Optimization of calcium concentration of saliva with phosphoryl oligosaccharides of calcium (POs-Ca) for tooth enamel recrystallization in the early stage of caries

Teeth are one of the most important organs. Without teeth, we cannot enjoy healthy life. Once we lose a tooth, it is never again regenerated. Furthermore it leads to reduced nutrient intake, and impairs our nutritional condition and might cause us to become bedridden. Thus one of the most important things in a long-lived society such as Japan is to maintain oral health, especially to retain healthy teeth, to safeguard a good quality of life.

Tooth enamel is chemically vulnerable to acid attack. Therefore after every meal, there is always the risk of tooth enamel dissolution by acid attack. Acidic food causes surface erosion. Acid from dental plaque, which is produced by cariogenic bacteria assimilating sugar, dissolves the subsurface of enamel (demineralization). Our study target is this early stage of caries, because subsurface lesions can be restored by remineralization and reversibly cured to restore healthy tooth enamel, whereas a cavity cannot be cured without surgical procedures. Only the supply of calcium and phosphate ions to saliva at adequate concentrations and optimal molar ratios is necessary to enhance the remineralization of the enamel lesions and the restoration of the microstructure of hydroxyapatite (HAp), the calcium salt that is the main component of enamel.

In a previous study [1], phosphoryl oligosaccharides of calcium (POs-Ca®), which is a highly soluble calcium material, was found to effectively remineralize tooth enamel lesions. It has already been elucidated that POs-Ca provides calcium ions to saliva in a bioavailable form without forming calcium-phosphate precipitates. However, the optimal dose of POs-Ca relative to phosphate ions for the remineralization of enamel lesions remains unknown. Thus, we investigated the optimal dose of POs-Ca for enhancing the remineralization of tooth enamel *in vitro* using transversal microradiography (TMR) [2]. First, POs-Ca added to artificial saliva was confirmed to be soluble in the presence of phosphate ions, whereas a proportion of calcium ions from $CaCl_2$ formed an insoluble salt in the presence of phosphate ions at pH 6.5 (Fig. 1). Therefore, POs-Ca was thought to have unique potential as bioavailable calcium in saliva even under non-acidic conditions. Then, the optimal Ca/P molar ratio was investigated. The greatest degree of enamel remineralization was observed at Ca/P molar ratio = 1.67 (Fig. 2). This is identical to the Ca/P molar ratio of HAp (Ca/P = 1.67). In contrast, an excess amount of POs-Ca (Ca/P molar ratio = 3.0) severely

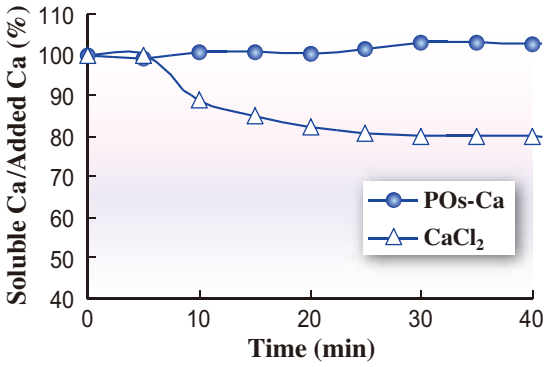

Fig. 1. Time courses of calcium ion concentrations for POs-Ca/$CaCl_2$-containing artificial saliva. The ratio of soluble calcium ions to added calcium was plotted against time from pH adjustment.

reduced the recovery rate of enamel remineralization. This result is consistent with a previous report that a Ca/P molar ratio of 1.67 provides the optimal remineralization rate [3]. An excessive Ca/P molar ratio due to POs-Ca addition might impair mineral exchange between solution and demineralized enamel in terms of kinetics, whereas increasing calcium thermodynamically enhances enamel remineralization.

Next, the effects of two calcium sources, POs-Ca and $CaCl_2$, on enamel remineralization at Ca/P molar ratio = 1.67 were compared. At this ratio, the mineral recovery rate for artificial saliva (AS) containing POs-Ca (24.2 ± 7.4%, n = 5) was significantly higher than that for AS containing $CaCl_2$ (12.5 ± 11.3%, n = 5) (mean ± SD, $p < 0.05$). Treatment with the POs-Ca solution restored the mineral to a depth of 100 μm. Whether free calcium released from POs-Ca itself passes into tooth enamel lesions is not known. However, POs-Ca may pass through the enamel pores because its average molecular weight is ~800, and the calculated molecular length of POs-Ca in the stretched form is < 4 nm [4].

However, TMR results cannot exclude the possibility that random calcium-phosphate precipitation fills the pore of demineralized enamel. Thus, wide-angle X-ray diffraction (WAXRD) analysis at beamline **BL40XU** was used in this study to analyze HAp crystal distributions and orientations. The 6 μm X-ray microbeam was produced by passing the X-ray through a pinhole. The tooth enamel sections were set before the detector. The distance from the sample to the detector was about 150 mm for WAXRD. The sample was moved so that the beam position was moved from surface to base in 5 μm

steps, and serial diffraction images of WAXRD were collected. From the WAXRD images, an arc-shaped spot corresponding to diffraction from the (100) plane of HAp was integrated. The plots of (100) intensity against depth from the surface for enamel remineralized with POs-Ca- and $CaCl_2$ solutions showed profiles (Fig. 3(b)) that indicate the recovery of crystallites. The orientation of the (100) reflection after remineralization was the same as that in healthy tooth enamel (Fig. 3(a)), indicating that the orientation of the regrown crystallites was also the same. Thus, WAXRD showed that the orientations of the HAp crystallites in the remineralized area of enamel treated with either calcium source were identical to those in intact enamel [5]. The recovery rate of HAp crystallites for AS containing POs-Ca (35.7 ± 10.9%, n=11) was also significantly higher than that for AS containing $CaCl_2$ (23.1 ± 13.5%, n=11) ($p<0.05$). These results indicate that most of the calcium and phosphate restored in tooth enamel lesions formed highly organized HAp crystallites, not random HAp crystals or other calcium-phosphate salts, regardless of whether the calcium source in the remineralization solution was POs-Ca or $CaCl_2$. The orientations of HAp crystallites influence the acid resistance and physical strength of the enamel surface, because the chemical nature of the plane perpendicular to the *c*-axis of the HAp, which is usually exposed on the tooth enamel, is different from that of the other planes [6]. Thus, the restoration of demineralized subsurface lesions of tooth enamel HAp implies that the physical and chemical stability of the surface of the lesion is improved by POs-Ca treatment.

WAXRD study confirmed the advantage of POs-Ca compared with other conventional calcium salts in enhancing the recrystallization of enamel and that it may be a beneficial material for maintaining healthy teeth.

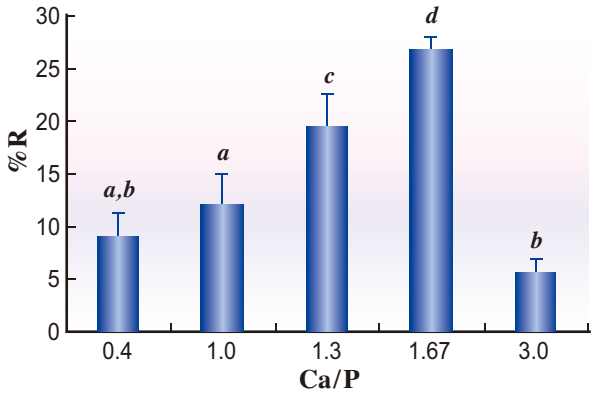

Fig. 2. Recovery rates of enamel treated with 0.4–3.0%-POs-Ca-containing artificial saliva. Histogram and bars represent mean and S.D. of recovery rates from each group, respectively. Each bar with a different letter indicates significant difference ($p < 0.01$, Scheffe's *post hoc* test).

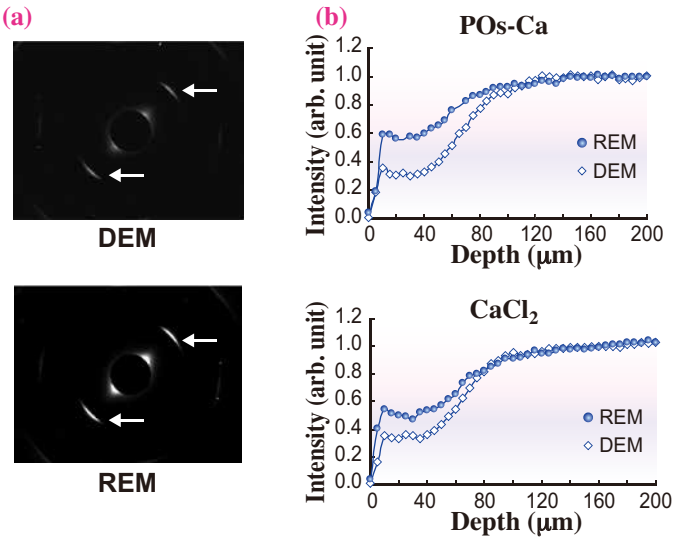

Fig. 3. Wide-angle X-ray diffractions of sound, demineralized, and remineralized zones in enamel treated with POs-Ca and $CaCl_2$. **(a)** Diffraction patterns in the DEM and REM zones at about 30 μm from the surface of POs-Ca-treated enamel. Exposure time was 300 ms. Arrows indicate the (100) equatorial reflections from the HAp crystallites. The direction of the c-axis of the HAp crystal is approximately perpendicular to the enamel surface. **(b)** Plots of (100) intensity against the depth from the enamel surface. The plots are averages of data from five samples from different teeth. Scans were made in six different areas in each of the DEM and REM zones of each enamel sample, and the results were averaged in each zone. To compensate differences in the thickness of the sample, the result from each area was normalized by the average intensity in the depth thickness 100–150 μm.

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