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Surface Roughness and Bacterial Colonization Of Two Types Of Zirconia (Cubic And Gradient) After Accelerated Hydrothermal Aging. (Comparative In-Vitro Study)

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ABSTRACT

Background: The oral cavity is a challenging environment that promotes the aging of dental restorations, causing surface roughness and bacterial adherence. Therefore, those properties of recently introduced gradient zirconia upon aging are under investigation. ***Aim:*** To assess the surface roughness and bacterial colonization of two types of zirconia (cubic and gradient) after accelerated hydrothermal aging. ***Materials and methods:*** Twenty-six disc-shaped samples (12mm X 1.2mm) were CAD/CAM fabricated. Samples were divided into two equal groups (n=13) according to the material type: **Group B**: cubic zirconia (BruxZir)and **Group I**: gradient zirconia (IPS e.max ZirCAD Prime). Finishing, polishing, and glazing of each sample was done followed by surface roughness measurement using a 3D non-contact profilometer. Samples were incubated in artificial saliva and bacterial suspension then total bacterial count of each disc was calculated using colony-forming units (CFUs/ml). ***Results:*** Regardless of aging IPS e.max ZirCAD Prime (0.2535± 0.0043) showed lower surface roughness than BruxZir (0.2552 ± 0.0037). Both types of zirconia showed an increase in surface roughness after aging (0.2581± 0.0017). Both types of zirconia; BruxZir (5.22 ± 0.81) and IPS e.max ZirCAD Prime (5.18 ± 0.57) showed an equal amount of bacterial colonization. Both types of zirconia showed an increase in bacterial colonization after aging (5.73, ± 0.46). ***Conclusion:*** Surface roughness of both materials increased due to aging. Both materials showed increase in bacterial counts after aging. There is a positive correlation between surface roughness and bacterial colonization.IPS e.max ZirCAD Prime can be used clinically as it provides a smoother surface than BruxZir.

Keywords: Accelerated hydrothermal aging, bacterial colonization, Gradient zirconia, surface roughness.

INTRODUCTION

Fixed prostheses have proved its efficiency in restoring missing teeth with durable restorations that achieve the goal of aesthetics and function.[1] The oral cavity is a challenging environment that is characterized by a humid nature, temperature, pH

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fluctuations and biting forces which can alter the surface properties of fixed restorations, making it an occupant for microbial flora.[2] Bacteria present in the oral cavity led to complications such as caries, secondary caries, periodontal diseases, and finally restoration failure. [2] The long-term success of any restoration is greatly affected by this complex environment. The surface texture of a restoration including its surface roughness, chemical composition, and shape detects the amount of bacterial adherence.[3]

Zirconia has been widely used in clinical daily practice due to its inert nature, high strength and biocompatibility.[4] Despite the great strength of zirconia (3Y-TZP) clinicians always faced the problem of opaqueness and decreased esthetics.[5] Different innovations were applied to solve this problem producing (4Y-PSZ) and (5Y-PSZ).[6] This was achieved by using a higher yttria content producing partially stabilized zirconia. The highly translucent zirconia provided the desired esthetics, but its strength was diminished.

Recently, an innovative technology produced the Gradient zirconia. It is a combination of 5Y-TZP in the occlusal or incisal area for good translucency and 3Y-TZP in the dentin area for high strength.[8] The outer surface of the translucent zirconia was infiltrated with feldspathic glass creating a glass-graded structure. This upgrade created natural esthetics and mechanical resilience to a high degree.[9]

Surface stresses encountered in the oral cavity induce tetragonal to monoclinic phase transformation putting the surface into compression.[10] This transformation (t-m) results in a volume expansion of 3% to 5%, which counteracts the external tensile stresses, so extra energy will be required for the crack to propagate.[11] This phenomenon is called stress induced-transformation toughening (t-m).[12,13]

In the absence of local stressors at the tip of an advancing crack, progressive and spontaneous phase transformation occurs gradually at low temperatures with the presence of water or oral secretions. [14] This also causes microcracks, that allow the entrance of the solvent molecules within the internal grains causing surface phase transformation to proceed deeper in the material.[15-17] Continuation of the following transformation allows for an increase in microcracks and finally causes fracture of the material. This transformation could be due to great particle size, low yttria content, or the presence of residual stress. The following process is named Low thermal degradation (LTD) or hydrothermal aging.[18]

Accelerated hydrothermal aging is an experimental method for simulating oral environmental conditions outside the patient's mouth.[19] It is carried out by autoclaving the samples.[20] This method has different protocols with varying temperatures, pressure, and duration. It is a negative phenomenon that can occur over time within a temperature range of 65–500° C.[10]

The surface texture of a restoration including its surface roughness, chemical composition, and shape detects its durability, longevity, esthetic success, and the amount of bacterial adherence.[21-24] Roughness can be defined as a complex of irregularities or little indentations that characterize a surface and can result in a non-uniform stress distribution having an influence on wetting, quality of adhesion, and brightness.[25,26] Several factors have reportedly been implicated to this issue such as surface-free energy, surface roughness, contact angle, and surface charge enhance bacterial adhesion.[27-29]

Studies emphasized that there was a positive correlation between surface roughness produced in response to aging and bacterial colonization.[23,30-32] However, gradient zirconia is a new material and its surface roughness and bacterial colonization after accelerated hydrothermal aging have not been measured yet in comparison to cubic zirconia. Thus, the aim of this study was to assess surface roughness and bacterial colonization after accelerated hydrothermal aging on two types of zirconia, cubic and gradient zirconia.

Two null hypotheses were suggested for this study, the first one was that there will be no statistical difference in surface roughness between cubic and gradient zirconia after accelerated hydrothermal aging and the second one was that there will be no difference in bacterial colonization of cubic and gradient zirconia after accelerated hydrothermal aging.

MATERIALS AND METHODS

Power analysis used surface roughness as the primary outcome. Based upon the results of **Elsherif et al. in 2020**[33]; G*Power Version 3.1.9.2 was used to calculate the effect size (d) which was 1.186 at alpha (α) level of (5%) and Beta (β) level of (20%); i.e. power = 80%.A total of twenty-six zirconia disc samples were constructed. They were divided into two equal groups (n=13), **Group B** BruxZir (Glidewell Laboratories, Irvine, USA) and **Group I** IPS e.max ZirCAD Prime (Ivoclar Vivadent, Liechtenstein).

For the purpose of samples standardization, samples were designed and manufactured using a 5-axis dental milling machine (SHERA ECO-MILL-CAM lemforde, Germany) following the manufacturer's instructions to produce samples with dimensions 12 mm diameter and 1.2 mm thickness.[33]

A digital caliper (INSIZE digital caliber, Insize measuring tool, India) was used to confirm the dimensions of the samples with 20-25% increase in size to compensate for sintering shrinkage.

Sintering finishing, polishing then glazing for all samples were performed according to manufacturer's instructions to ensure smooth surface which was critical and accomplished through using a surveyor with a straight handpiece and a gypsum base with a holder for the discs and attached to the surveyor. Polishing was carried out into two steps using (Eve Diacera Polishing kit Gmbh, Germany). First, a pre-polishing disc was used for 1 minute at a speed of 10,000 rpm by using a low-speed handpiece. Polishing was then performed using H8DC at an angle of 90 degrees for 1 minute.

For the glazing of the samples, a single layer of Dentsply Sirona Universal overglaze (High Flu Dentsply Sirona Prosthetics, New York, PA) was applied all over the disc surfaces using a ceramic thin brush to guarantee a uniform thickness.[34] Discs were then placed on a firing tray in (SUMMIT's Porcelain furnace IBEX Dental Technologies, USA). Firing parameters were done according to the manufacturer's recommendation.

Surface roughness measurement

Surface roughness for the samples was measured using a 3D-noncontact profilometer (U500x Digital Microscope, Guangdong, China). A 3D picture of the samples' surface profile was subsequently produced. For each sample, three 3D photos were taken at a 10 µm × 10 µm size in the middle and on each side. The average roughness (Ra) reported in µm, which are regarded as trustworthy indicators of surface roughness, were calculated using the WSxM software (version 5 develops 4.1, Nanotec, Electronica and SL).[35]

Bacterial colonization measurement

Artificial saliva was prepared from the following: albumin (Sigma, Sigma-Aldrich CO, St Louis, MO USA), methylcellulose, sodium carboxymethyl cellulose, hydroxypropyl methylcellulose, 0.062%potassium chloride, di-potassium hydrogen phosphate 0.034%, sodium fluoride 0.01%, Magnesium chloride 0.005%, Glucose, Methyl paraben, dextrose 4.69%2.

Streptococcus mutants (S.Mutants) were used in this study as a reference strain. S.

Mutants was seeded and cultured in nutrient broth medium at 37 C for 48 hours.[36]

Sterilized samples were placed in 26 well plates (1 sample per well). A pipette was used to transfer the bacterial suspension on each sample surface and then incubated at 37°C for 48 hours.[36]

Samples were incubated with 1 mL of an artificial saliva prior to adhesion assay in a thermo shaking device (WTB 35 MEMMERT GmbH + Co. KG) that mimicked oral shear stress for 2 h at 37C, to replicate the effect of a salivary pellicle on streptococcal adhesion.[36]

To evaluate the adherent microbial count on the material surface, samples were gently rinsed with phosphate-buffered saline (PBS) to remove non-adherent cells and placed into polypropylene tubes filled with 1 ml PBS. The attached bacteria were isolated by shaking virgously in a vortex.[37]

The desorbed suspension from the disc surfaces was appropriately diluted to 1:1000 using serial dilution technique by adding 1 ml of suspension to 999 ml of sterile water and spread on agar plates.[37] Cell colony numbers were counted, using digital colony counter by placing each agar plate on the illuminated pad and marking on the plate with a pen provided, the number of colony-forming units per 1 ml of the suspension (CFU/ml) was calculated to quantify the microbial adhesion.[38] For accelerated hydrothermal aging, all disc samples were placed in sterilization pouches, each disc was sealed a sterilization pouch in a steam autoclave (Clear autoclave class B, Clear. China).[39] The autoclave was set at temperature 134ºC and 2 bar pressure for 5 hours according to standard aging protocol which is equivalent to 15 years inside the oral cavity.[39]

After aging surface roughness and bacterial colonization measurements for all samples were repeated.

Statistical analysis

By evaluating the data distribution and utilizing tests of (Kolmogorov-Smirnov and Shapiro-Wilk tests) numerical data's normality was investigated. Due to the wide range of bacterial counts, logarithmic transformation of bacterial counts was carried out. The distribution of the data was normal (parametric) distribution. Mean and standard deviation (SD) values were used to present the data. Repeated measures ANOVA test was used to study the effect of zirconia type, aging, and their interactions on surface roughness and bacterial counts. While ANOVA test was significant, Bonferroni's post-hoc test was employed for pair-wise comparisons. The relation between surface roughness and bacterial counts was assessed using Pearson's correlation coefficient. The significance level was set at $P \leq 0.05$. Statistical analysis was carried out with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY:IBM Corp.

RESULTS

Surface roughness measurement

The results showed that whether before or after aging; IPS e.max ZirCAD Prime showed statistically significantly lower mean Ra than BruZir (*P*-value <0.001, Effect size = 0.54) and (*P*-value = 0.030, Effect size = 0.181), respectively.

Whether using IPS e.max ZirCAD Prime or BruxZir; there was a statistically significant increase in mean Ra after aging (*P*-value <0.001, Effect size = 0.926) and (*P*-value <0.001, Effect size = 0.913), respectively. **(Figure1&2)**

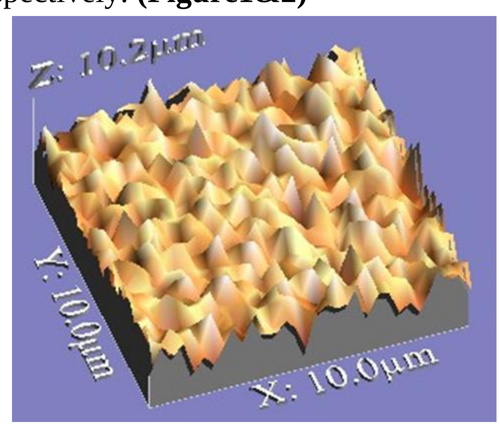

Figure (1): 3D non-contact profilometer interference microscope showing topographic micrograph of **IPS e.max ZirCAD Prime** disc sample before accelerated hydrothermal aging.

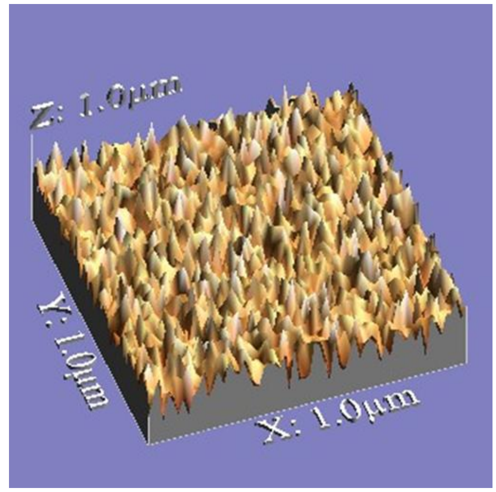

Figure (2): 3D non-contact profilometer interference microscope showing topographic micrograph of **IPS e.max ZirCAD Prime** disc sample after accelerated hydrothermal aging.

The mean and Standard deviation of different interactions of variables are shown in **figure 3** and **table 1**.

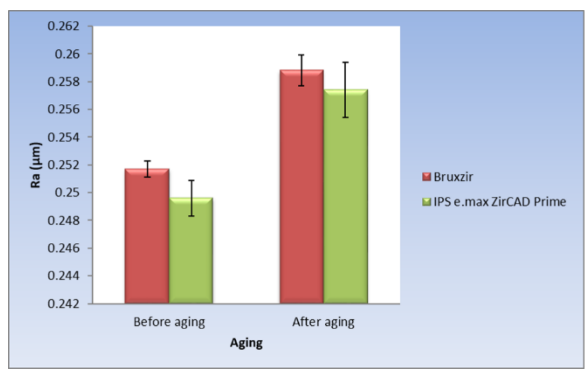

Figure (3): Bar chart representing mean and ±SD values for Ra with different interactions of variables.

Bacterial colonization measurement

The results showed that zirconia type (regardless of aging) had no statistically significant effect on mean Log_{10} CFU/ml of (*P*-value = 0.799, Effect size = 0.003).

Table (1): The mean, standard deviation (±SD) values and results of repeated measures ANOVA test for comparison between Ra (µm) of different variables interactions.

Aging	Bruxzir		IPS e.max ZirCAD Prime		*P*-value	Effect size *(Partial eta squared)*
	Mean	±SD	Mean	±SD		
Before aging	0.2517	0.0013	0.2496	0.0006	<0.001*	0.54
After aging	0.2588	0.002	0.2574	0.0011	0.030*	0.181
P-value	<0.001*		<0.001*			
Effect size *(Partial eta squared)*	0.913		0.926			

**: significant at p ≤ 0.05.*

Aging (regardless of zirconia type) had a statistically significant effect on mean Log_{10} CFU/ml of bacterial counts (*P*-value <0.001, Effect size = 0.759). The interaction between variables had no statistically significant effect on mean Log_{10} CFU/ml of (*P*-value = 0.089, Effect size = 0.116). **(Figure 4** and **Table 2)**

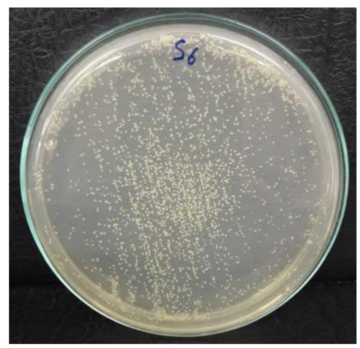

Figure (4): Agar plate representing colony forming units after aging.

Table (2): The mean, standard deviation (±SD) values and results of repeated measures ANOVA test for comparison between Log_{10} CFU/ml of bacterial counts with different interactions of variables.

Aging	Bruxzir		IPS e.max ZirCAD Prime		*P*-value	Effect size *(Partial eta squared)*
	Mean Log_{10} CFU	±SD	Mean Log_{10} CFU	±SD		
Before aging	4.59	±0.44	4.77	0.43	0.300	0.045
After aging	5.85	±0.54	5.6	0.33	0.176	0.075
P-value	<0.001*		<0.001*			
Effect size *(Partial eta squared)*	0.696		0.5			

**: significant at p ≤ 0.05.*

DISCUSSION

The demand for biocompatible and esthetic restorations among clinicians and patients over the last years lead to many advancements in dental materials and techniques of fabrication. Nowadays, CAD/CAM technology and materials including zirconia have gained popularity in the dental market due to their strength, esthetics and biocompatibility.[40]The newly innovations were concerned to upgrade the esthetic appeal of zirconia without compromising its strength.[41]

The continuous exposure of the restoration to complex humid oral environment leads to low thermal degradation (aging).[26] This aging process causes an increase in restoration's surface roughness affecting its physical and mechanical properties negatively.[42]

Previous studies have proved a positive correlation between surface roughness and bacterial colonization.[23,30,31,43] So, the clinical success of zirconia-based restorations seems to be dependent on its capacity to retain its smooth surface and to prevent bacterial adherence against aging process.

The purpose of this study was to evaluate the surface roughness and bacterial colonization of two types of CAD/CAM zirconia materials **Cubic** (BruxZir) and **Gradient** (IPS e.max ZirCAD Prime) after accelerated hydrothermal aging.

To provide information that was more closely related to the clinical scenario, the present analysis was an invitro study that provided a regulated and standardized process of fabrication and eliminated diverse oral cavity attributes.[4]

In this study **BruxZir** was selected as a control material. It is a 5 mol% yttria stabilized zirconia polycrystalline ceramic with approximately 50% cubic structure (5Y-ZP) monolithic zirconia material which was known for its good esthetics,strength.[44] **IPS e.max ZirCAD Prime** was also selected as it consists mainly of 2 layers, 5Y-TZP in occlusal or incisal area for good translucency, and 3Y-TZP in dentin area for high strength.[18,44] The outer surface of the translucent zirconia is infiltrated with feldspathic glass creating a glass graded structure.[42]This upgraded material combined the superior high strength of zirconia with natural esthetics.[42,43]

For the CAD/CAM blanks of **BruxZir** and **IPS e.max ZirCAD Prime** were designed with close simulation of optimum manufacturer conditions, which enabled the construction of discs with high intrinsic strength.[45]A 5-axis dental milling machine was used with the same milling burs recommended by the manufacturer with final thickness 1.2 mm and diameter 12 mm.[45,46] These dimensions were reported to be the suitable one for the 3D optical profilometer to record the readings easily **Elsherif et al. in 2020**.[33]

Before and after sintering, the dimensions of each sample were checked using a digital caliper to exclude any possible factors that might have an impact on the final results of surface roughness and bacterial colonization.[47]

Finishing and polishing were done for all samples to remove any irregularities and minor surface defects that can cause surface roughness and bacterial adhesion.[25] After polishing, all zirconia discs were glazed according to the manufacturer's instructions and to simulate the clinical condition using **Dentsply Sirona Universal Overglaze High Flu** as it is one of the most preferred glaze products and it is recommended by the manufacturer.[48,49] Glazing procedure can guarantee surface smoothness that prevents bacterial colonization and ensures biocompatibility.[50] **Toma et al. in 2023**[45] found that glazing adheres to cubic zirconia with high yttria content and large grain size forming a protective layer.

Saliva has a significant role in bacterial adhesion process. To standardize the variability of natural saliva due to variations in salivary secretions, different diets and variability in protein composition and content.[51] Artificial saliva was prepared in place of human entire saliva.[51] **Zupancic et al. in 2020**[51] used artificial saliva to test the in vitro adherence of bacteria to zirconia materials.

Surface roughness measurements were performed using a **3D optical non-contact profilometer interface**. This method was characterized by the absence of direct contact between the sample surface and the analyzing probe. It used laser beam illuminating the surface and measuring 3D profile of material, thus providing high quality and fully focused images.[52]

There is an importance for the measurement of bacterial colonization on ceramic surfaces as they are continuously exposed to oral conditions especially oral flora.[53] So, according to a study by **Vo et al. in 2015**[54] the selected reference strain was streptococcus mutants (S.Mutants) due to its strong ability to adhere and create biofilms. In order to simulate the influence of a salivary pellicle on streptococcal adhesion, samples were incubated in artificial saliva before the adhesion assay. [51]

Bacterial colonization was measured on zirconia surfaces before and after accelerated hydrothermal aging using a digital colony counter to avoid missing or double counting of colonies.by converting the number of colonies.[55] The obtained number was converted to (colony forming units per milliliter) CFU/mL according to recommended dilution. The obtained suspension was diluted to 1:1000 before aging and to 1:10000 after aging.[35]

To simulate further of oral conditions, samples were subjected to accelerated hydrothermal aging.[56] Standardization of aging procedure was done according to standard aging protocol of **ISO13356** by autoclaving zirconia samples of both groups at temperature of 134ºC and 2 bar pressure for 5 hours simulating 15 years serviceability.[57] This strategy was in accordance with another studies that mentioned the reliability of using autoclave to simulate oral conditions.[15,38,58]

The present study revealed that **IPS e.max ZirCAD Prime** showed significantly lower mean Ra before accelerated hydrothermal aging than **BruxZir**. These results may be attributed to the variation in composition of both materials, since the outer surface of **IPS e.max ZirCAD Prime** is infiltrated with feldspathic-glass that is not present in **BruxZir**. [15,41] The results showed that accelerated hydrothermal aging had a significant effect on both types of materials, **BruxZir** and **IPS e.max ZirCAD Prime** that led to surface roughness. These findings could be attributed to the low thermal degradation phenomenon, where tetragonal to monoclinic transformation occurred, thus decreasing its mechanical properties.[42] In cubic zirconia, phase transformation was not fully established leading to decreased mechanical properties.[59] This was attributed to the fact of cubic zirconia structure was more stable phase with reduced potential for stress-induced transformation toughening.[60] However, the generation of the monoclinic phase, even in small amounts, may be accompanied by grain pull-out and/or microcracks, which can be a fracture origin that reduce the strength and induce surface roughness of the material. [15-17]

The results of this study were in agreement with previous studies, where **Flinn et al. in 2017**[15] concluded that hydrothermal aging caused tetragonal to monoclinc transformation. **Elsherif et al. in 2020**[33] and **Choi et al. in 2020**[61] who concluded that accelerated hydrothermal aging caused an increase in surface roughness for both tetragonal and cubic zirconia.[61]

The results of the present study were in disagreement with **Hajhamid et al. in 2023**[62] and **Yan et al. in 2023**[63] who reported that accelerated hydrothermal aging had no significant effect on zirconia. These findings could be due to different type of materials used, sample size, time of aging and different sintering parameters.[64]

The results of the present study revealed that **IPS e.max ZirCAD Prime** showed lower mean Ra after accelerated hydrothermal aging than **BruxZir.**

Hence, the first null hypothesis that stated that there will be no statistical difference in surface roughness between **Bruxzir** and **IPS e.max ZirCAD Prime** after accelerated hydrothermal aging was rejected.

Before aging, the results of the bacterial colonization test revealed that there was no statistically significant difference in bacterial counts between the two zirconia types **BruxZir** and **IPS e.max ZirCAD Prime.**

The results of the bacterial colonization test revealed significant increase in bacterial counts after accelerated hydrothermal aging for both materials **BruxZir** and **IPS e.max ZirCAD Prime.**

These results might be attributed to the increased roughness values which provided more surface area for bacterial adhesion.[31] According to **Chen et al. in 2020**[43]**,** surface roughness offers irregular topography in which bacteria are strongly resisted to dissociation and provide favorable circumstances for the association. The results of surface roughness of this study were greater than 0.2 μm. This value of mean Ra was considered in study by **Dutra et al. in 2017**[65] to be the critical threshold value above which bacterial adhesion becomes possible.

The results of the present study were in agreement with **Siddiqui et al. in 2019**[30] who compared rough surface variants to smooth surface of zirconia, found that average adherent bacterial count tended to rise in rough surfaces. **Poole et al. in 2020**[23] who concluded a positive correlation between surface roughness and bacterial adhesion. **Matalon et al. in 2021**[66] who found a significant increase in surface roughness and bacterial adherence after aging of zirconia. **Abdalla et al. in 2021**[32] who proved that regardless of the type of ceramic, roughened ceramic surfaces aided in biofilm attachment.

The results of the present study were not in accordance with **Dutra et al. in 2017**[65] who found that bacterial adherence didn't increase with the increase of surface roughness after accelerated hydrothermal aging. This might be contributed to the use of different types of material and different aging time.

Results revealed that although bacterial colonization increased in both materials after accelerated hydrothermal aging, no significant difference between the two materials **BruxZir** and **IPS e.max ZirCAD Prime** in their total bacterial count was found.

Further explanation of results of this study could be attributed to the fact that roughness-induced surface energy that may change a material's inert potential and encourage the colonization of streptococcus mutants.[54] Materials differ in terms of hydrophilicity and surface texture due to changes in their chemical compositions, which may be connected to how well bacteria adhere to surfaces.[54] Also variability in bacterial species, and incubation environment might be implicated for the capacity of adhesion.[54] The composition of the salivary pellicle can also change the surface topography, because the enzymatic bacterial secretions such exopolysaccharides can transform sucrose into glucans that increase binding of S. mutants and alter the surface topography.[67]

Thus, the second null hypothesis that stated that there will be no difference in bacterial colonization of **Bruxzir** and **IPS e.max ZirCAD Prime** after accelerated hydrothermal aging was rejected.

The limitation of this study as it was conducted in vitro not in vivo when different oral conditions and variations of human beings as saliva, water, PH value, and temperature changes may vary in the amount of roughness and bacterial adherence. It also measured only the effect of accelerated hydrothermal aging on surface roughness and its effect on bacterial adherence without measuring the surface free energy and contact angle of the materials.

CONCLUSION

Within the limitations of this study, the following could be concluded:

1- Surface roughness of both BruxZir and IPS e.max ZirCAD Prime increased due to accelerated hydrothermal aging.

2- IPS e.max ZirCAD Prime showed lower surface roughness than BruxZir before and after aging

3- Both materials showed an increase in bacterial counts after accelerated hydrothermal aging as roughness exceeded the critical level of 0.2 mm.

4- There is a positive correlation between surface roughness and bacterial colonization.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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