

Evaluation of coronal leakage and pathway of dye leakage after obturation with various materials for open apical foramina

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The aim of this study was to investigate coronal leakage after obturation with mineral trioxide aggregate (MTA), resin-based sealer, and silicon-based sealer for open apical foramina and to evaluate pathway of leakage. Twenty-eight maxillary premolars were used, and instrumented to ISO size #80. Teeth were randomly divided into four groups as follows: Group A filled with MTA, Group B with gutta-percha and resin-based sealer, Group C with polymer-based material and resin-based sealer, and Group D with gutta-percha and silicon-based sealer. All samples were evaluated for coronal leakage with methylene blue solution and spectrophotometry. After leakage testing, samples were cut, and sections were observed. Dye leakage of Group A was significantly lowest among all groups at 15 days and 30 days. Defects which induced coronal leakage in resin-based sealer were observed at 7 mm from the apex. Coronal leakage after obturation with MTA for open apical foramina was significantly lower than resin-based sealer and silicon-based sealer.

Keywords: Coronal leakage, Mineral trioxide aggregate (MTA), Resin-based sealer, Silicon-based sealer

INTRODUCTION

The most important procedure of root canal treatment is mechanical shaping and chemical cleaning of the root canal system. After this procedure, tight filling of the root canal system is essential¹⁾. Gutta-percha and sealer are usually used for obturation²⁾. However, obturation with gutta-percha and sealer is not suitable for open apical foramina because of the risk of extrusion of root-filling materials³⁾ and deterioration of the long-term treatment outcome⁴⁾. Because of this risk, open apical foramina, such as those in immature teeth, and overenlargement of root canal ends are treated by apexification with calcium hydroxide⁵⁾. Apexification has a good clinical outcome; however, long-term application of calcium hydroxide for apexification increases the risk of root fracture⁶⁾. Hence, open apical foramina have recently been obturated with mineral trioxide aggregate (MTA). MTA was initially introduced as the material for perforation repair and root-end filling⁷⁾. It has advantageous properties, such as biocompatibility⁸⁾ and good sealing ability⁹⁾. MTA is also used for direct pulp capping¹⁰⁾ and the treatment of necrotic immature teeth¹¹⁾. Moreover, application of MTA has good clinical outcomes for complicated cases such as perforation¹²⁾, immature teeth¹³⁾, and root-end filling of apicoectomy¹⁴⁾. Some clinicians use MTA for obturation of open apical foramina¹⁵⁾. However, there are few investigations about coronal leakage after obturation with MTA in these cases¹⁶⁾.

On the other hand, dentin-adhesive materials are recently used for obturation. In this experiment, resin-based sealer (MetaSEAL™ (MS; Parkell, NY, USA) and Epiphany® SE™ (ES; Pentron Clinical Technologies,

CA, USA)) were used as dentin-adhesive materials. In manufacturer instruction, MS is recommended in the manner of the single-cone technique with gutta-percha point. ES is recommended using with polymer-based material (Resilon™ point (RP; Pentron Clinical Technologies)). ES is connected tightly with RP and reduces leakage¹⁷⁾. ES was used with RP in the manner of the continuous wave condensation technique.

The aim of this study was to investigate coronal leakage after obturation with MTA, resin-based sealer as dentin-adhesive materials, and silicon-based sealer as conventional materials for open apical foramina and to evaluate the pathway of dye leakage.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Tokyo Medical and Dental University (No. 600).

Tooth selection

Twenty-eight extracted single-rooted human maxillary premolars were used. They were then randomly divided into four groups of seven teeth each. Teeth were stored in distilled water after extraction and were confirmed to have a mature root apex, no root caries, and no fracture line under a VH-8000 digital microscope (Keyence, Osaka, Japan), which was set at ×10 magnification. Also, they were confirmed to have one root canal by use of cone beam computed tomography (FineCube; Yoshida Dental Trade Distribution Co., Ltd., Tokyo, Japan). The crowns were removed by a low-speed diamond cutting machine (IsoMet; Buehler Ltd., Lake Bluff, IL, USA) under running water, and the root lengths were standardized to 12 mm.

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Root canal preparation and obturation

The coronal third of the canal of each sample was prepared by Gates Glidden Drills #1-4 (Produits Dentaires S.A., Switzerland). The working length was determined by the insertion of a #10 K-file (Zipperer, München, Germany) until the tip of the file appeared through the apical foramen. Each sample was instrumented to ISO size #80 by the standardized method. In preparation, the root canal was filled with Six percent NaClO solution (Yoshida Pharmaceutical Co., Ltd., Tokyo, Japan), and irrigated with 2 mL of Six percent NaClO solution at each file change. After preparation, the root canal was rinsed again with 5 mL of the NaClO solution followed by 2 mL of 18% EDTA solution (Ultradent Products, UT, USA). The root canal was then finally rinsed with 5 mL of distilled water. Before obturation, the root canal was dried with #80 paper points (GC, Tokyo, Japan), which were inserted into the root canal one by one until the tip of the paper point was dry.

Group A

Mineral trioxide aggregate (Pro Root® MTA (MTA; DENTSPLY Tulsa Dental, TN, USA)) was mixed according to the manufacturer's instructions. A total of 0.25 g of MTA was mixed with 8.25×10^{-2} mL of distilled water on a glass plate. MTA was scooped and inserted into the root canal with an endodontic plugger (#2 Yamaura, Tokyo, Japan), and a #70 K-file was rotated counterclockwise 3 mm from the apex within the root canal. When MTA was extruded from the apex, the counterclockwise motion was finished. Then, MTA was carefully condensed with the endodontic plugger, avoiding the creation of voids. After filling, wet cotton pellets were placed on the coronal surface of the samples for 24 h.

Group B

The root canal was obturated with a #80 gutta-percha point (GP; GC) and MS using the single-cone technique. MS was mixed according to the manufacturer's instructions, then scooped and inserted into the root canal by using a #80 GP so that the whole root canal was filled with MS and #80 GP. The #80 GP was inserted to a working length, and surplus GP was removed 12 mm from the apex with a heated endodontic plugger. Finally, LED irradiation (DC Blue LEX, Yoshida Dental Trade Distribution Co., Ltd.) was applied for 20 s on the coronal side of the root canal.

Group C

The root canal was obturated with a #80 RP and ES using the continuous wave condensation technique. ES was inserted into the root canal with a #80 RP so that the whole root canal was filled with ES and RP. The #80 RP was inserted to a working length, and surplus RP was cut 12 mm from the apex with a heated endodontic plugger. Down packing was performed until 3 mm from the apex with a plugger (F, EIE/Analytic Technology, CA, USA) attached to a System B (EIE/Analytic Technology) at 125°C for 5 s. Back packing was then performed from the surface of the down packing with a needle (φ25 G; Obtura Spartan, MO, USA) attached to an Obtura II (Obtura Spartan) using Resilon pellets (Pentron) at 125°C. Finally, the LED irradiation was applied for 40 s on the coronal side of the root canal according to the manufacturer's instructions.

Group D

The root canal was obturated with a #80 GP and silicon-based sealer (RoekoSeal Automix®(RS; Coltene/Whaledent GmbH, Langenau, Germany)) as conventional materials using the continuous wave

Table 1 Compositions of filling materials in this study

Groups	Materials	Compositions
A (MTA)	Pro Root MTA (DENTSPLY Tulsa Dental, TN, USA)	tricalcium silicate, dicalcium silicate, tricalcium aluminate
B (GP+MS)	MetaSEAL (Parkell, NY, USA)	4-methacryloyloxyethyl trimellitate anhydride, functional methacrylate monomer, 2-hydroxyethyl methacrylate, zirconium oxide filler
C (RP+ES)	Epiphany SE (Pentron Clinical Technologies, CA, USA)	ethoxylated bisphenol A dimethacrylate, bisphenol A-glycidyl dimethacrylate, 2-hydroxyethyl methacrylate
	Resilon (Pentron Clinical Technologies)	polycaprolactone, urethane methacrylate
D (GP+RS)	RoekoSeal Automix (Coltene/Whaledent GmbH, Langenau, Germany)	polydimethylsiloxane, silicone oil, zirconium dioxide, hexachloroplatinic acid

Table 2 Materials, adhesiveness, and obturation methods about each group

Groups	Materials	Adhesiveness	Obturation methods
A	MTA	–	K-file and plugger
B	GP+MS	+	Single-cone
C	RP+ES	+	Continuous wave condensation
D	GP+RS	–	Continuous wave condensation

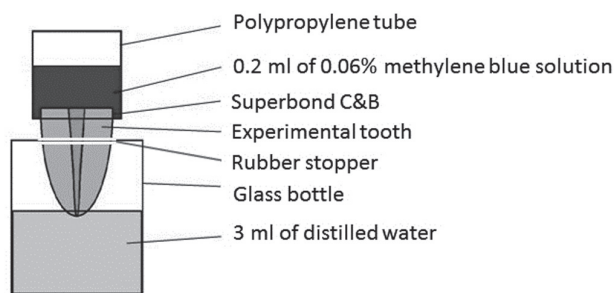


Fig. 1 Device for coronal leakage test.

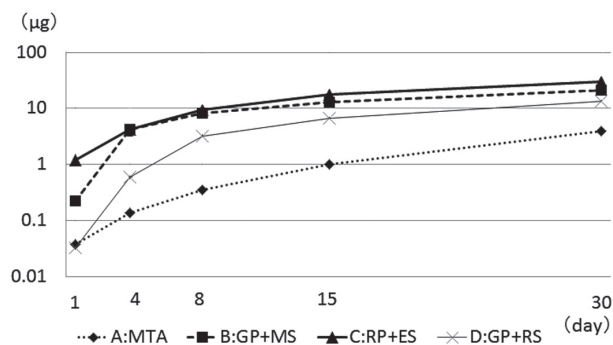


Fig. 2 Amounts of leaked methylene blue over time during the experimental course with the different root canal filling materials.

Table 3 Mean and standard deviation (SD) of leaked methylene blue over time in the course of experiment (µg).

Groups	1 day		4 days		8 days		15 days		30 days	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A	0.04 ^{Aa}	0.10	0.14 ^B	0.21	0.35 ^C	0.76	1.00 ^{Dcde}	1.31	3.92 ^{ABCDfgh}	2.06
B	0.22 ^A	0.26	4.21 ^B	4.44	8.19	7.59	12.93 ^c	10.85	21.14 ^{ABf}	13.25
C	1.18 ^{Aab}	1.30	4.18 ^B	4.56	8.64	8.78	14.00 ^d	11.17	25.71 ^{ABg}	22.46
D	0.04 ^{ABb}	0.09	0.78 ^{CD}	0.85	4.94 ^E	3.60	7.99 ^{ACFe}	4.22	17.38 ^{BDEFh}	5.52

The same letters behind mean are represented statistically significant differences ($p < 0.05$). Superscript letters of uppercase denote statistically significant differences among investigative days. Superscript letters of lowercase denote statistically significant differences among obturative materials.

condensation technique. RS was inserted into the root canal with a #80 GP so that the whole root canal was filled with RS and GP. The #80 GP was inserted to a working length, and surplus GP was removed 12 mm from the apex with a heated endodontic plugger. Down packing was performed until 3 mm from the apex with a plugger attached to a System B at 200°C for 5 s. Back packing was then performed from the surface of the down packing with a needle attached an Obtura II using GP pellets (Morita, Tokyo, Japan) at 200°C.

Table 1 showed compositions of filling materials in this study. Table 2 showed materials, adhesiveness, and obturation method about each group.

Coronal leakage test

After each procedure in all groups, all samples were stored in a thermostatic bath (Incubator IC43; Yamato Scientific Co., Ltd., Tokyo, Japan) kept at 37°C and 100% humidity for 1 week. Coronal leakage was evaluated according to the authors' previous study¹⁸⁾. Figure 1 shows the schematic device for the coronal leakage test. A polypropylene tube was attached at the coronal portion of each sample with resin cement (Super-Bond C&B, Sun Medical, Shiga, Japan). A total of 0.2 mL of 0.06% methylene blue solution (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) was poured into the polypropylene tube. Each polypropylene tube with the

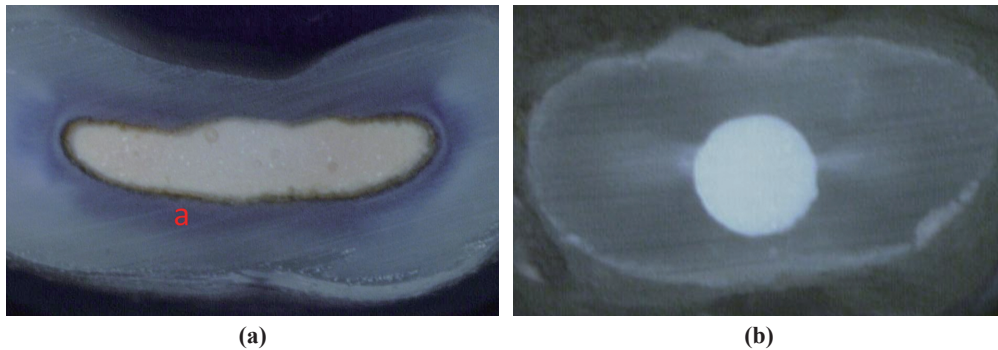


Fig. 3 (a) Representative section 7 mm from the apex in Group A. MTA was not dyed with methylene blue solution. Dentin in contact with MTA was dyed. a: Dentin dyed with methylene blue solution (b) Representative section 2 mm from the apex in Group A. Dentin and MTA were not dyed with methylene blue solution.

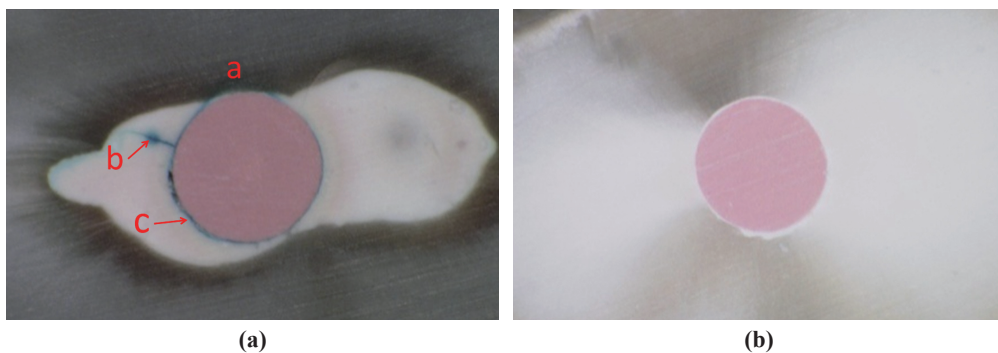


Fig. 4 (a) Representative section 7 mm from the apex in Group B. MS and GP were not dyed. The defect of MS, the gap between MS and GP, and the dentin in contact with MS or GP were dyed. a: Dentin dyed with methylene blue solution b: Methylene blue solution penetrated the MS defect c: Methylene blue solution penetrated the gap between MS and GP (b) Representative section 2 mm from the apex in Group B. Dentin, MS and GP were not dyed with methylene blue solution.

attached sample was closed with a rubber stopper and placed in a glass bottle, which was filled with 3 mL of distilled water. Two mm of the root apex was immersed in the distilled water in the glass bottle. The model was maintained in a thermostatic bath that provided 100% humidity at 37°C. Water in the glass bottle was collected for each measurement and replaced with fresh distilled water. The amount of methylene blue in each water sample was measured by absorbance at 630 nm using an Immuno-Mini NJ-2300 spectrophotometer (Nalge Nunc International, Rochester, NY, USA) at 1, 4, 8, 15, and 30 days. Distilled water was used as the standard solution. A regression curve equation was obtained as follows¹⁸⁾:

$$10^4 \cdot Y = X^3 - X^2 + 13 \cdot X + 0.4$$

where X is the absorbance and Y is the dye concentration. The concentration of each sample was

extrapolated using the regression curve. The amount of methylene blue was obtained from the concentration and accumulated over all time intervals. Data obtained were statistically analyzed using two-way ANOVA for obturative materials and investigative days and the Tukey-Kramer test at a 5% significance level.

Observation of sections

After the dye leakage test, investigation of the pathway of dye leakage was performed. Samples were cut perpendicular to the longitudinal axis 2 and 7 mm from the apex by the low-speed diamond cutting machine. Two sections were obtained from each sample. Each section was observed with the VH-8000 (×25) and showed by image processing software (Adobe® Photoshop® 7.0, Adobe Systems, CA, USA).

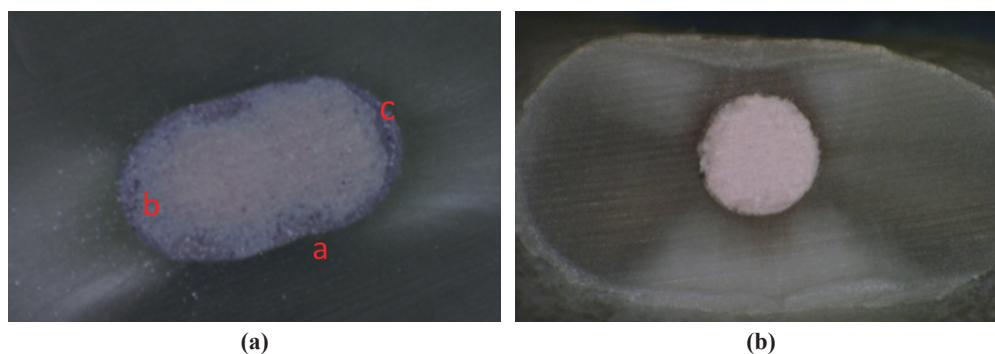


Fig. 5 (a) Representative section 7 mm from the apex in Group C. ES, RP, and dentin in contact with ES or RP were dyed.
a: Dentin dyed with methylene blue solution
b: RP dyed with methylene blue solution
c: ES dyed with methylene blue solution
(b) Representative section 2 mm from the apex in Group C. Dentin, ES and RP were not dyed with methylene blue solution.

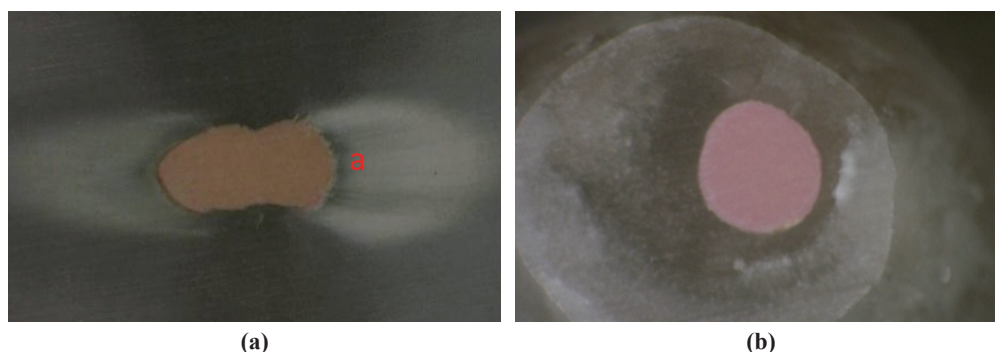


Fig. 6 (a) Representative section 7 mm from the apex in Group D. RS and GP were not dyed. Dentin in contact with RS or GP was dyed.
a: Dentin dyed with methylene blue solution
(b) Representative section 2 mm from the apex in Group D. Dentin, RS and GP were not dyed with methylene blue solution.

RESULTS

The mean amounts and standard deviation (SD) of dye leakage in all groups at the stipulated experimental time intervals are presented in Table 3 and Fig. 2. Two factors, obturative material and investigative day, significantly affected the amount of leakage in two-way ANOVA ($p < 0.0001$). There were no interactions. Concerning obturative materials, at 1 day, dye leakage in Groups A and D were significantly lower than dye leakage in Group C ($p < 0.05$). At 15 days and 30 days, dye leakage in Group A was significantly lowest among all groups ($p < 0.05$). As to investigative days, in Group A, dye leakage at 30 days was significantly highest among all groups ($p < 0.05$). In Groups B and C, dye leakage at 30 days was significantly higher than dye leakage at 1 day and 4 days ($p < 0.05$). In Group D, dye leakage at 30 days was significantly highest among all groups ($p < 0.05$), and dye leakage at 15 days was significantly higher than dye

leakage at 1 day and 4 days ($p < 0.05$).

Representative sections 7 and 2 mm from the apex in Group A are presented in Figs. 3a and 3b respectively. Representative sections 7 and 2 mm from the apex in Group B are presented in Figs. 4a and 4b respectively. Representative sections 7 and 2 mm from the apex in Group C are presented in Figs. 5a and 5b respectively. Representative sections 7 and 2 mm from the apex in Group D are presented in Figs. 6a and 6b respectively. Dentin was dyed with methylene blue solution at 7 mm section from the apex in all groups. However, dentin was not dyed at 2 mm section from apex in all groups. In Fig. 4a, the gap which was dyed with methylene blue solution was recognized in MS.

DISCUSSION

Coronal leakage

Coronal leakage in Group A was the lowest among

all groups at 15 days and 30 days (Table 3). MTA had better sealing ability than did resin-based sealer as dentin-adhesive materials and silicon-based sealer as conventional material under these experimental conditions. The major components of MTA are Portland cement, bismuth oxide, and gypsum¹⁹. Bismuth oxide is included for radiopacity, and gypsum is included to adjust the setting time²⁰. Important characteristics of MTA depend on Portland cement. The Portland cement component consists of tricalcium silicate ($3\text{CaO} \cdot \text{SiO}_2$), dicalcium silicate ($2\text{CaO} \cdot \text{SiO}_2$), and tricalcium aluminate ($3\text{CaO} \cdot \text{Al}_2\text{O}_3$)²¹. The main mechanism of Portland cement setting is hydration of these inorganic oxides, and expansion of Portland cement is induced in the process of Portland cement setting²². A unique property of MTA is the contribution to a tight seal between MTA and dentin²³. Because MTA required moisture to set²⁴, wet cotton pellets were placed on the coronal surface²⁵ after filling MTA in this experiment. The purpose of this experiment was to investigate the properties of filling materials. Therefore, temporary seal was not applied and samples were stored in a thermostatic bath kept at 37°C and 100% humidity as not to interact with filling materials.

Dentin-adhesive materials have advantageous properties, such as bonding to dentin. On the other hand, when dentin-adhesive materials polymerize, they cause shrinkage. Teeth used in this study were maxillary premolars having only one root canal. The canal shape of those at the sections perpendicular to the longitudinal axis of the root canal is oval²⁶, and oval canals usually have larger areas than those of round canals²⁷. Therefore, oval canals need to larger amount of sealers than round canals to fill the root canals. The effects of expansion and shrinkage were emphasized under these conditions. Expansion of MTA contributes to formation of a tight seal, and shrinkage of dentin-adhesive materials has the disadvantage of a tight seal because shrinkage of dentin-adhesive materials creates a gap between dentin and dentin-adhesive materials or between GP and dentin-adhesive materials. Group A obturated with MTA had better results than did Groups B and C obturated with dentin-adhesive materials and GP.

In Group D, silicon-based sealer was used as conventional materials. Group D obturated with RS and GP had the disadvantage of a tight seal between RS and dentin because RS did not adhere to dentin. However, RS expanded slightly during setting and penetrated into dentin tubules as did dentin-adhesive materials²⁸. There were no significant differences among Groups B, C, and D ($p > 0.05$) at 15 days and 30 days.

At 1 day, dye leakage in Groups A and D were significantly lower than dye leakage in Group C ($p < 0.05$). Group C was obturated by using RP+ES. ES was resin-based sealer and dual cure type materials (photo and chemical polymerization). According to the manufacturer's instructions, the LED irradiation was applied for 40 s on the coronal side of the root canal. However, application time of 40 s might be insufficient

in the condition of 12 mm root length. In the clinical condition, it may be necessary that application time is extended.

As to coronal leakage, there were significant differences among long investigative periods (Table 3). So this experimental period was sufficient.

The features of this investigation method about dye leakage is capability of employment of same sample in long time period non-destructively. It is very useful to investigate the relationship amount of leakage and pathway of the major leakage.

Observation of sections

Dentin, which was dyed with methylene blue solution, in contact with filling materials was observed for some sections in all groups (Figs. 3a, 4a, 5a, and 6a). One of the main pathways of coronal leakage was the gap between dentin and filling materials. MS had a defect like a fracture line (Fig. 4a). This line was dyed with methylene blue solution. Coronal leakage might be induced by this defect when this defect connects to the gap between MS and GP or between MS and dentin. Methacrylate resin is contained in MS. The materials containing methacrylate resin increase the amount of shrinkage²⁹. MS was scooped and inserted into the root canal not to make the void by using a #80 GP so that the whole root canal was filled with MS and #80 GP using single-cone technique. If MS is applied to fill the oval canals which need to large amount of sealers, MS will shrink and make defects like a fracture line which induce the leakage³⁰. Therefore, MS should be applied carefully to oval canals.

Methylene blue solution may pass the root canal from the coronal portion to the apical portion through various gaps. In the gap between dentin and filling materials, methylene blue solution penetrates into dentin tubules easier than do filling materials. Therefore, dentin in contact with filling materials was dyed with methylene blue solution³¹ (Figs. 3a, 4a, 5a, and 6a). It is speculated that there is a pathway around the dyed dentin³². Regarding sections of 2 mm from the apex in all groups (Figs. 3b, 4b, 5b, and 6b), dentin and root-filling materials dyed with methylene blue solution were not observed in any group. All root canals 2 mm from the apex were filled tightly at the apical portion. The sections perpendicular to the longitudinal axis of the root canal 2 mm from the apex were round, and core materials in Groups B, C, and D fit tightly to the round canal. Therefore, thickness of sealers was very thin, contributing to a tight seal³³. Dye leakage occurred in all samples. However, pathways of dye leakage were not observed at sections at 2 mm from apex in all groups. A methylene blue particle is very small (about $0.095 \mu\text{m}$)³⁴. Methylene blue could simulate the leakage behavior of endotoxin³⁵. Methylene blue particles penetrate into small gaps^{35,36} that cannot be observed under a digital microscope from coronal portion to apical portion. Therefore, dye leakage occurred in all samples even though pathways of dye leakage were not observed at some sections in this experiment.

Before MTA was commercially available, root canals

with open apical foramina, such as immature teeth, and overenlargement of root canal ends were filled with custom fitting materials³⁷⁾ or paste materials³⁸⁾. However, these procedures had poor clinical outcomes because root-filling materials were not filled tightly and were easily extruded from the apical foramina³⁾. Hence, apexification was performed by calcium hydroxide³⁹⁾. Apexification had a good clinical outcome⁴⁰⁾. However, apexification required a long treatment period. So, there were risks of reinfection and root fracture⁶⁾. MTA has good biocompatibility⁸⁾, and obturation with MTA for open apical foramina has an advantage against coronal leakage. In addition, obturation with MTA requires fewer appointments and shorter treatment periods than does apexification. Therefore, the risks of reinfection and root fracture could be reduced. MTA would be a material of choice for obturation of root canals with open apical foramina. However, it is difficult to retreat root canals obturated with MTA. Removal of MTA could be performed with ultrasonic instruments. However, removal of MTA cannot be completed in curved canals⁴¹⁾, and obturation with MTA increases the risk of root fracture because of expansion of MTA⁴²⁾. These characteristics of MTA as root-filling material should be noted.

CONCLUSION

Coronal leakage after obturation with MTA for root canals with open apical foramina was significantly lower than those with dentin-adhesive materials and silicon-based sealer.

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