In vitro antibacterial activity of endodontic sealers

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Summary Objectives. To evaluate the antibacterial activity of four endodontic sealers: one epoxy resin sealer (AH Plus), two zinc oxide eugenol (ZOE)-based sealers (Endométasone, Pulp Canal Sealer), and one sealer containing both ZOE and orthophenilphenol (Vcanalare).

Methods. A direct contact test (DCT) was performed. A 10 µl suspension of Enterococcus faecalis was placed on the test material 20 min, 24 h and 7 days after mixing. Bacteria were allowed to directly contact the sealers for 1 h at 37 °C. Bacterial growth was then spectrophotometrically measured every 30 min for 7 h, and again after 24 h as well.

Results. All freshly mixed sealers showed complete inhibition of bacterial growth. Similar results were obtained with the 24-h-old samples, with the exception of AH Plus. Vcanalare was the only sealer still inhibiting bacterial growth 7 days after mixing.

Conclusions. The antimicrobial activity of the tested sealers depends on the time interval between mixing and testing. All sealers exhibit bactericidal effect when freshly mixed, but only Vcanalare extended this effect until 7 days after setting.

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Introduction

The main objective of endodontic treatment is the elimination of microorganisms from the root canal system and the prevention of subsequent reinfection. Biomechanical cleaning and shaping, followed by the three-dimensional obturation of the root canal space, are common procedures used to achieve this goal. However, the presence of microorganisms has been reported even after thorough chemomechanical preparation of the root canal system.1–6 Microbial persistence and growth in dentinal tubules, lateral canals, and apical ramifications have also been proved.7–11 The residual organisms, together with those reentering from the oral cavity if the access cavity is not
sealed adequately,\textsuperscript{12,13} rapidly repopulate the empty canals between the appointments and can induce or sustain apical periodontitis.\textsuperscript{5,6,14} For this reason, the use of a sealer exhibiting antibacterial properties may be useful to decrease or avoid growth of these remaining microorganisms.

In the past decade, the antimicrobial activity of root canal sealers were assessed using the agar diffusion test (ADT). As this technique presented several limitations, in 1996 Weiss et al.\textsuperscript{15} described a direct contact test (DCT) assay designed to overcome them. The DCT has been used to evaluate the in vitro antibacterial activities of numerous endodontic sealers, such as the zinc-oxide-eugenol (ZOE-) and resin-based sealers, the ones containing calcium hydroxide, and the glass-ionomer-based endodontic sealing cements. On the other hand, there is scant research on the antimicrobial properties of the sealers that contain orthophenilphenol.

The aim of this study was to evaluate the antibacterial activities of four endodontic sealers using the DCT. The tested sealers included one recently introduced epoxy resin sealer (AH Plus; AHP), two ZOE-based sealers (Endométhasone and Pulp Canal Sealer; EM and PCP, respectively), and one sealer containing both ZOE and orthophenilphenol (Vcanalare; VC).

\textbf{Materials and methods}

The four endodontic sealers used in the study are shown in Table 1, together with the manufacturers. The antibacterial activities of the sealers were tested under three different conditions: (1) samples were used within 20 min after mixing (designated as fresh samples); (2) samples were prepared 24 h before testing and allowed to set in a humid atmosphere at 37 °C (designated as 24-h samples); (3) samples were allowed to set for 48 h in a humid atmosphere at 37 °C and then aged for 5 days in phosphate buffered saline (PBS) at 37 °C (designated as 7-day samples).\textsuperscript{16} The sealers were prepared according to the manufacturers’ instructions.

A collection strain of \textit{Enterococcus faecalis} (ATCC 29212; American Type Culture Collection, Rockville, MD) has been used in this study. Bacteria from frozen stock cultures were grown aerobically to late logarithmic or early stationary phase in brain heart infusion (BHI) broth (Oxoid Ltd, Basingstoke, UK) at 37 °C. Cells were harvested by centrifugation and resuspended in fresh medium. Inocula were prepared by adjusting the cell suspension to predetermined optical densities (OD) corresponding to 10\textsuperscript{8} CFU/ml.

The DCT has been performed following the method of Weiss et al. (1996),\textsuperscript{15} with minor modifications. Briefly, a 96-well microtiter plate was held vertically and the side wall of four wells was coated with freshly mixed sealer (Group A wells). Ten microliters of the bacterial inoculum (approximately 10\textsuperscript{6} bacteria) were placed on the test material, 20 min, 24 h and 7 days after mixing, each in a different plate. Plates maintained in vertical position were incubated in a humid atmosphere at 37 °C until evaporation of the suspension’s liquid was evident. This occurred within 1 h and ensured direct contact between bacteria and tested materials. BHI broth (250 μl) was added to each well and gently mixed for 2 min; a 50-μl inoculum was then transferred from Group A wells, respectively, to an adjacent set of four wells containing 200 μl of fresh medium (Group B wells). This resulted in two sets of four wells for each tested sealer, so that the bacterial growth could be monitored both in the presence and in the absence of the tested material. Two sets of uncoated wells (Groups A and B wells) were inoculated with identical volumes of bacterial suspension and served as positive control. The negative control consisted of two sets of wells containing uninoculated fresh medium (250 μl), one of which was coated with the test materials. Plates were incubated at 37 °C in a humid chamber. Bacterial growth was followed by densitometric measurement in a microplate reader (Multiskan MCC/340, Labsystems, Helsinki, Finland). The OD in each well at 600 nm was recorded every 30 min for 7 h, and therefore, after 24 h incubation. All experiments, carried out under aseptic conditions, were repeated three times to ensure reproducibility.

\textbf{Results}

The results of the DCT for freshly mixed, 24-h-old and 7-day-old endodontic sealers are shown in Figs. 1-3, respectively. Each point on the growth

\begin{table}
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\begin{tabular}{|l|l|}
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Sealers & Manufacturers \\
\hline
AH Plus (AHP) & Dentsply DeTrey GmbH, Konstanz, Germany \\
Endométhasone (EM) & Spécialités Septodont, Saint-Maur, France \\
Pulp Canal Sealer (PCS) & Kerr Corp., Romulus, Ml \\
Vcanalare (VC) & Vebas s.r.l., San Giuliano Milanese, Italy \\
\hline
\end{tabular}
\caption{Endodontic sealers and manufacturers.}
\end{table}
curve is the average of two OD measurements in four wells at any given time. Each curve includes 44 measurements taken within 24 h.

In Group A wells, where bacteria grew in the presence of the tested material, freshly mixed and 24-h samples of the four sealers showed complete inhibition of bacterial growth (Figs. 1 and 2). With the 7-day samples, only VC was completely inhibitory, whereas AHP, EM, and PCS were similar to the positive control (Fig. 3). The standard deviation of the OD measurements was calculated (Figs. 1–3) and never exceeded 5.2% of the mean.

In Group B wells, where transferred bacteria were incubated in the absence of the tested materials, measuring the short-term direct contact effect, results did not differ, except with the 24-h samples (Figs. 1-3). With these samples, in fact, EM, PCS and VC exhibited complete inhibition for the entire incubation period, whereas AHP, though inhibiting bacterial growth until the 7 h reading, was unable to kill all bacteria that could reach stationary growth phase after 24 h incubation.

Discussion

The use of an endodontic sealer with antibacterial properties may be advantageous, particularly when pulpal or periapical infections are present. Antimicrobial activity of root canal sealer, in fact, may help to eliminate residual microorganisms unaffected by chemomechanical preparation of the root canal system, therefore, improving the success rate of endodontic treatment.

The aim of this study was to evaluate the antibacterial activity of four endodontic sealers. These included a recently introduced epoxy resin sealer (AHP), two commonly used ZOE-based sealers (EM and PCS) and a ZOE-based sealer containing orthophenilphenol (VC). With the use of E. faecalis as a test organism, a DCT assay was
performed. *E. faecalis*, a facultative anaerobic microorganism, has been used in various studies of antibacterial properties because of its implication in endodontic failures and in primary root canal infections. *E. faecalis* is the most resistant species to eliminate from root canal and can survive in root-filled canals even without the support of other bacteria and with scant substrate. The DCT we performed is to date considered a valuable in vitro assay to study the antimicrobial properties of endodontic sealers and other solid dental materials. In the past years, ADT-based assays have been used in many studies to evaluate the antibacterial activity of several various dental materials. However, it is extremely difficult to accurately compare bacterial inhibition data, even for the same material, between different investigators with the ADT, because of the difficulties in controlling a large number of variables. The ADT is a relatively insensitive and semi-quantitative technique and does not distinguish between bactericidal and bacteriostatic effects of an agent, since this one cannot be removed from the agar. The results of ADT are also highly influenced by solubility and diffusability of the test agent through the agar, and therefore, this test is not suitable to assay water-insoluble materials. On the other hand, the DCT is based on measuring the effect of direct and close contact between microorganisms and the tested material on microbial outgrowth. Such quantitative and reproducible assay allows us to determine whether the data gathered from a specific material reflect bactericidal or just bacteriostatic effects, regardless of the diffusion rates of the active agents. Another aspect of the setup of the DCT includes the ability to follow bacterial growth, both in the presence and in the absence of the tested materials. Following the bacterial outgrowth in the presence of the tested material (Group A wells) is equivalent to measuring both the direct contact effect and the effect of those components which are capable of diffusing into the liquid medium, whereas following bacterial growth in the absence of the tested materials (Group B wells) measures the effect of the direct contact incubation period only. Moreover, the DCT can be used in standardized aging studies.

The sealers evaluated in this study by using the DCT showed different inhibitory effects depending on the time interval between mixing and testing. The EM and PCS sealers, both containing ZOE, were equally effective in inhibiting bacterial growth and exerted bactericidal effect until 24 h after mixing. The eugenol is a potent antimicrobial agent, and therefore, the activity of ZOE-based sealers may be attributable to the free eugenol released from the set materials. Our results confirm only in part previous findings that ZOE-based sealers possess a strong and persistent antibacterial activity. When the 7-day samples of both EM and PCS were tested, in fact, no antibacterial activity was found. On the contrary, after 7 days from mixing, the Roth’s cement, a commonly used ZOE-containing sealer, still exerted antibacterial activity, although to a lesser extent than the 24-h samples.

Similar findings were obtained with AHP, which appeared to possess a short-acting potent and diffusible antibacterial activity. With the 24-h samples, in fact, AHP completely inhibited bacterial growth only when bacteria were incubated in the presence of the sealer (Group A wells). This finding suggests that the 24-h samples of AHP were not effective in killing all *E. faecalis* organisms after the short-term direct contact allowed (1 h). However, such samples yet possess antimicrobial components capable of diffusing into the liquid...
media and exerting complete bactericidal activity. Our findings with the freshly mixed samples of AHP are in agreement with those of Kont Çobankara et al. who found complete inhibition of bacterial growth; older samples, however, were not tested in that or other studies.

Among the tested sealers, VC only exerted bactericidal effect lasting up to 7 days after mixing. There is no available data, to the best of our knowledge, regarding the antibacterial properties of endodontic sealers containing phenol compounds. It must be noted, however, that VC contains also eugenol, and this agent may synergically act with the orthophenilphenol, thus inducing a potent and persistent bactericidal effect. Due to its long-lasting antibacterial activity, VC may effectively supplement chemomechanical preparation in disinfection of the root canal space and may also be of benefit in the treatment of persistent or recurrent infections. Additional studies, however, are needed to evaluate the antimicrobial effects within dentinal tubules and biocompatibility of this sealer. Biocompatibility is important as antimicrobial properties when selecting a material for the endodontic therapy. As endodontic sealers may come into direct contact with the periapical tissues or may leach through dentin, only those sealers should be used which have been proved to possess an at least acceptable biocompatibility.

In conclusion, the present findings indicated that antimicrobial activity of the tested sealers depends on the time interval between mixing and testing. All sealers exhibited bactericidal effect when freshly mixed, but only VC extended its bactericidal effect until 7 days after setting. On the other hand, the other sealers tested lost antibacterial activity over time. With 7-day samples, EM and PCS did not show any antibacterial activity. AHP, just after 24 h from mixing, was unable to kill all E. faecalis cells during a 1 h contact period, but it still released water-soluble components that completely inhibited bacterial growth.

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References

20. Hancock 3rd HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in...