Effect of two storage solutions on surface topography of two root-end fillings

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Abstract
The effect of different storage solutions on surface topography of mineral trioxide aggregate (MTA) and new experimental cement (NEC) as root-end fillings was investigated. Twenty-four single-rooted teeth were cleaned, shaped and obturated in a same manner. After root-end resection, 3-mm deep root-end cavities were ultrasonically prepared. Samples were randomly divided into four test groups (A1-A2-B1-B2, n = 6). Root-end cavities in groups A and B were filled with MTA and NEC, respectively, and were then stored in 100% humidity for 24 h. The samples of groups 1 and 2 were, respectively, immersed in normal saline (NS) and phosphate buffer saline solutions for 1 week. The samples were imaged under stereomicroscope before and after immersion and were then investigated and analysed by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDXA). Results showed significant difference among studied groups. Surface topography of all samples was altered by crystal formation and precipitation on root-end fillings except for group A1 (MTA–NS). SEM and EDXA results showed that the composition and structure of precipitated crystals were comparable with that of standard hydroxyapatite. It was concluded that biocompatibility, sealing ability, and cementogenic activity of MTA and probably NEC may be attributed to this fundamental bioactive reaction.

Introduction
The goal of periradicular surgery is to seal the pathways of communication between the root canal system and surrounding tissues by means of a root-end cavity preparation, which is then filled using a root-end filling material (1). Numerous materials have been recommended to be used for this purpose. However, an ideal root-end filling material should be biocompatible, antibacterial, non-toxic, non-corrosive, non-resorbable, dimensionally stable, easy to handle, moisture indifferent, radiopaque, cost-effective, adaptable to the dentinal walls, and finally able to induce regeneration of the periodontal ligament complex, specifically cementogenesis over the root-end filling itself (2).

Various root-end filling materials are available in the market for surgical endodontic use, but none has presented the characteristics of an ideal material yet (3). Mineral trioxide aggregate (MTA) introduced in 1993 (4–5), has been tested as the most tissue-friendly root-end filling material (6). MTA consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite (7), and other mineral oxides, that is, bismuth oxide added as radiopacifier (8). When MTA is used as a root-end filling material, it causes significantly less inflammation than amalgam and Super-ethoxy benzoic acid (EBA), and cementum formation occurs directly over this material (6). While it was reported that the MTA cementogenesis mechanism is unclear (9), others reported that the storage of MTA as a root-end fillings in a simulated tissue fluid created a chemical bond between the material and tooth structure by formation of hydroxyapatite crystals, and they concluded that MTA is a bioactive material (10).
Because of the biocompatibility of this biomaterial, it has gained widespread use (11). Despite its excellent biocompatibility, MTA has a delayed setting time (3), poor handling characteristics (12), and is an expensive material.

Recently, the first author developed a new experimental cement (NEC) consisting of different calcium compounds (i.e. calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate and calcium chloride) which is compliant with the ISO 6876 standard for dental root canal sealing material. NEC has a different chemical composition than MTA but has similar clinical uses. This material is biocompatible, sets in an aqueous environment, has good handling characteristics, acceptable setting time (less than 1 h), forms an effective seal when used as root-end filling material and the results are comparable with MTA (13).

A good characteristic of a bioactive material, in contact with physiological or simulated body fluids such as phosphate buffer saline (PBS), is the ability of hydroxyapatite formation on their interfaces (14–15). Researchers reported that the characteristics of dental materials can be influenced with duration and the media of storage (16–18). Therefore, the aim of this study was to compare the ability of MTA and NEC in hydroxyapatite formation when these root-end filling materials are stored in normal saline (NS) or simulated tissue fluid composed of phosphate buffered saline solutions.

Methods and materials

Ethic Human Committees of Research School of Biomedical sciences, Australian National University, Canberra, Australia (Protocol: 2006/0211), and Dental Research Center, Shahid Beheshti University M.C., Tehran, Iran both approved this study.

Twenty-four freshly extracted human single-rooted teeth were used in this study. The teeth were decoronated and root canals were cleaned and shaped using standard step-back technique. After final irrigation, canals were dried and obturated with gutta-percha (Ariadent, Tehran, Iran) and Roth 801 root canal sealer (Roth International LTD, Chicago, IL, USA). Root-end resections were made by removing 3 mm of the apex at a 90°angle to the long axis of the root with a cylindrical carbide bur (D&Z, Wiesbaden, Germany) using a high-speed with-water spray coolant. The 3-mm deep class I root-end preparation was made using an ultrasonic power unit (miniPiezon, EMS, Nyon, Switzerland) with ultrasonic retrotips (DT-043, EMS, Nyon, Switzerland).

The cavities were dried and samples were randomly divided into two groups (A and B), each comprised of 12 roots. In group A and B cavities were filled with MTA (ProRoot MTA, Dentsply Tulsa Dental, Tulsa, OK, USA) and NEC cement, respectively, mixed according to manufacturers’ instructions. The excess material was removed and the roots were stored in an incubator at 37°C and 100% humidity for 24 h.

The samples of each group divided into two subgroups (A1, A2, B1 and B2) comprising six samples each. The samples of groups A1 and B1 were stored in NS solution while two other groups (A2 and B2) were stored in neutral PBS and all the samples were incubated at 37°C for 1 week. Before and after placement of each sample in appropriate solution, the root-end filling was photographed using a stereomicroscope (Wild, Heerbrugg-Leica, Heerbrugg, Switzerland) at x32 magnification.

For scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDXA) analysis, using a Dynavac CS300 (Dynavac, Wendouree, Australia) coating unit, all the samples were prepared and coated with ~20 nm of carbon. EDXA for elemental analyses was carried out on a JSM6400 (JEOL, Tokyo, Japan) scanning electron microscope, equipped with an Oxford Instruments light element dispersive spectrometer detector (Oxford Instruments, Eynsham, UK). All analyses were carried out at 15 kV and 1 nA of probe current, using well-characterised minerals as calibration standards (Astimex MINM25-53, Astimex Scientific Limited, Toronto, Ontario, Canada). Atomic number, absorption and fluorescence corrections were applied throughout (19). The results were analysed statistically using Kruskal-Wallis test.

Results

Stereomicroscope findings

Kruskal-Wallis test showed significant differences among studied groups \( P < 0.001 \). None of the samples of group A1 (MTA–NS) revealed alteration over the root-end fillings (Fig. 1a). In contrast, all the samples of groups A2 (MTA–PBS), B1 (NEC–NS) and B2 (NEC–PBS) showed white crystals formation and precipitation over the root-end filling materials, margin of the materials and superficial border of root-end cavities’ wall, and some extent of surrounding dentin surfaces as a white plaque (Fig. 1b–d).

Scanning electron microscopy findings

All the samples showed crystals formation and precipitation over the root-end filling materials, except group A1 (Fig. 2). The quantity of crystal precipitation on root-end fillings in the samples of group B1 was less than A2 and B2.
Energy dispersive X-ray analysis findings

X-ray spectrum of qualitative analysis of studied root-end fillings surfaces is shown in Figure 3. Quantitative composition data from EDXA of the test groups are shown in Table 1. EDXA indicated that MTA–PBS interaction surface contained mainly CaO and P₂O₅, with minor or trace amounts of Na, Cl, Mg, Al, Si and Bi, while the data showed absence of phosphorous ion in the component of MTA–NS interaction surface and also Si, Al, Mg and Bi present as the major components. The composition of NEC–PBS or NEC–NS interaction surfaces was similar to that of MTA–PBS except for Bi (Table 1).

Discussion

Healthy periradicular complex contains several discrete tissues including cementum, periodontal ligament and bone. The ability to enhance the regeneration of functional periradicular complex is a desirable property for any material used as root-end fillings. Ideally, any material used in periradicular surgery should result in not just the formation of new bone, but also periodontal ligament and cementum. New cementum may be formed adjacent to a few dental materials in contact with periodontal tissues (20). MTA has the ability to encourage hard-tissue deposition particularly cementogenesis (21,22).
Deposition of cementum against MTA may be due to a number of factors, such as sealing ability, biocompatibility or alkaline pH (22). Sarkar et al. (10) hypothesised that physico-chemical reactions of MTA may be attributed to good sealing ability and biocompatibility of this root-end filling material. To explain the physico-chemical basis of the biological properties of MTA they concluded that calcium ions released from MTA react with tissue phosphates yielding hydroxyapatite.

On the other hand, research studies revealed that MTA has the ability of calcium ion release during and after setting until a prolong time (9,23). The results of this study showed that MTA and NEC root-end fillings in contact with PBS solution, which contains phosphate ions, yielded the formation and deposition of hydroxyapatite crystals over the both materials. These findings are in agreement with Sarkar et al.’s (10) study and lead us to surmise that MTA and also NEC release sufficient calcium ions which can react with environmental phosphate and produce hydroxyapatite. Therefore, cementogenic or dentinogenic activity of MTA may be due to this physico-chemical reaction.

In contrast, no such process was found in MTA samples immersed in NS solution. Although the calcium ion release from MTA is an undoubted fact (9,23), the formation of hydroxyapatite crystals needs a sufficient amount of phosphate ions which release from the material in the lack of environmental sources. Torabinejad et al. (3) reported that phosphorous is a major element of crystalline phase of set MTA; however, their finding has not been supported by any other research studies (8,10,24,25) and also is not in agreement with our findings.

No current root-end fillings can provide a perfect tridimensional seal. Yet surgical endodontic management of the root end can result in successful outcomes, suggesting that an impermeable seal may not be an absolute prerequisite of root-end filling materials. It is possible to promote the formation of a double seal following periradicular surgery, incorporating a physical and biological...
covering over the root-end filling and surrounding dentin (26). The results of this study revealed that surface topography of NEC root-end filling samples immersed in NS solution was altered with white crystal formation and precipitation which was comparable with MTA and NEC samples stored in PBS. It is hypothesised that NEC provides the endogenous source of calcium and phosphate ions and after adequate increasing of ions’ concentration on the material-medium interface, chemical reactions of soluble Ca$^{2+}$ and PO$_4^{3-}$ facilitate and accelerate hydroxyapatite crystal formation as a double seal on this experimental root-end filling material.

It is well-known that different storage methods and media can influence dentine characteristics as well as physical properties of dental materials (27,28). In this study, neutral PBS solution was used and compared with NS solution. PBS is a buffer solution commonly used in biochemistry. It is a salty solution containing sodium chloride, sodium phosphate and potassium phosphate. This solution has many uses in medicine, because it is isotonic and non-toxic. Surface topography of MTA root-end fillings samples showed different behaviour according to ingredients of storage media. It seems that hydroxyapatite crystal formation and precipitation on the MTA in PBS, as an artificial tissue fluid, is more similar to clinical condition and can better explain the biocompatibility and good treatment outcomes of this material. Therefore, the storage of these kinds of samples in PBS solution as an artificial tissue fluid at 37°C can simulate more similar clinical conditions and results.

Conclusion

On the conditions of this experimental study, it is concluded that: (i) the ability of crystal deposition over MTA root-end fillings in PBS solution elucidate good treatment outcomes of this root-end filling via creation a double seal for sealing ability and hydroxyapatite formation for biocompatibility; (ii) NEC is able to produce hydroxyapatite with its endogenous as well as exogenous ions sources; and (iii) to obtain a more similar clinical condition it is suggested to store MTA filled teeth in PBS solution.

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