

## ***In Vitro* Effects of Four Porcelain Surface Treatment Methods on Adhesion of Lactobacilli Acidophilus**

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### **Abstract**

**Objective:** Adhesion of *Lactobacillus acidophilus* (*L. acidophilus*) to dental porcelain surface may lead to gingival inflammation and secondary caries. Surface roughness is among the factors affecting this adhesion. The purpose of this study was to evaluate the effects of four different surface treatment methods on adhesion of *L. acidophilus* to dental porcelain.

**Methods:** Sixty specimens (3x10mm) were fabricated of Noritake porcelain and divided into 4 groups (n=15) treated with one of the following four surface finishing techniques: 1. Auto-glazing; 2. Over-glazing; 3. Polishing with Kenda kit and 4. No surface treatment (non-glazed specimens). Specimens were inoculated with bacterial suspension containing  $1 \times 10^6$  colony forming units per milliliter (CFU/mL) and *L. acidophilus* adhesion to the surfaces was evaluated using a spectrophotometer. Data were analyzed using one-way ANOVA and Tukey's HSD test.

**Results:** The mean bacterial adhesion was 0.1440 (0.00429) to auto-glazed specimens, 0.0750 (0.00256) to over-glazed specimens, 0.1800 (0.00325) to polished specimens and 0.7064 (0.00408) to the non-glazed specimens. The differences in this regard among groups were statistically significant ( $p < 0.001$ ).

**Conclusion:** Over-glazed specimens caused the lowest and non-glazed specimens caused the highest bacterial adhesion. The glazed surfaces caused less adhesion than the polished surfaces.

**Key words:** Bacterial adhesion, Dental porcelain, *Lactobacillus acidophilus*, Surface treatment.

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### **Introduction:**

Adhesion of microorganisms such as *L. acidophilus* and *Streptococcus mutans* to tooth surfaces and dental porcelain can cause two main oral diseases namely dental caries and periodontal disease. Different restorative and prosthodontic techniques are used for restoration of carious or lost teeth. Amalgam and composite restorations and dental crowns are among the commonly performed restorative treatments. Adequate oral hygiene and decreasing bacterial

colonization around these restorations are especially important for long-term clinical service of these restorations (1). Evidence shows that porcelain surface roughness plays an important role in adhesion of microorganisms to dental porcelains (2, 3). Roughness of dental restorations decreases their optimal biological properties and enhances bacterial adhesion, plaque accumulation and inflammation of gingival tissue (4-6). Several techniques are available to decrease the surface roughness of ceramics. However, high prevalence of gingival

inflammation indicates that our knowledge is not sufficient regarding the best polishing technique (7, 8). Sonuglen *et al.* (2006) demonstrated that adhesion of microorganisms to different metal alloy surfaces depends on the form and surface roughness of metal alloy (9). Al-Marzok and Al-Azzawi (2009) demonstrated a significant association between surface roughness and adhesion of *S. mutans* (4).

Kantorski *et al.* (2009) showed that surface roughness and consequent adhesion of *S. mutans* to leucite-reinforced feldspathic porcelain was higher than that of fine grain feldspathic ceramic. The surface roughness and consequent adhesion of *S. mutans* to composites were similar to that in fine grain feldspathic porcelain (5). Thus, different porcelain surface treatments (smooth surfaces with and without glaze) may influence bacterial adhesion (10). Controversy exists regarding bacterial adhesion to glazed and polished surfaces (11, 2). Some studies have shown that polished surfaces are not different from glazed surfaces (12) and bacterial adhesion to both surfaces is the same. However, the efficacy of polishing depends on the type of ceramic and the polishing kit (13). Variability in results may be due to the type of porcelain and the polishing kit used (13).

On the other hand, in some cases, dentists need to polish the restoration surfaces to improve esthetics and correct contour. This may lead to a rougher surface with less optimal properties (3, 9, 14). Porcelain surfaces are smoothed to enhance their cleaning and decrease retention of microorganisms. This is a key factor in clinical service of restorations (5). Considering the gap of information about this topic, this study aimed to assess the effect of different porcelain surface treatments on adhesion of *L. acidophilus*.

## Methods:

Sample size was calculated to be 12 specimens in each group based on a previous study (4) and

minimum difference of 50% among groups considering type one error of 0.05 and type two error of 0.2 using NCCSS-Pass software. Considering the possible dropouts, a total of 15 specimens were evaluated in each group. Sixty porcelain discs were fabricated in this experimental study and divided into 4 groups of 15. Discs measuring 1cm in diameter and 3mm in thickness were punched out of red dental wax with 3mm thickness and placed on refractory stone for porcelain baking (Degussa, Frankfurt, Germany). After setting of stone and wax burn out, a mold was created with the above-mentioned dimensions for porcelain placement. Porcelain powder and liquid (Noritake, Tokyo, Japan) were measured in 1.3/0.3 ratio by a digital scale, mixed on a glass slab and incrementally applied to the mold (created by wax burn out). The mold was then placed inside a Phoenix-QC furnace (Ceramco, Chicago, USA) and baked under vacuum according to the manufacturer's instructions with an initial temperature of 600°C, heating rate of 45°C/minute and final temperature of 920°C. Specimens were divided into 4 groups. To ensure the thickness of the fabricated porcelain discs, gage (Juya Electronic, Tehran, Iran) with 1/10 mm accuracy was used. Their diameter was checked using a caliper with 1/1000 inch accuracy (Rahavard Co., Tehran, Iran).

Group 1 was considered as the control group and the specimens in this group were not glazed.

Group 2: Specimens in this group were auto-glazed. Fabricated specimens were heated at an initial temperature of 630°C with a heating rate of 50°C/minute and final temperature of 930°C. Specimens remained at the final temperature for 1 to 4 minutes and were then allowed to cool down in ambient air (8).

Group 3: Specimens in this group were over-glazed. The over-glaze liquid (Noritake, Tokyo, Japan) was applied to porcelain discs using a brush and the discs were heated at an initial temperature of 650°C for 5 minutes with a

heating rate of 50°C/min and a final temperature of 920°C. Specimens remained at this temperature for 1 minute followed by rapid cooling (9).

Group 4: Specimens in this group were polished. All surfaces of fabricated porcelain discs were polished with coarse and fine rubber discs (Kenda, Frankfurt, Germany) by a milling machine (Paramil 3, Dentaurem, USA) according to the manufacturer's instructions at 20,000 rpm under water coolant. After preparation, the surface roughness of all 4 groups was measured using a standard profilometer (V720 Phenom-World, Dilenburgstraat, Netherlands) (7). All groups were then cleaned with ultrasonic cleaner and then sterilized.

Standard strain *L. acidophilus* (PTCC1643) was obtained from the Scientific and Industrial Research Organization of Iran in lyophilized form and cultured in MRS agar medium (Merck, Germany). Specimens were placed in test tubes (Pyrex, USA) and inoculated with the bacterial suspension at a concentration of  $1 \times 10^6$  CFU/mL prepared at 0.5 McFarland concentrations (sodium sulfate solution) in the Microbiology Laboratory of Shahid Beheshti University. For this purpose, 350µL of this suspension was removed and added to porcelain specimens. Also, MRS liquid culture was used as the negative control. The suspension along with porcelain specimens immersed in it was incubated in an anaerobic jar at 37°C for 24 hours (Memmert, Germany). After incubation, porcelain specimens were removed from the tubes and rinsed with saline solution for 20 seconds. Discs were then placed in MRS liquid culture medium and all tubes were placed in an anaerobic jar and incubated at 37°C for 10 minutes. After completion of this time period, turbidity was noticed in the liquid culture medium. Some of this medium was transferred to a spectrophotometer (Cecil 1021, Cecil, UK).

Optical density (OD) for each specimen was measured at 625nm wavelength (indicative of the adhesion of *L. acidophilus* to porcelain) (pure MRS liquid was used as blank to read OD by spectrophotometer). Discs were removed from the medium and placed in MRS agar for bacterial culture. All media were incubated in anaerobic jar at 37°C for 24 hours. To confirm the results of spectrophotometer, colonies formed on MRS agar were counted using a colony counter (8, 9). To compare the adhesion of *L. acidophilus* to porcelain surfaces in the 4 groups, Tukey's test and one-way ANOVA were used.

## Results:

A total of 60 specimens in 4 groups were evaluated. Rate of bacterial adhesion to porcelain surfaces in the 4 groups is shown in Tables 1 and 2. As seen in Diagram 1, the highest and the lowest adhesion rate of *L. acidophilus* were noted in the non-glazed and the over-glazed groups, respectively.

The adhesion of *L. acidophilus* was significantly different among the 4 groups ( $p < 0.05$ ). Adhesion of *L. acidophilus* to non-glazed porcelain was higher than that in the other 3 groups and the difference between the non-glazed and over-glazed and auto-glazed groups was statistically significant ( $p = 0.003$ ).

The mean roughness was significantly different among the 4 groups ( $p < 0.05$ ) and the adhesion of *L. acidophilus* was significantly higher to polished porcelain compared to the other three groups and the difference in this regard between polished group and the over-glazed, non-glazed and auto-glazed groups was statistically significant ( $p = 0.003$ ).

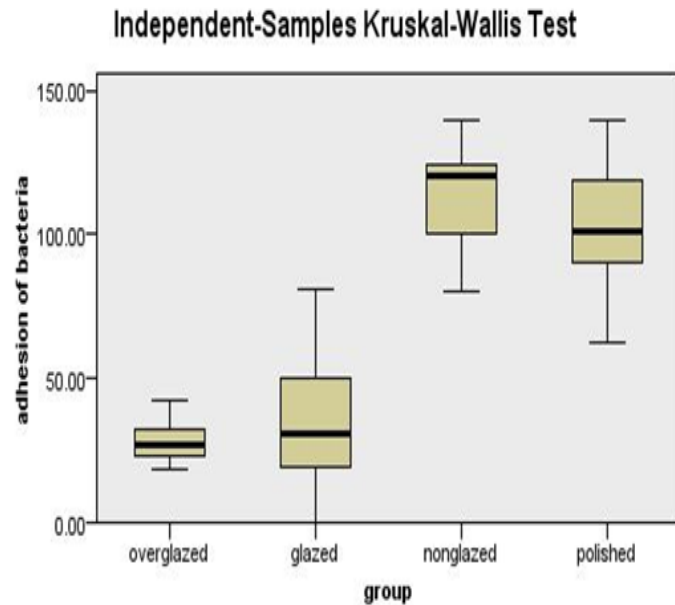
Groups were compared using one-way ANOVA and this test showed significant differences among groups in terms of bacterial adhesion and the mean roughness ( $p < 0.001$ ).

**Table 1- Descriptive statistics for the adhesion of *L. acidophilus* (OD) to porcelain surfaces in the 4 groups**

Groups OD	Number	mean	Standard deviation	95% confidence interval		Min	Max
				Minimum	Maximum		
Glazed	15	0.1440	0.00429	0.1416	0.1464	0.14	0.15
Overglazed	15	0.0750	0.00256	0.0736	0.0764	0.07	0.08
Polished	15	0.1800	0.00325	0.1782	0.1818	0.18	0.18
Nonglazed	15	0.7064	0.00408	0.7041	0.7087	0.70	0.71
Total	60	0.2764	0.25328	0.2109	0.3418	0.07	0.71

**Table 2- Descriptive statistics for the adhesion of *L. acidophilus* to porcelain surfaces in the 4 groups (based on colony count)**

Groups Adhesion rate	Number	mean	Standard deviation	95% confidence interval		Min	Max
				Minimum	Maximum		
Glazed	15	35	30.92	23.42	46.58	0	82
Overglazed	15	28	6.73	14.27	31.73	18	42
Polished	15	103	22.1	90.82	115.18	62	140
Nonglazed	15	115	16.2	106.03	123.97	80	140
Total	60	70	42.94	59.16	81.34	0	140



**Diagram 1- Independent-samples Kruskal-Wallis test**

**Table 3- Descriptive statistics for the roughness of porcelain specimens in the 4 groups**

Groups Roughness	Number	mean	Standard deviation	95% confidence interval		Min	Max
				Minimum	Maximum		
Glazed	15	0.67	0.004	0.67	0.68	0.67	0.68
Overglazed	15	0.48	0.003	0.48	0.48	0.47	0.48
Polished	15	1.7	0.004	1.693	1.698	1.69	1.7
Nonglazed	15	0.89	0.005	0.89	0.89	0.88	0.9
Total	60	0.93	0.47	0.81	1.05	0.47	1.7

Pairwise comparison of groups using Tukey's test revealed a significant difference among groups.

The results showed that over-glazed porcelain surfaces had the lowest and the non-glazed surface had the highest adhesion. Also, the auto-glazed surface caused significantly less adhesion than the polished surface ( $p < 0.01$ ).

### **Discussion:**

This study assessed the effect of 4 different porcelain surface preparation techniques on the adhesion of *L. acidophilus*. This study is among the few to assess the adhesion of *L. acidophilus* to over-glazed porcelain. The results showed that over-glazed porcelain surface had the lowest adhesion and the non-glazed surface caused the highest adhesion. Auto-glazed surface caused lower adhesion than the polished surface and this difference was significant. Factors affecting bacterial adhesion to porcelain surface can be divided into two groups. Group 1 includes factors related to the microorganisms such as the surface characteristics and culture medium and group 2 includes the characteristics related to the adhesion surface such as surface roughness, surface energy, and composition of material. One important characteristic of *L. acidophilus* is its ability to adhere to epithelial cells and different surfaces such as tooth surfaces and dental prosthetics (12).

The results of this study showed that non-glazed porcelain caused maximum adhesion and this finding is in accord with the results of Kantrosky *et al.* (2009) and Kawai and Urano (2001) (14, 15). This finding can be due to the porcelain surface structure. Non-glazed porcelain due to having porosities (created in the process of porcelain baking) on its surface has higher potential for bacterial adhesion and the scanning electron microscopic results confirm this finding (14, 15).

Several methods are available for correction of

ceramic surfaces such as 1. autoglazing, 2. Over-glazing and 3. Polishing (15).

The results of the current study showed that over-glazed porcelain caused minimum adhesion, which is in accord with the results of Karayazgan *et al.* in 2010 (16). Over-glaze is transparent porcelain with low melting temperature applied to the restoration surface. It is baked at a lower melting point than that of dentin and enamel porcelains. The over-glaze liquid flows into the porcelain surface cracks and prevents crack initiation. Over-glaze serves as a sealant and prevents crack propagation reaching the external surface. Thus, a smooth and uniform surface is created (17).

Thermal shrinkage of over-glaze is less than that of the underlying porcelain. As it cools down, compressive stresses are created in the over-glaze that prevents crack propagation. Over-glaze significantly affects crack propagation, porcelain surface smoothness and adhesion of microorganisms. The heat used for over-glazing causes an auto-glazed layer in the underlying porcelain. The auto-glazed layer may be formed as a distinct layer beneath the over-glaze. However, there is a higher possibility that the auto-glaze and the over-glaze layers merge and form a homogenous layer (18). However, it should be noted that the porcelain loses its ability for a natural glaze after several baking cycles. Thus, application of over-glaze to the surface of large restorations requiring repeated corrections may convert the porcelain to its crystalline form and confer a milky or cloudy appearance to it. This is called devitrification. It results in the loss of natural appearance of porcelain and no surface treatment can revive it (19). Thus, although this surface showed the lowest bacterial adhesion, this method should be used with caution in the clinical setting to maintain the surface texture and esthetics of porcelain restorations.

The results of this study showed that glazed porcelain caused lower adhesion than polished

porcelain. This finding was similar to that of Kantrosky *et al.* (2010), Kawai and Urano (2001) and Karayazgan *et al.* (2010) (14-16) but different from that of Sethi *et al.* (2013) (20). Polishing is also a highly popular technique (due to not requiring too much time and no need for laboratory equipment required for glazing) used for smoothing rough porcelain surfaces. Different manufacturers produce polishing kits, polishing burs and polishing pastes. Many studies have compared the efficacy of polishing and glazing and have reported controversial results. Such controversy is attributed to several factors such as the type of polishing kit used, duration of polishing, technician's hand pressure, device's operating speed, expertise of the operator, coarseness and grit of polishing kits, type of used porcelain, assessment methods and size of abrasive granules in polishing pastes. Thus, difference in results of our study and those of Sethi may be explained by the fact that all these conditions could not be possibly matched between the two studies and may explain the difference in results. Moreover, they used Ivoclar and Vita porcelain in their study; whereas, we used Noritake porcelain. This can also affect the results (20). Regarding the material characteristics, it should be noted that

the higher the surface energy and the rougher and the more hydrophilic the surface, the higher the microorganism adhesion (14, 21).

All studies on the adhesion of microorganisms to dental materials have stated that surface roughness significantly affects bacterial adhesion (15, 16). The results of this study showed that raw porcelain causes maximum adhesion. This is due to the surface structure of raw porcelain. Non-glazed porcelain due to the porosities caused on the porcelain surface in the process of baking has higher potential for bacterial adhesion (7, 10).

### Conclusion:

The results of this study showed that *L. acidophilus* had lowest adhesion to over-glazed and highest adhesion to non-glazed porcelain. Bacterial adhesion to auto-glazed porcelain surface was less than that to polished porcelain surface. Thus, over-glaze surface preparation technique ranks first followed by auto-glazing to prevent adhesion of *L. acidophilus* to porcelain surface.

### Conflict of Interest: "None Declared"

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