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# Adhesion of *Streptococcus mutans* and *Streptococcus sobrinus* to different types of self-ligating brackets

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## ABSTRACT

### Adhesion of *Streptococcus mutans* and *Streptococcus sobrinus* to different types of self-ligating brackets

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**Objective:** The adhesion capabilities of different types of self-ligating brackets were measured with respect to *Streptococcus mutans* and *Streptococcus sobrinus*. **Methods:** Five types of self-ligating brackets (Clippy-C; Mini Clippy; Clarity-SL; Speed; Damon 3) were used for the experiment group and composite resin brackets (Spirit-MB), metal brackets (Victory) and polycrystalline alumina brackets (Clarity) were used for the control group. In order to assess adhesion of bacteria to the brackets, the brackets were cultured for 3, 6 and 24 hours in media containing bacteria and 20% sucrose.

**Results:** There was no statistic difference in adhesion amount of *Streptococcus mutans* and *Streptococcus sobrinus* according to the types of brackets. A total adhesion amount according to bracket type was different. An extended incubation time increased adhesion amount. Observation under scanning electron microscope showed that *Streptococcus sobrinus* adhered more to Clippy-C and Victory rather than to Clarity-SL. **Conclusions:** Clarity-SL, a self-ligating esthetic bracket was confirmed to show lower bacterial adhesion to cariogenic bacteria, *Streptococcus mutans* and *Streptococcus sobrinus* group than other self-ligating brackets or conventional brackets, which suggests that proper use of self-ligating esthetic brackets might even be better in preventing tooth surface decalcification.

**Key words :** *Streptococcus mutans*, *Streptococcus sobrinus*, Self-ligating bracket, Bacterial adhesion

## I. Introduction

The oral environment provides a survivable condition for numerous normal floras to live

in harmony. Attachment of fixed orthodontic appliances such as a bracket destroys such balance. Fixed orthodontic appliances of complex form that are attached to the dental

surface for a long time make removal of dental plaques difficult and continued deposition of bacteria around the teeth results in increased adhesion of various bacteria to the bracket, eventually increasing the probability of dental caries. The use of oral orthodontic appliances causes changes such as lower pH and increased carbohydrate components, sudden increase organic acids produced by mutans streptococci within dental plaques depending on oral hygiene, food consumption, type and form of the appliance, and method of attachment.<sup>1, 2, 3)</sup> Streptococci are associated with dental caries are generally termed mutans streptococci (MS) and known species other than *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) that are most commonly found in human teeth include *Streptococcus ferus*, *Streptococcus maceus*, *Streptococcus downei*, *Streptococcus criceus*, *Streptococcus macacae*, *Streptococcus rattus*.<sup>4, 5)</sup> The studies of our predecessors indicates that *S. mutans* is found more often in most cases than *S. sobrinus* and is known to be the major cariogenic bacteria that cause enamel decalcification upon attachment of brackets.<sup>6)</sup> These two species are closely associated with early dental caries<sup>7)</sup> and information of their adhesive ability to the orthodontic appliances can provide useful information to prevent enamel decalcification which is one of the biggest problems during orthodontic treatment<sup>8)</sup>.

Comparisons in microbiological aspects such as the degree of adhesion of *S. mutans*

and *S. sobrinus* to brackets and orthodontic materials have been done in several studies but most of them were indirect measurements using radioisotope rather than measuring live bacteria<sup>9-15)</sup>. Varying results on the comparison of amount of bacterial adhesion are published due to such differences in experiment methods. Recently, various types of esthetic brackets are introduced in step with increasing desire for economical-cultural status. Especially, various esthetic brackets such as ceramic brackets composed of monocrystalline sapphires and polycrystalline alumina and polycarbonate brackets are marketed and it has come to a point where orthodontic ceramic brackets that could ease the discomfort that occurs from ligating orthodontic wires and it is believed that the frequency of its use will increase. Accordingly, there have been a lot of studies on the mechanical nature such as frictional resistance, adhesive strength and morphological characteristics but studies on bacterial adhesion are rather lacking especially orthodontic brackets. The objective of this study was to compare the effects of the properties or morphological characteristics of orthodontic brackets on adhesion of *S. mutans* and *S. sobrinus*.

## II. MATERIALS AND METHODS

The brackets used in this experiment were brackets for the first premolar with

Table 1. The used orthodontic brackets for study

	Commercial Name	Materials	Company	Characteristics
Self Ligating Brackets	Clarity-SL	Ceramic	3M Unitek, Monrovia, CA, USA	Nitinol spring clip
	Clippy-C	Ceramic	Tomy International Inc., Tokyo, Japan	Rhodium coating clip
	Damon 3	Composite + Metal	Ormco, Orange, CA, USA	Composite + Metal clip
	Speed	Metal	Strite Industries, Cambridge, Canada	Superelastic spring clip
	M-Clippy	Metal	Tomy International Inc., Tokyo, Japan	Chrome-cobalt clip
Conventional Brackets	Victory	Metal	3M Unitek, Monrovia, CA, USA	All metal
	Spirit-MB	Composite	Ormco, Orange, CA, USA	Composit + Metal slot
	Clarity	Ceramic	3M Unitek, Monrovia, CA, USA	Polycrystalline alumina + metal slot

Table 2. Adhesion amounts of bacteria according to bacteria, bracket type and incubation time

Bacteria	Incubation time(hr)		Brackets								Total
			Clarity-SL	Clippy-C	Damon 3	Speed	M Clippy	Victory	Spirit-MB	Clarity	
S. mutans	3	Mean	4.15 x 10 <sup>4</sup>	4.92 x 10 <sup>4</sup>	3.44 x 10 <sup>4</sup>	5.87 x 10 <sup>4</sup>	3.76 x 10 <sup>4</sup>	7.70 x 10 <sup>4</sup>	12.4 x 10 <sup>5</sup>	1.18 x 10 <sup>5</sup>	6.94 x 10 <sup>4</sup>
		S.D.	2.10 x 10 <sup>4</sup>	4.09 x 10 <sup>4</sup>	1.89 x 10 <sup>4</sup>	6.80 x 10 <sup>4</sup>	1.37 x 10 <sup>4</sup>	7.38 x 10 <sup>4</sup>	1.52 x 10 <sup>4</sup>	8.74 x 10 <sup>4</sup>	7.77 x 10 <sup>4</sup>
	6	Mean	3.39 x 10 <sup>5</sup>	1.07 x 10 <sup>6</sup>	5.35 x 10 <sup>5</sup>	8.80 x 10 <sup>5</sup>	4.27 x 10 <sup>5</sup>	4.82 x 10 <sup>5</sup>	6.86 x 10 <sup>5</sup>	8.32 x 10 <sup>5</sup>	6.57 x 10 <sup>5</sup>
		S.D.	2.34 x 10 <sup>5</sup>	1.19 x 10 <sup>6</sup>	3.04 x 10 <sup>5</sup>	3.18 x 10 <sup>5</sup>	1.18 x 10 <sup>5</sup>	3.15 x 10 <sup>5</sup>	4.44 x 10 <sup>5</sup>	5.29 x 10 <sup>5</sup>	5.36 x 10 <sup>5</sup>
	24	Mean	2.84 x 10 <sup>6</sup>	3.27 x 10 <sup>6</sup>	6.38 x 10 <sup>6</sup>	5.01 x 10 <sup>6</sup>	3.62 x 10 <sup>6</sup>	5.40 x 10 <sup>6</sup>	2.70 x 10 <sup>6</sup>	2.44 x 10 <sup>6</sup>	3.95 x 10 <sup>6</sup>
		S.D.	1.44 x 10 <sup>6</sup>	2.29 x 10 <sup>6</sup>	3.82 x 10 <sup>6</sup>	4.43 x 10 <sup>6</sup>	2.0 x 10 <sup>6</sup>	2.67 x 10 <sup>6</sup>	2.53 x 10 <sup>6</sup>	7.76 x 10 <sup>6</sup>	2.89 x 10 <sup>6</sup>
Subtotal	Mean	1.01 x 10 <sup>6</sup>	1.58 x 10 <sup>6</sup>	2.32 x 10 <sup>6</sup>	2.21 x 10 <sup>6</sup>	1.50 x 10 <sup>6</sup>	2.08 x 10 <sup>6</sup>	1.41 x 10 <sup>6</sup>	1.15 x 10 <sup>6</sup>	1.47 x 10 <sup>6</sup>	
S.D.	1.49 x 10 <sup>6</sup>	2.03 x 10 <sup>6</sup>	3.61 x 10 <sup>6</sup>	3.51 x 10 <sup>6</sup>	2.06 x 10 <sup>6</sup>	2.95 x 10 <sup>6</sup>	1.99 x 10 <sup>6</sup>	1.15 x 10 <sup>6</sup>	2.33 x 10 <sup>6</sup>		
S. Sobrinus	3	Mean	1.95 x 10 <sup>4</sup>	1.36 x 10 <sup>5</sup>	6.23 x 10 <sup>4</sup>	5.95 x 10 <sup>4</sup>	2.40 x 10 <sup>4</sup>	2.96 x 10 <sup>4</sup>	1.33 x 10 <sup>5</sup>	2.73 x 10 <sup>5</sup>	5.68 x 10 <sup>4</sup>
		S.D.	1.18 x 10 <sup>4</sup>	7.57 x 10 <sup>4</sup>	2.33 x 10 <sup>4</sup>	3.01 x 10 <sup>4</sup>	1.02 x 10 <sup>4</sup>	1.55 x 10 <sup>4</sup>	4.68 x 10 <sup>4</sup>	2.16 x 10 <sup>4</sup>	5.68 x 10 <sup>4</sup>
	6	Mean	1.31 x 10 <sup>5</sup>	5.79 x 10 <sup>5</sup>	1.76 x 10 <sup>5</sup>	2.11 x 10 <sup>5</sup>	3.15 x 10 <sup>5</sup>	1.84 x 10 <sup>5</sup>	3.33 x 10 <sup>5</sup>	2.52 x 10 <sup>5</sup>	6.95 x 10 <sup>5</sup>
		S.D.	1.31 x 10 <sup>5</sup>	1.91 x 10 <sup>5</sup>	1.64 x 10 <sup>5</sup>	9.10 x 10 <sup>5</sup>	2.42 x 10 <sup>5</sup>	2.54 x 10 <sup>5</sup>	1.12 x 10 <sup>5</sup>	3.72 x 10 <sup>5</sup>	1.24 x 10 <sup>6</sup>
	24	Mean	1.29 x 10 <sup>6</sup>	5.18 x 10 <sup>6</sup>	2.58 x 10 <sup>6</sup>	2.48 x 10 <sup>6</sup>	4.23 x 10 <sup>6</sup>	4.94 x 10 <sup>6</sup>	2.40 x 10 <sup>6</sup>	4.0 x 10 <sup>6</sup>	3.37 x 10 <sup>6</sup>
		S.D.	5.05 x 10 <sup>5</sup>	2.52 x 10 <sup>6</sup>	1.81 x 10 <sup>6</sup>	1.87 x 10 <sup>6</sup>	1.30 x 10 <sup>6</sup>	3.35 x 10 <sup>6</sup>	1.36 x 10 <sup>6</sup>	2.77 x 10 <sup>6</sup>	2.34 x 10 <sup>6</sup>
Subtotal	Mean	4.81 x 10 <sup>5</sup>	1.85 x 10 <sup>6</sup>	1.38 x 10 <sup>6</sup>	1.01 x 10 <sup>6</sup>	1.61 x 10 <sup>6</sup>	2.40 x 10 <sup>6</sup>	9.05 x 10 <sup>5</sup>	1.34 x 10 <sup>6</sup>	1.37 x 10 <sup>6</sup>	
S.D.	6.57 x 10 <sup>6</sup>	2.67 x 10 <sup>6</sup>	1.68 x 10 <sup>6</sup>	1.60 x 10 <sup>6</sup>	2.13 x 10 <sup>6</sup>	3.13 x 10 <sup>6</sup>	1.26 x 10 <sup>6</sup>	2.35 x 10 <sup>6</sup>	2.09 x 10 <sup>6</sup>		
Total	3	Mean	3.05 x 10 <sup>4</sup>	9.67 x 10 <sup>4</sup>	4.96 x 10 <sup>4</sup>	5.91 x 10 <sup>4</sup>	3.08 x 10 <sup>4</sup>	5.55 x 10 <sup>4</sup>	1.29 x 10 <sup>5</sup>	7.27 x 10 <sup>5</sup>	6.61 x 10 <sup>4</sup>
		S.D.	1.99 x 10 <sup>4</sup>	7.49 x 10 <sup>4</sup>	2.51 x 10 <sup>4</sup>	5.02 x 10 <sup>4</sup>	1.34 x 10 <sup>4</sup>	5.86 x 10 <sup>4</sup>	1.07 x 10 <sup>5</sup>	7.70 x 10 <sup>4</sup>	6.76 x 10 <sup>4</sup>
	6	Mean	2.25 x 10 <sup>5</sup>	8.27 x 10 <sup>5</sup>	1.15 x 10 <sup>5</sup>	5.82 x 10 <sup>5</sup>	3.66 x 10 <sup>5</sup>	1.22 x 10 <sup>6</sup>	5.26 x 10 <sup>5</sup>	5.42 x 10 <sup>5</sup>	6.76 x 10 <sup>5</sup>
		S.D.	2.07 x 10 <sup>5</sup>	8.45 x 10 <sup>5</sup>	1.28 x 10 <sup>5</sup>	4.22 x 10 <sup>5</sup>	1.93 x 10 <sup>5</sup>	1.94 x 10 <sup>6</sup>	3.71 x 10 <sup>5</sup>	5.28 x 10 <sup>5</sup>	9.54 x 10 <sup>5</sup>
	24	Mean	2.00 x 10 <sup>6</sup>	4.14 x 10 <sup>6</sup>	4.48 x 10 <sup>6</sup>	2.93 x 10 <sup>6</sup>	3.92 x 10 <sup>6</sup>	5.17 x 10 <sup>6</sup>	2.57 x 10 <sup>6</sup>	3.15 x 10 <sup>6</sup>	3.55 x 10 <sup>6</sup>
		S.D.	1.27 x 10 <sup>6</sup>	2.48 x 10 <sup>6</sup>	3.46 x 10 <sup>6</sup>	1.54 x 10 <sup>6</sup>	1.64 x 10 <sup>6</sup>	2.90 x 10 <sup>6</sup>	1.99 x 10 <sup>6</sup>	2.01 x 10 <sup>6</sup>	2.38 x 10 <sup>6</sup>
Total	Mean	7.30 x 10 <sup>5</sup>	1.71 x 10 <sup>6</sup>	1.83 x 10 <sup>6</sup>	1.25 x 10 <sup>6</sup>	1.56 x 10 <sup>6</sup>	2.24 x 10 <sup>6</sup>	1.05 x 10 <sup>6</sup>	1.24 x 10 <sup>6</sup>	1.45 x 10 <sup>6</sup>	
S.D.	1.14 x 10 <sup>6</sup>	2.34 x 10 <sup>6</sup>	2.78 x 10 <sup>6</sup>	1.61 x 10 <sup>6</sup>	2.06 x 10 <sup>6</sup>	3.00 x 10 <sup>6</sup>	1.56 x 10 <sup>6</sup>	1.81 x 10 <sup>6</sup>	2.13 x 10 <sup>6</sup>		

\* The adhesion amounts of cariogenic S. mutans and S. sobrinus was defined as the number of S. mutans and S. sobrinus attached to brackets.

\* Subtotal and total adhesion amounts represent mean values in each section of rows and columns.

.022 X .028 inch slots. Relatively similar form of maxillary first premolar bracket from several companies were used<sup>10</sup>.

5 self-ligating commercial brackets such as Clarity-SL, Clippy-C, Damon 3, Speed and M-Clippy were used and, 3 conventional orthodontic brackets such as Victory, Spirit-MB, Clarity brackets were used for this study (Table 1). *S. mutans* strain ATCC 25175 (Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea) and *S. sobrinus* strain ATCC 27351 (Bank of Oral Biofilm, Dental Research Institute, Seoul National University, Seoul, Korea) were used. The bacteria were suspended in brain heart infusion (BHI, Becton, Dickinson and Company, MD, USA) broth containing 20% glycerol and kept in a freezer of  $-70^{\circ}\text{C}$  for use. The bacteria kept in a freezer were aerobically cultured for 48 hours in BHI agar medium. One colony obtained from culture was inoculated to 10 ml BHI broth and aerobically cultured at  $37^{\circ}\text{C}$ . *S. mutans* and *S. sobrinus* were cultured for 17 hours and 12 hours, respectively. Optical density (OD) of culture media was measured at 570 nm and the numbers of bacteria were counted using standard curve between colony forming unit (CFU/ml) and OD at 570 nm of culture media. To harvest the bacteria for experiments, culture media were centrifuged for 5 minutes at 3,000 X g. Each bracket were each centered in a

well of 24-well plate by sterilized bracket tweezer and incubated 2 ml BHI broth containing  $1 \times 10^8$  bacteria and 20% sucrose for 3, 6 and 24 hours at  $37^{\circ}\text{C}$ . After indicated incubation period, bracket was pulled out of the wells and flushed 3 times for 10 seconds in phosphate-buffered saline solution (PBS, pH 7.2). Bacteria attached to bracket were isolated using vortexing and sonication. Each bracket was put in 1 ml PBS solution and vortexing and sonication were performed twice. Sonication was done for 15 seconds using ultrasonic cleaner (Branson Ultrasonic Corporation, Danbury, USA). The PBS solution containing bacteria was serially diluted, smeared on BHI agar and cultured for 3 days to count the number of bacteria. All test samples were counted 3 times and each experiment was repeated 6 times. The bacterial adhesion was compared by performing 3-way factorial analysis of variance (ANOVA) using SPSS program (SPSS Inc., Chicago, IL, USA) between bacteria, incubation time and bracket type and the Bonferroni t-tests at a significant level of  $\alpha = 0.05$  were used for the multiple comparison between the different groups. Brackets with adhering *S. mutans* and *S. sobrinus* were reapplied for SEM verification. The brackets were washed with PBS, fixed with 2% glutaraldehyde-paraformaldehyde. They were dehydrated in ethanol and infiltrated with and they were followed by Iso amyl acetate and subjected

Table 3. Results of statistical analysis for bacterial adhesion amount

Source	SS	DF	Mean of Square	F value	Comparison
Bacteria	$8.99 \times 10^{11}$	1	$8.99 \times 10^{11}$	0.48	
Bracket type	$4.73 \times 10^{13}$	7	$6.75 \times 10^{12}$	3.61	* Clarity-SL<Victory Clarity-SL<Damon 3 Spirit-MB<Victory
Incubation time	$6.25 \times 10^{14}$	2	$3.13 \times 10^{14}$	167.34	** 3<6<24hr
Bacteria * Incubation Time	$2.06 \times 10^{12}$	2	$1.03 \times 10^{12}$	0.551	
Bacteria * Bracket type	$1.39 \times 10^{13}$	7	$1.98 \times 10^{12}$	1.061	
Incubation Time * Bracket type	$4.86 \times 10^{13}$	14	$4.23 \times 10^{12}$	1.859	*

Statistical analysis was performed by using 3-way factorial ANOVA for adhesion amount of bacteria according to bacteria, bracket type and incubation time DF, Degree of freedom SS, Sum of Squares MS, Mean of Square Multiple comparison were done by Bonferroni t-test at a significance level of  $\alpha=0.05$ .

\* : statistically significant difference at  $p<0.05$

\*\* : statistically significant difference at  $p<0.001$

to Critical Point Dryer(HCP-2, Hitachi, Japan). They were coated with gold by ion sputter(IB-3 Eiko Japan) 6mA 6min and examined and photographed with a Scanning Electron Microscopy(FE SEM S-800, Hitachi, Japan) at the acceleration voltage of 20kV.

### III. RESULTS

The number of *S. mutans* and *S. sobrinus* attached to brackets was compared according to incubation time and bracket types. The bacterial adhesion amount was counted mean value as for 3, 6 and 24 hours for each bracket and time added together. The adhesion amount of *S. mutans* was higher than that of *S. sobrinus* but there was no significant difference (Table 2). There was a difference according

to the bracket type. The adhesion amount of *S. mutans* and *S. sobrinus* showed statistically significant difference depending on the bracket type. Clarity-SL was lower than Victory or Damon 3 bracket and that of Spirit-MB which is a resin bracket was lower than that of Victory(Fig. 1; Table 3). The total bacterial adhesion amount of *S. mutans* and *S. sobrinus* attached to the brackets increased with increasing incubation time of 3, 6 and 24 hours. There was no significant difference in bacterial adhesion depending on the bracket type for *S. mutans* at 3 hours. With *S. sobrinus*, there were differences between brackets at 3 hours with Clippy-C being higher than Clarity, M-Clippy and Clarity-SL and Spirit-MB showing higher bacterial adhesion than Victory, Clarity, M-Clippy and Clarity-SL(Table 4). There was no significant difference in adhesion of *S.*

mutans and *S. sobrinus* between brackets at 6 and 24 hours but the difference in adhesion between brackets was relatively bigger for *S. sobrinus* with increasing incubation period than for *S. mutans*. Even at 24 hr, there was no difference between brackets in both species, but comparison of

mean bacterial adhesion amount showed Damon 3 > Victory > Speed > M-Clippy > Clippy-C > Clarity-SL > Spirit-MB > Clarity for *S. mutans* and Clippy-C > Victory > M-Clippy > Clarity > Damon 3 > Speed > Spirit-MB > Clarity-SL for *S. sobrinus* (Fig. 2). A difference in the interaction effects was

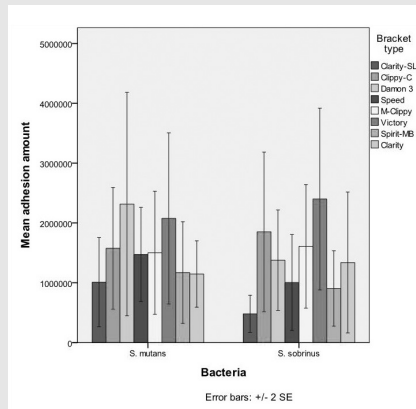


Fig 1. Adhesion amount of bacteria attaching to brackets according to bacteria, bracket type. The adhesion amount was calculated as a combined number of attached bacteria to brackets for 3, 6 and 24 hr

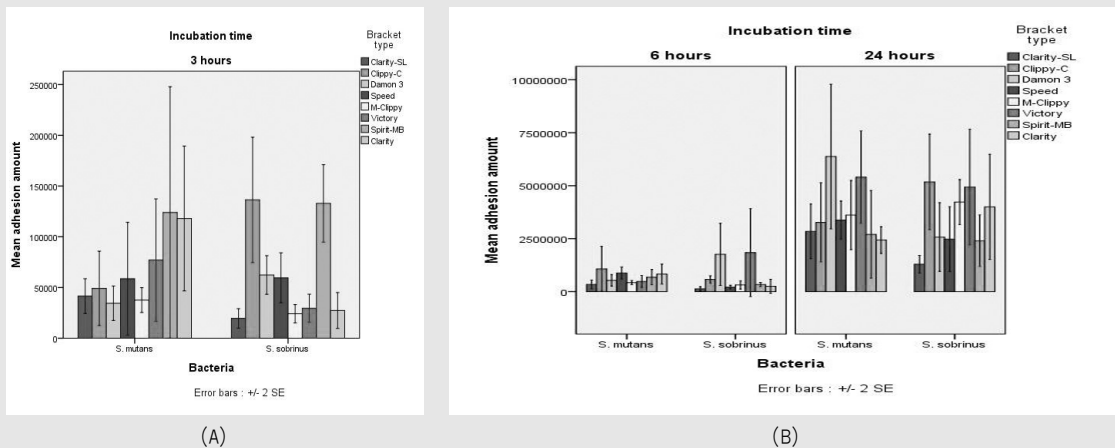


Fig 2. Mean adhesion amount of bacteria to brackets according to the incubation time at (A) 3 hr, (B) 6 hr and 24 hr. In *S. sobrinus*, the adhesion amount in the 3 hr-incubation time was high at the Clippy-C and the Spirit-MB

statistically significant only between bracket type and incubation times( $P>.05$ ). Pattern of adhesion of *S. sobrinus* to brackets incubated for 24 hr was observed under SEM. Clippy-C also showed a lot of *S. sobrinus* around the ion-coated cap(Fig. 3-B) and Victory which showed high bacterial adhesion showed multiple layers of bacteria clustered together all over the metal surface(Fig.3-G, 4-A). Nitinol clip, one of the major components of Clarity-SL which is a orthodontic bracket that showed low *S. sobrinus* adhesion, formed a

rounded, smooth contour and a niche around which bacterial adhesion was mainly observed and the smooth surface around the others of clip showed quite a low level of bacterial adhesion(Fig.3-A, 5). The resin parts of Spirit-MB and Damon 3 showed swelling and cracks, than it was smoothed out the outer surface. Partial bacterial adhesion was observed on the smooth surface of resin (Fig. 3-C, 4-B). The other brackets to *S. sobrinus* did not show much difference in the pattern of adhesion. *S. mutans* was observed to

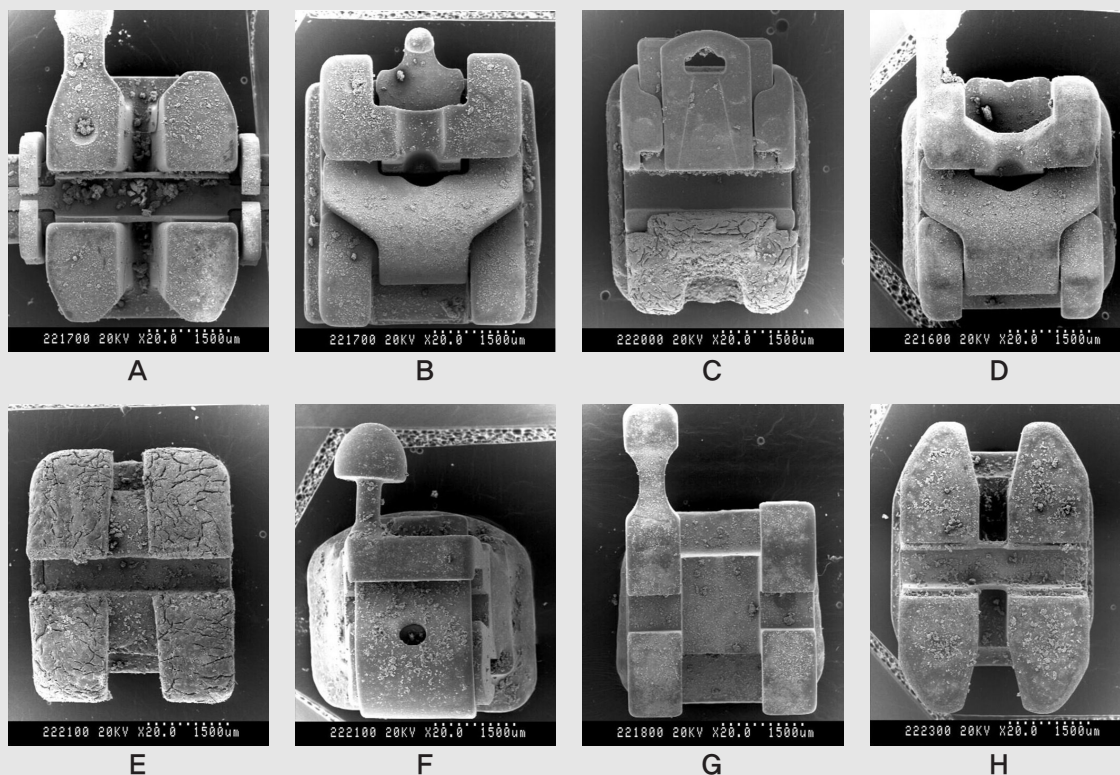


Fig. 3. The electron microscopy image of brackets removed from the wells containing *S. sobrinus* for 24 hr ( $\times 20$ ) (A) Clarity SL, (B) Clippy-C, (C) Damon 3, (D) Speed, (E) M-Clippy, (F) Victory, (G) Spirit MB, (H) Clarity

adhere more aggressively and in larger quantity than *S. sobrinus* and the data was not shown.

#### IV. DISCUSSION

Brackets that are attached to the enamel surface during long-term orthodontic treatment cause local changes in the oral environment due to changes in the free energy of the surface, surface charge, hydrophobicity, size, morphology and the properties of bracket adhesive, status changes in the residual adhesive. Consequently, brackets not only get to play an important role in the degree of bacterial adhesion and dental plaque accumulation but also increase the risk of dental caries. Papaioannou et al<sup>12)</sup> reported that more bacteria will be attracted to brackets with higher surface free energy and brackets consisting of metal cause special changes such as decrease of pH, increase of dental plaque and the number of *S. mutans*. Eliades et al<sup>14)</sup>, in the study on wettability of microbes to orthodontic appliances, reported that stainless steel has bigger surface tension than polycarbonate or alumina and, therefore, has bigger bacterial adhesion potential. Ahn et al<sup>15)</sup>, through the study on adhesion of *S. mutans* and *S. sobrinus* to brackets, reported that the amount of bacterial adhesion was in the order of plastic > metal, titanium > ceramic

and that the amount of bacterial adhesion increases with increase in the incubation time. Lim et al<sup>11)</sup>, in the study of *S. mutans* and *S. sobrinus* adhesion to bracket raw materials, reported that the amount of bacterial adhesion was larger with *S. mutans* than with *S. sobrinus* and ceramic materials showed higher bacterial adhesion than metal. They also reported rather low correlation of the surface energy and the amount of bacterial adhesion whereas other various factors such as surface roughness, surface charge, hydrophobicity were seen to have effects. This study including orthodontic brackets showed that the Clarity-SL has rather smaller bacterial adhesions than any other orthodontic brackets. The reasons why such various opinions are reported that differences in experimental method such as bacterial counting, components of media and condition of incubation and there could be errors from statistical processing of data with a small number of samples. In this study, both the experiment group and the control group showed significant increase in the adhesion of *S. mutans* and *S. sobrinus* with increase in the incubation time. There was a statistical difference of adhesion numbers of *S. sobrinus* between brackets. This implies that there were different adhesion mechanisms between brackets and there may have been interactions with the incubation time and bracket types. *S. mutans* showed higher

mean bacterial adhesion number than *S. sobrinus* but there was no statistical significance. Total adhesion amount of *S. mutans* and *S. sobrinus* showed a significant difference between brackets and Victory, the metal bracket or Damon 3, the orthodontic bracket combined metal and composite compartment was higher than Clarity-SL, the ceramic orthodontic bracket. Victory was higher than Spirit-MB, the resin bracket. As for the mean value of the bacterial adhesion, Clarity-SL showed the smallest bacterial adhesion level in both species. *S. sobrinus* showed significantly higher bacterial adhesion with Clippy-C and Spirit-MB when cultured for 3 hours, but other incubation times did not show any difference (Table 4). In order to confirm the differences between Victory, Spirit-MB, Clippy-C and Clarity-SL that show differences in bacterial adhesion,

bacteria were attached to each bracket under the same conditions for 24 hours and observed under SEM. Victory brackets composed of stainless steel and known to have the biggest surface free energy showed wide and multiple layers of bacteria (Fig. 4-A). Clippy-C is composed of ceramic part with alumina component and rhodium-coated clip, has relatively larger surface area than the other experiment group and has the shape of metal cap with a clip that has relatively larger surface area that covers the outer part which provides a convenient spot for bacterial adhesion (Fig. 3-B). Half of Damon 3 is metal and the rest consists of esthetic part of resin components and quite a lot of bacteria were attached to the metal side cover and a lot of bacteria were attached to the rough flank side due to thick width (Fig. 3-C). Spirit-MB composed of composite resins shows

Table 4. Results of one way ANOVA for adhesion amount of bacteria according to the incubation time

Bacteria	Incubation time(hr)	SS	MS	DF	F value	Sig	Comparison
<i>S. mutans</i>	3	$5.10 \times 10^{10}$	$7.28 \times 10^9$	7	1.25	.299	
	6	$2.27 \times 10^{12}$	$3.25 \times 10^{11}$	7	1.16	.349	
	24	$7.42 \times 10^{13}$	$1.06 \times 10^{13}$	7	2.12	.065	
<i>S. sobrinus</i>	3	$9.38 \times 10^{10}$	$1.34 \times 10^{10}$	7	9.95	.000	Clippy-C > Clarity, M-Clippy, Clarity-SL Spirit-MB
	6	$1.90 \times 10^{13}$	$2.71 \times 10^{12}$	7	2.08	.073	> Victory, Clarity, M-Clippy, Clarity-SL
	24	$7.59 \times 10^{13}$	$1.08 \times 10^{13}$	7	2.44	.037	

DF, Degree of freedom SS, Sum of Squares MS, Mean of Square

Multiple comparison were done by Bonferroni t-test at a significance level of  $\alpha=0.05$ .

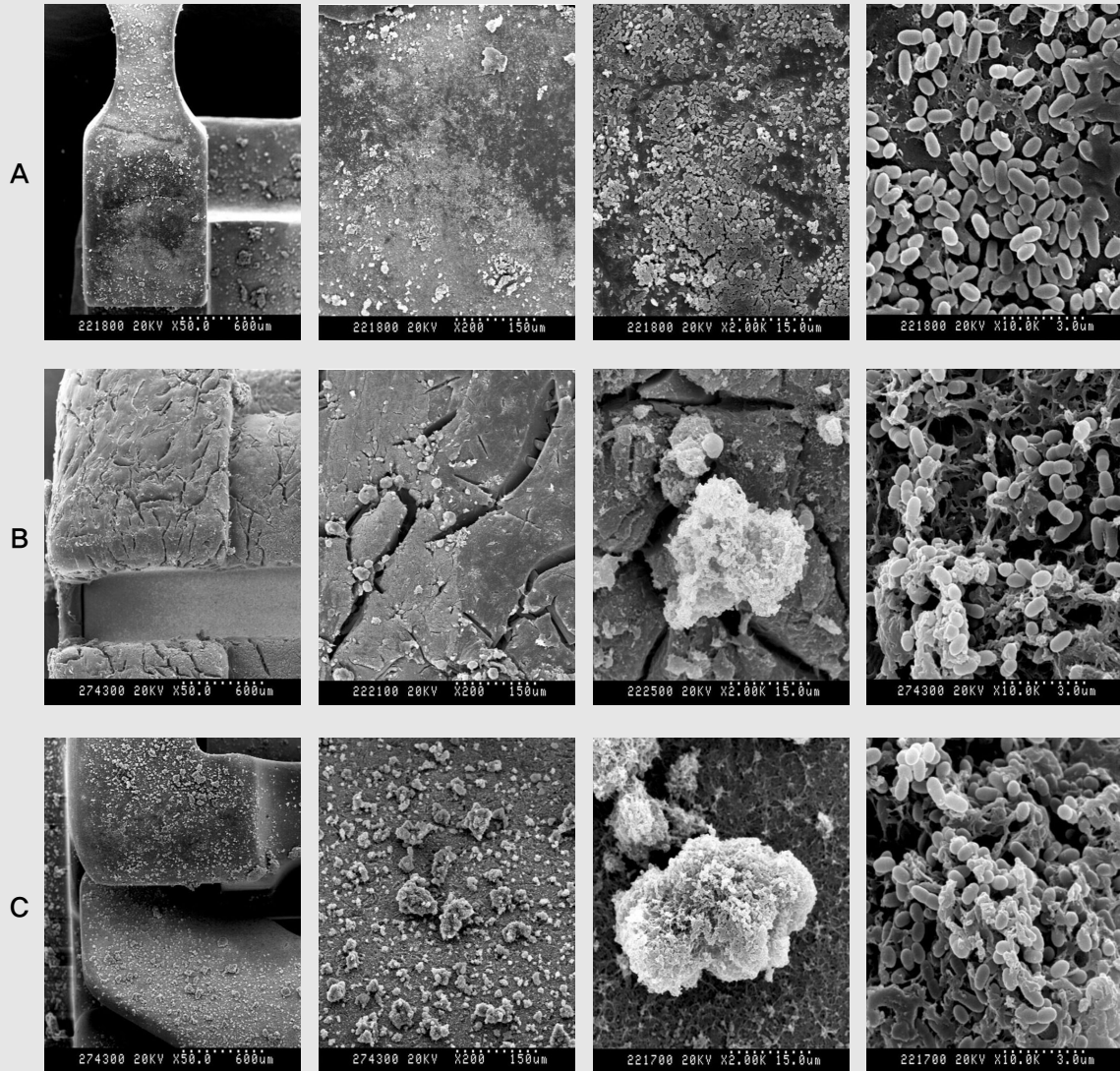


Fig. 4. The electroscopy image of Victory (A), Spirit-MB (B), Clippy-C (C) attached by *S. sobrinus* for 24 hr. Clippy-C have shown the highest adhesion amount of bacteria in the experimental group. Victory was accumulated and spread to a layer by layer. The surface of Spirit-MB was cracked and smoothened.

expansion and cracking when immersed in the bacterial solution due to hydrophilic characteristics and low tensile strength of the resin and shows less number of bacteria could be seen due to fracture and smoothing of resin materials (Fig. 3-E).

The resin part showed the same pattern as Spirit-MB and did not have a lot of bacteria adhesion. Speed, consisting of metal body and superelastic spring clip, has a large surface free energy and a large surface to attach to, so quite a lot of adhesion was

observed. M-Clippy is small but the metal cap that covers the orthodontic wire and a big hook, relatively large compared to the body, made the amount of bacterial adhesion not so different from other orthodontic brackets in spite of its relatively small size(Fig.3-F). Therefore, bacterial adhesion doesn't seem to depend solely on the surface area of brackets and changes in the properties of matter should be more considered with passing time. Clarity-SL that showed the smallest bacterial adhesion compared to the control

group has a streamline body with ceramic components and a nitinol clip that has a smooth surface in the shape of semicircle linked to the flank. It showed low bacterial adhesion pattern due to low surface energy of ceramic and the characteristics of the clip itself that has been designed to have smooth surface and semicircle shape (Fig.5). Bacterial adhesion around the clip was centered around the niche, the smooth curve of the edge showed lower bacterial adhesion and the existence of bacteria was observed to be quite looser than in Clippy-

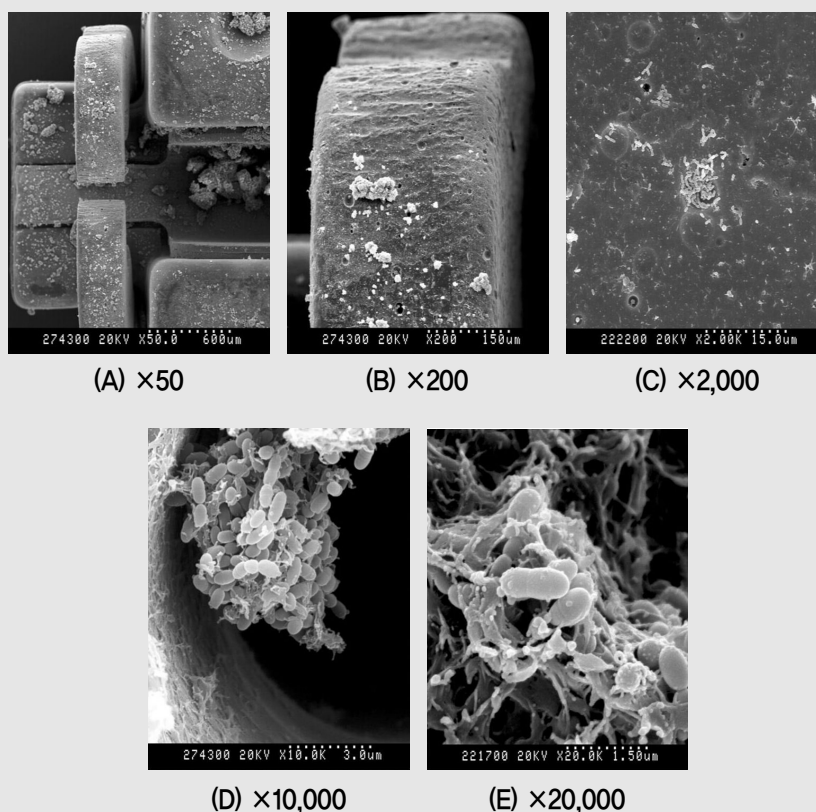


Fig 5. The electron microscopy image of the niche of nitinol clip on Clarity-SL attached by *S. sobrinus* for 24 hr. The smooth surface of nitinol clip interrupted from bacterial attachment and the adhesion affinity of bacteria on Clarity-SL was lower than any other brackets.

C. There are a lot of factors that affect the differences in the bacterial adhesion to brackets, but, in this experiment, it is noticeable how materials and shape that affect the surface free energy have such effects. It is believed that, in order to improve the shape so as to reduce the bacterial adhesion of orthodontic brackets, the shape of clip, which consists of nitinol, attached to the flank of brackets and the metal components should be minimized and made into a curve structure. It was found out that orthodontic brackets of such complex design that were believed to have more serious bacterial adhesion than general ceramic or metal brackets actually could have lower bacterial adhesion due to its characteristics and size. The results of this study could be used as a good piece of information in choosing a better suited bracket when starting fixed orthodontic treatment that requires consideration of both esthetic aspect and prevention of dental caries. There is a need to more data about this experiment in order to statistically objectify it and, based on this, continue the study on bacterial adhesion

according to using of main orthodontic wire and elastomer.

## V. CONCLUSION

The aim of this study is to understand the degree of adhesion to brackets of two major bacteria, *S. mutans* and *S. sobrinus* that cause the initial dental caries.

- Total bacterial adhesion amount of *S. mutans* and *S. sobrinus* showed Clarity SL was lower than Victory or Damon 3. That of Spirit MB was lower Victory.
- Clarity-SL showed the lowest mean bacterial adhesion amount among the orthodontic brackets
- The results of observation under SEM showed that the degree of bacterial adhesion was higher for Clippy-C and Victory for both species and it was rather low for Clarity-SL.

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