

Comparison of the Surface Hardness among 3 Materials Used in an Experimental Apexification Model under Moist and Dry Environments

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Abstract

Introduction: Procedures used in single-visit or multiple-visit approaches to apical barrier creation were used with an experimental apexification model to test the surface hardness of 3 materials. The purpose of this study was to examine the microhardness of the materials after setting in moist or dry conditions.

Methods: A simulated open apex and periapical environment model was created using polyethylene tubes placed into a porous block filled with phosphate-buffered saline. White ProRoot Mineral Trioxide Aggregate (MTA; Dentsply Tulsa Dental, Tulsa, OK), EndoSequence Root Repair Material (ESRRM; Brasseler USA, Savannah, GA), and Biodentine (BD; Septodont, Louisville, CO) were mixed and placed into the apical 4 mm of the tubes ($N = 15$). The moist group had a damp cotton pellet above the test materials (mineral trioxide aggregate or ESRRM) with Fuji II LC (GC America, Alsip, IL) sealing the coronal segment. The dry group had gutta-percha placed directly against the test materials with amalgam sealing the coronal segment. After 10 days of storage in 100% humidity at 37°C, samples were sectioned, and microhardness was independently measured at 2 mm and 4 mm from the apical end. Differences were assessed using analysis of variance and a Tukey post hoc test ($\alpha = .05$). **Results:** Analysis of variance analyses showed no significant effect of wet or dry conditions on resultant material hardness. A Tukey post hoc test showed that using ESRRM and BD would not result in a significant difference in hardness, but using MTA would result in statistically significant different hardness values when compared with ESRRM or BD. **Conclusions:** Either a moist or dry environment could allow hardening of materials; thus, both methods could be acceptable for clinical treatment procedures. (*J Endod* 2014;40:986–989)

Key Words

Biodentine, EndoSequence Root Repair Material, immediate apexification, microhardness, white mineral trioxide aggregate

Apexification treatment duration has evolved from multiple visits spread over many months to procedures requiring only a few visits or a single appointment. Historically, apexification was introduced by Kaiser in 1964 and popularized by Frank (1) and was performed with long-term use of calcium hydroxide to induce formation of an apical barrier in a necrotic immature tooth to allow for subsequent obturation (2). Newer materials, some of which incorporate moisture into the setting reaction, directly create the apical barrier. This results in a reduction time for completion and fewer appointments and avoids the long-term use of calcium hydroxide, which has been shown to weaken dentin and the subsequent increased risk of root fracture (3). ProRoot Mineral Trioxide Aggregate (MTA) (Dentsply Tulsa Dental, Tulsa, OK), EndoSequence Root Repair Material (ESRRM) (Brasseler USA, Savannah, GA), and Biodentine (BD) (Septodont, Louisville, CO) have been recommended for root-end fillings, pulp capping, pulpotomy, repair of perforations, canal obturation, and apical plug creation (4–6). Such materials should be nontoxic, noncarcinogenic, biocompatible, insoluble in tissue fluids, dimensionally stable, impervious to moisture, bacteriostatic, radiopaque, nonstaining, easily removed from the root, able to seal canals laterally as well as apically, and facilitate easy placement into the canal system (7–9). MTA, ESRRM, and BD have all been shown to show many of these “ideal” qualities as well as utility in various clinical procedures (4, 6, 10–14).

ProRoot MTA contains tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, and calcium sulfate dihydrate (15). Hydration of this powder results in a colloidal gel that has an initial pH of 10.2, which rises to as high as 12.5 after setting (16). MTA absorbs moisture from surrounding tissues, such as that found in the periapical environment, allowing the setting reaction to occur (17). MTA has a published set time of 165 ± 5 minutes (16). The setting process, described as a hydration reaction of tricalcium silicate and dicalcium silicate, gives the material strength (18). It has been shown that the compressive strength of MTA increases in the presence of moisture for up to 21 days (19). It has been recommended that moisture, in the form of a saturated cotton pellet, should be present inside the pulp chamber or in the root canal during the first 3 days of curing before further filling of the tooth is performed. Moistening of MTA during curing becomes important when the MTA obturation is to be exposed to dislodging forces (20). Flexural strength is decreased when MTA absorbs excess moisture (21). Indirect ultrasonic activation, at the time of placement, resulted in an MTA filling

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that is heavier by weight and denser when compared with hand condensation alone (22). The manufacturer's instruction is to place a moist cotton pellet in direct contact with the material until a follow-up appointment occurs; this should be no sooner than 4 hours after placement of the MTA. MTA is mixed to a 3:1 powder to sterile water ratio (23). Previous authors, in various experimental designs, have shown the setting of MTA without a moistened cotton pellet being required (24, 25).

ESRRM uses bioceramic technology to address some of the inconsistencies associated with MTA. ESRRM contains tricalcium silicate, dicalcium silicate, zirconium oxide, tantalum pentoxide, and calcium sulfate (26). This material is produced as a premixed product to provide the clinician with a homogeneous, predictable material. The bioceramic material is produced with nanosphere ($1 \times 10^{-3} \mu\text{m}$ at its greatest diameter) particles that allow the material to enter into the dentinal tubules and interact with the moisture present in the dentin, creating a micromechanical bond upon setting (27). According to the manufacturer, the bioceramic material is highly radiopaque and bright white in color, making the material readily identifiable on radiographs as well as easily visualized during clinical placement. A high alkaline pH is partially responsible for its antibacterial nature. The initial pH is 12.8, which steadily decreases over a 7-day period, giving the material excellent biocompatibility (27). ESRRM showed no negative influence on the cell survival of human dermal fibroblasts (5). The manufacturer recommends placing a moist cotton pellet in direct contact with the material, which should be left in place until a follow-up appointment (4 hours as a minimum). It has a working time of 30 or more minutes and a setting reaction initiated by moisture, with a final set achieved after 4 hours in normal conditions, but requiring up to 12 hours in dry conditions (26). ESRRM is dispensed in a premixed syringe and capillary tube for delivery.

BD, a new calcium silicate-based restorative cement with dentin-like mechanical properties, has been promoted for use as a coronal and root dentin substitute in a similar fashion as MTA (28). BD contains tricalcium silicate, dicalcium silicate, calcium carbonate, zirconium oxide, iron oxide, and calcium chloride as the accelerator (29). BD combines high mechanical properties with excellent biocompatibility and a short setting time of 12 minutes. Unlike MTA and ESRRM, the manufacturer of BD indicates no requirement for moisture to allow finalization of the setting reaction. BD is available as a mixable capsule, with predosed calcium chloride solution and powder, becoming activated after a 15-second trituration at 4000 cpm.

To date, the authors are unaware of any published studies that compared these materials when used in environments that are inconsistent with the manufacturers' instructions. Also, other than MTA, the authors are unaware of any literature reporting tests of these materials using an apical barrier procedure or model.

The purpose of this study was to use an experimental apexification model, an Oasis flower arrangement material (Smithers-Oasis, Kent, OH), and phosphate-buffered saline (7, 11) to determine the set or hardness of three materials in varied conditions. This would be measured using Vickers microhardness testing, with white MTA (wMTA) and ESRRM exposed to both a moist and dry environment, and BD to a dry environment.

Materials and Methods

Materials in the 3 groups were mixed according to manufacturer's instructions. A 3:1 powder to sterile water ratio of MTA was spatulated to proper consistency. ESRRM was extruded from a premixed syringe. BD was mixed and dispensed from a predosed capsule using an Optimix triturator (Kerr Corporation, Orange, CA) at 4000 cpm for 15 seconds. Once mixed, a 4-mm increment of material was inserted into simulated canal spaces, which consisted of polyethylene tubes (Hudson Extrusions

Inc, Hudson, OH) with dimensions of 10 mm in length and a 2-mm internal diameter. MTA was inserted incrementally using an amalgam carrier and then vertically compacted with a #80 endodontic plugger (B&L Biotech, Bala Cynwyd, PA). The material was then ultrasonically condensed using a ProUltra SINE #4 (Dentsply Tulsa Dental) tip applied to a #80 endodontic plugger, which was in contact with MTA. The activation unit was a P5 Booster Suprasson (Satelec, Merignac, France). ESRRM was directly injected to the proper length into the polyethylene tubes and then vertically compacted with a #80 endodontic plugger. After activation, an amalgam carrier was used to place the BD incrementally into the polyethylene tubes followed by vertical compaction with a #80 endodontic plugger.

A cotton pellet saturated with sterile water was placed directly on the materials to create a "wet" condition. A "dry" condition was created by placing nothing on the material followed by covering the previously placed materials with gutta-percha. This was accomplished by direct injection of thermoplastic gutta-percha using a Calamus unit (Dentsply Tulsa Dental). All tubes in the wet groups were coronally sealed above the cotton pellet with Fuji 2LC (GC America, Alsip, IL). This material was chosen to simulate the multiple-visit clinical technique of using a temporary restorative material as the coronal seal, whereas tubes in the dry groups were sealed above the gutta-percha with Tytin amalgam (Kerr Corporation) as would occur with a single-visit procedure.

To mimic periapical tissue conditions, all samples were then inserted into a porous Oasis material, which was previously saturated with phosphate-buffered saline. A graphical depiction of the experimental setup is shown in Figure 1.

The Oasis, with samples in place, was kept at 37°C and 100% humidity for 10 days. The polyethylene molds were then removed from the Oasis and sectioned at the junction of the test material and either gutta-percha or cotton pellet using the Accutom-5 (Struers, Cleveland, OH) cutting machine with a 4-inch diameter, 0.014-inch thick diamond blade. Samples were then polished sequentially with 600- and 2000-grit silicon carbide papers followed by 5 μm alumina paste.

Each sample was subjected to Vickers microhardness testing using the Micromet 5104 (Buehler, Lake Bluff, IL) at 2 locations (ie, 2 and 4 mm from the proposed apex). The test was performed with a square-based diamond pyramid indenter with a face angle of 136° at a load of 100–1000 g. The hardness value (HV) was calculated using

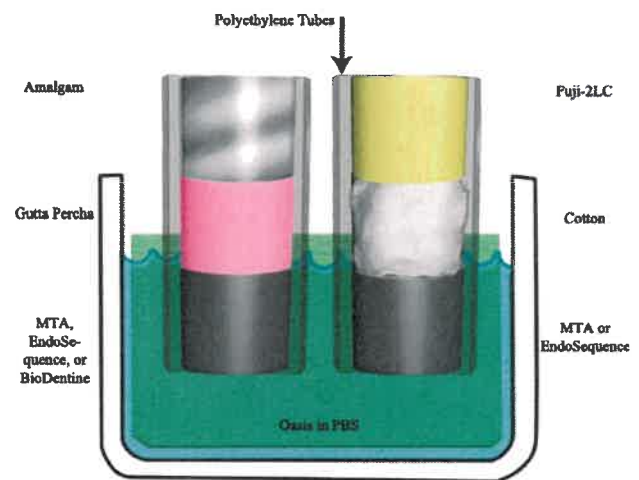


Figure 1. A depiction of the experimental apparatus. The design consisted of polyethylene tubes inserted into a PBS-soaked Oasis. Tubes representing a moist condition contained wMTA or ESRRM covered with a wet cotton pellet and a temporary filling material. Tubes representing a dry condition contained wMTA, ESRRM, or BD covered with gutta-percha and an amalgam filling material.

TABLE 1. Groups, Materials, Conditions, and Results from Vickers Hardness Testing (*) at 2 Different Levels

Group	N	Material	Condition	HV (N/mm ²) 4 mm*	HV (N/mm ²) 2 mm*
1	15	wMTA	Wet	103.30 ± 29.74	93.83 ± 40.42
2	15	wMTA	Dry	99.82 ± 17.20	87.36 ± 18.80
3	15	ESRRM	Wet	48.48 ± 51.64	44.38 ± 25.31
4	15	ESRRM	Dry	58.92 ± 33.54	31.73 ± 9.69
5	15	BD	Dry	50.61 ± 12.91	47.59 ± 11.55

BD, Biodentine; ESRRM, EndoSequence Root Repair Material; wMTA, white mineral trioxide aggregate.
Data were shown as average values and standard deviations.

the following formula: $HV = 0.1891 (F/d^2)$ in N/mm². The mean HV values for each of the 5 experimental groups are recorded in Table 1.

Statistical Analysis

The effects of different materials and conditions were analyzed using multiple analysis of variance (ANOVA) models. This helped to identify significant predictors. Finally, a Tukey post hoc test was used to compare the hardness between materials.

Results

The initial 3-way ANOVA model showed that the interaction between material and condition was not significant ($P > .59$). Deleting the interaction effect, a second 3-way ANOVA model found that “condition” was not a significant effect ($P > .40$). A final 2-way ANOVA model showed a significant effect for “material” ($P < .001$). Lastly, a Tukey post hoc test determined the presence of differences in hardness between the 3 materials. The results indicated no significant difference ($P > .75$) in hardness between BD and ESRRM but that MTA hardness was significantly higher than both BD and ESRRM ($P < .0001$).

Discussion

This study used phosphate-buffered saline-soaked Oasis material to simulate the periapical tissue environment. In this manner, an evaluation could be accomplished as to whether MTA and ESRRM could absorb adequate moisture from the surrounding “tissue” environment, or whether additional moisture would be necessary, such as that provided by placement of a moist cotton pellet, to allow proper setting (23, 30). If indeed, additional moisture were not required to achieve an adequate degree of hardness, this could support the clinical practice of performing such a procedure in a single visit. An additional goal was to determine if BD would achieve an adequate hardness in this dry environment.

Phosphate-buffered saline was used as part of the simulated periapical environment because the bioactivity of MTA has been attributed to its ability to produce surface apatite crystals when in contact with phosphate solutions (11). A Vickers hardness test was selected to assess the adequacy of the set of materials in 2 varied conditions. Prior publications remarked on its usefulness as an indicator of the setting process (31) and its capability to provide information on the effect of setting conditions and the strength of the materials tested (32). An example of the indentation of a sample surface, from which the measurements were taken for the hardness equation, is depicted in Figure 2.

It has been recommended that a moist cotton pellet be placed adjacent to the MTA before obturation of an immature tooth when the material is used as part of an apexification or apical barrier procedure (23). Our selection of experimental groups attempted to determine whether an alternative to this procedure could be clinically acceptable.

Similarly, with ESRRM, the manufacturer’s instructions suggest the placement of a wet cotton pellet to allow the proper moisture absorption, thus completing the setting reaction. As with MTA, validation of an alternate clinical approach could be useful to practitioners.

Manufacturer instructions for BD recommend that an immediate restoration be placed after insertion of the material. Our findings were contrary to those of some previous studies that stated that a moist cotton pellet is required after MTA placement (23, 33). Based on our data, either a moist or dry environment could allow hardening of materials; thus, both methods could be acceptable for treatment when the creation of an artificial barrier in an immature root is the expected outcome.

Our findings suggest that both MTA and ESRRM can absorb adequate moisture from a periapical environment to complete the setting reaction. In addition, BD appeared to also achieve hardening, which is apparently unaffected by the moisture in the simulated periapical environment. Within the limitations of this study, it would appear that single-visit apexification procedures are feasible. Nevertheless, because of the *ex vivo* nature of this experiment, the authors suggest that clinicians use caution when extrapolating the results.

The finding that wMTA showed significantly higher microhardness than the 2 other materials leads to potential implications unanswered in this study. Biologic effects could be altered based on the ability of a material to achieve an optimum set, such as the ability of cells to populate

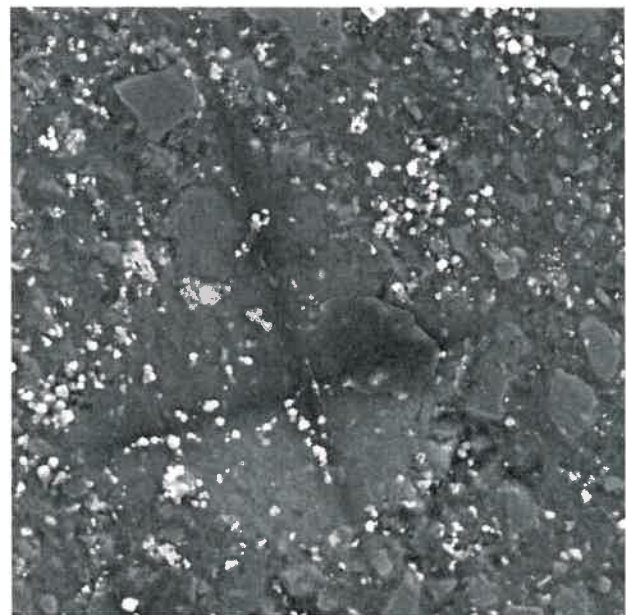


Figure 2. The surface of the representative sample with indentation from which measurements were taken to determine the Vickers hardness number.

or adhere to the surface of the material, or the ability of a material to create an impermeable seal with the surrounding interface. Such histologic or clinical questions would require additional investigation.

It was decided to test the material at the 4-mm level because that is a location where clinicians would be able to use an endodontic explorer to judge whether a material has achieved an adequate set. The hardness measurement at 2 mm from the proposed apex was made to further confirm that the center core of the material absorbed the necessary moisture from the environment to achieve a reasonable level of internal hardness. The data showed that the Vickers hardness number was less for all materials in the segment created at 2 mm from the proposed apex. It is believed that this occurred after initial surface solidification, which would subsequently allow internal aspects of the material to be exposed to less moisture. This would result in this internal aspect of each sample showing a lower HV. Based on observations of the materials after sectioning, all the samples appeared to be clinically “set.” In addition, it was noted that after the coronal cut of the ESRRM in the “dry” group, the gutta-percha seemed to have invaded the first millimeter of the test material. Because this is an injectable material, it necessarily has a higher flow rate and lower viscosity than MTA and BD. The effect of this on clinical outcomes will need to be investigated further.

Conclusions

Neither wet nor dry environments affected the hardness of ProRoot wMTA or ESRRM. ProRoot wMTA showed greater hardness in either environment than the other 2 materials studied. BD setting appeared to be unaffected by the artificial periapical condition. Either a moist or dry environment could allow hardening of materials; thus, both methods could be acceptable for treatment.

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

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