IN VITRO STUDY ON APICAL SEAL AND SEM MORPHOLOGY IN CANALS FILLED WITH RESIN-BASED ROOT CANAL SEALER AND SELF-ETCHING PRIMER

Sam-Hee Kang, Dong-Sung Park, Heyon-Mee You*, Tae-Seok Oh

Sungkyunkwan University School of Medicine, Department of Conservative Dentistry, The Institute of Oral Health Science, Samsung Medical Center

ABSTRACT

SELF-ETCHING PRIMER와 레진계 근관 SEALER 사용시 근관벽의 형태와 치근단 근관밀폐에 대한 실험적 연구

강삼희 · 박동성 · 유현미* · 오태석

성균관대학교 의과대학 삼성의료원 치과진료부 보존과

Gutta-percha와 근관sealer를 사용한 근관 충전은 가장 많이 이용되고 성공률이 높은 방법이지만, 현재 사용되는 모든 근관sealer는 미세누출을 나타낸다. 따라서 더 효과적인 근관 폐쇄를 이루기 위한 재료 및 방법들이 연구되어왔다. 이중 상아질 접착제는 근관 충전재와 함께 사용되어 미세누출을 감소시키지만, 근관 내에 사용하기에 술식이 복잡하고 기술이 요구된다.

본 연구의 목적은 gutta-percha와 레진계 근관 sealer로 충전한 근관에서 self-etching primer를 미리 도포한 경우와 도포하지 않은 경우의 치근단 미세누출을 비교하고, self-etching primer를 도포함에 있어 근관 내에 적용하는 방법에 따른 미세누출을 비교하는 것이다. 또한 근관sealer와 상아질 계면을 주사전자현미경으로 관찰하여, 이 결과를 미세누출과 관련시켜보고자 하였다.

36개의 발거된 사람의 단근치에서 치관부를 절단, 제거하고 ProFile로 근관 형성한 후, 무작위로 선택하여 4개의 군으로 분류하였다. 1군에서는 주사기와 30게이지 주사 바늘로 self-etching primer를 근관 내에 적용하였고, 2군에서는 self-etching primer를 paper point에 적셔 근관 내에 적용하였다. 3군에서는 self-etching primer를 적용하지 않았다. 1, 2, 3군의 치아를 gutta-percha와 AH26 sealer를 사용하여 continuous wave 충전법으로 충전한 후 치근단공 주위 3mm를 제외한 치근면에 nail polish를 2회 도포하였고, 4군(음성 대조군)은 치근면 전체에 도포하였다. 1군과 2군에서 각각 2개의 치아는 주사전자현미경적 관찰을 위해 준비하였다. 모든 치아를 Methylene Blue 수용액에 48시간동안 침적, 수세한 후 치아 장축에 평행하게 양분하여, 10배의 실물확대현미경 하에서 치근단 색소 침투를 관찰하였다.

Self-etching primer를 도포한 군과 도포하지않은 군 사이에는 평균 미세누출량에 유의한 차이가 없었다. 주사바늘로 적용한 군과 paper point로 적용한 군 사이에도 평균 미세누출량에 유의한 차이가 없었다. 상아질과 근관sealer계면의 주사전자현미경 관찰 결과 일부분에서 긴밀한 접착 관계를 나타내었고, 다른 부분에서는 간극을 나타내었다.

주요어 : Self-etching primer, 레진계 근관 sealer, 치근단 미세누출, 주사전자현미경

* 책임저자

Ⅰ. INTRODUCTION

One of the main objectives of endodontic treatment is total obliteration of the root canal system and development of fluid tight seal at the apical foramen[1-3]. Apical leakage adversely affects the success of endodontic treatment[2].

It is generally agreed that the use of gutta-percha cone with sealer is one of the most reliable methods for obturation of the root canal system[3,4]. The sealer fills in the irregularities and minor discrepancies between the gutta-percha cone and the canal wall[3,5] and it prevents apical leakage of the obturated root canal[6-9]. Although this method is highly successful, all obturated canals exhibit leakage[5,10] and most leakage occurs between the canal wall and the sealer[4,6].

The use of dentin bonding agents as endodontic filling materials has been reported in some of the studies[10-12]. Canals obturated with gutta-percha cones and dentin bonding agents[10,11] as well as with gutta-percha cones, resin-based root canal sealers and dentin bonding agents[12] have shown less leakage than canals obturated without dentin bonding agents. However, since these materials need relatively technique-sensitive and time-consuming steps, they are currently not used as endodontic filling materials.

The current generation of adhesive systems relies on the micromechanical bonding of resin to the etched dentin mediated by hydrophilic primer[13-19]. Primer molecules contain two functional groups ; a hydrophilic group and a hydrophobic group. The primer wets and penetrates the collagen meshwork, increases the surface energy and hence the wettability of the dentinal surface. As a result of this primer pre-treatment, a desirable bond site is formed for copolymerization in situ with the subsequently applied adhesive resin. Unfilled resins copolymerize with the primer to form an intermingling layer of collagen and resin, termed as the hybrid layer. Formation of the hybrid layer is thought to be the primary bonding mechanism for most current adhesive systems.

Considering the adhesion-promoting ability of primers[18] and the technical complexity of dentin bonding agents, primers alone instead of dentin bonding agents have the possibility of improving the adhesion of the resin-based root canal sealer in the root canal system.

If the sealing ability was assumed to be comparable, a technique which requires fewer steps would seem to be preferable. Self-etching primers containing acidic Phenyl-P monomers and HEMA could simplify the procedure[18,20,21].

The purposes of this study was to evaluate the effect of self-etching primer on apical seal of AH26 sealer and to compare the apical seal of the two different application methods : syringe and paper points. In addition, the state of dentin-gutta-percha interface was evaluated using a scanning electron microscope.

Ⅱ. MATERIALS AND METHODS

1. Preparation of Specimens

Thirty-six extracted teeth with single, straight root canals and mature apices were selected and extraneous soft tissue and calculus on the teeth were removed. They were decoronated at the cementoenamel junction and stored in saline.

2. Root Canal Instrumentation

The root canals were cleaned and shaped with ProFile (Maillefer, Switzerland) using crown-down technique with patency assured by periodic recapitulation with #10 k-file. Working lengths were established 1.0mm short of the apical foramen. Canals were irrigated with approximately 1.0ml of 5% NaOCl after using each file and then dried with paper points.

The prepared teeth were randomly divided into three experimental groups (12 teeth in Group, 1 and 2 and 10 teeth in Group 3) and one control group. Four teeth, two each from Groups 1and 2, were processed for scanning electron microscopic observation.

3. Root Canal Obturation

Group 1 : LB Primer (Kuraray, Japan) was mixed according to the manufacturer's instructions, placed

Table 1. Experimental groups

Group	Pretreatment / Application method	Obturation
1	LB Primer / syringe+Max-i-Probe	GP cone+AH 26
2	LB Primer / paper point	GP cone+AH 26
3	No	GP cone+AH 26

in the canals using a syringe and a 30-gauge endodontic needle(Max-i-probe, MPL Technology, USA) for 30 seconds and excess were removed by aspiration with a syringe and paper points. Canals were obturated with gutta-percha cone (Diadent, Korea) and AH 26 sealer (De Trey, Germany) by continuous wave of condensation technique. The sealer was mixed according to the manufacturer's instructions and introduced into the canal with the same gutta-percha cone, using the pumping movement.

Group 2 : LB Primer was placed in the canals using a soaked paper point (Diadent, Korea) and excess were removed by paper points. Then canals were obturated with gutta-percha cone and AH 26 sealer as described in Group 1.

Group 3 : Canals were obturated with gutta-percha cone and AH 26 sealer as described in Group 1. But LB Primer was not used.

Group 4 (negative control) : Two teeth were entirely covered with two coats of nail polish.

Instrumentation and obturation was done by one operator to reduce the inter-operator variability.

4. Microleakage Test

The access cavities of all teeth were filled with Cavit (ESPE, Germany) and teeth were left in saline for 48 hours to allow the sealer to set. After storage, the teeth were double-coated with nail polish with the exception of the apical 3mm. Specimens were placed in 2% methylene blue solution for 48 hours. The teeth were removed from the dye solution, washed, stored in water for 24 hours and dried with compressed air.

To evaluate the depth of the dye penetration, each tooth was sectioned longitudinally using a low-speed diamond disc, chisel and mallet. Microscopic images at 10-fold magnification were recorded (Urban Microscopes, Global Surgical corporation, USA) and captured. Linear dye penetration distance from the apex was double-blind measured using a digitalized image analyzing program by two different observers. Four teeth, two each from Groups 1 and 2 proved to be defective and excluded.

5. Scanning Electron Microscopic Observation

Two specimens from Group 1 and two specimens from Group 2 were longitudinally sectioned. Each specimen was mounted in aluminum stubs and sputter-coated with gold. The dentin-canal obturation material interfaces were observed under a scanning electron microscope (JSM-840, JEOL Ltd., Japan).

6. Statistical Analysis

A Mann-Whitney Rank Sum Test at the 0.05 level of significance was used to evaluate the statistical differences among the means of the groups.

Ⅲ. RESULTS

1. Microleakage Test

Table 2 and Fig. 1 show apical leakage values of the experimental groups. All specimens demonstrated dye penetration to varying degrees except for the specimens of Group 4 (negative control).

The mean of Group 1 (syringe-application) was 3.04±0.86mm and that of Group 2 (paper point-application) was 3.39±1.46mm. The difference between the two groups was not statistically significant ($p>0.05$).

Group 3 (no application of self-etching primer) showed mean leakage value of 2.92±1.30mm. The mean leakage values of primer-applied groups (Groups 1 and 2) were not significantly different

Table 2. Linear leakage measurements of experimental groups (mm)

Group	No.of specimen	Dye Penetration			
		Mean	Median	SD	Range
1	8	3.04	3.32	0.86	1.76~5.28
2	8	3.39	3.90	1.46	1.42~5.92
3	10	2.92	3.26	1.30	0.49~6.29

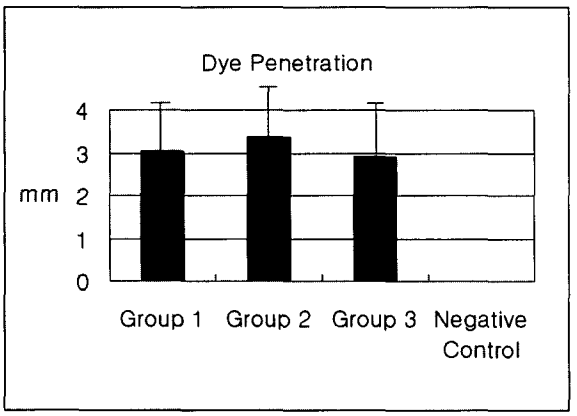

Fig. 1. The amount of dye penetration presented as mean±SD
There was no statistically significant differences among three experimental groups (p>0.05) .

from that of the non-primer group (Group 3) (p>0.05).

2. Scanning Electron Microscopic Observation

Meanwhile a close contact was found between canal obturation materials and dentin in some of the areas (Fig. 2), a resin-dentin interdiffusion zone was not observed. And AH26 sealer, penetrating the dentinal tubules was observed (Fig. 3). But gaps between the dentin and the canal obturation material were observed in other areas (Fig. 4) and close contact between them was not always observed along the entire canal (Fig. 5). Specimens of Groups 1 and 2 showed similar appearances.

Ⅳ. DISCUSSION

The dramatic improvement in the sealing of root canal system with dentin bonding agents was clearly demonstrated by Zidan *et al.*[10,11]. Leonard *et al.*[22] reported that a significantly better sealing and a hybrid layer formation were observed in the root canal system when using dentin bonding agents. However, in the present study using self-etching primers, there was no improvement in the apical seal of the root canal system.

Mannocci *et al.* reported that the resin-dentin interdiffusion zone was less frequently observed and the penetration of the adhesive resin into the dentinal tubules was not as deep with self-etching primers[23]. However, according to Zidan *et al.*, a material with a relatively low bonding strength could achieve an adequate seal in the case of root canal obturation [11]. Because the main volume of the canal is occupied with gutta-percha, polymerization shrinkage is minimal. Rather, very aggressive acid etching will expose the collagen so deeply that the adhesive resins may not penetrate completely, and this might cause an uninfiltrated weak collagenous layer of dentin that is susceptible to long-term degradation[13].

Mannocci and Ferrari[12] reported that adhesives will seal significantly better by eliminating the smear layer. Removal of smear layer opens the dentinal tubules, allowing obturation materials to enter, thereby increasing the mechanical retention and enhancing the sealability[24]. In the present study, EDTA was not used and the smear layer was not removed. However, self-etching primers have been reported to penetrate the smear layer and achieve micromechanical bonding of the smear layer to the underlying dentin[25]. The altered smear layer might be interpreted as iatrogenic surface irregularities.

Vichi *et al.*[26] reported that NaOCl treatment leads to removal of the collagen network and prevents hybrid layer formation. In fact, in the present study using NaOCl irrigation, typical hybrid layer was not observed under a scanning electron microscope. However, a close contact between the canal obturation materials and dentin could be seen. This observation suggests that hybrid layer formation is not a prerequisite for good sealing.

Scanning electron microscopic observation showed gaps between the dentin and gutta-percha cones occasionally. Water or residual solvents not completely evaporated might negatively affect the resin polymerization or preoccupy the space for the sealers. Also, the artificial changes might have been induced in SEM preparation.

Each of the researchers used his application method and so far, no study has evaluated the influence of the application methods of dentin bonding agents on the seal of root canal obturation. The present study compared two commonly used application methods and showed no significant difference in the apical leakage. Both syringe with endodontic needle and paper point did not assure that the primers were

delivered uniformly to the apical areas. The dentin walls of the root canals are not readily available for surface pretreatment compared with the accessible dentin walls in coronal cavities[11] and the number of dentinal tubules has also been decreased[22]. Therefore, the application of pretreatment materials to the root canal walls is very limited with the current methods.

Adhesion of sealer to dentin and gutta-percha may be important for the achievement of adequate sealing. However, the relation between the leakage and adhesion is complex and poorly understood[27,28]. Ørstavik *et al.* demonstrated the reasons for the difference in results between adhesion and leakage tests[27]. The interaction of the materials with the water vehicle and with the dye in the leakage test may influence the penetration of the dye. Moreover, adhesion as evaluated by the bond strength may not reflect the homogeneous adaptation of the material to the dentin and gutta-percha surfaces. Points of contact causing adhesion may be interspersed with voids, as shown in Fig. 5, permitting dye penetration in the leakage test.

Adhesion is more predictive of a material's retentive potential than of its sealing ability[28]. Thus, adhesion to dentin and gutta-percha does not appear to be an essential property of a sealer for obtaining an adequate sealing of the root canal system[29]. Rather, close contacts to the canal walls may affect the resistance to microleakage.

Another consideration regarding the use of self-etching primers as endodontic filling materials are their biocompatibility and periradicular tissue response to them. Bruce *et al.* [30] evaluated the cytotoxicity of primers and found them initially toxic, but their cytotoxicity was lost with time. Dentin bonding agents were tested as retrograde filling materials in humans and in most cases, the response obtained was healing with deposition of cementum and insertion of new sharpey's fibers[31]. Because the primers are covered with sealers, it is reasonable to suggest that clinically there would be little, if any, long-term contact between the primers and periapical tissue. The cytotoxicity may be clinically insignificant. It would be, however, potential for a cytotoxic effect to remain on the periodontal ligament by any excess material.

In conclusion, self-etching primers could improve the adhesion of AH26 sealer to dentin only in areas where these materials are applied. However, current application methods are not ensuring uniform application of these materials all over the canal walls. For clinical use of self-etching primers in endodontics, further studies are needed to develop a reliable application method along with the modification of the properties to fulfil the requirements of endodontic filling materials.

V. CONCLUSION

The purpose of this study was to determine whether self-etching primer application and its different application methods affect the apical sealing ability of resin-based root canal sealer, and to observe the sealer-dentin interface.

Thirty-six single rooted teeth were cleaned, shaped and obturated with AH26 sealer and gutta-pecha cone with or without LB Primer. LB Primer was applied either by a syringe or by paper points. After longitudinal sectioning, the apical microleakage was measured by the methylene blue dye penetration method, and the sealer-dentin interfaces were observed under a scanning electron microscope. The results were as follows.

1. Mean dye penetration of primer-applied groups were not significantly different from that of non-primer group ($p>0.05$).
2. Mean dye penetration of syringe-application group was not significantly different from that of paper point-application group ($p>0.05$).
3. The sealer-dentin interfaces showed close contacts in some areas but gaps in other areas.

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사 진 부 도

Scanning Electron Microscopic images of dentin-AH26 sealer-gutta-percha cone interfaces of the primer-applied groups

Abbreviations

D : dentin
S : AH 26 sealer
G : gutta-percha cone
SG : AH 26 sealer on gutta-percha cone

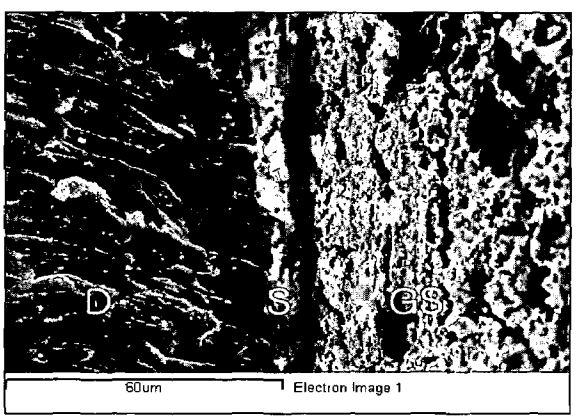

Fig. 2. SEM image showing close contact between dentin and AH 26 sealer (magnification : × 1000)

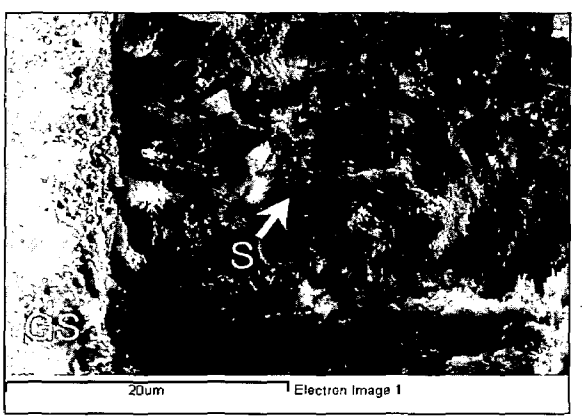

Fig. 3. SEM image showing penetration of AH 26 sealer (*arrow*) into the dentinal tubule about 20 μm deep (magnification : ×3000)

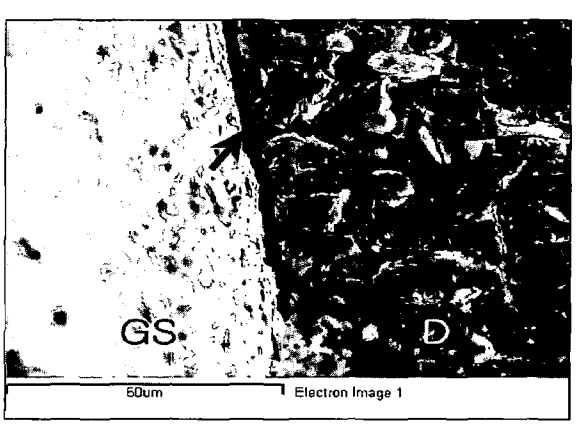

Fig. 4. SEM image showing a gap (*arrow*) of about 3μm in thickness between dentin and gutta-percha cone (magnification : ×1000)

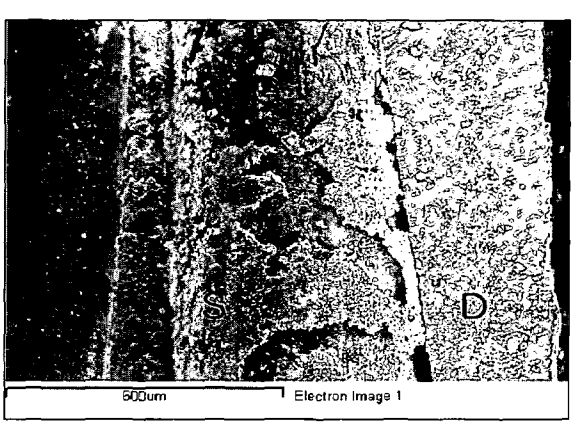

Fig. 5. SEM image showing gaps between dentin and gutta-percha cone along the canal circumference (magnification : ×100)