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Original Article

Effect of the crystalline structure of ceramic orthodontic brackets on the adherence of *Streptococcus mutans* and *Candida albicans*: An *in vitro* study

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ABSTRACT

Background: With recent increases in demand for the esthetic aspects of orthodontic treatments, the use of ceramic brackets has gained more popularity. Dental demineralization is a frequent, undesired effect of microbial biofilm adhesion to orthodontic appliances. The crystalline structure of ceramics results in different material properties, and its possible effect on microbial adhesion was investigated in this study.

Materials and Methods: This research was conducted experimentally and *in vitro*. Samples consisted of 40 monocrystalline and 40 polycrystalline brackets, further divided into two groups incubated with either *Streptococcus mutans* alone or *S. mutans* with *Candida albicans*. The culture medium was Tryptic Soy Broth with 20% sucrose. All samples were incubated at 37°C for 48 h. Macroscopic detachment of the formed biofilm would be the basis for adhesion scoring. The Mann–Whitney test was used to analyze the adhesion scores. In this study, a significance level of $P < 0.05$ was considered.

Results: The mean for adhesion score in *S. mutans* group was 1.85 ± 0.67 for the monocrystalline group and 2.35 ± 0.59 for the polycrystalline group (27% difference, $P = 0.035$). The adhesion score in *S. mutans* and *C. albicans* group was lower in the monocrystalline group (1.6 vs. 2.0) but was not statistically significant ($P = 0.108$).

Conclusion: This study showed that monocrystalline ceramic brackets had less overall microbial biofilm adhesion compared to polycrystalline ceramic brackets, especially when incubated with *S. mutans* alone. This observation might be explained primarily by lower surface roughness in monocrystalline ceramics.

Key Words: Bacterial adhesion, biofilm, ceramics, monocrystalline, orthodontic brackets, polycrystalline

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INTRODUCTION

There has been an increase in the number of patients seeking orthodontic treatment in recent years. With the ever-increasing emphasis on esthetic aspects of treatments, the demand for tooth-colored and highly esthetic brackets, such as ceramic brackets, has increased.[1] Ceramic brackets are divided into either monocrystalline or polycrystalline types based on their

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crystalline structure. While monocrystalline alumina ceramics have a surface roughness comparable to that of stainless steel, polycrystalline alumina ceramics have a rougher surface due to the fact that their surface is made up of numerous crystals.[2,3] The roughness of nonshedding surfaces in the oral cavity is directly associated with biofilm formation and plaque accumulation.[4] As a result of biofilm formation and the difficulty of maintaining proper oral hygiene and plaque control, approximately 50% of orthodontic patients suffer dental demineralization ranging from mild to severe.[5]

Streptococcus mutans is one of the predominant microorganisms in the oral cavity[6] and is widely regarded as one of the main pathogens in the process of dental caries.[7] *Candida albicans* is the dominant yeast in the oral cavity and the most abundant nonbacterial microorganism in the oral cavity.[8] The investigation of adhesion and biofilm formation by these organisms can be vital for the prevention of the often-irreversible carious lesions that may compromise the esthetics of the orthodontic treatment and the oral health of the patient.

Previous studies regarding biofilm formation on ceramic brackets have conflicting results and are sparse, especially regarding the crystalline structure of the ceramics. Most of the studies have generally compared ceramic brackets with steel brackets, with only a few distinguishing between different crystalline types and associated microbial adhesion. In their study, Brusca *et al*.[9] found that combined adhesion of *S. mutans* and *C. albicans* is lower in metals compared with the control, almost the same in ceramics compared with the control, and higher than the control in composites. van Gastel *et al*. found more bacterial colonies on ceramic brackets than conventional metal brackets.[10] On the other hand, Lindel *et al*.[11] and Saloom *et al*.[12] have found lower microbial plaque adhesion on ceramic brackets compared to metal brackets. The studies conducted by Papaioannou *et al*.,[13] Passariello and Gigola,[14] Thaweboon *et al*.,[15] and Lee *et al*.[16] have generally found no significant difference in microbial plaque adhesion between ceramic and metal brackets. Among the aforementioned studies, the ones conducted by Lee *et al*., Passariello and Gigola and Thaweboon *et al*. have investigated both monocrystalline and polycrystalline types of ceramic brackets. Lee *et al*.[16] stated that even though the surface roughness of monocrystalline brackets was lower than polycrystalline and even steel brackets, the biofilm adhesion was slightly higher in monocrystalline brackets compared to other groups, which was statistically insignificant. In the study conducted by Passariello and Gigola, it was found that the rate of biofilm formation in saliva on polycrystalline brackets was higher compared with monocrystalline brackets, but it was stated that this difference is not high enough to result in any clinically significant distinction between these two materials.[14] Thaweboon *et al*.[15] found significantly lower adhesion for *S. mutans* on monocrystalline brackets compared with polycrystalline ones.

Due to the mentioned controversies and conflicting results, we decided to specifically investigate the effect of the crystalline structure (monocrystalline vs. polycrystalline) of ceramic brackets on the adhesion of *S. mutans* and *C. albicans* in an *in vitro* environment. The results of this study would be beneficial in preventing and controlling the accumulation of these microorganisms in orthodontic patients and reducing their undesirable effects, such as dental caries.

MATERIALS AND METHODS

Study design

This *in vitro* study was designed to investigate the difference in adhesion of microbial plaque between monocrystalline and polycrystalline ceramic brackets. Thus, the primary objective of this study was to assess and compare the adhesion of *S. mutans* and *C. albicans* to monocrystalline and polycrystalline ceramic brackets. In order to compare different crystalline forms and bacterial compositions, half of the samples were monocrystalline and the other half were polycrystalline, and half of the samples contained *S. mutans* alone, while the other half contained both *S. mutans* and *C. albicans*. This resulted in four groups of samples in total [Table 1].

Brackets

The monocrystalline alumina bracket used was NeoCrystal Plus®, and the polycrystalline alumina

Table 1: Sample groups

Group	Microbial composition	Bracket type
1	*S. mutans*	Monocrystalline
2	*S. mutans*	Polycrystalline
3	*S. mutans* + *C. albicans*	Monocrystalline
4	*S. mutans* + *C. albicans*	Polycrystalline

S. mutans: Streptococcus mutans; C. albicans: Candida albicans

bracket was Illusion Plus®, both manufactured by Ortho Organizers® GmbH (Lindenberg, Germany). In order to homogenize samples, all brackets used were for the first maxillary premolar, without a hook, and with a 0.22-inch slot. Brackets were steam-sterilized before the beginning of microbial incubation.

Culture media and microbial composition

Microbial suspensions for the standard strains of *S. mutans* (ATCC-35668) and *C. albicans* (ATCC-10231) were acquired from the Pasteur Institute of Iran (Tehran, Iran). To prepare the culture medium, 5 ml of the suspension of Tryptic Soy Broth (TSB) and 20% sucrose were added to sterile glass test tubes. An appropriate bracket was placed inside the test tube using a sterile bracket holder, and then 0.5 ml of the appropriate microbial suspension was added to the test tube. In order to control for microbial contamination and the efficacy of the sterilization process, an additional test tube containing a bracket and culture medium (but no microbial suspension) was prepared to serve as the control.

Adhesion test

All test tubes were positioned with a 25° tilt from the ground and incubated for 48 h at 37°C in aerobic conditions.[12] On the conclusion of the incubation, TSB was removed from the test tubes, and a 0.1% safranin solution was used to stain the tubes for one minute. After the staining, safranin was removed, and the tubes were assessed for the adhesion of the biofilm.

The basis for the adhesion test in this study is the ability of the microorganisms to convert sucrose to glucan, which subsequently adheres to the glass surface of the test tubes.[12] To assess the adhesion, each test tube was repositioned from a tilted position to an upright position. The macroscopic manner in which the adhered biofilm and accompanying bracket would detach from the test tube would determine the adhesion score. If the biofilm and the bracket completely detach from the test tube, a score of one would be given. If a partial detachment was observed, a score of two would be given, and a score of three would be given if no visible detachment could be observed.[9] Sample preparations and incubations were carried out by a trained lab technician. Adhesion test scoring was done by two trained lab personnel, and in the event of disagreement between the two personnel, a third person would determine the final adhesion score (which did not happen).

Sample size and statistical analysis

The sample size was calculated using the G*Power v3.1.9.7 (Heinrich-Heine-Universität, Düsseldorf, Germany) software. Anticipating the need for a nonparametric Wilcoxon-Mann-Whitney test and considering an alpha value of 0.05, a statistical power of 80%, and an effect size of 1,[12] the required sample size for each group was calculated to be 18. To further enhance the power and make calculations easier, two additional samples were allocated to each group, resulting in 20 samples in each group and 80 samples in total.

The results are reported both quantitatively and qualitatively. The frequencies of different scores, mean, standard deviation, and coefficient of variation are reported wherever appropriate. The normality of the distribution of the adhesion scores was evaluated via Shapiro–Wilk and Kolmogorov–Smirnov tests, which indicated that the distribution is not normal in any of the groups. Therefore, the Mann–Whitney test was used to compare the adhesion scores across different ceramic groups. The statistical analyses were carried out using IBM SPSS Statistics v24 (IBM Corp., New York, USA).

RESULTS

The results consisted of 40 samples of monocrystalline and 40 samples of polycrystalline brackets, each divided into two groups: *S. mutans* alone, and *S. mutans* in addition to *C. albicans*.

The qualitative and quantitative results of the adhesion test can be found in Tables 2 and 3. Since the adhesion scores across groups did not have a normal distribution, the nonparametric Mann–Whitney test was used to compare adhesion scores. In the samples containing Streptococcus mutans, the mean adhesion score was 2.35 for the polycrystalline group and 1.85 for the monocrystalline group (27% difference), which was statistically significant ($P = 0.035$). In the samples containing both *S. mutans* and *C. albicans*, the mean adhesion score was higher in the polycrystalline group compared with the monocrystalline group (2.0 vs. 1.6), but this difference was not statistically significant ($P = 0.108$).

DISCUSSION

This study showed that the adhesion of *S. mutans* with or without *C. albicans* is lower in monocrystalline

Table 2: Qualitative results for the adhesion scores

Ceramic type	Microbial group					
	S. mutans			*S. mutans* and *C. albicans*		
	Adhesion score, frequency (%)					
	Low (1)	Medium (2)	High (3)	Low (1)	Medium (2)	High (3)
Monocrystalline	6 (30)	11 (55)	3 (15)	10 (50)	8 (40)	2 (10)
Polycrystalline	1 (5)	11 (55)	8 (40)	5 (25)	10 (50)	5 (25)

S. mutans: Streptococcus mutans; C. albicans: Candida albicans

Table 3: Quantitative results for the adhesion scores

Ceramic type	Adhesion score Microbial group			
	S. mutans		*S. mutans* and *C. albicans*	
	Mean±SD	CV	Mean±SD	CV
Monocrystalline	1.85±0.67	36	1.6±0.68	42
Polycrystalline	2.35±0.59	25	2.0±0.72	36
Statistical test‡	*P*=0.035§		*P*=0.108	

§Statistically significant, ‡Mann–Whitney test. SD: Standard deviation; CV: Coefficient of variation; *S. mutans: Streptococcus mutans; C. albicans: Candida albicans*

ceramic brackets compared with polycrystalline ones, even though this difference did not reach statistical significance when both organisms were present in the biofilm. The first factor contributing to this finding could be the difference in surface characteristics between these two ceramic groups. Lee *et al*. investigated the surface characteristics of the common materials used in orthodontic treatment and their effect on the adhesion of *S. mutans*.[16] Their samples included nine different materials (four orthodontic adhesives, three bracket types, hydroxyapatite brackets, and bovine incisors). Samples were evaluated by confocal laser microscopy for differences in surface characteristics and were incubated for 3 and 6 h with and without saliva. The findings showed significant differences in surface roughness, with monocrystalline ceramics showing the lowest surface roughness, followed by metals and polycrystalline ceramics. The lowest *S. mutans* adhesion was observed in polycrystalline ceramics, followed by metals and monocrystalline ceramics, although these differences in adhesion were not statistically significant.[16] A possible limitation in the extension of the results of this study is that the adhesion of biofilm was not investigated past 6 h of incubation time, even though the adhesion, accumulation, and maturation of microbial biofilm is a continuous process[17] that continues well beyond 6 h based on the culture medium and can result in significant changes.[18]

The aforementioned temporal limitation is addressed in the study by Passariello and Gigola, in which the *in vitro* adhesion of six strains of *S. mutans* to 15 different brackets was investigated. Each study group consisted of 12 brackets, which were incubated for 4 and 848 h at 37°C with microbial suspensions acquired from separate incubations in culture media and saliva. The results showed that the highest rate of growth of microbial biofilm adhesion among ceramics belonged to the polycrystalline brackets.[14] In the aforementioned study, brackets were fixed onto acrylic blocks via composites and orthodontic bonds. Although their methodology could better represent the environment encountered inside the oral cavity, the effect of the materials used to fixate the brackets cannot be ignored. The surface of orthodontic bonds and composites can act as a suitable surface for bacterial adhesion,[16] and as such, the presence of these materials in our study could alter the results. Due to these issues and since our aim was to isolate and investigate the effect of the crystalline structure of ceramic brackets on bacterial adhesion, we decided not to use this methodology. Even though this methodology could have better simulated the oral cavity, the pH of saliva, its contents, and their concentrations vary between different people, cannot be reliably controlled, and can alter the findings.

The currently available ceramic brackets are manufactured from alumina in either monocrystalline or polycrystalline form. Monocrystalline alumina has a lower surface roughness than polycrystalline alumina.[16] Rough surfaces facilitate microorganism adhesion via increased retention and available surface. In addition, the free surface energy is higher in polycrystalline alumina compared with monocrystalline alumina.[16] These differences in surface characteristics between polycrystalline and monocrystalline alumina can explain why *S. mutans* with or without *C. albicans* have lower adhesion to monocrystalline alumina. Higher surface roughness not only facilitates microbial adhesion but also hinders the sliding of the archwire in the bracket slot.[2] Monocrystalline alumina has a

higher surface hardness than polycrystalline forms, but if a crack develops, it can freely propagate through the entire crystal, often resulting in bulk fractures.[19] Polycrystalline forms show lower surface hardness but higher resistance to crack propagation due to their crystalline structure, which compresses the crack line and slows its propagation.[2] In addition to the aforementioned differences, there is also a difference in the esthetic aspect of these crystalline forms. While both forms display acceptable esthetics, monocrystalline alumina has higher translucency and can provide better esthetics, especially while observing from very close ranges.[2]

Although conducting this study in an *in vivo* setting would have been closer to clinical scenarios, defining appropriate and sufficient inclusion and exclusion criteria for this type of study would have been difficult. Not only would complete matching between samples have been difficult and require very large sample sizes, but homogenizing study settings between different samples would be very hard. Factors such as food intake, diet, hygiene habits, microflora variations, the saliva and its pH, contents, and their concentrations are unpredictable and different among individuals and can affect the results. As the primary objective of this study was to investigate the effect of the crystalline structure of the ceramics on microbial adhesion, we took advantage of conducting the study *in vitro* to exclude potential confounders, isolate the desired interactions, and reduce potential bias.

CONCLUSION

We found that monocrystalline ceramic brackets had less overall microbial biofilm adhesion compared to polycrystalline ceramic brackets, especially when incubated with *S. mutans* alone. This observation might be explained primarily by lower surface roughness in monocrystalline ceramics.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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