Application of cerium chloride to improve the acid resistance of dentine

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Abstract

OBJECTIVE: To investigate the effect of cerium chloride, cerium chloride/fluoride and fluoride application on calcium release during erosion of treated dentine. METHODS: Forty dentine samples were prepared from human premolars and randomly assigned to four groups (1-4). Samples were treated twice a day for 5 days, 30s each, with the following solutions: group 1 placebo, group 2 fluoride (Elmex fluid), group 3 cerium chloride and group 4 combined fluoride and cerium chloride. For the determination of acid resistance, the samples were consecutively eroded six times for 5 min with lactic acid (pH 3.0) and the calcium release in the acid was determined. Furthermore, six additional samples per group were prepared and used for EDS analysis. SEM pictures of these samples of each group were also captured. RESULTS: Samples of group 1 presented the highest calcium release when compared with the samples of groups 2-4. The highest acid resistance was observed for group 2. Calcium release in group 3 was similar to that of group 4 for the first two erosive attacks, after which calcium release in group 4 was lower than that of group 3. Generally, the SEM pictures showed a surface coating for groups 2-4. No deposits were observed in group 1. CONCLUSION: Although fluoride showed the best protective effect, cerium chloride was also able to reduce the acid susceptibility of dentine significantly, which merits further investigation.
Application of cerium chloride to improve the acid resistance of dentine

**Running title:** Cerium chloride to prevent calcium loss

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**Key words:** dentine, fluoride, cerium chloride, acid, erosion
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Introduction:

Loss of dental hard tissues due to caries has declined over the last decades\textsuperscript{1}. However, other causes of dental hard tissue loss or disintegration have entered into the focus of dentistry, e.g. erosion\textsuperscript{2}. Dental erosion is defined as tooth wear due to chemical dissolution of dental hard tissue not involving bacteria\textsuperscript{3}. As implied by this definition, acids or acidic metabolites play a major role in dental hard tissue loss for both disease entities.

For the prevention of caries, it is evident that fluoride promotes remineralization, inhibits demineralization of dental hard tissues and the use of fluorides in different forms and different application modes has caused a reduction in the prevalence of dental hard tissue loss due to caries over the last decades\textsuperscript{1,4-6}.

In 1977, Davis and Winter\textsuperscript{7} also reported that erosive tooth wear could be reduced by the use of fluoridated tooth pastes when applied before an erosive challenge. Since then numerous other in vitro and in situ studies have been performed to examine erosive tooth wear and its prevention, especially focussing on enamel\textsuperscript{8-13}. Studies dealing with dentine as a substrate are scarce\textsuperscript{14,15}.

Fluoride shows a protective effect against caries and erosion, but negative side effects such as fluorosis and toxicity have been reported\textsuperscript{16,17}. To avoid these negative side effects, Zhang et al. (1999) tested the use of lanthanides solutions and combinations of lanthanides with sodium fluoride solutions for the prevention of carious-like lesion development\textsuperscript{18}. The toxicity of rare earth elements such as lanthanum and cerium is lower than that of fluoride and they show a lower tendency to accumulate in the liver, kidney and brain\textsuperscript{19}. It was shown that the protective effects of the different lanthanides solutions were comparable with those of fluoride solutions\textsuperscript{18}. Furthermore, on cementum, the combination of lanthanides solutions with fluoride...
solution showed higher acid resistance compared to fluoride-only solution. The effect of cerium compounds on the demineralisation process is not known.

This in vitro study was designed to determine the effect of a cerium solution and combined cerium/fluoride solution on the prevention of mineral loss in dentine during acidic attacks. Furthermore, the presumed protective effect would be compared to the known protection of a commercial fluoride solution. The hypothesis of this study, in consideration of the results of Zhang et al. (1999)\textsuperscript{18}, was that the protective effect of a cerium solution against acidic attacks would be equal to that of a fluoride solution and that the combination of fluoride and cerium solution would show even better protection than the fluoride solution alone.

**Materials and Methods:**

**Sample preparation**

For the study, 40 dentine samples were prepared from ten human premolars that were extracted for orthodontic reasons and assigned to four experimental groups (1-4). Teeth were sectioned at the cementum-enamel junction with a water-cooled diamond disc and the pulp tissue was removed from the roots with endodontic files. The roots surfaces were cleaned by scaling and flattened for 2 min with Sof-Lex™ polishing discs (3 - 9 μm grit, 3M Espe, 3M AG, Rüschlikon, Switzerland) and water as coolant. The root surfaces were checked under an anatomic microscope to ensure they were free of cementum. From each root, four dentine samples were harvested by drilling with a water-cooled diamond trephine mill. The trephine mill had an inner diameter of 5 mm. From each root, two cylinders were harvested from the buccal and two from the palatal root surface, respectively. The first cylinder was harvested 1 mm apical from the cemento enamel junction, the second apical from the first one. The thickness of the cylinders was around 5 mm. The dentine cylinders were embedded
in acrylic resin. One sample of each root was assigned to one of the four experimental groups 1-4 (n=10 per group). The samples were stored under moist conditions (100% humidity) until use but maximum for 10 days.

**Study design and treatment**

The placebo solution for group 1 was prepared by mixing 0.10 g sodium benzoate with 99.90 g distilled water. For group 2, a commercially available amine (9250 ppm Olaflur and 750 ppm Dectaflur) fluoride solution (Elmex fluid, GABA International AG, Therwil, Switzerland) was used (pH 3.9). The cerium chloride solution in group 3 was composed of 10.00 g Cer(III)chlorid, 0.10 g sodium benzoate and 89.90 g distilled water (pH 4.94). The samples in group 4 were first treated with the group 2 amine fluoride solution (Elmex fluid) and then immediate application of the group 3 cerium chloride solution.

The individual solutions (groups 1 – 3) or solution combination (group 4) were applied to the dentine samples for 30 s under constant motion. After a 30 s pause, the respective solution was again applied for another 30 s. The samples were rinsed with distilled water to remove excess solution after the 60 s treatment time. The whole procedure was run once a day for 5 days. The samples were stored under moist conditions (100% humidity) between the application times. The application protocol of each solution based on a preliminary study (unpublished data).

**Determination of acid resistance**

For determination of the acid resistance of the dentine samples, the calcium released into the acid during an erosive attack (EA) was measured. For this determination 120 µl of lactic acid (pH 3.0) being applied for 5 min on each sample. After 5 min the acid was removed and new acid was applied. The acid was renewed five times so that the
Dentine was under erosive attack for 30 min in total. The acid used in each 5 min attack was collected and mixed with the same amount of water and strontium chloride (0.25%). Strontium chloride was added to mask the phosphate dissolved in the acid that might otherwise falsify the following measurement of calcium by atomic absorption spectroscopy (2380 Atomic Absorption Spectrophotometer, Perkin-Elmer, Schwerzenbach, Switzerland). Measurement was performed at 422.7 nm.

**EDS analysis and scanning electron microscopy**

To evaluate possible atomic interactions of the different solutions with the human dentine, X-ray energy-dispersive spectroscopy (EDS) analysis of the dentine surface was performed (SUPRA 50 VP and Genesis, Carl Zeiss GmbH, Oberkochen, Germany). For each group, an additional six dentine samples were prepared and treated with the respective solutions. Directly after application of the solutions, the samples were desiccated for 4 weeks in blue silica gel (Silica gel blue, Fluka Analytical, Buchs, Switzerland). On each sample, three areas of 200 x 200 µm were measured (15 kV, 100 s)\(^20\). The weight percentage of calcium, cerium, phosphor and fluoride were analysed stoichiometrically.

Furthermore, scanning electron microscopy pictures were taken of the dentine samples of each group after application of the respective solutions to determine if there was any kind of crystallisation or precipitation of the solutions with or on the dentine using a Supra 50 VP Scanning Electron Microscope (Carl Zeiss NTS, Oberkochen, Germany). Samples were treated as described above and sputter coated with gold before examination. SEM pictures were captured at a magnification of 10000x and an acceleration voltage of 5 kV.
Data presentation and statistical analysis of calcium release

For data presentation, the mean value and standard deviation of calcium in each 5 min acid fraction was calculated. Data analysis was performed using one-way ANOVA and Scheffe’s post hoc tests. Significance level was set at 95%.

Data presentation and statistical analysis of EDS analysis

For data presentation, the median and IQR of weight percentages of calcium, cerium, phosphor and fluoride were calculated. Data analysis was performed separately for each element using non-parametric one-way ANOVA and Mann-Whitney-U-tests as the standard deviation in the groups were significantly different. Significance level was set at p < 0.05.

Results:

Calcium release into the acid

The amount of calcium released into the lactic acid during each of the six erosive attacks (EA 1 – EA 6) for the four treatment groups is given in Table 1.

Statistically significant highest amounts of calcium released in the acid during each erosive attack (EA 1 – EA 6) were observed for the samples treated with the placebo solution (group 1). The amount of calcium released into the acid decreased statistically significant from EA 1 (6.6 ± 0.5 μg) and EA 2 (6.6 ± 0.5 μg) to EA 6 (5.6 ± 0.6 μg) in this group (p = 0.0365 and 0.0218, respectively).

The amounts of calcium released in the group treated with fluoride (group 2) were significantly lower during all erosive attacks compared in the respective amounts in all the other groups. For the six erosive attacks (EA 1 – EA 6) the amount of calcium released ranged from 0.3 ± 0.1 μg – 0.2 ± 0.0 μg, in group 2, which was not statistically significant different.
In group 3 (cerium chloride solution) the amount of calcium released in the acid increased with statistical significance from $0.8 \pm 0.3 \mu g$ for EA 1 to $2.6 \pm 1.0 \mu g$ for EA 3 ($p = 0.0008$) to $4.7 \pm 0.7 \mu g$ for EA 6 ($p < 0.0001$). During EA 1 and EA 2 the amounts of calcium released from the samples treated with the cerium chloride solution (group 3) showed no statistically significant difference ($p = 0.2268$ and $0.1781$) compared to the respective amounts of group 4 (fluoride/cerium chloride solution). Starting with EA 3, the amount of calcium released during the erosive attack was higher compared to that of group 4 (fluoride/cerium chloride solution).

For the samples treated with the fluoride/cerium chloride solution (group 4), a constant release of calcium ($1.1 \pm 0.2 \mu g$ - $1.5 \pm 0.4 \mu g$) in the acid could be observed during all erosive attacks (EA 1 - EA6). The calcium released during EA 1 - EA 6 of group 4 was significantly lower compared to the respective amounts of group 1 (placebo solution), but significantly higher compared with the respective amounts of group 2 (fluoride group).

**EDS analysis**

Results of EDS analysis are given in Table 2.

Highest weight percentage of surface phosphor was observed for the samples treated with the placebo solution (group 1). This was not significantly higher than after treatment with the cerium chloride solution (group 3) ($p = 0.9393$). Surface phosphor was significantly lower after treatment with fluoride (group 2) and fluoride/cerium chloride solution (group 4) as compared to samples treated with the placebo solution (group 1) ($p = 0.0015$ and $p < 0.0001$).

In group 4 (fluoride/cerium chloride solution) calcium content was significantly lower than in all other groups (groups 1 – 3). Weight percentages of calcium in groups 1 – 3 showed no statistically significantly difference.
Lowest surface fluoride was observed after treatment with the placebo solution (group 1) and the cerium chloride solution (group 3). The values of these two groups showed no statistically significantly difference ($p = 0.9885$). After treatment with the fluoride solution (group 2), the highest fluoride value was observed. The value of surface fluoride after treatment with fluoride/cerium chloride solution combination (group 4) was significantly lower compared to group 2 (fluoride group). In contrast, the fluoride value of group 4 (fluoride/cerium chloride solution combination) was higher compared to the values of groups 1 (placebo solution) and 3 (cerium chloride solution) ($p < 0.0001$ and $p < 0.0001$, respectively).

For surface cerium, the highest value was observed after treatment with the fluoride/cerium chloride solution combination (group 4). The values of surface cerium were not significantly different for the groups 1 – 3 (placebo solution, fluoride solution and cerium chloride solution).

**Scanning electron microscopy**

SEM pictures of all groups after application of the respective solutions are given in Figure 1.

Samples from groups 2 – 4 (fluoride-, cerium chloride- and fluoride/cerium chloride solution) showed a more or less pronounced smear layer, which could also be interpreted as coating or precipitation of cerium and/or fluoride compounds interacting with dentine on the surface. No such surface changes were observed for the samples in group 1 (placebo solution). The picture after placebo treatment showed the most distinct exposition of collagen fibres. These structures were less visible in the other representative images.

**Discussion:**
In the present study, dentine samples were prepared from human premolar roots. Various other studies concerning dentine erosion have used bovine dentine\textsuperscript{14,21,22}. An advantage of using bovine dentine is that it is easier to obtain a sufficient number of sound bovine teeth instead of human teeth\textsuperscript{23}. However, wear due to erosion is higher for human dentine\textsuperscript{24} so that human root dentine was used in the present study. Zhang et al. (1999)\textsuperscript{18} used dentine samples with intact cementum to test the preventive effect of lanthanides on root surface carious-like lesions. In the present study, the cementum was removed to replicate clinical relevance since the cementum of teeth with gingival recessions is lost to daily toothbrushing or dental professional activities like scaling, resulting in a cementum-free dentine surface\textsuperscript{25,26}.

In contrast to other investigations concerning erosion and prevention of erosion\textsuperscript{27-29}, no demineralization/remineralisation cycling with erosive agent and remineralisation agent, such as artificial or human saliva, was performed. The present study was performed as a primary study testing the possible preventive effect of cerium against dentine erosion. For the same reason, no simulation of dentine fluid pressure was performed.

In the present study, tooth wear due to erosion was determined by measuring the amount of calcium in the acid of the acidic attack. In an overwhelming number of studies\textsuperscript{30}, the measurement of erosive tooth wear has been performed by contact or optical profilometry\textsuperscript{31,32}. Measuring erosive tooth wear of dentine by profilometry is critical due to the remaining collagen matrix after erosion\textsuperscript{30}. This collagen matrix leads to an underestimation of the dentine wear because optical profilometry cannot differentiate between demineralised organic matrix and mineralised dentine. The stylus of contact profilometry will penetrate the organic matrix to an unknown depth. To avoid false reading, Ganss et al. (2007)\textsuperscript{30} suggested removing the organic matrix with collagenase. As the measurement of the calcium dissolved in the acid of the
acidic attack has been determined to be a sufficient method for the determination of mineral loss during erosion\textsuperscript{33} and has been used in various other studies\textsuperscript{13,14}, this method was also employed in the present study. The hypothesis of the present study had to be partially rejected. In contrast to the findings by Zhang et al. (1999)\textsuperscript{18} the protective effect of a fluoride solution was statistically significantly better than that of a cerium solution and the fluoride/cerium combination. This might be due to the different concentration of the fluoride solution used in the present study as compared to Zhang et al. (1999)\textsuperscript{18}. Zhang et al. (1999)\textsuperscript{18} used a 500 ppm sodium fluoride solution, while the concentration of the commercially available fluoride solution Elmex fluid in the present study was 10000 ppm. This higher fluoride concentration in the present study may have led to a better protective effect for the fluoride solution. While the cerium solution provided less protection on a relative scale, it still displayed a significant protective effect. In accordance with the findings by Zhang et al. (1999)\textsuperscript{18} the protective effect of the cerium chloride/fluoride solution combination was better than that of the cerium solution alone. The protective effect over all six erosive attacks of the fluoride solution was much better than that of the cerium solution. This might be hypothesized to be attributed to the penetration of the fluoride solution into the dentine through the dentine tubules while the penetration of the cerium solution was less pronounced. It was hypothesized, that the protective effect of the fluoride solution/ cerium solution combination would be as good as that of a fluoride solution alone, but in the present study this was not the case. The reason for this finding might be a rapid crystallisation of the cerium solution with the fluoride solution occurring on the dentine surface, hampering the penetration of the previously applied fluoride solution into the dentine. The EDS analysis shows a lower amount of surface fluoride after treatment with fluoride solution/cerium solution.
combination compared to the surface of samples treated only with the fluoride solution.

The mechanism for the protective effect of cerium against erosion can be found in the crystal structure of hydroxyapatite and its derivates found after cerium application. Due to the similar atomic radius of calcium and cerium and the higher electric charge valence of cerium, a replacement of calcium by cerium in the hydroxyapatite is conceivable. This assumption was verified by the EDS analysis performed in the present study. The ionic radii and the electric charge valence influence the stability of apatite and so the hydroxyapatite with cerium replacing calcium has a more stable crystal structure and a greater acid resistance due to the higher electric charge valence of the cerium.

The SEM images correlated well with the calcium release testing in the present study, showing a coating on the dentine surface for all three test groups and resulting in reduced calcium release during an erosive attack as compared to the placebo group, which showed no coating on the dentine surface. Whether these deposits are stable against mechanical challenges was not investigated in this evaluation, and should be determined in future experiments.

**Conclusion:**

By the results of the present study it can be concluded that the protective effect of cerium chloride alone is limited but the combined application of fluoride solution followed by a cerium chloride solution demonstrated quite promising results although distinctive protective effect was observed after fluoride application only. To substantiate this finding, further studies within a clinical framework should be performed.
References:


Figure Captions:

Tab. 1: Amount of calcium [µg] in the lactic acid of the six consecutive erosive attacks (EA 1 – EA 6) in the four treatment groups (Mean ± SD). Values of calcium within one treatment group for EA 1 – EA 6 that are not statistically significant different are marked with same capital letters (read horizontally). Values of calcium for the different treatment groups within the same erosive attack that are not different are marked with same lowercase letters (read vertically).

Tab. 2: Median of weight percentage [% Wt] of calcium, cerium, phosphor and fluoride in the dentine surface of the different groups (IQR) after application of the respective solutions. Comparisons are made within the elements (read vertically). Values that are not statistically significant different are marked with same lowercase letters.

Fig. 1 SEM pictures of dentine samples after treatment with the respective solutions.
Fig. 1
|                      | EA 1     | EA 2     | EA 3     | EA 4     | EA 5     | EA 6     | Mean  
|----------------------|----------|----------|----------|----------|----------|----------|-------
| **Placebo**          | 6.6 ± 0.5 | 6.6 ± 0.5 | 6.3 ± 0.6 | 6.1 ± 0.6 | 6.0 ± 0.7 | 5.6 ± 0.6 | 6.2 ± 0.4 |
|                      | a        | a        | AB a     | AB a     | AB a     | B a      | a     |
| **Fluoride**         | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 |
|                      | A b      | A b      | A b      | A b      | A b      | A b      | b     |
| **CeCl₃**            | 0.8 ± 0.3 | 1.6 ± 0.7 | 2.6 ± 1.0 | 3.6 ± 1.0 | 4.2 ± 0.9 | 4.7 ± 0.7 | 2.9 ± 1.5 |
|                      | A c      | AB c     | BC c     | CD c     | D c      | D c      | c     |
| **Fluoride + CeCl₃**  | 1.1 ± 0.2 | 1.1 ± 0.3 | 1.2 ± 0.3 | 1.3 ± 0.4 | 1.5 ± 0.4 | 1.5 ± 0.4 | 1.3 ± 0.2 |
|                      | A c      | A c      | A d      | A d      | A d      | A d      | d     |

Tab.1:
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<th>P</th>
<th>Ca</th>
<th>F</th>
<th>Ce</th>
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<td><strong>Placebo</strong></td>
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<td>40.41 (6.34)</td>
<td>0.02 (0.54)</td>
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<tr>
<td><strong>Fluoride</strong></td>
<td>13.17 (4.20)</td>
<td>35.66 (8.25)</td>
<td>20.66 (11.42)</td>
<td>0.01 (0.24)</td>
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<tr>
<td><strong>CeCl₃</strong></td>
<td>16.65 (1.11)</td>
<td>34.39 (4.54)</td>
<td>1.28 (0.89)</td>
<td>8.43 (5.64)</td>
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<tr>
<td><strong>Fluoride + CeCl₃</strong></td>
<td>11.45 (7.95)</td>
<td>26.21 (28.60)</td>
<td>12.06 (9.51)</td>
<td>12.96 (41.46)</td>
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Tab. 2: