

for either 4 or 6 days. They were then treated with remineralizing solutions for either 2 or 3 weeks at pH 7.0, 37°C. The level of remineralization was determined using contact microradiography. A 4-way analysis of variance (ANOVA) was used to evaluate significant differences among samples. The results showed that: (1) the contribution of demineralizing agent on subsequent remineralization was 29.6% to lesion depth (LD) and 22.7% to mineral loss (ΔZ) with a significant difference in both LD and ΔZ ($p < 0.001$) among groups; (2) duration of demineralization contributed 39.2% to the outcome of LD and 37.1% to ΔZ with a significant difference between groups ($p < 0.001$); (3) no significant difference was found with respect to either Ca/P concentration or duration of remineralization. It was concluded that duration and type of demineralizing agent are key factors affecting the outcome of this *in vitro* enamel demineralization and remineralization study.

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Effects of Ozone and Sodium Hypochlorite Treatments on Caries-like Lesions in Dentin

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Ozone is used in industry, medicine and dentistry because of its antimicrobial potential. For caries therapy, an additional beneficial effect of ozone has been proposed [Baysan and Lynch: *Am J Dent* 2004;17:56–60]: remineralization of lesions may be promoted by oxidation of demineralized dentin. The aim of this study was to compare the effects of ozone and another potent oxidizer – sodium hypochlorite (NaOCl) on demineralized dentin and on subsequent remineralization and demineralization of the treated lesions. Bovine dentin was demineralized in methylcellulose gel/lactic acid (pH 5). Part of each lesion was coated with nail varnish for baseline reference. The specimens were exposed for 60 s to ozone gas (HealOzone, KaVo Germany), NaOCl (10%) or water. Then the specimens were remineralized in 20 mM HEPES buffer (pH 7) containing 1.5 mM Ca and 0.9 mM phosphate for 2 or 8 days and subsequently demineralized (lactic acid, pH 5) for 2 days. Mineral content was assessed by transverse microradiography (TMR) in subgroups of samples right after the treatments, or following the subsequent re- and demineralization steps. No difference was found between ozone- and water-treated groups at any time point. After NaOCl treatment, the surface of the samples had ‘moth-eaten’ appearance. If not corrected for this bulk surface loss, NaOCl-treated samples remineralized significantly more than the other two groups. However, if the loss of the surface was included into the results then the beneficial effect of NaOCl on remineralization was lost. Additionally, after being remineralized, the NaOCl group demineralized significantly more than the ozone or water groups. We conclude that ozone does not affect mineral content of demineralized dentin, while concentrated sodium hypochlorite has detrimental effects on demineralized dentin. HealOzone unit provided by KaVo Nederland.

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Low Fluence CO₂ Laser (10.6 μm) Parameters for Caries Prevention

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Although CO₂ laser irradiation can decrease enamel demineralization, thermal damage to the surface is a common side effect. The occurrence of fissures and cracks may compromise *in vivo* application. Therefore, the aim of the present study was to find CO₂ laser (10.6 μm) parameters resulting in maximum caries-preventive effect with the lowest thermal damage. Five low fluences of 0.12, 0.29, 0.39, 0.50 and 0.60 J/cm² combined with high repetition rates of 500, 154, 167, 182, 187 Hz, respectively and 10 μs pulse duration were chosen for the experiments. 78 bovine enamel cubes were divided into 5 laser groups and one control. After treatment the samples were submitted to an 8-day pH-cycling regime. Demineralization was assessed by lesion depth measurements with a polarized-light microscope. The temperature rise at the enamel surface and the propagation into deeper layers were calculated using a finite element model. Surface morphology was evaluated by SEM. All laser groups resulted in statistically significant lower lesion depths than the control group (ANOVA; $p < 0.05$). Morphologically, the two lowest fluences resulted in no surface changes. The calculated temperature rise at 1.5 mm depth was less than 5°C in all groups. In the present *in vitro* study irradiation with 0.12 J/cm², 500 Hz and 2500 pulses of CO₂ laser increased enamel caries resistance without causing undesirable surface damage or excessive temperature rise.

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Effect of Er,Cr:YSGG Laser and Fluoride Application on Enamel Demineralization

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This study evaluated the influence of sub-ablative Er,Cr:YSGG laser and topical fluoride application on incipient caries development *in vitro*. One hundred and sixty human enamel slabs were randomly divided into eight equal groups: (1) untreated (control); (2–4) irradiated with Er,Cr:YSGG laser at 0.25, 0.50 and 0.75 W, respectively; (5) treated with acidulated phosphate fluoride (APF; 1.23% F) for 4 min; (6–8) pre-irradiated with Er,Cr:YSGG laser at 0.25, 0.50 and 0.75 W, respectively and subjected to APF application. All groups were submitted to pH-cycling simulating a cariogenic challenge, and after 10 cycles mineral loss (ΔZ) was measured in enamel. Calcium, inorganic phosphorus (P_i) and fluoride concentrations were also measured in the demineralizing

and remineralizing pH-cycling solutions. Data were analyzed by ANOVA ($\alpha = 0.05$). No differences in ΔZ were found between laser irradiation at 0.25 W, 0.50 W and controls, while after laser irradiation at 0.75 W (Group 4) and application of APF (Group 5) there was a significant decrease in ΔZ . Laser irradiation at 0.50 and 0.75 W prior to APF application there was a significantly lower ΔZ than in other groups. A significant increase in Ca and P_i concentrations was observed in demineralizing solutions compared to the initial concentrations, but no significant changes in Ca and P_i concentrations were detected between any groups. A significant decrease in fluoride concentration was observed in pH-cycling solutions of control and 0.25 W laser groups, while the association of laser at 0.50 and 0.75 W with fluoride promoted an increase of fluoride content in remineralizing solutions. In conclusion, Er,Cr:YSGG laser irradiation at 0.50 and 0.75 W, associated with fluoride, seems to be a good alternative for reducing enamel demineralization.

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Validation of pH-cycling Model of Enamel Demineralization to Determine Dose-response Relationship of Fluoride-releasing Materials

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There are pH-cycling models that allow to determinate the dose-response relationship in fluoride products, but they have not been validated for fluoride-releasing materials. The aim of this study was to validate a pH-cycling model to verify the dose-response relationship for fluoride-releasing materials, using bovine teeth. 60 bovine enamel blocks (4×3 mm) were selected through surface hardness (SH) at different distances from the enamel border (150, 300, 450 and 600 μm). 48 samples were made for the materials: composite resin Z100, Fluroshield, Vitremer and Vitremer $\frac{1}{4}$ diluted. The 12 remaining blocks were used as a control group. The specimens were submitted to a pH-cycling model with high cariogenic challenge. After pH-cycling, SH was again assessed to calculate the percentage change of SH (%SH). Next, enamel F concentration in enamel ($\mu\text{g F}/\text{mm}^3$) and F release to the pH-cycling solution ($\mu\text{g F}$) were measured. Cross-sectional hardness was measured and mineral loss (ΔZ) calculated. The data were analysed by ANOVA and Pearson correlation. The %SH was not significantly different between control and Z100 groups. Lower values were observed for Fluroshield, Vitremer and Vitremer $\frac{1}{4}$ diluted, these groups differing significantly ($p < 0.001$). Highest enamel F concentration and F release were found for Vitremer $\frac{1}{4}$ diluted ($p < 0.001$). There was an inverse relationship between %SH and enamel F concentration ($r = 0.921$), % SH and F release ($r = 0.998$), between ΔZ and enamel F concentration ($r = 0.987$) and ΔZ and F release ($r = 0.997$). A direct relationship was found between F release and enamel F concentration ($r = 0.995$) and also between %SH and the distance from the material ($r = 0.999$). The pH-cycling model allowed the verification of in

vitro dose-response relationship of fluoride-releasing materials, through %SH, fluoride present in enamel and in the pH-cycling solution.

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Monitoring Remineralization in an Artificial Caries Model Using Raman Spectroscopy and OCT: A Pilot Study

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Polarized Raman spectroscopy (PRS) and optical coherence tomography (OCT) are potential new tools for the detection and longitudinal monitoring of tooth surface demineralization and remineralization. Our previous studies showed that these methods could successfully monitor developing lesions in a pilot artificial caries model. The aim of the current study was to evaluate the utility of PRS and OCT for monitoring the reverse process, tooth surface remineralization. Experimental and control windows were created on axial surfaces of four extracted caries-free human premolars. All other tooth surfaces were covered with varnish. Control windows were covered with acid-resistant tape to prevent exposure while in solution. The teeth were subjected to aggressive demineralization solution for 1 day. Thereafter, three samples were placed in remineralization solution for 9 days, with one sample in water as control. Triplicate OCT and PRS measurements were obtained from all windows at days 0 and 1 of demineralization, and days 1, 2, 3, 5, 7, 9 of remineralization. Visual inspection, OCT depth images and PRS data revealed the formation of white spot lesions on experimental windows after demineralization. Visual images and OCT depth measurements from samples in remineralization solution showed inconsistent changes over time. However, Raman depolarization ratio (δ) of the main hydroxyapatite peak showed a progressive decrease with remineralization. At day 0, δ was 0.21 ± 0.06 and increased to 0.62 ± 0.07 after demineralization. Over time in remineralization solution, δ gradually reversed to 0.43 ± 0.10 by day 9. No significant changes were observed from control windows. This study suggests that PRS has potential for longitudinal monitoring of demineralization and remineralization. Improved data analyses of OCT data using optical attenuation coefficients are planned.

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