

## ***In vitro* antimicrobial activity of different gutta-percha points and calcium hydroxide pastes**

Atividade antimicrobiana *in vitro* de diferentes composições de cones de gutta-percha e pastas de hidróxido de cálcio

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**Abstract:** The aim of this study was to evaluate the antimicrobial activity of different trademarks and compositions of gutta-percha points and calcium hydroxide pastes used in endodontic therapy. The evaluated material consisted of gutta-percha points containing calcium hydroxide (Roeko™), gutta-percha points containing chlorhexidine (Roeko™), two conventional gutta-percha points (Endo Points™ and Roeko™) and two calcium hydroxide pastes (Calen™ and Calen/PMCC™). Antimicrobial tests included five species of microorganisms: *Escherichia coli* (ATCC10538), *Staphylococcus epidermidis* (ATCC12228), *Staphylococcus aureus* (ATCC6538), *Pseudomonas aeruginosa* (ATCC27853), and *Micrococcus luteus* (ATCC9341). The Agar diffusion method was employed. The plates were kept at room temperature for 2 h for prediffusion and then incubated at 37°C for 24 h. The triphenyltetrazolium chloride gel was added for optimization and the zones of inhibition were measured. Statistical evaluation was carried out using analysis of variance and Tukey Test. The obtained results showed that all microbial species used in the study were inhibited by the gutta-percha points containing chlorhexidine and by the calcium hydroxide pastes (Calen™ and Calen/PMCC™), with similar results ( $p > 0.05$ ). No antimicrobial activity was observed for the other groups. It was concluded that the gutta-percha points containing chlorhexidine presented antimicrobial activity, whereas the gutta-percha points containing calcium hydroxide did not.

**Descriptors:** Gutta-percha; Microbiology; Calcium hydroxide; Chlorhexidine.

**Resumo:** O objetivo deste estudo foi a avaliação da atividade antimicrobiana de diferentes marcas e composições de cones de gutta-percha e pastas à base de hidróxido de cálcio utilizados em endodontia. Os materiais avaliados foram: cones de gutta-percha contendo hidróxido de cálcio (Roeko™), cones de gutta-percha contendo clorexidina (Roeko™), duas marcas de cones de gutta-percha (Endo Points™ e Roeko™) e duas pastas à base de hidróxido de cálcio (Calen™ e Calen/PMCC™). Os testes antimicrobianos incluíram 5 espécies de microrganismos: *Escherichia coli* (ATCC10538), *Staphylococcus epidermidis* (ATCC12228), *Staphylococcus aureus* (ATCC6538), *Pseudomonas aeruginosa* (ATCC27853) e *Micrococcus luteus* (ATCC9341). O método empregado foi o de difusão em Ágar. As placas foram mantidas em temperatura ambiente por 2 horas para pré-difusão e então incubadas a 37°C por 24 horas. O gel de cloreto de trifeniltetrazólio foi acrescentado para otimização e as zonas de inibição foram medidas. A análise estatística foi realizada pela análise de variância e pelo teste de Tukey. Os resultados obtidos demonstraram que todas as espécies microbianas usadas foram inibidas pelos cones de gutta-percha com clorexidina e pelas pastas à base de hidróxido de cálcio (Calen™ e Calen/PMCC™), com resultados similares entre os materiais ( $p > 0.05$ ). Nenhuma atividade antimicrobiana foi observada para os demais grupos. Concluiu-se que os cones de gutta-percha com clorexidina apresentaram atividade antimicrobiana, enquanto os cones com hidróxido de cálcio não demonstraram esta propriedade.

**Descritores:** Guta-percha; Microbiologia; Hidróxido de cálcio; Clorexidina.

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## Introduction

The success of endodontic therapy of non-vital teeth is directly related to the elimination of root canal infection. Besides the cleaning effect of biomechanical preparation, further means such as intracanal medication and the use of filling materials with antimicrobial and sealing properties are of essential importance.<sup>2,8,11,14,18</sup>

Calcium hydroxide pastes, commonly used as intracanal medication, have presented antimicrobial action and ability to inactivate endotoxins which facilitate the healing process of periapical tissues.<sup>1,9,19,20</sup>

Chlorhexidine, used as an irrigating solution, may present substantivity,<sup>16,17,21</sup> has antimicrobial action and is able to inhibit most endodontic bacteria.<sup>1,3,4,10,13,17</sup>

With the aim of providing fuller antimicrobial action, gutta-percha points have been manufactured including several substances such as iodoform, chlorhexidine and calcium hydroxide. The antimicrobial effect of gutta-percha points containing calcium hydroxide was evaluated by Podbielski *et al.*<sup>12</sup> (2000). When compared to conventional gutta-percha points, the points containing calcium hydroxide showed greater antimicrobial action against *Enterococcus faecalis*, *Porphyromonas gingivalis*, and *Streptococcus intermedius*.

Barthel *et al.*<sup>1</sup> (2002) evaluated the antimicrobial action of gutta-percha points containing chlorhexidine or calcium hydroxide when compared to chlorhexidine gel and to calcium hydroxide paste. Conventional gutta-percha points showed smaller microbial inhibition.

The aim of this study was to evaluate *in vitro* the antimicrobial activity of conventional gutta-percha points, points containing calcium hydroxide or chlorhexidine, and two calcium hydroxide pastes.

## Material and Methods

The evaluated materials were: calcium hydroxide gutta-percha points (Roeko GmbH + Co., Langenau, Baden-Württemberg, Germany), gutta-percha points containing chlorhexidine (Roeko GmbH + Co., Langenau, Baden-Württemberg, Germany), two brands of conventional gutta-percha points (Roeko GmbH + Co., Langenau, Baden-Württemberg, Germany and

Endo Points Ind. e Com. Ltda., Rio de Janeiro, RJ, Brazil) and two calcium hydroxide based pastes (Calen and Calen/PMCC - S.S.White, Rio de Janeiro, RJ, Brazil). The morphotype and origin of the strains used as indicators of antimicrobial activity are presented on Table 1.

The inocula were obtained by suspending in saline the strains cultivated in MHb (Mueller-Hinton Broth – Difco™, Detroit, MI, USA), to a density of 0.5 on the Mc Farland scale.

Tests were performed using the plate diffusion method with MH media (Mueller-Hinton Medium – Difco™, Detroit, MI, USA) which was cooled to about 50°C, inoculated, and poured into 20 x 100 mm sterilized Petri dishes.

Paper points were involved with Calen™ or Calen/PMCC™ pastes (S.S.White, Rio de Janeiro, RJ, Brazil) and immediately applied. Afterwards, the tested cones were placed at equidistant points. The test was made in triplicate. Plates were kept at room temperature for two hours, so pre-diffusion of the material could occur, and then they were incubated at 37°C for 24 hours.

After the incubation period, amounts of 5 ml of 1% TTC gel prepared with agar (Difco™, Detroit, MI, USA) and 0.05% of triphenyltetrazolium chloride (TTC, Sigma™, St. Louis, MO, USA) were added to optimize the plates. After solidification occurred, they were incubated at 37°C for 30 minutes so the color could be developed to facilitate the measurement.

The biggest inhibition zone was measured with a millimeter ruler. Statistical evaluation was carried out using analysis of variance and Tukey Test, with a level of significance at 5% ( $p < 0.05$ ).

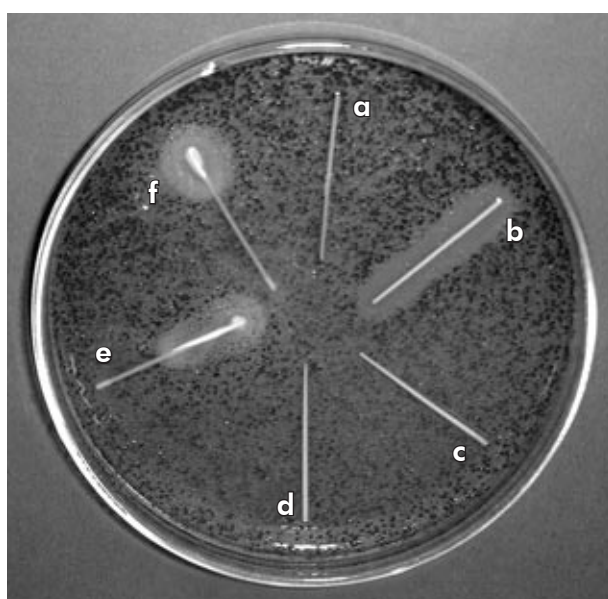
**Table 1** - Strains used as indicators of antimicrobiological activity – origin and morphotype.

Microorganisms	Origin	Morphotype
<i>Micrococcus luteus</i>	ATCC 9341	cg+
<i>Staphylococcus aureus</i>	ATCC 6538	cg+
<i>Staphylococcus epidermidis</i>	ATCC 12228	cg+
<i>Escherichia coli</i>	ATCC 10538	bg-
<i>Pseudomonas aeruginosa</i>	ATCC 27853	bg-

cg+: Gram-positive cocci; bg-: Gram-negative bacilli.

## Results

Figure 1 shows the inhibition zone after the use of the TTC gel. Table 2 shows the means and standard deviations of the diameter of the inhibition zone in millimeters. The results showed that all microbial species used in the study were inhibited by the gutta-percha points containing chlorhexidine and by the calcium hydroxide pastes (Calen™ and Calen/PMCC™), with similar results ( $p > 0.05$ ). No antimicrobial activity was observed in the other groups.



**Figure 1** - Inhibition zone after optimization of the culture medium with TTC gel for *Escherichia coli*. **a:**  $\text{Ca}(\text{OH})_2$  Point; **b:** Chlorhexidine gutta-percha point; **c:** Conventional Roeko gutta-percha point; **d:** Conventional Endo Points gutta-percha point; **e:** Paper point + Calen paste; **f:** Paper point + Calen/PMCC paste.

## Discussion

The agar diffusion method which was used in the present study is one of the most commonly employed methods among those that evaluate antimicrobial activity.<sup>1,5,7,15,22</sup> However, the following peculiarities should be taken into consideration when applying such method: the contact between the tested material and the agar; the weight, size, and molecular shape of the antimicrobial agent; the loading and concentration of the tested material; the agar gel texture; and the ionic concentration of the medium. All these are relevant factors to the material diffusion in agar. Some other factors that may limit the dynamics and variability of agar diffusion tests include the control and standardization of inoculum density, the moment at which the results are read, the choice of agar, the incubation temperature of the plates, and the reading of the inhibition zone.

The lack of standardization of these factors may be considered the main cause of the difference between the present results and those found in comparative studies about antimicrobial properties of endodontic materials.<sup>5</sup>

Because it was an *in vitro* experiment, in addition to the standardization of the above mentioned factors, it was possible to eliminate several *in vivo* variables.

The pre-incubation, which consisted of maintaining the culture media inoculated for about 2 hours at room temperature,<sup>6</sup> was an important factor to evince the antimicrobial activity of the calcium hydroxide pastes. Those results were in accordance with the results found by Leonardo *et al.*<sup>6</sup> (2000), who employed similar methodology. However, no antimicrobial activity for the gutta-percha points containing calcium hydroxide was observed.

**Table 2** - Means and standard deviations of the inhibition zones (in millimeters).

Material \ Microorganism	<i>M. luteus</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
$\text{Ca}(\text{OH})_2$ gutta-percha point	0	0	0	0	0
Chlorhexidine gutta-percha point	8.5 ± 1.0	5.5 ± 1.0	6.0 ± 0	8.5 ± 0.5	7.0 ± 0.5
Conventional a gutta-percha point	0	0	0	0	0
Conventional b gutta-percha point	0	0	0	0	0
Paper point + Calen paste	6.5 ± 1.5	6.0 ± 0.5	6.0 ± 0.5	6.0 ± 0.5	6.0 ± 0
Paper point + Calen/PMCC paste	8.5 ± 1.0	5.5 ± 1.0	7.5 ± 1.0	7.0 ± 1.0	6.0 ± 0

Means of triplicate measurements. a) Roeko GmbH + Co., Langenau, Germany. b) Endo Points Ind. e Com. Ltda., Rio de Janeiro, Brazil.

The optimization of the culture media with 1% TTC gel after the incubation period aimed at differentiating the growing bacterial colonies. This was a meaningful factor to the employed methodology. This procedure, through the changing in the medium color, facilitated the observation of the bacteria growth inhibition zone which could easily be misinterpreted with the material diffusion zone.

Barthel *et al.*<sup>1</sup> (2002) and Lenet *et al.*<sup>5</sup> (2000) showed that gutta-percha points containing chlorhexidine have a smaller antimicrobial effect than chlorhexidine in other vehicles. In the present study, however, those points were able to provide inhibition zones similar to those provided by the Calen™ and Calen/PMCC™ calcium hydroxide pastes. On the other hand, Lui *et al.*<sup>8</sup> (2004), when evaluating gutta-percha points containing chlorhexidine *in vitro*, did not find enough antimicrobial activity against *E. faecalis* inside infected dentine tubules. Szep *et al.*<sup>16</sup> (2003) found greater toxicity in gutta-percha points containing chlorhexidine than in calcium hydroxide or conventional gutta-percha points.

The antimicrobial action of materials containing calcium hydroxide depends on calcium hydroxide ionization and on the release of hydroxyl ions that promote an increase in the medium pH and its maintenance. Hydroxyl ions also have some action

on bacterial cells through the destruction of unsaturated fatty acids or phospholipids of the bacterial cytoplasmic membrane.<sup>12</sup>

Evaluation of the antimicrobial activity of materials containing calcium hydroxide in solid media presents some difficulties. The possible buffering of calcium hydroxide pH in this medium can harm its action. The antimicrobial effect of calcium hydroxide is directly related to its capacity of diffusion and alcalinization in the culture medium which can be decreased by the low solubility of the calcium hydroxide contained in the point.<sup>1</sup> This fact could explain the results obtained in this study that show that the gutta-percha points containing calcium hydroxide did not present antimicrobial activity on the tested strains.

## Conclusions

1. The gutta-percha points containing chlorhexidine (Roeko™) and the calcium hydroxide pastes (Calen™ and Calen/PMCC™) presented *in vitro* antimicrobial activity against all of the strains used in this study.
2. The calcium hydroxide points (Roeko™) and the conventional gutta-percha points (Endo Points™ and Roeko™) did not present antimicrobial activity.

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