

# Antimicrobial potential of various periodontal surgical dressings

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## Abstract

**Objective:** Periodontal surgeries expose surgical wounds to the oral cavity, bringing them into direct contact with the microorganisms present, thus increasing the risk of oral infections. This study evaluated the antimicrobial activity of surgical dressings used in periodontics using distinct methodologies against different microorganisms over several periods. **Methods:** The microorganisms (*Candida albicans*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, and a mixture) were tested using agar diffusion and direct exposure tests. The ANOVA on Ranks test was performed for statistical analysis, followed by the Tukey test. The significance level was set at  $\alpha = 5\%$ . **Results:** None of the materials showed inhibition zones against *S. aureus* and *S. mutans*. The TECHNEW dressing showed the largest inhibition zones against *C. albicans*, *E. faecalis*, and the microorganism mixture. After 1 day, only the TECHNEW and Lysanda dressings showed antimicrobial activity against all microorganisms. After 5 days, only the TECHNEW dressing effectively reduced the tested microorganisms. After 7 days, TECHNEW and COE-PACK dressings showed results similar to the negative control ( $p > 0.05$ ). **Conclusions:** No material was able to show inhibition zones against all the evaluated microorganisms. Regarding the direct exposure of dressings to microorganisms, only the TECHNEW cement was effective at 1, 5, and 7 days.

**KEYWORDS:** Microbiology; Dental Materials; Oral Surgery; Antimicrobial Agents.



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## Introduction

The oral cavity houses a complex community of microorganisms with over 700 species identified<sup>1,2</sup>. This microbiota establishes harmonious relationships with the host, maintaining homeostasis while exhibiting pathogenic potential. From an imbalance, different pathologies naturally develop<sup>3-6</sup>, including dental caries, endodontic infections, and periodontal diseases<sup>7,8</sup>.

The microbial diversity present in the oral cavity significantly increases the likelihood of infections at the surgical site, leading to complications that may result in postoperative morbidity and mortality. Therefore, effective strategies are necessary to prevent these adverse health conditions in patients<sup>9,10</sup>.

The relationship between microorganisms in the oral cavity, the blood supply to the periodontium, and the damage to the epithelial layer of the oral mucosa are factors that directly influence the establishment of transient bacteremia<sup>11</sup>. In oral surgeries, bacteremia rates can reach up to 80% of cases<sup>12</sup>. Another concerning factor regarding bacteremia is the patient's systemic condition, as patients with heart disease can develop more severe infectious conditions, such as infective endocarditis<sup>12</sup>.

Another important factor to highlight is the role that oral microorganisms play in the development of more severe systemic infectious conditions, with common findings of *Candida albicans*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, and other microorganisms associated with various pathologies not related to the oral cavity<sup>13-16</sup>.

Among the most frequent oral surgeries are periodontal surgeries, such as clinical crown lengthening, gingivectomies, and gingival grafts<sup>17-19</sup>. These, in turn, expose surgical wounds to the oral cavity and, consequently, to the microorganisms present, increasing the likelihood of oral infections<sup>20</sup>.

Surgical infections in periodontal surgery procedures can cause discomfort, complications, and pose risks to the patients'

health<sup>20</sup>. To reduce these risks, various therapeutic options, such as medications and dressings, are commonly used after periodontal interventions. Among these options, surgical dressings stand out as a protective barrier, preventing mechanical trauma, controlling postoperative bleeding, and reducing the risk of infections at the surgical site. Additionally, they contribute to the patient's comfort during the healing process<sup>21,22</sup>.

An ideal dressing should exhibit essential characteristics such as biocompatibility, antimicrobial activity, and physical protection of the surgical flap<sup>21,22</sup>. Considering the importance of the oral microbiota and its potential in developing oral infections, this study evaluated the antimicrobial activity of surgical dressings used in periodontics using distinct methodologies against different microorganisms over various periods.

## Materials and methods

### Materials assessed

Different materials were evaluated in the present study: Lysanda® (Lysanda Produtos Odontológicos LTDA-Vila Prudente, São Paulo, SP, Brazil), TECHNEW® (TECHNEW Com Ind LTDA, Rio de Janeiro, RJ, Brazil), Pericem® (Maquira SA, Maringá, PR, Brazil), and COE-PACK® (G.C, America, Inc. USA).

### Biological indicators

The microorganisms (*Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538, and *Streptococcus mutans* ATCC 25175), with different morphological, staining, and respiratory characteristics, as well as a mixture of these microorganisms, were used in this study.

The strains were inoculated into 7 mL of Brain Heart Infusion (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 24 hours. The microorganisms were cultivated on the surface of Brain Heart Infusion Agar (BHIA; Difco Laboratories,

Detroit, MI, USA), which had been previously distributed into test tubes and sterilized at 121°C for 20 minutes. After 24 hours of incubation at 37°C under appropriate respiratory conditions for the indicator microorganisms, microbial cells were suspended in 0.5% physiological solution (Halex Istar, Goiânia, GO, Brazil) and sterilized. For all microorganisms, the test suspension was adjusted, with the same diluent, to the first tube of the MacFarland scale, at an approximate concentration of  $3 \times 10^8$  cells per mL.

For the preparation of the mixture, a 1.0 mL aliquot was taken from each pure microbial suspension and transferred to a test tube, thereby obtaining the experimental mixture containing *C. albicans*, *E. faecalis*, *S. aureus*, and *S. mutans*.

#### Agar diffusion test

In the agar diffusion test, 40 Petri dishes containing 20 mL of Brain Heart Infusion Agar (BHIA; Difco Laboratories, Detroit, MI, USA) were inoculated with 0.1 mL of the microbial suspension using sterilized swabs. The inoculum was spread across the surface of the culture medium to achieve confluent growth. In each plate containing BHIA, 5 cavities were made, each measuring 4 mm in depth and 4 mm in diameter, using a copper coil. These cavities were then filled with the tested products. For each substance tested, 10 repetitions were performed.

The plates were kept at room temperature for 1 hour, then incubated at 37°C for 48 hours. The diameters of the zones of microbial inhibition were measured using a digital caliper (Mitutoyo, São Paulo, SP, Brazil).

The positive control group consisted of three plates inoculated with each microorganism: *C. albicans*, *E. faecalis*, *S. aureus*, *S. mutans*, and a mixture of these microorganisms. The negative control group consisted of three plates containing agar without inoculation, under identical incubation periods and conditions.

### Direct exposure test

In the direct exposure test, one hundred eighty sterilized absorbent paper cones (Tanari, Tanariman Indústria, Ltda, Manacaru, AM, Brazil) were immersed in the microbial suspensions for 5 minutes. After this period, the contaminated paper cones were placed in Petri dishes and covered with one of the five tested substances. At 1 day, 5 days, and 7 days intervals, sixty paper cones were removed from contact with periodontal dressings, transported individually, and immersed in 5 mL of Letheen Broth (LB, Difco Laboratories, Detroit, MI, USA), followed by incubation at 37°C for 48 hours.

Subsequently, a subculture was performed. A 0.1 mL aliquot from each sample was transferred individually into 5 mL of Brain Heart Infusion (BHI; Difco Laboratories, Detroit, MI, USA), followed by another incubation period under identical conditions.

Forty-eight hours after subculturing, microbial growth was evaluated by the turbidity of the culture medium and by optical density using a UV spectrophotometer (Spectrophotometer Model Nova 1600 UV, Piracicaba, SP, Brazil) set at a wavelength of  $\lambda = 600$  nm (nanometers), which corresponds to an absorbance of 0.137 nm.

The positive control group consisted of 15 sterilized absorbent paper cones. These cones were contaminated for 5 minutes and then immersed individually in 7 mL of Letheen Broth (LB, Difco Laboratories, Detroit, MI, USA) and incubated under the same conditions. The negative control group consisted of 15 sterilized, non-contaminated paper cones, which were immersed individually in the culture medium and incubated under the same conditions as previously described.

### Statistical analyses

Statistical analysis was performed using the SigmaPlot 12.0™ software (Chicago, IL, USA). The normality of the data was verified using the Shapiro-Wilk test. ANOVA on Ranks test was

performed, followed by the Tukey test. Results were considered statistically significant when the probability was less than 5% ( $p < 0.05$ ).

## Results

The results of the agar diffusion test are described in Table 1. The Lysanda® paste showed no inhibition zones against all the bacterial solutions tested. None of the materials exhibited inhibited zones against the microorganisms *S. aureus* and *S. mutans*. The TECHNEW® dressing showed the largest inhibition zones against *C. albicans*, *E. faecalis*, and the microorganism mixture.

The results of the direct contact test are described in Tables 2, 3, and 4. On the 1 day, only the TECHNEW® and Lysanda® dressings showed statistically similar results to the negative control against all microorganisms. The COE-PACK® dressing showed no activity against *S. aureus* and *E. faecalis*. The Pericem® dressing showed no activity against *S. aureus* on day 1.

At 5 days, only the TECHNEW® dressing showed statistically similar results to the negative control against all microorganisms. Similar to the 1 day, the COE-PACK® dressing showed no antimicrobial activity against *S. aureus* and *E. faecalis*. The Lysanda® paste showed no significant antimicrobial activity against *C. albicans*. The Pericem® dressing showed no significant antimicrobial activity against the microorganisms *S. aureus* and *E. faecalis*.

TABLE 1 · Results of the agar diffusion test

Microorganisms	Surgical periodontal dressings (median and interquartile range)*				P value
	Lysanda	TECHNEW	Pericem	COE-PACK	
<i>C. albicans</i>	0 (0.00-0.00) <sup>Aa</sup>	13 (12.00-14.00) <sup>Bab</sup>	0 (0.00-0.00) <sup>Aa</sup>	9 (8.00-9.00) <sup>Ba</sup>	< 0.001
<i>E. faecalis</i>	0 (0.00-0.00) <sup>Aa</sup>	15 (15.00-16.25) <sup>Ba</sup>	5 (5.00-7.25) <sup>Cb</sup>	6 (5.00-7.00) <sup>Ca</sup>	< 0.001
<i>S. aureus</i>	0 (0.00-0.00) <sup>Aa</sup>	0 (0.00-0.00) <sup>Ab</sup>	0 (0.00-0.00) <sup>Aa</sup>	0 (0.00-0.00) <sup>Ab</sup>	1.00
<i>S. mutans</i>	0 (0.00-0.00) <sup>Aa</sup>	0 (0.00-0.00) <sup>Ab</sup>	0 (0.00-0.00) <sup>Aa</sup>	0 (0.00-0.00) <sup>Ab</sup>	1.00
Mixture	0 (0.00-0.00) <sup>Aa</sup>	15 (14.00-17.00) <sup>Ba</sup>	5 (5.00-7.25) <sup>Cb</sup>	6 (5.00-7.00) <sup>Ca</sup>	< 0.001
P value	1.00	< 0.001	< 0.001	< 0.001	

\*Capital letters indicate comparisons in the lines. Lower letters indicate comparisons in columns. Different letters indicate statistical differences ( $P < 0.05$ ).

At the 7 day, the TECHNEW® and COE-PACK® dressings showed statistically similar results to the negative control. The Pericem® dressing showed no significant antimicrobial activity against *C. albicans* and *S. aureus*. The Lysanda® paste showed results similar to the negative control against all microorganisms, except for *C. albicans*.

TABLE 2 · Direct contact test (1-day period)

Microorganisms	Surgical periodontal dressings (mean and standard deviation)*						P value
	Lysanda	TECHNEW	Pericem	COE-PACK	Positive control	Negative control	
<i>C. albicans</i>	0.091 ± 0.041 <sup>Ab</sup>	0.051 ± 0.035 <sup>ACa</sup>	0.032 ± 0.012 <sup>ACab</sup>	0.052 ± 0.010 <sup>ACa</sup>	0.264 ± 0.023 <sup>Ba</sup>	0.000 (0.00-0.00) <sup>Ca</sup>	< 0.001
<i>E. faecalis</i>	0.004 ± 0.005 <sup>Ab</sup>	0.035 ± 0.039 <sup>ABa</sup>	0.095 ± 0.133 <sup>ABabc</sup>	0.186 ± 0.027 <sup>Bb</sup>	0.552 ± 0.019 <sup>Bb</sup>	0.000 (0.00-0.00) <sup>Ab</sup>	< 0.001
<i>S. aureus</i>	0.026 ± 0.008 <sup>Ab</sup>	0.026 ± 0.021 <sup>Ab</sup>	0.214 ± 0.047 <sup>Bac</sup>	0.194 ± 0.029 <sup>Bb</sup>	0.190 ± 0.020 <sup>Bc</sup>	0.000 (0.00-0.00) <sup>Ab</sup>	< 0.001
<i>S. mutans</i>	0.074 ± 0.065 <sup>Ab</sup>	0.017 ± 0.003 <sup>Ab</sup>	0.005 ± 0.002 <sup>Ab</sup>	0.007 ± 0.006 <sup>Ab</sup>	0.192 ± 0.020 <sup>Bc</sup>	0.000 (0.00-0.00) <sup>Ab</sup>	< 0.001
Mixture	0.104 ± 0.138 <sup>ABCa</sup>	0.057 ± 0.006 <sup>ABCa</sup>	0.242 ± 0.048 <sup>ABCc</sup>	0.121 ± 0.029 <sup>ABCc</sup>	0.544 ± 0.030 <sup>Bb</sup>	0.000 (0.00-0.00) <sup>Ca</sup>	0.013
P Value	0.401	0.338	0.004	< 0.001	< 0.001	1.00	

\*Capital letters indicate comparisons in the lines. Lower letters indicate comparisons in columns. Different letters indicate statistical differences (P < 0.05).

TABLE 3 · Direct contact test (5-day period)

Microorganisms	Surgical periodontal dressings (mean and standard deviation)*						P value
	Lysanda	TECHNEW	Pericem	COE-PACK	Positive control	Negative control	
<i>C. albicans</i>	0.047 ± 0.018 <sup>ADa</sup>	0.008 ± 0.007 <sup>ACa</sup>	0.037 ± 0.027 <sup>ADCa</sup>	0.076 ± 0.014 <sup>Da</sup>	0.103 ± 0.018 <sup>BDa</sup>	0.000 (0.00-0.00) <sup>Ca</sup>	< 0.001
<i>E. faecalis</i>	0.011 ± 0.002 <sup>Ab</sup>	0.012 ± 0.004 <sup>ABb</sup>	0.299 ± 0.050 <sup>Ba</sup>	0.285 ± 0.013 <sup>Ba</sup>	0.403 ± 0.006 <sup>Cab</sup>	0.000 (0.00-0.00) <sup>Ab</sup>	< 0.001
<i>S. aureus</i>	0.018 ± 0.017 <sup>ACab</sup>	0.020 ± 0.012 <sup>ACab</sup>	0.180 ± 0.150 <sup>ABa</sup>	0.200 ± 0.169 <sup>Ba</sup>	0.196 ± 0.004 <sup>Bab</sup>	0.000 (0.00-0.00) <sup>Ca</sup>	0.038
<i>S. mutans</i>	0.018 ± 0.007 <sup>Ab</sup>	0.024 ± 0.008 <sup>ABb</sup>	0.182 ± 0.146 <sup>Aa</sup>	0.102 ± 0.162 <sup>Aa</sup>	0.197 ± 0.020 <sup>ABb</sup>	0.000 (0.00-0.00) <sup>Ab</sup>	0.077
Mixture	0.049 ± 0.009 <sup>ABCa</sup>	0.037 ± 0.011 <sup>ABCb</sup>	0.100 ± 0.076 <sup>ABCa</sup>	0.127 ± 0.015 <sup>ABCa</sup>	0.429 ± 0.010 <sup>Bb</sup>	0.000 (0.00-0.00) <sup>Ca</sup>	0.015
P value	0.007	0.025	0.088	0.181	0.012	1.00	

\*Capital letters indicate comparisons in the lines. Lower letters indicate comparisons in columns. Different letters indicate statistical differences (P < 0.05).

TABLE 4 · Direct contact test (7-day period)

Microorganisms	Surgical periodontal dressings (mean and standard deviation)*						P value
	Lysanda	TECHNEW	Pericem	COE-PACK	Positive control	Negative control	
<i>C. albicans</i>	0.165 ± 0.0120 <sup>Aa</sup>	0.042 ± 0.003 <sup>Ba</sup>	0.125 ± 0.0134 <sup>ACab</sup>	0.061 ± 0.062 <sup>BCa</sup>	0.161 ± 0.021 <sup>Aa</sup>	0.000 (0.00-0.00) <sup>Ba</sup>	< 0.001
<i>E. faecalis</i>	0.019 ± 0.012 <sup>Ab</sup>	0.031 ± 0.022 <sup>Ab</sup>	0.031 ± 0.031 <sup>Aa</sup>	0.004 ± 0.002 <sup>Aa</sup>	0.309 ± 0.020 <sup>Bb</sup>	0.000 (0.00-0.00) <sup>Ab</sup>	< 0.001
<i>S. aureus</i>	0.010 ± 0.00 <sup>Ab</sup>	0.001 ± 0.001 <sup>Ab</sup>	0.274 ± 0.006 <sup>Bb</sup>	0.040 ± 0.060 <sup>Aa</sup>	0.190 ± 0.008 <sup>Cc</sup>	0.000 (0.00-0.00) <sup>Ab</sup>	< 0.001
<i>S. mutans</i>	0.120 ± 0.173 <sup>ABCab</sup>	0.016 ± 0.011 <sup>ABCab</sup>	0.115 ± 0.131 <sup>ABCab</sup>	0.003 ± 0.001 <sup>ABCa</sup>	0.198 ± 0.007 <sup>Bc</sup>	0.000 (0.00-0.00) <sup>Ca</sup>	0.014
Mixture	0.115 ± 0.010 <sup>ABCab</sup>	0.036 ± 0.021 <sup>ABCab</sup>	0.046 ± 0.051 <sup>ABCa</sup>	0.056 ± 0.025 <sup>ABCa</sup>	0.398 ± 0.005 <sup>Bd</sup>	0.000 (0.00-0.00) <sup>Ca</sup>	0.012
P value	0.042	0.039	0.007	0.296	< 0.001	1.00	

\*Capital letters indicate comparisons in the lines. Lower letters indicate comparisons in columns. Different letters indicate statistical differences (P < 0.05).

## Discussion

A critical property of periodontal surgical dressings is their ability to exhibit antimicrobial activity against resistant microorganisms prevalent in the oral microbiota. The results showed that in this study, all evaluated materials (Lysanda, TECHNEW, Pericem, and COE-PACK) demonstrated antimicrobial efficacy against at least one tested microorganism in both in vitro assays. The only exception was Lysanda paste, which failed to exhibit inhibition in the agar diffusion test.

Agar diffusion and direct contact tests are commonly used in dentistry to assess the antimicrobial activity of dental materials<sup>23-25</sup>. Both tests have limitations. The agar diffusion test is dependent on the material's ability to diffuse into the medium<sup>26</sup>, whereas the direct contact test is a quantitative analysis that, due to its higher reliability, is ideal for complementing the agar diffusion test<sup>27</sup>.

The microorganisms were selected because they are important in oral diseases and are frequently found in the oral cavity. All of them have pathogenic potential, which can lead to tissue destruction, alter the host's immune response, and disrupt microbial host homeostasis<sup>28</sup>. Studies have stated that these microorganisms in the oral cavity can, to varying degrees, invade epithelial cells, induce immune responses in gingival epithelial cells, leading to cytokine secretion, and establish an inflammatory process<sup>29</sup>.

*C. albicans* is characterized by its tolerance to antimicrobial therapy, highlighting the importance of research focused on preventing and controlling these clinical microbial communities<sup>30</sup>. In the present study, the tolerance of *C. albicans* to antimicrobial substances was observed. In the agar diffusion test, Lysanda paste did not show inhibition zones against *C. albicans*. This result was also found in the direct contact test at the 5- and 7-day periods for the same material and the 7 days for the Pericem dressing.



*Enterococcus faecalis* is a microorganism commonly found in the oral microbiota and is associated with the development of infective endocarditis<sup>31</sup>, as well as being one of the agents responsible for apical periodontitis<sup>32</sup>. It can also survive in environments with few nutrients and highly alkaline conditions, making it resistant to various types of medication<sup>33</sup>. In the present study, on the 1 day, COE-Pack dressing showed no activity against *E. faecalis*, a characteristic also observed during the 5-day period for both Pericem and COE-PACK, which can be explained by the microorganism's high resistance<sup>34</sup>.

*Staphylococcus aureus* is a microorganism usually found in acute dental alveolar infections<sup>35</sup>. In this study, none of the materials in the agar diffusion test showed inhibition zones against *S. aureus*. Additionally, the COE-PACK and Pericem dressings showed no activity in the direct contact test at the 1- and 5-day periods, and at the 7-day period, the Pericem dressing remained ineffective. These factors can be explained by the bacteria's high resistance, which is related to their ability to produce enzymes that neutralize antimicrobial substances<sup>36</sup>.

*Streptococcus mutans* is considered one of the main etiological agents of dental caries<sup>37</sup> and is also cited in the literature as a cause of bacteremia and endocarditis<sup>38</sup>. In the present study, all the materials exhibited antimicrobial activity against *S. mutans* in the evaluated periods.

It is important to note that despite the characteristics of the microorganisms previously presented, the microbiota of the oral cavity is complex both quantitatively and qualitatively<sup>2</sup>. Therefore, it is essential to assess the behavior of materials against contamination by multiple bacteria, as was evaluated in the present study, which demonstrated that despite the difficulty of some materials in acting against isolated strains, all the materials, regardless of the period and antimicrobial test, showed activity against the mixed microorganisms.

Although this study is an *in vitro* analysis, experimental studies remain an important part of preclinical research. However, care should be taken when extrapolating results to clinical conditions in humans.

## Conclusions

None of the materials were able to present inhibition zones against all the evaluated microorganisms. Regarding the direct exposure of the dressings to the microorganisms, only the TECHNEW dressing showed activity against all the microorganisms at the 1, 5, and 7-day periods.

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## Análise das propriedades antimicrobianas de diferentes cimentos periodontais

### Resumo

**Objetivo:** As cirurgias periodontais expõem feridas cirúrgicas à cavidade oral, colocando-as em contato direto com os microrganismos presentes, o que aumenta o risco de infecções orais. Este estudo avaliou a atividade antimicrobiana de cimentos cirúrgicos utilizados em periodontia, empregando metodologias distintas contra diferentes microrganismos ao longo de vários períodos. **Métodos:** Os microrganismos (*Candida albicans*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans* e uma mistura) foram testados por meio dos testes de difusão em ágar e exposição direta. Para a análise estatística, foi utilizado o teste ANOVA on Ranks, seguido pelo teste de Tukey. O nível de significância adotado foi  $\alpha = 5\%$ . **Resultados:** Nenhum dos materiais testados apresentou zonas de inibição contra *S. aureus* e *S. mutans*. O cimento TECHNEW apresentou as maiores zonas de inibição contra *C. albicans*, *E. faecalis* e a mistura de microrganismos. Após 1 dia, apenas os cimentos TECHNEW e Lysanda mostraram atividade antimicrobiana contra todos os microrganismos. Após 5 dias, somente o cimento TECHNEW reduziu efetivamente os microrganismos testados. Após 7 dias, os cimentos TECHNEW e COE-PACK apresentaram resultados semelhantes ao controle negativo ( $p > 0,05$ ). **Conclusões:** Nenhum material foi capaz de apresentar zonas de inibição contra todos os microrganismos avaliados. Em relação à exposição direta dos cimentos aos microrganismos, apenas o cimento TECHNEW foi eficaz nos períodos de 1, 5 e 7 dias.

**PALAVRAS-CHAVE:** Microbiologia; Materiais Dentários; Cirurgia Oral; Agentes Antimicrobianos.

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