©Operative Dentistry, 2018, **43-3,** 315-325

Monolithic Ceramics: Effect of Finishing Techniques on Surface Properties, Bacterial Adhesion and Cell Viability

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Clinical Relevance

Clinical procedures should be performed with caution considering that rough surfaces are directly related to higher bacteria adhesion. However, a polishing procedure may lead to a temporary inflammatory tissue reaction.

SUMMARY

Introduction: This study evaluated the morphology, biofilm formation, and viability of human gingival fibroblasts in contact with two monolithic ceramics after two different finishing techniques: polishing or glazing. For this, 92 blocks (4.5 × 4.5 × 1.5 mm) of each ceramic were made using high translucency zirconia partially stabilized by yttrium (YZHT) and lithium silicate reinforced by zirconium (ZLS).

Methods and Materials: Blocks were sintered and then divided into glazing (g) or polishing (p) surface finish. Surface roughness (Ra and RSm) was evaluated through a contact rugosimeter and profilometry. Specimens were contaminated for heterotypic biofilm formation with *Streptococcus mutans*, *Streptococcus*

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DOI: 10.2341/17-011-L

***sanguinis* and *Candida albicans* for 16 hours. Biofilm was quantified by counting the colony forming units (CFU/mL) and analyzed by scanning electron microscopy (SEM). Fibroblast viability was evaluated by MTT assay. Surface free energy (SFE) was also determined. Roughness data were evaluated using nonparametric tests, while SFE, MTT and CFU results were evaluated by analysis of variance and Tukey test, and MTT data were also submitted to *t*-test (all, α=0.05).**

Results: Results showed that polished samples presented a lower high profile mean ($p<0.001$); however, YZHTg presented less space between defects ($p=0.0002$). SFE showed that YZHT presented higher SFE than ZLS. Profilometry evidenced more homogeneity on polished surfaces. The interaction of finishing technique and microorganisms influenced the CFU ($p=0.00$). MTT assay demonstrated initial severe cytotoxic behavior for polished surfaces. SEM images showed homogeneous surfaces, except for glazed YZHT.

Conclusion: Glazed surfaces have a greater roughness and tend to accumulate more biofilm. Polished surfaces have higher SFE; however, they are temporarily cytotoxic.

INTRODUCTION

Ceramics have become an alternative material for the manufacture of dental prostheses due to their esthetics and long-term proven resistance. With these advantages, their restorative techniques have greatly developed over time.[1] Zirconia partially stabilized by yttrium (YTZP) does not present a glass phase; however, it contains a highly crystalline phase and low translucency,[2] which confers opacity to visible light and the need for veneering infrastructures with esthetic ceramics.[3] Monolithic materials appeared with the purpose of combining adequate translucency and excellent mechanical properties,[4,5] aiming to overcome failures due to chipping of the veneering ceramic, decreasing clinical time and restoration costs.[6]

Recently, new formulations of zirconia reinforced lithium silicate (ZLS) ceramic materials have been introduced in the market (Celtra Duo, Dentsply, Konstanz, Baden-Württemberg, Germany; Suprinity, Vita Zahnfabrik), joining the existing group of ceramics and expanding the possibility of ceramic use in different clinical situations. According to the manufacturer, Vita Suprinity contains 56% to 64% SiO_2, 15% to 21% LiO_2, 1% to 4% K_2O, 3% to 8% P_2O_5, 1% to 4% Al_2O_3, 0% to 4% CeO_2, and 0% to 6% pigments, in addition to 10% zirconia, thereby presenting superior properties to lithium disilicate (LD), such as fracture toughness (2.31 ± 0.17 MPa $m^{0.5}$), flexural strength (443.63 ± 38.90 MPa), and elastic modulus (70.44 ± 1.97 GPa). As disadvantages, ZLS proved to be harder (6.53 ± 0.49 GPa) and more friable (2.84 ± 0.26 $\mu m^{-1/2}$) than LD.[7] When used as an infrastructure, it is possible to leave zirconia exposed to oral medium due to its biocompatibility and lower tendency to accumulate oral biofilm.[8,9] However, the outcome of exposure of monolithic crowns of high translucency zirconia partially stabilized by yttrium (YZHT) and ZLS to oral fluids is not fully elucidated.

Bacterial adhesion to a substrate and the initial biofilm composition is related to topography,[10-12] surface hydrophobicity,[13,14] and communication between existing microorganisms.[15] If the surface is hydrophilic, a water pellicle will be present, making direct contact between the hydrophobic microorganism and the substrate difficult. *Streptococcus* is one of the first colonizers of initial supragingival biofilm in the first 8 hours[16] and is present in greater quantity in the oral biofilm.[17] Due to technological and clinical advances, ceramics tend to promote excellent marginal adaptation, finishing, and polishing. Zirconia has a less homogeneous surface compared with other materials because of pores resulting from the sintering process[18] or defects caused by polishing; these defects are due to the larger grains found in zirconia, and those grains susceptibility to being exposed during polishing.[19] Bacteria present in the oral cavity naturally tend to adhere to ceramic materials or to the interface between tooth and restoration,[11] the cervical third of the proximal surface, and along the gingival margin, where they are protected from mechanical action.[20] Oral biofilm is one of the best described microbial systems,[21] so it is well known that there is a mechanism for bacterial adherence and biofilm formation. On solid surfaces such as enamel, the ability to aggregate, the order of appearance of the microorganisms[17] and the environment[21] are important factors in oral biofilm formation. There is no consensus about the finishing technique that promotes the best surface smoothness in ceramics.[22,23] In the same way, to the knowledge of the authors, no studies have evaluated the interaction between different finishing techniques on the surface properties of these new materials, the formation of oral biofilm, or human gingival fibroblast (FMM-1)

viability when in contact with these monolithic ceramics.

Therefore, this study aimed to evaluate the influence of two finishing techniques (polishing or glazing) on the surface properties of two monolithic ceramics, as well as initial heterotypic biofilm formation *in vitro* and human gingival fibroblast (FMM-1) viability in contact with these ceramics. The null hypothesis was that surfaces resulting from polishing or glazing do not influence bacterial adhesion or FMM-1 cell viability.

METHODS AND MATERIALS

Vita YZ HT (YZHT; Vita Zahnfabrik, Bad, Säckingen, Germany; batch number 48980) and Vita Suprinity (ZLS; Vita Zahnfabrik; batch number 49142) were cut with a diamond disk in a cutting machine (Isomet 1000, Precision Sectioning Saw, Buehler, Lake Bluff, IL, USA) under constant cooling. In total, 92 specimens of each material were obtained, which were then sanded to standardize their dimensions in an automatic polisher (EcoMet/AutoMet250, Buehler) using sandpapers of decreasing grit up to #1200 (30 seconds per grit), and under water cooling. After cleaning in an ultrasonic bath with isopropyl alcohol (5 minutes), the specimens were sintered in their specific ovens. The final dimensions for both materials were 4.5 × 4.5 × 1.5 mm. Half of the blocks received a thin layer of Vita Akzent Spray HT glaze (Vita Zahnfabrik; batch number E33820) on its working side. The other half was submitted to the two step polishing protocol suggested by the manufacturer for both monolithic ceramics (VITA Suprinity Polishing Set clinical; batch number E6510). The specimens were randomly divided into four groups according to material (YZHT or ZLS) and finishing technique (g = glazing; or p = polishing), namely: YZHTg, YZHTp, ZLSg, ZLSp (Figure 1).

Surface Roughness (SR)

Twenty specimens from each group were analyzed by a contact rugosimeter (SJ 400, Mitutoyo, Tokyo, Japan) and a digital optical profiler (Wyko, ModelNT 1100, Veeco Instruments Inc, Tucson, AZ, USA). For roughness, five measurements were performed for each specimen in 5 random different areas with a read length of 3 mm and speed of 0.2 mm/s. The analysis was performed following ISO 4287-1997 standards, with Gaussian Filter and cut-off wavelength value of 0.8 mm. Average values were calculated for each sample, and the mean Ra and RSm (μm) values were obtained. For profilometry, specific software (WykoVision 32, Veeco Instruments Inc) was used for three-dimensional parameter measurements at 20× magnification in an area of 301.3 × 229.2 μm of two samples from each group.

Surface Free Energy (SFE)

Five samples (14×12×1.5 mm) from each group (obtained in the same way as described before) were used in conducting the SFE analysis by goniometer. An optical tensiometer (TL 1000, Theta Lite, OneAttension, Biolin Scientific, Lichfield, UK) was used to measure the mean contact angle (CAm) on five different areas by the sessile drop technique. Two liquids with different surface tensions were used: distilled water and diiodomethane,[24] at room temperature. In this technique, a graduated syringe (Gastight Syringes #1001 – 1ml, Hamilton, Reno, NV, USA) with a hydrophobic needle deposits a drop, and after 5 seconds the CAm is calculated with 60 images per second over 10 seconds. The SFE (mJ/m^2) was calculated according to the method proposed by Owens and Wend[25] using the harmonic average formula (equations 1 and 2) and information relating to the liquids[24]. The CAm was replaced to isolate the dispersive and polar constants of each solid. The sum of these constants correspond to the SFE (W or γ).

$$W_{12A} = \gamma_{1A}(1 + \cos\theta_A) = \frac{4\gamma_{1A}^d\gamma_2^d}{\gamma_{1A}^d + \gamma_2^d} + \frac{4\gamma_{1A}^P\gamma_2^P}{\gamma_{1A}^P + \gamma_2^P} \quad (1)$$

$$W_{12B} = \gamma_{1B}(1 + \cos\theta_B) = \frac{4\gamma_{1B}^d\gamma_2^d}{\gamma_{1B}^d + \gamma_2^d} + \frac{4\gamma_{1B}^P\gamma_2^P}{\gamma_{1B}^P + \gamma_2^P} \quad (2)$$

Where: γ corresponds to the SFE of the liquid, $\cos\theta$ = cosine of the liquid CAm, respectively for diiodomethane (1A) and water (1B). γ^d correponds to the dispersive energy and γ^p, to the polar energy. $\gamma_2{}^d$ and $\gamma_2{}^p$ correspond to the solid energies.

Colony Forming Units (CFUs)

Standard suspensions of *Streptococcus* (UA 159), *Streptococcus sanguinis* (ATCC 35688), and *Candida albicans* (ATCC 18804) were prepared containing 10^6 cells/mL (24 hours, 37°C). *Streptococcus* was cultured under microaerophilic conditions on brain heart infusion (BHI) broth supplemented with 15% glucose, and *C albicans* was cultured for 18 hours at 37°C in yeast nitrogen base broth (YNB; Difco, Detroit, MI, USA) supplemented with 100 mM of glucose. After incubation, the growth was suspended in a sterile physiological solution (0.9% sodium chloride [NaCl]),

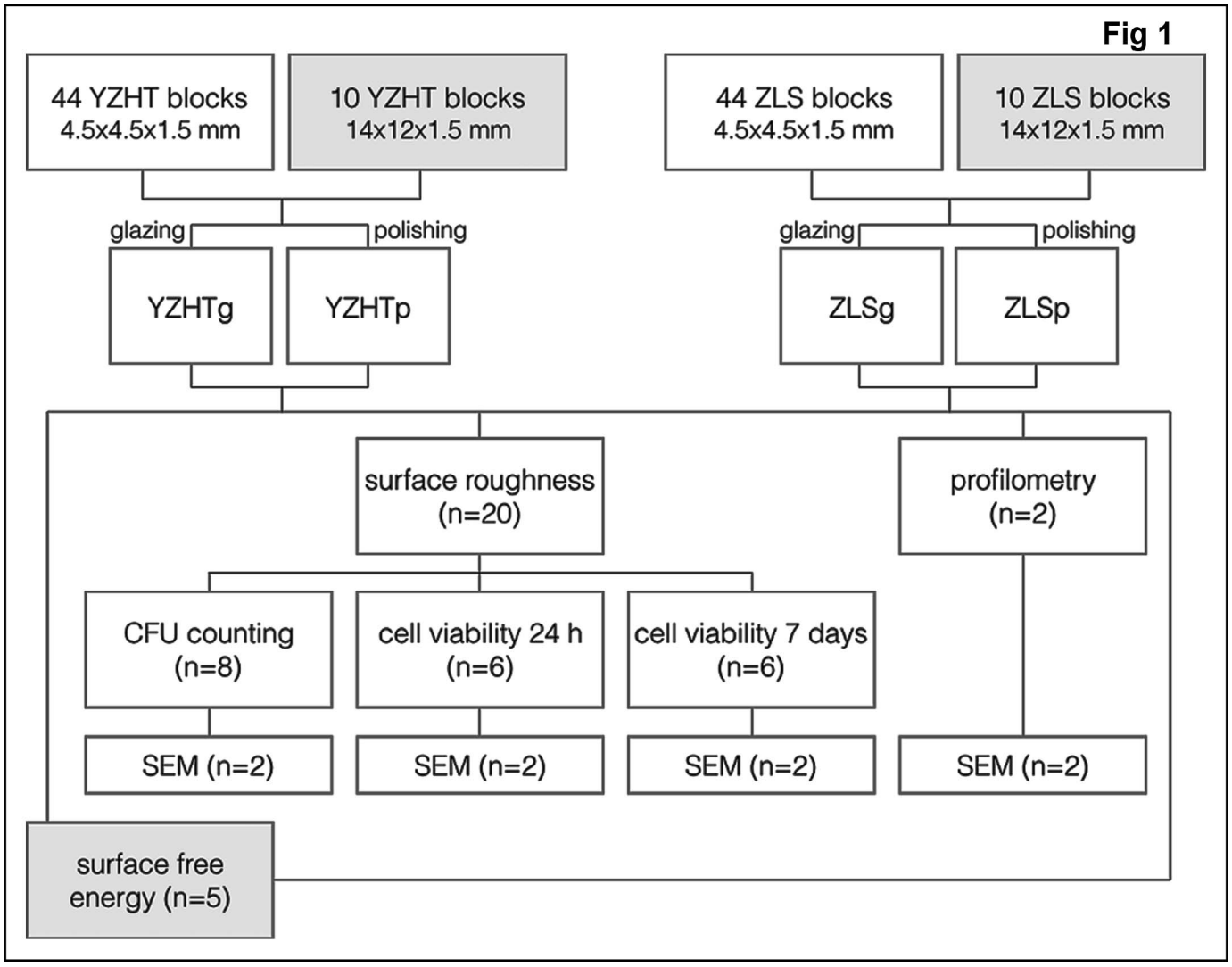

Figure 1. *Flow chart of tested samples. After nondestructive analysis (surface roughness and profilometry), samples were reused. In the surface free energy analysis, 14 × 12 × 1.5 mm blocks were used (grey boxes).*

and the number of cells in suspension was counted in a spectrophotometer (B582 – Micronal, São Paulo, Brazil). The parameters of optical density and wavelength used were 0.620 and 398 nm for *S mutans*, 0.560 and 398 nm for *S sanguinis* and 0.284 and 530 nm for *C albicans,* respectively. These parameters were previously established using a standard curve for CFU vs absorbance. Eight specimens of each group were sterilized in laminar flux (15 min each side), and each one was then distributed on a sterile 96-well polystyrene tissue culture plate. Next, each plate was contaminated with the association of all microorganisms (16.5 μL of each microorganism suspension, 70 μL of YNB and 30 μL of BHI supplemented with 1% of glucose). After this period, samples were removed and washed with sterile 0.9% NaCl in order to remove weakly bonded cells. The samples were individually placed in tubes with 10 mL of sterile 0.9% NaCl and sonicated (Sonoplus HD 2200, 30 W, Bandelin Eletronic, Berlin, Germany) for 30 seconds to disperse the biofilms. The suspension obtained was diluted 10^{-3} times for *C albicans* and 10^{-5} times for *Streptococcus*. Aliquots of 0.1 mL were seeded in duplicate onto petri plates with selective medium for each microorganism, as follows: Mitis Salivarius agar (Difco) for *S sanguinis*, Mitis Salivarius agar (Difco) with 0.2 UI/mL of bacitracin (União Química, Sao Paulo, Brazil) and sucrose (MSBS) for *S mutans*, and Sabouraud dextrose agar with 50 mg/L of clorafenicol (União Química) for *C albicans*. The plates were incubated for 16 hours at 37°C in a CO_2 chamber. Then, the plates with 30 to 300 typical

Table 1: *Mean Values (μm) ± SD, 95% CV, Median (μm), Kruskal-Wallis Analysis Results (p-value and Kruskal-Wallis Statistic), and Homogeneous Groups from the Dunn Test for Roughness Values in Ra and RSm Parameters*[a]

Material	Ra			RSm		
	Mean ± SD	95% CV	Median	Mean ± SD	95% CV	Median
YZHTg	2.37 ± 0.97	40.85%	2.45[A]	128.5 ± 51.92	40.49%	109.0[B]
YZHTp	0.58 ± 0.23	39.82%	0.55[C]	103.2 ± 101.60	98.48%	215.0[A]
ZLSg	0.96 ± 0.36	37.21%	1.00[B]	258.1 ± 112.40	43.55%	244.6[A]
ZLSp	0.33 ± 0.18	55.03%	0.31[C]	73.40 ± 49.40	67.30%	240.4[A]
Kruskal Wallis	55.65			19.37		
p value	<0.001			0.0002		

Abbreviations: CV, coefficient of variation; SD, standard deviation; YZHTg, zirconia partially stabilized by yttrium with glazing; YZHTp, zirconia partially stabilized by yttrium with polishing; ZLSg, zirconia reinforced lithium silicate with glazing; ZLSp, zirconia reinforced lithium silicate with polishing.
[a] *Groups with similar letters do not present statistical difference.*

colonies were counted and mean values of CFU/mL were obtained.

FMM-1 Cell Viability Assay

Cell viability was determined by measuring mitochondrial function based on its capability to reduce MTT (3-[4.5-dimethyl-thiazol-2-yl-] -2.5-diphenyl tetrazolium bromide) into a colored formazan product. Cell viability was quantified by dissolving MTT (Sigma, St Louis, MO, USA) in 0.1 N NaOH (6.25 v/v%) in DMSO (dimethyl sulfoxide), and cell survival was expressed as a percentage in relation to control group (=100%) consisting of plates without ceramic material. The standard curve was evaluated to convert optical density values to the number of viable cells, using cell densities of 2×10^4 cells/well. Twelve samples from each group were used to evaluate cell viability after 24 hours and 7 days. The medium was replaced every 48 hours over the 7 days. The cell monolayer at the bottom of the wells was washed with 500 mL of PBS. Then, 500 μl of MTT solution (0.5 mg/mL PBS) was added to each well. The plates were incubated (1 hour at 37°C) in the absence of light and supernatants were discarded. After the wells were washed with 500 μl PBS, the plates were incubated in DMSO solution (10 min, 37°C) and shaken on an orbital table (10 minutes). Finally, 100 μL of supernatant from each well was placed in triplicate in a 96-well plate and read at 570 nm (EL808IU, Biotek, Winooski, VT, USA).

Scanning Electron Microscopy (SEM)

Polished and glazed surfaces free of microorganisms, as well as contaminated specimens with initial biofilm and with FMM-1 cells, were observed and photographed by SEM (Inspect S 50 – FEI Company, Brno, Czech Republic), operating at 15 kV. Samples with cells or microorganisms were fixed for 1 hour in 2.5% glutaraldehyde, dehydrated in several ethanol washes (10%, 25%, 50%, 75%, and 90% for 20 minutes and 100% for 1 hour) and dried overnight in a bacteriologic incubator at 37°C. All samples received a gold coat in a low-pressure atmosphere using an ion sputter coater (Polaron SC 7620 Sputter Coater, Quorum Technologies, Newhaven, UK).

Statistical Analysis

Once the normality of the data using Kolmogorov-Smirnov test was confirmed, SFE results (mN/m) were statistically analyzed by two-way analysis of variance (ANOVA), CFU data (in log10) and MTT (in %) were analyzed by three-way ANOVA, all with $\alpha = 0.05$, using Minitab software (Minitab 17 for Windows, 2004, State College, Pennsylvania, USA). Tukey test was used to detect differences (α=0.05). Because the distribution was not normal, roughness data (μm) were submitted to Kruskal-Wallis, Dunn, and Mann-Whitney tests (all, α=0.05). Images obtained by profilometry and SEM were qualitatively analyzed.

Results

SR

Table 1 presents the descriptive statistical analysis for roughness data. The Kruskal-Wallis test showed that both roughness parameters were influenced by finishing techniques and material ($p<0.05$; Table 1). The YZHTg group showed higher (Ra) and less spaced (RSm) grooves on average compared with the others. The Mann Whitney test indicated that finishing technique only influenced the Ra parameter, while material influenced both parameters (Table 2). Three-dimensional profilometry images (Figure 2) emphasize the statistical differences observed by SR between glazed and polished ceramics. Glazed surfaces are rougher than polished

Table 2: *Descriptive Statistics (Median, in μm), Results of Mann-Whitney Analysis (p-Value and W statistic) of Roughness Values for Material and Finishing Technique Isolated Factors*[a]

Parameters	Polishing	Glazing	Surface		YZHT	ZLS	Material	
	Median	Median	*p* value	W	Median	Median	*p* value	W
Ra	0.40	1.30	**0.0000**	937	1.00	0.50	**0.004**	438
RSm	215.0	167.40	0.52	1.686.0	186.3	241.0	**0.027**	493.0

Abbreviations: YZHT, zirconia partially stabilized by yttrium; ZLS, zirconia reinforced lithium silicate.
[a] *Bold p values were statistically significant.*

surfaces. The YZHTg surface was more heterogeneous than ZLSg. For polished surfaces, ZLS was more homogeneous but the presence of polishing grooves was noted on YZHT ceramic.

SFE

CAm data and the resulting SFE are described in Table 3. Both glazed and polished ceramics presented a predominantly hydrophilic behavior. The interaction between material and finishing technique influenced the SFE (p=0.001), and Tukey test showed that YZHT (glazed or polished) presented higher SFE than ZLS, while ZLSp and ZLSg were similar to each other.

CFU/mL

Three-way ANOVA showed a statistical difference for the interaction between finishing technique and microorganism. The mean values of CFU transformed into log 10 base for all experimental groups according to the interaction are shown in Table 4. *C albicans* formed fewer CFUs per milliliter on ceramics, with statistically higher adhesion on glazed surfaces.

Cell Viability Assay

The MTT assay results indicated that FMM-1 cells in early contact with the ceramics (24 hours) or with longer exposure (7 days) did not cause enough damage to characterize them as cytotoxic materials, except for the polished groups, which presented cellular viability lower than 50% after 24 hours, thus characterizing them with severe cytotoxicity, according to the International Organization of Standardization 10993-5.[26] Student t-test showed difference in cell viability between both evaluated periods (24 hours and 7 days) and control group considered 100%, (p<0.05). Three-way ANOVA showed that only period of contact influenced cell growth on the ceramics (p=0.00). Tukey test identified that ceramics in contact with the cells over 7 days had a higher number of viable cells compared with the 24-hour period, regardless of material or surface treatment. The YZHTg group was the only one that presented a decrease in mean cell viability (5.1%) between 24 hours and 7 days, whereas the others presented variable increases (Figure 3).

SEM

The surface micrographs of sterile (1000×, Figure 2, middle column) and contaminated materials (3000×, Figure 2, right column) allowed for observing different surface patterns. The glaze layer on YZHT ceramic was less homogeneous than on ZLS. The

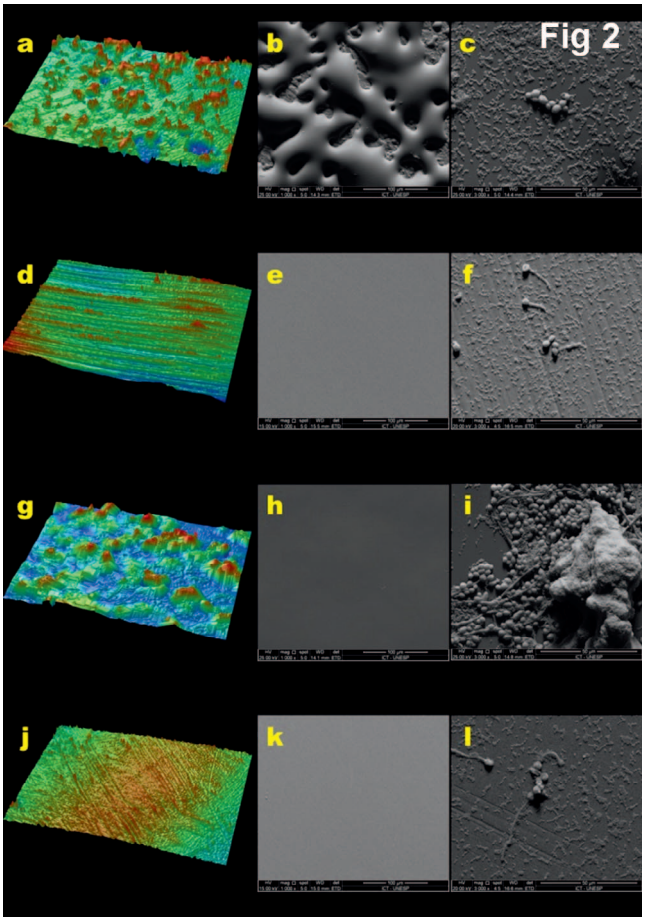

Figure 2. *Images from three-dimensional profilometry, SEM of the surfaces and SEM of contaminated surfaces, respectively, for the groups (a-c) YZHTg, (d-f) YZHTp, (g-i) ZLSg, and,(j-l) ZLSp.*

Table 3: *Mean Contact Angle ± SD for Water and Diiodomethane, Dispersive (γ_d, in mN/m) and Polar (γ_p, in mN/m) components and Respective Surface Free Energy (γ_T, in mN/m) of the Ceramics With Evaluated Finishing Techniques*[a]

Material	Mean Contact Angle		Components		
	Water Mean ± SD (°)	Diiodomethane Mean ± SD (°)	γ_d (mN/m)	γ_p (mN/m)	γ_T (mN/m)
YZHTg	51 ± 11	47 ± 2	37.5	38.0	75.5[B]
YZHTp	86 ± 10	54 ± 5	33.2	57.4	90.6[C]
ZLSg	32 ± 24	49 ± 7	37.5	33.0	71.5[A]
ZLSp	19 ±4	53 ± 5	34.0	39.0	73.0[AB]

Abbreviations: SD, standard deviation; YZHTg, zirconia partially stabilized by yttrium with glazing; YZHTp, zirconia partially stabilized by yttrium with polishing; ZLSg, zirconia reinforced lithium silicate with glazing; ZLSp, zirconia reinforced lithium silicate with polishing.
[a] Identical upper case letters represent absence of statistical difference by Tukey test (α=0.05).

roughness pattern generated by the polishing technique was homogeneous and similar between materials. The presence of *Streptococcus* and *C albicans* was observed on contaminated samples. An increase in the number of FMM-1 cells adhered to the materials' surface submitted to the MTT assay was observed in relation to time (Figure 4), independent of the surface morphology.

DISCUSSION

This study aimed to evaluate the interaction between surface properties and biofilm formation for the viability of human gingival fibroblasts. The results rejected the null hypothesis. The clinical long-term success of dental ceramics depends on their physical properties, manufacturing process, laboratory manufacturing technique, and clinical procedures. The composition of the material, as well as its surface structure, can influence the initial bacterial adhesion and compromise dental health.[10,12] Ceramics are attractive restorative materials due to their esthetic quality and biocompatibility; the smooth surfaces minimize oral biofilm accumulation.[13]

Table 4: *Mean Values ± SD in log10 of the Amount of CFU and Homogeneous Groups According to Tukey test for the Interaction Finishing technique*Microorganism* [a]

Finishing Technique *Microorganism	Mean ± SD (log10)
Glazed**mutans*	7.72 ± 0.13[A]
Glazed**sanguinis*	7.60 ± 0.19[A]
Polished**sanguinis*	6.60 ±0.31[B]
Polished**mutans*	6.55 ± 0.27[B]
Glazed**candida*	5.24 ± 0.23[C]
Polished**candida*	0.26 ± 1.52[D]

Abbreviations: CFU, colony forming unit; log10, log of base 10; SD, standard deviation.
[a] Identical upper case letters indicate absence of statistically significant difference.

For roughness analysis and qualitative assessments, high translucency zirconia (YZHT) presented a rougher profile than lithium silicate reinforced by zirconia (ZLS). Results from profilometry and SEM analyses corroborate YZHTg as having the highest absolute mean height of irregularities along the profile. Regardless of ceramic material, glaze application resulted in rougher surfaces. Another fact contributing to greater roughness in YZHTg may be the chemical union between glaze and zirconia, where the glaze has accumulated in islands. This accumulation causes unevenness between surface and glaze, resulting in higher Ra values for YZHT. The glaze layer on vitreous ceramics is distributed more evenly, increasing the spacing between peaks and valleys (higher RSm).

ZLSp was more hydrophilic regarding the mean contact angle (CAm) between water and ceramic. According to Shirtcliffe and others,[14] a surface with CAm to water between 0 and 180° is characterized as partially hydrophilic. A surface with a hydrophobic tendency may have this feature raised by increasing the roughness,[13,14] affecting its wettability and thus favoring bacterial retention.[13] This was observed for ZLS which presented high values of Ra and higher CAm to water when glazed. Therefore, it is important that clinicians have knowledge about the consequences of inadequate procedures that result in rougher surfaces,[27] which may be contaminated with impurities or modified by exposure to changes in temperature, which in turn may increase SFE.[28] According to Anusavice,[29] SFE is directly associated with adhesion. Thus, YZHTp may be suggested as the condition that results in a better adhesive property.

The presence of glaze on the surface does not prevent the formation of dental biofilm, as observed in a previous study[23] that compared *C albicans* adhesion on a porcelain surface without surface treatment, glazed and polished. The authors verified

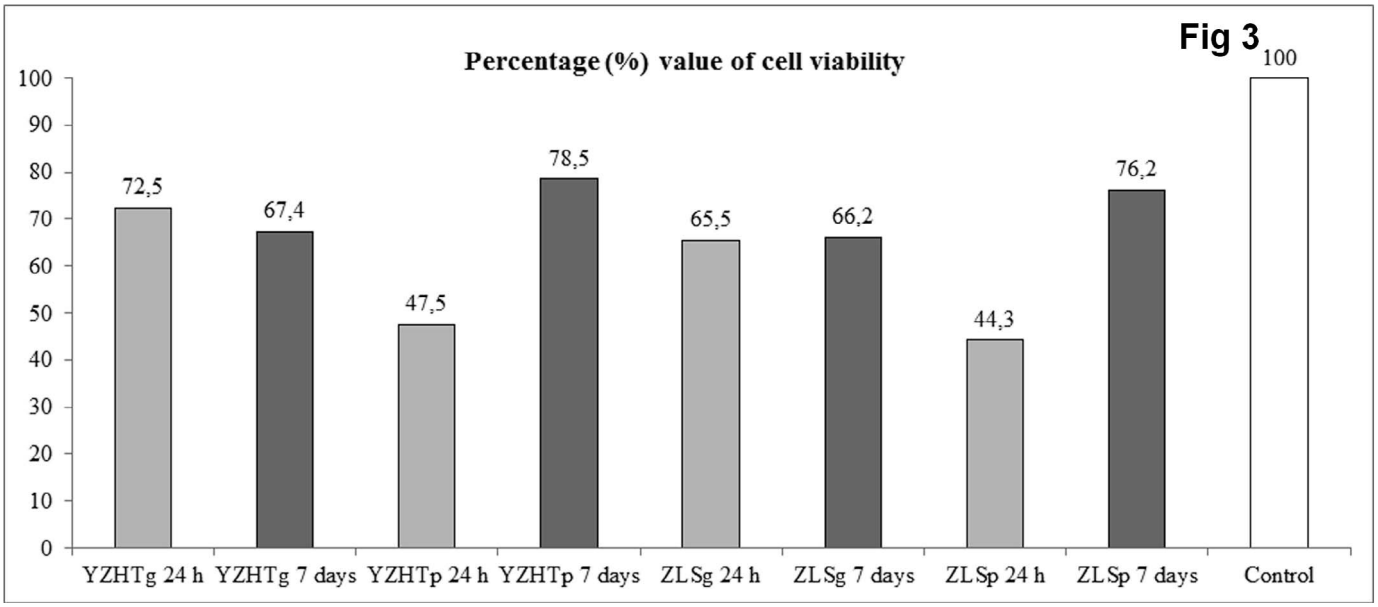

Figure 3. *Bar graph for percentage of viable cells through MTT assay at 24 hours and 7 days.*

that the glazed surface had a lower value of bacterial adhesion compared with the surface without superficial treatment, but there was no significant difference from the polished group.[23] In mature biofilm, the adherence of microorganisms occurs on other layers of microorganisms. Therefore, this study simulated *in vitro* environmental conditions for the formation of an initial biofilm in order to evaluate the interaction between microorganisms and the surface of the materials. Recent studies have validated different periods up to 24 hours for the formation of such biofilms, using two or more microorganisms.[30,31]

In this study, we examined the adherence of an initial colonizer (*S sanguinis*), a colonizer associated with the development of carious lesions (*S mutans*), and finally, a colonizer related to caries, periodontal diseases, and candidiasis (*C albicans*). The availability of studies evaluating bacterial adhesion to monolithic ceramics is scarce. The adhesion of *S mitis* and *Prevotella nigrescens* on Metoxit AG zirconia (High Tech Ceramics, Thayngen, Switzerland), for example, is lower than on titanium used in manufacturing dental implants (Goodfellow Cambridge Limited, Huntingdon, UK).[31] A previous study verified that glazed Lava zirconia (3M ESPE, St. Paul, MN, USA) presented greater roughness compared with the polished surface, and there was a tendency toward biofilm accumulation.[8] When microorganisms were compared to each other, a greater growth of *Streptococcus* was observed independent of the surface type. Also, a greater formation of colonies on glazed surfaces was observed in comparison to polished surfaces, corroborating a previous study.[8] This may be associated with the fact that *S sanguinis* facilitates the growth of other *Streptococcus* that grow in a similar way, which can be justified by their hydrophobic nature.[31,32] *C albicans* also presents a hydrophobic characteristic; however, its smaller growth may be associated with the fact that *Streptococcus* is a commensal microorganism,[21] where both species are associated with benefits for one of them without harming the other. In the ceramic structure, *C albicans* acts as a facilitator for the adherence of *S mutans*.[33] The low growth of *C albicans* on polished surfaces can be justified by its difficulty in adhering to very smooth surfaces compared with *S mutans*,[34] since the second produces a water-insoluble substance that facilitates the adherence of these microorganisms to a smooth substrate.[35] Different from rough surfaces, polished surfaces do not accumulate many nutrients. This dispute over scarce food, as well as the lack of space and negative effect of metabolites from bacteria, can also justify a competition between *C albicans* and *Streptococcus*. The interaction between *S mutans* and *C albicans* is given by mutualism, where microorganisms benefit, resulting in mutual dependence.[36] It is interesting to observe the small number of *C albicans* colonies because healthy tissue free of fungus in restorations where the dental preparation has contact with the gingival tissue is necessary. A lower number of *C albicans* colonies on smooth surfaces has also been reported for other materials

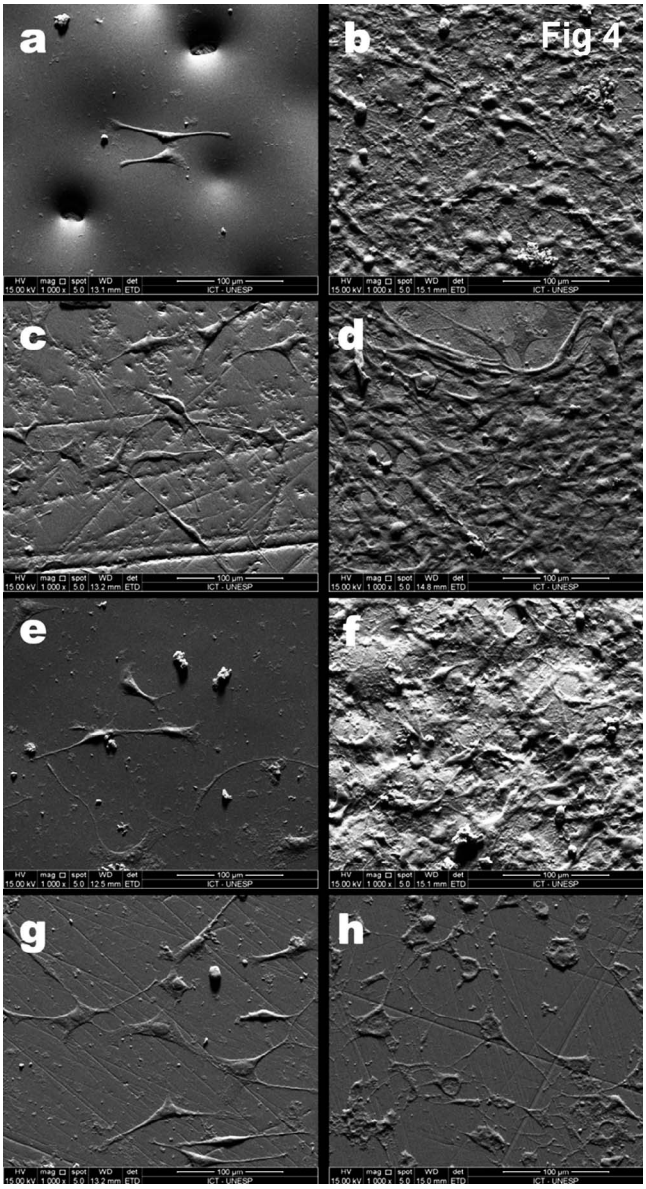

Figure 4. *SEM images of FMM-1 cells in contact with the ceramics YZHTg (a-b), YZHTp (c-d), ZLSg (e-f), and ZLSp (g-h) for the periods of 24 hours and 7 days, respectively. Magnification of 1000×.*

used in the oral cavity.[35] The results show that a higher number of microorganisms adhered to the roughened surfaces, presenting less SFE than polished surfaces. In this way, the roughness seems to be the main factor related to biofilm formation. Considering smooth surfaces, it is suggested that SFE may be the main factor associated with initial bacterial adherence.[31,34]

It is possible to verify the presence of *Streptococcus* and *C albicans* (Figure 2, right column) in the SEM images. The ceramics were colonized with a thin biofilm filled with cellular agglomerates of similar size and morphology, with an emphasis on the extensive colonization by *Streptococcus*, and it was not possible to distinguish *Streptococcus*. The amorphous substance is an important factor in the relationship of all studied microorganisms. In Figure 2 (left column), SEM images show this amorphous matrix involving the species, visibly larger on ZLSg. This matrix may be associated with the adhesion of *C albicans* to the biofilm. The presence of this fungus corroborates the assertion that this facilitates the adherence of *S mutans*, and may be associated with an increased risk of caries. The results show that both materials under both finishing techniques, can be considered moderately cytotoxic[26] to the growth of human gingival fibroblasts (FMM-1), since all groups presented cellular viability between 50% and 79%. The initial (24 hour) cytotoxicity of polished groups may be related to the release of some substance at this initial time, reducing its cytotoxic effect after 7 days. This initial cytotoxicity may occur if the cells do not present sufficient immediate defense to some remnant of the polishing procedure. Over time, the cells enhance their defense mechanisms and become capable of protecting themselves from the aggressor. Therefore, future studies evaluating which substances are released causing tissue damage are important.

CONCLUSION

ZLS resulted in lower mean roughness profile and more spaced defects regardless of surface finishing. Polished surfaces were less rough and presented higher SFE, but they also showed severe initial cytotoxicity when in contact with FMM-1 cells. However, they were inert in the long term.

Acknowledgements

The authors would like to thank the Foundation for Research Support of the State of São Paulo (FAPESP) for the scholarship, through the process numbers 2015/13322-1 and 2014/19357-9. The authors also would like to thank Vita Zahnfabrik and Wilcos from Brazil and CAPES (Coordination for Improvement of Higher Education Staff) for their support.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institute of Science and Technology, São Paulo State University, Brazil.

Conflict of Interest

The authors of this manuscript 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.

(Accepted 5 April 2017)

REFERENCES

1. Denry I, & Kelly JR (2008) State of the art of zirconia for dental applications *Dental Materials* **24(3)** 299-307.
2. Shenoy A, & Shenoy N (2010) Dental ceramics: an update *Journal of Conservative Dentistry* **13(4)** 195-203.
3. Baldissara P, Llukacej A, Ciocca L, Valandro FL, & Scotti R (2010) Translucency of zirconia copings made with different CAD/CAM systems *Journal of Prosthetic Dentistry* **104(1)** 6-12.
4. Denry I, & Kelly JR (2014) *Emerging ceramic-based materials for dentistry Journal of Dental Research* **93(12)** 1235-1242.
5. Amer R, Kürklü D, Kateeb E, & Seghi RR (2013) Three-body wear potential of dental yttrium-stabilized zirconia ceramic after grinding, polishing, and glazing treatments *Journal of Prosthetic Dentistry* **112(5)** 1151-1155.
6. Stawarczyk B, Özcan M, Schmutz F, Trottmann A, Roos M, & Hämmerle CH (2013) Two-body wear of monolithic, veneered and glazed zirconia and their corresponding enamel antagonists *Acta Odontologica Scandinavica* **71(1)** 102-112.
7. Elsaka SE, & Elnaghy AM (2016) Mechanical properties of zirconia reinforced lithium silicate glass-ceramic *Dental Materials* **32(7)** 908-914.
8. 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.
9. Scotti R, Zanini Kantorski K, Scotti N, Monaco C, Valandro LF, & Bottino MA (2006) Early biofilm colonization on polished- and glazed-zirconium ceramic surface. Preliminary results *Minerva Stomatologica* **55(9)** 493-502.
10. Anami LC, Pereira CA, Guerra E, Assunção e Souza RO, Jorge AO, & Bottino MA (2012) Morphology and bacterial colonisation of tooth/ceramic restoration interface after different cement excess removal techniques *Journal of Dentistry* **40(9)** 742-749.
11. Vo DT, Arola D, Romberg E, Driscoll CF, Jabra-Rizk MA, & Masri R (2015) Adherence of *Streptococcus mutans* on lithium disilicate porcelain specimens *Journal of Prosthetic Dentistry* **114(5)** 696-701.
12. Pereira SMB, Anami LC, Pereira CA, Souza ROA, Kantorski KZ, Bottino MA, Jorge AOC, & Valandro LF (2016) Bacterial colonization in the marginal region of ceramic restorations: Effects of different cement removal methods and polishing *Operative Dentistry* **41(6)** 642-654.
13. Aksoy G, Polat H, Polat M, & Coskun G (2006) Effect of various treatment and glazing (coating) techniques on the roughness and wettability of ceramic dental restorative surfaces *Colloids* and Surfaces B: *Biointerfaces* **53(2)** 254-259.
14. Shirtcliffe NJ, McHale G, Atherton S, & Newton MI (2010) An introduction to superhydrophobicity *Advances in Colloid and Interface Science* **161(1-2)** 124-138.
15. 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.
16. Meier R, Hauser-Gerspach I, Lüthy H, & Meyer J (2008) Adhesion of oral streptococci to all-ceramics dental restorative materials in vitro *Journal of Materials Science* **19(10)** 3249-3253.
17. Burgers R, Gerlach T, Hahnel S, Schwarz F, Handel G, & Gosau M (2010) In vivo and in vitro biofilm formation on two different titanium implant surfaces *Clinical Oral Implants Research* **21(2)** 156-164
18. Grenade C, De Pauw-Gillet MC, Gailly P, Vanheusden A, & Mainjot A (2016) Biocompatibility of polymer-infiltrated-ceramic-network (PICN) materials with human gingival fibroblasts (HGFs) *Dental Materials* **32(9)** 1152-1164.
19. Al-Haj Husain N, Camilleri J, & Özcan M (2016) Effect of polishing instruments and polishing regimens on surface topography and phase transformation of monolithic zirconia: An evaluation with XPS and XRD analysis *Journal of the Mechanical Behavior of Biomedical Materials* **28(64)** 104-112.
20. Kidd EA, & Fejerskov O (2004) What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms *Journal of Dental Research* **83(Special Issue C)** C35-C38.
21. Kolenbrander PE, Palmer RJ Jr, Periasamy S, & Jakubovics NS (2010) Oral multispecies biofilm development and the key role of cell-cell distance *Nature Reviews Microbiology* **8(7)** 471-480.
22. Hmaidouch R, Müller WD, Lauer HC, & Weigl P (2014) Surface roughness of zirconia for full-contour crowns after clinically simulated grinding and polishing *International Journal of Oral Science* **6(4)** 241-246.
23. Lawaf S, Azizi A, Farzad A, & Adimi P (2016) Effect of surface treatments of porcelain on adhesion of *Candida albicans General Dentistry* **64(4)** e1-e4.
24. Combe EC, Owen BA, & Hodges JS (2004) A protocol for determining the surface free energy of dental materials *Dental Materials* **20(3)** 262-268.
25. Owens DK, & Wendt RC (1969) Estimation of the surface free energy of polymers *Journal of Applied Polymer Science* **13(8)** 1741-1747.
26. International Organization for Standardization (1999) *Biological Evaluation of Medical Devices – ISO 10993, Part 5: Tests for In Vitro Cytotoxicity* International Organization for Standardization, Geneva, Switzerland.
27. Schmitter M, Lotze G, Bömicke W, Rues S (2015) Influence of surface treatment on the in-vitro fracture resistance of zirconia-based all-ceramic anterior crowns *Dental Materials* **31(12)** 1552-1560.
28. Craig RG (1993) *Restorative Dental Materials Applied Surface Phenomena* **9th edition** Mosby, St Louis, MO.
29. Anusavice KJ (1996) *Phillips' Science of Sental Materials* **10th edition** WB Saunders, Philadelphia, PA.
30. de Avila ED, Avila-Campos MJ, Vergani CE, Spolidório DM, & Mollo Fde A Jr (2016) Structural and quantitative analysis of a mature anaerobic biofilm on different

implant abutment surfaces *Journal of Prosthetic Dentistry* **115(4)** 428-436.

31. Yuan C, Wang X, Gao X, Chen F, & Liang X, Li D (2016) Effects of surface properties of polymer-based restorative materials on early adhesion of *Streptococcus mutans* in vitro *Journal of Dentistry* **54** 33-40.
32. Mukasa H, & Slade HD (1973) Mechanism of adherence of *Steptococcus mutans* to smooth surfaces *Infection and Immunity* **8(4)** 555-62.
33. Barbieri DSV, Vicente VA, Fraiz FC, Lavoranti OJ, Svidzinski TIE, & Pinheiro RL (2007) Analysis of the in vitro adherence of *Streptococcus mutans* and *Candida albicans Brazilian Journal of Microbiology* **38(4)** 624-631
34. Tari BF, Nalbant D, Dogruman AF, & Kustimur S (2007) Surface roughness and adherence of *Candida albicans* on soft lining materials as influenced by accelerated aging *Journal of Contemporary Dental Practice* **8(5)** 18-25.
35. Satpathy A, Dhakshaini MR, & Gujjari AK (2013) An evaluation of the adherence of *Candida albicans* on the surface of heat cure denture base material subjected to different stages of polishing *Journal of Clinical and Diagnostic Research* **7(10)** 2360-2363.
36. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Arends J, Darius PL, & van Steenberghe D (1998) The influence of surface free-energy on planimetric plaque growth in man *Journal of Dental Research* **68(5)** 796-799.