

Original Article

## Correlation in Inorganic Ion Concentration between Saliva and Plaque Fluid

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The composition and the concentration of inorganic ions in dental plaque significantly influence the initiation and the development of dental caries through altering the degree of saturation of the aqueous phase surrounding the dental enamel. In order to know how plaque is affected by saliva, the composition and the concentration of inorganic ions in saliva and plaque fluid were investigated. The ionic concentrations of sodium and chlorine had similar values between plaque fluid and saliva. However, the concentrations of the inorganic ions such as ammonium, potassium, magnesium, calcium, and phosphate were significantly different between plaque fluid and saliva. This meant that the saliva and plaque fluid were different in its inorganic composition presumably reflected by the metabolic activity of bacteria in the plaque. On the other hand, as for the correlation coefficients between plaque fluid and saliva composition, statistically significant correlation was observed in ions such as sodium, ammonium, potassium, magnesium, and chlorine but not in calcium, phosphate, or in pH values. This was possibly due to the fact that saliva was the main source of supply of these ions. However as for calcium and phosphate, no close correlation was found possibly because they could be supplied also through

tooth enamel dissolution. The discrepancy of the results with former studies on this point was speculatively explained by the difference of the plaque age used. It was considered reasonable that the pH value was independent, as it is mainly decided by the activity of the bacteria in the plaque.

**Key words:** plaque fluid, saliva, inorganic ion

### Introduction

Incipient caries are produced on the enamel surface under the dental plaque. The composition of plaque significantly influences the initiation and the development of dental caries through altering the degree of saturation of the aqueous phase surrounding the dental enamel<sup>1</sup>. Tatevossian et al.<sup>2</sup> showed that ingestion of sugar containing inorganic phosphate did not consistently raise the inorganic phosphate of the plaque. Ashley<sup>3</sup> also reported that the effect on plaque phosphorous composition by sweets-ingestion supplemented with 3% dicalcium phosphate dihydrate was not significantly different from that of exposure to the sweets without dicalcium phosphate dihydrate. These results suggest that the composition of plaque is not very easily influenced by its environment. However, it will be advantageous in preventing dental caries if we can get information concerning the possible modification of the plaque composition by saliva which is always adjacent to the plaque. The relationship between human saliva and dental plaque has mostly

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been studied on calcium and phosphorus concentrations<sup>4,5,6</sup>. Matsuo *et al.*<sup>6</sup> reported a very strong correlation in the ionized and total calcium concentrations between whole saliva and simultaneously collected plaque fluid. Ashley *et al.*<sup>5</sup> found a statistically significant relationship between salivary and plaque concentrations of both calcium and phosphorus collected from children, however, these relationships were not shown in the young adults. These results indicate that the relationship in the inorganic composition between human saliva and dental plaque is not very conclusive. The aim of this study was to investigate the relationship between saliva and plaque fluid of other inorganic ions such as sodium, ammonium, potassium, magnesium, chlorine in addition to calcium and phosphate.

### Materials and methods

#### Plaque fluid collection and Analysis

Plaque samples were collected from 9 Japanese male students aged 23-25. Prior to plaque collection, all subjects refrained from oral hygiene for 48 hr and fasted overnight. No restrictions were imposed on diet during the 48 hr. On the following morning before breakfast, supragingival plaque samples were collected from each subject with an excavator paying attention not to carve the enamel surface and also not to contaminate them with saliva. The collected plaque was stored in an ultrafiltration tube (pore size : 0.45  $\mu$ m, Milipore, MA., USA) and kept in ice temperature until it was centrifuged at 5000 g for 30 min at 4 °C. The isolated plaque fluid was kept in mineral oil in order to prevent evaporation until it was used for analysis. 1.0  $\mu$ l of the plaque fluid was used for pH measurement under 100% humidity using a microelectrode (Microelectrodes, Inc., NH., USA). Another 1.0  $\mu$ l was diluted 101 times and analyzed for inorganic ions using ion chromatography (Dionex Corp., CA., USA). Analysis of inorganic cations was carried out on an IonPac CS12 column and a CSRS-I cation micro-membrane suppressor with the eluent of 20 mmol of methane sulfonic acid. For analysis of inorganic anions an IonPac AS4A-SC column and an ASRS-I anion micro-membrane suppressor was used. The eluent was a mixed solution of 1.8 mmol sodium carbonate and 1.7 mmol sodium hydrogencarbonate.

#### Saliva collection and analysis

Prior to plaque collection subjects were asked to be seated in a relaxed position, allowing the saliva to accu-

mulate in the floor of the mouth. The accumulated saliva was spat into a polypropylene test tube (10 ml, Iwaki, Tokyo, Japan) until at least 0.5 ml of saliva was collected. The collected saliva was kept in ice temperature until it was processed for pH and inorganic ion measurement. The pH of the saliva was measured by inserting the microelectrode directly to the collected saliva. The saliva was transferred to the ultrafiltration tube and centrifuged at 5000 g for 30 min at 4 °C. 1.0  $\mu$ l of the ultrafiltrated saliva was diluted 101 times and analyzed for inorganic ions using ion chromatography in the same way as with the plaque fluid sample.

#### Statistics

The statistical significance of differences in each inorganic ion concentration and pH between plaque fluid and saliva were evaluated by paired t-test. The correlation of each component between plaque fluid and saliva was evaluated using Pearson's correlation coefficient test.

### Results

Mean values and standard deviations of inorganic ion concentrations and pH in plaque fluid and saliva are summarized in Table 1. The mean values of each component were statistically different between plaque fluid and saliva except sodium and chlorine ions. The sodium ion concentration and that of chlorine ion had similar values between plaque fluid and saliva. The individual concentrations of each component in saliva were plotted against those of plaque fluid in Fig 1. The vertical axes indicate concentrations in saliva and the horizontal axes indicate those in plaque fluid. Table 2 gives the correlation coefficients of each component

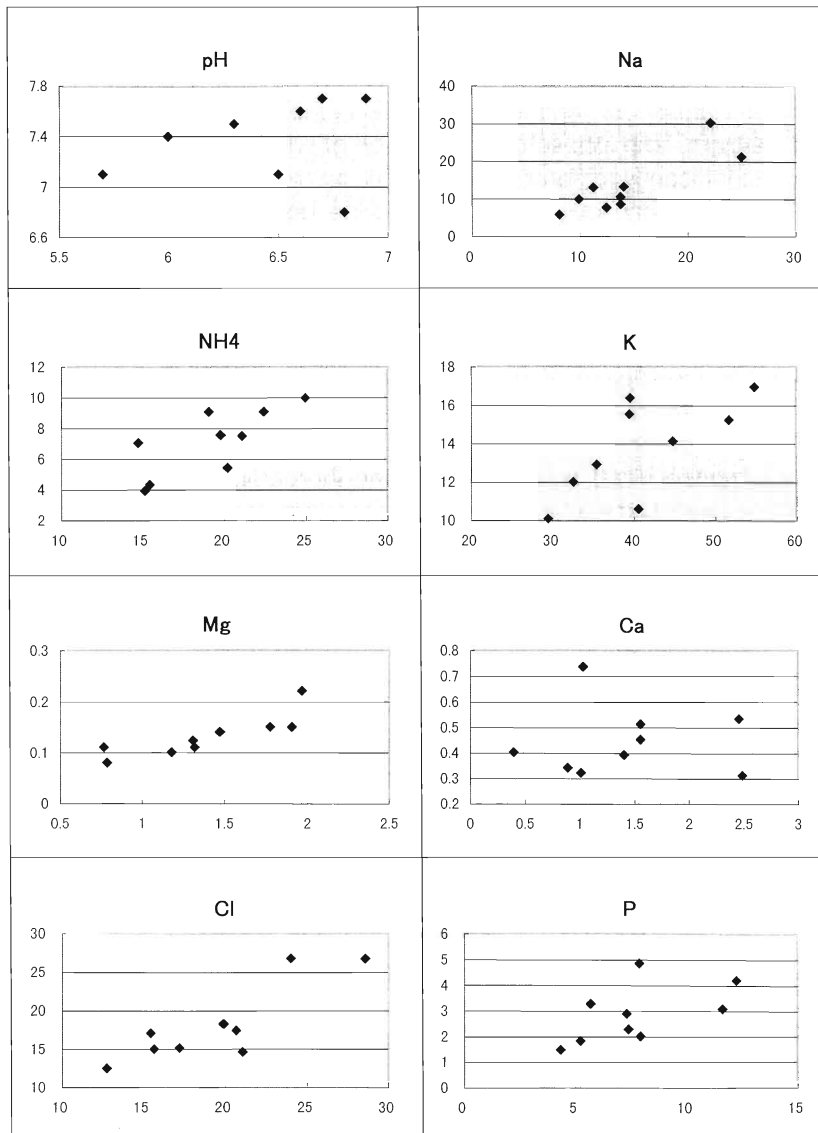
**Table 1.** Concentration of each component in plaque fluid and saliva

	Plaque fluid		Saliva		
	Mean	SD	Mean	SD	
pH	6.4 $\pm$ 0.4		7.4 $\pm$ 0.3		*
Na	14.5 $\pm$ 5.2		13.4 $\pm$ 7.3		NS
NH <sub>4</sub>	19.2 $\pm$ 3.3		7.1 $\pm$ 2.0		*
K	40.9 $\pm$ 7.9		13.8 $\pm$ 2.4		*
Mg	1.4 $\pm$ 0.4		0.13 $\pm$ 0.04		*
Ca	1.4 $\pm$ 0.7		0.4 $\pm$ 0.1		*
Cl	19.5 $\pm$ 4.6		18.2 $\pm$ 4.9		NS
P	7.7 $\pm$ 2.5		2.9 $\pm$ 1.1		*

\* : Statistically significant

NS: not significant

§ : Concentrations are expressed in mmol/l.



**Fig 1.** The individual concentrations of each component in saliva were plotted against those of plaque fluid. The vertical axes indicate concentrations in saliva and the horizontal axes indicate those in plaque fluid.

between saliva and plaque fluid. All coefficients were positive, and statistically significant correlation was observed in sodium, ammonium, potassium, magnesium, chlorine ions but not in pH, calcium and phosphate.

### Discussion

According to the data reported by Tatevossian et al<sup>2</sup>, the contents of the plaque fluid in preformed plaque can not be invariably raised by modifying its environment. The data obtained in this experiment supported this result. If saliva can permeate plaque easily, their com-

**Table 2.** Correlation coefficients of each component between saliva and plaque fluid

	Correlation coefficients	
pH	0.232	NS
Na	0.856	*
NH <sub>4</sub>	0.753	*
K	0.707	*
Mg	0.846	*
Ca	0.004	NS
Cl	0.856	*
P	0.567	NS

\* : Statistically significant

NS : not significant

position should resemble each other. However, significant differences were found between the composition of plaque fluid and saliva collected from the same person; concentrations of ions such as ammonium, potassium, magnesium, calcium, and phosphate as well as pH values were significantly different from each other. This result means that even though plaque exists adjacent to saliva, plaque fluid has its unique inorganic ion composition, which is different from that of saliva. In order for the disparity to be kept, there must be some mechanism of active transportation between saliva and plaque. The metabolism of bacteria in plaque may be one of the possible mechanisms. *Streptococcus sarivarius* in plaque, for example, has urease activity<sup>7</sup> which decompose urea, and consequently ammonium ion will be produced in plaque. The close correlation between saliva and plaque fluid in sodium, ammonium, potassium, magnesium and chlorine concentrations indicates that saliva supplies inorganic ions to plaque by some unknown mechanism, possibly through bacterial metabolism.

No correlation was found in calcium and phosphate concentration between saliva and plaque fluid. This result is inconsistent with that of Matsuo *et al.*<sup>6</sup>. According to Jenkins<sup>8</sup> salivary calcium and phosphate may be precipitated and included in plaque, along with salivary protein, during the plaque formation. It is probable that the composition and concentration of inorganic ions in the incipient plaque might resemble that of saliva and this resemblance might gradually be modified afterwards by the environment which includes the dissolution of enamel surface by acids from bacteria. Matsuo *et al.* used 24 hr plaque which was fresher than that which was used in this study. The age of the plaque may be a possible reason for the discrepancy between these two studies. The same explanation can be made about the results by Ashley *et al.*<sup>5</sup>. They found significant correlation of calcium concentration between saliva and plaque fluid in children to whom no restrictions on diet and oral hygiene were imposed. The plaque is expected to be rather fresh because of tooth brushing. In contrast, they found no correlation in young adults who abstained from oral hygiene for 48 hr. The plaque was rather old and the results were similar to the present experiment. As for pH, it is reasonable that there was no relation and also a large difference between plaque fluid and saliva, because pH of the plaque fluid is decided mainly through the acid production of micro-organisms in the plaque.

Concerning the permeability in plaque, there exists

an inconsistency among findings. The pH of plaque fluid decreases soon after exposure of the plaque to sucrose or other sugar solutions<sup>9,10</sup>. This decrease of pH in the plaque fluid is caused through the fermentation of sugar by micro-organisms in the plaque. As the pH in the plaque decreases when it is measured even at the bottom of the plaque layer using an antimony electrode buried in the plaque<sup>11</sup>, sugar must have permeated into the plaque in a short time after rinsing. In studies on diffusion through plaque, Melsen *et al.*<sup>12</sup> found that calcium chloride penetrated plaque much more significantly than glucose did. On the other hand Tatevossian *et al.*<sup>13,14</sup> reported the opposite finding. Thus, the discrepancy in permeability between sugar and inorganic ions cannot be explained rationally by these findings at present.

As it is impossible to distinguish plaque completely from saliva, the possibility of contamination of the plaque by saliva at the time of plaque collection cannot be denied. The composition of the plaque fluid might have been affected by the contaminated saliva. However the amount of contamination can be estimated to be very small, because careful attention was paid at the time of plaque collection and also there were big differences of concentration in several ions between saliva and plaque fluid.

The fluctuation and circadian variations of calcium and phosphate concentrations in saliva are reported by Larsen *et al.*<sup>15</sup> and Kavanagh and Svehla<sup>16</sup>. Similar tendencies are observed also in our laboratory even in the inorganic ions other than calcium and phosphate<sup>17</sup>. In spite of the possible fluctuation of concentration in the saliva, a strong correlation was found in many kinds of inorganic ions between plaque fluid and saliva in this experiment. This result suggests that significant individual differences beyond the fluctuation exist in salivary inorganic ion concentration.

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