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Original Research

Surface Characteristics of Different Dental Cements after Incubation with Adherent and Non-adherent Strains of Streptococcus Mutans

Características da Superfície de Diferentes Cimentos Dentários após Incubação com Cepas Aderentes e Não Aderentes de Streptococcus Mutans

Características Superficiales de Diferentes Cementos Dentales tras la Incubación con Cepas Adherentes y no Adherentes de Streptococcus Mutans

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Abstract

The aim of this study was to evaluate surface characteristics and Knoop hardness of two resin cements after incubation with adherent and non-adherent strains of *Streptococcus mutans.* The ATCC 25175 strain and a mutant isolated due to its low biofilm forming capacity and normal acidogenic capacity were used. Disks with 5 mm diameter and 2 mm thickness were fabricated with RelyX U200 and Allcem following the manufacturer's instructions. Specimens were incubated in TSB medium with the adherent or non-adherent strains at 37ºC for 30 days, changing the culture medium every day, whereas control group specimens were incubated with sterile culture medium. Then, all specimens were subjected to microhardness Knoop. Representative specimens were examined using scanning electron microscopy (SEM). Tukey multiple comparisons test and unpaired t-test was used to compare two groups (α = 0.05). The resin cement RelyX U200 do not presented degradation or microhardness alteration under conditions tested. On the other hand, the resin cement Allcem presented more organic matrix degradation, exposing more filler particles after incubation with adherent strain than after incubation with the non-adherent strain. Microhardness of Allcem was smaller after incubation with ATCC strain than with non-adherent strain, and both presented smaller microhardness than the specimens of the control group. In conclusion, the resin cement RelyX U200 was not affected by the medium incubation with both adherent or non-adherent bacteria. The resin cement Allcem was more affected by the adherent bacteria strain then non-adherent regarding to surface topography.

Descriptors: Cimentos de Resina; Dureza; Streptococcus mutans.

Resumo

O objetivo deste estudo foi avaliar as características de superfície e a dureza Knoop de dois cimentos resinosos após incubação com cepas aderentes e não aderentes de Streptococcus mutans. A cepa ATCC 25175 e um mutante isolado devido à sua baixa capacidade de formação de biofilme e capacidade acidogênica normal foram utilizados. Discos com 5 mm de diâmetro e 2 mm de espessura foram fabricados com RelyX U200 e Allcem seguindo as instruções do fabricante. Os espécimes foram incubados em meio TSB com as cepas aderentes ou não aderentes a 37 °C por 30 dias, trocando o meio de cultura todos os dias, enquanto os espécimes do grupo controle foram incubados com meio de cultura estéril. Em seguida, todos os espécimes foram submetidos à microdureza Knoop. Os espécimes representativos foram examinados usando microscopia eletrônica de varredura (MEV). O teste de comparações múltiplas de Tukey e o teste t não pareado foram usados para comparar dois grupos (α = 0,05). O cimento resinoso RelyX U200 não apresentou degradação ou alteração de microdureza sob as condições testadas. Por outro lado, o cimento resinoso Allcem apresentou maior degradação da matriz orgânica, expondo mais partículas de carga após a incubação com a cepa aderente do que após a incubação com a cepa não aderente. A microdureza do Allcem foi menor após a incubação com a cepa ATCC do que com a cepa não aderente, e ambos apresentaram menor microdureza do que os espécimes do grupo controle. Em conclusão, o cimento resinoso RelyX U200 não foi afetado pela incubação do meio com bactérias aderentes ou não aderentes. O cimento resinoso Allcem foi mais afetado pela cepa de bactérias aderentes do que não aderentes em relação à topografia da superfície.

Descritores: Cimentos de Resina; Dureza; Streptococcus mutans.

Resumen

El objetivo de este estudio fue evaluar las características superficiales y la dureza Knoop de dos cementos de resina después de la incubación con cepas adherentes y no adherentes de Streptococcus mutans. Se utilizó la cepa ATCC 25175 y un mutante aislado debido a su baja capacidad de formación de biopelículas y capacidad acidogénica normal. Se fabricaron discos de 5 mm de diámetro y 2 mm de espesor con RelyX U200 y Allcem siguiendo las instrucciones del fabricante. Las muestras se incubaron en medio TSB con las cepas adherentes o no adherentes a 37 °C durante 30 días, cambiando el medio de cultivo todos los días, mientras que las muestras del grupo control se incubaron con medio de cultivo estéril. Luego, todas las muestras se sometieron a microdureza Knoop. Las muestras representativas se examinaron mediante microscopía electrónica de barrido (SEM). Se utilizó la prueba de comparaciones múltiples de Tukey y la prueba t no pareada para comparar dos grupos (α = 0,05). El cemento de resina RelyX U200 no presentó degradación ni alteración de la microdureza en las condiciones probadas. Por otro lado, el cemento de resina Allcem presentó una mayor degradación de la matriz orgánica, exponiendo más partículas de relleno después de la incubación con la cepa adherente que después de la incubación con la cepa no adherente. La microdureza de Allcem fue menor después de la incubación con la cepa ATCC que con la cepa no adherente, y ambas presentaron una microdureza menor que las muestras del grupo control. En conclusión, el cemento de resina RelyX U200 no se vio afectado por la incubación del medio con bacterias adherentes o no adherentes. El cemento de resina Allcem se vio más afectado por la cepa bacteriana adherente que por la no adherente con respecto a la topografía de la superficie.

Descriptores: Cementos de Resina; Streptococcus mutans.

INTRODUCTION

Some clinical problems such as tooth deterioration, calculus formation, and gingivitis are among the drawbacks caused by bacteria that adhere to tooth structure and restorative materials[1-3]. The dominant microorganism in the population of bacteria that form the dental plaque is *Streptococcus mutans*. Due also to its adhesion capacity, this bacterium contributes to most of the process of development for dental caries and periodontal disease in patients with active caries, making this microorganism the main responsible for dental loss[4-7].

The adhesion process is mediated by a series of enzymes, including those that use sucrose to produce adhesive extracellular glucans, responsible for keeping the bacterial biofilm adhered to the tooth surface[8]. Another feature related to the pathogenicity of this bacterium is the metabolic acid generation, making it able to attack the dental enamel, initiating the caries process. Acid is generated as a by-product of fermentative processes, in which carbohydrates such as glucose are degraded into organic acids to release energy for cellular activities[6].

Cementing procedure plays a crucial role when applying indirect restorations of all types[9]. In this sense, the cementing procedure is critical and involves some steps that could jeopardize the final quality of the restoration, if they are not carefully followed and carried out. Moreover, a cemented restoration has a cement line that stays many times at the cervical area, which relates directly to the soft tissue[10]. It has been shown that the type and properties of a cement agent influence bacterial adhesion; since different cement agents have different composition, surface characteristics and free energy[11]. It has been shown that different restorative and cement materials present differences in biofilm formation[12]. In the same sense it has been shown that material with higher surface free energy exhibits more biofilm formation compared to those with lower surface free energy[13]. However, not only the surface energy, but also the roughness of a surface, influences microbial adherence[14,15]. From a clinical standpoint, a cement line is very difficult and, in some cases, impossible to polish when it is between two teeth. Thus, without polishing the surface is rougher and roughness seems to be more important to the accumulation of a biofilm, whereas the surface free energy impacts more surfaces with similar pattern of roughness[16].

Thus, the aim of this study *in vitro* was to evaluate surface characteristics of two resin cements after incubation with adherent and non-adherent strains of *Streptococcus mutans* through Knoop hardness and Scanning Electron Microscopy (SEM). The hypothesis was that the surface characteristics and Knoop hardness of resin cements would not be affected after incubation with adherent strain of *Streptococcus mutans* compared to non-adherent.

MATERIAL AND METHOD

The resin cement RelyX U200 (3M ESPE, St Paul, MN, USA) and Allcem (FGM, Joinville, SC, Brazil) were use in this study. The present study was conducted according to the experimental design illustrated in Figure 1.

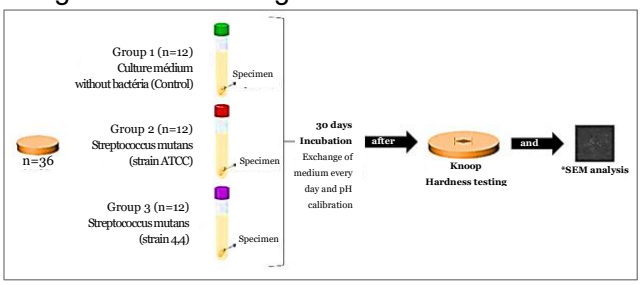

Figure 1. Design study.
*Note: SEM (Scanning Electron Microscopy)

- *Specimens' preparation*

The resin cements RelyX U200 (3M ESPE) and Allcem (FGM) were mixed following manufacturer's instructions and placed into a polymer mold (5mm in diameter x 2mm deep) (Figure 2 (1)). A transparent polyester strip was then placed over the filled mold with resin cements and a controlled static load of 235 g was applied for 60 s on a microscope slide using a 10 mm diameter ball (Figure 2 (2)). The excess cement was removed with a microbrush. The load and the microscope slide were removed and resin cement was exposed to a LCU source (Bluephase, Ivoclar Vivadent AG, Schaan, Liechtenstein) having an irradiance of 1,000 mW/cm^2 as measured using a curing radiometer (Model 100, Demetron Research Corporation, Danbury, CT) (Figure 2 (3)). The light guide ended with the polyester strip. After removing the mold, the specimens were observed by optical microscopy at 40 x magnification (Olympus Corp, Tokyo, Japan) to check if surfaces were smooth and free from any pores of discontinuity. Defective specimens were discarded and replaced with a new one. All specimens were stored in distilled water at 37°C for 24 h. A total of 36 specimens were made for each resin cement and randomly divided into 3 groups (n=12): Group 1 - Culture medium without bacteria (Control); Group 2 - *Streptococcus mutans* (strain ATCC); and, Group 3 - *Streptococcus mutans* (strain 4.4).

- *Bacterial strain*

In this study, ATCC25175 strain of *Streptococcus mutans*, obtained from Fundação André Toselo (Campinas, SP, Brazil) and *S. mutans* strain (strain 4.4) were used. Strain 4.4 was isolated in the Microbiology and Biochemistry laboratory due to its normal acidogenic capacity and reduced capacity of biofilm formation.

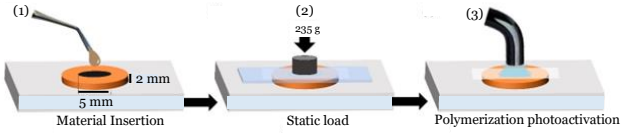

Figure 2. Specimens' preparation. (1) Material insertion into mold. (2) Placement of transparent polyester strip and microscope slide with static load. (3) Photoactivation of material polymerization with manufacturer's recommended time.

- *Cultivation of the bacteria*

Bacterial cells were stocked at -20ºC in 40% glycerol: water (vol/vol) and cultured on 5 mL of Tryptic Soy Broth (TSB) from Difco (Sparks, MD, USA), prepared according to the manufacturer instructions (pH 7) and autoclaved at 121ºC during 15 min prior to use. Growth was in a candle jar with anaerobic environment (obtained after lighting a candle inside the jar to consume oxygen), at 37ºC, as previously described by de Menezes et al.[6].

- *Specimens' incubation with S. mutans*

Each specimen was incubated in 5 mL TSB medium with the adherent strain ATCC 25175 or non-adherent strain 4.4 of *S. mutans* at 37ºC for 30 days. The medium was changed every day and the pH of the culture medium was measured with a calibrated pH meter at 25 ° C in each change. For the control groups, specimens were incubated with sterile culture medium (6).

- *Knoop hardness testing*

After exposure to the culture medium for 30 days, the specimens were washed with distilled water using a triplex syringe operated at a pressure of 45 psi and air dried. Knoop microhardness measurements were performed using a microhardness tester (HMV-2; Shimadzu Corp., Tokyo, Japan) under a load of 50 g applied for 15 s. Three indentations were made in each specimen at depth of 100 µm from the top surface at a distance of 1 mm between them. The average value of the three readings was recorded as the Knoop hardness for that specific specimen.

- *Scanning Electron Microscopy (SEM) analysis*

In order to observe the surface characteristics, representative specimens of each resin cement and culture medium were sputter coated with gold (Balzers-SCD 050, Balzers Union, Aktiengeselischaft, Furstentun, Liechtenstein) for 180 seconds at 40 mA. The specimens were then mounted on coded brass stubs and examined using SEM (LEO 435 VP, Cambridge, UK), operated at 20 Kv, by a single operator. Specimens were examined under magnifications of 3000x.

- *Statistical analysis*

Data were tested for normality (D'Agostino & Pearson). Multiple comparisons (between groups) were performed using the Tukey multiple comparisons test, whereas unpaired t-test was used to compare two groups (between the two cements tested) (α = 0.05).

RESULTS

- *Knoop Hardness*

Significant difference in Knoop hardness for resin cements was detected only after incubation with the non-adherent strain ($p<0.05$) (Table 1). For the results obtained for strain 4.4, the mean values of Knoop hardness of the resin cement RelyX U200 was significantly higher than resin cement Allcem ($p<0.05$). No statistical difference was found between RelyX U200 and Allcem for control group and strain ATCC ($p>0.05$). The mean values of Knoop hardness obtained for control group for resin cement Allcem was significantly higher than strain ATCC and strain 4.4 ($p<0.05$). Strain ATTC was significantly higher than strain 4.4 ($p<0.05$). For resin cement RelyX U200 no statistical difference was found among control group, strain ATCC and strain 4.4 ($p>0.05$).

Table 1 - Knoop hardness means ± standard deviation for two resin cements (RelyX U200 and Allcem) after submitted to two bacterial culture mediums.

Resin Cements	Control group	Strain ATCC	Strain 4.4
RelyX U200	55.5 ± 7.8 Aa	52.2 ± 11.0 Aa	54.0 ± 8.9 Aa
Allcem	62.2 ± 7.2 Aa	49.7 ± 16.8 Ba	41.0 ± 5.8 Cb

Values followed by the same lower-case within the same column (unpaired t-test) and upper-case (Turkey's multiple comparisons test) in the same row are statistically similar (α = 5%).

The mean values of pH are shown in Figure 3. After incubation with adherent and non-adherent strains, the pH became acidified compared to the control group ($p<0.05$), No difference was observed between the two strains, for both resin cements ($p>0.05$). No statistical difference was found between RelyX U200 and Allcem for all groups ($p>0.05$).

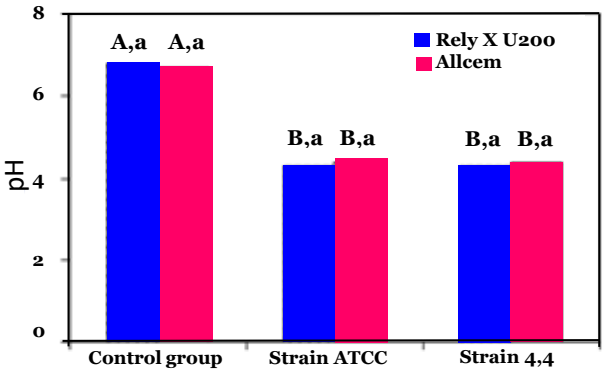

Figure 3 - Average hydrogen potential (pH) after every 24 hours during the 30-day incubation period.

Scanning Electron Microscopy

SEM images of RelyX U200 and Allcem after incubation, adherent strain ATCC and non-adherent strain 4.4 are shown in Figure 4. The resin cement RelyX U200 did not show any surface degradation was observed after incubation under all conditions tested (Figures 4A, 4B and 4C). For Allcem resin cement no surface degradation was

observed after incubation with culture medium (Figure 4D). Figure 4E shows greater organic matrix degradation, exposing more filler particles, when incubation was done with wild type adherent strain. On the other hand, the image in Figure 4F presented smaller degradation after incubation with non-adherent strain.

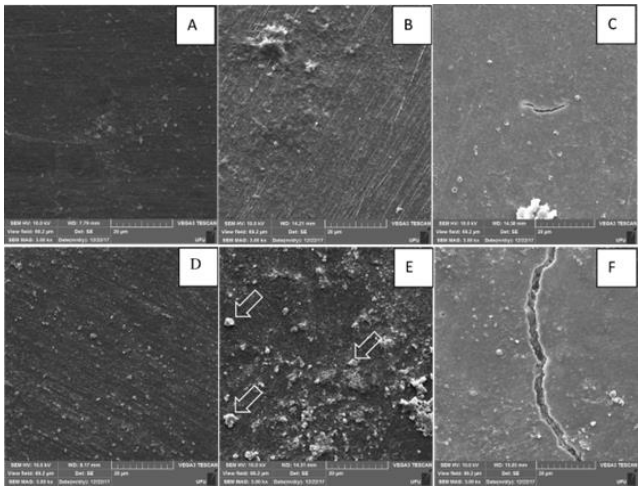

Figure 4. SEM images (2000 x magnification) resulting from incubation with *Streptococcus mutans* on resin cements surface RelyX U200 (A-C) and Allcem (D-F). (A and D) control - culture medium without bacterium; (B and E) *Streptococcus mutans*, adherent strain ATCC; and (C and F) *Streptococcus mutans*, non-adherent strain 4.4. Arrows indicate filler particles exposed (Figure 1E).

DISCUSSION

In the present study, the surface of the resin cement RelyX U200 was not altered by the incubation with both adherent and non-adherent strain e *S. mutans* (Figures 4A, 4B and 4C), in agreement with the microhardness maintenance of this cement in the different incubation conditions (Table 1). The resin cement Allcem showed surface degradation after incubation with non-adherent 4.4 strain and a higher degradation after incubation with adherent ATCC strain, suggesting that the adhesivity promote the degradation (Figures 4D, 4E and 4F) as observed earlier[7]. On the other hand, the microhardness values to Allcem resin cement were higher when incubated with the adherent strain than non-adherent strain (Table 1), regardless the pH after incubation with adherent or non-adherent strains was the same (Figure 3). Thus, the results indicated that the hypothesis was partially accepted.

The microhardness reduction of the resin cement Allcem was also observed in relation to the resin cement RelyX U200 in the same condition of incubation, that is, with non-adherent strain 4.4 (Table 1), suggesting that the Rely X U200 is more acid resistant compared to Allcem resin cement. Though, it could be inferred that the organic matrix of this cement is more prone to acid attack, compared to RelyX U200.

The composition of the cements evaluated is different regarding amount of chemicals and photo-initiators molecules, monomeric distribution that includes dissimilar ratio of high and low molecular weight monomers. Furthermore, the filler content is also different, thus, all these could explain the results. Nevertheless, as manufacturers do not provide the composition and distribution of resin cement components, care must be taken not to extrapolate the results directly to the clinic. On the other hand, as Allcem showed less microhardness and its organic matrix was more washed away compared to RelyX U200, it could then be inferred that the cross-link rate in the U200 monomeric chain was more resistant to being broken by the studied incubation media. Furthermore, the RelyX U200 filler particles are more rounded, and it could have better distribution[17]. In this way, less internal stresses would lead to a better behavior between organic matrix and particulate charge. As both studied cements had the same color and were photo-activated in the same way, that is, with the same energy density trying to simulate the clinical condition as closely as possible, it could be thought that the cement line exposed in the oral cavity would be more prone to degrade bringing more problems of accumulation of residues and retention of plaque, as well as degradation.

Regarding the medium studied an explanation for the differences in microhardness between strains for Allcem resin cement can be given by the fact that the medium that degraded more organic matrix, as seen in the SEM micrographs (ATCC) exposed more filler particles on the surface. Thus, as the diamond tip of the microhardness reached more exposed particles, the microhardness was greater. On the other hand, the incubation with 4.4 strain, which degraded less organic matrix resulted in less microhardness because the organic matrix has a lower microhardness than the filler particles. Moreover, the microhardness of Allcem resin cement after incubation in control conditions was almost unchanged as shown by SEM, thus, the microhardness was higher.

Considering that 4.4 strain acidifies the medium in a similar way to the ATCC strain (Table 1), we can suggest that considering surface characteristics, the adhesion itself is the main cause of damage, due to the acid environment created by fermentation. In fact, we know that, *in vivo*, the more adherent strains are more cariogenic than the less adherent ones[7]. Furthermore, the adhesion capacity seems to interfere in the disintegration of the Allcem's organic matrix, since the specimens surface incubated with adherent strain were more affected than those incubated with less adherent strain. Future studies should be carried out to investigate other types of resin cements, mechanical properties and aging.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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