Cariostatic Effect of Hydroxyapatite-containing Dentifrices
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Key words: hydroxyapatite, dentifrice, caries prevention, remineralization, artificial caries lesion

Introduction
Hydroxyapatite is one of the inorganic constituents of biological hard tissue. In medicine, it has commonly been applied in orthopedics in artificial bone and artificial cartilage, while in dentistry, it has widely been used not only in artificial tooth roots for implant-supported prostheses, but also as bone graft material for the restoration of alveolar bone resorbed due to periodontitis; moreover, apatite cement has been developed to restore caries lesions. To date, it has been gaining wide attention as a substitute material for both teeth and bone.

As dental caries starts with the loss of hydroxyapatite, an inorganic constituent of teeth, to repair such early caries lesions with apatite offers the advantage of healing the tooth with the same inorganic component as its original tissue.

Hydroxyapatite has been shown to have the potential to remineralize micro defects in apatite pellets or artificially developed enamel caries lesions and is known to have a high adsorption capacity for glucans and proteins produced by S. mutans. Therefore, apatite, if used in dentifrice, can help remineralize caries lesions and inhibit plaque formation by binding with salivary proteins to enhance the tooth surface cleaning effects of the dentifrice.

Based on such findings, hydroxyapatite can be expected to play an effective role in caries prevention as a dentifrice ingredient, besides the various agents currently added to dentifrice as supplementary ingredients effective for the prevention of caries and periodontal disease.

In this study, the effects of hydroxyapatite-containing dentifrice on caries prevention reported in our previous investigations, as well as those by other researchers, are reviewed and summarized.

I. The effects of hydroxyapatite-containing dentifrice on caries prevention
Two available reports aimed to investigate this effect: a one-year study in elementary schoolchildren by Shimura et al. and a three-year cohort study by the authors.

1. One-year study in elementary schoolchildren
Fourth, fifth, and sixth grade elementary school children (n = 510) participated and half were asked to brush their teeth before school lunch every school day with a dentifrice containing 5% hydroxyapatite. [Note: Of the 510 children participating in the study, 254 used the hydroxyapatite dentifrice and the remaining 256 used an identical dentifrice without hydroxyapatite, as controls.] The schoolchildren received tooth-brushing instructions and oral health guidance once every three months and replaced their toothbrush with a new one roughly once every two months. Their teeth surfaces were examined for caries at baseline and after one year,
the percentage of teeth surfaces affected by caries was investigated. OHI was used to assess the oral hygiene status of the six upper anterior teeth of fifth grade and sixth grade children.

The result showed the percentages of teeth newly affected by caries on the buccal surface in the test group were 3.09% and 7.47%, for previously erupted teeth and newly erupted teeth respectively. [This compared with 4.51% and 8.40% respectively for controls.] On the buccal surfaces, a significant percentage of caries inhibition of 26.42% at the 0.01 significance level, was observed in the test group that used the hydroxyapatite-containing dentifrice for 1 year compared with controls (Table 1). The percentage of proximal teeth surfaces newly affected by caries was very low in both groups and the percentage of caries inhibition of 21.13% for the proximal surfaces in the test group was not significantly different compared with controls (Table 2).

The examinations of oral hygiene status in fifth and sixth grade children revealed a decreased amount of plaque deposit after 1 year from baseline. The decreases in the plaque index per tooth over that 1 year were 0.49 and 0.41 for the test group and the control group respectively, indicating a slightly higher plaque removal performance in the test group.

Shimura et al. suggested the absorption and removal of dextran by hydroxyapatite as the reason for the effect of hydroxyapatite-containing dentifrice on caries prevention but they also considered the possibility of remineralization of the enamel by hydroxyapatite.

2. Cohort study in elementary schoolchildren

A 3-year cohort study was carried out among 181 elementary schoolchildren in fourth grade (92 boys and 89 girls, aged 9 years) (Table 3) who were put through a tooth-brushing regimen using a dentifrice containing or not containing hydroxyapatite after school lunch to evaluate the effect of hydroxyapatite on caries prevention. The test dentifrice used in this study was the same commercially available dentifrice (Apato) as the one used in the investigation by Shimura et al., and it contained 5% synthetic hydroxyapatite and DCPD as an abrasive.

Specifically prepared dentifrices and toothbrushes were provided to all the elementary schoolchildren. The apatite-containing dentifrice was given to 89 children in the apatite group while the same dentifrice without apatite was given to 92 children in the control group. They were instructed to brush their teeth using the dentifrices after every school lunch.

An intraoral examination was carried out in May each year and in March of their final year, for children who were graduating. Effectiveness was determined based on 1) DMFT indices of all erupted teeth and 2) the incidence of the development of new caries during the study period, both of which were evaluated separately for (1) intact teeth at baseline and (2) teeth newly erupted during the study period. $X^2$ test was used to determine the significant differences.

The results obtained were as follows.
1) DMFT indices for all erupted teeth

At baseline, the fourth grade boys in both groups had only a slightly different total number of erupted teeth whereas by the sixth grade, boys in the control group had a larger total number of erupted teeth by about one than those in the test group when examined in March. Girls also showed a similar tendency (Fig. 1, Table 4).

The DMFT indices obtained at baseline for boys in the apatite group and the control group were 2.31 and 1.57, respectively, which increased to an almost identical number after 3 years in both groups (Fig. 1, Table 4). The annual increments of DMFT indices in each of the first two years showed no remarkable difference between the two groups. Over 3 years, however, those of the apatite group and the control group had grown by 0.29 and 0.96, respectively, and were significantly different.

As for girls, the difference in the DMFT indices between the two groups of girls at baseline was small but widened over the years. The annual increments of DMFT indices of girls in the apatite group were significantly smaller than those of the control group and the increment over 3 years was less than half that of the control group (Fig. 1, Table 4). As a result, the percentage of caries inhibition for boys and girls in the test group calculated by means of DMFT indices was 35.86% and 55.93%, respectively.

2) Incidence of newly developed caries during the study period

(1) Intact teeth at baseline

The incidence of newly developed caries for intact teeth at baseline was comparable for boys in both groups while that of girls in the apatite group greatly decreased after only 1 year compared to that of the control group with a statistically significant difference (p < 0.001) and thus the efficacy of the hydroxyapatite toothpaste for caries inhibition was indicated (Fig. 2, Table 5).

(2) Newly erupted teeth during the study period

The number of newly erupted teeth during the study period was almost the same in both boys and girls. During the first year, boys in the apatite group developed no caries, and those in the control group developed only a few. However, 3 years later, the incidence of caries for the control group and the apatite group was 9.16% and 1.56%, respectively, with a statistically significant difference (p < 0.001), and the effect of apatite on caries inhibition was indicated (Fig. 2, Table 6). As was observed in boys, girls in the apatite group developed no caries in the first year. Similarly, the incidence of caries 3 years after the baseline examination was 15.60% and 5.00% for the control group and the apatite group, respectively. The statistically significant difference (p < 0.001) indicated the caries inhibitory effect of brushing with apatite-containing dentifrice.

The conclusions are summarized as follows. Dentifrice containing 5% synthetic hydroxyapatite was used for brushing the teeth after school lunches for three years by fourth grade elementary schoolchildren, and the effect on the prevention of caries was investigated and the following results were obtained.

① Lower DMFT indices for all erupted teeth in the boys’ and the girls’ groups that used the apatite-containing dentifrices were obtained compared with controls, and the percentages of caries inhibition in boys and girls were 35.86% and 55.93%, respectively, which indicated the statistically significant effect of apatite on the prevention of caries.

② The incidence of caries developing in teeth that were intact at baseline was not significantly
different in the boys of both groups, however a significant difference between groups appeared in girls from the first year, and indicated the efficacy of apatite for the prevention of caries.

③ The incidence of caries developing in teeth newly erupted during the study period was lower than that for erupted intact teeth at baseline in both boys and girls, and a statistically significant effect on caries prevention in newly erupted teeth was evident.

All the above conclusions demonstrated the high effectiveness of brushing with apatite-containing dentifrice for the prevention of new caries.

II. The caries prevention mechanism of hydroxyapatite-containing dentifrice

The caries preventive action of hydroxyapatite-containing dentifrice is considered to be attributable to hydroxyapatite’s properties, including (1) filling of micro decalcified lesions in the enamel surface, (2) promoting the remineralization of initial caries lesions, (3) physical polishing of the tooth surface, (4) capacity to adsorb polysaccharides (glucans) produced by S. mutans, and (5) capacity to adsorb proteins. Among those properties, (1), (2), and (3) are actions on the teeth while (4) and (5) are actions inhibiting plaque adhesion.

1. Interaction between microcrystalline hydroxyapatite and apatite ceramic surface

Micro scratches on the enamel surface are believed to promote initial demineralization in caries lesions. Any measure of artificial rapid repair of such micro demineralized lesions might contribute to preventing further progress into evident caries. Based on the concept of artificial repair of micro defects on the enamel surface by filling the large part with hydroxyapatite microparticles and the residual gap with possible deposition of minerals, including Ca++ and HPO₄⁻³ from saliva (microfilling theory) (Fig. 3), Kuboki et al. investigated the amount of hydroxyapatite (HAP) and brushite (BRU) adsorbed on hydroxyapatite pellets after exposing the pellets to slurries of these minerals.

The results showed much more rapid adsorption of HAP microparticles onto HAP pellets than for BRU, as well as adsorption in much greater amounts, increasing to twenty-fold in 5 minutes and to over one-hundred-fold in 40 minutes. Thus, HAP’s potential to fill micro defects in apatite ceramics was indicated.

The researchers also mentioned the necessity for further investigation in future, including chemical and morphological analysis and assessing the effects of a remineralizing solution on micro demineralized lesions prepared by exposure to acids and other agents.

2. Remineralization of artificially produced caries lesions by hydroxyapatite

Following the experimental filling of micro defects with hydroxyapatite, which indicated the possibility of remineralization, the effect of synthetic hydroxyapatite added to a dentifrice on the remineralization of initial caries was investigated in this study.

1) Material and methods (outline)

Four enamel blocks each were prepared from extracted human impacted third molars and enamel windows
3 mm in diameter were created on the surfaces. These specimens were immersed in 50% synthetic hydroxyapatite (HAP) saturated lactate buffer (pH4.5) at 37°C for 72 hours to create artificial caries lesions. Experiments were performed in 3 groups: 1) specimens demineralized and untreated (control group), 2) specimens demineralized and immersed in artificial saliva (saliva group), and 3) specimens demineralized, immersed in a solution of HAP for dentifrice use and then in artificial saliva (HAP group). The HAP solution used in this study was a slurry prepared by mixing synthetic hydroxyapatite with water. The specimens were suspended in the HAP solution at 37°C for 55 hours, then similarly immersed in the artificial saliva at 37°C for 24 hours. The following observations were carried out.

(1) Scanning electron microscope (SEM) observation

According to the conventional method, specimens were dehydrated with ethanol, sputter-coated with a gold layer using an ion sputter (JEC-1100, JEOL), and examined by SEM (JSM-35C, JEOL) at a magnification of 3,000 X.

(2) Observation by polarized light microscopy (PLM) and contact microradiography (CMR)

PLM and CMR examinations were performed to investigate remineralization of artificial caries lesions. According to the conventional method, specimens were dehydrated with ethanol, embedded in a polyester resin (BPS resin, Kyoto Kagaku), sliced perpendicularly to the enamel surface into thin sections (about 80 µm), and observed by polarized light microscopy (POH, Nikon) at a magnification of 25 X. CMR was performed using a soft X-ray apparatus (SRO-M50, SOFRON).

(3) Microfocus X-ray diffractometry

Microfocus X-ray diffractometry was used to identify the substances in areas of artificial caries lesions and to evaluate crystallinity. As previously described, polished thin sections were prepared and a micro Laue pattern was obtained using a microfocus X-ray diffraction unit (Micro Flex, Rigaku) and a microbeam X-ray camera (Microlauecamera, Rigaku). Films were diagrammatically analyzed to identify substances and evaluate crystallinity. For the evaluation of crystallinity, the width of the (310) diffraction line in the direction of the a-axis and the half-width of the (002) diffraction line in the direction of the c-axis were measured.

(4) Analytical electron microscope observation

An electron microscope (JEM-1200EX, JEOL) was used with an accelerating voltage of 25 kV and the Energy Dispersive X-ray Micro Analyzer (TN-2000, Tracor Northern) was connected to obtain SEM cross-sectional images of artificially created caries lesions at a magnification of 800 X, to determine the concentrations of Ca and P by line analysis at a magnification of 800 X, and to calculate the Ca/P molar ratio at each point, by point analysis, at a magnification of 12,000 X. Enamel specimens were prepared as thin sliced sections of about 80 µm, embedded in EPON 812 resin, polished, and sputter-coated with carbon.

2) Experimental results

(1) Scanning electron microscope (SEM) observation

In the control group, the enamel surface of artificial caries lesions was considerably coarse and demineralized compared to the intact surface, whereas in the saliva group considerable deposits on the whole surface were apparent. In the HAP group a larger amount of minute deposits on the enamel surface of caries
lesions was observed, smoothing the enamel surface. (Fig. 4a-d).

(2) Observation by polarized light microscopy (PLM) and contact microradiography (CMR)

The PLM image of the control group showed a coarse demineralized surface layer of enamel as the caries lesion (Fig. 5a). In the corresponding CMR image, thin and indistinct remineralization of the surface layer and subsurface decalcification were observed (Fig. 5d). A thicker layer on the calcified surface was found in the PLM image of the saliva group compared to the control group, which indicated the promotion of remineralization by immersion in artificial saliva (Fig. 5b). Similarly, the CMR image showed an increased thickness of the calcified surface layer (Fig. 5e). The PLM image of the HAP group demonstrated a significantly increased remineralized surface layer compared with the saliva group as well as a reduced decalcified layer (Fig. 5c). Similarly, the thickened remineralized surface layer and reduced decalcified layer were also identified in the CMR image (Fig. 5f).

(3) Microfocus X-ray diffractometry

Substance identification of products in the area of the artificial caries lesions revealed that no other calcium phosphate but hydroxyapatite was present in the artificial caries lesions of each specimen (Fig. 6). An analysis of the crystalline structure in the direction of the a-axis demonstrated lowered crystallinity in all three groups compared to healthy enamel while that in the direction of the c-axis indicated the highest reduced crystallinity in the control group and an increased crystallinity in the HAP group compared to healthy enamel.

(4) Analytical electron microscope observation

① SEM observation of cross-section image of artificial caries lesions

A coarse surface appearance was observed in the control group. Considerable restoration at a deeper level was shown in the saliva group, and further restoration caused by advancing remineralization was suggested by the indistinguishable margin of the lesion in the HAP group (Fig. 7).

② Ca and P concentrations by linear analysis

Linear analysis (qualitative analysis) of CaKα and PKα was performed along the white line in the center of the cross-section image of the thin enamel section (Fig. 8). The peak heights of both CaKα and PKα were almost the same from the surface layer to the inner layer in healthy enamel (Fig. 8a). In the control group, more reduced heights of CaKα and PKα were apparent in the artificial caries lesions (seen on the left) compared to the healthy enamel located deeper (on the right side of the illustration) and demineralization was suggested (Fig. 8b). The peak of CaKα and PKα in the saliva group was higher in the surface layer than in the control group, which indicated remineralization (Fig. 8c). The HAP group showed a rather smaller and less evident difference in peak heights between the lesion and healthy enamel compared to the control group (Fig. 8d).

③ Ca/P molar ratio analysis

The Ca/P molar ratio was determined by point analysis (semi-quantitative analysis) using an analytical electron microscope (Fig. 9). In all groups, the Ca/P molar ratios were high in the surface enamel layer, ranging from 1.46 to 1.48, but low in the inner layer. In the inner layer, those in the HAP group ranged from 1.40 to 1.43 and followed a similar pattern to those of healthy enamel, which ranged from 1.41 to 1.44. Meanwhile, a low Ca/P molar ratio of 1.37 to 1.39 was measured in the immediate subsurface layer in the
control group and the saliva group.

3) Discussion
Recently, awareness that enamel white spot lesions often naturally disappear has inspired many attempts to prevent the progression of initial enamel caries (white spot lesions) or to remineralize them by means of fluoride application and plaque control.\textsuperscript{18, 19} These attempts aim to artificially promote remineralization of subsurface demineralized areas (initial caries lesions) clinically observed as enamel white spot lesions, to restore them to a state close to healthy enamel.

Remineralization here refers to the new precipitation and deposition of calcium phosphate salts within the tooth substance after subsurface mineral loss has occurred due to partial demineralization by acid challenge. Saliva provides a natural source of calcium and phosphate ions for remineralization of surface enamel while calcium and phosphate ions dissolved out of the enamel participate in the remineralization of the inner layer. Remineralization is not a one-way process but alternates with demineralization to progress simultaneously.\textsuperscript{20}

Kuboki et al.\textsuperscript{2} found that HAP has a greater capacity for adsorption into micro defects on the HAP pellet than dental grade dibasic calcium phosphate (DCPD) and suggested the potential of HAP to remineralize HAP pellets.

In the present study, human enamel was used to artificially create caries (artificial white spots) and then treated with HAP for dentifrice use to investigate one of HAP’s actions, namely its promotion of remineralization, by scanning electron microscopy (SEM), polarized light microscopy (PLM), contact microradiography (CMR), microfocus X-ray diffractometry, and analytical electron microscopy.

Under the conditions of this study, CMR and PLM observations confirmed the occurrence of remineralization of artificial caries to some extent by artificial saliva following immersion into it of artificially created caries samples. The artificial saliva described by Birkeland\textsuperscript{17} contains 1 mM CaCl\textsubscript{2} and 3 mM NaH\textsubscript{2}PO\textsubscript{4}, and thus it could work as a remineralizing solution promoting the remineralization of caries lesions.

However, treatment of artificial caries samples with HAP solution prior to immersion in artificial saliva further promoted their remineralization, as witnessed by less distinguishable subsurface lesions than those in samples only immersed in artificial saliva, a more thickened surface remineralized layer, and a similar birefringence to that of healthy enamel. This may be explained by either precipitation and deposition of new crystals with a similar refractive index to those of healthy enamel, or by the alignment and reorientation of the enamel’s remaining original crystals. If the latter is correct, a large difference in X-ray diffraction pattern should not be noticed before and after remineralization. However, examination of crystallinity in the direction of the c-axis by microfocus X-ray diffractometry revealed that the crystallinity of artificial caries lesions in the HAP group was evidently higher than that of healthy enamel (Fig. 4). In addition, no other calcium phosphate salts but HAP were detected upon identification of substances within the treated artificial caries lesions. X-ray powder diffraction analysis revealed that the HAP for dentifrice used in this study had similar crystallinity to dentin. The Ca/P molar ratio was 1.606, and the Ca and P concentrations of the HAP treatment solution were 4.28 and 2.24 ppm, respectively.

By all these findings and observations, the precipitation and deposition of crystals with higher crystallinity
than the original healthy enamel due to remineralization within the artificial caries lesion can be supported. The rough and coarse surface of artificially produced fresh caries lesions observed in cross-section image by SEM was considerably restored by immersion of the samples in artificial saliva. Furthermore, expansion of the treatment to use HAP solution followed by immersion in artificial saliva further promoted this remineralization to make the artificial caries lesions no longer distinguishable.

The Ca/P molar ratios at each point on the surface of the enamel section in each group were determined by point analysis using an analytical electron microscope. Those of the HAP group followed the same pattern as healthy enamel and underlined the effect of HAP treatment for promoting remineralization of artificial caries lesions. The Ca/P ratios obtained in this study (1.46-1.48 and 1.41-1.44 for the surface and inner layer of healthy enamel respectively) were actual calculation results using an EDX computer and are lower than the theoretical values of human enamel HAP.

All the observations listed above suggest that HAP for dentifrice use is not only deposited onto the enamel surface of artificial caries lesions but also helps promote remineralization within such lesions and increases the crystallinity of deposited HAP through remineralization.

4) Conclusion

The purpose of this study was to examine the effect of synthetic hydroxyapatite for dentifrice use on remineralization of initial caries lesions. Artificial caries lesions were formed on specimens of human enamel according to the method described by Moreno, then treated with synthetic hydroxyapatite for dentifrice use. Their structures and condition before and after treatment were observed by polarized light microscopy, contact micro-radiography, scanning electron microscopy, microfocus X-ray diffractometry and analytical electron microscopy, and the following results were obtained.

(1) Scanning electron microscopic images of the surfaces of artificial caries lesions showed notable deposits of fine particles on the enamel surfaces in the apatite-treated group, and the surfaces were smoother as compared with the control group.
(2) Polarized light microscopy and contact micro-radiography demonstrated a reduction in both the thickness and radio transparency of artificial caries lesions in the apatite-treated group.
(3) Microfocus X-ray diffraction pattern analysis revealed that hydroxyapatite in the HAP group had higher crystallinity in the direction of the c-axis than that of healthy enamel.
(4) The Ca/P molar ratio of artificial caries lesions determined by point analysis using analytical electron microscopy was lower than that of healthy enamel.
(5) Artificial caries lesions were partially remineralized by immersion in artificial saliva while in the apatite group significantly enhanced remineralization was observed.

With all above observations, the effectiveness of hydroxyapatite for dentifrice use to promote the remineralization of not only the surface layer of artificial caries lesions but also the subsurface decalcified layer was confirmed. Hydroxyapatite-containing dentifrice can therefore be expected to contribute to the remineralization of initial caries.
3. Other caries preventive actions of HAP

1) Adsorption of polysaccharides and proteins

The adsorption capacity of HAP for extracellular polysaccharides produced by *S. mutans* has been suggested by Pearce and that for glucans produced by *S. mutans* has been confirmed by Shimura et al. The potential for HAP to reduce the volume of dental plaque adhesion has also been suggested. Moreover, HAP adsorption of salivary proteins as well as lipoteichoic acid, which plays an important role in the adhesion and aggregation of bacteria in dental plaque, has been reported.

2) Tooth surface abrasion

Aoki et al. investigated tooth surface abrasion by HAP and concluded that HAP micro particles in gel form or in sizes less than 400 mesh were only weakly abrasive, and the results of a comparative study with a commercially available dentifrice indicated little risk of the synthetic apatite-containing dentifrice causing enamel abrasion. Nakano et al. used optical interferometry to measure the enamel surface abrasivity of the HAP dentifrice in comparison with two other commercially available dentifrices and found that it demonstrated the least abrasivity. The researchers postulated that this was attributable to HAP’s mineralization effect on the enamel surface.

Summary

Hydroxyapatite-containing toothpastes have been shown clinically to have caries preventive effects. These can be attributed to HAP’s various functions, including (1) filling of micro decalcified lesions in the enamel surface, (2) promoting the remineralization of initial caries lesions, (3) physical polishing of the tooth surface, (4) adsorption capacity for polysaccharides produced by *S. mutans*, and (5) adsorption capacity for proteins. Among those actions, one critical to the teeth is that of promoting remineralization of the initial caries lesion. HAP’s adsorption capacity for polysaccharides and proteins may be another important function enhancing oral prophylaxis and improving the intraoral environment.
Table 1  Percentage of teeth newly affected by caries on the buccal surface (Shimura 1982)  

<table>
<thead>
<tr>
<th>Grade</th>
<th>Object</th>
<th>n</th>
<th>Previously erupted teeth</th>
<th>Newly erupted teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fourth</td>
<td>Test group</td>
<td>83</td>
<td>61/1588=3.84%</td>
<td>9/360=2.50%</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>86</td>
<td>73/1606=4.55%</td>
<td>14/388=3.61%</td>
</tr>
<tr>
<td>Fifth</td>
<td>Test group</td>
<td>86</td>
<td>36/2056=1.75%</td>
<td>21/358=5.86%</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>85</td>
<td>71/2035=3.49%</td>
<td>20/378=5.33%</td>
</tr>
<tr>
<td>Sixth</td>
<td>Test group</td>
<td>85</td>
<td>86/2271=3.79%</td>
<td>49/339=14.45%</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>85</td>
<td>123/2277=5.40%</td>
<td>56/309=16.97%</td>
</tr>
<tr>
<td>Total</td>
<td>Test group</td>
<td>254</td>
<td>183/5913=3.09%</td>
<td>179/1057=7.47%</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>256</td>
<td>267/5918=4.51%</td>
<td>90/1072=8.40%</td>
</tr>
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</table>

Percentage caries inhibition in the test group:

Previously erupted teeth: 31.49% (X²-test, p<0.01) (a)/(b)
Newly erupted teeth: 11.07% (X²-test, p<0.01) (c)/(d)
Total: 26.42% (X²-test, p<0.01) (183+179)/(5913+1057) ÷ (267+90)/(5918+1072)

Table 2  Percentage of teeth newly affected by caries on the proximal surfaces (previously erupted tooth) (Shimura 1982)  

<table>
<thead>
<tr>
<th>Grade</th>
<th>Object</th>
<th>n</th>
<th>Previously erupted teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fourth</td>
<td>Test group</td>
<td>83</td>
<td>52/2378=2.19%</td>
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<tr>
<td></td>
<td>Control group</td>
<td>86</td>
<td>48/2424=1.98%</td>
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<tr>
<td>Fifth</td>
<td>Test group</td>
<td>86</td>
<td>24/3176=0.76%</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>85</td>
<td>48/3148=1.52%</td>
</tr>
<tr>
<td>Sixth</td>
<td>Test group</td>
<td>85</td>
<td>26/3516=0.74%</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>85</td>
<td>34/3558=0.96%</td>
</tr>
<tr>
<td>Total</td>
<td>Test group</td>
<td>254</td>
<td>(a) 102/9070=1.12%</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>256</td>
<td>(b) 130/9130=1.42%</td>
</tr>
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Percentage caries inhibition in the test group: 21.13% (X²-test, p<0.01) (a) / (b)

Table 3  Object and number

<table>
<thead>
<tr>
<th>Object</th>
<th>Total</th>
<th>Boys</th>
<th>Girls</th>
</tr>
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<tbody>
<tr>
<td>Test group</td>
<td>89</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Control group</td>
<td>92</td>
<td>47</td>
<td>45</td>
</tr>
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Three-year cohort study (Kani 1989)
Fig 1  Average number of erupted teeth and average DMFT index

6#: Final examination in March for sixth grade graduating children  (Kani 1989)  

Table 4   Average number of erupted teeth and average DMFT index  (Kani 1989)  

<table>
<thead>
<tr>
<th>Object</th>
<th>Grade</th>
<th>% caries inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test group</td>
<td>(a) 2.31 (12.69)</td>
<td>2.76 (16.40)</td>
</tr>
<tr>
<td>Control group</td>
<td>(b) 1.57 (12.66)</td>
<td>2.11 (16.25)</td>
</tr>
<tr>
<td>Girls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test group</td>
<td>(a) 2.68 (15.70)</td>
<td>2.91 (19.47)</td>
</tr>
<tr>
<td>Control group</td>
<td>(b) 2.49 (15.38)</td>
<td>3.33 (20.11)</td>
</tr>
</tbody>
</table>

6#: Final examination in March for sixth grade graduating children

Average number of erupted teeth

* Based on increment in DMFT index after 3 years: [(c) –(a)] / [(d) –(b)]
Fig 2  Incidence of new caries (new DMFT rate)  (Kani 1989)\(^{14}\)

(1) Previously erupted healthy teeth at the start of the study
(2) Teeth newly erupted during the study
※p<0.05
※※※※p<0.001

Table 5  Incidence of new caries (new DMFT rate) (Kani 1989)\(^{14}\)

Newly developed caries for intact teeth at baseline

<table>
<thead>
<tr>
<th>Study year</th>
<th>Object</th>
<th>BOYS</th>
<th>GIRLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of teeth</td>
<td>New DMFT</td>
<td>New DMFT rate</td>
</tr>
<tr>
<td>1</td>
<td>Test group</td>
<td>467</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>488</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Test group</td>
<td>467</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>488</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>Test group</td>
<td>467</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>488</td>
<td>49</td>
</tr>
</tbody>
</table>

※※※※p<0.001
Table 6  Incidence of new caries (new DMFT rate)  (Kani 1989)\textsuperscript{14}

<table>
<thead>
<tr>
<th>Study year</th>
<th>Object</th>
<th>BOYS</th>
<th></th>
<th>GIRLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of teeth</td>
<td>New DMFT</td>
<td>New DMFT rate</td>
</tr>
<tr>
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<td>Test group</td>
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<td>0.00</td>
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<td>Test group</td>
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<td>1.44</td>
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<td>Control group</td>
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<tr>
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<td>Control group</td>
<td>404</td>
<td>37</td>
<td>9.16</td>
</tr>
</tbody>
</table>

※p<0.05  ※※※※p<0.001

Fig 3  Graphic illustration of micro filling theory  (Kuboki 1987)\textsuperscript{2}

1. Microscopic enamel surface fissures
2. Filling of micro defects with hydroxyapatite
3. Remineralization of subsurface demineralized areas with mineral ions.

Fig 4  Scanning electron microscope (SEM) observation of artificially produced caries on the enamel after different treatments  (Kani 1991)\textsuperscript{6}

a. Healthy enamel surface  c. Saliva group (artificially produced caries followed by immersion in artificial saliva)
b. Control group (artificially produced caries only)  d. HAP group (artificially produced caries followed by treatment with HAP and then immersion in artificial saliva)
Fig 5  Polarized light microscopy (PLM) (left) and contact microradiography (CMR) (right) observation of artificially produced caries on the enamel after different treatments (Kani 1991).  

a,d Control group  b,e Saliva group  c,f HAP group

Fig 6  Microfocus X-ray diffractometry observation of artificially produced caries on the enamel after treatment with HAP and then artificial saliva.  

HAP group (Kani 1988)
Fig 7  SEM observation of cross-section images of artificial caries lesions after different treatments  (Kani 1991)

a. Healthy enamel  c. Saliva group
b. Control group          d. HAP group

[Note: group b. and c. illustrations were mis-named in the original publication]

Fig 8  Analytical electron microscope observation of artificial caries lesions after different treatments, showing Ca and P concentrations by linear analysis  (Kani 1991)

a. Healthy enamel  c. Saliva group
b. Control group          d. HAP group

[Note: group b. and c. illustrations were mis-named in the original publication]


Fig 9  The Ca/P molar ratio of the artificial caries lesions from the enamel surface layer to the inner layer after different treatments (Kani 1991)
6) Pearce E: Adsorption of Streptococcal Extracellular Polysaccharides by Hydroxyapatite.  


8) Bolton R: Adherence of oral streptococci to hydroxyapatite in vitro via glycerolteichoic acid.  


Dental Outlook : 112-117, 1982.*

Shikaigaku 49: 531-549, 1986.*


17) Birkeland J: The effect of pH on the interaction of fluoride and salivary ions.  


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