detail elsewhere (Hirotomi et al. 2002, Ogawa et al. 2002). A written invitation was sent to all individuals aged 70 years ($n = 4542$; 2099 males and 2443 females) who were registered as the citizens of Niigata city, Japan in 1998 to take part in this survey once the Ethical Committee of the School of Dentistry, Niigata University, approved the research protocol of the survey. After sending a second request, 79.5% (3695) of the population consented to participate in the survey. Having considered the resources available, out of the positive respondents 600 individuals were randomly recruited into a cross-sectional community-based study so as to have an approximately equal number of males (306) and females (294). Informed consent was obtained from all subjects prior to the investigation.

Four calibrated dentists conducted the intra-oral examination involving assessment of CAL, probing depth (PD) and bleeding on probing (BOP) using mouth mirrors and pressure-sensitive TPS Probe® (Vivacare, Schaan, Liechtenstein) under artificial light. All teeth present including third molars were probed at six sites per tooth, namely, mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and mesio-lingual and the recordings were rounded up to the nearest whole millimetre. Calibration of the examiners was carried out before and during the survey and the examiner consistency ranged from 0.56 to 0.92 as indicated by κ statistic. Information pertaining to smoking habits as well as oral hygiene practices was obtained by means of a personal interview, whereas blood samples were sent to the laboratory in order to evaluate the serum vitamin C levels using high-pressure liquid chromatography (HPLC) method and random blood sugar levels.

Statistical analyses were carried out by means of STATA statistical software package. Throughout the analysis, CAL, the dependent variable, was considered as a continuous variable and the unit of analysis was the subject. Among independent variables, serum vitamin C level was a continuous variable while gender (male:female), smoking status (current smoker: ex-smoker: non-smoker), diabetic status (random blood sugar (RBS) <140 mg/dl: $\geqslant 140$ mg/dl), frequency of tooth cleaning (<2/day: $\geqslant 2$/day) and the number of teeth present (<20 teeth: $\geqslant 20$ teeth) were treated as categorical variables. To compare the difference between two means Student's *t*-test was employed while one-way ANOVA combined with Bonferroni's test was used where necessary to compare more than two means. Moreover, the association between two continuous variables was determined by means of Pearson's correlation technique. Finally, having excluded the presence of multicolinearity, the independent variables that showed significant relationships with CAL at bivariate level were included in a multiple linear regression analysis to identify the independent effect of serum vitamin C level on CAL while controlling for other confounding factors. The level of statistical significance was fixed at $p \leqslant 0.05$.

Results

The current analysis was limited to 413 dentate subjects in whom the data for CAL as well as serum concentrations of vitamin C were available. The serum levels of vitamin C ranged from 0.2 to 22.6 mg/l with a mean of 7.21 (results not shown). Table 1 shows the periodontal characteristics of the sample. Accordingly, the mean CAL was 3.26 mm (SD = 1.05) while the mean PD was 2.1 mm (SD = 0.58). The mean percentage of BOP per person was 7.3 (SD = 8.6). It was also observed that 62.6% of subjects had at least one site with CAL of $\geqslant 6$ mm, whereas almost one-third (33%) of the sample exhibited PD of $\geqslant 6$ mm at least on one site (results not shown). Indeed, there was no significant relationship between serum vitamin C levels and either PD or BOP (results not shown) and consequently, it was decided to confine the present analysis to explore the relation between the independent variables including serum vitamin C concentration and CAL. Table 2 depicts the associations between CAL and the independent variables including gender, smoking status, diabetic status, brushing frequency, the number of teeth present and serum vitamin C concentrations. As revealed by Student's *t*-test, males had significantly greater CAL (mean = 3.54 mm; SD = 1.2) than females (mean = 2.96 mm; SD = 0.8 mm) while those who had 20 or more teeth showed significantly lower CAL (mean = 2.92 mm; SD = 0.8) compared with the subjects with <20 teeth (mean = 3.69 mm; SD = 1.2). It is also apparent that CAL was significantly higher in the

Table 1. Periodontal characteristics of the sample

Variable	Mean	SD
CAL (mm)	3.26	1.05
PD (mm)	2.10	0.58
BOP (%)	7.30	8.60

CAL, clinical attachment loss; PD, probing depth; BOP, bleeding on probing

Table 2. Relationships between CAL and independent variables at bivariate level

Independent variables	CAL Mean (SD)	p
Gender*		
male ($n = 215$)	3.54 (1.2)	<0.00005
female ($n = 198$)	2.96 (0.8)	
Number of teeth present*		
<20 ($n = 184$)	3.69 (1.2)	<0.00005
$\geqslant 20$ ($n = 229$)	2.92 (0.8)	
Brushing frequency*		
<2/day ($n = 145$)	3.40 (1.1)	<0.05
$\geqslant 2$/day ($n = 268$)	3.20 (1.0)	
Smoking status†		
current smoker ($n = 71$)	3.82 (1.3)	<0.00005‡
ex-smoker ($n = 137$)	3.45 (1.1)	
non-smoker ($n = 205$)	2.95 (0.8)	
Diabetic status*		
RBS <140mg/dL (n = 356)	3.22 (0.1)	<0.05
RBS $\geqslant 140$ mg/dl ($n = 57$)	3.52 (0.2)	
Serum ascorbic acid§	$r = -0.23$	<0.00005

CAL, clinical attachment loss; RBS, random blood sugar. *Student's *t*-test.
†One-way ANOVA.
‡Bonferroni's test: $3.82 > 3.45 > 2.95$ ($p < 0.05$).
§Pearson's correlation.

Table 3. Multiple linear regression model for CAL with significant variables

Independent variables	Coefficient	SE	p	95% CI	
Serum ascorbic acid	− 0.04	0.02	<0.05	− 0.06	− 0.005
Current smoker	0.57	0.17	<0.005	0.24	0.92
Gender (male = 0)	− 0.30	0.04	<0.05	− 0.58	− 0.01
Teeth present (<20 = 0)	− 0.73	0.09	<0.0005	− 0.92	− 0.55
Constant	3.83	0.25	<0.0005	3.46	4.20

$R^2 = 0.26$; $p < 0.00005$; SE, standard error; CI, confidence interval.

subjects who brushed their teeth <2/day than in those who used a toothbrush ⩾2/day. One-way ANOVA combined with Bonferroni's post hoc test disclosed that current smokers had significantly worse CAL (mean = 3.82 mm; SD = 1.3) in comparison with both ex-smokers (mean = 3.45 mm; SD = 1.1) and non-smokers (mean = 2.95 mm; SD = 0.8), whereas subjects with RBS <140 mg/dl showed significantly lower CAL than those who had RBS⩾ 140 mg/dl. Furthermore, there was an inverse relationship between serum vitamin C concentration and CAL as indicated by Pearson's correlation technique ($r = -0.23$; $p < 0.00005$).

All the independent variables that demonstrated significant effects on CAL at bivariate level, namely, serum vitamin C, smoking status, diabetic status, gender, toothbrushing frequency and the number of teeth present were included in a multiple linear regression analysis and the variables that remained significant in the final model are shown in Table 3. Accordingly, it was found that serum vitamin C had a significant effect on CAL (correlation coefficient = − 0.04; $p < 0.05$), which was independent of the other covariates including smoking and random blood sugar levels. The independent variables in the final model explained 26% of the variance in CAL ($R^2 = 0.26$).

Discussion

The findings of this cross-sectional study suggested that there was a weak but significant association between the level of serum vitamin C and periodontitis as measured by CAL notwithstanding the effect of established risk factors for periodontitis such as smoking and diabetes mellitus in this elderly population. In other words, we observed an inverse independent relationship between serum vitamin C concentration and CAL – the lower the level of serum vitamin C the higher was the periodontal attachment loss. This was indicated by the relatively smaller correlation coefficient of serum vitamin C (correlation coefficient = − 0.04): CAL in subjects with lower serum vitamin C levels would only be 4% greater compared with those who had higher serum vitamin C concentrations regardless of other covariates.

Notwithstanding the fact that our study was confined only to the elderly and that we evaluated serum ascorbic acid concentration instead of dietary intake of vitamin C, the present findings may be comparable to those of others (Ismail et al. 1983; Nishida et al. 2000) who observed a weak albeit statistically significant relationship between dietary vitamin C and periodontal disease in the US adults. In particular, the latter (Nishida et al. 2000) found that even after controlling for the effects of age, gender, smoking and gingival bleeding, the level of periodontitis in subjects with a lower dietary intake of vitamin C was 1.19 times greater than that of individuals with a higher intake of vitamin C while the former (Ismail et al. 1983) did not adjust for such factors. More recently, Pussinen et al. (2003) who investigated the relation between plasma vitamin C levels and serology of periodontitis in Finnish and Russian men observed that the antibody levels to *Porphyromonas gingivalis* were inversely correlated with plasma vitamin C concentrations ($r = -0.22$; $p < 0.001$) and this association remained significant in a linear regression model even after controlling for confounding factors. Accordingly, they concluded that lower concentrations of plasma vitamin C might increase the risk of periodontitis, which is in accord with the present findings.

Various researchers have proposed several plausible biological mechanisms while attempting to explain how ascorbic acid could affect the healthy tissues in humans as well as in animals (Goetzl et al. 1974, Alfano et al. 1975, Boxer et al. 1979, Dallegri et al. 1980, Alvares et al. 1981, Alvares and Siegel 1981, Berg et al. 1983, Leggot et al. 1986, Jacob et al. 1987, Nakamoto et al. 1984). It has been established that ascorbic acid plays a major role in the synthesis of collagen, especially the hydroxylation process, helix formation and cross-linking of collagen molecules (Alfano et al. 1975, Berg et al. 1983). Collagen is undoubtedly an essential component of human tissues including periodontium and required in wound healing as well as periodontal regeneration and maintaining the integrity of the gingival vasculature. Also, there are several lines of evidence to suggest that vitamin C affects chemotaxis as well as phagocytosis of polymorphonuclear leucocytes and thereby influences the host-immune reactions (Alfano et al. 1975, Boxer et al. 1979, Dallegri et al. 1980, Patrone et al. 1982). Moreover, some researchers have hypothesized that ascorbic acid might express an antihistamine effect through direct detoxification of histamine or indirectly affecting the histamine breakdown and this in turn would retard gingival inflammation (Nakamoto et al. 1984) whereas others (Alfano et al. 1975, Alvares and Siegel 1981) reported that the deficiency in vitamin C levels could be linked to increased permeability of gingival mucosa, which allows easy passage of microbial and other noxious products into the periodontium. It has also been shown that ascorbic acid demonstrates antioxidant properties and therefore is considered one of the constituents of antioxidant defence mechanism in human body (Nishida et al. 2000). Tobacco, especially, cigarette smoke contains various oxidants that cause tissue damage and consequently smokers do require a higher serum concentration of vitamin C than non-smokers do (Kallner et al. 1981, Nishida et al. 2000). Moreover, given that avitaminosis C and diabetes mellitus share some common pathological characteristics such as raising of oxidant stress (Schmidt et al. 1996) and collagen degradation (Kjersem et al. 1988) in gingival tissues, it has been hypothesized that vitamin C might play a critical role in the aetiology and/or progression of periodontitis in type I diabetics (Aleo 1981, Nishida et al. 2000). In this connection, it is also noteworthy that both diabetes as well as smoking, which are regarded as well-established risk factors for periodontitis, may contribute to oxidative tissue damage and given the antioxidant properties of vitamin C, it might act as a potential moderator in both smoking– and diabetes–periodontal relationships – this would be an interesting hypothesis to be tested in future investigations. Although exploring such biological mechanisms and/or hypotheses was beyond the scope of our study, the association between serum vitamin C levels and CAL that was observed even after controlling for known risk factors such as smoking and diabetes mellitus in the present study could be explained on the basis of these mechanisms. This is further augmented by the fact that such mechanisms could be connected to pathogenesis of periodontal disease, which is of inflammatory nature and which may be mediated through the tissue damage caused by interaction of microbial noxious products and host-immune response. However, it should also be highlighted that the observed relationship is rather weak and that these biological phenomena involving vitamin C have neither been clearly understood nor well defined (Leggot et al. 1986, Nishida et al. 2000, Pussinen et al. 2003).

This study population comprised non-institutionalized elderly people who were active, living independently and willing to participate in the survey. It has been shown that the elderly who are institutionalized, less active and dependent are at a higher risk for periodontal disease than those who are active and independent (Hirotomi et al. 2002, Ogawa et al. 2002). Besides, the mean serum concentration of vitamin C in this sample was rather high and the serum vitamin C level in only about 4% of subjects (results not shown) was below the reference range for Japanese elderly (Sakai et al. 1998). In this context, the current sample might be considered a biased one and therefore the findings should be interpreted with caution.

In conclusion, the results suggest that the serum vitamin C levels in this elderly population weakly correlate with periodontitis as evaluated by CAL notwithstanding the effects of smoking, diabetes mellitus, gender, oral hygiene practices or the number of teeth present. Moreover, considering the cross-sectional nature of the study design it was almost impossible for us to ascertain lifetime changes in either the vitamin C intake or serum vitamin C levels in this population. Because of these facts and also given the relatively low correlation observed between serum vitamin C and CAL in the current analysis, we could neither confirm an unambiguous cause–effect relationship between serum vitamin C and periodontitis nor a substantial beneficial effect of vitamin C on periodontal health. All in all, the association observed here could not be a straightforward one but it would be plausible that the serum vitamin C might be inflicting a moderating influence on periodontitis through the established risk factors such as smoking and diabetes, as it was mentioned hitherto. Consequently, it warrants further investigations, in particular, longitudinal studies and experimental designs to explore the actual role of vitamin C in the aetiology and/or progression of periodontal disease. It should also be highlighted that this elderly cohort will be followed up for several years and thus, we intend to analyse the serum vitamin C–periodontal relationship prospectively, in the same population.

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References

Aleo, J. J. (1981) Diabetes and periodontal disease. Possible role of vitamin C deficiency: an hypothesis. *Journal of Periodontal Research* **52**, 251–254.

Alfano, M. C., Miller, S. A. & Drummond, J. F. (1975) Effect of ascorbic acid deficiency on the permeability and collagen biosynthesis of oral mucosal epithelium. *Annals of New York Academy of Science* **258**, 253–263.

Alvares, O., Altmon, L. C., Springmeyer, S. & Ensign, W. (1981) The effect of subclinical ascorbic acid deficiency on periodontal health in non-human primates. *Journal of Periodontal Research* **16**, 628–636.

Alvares, O. & Siegel, I. (1981) Permeability of gingival sulcular epithelium in the development of scorbutic gingivitis. *Journal of Oral Pathololgy* **10**, 40–48.

Barros, L. & Witkop, C. J. (1963) Oral and genetic study of 160 Chileans. III. Periodontal disease and nutritional factors. *Archives of Oral Biology* **8**, 195–207.

Berg, R. A., Steinmann, B., Rennard, S. I. & Crystal, R. G. (1983) Ascorbate deficiency results in decreased collagen production: unhydroxylation of proline leads to increased intercellular degradation. *Archives of Biochemistry and Biophysiology* **226**, 681–686.

Boxer, C. A. L., Vanderbilt, B., Bonsib, S., Jersild, R., Yang, H. H. & Baehner, R. L. (1979) Enhancement of chemotactic response and microtubule assembly in human leucocytes by ascorbic acid. *Journal of Cellular Physiology* **100**, 119–126.

Dallegri, F., Lanzi, G. F. & Patrone, F. (1980) Effects of ascorbic acid on neutrophil locomotion. *International Archives of Allergy and Applied Immunology* **61**, 40–45.

Enwonwu, C. O. (1972) Epidemiological and biochemical studies of necrotizing ulcerative gingivitis and noma (cancrum oris) in Nigerian children. *Archives of Oral Biology* **17**, 1357–1371.

Enwonwu, C. O. & Endozein, J. C. (1970) Epidemiology of periodontal disease in Western Nigerians in relation to socio-economic status. *Archives of Oral Biology* **15**, 1231–1244.

Goetzl, E. J., Wasserman, S. I., Gigli, I. & Austen, K. F. (1974) Enhancement of random migration and chemotactic response of human leukocytes by ascorbic acid. *Journal of Clinical Investigation* **53**, 813–818.

Hirotomi, T., Yoshihara, A., Yano, M., Ando, Y. & Miyazaki, H. (2002) Longitudinal study on periodontal conditions in healthy elderly people in Japan. *Community Dentistry and Oral Epidemiology* **30**, 409–417.

Ismail, A. M., Burt, B. A. & Eklund, S. A. (1983) Relation between ascorbic acid intake and periodontal disease in the United States. *Journal of American Dental Association* **107**, 927–931.

Jacob, R. A., Omaye, S. T., Skala, J. H., Leggot, P. J., Rothman, D. L. & Murray, P. A. (1987) Experimental vitamin C depletion and supplementation in young men. Nutrient interactions and dental health effects. *Annals of New York Academy of Science* **498**, 333–346.

Kallner, A. B., Hartman, D. & Horning, D. H. (1981) On the requirements of ascorbic acid in man: Steady-state turnover and body pool in smokers. *American Journal of Clinical Nutrition* **34**, 1347–1355.

Kjersem, H., Hislted, J., Madsbad, S., Wandall, J. H., Johansen, K. S. & Borregaard N. (1988) Polymorphonuclear leucocyte dysfunction during short-term metabolic changes from normo- to hyperglycemia in type I (insulin dependent) diabetic patients. *Infection* **16**, 215–221.

Leggott, P. J., Robertson, P. B., Rothman, D. L., Murray, P. A. & Jacob, R. A. (1986) The effect of controlled ascorbic acid depletion and supplementation on periodontal health. *Journal of Periodontology* **57**, 480–485.

Melnick, S. L., Alvarez, J. O., Navia, J. M., Cogen, R. B. & Roseman, J. M. (1988) A case control study of plasma ascorbate and acute necrotizing ulcerative gingivitis. *Journal of Dental Research* **67**, 855–860.

Nakamoto, T., McCroskey, M. & Mallek, H. M. (1984) The role of ascorbic acid deficiency in human gingivitis – a new hypothesis. *Journal of Theortical Biology* **108**, 163–171.

Nishida, M., Grossi, S. G., Dunford, R. G., Ho, A. W., Trevisan, M. & Genco, R. J. (2000) Dietary vitamin C and the risk for periodontal disease. *Journal of Periodontology* **71**, 1215–1223.

Ogawa, H., Yoshihara, A., Hirotomi, T., Ando, Y. & Miyazaki, H. (2002) Risk factors for periodontal progression among elderly people. *Journal of Clinical Periodontology* **29**, 592–597.

Patrone, F., Dallegri, F., Bonvini, E., Minervini, F. & Sacchetti, C. (1982) Effects of ascorbic acid on neutrophil function. Studies on normal and chronic granulomatous disease neutrophils. *Acta Vitaminologica Enzymologica* **4**, 163–168.

Pussinen, P. J., Laatikainen, T., Alfthan, G., Asikainen, S. & Jousilahti, P. (2003) Periodontitis is associated with a low concentration of vitamin C in plasma. *Clinical and Diagnostic Laboratory Immunology* **10**, 897–902.

Rubinoff, A. B., Latner, P. A. & Pasut, C. A. L. (1989) Vitamin C and oral health. *Journal of Canadian Dental Association* **55**, 705–707.

Russel, A. L., Leatherwood, E. C., Consolazio, C. F. & Van Reen, R. (1965) Periodontal disease and nutrition in South Vietnam. *Journal of Dental Research* **44**, 775–782.

Sakai, N., Yokoyama, T., Date, C., Yoshiike, N. & Matsumura, Y. (1998) An inverse relationship between serum vitamin C and blood pressure in a Japanese community. *Journal of Nutritional Science and Vitaminology (Tokyo)* **44**, 853–867.

Schmidt, A. M., Weidman, E., Lalla, E., Yan, S. D., Hori, O., Cao, R., Brett, J. G. & Lamster, I. B. (1996) Advanced glycation end-products (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *Journal of Periodontal Research* **31**, 508–515.

Shannon, I. L. (1973) Significant correlations between gingival scores and ascorbic acid status. *Journal of Dental Research* **52**, 394.

Simon, J. A. & Hudes, E. S. (2001) Relation of ascorbic acid to bone mineral density and self-reported fractures among US adults. *American Journal of Epidemiology* **154**, 427–433.

Waerhaug, J. (1958) Effect of C-avitaminosis on the supporting structures of the teeth. *Journal of Periodontology* **29**, 87.

Woolfe, S. N., Kenney, E. B. & Hume, W. R. (1980) Ascorbic acid and periodontal disease: a review of the literature. *Journal of Western Society of Periodontology* **28**, 44.

Woolfe, S. N., Kenney, E. B., Hume, W. R. & Carranza, F. A. (1984) Relationship of ascorbic acid levels of blood and gingival tissue with response to periodontal therapy. *Journal of Clinical Periodontology* **11**, 159.

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Original

Characteristics and willingness of patients to pay for regular dental check-ups in Japan

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Abstract: The purpose of this survey was to investigate the relationship between demographic characteristics and willingness of patients to pay for regular dental check-ups in Japan. Questionnaires were distributed at private dental offices and collected via postage-paid return envelopes addressed to the center of the study groups. Questions focused on demographics and willingness to pay for regular check-ups. Five thousand one hundred thirty-two questionnaires were collected (response rate 56.8%). The 3 groups most likely to have regular dental check-ups were found to be the under 20s, 50 to 59 year olds and civil servants. Of these groups, civil servants were found to be the most likely of all to have regular check-ups. More females than males were represented in the sample. More than 60% of the patients responded that they would be willing to pay for regular check-ups if the cost were less than 2,000 yen (about $ 20). However, no statistically significant differences were observed in relation to household income. The results suggested that participation in regular dental check-ups might be related to gender and age, but not to household income. (J. Oral Sci. 46, 127-133, 2004)

Key words: regular check-ups; willingness to pay; household income.

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Introduction

The decline of the prevalence of dental caries is a worldwide trend (1). This tendency has also been observed in Japan. The national survey for oral diseases, carried out every six years, indicates a tendency toward the decline of dental caries in the deciduous teeth and permanent teeth of the young adult population (2). The services provided by dental professionals have therefore gradually changed from the treatment of dental caries to the provision of regular check-ups and professional preventive programs. However, an increase in periodontal disease has nevertheless been observed (3,4). In Japan, for example, according to a survey in 1999, 72.9% of subjects aged 5 years and older had periodontal disease (2). Prevalence was also found to increase with age, with 88.4% of subjects in the 45- to 54-year-old range having periodontal disease (2). Another survey showed that only 54.4% of patients visit clinics to treat periodontal disease (5). Consequently, there is still a need for regular check-ups and dental care to prevent dental caries (6,7). Furthermore, the need to prevent and treat periodontal disease is high. However, these needs are not always attended to at the dental clinic visited.

The effect of regular check-ups on the prevention of dental caries has not been clearly established. It has been shown that decreasing the frequency of regular check-ups does not lead to an increase in dental caries (8). Another report showed that the incentive of a capitation system reduced dental caries, and the number of deep periodontal and bleeding pockets (9,10). This difference may be derived from the extent of dental care provided at regular check-ups. In Japan, regular check-ups for post-adolescents are not provided by the public health service. Subjects must therefore obtain these services at private dental clinics, even though Japanese health insurance does not cover the check-ups. The national average percentage of regular check-ups per clinic was less than 5% (7); a figure we consider to be less than satisfactory.

The purpose of this survey was to investigate the relationship between demographic characteristics and willingness of patients to pay for regular dental check-ups in Japan. The goals of our study were to investigate and improve the skills of dentists and dental hygienists working in private dental clinics to better prevent oral diseases and encourage regular check-ups in association with professional preventive programs. In particular, this investigation focused on the factors that discourage persons from attending regular check-ups and strategies to overcome them. We also focused on the difference in characteristics between persons who had regular check-ups and those who did not. In this study, we selected dental clinics that routinely perform regular check-ups and provide professional preventive programs in Japan.

Materials and Methods

Study population

Thirty-nine private dental clinics in 15 prefectures throughout Japan participated in this study. These private dentists were members of study groups of the Japan Health Care Dental Association. The questionnaires were distributed over a set period of time to all visiting patients in the waiting room of the dental clinics. Patients returned the questionnaires to the study group center using a postage-paid return envelope. A total of 9,024 questionnaires were distributed, of which 5,132 were returned (response rate 56.8 %). The sample population comprised 1,901 men (37.0%), 3,044 women (59.3%) and 187 unknown (3.6%). It should be noted that the samples surveyed in this study comprised of patients visiting dental clinics where regular check-up systems are in place.

Table 1 Questionnaires used in this study

	Gender		Age (years)		Occupation		Household income per month (yen)	Regular check-ups experience		Desired cost for regular check-ups (yen)
1	Male	1	- 9	1	Office workers	1	less than 200000	Yes	1	1000
2	Female	2	10 - 14	2	Civil servant	2	200000-300000	No	2	2000
		3	15 - 19	3	Self-employed	3	300000-400000		3	3000
		4	20 - 29	4	Housewife	4	400000-500000		4	4000
		5	30 - 39	5	Student	5	more than 500000		5	5000
		6	40 - 49	6	Part-time job				6	7000
		7	50 - 59	7	No occupation				7	10000
		8	60 - 69	8	Others				8	20000
		9	70 -							

	Reasons for visiting clinic		Reason for selecting dental office		Information for selecting dental office
1	Tooth or gum disease	1	Location	1	Specialist
2	Denture problem	2	Personal dental office	2	Technological assessment
3	Health care	3	Technical competence of dentist	3	Cost of treatment
4	Trauma	4	No waiting time	4	Good reputation
5	Others	5	Explanation of treatment	5	Staff
		6	Office open late or on holidays	6	Background of the dentist
		7	Recommendation from associate	7	Others
		8	Recommendation from doctor*		

"Regular check ups" refers to attendance at the dental office for the purpose of the maintaining a healthy oral condition.
* Doctor refers to a medical doctor or another dentist.

Table 2 Demographic characteristics of participants who make regular or infrequent visits for check-ups

(A)

Gender	Regular visitors		Infrequent visitors		No answer		Total	
	n	%	n	%	n	%	n	% (% of Total)
Male	779	41.0	1096	57.7	26	1.4	1901	100 (37.0)
Female	1520	49.9	1475	48.5	49	1.6	3044	100 (59.3)
Unknown	83	44.4	89	47.6	15	8.0	187	100 (3.6)
Total	2382	46.4	2660	51.8	90	1.8	5132	100

(B)

Age (years)	Regular visitors		Infrequent visitors		No answer		Total	
	n	%	n	%	n	%	n	% (% of Total)
- 9	23	69.7	10	30.3	0	0	33	100 (0.6)
10 - 14	142	70.6	56	27.9	3	1.5	201	100 (3.9)
15 - 19	32	31.7	68	67.3	1	1.0	101	100 (2.0)
20 - 29	128	35.2	232	63.7	4	1.1	364	100 (7.1)
30 - 39	331	47.5	364	52.2	2	0.3	697	100 (13.6)
40 - 49	406	51.9	376	48.0	1	0.1	783	100 (15.3)
50 - 59	435	44.7	530	54.5	8	0.8	973	100 (19.0)
60 - 69	385	42.4	492	54.1	32	3.5	909	100 (17.7)
70 -	202	35.6	339	59.7	27	4.8	568	100 (11.1)
No answer	298	59.2	193	38.4	12	2.4	503	100 (9.8)
Total	2382	46.4	2660	51.8	90	1.8	5132	100

(C)

Occupation	Regular visitors		Infrequent visitors		No Answer		Total	
	n	%	n	%	n	%	n	% (% of Total)
Office worker	412	38.6	643	60.3	11	1.0	1066	100 (20.8)
Civil servant	139	55.4	111	44.2	1	0.4	251	100 (4.9)
Self-employed	205	45.3	238	52.5	10	2.2	453	100 (8.8)
Housewife	627	50.2	598	47.9	23	1.8	1248	100 (24.3)
Student	349	60.0	227	39.0	6	1.0	582	100 (11.3)
Part-time job	181	45.4	215	53.9	3	0.8	399	100 (7.8)
Unemployed	241	37.4	388	60.2	15	2.3	644	100 (12.5)
Others	116	46.6	130	52.2	3	1.2	249	100 (4.9)
No answer	112	46.7	110	45.8	18	7.5	240	100 (4.7)
Total	2382	46.4	2660	51.8	90	1.8	5132	100

(A) Gender. (B) Age group. (C) Occupation.
All of these factors were correlated with the likelihood of attending regular check-ups, based on chi-square tests (P values were all less than 0.001). All the chi-square tests excluded "No answer," "Undetermined," and "Unknown" categories. "Regular visitors" refers to participants who answered yes to "Regular check-up experience". "Infrequent visitors" refer to those answering no to "Regular check-up experience".

Table 3 Reasons for visiting the dental office, reasons for selecting the dental office and information for selecting the dental office

(A)

Reason for visiting clinic	Regular visitors		Infrequent visitors		No answer		Total	
	n	%	n	%	n	%	n	% (% of Total)
Tooth or gum disease	892	34.3	1677	64.4	35	1.3	2604	100 (50.7)
Denture problem	178	28.1	440	69.4	16	2.5	634	100 (12.4)
Health care	1146	78.9	295	20.3	11	0.8	1452	100 (28.3)
Trauma	13	41.9	18	58.1	0	0	31	100 (0.6)
Others	135	37.4	218	60.4	8	2.2	361	100 (7.0)
No answer	18	36.0	12	24.0	20	40.0	50	100 (1.0)
Total	2382	46.4	2660	51.8	90	1.8	5132	100

(B)

Reason for selecting dental office	Regular visits		Infrequent visits		No answer		Total	
	n	%	n	%	n	%	n	% (% of Total)
Location	346	34.6	636	63.5	19	1.9	1001	100 (19.5)
Personal dental office	1150	58.0	806	40.6	27	1.4	1983	100 (38.6)
Technical competence of dentist	296	46.1	335	52.2	11	1.7	642	100 (12.5)
No waiting time	16	40.0	23	57.5	1	2.5	40	100 (0.8)
Explanation of treatment	59	49.6	60	50.4	0	0	119	100 (2.3)
Office open late or on holidays	0	0	4	100.0	0	0	4	100 (0.1)
Recommendation from associate	429	37.3	705	61.4	15	1.3	1149	100 (22.4)
Recommendation from doctor*	53	55.2	43	44.8	0	0	96	100 (1.9)
No answer	33	33.7	48	49.0	17	17.3	98	100 (1.9)
Total	2382	46.4	2660	51.8	90	1.8	5132	100

(C)

Information for selecting dental office	Regular visitors		Infrequent visitors		No answer		Total	
	n	%	n	%	n	%	n	% (% of Total)
Specialist	728	48.9	741	49.8	20	1.3	1489	100 (29.0)
Technological assessment	526	46.1	600	52.5	16	1.4	1142	100 (22.3)
Cost of treatment	112	38.2	177	60.4	4	1.4	293	100 (5.7)
Good reputation	805	45.9	932	53.1	17	1.0	1754	100 (34.2)
Staff	69	46.3	77	51.7	3	2.0	149	100 (2.9)
Background of the dentist	25	46.3	29	53.7	0	0	54	100 (1.1)
Others	52	55.3	40	42.6	2	2.1	94	100 (1.8)
No answer	65	41.4	64	40.8	28	17.8	157	100 (3.1)
Total	2382	46.4	2660	51.8	90	1.8	5132	100

(A) A high proportion of regular visitors visited dental offices for health care.
(B) 63.5%of infrequent visitors selected the dental office by location. There was little difference observed in the influence of the factors of technique, waiting time and explanation. * Doctor refers to a medical doctor or another dentist.
(C) There was little difference between regular and infrequent visitors with regard to the information used for dental office selection.

Questionnaires

The questionnaires consisted of items relating to demographics, regular check-up experience, desired cost of regular check-ups, reason(s) for visiting the dental clinics, the main reasons for dental clinic selection, information used for dental clinic selection, and monthly household incomes. All questionnaires were presented in a forced-choice format. (Table 1). In the case of patients aged 12 years and below the questionnaires were completed by family members.

Statistical analysis

To investigate the differences between those who visited clinics regularly (Regular check-up experience, Yes) and those who did not (Regular check-up experience, No), chi-square tests for the nominal scales and two-way ANOVA for ordinal scales were used. In addition, multiple logistic regression analysis was used to calculate the odds ratios for regular visitors to control for confounders. These analyses were performed using SPSS ver. 11.0 (SPSS, Tokyo, Japan).

Results

The number of patients who reported visiting clinics regularly (Regular check-up experience, Yes) was 2,382 (46.4%), and their demographic characteristics are shown in Table 2. There were more female regular visitors (49.9%) than male visitors (41.0%) (Table 2A). When comparing by age group, many of the regular visitors were found to be aged under 14 years. In contrast, a high proportion of the 15- to 29-year-old group did not visit regularly. From age 15 to 49, the proportion of infrequent visitors (Regular check-up experience, No) decreased, then from age 50 and above, it increased. (Table 2B) When comparing by occupational group, the proportion of infrequent visitors was especially high in office workers (Table 2C).

The three major reasons for attending dental clinics were tooth or gum disease, routine health care, and denture

Table 4 Willingness of regular and infrequent visitors to pay for regular check-ups

(A)

Desired cost for regular check-ups (yen)	Regular visitors		Infrequent visitors		No answer		Total	
	n	%	n	%	n	%	n	%
1000	673	29.4	930	36.4	18	37.5	1621	31.6
2000	923	40.3	1022	40.0	17	35.4	1962	38.2
3000	501	21.9	439	17.2	9	18.8	949	18.5
4000	27	1.2	14	0.5	0	0.0	41	0.8
5000	133	5.8	106	4.1	2	4.2	241	4.7
7000	7	0.3	6	0.2	0	0.0	13	0.3
10000	17	0.7	25	1.0	1	2.1	43	0.8
20000	7	0.3	13	0.5	1	2.1	21	0.4
No answer	95	4.2	104	4.1	42	37.5	241	4.7
Total	2288	100	2555	100	48	100	5132	100

(B)

Desired cost of treatment	less than 1,000		less than 2,000		more than 2,000		No answer		Total	
Household income	n	%	n	%	n	%	n	%	n	%
less than 200,000	181	11.2	139	7.1	55	4.2	116	48.1	491	9.6
200,000 - 300,000	353	41.5	339	17.3	148	17.4	11	1.3	851	16.6
300,000 - 400,000	355	21.9	459	23.4	277	21.2	26	10.8	1117	21.8
400,000 - 500,000	206	12.7	287	14.6	184	14.1	31	12.9	708	13.8
more than 500,000	280	17.3	459	23.4	444	33.9	37	15.4	1220	23.8
No answer	246	15.2	279	14.2	200	15.3	120	49.8	845	16.5
Total	1621	100	1962	100	1308	100	241	100.0	5132	100

(A) Willingness of regular and infrequent visitors to pay for regular check-ups. More than 60% of patients were willing to pay for regular check-ups if the cost were less than 2,000 yen. There was little difference between regular and infrequent visitors.
(B) Willingness to pay and household income per month. There were no clear differences in the willingness to pay based on household income.

Table 5 Ratios for regular check-up visits

	Odds Ratio	95% CI	*P*-value
Gender			
Male	0.65	0.77 - 0.55	< 0.01
Age group			
-9	5.22	1.91 - 14.27	< 0.01
10-19	7.39	3.11 - 17.53	< 0.01
20-29	1.22	0.48 - 3.10	0.68
30-39	1.01	0.70 - 1.46	0.95
40-49	1.31	0.97 - 1.77	0.08
50-59	1.59	1.18 - 2.13	0.00
60-69	1.31	0.99 - 1.74	0.06
70-	1.38	1.05 - 1.80	0.02
Occupation			
Office worker	0.97	0.67 - 1.41	0.89
Civil servant	1.74	1.12 - 2.70	0.01
Self-employed	1.24	0.83 - 1.85	0.29
Housewife	1.30	0.90 - 1.88	0.17
Student	0.66	0.29 - 1.51	0.33
Part-time job	1.02	0.68 - 1.54	0.91
Unemployed	1.02	0.68 - 1.53	0.94
Household income			
less than 200,000	0.91	0.68 - 1.23	0.55
200,000 - 300,000	1.14	0.69 - 1.91	0.61
300,000 - 400,000	1.15	0.66 - 1.99	0.62
400,000 - 500,000	1.14	0.65 - 1.99	0.64
more than 500,000	1.36	0.79 - 2.36	0.27

We included all the demographic factors for logistic regression analysis of regular check-up visits.
The results indicated that men were inclined not to be regular visitors.
Persons aged under 20, persons aged 50 to 59, and civil servants were inclined to be regular visitors.
However, no statistically significant differences were observed with regard to household income.

problems in this study. The proportion of infrequent visitors was high for tooth or gum disease, and was higher then regular visitors for denture problems (Table 3A).

The major reasons for dental clinic selection were personal dental office (1,983; 38.6%), recommendation from an associate (1,149; 22.4%), and location (1,001; 19.5%). Infrequent visitors were more likely than regular visitors to include recommendations from an associate and location as reasons for dental clinic selection (Table 3B).

Information used for selecting a dental clinic included good reputation, specialist, and technological assessment. There was considerable variation for both regular and infrequent visitors with respect to the information used for dental clinic selection (Table 3C).

As shown in Table 3A, most visitors were willing to pay less than 2,000 yen (about $ 20). The proportion of regular visitors was not large in any of the groups. The difference was statistically significant based on two-way ANOVA. Table 4B compares the willingness of using household income to pay. As household income increased, the number of persons willing to pay less than 1,000 yen (about $ 10) decreased. In contrast, the number of persons willing to pay less than 2,000 yen (about $ 20) and the number of persons willing to pay more than 2,000 yen increased.

To control for confounders, we used all the demographic factors to calculate the odds ratio of the persons who visited regularly for check-ups. As shown in Table 5, men tended not to visit clinics regularly. Relatively more persons under 20 years old, 50- to 59-year-olds and civil servants visited clinics regularly. No statistically significant difference was observed in relation to household income. When comparing by occupation, the relatively high proportion of students who visit clinics regularly was found to be not statistically significant.

Discussion

The present survey demonstrated two important issues that should be improved in order to promote the willingness of patients to pay for regular dental check-ups in Japan. First, the environmental considerations for adult working males should be improved. According to our survey, the majority of those not receiving regular check-ups were male (Table 2A). The age group of 50 - 59 years comprised of more persons who did not visit clinics regularly than those who did. Logistic regression analysis revealed statistically significant differences for males. In the age groups for 20 to 49, there were no regular visitors and occupation was not always associated with regular attendance. However, as the prevalence of periodontal disease is high in this population (1), regular check-ups should be encouraged so as to minimize the prevalence of periodontal disease. One of the most important reasons deterring adult males from regular check-ups was the high opportunity cost. The survey of factors influencing regular check-ups revealed that the main criteria for selecting clinics included, location, appointment times for visits to the clinics, and whether the clinic held late hours. To meet these demands, clinics should be open for late hours and on weekends.

A method of payment for regular check-ups is also necessary. In this survey, 73.3% of the patients answered that they were willing to pay less than 2,000 yen (about $ 20) for regular check-ups. The national average hourly fee for a dental hygienist in Japan is 1,938 yen (about $ 19)(13). At this hourly rate, the treatment in professional preventive programs should be completed over 20 or 30 minutes. These facts indicate that professional preventive programs costing less 2,000 yen might have not profits in dental clinics, because the current insurance system in Japan does not provide for dental health maintenance. The system improved slightly in 2002, to cover regular check-ups, but only for patients with periodontal disease after treatment. This change may well promote regular check-ups.

In conclusion, the present survey indicates that the likelihood of attending regular dental check-ups is related to gender and age, but not to household income.

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References

1. Sheiham A (1984) Changing trends in dental caries. Int J Epidemiol 13, 142-147
2. Health Policy Bureau Ministry Health and Welfare, Japan (2001) Report on the survey of dental disease 1999. Oral Health Association, Tokyo, 1-178 (in Japanese)
3. Borrell LN, Burt BA, Gillespie BW, Lynch J, Neighbors H (2002) Periodontitis in the United States: beyond black and white. J Public Health Dent 62, 92-101
4. Griffiths GS, Duffy S, Eaton KA, Gilthorpe MS, Johnson NW (2001) Prevalence and extent of lifetime cumulative attachment loss (LCAL) at different thresholds and associations with clinical variables: changes in a population of young male military recruits over 3 years. J Clin Periodontal 28, 961-969
5. Policy document: the dental needs of children 1990. Br Dent J 168, 79-81
6. Crossner CG, Unell L (1986) A longitudinal study of dental health and treatment need in Swedish teenagers. Community Dent Oral Epidemiol 14,10-14
7. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare Japan (1998) Comprehensive Survey of Living Condition of the People on Health and Welfare. Health and Welfare Statistics Association, Tokyo (in Japanese)
8. Karkkainen S, Seppa L, Hausen H (2001) Dental check-up intervals and caries preventive measures received by adolescents in Finland. Community Dent Health 18,157-161
9. Holloway PJ, Clarkson JE (1994) Cost benefit of prevention in practice. Inter Dent J 44, 317-322
10. Mellor AC, Blinkhorn AS, Hassall DC, Holloway PJ, Worthington HV (2000) An assessment of capitation in the General Dental Service Contact 2.Patterns of treatment provided to regularly attending patients. Br Dent J 182,460-464
11. Okada M, Kuwahara S, Kaihara Y, Ishidori H, Kawamura M, Miura K, Nagasaka N (2000) Relationship between gingival health and dental caries in children aged 7-12 years. J Oral Sci 42, 151-155
12. Zickert I (2000) Disease activity and need for dental care in a capitation plan based on risk assessment. Br Dent J 189, 480-486
13. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare Japan (2002) Basic Survey on Wage Structure 2000. Rodohorei Kyokai, Tokyo, 64-65 (in Japanese)

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Original

Feasibility of eradication of mutans streptococci from oral cavities

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Abstract: Objectives: Dental caries prevention programs using chlorhexidine (CHX) have been proposed, but CHX's effect in reducing levels of mutans streptococci (*S. mutans* and *S. sobrinus*) appears to last for only a few months. The aim of this study was to attempt to eradicate mutans streptococci from the oral cavity using intensive professional mechanical tooth cleaning (PMTC) and topical application of CHX in custom-made trays. Methods: Seven adult dentate subjects participated in this study (mean age 53.7 +/- 5.6, age range 46 to 62, mean DMFT, 9.1 +/- 4.2). For each subject, PMTC was carried out eight times within ten days. After each PMTC, 1% CHX was applied twice to the tooth surface using custom-made trays. In addition, as home treatment, subjects were required to carry out tooth brushing three times a day, and apply 0.2% CHX in custom trays after brushing in the morning and evening. In addition, subjects rinsed with 0.2% CHX solution after lunch. Salivary levels of mutans streptococci were evaluated using Dentocult-SM at baseline and on days 9, 20, 70, 120. Results: Mutans streptococci were eradicated by day 120 from 4 of the 7 seven subjects participating in this study. Those 3 subjects still harboring mutans streptococci exhibited deep periodontal pocketing. Conclusions: Eradication of mutans streptococci from the oral cavity is feasible using a combination of CHX application in custom-made trays and intensive PMTC. (J. Oral Sci. 46, 179-183, 2004)

Keywords: mutans streptococci; chlorhexidine; eradication.

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Introduction

It is generally accepted that harboring mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) is a risk factor for dental caries. A number of in-vitro studies have demonstrated mechanisms by which these bacteria play a role in dental caries formation (1). In this regard, the production of water-insoluble glucans on the tooth surface seems a particularly important virulence factor (2-4).

Dental plaque is a biofilm, and the surface of it is covered with the matrix structure of dextran (5-8), which is resistant to penetration by most anti-microbial agents.

Chlorhexidine (CHX) is a topical anti-microbial agent that is effective in the elimination of mutans streptococci from the oral cavity (9). Application of CHX is performed using a rinsing solution, dentifrice, varnish on the tooth surface or gel application in individual trays. Of these methods, varnish application to the tooth surface and gel application using individual trays have been shown to be most effective (10,11) since they permit a high concentration of CHX to be maintained on the tooth surface. However, even using these methods, this agent is still unable to penetrate the biofilm (11). Eradication of mutans streptococci using CHX has been attempted in the clinical trial setting, but re-growth of the bacteria is generally observed within two to three months (12). There are no reports of permanent eradication of mutans streptococci from the oral cavity. Moreover, attempting to eradiate these bacteria using high concentrations of CHX over an extended time frame may result in local adverse effects on the oral mucosa (13,14).

We propose use of a combination of professional mechanical tooth cleaning (PMTC) and a dental drug delivery system (3DS) with CHX for elimination of mutans streptococci from the oral cavity (15,16). 3DS consists of drug retainers that contact the tooth surface and permit contact of the anti-microbial drugs directly with the tooth surface. Since mutans streptococci do not have receptors to permit adherence to the oral mucosa (17), it is not necessary to eradicate them from areas where teeth are absent. It was hypothesized PMTC would destroy the biofilm structure and permit the delivered CHX to reach the mutans streptococci remaining in micro-colonies.

Materials and Methods

Subjects and clinical examination

This study was approved by the ethical committee of the National Institute of Infectious Diseases of Japan. Members of the institute and co-researchers of this study were recruited, and all subjects gave written informed consent for participation in the study. In total, seven adult male subjects participated, with mean age 53.7 ± 5.6 years and mean DMFT 9.1 ± 4.2. Probing depths were measured a six sites per tooth using a WHO probe. Among the seven subjects, 2 subjects had deep periodontal pockets (> 4 mm). All subjects exhibited pockets of 3 mm.

Clinical and sampling procedures

Levels of salivary mutans streptococci were determined at baseline using Dentocult-SM strip methods (Orion Diagnostica, Finland). Alginate impressions were then taken and maxillary and mandible casts prepared. A polypropylene sheet (3.0 mm disk for mouth guard soft, Keystone, New Jersey, U.S.A.) was vacuum-adapted to each cast with a vacuum-forming machine (VACCUM ADAPTER I, Keystone). Vacuum-adapted drug retainers were individually fabricated to cover the complete arch of the dentition. The drug retainer was trimmed to be approximately 1.0 mm apical to the gingival margin.

Before CHX application, PMTC was carried out eight times within 10 days on each subject to remove the tooth biofilm. By the PMTC, dental plaque was disclosed prior to its removal using rubber cups with polishing paste (Prophy Paste;(RDA170); CCS Cleanchemical, Vasby, Sweden). The remaining inter-dental plaque was removed using dental floss and polishing using Eva chips with polishing paste. Complete plaque removal was confirmed by further disclosing. The tooth surface was varnished with a 0.2% NaF solution (FULORIDENT GEL, Stone Pharmaceuticals, Philadelphia, USA).

After each PMTC, 1% CHX gel (CORSODYL Zahngel, Smithkline Beecham, Thorishaus, Switzerland) was injected into those periodontal pockets deeper than 4 mm. This gel was also applied using a dental drug retainer for 5 minutes. During the 10 days after PMTC, subjects also applied 0.2% CHX gel (Plakout, Howe-Neos Dental, Bioggio, Switzerland) twice a day after tooth brushing (morning and evening) using a custom-made tray. In addition, after lunch, tooth brushing and mouth rinsing using 0.2% CHX mouth rinse was performed. Salivary mutans streptococci levels were determined using the Dentocult-SM system at days 9, 20, 70, 120. On days 70 and 120, bacteria were also cultured to determine salivary mutans streptococci levels.

Microbial procedures and saliva sampling

Salivary mutans streptococci and Lactobacillus were counted using a commercially available mutans streptococci evaluation kit, Dentocult-SM (Orion Diagnostica, Epsom, Finland). The levels were classified according to the manufacturer's instructions, that is: level 0 - 1: < 100,000 colony forming units (CFU) mutans streptococci /ml saliva; level 2: 100,000 < CFU/ml < 1,000,000; and level 3: > 1,000,000 CFU/ml.

On days 70 and 120, mutans streptococci were also cultured. Paraffin-stimulated whole saliva samples were collected for 5 minutes. Saliva samples of 50 μl were then sonicated by ultrasonic dispersion (60 power output) for 10 seconds and spread onto Mitis-Salivarius agar (MS, Gibco, Tokyo, Japan) plates for growth of streptococci, and onto improved Mitis-Salivarius agar plates containing 0.02 M bacitracin (Wako Pure Chemicals, Osaka, Japan) (MSB) for selective growth of mutans streptococci (18), using an EDDY JET spiral system (Gunze Sangyo, Tokyo,

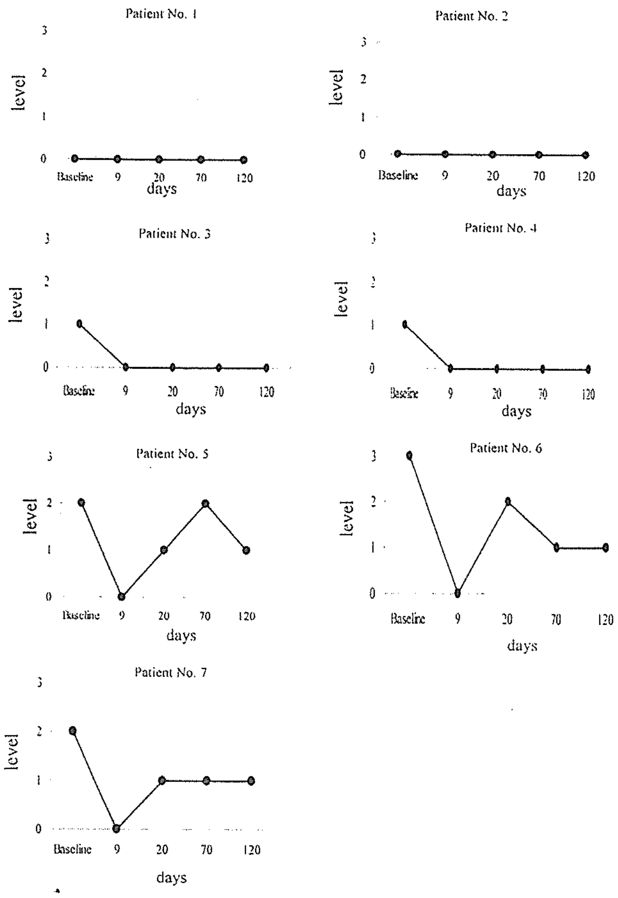

Fig. 1 Evaluation of the salivary levels of mutans streptococci by Dentocult SM.

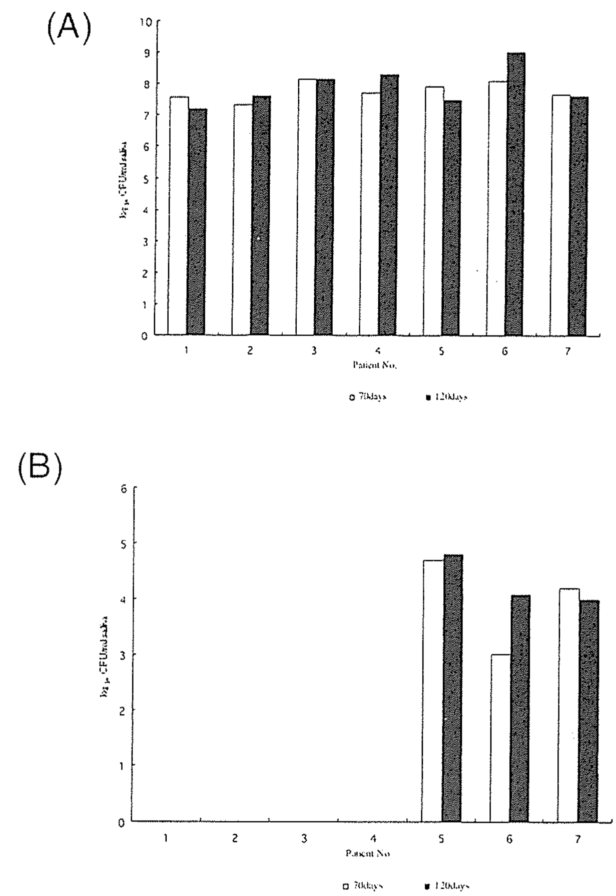

Fig. 2 Confirmation of the eradication of mutans streptococci from the oral cavity by culture.

Japan). After 48 hours anaerobic incubation, colonies were counted and the number of bacteria per ml of whole saliva calculated.

Statistical analysis

Mann-Whitney's *U*-tests were used for the evaluation of baseline differences between subjects in whom mutans streptococci was eradiated and those still harboring mutans streptococci.

Results

Figure 1 shows the mutans streptococci levels evaluated using the Dentocult SM. Two subjects with level 0 (no mutans streptococci detected)at baseline also had level 0 throughout the study. In addition, two subjects with level 1 at baseline decreased to the 0 level after 120 days. Three subjects above level 2 at baseline decreased to level 0 by day 9, and recovered to level 2 by day 120.

Figure 2 shows the mutans streptococci levels evaluated by the improved MSB culture system at 70 (A) and 120 days (B). Eradication of mutans streptococci was observed in three subjects.

Table 1 Baseline clinical characteristics (mean ± SD) of subjects according to success of mutans streptococci eradication methods

	Eradicated (n = 4)	Not eradicated (n = 3)	*P*-value
DMFT	10.00 ± 4.97	8.00 ± 5.29	0.31
Probing depth (% of pockets)			
> 3 mm	49.85 ± 19.92	54.76 ± 45.07	0.78
> 4 mm	6.40 ± 2.40	18.85 ± 18.46	0.70
> 5 mm	1.64 ± 0.89	6.94 ± 9.57	0.71
> 6 mm	0.89 ± 1.03	2.18 ± 3.78	0.58

The baseline characteristics of subjects in whom mutans streptococci were eradicated and those exhibiting mutans streptococci re-growth are shown in Table 1. There was a tendency for those subjects with deep periodontal pockets to exhibit streptococci regrowth, although no statistically significant difference was observed.

Discussion

The results of the present study demonstrate that 3DS used in combination with intensive PMTC is effective in reducing salivary levels of mutans streptococci and in eradicating mutans streptococci. A number of dental caries preventive programs have been described (for review see Lewis et al.;19), including tooth cleaning, and fluoride or CHX application. Tooth cleaning and tooth brushing instruction have some benefit in caries prevention, although this benefit appears minor (20). A combination of oral hygiene instruction and fluoride application appears more effective (21), and if CHX is used in addition, an even greater favorable effect is evident (11). However, these preventive measures do not appear to result in complete inhibition of new dental caries.

Complete removal of dental plaque can be challenging, even in the case of chemical plaque removal (22). Tooth brushing in combination with tooth brushing instruction is also of questionable value, especially on tooth surfaces prone to caries (23). PMTC has been suggested as being one of the most effective methods for plaque removal, and PMTC performed by dental hygienists has been demonstrated to suppress dental caries (24,25) and reduce salivary levels of the mutans streptococci without the use of anti-microbial agents (26).

Elimination of mutans streptococci using CHX has been attempted in a number of studies, but the mean levels of these bacteria returned to baseline levels within two weeks when a rinsing solution was used (27), within 4 weeks using gel application or within 12 weeks using varnish (28). The results of these studies suggest that eradication of mutans streptococci from the oral cavity is not feasible. However, these results might be a consequence of inadequate plaque removal prior to CHX application. The dental plaque biofilm in which mutans streptococci are found (8) is resistant to anti-microbial agent penetration (29). Using the system described in the current study, pre-treatment of the tooth surface using PMTC to remove the biofilm appears to permit CHX to function more effectively in mutans streptococci removal.

The use of either PMTC or anti-microbial agents alone has some benefit in preventing dental caries, but is not effective in eradication of mutans streptococci. Using the system described in this study, that is complete removal of dental plaque by intensive PMTC and frequent applications of CHX within a short period of time, in some cases results in the eradication of mutans streptococci from the oral cavity. However, even using this system, mutans streptococci were eradicated from only 4 of 7 subjects.

Baseline differences in the periodontal condition between subjects in whom mutans streptococci were eradicated and those in whom they were not eradicated were not seen. This may be the result of the small sample size, and thus insufficient power to detect differences. However, in general, those subjects where mutans streptococci were not eradicated had deeper periodontal pockets. These results suggest periodontal treatment might be advisable prior to use of anti-microbial agents.

In conclusion, using appropriate methods, eradication of mutans streptococci from the oral cavity is feasible, but is not consistently achieved.

References

1. Kuramitsu HK (1987) Recent advances in defining the cariogenicity of mutans streptococci: molecular genetic approaches. Eur J Epidemiol 3, 257-260
2. Inoue M, Smith EE (1980) Specific inhibition of glucosyltransferase of *Streptococcus mutans*. Carbohydr Res 80, 163-177
3. Montville TJ, Cooney CL, Sinskey AJ (1977) Measurement and synthesis of insoluble and soluble dextran by *Streptococcus mutans*. J Dent Res 56, 983-989
4. Takada K, Shiota T, Curtiss R, Michalek SM (1985) Inhibition of plaque and caries formation by a glucan produced by *Streptococcus mutans* mutant UAB108. Infect Immun 50, 833-843
5. Shu M, Wong L, Miller JH, Sissons CH (2000) Development of multi-species consortia biofilms of oral bacteria as an enamel and root caries model system. Arch Oral Biol 45, 27-40
6. Sissons CH, Wong L, Shu M (1998) Factors affecting the resting pH of in vitro human microcosm dental plaque and *Streptococcus mutans* biofilms. Arch Oral Biol 43, 93-102
7. Rose RK, Turner SJ (1998) Extracellular volume in streptococcal model biofilms: effects of pH, calcium and fluoride. Biochim Biophys Acta 1379, 185-190
8. Marsh PD, Bradshaw DJ (1997) Physiological approaches to the control of oral biofilms. Adv Dent Res 11, 176-185
9. Emilson CG (1994) Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 73, 682-691
10. Emilson CG., Gisselsson H, Birkhed D (1999) Recolonisation pattern of mutans streptococci after

suppression by three different modes of chlorhexidine gel application. Eur J Oral Sci 107, 170-175
11. van Rijkom HM, Truin GJ, van't Hof MA (1996) A meta-analysis of clinical studies on the caries-inhibiting effect of chlorhexidine treatment. J Dent Res 75, 790-795
12. Pratten J, Barnett P, Wilson M (1998) Composition and susceptibility to chlorhexidine of multispecies biofilms of oral bacteria. Appl Environ Microbiol 64, 3515-3519
13. Hepso HU, Bjornland T, Skoglund LA (1988) Side-effects and patient acceptance of 0.2% versus 0.1% chlorhexidine used as post-operative prophylactic mouthwash. Int J Oral Maxillofac Surg 17, 17-20
14. Mackenzie IC, Nuki K, Loe H, Schiott CR (1976) Two years oral use of chlorhexidine in man. V. Effects on stratum corneum of oral mucosa. J Periodontal Res 11, 165-171
15. Takeuchi H, Senpuku H, Matin K, Kaneko N, Yusa N, Yoshikawa E, Ida H, Imai S, Nisizawa T, Abei Y, Kono Y, Ikemi T, Toyoshima Y, Fukushima K, Hanada N (2000) New dental drug delivery system for removing mutans streptococci from the oral cavity: effect on oral microbial flora. Jpn J Infect Dis 53, 211-212
16. Takeuchi H, Fukushima K, Senpuku H, Nomura Y, Kaneko N, Yano A, Morita E, Imai S, Nisizawa T, Kono Y, Ikemi T, Toyoshima Y, Hanada N (2001) Clinical study of mutans streptococci using 3DS and monoclonal antibodies. Jpn J Infect Dis 54, 34-36
17. Caufield PW, Dasanayake AP, Li Y, Pan Y, Hsu J, Hardin JM (2000) Natural history of *Streptococcus sanguinis* in the oral cavity of infants: evidence for a discrete window of infectivity. Infect Immun 68, 4018-4023
18. Hanada N, Nomura Y, Takeuchi H, Sennpuku H, Ida H, Yosikawa E, Kumagai T (2001) New dental drug delivery system for removing Mutans Streptococci. J Dent Res 80, 567 (abstract)
19. Lewis DW, Ismail AI (1995) Periodic health examination, 1995 update: 2. Prevention of dental caries. The Canadian task force on the periodic health examination. CMAJ 152, 836-846
20. Curnow MM, Pine CM, Burnside G, Nicholson JA, Chesters RK, Huntington E (2002) A randomized controlled trial of the efficacy of supervised toothbrushing in high-caries-risk children. Caries Res 36, 294-300
21. Axelsson P, Kristoffersson K, Karlsson R, Bratthall D (1987) A 30-month longitudinal study of the effects of some oral hygiene measures on *Streptococcus mutans* and approximal dental caries. J Dent Res 66, 761-765
22. Binney A, Addy M, Newcombe RG (1993) The plaque removal effects of single rinsings and brushings. J Periodontol 64, 181-185
23. Bellini HT, Arneberg P, von der Fehr FR (1981) Oral hygiene and caries. A review Acta Odontol Scand 39, 257-265
24. Poulsen S, Agerbaek N, Melsen B, Korts D., Glavind L, Rolla G (1976) The effect of professional toothcleansing on gingivitis and dental caries in children after 1 year. Community Dent Oral Epidemiol 4, 195-199
25. Klock B (1984) Long-term effect of intensive caries prophylaxis. Community Dent Oral Epidemiol 12, 69-71
26. Kristoffersson K, Axelsson P, Bratthall D (1984) Effect of a professional tooth cleaning program on interdentally localized *Streptococcus mutans*. Caries Res 18, 385-390
27. Ullsfoss BN, Ogaard B, Arends J, Ruben J, Rolla G., Afseth J (1994) Effect of a combined chlorhexidine and NaF mouthrinse: an in vivo human caries model study. Scand J Dent Res 102, 109-112
28. Pienihakkinen K, Soderling E, Ostela I, Leskela I, Tenovuo J (1995) Comparison of the efficacy of 40% chlorhexidine varnish and 1% chlorhexidine-fluoride gel in decreasing the level of salivary mutans streptococci. Caries Res 29, 62-67
29. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284, 1318-1322

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Application of Phosphoryl Oligosaccharides of Calcium (POs-Ca) for Oral Health

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Abstract: Phosphate ester groups are known to link to some glucosyl residues in starch molecules. We have prepared phosphoryl oligosaccharides (POs) from potato starch hydrolysates. The POs were composed of two fractions, PO-1 and PO-2. Fraction PO-1 was the main fraction, and it was composed of maltotriose, maltotetraose, and maltopentaose to which one phosphoryl group was attached. Fraction PO-2 was predominantly composed of maltopentaose and maltohexaose to which at least two phosphoryl groups were attached. POs had the ability to form a soluble complex with calcium and had an inhibitory effect on the formation of a calcium-phosphate precipitate. Based on the function of the POs, described above, we applied the POs of calcium (POs-Ca) as a food ingredient. POs-Ca was an advantageous food ingredient as a soluble calcium source. In relation to prevention of dental caries, POs cannot be fermented by cariogenic microorganisms or mutans streptococci, and they reduce the fall in plaque pH *in vitro*. Moreover, POs-Ca effectively enhanced the remineralization of enamel lesions. The aim of this study was to develop the application of POs-Ca for dental health to the enamel remineralization through the chewing of a sugar-free gum.

Key words: phosphoryl-oligosaccharides, saliva, remineralization, enamel, chewing-gum

The wide distribution of the ester phosphorus is observed in starches from various sources. Potato starch is known to contain an esterified phosphoryl group amoung its components.[1,2] Takeda and Hizukuri have reported that the phosphate groups were located mostly in the B-chain of amylopectin, whereas the phosphorylation of amylose was very little.[3] Potato amylopectin contains 100–1000 ppm of the ester phosphorus.[2] Furthermore, approximately 60% to 70% of the phosphate groups were linked to C-6 of the glucosyl residues, almost all the rest being linked to C-3 and a very small part possibly being linked to C-2 of the glucosyl residues.[2] Our attention was focused on the utilization of the esterified phosphoryl group in potato starch, and we succeeded in preparing new phosphoryl oligosaccharides (POs) from the starch hydrolysate.[4] In this article, we introduce our recent achievements in a new function of the oligosaccharides focusing on the application for soluble calcium and the effect of remineralization on enamel lesion.

Structure and characterization of POs.

We developed a method of producing POs from potato starch, using bacterial liquefying α-amylase (BLA) [EC 3.2.1.1], glucoamylase (GA) [EC 3.2.1.3] and pullulanase [EC 3.2.1.41]. The actions of the amylolytic enzymes were hindered by the phosphoryl groups linked to the glucosyl residues, and POs were obtained as indigestible components by the enzymes. The components of the POs were analyzed by high-performance anion-exchange chromatography and pulsed amperometric detector system.[4] The substance-linked phosphoryl group is detected by the system at different retention times according to the number and the positions of phosphate groups linked to each molecule. The POs were fractionated into two fractions; PO-1 and PO-2.[4,5] Fraction PO-1 was the major component of POs and was composed of maltotriose, maltotetraose and maltopentaose to which one phosphoryl

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Abbreviations: POs, phosphoryl oligosaccharides; POs-Ca, phosphoryl oligosaccharides of calcium; Ca, calcium; P, phosphate; BSA, bacterial saccharifying α-amylase; GA, glucoamylase; BLA, bacterial liquefying α-amylase; PPA, porcine pancreatic α-amylase; HSA, human saliva α-amylase; TAA, Taka-amylase A; Glc-6-P, D-glucose-6-phosphate; 6^2-phosphoryl maltose, *O*-6-phosphoryl-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranose; 6^3-phosphoryl maltotriose, *O*-6-phosphoryl-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose; 6^2-phosphoryl maltotriose, *O*-α-D-glucopyranosyl-(1→4)-*O*-6-phosphoryl-α-D-glucopyranosyl-(1→4)-D-glucopyranose; 6^3-phosphoryl maltotetraose, *O*-α-D-glucopyranosyl-(1→4)-*O*-6-phosphoryl-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose; 6^4-phosphoryl maltopentaose, *O*-α-D-glucopyranosyl-(1→4)-*O*-6-phosphoryl-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose; 3^3-phosphoryl maltotetraose, *O*-α-D-glucopyranosyl-(1→4)-*O*-3-phosphoryl-α-D-glucopyranosyl- (1→4) -*O*-α-D-glucopyranosyl- (1→4) -D-glucopyranose; 3^4-phosphoryl maltopentaose, *O*-α-D-glucopyranosyl-(1→4)-*O*-3-phosphoryl-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose.

group is attached.[5] Fraction PO-2 was predominantly composed of maltopentaose and maltohexaose to which at least two phosphoryl groups were attached.[6] The average degree of polymerization of dephosphorylated PO-1 and PO-2 was evaluated to be 4.02 and 5.82, respectively.[4] The detailed structure of the components of the PO-1 fraction was analyzed by using the different hydrolytic properties of bacterial saccharifying α-amylase (BSA) and GA on the phosphoryl oligosaccharides. The limiting cleavage points of BSA on PO-1 components were the same sites as those of porcine pancreatic α-amylase (PPA) and human saliva α-amylase (HSA), as reported by Takeda *et al.*[7] The phosphate group linked at C-6 of the glucosyl residue was detected as the content of the Glc-6-P after acid hydrolysis. The spectrometric analysis by ^{13}C-NMR also distinguished the phosphate groups linked at C-3 and C-6 of the glucosyl residues, respectively. In conclusion, the PO-1 fraction was made up of oligosaccharides phosphorylated at C-3 (3^3-phosphoryl maltotetraose and 3^4-phosphoryl maltopentaose) and oligosaccharides phosphorylated at C-6 (6^3-phosphoryl maltotriose, 6^2-phosphoryl maltotriose, 6^3-phosphoryl maltotetraose, and 6^4-phosphoryl maltopentaose) (Fig. 1).[5] In the case of the PO-2 fraction, a little of these enzymes' treatment-resistant PO-2 remained. It clearly indicated that two of the phosphoryl groups attached to C-6 and C-3 existed in PO-2 components.[6] From these results, the possible structures of PO-2 components were as shown in Fig. 1.

The POs can form solubilized complexes with Ca and iron.[4,8] The inhibitory effect of POs on the formation of Ca-P precipitate was dependent upon the covalently bound phosphoryl groups in the molecule.[4,8-10] POs-bound calcium was thought an advantageous food ingredient as a soluble calcium source.[11] In addition, POs can not be metabolized by cariogenic bacteria as mutans streptococci, as is true for xylitol, and the preventive effect was shown on reducing the fall in plaque pH despite the buffering power sucrose-dependent fermentation.[12] Sugar alcohols such as xylitol are widely used as sweeteners in chewing-gum to prevent dental caries and to promote remineralization.[13,14] The POs can also reduce the amount of artificial plaque and demineralization on enamel, even in the presence of sucrose.[15] Furthermore, the effects of POs on remineralization of caries-like lesions in enamel were examined *in vitro*.[16] The results showed the possibilities that POs may have a synergistic effect with fluoride on the rate of remineralization.[16] Based on the previously revealed features of POs, we examined the effect of remineralization on enamel by a chewing gum containing a calcium salt of POs (POs-Ca).

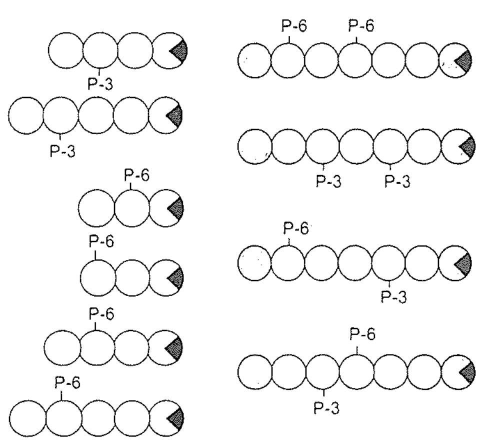

Fig. 1. Structure of phosphoryl oligosaccharides.

Symbols: P-3 and P-6, phosphoryl groups linked at C-3 and C-6 of glucosyl residues; ○, glucosyl residue; ○, glucosyl residue which possibly exists; ◐, reducing end.

Application of POs-Ca for oral health.

The effects of daily application of a sugar-free chewing gum containing 2.5 wt% POs-Ca on remineralization of enamel were examined. POs-Ca was prepared as 5 wt% calcium in the molecule. The gum was concluded to be a non-cariogenic product since it was proven by intraoral plaque pH-telemetry tests in four human volunteers not to depress the pH of interdental plaque below 5.7 by bacterial fermentation, either during consumption or during a period of 30 min following consumption by the general method of the Association for Toothfriendly Sweets.[17] First, the effect of the gum containing POs-Ca on remineralization of caries-like lesions in enamel was examined by using a human saliva immersing (HSI) test.[18] The HSI-test would be a useful system for detection and evaluation of the remineralization effect using human saliva, since it is easy to control condition for the test and light demands are made on volunteers, making it preferable to an intraoral study. The results suggested that the HSI-test and intraoral study have relevance to the effect on enamel remineralization of POs-Ca.[18-20] It is thought that human saliva plays some important role in oral health.[21] In particular, stimulated saliva for chewing would have some influence on remineralization.

1. Salivary Assessment.

We produced two types of sugar-free chewing gum (tablet type) for the experiments. One contained 2.5% POs-Ca (POs-Ca (+) gum) and 46% xylitol, and the other contained 48.5% xylitol without POs (POs-Ca (−) gum). The average weight of each chewing gum tablet was about 1.5 g. All saliva stimulated while chewing 2 tablets of POs-Ca (+) gum or POs-Ca (−) gum was collected from 12 healthy adult volunteers (6 males and 6 females; mean age=29.9 y old). Each volunteer chewed 2 pieces of gum for 20 min and the whole saliva was collected for the first 10 min (Fs) and last 10 min (Ls) separately. Demineralized bovine enamel slabs were immersed in the Fs for 10 min and subsequently in the Ls for 10 min at 37°C. Immediately after the salivary treatments, the enamel slabs were rinsed with deionized water. This procedure was repeated 4 times a day for 4 days. During the study period, no fluoride agent was used and great care was taken not to dry the enamel disk samples. The human saliva was used in the HSI-test within 1 h after sampling. Salivary volume and mineral contents were compiled in Table 1. The volume and pH of saliva from each volunteer were measured immediately after sampling. Subsequently, an aliquot of the saliva was centrifuged (10,000 $\times g$) for 5 min and the supernatant was prepared 0.1 N HCl solution with addition of 1 N HCl solution. After re-centrifugation, the supernatant was filtrated with an ultra filter (0.45 μm). The filtered saliva was assayed for the

Table 1. Analysis of volumes and mineral contents in saliva.

	POs-Ca	Fs		Ls		P^b
		Means±SD	P^a	Means±SD	P^a	
Salivary volume (mL)	+	20.34±4.13	ns	9.35±3.24	ns	**
	−	20.74±4.43		9.65±3.35		**
Ca (mM)	+	6.29±2.44	**	1.72±0.27	*	**
	−	1.69±0.41		1.39±0.37		ns
P (mM)	+	5.62±1.41	ns	6.22±1.31	ns	ns
	−	6.15±1.35		6.49±1.15		ns
Ca/P	+	1.12±0.31	**	0.27±0.05	*	**
	−	0.28±0.08		0.22±0.05		ns

Fs, collected whole saliva for the first 10 min; Ls, collected whole saliva for the last 10 min. ᵃ*p* for POs-Ca (+) *vs.* POs-Ca (−), ᵇ*p* for FS *vs.* LS. ns, not significant; $^{**}p<0.0001$, $^{*}p<0.05$.

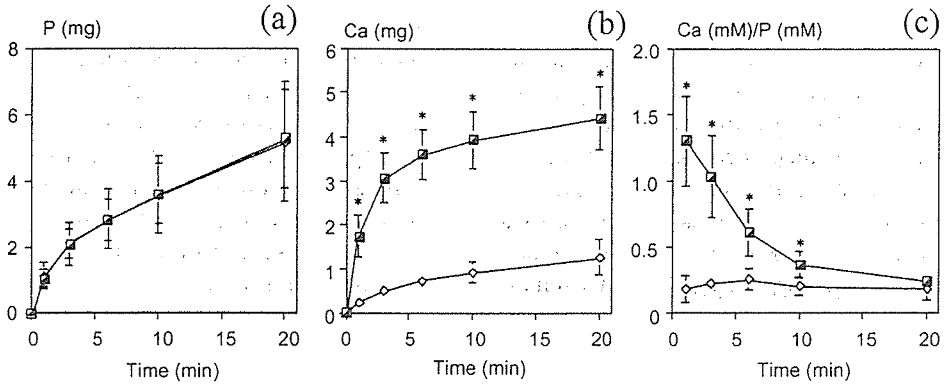

Fig. 2. Time course of salivary soluble phosphate (a), calcium (b), and Ca/P ratio during chewing gum with (◪) or without (◇) POs-Ca.

Human whole saliva was collected from 17 healthy adult volunteers (9 males and 8 females; mean age=29.0 y old) by chewing 2 tablets of POs-Ca (+) gum or POs-Ca (−) gum. Each volunteer chewed the tablets for 20 min and the whole saliva was collected. Vertical bars, SD.

concentrations of inorganic phosphate (P) and calcium (Ca). The concentrations of P and Ca were measured by the methods using molybdenum reagent[22] and by *o*-cresolphthalein complexion (OCPC) method using the calcium-C-test (Wako Pure Chemical, Japan), respectively. The salivary volume of Fs and Ls secreted by chewing of POs-Ca (+) gum and that by POs-Ca (−) gum were nearly equal values, and no significant difference was observed. The concentration of Ca in Fs in POs-Ca (+) gum was much higher than that of POs-Ca (−) gum ($p<0.0001$). However, the Ca concentration of Ls in POs-Ca (+) gum was similar to that of Ls and Fs from POs-Ca (−) gum. The difference in chewing gum did not largely influence the concentration of P in saliva samples. Neither gum included any ingredients containing P. Human saliva includes abundant P, compared with Ca. The time course of soluble Ca and P during 20 min was measured in chewing each gum (Fig. 2a, b). In the experiment, human whole stimulated saliva was collected from 17 healthy adult volunteers (9 males and 8 females; mean age=29.0 y old) including the former 12 volunteers during chewing 2 tablets of POs-Ca (+) gum or POs-Ca (−) gum. The results indicated that most Ca in POs-Ca (+) gum was extracted into saliva within the first 10 min. However, the concentration of P is almost fixed in saliva and increases in proportion to the amount of saliva. The Ca/P ratio values in Fs from POs-Ca (+) gum (1.12±0.31) were significantly higher than the values in the other ($p<0.0001$). Especially at the beginning, the Ca/P ratio ranged to 1.67, which is the value of hydroxyapatite in enamel (Fig. 2c). The pH of saliva was measured at 1, 3, 6, 10 and 20 min during saliva sampling from 17 volunteers. The pH was about 7.0 at the beginning, rose to about 7.5 in the first 6 min, and thereafter remained around 7.5 during the chewing period in both cases. After human salivary treatments, planoparallel sections of about 500 μm thickness were cut from the enamel samples using a water-cooled diamond coated saw (Isomet, Buhler, USA). These sections were ground planoparallely on a wet 800-grit abrasive paper to a thickness of about 200 μm. The sections were fitted on a high resolution positive film (Fuji, Japan) together with an aluminum step wedge and microradiographed (PW-1830, Philips, The Netherlands) by Cu-Kαx-ray generated at 25 kV and 25 mA for 24 s. The films were developed, fixed, and rinsed under standardized conditions. The degree of remineralization was evaluated on digitized microradiographic images by combined means of computer-assisted videodensitometry (CAV) and a mineral distribution analysis program (MDA) developed by Inaba *et al.*[23,24] Finally, the mineral distribution parameters, namely