

Suppression of Demineralization by Inorganic Polyphosphates with Optimum  
Chain Length for Stain Removal and Prevention of Stain Deposition

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Suppression of Demineralization by PolyP

## **Abstract**

**Purpose:** Inorganic polyphosphates (polyPs) bind to tooth surface and aid in removing stain and preventing stain deposition. In this study, we investigated whether the degree of polymerization of polyP affects the stain removal and prevention of stain deposition on the tooth surface. We also investigated whether polyP bound to the tooth surface suppresses demineralization.

**Methods:** Hydroxyapatite powders stained with tea and coffee extracts were placed in centrifuge tubes, and purified water or polyPs solution of various chain lengths was added to it. The solution was mixed and then centrifuged to separate the hydroxyapatite. Further, unstained hydroxyapatite powder was mixed with polyPs solution or purified water in the same manner, and dye them with tea and coffee. Color of the hydroxyapatite powder for each sample was scanned using an image scanner, and the color density was measured to evaluate the extents of stain removal and prevention of deposition.

An ivory plate was then sanded to prepare a smooth specimen. Each specimen was immersed in 1% polyP9.7-Salivert solution or Salivert for 10 min and then washed with water and dried. The specimen was then treated with 40% phosphoric acid for 20 min. After this, the phosphoric acid was washed off with water, and the specimen was dried. Cross-sectional depth of the demineralized portion after drying was observed with an optical microscope.

Results: PolyP with a degree of polymerization of 9.7 (polyP9.7), was highly effective in removing stain and preventing deposition. PolyP9.7 is more effective in removing stain and preventing deposition than tripolyphosphate, a polymer of three phosphate residues commonly used in dentifrices, and polyP, which has a degree of polymerization of 60. In addition, demineralization was suppressed on the ivory surface treated with polyP9.7 upon phosphoric acid etching.

Conclusion: PolyP9.7 is highly effective in removing stain and preventing deposition on the tooth surface and can suppress the demineralization by binding to the tooth surface.

**Key words:** demineralization, polymer size, polyP, stain control

## Introduction

Inorganic Polyphosphate (polyP) is a polymer composed of phosphate and has various physiological functions in living organism<sup>1)</sup>. The precise physiological function of polyphosphates depends on its molecular weight. For instance, long chain polyP has a dramatic effect of the suppression of bone resorption and the expression of iNOS<sup>2)</sup>, while polyP with 60 to 100 phosphate residues are found to promote blood clotting<sup>1,3)</sup>. It has also been found that long chain polyP with a degree of polymerization of 100 or more have intestinal tract-protective effects<sup>4)</sup>. PolyPs with an average length of 150 phosphate residues have been shown to be lipopolysaccharide (LPS) -induced lethal and able to cure mice and increase their chances of survival from LPS-induced shock. They can inhibit macrophage recruitment to the liver and lungs, thus protecting against tissue injury<sup>5)</sup>.

In the field of dental science, polyP turned out to aid in removing stains on the dental surface while exerting an anti-deposition effect on the stains. However, these effects are dependent on the molecular weight of PolyP<sup>1)</sup>. Ultraphosphate, a highly condensed polyP with a three-dimensional structure, has been shown to be particularly capable of removing stains and preventing deposition<sup>6)</sup>.

In this study, we used the powder form of hydroxyapatite, which is a constituent of the enamel, to assess the degree of polymerization (molecular

weight) of polyP that is effective in removing stain and preventing deposition.

In addition, the polyP coating on the tooth surface may protect the tooth surface not only from the effect of stains but also from the effect of other chemicals.

Particularly, it may be possible to suppress the demineralization of the tooth surface by an acidic substance or the like by coating. Therefore, we evaluated whether acid-induced demineralization could be suppressed when the surface was pretreated with a high-coating-force molecular-weight polyP, using the ivory plates obtained after the formation of dentin impressions.

## **Materials and Methods**

### **1. Materials**

Materials and reagents used in experiments are listed in Table 1. Sodium polyP of various molecular weights was prepared by separating sodium metaphosphate (Taihei Chemical Industry, Osaka, Japan) based on their molecular weights and differences in the solubility in ethyl alcohol<sup>7)</sup>. Consequently, three types of polyP samples with average degrees of polymerization of 8.1 (polyP8.1), 9.7 (polyP9.7), and 18 (polyP18) were obtained. PolyP60, with an average degree of polymerization of 60 (i.e., 60 phosphate residues), was prepared as described<sup>7)</sup>. Tripolyphosphate, with a degree of polymerization of 3, was purchased from Taihei Chemical Industry,

Osaka, Japan. Sodium pyrophosphate, which was used as the molecular weight marker, was purchased from Fujifilm Wako Pure Chemical Corporation, Osaka, Japan. Hydroxyapatite powder used in stain removal and anti-deposition studies was prepared by BioRad (BioRad Japan, Tokyo, Japan). Commercially available tea and coffee were used to color hydroxyapatite. Artificial saliva, Saliveht Aerosol (Saliveht) was manufactured by Teijin Pharma Co., Ltd, Tokyo, Japan. A 40 mm x 30 mm square-shaped ivory plate (Zogedo, Registration Number 00427, Kyoto, Japan) was used to evaluate the efficiency of the suppression of demineralization. The ivory plates were polished in the order #80, #120 and #240 abrasive paper, and the entire plate was smoothened before being used in the experiment. Phosphoric acid (40% (w/w), Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) was used for etching the dentin discs. To evaluate the suppression of demineralization, polyP9.7 was dissolved in Saliveht to a final concentration of 1% (w/v).

## **2. Determination of the degree of polymerization of polyP**

The degree of polymerization of polyP8.1, polyP9.7, and polyP18 were measured by gel filtration chromatography using a high performance liquid chromatograph (Ohpak SB-803 HQ, 8 mm x 300 mm, Shodex gel filtration column). The mobile phase was 0.1 M NaCl; the flow rate and column

temperature were maintained at 1 mL/min and 25 ° C, respectively. Signals were detected by a refractive index detector. Molecular weight was analyzed using polyP with a degree of polymerization of 60, 14, 3 (tripolyphosphate), and 2 (sodium pyrophosphate) as molecular weight markers. The respective elution times were 11.87, 12.81, 13.85, and 14.16 min. The elution times in the gel-filtration chromatography of polyP8.1, polyP9.7, and polyP18 were 13.17, 13.05, and 12.63 min, respectively. The logarithm of these values and the molecular weight markers were plotted to obtain the line, and the degree of polymerization of polyP was calculated from the approximate formula.

### **3. Evaluation of stain removal and prevention of stain deposition using hydroxyapatite powder**

Concentrated tea 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. For testing the stain removal efficiency, 30 mg of hydroxyapatite powder that was stained by concentrated tea and coffee solution was put into a 2 mL centrifuge tube, and 1 mL of each test solution containing purified water (negative control) or polyP of

various chain lengths was added into the tubes. Then, the centrifuge tube was tipped over for 1 min for mixing the components, and the hydroxyapatite was separated from polyP solution by centrifugation ( $1,500 \times g$  for 2 min). After the supernatant was removed, 10 mL of purified water was added to the hydroxyapatite pellet, and the pellet was washed. After repeating the washing step, each suspension was transferred into a 96-multi-well plate, and the color of hydroxyapatite powder was scanned from the bottom of the multi-well plate using an image scanner. Then, the image was inverted to be negative, and the color density was calculated using an image analysis program.

For testing the prevention of stain deposition, 30 mg of hydroxyapatite powder was put into a 2 mL centrifuge tube, and 1 mL of each test solution containing or purified water (negative control) or polyP of various chain lengths was added into the tubes. The tube was inverted to mix the components and then centrifuged at  $1,500 \times g$  for 2 min. The supernatant was removed and the resultant hydroxyapatite pellets were washed with 2 mL of purified water. The supernatant was removed again. This washing step was repeated twice.

Following this, 1 mL of concentrated tea and coffee extract was added to each tube, and the tube was inverted for 1 min. After the supernatant was removed, the hydroxyapatite pellet was washed with 2 mL of purified water. Finally, 2 mL of purified water was added to the hydroxyapatite pellet and inverted for 1 min



to prepare a hydroxyapatite suspension. Then, the suspension was transferred into a 96-multi-well plate, and the color of the hydroxyapatite powder was scanned from the bottom of the multi-well plate using an image scanner. The extent of prevention of stain deposition was calculated from the scan data. Statistics of each result were verified by Tukey's test.

#### **4. Suppression of demineralization by PolyP9.7**

The process of etching on dentin discs is shown in Fig. 1. The dentin discs were sectioned in half and immersed either in only 1% (w/v) polyP9.7-Salivest solution or Salivest solution for 10 min. Then, they were washed with water and dried. After drying, holes of 6 mm diameter were drilled in the lids of 1.5 mL microtubes, which were fixed on the dentin plane by UV resin. The indented part of the lids were filled with approximately 0.2 mL of 40% (w/w) phosphoric acid and etched at room temperature for 20 h. Etching was performed on identical ivory plates in triplicate. After etching, the remaining 40% (w/w) phosphoric acid was washed out with water, and the lid of the microtube was removed and dried. Following this, cross-sectional shapes and demineralized depths of the demineralized areas were observed using light microscopy (VHX-5000, KEYENCE, Osaka, Japan) at  $200\times$  magnification. Depth of the three center sites of the measuring point and six edge sites per spot were

measured as indicated in Fig. 1, and the average of each was considered as the depth of the demineralized area. Statistics of each result were verified by t-test.

## Results

### 1. Effect of polyP chain length on stain removal

The efficiency of prevention of stain deposition is shown in Fig. 2 (a). Short chain polyP9.7, with 9.7 phosphate residues, was the most effective in removing stains. The stain removal activity was low with tripolyphosphate, and almost no stain removal activity was observed with polyP60, polyP18 also showed considerably weaker stain removal activity ( $p < 0.05$ ). Thus, the optimum chain length for effective stain removal was 9.7 phosphate residues.

The efficiency of prevention of stain deposition is shown in Fig. 2 (b). Similar to that observed for the stain removal activity, polyP9.7 was found to be the ideal polymer for preventing stain deposition. Both polyP8.1 and polyP18 generated a polyphosphate chain of 9.7 residues, which was almost equally effective in preventing deposition ( $p < 0.05$ ). Therefore, the anti-deposition properties of polyP with 8.1 to 18 residues differed only slightly. Tripolyphosphate was only fourth in the sequence as an effective material for preventing deposition, with the maximum anti-deposition properties exhibited by polyP9.7. Poly60 was about ninety percent effective. Thus, the anti-deposition efficacy of

tripolyphosphate, which is commonly used in common dentifrices, is considerably lower than that of polyP having a degree of polymerization of 8.1 or more ( $p < 0.05$ ).

## **2. Efficiency of suppression of demineralization by polyP9.7 using ivory plates**

Fig. 3 shows the cross-sectional images of the demineralized dentin analyzed by light microscopy, and Fig. 4 shows the results of depth measurement. The depth of the demineralized part was evaluated by connecting the highest parts at both ends of the hole with a line, setting a measuring point between the central part and both ends, and measuring the length of the perpendicular line dropped from the connected line to the measuring point. Cross-sectional images of the dentin demineralized in 1% (w/v) polyP9.7-Salivert solution and Salivert showed raised features in the central part. Thus, the center and edge were analyzed separately. The results showed that the demineralization depth was significantly shallower in the spots treated with polyphosphate in both center (3 points) and edge (6 points) ( $p < 0.05$ ).

## **Discussion**

PolyP, which is commonly used in dentifrices, is mainly a tripolyphosphate. It is a short molecule with only three phosphate residues. However, polyP longer

than tripolyphosphate has been shown to be more effective in removing stains and preventing deposition<sup>1)</sup>. However, very long polyPs were found to be less effective in removing stains and preventing deposition. In this study, we prepared polyP with finely divided molecular weights and determined the molecular weight (or degree of polymerization) that was most effective for removing stain and preventing deposition (Table 2).

The mechanism of stain removal and prevention of deposition is believed to involve the binding of the negatively charged polyphosphate to the positively charged portion of apatite by its charge in the enamel surface<sup>1,6,8,9)</sup>. It is believed that the binding of polyP to the apatite on the tooth surface is stronger than that of the colored material from which the stain is derived to the apatite. Thus, the stain is easily removed by polyP<sup>8)</sup>. It is considered that the strength of the bond between apatite and polyphosphate depends on the molecular weight of polyP, and polyP of a specified degree of phosphate polymerization is highly effective in removing stains. The findings of this study suggest that a polymer with only 3 residues or 18 or more residues has weaker binding to apatite compared to polyP in which the degree of polymerization is 8 to 10. In polyP with a degree of polymerization of 60, the binding to apatite might be rather weak, as apparent from the low stain removal activity. In addition, it is considered that the deposition is prevented because polyP bonded with apatite continues to bind to

the tooth surface and coats it, and the polyP coating does not allow any new coloring material to bind to the tooth surface. The anti-deposition and stain removal activities of tripolyphosphate are also low because of its relatively weak binding to apatite. However, polyP in which the degree of polymerization ranged from 8 to 18 were equally effective in preventing the deposition, which was different from the case of stain removal. PolyP60 of polymers was also significantly lower than that of stain - removed polymers, but it did not decrease to the level where little effect was observed as in the case of stain - removed polymers. These results suggest that the binding affinity between polyP and apatite depends on the molecular weight of polyP and is not affected as much as in the case of elimination, in terms of the anti-deposition efficacy of polyP with different molecular weights.

Ultraposphate which is highly condensed phosphate polymer and its negative charge density is higher than that of linear polyP also has both strong stain removal and anti-deposition activities<sup>6)</sup>. This suggests that the binding affinity between polyP and apatite is dependent on the level of negative charge of polyP molecule. However, longer chain polyP which charge is higher than shorter chain does not have higher activity in stain removal and anti-deposition. Taking into account of this result, the binding affinity could not simply be explained by the level of the negative charge. Some different mechanism might be involved in

binding between polyP and apatite.

Our results suggest that the shortest polyP, tripolyphosphate, has low stain-removal efficiency as well as low anti-deposition activity and is not optimal for use in dentifrices. It would be more effective to use polyP with a degree of polymerization of about 9 to 10 in dentifrices.

Previously, Ogawa et al. showed that polyP treated enamel surface mildly demineralized by K-etchant by SEM observation whereas severe demineralization was observed in enamel surface which was treated by artificial saliva. This report also showed that polyP inhibited  $\text{Ca}^{2+}$  release from enamel surface<sup>10</sup>). In this study, we confirmed this previous report and polyP9.7, which had the highest stain removal and anti-deposition efficacies, significantly suppressed the demineralization by phosphate by coating the dentin (Table 3). By utilizing the fragments of extra ivory remaining after creating the ivory stamp, ivory plates larger than human dentin could be used for the experiment, and the demineralization reaction could be reproduced in a uniform ivory surface. It is considered that by pretreating the surface of this ivory plate by polyP, the surface is coated, and demineralization due to phosphate is suppressed. This reveals that the coating with polyP not only prevents the deposition of stains but also suppresses the acid-induced demineralization. This also indicates the possibility of suppressing the demineralization of the tooth surface by using a

dentifrice containing polyP with degree of polymerization of 9 to 10, which binds more strongly to the tooth surface. Therefore, dentifrices containing polyP with a degree of polymerization of 9 to 10 may be preferred to prevent caries.

## **Conclusion**

The results of this study suggest that polyP9.7 is highly effective in removing stain and preventing stain deposition on the tooth surface. It also suppresses demineralization by binding to the tooth surface. In future, polyP9.7 is expected to be an effective material for preventing caries.

## **Conflict of Interest**

The authors declare that there are no potential conflicts of interest with regard to the publication of this article.

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ステイン除去および沈着防止に最適な鎖長の無機ポリリン酸による脱灰抑制効果

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## 抄録

目的：ポリリン酸塩(polyP)は、リン酸塩が鎖状に重合された高分子物質であり、生体内に存在し様々な生理学的機能を有していることがわかっている。歯科領域では、歯の表面に結合して着色を落とし沈着を防止することが知られている。本研究では、polyP の重合度によって歯の表面の着色除去および付着防止の効果が異なるかどうかについて検討を行った。また、歯の表面に結合した polyP が酸による脱灰を抑制するかどうかについても検討を行った。

材料と方法：紅茶とコーヒー抽出液で染色されたハイドロキシアパタイト粉末を遠心チューブに入れ、様々な鎖長の polyP または精製水を入れて溶液を作製した。1 分間混合し、1500×g で 2 分間の遠心分離によって溶液を分離させ上澄みを除去した。さらに、染色していないハイドロキシアパタイト粉末を同様の方法で polyP または精製水を入れて溶液を作製し、混合後遠心分離によって溶液を分離させ上澄みを除去した。そして茶とコーヒー抽出液で染色した各々を精製水で洗浄し各懸濁液を 96 マルチウェルプレートに移した。イメージスキャナーを使用して各ハイドロキシアパタイト粉末の色をスキャンし、色濃度を計測して着色の除去効果と沈着防止効果を評価した。

次に、象牙のプレートを耐水研磨紙によって試片を滑沢にした。重合度 9.7 の polyP (polyP9.7) をサリベートに溶解して 1%に調整した溶液またはサリベ

トに 10 分間浸漬後、水洗乾燥させた。その後試片を 40%リン酸で 20 分間処理後、水洗乾燥させた。乾燥後脱灰部分の断面深さを光学顕微鏡により観察した。

結果：polyP9.7 は、着色の除去および沈着防止の両方に高い効果を示した。歯磨剤に一般的に使用される 3 つのリン酸塩残基が重合したトリポリリン酸塩と重合度 60 の polyP では、polyP9.7 前後の分子量の polyP と比較して着色の除去および沈着防止効果の両方が低いことがわかった。また、polyP9.7 で処理された象牙の表面は、リン酸エッチングによる脱灰が抑制された。

結論：本研究の結果、polyP9.7 は歯面への着色の除去および沈着防止の両方に高い効果を示し、歯の表面に結合することで脱灰を抑制することが示唆された。

キーワード：ポリリン酸，ポリマーサイズ，着色防止，脱灰

## Figure legends

### **Fig. 1 Demineralization using ivory plates**

A rectangular ivory plate was used. Artificial saliva was applied in half of the plate, and artificial saliva containing 1% polyP9.7 was applied in the other half. Subsequently, a 6-mm diameter hole was placed on the lids of the microtubes so that a certain area could be treated with phosphate. Phosphate solution (40%) was added to each hole to perform etching at room temperature for 20 h. The plates were then washed with water to measure the depth of the hole formed in the etched portion by light microscopy (VHX-5000). For the hole formed by demineralization, an image of the cut surface was obtained from three directions in A, B, and C (see Fig. 3); 3 points near center and at both ends were set as the measuring point on the cut surface, and the depth between them was measured.

### **Fig. 2 Evaluation of stain removal and prevention of deposition using hydroxyapatite**

(a) Stain removal experiment and (b) Experiment to evaluate the prevention of deposition. Water in (a) is that in which hydroxyapatite powder dyed by tea and coffee concentrate is washed with water only, and water in (b) is that in which uncolored hydroxyapatite is washed with water only and then treated with tea and coffee concentrates. The stain removal rate is shown as the removal ratio,

with water set at 0% removal rate. Therefore, higher the value, lighter will be the color. Water was set at 0% of the rate for color protection in (b), and the value was calculated so that the value corresponding to less coloration became higher.

**Fig. 3 Cross-sectional view of a hole formed by demineralization on an ivory plate.**

Cross-sections of the hole formed by demineralization shown in Fig. 1 were observed by microscopy. (a) is the part pretreated with polyphosphate and (b) is the part pretreated with only the artificial saliva of the control. The depths of center and edge 2 points were measured by optical microscopy (VHX-5000).

**Fig. 4 Suppression of demineralization by polyP**

Depth of the demineralized area was significantly shallower in the dentin pretreated with polyP9.7 at both center and edge measuring points, as shown in Fig.1 and 3.

Fig. 1

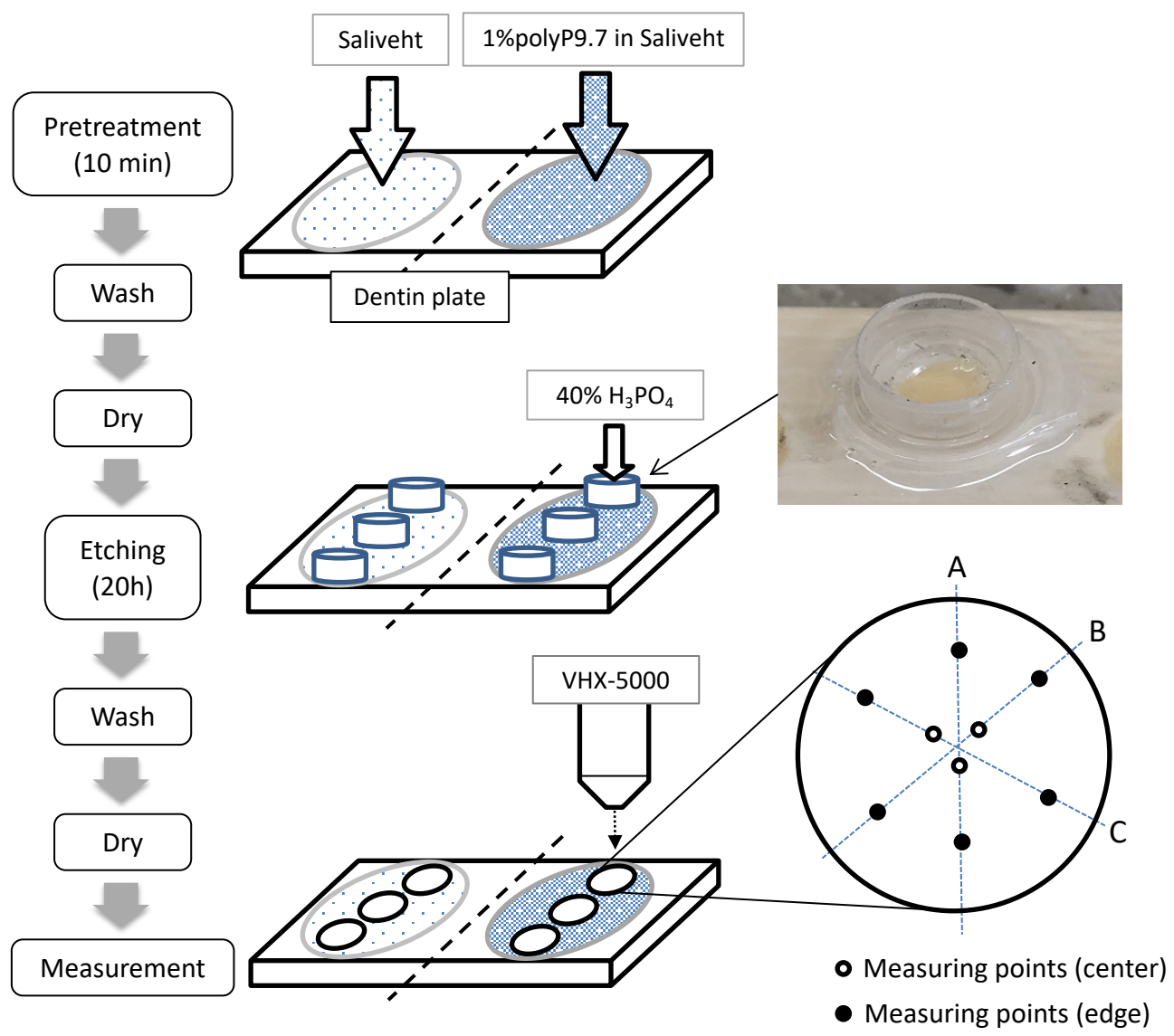
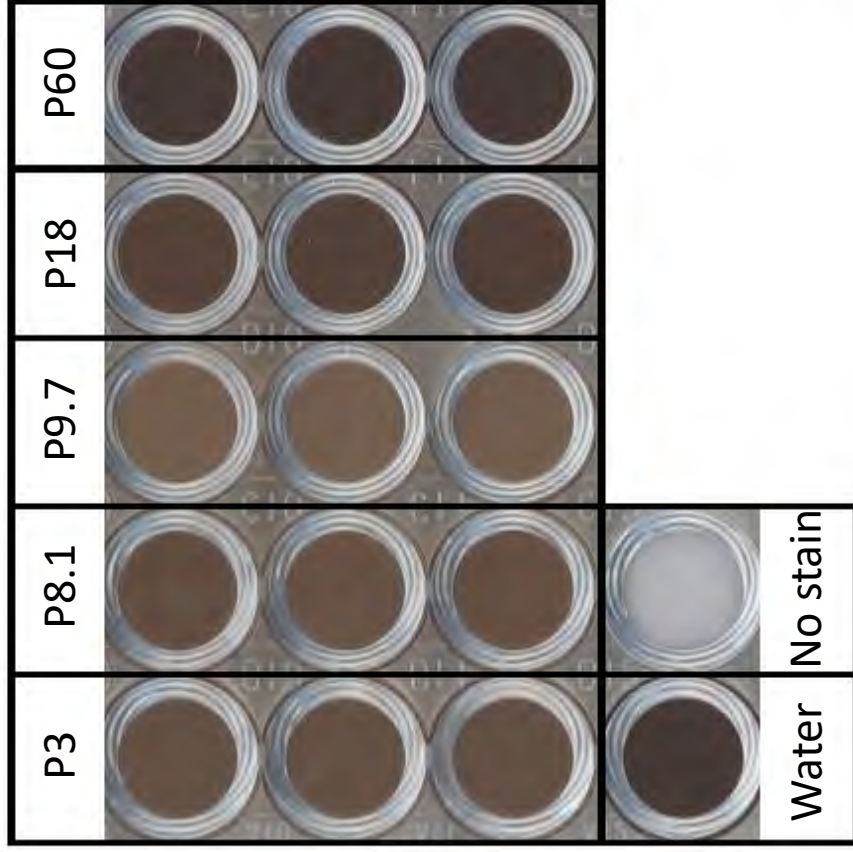


Fig. 2

**(a) Stain removal**



**(b) Prevention of stain deposition**

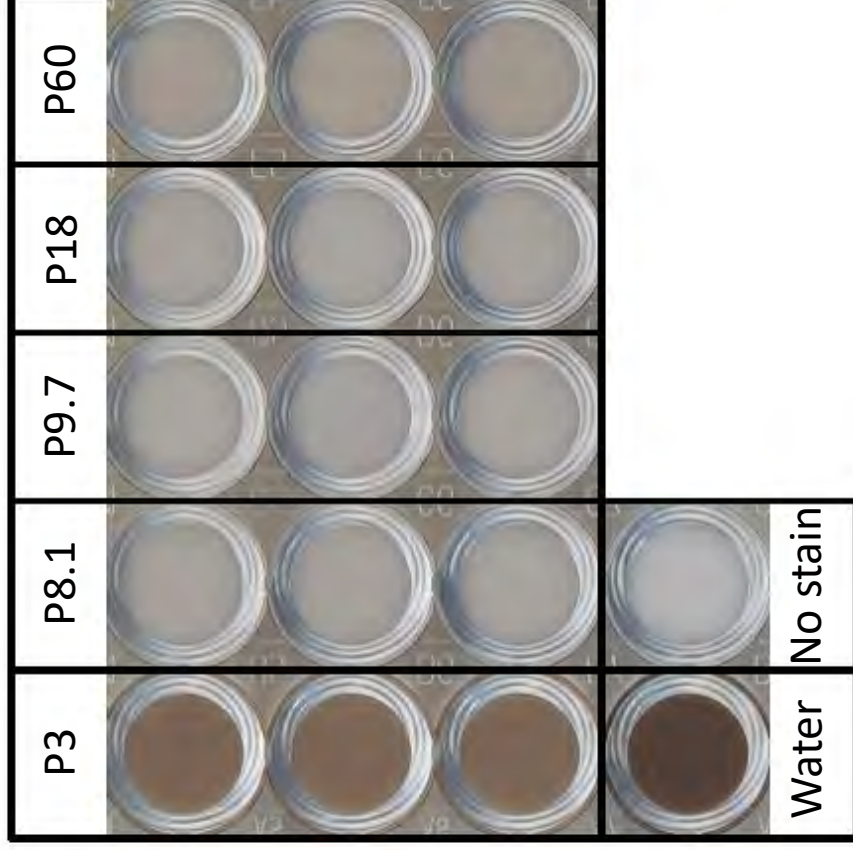




Fig. 3

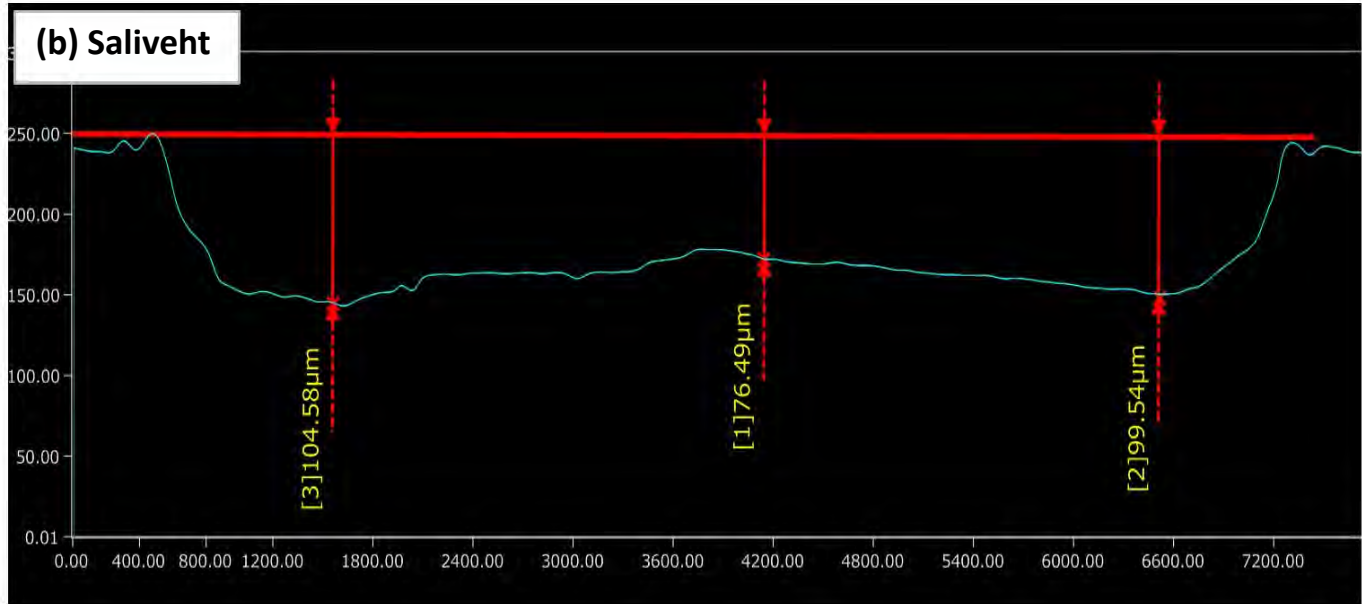
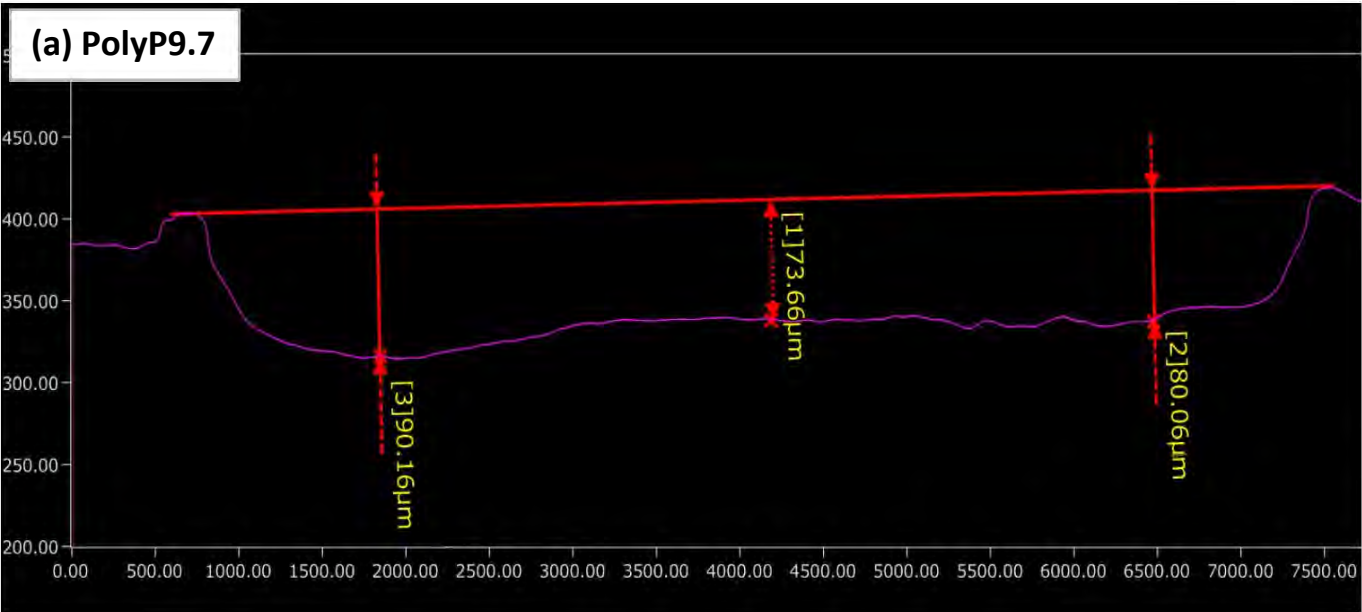
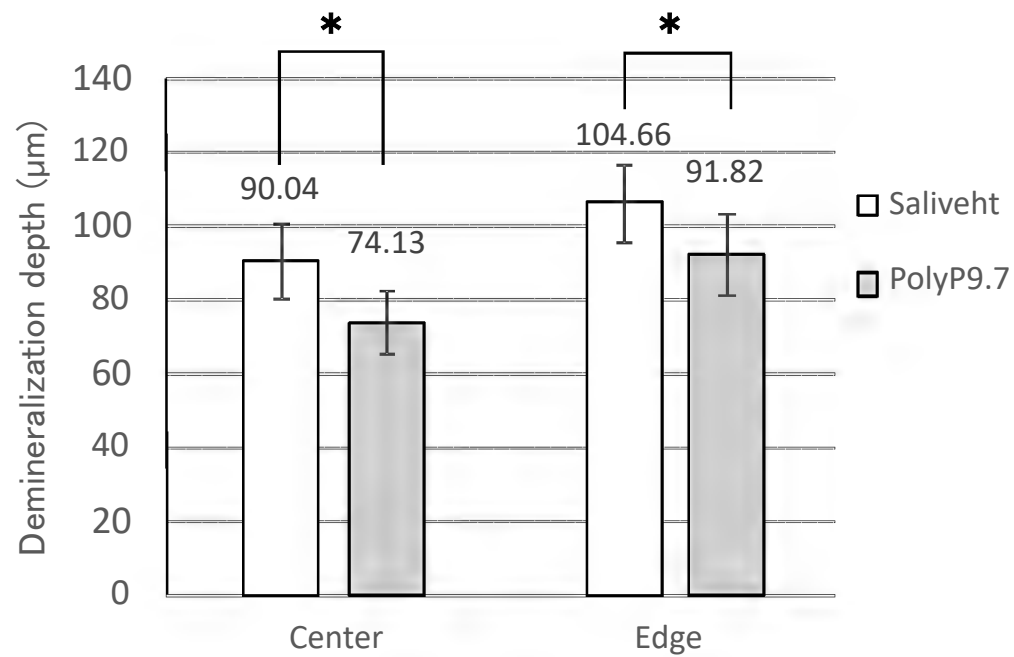


Fig. 4



\* t-test :  $p < 0.05$

Table 1 Materials and reagents used in experiment

Materials	Working concentrations	Company
Sodium metaphosphate	polyP8.1	Taihei Chemical Industry, Osaka, Japan
	polyP9.7	
	polyP18	
	polyP60	
Tripolyphosphate		
Sodium pyrophosphate	1%(w/v)	Fujifilm Wako Pure Chemical Corporation, Osaka, Japan
Hydroxyapatite powder	—	BioRad Japan, Tokyo, Japan
Lipton tea bag	—	Unilever Japan K.K., Tokyo, Japan
Nescafe gold blend coffee	—	Nestle Japan,Ltd., Hyogo, Japan
Saliveht Aerosol	—	Teijin Pharma Co., Ltd, Tokyo, Japan
Dentin plate	—	Zogedo, Kyoto, Japan
Phosphoric acid	40% (w/w)	Fujifilm Wako Pure Chemical Corporation, Osaka, Japan
Whatman No. 5B filter paper	—	Whatman International Co., Ltd., Maidstone, UK

Table 2 Stain removal rate (a) and stain deposition prevention rate (b) by various polyP using hydroxyapatite

Samples	(a) Removal				(b) Prevention			
	Removal ratio (%)	Mean	SD	Significance $p < 0.05$	Prevention ratio (%)	Mean	SD	Significance $p < 0.05$
Water	0.0				0.0			
No stain	100.0				100.0			
PolyP3	19.4	19.8	0.36		31.9	33.1	0.98	
	20.0				33.7			
	20.0				33.6			
PolyP8.1	19.1	20.3	1.25		82.7	83.0	1.07	
	21.5				82.1			
	20.2				84.2			
PolyP9.7	30.7	30.8	0.68		85.0	84.6	1.03	
	31.5				83.4			
	30.2				85.4			
PolyP18	7.9	7.1	1.00		83.1	83.8	1.37	
	7.5				85.4			
	6.0				83.0			
PolyP60	0.0	0	0		76.2	76.2	0.97	
	0.0				77.2			
	0.0				75.3			

The image shown in Fig. 2 was quantified and statistical processing by Tukey's test was performed (n=3). The two samples that were significantly different were shown in significance section by the line.

Table 3 Suppression of demineralization by 1%polyP9.7

(a) PolyP9.7

	Demineralization depth (μm)		
Measuring point	Center	Edge	
A	60.43	71.75	77.11
	73.66	80.06	90.16
	63.75	82.5	75.75
B	78.48	97.1	110.16
	69.85	95.85	98.71
	79.21	103.05	100.27
C	70.92	81.96	88.28
	84.14	92.85	103.09
	86.73	102.48	101.6
Mean	74.13	91.82	
SD	8.35	11.00	

(b) Saliveht

	Demineralization depth (μm)		
Measuring point	Center	Edge	
A	76.49	99.54	104.58
	90.96	102.27	76.62
	80.46	100.9	97.37
B	102.76	119.82	116.34
	101.23	112.27	116.13
	90.83	107.22	106.91
C	82.19	102.06	91.33
	79.96	99.93	102.33
	105.52	105.25	123.09
Mean	90.04	104.66	
SD	10.36	10.55	

Depth of demineralized dentin in each measuring point shown in Fig.1 was measured by light microscopy (VHX-5000) .