
Cytotoxicity evaluation of endosequence root repair material

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Objective. The purpose of this study was to evaluate the cytotoxicity of EndoSequence Root Repair Material (Brasseler USA, Savannah, GA) and compare it with gray and white MTA.

Study design. Samples of 2 mg freshly mixed or set gray MTA (GMTA), white MTA (WMTA), EndoSequence Root Repair Material (ERRM), and AH26 were eluted with 300, 600, and 1,000 μ L cell culture medium for 24 and 72 hours. L929 cells were seeded into 96-well plates at 3×10^4 cells/well and incubated with 100 μ L elute from each elute group. Cells cultured only with culture medium served as negative control. AH26 was used as positive control. After 24 hours' incubation, cell cytotoxicity was evaluated by MTT assay. Cell viability was calculated as percentage of the control group. The results were analyzed with 1-way analysis of variance.

Results. For both set and fresh samples, there were no significant cell viability differences among GMTA, WMTA, and ERRM. Cell viability in the AH26 group was less than in all of the other 3 materials.

Conclusion. This study suggests that ERRM may have cell viability similar to GMTA and WMTA in both set and fresh conditions. (*Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109:e122-e125)

In the U.S., it is estimated that more than 24 million endodontic procedures are performed annually, and up to 5.5% of these procedures are apical surgery, perforation repair, and apexification procedures.¹⁻² Mineral trioxide aggregate (MTA) is a cement composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, calcium sulfate, and bismuth oxide. It is used as a root-end filling material and for the sealing of communications between the root canal system and the surrounding tissues.³⁻⁷ It is also used for creating apical plugs, furcation repair, and perforation repair.⁸⁻¹² Currently, MTA is a commonly used root-end filling material.^{6,13,14}

MTA is mixed with sterile water in a 3:1 (w/v) ratio according to manufacturer's instruction. Setting time of MTA is 3-4 hours.^{2,15,16} It sets in a hydration reaction of tricalcium silicate ($3\text{CaO}\cdot\text{SiO}_2$) and dicalcium silicate ($2\text{CaO}\cdot\text{SiO}_2$). Upon mixing, it forms a colloidal gel, which solidifies into a hard structure in the presence of moisture.² In apical surgery applications moisture is provided from surrounding tissues. The pH of MTA rises from 10.2 to 12.5 3 hours after mixing.^{1,15}

Many clinicians subjectively report that the handling properties of MTA are less than ideal. The consistency is difficult to maintain owing to the setting reaction which results in desiccation of the MTA/water mixture. Long setting time of MTA makes it less than ideal for creating an apical plug, repairing a perforation and obturating the tooth in a single visit, or perforation repair. Several reports have been published on attempts to improve the handling characteristics of MTA.^{7,28}

Recently, a new root repair material has become available on the market: EndoSequence Root Repair Material (ERRM; Brasseler, Savannah, GA). According to the manufacturer, it is composed of calcium silicates, monobasic calcium phosphate, zirconium oxide, tantalum oxide, proprietary fillers, and thickening agents. The manufacture claims that the material is biocompatible and hydrophilic. According to the manufacturer, ERRM has a high pH, although no further details are given on pH. ERRM has a strength of 70-90 MPa. The material also has excellent radiopacity. The working time of ERRM is 30+ minutes. ERRM is available in a preloaded syringe with a moldable putty form. The preloaded syringe also has intracanal tips that can be bent to facilitate placement in clinical situations.

So far, no studies regarding EndoSequence Root Repair Material have been published. The purpose of the present experiment was to evaluate the cytotoxicity of ERRM and compare it with gray MTA (GMTA), white MTA (WMTA), and AH26.

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Received for publication Sep 11, 2009; returned for revision Nov 13, 2009; accepted for publication Nov 14, 2009.

1079-2104/\$ - see front matter

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doi:10.1016/j.tripleo.2009.11.028

MATERIALS AND METHODS

L929 mouse fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in Eagle minimum essential medium (ATCC) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) and 1% antibiotic/antimycotic cocktail (300 U/mL penicillin, 300 mg/mL streptomycin, and 5 mg/mL amphotericin B; Gibco BRL, Gaithersburg, MD) under standard cell culture conditions (37°C, 100% humidity, 95% air/5% CO₂).

The materials tested consisted of GMTA (ProRoot; Dentsply Tulsa Dental, Johnson City, TN), white MTA (ProRoot), and ERRM. AH26 root canal sealer (Dentsply Tulsa Dental) served as positive control, because fresh AH 26 has the most cytotoxicity when freshly mixed.

The cytotoxicity of the different materials was evaluated in 2 ways. In one set of experiments, set materials were used. Materials were mixed according to manufacturer’s instruction, placed into the 48-well plates at 2 mg/well using micropipette tips, and incubated for 72 hours in a cell culture incubator to allow the materials to become set. In another set of experiments, freshly (immediately) mixed materials were placed into the 48-well plates at 2 mg/well. The freshly mixed and set materials were incubated with 3 different amounts of cell culture medium, 300 μL, 600 μL, and 1,000 μL, for 24 and 72 hours.

For the cell cytotoxicity assay, L929 cells were seeded into 96-well plates at 3 × 10⁴ cells/well and incubated for 24 hours to allow attachment. Then 100 μL of the material elute from different elute groups was placed into the culture wells. Cells with 100 μL culture medium served as a control group. After an incubation period of 24 hours, cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer’s instructions (ATCC).

Cell viability was calculated as percentage of the control group, and the results were analyzed with 1-way analysis of variance. Post hoc tests were done with Scheffé test. Each experiment was repeated 3 times.

RESULTS

Fresh material

When cells were cultured with 1-day elute of the freshly mixed materials, there were no significant cell viability differences among GMTA, WMTA, and ERRM in the 300 μL and 600 μL elute groups (Fig. 1). ERRM had more cell viability than GMTA in the 1,000 μL groups (Fig. 1). There were no cell viability differences between GMTA and WMTA or between WMTA and ERRM in the 1,000 μL groups. AH26 had less cell viability than all of the other materials in all of the elute groups (Fig. 1).

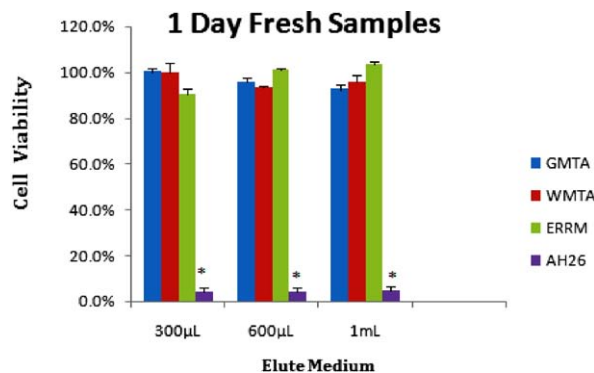


Fig. 1. Cell viability of L929 cells after culture with 1-day elute of fresh GMTA, WMTA, ERRM, and AH26.

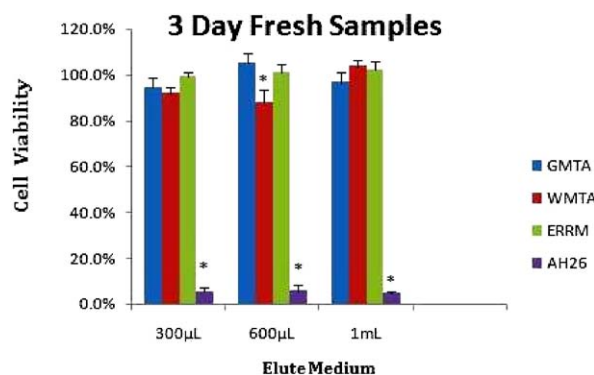


Fig. 2. Cell viability of L929 cells after culture with 3-day elute of fresh GMTA, WMTA, ERRM, and AH26.

When cells were cultured with 3-day elute of the freshly mixed materials, there were no significant cell viability differences among GMTA, WMTA, and ERRM in the 300 μL and 1,000 μL elute groups (Fig. 2). GMTA had more cell viability than WMTA in the 600 μL groups (Fig. 2). There were no cell viability differences between GMTA and ERRM or between WMTA and ERRM in the 600 μL groups. AH26 had less cell viability than all of the other materials in all of the elute groups (Fig. 2).

Set material

When cells were cultured with 1-day and 3-day elute of the set materials, there were no significant cell viability differences among GMTA, WMTA, and ERRM in all of the elute groups (Figs. 3 and 4). AH26 had significantly less cell viability than all of the other materials in all of the elute groups (Figs. 3 and 4).

In summary, ERRM had a cytotoxicity similar to MTA.

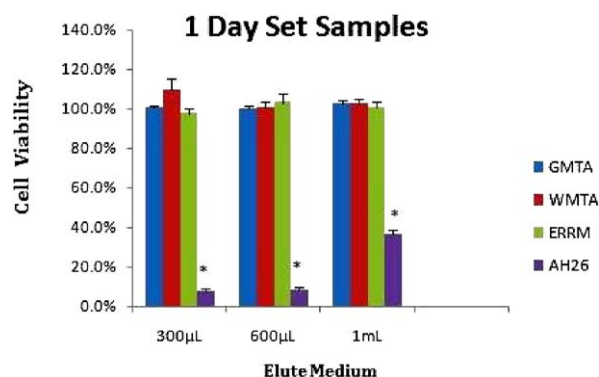


Fig. 3. Cell viability of L929 cells after culture with 1-day elute of set GMTA, WMTA, ERRM, and AH26.

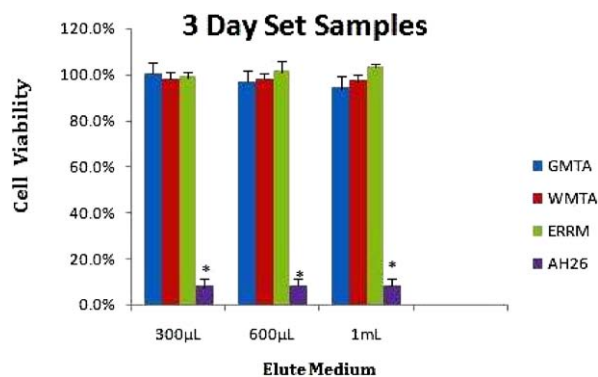


Fig. 4. Cell viability of L929 cells after culture with 3-day elute of set GMTA, WMTA, ERRM, and AH26.

DISCUSSION

ERRM is described by manufacturer as a bioceramic material. Bioceramic materials are “ceramic products or components employed in medical and dental applications, mainly as implants and replacements, which have osteoinductive properties.”³¹ Many materials used today in dentistry are considered to be bioceramics, such as zirconia, hydroxyapatite, tricalcium phosphate, tricalcium silicate, and dicalcium silicate.³² According to this definition, both GMTA and WMTA are considered to be bioceramics.

In the present study, L929 mouse fibroblasts were placed in the prepared elute of the tested materials. L929 is easy to prepare and culture, provides more reproducible results, and is routinely used for cytotoxicity studies.^{7,17,33-35} The MTT assay is also a standard assay to evaluate the cytotoxicity of endodontic materials.^{7,17,33-35} The elution of the root-end filling material mainly tests whether the material will leach cytotoxic substances which inhibit cell activity and growth. The advantage of this method is not only quantitative and reproducible, but also to test fresh and set material at the various stages. A series of elution with various times could provide the cytotoxic degree of the tested material. However, different materials may have different elution processes, so the elution volume and elution time should be carefully considered.

The cytotoxicity of MTA has been investigated in many studies. The results showed MTA is biocompatible and is not genotoxic.^{2,17-21} MTA was biocompatible when tested with human periodontal ligament fibroblasts for cell viability, apoptosis, and mitochondrial dehydrogenase activity.^{7,22,23} Similar results were obtained when MTA and glass ionomer were tested with mouse and human periodontal ligament fibroblasts.^{7,24} Several studies have also shown that there was no cytotoxicity difference between GMTA and WMTA both in vivo and in vitro.^{29,30} In vivo, Parirokh et al. mechanically exposed pulps in beagle dogs

and histologically observed inflammatory response for 2 weeks. The results showed no difference in inflammatory reaction between the cements. The author also found that inflammatory cells decreased after 2 weeks in both cement groups.²⁹ In vitro, genotoxicity of GMTA and WMTA on Chinese hamster ovary K-1 cells was tested using single-cell gel (comet) assay. The comet assay measures DNA damage. Cytotoxicity was also measured using trypan blue staining. Neither GMTA nor WMTA caused DNA breakdown or caused cell death.³⁰

ERRM showed cell viability similar to GMTA and WMTA. During setting, ERRM released a clear fluid. In this experiment, the fluid was not removed and medium was directly added to the set material. When MTA was used as a root-end filling material, formation of cementum and periodontal ligament fibers was observed on its surface.^{3,4} Whether ERRM has osteoinductive properties need to be further studied.

AH26 is a resin-based sealer. Several studies have shown AH26 to be cytotoxic to different cell lines.²⁵⁻²⁷ AH26 was used in the present experiment as a negative control. AH26 was shown to be cytotoxic to Chinese hamster V79 cells. Set AH26 was cytotoxic in doses of $>55.7 \mu\text{g/mL}$.²⁵ AH26 and AH Plus demonstrated both cytotoxicity and genotoxicity in vitro, when tested with rat cerebral astrocyte cell culture.²⁶ When used with human cervical carcinoma (HeLa) cells and mouse skin fibroblasts (L929), AH Plus was found to be more toxic than AH26.²⁷ AH Plus-Jet toxicity was evaluated using rat osteosarcoma cells and MTT assay. The authors found AH Plus-Jet to be severely toxic to cells during the first 72 hours, but toxicity decreased over 5 weeks.³⁶ The results of the present experiment are in agreement with these earlier studies.

In summary, the results of the present experiment show that ERRM had a cytotoxicity similar to MTA in both freshly mixed and set conditions. Other properties of

ERRM, such as sealing ability, solubility, in vivo endodontic usage, and so on, need to be investigated in the future.

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