**Medical Applications** 

# Microbeam X-ray Fluorescence Analysis of Zinc Concentration in Remineralized Enamel

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## **Introduction**

It has long been said that zinc compounds, such as zinc chloride and zinc citrate are beneficial for teeth, and that zinc oxide inhibits dentine demineralization.<sup>1</sup> However, the mechanisms by which Zn inhibits dentine demineralization are not fully resolved.

The present study was designed to investigate the Zn incorporation and concentration in a remineralized enamel surface. The experiment was performed by synchrotron radiation induced X-rays focused into a small beam spot. The XRF from the sample was detected with a Si(Li) detector. For the purpose of trace element detection, synchrotron radiation analyses bring sub-micron resolution and detection limits of the ppm range. We hypothesized that the distribution and concentration of Zn in mineral deposition could be elucidated using calciumcontaining chewing gum to accelerate the remineralization.

# Materials and methods

Two different types of calcium phosphate-containing xylitol chewing gums on the market were examined with at least one week as a rest period between treatments. One was Xylitol<sup>®</sup> (Lotte Co.; L gum), which contained xylitol, funoran and calcium hydrogen phosphate. The other was Poscam<sup>®</sup> (Glico Co.; G gum), which contained xylitol and calcium phosphorylated oligosaccharide. For the pellet gum study, the subjects set the appliance in the mouth chewed for 10 minutes and the appliances were retained for another 10 minutes in a subject's mouth; 5 times daily for seven days (sufficient for quantitative comparison using energy dispersive X-ray microanalysis (EDX)).<sup>2</sup> This *in situ* study was carried out by the double blind method.

The experiment was performed on a beam line (BL)-4A (the station for XRF analyses) at the Photon Factory, High Energy Acceleration Research organization (KEK) in Tsukuba, Japan. Synchrotron radiation from a bending magnet was monochromatized using a synthetic multiplayer monochromator and X-rays were focused into a small beam spot of about 4  $\mu$ m diameter using a Ptcoated ellipsoidal mirror. The sample was moved by an xy-z stepping motor (1 mm) while being observed by the optical microscope. The XRF from the sample was detected with a Si(Li) detector (Princeton Gamma-Tech. Inc., Princeton, NJ, USA). X-ray beam energy was tuned to be 14.2 keV to detect Zn. Zn spectrum was analyzed at 6 points, the 10  $\mu$ m step of interval from the superficial layer (0  $\mu$ m deep) to the inner layer (50  $\mu$ m deep) and in 5 files (100  $\mu$ m interval) in each piece. A total of 59 pieces (18 pieces for L gum, 23 pieces for G gum, 4 pieces for control without gum- chewing, 7 pieces for only demineralization and 7 pieces for intact enamel) were measured. The standard reference material was used during the calibration process. The area density of each spot was precisely determined by comparing it to that of a reference thin film.

## **Results and Discussion**

A high concentration of Zn was detected in a superficial area 10  $\mu$ m deep of the sectioned enamel after gum chewing. This concentration increased over that in the intact enamel. The Zn concentration in the remineralized enamel after either L (p<0.01) or G (p<0.05) gum chewing was significantly higher than that in the control group without gum chewing. The average Zn concentration in the remineralized enamel after chewing the two types of gum was not significantly higher than that in the intact enamel. The present experiments<sup>3</sup> suggest that Zn is effectively incorporated into remineralized enamel through the physiological processes of mineral deposition in the oral cavity through gum-chewing.

#### **References**

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