Ultraphosphate, a potent stain control agent that is effective for both stain removal and prevention of stain deposition

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Polyphosphate is a phosphate polymer which is effective for stain removal and prevention of stain deposition. Ultraphosphate belongs to the polyphosphate group and has a highly branched mesh-like structure. To evaluate stain control ability of ultraphosphate, we used HAP powder, glass-ionomer cement and detached human teeth for models of in vitro stain control experiments. When using HAP powder, the stain removal ability of ultraphosphate was the highest among common chelating agents. In addition, ultraphosphate efficiently removed stain and prevented stain deposition on glass-ionomer cement at 20°C and 37°C. Finally, ultraphosphate removed coffee stain from human teeth surface efficiently and the color difference ($\Delta E^*_{ab}$) before and after ultraphosphate treatment was changed dramatically from 59.4 to 8.3. Similarly, the $\Delta E^*_{ab}$ value of human teeth treated with ultraphosphate before coffee treatment was only 9.9, while the value without ultraphosphate pre-treatment was 21.2. These results indicate that ultraphosphate is a potent agent for stain control.

Keywords: Polyphosphate, Stain, Ultraphosphate, Whitening

INTRODUCTION

Inorganic polyphosphates (poly(P)) are polymers that are widely found in living organisms and natural foods. They have been used as a food additive all over the world, and have been subjected to many safety tests1). Recently, poly(P) was shown to have many biological functions such as in bone regeneration2), collagen production3), regulation of gene expression4), regulation of translation fidelity5) and anti-bacterial and anti-fungal properties6). In the dental field, poly(P) removes stain and prevents stain deposition on teeth surfaces by its chelating properties5). In addition, it has been reported that poly(P) is clinically effective in treating periodontal diseases8).

Conventionally, some attempts to add salts of poly(P) to an oral composition such as dentifrice have been made in order to remove stains on teeth. For example, an oral composition has been reported in which poly(P) is used together with menthol or anethole9), pyrophosphates10), and natural scrubbing agents11). However, these oral compositions and tooth cleaning materials had some disadvantages, as the stain on the tooth surface could not be completely removed with a toothbrush. Accordingly, there has been a desire to develop a stain remover that can not only effectively remove stains from the tooth surface without any damage, but also efficiently prevent stain deposition on the tooth surface.

Ultraphosphates are known to possess antibacterial properties, and have conventionally been used as a food preservative12,13). It was also reported that ultraphosphates inhibit color change of a composition containing tannin14). However, it has not yet been reported whether ultraphosphates are capable of stain removal.

Ultraphosphate is a kind of condensed phosphate that is generated by polymerizing two or more PO₄ tetrahedas to share an oxygen atom included in other PO₄ tetrahedas15). The condensed phosphates are classified into poly(P), metaphosphates and ultraphosphates based on the molar ratio of M₂O/P₂O₅ (M represents a monovalent metal typified by an alkali metal). The poly(P) has an M₂O/P₂O₅ molar ratio (R) satisfying 2> R > 1, and is a linear compound represented by Formula (I):

$$M_{2n}P_{m}O_{3m+1}$$

(m represents an integer of 2 or greater.) The metaphosphate has an M₂O/P₂O₅ molar ratio (R) of 1, and is a cyclic or extremely long linear compound represented by Formula (II):

$$(MPO_4)_n$$

Color figures can be viewed in the online issue, which is available at J-STAGE.

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The above-mentioned poly(P) and metaphosphate together are referred to as poly(P).

The ultraphosphate has an M$_2$O/P$_2$O$_5$ molar ratio (R) satisfying 1>R>0, and is a compound represented by Formula (III):

\[ \frac{x}{2} \text{M}_2\text{O} \cdot \frac{y}{2} \text{P}_2\text{O}_5 \]  

(Both x and y represent a positive integer satisfying 0<x/y<1.)

Ultraphosphate has a crosslinked mesh-like structure including a branched PO$_4$ group in the molecule, and the specific structure is shown in Fig. 1. Ultraphosphate is also known as a strong chelating agent$^{15}$.

In this study, we investigated whether ultraphosphate is an efficient stain remover compared to other conventional agents using hydroxyapatite (HAP) powder, glass ionomer cement and detached human teeth. We confirmed that ultraphosphate is the most effective not only in removing stains from the tooth surface but also in the prevention of stain deposition.

**MATERIALS AND METHODS**

**Materials**

Materials and reagents used in experiments are listed in Table 1. Tea extract was prepared as follows: 4 g of tea leaves (2 tea bags) were immersed in 200 mL of boiled purified water for 10 min, and this liquid was filtered through Whatman No. 5B filter paper (Whatman International Co., Ltd., Maidstone, UK). Coffee solution was prepared by dissolving 1 g of commercial instant coffee powder into 14 mL of hot water at 100°C.

### Table 1  Materials and reagents used in experiment

<table>
<thead>
<tr>
<th>Materials</th>
<th>Working concentrations</th>
<th>Grade and characteristics</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium ultraphosphate</td>
<td>1w/v% for HAP experiments (Fig. 2), 10w/v% for glass ionomer cement (Figs. 3 and 4) and human teeth experiments (Fig. 5)</td>
<td>Food-additive grade</td>
<td>Mitejima Chemical Co., Ltd. (Osaka, Japan)</td>
</tr>
<tr>
<td>Sodium hexametaphosphate</td>
<td>1 w/v%</td>
<td>Food-additive grade with average chain length around 30 phosphate residues</td>
<td>Taihei Chemical Industrial Co., Ltd. (Osaka, Japan)</td>
</tr>
<tr>
<td>Phytic acid</td>
<td>1 w/v%</td>
<td>Reagent grade</td>
<td>Tsuno Fine Chemicals Co., Ltd. (Wakayama, Japan)</td>
</tr>
<tr>
<td>EDTA-2Na</td>
<td>1 w/v%</td>
<td>Reagent grade</td>
<td>Wako Pure Chemical Industries, Ltd. (Osaka, Japan)</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>1 w/v%</td>
<td>Reagent grade</td>
<td></td>
</tr>
<tr>
<td>Malic acid</td>
<td>1 w/v%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite (HAP) powder</td>
<td>—</td>
<td>Chromatography grade</td>
<td>Bio-Rad Laboratories Inc. (Tokyo, Japan)</td>
</tr>
<tr>
<td>The glass-ionomer cement (FUJI I)</td>
<td>—</td>
<td></td>
<td>GC Corp. (Tokyo, Japan)</td>
</tr>
<tr>
<td>Lipton tea bag</td>
<td>—</td>
<td>Commercial product</td>
<td>Unilever Japan K.K. (Tokyo, Japan)</td>
</tr>
<tr>
<td>Nescafe gold blend coffee</td>
<td>—</td>
<td>Commercial product</td>
<td>Nestle Japan, Ltd. (Hyogo, Japan)</td>
</tr>
</tbody>
</table>
To evaluate the effect of ultraphosphate on stain removal and prevention of stain deposition, HAP powder was used as an artificial tooth model. Original protocols for stain removal and prevention of stain deposition test were described in previously. For stain removal, each 150 mg of HAP powder was pre-washed with 10 mL of purified water in a 15-mL centrifuge tube, and HAP powder was collected by centrifugation at 1,500×g for 2 min. Five milliliters of tea extract was added to the HAP pellet, and the tube was inverted several times to immerse HAP in the tea extract, then the supernatant was removed. To remove excessive tea stain, 10 mL of purified water was added to HAP, and the tube was inverted for 1 min. The supernatant was discarded after centrifugation. This washing procedure was repeated twice in total. One-milliliter of each test solution (1 w/v%, pH 7.0) or purified water was added to the pre-stained HAP and each tube was inverted for 1 min to immerse HAP in each solution. After the supernatant was removed, each tube was washed with 2 mL of purified water for 1 min and the supernatant was discarded. Finally 2 mL of purified water was added to each tube and inverted for 1 min to make an HAP suspension. Then each suspension was transferred into 96-multi-well plate and the color of each HAP powder was scanned from the bottom of the plate using an image scanner. The ratio of the prevention of stain deposition was calculated and statistically analyzed in the same manner as mentioned above.

For testing the prevention of stain deposition, each 30 mg of HAP powder was put into a 2-mL centrifuge tube, and 1 mL of each test solution or purified water was added into tubes. The tube was inverted to mix and centrifuged at 1,500×g for 2 min to remove the supernatant. The resultant HAP pellets were washed by 2 mL of purified water and the supernatant was removed. This washing procedure was repeated twice in total, then 1 mL of the tea extract was added to each tube and the tube was inverted for 1 min. After the supernatant was removed, each HAP pellet was washed by 2 mL of purified water. Finally 2 mL of purified water was added to each HAP pellet and inverted for 1 min to make a HAP suspension. Then each suspension was transferred into 96-multi-well plate and the color of each HAP powder was scanned from the bottom of the multi-well plate using an image scanner. The ratio of the prevention of stain deposition was calculated and statistically analyzed in the same manner as mentioned above.

Preparation of glass-ionomer cement slice
A stainless steel mold (outline form of the denture base was 15 mm/ internal diameter of 7 mm/thickness of 1.5 mm) was used for preparing a glass-ionomer cement slice. The mold was fixed on a mixing slab, and the glass-ionomer cement was mixed for 20 s in the standard powder liquid ratio. Then, the glass-ionomer cement was injected and pressed for 5 min to form the slice. After removing a piece of the slice from the mold, it was immersed in distilled water for 24 h at room temperature (20°C). To remove the residual chemicals, the slice was washed with neutral detergents, and both sides of the slice were ground with #220 water-resistant paper. Further ultrasonic washing was carried out for 10 min with water.

Evaluation of stain removal and prevention of stain deposition of the glass ionomer cement slice
The evaluation protocol of stain removal and prevention of the stain deposition of the glass ionomer cement slice is summarized in Table 2. C1, U1, C3 and U3 groups were conducted in order to evaluate the stain removal efficiency. A glass-ionomer cement slice was immersed in coffee solution for 72 h, and the slice was dried in

### Table 2 The scheme of stein control experiments using glass ionomer cements

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Temperature</th>
<th>Ultraphosphate</th>
<th>Coffee</th>
<th>Washed with H₂O</th>
<th>H₂O</th>
<th>Ultraphosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td></td>
<td>—</td>
<td>72 h</td>
<td>—</td>
<td>0–72 h</td>
<td>—</td>
</tr>
<tr>
<td>U1</td>
<td>20°C</td>
<td>—</td>
<td>72 h</td>
<td>—</td>
<td>—</td>
<td>0–72 h</td>
</tr>
<tr>
<td>C2</td>
<td>24 h</td>
<td>72 h</td>
<td>10 s</td>
<td>0–72 h</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>U2</td>
<td></td>
<td>24 h</td>
<td>72 h</td>
<td>10 s</td>
<td>0–72 h</td>
<td>—</td>
</tr>
<tr>
<td>C3</td>
<td>37°C</td>
<td>—</td>
<td>72 h</td>
<td>—</td>
<td>0–300 min</td>
<td>—</td>
</tr>
<tr>
<td>U3</td>
<td></td>
<td>—</td>
<td>72 h</td>
<td>—</td>
<td>—</td>
<td>0–300 min</td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td>—</td>
<td>72 h</td>
<td>10 s</td>
<td>0–300 min</td>
<td>—</td>
</tr>
<tr>
<td>U4</td>
<td></td>
<td>24 h</td>
<td>72 h</td>
<td>10 s</td>
<td>0–300 min</td>
<td>—</td>
</tr>
</tbody>
</table>
a personal incubator (PIC-100, As One Corp., Osaka, Japan) at 37°C. After drying, the slice was washed with mineral water (HAWAII water, Toell, Inc. Kanagawa, Japan) for a maximum of 72 h (C1 group) or 300 min (C3 group). Similarly, another slice was immersed in coffee solution for 72 h, dried, then washed with a sodium ultraphosphate solution (10 w/v%, pH 6.8) for a maximum of 72 h (U1 group) or 300 min (U3 group).

In order to evaluate the efficiency of the prevention of stain deposition, C2, U2, C4 and U4 groups were conducted. A glass-ionomer cement slice was immersed in coffee solution for 72 h, and the slice was washed for 10 s with water. Then, the slice was washed with mineral water for a maximum of 72 h (C2 group) or 300 min (C3 group). After the slice was immersed in sodium ultraphosphate solution (10 w/v%, pH 6.8) for 24 h, it was soaked in coffee solution for 72 h, and dried. After the slice was washed with water for 10 s, the slice was immersed again in mineral water for a maximum of 72 h (U2 group) or 300 min (U4 group). All experimental steps other than drying process was done at room temperature (20°C) in C1, U1, C2 and U2 groups and at 37°C in C3, U3, C4 and U4 groups.

The color tone of the glass-ionomer cement slice in each treatment group was measured by the dental colorimeter (ShadeEye NCC, SHOFU, Inc., Kyoto, Japan) on a black sheet. The \( L^*a^*b^* \) value of the slice that was immersed in only mineral water was applied as a blank, and color tone differences, the \( \Delta E^*ab \) values, of each group were compared based on this value.

**Preparation of tooth sample**

The experiment using tooth was approved by the Showa University School of Dentistry Bioethical Committee (approval number 2012-1). The detached incisors were cleaned after removing soft tissue. The cervical area was cut under irrigation with a diamond point (FG105R, SHOFU, Inc., Kyoto, Japan), and washed by ultrasonication for 10 min. The tooth was embedded in a standard mold with low viscosity epoxy resins (SpeciFix-20, Marumoto Strauss KK, Tokyo, Japan). After hardening of the resin, the tooth was immersed in a standard mold with low viscosity epoxy resins (SpeciFix-20, Marumoto Strauss KK, Tokyo, Japan). The tooth sample was immersed in coffee solution for 72 h. After drying, the tooth sample was washed with mineral water for a maximum of 300 min (CT group). Similarly, the tooth sample was immersed in coffee solution for 72 h, and dried, then washed with a sodium ultraphosphate solution (10 w/v%, pH 6.8) for a maximum of 300 min (UT1 group). The tooth sample was immersed in sodium ultraphosphate solution (10 w/v%, pH 6.8) for 24 h, and the sample was washed for 10 s with water. Then, the sample was washed with mineral water for a maximum of 300 min (UT2 group).

The color tone of the tooth sample in each treatment group was measured by the dental colorimeter (ShadeEye NCC, SHOFU, Inc., Kyoto, Japan) on a black sheet. Using the \( L^*a^*b^* \) value of the sample that was immersed only in mineral water as a blank, color tone differences, the \( \Delta E^*ab \) values, of the CT group, UT1, and CT group and UT2 group were compared.

**Calculation of \( \Delta E^*ab \) value**

Color tone difference was evaluated by calculating \( \Delta E^*ab \) values with the following equation.

\[
\Delta E^*ab = \sqrt{(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2}
\]

Student’s \( t \)-test was applied for statistical analysis with \( p<0.05 \) indicating a significant difference.

**RESULTS**

**Comparison of various stain control agents**

Comparing the efficiency of stain removal between sodium ultraphosphate and some other chelating agents, sodium ultraphosphate had the highest ability at neutral pH. As shown in Fig. 2(a) and 2(b), the stain removal efficiency of sodium ultraphosphate was the highest among sodium phytic acid, sodium malic acid, sodium hexametaphosphate, sodium citrate and EDTA disodium salt at pH 7.0. The concentration of each reagent was 1 w/v%. The stain residual ratio of sodium ultraphosphate was much lower than that of malic acid (96.7%) and phytic acid (82.5%), but the

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Ultraphosphate</th>
<th>Coffee</th>
<th>Washed with H₂O</th>
<th>H₂O</th>
<th>Ultraphosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>—</td>
<td>72 h</td>
<td>10 s</td>
<td>0–300 min</td>
<td>—</td>
</tr>
<tr>
<td>UT1</td>
<td>—</td>
<td>72 h</td>
<td>10 s</td>
<td>—</td>
<td>0–300 min</td>
</tr>
<tr>
<td>UT2</td>
<td>24 h</td>
<td>72 h</td>
<td>10 s</td>
<td>0–300 min</td>
<td>—</td>
</tr>
</tbody>
</table>
Efficiency of stain removal and prevention of stain deposition in various chelating agents. Images of color differences of HAP powder are shown in panel (a). The stain residual ratio after stain removal and prevention of stain deposition experiments are shown in panel (b) and (c), respectively. Percentage of darkness of HAP powder when the stained powder that was washed or pre-treated by water was calculated as 100%. The color of the HAP powder that was not treated by tea extract was set at 0%. “Removal” and “Prevention” described in panel (a) represent the results of stain removal and prevention of stain deposition experiments, respectively. “Ultra” and “Hexameta” in panel (b) and (c) represent ultraphosphate and hexametaphosphate, respectively. Numbers stated above bars in panel (b) and (c) represent percentages of stain residual ratio and stain deposition ratio, respectively. Asterisks represent significant differences between two indicated groups connected by lines (n=3, Turkey’s test with 95% confidence intervals).

Regarding the efficiency of prevention of stain deposition, little difference was observed between sodium hexametaphosphate and sodium ultraphosphate. In addition, there is no significant difference between sodium ultraphosphate and following two agents, sodium citrate and phytic acid. EDTA-2Na and sodium malic acid were much less capable of preventing stain deposition comparing sodium ultraphosphate and had significantly lower efficiency than sodium ultraphosphate (Turkey’s test with 95% confidence intervals) (Fig. 2(a) and 2(c)). This means that both sodium ultraphosphate and sodium hexametaphosphate seems to be better than the other agents in terms of efficiency for the prevention of stain deposition, whereas the margin of difference of the efficiency for the prevention of stain deposition between ultraphosphate and other agents are less than that of stain removal efficiency.

Effect of sodium ultraphosphate on stain removal and prevention of stain deposition in a glass-ionomer cement slice
The results of stain removal and prevention of stain deposition by sodium ultraphosphate at room temperature (20°C) are shown in Fig. 3(a). The $\Delta E^{*}ab$ values of stain removal and prevention of stain deposition experiments are summarized in Fig. 3(b) and 3(c), respectively. For stain removal, the color difference between the C1 and U1 groups before washing with mineral water or sodium ultraphosphate were 73.9 and 73.3, respectively. After washing with mineral water (C1 group), the color difference decreased to 50.2, 44.7 and 43.3 at 24, 48 and 72 h, respectively. However, after washing with sodium ultraphosphate (U1 group), the...
Fig. 3  Efficiency of stain removal and prevention of stain deposition of glass-ionomer cement at 20°C.
Images of glass-ionomer cement are shown in panel (a). The color tone difference after stain removal and prevention of stain deposition experiments are shown in panel (b) and (c), respectively. Asterisks represent significant differences between C1 and U1 in panel (b) or C2 and U2 in panel (c) (n=10, p<0.05, Student’s t-test).

The results of stain removal and prevention of stain deposition by sodium ultraphosphate at 37°C are shown in Fig. 4(a). The ΔE*ab values of stain removal and prevention of stain deposition experiments are summarized in Fig. 4(b) and 4(c), respectively. For stain removal, the color differences between the C3 and U3 groups before washing with mineral water or sodium ultraphosphate were 69.7 and 74.0, respectively. After washing with mineral water (C3 group), the color differences decreased to 37.5 and 25.1 at 15 and 300 min, respectively. However, after washing with sodium ultraphosphate (U3 group), the color difference dramatically decreased to 7.0 and 4.8 at 15 and 300 min, respectively. The color difference between the C3 and U3 groups after washing with mineral water or sodium ultraphosphate was significantly different.

As for the prevention of stain deposition, the color difference in the C4 group that had no pretreatment with sodium ultraphosphate was 68.8, whereas the color difference in the U4 group that was treated with sodium ultraphosphate was 12.1. The color difference in the C3 group changed to 12.4 after immersion in water for 300 min, whereas the color difference in the U4 group changed to 4.1 after immersion in water for 300 min. All values of the color differences between the C4 and U4 groups at each time point were significantly different.

Effect of sodium ultraphosphate on stain removal and prevention of stain deposition in human tooth
The photographs of a representative sample comparing the color differences of tooth samples are shown in Fig. 5(a). The ΔE*ab values of stain removal and prevention of stain deposition experiments are summarized in
Fig. 4  Efficiency of stain removal and prevention of stain deposition of glass-ionomer cement at 37°C. Images of glass-ionomer cement are shown in panel (a). The color tone difference after stain removal and prevention of stain deposition experiments are shown in panel (b) and (c), respectively. Asterisk represents significant difference between C3 and U3 in panel (b) or C4 and U4 in panel (c) ($n=10$, $p<0.05$, Student’s $t$-test).

Fig. 5  Efficiency of stain removal and prevention of stain deposition of human teeth. Images of dental enamel are shown in panel (a). The color tone difference after stain removal and prevention of stain deposition experiments are shown in panel (b) and (c), respectively. Asterisk represents significant difference between CT and UT1 in panel (b) or CT and UT2 in panel (c) ($n=8$, $p<0.05$, Student’s $t$-test).
Fig. 5(b) and 5(c), respectively. The color differences in the CT and UT1 groups before sodium ultraphosphate washing were 61.2 and 59.4, respectively, which were not significantly different. The color differences in the CT group after immersion in water for 15 and 300 min were 49.8 and 21.2, respectively, whereas the color differences in the UT1 group after immersion in ultraphosphate for 15 and 300 min were 15.4 and 8.3, respectively. The color difference values of the UT1 group after washing were significantly lower than those of the CT1 groups.

Regarding the prevention of stain deposition, the color difference in the UT2 group pretreated with sodium ultraphosphate decreased from 27.9 to 9.9 after immersion in water for 300 min, while the color difference in the CT group decreased from 61.2 to 21.2. All values of the color differences between the CT and UT2 groups at each time point were significantly different.

**DISCUSSION**

Many agents are used for removing stains from tooth surfaces. Although abrading agents efficiently remove stains, they cause scratches on the tooth surface. These scratches could allow new stain deposition. Therefore, more efficient stain removing agents other than abrading agents, especially new chelating agents, need to be developed.

Comparing the efficiency of stain removal of some chelating agents, sodium ultraphosphate had the highest ability at neutral pH. Our results clearly indicate that sodium ultraphosphate was not only the best agent for stain removal among all the other chelating agents used in this experiment, but also relatively effective agent for prevention of stain deposition. The method for evaluating efficiency of stain removal and prevention of stain deposition using HAP are simple and easy *in vitro* method that originally developed by Baig et al. 16).

The basic ingredient of glass-ionomer cement is a calcium aluminosilicate glass containing 34.5% CaF$_2$ and it is known to have good adhesion properties to teeth and sustained release of fluoride when used as dental cement 17). Poly(P) groups including ultraphosphate have strong affinity for calcium. Therefore, it is thought that ultraphosphate also binds to glass-ionomer cement that has a calcium component. Moreover, ultraphosphates are strong cation chelating agents, and the electron density of an intra-molecular minus ion could be higher than that of other molecules. Ultraphosphates bind to the glass-ionomer cement slice, and can replace stains that are bound to its surface. Once the ultraphosphates bind to its surface, it prevents further stain deposition since they cover the surface of the glass-ionomer cement slice.

In the present experiment, we used FUJI-I as conventional luting glass ionomer cement for substitute tooth samples. Although other types of conventional glass ionomer cement, such as restorative glass ionomer cement, are available 18), the main composition and curing mechanism are almost the same between luting and restorative glass ionomer cement 19-21). As the curing reaction, polyacrylic acid is dissolved in aluminosilicate glass powder to release calcium, aluminum, sodium, and other positive ions 22). Thus we speculate that ultraphosphates could act specifically on calcium of glass ionomer cement, and effective to stain control. Since releasing calcium from aluminosilicate glass powder is common phenomenon on its curing reaction, effect of ultraphosphate on luting and restorative glass ionomer cement could be similar.

Compared to other luting cements, glass ionomer cement has strong disintegration 23). It has been suggested that water sensitivity at the initial stages of curing causes disintegration of the water contact surface matrix, resulting in the loss of unreacted glass particles in the core 24). In the present experiment, we immersed samples into distilled water immediately after cement curing. It has been reported that a porous and colored layer appears on the surface when immersed into water immediately after curing 25). The test samples used in the present experiment were such that coffee could penetrate the surface relatively easily, suggesting that we obtained good data.

Moreover, sodium ultraphosphate is effective in both stain removal and prevention of stain deposition at room temperature (20°C). To simulate body environment, we performed another experiment at 37°C (Fig. 4). Of note, at 37°C, 15 min was enough to remove 90.5% of the stain from the surface of the glass-ionomer cement slice. This suggests that sodium ultraphosphate could be a potent stain remover when it was used at ordinary oral temperature. In addition, only 30.4% (20°C) and 17.6% (37°C) of the stain remained in glass-ionomer cement that had been pre-treated with sodium ultraphosphate just after coffee treatment comparing to the ΔE*$^ab$ values without pre-treatment, which was set at 100%. The ability to prevent stain deposition at 37°C was likely to be stronger than that at 20°C. This means sodium ultraphosphate works more efficiently at body temperature.

As in glass-ionomer cement, sodium ultraphosphate is highly effective in stain removal as well as prevention of stain deposition in human teeth. In the UT1 group, stain deposited on the enamel surface was clearly removed after 15 min on washing with sodium ultraphosphate. In addition, the color change in enamel cracks also diminished depending on the washing time with sodium ultraphosphate, and stain deposition within dentin was also removed. On the other hand, in the CT group, stain deposited on the enamel surface was not efficiently removed compared to the UT1 group. The coffee that had penetrated deep into the enamel crack remained, causing color change of dentin. In the UT2 group, it is likely that stain was deposited before washing with water, but it was easily removed by immersion in water. Thus, pre-treatment with sodium ultraphosphate prevents stain deposition and penetration of coffee color into enamel cracks.

The high efficiency of stain removal and prevention
of stain deposition by sodium ultraphosphate may result from its highly condensed negative charge. Ultraphosphate could bind to the teeth surface by its highly negative charge and repel the stain since the binding affinity between teeth surface and ultraphosphate is much greater than that between teeth surface and stain. Also, once ultraphosphate bind to the teeth surface, it prevents newly binding between teeth surface and stain by ultraphosphate coating. These characteristics are common in phosphate polymers such as polyphosphate and hexametaphosphate. Since the detailed mechanism how ultraphosphate efficiently remove stain and prevent stain deposition has not been clear, further analysis should be necessary. These results clearly indicate that sodium ultraphosphate may be a potential stain control ingredient of toothpastes. The most important thing is that sodium ultraphosphate can remove stain without brushing. This means that sodium ultraphosphate would also be useful for liquid-type oral care products, such as mouthwash and dental rinse. Moreover, this ability of sodium ultraphosphates would prevent stain deposition and help teeth maintain their natural white color. Sodium ultraphosphate could be used as a professional whitening agent to enhance the bleaching effect of hydrogen peroxide and/or carbamide peroxide. Development of a novel teeth-bleaching system incorporating sodium ultraphosphate is expected.

REFERENCES