

EVALUATION OF ANTIMICROBIAL EFFECT OF ROOT CANAL SEALERS

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ABSTRACT

Endodontic sealers are used to fill the irregularities of root canals and to overcome the poor sealing ability of core root canal filling materials. Some sealers possess antibacterial effect and may eliminate microorganism left in the root canal system after completed cleaning and shaping procedures. Many studies have reported the antimicrobial activity of root canal sealers, but different techniques and methodologies have been used. The aim of this review is to review three of the most common techniques used for testing the antimicrobial effect of endodontic sealers. The Agar Diffusion Inhibitory Test, until recently, has been considered the standard method. It has some limitations and the examiner needs to consider and control many variables such as molecular weight of the material, temperature of the medium and the solubility of the endodontic sealer. The Direct Contact Test is able to measure the antimicrobial effect regardless of the solubility and the diffusability of the antimicrobial components of the sealers. A third technique is used to evaluate the disinfection of dentinal tubules using different root canal sealers. It is called Dentinal Tubule Penetration Effect. Reproducibility, functionality, reliability and simplicity are important requirements for better assessment of the antimicrobial effect of endodontic sealers. According to the recently published literature, Direct Contact Test appears to be the most reliable and is mostly used for evaluating the antimicrobial effect of endodontic sealers. For more accurate results it has been suggested to utilize more than one technique when evaluating the antimicrobial effect of root canal sealers.

Key words: Antibacterial, Antimicrobial, Endodontic sealer, Endodontic cement

INTRODUCTION

The main aim of root canal treatment is to eliminate microorganisms from the root canal system and to prevent reinfection.^{1,2} To achieve this goal, combination of chemical and mechanical cleaning and shaping techniques are used. Those are followed by filling of the canal system with appropriate filling material and proper sealer. With all the advances in endodontic techniques, we still lack a perfect system that can render complete eradication of microorganism from the root canal system. Dalton et al (1998) compared endodontic rotary files preparation to manual step back technique. Their findings showed that none of the two systems resulted in bacteria free canals.³ Several other studies have shown the presence of microorganism even after a comprehensive chemo-mechanical preparation of a complex root canal system.⁴⁻⁸

Gutta-percha is still the material of choice to fill root canals even though it has poor sealing ability and lacks adequate antibacterial properties, which necessitates the use of an endodontic sealer. To prevent bacterial growth, ideally, obturation materials and sealers should have antimicrobial properties.⁹ Later on, bactericidal or bacteriostatic activity of root canal sealers became one

of the ideal requirements which were established by Grossman.¹⁰ Many investigators have reported that endodontic sealers possess antimicrobial effect.¹¹⁻¹³ Many authors have suggested filling the root canal system using a sealer with bactericidal effect, which is expected to eradicate the remaining microorganisms and overcome persistent residual infection.¹² Theoretically this would improve the outcome of treatment, yet we lack randomized clinical studies, or animal studies, evaluating the antimicrobial effect of sealers on the prognosis of root canal treatments.

Many studies have reported on the antimicrobial activity of root canal sealers, but different techniques and methods have been used for testing single cements or comparing multiple ones.^{14,15} There is no single and updated review comparing techniques used for testing the antimicrobial effect of root canal sealers, therefore the aim of this paper is to review techniques used to test antimicrobial effect of endodontic sealers and to highlight the strengths and weaknesses of tests used.

1- Agar Diffusion Inhibitory Test

The Agar Diffusion Inhibitory Test (ADT), or Lawn's technique has been used for long time as the standard

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test for antibacterial activity of dental materials.¹⁶ To test the antimicrobial effect of setting materials including root canal sealers, fixed diameter wells are prepared in an agar plate. A suspension of bacteria of choice is placed onto the surfaces of blood agar plates. Small wells are cut out of the agar plate, which are then filled with the sealer of choice. The zone of inhibition is measured as an indication of the antibacterial effect of that sealer. Grossman in 1980 tested eleven different root canal sealers within aerobic conditions. He used human saliva or *Bacillus Subtilis* and showed that Zinc Oxide Cement containing formaldehyde reported the largest inhibition zone after 24 hours of incubation.¹⁷ Ten days after incubation there was no differences between the sealers. A variation of this technique is placement of absorbent paper points mixed with the sealer of choice on the agar plate instead of using wells.¹⁸ It's noteworthy to mention the difficulty in comparing the results of various studies using Agar Diffusion Inhibitory Test due to the usage of different strains and different growth media. This has been emphasized in a literature review focusing on the antibacterial properties of dental materials.¹⁹ The ADT lacks the ability to test and compare the viability of microorganisms used. Inability to distinguish between bacteriostatic and bactericidal effect of the root canal sealer material, is another drawback of this method. In addition, ADT requires the control and standardization of many factors to be able to obtain accurate results. Barry in 1976, discussed the most important variables and dynamics affecting Agar Diffusion Inhibitory Test results.²⁰ The author stated that the contact of the materials tested and the surrounding gel is important. Secondly, the molecular weight of the antimicrobial material tested should be of size and shape small enough to pass through a gel medium. It is important to mention that the diffusion rate of the antimicrobial agent in the tested material depends on many variables: A) charge and concentration of the antimicrobial agent in the medium, B) viscosity of the agar gel, C) temperature and ionic concentration of the material tested.^{16,19,20} Another important factor is time, which has to be sufficient for the antimicrobial agent to diffuse into the agar before a specific density of the microorganisms is reached. The zone of inhibition can be measured as soon as the microorganisms begin to multiply. To be able to produce reliable and reproducible results we need to control the inoculum density.²⁰ The Agar medium type is as important as all the previously mentioned critical factors. It has a major role in the discrepancy between the investigations done using same root canal sealer and different media. If an investigator is using an agar medium that promotes slow growth this may lead to false reading of antimicrobial effect. While the microorganisms are slowly growing, the antibacterial agent has more diffu-

sion time, thus a bigger zone of inhibition will form.¹⁴ Investigators should pay attention to the volume of the agar medium which should be similar between different plates. The thickness of the agar should be enough to allow smooth diffusion of the antimicrobial agent to the medium, too large or too little volume will result in false negative or false positive readings.²⁰ Many researchers have neglected this important point and failed to mention this in their methodology. Some studies have used 5mm thickness.^{11,21} But it has previously been reported that the optimal thickness of the agar medium is 4mm.^{14,20,22}

It is critical to control the temperature of the plates when using the ADT technique, not fulfilling this condition will lead to variation in the results and inability to reproduce the results or to compare them to other experiments. Cooper et al 1952, reported that any delay in raising of the temperature of the plate will effectively slow the growth of organisms and produce larger inhibition zones than optimum condition. The commonly used temperature with Agar Diffusion Test is 37°C. Size and number of specimens per plate can also be a critical variable during the use of this technique.

Solubility of the tested material is another critical factor that may affect the ADT inhibition zone. Soluble materials work well with the ADT.^{23,24} But, when testing a low water soluble and low diffusible material like calcium hydroxide, the ADT may result in false negative results. On the contrary, a material that results in dramatic inhibition of the ADT may fail as a permanent dental restoration. Due to its solubility sensitivity, it was suggested that ADT may be a better technique for testing materials indicated for temporary treatments or inter-appointment medications. Despite all the mentioned factors and limitations of the ADT, this technique is still being used.^{25,26}

Scientists have come up with alternative techniques to overcome the limitations encountered by the ADT, such as: Direct Contact Test (DCT), Dentinal Tubule Penetrating Effect, Dilution in Broth¹⁵, Survival Time,^{27,28} Modification Cavity method.²⁹ The DCT and Dentinal Tubule Penetrating Effect techniques are most commonly used and will be discussed in detail.

2- Direct Contact Test (DCT)

In the mid-nineties a new technique was introduced to overcome the disadvantages of ADT and was named Direct Contact Test (DCT) for antimicrobial evaluation of different materials.²⁵ The DCT aims to test the antibacterial effect when there is direct contact between the tested material and the target material and the target microorganism. One of the advantages of this technique is the ability to measure the anti-

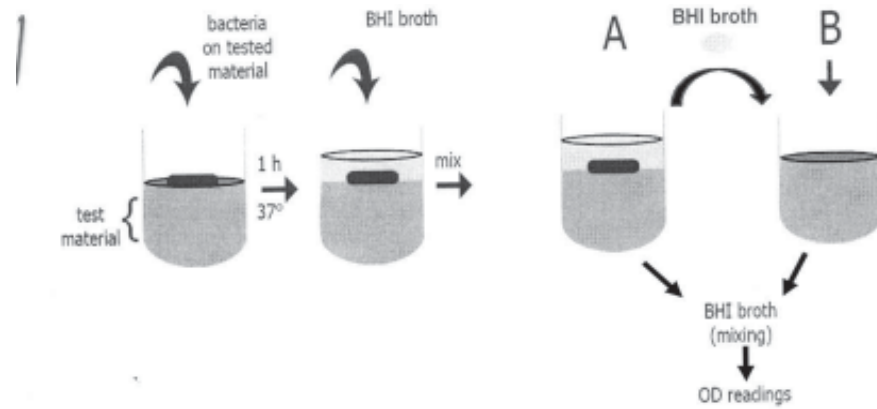


Fig 1: **Schematic representation of wells used for Direct Contact Test.** Specific Bacteria are added in-direct contact with the sealer of choice. Growth medium is then added to the well. Bacterial outgrowth is monitored in the presence (A) or absent (B) of the sealer using a spectrophotometer. BHI, Brain Heart Infusion; OD, Optical Density.

icrobial effect regardless of the solubility and the diffusability of the antimicrobial components of the sealers. The DCT has been shown to work well with less water-soluble materials. Therefore, the DCT proved to be better method to test the antibacterial effect of calcium hydroxide sealers.³⁰

As shown in Figure 1, the DCT is based on measuring the turbidity of material growth by means of microtiter plates. A temperature controlled spectrophotometer is used and each well is read every 30 minutes. To guarantee a homogeneous bacterial cell suspension, auto mixing of the well content is done routinely. The micro plate is held vertically and the tested material must be applied to the well carefully to avoid touching the floor of the well which will lead to false positive results. Afterward, the bacteria mixture is added to the site. After a certain incubation time, bacterial growth can be monitored.

The Direct Contact Test relies on the direct and close contact between the tested material and test microorganism, it is virtually independent of the diffusion properties of both the tested material and the media. DCT has been recommended for the evaluation of restorative material and endodontic sealers.²⁵ Not being affected by the inoculum size, the results of DCT are more quantitative and reproducible when compared to those of the ADT. A temperature controlled spectrophotometer is used to control the temperature in DCT. Moreover, the DCT is able to monitor the growth of test microorganism in the presence or absence of the tested sealer. Studying the bacterial growth in presence of tested material will allow studying of both the direct contact effect and the effect of those particles tested while diffusing into the liquid medium. On the other hand, when the tested material is absent, the examiner will be able to test and evaluate the effect of direct contact incubation period only as a

control.²⁵ It is noteworthy to mention that the risk of contamination of the samples is one of the draw backs in this technique; some studies have overcome this problem by using test microorganism with appropriate selective growth media.^{31,32}

3- Dentinal Tubule Penetrating Effect

In 1987, Haapasalo and Orstavik proposed a new technique to test the infection and disinfection of dental tubules.³³ Their aim was to develop an in vitro method to test different types of root canal materials by means of infected tubules under controlled conditions. The method uses freshly extracted intact bovine incisors. After disinfection with 0.5% NaOCl, the apical part and the two thirds of the crown are cut off, then the canal is widened to 2 mm in diameter. Later on, the root cementum is removed leaving a cylindrical specimen which then is cut into sections of 4 mm. The dentin blocks are kept in sterile water during all procedures to avoid dehydration. The smear layer is removed by using 17% Ethylene Diamine Tetraacetic Acid ultrasonic cleaning bath and NaOCl 5.25% to allow the opening of the dentinal tubules. All sections are sterilized by autoclaving the specimens in water for 15 minutes at 121°C.

Then sections are moved to yeast extract glucose broth in which they are incubated for 24 hours to test their sterility. A decided number of sections are subjected to preselected test bacteria which are allowed to infect the tubules during 3 weeks. The long time period is necessary to allow bacteria to penetrate into the tubules. Strict asepsis is necessary to avoid contamination. After placing a varnish on the outer surface, the test material is placed in the canal lumen and left for incubation during different time intervals. At the termination of the study, the tested material is removed with a sequence of sterile round burs to remove dentin cuttings from the lumina. The cuttings samples are

collected and placed in brain heart infusion broth in sterile tubes and incubated for certain periods of time. Finally, the optical density of the broth is measured using a spectrophotometer to evaluate the presence of organisms and the antibacterial effect of the tested root canal sealer. Later on, the technique has been modified e.g. instead of using bovine teeth, human teeth have been used.³³⁻³⁵

In conclusion, antibacterial component of endodontic sealers may be an essential factor to prevent the continuous growth of remaining bacteria in the canal. Many techniques are available for testing antimicrobial effect of endodontic sealers. Fulfilling the previously mentioned requirements and according to the recently published papers.^{9,30,36,37} Currently, Direct Contact Test appears to be the most reliable and mostly used for evaluating the antimicrobial effect of endodontic sealers. For more accurate results it has been suggested to utilize more than one technique when evaluating antimicrobial effect of root canal sealers.^{25,38}

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