

Grinding With Diamond Burs and Hydrothermal Aging of a Y-TZP Material: Effect on the Material Surface Characteristics and Bacterial Adhesion

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Clinical Relevance

Finishing of Y-TZP restorations with diamond burs altered the material surface characteristics, but neither the grinding nor an aging condition affected biofilm formation.

SUMMARY

The aim of this study was to evaluate the effect of grinding with diamond burs and low-temperature aging on the material surface characteristics and bacteria adhesion on a yttrium-stabilized tetragonal zirconia polycrystalline (Y-TZP) surface. Y-TZP specimens were made from presintered blocks, sintered as recom-

mended by the manufacturer, and assigned into six groups according to two factors—grinding (three levels: as sintered, grinding with extra-fine diamond bur [25-μm grit], and grinding with coarse diamond bur [181-μm grit]) and hydrothermal aging—to promote low-temperature degradation (two levels: presence/absence). Phase transformation (X-ray diffractometer), surface roughness, micromorphological patterns (atomic force microscopy),

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and contact angle (goniometer) were analyzed. Bacterial adhesion (colony-forming units [CFU]/biofilm) was quantified using an *in vitro* polymicrobial biofilm model. Both the surface treatment and hydrothermal aging promoted an increase in *m*-phase content. Roughness values increased as a function of increasing bur grit sizes. Grinding with a coarse diamond bur resulted in significantly lower values of contact angle ($p < 0.05$) when compared with the extra-fine and control groups, while there were no differences ($p < 0.05$) after hydrothermal aging simulation. The CFU/biofilm results showed that neither the surface treatment nor hydrothermal aging simulation significantly affected the bacteria adherence ($p > 0.05$). Grinding with diamond burs and hydrothermal aging modified the Y-TZP surface properties; however, these properties had no effect on the amount of bacteria adhesion on the material surface.

INTRODUCTION

Zirconia-based ceramics are a contemporary option for fixed dental prostheses, dental implants and abutments¹ because of their esthetic and superior mechanical strength.^{2,3} Among the different types of zirconia-based ceramics, yttrium-stabilized tetragonal zirconia polycrystalline (Y-TZP) has been highlighted.^{4,5} Y-TZP ceramic shows high biocompatibility, chemical stability, and a fracture strength/toughness higher than other ceramic systems.⁶ More recently, it has been used to produce monolithic zirconia crowns in posterior teeth.⁷

Zirconia is a polymorphic material that has three crystalline forms that are stable at different temperatures: monoclinic (*m*: up to 1170°C), tetragonal (*t*: above 1170°C and up to 2370°C), and cubic (*c*: above 2370°C).⁸ Phase transformation from monoclinic to tetragonal zirconia ($m \rightarrow t$) occurs during the sintering process and is associated with a volume decrease of approximately 4%. After sintering, stabilizing oxides (ie, Y_2O_3) are added to pure zirconia, keeping the tetragonal form stable at room temperature and avoiding the deleterious effects of volume expansion during the cooling process due to $t \rightarrow m$ transformation.⁶

Some factors associated with the clinical use of Y-TZP may also induce a $t \rightarrow m$ transformation of this material, such as intermittent mechanical loading (stress) and corrosion in the presence of humidity (low-temperature degradation [LTD]).^{9,10} Additionally, stress concentration with subsequent phase

transformation will occur to Y-TZP after adjustment of the Y-TZP surface (outer or intaglio) by grinding and/or polishing.¹¹ These procedures introduce different types of damage to the Y-TZP surface, such as scratches and cracks of various depths, from the surface toward the subsurface of the material.^{12,13}

These damages to the Y-TZP surface (scratches and cracks) may be limited by a phenomenon known as transformation toughening. The $t \rightarrow m$ transformation associated with localized volumetric expansion results in compressive stresses at an existing crack that counteract tensile stresses in this region and limit crack propagation. However, the increase in the transformation area may also result in material loss (grain pullout), a rougher surface, and a higher incidence of cracks, all of which decrease the material strength.¹⁴

Moreover, grinding with diamond burs produces a modification of the surface characteristics of the Y-TZP material, and this might increase bacterial adhesion¹⁵⁻¹⁹ and favor the incidence of secondary caries and periodontal inflammation,²⁰ relevant aspects of the longevity of restorations. The restoration surface properties, such as roughness and the surface free energy, seem to play a key role in this process.²¹ The surface free energy influences the acquired film formed over the restorative surface.^{22,23} The increase in free energy of the substrate surface can result in a higher plaque growth rate and plaque retention capacity of the surface and the selection of specific organisms.²¹ Regarding the surface roughness, previous studies suggest that the biofilm is formed in larger amounts and more rapidly on rough surfaces when compared to smooth surfaces.²³ *In situ* studies using scanning electron microscopy revealed that the initial adhesion of microorganisms starts on irregularities and sequentially expands to the rest of the surface.¹⁹ Additionally, previous studies have demonstrated a positive association between the amount of biofilm and the surface roughness in different dental materials, such as ceramics, composite resin, acrylic resin, and titanium.^{18,24,25}

Although there is evidence regarding the influence of surface characteristics on bacteria adhesion to restorative materials and the importance of these factors on the longevity of prosthetic restorations, there are no studies that have investigated the effects of grinding with diamond burs and hydrothermal aging (Y-TZP under LTD) on bacterial adhesion (biofilm formation) on a Y-TZP surface. These conditions can be clinically relevant when utilizing a Y-TZP ceramic for implant abutments,

Table 1: Study Groups

Groups	Surface Treatment	Low-Temperature Aging
Control Control aging	As sintered (untreated)	Without With
Coarse Coarse aging	Coarse diamond bur #3101G (average grit size 181 μm)	Without With
Xfine Xfine aging	Xfine diamond bur #3101FF (average grit size 25 μm)	Without With

which are placed subgingivally and at areas close to gingival tissues (marginal and connector zones of fixed prostheses). Thus, the present study aimed to evaluate the effect of grinding with diamond burs and hydrothermal aging on the material surface characteristics (*m*-phase transformation, surface roughness, superficial topography, and surface free energy) and bacteria adhesion on a Y-TZP ceramic surface. The null hypothesis (H_0) was that grinding with diamond burs of different grit sizes and hydrothermal aging conditions would yield equivalent bacteria adhesion on the Y-TZP surface.

METHODS AND MATERIALS

Specimen Preparation

Y-TZP specimens (In-Ceram YZ, Vita Zahnfabrik, Bad Sackingen, Germany) were prepared from prefabricated blocks. For the complementary analysis of surface characterization, specimens were manufactured with a final size of $14 \times 14 \times 2$ mm, while for the microbiological evaluation with an *in vitro* biofilm formation model, specimens were used with a final size of $7 \times 6 \times 2$ mm.

To remove the cutting irregularities, the presintered specimens were polished with 1200-grit SiC paper and cleaned in an ultrasonic bath (1440 D, Odontobras, Ribeirão Preto, Brazil) using 78% isopropyl alcohol for 10 minutes. Then the specimens were sintered as recommended by the manufacturer (Zyrcomat T, Vita Zahnfabrik).

Experimental Groups

After sintering, the Y-TZP specimens were allocated into six groups according to two factors: grinding with diamond burs and low-temperature aging to simulate LTD, as shown in Table 1.

Surface Treatment

Specimens from the control groups (control and control aging) remained untreated after the sintering process. For the other groups, a single trained

operator performed the grinding procedures using diamond burs (Xfine #3101FF, 25- μm grit size, and coarse #3101G, 181- μm grit size, KG Sorensen, Cotia, Brazil) coupled with a low-speed motor (Kavo Dental, Biberach, Germany) associated with a contra-angle hand piece (T2 REVO R 170 contra-angle hand piece up to 170,000 rpm, Sirona, Bensheim, Germany) under constant water cooling ($\cong 30$ mL/min). The diamond bur was replaced after each specimen.

A marking with permanent marking pen (Pilot, São Paulo, Brazil) was made over the entire surface of each specimen prior to the grinding procedures. Afterward, the specimens were fixed to a device that ensured parallelism between the specimen and diamond bur. Grinding was carried out by similar horizontal movements until the pen mark was eliminated. This protocol standardized the grinding thickness while ensuring that the entire specimen surface was subjected to bur grinding.²⁶

Low-Temperature Aging

The hydrothermal aging was simulated in an autoclave (Sercon HS1-0300, no. 1560389/1) at 134°C under 2 bars for 20 hours.²⁷

Phase Analysis by X-Ray Diffraction

Quantitative analysis of phase transformation was conducted (one specimen per group) to determine the relative amount of *m*-phase and depth of the transformed layer under each condition. This analysis was performed using an X-ray diffractometer (Bruker AXS, D8 Advance, Karlsruhe, Germany). Spectra were collected into the 2 θ , with a range of 25-35 degrees, at a step interval of 1 second and step size of 0.03 degrees. The amount of *m*-phase was calculated using the method introduced by Garvie and Nicholson:²⁸

$$X_M = \frac{(-111)_M + (111)_M}{(-111)_M + (111)_M + (111)_T} \quad (1)$$

where $(-111)_M$ and $(111)_M$ represent the intensity of the monoclinic peaks ($2\theta = 28$ degrees and $2\theta = 31.2$ degrees, respectively) and $(101)_T$ indicates the intensity of the respective tetragonal peak ($2\theta = 30$ degrees). The volumetric fraction of the *m*-phase was calculated according to Toraya and others:²⁹

$$F_M = \frac{1.311 \cdot X_M}{1 + 0.311 \cdot X_M} \quad (2)$$

The depth of the transformed layer was calculated based on the amount of the *m*-phase, considering

that a constant fraction of grains had symmetrically transformed to the *m*-phase along the surface, as described by Kosmac and others:³⁰

$$PZT = \left(\frac{\sin \theta}{2\mu} \right) \left[\ln \left(\frac{1}{1 - FM} \right) \right] \quad (3)$$

where $\theta = 15$ degrees (the angle of reflection), $\mu = 0.0642$ is the absorption coefficient, and FM is the amount of *m*-phase obtained using equations 1 and 2.

Surface Roughness and Micromorphological Analysis

Y-TZP specimens were evaluated for quantitative (10 specimens per group) and qualitative (two specimens per group) analysis of the micromorphological pattern generated by the grinding procedure. Specimens were analyzed using a surface roughness tester (Mitutoyo SJ-410, Tokyo, Japan) and atomic force microscopy (AFM, Agilent Technologies 5500 equipment, Chandler, AZ, USA), respectively.

For the roughness analysis, four measurements were made for each specimen (two following the grinding direction and two in the opposite direction) according to the ISO 1997 parameters (R_a , arithmetical mean of the absolute values of peaks and valleys measured from a medium plane [μm], and R_z , average distance between the five highest peaks and five major valleys [μm])³¹ with a cutoff ($n=5$) of λC 0.8 mm and λS 2.5 μm . After that, the arithmetic mean of all measurements from each specimen was obtained.

Afterward, two specimens of each group were randomly selected for qualitative analysis of superficial topography using AFM. First, all selected specimens were submitted to the cleaning protocol in an ultrasonic bath as previously described. The AFM images were obtained by noncontact methodology and specific probes from an area of $20 \times 20 \mu\text{m}$ (PPP-NCL probes, Nanosensors, force constant = 48 N/m) and evaluation using specific computer software (Gwyddion version 2.33, GNU, Free Software Foundation, Boston, MA, USA).

Contact Angle

The contact angle was measured (10 specimens per group) using the sessile drop technique and a goniometer (DSA30S, Drop Shape Analyzer, KRÜSS, Hamburg, Germany) associated with a computer device using specific software (Advanced Drop Shape Analysis, KRÜSS). For the contact angle measurement, a syringe was used to place a drop (10 μL) of preselected liquid (deionized water) on the

treated surface of the specimen, and the contact angle (angle between the drop and the surface plane) was measured after 5 seconds.³² The software carried out five measurements, and the average value from each specimen was calculated.

Biofilm Model

In vitro biofilms were grown using the Amsterdam Active Attachment (AAA) model.³³ This model consisted of a custom-made stainless-steel lid with 24 clamps in which the substratum was fixed.

Saliva Collection

Stimulated saliva was previously collected from a single donor (DAMD) who refrained from dental hygiene for 24 hours before the collection procedure. The saliva was diluted twofold with 60% sterile glycerol to protect the bacterial cells from cryo-damage and stored at -80°C .

Initial Bacterial Attachment

The inoculation medium for the polymicrobial biofilms was 50-fold diluted saliva in a semidefined medium³⁴ with 0.2% sucrose and 50 mmol/L PIPES at pH 7.0.

Y-TZP specimens (six specimens per group) were fixed in the lid clamps and placed onto standard polystyrene 24-well plates (multiwell plates, Greiner Bio One, Alphen aan den Rijn, Netherlands). Biofilms were produced by adding 1.7 mL of the inoculation medium to each well, and the model was subsequently incubated anaerobically (10% CO_2 , 10% H_2 , 80% N_2) at 37°C for 6 hours.

Determination of Colony-Forming Units

After allowing for biofilm growth, the specimens with the biofilms were removed from the lid and transferred into 2-mL cysteine peptone water. The biofilms were dispersed by sonication for 2-minutes, 1-second pulsations at an amplitude of 40 W (Vibra Cell, Sonics & Materials Inc, Newtown, CT, USA) and vortex mixing for 30 seconds, and then a series of dilutions were made.

The polymicrobial biofilm suspensions were plated on tryptic soy agar blood plates for total counts. Plates were incubated for 96 hours at 37°C under anaerobic conditions (10% CO_2 , 10% H_2 , 80% N_2).

Data Analysis

Statistical analysis was executed using SPSS 18. Roughness (R_a and R_z) and contact angle data were

Table 2: X-Ray Diffractometry Analysis (F_m , % of Monoclinic Phase; PTZ, Depth of Transformed Layer), Roughness (Ra and Rz), and Contact Angle Results for Grinding and Aging Factors

Groups	Diffractometry Analysis ^a		Ra (μm) ^b	Rz (μm) ^b Mean (SD)	Contact Angle ^b
	F_m (%)	PTZ (μm)			
Control	0.00	0.00	0.13 (0.02) A	1.17 (0.19) A	81.02 (9.83) A
Control aging	54.38	3.97	0.14 (0.02) A	1.32 (0.27) A	59.55 (8.30) CD
Xfine	8.93	0.47	0.70 (0.21) B	4.56 (0.94) B	75.88 (11.90) AB
Xfine aging	12.72	0.68	0.53 (0.11) C	3.47 (0.65) C	67.71 (9.01) BC
Coarse	10.66	0.57	1.16 (0.14) D	6.87 (0.71) D	53.75 (7.27) D
Coarse aging	19.95	1.12	0.99 (0.08) E	6.11 (0.54) D	60.01 (14.12) CD

^a Diffractometry analysis: F_m , % of monoclinic phase; PTZ, depth of transformed layer.

^b Two-way ANOVA and Tukey test. Same letters show no statistical difference between the groups ($p > 0.05$). Different letters represent differences between groups ($p < 0.05$).

analyzed using two-way analysis of variance (ANOVA) considering two factors (grinding and aging) and the interaction of both factors. Colony-forming units (CFU)/biofilm counts were compared using one-way ANOVA and the Tukey test. All statistical tests were performed considering a 5% significance level.

RESULTS

Phase Analysis

Surface treatment alone promoted an increase in the *m*-phase content and transformation depth, showing higher values for both the bur grit sizes (Xfine and coarse) when compared to the control (Table 2). Furthermore, all groups showed a higher amount of *m*-phase content and transformation depth after hydrothermal aging, and these differences were more pronounced for the as-sintered control group (*m*-phase from 0% to 54% and depth from 0 to 3.97 μm , respectively).

Surface Roughness and Micromorphological Analysis

The bur grit size directly affected the Ra and Rz parameters on the material surface (Table 2). These results showed an increase ($p < 0.05$) in the roughness parameters as a function of increasing bur grit size. Additionally, there was an effect of hydrothermal aging on the roughness parameters for the treated groups (Xfine and coarse groups), with a decrease ($p < 0.05$) of the Ra parameter after aging for the Xfine (0.70 to 0.53 μm) and coarse (1.16 to 0.99 μm) groups. There was no difference between control groups, either with or without aging ($p > 0.05$).

Micromorphological analysis showed that grinding with a diamond bur (Xfine and coarse) resulted in similar surface patterns, with scratches parallel to

the direction of the grinding tool motion and a depth proportional to the grit size of the diamond bur used. The untreated surface showed a distinct micromorphological pattern, with a smoother surface where superficial Y-TZP grains can be seen.

Contact Angle Measurements

The data from contact angle measurements indicated that the surface treatment alone also modified the surface free energy (Table 2). This result indicates that the specimens ground with the coarse diamond bur had significantly lower values of contact angle measurement ($p < 0.05$) when compared with the Xfine and control groups. Moreover, hydrothermal aging significantly affected ($p < 0.05$) the contact angles values between the control groups (81 to 59 degrees), but no difference ($p > 0.05$) was observed between the Xfine and coarse groups. When only the aged groups were compared, the contact angle values showed no significant differences ($p > 0.05$) between the groups.

Bacteria Adherence

The bacteria adherence was evaluated using an *in vitro* model of biofilm formation. The CFU/biofilm results showed that neither the surface treatment nor hydrothermal aging simulation significantly affected ($p > 0.05$) bacteria adherence on the material surface (Figure 2).

DISCUSSION

In the present study, grinding with diamond burs (Xfine and coarse) promoted higher *m*-phase content when compared to the as-sintered condition (control). Additionally, the grinding procedures altered the superficial topography, roughness, and surface free energy of the Y-TZP ceramic. Regarding aging,

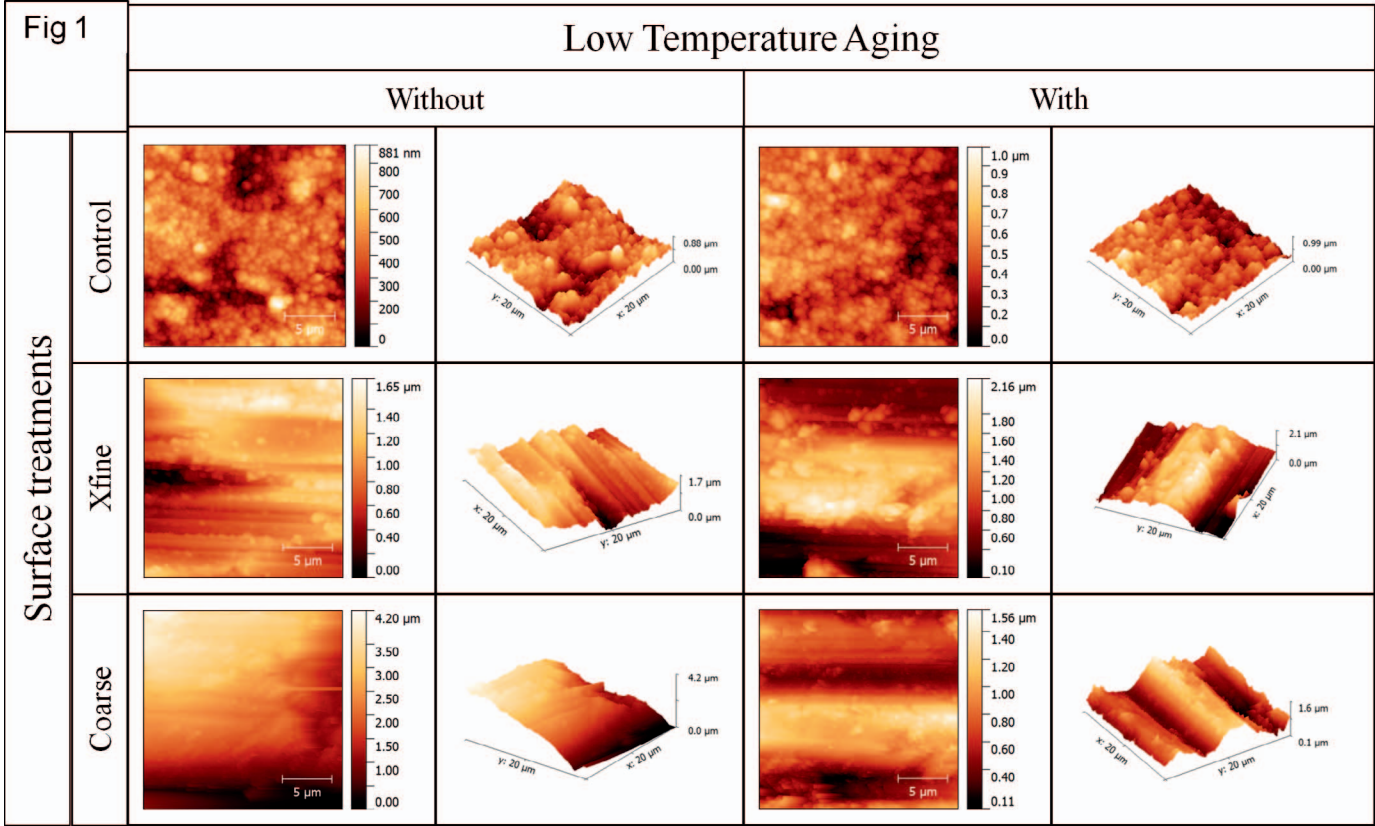


Figure 1. Atomic force micrographs from zirconia samples of different groups considering the two factors (grinding and aging). It can be noticed that the grinding procedures promoted surface alterations compared to the as-sintered group and that the low-temperature aging did not change the micromorphological pattern.

distinct effects were observed depending on the presence/absence of grinding. However, despite these differences observed regarding the surface treatment, no significant effect was observed on the bacterial adhesion to Y-TZP surface using an *in vitro* model of biofilm formation.

As aforementioned, some conditions associated with the clinical use of Y-TZP may induce phase transformation ($t \rightarrow m$), such as intermittent loading, humidity, and adjustment by grinding of the Y-TZP surface.¹¹ In this study, the clinical adjustment was simulated by grinding using diamond burs with different grit sizes (Xfine and coarse), and LTD was artificially induced by hydrothermal aging. In agreement with the literature,^{26,35-36} the current data indicate that grinding increased the *m*-phase content (control: 0%; Xfine: 8.9%; coarse: 10.6%), and it decreased the susceptibility of Y-TZP to phase transformation during aging (control aging: 54.3%; Xfine aging: 12.7%; coarse aging: 19.9%). Muñoz-Tabares and Anglada³⁷ stated that grinding induces a recrystallization of a very thin surface layer of tetragonal nanograins from the

highly deformed surface, whose size is smaller than the critical size for phase transformation in a humid environment, such that this process may decrease Y-TZP susceptibility to $t \rightarrow m$ transformation.

Surface topography (AFM images) and roughness examinations (Ra and Rz parameters) were conducted to evaluate the direct effect of grinding on the Y-TZP surface. Roughness results from nonaged groups showed that Ra and Rz values increased with increasing bur grit sizes, and these differences among groups can also be observed in the surface topography images obtained using AFM (Figure 1). The as-sintered condition (control) presented a smoother topographical pattern (zirconia grains at the surface can be seen), and that grinding, regardless of grit size, changed this pattern by introducing scratches and promoting deformations in the direction of the bur movement.

Previous studies have suggested that the increase in the transformation area ($t \rightarrow m$) would result in material loss (by grain pullout) and increasing surface roughness.^{10,14,37,38} However, even with the

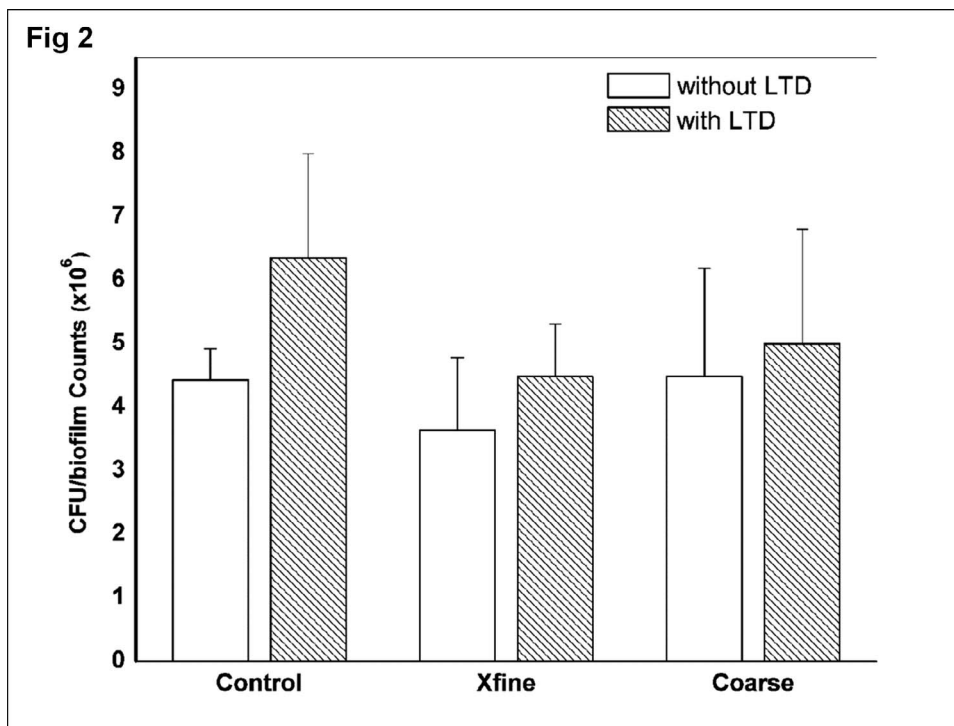


Figure 2. CFU/biofilm counts of bacteria grown in vitro on zirconia surfaces. Two-way ANOVA was performed considering the two factors (grinding and aging) and showed no significant differences between the experimental groups ($p > 0.05$). Error bars show the standard deviation from the average value.

higher *m*-phase content presented after aging for all groups of this study, higher roughness values did not present as a result. Both the Xfine and the coarse groups had lower Ra (Xfine: 70 to 53 μm ; coarse: 1.16 to 0.99 μm) and Rz (Xfine 4.56 to 3.47 μm ; coarse: 6.87 to 6.11 μm) values after hydrothermal aging, and the difference between the control groups was not significant ($p > 0.05$), even with an extensive increase in *m*-phase (0% to 54%). On the other hand, Deville and others³⁹ stated that $t \rightarrow m$ transformation is triggered preferentially on surrounding areas of superficial defects and residual stress concentration. Thus, it is possible to hypothesize that effects of aging (ie, grain pullout) on ground surfaces occur initially around the highest topographical grains (superficial layer), which are also more susceptible to water contact, resulting in a less rough surface when compared to nonaged ground surfaces. This fact could also be indicative that aging by autoclave for 20 hours at 134°C with 2 bars of pressure was not significant enough to promote the deleterious effects described by Lughy and Sergio¹⁴ on the Y-TZP ceramic used here.

Moreover, the effect of surface roughening on the material surface wettability has been previously reported.⁴⁰ In this study, the grinding effect on the contact angle analysis of the Y-TZP surface was observed only for the coarse group, which presented higher surface free energy than the Xfine and control

groups. Additionally, it is important to notice that, after aging, there was no difference between the groups regardless of the presence or absence of surface treatment.

The relationship between material surface characteristics and bacteria adhesion has been studied extensively;^{40,41} however, few studies have been performed on ground Y-TZP. The understanding of bacteria-surface interactions and how grinding using diamond burs and aging affect biofilm accumulation becomes an important tool for biofilm control and a relevant aspect to preview the longevity of Y-TZP restorations and implant abutments. Regarding the surface characteristics, previous studies have reported that roughness and surface free energy seem to play an important role in the process of bacteria adhesion on restorative surfaces.⁴⁰⁻⁴² Quirynen and Bollen²³ found that increased surface free energy attracts more bacteria when compared to more hydrophobic surfaces. Likewise, Al-Radha and others⁴³ concluded that the influence of surface free energy on initial bacterial adhesion to smooth implant materials *in vitro* appears to be the most important factor, in addition to the material type. However, these studies have compared materials with similar patterns of surface roughness. When both the roughness and the surface free energy were evaluated together, the influence of surface roughness on the accumulation and compo-

sition of biofilm is more important than the influence of surface free energy.⁴⁴

In general, an increase in surface roughness promotes an increase in bacterial attachment due to the initial adhesion of bacteria at locations where they are sheltered against shear forces⁴⁰ and also because roughening of the surface increases the contact area between the material surface and bacterial cells available for adhesion.⁴⁵ It is accepted that an increase in surface roughness above a threshold of 0.2 μm facilitates biofilm formation on restorative materials, while bacterial adhesion to surfaces below the threshold of 0.2 μm cannot be reduced.⁴⁶ On the other hand, while both the Xfine and the coarse groups presented Ra values higher than the threshold of 0.2 μm (0.70 and 1.16 μm , respectively), they did not present an increase in bacterial adhesion when compared with the control group (0.13 μm). Hence, it is possible to hypothesize that the range of surface roughness observed in our results is not the main factor for promoting bacterial adhesion on the Y-TZP ceramic *in vitro* and that this low susceptibility to bacterial adhesion can be considered an advantage of this material. This result is in agreement with other studies that indicated that bacteria adhesion cannot be fully explained by small differences in the surface roughness and surface free energy.^{47,48}

This inconsistency regarding the effect of surface characteristics on bacteria adhesion on material surface may be explained mainly by 1) characteristics derived from the distinctive materials, such as material chemical composition; 2) the range of roughness promoted on the material surface; and 3) culture conditions used in the tests. In relation to culture conditions, this study evaluated a complex *in vitro* polymicrobial biofilm consisting of diluted-saliva inoculation medium, which differs from other studies with similar purposes that used a single-specimen biofilm, with less varied modes of attachment and without a significant degree of interspecies interactions.⁴⁹ The protocol of 6 hours of biofilm growth was chosen in order to evaluate early bacteria adhesion. Additionally, the current study evaluated bacteria adhesion on a Y-TZP surface using the AAA model,³³ a validated and extensively studied polymicrobial model of biofilm formation *in vitro*. However, the use of the AAA model can be considered a limitation of this study, as this *in vitro* model does not simulate some factors from a typical oral environment, such as low shear forces, which can limit the roughness effect on bacteria adherence capacity.

The findings of the current study indicate that grinding with diamond burs and hydrothermal

aging modify the surface properties (ie, *m*-phase content, surface roughness, and surface free energy) of the assessed Y-TZP material; however, those properties/characteristics did not significantly affect bacterial adhesion when using the AAA model of *in vitro* biofilm formation. These results suggest that the Y-TZP ceramic may have low susceptibility to bacterial adhesion regardless of the surface condition. However, even if our results have shown no differences between the control and other groups with regard to bacterial adhesion, the surface roughness may affect other properties of the material, such as its mechanical behavior and wear of antagonist teeth, so a smoother surface is clinically preferable. Thus, when clinical grinding is necessary, it should be made using extra-fine diamond burs followed by polishing.⁵⁰ Further studies should be performed to provide additional information regarding the behavior of this material using biofilm models that simulate clinical conditions and/or clinical studies to better understand the influence of these factors on the longevity of the prosthetic restorations.

CONCLUSION

- Grinding with diamond burs and hydrothermal aging promoted *m*-phase content, surface roughness, and surface free energy alterations of the assessed Y-TZP material.
- Bacterial adhesion was not affected by grinding with different diamond burs.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Federal University of Santa Maria, Brazil.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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REFERENCES

- Wenz HJ, Bartsch J, Wolfart S, & Kern M (2008) Osseointegration and clinical success of zirconia dental implants: A systematic review *International Journal of Prosthodontics* **21**(1) 27-36.
- Denry I, & Kelly JR (2008) State of the art of zirconia for dental applications *Dental Materials* **24**(3) 299-307 DOI: 10.1016/j.dental.2007.05.007.
- Denry I, & Kelly JR (2014) Emerging ceramic-based materials for dentistry *Journal of Dental Research* **93**(12) 1235-1242 DOI: 10.1177/0022034514553627.
- Kelly JR, & Denry I (2008) Stabilized zirconia as a structural ceramic: An overview *Dent Mater* **24**(3) 289-298 DOI: 10.1016/j.dental.2007.05.005.
- Kosmac T, Oblak C, Jevnikar P, Funduk, N & Marion L (1999) The effect of surface grinding and sandblasting on flexural strength and reliability of Y-TZP zirconia ceramic *Dental Materials* **15**(6) 426-433.
- Piconi C, & Maccauro G (1999) Zirconia as a ceramic biomaterial *Biomaterials* **20**(1) 1-25.
- Sabrah AH, Cook NB, Luangruangrong P, Hara AT, & Bottino MC (2013) Full-contour Y-TZP ceramic surface roughness effect on synthetic hydroxyapatite wear *Dental Materials* **29**(6) 666-673 DOI: 10.1016/j.dental.2013.03.008.
- Guazzato M, Quach L, Albakry M, & Swain MV (2005) Influence of surface and heat treatments on the flexural strength of Y-TZP dental ceramic *Journal of Dentistry* **33**(1) 9-18 DOI: 10.1016/j.jdent.2004.07.001.
- Chevalier J, Gremillard L, & Deville S (2007) Low-temperature degradation of zirconia and implications for biomedical implants *Annual Review of Materials Research* **37** 1-32.
- Kobayashi K, Kuwajima H, & Masaki T (1981) Phase change and mechanical properties of ZrO₂-Y₂O₃ solid electrolyte after aging. *Solid State Ionics* **3/4** 489-495.
- Aboushelib MN, Feilzer AJ, & Kleverlaan CJ (2009) Bridging the gap between clinical failure and laboratory fracture strength tests using a fractographic approach *Dental Materials* **25**(3) 383-391 DOI: 10.1016/j.dental.2008.09.001.
- Ban S, Sato H, Suehiro Y, Nakanishi H, & Nawa M (2008) Biaxial flexure strength and low temperature degradation of Ce-TZP/Al₂O₃ nanocomposite and Y-TZP as dental restoratives *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **87**(2) 492-498 DOI: 10.1002/jbm.b.31131.
- Papanagiotou HP, Morgano SM, Giordano RA, & Pober R (2006) In vitro evaluation of low-temperature aging effects and finishing procedures on the flexural strength and structural stability of Y-TZP dental ceramics *Journal of Prosthetic Dentistry* **96**(3) 154-164 DOI: 10.1016/j.prosdent.2006.08.004.
- Lughi V, & Sergo V (2010) Low temperature degradation "aging" of zirconia: A critical review of the relevant aspects in dentistry *Dental Materials* **26**(8) 807-820 DOI: 10.1016/j.dental.2010.04.006.
- Auschill TM, Arweiler NB, Netuschil L, Brex M, Reich E, & Sculean A (2001) Spatial distribution of vital and dead microorganisms in dental biofilms *Archives of Oral Biology* **46**(5) 471-476.
- Azevedo SM, Kantorski KZ, Valandro LF, Bottino MA, & Pavanelli CA (2012) Effect of brushing with conventional versus whitening dentifrices on surface roughness and biofilm formation of dental ceramics *General Dentistry* **60**(3) e123-e130.
- Brentel AS, Kantorski KZ, Valandro LF, Fucio SB, Puppini-Rontani RM, & Bottino MA (2011) Confocal laser microscopic analysis of biofilm on newer feldspar ceramic *Operative Dentistry* **36**(1) 43-51 DOI: 10.2341/10-093-LR.
- Kantorski KZ, Scotti R, Valandro LF, Bottino MA, Koga-Ito CY, & Jorge AO (2009) Surface roughness and bacterial adherence to resin composites and ceramics *Oral Health and Preventive Dentistry* **7**(1) 29-32.
- Scotti R, Kantorski KZ, Monaco C, Valandro LF, Ciocca L, & Bottino MA (2007) SEM evaluation of in situ early bacterial colonization on a Y-TZP ceramic: A pilot study *International Journal of Prosthodontics* **20**(4) 419-422.
- Axelsson P, & Lindhe J (1978) Effect of controlled oral hygiene procedures on caries and periodontal disease in adults *Journal of Clinical Periodontology* **5**(2) 133-151.
- Quirynen M (1994) The clinical meaning of the surface roughness and the surface free energy of intra-oral hard substrata on the microbiology of the supra- and subgingival plaque: Results of in vitro and in vivo experiments *Journal of Dentistry* **22**(Supplement 1) S13-S16.
- Nassar U, Meyer AE, Ogle RE, & Baier RE (1995) The effect of restorative and prosthetic materials on dental plaque *Periodontology 2000* **8** 114-124.
- Quirynen M, & Bollen CM (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man: A review of the literature *Journal of Clinical Periodontology* **22**(1) 1-14.
- Castellani D, Bechelli C, Tiscione E, Lo Nostro A, & Pierleoni PP (1996) In vivo plaque formation on cast ceramic (Dicor) and conventional ceramic *International Journal of Prosthodontics* **9**(5) 459-465.
- Kantorski KZ, Scotti R, Valandro LF, Bottino MA, Koga-Ito CY, & Jorge AO (2008) Adherence of *Streptococcus mutans* to uncoated and saliva-coated glass-ceramics and composites *General Dentistry* **56**(7) 740-747 quiz 748-749, 768.
- Pereira GK, Amaral M, Simoneti R, Rocha GC, Cesar PF, & Valandro LF (2014) Effect of grinding with diamond-disc and -bur on the mechanical behavior of a Y-TZP ceramic *Journal of the Mechanical Behavior of Biomedical Materials* **37** 133-140 DOI: 10.1016/j.jmbbm.2014.05.010.
- Chevalier J, Cales B, & Drouin JM (1999) Low-temperature aging of Y-TZP ceramics *Journal of the American Ceramic Society* **82**(8) 2150-2154.
- Garvie RC, & Nicholson PS (1972) Phase analysis in Zirconia systems *Journal of the American Ceramic Society*. **55**(6) 303-305.
- Toraya H, Yoshimura M, & Somya S (1984) Calibration curve for quantitative analysis of the monoclinic tetragonal ZrO₂ system by X-ray diffraction *Journal of the American Ceramic Society* **67**(6) 119-121.

30. Kosmac T, Wagner R, & Claussen N (1981) X-ray determination of transformation depths in ceramics containing tetragonal ZrO₂ *Journal of the American Ceramic Society* **64**(4) c72-c73.
31. Standardization IOF (1997) ISO 4287 Geometrical Product Specifications (GPS)—Surface texture: Profile method, terms, definitions and surface texture parameters. Geneva: International Organization for Standardization.
32. Venturini AB, Prochnow C, Rambo D, Gundel A, & Valandro LF Effect of hydrofluoric acid concentration on resin adhesion to a feldspathic ceramic *Journal of Adhesive Dentistry* **17**(4) 313-320.
33. Exterkate RA, Crielaard W, & Ten Cate JM (2010) Different response to amine fluoride by *Streptococcus mutans* and polymicrobial biofilms in a novel high-throughput active attachment model *Caries Research* **44**(4) 372-379 DOI: 10.1159/000316541.
34. McBain AJ, Sissons C, Ledder RG, Sreenivasan PK, De Vizio W, & Gilbert P (2005) Development and characterization of a simple perfused oral microcosm *Journal of Applied Microbiology* **98**(3) 624-634 DOI: 10.1111/j.1365-2672.2004.02483.x.
35. Amaral M, Valandro LF, Bottino MA, & Souza RO (2013) Low-temperature degradation of a Y-TZP ceramic after surface treatments *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **101**(8) 1387-1392 DOI: 10.1002/jbm.b.32957.
36. Pereira GK, Amaral M, Simoneti R, Rocha GC, Cesar PF, & Valandro LF (2015) Effect of grinding with diamond-disc and -bur on the mechanical behavior of a Y-TZP ceramic *Journal of the Mechanical Behavior of Biomedical Materials* **37** 133-140.
37. Muñoz-Tabares JA (2012) Hydrothermal degradation of ground 3Y-TZP *Journal of the European Ceramic Society* **32**(2) 325-333.
38. Kim HT, Han JS, Yang JH, Lee JB, & Kim SH (2009) The effect of low temperature aging on the mechanical property & phase stability of Y-TZP ceramics *Journal of Advanced Prosthodontics* **1**(3) 113-117 DOI: 10.4047/jap.2009.1.3.113.
39. Pereira GK, Venturini AB, Silvestri T, Dapieve KS, Montagner AF, Soares FZ, & Valandro LF (2015) Low-temperature degradation of Y-TZP ceramics: A systematic review and meta-analysis *Journal of the Mechanical Behavior of Biomedical Materials* **55** 151-163.
40. Deville S, Chevalier J, & Gremillard L (2006) Influence of surface finish and residual stresses on the ageing sensitivity of biomedical grade zirconia *Biomaterials* **27**(10) 2186-2192.
41. Teughels W, Van Assche N, Sliepen I, & Quirynen M (2006) Effect of material characteristics and/or surface topography on biofilm development *Clinical Oral Implants Research* **17**(Supplement 2) 68-81.
42. Song F, Koo H, & Ren D (2015) Effects of material properties on bacterial adhesion and biofilm formation *Journal of Dental Research* **94**(8) 1027-1034.
43. Quirynen M, Van der Mei HC, Bollen CM, Van den Bossche LH, Doornbusch GI, van Steenberghe D, & Busscher HJ (1994) The influence of surface-free energy on supra- and subgingival plaque microbiology: An in vivo study on implants *Journal of Periodontology* **65**(2) 162-167 DOI: 10.1902/jop.1994.65.2.162.
44. Al-Radha AS, Dymock D, Younes C, & O'Sullivan D (2012) Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion *Journal of Dentistry* **40**(2) 146-153.
45. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, & van Steenberghe D (1990) The influence of surface free energy and surface roughness on early plaque formation: An in vivo study in man *Journal of Clinical Periodontology* **17**(3) 138-144.
46. Anselme K, Davidson P, Popa AM, Giazson M, Liley M, & Ploux L (2010) The interaction of cells and bacteria with surfaces structured at the nanometre scale *Acta Biomaterialia* **6**(10) 3824-3846.
47. Bollen CM, Lambrechts P, & Quirynen M (1997) Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature *Dental Materials* **13**(4) 258-269.
48. Hahnel S, Rosentritt M, Handel G, & Burgers R (2009) Surface characterization of dental ceramics and initial streptococcal adhesion in vitro *Dental Materials* **25**(8) 969-975.
49. Rimondini L, Cerroni L, Carrassi A, & Torricelli P (2002) Bacterial colonization of zirconia ceramic surfaces: An in vitro and in vivo study *International Journal of Oral and Maxillofacial Implants* **17**(6) 793-798.
50. Hojo K, Nagaoka S, Ohshima T, & Maeda N (2009) Bacterial interactions in dental biofilm development *Journal of Dental Research* **88**(11) 982-990.
51. Rashid H (2014) The effect of surface roughness on ceramics used in dentistry: A review of literature *European Journal of Dentistry* **8**(4) 571-579.