

## ANTIMICROBIAL ACTIVITY OF DIFFERENT DENTAL CEMENTS ON *AGGREGATIBACTER ACTINOMYCETEMCOMITANS*

### ATIVIDADE ANTIMICROBIANA DE DIFERENTES CIMENTOS ODONTOLÓGICOS SOBRE *AGGREGATIBACTER ACTINOMYCETEMCOMITANS*

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#### ABSTRACT

**Objectives:** The objective of this study was to evaluate, *in vitro*, the possible antimicrobial activity against the *A actinomycetemcomytans* of three dental cements: glass-ionomer cement, zinc phosphate cement and resin cement. **Material and Methods:** Strains of *A actinomycetemcomitans* ATCC 29522 were used. The microorganism was grown in BHI Agar and transferred to tubes containing sterile saline solution. The suspension was calibrated to a similar turbidity to the 0.5 tube from McFarland scale. A base layer consisting of 20 ml of BHI agar was placed in sterile Petri 90 x 15 mm plates. After solidification, was added 0,1 uL of microbial suspension, and three wells with 6 mm in diameter and 1mm deep were made, the wells were filled with cements with zinc phosphate cement, glass-ionomer cement and resin cement indicated for permanent cementation for fixed prostheses immediately after handling and positive (chlorhexidine) and negative (saline) controls. **Results:** After 48 hours the presence or absence of inhibition halo of microbial growth was analyzed around the specimens. **Conclusion:** Zinc phosphate cement and glass ionomer cement tested showed antibacterial activity against *A actinomycetemcomitans* unlike resin cement.

**Descriptors:** Periodontium • Dental Cements • Periodontal Diseases

#### RESUMO

O objetivo deste estudo foi avaliar *in vitro* a ação antibacteriana de três cimentos odontológicos utilizados para fixação de próteses fixas: ionômero de vidro (Vitro CemR), fosfato de zinco (Cimento LSR) e cimento resinoso (Dual CementR) ao microrganismo *Aggregatibacter actinomycetemcomitans* ATCC 29522. A espécie foi ajustada a 0,5 de MacFarland. Foi realizado o teste de difusão em ágar em triplicata, em meio BHI onde 0,1 µL foi semeado por placa. Um corpo de prova em formato de disco de cada material foi colocado em poços de 6mm de profundidade. Como controles positivo e negativo foram utilizados clorexidina e soro fisiológico respectivamente. Após a distribuição dos corpos de prova, as placas foram incubadas a 37°C por 48 horas. O halo de inibição formado foi mensurado. O cimento resinoso não apresentou atividade antibacteriana, diferentemente do fosfato de zinco e ionômero de vidro, que apresentaram positividade em todas as amostras, havendo diferença entre eles com o fosfato de zinco apresentando maior atividade. Cimentos de fosfato de zinco e ionômero de vidro possuem atividade antimicrobiana, diferentemente dos cimentos resinosos.

**Descritores:** Periodonto • Cimentos Dentários • Doenças Periodontais

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## INTRODUCTION

The teeth loss in adults is strongly associated with the periodontal disease which is the leading cause of teeth loss<sup>1</sup>. It consists of a chronic inflammatory process resulting in the loss of the tooth support.

Aggressive periodontitis consists of a rare type of periodontal disease; it affects approximately 1% of the adult population. The patient presents rapid loss of periodontal attachment and severe destruction of the alveolar bone. On clinical examination it is observed that the amount of biofilm is not proportional to the destruction degree of periodontal tissues, but there is a direct relation with the founded amount of *Aggregatibacter actinomycescomitans*<sup>2-10</sup>.

Microbiological and host factors are determinants of pathogenicity of the microorganism. The colonization by *A actinomycescomitans* is facilitated by several factors of virulence, facilitators of colonization, invasion and destruction of periodontal tissues<sup>3,5,6</sup>.

Patients who are conventional fixed prostheses users present an additional risk to the colonization by periodontopathogenic microorganisms, which is the union interface between the prosthesis and the teeth. Different cements are used for the union of the prostheses to the teeth<sup>11</sup>.

The objective of this study was to evaluate, *in vitro*, the possible antimicrobial activity against the *A actinomycescomitans* of three dental cements: glass-ionomer cement, zinc phosphate cement and resin cement.

## METHODS

**Microorganisms Reactivation**

Strains of *A actinomycescomitans* ATCC 29522 were used. The microorganisms were obtained on the Reference Materials Laboratory of the National Institute for Quality Control in Health (*Oswaldo Cruz Foundation - Fiocruz, Rio de Janeiro, RJ, Brazil*). The reactivation was performed in heart and brain infusion broth - BHI (DIFCO, Detroit, Michigan, USA) at 37°C. Microorganism suspensions were prepared in saline solution, under  $1.5 \times 10^6$  ml microorganism<sup>-1</sup> concentration, consis-

tent with the MacFarland scale.

The microorganism was grown in BHI Agar (Difco®, Detroit, Michigan, USA) at 37°C for 48h. After growing, the microorganism was removed from the surface of the plaques with the sterile swabs help, and transferred to tubes containing sterile saline solution. The suspension was calibrated to a similar turbidity to the 0.5 tube from McFarland scale, which corresponds to  $10^6$  cells/ml; proven by the reading through spectrophotometer (*Micronal S/A - São Paulo, SP, Brazil*).

**Antimicrobial Activity Analysis**

A base layer consisting of 20 ml of BHI agar (Difco, Detroit, USA) was placed in sterile Petri 90 x 15 mm plates. After solidification, was added 0,1 uL of microbial suspension, and three wells with 6 mm in diameter and 1mm deep (one for each material) were made by removing agar in equidistant points, using sterile instruments.

The wells were filled with cements with zinc phosphate cement, glass-ionomer cement and resin cement indicated for permanent cementation for fixed prostheses immediately after handling and positive (chlorhexidine) and negative (saline) controls.

The Petri plaques were taken to a bacteriological incubator at 37°C under microaerophilic conditions. After 48 hours the presence or absence of inhibition halo of microbial growth was analyzed around the specimens. When present, its diameter was measured with good lighting conditions, with the aid of an electronic digital caliper Stainless Steel - Digimess®, Hong Kong, China). In positive and negative controls the presence or absence of microbial growth was analyzed.

**Statistical analysis**

Kolmogorov-Smirnov test demonstrated that the data of the variable "inhibiting halo" showed normal distribution.

ANOVA test one criterion, followed by the post hoc Tukey test, was used to evaluate the existence of differences in the variable "inhibition halo" between each one of the evaluated cements: zinc phosphate cement(C1), glass ionomer cement (C2) and resin cement(C3).

The significance level was set at 5%.

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The analyses were performed using GraphPad Prism 1.5 software (San Diego, CA, USA).

## RESULTS

The Table 1 presents the inhibition halo formed for the tested cements as well as for positive and negative controls.

ANOVA test one criterion showed the presence of difference in the "inhibiting halo" variable between the cements. D'agostinho Tukey & Pearson test showed that "inhibiting halo" was: higher in C1 when compared to C2 ( $p < 0.05$ ), and C3 ( $p < 0.05$ ); higher in C2 when compared to C3 (resin cement) ( $p < 0.05$ ).

## DISCUSSION

The periodontal insertion loss has been responsible for the early loss of teeth in

a significant range of the population(10). Several microorganisms participate in the etiology of the periodontal disease<sup>11-14</sup>. Prevention methods need to be widely used aiming to control this disease, as offering orientation and oral hygiene prac-

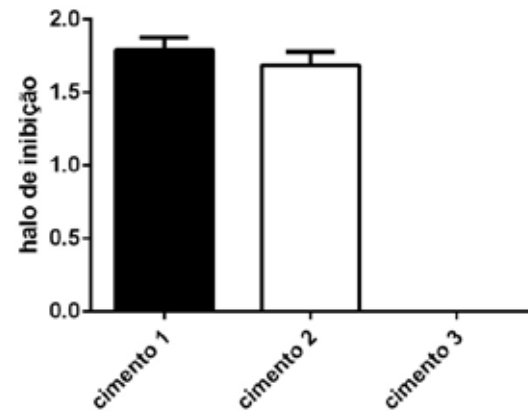


Table 1 - Mean, standard deviation and comparison between value groups of the variable "inhibiting halo".

C1	C2	C3	Valor de p
1.79 ± 0.08	1.68 ± 0.09	0.00 ± 0.00	<0,05 <sup>1,2,3</sup>

\* P value obtained by ANOVA one criterion followed by the post hoc Tukey.

<sup>1</sup> C1 versus C2

<sup>2</sup> C1 versus C3

<sup>3</sup> C2 versus C3



Figure 1 - Mean and standard deviation of the variable values "inhibiting halo"



tices<sup>12</sup>, and the use of specific antimicrobials.

In the classification of the periodontal diseases, the aggressive periodontitis is characterized by a rapid insertion loss in patients that do not present a biofilm compatible with the evolution of the disease, and that present themselves without systemic changes<sup>15</sup>.

*A actinomycescomitans* participates in the etiology of the periodontitis aggressive<sup>5</sup>, so the control of this microorganism becomes fundamental in the prevention and treatment of this disease.

The restoration and the maintenance of the periodontal health are fundamental to obtain success in fixed prostheses<sup>11</sup>. The use of fixed prostheses on natural teeth presents a space between the tooth and the prosthesis that is filled with cement and is called cementation line. This space is externalized in the cervical margins of the crowns, usually extending subgingivally to a 0.5 mm limit. The searching for the best possible adaptation has always been the goal for prosthodontists, as through that space bacterial infiltration can occur, causing decay and the periodontal disease.

Dental cements can actively participate in the prevention of periodontal disease by presenting antimicrobial activity. The results of this study were very useful from a scientific point of view, given that they show statistically significant values for the tested cements, with positivity zinc phosphate cement and glass ionomer cement and with negativity for the resin cement for antibacterial activity. The founded values corroborate to those from Kla1 *et al*<sup>6</sup> (2014) regarding the zinc phosphate cement and the resin cement, but not in relation to the glass ionomer cement.

In the present study, zinc phosphate cement presented the highest antibacterial activity among the tested cements. That activity is certainly due to the presence of oxides in the composition of this material. This characteristic can be an indication for the use of such material at with risk patients to the periodontal disease.

Although it is the most studied material among the tested sealers, the resin cement

did not present antibacterial activity, which is in line with the other studies described in the literature<sup>17, 18</sup>

The glass ionomer cement tested showed antibacterial activity against *A actinomycescomitans*. This result is in accordance with the results from<sup>19</sup>.

This study show positive antibacterial activity for glass ionomer cements, and attribute this activity to fluoride release. This release occurs both in fresh state and after the polimerization, being more significant in the fresh state.

The glass ionomer pH also favors the antibacterial activity of this material<sup>20</sup>. Authors present the results of studies that establish a correlation between low pH and fluoride release, that is, the lower the pH, the lower the fluoride concentration needed to develop antibacterial activity<sup>20</sup>. In the current study the antibacterial action of the glass ionomer cement was satisfactory with respect to *A actinomycescomitans*, what suggests that the use of cement, besides of the zinc phosphate cement, is also suitable for the cementation of fixed prostheses in patients with a history of periodontal disease.

The cements were evaluated in this study for a 48 hours period. Other studies with alternative methods, and for longer periods of time are needed to confirm the findings, however, the authors consider important the obtained results, because there were statistical differences between the tested cements with two materials presenting positive antimicrobial activity and one of them presenting negative activity, what makes this study important to be added to others in this important area of study of great clinical relevance in dental practice.

## CONCLUSION

Considering the limitations of this study, we concluded that zinc phosphate cement and glass ionomer cement tested showed antibacterial activity against *A actinomycescomitans* unlike resin cement and zinc phosphate cement showed the highest antibacterial activity among the evaluated materials, being statistically superior to the others.

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