the high-R lesions' greater porosity. A very

* Tel.: +1 317 274 3983; fax: +1 317 274 5425.
E-mail address: flippert@iupui.edu.
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recent in situ study[6] found that, rather surprisingly, high- and low-R lesions showed opposite behaviours under virtually identical conditions, with the high-R lesions exhibiting some remineralization, whereas low-R lesions further demineralized. These studies have highlighted that more research is required in this area to further our understanding of the importance of the R value.

The role of Sr in caries prevention has attracted great interest in the research community in the past. A number of epidemiological studies[7–9] suggested that elevated Sr concentrations ([Sr]) in drinking water are directly correlated with decreases in caries incidence. However, the Sr caries relationship is by no means straightforward. Curzon et al.[7] suggested an ''optimum [Sr]'', as caries incidence tended to increase (again) with increasing [Sr]. The same authors also reported differences in Sr response between lifelong residents and immigrants, suggesting topical and systemic Sr effects. While mechanistic studies[10–12] have failed to support earlier epidemiological studies as Sr was shown to enhance apatite solubility as it destabilizes the apatite crystal structure, Sr-substituted apatite was, however, also shown to act as a template for nucleation and to promote apatite growth.[13] Indeed, a very recent in vitro study,[14] investigating remineralization of caries lesions in the presence of Sr and fluoride (F), found not only synergistic Sr+F effects but also an enhancement in lesion remineralization by Sr. An earlier in situ de-/remineralization study[15] also demonstrated superior anti-caries benefits of a Sr-supplemented F dentifrice vs. a F dentifrice control (at identical [F]). While the data on Sr addition per se is compelling, little attention has been paid to studying lesion de- or remineralization in systems where Ca has been partially replaced by Sr, as these studies would not only allow for a better evaluation of potential caries preventative effects of Sr, but they would also provide a valuable mechanistic insight. Furthermore, it would be advantageous to study Sr effects under plaque fluid-like conditions, as these systems resemble most closely those found at the sites of caries occurrence.

Therefore, the aim of the present in vitro study was to study the effects of lesion baseline characteristics and different Sr:Ca ratio in PF on lesion de- and remineralization.

2. Materials and methods

2.1. *Enamel specimen preparation*

Enamel specimens were obtained from bovine teeth. Tooth crowns were cut into 5 mm × 3 mm specimens using a Buehler Isomet low-speed saw. The teeth were stored in deionized water saturated with thymol during the sample preparation process. Specimens were ground and polished to create flat, planar parallel dentine and enamel surfaces using a Struers Rotopol 31/Rotoforce 4 polishing unit (Struers Inc., Cleveland, PA, USA). The dentine side of the specimens was ground flat to a uniform thickness with 500-grit silicon carbide grinding paper. The enamel side of the specimen was serially ground using 1200, 2400 and 4000 grit paper. The specimens were then polished using a 1 μm diamond polishing suspension on a polishing cloth until the enamel surface had a minimum of a 5 mm × 2 mm highly polished facet across the specimen. Resulting specimens had a thickness range of 1.7–2.2 mm. The specimens were assessed under the Nikon SMZ 1500 stereomicroscope at 20× magnifications for cracks, hypomineralized (white spots) areas or other flaws in the enamel surface that would exclude them from use in the study. An experimental window, measuring approximately 5 mm × 1.7 mm, was created on the specimens using acid-resistant nail varnish (Sally Hansen Advanced Hard As Nails Nail Polish, USA), leaving sound enamel areas on either side. Prepared specimens were stored at 100% relative humidity at 4 °C until use.

2.2. *Artificial caries lesion creation*

In vitro incipient caries lesions were prepared using the following three methods as (unpublished) pilot studies revealed significant differences in the resulting lesions' mineral distributions:

For MeC, the method used by ''Laboratory D'' as described by ten Cate et al.[16] was employed. Sound enamel specimens were demineralized at pH 4.6 in 8% methylcellulose (aqueous, 1500 cP, 63 kDa; 'M0387', Sigma–Aldrich, USA) covered with an equal volume of 0.1 M lactic acid, pH adjusted with KOH. Neither the gel nor the demineralization solution was replaced during the seven day demineralization period.

For CMC, sound enamel specimens were immersed in a demineralization solution containing 0.1 M lactic acid, 4.1 mM Ca (as $CaCl_2 \times 2H_2O$), 8 mM PO_4 (as KH_2PO_4) and 1% (w/v) carboxymethylcellulose (''high viscosity'', ''C5013'', Sigma–Aldrich, USA), pH adjusted to 5.0 using KOH. The solution was not stirred or replaced during the seven day demineralization period.

For SOLN, sound enamel specimens were immersed in a demineralization solution which was identical to the one used for CMC, but lacking carboxymethylcellulose. It contained 0.1 M lactic acid, 4.1 mM Ca (as $CaCl_2 \times 2H_2O$), 8 mM PO_4 (as KH_2PO_4), pH adjusted to 5.0 using KOH. The solution was not stirred or replaced during the seven day demineralization period.

Lesions were created at 20 ml gel and/or 20 ml solution per specimen and at 37 °C.

2.3. *Transverse microradiography (TMR)*

Sections, one per specimen and approximately 100 μm in thickness, were cut from the centre of the specimens and across the lesion window and sound enamel areas after lesion creation (lesion baseline) using a Silverstone–Taylor Hard Tissue Microtome (Scientific Fabrications Laboratories, USA). Thus, a matched pair of specimens was obtained from each lesion. After sectioning, a coloured nail varnish was used to cover the cut surfaces of the specimens, serving as a reference point. Post-treatment, another section was cut from each half of the specimen and from the same side the baseline section was cut from (i.e. the coloured side). The sections were mounted, with an aluminium step wedge, on high resolution glass plate Type I A (Microchrome Technology Inc., San Jose, CA) and X-rayed at 20 kV and 30 mA at a distance of 42 cm for 65 min. The film was developed in Kodak d-19 developer for 3 min, placed in a stop bath (Kodak 146-4247) for 45 s, and then

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Table 1 – Composition and chemical properties of plaque fluid-like test solutions.

	SrCa0	SrCa1/3	SrCa1	SrCa3
Lactic acid	30	30	30	30
$CaCl_2 \times 2H_2O$	5.5	4.125	2.75	1.375
$SrCl_2 \times 6H_2O$	0	1.375	2.75	4.125
KH_2PO_4	9.4	9.4	9.4	9.4
KCl	63	63	63	63
NaN_3	3.08	3.08	3.08	3.08
F (as NaF)	1	1	1	1
pH	5.5	5.5	5.5	5.5
Sr:Ca molar ratio	0	1/3	1	3
DS_{HA}	3.50	2.98	2.54	1.73
DS_{OCP}	1.12	0.97	0.84	0.60
DS_{BR}	0.96	0.83	0.73	0.51
DS_{FA}	14.56	12.41	10.62	7.22
DS_{CaF_2}	0.36	0.33	0.30	0.24

All concentrations are in mmol/l, except for F which is in ppm.

fixed (Kodak 146-4106) for 3 min. All plates were then rinsed in deionized water for 15 min and air-dried. Microradiographs were examined with a Zeiss EOM microscope in conjunction with the TMR software v.3.0.0.11. Sound enamel was assumed to be 87% (v/v) mineral. Only lesions exhibiting an intact surface zone were included in the study. To allow for appropriate study comparisons, lesions were randomized among treatment solutions by lesion type and based on ΔZ_{base}.

2.4. *Plaque fluid-like solutions (PF)*

A total of four different solutions, varying only in their Sr:Ca molar ratios were prepared. Table 1 highlights their composition and chemical properties. Specimens were exposed to these solutions (20 ml per specimen) at 37 °C for seven days with the solutions being renewed after day three. The solutions were not stirred.

2.5. *Calculation of saturation with respect to calcium phosphate and fluoride phases*

The respective degrees of saturation with respect to hydroxyapatite (DS_{HA}), octacalcium phosphate (DS_{OCP}), brushite (DS_{BR}), fluorapatite (DS_{FA}) and calcium fluoride (DS_{CaF2}) of PF were calculated using a computer program.[17]

2.6. *Study variables*

For better clarity, all reported variables are summarized below:

ΔZ – integrated mineral loss (product of lesion depth and the mineral loss over that depth)

L – lesion depth (83% mineral; i.e. 95% of the mineral content of sound enamel)

R – ratio of integrated mineral loss to lesion depth ($\Delta Z/L$)

SZ_{max} – maximum mineral density at the lesion surface zone

LB_{min} – minimum mineral density in the lesion body

Changes in variables were calculated as follows:

$\Delta\Delta Z^* = \Delta Z_{base} - \Delta Z_{post}$ (primary variable)

$\Delta L = L_{post} - L_{base}$

$\Delta R = R_{post} - R_{base}$

$\Delta SZ_{max} = SZ_{max,post} - SZ_{max,base}$

$\Delta LB_{min} = LB_{min,post} - LB_{min,base}$

* – indicative of remineralization if >0, or further demineralization if <0.

2.7. *Statistical analysis*

ANOVA was used to compare the effects of lesion type (MeC, CMC, SOLN) and Sr:Ca ratio (0; 1/3; 1; 3) on $\Delta\Delta Z$, ΔL, ΔR, and ΔSZ_{max}. The ANOVA models included terms for lesion type, Sr:Ca ratio, and lesion type – by –Sr:Ca ratio interaction. The software SigmaPlot 11.0 (Systat Software, Inc., Germany) was used to analyse the data.

3. Results

The results of the TMR analysis of lesions at baseline are given in Table 2, and the different lesions' mineral distributions are shown in Fig. 1. While MeC and CMC had similar ΔZ_{base}, SOLN were more demineralized. All lesions varied in at least one parameter, with the biggest differences observed between MeC on one hand and CMC and SOLN on the other, especially in terms of mineral distribution. MeC were shallower, approximately half as mineralized in the surface zone and had a higher R value than the other lesion types. SOLN were similar to CMC as both lesion types had similar R values; however, SOLN were deeper and less mineralized in the lesion body than CMC. MeC exhibited lesion mineral distributions representative of surface softened lesions, lacking a pronounced surface layer. Therefore, LB_{min} measurements were not conducted on these lesions.

All solutions were supersaturated with respect to HA (Table 1). DS values for Sr-containing PF were lower as [Ca] were lower and as the present software did not allow for DS calculations of Sr-substituted CaPi's.

Statistical analysis of the $\Delta\Delta Z$ data revealed no interaction between lesion type and Sr:Ca ratio ($p = 0.705$). However, both lesion type ($p < 0.001$) and Sr:Ca ratio ($p = 0.039$) were shown

Table 2 – Lesion parameters at baseline.

Lesion type	n	ΔZ [vol.% min × μm]	L [μm]	R [vol.% min]	SZ_{max} [vol.% min]	LB_{min} [%min]
MeC	22	2547 ± 65^A	74.3 ± 2.4^A	34.7 ± 0.9^B	28.6 ± 1.4^A	n/a
CMC	23	2671 ± 82^A	96.5 ± 2.0^B	27.7 ± 0.7^A	54.6 ± 1.2^B	45.1 ± 1.3^B
SOLN	18	3238 ± 127^B	111.8 ± 4.7^C	29.2 ± 0.9^A	56.6 ± 1.7^B	37.0 ± 1.3^A

The results are means ± standard error. Different superscript letters indicate statistically significant differences within variable between lesion types ($p < 0.05$).

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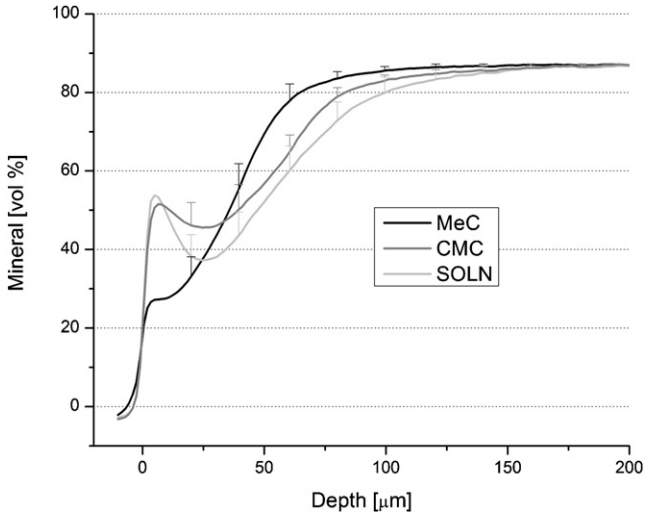

Fig. 1 – Average mineral distribution of lesions at baseline. For better clarity, error bars (standard deviations) are given at 20-µm intervals.

to affect ΔΔZ. Consequently, the data will be reported and discussed separately.

As all lesions were randomized among treatment solutions to achieve equally balanced groups with respect to ΔZ, L, R and SZ_{max} only post-treatment data are shown for the Sr:Ca ratio groups. The ΔΔZ data by lesion type and treatment are shown in Table 3. Post-treatment data for all other lesion parameters are shown in Table 4. As each lesion yielded a pair of matched specimens for the solution treatments (see "Section 2"), the "n" post-treatment should obviously be double that of baseline for each lesion type. Several specimens did, however, not yield a suitable section post-treatment; hence the sum of all post-treatment data points is lower than "baseline $n \times 2$".

For better transparency, ΔΔZ means and standard deviations are shown for all groups, despite the aforementioned lack of interaction between study factors. This was, however, omitted for the other lesion parameters as ΔΔZ was the primary variable. MeC demineralized further and also showed an increase in L. CMC and SOLN showed some remineralization, with the differences in ΔΔZ between MeC on one hand and CMC and SOLN on the other being of statistical significance. No differences in ΔL and ΔR between lesion types were found. Considering SZ_{max}, MeC and CMC exhibited an increase, whereas SOLN showed a decrease, with the difference between groups being of statistical significance. Both CMC and SOLN showed an increase in LB_{min} which was not different between lesion types, but of statistical significance in relation to their respective baseline values.

Figs. 2–4 show the average mineral profiles of MeC (Fig. 2), CMC (Fig. 3) and SOLN (Fig. 4) post-treatment and at baseline. No change in mineral distribution can be noted for MeC. Demineralization appeared to occur solely beyond the original lesion. CMC exhibited some changes, as the lesion surface zone appeared to have moved inwards. Remineralization within the lesion body can be seen as well as some demineralization beyond the original lesion. SOLN also showed several changes post-treatment. Similar to CMC, the lesion surface zone moved inwards and the lesion body remineralized. However, the degree of surface zone mineralization was somewhat lower post-treatment vs. baseline.

Table 3 – Change in lesion volume (ΔΔZ) post-treatment for all experimental groups.

Lesion type	Treatment				Means	n
	SrCa0	SrCa1/3	SrCa1	SrCa3		
MeC	−457 ± 122	−85 ± 101	−247 ± 90	−198 ± 108	$-247 \pm 76^{A*}$	40
CMC	85 ± 207	412 ± 144	44 ± 107	−134 ± 212	102 ± 77^{B}	41
SOLN	44 ± 148	366 ± 89	−27 ± 192	208 ± 283	148 ± 84^{B}	33
Means	-109 ± 87^{a}	231 ± 93^{b}	-76 ± 89^{ab}	-42 ± 97^{ab}		
n	31	28	30	25		

The results are least square means ± standard error. For better clarity, all ΔΔZ data are shown without the appropriate unit [vol.% × µm]. Different superscript letters indicate statistically significant differences between lesion types (capital) and treatments (small) ($p < 0.05$).
* Indicates a statistically significant change from lesion baseline ($p < 0.05$).

Q3 **Table 4 – Change in lesion parameters post-treatment by lesion type and treatment.**

	ΔL [µm]	ΔR [vol.% min]	ΔSZ_{max} [vol.% min]	ΔLB_{min} [%min]
Lesion type				
MeC	$9.0 \pm 2.0^{A*}$	-0.8 ± 0.6^{A}	2.9 ± 1.2^{B}	n/a
CMC	3.8 ± 2.1^{A}	-1.8 ± 0.6^{A}	3.3 ± 1.2^{B}	$6.0 \pm 0.9^{A*}$
SOLN	2.5 ± 2.3^{A}	-1.9 ± 0.6^{A}	-2.0 ± 1.3^{A}	$4.3 \pm 1.0^{A*}$
Treatment				
SrCa0	5.9 ± 2.3^{a}	-0.6 ± 0.6^{b}	0.8 ± 1.3^{ab}	3.4 ± 1.2^{a}
SrCa1/3	3.6 ± 2.5^{a}	-3.6 ± 0.7^{a}	5.8 ± 1.4^{b}	7.7 ± 1.3^{a}
SrCa1	4.5 ± 2.4^{a}	-0.7 ± 0.7^{b}	-1.4 ± 1.3^{a}	5.0 ± 1.2^{a}
SrCa3	6.4 ± 2.6^{a}	-1.1 ± 0.7^{ab}	0.5 ± 1.5^{ab}	4.4 ± 1.4^{a}

The results are least square means ± standard error. Different superscript letters indicate statistically significant differences between lesion types (capital) and treatments (small) ($p < 0.05$).

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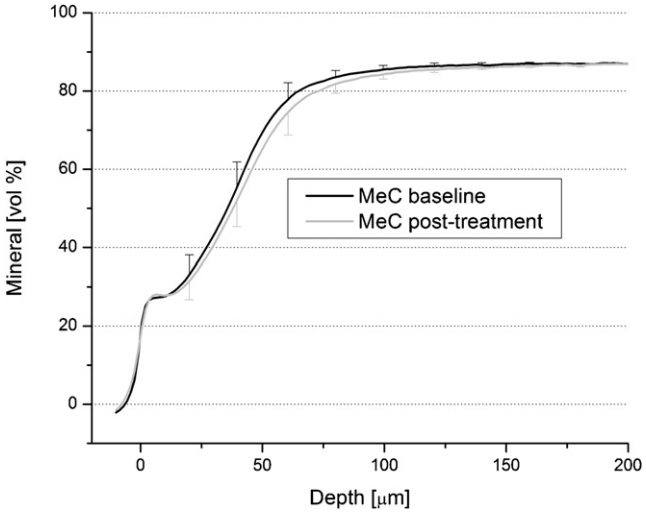

Fig. 2 – Average mineral distribution for MeC lesions at baseline and post-treatment. For better clarity, error bars (standard deviations) are given at 20-μm intervals.

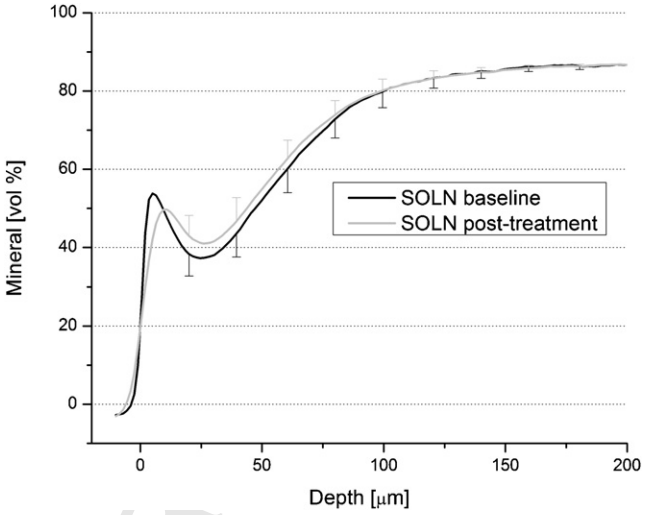

Fig. 4 – Average mineral distribution for SOLN lesions at baseline and post-treatment. For better clarity, error bars (standard deviations) are given at 20-μm intervals.

Within Sr:Ca ratio, SrCa1/3 was the only group showing remineralization, whereas other groups exhibited some demineralization. SrCa1/3 was superior to SrCa0 in terms of preventing de- and promoting remineralization. No other differences in $\Delta\Delta Z$ between groups were found. SrCa1/3 also showed (numerically) the biggest change in ΔR, ΔSZ_{max} and ΔLB_{min}. However, differences between groups in general were relatively small.

4. Discussion

The chosen study design of continuously exposing caries lesions to solutions of (near) constant composition is common practice in the investigation of de- and/or or remineralization processes,[14,18,19] although it is, admittedly, somewhat removed from the in vivo situation, where de- and remineralization periods alternate frequently. However, these "simplistic" models allow for a better mechanistic understanding of certain facets in the caries process and are therefore justifiable. Lesion protocols were chosen based on preliminary studies. MeC have been employed in many previous studies,[4,16] whereas CMC and SOLN were chosen to represent lesions with more pronounced although similar surface layers, yet different degrees of lesion body mineralization (Table 2). While there is no consensus among researchers with regards to what constitutes a suitable in vitro caries lesion, the three chosen protocols are representative of the broad spectrum of available laboratory lesions.

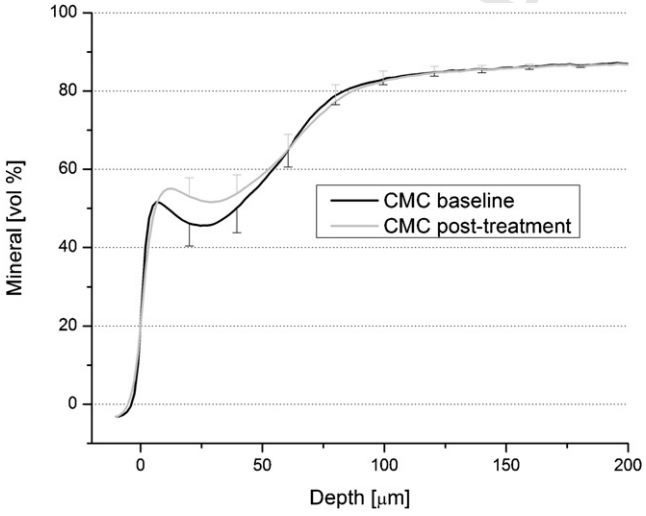

Fig. 3 – Average mineral distribution for CMC lesions at baseline and post-treatment. For better clarity, error bars (standard deviations) are given at 20-μm intervals.

The results of the present study, with respect to the high-R lesion group, MeC, are somewhat different compared to two previous studies on this lesion type – a QLF study[20] employing almost identical PF formulations/solutions, and a TMR study[18] using MeC-type acid gels as PF surrogates. In the previous studies, remineralization was observed in the presence of F, and in the study by Lynch et al.[18] even at lower pH, [Ca] and [Pi]; i.e. at lower DS_{HA} and DS_{FA}. In the present study, however, MeC showed demineralization. The reason for this discrepancy is not clear, especially considering that all PF were supersaturated with respect to HA and FA. Differences in the respective solubility of the bovine hard tissue samples employed may perhaps explain this discrepancy. It is worth noting that in the present study no lamination of lesions was observed (regardless of lesion type), which is in stark contrast to the study by Lynch et al.[18] According to Driessens et al.,[21,22] laminations do not result from reprecipitation of CaPi's of lesser solubility within the lesion. They do occur, however, because the mineral is protected from local dissolution early in the process. Therefore, the lack of laminations observed in the present study may indicate that the bovine enamel substrate of the present study is inherently more soluble

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than the enamel of the earlier, aforementioned studies and therefore behaved differently. This may explain the shift towards net demineralization for MeC in the present study. However, one further explanation for the lack of lamination observed in the present study could be simply that the PF pH was too high at 5.5[23] (Lynch et al.[18] studied in the pH range 4.8–5.2). In this case, no explanation for the observed discrepancy can be provided. It must also be mentioned that laminations are more likely to occur when lesions are ''cycled'' through alternating de- and remineralization periods.[e.g.24]

The results for CMC and SOLN (both low-R lesions) in comparison with MeC are intriguing, as the low-R lesions tended to remineralize, therefore showing opposite behaviour compared with MeC. Strictly speaking, SOLN cannot be compared with CMC and MeC as differences in ΔZ_{base} existed which were shown by various investigators[3,4] to predispose lesions to their subsequent de-/remineralization behaviour. Regardless, MeC and CMC results are in disagreement with a recent in situ study,[6] reporting that under virtually identical conditions and in the presence of F, high-R lesions remineralize and low-R lesions further demineralize. Furthermore, Lynch et al.[5] reported that high-R lesions are more responsive to remineralizing treatments than low-R lesions because of the high-R lesions' greater porosity. High- and low-R lesions were somewhat comparable between all studies, although different protocols were used for low-R lesion creation in the present study, which is unlikely to be accountable for the observed discrepancy. Again, no clear explanation can be provided, as previous studies and the current knowledge of the relative importance of various lesion parameters would have predicted opposite results. To the author's knowledge, the present study was the first in vitro study comparing the response of low- and high-R lesions to PF, clearly showing that further research is needed in this area, as at present, a discrepancy between in situ and in vitro data is evident.

Two further important aspects must be noted (a) the overall change in $\Delta Z/\Delta\Delta Z$ was relatively low (max. 10%, observed for MeC) and (b) the present study has been conducted on bovine enamel, whereas the discussed in situ study was conducted on human enamel. With respect to (b), it is very unlikely that the choice of substrate influenced the outcome of the study as bovine and human enamel have been shown to behave similarly qualitatively but not necessarily quantitatively in many previous studies.[4,25] While differences can be expected, they are most likely only of a quantitative nature. Nonetheless, extrapolation to human enamel is by no means straightforward and has to be viewed with caution, especially considering the absence of comparable in situ data on the two tissue substrates. With respect to (a), these relatively small changes (which were also observed for the Sr:Ca ratio data which will be discussed later) may potentially give rise to over-interpretation of the data. However, these differences were of statistical significance for MeC, and further significant changes in lesion parameters and mineral distribution were observed, especially for CMC and SOLN. With respect to SOLN, it appears that the lesion surface underwent demineralization (in contradiction to CMC), whereas the lesion body remineralized. As can be seen in Fig. 1, the addition of carboxymethylcellulose to the partially saturated lactic acid solution did not also reduce the amount of demineralization at baseline (in comparison to SOLN), it also produced lesions with a wider surface zone and a more mineralized lesion body, suggesting that the carboxymethylcellulose acts a ''barrier'', capable of retarding the lesion outward flow of Ca and Pi ions, which in turn can concentrate near the lesion surface and (re)precipitate again. As SOLN were slightly more mineralized at the surface but had a narrower surface zone than CMC, SOLN may have been more prone for net de- rather than remineralization at the surface. This is also highlighted by the fact that the lesion surface zone move inwards for both CMC and SOLN, but not for MeC, which were considerably less mineralized at baseline and therefore offered less mineral per se for further demineralization, and likely also mineral that was inherently less soluble.

The results for the various Sr:Ca ratios in PF tested are in line with the data reported on potential caries preventative effects of Sr, as the partial substitution of Sr for Ca at a 1:3 molar ratio proved beneficial in retarding further demineralization and promoting remineralization of early caries lesions in comparison with the Sr-free, Ca only control. However, a further increase in the Sr:Ca ratio did only provide numerical benefits vs. Ca alone. While Sr incorporation into the apatite lattice causes an expansion in both *a*- and *c*-axes[26] due to Sr's larger atomic radius,[27] leading to higher apatite solubility,[12] Sr has also the ability to stabilize apatite pre-cursor phases, such as OCP.[28] Furthermore, Pan et al.[13] postulated that nucleation of Sr-substituted apatite is easier than that of apatite, and that this may act as a template for apatite growth. This would explain the results of Thuy et al.,[14] who demonstrated enhanced in vitro remineralization of caries lesions in the presence of Sr and F compared to F alone. In addition, Nelson et al.[29] reported that Sr can at least partially offset the paracrystalline disorder in apatite induced by carbonate, and Sr and F in combination were shown to improve the crystallinity of carbonated apatite to a greater extent than by Sr or F alone, suggesting synergistic effects between Sr and F in low-carbonated apatite, which is very similar to enamel.[30] Featherstone et al.[31] postulated that Sr is incorporated into Ca-deficient areas of enamel, which were related to carbonate inclusion. Therefore, the results of the present study are in general agreement with the aforementioned literature. The earlier proposed[7] ''optimum [Sr]'' will, however, not be entertained as the present study was limited to one PF composition. Further work employing a wider pH range (4.97–5.45 has been reported for plaque fluid)[32,33] and different [Ca], [P] and [F] would be required to draw such conclusions.

Structure, porosity and inherent solubility of caries lesions are important factors that determine the lesion's behaviour to de- and or remineralization challenges,[34,35] which was elaborated on very recently.[6] During lesion formation, there is preferential dissolution of the least stable fractions, especially those associated with the impurities Mg and CO_3.[36] Within the developing lesion, this can lead to levels of supersaturation which in turn will allow (re)precipitation of mineral phases different to the original enamel mineral and possibly close to the lesion-demineralization medium interface. These mineral phases, in turn, can affect secondary de- and remineralization processes. Therefore, differences in chemical composition in addition to structural differences between lesions may explain the results of the present study.

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It should also be mentioned here that a comparison between artificial caries lesions (as employed in the present study) and natural white spot lesions is not straightforward. The latter contain considerably more protein (see below), form over a substantially longer period of time (assuming similar severity) and are subject to appreciably higher fluctuations in pH and F, Ca and Pi concentrations during their development whereas laboratory lesions are not. Likewise, differences in pore structure between and within natural and artificial caries lesions (this was not investigated in the present study) may exist depending on the rate of lesion formation,[37] thereby further complicating the matter. Our understanding of these properties, processes and consequences, however, is rather poor, as highlighted recently,[6] warranting further research.

One further aspect, which is rarely considered, is the role of organic material in the caries process. Previous studies[38,39] were able to determine the relative distribution of proteins (predominantly salivary amylase and albumin) in various zones of white spot lesions, and albumin in particular has been postulated to affect both de- (favourably) and remineralization (adversely).[40,41] Apart from exogenous proteins, endogenous proteins need to be mentioned and in particular insoluble enamel tuft proteins. These proteins are present in both human and bovine enamel and were found to be predominantly associated with the dentine–enamel junction. They are, however, also found throughout the enamel and may constitute a large part of the interprismatic organic material.[42] As an initial caries attack will result mainly in mineral loss from interprismatic areas and from the prism peripheries,[43] these tuft proteins may control diffusion as well as reprecipitation of mineral phases from ions dissolved at the advancing front, thereby impacting mineral distribution within the lesion. However, the endogenous organic content of lesions is unlikely to affect the overall caries process to the same extent exogenous proteins do, although comparative and or mechanistic studies in general are sparse. Furthermore, salivary or plaque-derived proteins may also play a role in lesion arrest. Their importance in lesion surface layer formation has been reported,[44] and it is very likely their accumulation in caries lesions and especially near the surface can lead to a reduction in pore size and volume, thereby controlling inward and outward diffusion of ions and ultimately resulting in lesion arrest.[45] Nonetheless, this area in general warrants further, clarifying research.

In general, it would have been beneficial to extend the experimental period beyond seven days, as differences between lesion types and Sr:Ca ratios may have been more pronounced. However, the extrapolation of in vitro data to the clinical situation is not straightforward as seen in the present study. Furthermore, an extension of the treatment period until considerably bigger differences could have been observed would have further limited the physiological relevance and therefore, the present results may be accurate to the extent that partial Sr for Ca substitution in PF affects de- and remineralization processes, but only to a relatively small extent.

Clearly, more research is needed to further our understanding of the impact of various lesion parameters and potential anti-cariogenic benefits of agents other than F. In conclusion, high-R lesions are more prone to demineralize under PF-like conditions than low-R lesions under the conditions of the study. In addition, partial Sr for Ca substitution in PF was shown to enhance remineralization and to retard further demineralization of early caries lesions.

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Conflict of interest statement

The author declares that there is no conflict of interest.

Ethical approval

Not required.

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