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Original Article

Surface topography and bacterial adhesion of CAD/CAM resin based materials after application of different surface finishing techniques

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Aim: This study evaluated the surface topography and bacterial adhesion of a hybrid ceramic and a nano ceramic resin composite after different surface finishes. **Methods:** Hybrid ceramic (Vita Enamic, VITA - EN) and nano ceramic resin composite (Lava Ultimate, 3M/ESPE - LV) blocks of 12 x 14 x 18 mm were cut into 1 mm slices. Each slice was divided into four specimens (6 x 7 mm) that were randomly allocated into 4 groups (n=8) according to the surface finishing: CTL - without surface finish (control); DB - wear with a diamond bur; VT - polishing system for hybrid ceramic (VITA); and DD - polishing system for ceramics (Dedeco). The specimens were analyzed regarding surface roughness parameters (Ra, Rz, Rq), sterilized and subjected to bacterial adhesion. Representative specimens from each group were observed by SEM and Confocal Laser Scanning Microscopy. Data were submitted to two-way ANOVA and Tukey's test (α=0.05). **Results:** EN had lower surface roughness and bacterial adhesion than LV ($p<0.05$), regardless of the surface finish. The highest values for all roughness parameters was observed in LVDB group, differing from the other groups, which were not significantly different. Smaller bacterial adhesion values (CFU/mL) were observed for ENDD and ENVT, which differed significantly from the other groups, except ENCTL. For LV groups there was no significant difference between the different surface finishes ($p>0.05$). The type of material and surface finish system significantly interfered with surface roughness parameters and bacterial adhesion. The hybrid ceramic performed better after polishing than the nano-ceramic resin. **Conclusion:** An adequate finishing/polishing technique should always be performed after any kind of adjustment to indirect restorations made with these materials tested.

Keywords: bacterial adhesion, ceramics, composite resins, dental polishing.

INTRODUCTION

All exposed surfaces in the oral cavity are coated with a salivary pellicle that enables microbial adhesion and may cause damage to teeth and restorative materials[1]. Failures of all-ceramic restorations are due to several factors, such as fracture of the restoration, marginal discoloration, and secondary caries[2]. Particularly, discoloration and secondary caries involve cariogenic microorganisms[3].

Ceramic restorations with rough surface finishes may result in increased wear of the antagonist teeth and bacterial adhesion; they can also lead to tooth decay and periodontal disease[4]. In contrast, well-polished restorations show less wear on the opposing tooth, lower bacterial adhesion, improved color stability, and suitable optical properties[5].

Thus, the final surface polishing in ceramic restorations should ideally remain intact. However, in some cases, it is necessary to make adjustments to ceramics; this requires new polishing procedures. Some studies have found that manual final finishing has better clinical performance than glaze in regards to surface roughness and shade matching[6,7].

While a wide variety of restorative materials are available, new materials compatible with dental structure and function are still necessary. Ceramics have several advantages, such as a high flexural strength and excellent color stability; however, they also have disadvantages, such as opposing tooth wear (when ceramics are not properly polished) and requiring a minimum thickness to prevent fracture[8].

Hybrid materials, such as those based on a ceramic network infiltrated with polymers and resins with ceramic nanoparticles, have been developed to minimize the disadvantages of ceramics. These materials exhibit promising characteristics, such as mechanical properties similar to teeth, such as elastic module similar to dentin[9]. They can be applied in thin layers but are still strong enough to prevent material cracks. These new materials are applicable for minimally invasive restorations, the treatment of young patients, patients suffering from hereditary diseases (i.e., imperfect amelogenesis), and patients suffering from bruxism and dental erosion[10].

VITA Enamic® is a hybrid material with a combination of ceramic and polymeric properties. It is a ceramic network infiltrated with resin. The filler particles (silica based) confer optical characteristics, while the monomers (i.e., Bis-GMA, UDMA, UTMA, and Bis-EMA) determine the organic contents. This material is manufactured in two steps: first, a network of pre-sintered ceramics is produced and conditioned by a binding agent, and then, the network is infiltrated with a polymer by capillary action[11].

Lava Ultimate® is a nano-ceramic resin based material composed of about 80% nano-ceramic particles that form a nano-cluster of silica and zirconia, which are wrapped in a highly polymerized polymer matrix[12].

There is no standard protocol for finishing ceramic restorations and there have been limited studies on the effects of different finishing techniques on the surface topography and bacterial adhesion of these new hybrid materials[13]. Thus, the objectives of this study were to evaluate the surface topography and bacterial adhesion of a hybrid ceramic and a nano-ceramic resin after different surface finishing techniques.

The null hypotheses of this study are: there is no difference in surface roughness or bacterial adhesion between materials, and there is no difference in surface roughness or bacterial adhesion among finishing techniques.

MATERIAL AND METHODS

Specimen preparation

Blocks (12 × 14 × 18 mm) of hybrid ceramic VITA Enamic® (VITA Zahnfabrik, Bad Säckingen, Germany) and nano-ceramic resin Lava Ultimate® (3M ESPE, St. Paul, MN, USA) were purchased. The blocks were sectioned (IsoMet 1000, Buehler, Lake Buff, USA) into eight 1-mm slices, and each slice was divided into 4 specimens (6 x 7 mm). The specimens were randomly assigned into 4 surface finishing technique groups (n = 8): CTL - no finish (control); DB - worn with diamond bur (#3203, JOTA, Ruthi, Switzerland); VT - polished using hybrid ceramic system (VITA Zahnfabrik, Bad Säckingen, Germany); and DD - polished using ceramic system (Dedeco International Inc, Long Eddy, NY, USA).

The specimens were placed on a glass slide fixed with wax, and the finishing techniques were performed using a high-speed turbine with diamond bur (#3203, JOTA) for the DB group, or a straight hand piece for the VT and DD groups. All instruments were intermittently placed on the specimen surface in one direction for 10 s. The same operator performed all procedures. Surfaces were gently washed and dried after completing the surface finishing procedures.

Surface roughness

Eight specimens from each group were gently dried with absorbent paper and fixed to a glass slide with wax. Surface roughness was analyzed with a surface roughness-measuring instrument (Surftest SJ-210P, Mitutoyo, Tokyo, Japan) equipped with a 2-μm radius diamond needle. The needle moved at a constant speed (0.5 mm/s) under a 0.7 mN load. To maximize filtration of surface waviness, three readings were recorded for each specimen at different parallel positions (2 mm apart) with a length of 2.5 mm and a cut-off of 0.25 mm. The average of the three readings was used as the roughness value for each specimen.

In this study, it was assessed three roughness parameters: average roughness (Ra), the arithmetical average value of all absolute distances of the roughness profile from the centerline within the measuring length the average of peaks and valleys recorded in each sampling length (Rz), and the effect of the profile values that deviate from the average (Rq). Means and standard deviations of Ra, Rz, and Rq were calculated.

After the analysis of surface roughness, specimens were sterilized in ethylene oxide under the following conditions: EtO, 500-750 mg/L; temperature, 50-60°C, humidity 40-90%, 180 minutes exposure time over 4 hours of incubation.

Bacterial adhesion

A 3 mL suspension of *Streptococcus mutans* (ATCC 35688), adjusted to the MacFarland scale #2 (~6×10^8 cells/mL), was sequentially diluted by the addition of sterile physiological solution (0.9% sodium chloride). The number of cells in suspension was measured via spectrophotometry (UV-VIS Double beam with scanning, QUIMIS, Curitiba, Brazil); specifically, optical density (0.135 nm) and wavelength (0.303 nm) were quantified. Colony forming units (CFU) were calculated against reference standards (R^2 = 0.9214).

Bacterial adhesion was performed in an aseptic environment in a laminar airflow chamber. Specimens from each group received 20 µL of standard *S. mutans* suspension on the center of their surface and incubated at 37°C for 1 hour. The specimens were removed, rinsed twice with sterile distilled water to remove materials with low adhesion, placed in individual tubes with 1.5 mL of sterile saline (0.9% NaCl), and sonicated in a vortex mixer (Vortex mixer, Quimis, Diadema, SP, Brazil) for 30 seconds to disperse the adhered bacteria. Samples (1 mL) were measured on the spectrophotometer to obtain average CFU/mL values.

Scanning electron microscopy (SEM) analysis

One specimen from each group was subjected to bacterial adhesion analysis by scanning electron microscopy. The specimens were fixed in 10% formaldehyde for 1 h, dehydrated in successive baths of increasing concentrations of ethanol, and dried in a bacteriological incubator at 37°C for 24 hours. The specimens were then mounted on aluminum stubs using a copper tape and coated with gold. Surface topography was observed and photographed using a scanning electron microscope (Feg Quanta 450, FEI, Oregon, USA) operating at 15 kV with 1000 and 5000 magnification.

Confocal laser scanning microcopy (CLSM) analysis

Three samples from each group were removed from the growth medium and stained for Live/Dead BacLight bacterial viability and cell counting using two fluorescent nucleic acid dyes: SYTO 9, which penetrates bacterial membranes and stains cells green; and isopropidium iodide, which penetrates cells with damaged membranes and stain cells red. The dyes were each diluted in sterile saline (0.9% NaCl) in an opaque container at a ratio of 10 mL of saline solution per 4 µL of dye. They were then placed on each specimen and incubated for 15 minutes under light protection. Finally, the specimens were washed with sterile saline solution to remove excess dye and non-adherent bacteria.

The surface of each sample was analyzed by CLSM (Nikon Eclipse Ti, Curitiba, Brazil) using excitation wavelengths of 488 nm and 535 nm for SYTO 9 and isopropidium iodide, respectively, and light emission between 500 and 560 nm. Samples were observed via optical lenses at 20X and 63X magnification. HR NIS-Elements software was used to check for viable bacteria on the surface.

Three images in different areas were obtained via CLSM for each group. Since it was not possible to quantify the biofilm volume or thickness in this study, a score was established for each image according to the presence of viable bacteria (stained green): no viable bacteria in the entire image (Score of 0); presence of viable bacteria in up to 25% of the image area (Score of 1); presence of viable bacteria in 50% of the image area (Score of 2); presence of viable bacteria by more than 50% of the image area (Score of 3), and; presence of viable bacteria covering the entire image area (Score of 4).

All images were evaluated by a qualified examiner twice (7-days apart). The Kappa test score was 0.864 ($p<0.001$), indicating excellent agreement between duplicate examinations.

Statistical analysis

Data were analyzed using the Statistica software version 10.0. The homogeneity of variances and normal distribution of the data was checked by Kolmogorov-Smirnov and Levene tests. The dependent variables of the study were as follows: surface roughness (continuously quantitative), bacterial adhesion (continuously quantitative), and viable bacteria (categorically qualitative). The independent variables in the study were the different materials (i.e., hybrid ceramic (VITA Enamic - EN) and nano ceramic resin (Lava Ultimate - LV)) and the different surface finishing techniques (i.e., CTL, DB, VT, and DD).

Roughness and bacterial adhesion data were analyzed using analysis of variance (two-way ANOVA) considering the factors "material" and "surface finish", as well as the Tukey test ($\alpha = 0.05$).

The scores for viable bacteria were compared among groups using the chi-square test (5% significance level).

RESULTS

Surface roughness

The mean surface roughness values ranged from 0.13 to 1.45 µm, 0.83 to 7.59 µm, and 0.17 to 1.84 µm for Ra, Rz, and Rq, respectively (Table 1). Analysis of variance (two-way ANOVA) showed a statistically significant difference for the factors "material" ($p = .021$), "finish" ($p = .000000$), and interaction "material*finish" ($p = .0000001$) for all roughness parameters (Ra, Rz and Rq).

Table 1. Mean and standard deviation of roughness (µm) and bacterial adhesion (CFU/mL) for the different groups (n=8).

Material	Finish	Ra (µm)	Rz (µm)	Rq (µm)	Bacterial adhesion (UFC/mL)
Vita Enamic (EN)	CTL	0.32 (±0.12) a	2.21 (±0.79) a	0.42 (±0.16) a	2.40×10^8 ($\pm 2.5 \times 10^7$) ab
	DB	0.48 (±0.16) a	2.85 (±0.68) a	0.61 (±0.18) a	2.58×10^8 ($\pm 2.9 \times 10^7$) b
	VT	0.31 (±0.15) a	1.75 (±0.71) a	0.39 (±0.18) a	2.22×10^8 ($\pm 2.0 \times 10^6$) a
	DD	0.16 (±0.03) a	1.12 (±0.16) a	0.22 (±0.03) a	2.20×10^8 ($\pm 2.0 \times 10^6$) a
Lava Ultimate (LV)	CTL	0.23 (±0.03) a	1.68 ± (0.24) a	0.31 (±0.04) a	2.60×10^8 ($\pm 1.7 \times 10^7$) b
	DB	1.45 (±0.78) b	7.59 (±3.97) b	1.84 (±0.95) b	2.73×10^8 ($\pm 4.0 \times 10^6$) b
	VT	0.13 (±0.03) a	0.83 (±0.21) a	0.17 (±0.04) a	2.70×10^8 ($\pm 1.0 \times 10^6$) b
	DD	0.15 (±0.02) a	0.91 (±0.12) a	0.18 (±0.03) a	2.70×10^8 ($\pm 1.0 \times 10^6$) b

* Values followed by the same letters are statistically similar ($p>0,05$).

According to Tukey's test, the greatest roughness parameter value was observed for the LVDB group, where the nano-ceramic resin (LV) was ground with a diamond bur (DB); this value was different from the other groups, which were not significantly different from each other.

A significant difference was observed for "material" in regards to Ra ($p = .021$). The hybrid ceramic (EN) had a lower average surface roughness (Ra) than the nano-ceramic resin (LV) regardless of surface finishing. There was significant difference in "finish" between groups ($p = .0000001$). The mean values of surface roughness (Ra) for the different treatments were as follows: DD = VT = CTL < DB. The groups treated with diamond bur (DB) showed higher average surface roughness (Ra) compared with other groups, which did not differ regardless of the material (i.e., hybrid ceramic (EN) or nano ceramic resin (LV)).

Bacterial adhesion

Analysis of variance (two-way ANOVA) showed statistically significant differences for the factors "material" ($p = .000001$), "finish" ($p = .034$), and the interaction "material*finish" ($p = .043$) (Table 2).

Lower bacterial adhesion (CFU/mL) was observed for the polished hybrid ceramic (ENDD and ENVT) than the other experimental groups; these two groups had levels similar to the control group (ENCTL). There was no significant difference between surface finish for the nano-ceramic resin groups.

When the factors were considered individually, there was significant difference for the factor "material" ($p = .00001$). The hybrid ceramic (EN) showed less bacterial adhesion than the nano-ceramic resin (LV) regardless of surface finish. There was significant difference among groups for the factor "finish" ($p = .034$). The mean values of bacterial adhesion to the groups polished with the Dedeco system (DD) were lower than the groups treated with the diamond bur (DB). The group polished with the vita system (VT) and the control group had intermediate values that were not different from the other groups regardless of the material used (i.e., hybrid ceramic or ceramic nano-resin).

SEM analysis

The SEM images of the materials' surfaces before bacterial adhesion show that the polishing methods produced a regular and smoother surface for both materials.

The SEM images also show that all surfaces of the hybrid ceramic (EN) showed bacterial adhesion with higher cellular accumulation (*S. mutans*) in the regions of higher surface irregularity (Fig. 1). It is also evident that all surfaces of the nano-ceramic resin show similar bacterial adhesion (Fig. 2).

CLSM analysis

There was a statistically significant difference in bacterial viability between the experimental groups (Fig. 3, chi-square = 66.69, $p < 0.001$). There was a similar distribution found for the two materials. All of the group finished with the diamond bur (DB) scored 4 (i.e., the entire image area had viable bacteria). The other groups (i.e., polishing and control) were mostly scored 1 (i.e., less than 25% of viable bacteria in the image). Figure 4 shows representative images of scores found for both materials (ENDB and LVDB – score 4, and ENDD and LVDD – score 1).

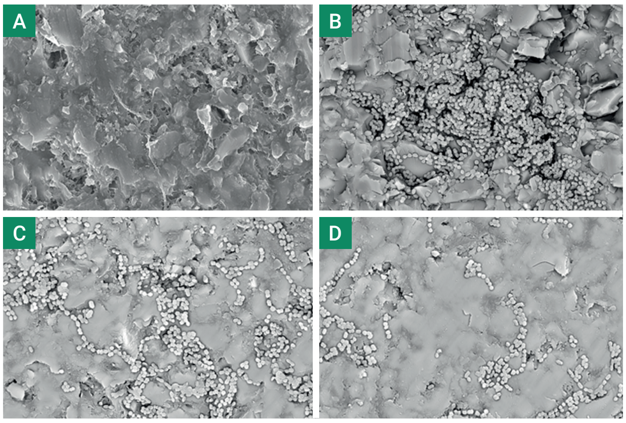

Figure 1. Photomicrographs of the surfaces of hybrid ceramic (EN) after different finishes and bacterial adhesion. Control (A), diamond bur (B), Vita polishing system (C), and Dedeco polishing system (D) (5000 X).

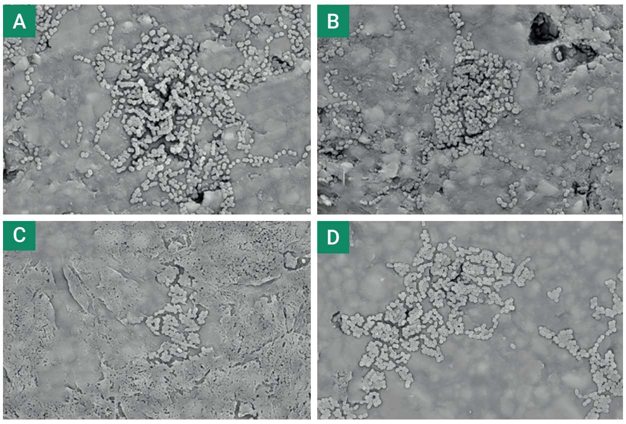

Figure 2. Photomicrographs of surfaces of nano ceramic resin (LV) after different finishes and bacterial adhesion. Control (A), diamond bur (B), Vita polishing system (C), and Dedeco polishing system (D) (5000 X).

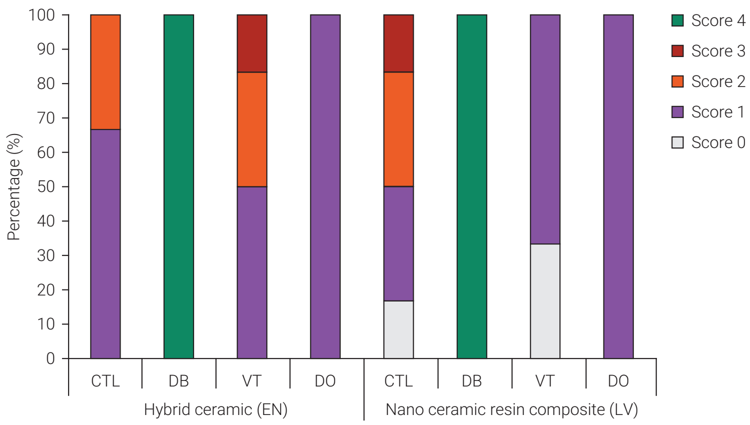

Figure 3. Scores attributed in different groups.

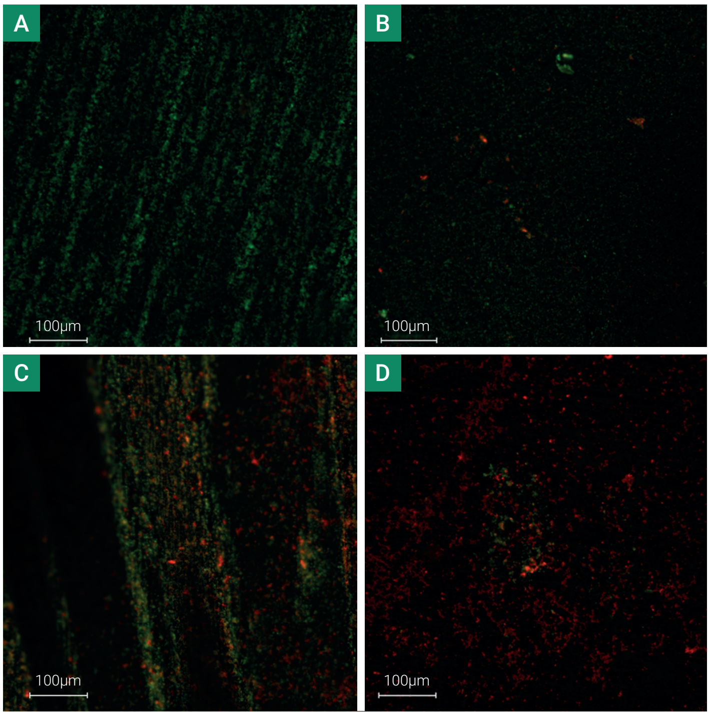

Figure 4. Representative images of scores found for both materials after staining using Live/Dead BacLight bacterial viability and cell counting method. Viable/Live bacteria stained in green and Dead bacteria stained in red. (A) Hybrid ceramic treated with diamond bur (ENDB); (B) Hybrid ceramic polished with Dedeco system (ENDD); (C) Nano ceramic resin composite treated with diamond bur (LVDB); and (D) Nano ceramic resin composite polished with Dedeco system (LVDD).

DISCUSSION

According to the results, the first null hypothesis should be rejected. There was a significant difference between the materials regarding the surface roughness and bacterial adhesion,,the hybrid ceramic (EN) had a lower roughness and lower bacterial adhesion than the nano-ceramic resin (LV). These differences could be related to materials composition and microstructure. VITA Enamic is a hybrid material (ceramic/resin) with a porous three-dimensional structure of feldspathic ceramic infiltrated with resin. Lava Ultimate is a resin composite with nano-zirconia particles, cost effective, easy to repair and is also for machining in CAD/CAM systems[14]. Although both materials include resin, their microstructures differ significantly; this is evident in scanning electron microscopy images (Fig. 1, 2). The flexural strength and modulus of elasticity of these materials are similar to dentin and lower than ceramics; this makes them more suitable for indirect restorations[15].

Other studies have also shown that ceramic has lower roughness and bacterial adhesion than resin composites[16-18]. Awad et al.[19] defined an ascending order of surface roughness for restorative materials: ceramic, feldspathic ceramic, hybrid ceramics, resin-based composites, and polymethylmethacrylate (PMMA). However, Fasbinder and Neiva[20] observed a lower surface roughness in a nano-ceramic resin material than in a hybrid ceramic.

Regarding the finishing techniques, the second null hypothesis should also be rejected. There was also a difference in surface roughness and bacterial adhesion among surface finishing techniques (DB groups presented higher roughness and bacterial adhesion). The superficial characteristics of the materials interfere in the bacterial adhesion, while treatment/polishing also influences the surface roughness and subsequently affects bacterial adhesion[21]. Different polishing materials are available; in this study, two polishing systems were selected: the VITA polishing kit, which was specially developed for Vita Enamic, and; the Dedeco kit, which is used to polish ceramics worldwide. Both kits were used according to the manufacturer's recommendations.

The surfaces of the polished specimens had values similar to the control group for both materials, and there were no differences between the different polishing kits (i.e., DD or VT). The SEM images show that polishing produces a regular and smooth surface on both materials. For the hybrid ceramic (EN), the ceramic polishing system (DD) produced a more regular and smooth surface compared to the other system (VT); this conforms to recommendations by hybrid ceramic manufacturers. Both polishing systems produced regular and smooth surfaces on the nano-ceramic resin (LV).

SEM images revealed that bacteria adhered to the all hybrid ceramic (EN) specimens with the highest biofilm accumulations. *S. mutans* were observed in areas with higher surface irregularities, such as those present in the DB group; in these cases the bacteria were deposited in cracks and depressions present in the material. The group finished with the Dedeco system (DD) also had biofilm accumulation; however, it was at a lower intensity than the other groups. CLSM images showed that, independent of the material, the greatest concentration of viable bacteria was present in the groups finished with the diamond bur (DB). The other groups (i.e., control and polished) had comparable distributions of viable bacteria on the surface. The CLSM images confirmed the distribution of bacteria observed in the SEM images and also enabled analysis of bacterial viability[2,22]. Images from the hybrid ceramic (EN) have higher amounts of green coloring; this indicates that there may be an interaction between the dye (SYTO 9) and the material. Thus, the visualization of bacterial adhesion may be more complex.

According to Bollen et al.[23], the roughness values (Ra) of the intraoral hard tissue should be approximately 0.2 μm or lower to limit bacterial adhesion. It was observed surface roughness values (Ra) close to or smaller than 0.2 μm for both materials except for the samples finished with the diamond bur (DB). Thus, the surfaces subject to polishing techniques are considered clinically acceptable. The highest surface roughness values (Ra, Rz, and Rq) were observed in the nano-ceramic resin (LV) finished with the diamond bur (LVDB); these values differed significantly from the other groups.

The polishing technique, independent of the selected system (i.e., VT or DD), resulted in a more regular and smooth surface with less accumulation of biofilm for the hybrid ceramic (EN) (Fig. 1). Similar data were recently found by Vo et al.[21], who assumed that the surface roughness of a lithium disilicate-based material was a critical factor for *S. mutans* adherence. Thus, an additional polishing of this surface leading to lower surface roughness is expected to reduce bacterial adherence.

Clinically, the superficial characteristics of restorative materials and biofilm adhesion on these surfaces are related to the degradation of these restorative materials, the development of recurrent caries lesions, and gingival inflammation[24]. Thus, bacterial adhesion to restorative materials interferes with clinical performance[23]. Here, we show that the surface characteristics of restorative materials should be taken into consideration during the selection of these materials. For the CAD/CAM resin-based materials tested, if mouth adjustments are necessary using diamond burs or after CAD/CAM procedures, the use of specific systems for finishing and polishing of indirect restorations is required[7].

In conclusion, the type of the material and the finishing technique significantly influence surface roughness and bacterial adhesion. The hybrid ceramic performed better after polishing than the nano-ceramic resin. Treatment with a diamond bur increased surface roughness and bacterial adhesion. An adequate finishing/polishing technique should always be performed after any kind of adjustment to indirect restorations made with these materials (hybrid ceramic or nano-ceramic resin).

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