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# An in vitro evaluation of surface roughness, color stability and bacterial accumulation of lithium disilicate ceramic after prophylactic periodontal treatment

Avaliação in vitro da rugosidade superficial, estabilidade de cor e acúmulo bacteriano em cerâmica de dissilicato de lítio após tratamento de profilaxia periodontal

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## ABSTRACT

**Objective:** To evaluate the effect of scaling procedures using different ultrasonic tips on the surface roughness, color stability and bacterial accumulation of lithium disilicate ceramic. **Material and Methods:** Scaling procedure was carried out using ultrasonic scaler (Satalec, Acteon, North America) with stainless-steel tip (US), titanium tip (UT) and plastic tip (UP), on disc shaped lithium disilicate samples cemented into a cavity prepared onto the labial surface of freshly extracted bovine teeth (10 samples per group). The samples were stored in coffee solution in an incubator at 37°C for 12 days, which is equivalent to 1 year of coffee consumption. The surface roughness was measured before and after the scaling procedure using a profilometer and atomic force microscopy. The color parameters were measured before and after scaling and staining procedures using VITA Easyshade Advance 4.0 according to the CIE L*a*b* color order system. The samples were then incubated with Streptococcus mutans (S. mutans) suspension. After incubation, the plates with 30 to 300 typical colonies of S. mutans were counted in a colony counter and mean values of colony forming units were obtained (CFU/mL). **Results:** The titanium scaling tip showed a statistically significant higher mean values of change in surface roughness ΔRa and bacterial count than the plastic scaling tip. Color changes (ΔE) were not a statistically significant among the groups. The results showed a statistically significant positive (direct) correlation between surface roughness

## RESUMO

**Introdução:** Avaliar o efeito de procedimentos de raspagem com diferentes pontas de ultrassom na rugosidade superficial, estabilidade de cor e acúmulo bacteriano em cerâmica de dissilicato de lítio. **Material e Métodos:** O procedimento de raspagem foi realizado usando um aparelho de ultrassom (Satalec, Acteon, América do Norte) com ponta de aço inoxidável (US), ponta de titânio (UT) e ponta de plástico (UP), em amostras de dissilicato de lítio em forma de disco cimentadas em uma cavidade preparada na superfície vestibular de dentes bovinos recém-extraídos (10 amostras por grupo). As amostras foram armazenadas em solução de café em incubadora a 37 ° C por 12 dias, o que equivale a 1 ano de consumo de café. A rugosidade da superfície foi medida antes e após o procedimento de raspagem usando um perfilômetro e um microscópio de força atômica. Os parâmetros de cor foram medidos antes e depois dos procedimentos de raspagem e armazenagem no café usando VITA Easyshade Advance 4.0 de acordo com o sistema de ordem de cores CIE L*a*b*. As amostras foram incubadas com suspensão de Streptococcus mutans (S. mutans). Após a incubação, as placas com 30 a 300 colônias típicas de S. mutans foram contadas em contador de colônias e obtidos os valores médios das unidades formadoras de colônias (UFC / mL). **Resultados:** A ponta de titânio mostrou valores estatisticamente maiores de mudança na rugosidade da superfície ΔRa e contagem de bactérias do que a ponta de raspagem de plástico. A mudança de cor (ΔE) não foi estatisticamente significativa entre os grupos. Os resultados mostraram uma correlação positiva (direta) estatisticamente significativa entre rugosidade

and color change (p = 0.012) and also between surface roughness and bacterial count (p = 0.00). **Conclusion:** Within the limitations of this study, titanium scaling instruments cause irreversible surface alterations of lithium disilcate ceramics which was in direct correlation to the color changes and bacterial accumulation; therefore, dentists should proceed with caution when scaling lithium disilicate surfaces. The findings of the current study may indicate the need for instruments or equipment that can remove plaque and calculus without causing surface damage.

## KEYWORDS

Surface properties; Color; Bacterial adhesion; Ultrasonics; Dental scaling; Ceramics.

superficial e alteração de cor (p = 0,012) e também entre rugosidade superficial e contagem bacteriana (p = 0,00). **Conclusão:** Dentro das limitações deste estudo, os instrumentos de raspagem de titânio causam alterações irreversíveis na superfície das cerâmicas de dissilicato de lítio que estão em correlação direta com as mudanças de cor e o acúmulo de bactérias. Portanto, os dentistas devem proceder com cautela ao realizar raspagem em superfícies de dissilicato de lítio. Os resultados deste estudo podem indicar a necessidade de instrumentos ou equipamentos que possam remover a placa e cálculo sem causar danos à superfície.

## PALAVRAS-CHAVE

Propriedades de superfície; Cor; Aderência bacteriana; Ultrassom; Raspagem dentária; Cerâmica.

## INTRODUCTION

Ceramics are the material of choice for patients with high esthetic demands owing to their good physical and optical properties and to their ability to match natural dentition. The recent developments of ceramic systems and processing techniques enabled the treatment of teeth in both the anterior and posterior areas restoring form, function and esthetic excellence with metal-free restorations.

Each time esthetic procedures are considered, gingival health should also be evaluated. Initially, dental prophylaxis and periodontal treatment, including scaling and root planning, should be performed. Ultrasonic scaling have become the most widely used methods among dental surgeons and oral hygienists, due to the decreased time requirement and ease of application in comparison with hand instrumentation and various studies have confirmed that the two techniques yield similar results [1].

Surface roughness of the dental tissues is one of the most described alterations in the literature after periodontal instrumentation. The cumulative effect of minor substance removal per instrumentation performed over the years may lead to severe damage of the dental tissues and existing restorations over time which may increase surface roughness [2] and cause evident unesthetic color changes.

Unfortunately, there is limited information concerning the effects of periodontal instrumentation on lithium disilicate pressable ceramics. And hence the purpose of this study was to evaluate the effect of different scaling methods on surface roughness and color stability and bacterial accumulation of lithium disilicate ceramics.

## MATERIALS AND METHODS

Forty bovine anterior teeth were collected. All soft tissue was removed until teeth were visually clean. The teeth were decapitated 2 mm below the cemento-enamel junction using tapered diamond stone with copious coolant to remove the root. The labial surfaces of the teeth were flattened using a cylinder diamond stone to obtain a flat area of 1x1 cm2. The teeth were mounted in acrylic (Acrostone Dental Factory, Egypt) blocks using PVC tube of 1-inch inner diameter and 1.5 cm thickness. Then a cavity of 5 mm diameter and 0.5 mm depth was prepared

on the flat surface of the bovine teeth using wheel diamond stone (Komet Dental, Gebr. Brasseler).

Forty disc shaped lithium disilicate (IPs e.max Press, Ivocalr Vivadent, Inc) samples of 0.5 mm thickness and 5 mm diameter were constructed according to the manufacturer's instructions. The fitting surfaces of the ceramic discs were etched using hydrofluoric acid (9.5 %) (BISCO, Inc, America) porcelain etchant followed by porcelain primer (Pre-Hydrolized Silane Primer) (BISCO, Inc, America) application according to the manufacturer's instructions. The cavities on the teeth surfaces were acid-etched using phosphoric acid (37 %) (Eco-Etch, Ivocalr Vivadent) followed by the bonding agent (TE-Econom Bond, Ivocalr Vivadent) according to the manufacturer's instructions. Finally, the discs were cemented into the cavities using Variolink N clear (Ivocalr Vivadent, Inc).

The samples were divided into four groups (10 samples per group); i) C: Control group (no scaling), ii) US: Ultrasonic stainless-steel tip (NSK), iii) UT: Ultrasonic titanium tip (Woodpecker), iv) UP: Ultrasonic plastic tip (NSK).

A specially designed scaling apparatus (Figure 1) was designed and fabricated to standardize the scaling procedure [3]. The samples were mounted onto one side of the double-pane balance and attached in place using screws. Scaling procedure was carried out using an ultrasonic scaler handpiece (Satalec, Acteon, North America) at intermediate power setting (level 5 of 14 grades). The ultrasonic scaling tips were angled 90° relative to the surface of sample. A constant force of 30 g was applied to the ultrasonic scalar tip by the vertical movement of a counterweighed balance. A standardized 5 mm horizontal movement and three consecutive cycles of 20 seconds each of the ultrasonic handpiece at a speed of 2 Hz was achieved and operated by the control box [3].

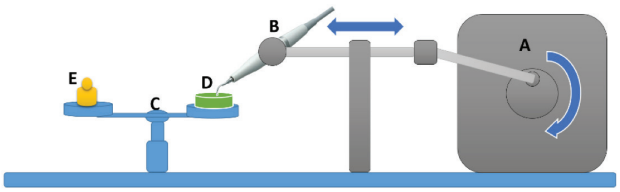


**Table I -** Diagramatic drawing showing the scaling apparatus.

The surface roughness of samples was measured before and after the scaling procedure using the profilometer; Surface Roughness Tester TIME3202 (TR220) (Landmark Industrial Inc, USA), at cut off 0.25 mm, number of cuts 1 and range ± 40 $\mu$m. Measurements were made at three different regions (in the middle and sides) were evaluated in each sample to determine the surface roughness (Ra) values, and averaged to determine the mean values. Then the ceramic surface was analyzed using Atomic force microscopy using AutoProbe CP-Research (Thermomicroscope, Bruker Nano Inc., USA) operated in contact mode using nonconductive silicon nitride probe, at scan area of 25 $\mu$m, scan rate of 1 Hz and number of data points 256 x 256 $m^2$ was using proscan 1.8 software for controlling the scan parameters and IP 2.1 software for image analysis.

Staining procedure: Staining procedure was carried out using coffee solution (Nescafé Classic; Nestlé Egypt). The coffee solution was prepared according to the manufacturer's instructions by using 3.6 g of coffee and 300 mL of hot water. The solution was stirred for 10 minutes and passed through filter paper (Melitta; Melitta Haushaltsprodukte GmbH & Co Kg). In the interval between the 2 color measurements, all specimens were stored in coffee solution in an incubator (Model B 28, BINDER GmbH) at 37°C for 12 days, which is equivalent to 1 year of coffee consumption. [4,5] The solution was stirred every 12 ± 1 hours. After 12 days, the specimens were washed with tap water and dried with tissue paper.

The color parameters of each specimen were measured before and after the scaling

and staining procedures using VITA Easyshade Advance 4.01 (VITA shade, VITA made, VITA) according to the CIE L*a*b* color order system.

Bacterial accumulation test: Initially, a standard suspension of S. mutans was prepared. As the S. mutans preferentially colonizes the pits and fissures of the occlusal surface of teeth, a swab was taken from an oral cavity of bad oral hygiene using sterile loop in laminar air flow, then it was spread on sheep blood agar and incubated for 24 hours at 37 °C in a CO2 chamber. After isolation, the cultured S. mutans was seeded onto a brain heart infusion (BHI) agar and incubated for 24 hours at 37 °C in a CO2 chamber. Then, the growth was suspended in sterile physiological solution [0.9% sodium chloride (NaCl)] to obtain a standard suspension containing 106 cells/ml. The number of cells in suspension was counted in a spectrophotometer. The parameters of optical density and wavelength used were, respectively, 0.620 and 398 nm. These parameters were previously established using a standard curve for CFU vs. absorbance.

Biofilm adhesion was performed in an aseptic environment. The broth used for adherence contains 20 g tripticase, 2 g NaCl, 3 g K2HPO4, 2 g KH2PO4, 1 g K2CO3, 120 mg MgSO4, 15 mg MnSO4, and 50 g C6H8O7 dissolved in 1000 mL of distilled water. The broth was sterilized by autoclaving it at 121 °C for 15 minutes [6]. The samples were placed in a sterile 24-well culture plate then 1.5 mL of broth and 0.1 mL of standardized S. mutans suspension were added to the samples. The plates were sealed and incubated at 37 °C for 24 hours in a CO2 chamber. Samples were then removed and washed twice with sterile physiological solution (0.9% NaCl) in order to remove loosely bound material. The samples were then placed in plates with 3 mL of sterile physiological solution (0.9% NaCl) and sonicated for 30 seconds to disperse the biofilms. The suspension obtained was diluted 100, 1000, and 10,000 times and aliquots of 0.1 mL were seeded in duplicate onto brain heart infusion (BHI) agar and incubated for 48 h at 37 °C in a CO2 chamber.

After incubation, the plates with 30 to 300 typical colonies of S. mutans were counted in a colony counter and mean values of colony forming units were obtained (CFU/mL).

## RESULTS

Data was analyzed on an IBM® (IBM Corporation, NY, USA) personal computer, using Statistical Package for Special Science (SPSS)® (SPSS, Inc., an IBM Company) software computer program version 22. Data were described as mean ± standard deviation (SD) for quantitative (Numerical) variables. One-Way ANOVA followed by Tukey's post-hoc test were was used for comparison of quantitative variables among more than two independent groups. Dunnett's test was used to compare the control group with the other study groups. Correlation between continuous variables was performed using pearson's correlation coefficient. The significance level was set at $p \leq 0.05$.

The results showed that the mean values of ΔRa of the stainless-steel tip showed no statistically significant difference from that of the titanium and the plastic tips. While the titanium scaling tip showed a statistically significant higher mean values of ΔRa than the plastic scaling tip (Table I). AFM showed that scaling using ultrasonic stainless-steel tip clearly resulted in scraping of the ceramic surfaces and loss of their original texture, leading to increased surface roughness, they showed many shallow irregularities. Ultrasonic titanium tip showed more aggressive and deeper scratches than the ultrasonic stainless-steel tip. Scaling using plastic tip did not appear to markedly affect the ceramic surfaces, it showed the least surface alterations (Figure 2-5).

There was no statistically significant difference among mean values of ΔE of the three tip materials and the control group. However,

for the bacterial accumulation, the plastic groups showed the statistically significant least mean values of bacterial count. The stainless-steel and the titanium groups showed the statistically significant highest mean values of the bacterial count. All groups were statistically significant different from the control group.

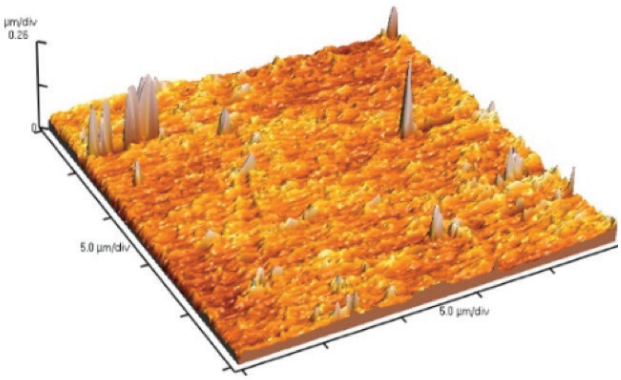


**Figure 2 -** AFM of Control group.

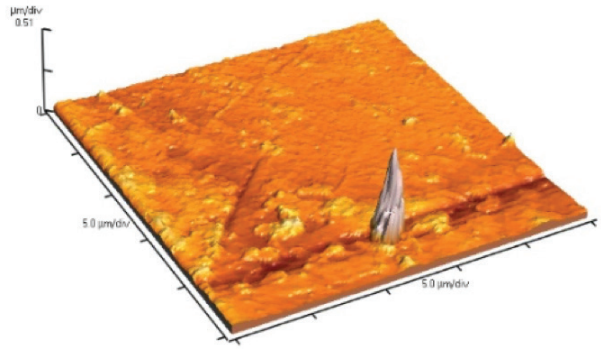


**Figure 3 -** AFM of US.

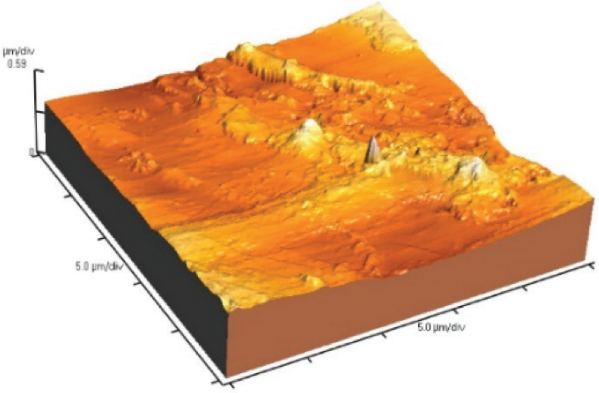


**Figure 4 -** AFM of UT.

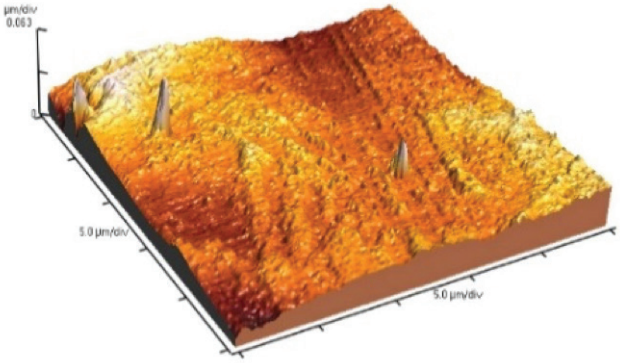


**Figure 5 -** AFM of UP.

## DISCUSSION

Based on their strength, longevity, conservative nature, biocompatibility and esthetics, veneers have been considered one of the most viable treatment modalities since their introduction in 1983 [7]. Esthetic veneers in ceramic materials demonstrate excellent clinical performance and as materials and techniques have evolved, veneers have become one of the most predictable, most esthetic and least invasive modalities of treatment. Studies that followed ceramic veneers for up to 15 years reported total failures of only7%, equaling approximately a 1% chance of failure per year [8,9].

However, the challenge with laminate veneers is to achieve ideal esthetics including color matching and subsequent color stability [10]. Although ceramics are considered to be color stable, discoloration of esthetic restorations may occur due to intrinsic or extrinsic factors. Intrinsic factors include changes within the material itself, while extrinsic factors involve adsorption or absorption of stains from the oral cavity. The smoothness of the surface of the restoration is one of the factors affecting extrinsic staining [11].

Periodontal prophylactic treatment is performed using supra- and subgingival scaling procedures to disrupt and thoroughly remove biofilm, calculus deposits, periodontal pathogens and deposits. Instrumentation options include hand scalers and ultrasonic scalers. Ultrasonic

scalers have a similar or greater efficiency than hand instruments in the removal of plaque and calculus on the surfaces of dental materials [12,13]. Accordingly, ultrasonic scalers have become an established tool for the removal of dental plaque and calculus. The cleaning procedures, especially using ultrasonic scaler, can increase surface roughness of dental restorations [14,15].

Recently, different types of nonmetallic instruments and ultrasonic tips such as rubber cups, plastic curettes, titanium curettes and air-power abrasive systems have been introduced as a substitute for implant maintenance to avoid alterations of the implant surfaces and they have been recommended for use in removing plaque from implants rather than metallic instruments [16-18]. These novel instruments may also be used with all-ceramic restorations in order to minimize the possible surface alterations that could result due to the use of conventional periodontal prophylactic instruments.

Accordingly, in the current study in order to determine which instrument is most appropriate for use on lithium disilicate surfaces; the effect of different scaling methods and instruments; (stainless-steel, titanium and plastic) on surface roughness and color stability of lithium disilicate ceramic was investigated.

The results of ΔRa readings indicated that scaling using ultrasonic titanium tip showed the highest mean value of ΔRa readings which was not statistically significantly different from the stainless-steel tip. However, it was statistically significantly different from the plastic tip. This may be attributed to the increased hardness of the titanium instruments (~751.9 MPa) compared to the stainless-steel instruments (~591.6 MPa)[19] Ito A et al. (1991) reported that the Vickers hardness of titanium nitride was 1170 and Nukata K et al. (1995) reported it to be 1370 [20] . The results of the profilometry were in agreement with the observations made from the atomic force microscopy which showed that lithium disilicate surfaces treated by stainless-steel and titanium tips revealed multiple large scratches while the scaling method based on a plastic instruments caused the least alterations.

Concerning color stability, the color change of the control (ΔE = 2.638) and the plastic (ΔE = 2.765) groups) recorded clinically acceptable value (ΔE > 3.5) while the titanium (ΔE = 4.431) and the stainless-steel (ΔE = 4.451) groups recorded clinically unacceptable values. These results were in accordance with the records of surface roughness indicating direct positive correlation between the surface roughness and the ΔE. This may be attributed to the removal of the surface glaze and the inferior ability of rough surface to reflect light. In 1981, Obregon et al. [21] stated that surface gloss texture and roughness can alter the color perception of porcelain restorations. Also, Kim et al. (2003)[22] found that color differences due to the surface conditions were large enough to be visually perceivable when measured with spectrophotometer. These results are in accordance with Motro PF et al. (2012)[10] who demonstrated that surface roughness affected the stainability of the IPS e.max Ceram (Ivoclar Vivadent) feldspathic ceramic and Akar GC et al. (2014)[23] who reported statistically significant differences in the surface-finishing protocols applied for the Lithium disiliacte ceramic systems and the Δ Ra, Δ E and translucency parameters. The smoothest surfaces and the lowest Δ E values were seen with the autoglazed method and the highest values were obtained with the polishing with adjustment kit plus diamond polishing paste method. Also, Gawriołek M et al. (2012)[11] reported that unpolished composite and ceramic specimens with a more developed surface have lower color stability.

Also, there was a direct positive correlation between bacterial accumulation and surface roughness. These observations may be attributable to the easier initial bacterial adhesion to and its more difficult removal from rough surfaces [24-26]. All groups showed statistically significant difference from the

control group. The highest mean value of bacterial accumulation was recorded for the titanium group (270 CFU/mL) where the treated surface presented the highest mean ΔRa values using profilometer and the deepest scratches according to atomic force microscopy.

These results were in accordance with the results of Quirynen et al. 1996 [27] who showed that bacterial colonization on rough titanium surfaces is greater than that on smooth surfaces and that reduction of surface roughness below a threshold value of Ra = 0.2 lm seems to have no further effect on quantitative and qualitative bacterial adhesion and colonization.

This was also in agreement with the results of Aykent et al. (2010)[28] who stated that the amount of viable S. mutans on restorative surfaces after different finishing techniques was correlated with surface roughness. The highest bacterial adhesion was observed on the surfaces finished with diamond rotary cutting instruments which were the surfaces with the highest surface roughness. This finding supports results of previous studies that found increased dental plaque formation on rough surfaces [29-31].However, some studies have not found a correlation between bacterial adhesion and surface roughness [32,33].

These varying results with regard to bacterial adhesion reinforce the idea that multiple factors influence bacterial adhesion other than surface roughness.[26,31,34]

Further studies, including clinical trials, of the effects of periodontal instrumentation onto ceramic restorations and into the most desirable approach for intraoral debridement of ceramic restorations would be desirable to clarify the significance of the observations made in this in vitro study.

## CONCLUSIONS

The surface roughness of lithium disilicate ceramics can be increased due to prophylactic periodontal treatment. Plastic based periodontal instruments have the least statistically significant effect on lithium disilicate ceramic surface roughness. There is a positive correlation between surface roughness and color stability of lithium disilicate ceramics.

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