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Bacterial Adhesion on Lithium Disilicate Ceramic Surface Exposed to Different Hydrofluoric Solutions

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Received: 18 July 2018; Accepted: 15 August 2018; Published: 19 August 2018

Abstract: The effect of Fluorine solutions on the surface and bacterial adhesion of lithium disilicate is a concern. The aim was to evaluate the surface roughness and the adhesion of *Streptococcus sanguinis* on lithium disilicate ceramic, under the influence of different solutions containing Fluorine. Forty lithium disilicate (IPS e.max Press Impulse) discs (2.5 × 5 mm) was divided into 4 groups (n = 10): artificial saliva (Group AS), 0.2% sodium fluoride (Group NaF), 1.23% acidulated phosphate fluoride gel (Group APF), and mouthwash (Group MW). Roughness analyses were performed before and after the immersion. The surface aspect was evaluated by scanning electron microscopy (SEM) and the adhesion of *Streptococcus sanguinis* were evaluated after immersion in the solutions. The data obtained were submitted to the analysis of variance (ANOVA) and the Tukey test ($\alpha = 0.05$). The Group APF presented a bigger roughness (3.263), statistically different to the other solutions. The bacterial adhesion in the Group APF (5.85) presented statistical difference to the other solutions. The SEM micrographs showed a rougher surface in Group APF. The 1.23% APF gel promoted major surface roughness and bacterial adhesion and could be inadequate for the use of patients with lithium disilicate ceramic restorations. Clinical significance: The Fluorine solution can affect the lithium disilicate ceramics, generating a rough and non-esthetic surface. This altered surface could be susceptible to bacterial adhesion, being directly related with periodontal health, the longevity of the restoration and the success of the rehabilitation.

Keywords: ceramics; biofilms; fluoride; Acidulated Phosphate Fluoride; bacterial adhesion

1. Introduction

The growing valorization of appearance exhibited in current society promotes development of restorative materials with excellent esthetic properties [1]. Therefore, ceramic dental restorations have gained wide popularity since they closely resemble natural teeth, principally when integration and balance exist between color, format and surface texture [1,2].

Dental ceramic is broadly used in indirect restoration fabrication, such as in single-unit total or multiple crowns [3], implant-supported prostheses, and ceramic fragments (laminated veneers or dental contact lenses) [4]. Among the available ceramics on the market, lithium disilicate stands out

from the other glass ceramics since its composition (fluorapatite crystals [$Ca_{10}(PO_4)6F_2$] and leucite [K_2O-Al_2O_3-$4SiO_2$]) grants high resistance and the ability to reflect light very naturally [4–7]. A recent retrospective study [8] of 43 partial and total lithium disilicate restorations demonstrated a 97.7% of cumulative survival rate, and 94.2% of cumulative success rate.

However, the surface of these restorations can be susceptible to erosive solutions, or even those that fluoride-containing in their composition, which could damage the surface of such restorations, promoting an increase in roughness [9,10]. It is known that restoration surface integrity is related to the clinical success of the rehabilitation treatment [6]. Some solutions used in oral hygiene can affect the properties of some restorative materials [11–13]. However, literature is scarce when assessing the influence of such solutions on lithium disilicate ceramics and their influence on bacterial adhesion. Nonetheless, it is known that the accumulation of bacterial plaque is proportional to the surface roughness of the material, being a direct relationship with periodontal health, and consequently, the longevity of the restoration [1,9].

As such, the present study evaluated the influence of different fluoride-containing solutions on the surface roughness of lithium disilicate ceramics, and the influence on the bacterial adhesion. The null hypothesis is that there would be no difference in the roughness and bacterial adhesion of the solutions tested.

2. Materials and Methods

Forty lithium disilicate discs (IPS e.max Press Impulse; Ivoclar Vivadent, Schaan, Liechtenstein), with 2.5 mm thickness and 5 mm diameter, were fabricated using the lost-wax technique [13] For this, 40 auto-polymerized resin specimens (Duralay; Reliance Dental Mgf. Co., Alsip, IL, USA) were initially fabricated with the same diameters, but with greater thickness. After polishing in a semiautomatic polishing machine (Aropol-2V; Arotec, Cotia, SP, Brazil), under constant irrigation with 240, 400, 800, and 1200 granulated sandpaper (CarbiMet 2; Buehler, Lake Bluff, IL, USA), the acrylic resin specimens obtained the desired 2.5 mm thickness. Subsequently, these acrylic resin specimens were transformed, by the injection method, into lithium disilicate specimens by using the lost-wax technique.

The ceramic discs were divided in 4 groups (n =10), according to the solution in which they were immersed (Group AS, Group NaF, Group APF, Group MW). The study design with the groups, solutions composition, pH and immersion period was described in Table 1. All the solutions were changed every 12 h for renewal and prevention of evaporation [13,14]. After the immersion in the fluoridated solutions, the discs were maintained in artificial saliva until the moment of the analysis, in a total of 21 days of immersion of the all groups. One specimen of each group was evaluated by scanning electron microscopy (SEM) with an increase of 300× after immersion in the solutions. The adhesion of Streptococcus sanguinis (S.sanguinis) was evaluated by a 71 microbiological analysis performed in triplicate, in three independent experiments (n = 9) after the 72 immersion in the solutions.

2.1. Surface Analysis

The surface roughness reading was performed by means of a profilometer (Dektak d-150; Veeco, Plainview, New York, NY, USA). The readings were performed before and after the immersion in the fluoridated solutions in 9 specimens of each group. The profilometer measuring point performed the reading 3 times (initially being positioned on the specimen surface center, and subsequently to the right and left of the first reading), and the total reading extension was 6 mm [21]. The Rt values (total height of the roughness profile) were measured by using a cut off of 500 µm at a constant time of 12 s [13,22]. The scanning electron microscopy (SEM) (n = 1) was used with a magnification of 300× to evaluate the surface morphology of the lithium disilicate discs after immersion in the solutions.

Table 1. Study design, groups distribution, solutions composition, pH and immersion period.

Groups	Composition	pH	Immersion Period
Group AS (artificial saliva)	[KCl (0.4 g L^{-1}), NaCl (0.4 g L^{-1}), $CaCl_2$-$2H_2O$ (0.906 g L^{-1}), NaH_2PO_4-$2H_2O$ (0.690 g L^{-1}), Na_2S-$9H_2O$ (0.005 g L^{-1})]	5.5 [15]	21 days in a bacteriological incubator (37 ± 1 °C)
Group NaF	0.2% NaF	5.14 [16]	73 h (simulating 3 daily brushings with fluoride toothpaste of 2 min each for a period of 2 years) [13]
Group APF	1.23% APF gel (fluoride-containing of 12,300 parts per million (ppm) with 0.34% hydrofluoric acid (HF), 2% NaF, and 0.98% phosphoric acid)	3 to 3.5 [17]	48 h (simulating 4 min of daily use over a period of 2 years) [13,18]
Group MW (mouthwash)	Thymol, eucalyptol, methyl salicylate, menthol, water, sorbitol solution, alcohol (30%), poloxamer 407, benzoic acid, mint and mint essences, sodium saccharin, sodium benzoate, green dye 3.	4.35 [19]	12 h (simulating 1 min of daily use over a period of 2 years) [13,20]

2.2. Microbiological Analysis

After the immersions were performed, the lithium disilicate discs were sterilized in gamma radiation (14.5 ± 0.05 kGy) for the subsequent performance of microbiological analysis [23], which was performed in triplicate, in three independent experiments (n = 9). The *Streptococcus sanguinis* IAL 1832 strain was used. The microorganism was maintained with 20% glycerol, frozen to −80 °C until the beginning of the test. For the performance of the inoculation and growth conditions, 100 µL of the microorganism culture was mixed with 5 mL of 1% glucose-supplemented brain heart infusion (BHI) broth (Difco Laboratories, Becton, Dickinson and Company, Detroit, MI, USA), and incubated overnight in 10% CO_2 (v/v) supplemented with 1% glucose at 37 °C for 6 hours (exponential growth phase) without agitation. The *S. sanguinis* cells were collected after centrifugation (6000× *g* for 5 min at 4 °C), washed 2 times with buffered saline solution (PBS; composition: NaCl 8 g/L, KCl 0.2 g/L, KH_2PO_4 0.2 g/L, $NaH_2PO_4 \cdot 2H_2O$ 1.41 g/L; pH 7), and resuspended in BHI broth to adjust the inoculum. A spectrophotometer (Spectronic 20; Bausch & Lomb, Rochester, New York, NY, USA) was used to adjust the inoculum at 600 nm (OD = 1 ± 0.02), aiming to achieve a final suspension of 10^7 cells/mL.

The lithium disilicate discs were covered with a film of human saliva, before the development of the biofilm, to simulate the oral cavity. The total non-stimulated saliva was collected and donated by 2 healthy volunteers that conceded their consent and signature, after approval of the project from the Ethics in Research Committee (Human Ethics in Research Committee of the Aracatuba Dental School—UNESP, N°69008816.3.0000.5420). Quantities of the saliva were united and centrifuged under 10,000× *g* for 10 min at 4 °C. The supernatant was sterilized by filtration and used immediately. The specimens were collected individually in a 24-well culture plate containing 1 mL of human saliva under aseptic conditions. Subsequently, they were incubated with agitation at 37 °C for 2 h, aiming to form the salivary film. The saliva covered discs were transferred to wells of another 24-well plate with 100 µL of *S. sanguinis* cell suspension (10^7 cells/mL) and 900 µL of 1% glucose-supplemented BHI broth. The plates were incubated in 10% CO_2 (v/v) at 37 °C for 1.5 h (adhesion phase) [24]. Subsequently, the discs were washed in 2 mL of 0.9% NaCl solution to remove the non-adhered cells. Finally, they were transferred to a 24-well culture plate containing fresh 1% glucose-supplemented BHI broth and incubated in 10% CO_2 at 37 °C for 48 h, with the culture medium renewed every 24 h [24].

For the live cell count, the discs were washed 2 times in a 0.9% NaCl solution and then immersed in 3 mL of 0.9% NaCl and sonicated at 7 W for 30 s (Sonifier, Branson Ultrasonics Corporation, Newtown,

CT, USA) to disorganize the biofilm structure. The resulting suspension was used to analyze the biofilm by means of the serial dilution in 0.9% NaCl, with 20 µL of the specimens plated in triplicate in Trypticase Soy Broth (TBS) (Difco Laboratories). The TBS plates were incubated in 10% CO_2 at 37 °C for 48 h, for the subsequent number count of the colony forming units (CFU) per mL [24].

2.3. Statistical Analysis

The surface roughness data were submitted to the two-way repeated measures variance of analysis (ANOVA) (factor 1: immersion solution; factor 2: period), while the microbiological data were submitted to the 1-way ANOVA (factor: immersion solution). The Tukey HSD post-hoc test was used with 5% significance. All tests were conducted with a 5% significance level (SPSS v. 20.0; SPSS Inc.). The SEM micrographs were compared visually.

3. Results

The immersion solution and the interaction between period and immersion interfere with the surface roughness (Rt) ($p < 0.001$) (Table 2).

Table 2. Two-way repeated measures analysis of variance (ANOVA) for ceramic roughness (Rt).

	SS	*df*	MS	F	*p*
Immersion solution	3.442	3	1.147	7.198	0.001 *
Period	0.468	1	0.468	2.773	0.106
Period x Immersion solution	5.411	3	1.804	10.676	<0.001 *
Error	5.406	32	0.169		

* $p < 0.05$ denotes significant statistical difference.

The discs immersed of Group APF presented a statistically significant increase in roughness, in relation to the evaluation of periods analyzed (before and after immersion) (Table 3).

Table 3. Mean values (standard deviation) of ceramic surface roughness (Rt) for each immersion solution, in different periods evaluated.

Groups	Initial Rt (µm)	Final Rt (µm)
Group AS	2.2 (0.4) Ba	2.1 (0.4) Ba
Group NaF	2.4 (0.3) ABa	2.3 (0.3) Ba
Group APF	2.2 (0.2) Bb	3.3 (0.6) Aa
Group MW	2.5 (0.5) Aa	2.3 (0.4) Ba

Means followed by same uppercase letter in column and lowercase letter on line do not differ ($p > 0.05$) with Tukey test.

By means of Figure 1, it is possible to observe that the lithium disilicate ceramic surface of Group APF presented bigger surface irregularities when compared with the other groups, corroborating with the roughness results (Table 3).

By means of Table 4, it is possible to observe that the immersion solution potentiated the bacterial adhesion on the ceramic ($p < 0.001$), presenting bigger bacterial adhesion mean values on the ceramic surface of Group APF, as demonstrated in Table 5.

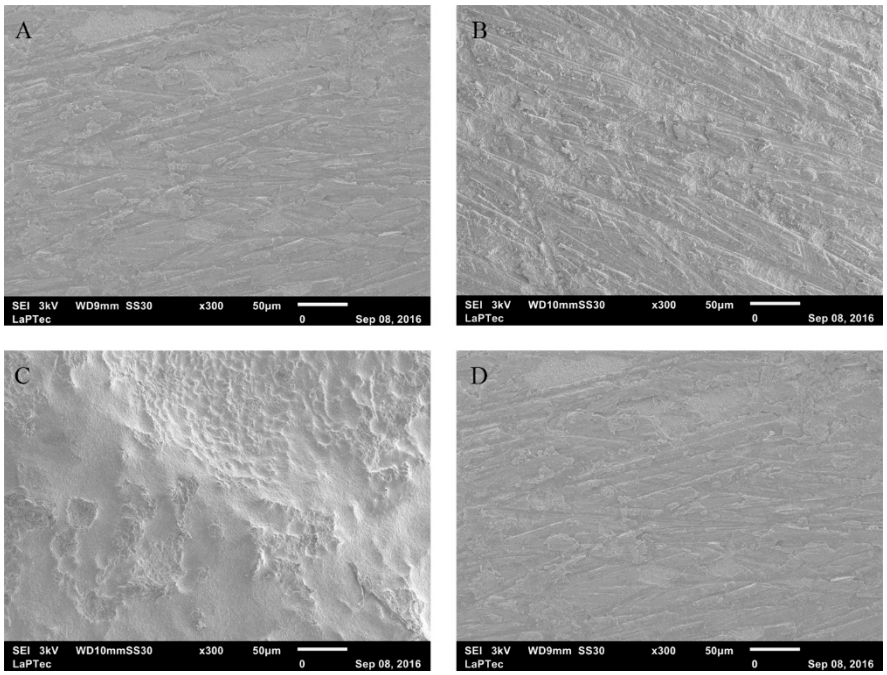

Figure 1. SEM representative micrographs of lithium disilicate after immersion in different Fluorine solutions. (**A**) Group AS; (**B**) Group NaF; (**C**) Group APF; (**D**) Group MW.

Table 4. One-way analysis of variance (ANOVA) for bacterial adhesion on ceramic surface.

	SS	*df*	MS	F	*p*
Immersion solution	7.712	3	2.571	11.392	<0.001 *
Error	7.221	32	0.226		
Total	946.855	36			

* $p < 0.05$ denotes significant statistical difference.

Table 5. Mean values (standard deviation) of ceramic surface bacterial adhesion according to immersion solution used.

Groups	Bacterial Adhesion (log CFU/mL)
Group AS	4.5 (0.5) B
Group NaF	4.5 (0.5) B
Group APF	5.9 (0.6) A
Group MW	4.6 (0.3) B

Means followed by same uppercase letter in column do not differ ($p > 0.05$) with Tukey test.

4. Discussion

The null hypothesis of the study was rejected since there was significant statistical difference between the groups, demonstrating that the lithium disilicate ceramic in contact with 1.23% APF gel presents greater values of roughness and bacterial adhesion.

The literature reports that ceramic materials in solutions with acidic conditions could accelerate the aging by means of cracks in the prosthesis structure [25]. Acidulated fluoride gel is commonly used to prevent dental caries [25], with its professional application being recommended every three months in patients with a high index of caries [9]. However, this gel presents low pH, which could directly influence the longevity of ceramic restorations in the oral cavity. The presence of ceramic restorations in the oral cavity of patients is generally disregarded in performing the use of APF gel

during a clinical session [9]. In this study, the continuous period of immersion was used aiming to reproduce the cumulative effect of the acidic solutions, simulating 2 years of hygiene habits [13,18,20].

Commercial APF gel at 1.23% has a fluoride-containing of 12,300 parts per million (ppm) with 0.34% hydrofluoric acid (HF), 2% NaF, and 0.98% phosphoric acid, which results in a low pH, around 3.5 to 3.9 [17,18]. Hydrofluoric acid has been associated with the dissolution of silica in dental bioglass ceramics [25]. Our results corroborate with such statements, since only the lithium disilicate discs immersed in 1.23% APF gel presented an increase in surface roughness values. In addition, the immersion could have caused an elevated dissolution of the glass matrix of the lithium disilicate discs, exposing the crystal phase [13].

The increase in roughness caused by the contact of the ceramic with the APF gel could cause an increase in bacterial plaque adhesion and alteration of the restoration color, affecting the esthetic quality of the rehabilitation [25]. In the present study, bacterial adhesion was evaluated, since the lithium disilicate discs in contact with 1.23% APF gel presented greater roughness values and bacterial adhesion, demonstrating the direct relation between these two factors. Since it is a primary etiologic factor of periodontal diseases, bacterial adhesion could lead to clinical failure by favoring the medium for tooth decay and damaging gingival health, altering the pink esthetic of the rehabilitations.

Additionally, the lithium disilicate discs used in the study were fabricated with the e.max Impulse System, recommended by the manufacturer for laminated and ceramic veneers. This rehabilitation is highly esthetic and require prostheses with color, texture, and an adequate format [26] and healthy soft tissue [27]. The increase in roughness could induce color alterations in the restorations [28] and cause an inflammation in the adjacent soft tissues [29], leading to failure of these esthetic restorations.

The *S. sanguinis* microorganism was used in this study for being responsible for the initial formation of dental biofilm [30] and being frequently encountered in oral mucosa [31]. This bacterium is responsible for biofilm formation which causes periodontal problems and tooth decay [23]. Therefore, it is necessary to diminish factors that lead to the adhesion of these first-colonizer bacteria, thus avoiding the maturation of the biofilm which could develop into oral diseases and clinical failures of ceramic restorations.

Despite the problems inherent in APF gel, it is one of the most used products by dental surgeons in controlling dental caries, demonstrating satisfactory clinical results [32,33]. However, the authors suggest that professionals take more precautions during the application of this product, avoiding contact with ceramic restorations. In this way, dental caries is prevented and the lithium disilicate prosthesis surface is preserved.

The other acidic solutions tested did not demonstrate a statistically significant difference in relation to roughness or bacterial adhesion. Therefore, the frequent use of these products in the prevention of tooth decay can be recommended. These solutions are used by the patient in products such as toothpastes and mouthwash [13].

The present study presents limitations by being in vitro, which simulates the application of the fluoridated solutions in a static way. Thus, clinical studies are necessary to evaluate the use of the tested solutions in the oral cavity.

5. Conclusions

Taking the limitations of this study into consideration, the exposure of lithium disilicate ceramic restorations to 1.23% APF gel increases surface roughness and bacterial adhesion, which can be harmful to the oral health of patients and could be inadequate for the use of patients with lithium disilicate ceramic restorations.

Author Contributions: D.M.d.S. and M.C.G. conceived and designed the experiments; E.V.F.d.S., R.A.d.M., A.O.M. and B.C.Z.M. performed the experiments; E.C.R. and V.A.R.B. provided important equipment for the experiments; all the authors analyzed the data; S.B.B., E.V.F.d.S. and R.A.d.M. wrote and review the paper.

Funding: This research was funded by Research Support Foundation of the State of Sao Paulo—FAPESP grant number 12/18880-4.

Conflicts of Interest: The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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