Cytotoxicity Comparison of Three Current Direct Pulp-Capping Agents With a New Bioceramic Root Repair Putty

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Abstract

Introduction: The purpose of this *in vitro* study was to compare the cytotoxicity of white mineral trioxide aggregate cement (AMTA, MTA-Angelus), Brasseler Endosequence Root Repair Putty (ERRM), Dycal, and Ultra-blend Plus (UBP) by using human dermal fibroblasts and a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **Methods:** Cultured adult human dermal fibroblasts were exposed to multiple concentrations of material elutes. The test material samples were immersed and incubated in the culture medium for 2, 5, or 8 days at 37°C. The cytotoxic effects were recorded by using an MTT-based colorimetric assay. Positive and negative controls were used. The results were statistically examined by one-way analysis of variance and Tukey post tests. **Results:** The cell viability of cultures exposed to all dilutions of AMTA, ERRM, and UBP was statistically similar to the negative control at 2 and 5 days. Only the Dycal-exposed specimens exhibited a statistically significant increase in cytotoxicity at the 2 initial evaluation periods. After exposure to the 8-day elutes, the respective percentage of cell survivability was 91% (Brasseler), 88% (MTA-Angelus), 76% (Ultra-blend Plus), and 37% (Dycal). **Conclusion:** From the data in this *in vitro* study, AMTA, ERRM, and UBP had statistically similar adult human dermal fibroblast cytotoxicity levels. Relative to the negative control, only Dycal was shown to have a statistically significant cytotoxic effect to adult human dermal fibroblasts at all tested intervals. *(J Endod 2012;■:1–4)*

Key Words

Bioceramic root repair material, biocompatibility, calcium hydroxide, direct pulp cap materials, MTA, MTT-assay, vital pulp therapy

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As the specialty of endodontics continues to evolve in the twenty-first century, there appears to have been a shift in our collective biologic focus. Understanding the human body's oral regenerative potential is an area of current confusion and considerable exploration. Perhaps lost in this current perspective is the appreciation of material developments in the vital pulp therapy arena. Maintaining pulpal vitality to permit continued odontogenic development and maturity is a valuable component of operative and endodontic treatment. A reasoned strong argument can be made that the ideal endodontic obturation material is the vital asymptomatic pulp itself.

Historically, vital pulp therapy was first considered by performing pulp amputation at the root orifices and placing calcium hydroxide. The aim was to form dentin over the surface of the cut wound and wall off the pulp from the cavity. It was reported in an initial study of vital pulp therapy that 71% of 150 cases showed no radiographic changes at checkup (1). Today, vital pulp therapy is still considered a successful treatment option (2). Calcium hydroxide has been considered the standard of care because of beneficial properties such as induction of mineralization, high pH, and low cytotoxicity (3–5). However, some of the limitations reported include dissolution over time, mechanically weak, and presence of tunnels in the dentin barrier (6–8). In addition, the handling properties are less than ideal (9). An improvement in handling characteristics was obtained with a light-curable calcium hydroxide cement, Ultra-blend Plus (Ultradent Products, Inc, South Jordan, UT) (UBP). In an *in vivo* study, UBP induced a similar tissue response as conventional calcium hydroxide cement (10). It has been reported that mineral trioxide aggregate (MTA) was clinically easier to use and resulted in less pulpal inflammation and more predictable hard tissue barrier formation than calcium hydroxide. Therefore, MTA or similar products should be the material of choice for direct pulp-capping procedures instead of calcium hydroxide (9). A new material that has been released to the market as an alternative to MTA might be a good candidate. Brasseler USA (Savannah, GA) has formulated a bioceramic material for root repair needs. Currently there is limited research on the Endosequence Root Repair Material (ERRM). It has mainly been evaluated for use as a root-end filling material. Its properties include exceptional stability, high mechanical bond strength, high pH, radiopaque, and hydrophilic setting properties, and it is premixed (11). The purpose of this *in vitro* study was to compare the cytotoxicity of white MTA cement (MTA-Angelus, Angelus, Londrina, PR, Brazil) (AMTA), ERRM, Dycal, and UBP by using human dermal fibroblasts and a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Materials and Methods

The 4 materials included in this study were MTA-Angelus, Endosequence Root Repair Material, Dycal, and Ultra-blend Plus. All materials were prepared according to the manufacturer's instructions (12–15). The Brasseler USA product was packaged premixed by the manufacturer and does not require preparation before use. UBP was packaged in a syringe with no preparation needed. AMTA was prepared with a powder-to-liquid ratio of 3:1 and mixed on a glass slab for 1 minute. Dycal was mixed at a 1:1 base-to-catalyst ratio on a glass slab.

The samples were placed into plastic wells with a diameter of 5 mm and a thickness of 6 mm. The AMTA samples were carried to the molds with an amalgam plugger and hand condensed with a standard amalgam condenser. ERRM was carried to the molds with the beaver-tail end of a Glick hand instrument (Vista Dental Products, Racine, WI) and hand condensed with an amalgam condenser. Dycal was carried to the molds and contoured with the beaver-tail end of a Glick hand instrument. UBP was dispensed into the molds via the tips provided by the manufacturer. Only AMTA was covered with a sopping wet cotton pellet before placement in the incubator according to the manufacturer's instructions for direct pulp caps. The samples were stored at 37°C in a chamber of 100% relative humidity for 1 week.

Cytotoxicity

Cytotoxicity can be determined by using an MTT-based colorimetric assay. This assay detects living cells only, which allows a measurement of cytotoxicity, proliferation, or activation (16). After sterilization of the samples by UV radiation, 2 cylinders of each material were immersed in 8 mL of culture medium (dermal fibroblast medium formulated by Zen-Bio, Inc, Research Triangle Park, NC) in a 15-mL conical tube. The cylinders were incubated in the culture medium for 2, 5, or 8 days at 37°C to allow the soluble components to leach from the samples into the medium. The medium was filtered through 0.2-μm syringe filters to remove any particulate matter before use, especially for the case of Dycal. The medium conditioned by each of the 4 materials was then diluted with fresh culture medium as follows: no dilution ("full-strength"), 1:2 dilutions, and 1:5 dilutions.

Adult human dermal fibroblasts, pre-plated in wells of a 48-well tissue culture plate, were purchased from Zen-Bio. Fibroblasts are a typical model cell used in cytotoxicity assays because of their ease of growth (17, 18). After delivery, fibroblasts were re-acclimated to culture conditions of 37°C and 5% CO_2 for 24 hours. One milliliter of culture medium of each material and dilution combination was then added to 10 wells in the tissue culture plate (n = 10 per combination). As a negative control, cells were cultured in medium only, and as a positive control, cells were cultured in medium containing 0.010% chlorhexidine (11, 19).

After 24 hours in culture, 0.1 mL MTT solution (Cell Growth Determination Kit MTT Based; Sigma-Aldrich, St Louis, MO) was added to each well, and cells were incubated for an additional 3 hours. The resulting MTT formazan crystals were dissolved by removing the culture medium and adding 1 mL MTT solvent to each well. The plate was shaken at room temperature for 10 minutes to dissolve the crystals. Two hundred–microliter samples (n = 3) were transferred to wells of a 96-well microtiter plate, and the absorbance at 570 nm (A_{570nm}) was determined by using a Vmax microtiter plate reader (17, 18).

The mean and standard error of the mean absorbance for each treatment were calculated from the triplicate samples. For comparisons across all conditions of material type and culture medium dilutions, the absorbance values of the negative control of fibroblasts cultured in medium only were set arbitrarily at 100% cell viability. All absorbance values were standardized to the negative control value. Statistical analysis of the data was performed by using one-way analysis of variance and Tukey multiple comparison post test, with significance of $P < .05$.

Results

Human dermal fibroblasts were treated with soluble products leached into culture medium from 4 dental materials for 24 hours. When examining fibroblast viability in culture medium containing soluble products that had leached from the materials after 2 days, only Dycal showed significant toxicity compared with control group (Fig. 1*A*). For the 2-day soluble products treatment, AMTA, ERRM, UBP, and Dycal resulted in cell viabilities of 105.3% ± 4.3%, 94.1% ± 3.8%, 94.3% ± 3.2%, and 82.2% ± 0.1%, respectively, compared with control arbitrarily set to 100%. Similar results were observed for dermal fibroblasts treated with soluble products from materials that had leached into culture medium for 5 days (Fig. 1*B*). For the 5-day soluble products treatment, AMTA, ERRM, UBP, and Dycal had cell viabilities of 99.7% ± 4.6%, 100.5% ± 3.3%, 102.4% ± 2.6%, and 77.1% ± 2.3%, respectively, compared with 100% control group. Data from use of the full-strength culture medium are shown for Figure 1. The 1:2 and 1:5 dilutions of the culture medium containing the soluble products for AMTA, ERRM, and UBP gave similar results.

The soluble products that had leached from all 4 materials after 8 days in culture proved to be cytotoxic to dermal fibroblasts (Fig. 1*C*). For the 8-day soluble products treatment, AMTA, ERRM, UBP, and Dycal had cell viabilities of 87.7% ± 2.1%, 90.8% ± 1.8%, 75.8% ± 2.9%, and 36.6% ± 1.5%, respectively, compared with 100% control group. Dycal resulted in significantly higher levels of cytotoxicity than

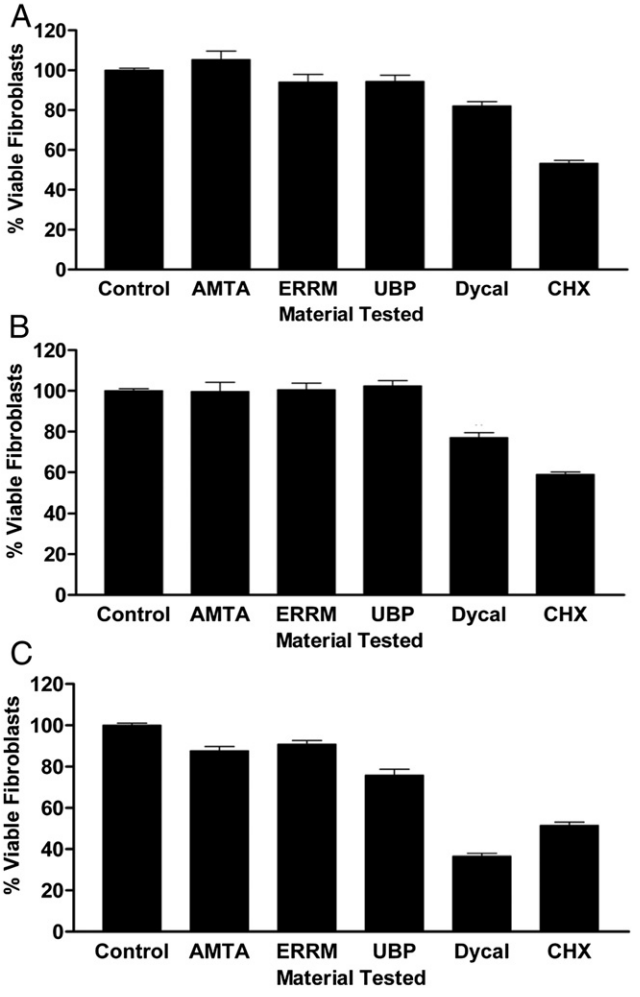

Figure 1. Cytotoxicity of 4 endodontic materials expressed as percentage of viable dermal fibroblasts present after exposure compared with the negative control group set at 100%. (*A*) Two-day soluble products of Dycal are significantly more cytotoxic than other materials. (*B*) Five-day soluble products of Dycal are significantly more cytotoxic than other materials. (*C*) Eight-day soluble products of all materials are significantly more cytotoxic than the control group. One-way analysis of variance, $P < .05$. Mean ± standard error of the mean.

chlorhexidine, a compound that has been shown to be highly toxic to cultured fibroblasts (11, 19).

The effect of dilution on the cytotoxicity of the Dycal soluble products is shown in Figure 2. For the 2-day soluble products, the cell viability for Dycal full-strength, 1:2, and 1:5 dilutions was 82.3% ± 1.6%, 97.3% ± 1.3%, and 96.6% ± 2.6%, respectively, compared with 100% control group. Although the full-strength Dycal soluble products showed a significant amount of cytotoxicity, the diluted solutions were not toxic to dermal fibroblasts (Fig. 2*A*). For the 5-day soluble products, the cell viability for Dycal full-strength, 1:2, and 1:5 dilutions was 76.8% ± 1.8%, 86.0% ± 1.5%, and 89.8% ± 3.4%, respectively, compared with control. All dilutions were significantly more toxic to dermal fibroblasts than the control group (Fig. 2*B*).

Discussion

Direct pulp capping has been shown to be an acceptable mode of treatment, with a reported success rate of 72.9%–95.4%. A direct comparison of a weighted pooled success rate when using MTA and calcium hydroxide has shown no statistically significant difference, but MTA was superior to calcium hydroxide with an indirect comparison (1). Mente et al (20) showed that the difference in the success rates of MTA compared with calcium hydroxide as a direct pulp-capping agent might be clinically relevant. Although results only indicated a borderline statistically significant difference with 78% success rate for MTA and 60% for calcium hydroxide, it was hypothesized that the lack of significance was due to the study's small sample size.

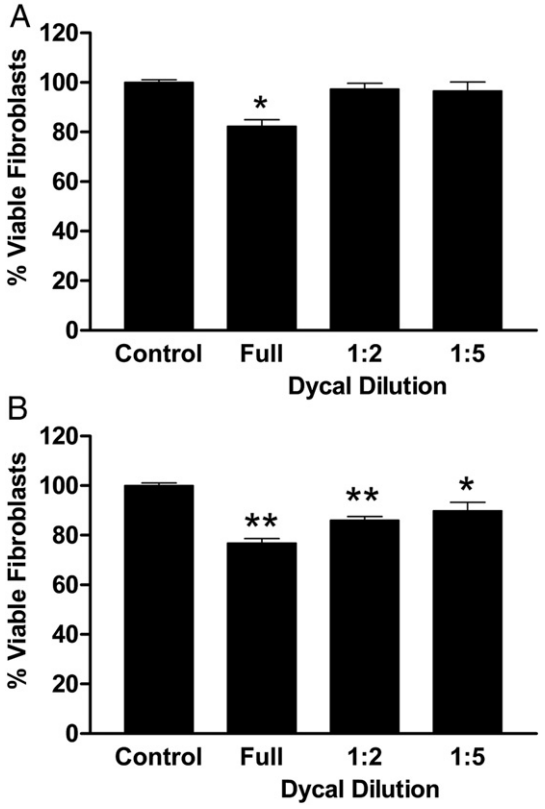

Figure 2. Cytotoxicity of Dycal dilutions expressed as percentage of viable dermal fibroblasts present after exposure compared with the negative control group set at 100%. (*A*) Two-day soluble products of Dycal are significantly more cytotoxic only when tested at full-strength. (*B*) Five-day soluble products of Dycal are significantly more cytotoxic than control at full-strength and when diluted. One-way analysis of variance, $P < .05$.

In the present study, Dycal and AMTA were used as conventional pulp-capping materials. It has been previously reported that MTA showed less inflammation and more predictable results than Dycal after pulp capping (9). ERRM had significantly less cell viability than AMTA at 24 hours according to Damas et al (11). The results from the current study showed that over time, ERRM was more toxic than AMTA at 48 hours as well but was equal at 5 days and less toxic at 8 days. In the present study, Dycal showed a statistically significant difference in cytotoxicity compared with the negative control at all tested intervals. This could be the reason for those earlier reported findings on inflammation and success. With this study's data, ERRM and UBP showed no statistically significant difference in cytotoxicity when compared with the negative control and AMTA. It could be considered these contemporary materials are viable options for use as direct pulp-capping materials.

The foundation to success for vital pulp therapy could be strict case selection and proper treatment protocol. The systematic review by Aguilar et al (2) shows the best current evidence regarding vital pulp therapy on vital permanent teeth with cariously exposed pulps. The review shows that vital pulp therapy can be an effective mode of treatment but did conclude that partial and full pulpotomy is more predictable than direct pulp capping. The authors explained it to be a function of complete removal of the infected tissue or damaged dentin-pulp complex. Currently there is no reliable method to determine the extent of this damage. A possible solution to this dilemma is to repair the damaged dentin-pulp complex. Rakkiettiwong et al (21) have demonstrated the retained effect of transforming growth factor beta-1 in a novel material that could be used as a vital pulp therapy material. This growth factor plays a key role in regeneration and repair of the pulp-dentin complex (22).

Various studies have reported an increased success rate when a permanent restoration is placed within 2 days of the pulp-capping procedure (20, 23, 24). This supports the importance of a good seal to prevent bacterial ingress. The ability of the pulp-capping material itself to prevent leakage is a viable factor to be investigated. There are limited leakage studies on pulp-capping materials. The effectiveness of different thicknesses of MTA as a coronal seal in deciduous teeth has been evaluated. Leakage was present at all thicknesses tested, 1–4 mm (25). Another study reported that MTA had leaked significantly less than glass ionomer with a 4-mm intracoronal seal (26). Further studies are necessary to evaluate the bacterial leakage of MTA, conventional and light-curable calcium hydroxide, and ERRM to find out whether the latest materials are superior.

Conclusion

From the data in this *in vitro* study, MTA-Angelus, Brasseler Endosequence Root Repair Putty, and Ultra-blend Plus had statistically similar adult human dermal fibroblast cytotoxicity levels. Relative to the negative control, only Dycal was shown to have a statistically significant cytotoxic effect on adult human dermal fibroblasts at all tested intervals. ERRM and UBP did not negatively influence cell survival and might thus be further considered for histologic pulp-capping studies.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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