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A study of the formation of artificial plaque on orthodontic brackets

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국문초록

교정용 브라켓상의 인공치태 형성에 대한 연구

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구강내의 교정 장치는 미생물 전파에 있어서 다양한 장소를 제공한다. 치태는 치아 우식증 발생에 있어 매우 큰 역할을 하며 미생물, 비세포성 물질로 구성되어 있다. 본 연구의 목적은 생체외에서 교정용에서의 인공 치태 생성에 영향을 주는 요인을 평가하는 것이다.

Streptococcus mutans type c는 CO_2 incubator내의 37℃에서 brain heart infusion broth에서 배양 되었다. 중절치에 사용하는 9개의 .018″×.025″ standard edgewise brackets을 3개씩 비이커의 배양액에 매달았다. 3개 비이커의 배양액 pH는 각 각 pH 5.5, 7.0 그리고 8.5로 조절되었다. 5시간 후에 비이커에서 각각의 bracket을 꺼내서, bracket의 평균 무게를 측정하였다. 배지의 stirring effect를 측정하기 위하여 3개씩 .018″×.025″ standard edgewise brackets을 2개의 비이커에 위치시켰다. 12개의 brackets을 $CaCl_2$(0.25, 1.0, 4.0 그리고 16.0mM). KCl(2.5, 10, 40 그리고 160mM) 그리고 $MgCl_2$(0.1, 0.4, 1.6, 그리고 6.4mM) 용액에 각각 매달았다. 6개의 .018″×.025″ standard edgewise brackets, 6개의 .022″×.028″ Roth brackets과 6개의 .022″×.028″ Broussard brackets을 각각의 비이커 내에 매달았다.

배양액 내에서 5시간 동안 배양한 후 각각의 brackets을 근사값의 milligram 단위로 측정하였다. 그룹 사이의 차이는 Mann-Whitney와 Kruskal-Wallis tests를 이용하여 비교하였다. p value<0.05의 조건에서 이들의 차이는 통계학적 유의성을 갖는다. 5시간 동안 pH 5.5에서 배양된 Streptococcus mutans에 의해 형성된 인공 치태는 pH 7.0 이나 pH 8.5에서 배양된 것보다 작았다($p<0.05$). 인공 치태는 배양하는 동안 저어졌을 때 더 많이 형성되었다($p<0.05$).

결론적으로 bracket에 형성된 인공 치태는 좀 더 높은 알칼리성 배지에서 배양됨으로써, 그리고 배양 동안 배지를 저어줌으로써 유의성있게 증가하였다. 그러나 배지의 $CaCl_2$, KCl, $MgCl_2$의 농도와 상업적으로 다른 종류의 이용 가능한 교정용 bracket에 대해서는 유의한 차이가 없었다.

주요어 : 치태, 교정용 브라켓, pH, *S. mutans*

Ⅰ. INTRODUCTION

Dental plaque plays a great role in cariogenicity, and consists of microbes and non-cellular materials[1-4]. Glucan, which is extracellularly synthesized from sucrose by glucosyltransferase, acts as the basic material of dental plaque. There are two constituents in glucan, water-soluble dextran and water-insoluble mutan. Mutan is the more important constituent in the formation of dental plaque and dental caries. Mutan is mostly synthesized by *Streptococcus mutans*. *Streptococcus mutans* has been known to enhance dental caries in the human oral cavity[5-9]. *Streptococcus mutans* forms colonies in retentive sites on human tooth surfaces, but does not form on the oral mucous membrane. *Streptococcus mutans* needs a tooth to form a colony. So we could see few numbers of *Streptococcus mutans* in the oral cavities of newborns or edentulous adults. The primary source of *Streptococcus mutans* that affects children has been thought to be the mother of that child. When *Streptococcus mutans* are suppressed in the oral cavity of the mother, the number of colonies of *Streptococcus mutans* in the oral cavity of the child could be reduced[10,11]. Strains of *Streptococcus mutans* are serologically divided into eight types. Among these serotypes, strains of serotype c are most frequently isolated from people from different countries[5].

Severe dental caries are sometimes present in the oral cavities of children who have eaten food-containing sucrose[12]. Sucrose and the aggregation of *Streptococcus mutans* produce acid[5,6,13,14].

Orthodontic appliances in the oral cavity offer multiple sites for microbial propagation. The purpose of this study is to evaluate the factors that effect the formation of plaque on orthodontic brackets.

Ⅱ. MATERIALS AND METHODS

Streptococcus mutans type c was cultured in brain heart infusion broth (BHI broth, Difco, Detroit, MI, USA) at 37°C in a $C0_2$ incubator. Brain heart infusion broth is a nutrient in which *Streptococcus mutans* growth is optimized.

The effect of pH on the formation of artificial plaque was determined. Nine central incisor .018″ × .025″ standard edgewise brackets (146-13, TOMY international inc. Japan) were suspended in three beakers each containing BHI broth containing 0.5% yeast extract and 10% sucrose. The pH of the broth in the different beakers was adjusted to either pH 5.5, 7.0 or 8.5. The media in each beaker was inoculated with 2.5×10^6 *Streptococcus mutans* cells per ml. Three brackets were immersed in the broth of each beaker. The beakers were placed into a $C0_2$ incubator at 37-degree Celsius. After five hours the brackets were removed from the beakers and the average weight of the brackets in each medium was determined.

The effect of stirring of the culture media was determined. Three .018″ × .025″ standard edgewise brackets were suspended into each of two beakers containing broth. The broths were inoculated with 2.5×10^6 *Streptococcus mutans* cells per ml, and the broths were incubated for 5 hours at 37°C. During in-

cubation the broth in one beaker was stirred and broth in the other beaker was unstirred. After incubation, the brackets were removed from their respective broths and weighed using a balance. Weights were determined to the nearest one hundredth of a milligram. The weight used was the average weight of the three brackets in each broth.

In order to determine the effects of $CaCl_2$ on the colonization of *Streptococcus mutans*, the concentration of $CaCl_2$ in four different beakers was adjusted to either 0.25, 1.0, 4.0 or 16.0 mM. Similarly, four other beakers of broth were used to determine the effects of KCl on the colonization of *Streptococcus mutans*. The concentration of KCl in each beaker was adjusted to 2.5, 10, 40 or 160 mM. Four different beakers of broth were used to determine the effects of $MgCl_2$, on the colonization of *Streptococcus mutans*. The concentration of $MgCl_2$ in each beaker was adjusted to 0.1, 0.4, 1.6 or 6.4mM. The broth in each beaker was inoculated with 2.5×10^6 cells of *Streptococcus mutans* per ml. The beakers containing the broths and brackets were placed in an incubator for five hours at 37° C. The beakers were then removed from the incubator and the weight of each bracket was determined. Weights were determined to the nearest one hundredth of a milligram. The weight used was the average weight of the three brackets from each broth.

Different commercially available orthodontic brackets were used to evaluate the colonization of the *Streptococcus mutans* on different types of brackets. Five .018″ × .025″ standard edgewise brackets (146-13, TOMY International Inc. Japan), were suspended within the broth of one beaker. The broth was inoculated with 2.5×10^6 *Streptococcus mutans* cells per ml, and incubated for five hours at 37° C. In a second beaker, five .022″ × .028″ Roth brackets (A-5570, RMO Inc., Denver. Colorado, USA) were suspended and in a third beaker, five .022″ × .028″ Broussard brackets (A-5075, RMO Inc., Denver, Colorado, USA) were suspended. After incubation, brackets were removed from their respective broths and weighed using a balance. The weight used was the average weight of the five brackets of each type.

Table 1. Artificial Plaque Formation by pH (unit: mg)

PH	5.5	7.0	8.5
Plaque	0.06	0.06	0.08
Formation	0.04	0.09	0.06
(mg)	0.03	0.08	0.08
Mean	0.04	0.08	0.07
S.D.	±0.02	±0.01	±0.01

*** : p<0.001

Ⅲ. RESULTS

The amount of plaque that formed on the bracket after culturing was measured to the nearest milligram. Values were compared to the weight of the brackets as supplied by the manufacturer. Differences between groups were compared with the Mann-Whitney and the Kruskal-Wallis tests. Differences were considered statistically significant at p value <0.05.

The effect of pH on the formation of artificial plaque was determined. The artificial plaque that was formed by culturing *Streptococcus mutans* at pH 5.5 for 5 hours, was significantly less than that cultured at pH 8.5 or pH 7.0 ($p < 0.001$). The average weight of the plaque adhering to each bracket surface at pH 5.5 was 0.04 mg. The average weight at pH 7 increased

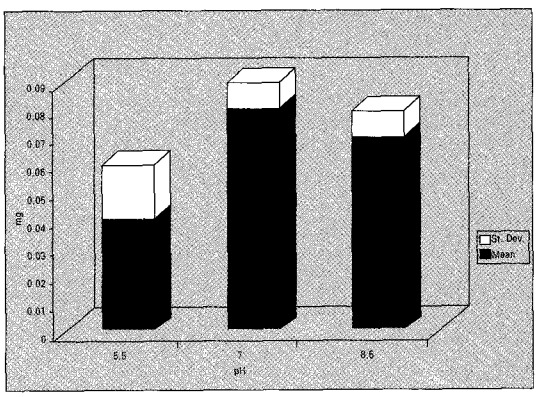

Fig. 1 Artificial Plaque Formation by pH

Table 2. Artificial Plaque Formation by Stirring (unit: mg)

	Stirring	No stirring	
Plaque	0.06	0.01	
Formation	0.09	0.01	
(mg)	0.08	0.01	
Mean	0.08	0.01	***
S.D.	±0.01	±0.00	

*** : $p<0.001$

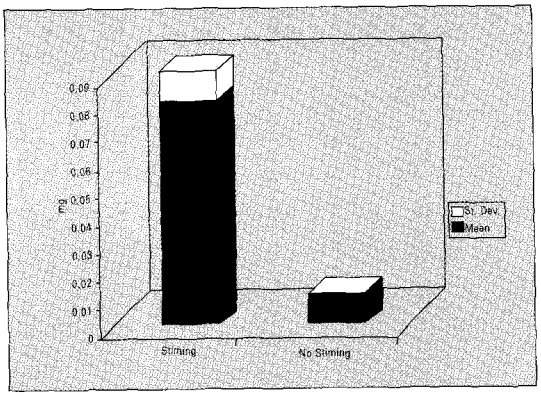

Fig. 2 Artificial Plaque Formation by Stirring

Table 3. Artificial Plaque Formation by $CaCl_2$ Concentration (unit: mg)

$CaCl_2$ Concentration	0.25mM	1mM	4mM	16mM	
Plaque	0.08	0.04	0.08	0.06	
Formation	0.05	0.11	0.07	0.04	
(mg)	0.09	0.10	0.10	0.06	
Mean	0.07	0.08	0.08	0.05	NS
S. D.	±0.02	±0.04	±0.01	±0.01	

NS : non significant

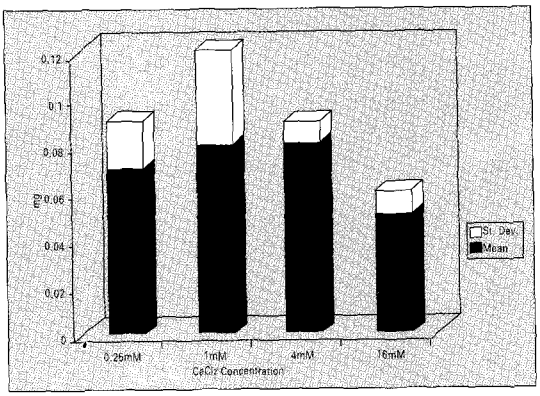

Fig. 3 Artificial Plaque Formation $CaCl_2$ Concentration

Table 4. Artificial Plaque Formation by KCl Concentration (unit: mg)

KCl Concentration	2.5mM	10mM	40mM	160mM	
Plaque	0.09	0.08	0.04	0.06	
Formation	0.07	0.07	0.07	0.04	
(mg)	0.09	0.07	0.10	0.06	
Mean	0.07	0.07	0.07	0.05	NS
S. D.	±0.01	±0.00	±0.03	±0.01	

NS : non significant

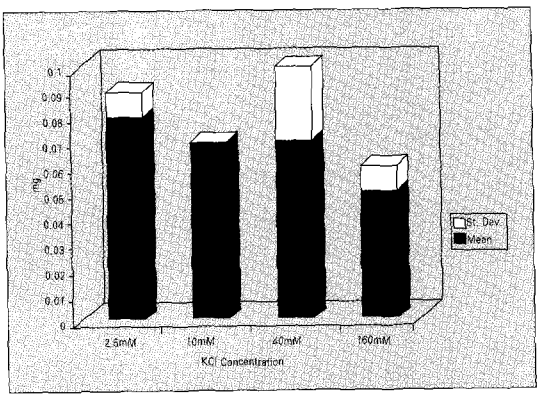

Fig. 4 Artificial Plaque Formation by KCl Concentration

to 0.08mg and the weight at pH 8.5 was 0.07mg (Table 1, Fig. 1).

Stirring also increased the number of colony forming units of artificial plaque. More artificial plaque formed when the media was stirred during incubation. In the stirred medium, 0.08mg of plaque formed whereas plaque formation in the unstirred mediums was 0.01 mg. This difference was significant ($p<0.001$) (Table 2, Fig. 2).

Effects of Ca, K, and Mg ion concentration in the media were evaluated. The amount of artificial plaque was not statistically different irrespective of the

Table 5. Artificial Plaque Formation by $MgCl_2$ Concentration (unit: mg)

$MgCl_2$	0.1mM	0.4mM	1.6mM	6.4mM	
Plaque	0.08	0.09	0.09	0.09	
Formation	0.13	0.09	0.07	0.06	
(mg)	0.09	0.07	0.09	0.09	
Mean	0.10	0.08	0.09	0.08	NS
S. D.	±0.03	±0.01	±0.01	±0.02	

NS : non significant

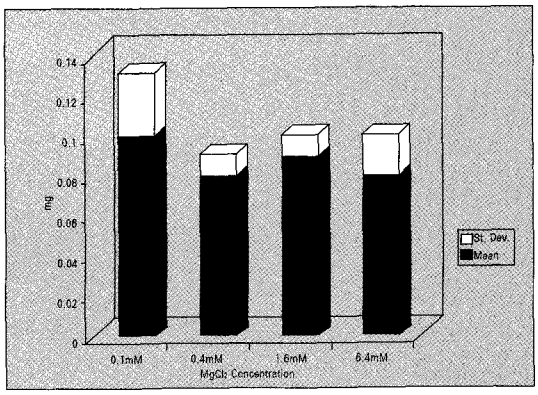

Fig. 5 Artificial Plaque Formation by $MgCl_2$ Concentration

Table 6. Artificial Plaque Formation by Bracket Shape(unit: mg)

Bracket	Standard	Roth	Broussard	
Plaque	0.05	0.10	0.06	
Formation	0.08	0.06	0.06	
(mg)	0.08	0.06	0.08	
	0.07	0.04	0.04	
	0.08	0.06	0.06	
Mean	0.07	0.06	0.06	NS
S. D.	±0.01	±0.02	±0.01	

NS : non significant

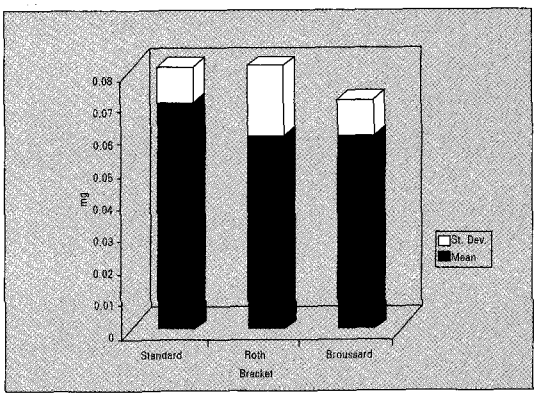

Fig. 6 Artificial Plaque Formation by Bracket Shape

concentration of $CaCl_2$, KCl and $MgCl_2$ in the media (Table 3, 4, 5, Fig. 3, 4, 5).

After 5 hours incubation the amount of plaque growth was not dependent on bracket shape. The weight of the artificial plaque that formed on the .018″ × 025″ standard edgewise (146-13, TOMY International Inc., Japan), the .022″ × .028″ Roth brackets (A-5570 RMO Inc., Denver, Colorado, USA), and .022″ × .028″ Broussard (A-5075, RMO Inc., Denver, Colorado, USA) brackets was not significantly different (Table 6, Fig. 6).

Ⅳ. DISCUSSION

Dental caries is one of the most prevalent dental diseases and the main cause of tooth loss in children. *Streptococcus mutans* is a primary pathogen in dental caries[15,16]. *Streptococcus mutans* is an anaerobe, resides in the acquired enamel pellicle on the tooth's surface and degrades the carbohydrates[5], especially glucose and fructose etc. from digested foodstuffs. The enamel surface is decalcified by the extracellular releasing of lactic acid obtained from metabolic pathway of the carbohydrates. This bacterium adheres to the tooth surface[17]. The edentulous newborns and infants with few teeth do not harbor *Streptococcus mutans*. As the number of erupted primary teeth increased, there is a gradual increase of early colonization of *Streptococcus mutans* on the interproximal surfaces of primary teeth[18].

Strains of *Streptococcus mutans* are divided serologically into eight groups, and among these serotypes, the C strains are most frequently isolated in man in many countries[5].

Toda et al.[19] and Loimaranta et al.[20] investigated the ultrastructure of the extracellular polysaccharide pro-

duced by *S. mutans*. They revealed that the polysaccharide consisted of three structural components; fructan in a globular structure, dextran in a single-stranded filament and mutan in a double-stranded fibril. On the other hand, two kinds of structures, a globular body and an amorphous substance, were observed by scanning electron microscopy.

Mutan that is water-insoluble glucan enhances the accumulation of *S. mutans* and other microbes on the tooth surface. Dextran and fructan that is water-soluble glucan are considered to provide extracellular energy storage for microbes. Extracellular glucan is formed by *Streptococcus mutans*, other Streptococcus species and Lactobacillus species[12,21-25]. So, these microbes adhere to tooth surfaces and affect the formation of dental caries.

A variety of factors could affect the formation of artificial plaque. The effect of pH, stirring, concentration of $CaCl_2$, KCl, $MgCl_2$, and bracket shape was considered in this study. At first in regard to effect of pH, little artificial plaque was formed at pH 5.5(Table 1, Fig. 1). *Streptococcus mutans* growth did not increase under acidic condition but increased growth in alkaline medium was observed. Metabolic products such as lactic acid produced by bacteria neutralize the alkaline medium. Stirring the medium enhanced the growth rate of *Streptococcus mutans* increasing the formation of plaque (Table 2, Fig. 2). A lot of artificial plaque eventually will form if more microbes including *Streptococcus mutans* exist on tooth surface.

Mattingly and his colleagues[26] reported that *Streptococcus mutans* showed a distinct tendency to increase in case bracket was attached, compared with the opposite case. Abe[27] reported that *Streptococcus mutans* increase in case a patient has dental caries, but there is none of report on the relation between the shape of bracket and the amount of *Streptococcus mutans* formation

In this study the amount of *Streptococcus mutans* formation according to three different types of brackets, .018″ × .025″ standard edgewise (146-13, TOMY International Inc. Japan), .022″ × .028″ Roth (A-5570, RMO Inc. Denver. Colorado, USA), and .022″ × .028″ Broussard (A-5075, RMO Inc., Denver, Colorado, USA) was compared but they did not show any statistically significant differences. According to the result the amount of *Streptococcus mutans* formation seems to be more related to whether bracket is attached or not than to the shape. Further research is required.

V. CONCLUSION

In conclusion, the artificial plaque that formed on the orthodontic brackets was significantly increased by culturing in a more alkaline broth and by stirring the media during incubation. Differences in the weight of artificial plaque formed on the brackets after 5 hours incubation were not statistically significant when cultured in broths that contained different concentrations of $CaCl_2$, KCl or $MgCl_2$. The amount of artificial plaque that formed on the .018″ × .025″ standard edgewise, the .022″ × .028″ Roth brackets, and the .022″ × .028″ Broussard brackets, were not significantly different from each other after 5 hours incubation.

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