Antibacterial Effects of Various Root Filling Materials

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Summary

The aim of this study was to evaluate the antimicrobial properties of three endodontic sealers (AH 26, Ketac-Endo and Apexit) using Direct Contact Test (DCT) and Agar Diffusion Test (ADT). DCT is based on measuring the effect of close contact between test bacteria and tested material, while ADT measures the areas of bacterial growth inhibition and consequently the incidence of microbial resistence of bacteria.

The results of DCT showed that all tested materials, apart from Apexit, have antibacterial activity. The diameters of the bacterial growth inhibition zones in ADT demonstrated by Ketac-Endo (x=12.26 ± 1.76 mm), AH 26 (x=5.67 ± 0.66 mm) and Apexit (x=0.80 ± 0.29 mm).

Key words: *antibacterial activity, filling materials, root canal*

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Introduction

Microorganisms and their products are the most frequent etiologic factors in dental, pulpal and periapical pathology. In the normal oral flora, there are about 300 different bacterial species, a great number of which can colonize the root canal. Failure, during and after endodontic treatment are linked to the presence of bacteria in the root canal (1,2). The bacterial colonisation can be attributed to infection of the root canal and dentin tubulus before or during the treatment or to the coronary gap being re-entered by pathogenic bacteria after treatment. In many studies particular importance in the case of root canal infection is given to anaerobic bacteria, which are able to survive in conditions of longlasting infection, with scarce or almost non -oxygen supply (2,3,4). For this reason removal of these bacteria during treatment, growth inhibition of the residual bacteria and prevention of their re-entry, are considered to be key factors of successful endodontic treatment (5). Concerning the last of these requirements, the root canal must be completely sealed with suitable filling material. An ideal root filling material should produce a complete apical seal, should be nontoxic, tolerated by the periradicular tissues, nonresorbable, dimensionally stable, easy to manipulate and radiopaque (6). It

also should be bactericidal or bacteriostatic. The main objectives of endodontic therapy are to eliminate bacteria from the root canal and prevent regrowth of residual microorganisms.

The aim of this study was to evaluate the antibacterial activity of three endodontic sealers: AH 26, Apexit and Ketac-Endo cement. The antimicrobial properties were evaluated by DCT and the results compared to those obtained by ADT.

Materials and method

Three root canal sealers: Apexit (Vivadent, Schaan Liechtenstein), AH 26 (Dentsply DeTrey, Germany) and Ketac-Endo (ESPE GmbH, Seefeld, Germany) were analysed in this study. The sealer were prepared according to the manufacturer´s recommendation. Tested materials were examined 20 min after mixing.

Enterococcus faecalis was obtained from the Department of Microbiology, Medical Faculty University of Rijeka.

DCT was based on quantitative determination of bacterial growth in 96-well microtiter plates. One side of the plate's surface was coated with freshly mixed tested materials. 10 µl bacterial suspension (cca 10^6 bacteria) was placed on the tested materials. While the plate remained in vertical position, wells were inspected for evaporation of the suspension liquid, which occurred within one hour at 37°C. This ensured direct contact between bacteria and the tested material. Brain heart "BHI" broth (250 µl) was added with a micropipete of 100 µl in each sample and gently mixed for two min. (tenfold dilution). Two sets of four uncoated wells in the same microtitter plate served as positive control. The negative control consisted of a set of 4 wells coated with the tested materials.

For the ADT, 200 µl of bacterial suspension was spread on BHI agar plates. Freshly mixed material (0.13 g) was punched into the agar. After incubation at 37°C for 24 h, the agar plates were examined for bacterial growth inhibition. Following the incubation period, the diameters of bacterial growth inhibition zones were measured in milimeters on two different locations for each sample.

Results

The ADT showed that freshly mixed Ketac-Endo exhibit a markedly wider inhibition zone after three measurements ($x=12.26 \pm 1.76$ mm) compared with AH 26 ($x=5.67 \pm 0.66$ mm) and Apexit ($x=0.80 \pm 0.29$) (Table 1.).

The results of DCT showed that tested materials, apart from Apexit, have antimicrobial effect. After three measurement, in the samples with freshly mixed Apexit bacterial growth was $x=4.96 \times 10 \pm 0.25 \times 10^3$ CFU, whereas the freshly mixed samples of other materials showed complete inhibition of bacterial growth (Table 2).

Discussion

Numerous studies of root canal filling material have been done by using DCT and ADT in order to detect their antimicrobial properties (1,8,9). In this study both methods have been applied for testing these effects of Apexit, AH 26 and Ketac-Endo and obtained different results. The use of ADT has shown that of these three root sealers, after 24 hour incubation, Ketac-Endo creates the widest zone of bacterial growth inhibiton. The ADT technique is very often used in estimating the antimicrobial effects. However its reliability depends on the diffusion degree of the hard agar medium on the components present in each material tested (10). This technique is also insufficiently sensitive and semiquantitive because it cannot discern bactericidial from bacteriostatic material effect.

Therefore the DCT test has been used to verify the obtained results. Material testing by using DCT is based on the close contact of the tested material and tested bacteria, and is almost entirely isolated from the diffusion properties of the tested materials (11). Results of the study have proved that the tested materials, apart from Apexit, have a antimicrobial effect. Such ineffectiveness of a single calcium hydroxide material depends on the physical and chemical characteristics of the ingredients present (12). Shalhav et al. (13) have shown that Ketac -Endo contains a very strong antibacterial effect. It is possible that the antibacterial ingredients of Ketac-Endo have better diffusion and fusibility in water. An investigation with AH 26 made by Al

-Kathib et al. (11) showed the existence of satisfactory antibacterial activity of this material on tested bacteria (Streptococcus mutans i Staphylococcus aureus). This is due to the oligodynamic effect of the metal ions (14).

Examination of the antimicrobial effect of three endodontic sealers; Ketac-Endo, AH 26 and Apexit using ADT and DCT, showed that Ketac-Endo has the best antibacterial activity. The zones of bacterial growth inhibition were 12.26 ± 1.76 mm for Ketac-Endo, 5.67 ± 0.66 mm for AH 26 and 0.80 ± 0.29 mm for Apexit.

Results of DCT confirmed antibacterial activity of the experimental materials, apart from Apexit. It is proposed that more than one assayable method should be used in the process of evaluation of the antimicrobial properties of dental materials in particular endodontic sealers.