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Effect of tricalcium silicate (Ca_3SiO_5) bioactive material on reducing enamel demineralization: An *in vitro* pH-cycling study

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ABSTRACT

Objectives: The aim of this study was to investigate the effect of Ca_3SiO_5 on reducing enamel demineralization under pH-cycling conditions.
Methods: Forty bovine enamel samples were treated under four conditions: group 1, double distilled water (negative control); group 2, 1000 ppm F (as NaF, positive control); group 3, Ca_3SiO_5 slurry; and group 4, Ca_3SiO_5–F slurry (Ca_3SiO_5 with 1000 ppm F aq.). All the specimens were treated with treatment materials 4 times each day. Samples in groups 1 and 2 were soaked in test solutions for 2 min and samples in groups 3 and 4 were painted in treatment slurry for 2 min. At times between treatments, they were immersed in citric acid solution 3 times a day and 15 s each time. All the procedures were repeated for 7 days. Knoop microhardness, scanning electron microscopy (SEM), X-ray diffraction (XRD) and atom force microscopy (AFM) were used to examine samples.
Results: After treatment for 7 days, enamels in all the groups were significantly softened. The extents of microhardness reduction were 52.3%, 28.5%, 28.5% and 20.2% for groups 1, 2, 3 and 4, respectively. Samples in the negative control group showed a typical acid etched pattern while enamels in other groups were relatively compact. There was no significant difference between samples treated with Ca_3SiO_5 and F. The combination of Ca_3SiO_5 with F showed the best effect on reducing enamel demineralization.
Conclusions: Ca_3SiO_5 is an effective material against enamel demineralization alone but in combination with F a better anti-demineralization effect may be obtained.

1. Introduction

Dental demineralization is the loss of tooth substance through acidic dissolution. The main acid sources that cause dental demineralization are products of bacteria metabolism and acidic foods or drinks.[1,2] The presence of dental diseases caused by demineralization, such as tooth erosion, tooth sensitivity and tooth wear, is growing steadily,[3–5] probably due to the increase of the consumption of soft drinks.[6] It has been suggested that clinical management of tooth demineralization caused diseases should focus on early detection and prevention before a restorative approach is applied.[5,7] Several materials such as fluoride,[8] casein phosphopeptide amorphous calcium phosphate(CPP-ACP)[9] and *Galla chinensis*[10] have been used to reduce dental demineralization in experimental

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studies and clinical applications. Nowadays, fluoride is still the most effective material to protect enamel against demineralization. The mechanism of the protective effect of fluoride is that it can partially substitute –OH of hydroxyapatite and form fluoride substituted hydroxyapatite which is more stable under acid conditions.[11] Continuing efforts are made by manufacturers to improve fluoride toothpaste by combining micro-calcium carbonate particles into a calcium carbonate/SMFP toothpaste in order to enhance calcium delivery and improve enamel surface hardness.[12–14]

In recent years, bioactive materials including bioactive glasses, glass–ceramics and bioceramics have attracted even more attention as bone substitute materials because of their excellent biocompatibility and bioactivity both *in vitro* and *in vivo*.[15–18] When soaked in simulated body fluid, bioactive materials can induce the formation of a carbonate-containing hydroxyapatite layer, which has similar composition as the main mineral constitute of bone and teeth.[19,20] Apart from the application of bone substitute materials, bioactive materials have also been used in dental research.[21–23] Wang et al.[24] and Bakry et al.[25] reported that bioactive glasses could induce the formation of an apatite layer on dentine which occluded exposed dentine tubules. Dong et al.[26] reported that Ca_3SiO_5, as one of the bioceramics, could be used to occlude exposed dentine tubules. Further study showed that when Ca_3SiO_5 was applied on etched enamel, the remineralization process was enhanced and an apatite layer formed on the enamel surface.[27] It was found by SEM on cross sectioned samples that after soaking in artificial saliva for 1 day, a new apatite layer of about 250–300 nm was formed, and after soaking for 7 days, the apatite layer can reach to 1.7–1.9 μm in thickness. However, it was not clear how effective this newly formed apatite layer induced by Ca_3SiO_5 would be in reducing tooth demineralization under clinically relevant pH-cycling conditions. Compared with conventional fluoride treatment, the protective ability of Ca_3SiO_5 against tooth demineralization under pH-cycling conditions needs to be addressed in a quantitative way.

The aim of this study was to investigate the effect of Ca_3SiO_5 on reducing enamel demineralization by using an *in vitro* pH-cycling process. The microhardness of enamel surface was measured at different pH-cycling time points for all the samples. Double distilled water and 1000 ppm fluoride were employed as negative and positive control, respectively. The null hypothesis was that Ca_3SiO_5 had no significant effect on reducing enamel demineralization.

2. Materials and methods

2.1. Preparation of Ca_3SiO_5 powders

Ca_3SiO_5 was prepared by the sol–gel method according to a previous report.[28] Briefly, 0.5 mol $Si(OC_2H_5)_4$ (Lingfeng, Shanghai, China) and nitric acid as a catalyst were added to 200 mL water under continuous stirring. Then, 1.5 mol $Ca(NO_3)_2{\cdot}4H_2O$ (Shanghai Chemical, Shanghai, China) was added into the solution. The molar ratio of $Ca(NO_3)_2{\cdot}4H_2O/Si(OC_2H_5)_4$ was 3. The solution was kept at 60 °C until gelation occurred. The obtained gel was then dried at 120 °C for 36 h and calcined at 1400 °C for 8 h. The resultant powders were ground and sieved to 300 mesh before use.

2.2. Preparation of enamel samples

Twenty bovine teeths from 3-year-old cows were used in this study. They were cleansed and then stored at 4 °C in water until used. A diamond-coated band saw (SYJ-150; Kejing, Shenyang, China) was used to cut the teeth. After the roots were separated from the crowns, samples were cut longitudinally and each half was cut into sections measuring 6 mm × 6 mm × 3 mm. The labial surfaces of these were polished by silicon carbide paper (Norton a257; Norton, Canada) with different roughness (180, 400, 800, 1200, 1500 and 2500 grit) under water irrigation and then cleaned ultrasonically in distilled water to remove the residue. The polishing procedure removed the outer enamel layer with a thickness of about 150 μm.

2.3. pH-cycling process

The samples were randomly divided into four treatment groups (10 specimens per group): group 1, double distilled water (DDW, negative control); group 2, 1000 ppm fluoride aq. (F, positive control); group 3, Ca_3SiO_5 slurry (Ca_3SiO_5, Ca_3SiO_5 powders were mixed with water with L/P ratio of 3 mL/g); and group 4, Ca_3SiO_5 with 1000 ppm fluoride (Ca_3SiO_5–F, Ca_3SiO_5 powders were mixed with 1000 ppm fluoride with L/P ratio of 3 mL/g). The pH-cycling procedure was as follows: samples from different groups were treated 4 times with the corresponding treatment materials at 9:00, 13:00, 17:00 and 21:00 each day for 2 min each time. Samples in groups 1 and 2 were immersed in 10 mL corresponding test solutions during treatment time. Samples in group 3 were adhered on the bottom of a Petri dish with duplex adhesive tape firstly. After the treatment slurry (10 mL DDW and 3.3 g Ca_3SiO_5 powders mixed together) was poured into the Petri dish, they were painted gently with a soft painting pen for 2 min. The difference between groups 4 and 3 was that 10 mL 1000 ppm F was used instead of DDW. When each treatment was completed, the samples were rinsed with 20 mL distilled water three times. At times between treatments, they were etched with citric acid solution (0.1 M, pH = 1.85) three times each day at 11:00, 15:00 and 19:00 for 15 s each time. During the remaining time the samples were put in four polyethylene bottles which contained 10 mL artificial saliva (pH = 7.0)[29] and kept at 37 °C. After pH-cycling for 7 days, the samples were gently washed with water 3 times and then stored in a fridge (4 °C) before further analysis.

2.4. Microhardness evaluation

Microhardness of samples was measured using a microhardness tester (HX1000-TM; Taiming, Shanghai, China) with a Knoop diamond indenter under 50 g load for 15 s. Five indentations were made per test for each specimen in different regions to avoid residual stress. Knoop microhardness of the samples in all the groups was measured before pH-cycling (baseline surface microhardness), and after pH-cycling for different times (day 1, day 3 and day 7).