Effects of Blood Contamination on Sealing Ability and Microhardness of Mineral Trioxide Aggregate Used as a Root-end Filling Material

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Abstract

Purpose: The purpose of this study was to evaluate the influence of various contaminants on the apical sealing ability and microhardness of mineral trioxide aggregate (MTA) in extracted teeth ex vivo.

Methods: The root canals of 51 single-rooted human teeth were prepared and filled with laterally condensed gutta-percha cones and a sealer. After root surface coating, apicoectomy and preparation of apical cavities, the teeth were randomly divided into 5 groups. Group 1 was contaminated with distilled water (control), group 2 with saline solution, group 3 with adrenalin solution, group 4 with blood, and group 5 with EDTA solution. After filling with MTA, the teeth were subjected to a leakage test. The teeth samples were then bisected and subjected to a microhardness test, and observed by stereoscopy and scanning electron microscopy.

Results: A hermetic seal was observed morphologically in group 1. However, partial sealing and gaps between dentin and MTA or lacunae in MTA were observed in groups 2–5. Group 1 showed significantly less leakage than the other groups, but there was no significant difference among the experimental groups. The microhardness of group 5 was significantly less than that of group 1.

Conclusion: The results suggested that contamination of MTA while setting decreased the sealing ability of MTA.

Key words: Contamination, Mineral trioxide aggregate, Sealing ability
Introduction

When nonsurgical endodontic therapy is unsuccessful or impossible, endodontic surgery is indicated to save a tooth. This procedure consists of exposure of the involved apex, apicoectomy, preparation of cavity at the resected root-end, and insertion of a root-end filling material in the prepared cavity. The aim of placing a root-end filling material is to develop an apical seal at the end of the resected root\(^1\). An ideal root-end filling material should adhere and adapt to the dentinal walls of the root-end preparation, prevent leakage of microorganisms and their by-products into the peri-radicular tissues, and be biocompatible. In addition, the material should be insoluble in tissue fluids, dimensionally stable, and susceptible to the presence of moisture\(^3\).

Mineral trioxide aggregate (MTA), which was first developed by Torabinejad et al., has been successfully used in many endodontic applications such as root-end filling, apexification, pulpotomy, and pulp therapy, because of its unique biocompatibility, antibacterial nature, sealing ability, and its capacity to promote hard tissue formation\(^3,4\)\(^a\). However, MTA is clinically difficult to use because of its granular consistency, slow setting time, and initial looseness. Many attempts have been made to improve the handling properties of MTA by adding calcium compounds as a setting accelerator or other materials to enhance viscosity\(^3,5\)\(^b\).

Microleakage of the root canal or root end is defined as the passage of bacteria, fluids, or chemical substances between the tooth and the filling material of the root canal. Leakage through an obturated root canal or root end is expected to take place at the interfaces between sealer and dentin, sealer and gutta-percha or through voids within the sealer, or between root-end filling materials and dentin. Microleakage in the root canal or root end is a complex subject because of the many variables that might influence leakage, such as the root canal filling technique, the physical and chemical properties of the sealer used, and the presence of a smear layer\(^6\).

The root-end cavities are often contaminated during apical surgery, for example by blood, adrenalin as a hemostat or EDTA for removing the smear layer, but the influence of such contamination on the sealing ability and microhardness of MTA used as a root-end filling material has been unclear. The purpose of this study was to evaluate the influence of various contaminants on the apical sealing ability and microhardness of MTA using extracted teeth ex vivo.

Materials and Methods

1. Tooth selection

All experiments in this study complied with the guidelines of the Ethical Review Board of Ohu University (Approval No. 40). Fifty-one single-rooted anterior and premolar human teeth were used in this experiment and stored in 10% formalin (Wako Pure Chemical Industries). Before the experiment, samples were thoroughly washed with distilled water. Preoperative radiographs confirmed the absence of multiple canals, calcifications, or severe apical curvatures. The teeth were decoronated, and the roots were cleaned with an ultrasonic device and hand scaler. The canal orifices were enlarged using a Peeso Reamer (Mani).

2. Root canal preparation and root canal filling

A #15 K file was inserted into the root canal until the visible level reached the apical foramen. The working length of each root canal was established at 1 mm short of the apical foramen. After enlarging the root canals to a #50 K file, the rest of the canal was cleaned and shaped using a standard step-back technique; 10% sodium hypochlorite (NaClO) was used as an irrigant. The instrumented canals were dried with absorbent paper points and obturated with laterally condensed gutta-percha and Grossman sealer (Canals, Showa Yakuhin Kako).

3. Root-end resection and root-end preparation

Dual coats of nail polish were applied to the external surface of each root, and root-end resection was performed by removing 3 mm from the apex, at a 90-degree angle to the long axis of the root, using a #701 fissure burr (Shofu) in a high-speed hand piece with water coolant. Apical cavity preparations were made in each of the roots. An E32D tip (Nakanishi) on an ultrasonic device with distilled water as coolant was used to create cavities. The cavities were enlarged and deepened to approximately 3 mm.

4. Root-end filling

MTA (ProRoot MTA, Dentsply Tulsa Dental, USA) was mixed with water to a putty consistency at a powder: distilled water ratio of 1 g: 0.35 mL. According to each group, extra liquid content was added (Table 1), and MTA was further mixed. After the cavities of each group were dried completely using an air syringe, they were filled with the mixed MTA which was condensed into the preparations using a small plugger.

The teeth were randomly divided into 5 groups (n=9,
Table 1. Group 1 served as the control, and distilled water (0.1 ml) was added. Saline solution (0.1 ml) was added in group 2, adrenalin solution (1 mg/ml, Bosmin, Daiichi-Sankyo, 0.1 ml) was added in group 3, blood (horse blood, Nippon Biostest Lab, 0.1 ml) was added in group 4, and 15% EDTA solution (pH 7.2 with NaOH, Dojindo Lab, 0.1 ml) was added in group 5. Of the remaining 6 specimens, 3 specimens were used as negative controls and were coated with nail polish after apicoectomy to confirm the absence of leakage, and the others were used as positive controls and were left uncoated with nail polish after apicoectomy and cavity preparation to confirm complete leakage.

5. Leakage test and microhardness test

After being filled with MTA, specimens were dried for 5 min at room temperature (25°C), and then were immersed in rhodamine B dye solution (Muto Chemical) at 37°C for a dye leakage test lasting 7 days. After the leakage test, the teeth were washed with distilled water, longitudinally bisected with a slow-speed diamond saw (IsoMet, Buehler, USA) and observed by stereoscopy (SMZ-10, Nikon). Then, the MTA filling materials of 5 randomly selected specimens from each group were subjected to a microhardness test (HMV-2ADW, Shimadzu).

The sealing status between dentin and MTA was carefully examined and photographs were taken. Final evaluation of leakage was performed after removing the filled MTA. The degree of apical leakage was measured by a technician who was not informed of the true nature and purpose of the experiments. Thus, the evaluation of leakage degree was kept blind. The mean and standard deviation (SD) of each group were calculated.

6. Scanning electron microscopic (SEM) observation

After each sample was observed by stereoscopy, they were progressively dehydrated using graded concentrations of aqueous ethanol (70%, 80%, 90% and 100%) for 24 h at each concentration. After dehydration, the samples were dried and sputter-coated with platinum ion (Ion sput-

Table 1  Extra added liquid contents in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Added liquid content (0.1 ml volume)</th>
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<tbody>
<tr>
<td>1</td>
<td>Distilled water</td>
</tr>
<tr>
<td>2</td>
<td>Saline solution</td>
</tr>
<tr>
<td>3</td>
<td>Adrenalin solution (1 mg/ml)</td>
</tr>
<tr>
<td>4</td>
<td>Blood (horse)</td>
</tr>
<tr>
<td>5</td>
<td>EDTA (15% (w/v), pH 7.2)</td>
</tr>
</tbody>
</table>

der, E-1010, Hitachi). The samples were observed by SEM (S-3000, Hitachi) at an accelerating voltage at 25 kV.

7. Statistical analyses

The nonparametric data was analyzed by Kruskal-Wallis test among 5 groups, and Mann-Whitney U test between 2 groups. All statistical analyses were set with a significance level of $\alpha=0.05$.

Results

Figure 1 shows representative stereomicroscopic photographs from each group. A hermetic and complete tight seal between dentin and MTA was observed in almost all samples in group 1. On the other hand, partial loose sealing with occasional gaps between dentin and MTA was observed in almost all samples in groups 2–5. Figure 2 shows representative SEM micrographs of MTA surfaces from each group. Compared with group 1, relatively large lacunae were observed in other groups.

The results of leakage degree in each group are shown in Table 2. The leakage degree in group 1 was significantly different ($p<0.05$), but there was no significant difference among other groups except group 1. No leakage was observed in any samples of the negative control, and leakage was observed in the full length of the cavity wall in all samples of the positive control.

The results of microhardness test are shown in Table 3. Group 1 (control group) was the hardest with the highest value, and the lowest value group was group 5. There was a significant difference between these two groups ($p<0.05$), but no significant difference among the 5 groups.

Discussion

Endodontic surgery has now evolved into endodontic microsurgery\(^5\). In modern endodontic surgery, the most commonly used root-end filling materials are intermediate restorative material (IRM), Super EBA, and MTA\(^6,13\). Similar successful outcomes have been observed with all three filling materials\(^10\). The application of MTA and IRM as root-end fillings in surgical endodontics had the same clinical effectiveness, with high rates of success (92% and 86%, respectively)\(^11\). On the other hand, results from a previous investigation indicated that MTA exhibits significantly less dye leakage in comparison with Super EBA and IRM\(^4\). It appears that MTA is one of the root-end filling materials most resistant to dye penetration. However, dif-
Contamination on Sealing Ability of MTA

**Fig. 1** Representative stereomicroscopic photographs from each group

Arabic numerals show the group number. Interface between dentin (D) and MTA (M) was observed, and hermetic seal (arrow) between D and M was observed in group 1 (bar = 0.1 mm, original magnification × 20).

**Fig. 2** Representative SEM micrographs from each group

Arabic numerals show the group number. A few small lacunae (arrows) were observed in some groups (one scale = 10 μm, original magnification × 500).
different factors influence the leakage on sealing by MTA, which include dentinal wall thickness, dye pH, type of dye, pretreatment with chelating agents, tooth storage environment before the experiment, and setting status of MTA before its placement in the dye. There are also many factors that influence the sealing ability of MTA. Smear layer is one of these factors, and it was reported that the apical microleakage of MTA is less when the smear layer is present than when it is absent. The influence of acidic or alkaline environment on sealing ability was also investigated. However, there have been only a few reports on the effect of blood, saline, adenalin, and EDTA on the sealing ability of MTA used as a root-end filling material.

Contamination of MTA by blood, saline, and adenalin while setting simulates the clinical conditions generally encountered during surgery. The method used in this study did not correctly simulate the clinical situation, but we used it as a reproducible experimental model. The effect of blood on the sealing ability of root-end filling materials, self-etching adhesives or dentin bonding agent was investigated, and contamination by blood was shown to affect the seal.

The sealing ability of MTA used as a root-end filling material has been evaluated by leakage investigations such as dye leakage, fluid filtration, protein leakage, and bacterial penetration. Numerous dye leakage studies that employed methylene blue, fuchsin, rhodamine B, silver nitrate, India ink, and Pelikan ink, have been performed on MTA. Dye penetration method was used in this study, because it is the oldest and most popular method. Fluorescent dyes are particularly useful as tracers of leakage around dental materials for several reasons: they are detectable in dilute concentrations, sensitive to ultraviolet light, easy to photograph, permit reproducible results, are inexpensive, contrast sharply with the natural fluorescence of teeth, require short immersion periods, permit direct observation of the total marginal leakage, and are non-toxic. Removal of root-end filling materials from their respective cavities provided the examiners with a three-dimensional view of the extent of the dye leakage. Dye penetration into the interface between the root-end filling materials and dentinal walls was uneven, with deeper penetration compared to the blood-contaminated samples at 7 days, but there was no description of the effect of blood on the sealing ability of MTA.

Previous investigations reported that the presence or absence of blood did not affect the sealing ability of MTA, or that no significant difference in bacterial penetration was noted between MTA contaminated with blood and that with saline compared to unoinfected specimens. The results in this study showed a significant difference in the leakage test, but no significant difference in the microhardness test. From these results, it was suggested that the effects from contamination are still unclear, but are dependent on the volume of contamination or other unknown factors.

With regard to adenalin, there have been no reports on its effect on the sealing ability of MTA. The effect of a local anesthetic, 2% lidocaine HCl with 1:100,000 adenalin, on the displacement test of MTA used for furocacin perforation repair was reported, and the use of lidocaine displayed significantly less resistance to displacement than the control (sterile water) at 7 days. On the other hand, it was reported that EDTA might influence MTA setting and hardness. The higher leakage in the group with the smear layer removed might be due to the reaction between MTA and residual EDTA inside the root canal. In the present study, contamination by adenalin or EDTA did not significantly affect the sealing by MTA compared to the saline-contaminated group.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Results of leakage degree of each group</th>
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<tbody>
<tr>
<td>Group</td>
<td>Leakage degree (mm)</td>
</tr>
<tr>
<td>1</td>
<td>1.11±0.58</td>
</tr>
<tr>
<td>2</td>
<td>2.06±0.79*</td>
</tr>
<tr>
<td>3</td>
<td>1.77±0.55*</td>
</tr>
<tr>
<td>4</td>
<td>2.13±0.40*</td>
</tr>
<tr>
<td>5</td>
<td>2.12±0.67*</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation (SD).
*: shows a significant difference compared to group 1.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Results of microhardness test</th>
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<tbody>
<tr>
<td>Group</td>
<td>Microhardness value</td>
</tr>
<tr>
<td>1</td>
<td>17.48±0.47</td>
</tr>
<tr>
<td>2</td>
<td>14.98±0.98</td>
</tr>
<tr>
<td>3</td>
<td>15.38±2.36</td>
</tr>
<tr>
<td>4</td>
<td>16.99±0.91</td>
</tr>
<tr>
<td>5</td>
<td>12.86±2.74*</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation (SD).
*: shows a significant difference compared to group 1.
tration in some areas than others. The furthest extent of linear dye penetration was used to represent the deepest gap between the root-end filling materials and the dentinal walls. Although the dye molecules are much smaller than bacteria, the existence of dye leakage indicates the presence of a potential gap for bacterial penetration.

Ensuring a complete seal of the root-end resected tooth after periapical surgery is of the most importance to increase the potential for endodontic success. It seems that MTA as a root-end filling material is a viable option; however, the results of the leakage study after MTA filling might be dependent on the contamination by drug or blood. Further studies are required to determine the ideal conditions for using MTA as a root-end filling material.

**Conclusion**

From the results of this study, it was unclear if blood directly affected the sealing ability of MTA, but contamination of MTA while setting seemed to decrease the sealing ability of MTA.

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逆根管充填材として使用した mineral trioxide aggregate の
辺縁封鎖能と硬度における血液污染物の影響

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抄録
目的：本研究の目的は、抜去歯を使用して MTA (mineral trioxide aggregate) の根尖部辺縁封鎖能と硬度におけるさまざまな污染物の影響を評価することである。

材料と方法：51 本のヒト単根歯に対して、ガラスバーチャポインタとシーラーを用いて側方加圧根管充填を行った。歯根面をコーティングした後に、歯根尖切除法と根尖部において窩洞形成を行い、無作為に 5 群に分
類した。1 群は蒸留水 (対照)、2 群は生理食塩液、3 群はアドレナリン、4 群は血液および 5 群は EDTA 溶液で汚染させ、污染物を混入した MTA で充填した後、浸漬し漏洩試験を行った。試料は 2 等分し、微小硬さ
試験を行い、実体顕微鏡および走査電子顕微鏡による観察を行った。

結果：1 群は形態学的に密封された辺縁封鎖が観察された。しかし、2～5 群の一部には部分的な辺縁封鎖とい
象牙質と MTA 間または MTA 中に空隙が観察された。1 群はほかの群に比較して有意に漏洩は少なかったが、
すべての実験群では有意差は認められなかった。5 群の微小硬さは 1 群と比較すると有意に低かった。

結論：MTA の硬化時における污染物の混入は、辺縁封鎖能を低下させることが示唆された。

キーワード：污染物、MTA（mineral trioxide aggregate）、辺縁封鎖能

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