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Laboratory Research

Effects of Manufacturing and Finishing Techniques of Feldspathic Ceramics on Surface Topography, Biofilm Formation, and Cell Viability for Human Gingival Fibroblasts

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Clinical Relevance

For both stratified and machined porcelains, polishing as a finishing technique promotes smoother surfaces with higher surface free energy and decreasing microorganism adhesion.

SUMMARY

Purpose: Feldspathic ceramic restorations can be obtained by different techniques (stratification or computer-aided design/computer-aided manufacturing [CAD/CAM] blocks) and finishing procedures (polishing or glaze application). This study evaluated the effects of techniques and finishing procedures on surface properties, biofilm formation, and viability of human gingival fibroblasts (FMM-1) in contact with these materials.

Methods and Materials: Ceramic specimens were obtained through a stratification technique (Vita VM9) and from CAD/CAM blocks (Vita Blocs Mark II; both Vita Zahnfabrik) and

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their surfaces were finished by polishing (ceramisté diamond rubbers + polishing paste; "p" subgroups) or glaze spray application + sintering ("g" subgroups). Roughness (Ra and RSm parameters) and surface free energy (SFE) were measured. Early biofilm formation of *Streptococcus mutans*, *Streptococcus sanguinis*, and *Candida albicans* was evaluated by counting colony-forming units (CFU). MTT (3-[4.5-dimethyl-thiazol-2-yl-]-2.5-diphenyl tetrazolium bromide) cytotoxicity test evaluated cellular viability for the growth of FMM-1 after 24 hours and seven days of contact. Scanning electron microscopy (SEM) and three-dimensional optical profilometry were performed to qualitatively analyze the surface. Data were analyzed by analysis of variance, Tukey test, and *t*-test (all α=0.05).

Results: Polished samples presented lower roughness (Ra, p=0.015; RSm, p=0.049) and higher SFE (p=0.00). *Streptococci* had higher CFU in all groups, but the CFU of *C albicans* was lower for polished samples. Biofilm formation was influenced by the interaction of all factors (p=0.018), and the materials showed no cytotoxicity to FMM-1 growth.

Conclusions: Polishing resulted in the lowest values for surface roughness and higher SFE values. Polished ceramics showed less *C albicans* adherence while the adherence of *Streptococci* was greater than *C albicans* in all conditions.

INTRODUCTION

Feldspathic ceramics can be used to obtain partial or full restorations and for veneering metal or ceramic substructures. These restorations can be prepared by various techniques, such as conventional stratification or the computer-aided design/computer-aided manufacturing (CAD/CAM) technique, in which restorations are obtained from milling ceramic blocks. The final product may have different properties, such as porosity[1] and residual stress and grain size,[2] which influence the physical characteristics of the restorations.

Different particle sizes, porosity, and impurities present in the material can influence the surface characteristics that are in intimate contact with the periodontal tissues. For the success of rehabilitation treatment, it is essential to know the behavior of the restorative materials in relation to the microbiology of the oral environment.[3]

There is no consensus about the best surface treatment to obtain smooth feldspathic surfaces. Many ceramists advocate polishing rather than glazing, to better control the surface gloss of ceramic restorations.[4-7] There is little information about CAD/CAM ceramics.[8] Some authors have found that the initial softness of a glazed surface was superior than a polished surface.[9,10] Roughness and surface free energy (SFE) have important roles in bacterial adhesion[8-13] and consequent early biofilm formation.[14-16] Interactions between bacteria and the surfaces of the prosthetic device are thought to be based on a variety of forces, including the Lifshitz–van der Waals, electrostatic, and hydrophobic forces and various specific receptor-ligand interactions.[17] However, other studies have reported greater adherence of microorganisms on hydrophobic surfaces[18,19] and divergent results in which no significant relationship was observed between SFE and microbial adhesion are also described in the literature.[20,21] The bacterial community in the oral environment is very broad, and *in vitro* studies have revealed that interaction of *Streptococcus mutans* and *Candida albicans* may favor the formation of heterotypic biofilm.[12]

The biocompatibility of feldspathic ceramics is well accepted by dentists.[23] However, there are not enough studies on how CAD/CAM ceramics can influence fibroblast cells. These cells are responsible for creating soft tissues that can affect the esthetic result. In studies, the CAD block feldspathic ceramics demonstrated a significant decrease in cell viability in this type of cells.[24]

The good relation between feldspathic ceramic and fibroblast cells is due to Al^{+3} and K^{+} ions released upon degradation of feldspathic ceramics, which leads to lower cytotoxicity.[23] However, information on surface characteristics and formation of biofilms on materials is scarce, particularly with regard to the impact of intraoral polishing and the new spray glaze with respect to bacterial adherence and cellular viability.[8] Therefore, this study aimed to evaluate the oral biofilm formation and human gingival fibroblasts (FMM-1) viability on the surface of two feldspathic ceramics manufactured and finished by different techniques.

METHODS AND MATERIALS

Preparation of the Specimens

Vita Mark II blocks (Vita Zahnfabrik, Bad Säckingen, Germany, batch 34700) were cut into smaller blocks (4.5 × 4.5 × 1.5 mm) with a diamond disk under constant water cooling using a cutting machine. Vita VM9 stratified ceramic powder and

modeling liquid (Vita Zahnfabrik, batch 41810) were mixed following the manufacturer's recommendations and applied into a matrix. The excess liquid was removed, and the blocks were sintered in Vacumat 6000 MP (Vita Zahnfabrik). All samples were polished until reaching standardized dimensions (4.5 × 4.5 × 1.5 mm) in an automatic polisher (EcoMet/Auto Met 250, Buehler, IL, USA) with granulated sandpaper decreasing up to No. 1200 (30 seconds per sandpaper) and under water cooling. The samples were cleaned in an ultrasonic bath with isopropyl alcohol. Half of the samples received a layer of Vita AKZENT Plus–Glaze LT Spray ("g" subgroups; Vita Zahnfabrik, batch 36300). The other half was polished with Ceramisté PM ("p" subgroups; Standard, Ultra and Ultra II, Shofu Kyoto, Japan, batches 0511125, 0113203, 0113203) in three steps (standard for finishing, ultra for polishing, and ultra II for superpolishing) and then followed by diamond paste (Diamond Excel, FGM, Joinville, Brazil, batch 130616) on a felt disc (Diamond, FGM, batch 231115) using a handpiece at slow speed, for 20 seconds each. Next, the specimens were randomly divided into four groups according to the "manufacture technique" (stratification technique [ST] or CC [CAD/CAM]) and "surface finishing treatment" (p=polished or g=glazed).

Surface Roughness

For an effective evaluation of the roughness, measurement of more than one roughness parameter is recommended.[25] Ra (average roughness) and RSm (mean width of the roughness profile elements) parameters were evaluated by a surface roughness tester (SJ 400, Mitutoyo, Tokyo, Japan). A mean value was obtained for each sample (n=20) from three readings performed at the center of the samples at 0.2 mm/s speed, following ISO 4287-1997, with a Gaussian filter and a cutoff value of 0.8 mm.

Surface Free Energy

For SFE, larger samples (14 × 12 × 1.5 mm) of each group were obtained as previously described. A tensiometer (TL 1000, One Attension, Lichfield, Staffordshire, UK) was used to measure the mean contact angle in five distinct areas of each sample with the sessile drop technique, using two different liquids: water (polar liquid) and diiodomethane (dispersive liquid). The SFE was calculated using the method proposed by Owens and Wendt[26] along with the harmonic average equation (1). With the cosine of the contact angle for each liquid, we can calculate the components of surface energy of a solid surface. Replacing the known values of the liquids, starting with diiodomethane values (γ^L=50,811, $\gamma^L{}_P$=0, $\gamma^L{}_d$=50) then, water values (γ^L=72.8, $\gamma^L{}_P$=51, $\gamma^L{}_d$=21.8)[27] to obtain the γ^Sd and γ^Sp values. The SFE of the solid can be calculated by equation 2.

$$\gamma^L \cdot [\cos(\theta) + 1] = \frac{4\gamma_d^S \cdot \gamma_d^L}{\gamma_d^S + \gamma_d^L} + \frac{4\gamma_p^S \cdot \gamma_p^L}{\gamma_p^S + \gamma_p^L} \quad (1)$$

$$\gamma^{\mathbf{T}} = \gamma_{\mathbf{d}}^{\mathbf{S}} + \gamma_{\mathbf{p}}^{\mathbf{S}} \quad (2)$$

where $\gamma^{\mathbf{T}}$ corresponds to the SFE and γ_p and γ_d correspond to the polar and dispersive components, respectively.

Colony-Forming Units

Specimens were sterilized in a flow chamber under ultraviolet light (15 minutes each side). The biofilms were composed of standard strains of *Streptococcus mutans* (ATCC 35688), *Streptococcus sanguinis* (ATCC 10556), and *Candida albicans* (ATCC 18804). Standard suspensions were suspended in sterile physiological solution (0.9% NaCl), and the number of cells was calculated through a spectrophotometer (B582, Micronal, Sao Paulo, Brazil). Each sample was individually distributed on each plate from a 96-well sterile plate (Costar Corning, Corning, NY, USA) containing 150 μL of sterile BHI broth supplemented with 1% sucrose and 16.5 μL standardized suspension of each microorganism: *S mutans*, *S sanguinis*, and *C albicans*. Then, the plates were incubated at 37°C in a CO_2 chamber for 48 hours. Each specimen was washed by replacing 200 μL of the broth from each well with sterile physiological solution, and the plates were shaken for 5 minutes (Orbital Shaker, Solab, Piracicaba, Brazil). Samples of each group were individually transferred to falcon tubes with 10 mL of sterile saline solution (NaCl 0.9%) and were sonicated (Sonoplus HD 2200, 50 W, Bandelin Eletronic, Berlin, Germany) for 30 seconds to remove loose bacteria from the surface. Aliquots with dilutions of 10^{-1} to 10^{-4} were performed until *C albicans* was seeded at 10^{-3} and *Streptococcus* at 10^{-4}. Next, the suspensions were seeded in duplicate in Petri plates with a selective medium for each microorganism and then were incubated at 37°C for 16 hours. The plates containing colonies were counted, and the number of colony-forming units per milliliter (CFU/mL) was determined and transformed to log10.

Table 1: *Mean ± Standard Deviation (SD), Coefficient of Variance (CV), and Homogeneous Groups From Tukey Test of the Roughness Data According to the Ra and RSm Parameters*[a]

Groups	Ra		RSm	
	Mean ± SD, μm	CV, %	Mean ± SD, μm	CV, %
CCp	0.3 ± 0.1^{C}	43.3	108.9 ± 38.9^{B}	35.7
CCg	1.6 ± 0.7^{B}	19.2	168.6 ± 44.0^{A}	26.1
STp	0.3 ± 0.2^{C}	59.7	123.3 ± 64.5^{B}	52.3
STg	2.0 ± 0.7^{A}	35.6	135.8 ± 59.3^{AB}	43.7

Abbreviations: CCp, CAD/CAM and polished surface; CCg, CAD/CAM and glazed surface; STp, stratification technique and polished surface; STg, stratification technique and polished surface.
[a] *Different uppercase letters represent statistically significant difference within the same parameter (column).*

Cytotoxicity Assay

Human gingival fibroblasts (FMM-1) were cultured in Dulbecco's Modified Eagle Medium (DMEM high glucose, GlutaMAXTMSupplement, pyruvate, Gibco, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin streptomycin (LGC, Cotia, Brazil). Two sterilized samples per well (24-well plate) were placed with 1 mL of the suspensions containing 2×10^4 cells. The plates were incubated at 37°C in a humid atmosphere containing 5% CO_2 for 24 hours or seven days. After these periods, cell viability was measured by MTT assay (3-[4.5-dimethyl-thiazol-2-yl-]-2.5-diphenyl tetrazolium bromide; Sigma, St Louis, MO, USA). The supernatant from each well was discarded, and the ceramics were removed from the wells. The cell monolayers at the bottom of the wells were washed with 500 μL of phosphate-buffered saline (PBS) and 500 μL of MTT (0.5 mg/mL PBS) solution was applied to each well. The plates were incubated for one hour at 37°C in a completely dark environment. Then, the supernatant was discarded, and the wells were washed with 500 μL PBS. Next, 500 μL of DMSO was added, and the plates were incubated for 10 minutes and shook on an orbital table for 10 minutes. Then, 100 μL of the supernatant from each well was removed for further optical density reading in a spectrophotometer using 570-nm wavelength (EL808IU, Biotek, Winooski, VT, USA). Cell viability was expressed as a percentage of the control (=100%) that remained on plates without ceramic material, according to the International Organization of Standardization 10993-5:1999.[28]

Qualitative Analysis

Sample topography was evaluated by scanning electron microscopy (SEM) and optical profilometry. SEM (Inspect S50, FEI Company, Brno, Czech Republic) was performed on sterile and contaminated samples and on samples with FMM-1 cells after each evaluation period. Specimens with oral biofilm or cells were fixed in 2.5% glutaraldehyde solution for one hour and dehydrated in increasing series of ethyl alcohol baths for 30 minutes (10%, 25%, 50%, 75%, 80%, 90%, and 100%). Then, specimens were metallized with gold alloy (Emitech SC 7620, Sputter Coater, Laughton, UK) and evaluated at a magnitude of 2000× to 3000×. Profilometry was evaluated on two sterile samples from each group in the area of 301.3 × 229.2 μm (Wyko, NT 1100, Veeco, Tucson, AZ, USA).

Statistical Analysis

Surface roughness (Ra and RSm parameters) and SFE data were analyzed by two-way analysis of variance (ANOVA) followed by Tukey test. CFU and MTT assay data were analyzed by three-way ANOVA followed by Tukey test. MTT assay was also evaluated by *t*-test. All tests were performed with $\alpha = 0.05$.

RESULTS

Surface Roughness

The interaction between "Manufacture Technique × Finishing Treatment" significantly influenced both roughness parameters (Ra, $p=0.015$; RSm, $p=0.049$). Tukey test presented glazed groups showing higher mean values of Ra and RSm parameters. In summary, polished surfaces presented decreased roughness values, suggesting lower but more frequent defects (Table 1).

Surface Free Energy

Surface finishing treatment also influenced SFE ($p=0.00$), and Tukey test demonstrated that the polished groups showed higher SFE (γT). The polar component was higher than the dispersive component for all tested groups (Table 2).

Colony-Forming Units

The interaction among "Manufacture Technique × Finishing Treatment × Microorganisms" statistically influenced the adherence of microorganisms on the materials' surfaces ($p=0.018$). The adherence of *S mutans* and *S sanguinis* ranged from 6.38^{A} to 7.29^{A} CFU/mL(log10), and it was statistically similar among all tested groups and superior than Candida. Glazed samples presented higher adherence of *C albicans* than polished groups, with mean CFU

Table 2: *Mean Contact Angle Values ± Standard Deviation (SD) for Water and Diiodomethane, Dispersive (γ_d) and Polar (γ_p) Components and Respective SFE (γ_T)*[a]

Group	Mean Contact Angle		Components		
	Water Mean ± SD, °	Dioodomethane Mean ± SD, °	γ_d, mN/m	γ_p, mN/m	γ_T, mN/m
CCp	60.8 ± 6	43.1 ± 7	18.5 ± 1	46.6 ± 3	65.1 ± 6[A]
CCg	41.5 ± 6	37.5 ± 5	17.7 ± 3	38.6 ± 3	56.3 ± 4[B]
STp	66.0 ± 5	46.9 ± 5	26.8 ± 3	41.1 ± 2	67.9 ± 4[A]
STg	33.8 ± 6	36.4 ± 3	15.6 ± 3	36.8 ± 3	52.4 ± 2[B]

Abbreviations: CCp, CAD/CAM and polished surface; CCg, CAD/CAM and glazed surface; STp, stratification technique and polished surface; STg, stratification technique and polished surface.
[a] *Different uppercase letters represent statistically significant difference among experimental groups.*

adherence of 0[D] CFU/mL(log10) for CAD/CAM and polished surface (CCp), 4.78[B] CFU/mL(log10) for CAD/CAM and polished surface (CCg), 1.55[C] CFU/mL(log10) for stratification technique and glazed surface (STp), and 4.67[B] CFU/mL(log10) for CAD/CAM and polished surface (STg).

Cytotoxicity Assay

The absorbance means resulting from the MTT test in relation to the control (considered 100%) is shown in Table 3. Student *t*-test showed that polished groups for seven days were not statistically different from the control. All groups were noncytotoxic (with viability higher than 50%). Three-way ANOVA showed that the interaction of "Time × Finishing Treatment" statistically influenced viability (p=0.00), and Tukey test identified that the highest viability values occurred at 24 hours for glazed samples. Viability decreased after seven days.

Table 3: *Cell Viability Percentage in Relation to the Control, Considered 100%, and Absorbance Means ± Standard Deviation (SD), in Optical Density, and Homogeneous Groups From Tukey Test of the Interaction Between Time × Surface Effects*[a]

Group	Cell Viability, %		Absorbance	
	24 h	7 d	24 h	7 d
Polished				
CCp	85.91	86.11	0.083 ± 0.01[B]	0.069 ± 0.00[C]
STp	95.26	71.77		
Glazed				
CCg	152.00	69.11	0.143 ± 0.02[A]	0.064 ± 0.00[C]
STg	160.75	75.69		

Abbreviations: CCp, CAD/CAM and polished surface; CCg, CAD/CAM and glazed surface; STp, stratification technique and polished surface; STg, stratification technique and polished surface.
[a] *Different uppercase letters indicate statistical difference of absorbance among groups.*

Qualitative Analysis

Representative three-dimensional (3D) images of profilometry (left column) and micrographs of sterile (central column) and contaminated surfaces (right column) are presented in Figure 1. In SEM micrographs of biofilm, *C albicans* is more prevalent than the bacteria, and it is possible to identify its hyphae (Figure 1I). Figure 2 shows SEM micrographs of cell viability. FMM-1 fibroblasts grew independent of porosities. The 24-hour period (Figure 2, left column) shows the presence of fibroblasts. In the seven-day period (Figure 2, right column), there are cells adhered to the glazed surfaces arranged in multilayers and with higher extracellular matrix production (Figures 2D,H).

DISCUSSION

To improve their material properties, ceramics are subjected to treatments for smooth surfaces, as lower surface roughness is associated with improved patient comfort as well as esthetic and biological aspects.[29,30] With the Ra parameter, commonly used in the scientific literature, it is observed that different profiles can result in the same measure of average profile amplitude, that is, in the same Ra value. This parameter (or any parameter of amplitude) does not faithfully characterize a surface, and therefore, the relation with other parameters is necessary, for example, the parameter of spacing (RSm).[25] The roughness of the evaluated feldspathic ceramics was influenced by the interaction of surface finishing treatment and manufacturing techniques. Our findings are similar to those of Han and others,[18] who evaluated only the Ra parameter and observed the lowest roughness values for polished samples. For Sarikaya and Guler,[19] the roughness of polished samples varied according to the manufacturing technique (stratification and CAD/CAM). The authors evaluated porcelains obtained by different techniques (VMK 95 and Vitablocs Mark II, Vita Zahnfabrik, Bad Säckingen, Germany; Ceramco III,

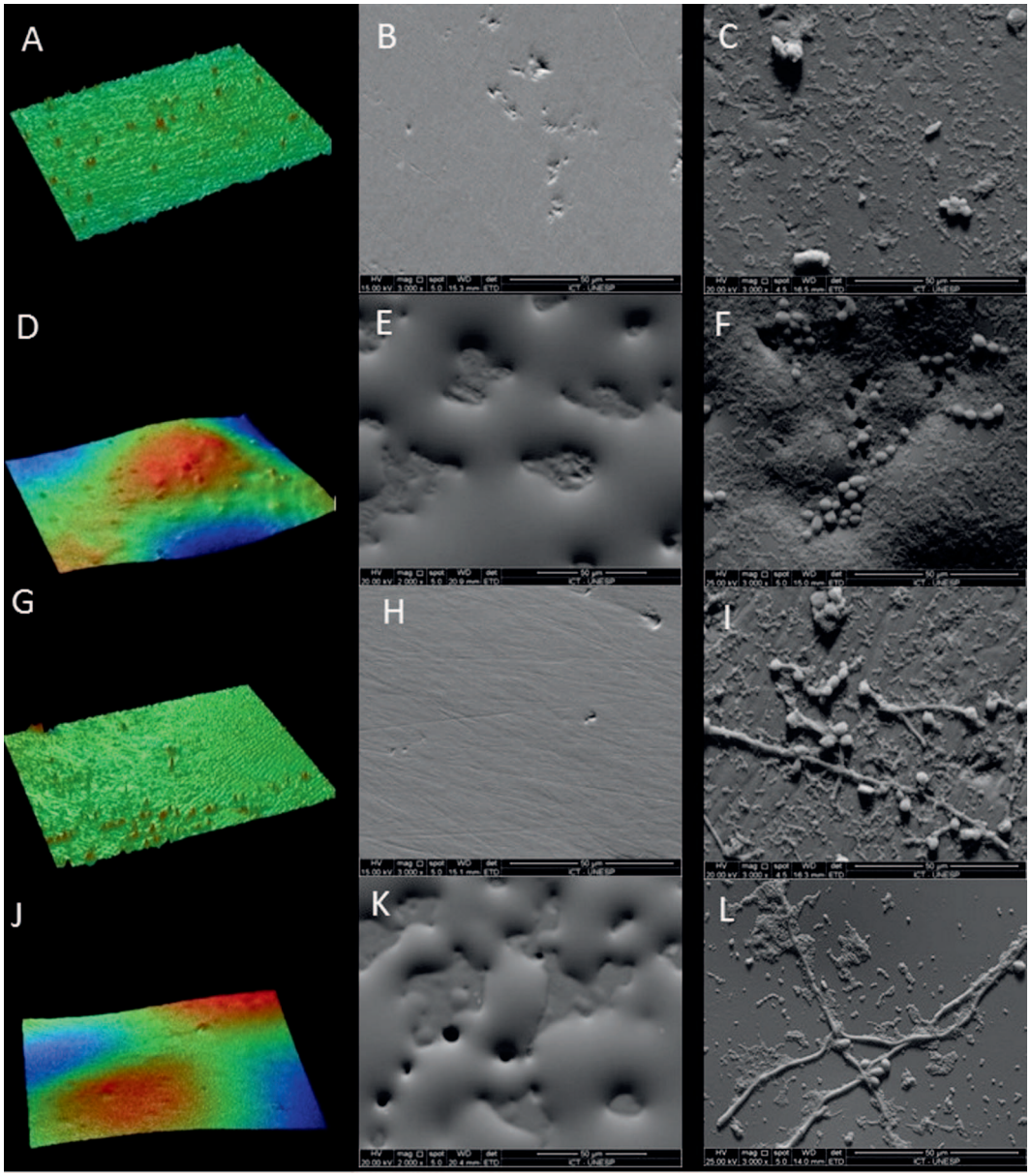

Figure 1. *Representative micrographs. Left column, three-dimensional images of optical profilometry. Central column, micrographs of sterile samples. Right column, samples contaminated with heterotypic biofilm. (A–C): CCp. (D–F): CCg. (G–I): STp. (J–L): STg.*

Degudent GmbH, York, PA, USA; Matchmaker MC, Schottlander, Letchworth, UK) and used glaze as a paste. They also verified an increase of mean roughness by applying glaze on VMII compared with some polishing techniques for this material and concluded that the evaluated polishing techniques were similar to each other because of the hardness of the ceramic blocks.[19] In the present study, the glaze spray application resulted in a higher average amplitude profile for the stratified ceramic (VM9, ST group) when compared with CAD/CAM blocks (VMII, CC group).

Glazing is not a requirement of manufacturers but rather an alternative to polishing. It is performed by applying a thin layer of ceramic paste with low melting temperature on the ceramic surface, followed by sintering at a temperature lower than the firing of ceramics.[20,21] The glaze used in this study was sprayed and thus deposited ceramic powder particles on the ceramic surface, but the distribution of material depends on the distance, pressure, and duration of the application. In theory, the glaze application should fill the surface irregularities, as it is initially a liquid material.

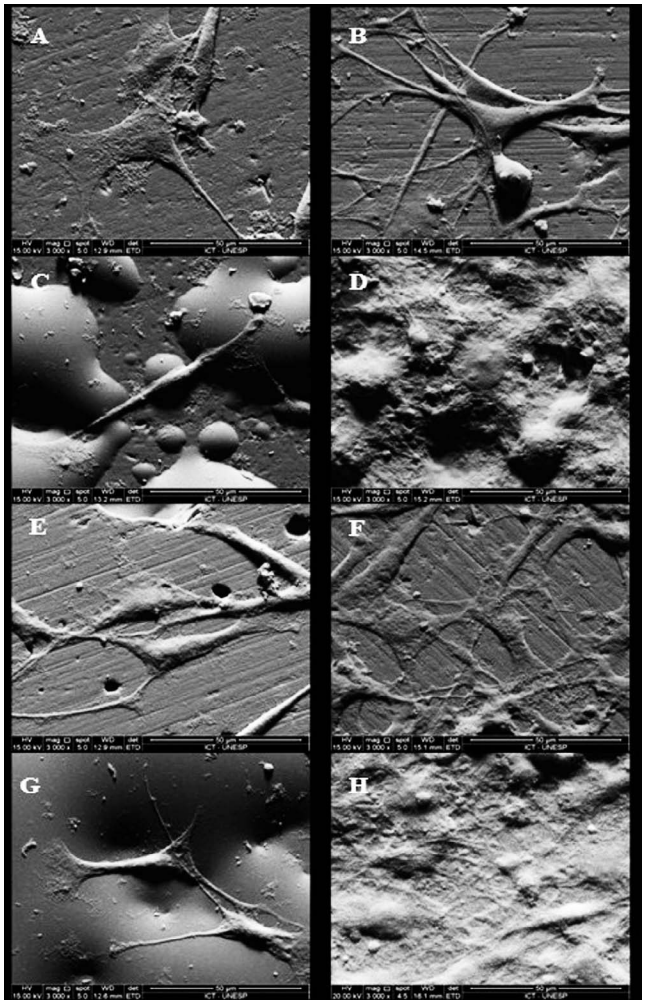

Figure 2. *Representative micrographs. Left column, 24 hours of cell growth. Right column, seven days. (A, B): CCp. (C, D): CCg. (E, F): STp. (G, H): STg.*

However, glazing does not always result in flat and smooth surfaces but in waved surfaces with numerous irregularities due to the nonhomogeneity of the application and to gaps in the ceramic's surface. In this study, topographic analysis performed by SEM and profilometry showed that polished surfaces presented fine scratches resulting from the polishing and glazed surfaces presented greater irregularities due to glaze distribution, which accumulated in "islands" on the materials. There are few studies on the glaze spraying technique applied to feldspathic ceramics or on the interaction between different glazes and materials and their subsequent performance in the oral cavity. A previous study found that glaze spraying resulted in greater roughness than the paste version but with similar final brightness.[8]

Despite the manufacturing technique not influencing SFE, surface finishing treatment was significant, and polished ceramics resulted in greater SFE.

According to the literature, the average values of SFE for *S mutans*, *S sanguinis*, and *C albicans* are, respectively, 48.4, 47.7, and 40.1 mNm; however, these values vary among different strains.[31] According to Minagi and others,[32] the closer the SFE of the material and microorganism, the greater the likelihood of adhesion. The gram-negative bacterial cells have predominantly higher SFE (35 to 65 mNm,) whereas some gram-positive bacterial cells have high (35 to 65 mNm) or low (0 to 25 mNm) values.[31] Evidence suggests that the presence of polysaccharides on the cell's surface of gram-positive bacteria, such as *S mutans* and *S sanguinis*, tends to make the bacterial cell more hydrophilic. Other studies have demonstrated that SFE has an important role in the early adhesion stages of *C albicans*, and substrates with greater SFE induce greater adherence by *C albicans*.[33] In our study, the surface energy in all groups was higher than 52.0 mN/m and was not as relevant as roughness.

Studies have found a positive relation between the roughness of the material and the amount of adhered bacteria.[15,34] Surface energy values depend primarily on the chemical composition of the interactors at each interface, and an SFE analysis can be performed on smooth or rough surfaces. The roughness tends to accentuate the wettability characteristic of the surface. It is known that roughness values less than 0.2 μm have less relation to bacterial colonization than superficial properties. On the other hand, rough surfaces provide physical protection to the bacteria, as well as increased contact area for bacterial adhesion.[11,35,36] In this study, the average roughness of polished ceramic was 0.3 μm, which probably hindered the adherence of *C. albicans*, which measured from 2 to 6 μm.[37] This might have contributed to the lower CFU number of *C albicans* on polished samples, as they probably shed from the surface of the specimens during the wash, since the presence of *C albicans* can be checked by SEM.

MTT analysis showed that all groups had no cytotoxicity. The surface finishing treatment and evaluation period had an influence on the cell viability of fibroblasts. In the 24-hour period, glazed ceramics had higher cell viability compared with the polished groups. The cell viability of glazed samples was higher than the control group in the 24-hour period, representing a good initial relationship

between material and cells.[38,39] Cell viability was reduced after seven days, but it was similar, regardless of the surface finishing treatment. The micrographics show greater cell growth on glazed samples after seven days (Figure 2D,H) when compared with polished ones (Figure 2B,F). This can be justified by the fact that fibroblasts have good affinity for rough surfaces (glazed samples, higher Ra, higher RSm), as they anchor better and proliferate more. However, this excessive proliferation represented by the provision of these cells in multilayers can culminate in cell death, and SEM fibroblast images can generate some confusion in interpretation since the MTT test showed quantification of viable cells present in the supernatant liquid where the sample had been in contact with fibroblasts, while SEM features cells (viable or nonviable) on the material's surface. The integrity of the fibroblastic cells is important for the health of the tissues and around prosthetic restorations, since these cells are responsible for tissue maintenance and offer a buffer that serves as a protective barrier.[40,41]

CONCLUSION

Based on the results of the present study, it can be concluded that polishing compared with glaze spray treatment resulted in the lowest values for surface roughness and higher SFE values. The adherence of *Streptococcus* was superior in all conditions to *C albicans*, and polished ceramics adhered even less *C albicans*. More studies are necessary to understand which properties interact with the formation of biofilm and other tests for cellular viability of human gingival fibroblasts after seven days.

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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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