

Antibacterial effect of four endodontic cements against *Enterococcus faecalis* ATCC 29212. An *in vitro* study.

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Abstract: Objective: To compare the *in vitro* antibacterial effect of the root canal cements Endobalsam®, Top Seal®, Apexit® and Endofill® against *Enterococcus faecalis* ATCC 29212. Materials and method: Eighty-five applications of cements on *Enterococcus faecalis*, cultured *in vitro* on solid media in Petri dishes, were analyzed. Five groups were evaluated: four for each cement, and the fifth for the positive control (amoxicillin). The antibacterial effect was measured by the diameters of the bacterial inhibition halos at 24 hours, 48 hours, and seven days. Student's *t-test*, ANOVA and the Tukey *test* were used for the statistical analysis. Results: No statistically significant differences were found at 24 hours ($p>0.05$); at 48 hours and seven days, Endofill® and Apexit® had the greatest effect ($p<0.05$); finally, on day 7 only Endofill® showed an effect similar to the positive control ($p>0.05$). Conclusion: *Enterococcus faecalis* ATCC 29212 was susceptible to all cements. Endofill® had greater *in vitro* antibacterial effect than Apexit®, Top Seal® and Endobalsam®.

Keywords: Antibacterial effect; endodontic cement; *Enterococcus faecalis*.

INTRODUCTION.

One of the most important objectives of endodontic treatment is the complete elimination of microorganisms from the root canal. This is accomplished by using different cleaning mechanisms and filling agents with antibacterial activity.¹

However, a perfect filling cement had not been found yet. Current canal filling cements made from plastic resin, glass ionomer, silicone, calcium hydroxide, eugenol zinc oxide, and eugenol-free zinc oxide, exhibit varying antibacterial properties.²

Despite the antibacterial activity of the cements, some microorganisms such as *Enterococcus faecalis* develop resistance, leading to the failure of root canal treatments.^{3,4} *Enterococcus faecalis* is not normally found in primary apical periodontitis and its presence in acute dental infections is not common.⁵ However, its incidence in teeth affected by apical periodontitis has been clearly established, and it has been strongly associated with endodontic failures.^{4,6}

Several studies have been conducted in order to identify a filling cement with antibacterial properties.^{1,2,7} However, it is necessary to assess the antibacterial characteristics of the currently available filling cements against *Enterococcus faecalis*. Additionally, the general antibacterial effect of some cements such as Endobalsam® remains unclear.

The aim of this study was to compare the *in vitro* antibacterial property

of canal filling cements Endobalsam®, Top Seal®, Apexit® and Endofill® against *Enterococcus faecalis* ATCC 29212.

MATERIALS AND METHODS.

Study design and samples

An *in vitro* study was carried out, including 85 applications of the filling cements Endobalsam® (Universidad Peruana Cayetano Heredia, Lima, Peru), Top Seal® (Dentsply Maillefer, Ballaigues, Switzerland), Apexit® (Ivoclar Vivadent, Schaan, Liechtenstein) and Endofill® (Produits Dentaires SA, Vevey, Switzerland) placed on *Enterococcus faecalis* ATCC 29212, cultured *in vitro* on solid media in Petri dishes.

Five groups (n=17 each) were formed, four for the cements under study and the fifth for the positive control (a 25µg amoxicillin disc, Jampar®, Biolabtest Laboratory, Peru). The order of placement of the first five applications in the Petri dishes was randomly assigned.

The present study was approved by the Permanent Research Committee of the School of Medicine at Universidad Nacional de Trujillo-Perú (Code RD47707EPG).

Procedures

A fresh bacterial liquid culture diluted to 0.5 McFarland turbidity was inoculated on blood agar in Petri dishes by swabbing the whole surface of the agar with a saturated swab; the plates were incubated for five minutes in order for the agar absorb the suspension.

The four cements and the control were then placed in each Petri dish.

Cements were prepared according to the manufacturer's instructions, and then placed on the agar with the help of sterile tuberculin syringes to fill four 5mm-diameter perforations; one perforation for each cement, while the positive control disc did not need a perforation.

The 17 Petri dishes were placed in the incubator at 37°C in micro-anaerobiosis. Observations were made at 24 hours, 48 hours and 7 days, to assess the effect of the cements over time.

The antibacterial effect was measured by the diameters of the bacterial inhibition halos produced by each sample. Each cement application was performed by a single observer, who was blinded with respect to the order of the cements, as they had been assigned codes.

Statistical analysis

Data were analyzed with the statistical program SPSS 18 (IBM, USA). One-way ANOVA, Student's *t-test*, and Tukey *test* were used for multiple comparisons. All the analyses considered a level of significance $p < 0.05$.

RESULTS.

Table 1 shows the comparison of the *in vitro* antibacterial effect of the canal filling cements Endobalsam®, Top Seal®, Apexit® and Endofill® against the *Enterococcus faecalis* ATCC29212 strain.

Table 1. Comparison of the *in vitro* antibacterial effect of the canal filling cements Endobalsam®, Top Seal®, Apexit® and Endofill® against the *Enterococcus faecalis* ATCC 29212 strain.

Cement	Diameters of inhibition halos (mm)						p-value**
	24 hours		48 hours		Day 7		
	mean	SD	mean	SD	mean	SD	
Endobalsam®	7.68 ^a	2.69	8.00 ^a	2.58	9.15 ^a	2.33	0.217
Top Seal®	7.77 ^a	2.20	8.32 ^a	1.80	8.88 ^{ab}	1.67	0.242
Apexit®	9.06 ^{aA}	1.85	9.94 ^{abA}	1.75	14.21 ^{bcB}	6.82	0.002
Endofill®	10.94 ^{aA}	4.89	12.21 ^{bb}	4.37	16.88 ^{cdC}	8.31	0.017
Amoxicilina	19.06 ^b	5.19	20.65 ^c	4.89	21.65 ^d	4.92	0.323
p-value*	<0.001		<0.001		<0.001		

*ANOVA; SD, standard deviation; different letters indicate statistically significant differences (Tukey *test*).

** ANOVA of repeated measures, different capital letters indicate statistically significant differences (Student's *t* for paired groups).

DISCUSSION.

Although the success of root canal therapy is directly related to the elimination of microorganisms, the different procedures employed do not result in complete sterility of the root canal. Therefore, antimicrobial agents have been added to canal filling cements in order to improve their antibacterial characteristics and reduce the complex microbial load occurring in root canals.²

Top Seal® had a lower antibacterial effect than Apexit® and Endofill® in the three observations made in agreement with Anumula *et al.*¹ who reported low antimicrobial activity of resin-based cements against *Enterococcus faecalis*. Apexit® showed the second highest antibacterial effect, being significantly higher than Top Seal® and Endobalsam®. Its antibacterial action is attributed to the amount of calcium hydroxide that it contains, related to its alkaline pH, which prevents the development of bacteria.² Endobalsam®, despite having a lower antibacterial effect, maintained its activity, which even increased slightly, until day 7. The lower antibacterial effect of this cement is probably due to the Balsam of Peru, which has been shown to have mainly anti-inflammatory activity and bacteriostatic action.⁸

In the present study, Endofill® (based on zinc oxide - eugenol) had the largest inhibition halo diameter. These results were similar to those reported by Haghgoo *et al.*⁹

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The antibacterial action of Endofill® is directly related to the presence of eugenol, which has a powerful antibacterial effect due to its low pH, its affinity for plasma membranes and its activity at interrupting oxidative phosphorylation. In addition, eugenol plus zinc oxide in contact with water undergoes hydrolysis and releases zinc hydroxide. The latter has bactericidal properties that last for 1 or 2 weeks and then diminishes progressively.¹⁰ This would explain the increase in the diameters of inhibition halos of Endofill® at 24 and 48 hours and their increase until the seventh day.

The obvious limitation to making clinical projections of the present study is the fact that it is *in vitro* study, so *in vivo* designs are suggested to complement the reported findings. Despite this, our results indicate that the antibacterial effect of the studied cements keeps increasing up to the seventh day, particularly Apexit® and Endofill®. In addition, the latter showed a more promising effect due to the fact that after seven days, its effect was comparable to that of amoxicillin.

CONCLUSION.

Enterococcus faecalis ATCC 29212 was sensitive to all cements. Endofill® had greater *in vitro* antibacterial effect than Apexit®, Top Seal® and Endobalsam®.

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