Antimicrobial activity of eight root canal sealers before and after setting

Denny Y Fang*, WooCheol Lee*, Chern H Lai**
*Department of Endodontics**, *Department of Periodontics & Microbiological Testing Laboratory***,
University of Pennsylvania, School of Dental Medicine

국문초록

8종 root canal sealers의 경화 전, 후의 항균효과에 관한 연구

이우철 · Denny Y Fang · Chern H Lai
펜실베니아

항균 활성도는 Root canal sealer가 갖추어야 할 필수요소 중 하나이다. 본 연구는 최근 임상술식에 사용되고 있는 8종의 root canal sealer의 근관내 혐기성 세균에 대한 항균효과를 알아보기 위해 시행되었다. 또한 본 연구에서는 혼합직후의 경화되지 않은 sealer와 경화 7일 후 sealer의 항균효과도 비교하였다. 항균효과 측정을 위해 사용된 균주는 최근 실패한 근관치료 증례에서 배양되어 보고된 바 있는 *Enterococcus faecalis*와 근관내 주요 감염균인 그램음성 혐기성 세균인 *Staphylococcus aureus*를 대상으로 하였고, Agar diffusion test 방법을 사용하였다. 실험방법으로는 2개의 paper disk에 신선하게 혼합한 각각의 sealer를 도포하여 한개의 disk는 즉시 실험에 사용하고 다른 한개의 disk는 일주일간 혐기성 배양기에서 경화시킨다음 사용한다. 각각의 균주를 Brucellar blood agar plate 에 접종한 다음, sealer가 도포된 paper disk를 plate상에 올려놓는다. 대조군으로는 식염수에 침윤시킨 disc를 같은 방법으로 각 실험단계에 사용한다. 각 plate를 혐기성 배양기에서 48시간동안 배양한 뒤, 실험에 사용한 sealer의 항균효과를 6mm paper disk를 둘러싼 inhibition zone을 측정하여 평가한다. Fisher's PLSD 분석방법 결과 *E. faecalis* 에 대하여 경화 전과 후의 AH26모두 경화 전과 후의 Roth 801, Dentalis, Apexit, AH Plus, RSA 그리고 경화 후의 MCS보다 유의성있게강한 항균효과를 나타내는 것으로 보고되었으며, 경화 후의 AH26은 경화 전의 AH 26, 경화 전의 Ketac Endo, 경화 전의 MCS보다 통계학적으로 유의성이 있는 항균작용을 하는 것이 관찰되었다 ($p<0.05$). 경화 후Roth 801, 경화 전과 후의 Dentalis, AH plus, Apexit, RSA는 *E. faecalis*에대한 항균효과를 나타내지 못하였다. *S. aureus*에 대하여 경화후의 AH26이 경화 전과 후의 Roth 801, Apexit, AH Plus, RSA보다 유의성있는 항균효과를 보이는 것을 발견할 수 있었고, 경화 전의 AH 26이 경화 후의 AH plus보다 나은 항균효과를 나타냄을 알 수 있었다. 또, 경화 전과 후의 Apexit, 경화 후의 AH Plus, 경화 전과 후의 RSA에서는 *S. aureus*에 대한 항균작용이 발견되지 않았다. 본 실험의 결과 AH26이 가장 강한 항균 작용을 갖는 것을 알 수 있었으며, 각 sealer의 경화 전과 후의 항균효과는 AH26이 경화 전보다 경화 후에서 더 강한 항균효과를 나타내는 것 이외에는 효과의 차이가 없었다.

I. Introduction

The goal of endodontic treatment is to eliminate or to lower the bacterial concentration gradient from the root canal and to prevent reinfection[1,2]. In addition to cleaning and shaping procedure, root canal filling material may play a critical role in destroying bacteria which remained in the root canal system. Killing bacteria by root canal filling procedure is either done by the hermetic obturating seal or by the direct bactericidal properties of the obturating materials. Several endodontic sealers have been found to possess antibacterial properties depending on their chemical components, such as calcium hydroxide, eugenol, and fluoride[3-5]. Different types of sealers have been introduced in the market and several studies have been done to evaluate antimicrobial effect of those sealers[6]. However, data were limited

to the initial antimicrobial activity only because the highest microbial inhibition is thought to occur immediately after the sealer has been mixed and to decrease as it becomes hard. Therefore, it is important to compare the antibacterial activities of endodontic sealers before and after setting.

The purpose of this study is to compare the antimicrobial potential among the eight commercially available sealers by using the agar diffusion test. The bacterial species tested against were *Enterococcus faecalis and Staphylococcus aureus*. This study also compared the freshly mixed and one-week set to evaluate if the antimicrobial effect sustained once the sealers have completely set.

Ⅱ. Materials and Methods

The sealers used in this study were : Roth 801 (Roth Int., Chicago, IL), MCS (Lone Star Tech., Westport, CT), Dentalis (DiaDent Group Int., B.C., Canada), Apexit (Vivadent Ets., Schaan, Liechtenstein), Ketac Endo (ESPE, Norristown, PA), AH 26 (Dentsply De Trey, Konstanz, Germany), AH Plus (Dentsply De Trey, Konstanz, Germany), and RSA (Roeko, Langenau, Germany). The chemical components of each sealer were listed in Table 1. Each of the eight sealers was mixed according to its manufacture's instruction. Each sealer was divided into two groups; dry and wet group. The dry group was the sealer-coated paper disk (Becton Dickinson, Cockeysville, MD), which was set and stored in a sterile petri dish for one week. The wet group was the paper disk coated with the freshly mixed sealer prior to the agar diffusion experiment.

An agar diffusion test was used to evaluate the bacterial inhibition of each sealer. Two facultative anaerobes, *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 29213) were tested. All the straines were obtained from the Department of Periodontics & Microbiological Testing Laboratory, University of Pennsylvania, School of Dental Medicine. 200㎕ of bacterial suspension (McFarland

Table 1. Chemical component of each sealer used in this study

Sealer	Chemical component
Roth 801	Liquid : eugenol Powder : Staybelite resin, zinc oxide, sodium borate anhydrous, barium sulfate, bismuth subcarbonate NF, hydrogenated rosin
MCS	Liquid : eugenol Powder : zinc oxide, sodium borate, barium sulfate, bismuth subcarbonate, hydrogenated rosin, iodoform
Dentalis	Liquid : eugenol Powder : calcium hydroxide, zinc oxide, iodoform, others
Apexit	Base : calcium hydroxide, hydrogenated colphony, silicon dioxide, paraffin oil, calcium oxide, tricalcium phosphate, polydimethylsiloxane, zinc stearate, alkyl ester of phosphoric acid, pigments Activator : Trimethylhexanedioldisalicylate, bismuth carbonate basic, bismuth oxide
Ketac endo	Poly Maleinate Glass ionomer
AH 26	Powder : Bismuth oxide, methenamine Resin : bisphenol-A-diglycidylether
AH Plus	Paste A : Epoxy resin, calcium tungstate, zirconium oxide, aerosol, iron oxide Paste B : Adamantane amine, N,N'-dibenzyl-5-oxanonane-diamine-1,9, TCD-diamine, calcium tungstate, zirconium oxide, silicone oil
RSA	Polydimethylsiloxane, silicone oil, paraffin-based oil, hexachloroplatinicacid, zirconium oxide

Standard 0.5=150×10^6 cells) were spread on Brucellar blood agar plates. Both groups of sealer incorporated paper disks, dry and wet, were placed in the center of the plates. As a control, a disk saturated with normal saline solution was placed on the plate for each series of experiment. After 35℃ incubation in an anaerobic chamber containing a mixture of gas with 80% N_2, 10% CO_2 and 10% H_2 for 48 hours, the agar plates were examined for bacterial growth inhibition. The diameter of the inhibition zone formed beyond the 6mm paper disk was measured by millimeters. Greater diameter of zone of inhibition was interpreted to indicate greater antimicrobial activity of the involved sealers. For consistency of the result, each sealer was retested for three times.

The Fisher's PLSD analysis was used to detect any significant difference between two different sealers and the conditions (between dry and wet) of each sealer.

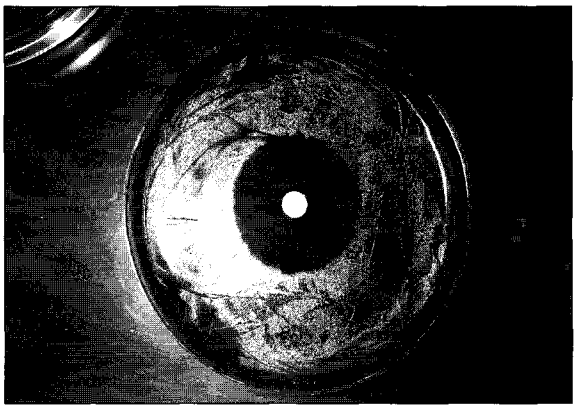

Fig. 1. Inhibition zone produced by dry AH26 against *Enterococcus faecalis.*

Ⅲ. Results

Control

The saline saturated disk did not exhibit zones of inhibition when tested against either *E. faecalis or S. aureus.*

Antimicrobial activity against *E. faecalis*

Fig. 2 showed the mean inhibition diameter of the wet and dry group sealers against *Enterococcus faecalis.* The Fisher's PLSD analysis found both one-week set (dry) and freshly mixed (wet) AH26 have significantly higher antimicrobial activity than the dry and wet Roth 801, dry MCS, dry and wet Dentalis, dry and wet Apexit, dry Ketac Endo, dry and wet AH Plus, and the dry and wet RSA ($p<0.05$). Only the dry AH26 has significantly greater antimicrobial activity than the wet AH 26, the wet Ketac Endo and the wet MCS. Fig. 1 demonstrated the inhibition zone produced by dry AH26

The dry Roth 801, dry and wet Dentalis, dry and wet Apexit, dry and wet AH Plus, and dry and wet RSA have no antimicrobial activity against *E. faecalis.*

Antimicrobial activity against *S. aureus*

Fig. 3 showed the mean inhibition diameter of the wet and dry group sealers against *Staphylococcus aureus.* The Fisher's PLSD analysis found the dry AH26 has significantly better antimicrobial activity than the dry and wet Roth 801, dry and wet Apexit, dry and wet AH Plus, and the dry and wet RSA ($p<0.05$). And the wet AH26 is significantly better than the dry AH Plus. The dry and wet Apexit, dry

Fig. 2. Mean inhibition diameter (mm) of the wet and dry group sealers against the *Enterococcus faecalis.*

sealer	wet	dry	sealer	wet	dry
Roth 801	1	0	Ketac Endo	1.5	1
MCS	1	0.5	AH 26	3.2	6
Dentalis	0	0	AH plus	0	0
Apexit	0	0	RSA	0	0

Fig. 3. Mean inhibition diameter (mm) of the wet and dry group sealers against the *Staphylococcus aureus.*

sealer	wet	dry	sealer	wet	dry
Roth 801	2.3	0.5	Ketac Endo	3	2.5
MCS	2	1	AH 26	6.5	8.7
Dentalis	2	3.2	AH plus	5.3	0
Apexit	0	0	RSA	0	0

AH Plus, and dry and wet RSA have no antimicrobial activity against *S. aureus*.

Ⅳ. Discussion

This study demonstrated eight different sealers in their antimicrobial capability by using the agar diffusion test. The bacterial strains tested against were *E. faecalis* and *S. Aureus*. These bacteria were selected because they were highly resistant to calcium hydroxide treatment [8,9]. They both can survive in a harsh environment. Especially, *E. faecalis* is able to survive longer than seven days without any nutrient [10]. *E. faecalis* is also a common single isolate from the canals of the refractory cases [9]. When tested against *E. faecalis*, with the release of formaldehyde as an antimicrobial agent [7,11], AH26 has significantly stronger bacterialcidal effect than Roth 801, MCS, Dentalis, Apexit, Ketac Endo, AH Plus, and RSA. The sealers, Dentalis, Apexit, AH Plus and RSA, do not provide any antimicrobial effect against *E. faecalis*. There is no significant difference between the freshly mixed sealer and the one-week set sealer in terms of its sustaining antimicrobial effect. Except in the group of AH26, the one-week set sealer has significantly higher bacterialcidal effect than the freshly mixed. We assume that antimicrobial component in the AH26 may release in higher concentration once the sealer has completely set. This is a similar phenomenon to Al-Khatib's [5] experiment of agar diffusion test against *Bacteroids endodontalis* on 7-days and 35-days.

When tested against the weaker bacteria strain *S. aureus* as a comparison to the E. faecalis group, most of the sealers perform equally well in their antimicrobial activity except Apexit and RSA sealer groups. The RSA sealer does not provide any antimicrobial capability because none of its chemical components has bacterialcidal effect. The chemical component of the RSA sealer is very similar to the silicone based Endo-Fill sealer which Görduysus[12] also found no antimicrobial capability. The fact that Apexit did not show any microbial inhibition corroborates those observed by Duarte et al.[13] who found that Apexit produced least alkaline pH and calcium ion compared with other two calsium hydroxide-based sealers such as sealapex and sealer 26. Optimal pH for growth of *S. aureus* is between 7 and 7.5 with a 4.2 to 9.3 range. The result in this study confirmed that alkaline pH produced by Apexit did not exceed this value, thus allowing this microorganism to grow[7]. Glass ionomer cement possess strong antibacterial properties[14,15], it is mainly due to a fluoride and other ingradients. Ketac Endo is a newly developed glass ionomer-based sealer. According to the recent studies [16-18], this sealer was superior in its ease of manipulation, radiopacity, and setting time compared to Grossman's sealer as well as its adaptation to the canal walls. The data of the current study indicated that Ketac Endo did possess antibacterial activity against both tested microorganisms. Our data also consist with Siqueira's findings[6] that ZOE-based sealers demonstrate a strong bacterial inhibition, which seems related to a high concentration of eugenol.

The Agar diffusion test used to evaluate antibacterial activity of sealers is the most commonly used technique[6,15]. This method is relatively insensitive and data are highly dependent on molecular size and the diffusion constant of the antimicrobial component, inoculum size, incubation time, and degree of material-agar contact[19]. Moreover, since the antimicrobial substance must diffuse through the aqueous agar medium, only water soluble agents can be tested. Therefore, Agar diffusion technique has some type of limitation and the interpretation of data obtained by this test should be reevaluated.

AH 26 and AH Plus are basically same material. The difference between them lies in the presence of silicone and aerosol in the fomula as well as the elimination of formaldehyde release from the latter material. Even though this study proved the superiority of antimicrobial acitivity of the AH26 sealer over the others, its cytotoxicity effect from the release of the formaldehyde cannot be neglected[20]. Careful usage and manipulation of this sealer should be considered. It is recommended that maintain the sealer inside the canal system and contact with the periapical tissue as little as possible.

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