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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 (Endomèthasone, 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.

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

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sealed adequately,[12,13] rapidly repopulate the empty canals between the appointments and can induce or sustain apical periodontitis.[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.[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 (Endomèthasone and Pulp Canal Sealer; EM and PCP, respectively), and one sealer containing both ZOE and orthophenilphenol (Vcanalare; VC).

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).[16] The sealers were prepared according to the manufacturers' instructions.

Table 1 Endodontic sealers and manufacturers.

Sealers	Manufacturers
AH Plus (AHP)	Dentsply DeTrey GmbH, Konstanz, Germany
Endomèthasone (EM)	Spécialités Septodont, Saint-Maur, France
Pulp Canal Sealer (PCS)	Kerr Corp., Romulus, MI
Vcanalare (VC)	Vebas s.r.l., San Giuliano Milanese, Italy

A collection strain of *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 10^8 CFU/ml.

The DCT has been performed following the method of Weiss et al. (1996),[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 10^6 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.

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