

1998

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## Recommended Citation

Lopez, Arturo James, "The Adhesion of Streptococcus sa/ivarius and Staphylococcus aureus to Five Dental Composite Resins" (1998). *Senior Research Projects*. 106.

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The Adhesion of *Streptococcus salivarius* and *Staphylococcus aureus*  
to Five Dental Composite Resins

April 1998

Southern Scholars Senior Project

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## ABSTRACT

Resin composites are used for anterior esthetic restorative procedures. Breakdown areas between cavity preparations and restorative materials can provide potential sites of reinfection. Reducing the marginal breakdowns by using effective composite resins is important to reduce the amount of recurrent caries.

Each composite type was analyzed for bacterial adherence after bacterial exposure by microscopically counting them after staining. The purpose of this experiment was to measure and observe the ability of *Streptococcus salivarius* and *Staphylococcus aureus* to adhere to five different resin composites (APH, Charisma, Herculite, Silux, and Z-100) using an *in vitro* assay.

It was found that there is a great ability for bacteria to colonize and adhere to resin composites after bacterial exposure. Furthermore, the amount of adherence varied at the same bacterial exposure time as well as over varying exposure times. The amount of bacterial adherence on a single composite sample was not uniform in adherence. The large standard deviations obtained from the bacterial counts indicated a large degree of variance of bacterial adhesion on a single composite resin for all the resins tested. The Z-100 composite had the most overall bacterial adherence, and the Herculite composite had the least adherence.

## INTRODUCTION

Resin composites are selected as primary material for restorative procedures in current dental practice. For example, if a tooth has been chipped or two teeth are separated in the anterior portion of the mouth, resin composites are generally used to esthetically restore the anatomy of the teeth. Despite its usefulness, the deterioration of resin composite materials has been associated with recurrent dental caries (5). Potential reinfection can occur in breakdown areas between cavity preparations and restorative materials. Since potential reinfection can occur in these areas, an ideal resin composite would reduce bacterial adherence.

It would be advantageous if these materials possessed antimicrobial activity. Restorative materials have demonstrable *in vitro* bactericidal abilities. This activity may be due to the release of high concentrations of fluoride ions, or an initially low surface pH (5). There are fluoride-containing composite resins that are available. The inference from this is that, over time, the fluoride could reduce the formation of recurrent caries.

Specialized biomolecules have evolved to serve as primary adhesins to inert surfaces and the molecular architecture of extracellular components seem to be fashioned for adhesion or detachment from inert surfaces (2). It has been shown that after the primary adhesins bind, the adherence of bacteria to surfaces is a primary step in plaque formation (3,4,6). Studies have shown that plaque and gingivitis occur adjacent to resin composites (8). Also, it has been shown that bacteria associated with plaque is able to bind to a greater extent to a resin composite (P-10) than to an amalgam (Dispersalloy) and a glass-polyalkenoate (ionomer) (7). With this research in mind, it is important to realize that biofilms form on composite resins, therefore good restorative materials are needed to prevent or at least reduce the amount of recurrent caries.

Whether or not certain kinds of resin composites induce bacterial adherence is an important question to dentists and patients. Any surface is prone to bacterial adherence, but exactly how much adherence occurs on each composite resin should be compared. As a result, the purpose of this study is to observe and measure the *in vitro* adherence of two prevalent oral bacteria, *S. salivarius* and *S. aureus*, to five different resin composites.

## MATERIALS AND METHODS

### **Bacteria, chemicals, and media**

*Staphylococcus aureus* and *Streptococcus salivarius* were purchased from WARD'S Natural Science Establishment, Inc., Rochester, New York. Chemicals used were reagent grade chemicals and purchased from Sigma Chemical Company, St. Louis, MO. Tryptic soy broth (TSB) and tryptic soy agar (TSA) were purchased from Difco Laboratories, Detroit, MI.

### **Composite test materials**

The composite resins used in this experiment were Herculite (Kerr A2), Z-100 (3M B3 5905 AP), Charisma (B30 322), APH (Caulk 4.391.590), and Silux (3M 55002G). They were obtained from Dr. Roger Hall in Cleveland, TN.

### **Composite preparation**

Composite resins were prepared at Dr. Hall's dental office with his assistance. Glass slides (Erie Scientific, Portsmouth, NH.) were washed with 70% ethanol in a Sterngold ultrasonic cleanser for ten minutes. The area on each slide where the composite was mounted was sand blasted with 90 micron sized sand at 90 psi for approximately 10 seconds and the slide resonicated with 70% ethanol. The composites were mounted according to Dr. Hall's specifications.

A lexan surface (6 mm thick) was used to flatten the composite to 0.5 mm using a copper wire spacing template. A milar strip was used between the composite and the lexan surface to keep composite surfaces pure (Fig. 1).

### **Slide cultures**

Each slide was placed in 95% ethanol for five minutes and then air dried prior to bacterial exposure. Each slide was placed in a sterile petri plate covered with  $1 \times 10^6$  bacteria per mL for a total volume of 18 ml to completely cover the slide and composite sample. The slides were incubated with bacterial culture at 37°C for 4, 8, and 12.5 hours. After incubation, the samples were rinsed with 10 ml of double deionized H<sub>2</sub>O and stored for staining. One bacterial culture was used to expose all the samples pertaining to a single time period.

### **Staining**

The biofilm was heat fixed, Gram stained, and examined microscopically at 1000X with oil immersion.

### **Counting methods**

The samples were viewed under low power to find the areas of greatest and least amount of bacterial adhesion. Then, all the bacteria present in five different fields of view were counted and examined. A single bacterium as well as a colony of bacteria were counted as one for all the samples. Often, a colony of bacteria was found which seemed to come from a single bacteria. Colonies such as these were counted as one, and not as the total number of bacteria in the colony.

### **Statistical analysis**

The standard deviation of the five different fields of view for each composite sample was obtained.

## Results

It was determined from a pilot study that a biofilm began to form within four hours of exposure to the bacteria, but before three hours, few bacteria adhered to the composite resin surface. For this reason 4, 8, and 12.5 hour exposure times were chosen to determine the degree of bacterial adhesion on the five different composite resins.

There was a great degree of variance of *S. aureus* adhesion to the composite resins from the 4 hour to the 12.5 hour exposure. For example, the 4 hour exposure to APH, Charisma, and Herculite had more bacterial adherence than the 12.5 hour exposure (Fig. 2). The adherence did not increase with time for any of the composite resins. The Z-100 resin did increase in bacterial adherence from 8 to 12.5 hours (100 to 440), but decreased from 4 to 8 hours (200 to 100) of exposure (Fig. 2). The Herculite and Silux resins did not show a gradual increase in bacterial adherence, but showed an increase of adherence between 4 and 8 hours and then a steep decrease in adherence between 8 and 12.5 hours (Fig 2). The Silux resin was very similar in that it increased in adherence dramatically between 4 and 8 hours of exposure and then decreased from 8 to 12.5 hours of exposure.

The average of the amount of adherent bacteria of all three exposure times of *S. aureus* on each composite resin tested was obtained (Fig. 3). From the five resins tested, the Z-100 composite showed the most bacterial adherence (370) while Herculite showed the least (165) (Fig 3). APH at 180, Silux at 180, and Herculite at 165 had close results and were lower in adhesion than Charisma (270) and Z-100 (370) (Fig. 3).

In the three different timed exposures of *S. salivarius*, the APH resin gave the same results as with the *S. aureus* exposure in that it decreased in bacterial adherence with time (Fig. 4). Charisma, Silux, and Z-100 increased in bacterial adherence with time. For example, 50 bacteria adhered to the Z-100 resin at 4 hours, 175 at 8 hours, and

450 at 12.5 hours (Fig. 4). The Herculite resin was the only one that had different results by increasing adherence at 8 hours and then decreasing adherence at 12.5 hours of exposure (Fig. 4).

The average amount of adherent *S. salivarius* to each composite resin of the three timed samples were obtained (Fig. 5). Herculite had the least amount of bacterial adherence than the rest of the composite resins. On the other hand, Silux and Z-100 had the greatest amount of bacterial adhesion.

Evidently, *S. aureus* and *S. salivarius*, adheres least on the Herculite resin than on the other four composite resins (Fig. 4 and 5). In contrast, *S. aureus* and *S. salivarius* are attracted to the Z-100 composite resin (Fig. 4 and 5).

The standard deviations (STD) from the results of the three timed exposures to *S. aureus* were calculated. Within a single composite resin sample, there was a great degree of variance of bacterial adhesion. Some areas had few adherent bacteria, while others had many adherent bacteria in the same sample. This observed variance gave high standard deviations from the mean for some composite samples (Table 1 and 2). For example, the Z-100 composite showed a high STD for the 12.5 exposure (Table 1).

The standard deviations from the results of the three timed exposures to *S. salivarius* were obtained. In the 12.5 hour exposure, large standard deviations are evident for all the composite resins except for Herculite (Table 2). There was much variance of bacterial adherence observed in the Silux, APH, Z-100, and Charisma resins. The variance in the amount of bacteria counted within individual samples resulted in large STD values for these composite resins (Table 2).



## DISCUSSION

This *in vitro* assay on bacterial adhesion to composite resins has confirmed the previously held view that bacteria form a biofilm after a certain period of time (3). *S. salivarius* and *S. aureus* were found to begin to adhere and colonize on all five composite resins tested within four hours of exposure to the bacteria. It is evident that the bacteria adhere in different amounts to the different composite resins (Fig. 3 and 5).

Charisma and Z-100 resins induced the most bacterial adhesion while Herculite induced the least amount of bacterial adhesion when exposed to *S. aureus* (Fig. 3). Similarly, when exposed to *S. salivarius*, the Z-100 resin had the most bacterial adherence while the Herculite composite resin had the least (Fig. 5). Overall, the Z-100 composite resin had the greatest bacterial adherence while the Herculite composite resin had the lowest bacterial adherence. It is evident, within the parameters of this assay, that the Z-100 resin would not be desirable for dental use, while the Herculite composite resin would be. It has been observed from published literature that bacteria adhered least to the Herculite substratum in most instances when compared to the APH and Z-100 composite resins (3).

The amount of bacterial adhesion was expected to either increase or decrease with exposure time to bacteria. The decrease in bacterial adhesion with increasing time of the APH composite may indicate that the APH resin has an antimicrobial mechanism to prevent the adhesion of bacteria with time (4). The increase and decrease in bacterial adhesion to Herculite and Silux resins with time can be due to a late onset of antimicrobial activity in these resins (4).

More continual increases and decreases in bacterial adhesion were observed from the *S. salivarius* exposure. This kind of increasing growth patterns was more of what was expected from this *in vitro* assay. The results of the APH and Herculite resins can be attributed to an antimicrobial ability to reduce bacterial adherence (4).

There was a great degree of variance in bacterial adherence observed among the same and different composite resins. The standard deviations obtained indicated much variance in the amount of bacteria in different areas on the same composite resin. Published literature has stated that surface roughness, positive charges, and negative charges contribute to the variance in bacterial adhesion observed (1). These factors are what seems to be the major reasons for the variation of bacterial adhesion observed on composite resins.

In conclusion, it was found that *S. aureus* and *S. salivarius* adhere to composite resin surfaces after a 4 hour exposure time. The bacteria adhere in varying amounts among the same composite resins and also among different composite resins. Overall, it was evident that the Herculite and APH resins had the least amount of bacterial adherence, while the Z-100 composite resin was found to have the most.

Further research can be done by exposing composite resins to different bacterial strains that are common in the oral cavity. Also, the same bacterial culture solution could be used to expose the composite resins for a total of 12 hours instead of using a separate bacterial culture solution for each different time. Other surface features can be analyzed to determine whether or not they induce or reduce bacterial adhesion to the composite resin surface.

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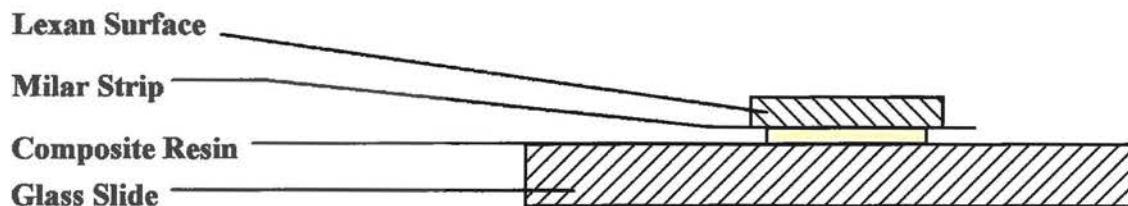


Figure 1. Schematic of a composite resin sample preparation.

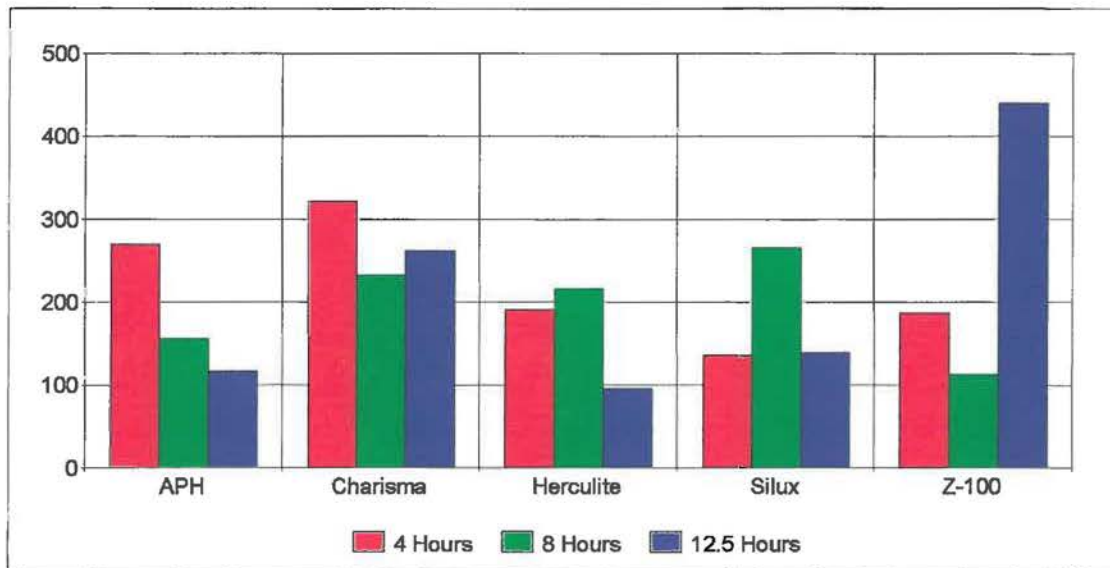


Figure 2. Adhesion of *S. aureus* to the five composite resins during 4, 8, and 12.5 hour incubation. Results are the average of two experiments.

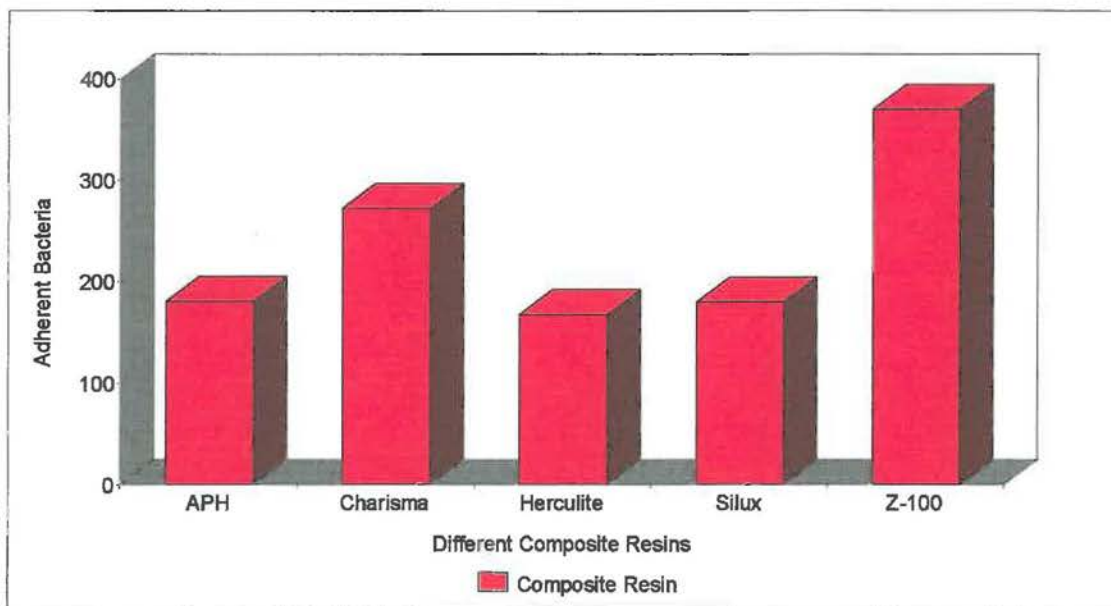


Figure 3. The average adherent bacteria of all three exposure times of *S. aureus* on each composite resin tested. Results are the average of two experiments.

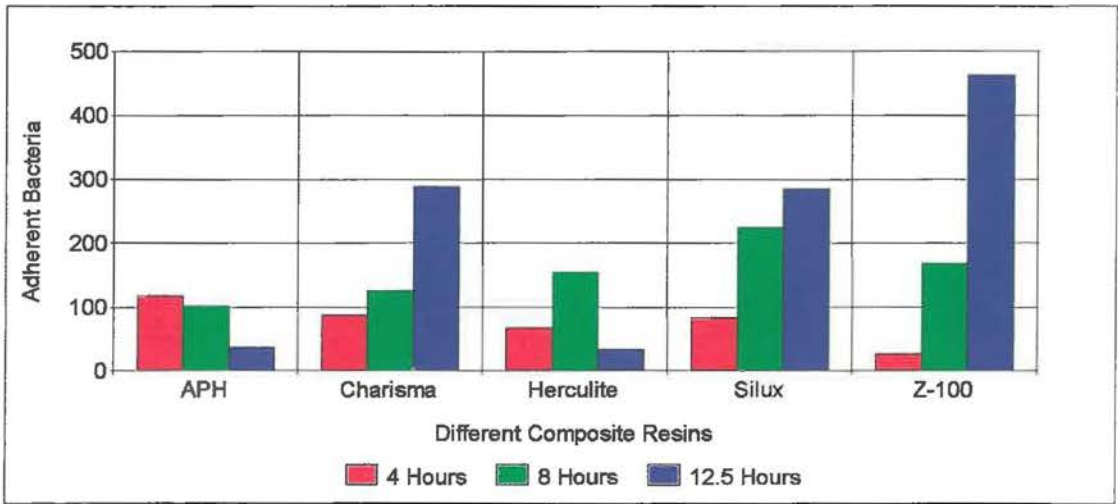


Figure 4. Results of three different timed exposures to *S. salivarius* to the five composite resins. Results are the average of two experiments.

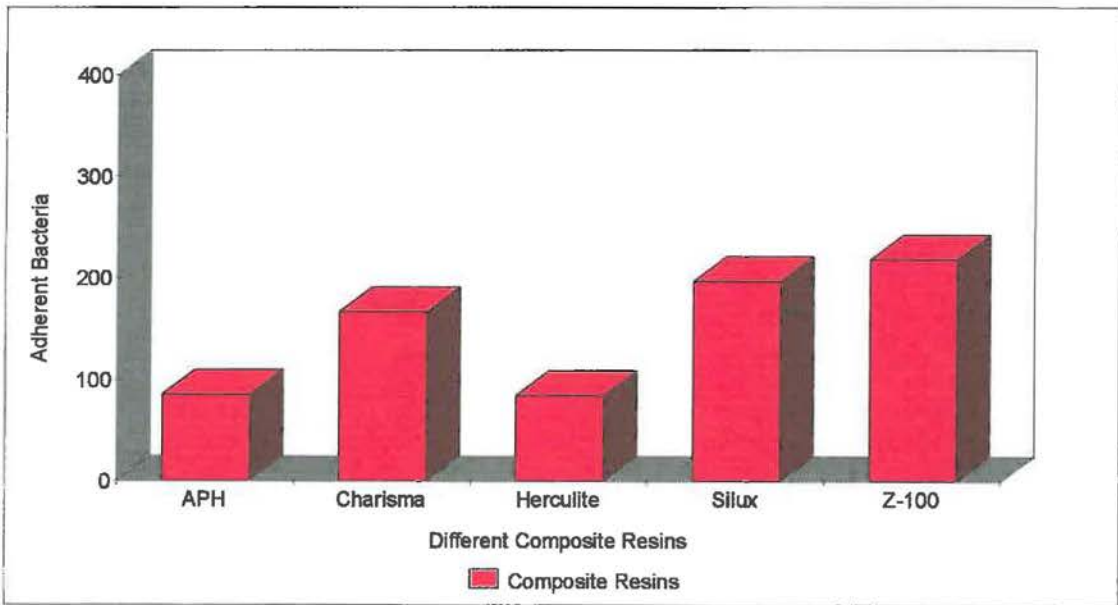


Figure 5. The average amount of adherent *S. salivarius* to each composite resin of three timed samples. Results are the average of two experiments.

<i>Composite Type</i>	<i>Time</i>		
	<i>4 Hour</i>	<i>8 Hour</i>	<i>12.5 Hour</i>
APH:	327 ± 56.3	156 ± 50.1	117 ± 9.6
Charisma:	323 ± 28.8	233 ± 54.0	262 ± 69.5
Herculite:	178 ± 94.5	217 ± 84.1	96 ± 26.2
Silux:	110 ± 24.5	266 ± 111.7	139 ± 72.9
Z-100:	195 ± 40.8	113 ± 17.2	440 ± 445.8

Table 1. List of means and standard deviations (STD) obtained from the results of the timed exposures to *S. aureus*. Results are the average of two experiments.

<i>Composite Type</i>	<i>Time</i>		
	<i>4 Hour</i>	<i>8 Hour</i>	<i>12.5 Hour</i>
APH:	151 ± 17.8	100 ± 50.5	460 ± 25.0
Charisma:	46 ± 21.4	126 ± 35.5	289 ± 510.1
Herculite:	40 ± 21.5	154 ± 191.0	33 ± 26.1
Silux:	64 ± 6.9	224 ± 120.3	285 ± 549.0
Z-100:	4 ± 1.2	168 ± 49.7	462 ± 667.1

Table 2. List of means and standard deviations (STD) obtained from the results of the timed exposures to *S. salivarius*. Results are the average of two experiments.