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Effect of phosphate buffer saline on coronal leakage of mineral trioxide aggregate

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Abstract: This study was carried out to compare the bacterial leakage of MTA used as a root-end filling material when it was kept in phosphate buffer saline (PBS) or normal saline. In this study, 72 freshly extracted teeth were used. The roots were randomly divided into four experimental groups of 15 each (groups I and II gutta-percha obturation + MTA, groups III and IV only MTA) and two positive and negative control groups of six each. The samples in groups I and III were kept in normal saline for 1 month while the samples in groups II and IV were kept in PBS. *Enterococcus faecalis* was used for determination of the bacterial penetration. Kaplan-Meier survival curve and χ^2 test were employed for data analysis. The obturated samples with root-end filling showed significantly longer duration of resistance to bacterial penetration than canals without obturation ($P < 0.05$). The roots that were placed in PBS (groups II and IV) showed significantly less bacterial penetration in comparison with the roots that were stored in normal saline ($P < 0.05$). In conclusion, MTA, which acts as a bioactive material, should be placed in a synthetic tissue fluid before any leakage evaluation. (J Oral Sci 51, 187-191, 2009)

Keywords: bacterial penetration; bioactive material; mineral trioxide aggregate; MTA; PBS; root-end filling.

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Introduction

A number of materials are used as root-end filling materials in endodontic surgery including amalgam, gutta-percha, composite resins, glass ionomers, IRM, and SuperEBA (1-5). Recently, mineral trioxide aggregate (MTA) has also been suggested as a root-end filling material (4,6). In addition to its sealing ability (1-3,7,8), MTA is a biocompatible material (4,6,9,10). Studies in experimental animals show that MTA causes significantly less inflammation than amalgam and SuperEBA (4,10). More importantly, cementum bridge formation occurs directly over the MTA when it is used as a root-end filling material (4,6).

In two separate cell culture studies, Koh et al. (11,12) added MTA to osteoblast cell cultures. They observed that osteoblasts produce IL-1α, IL-1β, IL-6, and macrophage colony stimulating factor in the vicinity of MTA. Meanwhile, the amounts of osteocalcin and alkaline

phosphatase increased in the cell culture environment. Other cell culture studies have reported the production of IL-4, IL-10 (13) and bone morphogenic protein 2 in the vicinity of MTA (14).

One of the characteristics of a bioactive material is its ability to form an apatite-like layer on its surface when it comes in contact with physiological fluids *in vivo* (15) or with simulated body fluids such as phosphate buffer saline (PBS) (16). Sarkar et al. (17) used MTA as a root canal filling material and kept their samples in PBS. They reported that calcium from MTA reacts with phosphorus in PBS, producing hydroxyapatite. Similar results were obtained by Bozeman et al. (18).

Several studies on bacterial leakage employed saline (2,5) or 100% humidity (19,20) as a medium during experiments when MTA was used as a root-end filling material (5,19-23). So far, the effect of PBS on bacterial leakage of MTA when used as a root-end filling material has not been examined. The purpose of this study was to determine the effect of PBS on coronal leakage of MTA when used as a root-end filling material.

Materials and Methods

Seventy-two freshly extracted human single-rooted teeth that were extracted either due to periodontal problems or prior to orthodontic treatment were used in this study. After tooth decoronation, the remaining root was ground in a coronoapical direction with a No.701 fissure bur (Diatech, Heerbrugg, Switzerland) mounted on a high speed handpiece to develop a root sample 18 mm in length. The root canal in each sample was instrumented with a crown down technique.

The roots were then randomly divided into four experimental groups of 15 each and two control groups of six each. The roots in groups I and II were obturated with gutta-percha and AH26 (Dentsply-GmbH, Konstanz, Germany) using the lateral condensation technique while the root canals in groups III and IV were left unfilled. After resecting the apical 3 mm of each root using a No.701 fissure bur (Diatech) mounted on a high-speed handpiece under copious water spray, a root-end cavity was prepared with an ultrasonic diamond coated retro-tip to a depth of 3 mm (Piezon-EMS, Vallée de Joux, Switzerland).

Once complete, the apical preparation was irrigated with 1 ml of normal saline and dried with paper points. Grey MTA (Pro Root MTA, Dentsply Tulsa Dental, Tulsa, OK, USA) was prepared according to the manufacturer's instruction with a powder-to-liquid ratio of 3:1. The root-end cavity preparations in groups I and II were filled with grey MTA. In root canals of groups III and IV, after placing a tight fitting hand plugger 3 mm short of the apical opening in each prepared canal, the root-end cavities were filled with grey MTA. The samples in groups I and III were placed in normal saline while the samples in groups II and IV were placed in PBS (pH = 7.4).

The specimens were incubated at 37°C for 1 month. They were then observed under a stereomicroscope (Wild Heerbrugg, Gais, Switzerland) at a magnification of ×6.4 to ×32. The external surfaces of the specimens were covered with two layers of nail varnish (Arcancil, Paris, France) from the coronal edge to 1 mm short of the root resected area. All samples in the negative control group (six roots) were covered completely with two layers of nail varnish including the apical portion of the root and resected area. Positive control samples consisted of six roots with unfilled root canal and root-end preparation. The roots were inserted individually into Ependorff tubes with the root apex protruding through the cut end of the tube. The coronal and middle portion of each specimen was sealed with cyanoacrylate glue to prevent leakage at the connection. Care was taken to ensure that no cyanoacrylate glue covered the coronal end of the root.

The system was sterilized using ethylene oxide gas and placed in a cryo sterile bottle containing 3 ml sterile Brain Heart Infusion (BHI) (Merk, Darmstadt, Germany) with phenol red ensuring that the apical portion of the root was immersed in liquid. The coronal chamber was inoculated with 0.5 ml of BHI containing approximately 10^9 bacteria/ml *Enterococcus faecalis* (*ATCC 8213*) using a sterile syringe and 22-gauge needle. The medium with microorganisms was changed every 3 days. The system was stored in an incubator at 37°C, and color change of the culture (red to yellow) in the apical chamber was checked every day for 3 months. The time taken for this to occur was recorded as an indicator of coronal contamination. Cultures from the apical chamber were streaked onto blood agar culture plates and incubated under aerobic and anaerobic conditions. Microorganisms were identified by colony morphology, Gram stain and, in the case of *E. faecalis*, by biochemical tests.

The mean leakage (by number of days) for the samples was compared using Kaplan-Meier survival analysis and χ^2 tests. The significance level was set at 0.05.

Results

Stereomicroscopic observation of the samples that were kept in PBS showed the presence of a white crystalline layer over MTA. This layer was absent in the samples that were kept in normal saline.

All samples in the positive control group exhibited bacterial leakage within 24 to 48 h, whereas the lower chamber of the negative control roots remained uncon-

taminated throughout the experiment.

The estimated mean and the confidence interval of the number of days in which the leakage occurred based on the results of survival analysis, classified by the type of solution and material, are shown in Table 1. Among all groups, the samples in group III showed the lowest average time for bacterial leakage (39.13 ± 8.65 days). In contrast, the samples in group II showed the highest average time for bacterial leakage (73.85 ± 6.52 days). The roots in group III showed significantly more bacterial leakage in comparison with the roots in group IV ($P < 0.05$). The roots in group I showed significantly less bacterial penetration in comparison with roots in group III ($P < 0.05$). Meanwhile, there was a significant difference between groups I and II in bacterial penetration ($P < 0.05$). There was no significant difference between groups I and IV ($P > 0.05$). The difference between the groups that were obturated with gutta-percha (I, II) and root canal sealer and those without root canal obturation (III, IV) was significant ($P < 0.05$). The roots that were immersed in PBS (groups II and IV) showed significantly less bacterial penetration in comparison with the roots that were placed in normal saline (groups I and III) ($P < 0.05$) (Figs. 1 and 2). The amount of positive culture in the different groups was as follows: group III > group I > group IV > group II.

Discussion

Previous bacterial leakage studies on MTA used different storage media after using the material as a root-end filling. Some of the studies published did not mention the storage media (21-23) while others used either saline (2,5) or 100% humidity with tap water or saline (24,25). In this study, we placed MTA in PBS storage medium that resulted in formation of crystals over MTA and caused a significant decrease in coronal bacterial penetration. Since the composition of tap water may vary in different geographic areas, we used normal saline as our control medium.

In the present study, the roots that were kept in PBS showed significantly less bacterial penetration ($P < 0.05$) compared to those placed in saline. Martin et al. (26) used MTA as an orthograde apical plug of the teeth. Results of their fluid filtration study indicated that the teeth that were immersed in PBS after inserting an apical plug with MTA exhibited significantly less leakage. Despite the use of a bacterial penetration model in the present study, our results corroborate those of Martin et al. (26).

Studies examining bacterial penetration of root-end filling materials have been performed with and without canal obturation. A number of studies evaluated root-end filling materials without root canal obturation (20-22). Others have used samples of root-end filling with (23) or

Table 1 The estimated mean and its confidence interval of the number of days during which the leakage occurred

Groups	Mean	95% CI
Saline, without gutta-percha (Group I)	63.86	52.68-75.04
Saline, gutta-percha (Group II)	73.9	61.1-86.6
PBS, without gutta-percha (Group III)	39.1	22.2-56.1
PBS, gutta-percha (Group IV)	60.2	43.1-77.3
All samples with gutta-percha	67.1	56.1-78.0
All samples without gutta-percha	46.8	34.6-59.1
All samples in Saline	49.3	36.6-62.0
All samples in PBS	63.9	52.7-75.0

CI: Confidence interval, PBS: Phosphate buffered saline

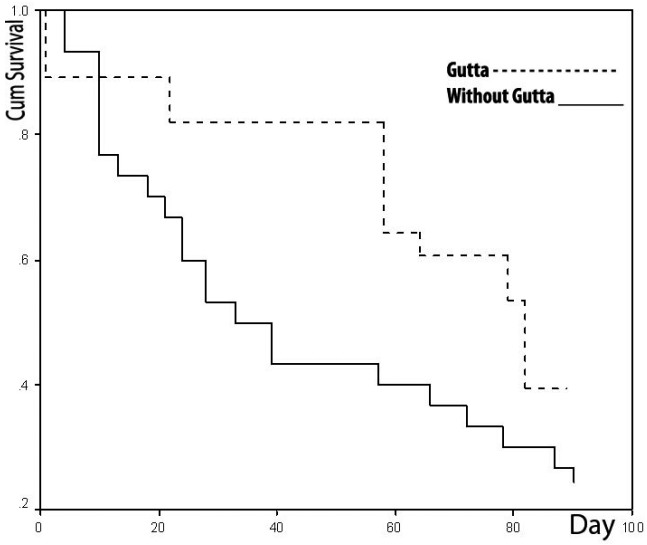

Fig. 1 Plot of Kaplan-Meier survival analysis during study in the teeth with or without gutta-percha obturation.

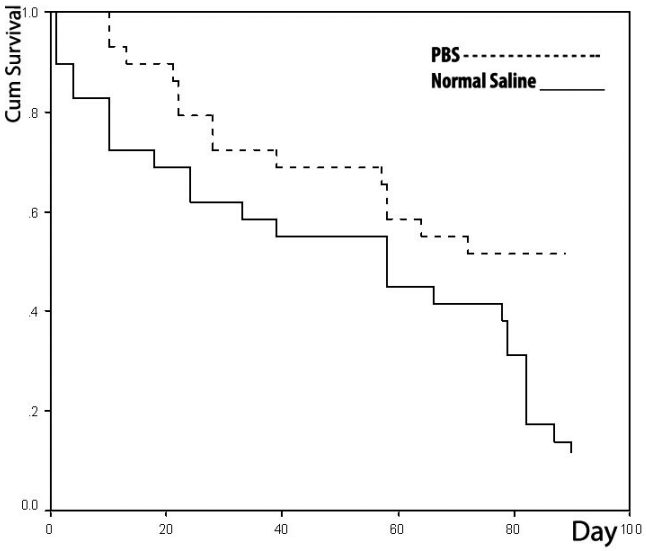

Fig. 2 Plot of Kaplan-Meier survival analysis during the study for the teeth that were kept in either PBS or normal saline.

without canal obturation (27). Malcic et al. (27) in their study have shown that after root-end resection of the apex, the samples that were filled both coronally and apically with IRM leaked significantly less than the samples filled only with an orthograde approach. They stated that this finding indicates the importance of total root canal obturation before root-end filling in surgical endodontics. Our results confirm the finding of their study and the importance of complete coronal leakage before placement of root-end filling materials (27). Although at the end of the study, roots in group I showed no significant difference in bacterial penetration in comparison to group IV, it can be assumed that the importance of storage media is as important as root canal obturation on inhibition of bacterial penetration when MTA is used as root-end filling. Although they were insignificant, our results show that roots with canal obturation that were placed in normal saline (group I) showed more bacterial penetration than the roots without gutta-percha canal obturation that were placed in PBS (group IV).

Placement of MTA in PBS storage medium not only resulted in a significant decrease in coronal bacterial leakage, it also caused formation of crystals over MTA. Presence of these crystals might be an important phenomenon in biological responses such as cementum formation after using MTA as a root-end filling material. MTA has been shown to exhibit a good apical seal and low cytotoxicity (1,11,19,28). In addition, cementum has been found growing in contact with MTA when it is used as a root-end filling material (4,6,10,29). Many researchers suggested that cementum formation over MTA might be due to formation of hydroxyapatite when the material comes into contact with tissue fluids (17,18).

Based on the results of this study, it appears that placing MTA in PBS storage medium results in formation of crystals over MTA and causes a significant decrease in bacterial penetration. This may indicate that future *in vitro* studies should use PBS as a storage medium to produce an environment similar to that of an *in vivo* condition. Mineral trioxide aggregate (MTA) is introduced as the most biocompatible root-end filling material (30). It is also known as a bioactive material that is hard tissue inductive and conductive (31,32). Recently, many investigators have tried to improve MTA's handling characteristics, antibacterial properties, and physical properties and to compare the material's sealing ability to that of new root-end filling materials (4, 24,25,32-34). As a calcium silica based material, MTA can develop hydroxyapatite on its surface in a synthetic tissue fluid such as phosphate buffer saline (PBS) (17,18). Results of the present study show that this layer improves MTA sealing ability. For this reason, we suggest that further *in vitro* studies evaluating the sealing ability of newly modified MTA or comparing the material with other root-end filling materials should use PBS as a media to simulate the environment in the human body, thus producing more clinically relevant results.

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