# Evaluation of Ultrasonically Placed MTA and Fracture Resistance with Intracanal Composite Resin in a Model of Apexification

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**The purpose of this study was to evaluate whether intracoronal delivery of an apical barrier of mineral trioxide aggregate (MTA) placed ultrasonically, non-ultrasonically, or ultrasonically with the addition of an intracanal composite resin provided a better seal against bacterial leakage. A second purpose was to determine whether intracanal composite resin or gutta-percha and sealer placed against an apical barrier of MTA provided greater resistance to root fracture. In a standardized in vitro open apex model, MTA was placed as an apical barrier at a thickness of 4 mm, with and without ultrasonic vibration. The barriers were challenged with bacteria exposure within a leakage model, and fracture resistance was assessed with increasing forces applied via an Instron machine. After 45 days, the addition of ultrasonics significantly improved the MTA seal, compared with the non-ultrasonics treatment (Kruskal Wallis nonparametric ANOVA with Dunn multiple comparison test p < 0.05). Bacterial leakage occurred in 6 (33%) of 18 in the non-ultrasonic MTA group, 2 (11%) of 18 in the ultrasonic MTA group, and 1 (6%) of 18 in the ultrasonic MTA-composite group. There were no significant differences at 90 days. A 4-mm thickness of MTA followed with an intracanal composite resin demonstrated a significantly greater resistance to root fracture than MTA followed with gutta-percha and sealer (one-way ANOVA with Newman-Keuls multiple comparison test, p < 0.01). The MTA–gutta-percha group was not significantly different than the MTA unrestored positive control.**

Trauma or deep caries in the immature tooth can result in pulpal necrosis with incomplete root formation, a clinical situation representing a significant endodontic and restorative challenge. The divergent open apex limits effective debridement and control of obturation materials (1). Apexification procedures using calcium hydroxide have been historically used to induce apical closure (2). However, a recent study by Andreasen et al. (3) showed that immature roots, which had calcium hydroxide placed within the root canals of immature teeth over 1 yr, had a 50% reduction in strength versus the controls. Thus, an alternative treatment to long-term apexification with calcium hydroxide may offer a better long-term prognosis.

Several studies investigated treating the divergent open apex using mineral trioxide aggregate (MTA) as an apical barrier (4–7). Shabahang et al. (4) demonstrated that the MTA apical barrier induced apical hard tissue formation. In addition, two clinical apexification cases were published demonstrating radiographic success at 6 months and 2 yr (5). Witherspoon and Ham (6) described the use of ultrasonics to aid the placement of MTA, with radiographic success seen at 6 months post-treatment. However 91% of the MTA apical barriers in an in vitro model of apexification had bacterial leakage by day 10 (7). The MTA was delivered without ultrasonics, and the authors concluded that the intracanal delivery technique led to the leakage and not the MTA.

In addition to the difficulties in treating the underdeveloped open apex, the immature root is prone to fracture, especially in the cervical area, due to its thin radicular dentin walls (8). Several studies have suggested using internal bonding procedures to strengthen the weakened immature root (9–13). Pene et al. (12) demonstrated a significant increase in fracture resistance with intracanal composite resin in the immature root. Similarly, Goldberg et al. (13) significantly reinforced the immature root with a resin glass ionomer. However, no study has evaluated whether the combination of ultrasonic delivery of intracoronal MTA with intracanal composite has any effect on root resistance to fracture.

The combination of an apical barrier of MTA and subsequent internal bonding with composite resin may decrease treatment time, increase fracture resistance, and hopefully improve the long-term prognosis of the pulpless immature tooth. The goal of our investigation was to test the hypothesis that an alternative technique may improve the seal of MTA by using ultrasonics to aid in the flow, settling, and compaction of MTA in the divergent open apex. To test this treatment option, we used a reproducible, standardized, in vitro model developed by Hachmeister et al. (7) to simulate the structurally weakened root and divergent apex of the

immature tooth. Therefore, the purpose of this study was to evaluate whether an apical barrier of MTA placed ultrasonically, non-ultrasonically, or ultrasonically with the addition of an intracanal composite resin provided a better seal against bacterial leakage. A second purpose was to determine whether intracanal composite resin or gutta-percha and sealer placed against an apical barrier of MTA had a higher resistance to fracture.

## MATERIALS AND METHODS

Sixty-eight extracted human single-rooted mandibular premolars and 36 intact maxillary central incisors were used for this study. The teeth were radiographed and examined under magnification for fractures and stored in 10% formalin. A coronal access was made with a #331-L bur (SSWHITE, Lakewood, NJ) and an Endo-Z Bur (Dentsply Maillefer, Tulsa, OK) in a high-speed handpiece to allow straight-line access. Pulp tissue, if any, was removed with barbed broaches. Two millimeters of the apical tip of each root was resected with a #57 bur (SSWHITE, Lakewood, NJ) to remove any apical deltas and standardize the canal exit to the center of the tooth. Working length was determined visually with stainless steel hand files (Dentsply Maillefer, Tulsa, OK) through the canal until the tip was flush with the apical surface. The canals were instrumented with a #2 Peeso reamer (Dentsply Maillefer) for the premolars and a #3 Peeso reamer (Dentsply Maillefer) for the incisors to the working length. A divergent open apex was prepared to a size of 1.24 mm at the foramen by retrograde apical preparation using a #8 (0.60) Profile Series 29 .04 taper (Dentsply Tulsa Dental, Tulsa, OK) inserted to the length of the cutting blade (D = 16 mm). The teeth were kept in moist gauze during preparation, and 5.25% NaOCl was used for irrigation. Calcium hydroxide (UltraCal, Ultradent, South Jordan, UT) was delivered into all the canals with an injectable syringe tip (Ultradent). The teeth were stored at 37°C and 100% humidity for 7 days. The calcium hydroxide was removed with stainless steel files (Dentsply Maillefer) and 5.25% NaOCl irrigation. The prepared teeth were radiographed and stored in saline.

### Leakage Study

Fifty-four premolars were randomly divided into three experimental groups. In groups 1 (n = 18) and 3 (n = 18), a 4-mm apical barrier of MTA was placed into the canals using ultrasonics. In group 2 (n = 18), a 4-mm apical barrier of MTA was placed into the canals without ultrasonics.

Teeth were inserted into wet, flower-arrangement foam (Aquafoam; Syndicate Sales Inc, Kokomo, IN) to the cementoenamel junction (CEJ). The insertion angle included a 20-degree facial tilt directly perpendicular to the bench-top to simulate tooth position when working clinically. A #7 hand condenser was placed into the canal to length, in order to make sure no foam material was wedged into the canal space. The MTA was mixed according to manufacturer's directions (ProRoot; Dentsply Tulsa Dental). A messing gun (EndoGun; Medidenta, Woodside, NY) provided with the ProRoot kit was used to place the material as close to the apex as possible. In groups 1 and 3, a Mini-Endo ultrasonic unit (SybronEndo, Orange, CA) with a ball-like tip (File Adapter; Spartan) at 50% power was used to apply ultrasonic vibration to a #7 condenser to flow, settle, and compact the MTA apically (Fig. 1a). In group 2, only hand condensation with a #7 condenser was used. Radiographs were taken to ensure proper placement and increment thickness (4.0 ± 0.5 mm; Fig. 1b). Any residual MTA left on the canal walls coronal to the 4-mm barrier was removed with a #50 stainless hand file (Dentsply Maillefer) and paper points. Moistened paper points were placed in the canals to aid in the setting of the MTA. The access openings were covered with moist cotton pellets and wrapped in moist gauze. All specimens were stored at 37° and 100% humidity for 7 days. After that time, the material was tested with paper point pressure to assure an adequate set. In group 3, the canal was etched with 38% phosphoric acid (Etch-Rite; Pulpdent Corp., Watertown, MA), rinsed, dried, and lightly coated with a self-cured dentin bonding agent (All-Bond 2; Bisco, Schaumburg, IL) using a microbrush (Bisco). The canal was then backfilled with a flowable self-cured composite, (BISFIL 2B; Bisco), delivered with a C/R EZ Centrix syringe (Centrix, Shelton, CT) through a needle tube (Centrix) from the MTA barrier to within 2 mm of the cavosurface margin.

Fourteen teeth served as the controls. For the positive control group, six were obturated with a single cone of gutta-percha without sealer. For the negative control group, eight had the entire root sealed with sticky wax (Kerr, Romulus, MI). To prevent bacterial leakage through the root surfaces, the roots of all teeth were coated (except over the apex) with two coats of nail varnish. The apices of the negative control teeth were also sealed with nail varnish and wax.

The upper portion of the leakage apparatus was assembled by cutting 2 cm off the end of a 1.5-ml polypropylene microcentrifuge tube (Intermountain Scientific Corporation, Kaysville, UT). The tooth was inserted into the tube with the root protruding. The tube end was heat softened to closely adapt it to each root. The microcentrifuge tube/root interface and the remaining root, except for the apical 1 mm, were then sealed with sticky wax. An 11-mm circular opening was cut in the plastic lid of a scintillation vial. The tooth/tube assembly was inserted into the lid hole and sealed with sticky wax, and all groups were sterilized overnight twice with ethylene dioxide. All specimens were then screwed into sterile 20-ml scintillation vials (Kimble Glass Inc, Vineland, NJ) containing enough sterile brain heart infusion broth (BHI) (Difco/Benton Dickinson, Sparks, MD) to cover the tooth apex to a depth of 1 mm.

Using a micropipette, the canals of all teeth were filled with 15 $\mu$l of sterile BHI and then incubated at 37°C for 72 hr to check the sterility of the model. After that time, 15 $\mu$l of bacterial suspension was placed into the canals of all the experimental and eight of the control teeth. The canals of two teeth from each of the control groups were filled with 15 $\mu$l of sterile BHI to test for continued sterility of the model throughout the experiment. Fresh bacterial suspension was added to the canals every 3 to 5 days. Turbidity of the BHI broth in the scintillation vial indicated leakage through the apical barrier. Cultures were taken from the turbid broth and streaked on BHI agar plates and incubated at 37°C to examine colony morphology. A 1-ml sample was also frozen at −80°C in glycerol for later evaluation by polymerase chain reaction (PCR). To determine which bacteria were in the leakage samples, the frozen bacteria were thawed, DNA was prepared, and a portion of the 16S rRNA genes present was amplified by PCR with universal primers (14). The amplification products were then cut with a set of restriction endonucleases, and the resulting pattern was examined on agarose gels to determine which bacteria had been present in the original frozen samples. They were also inspected microscopically (Nikon 40×) for cell morphology.
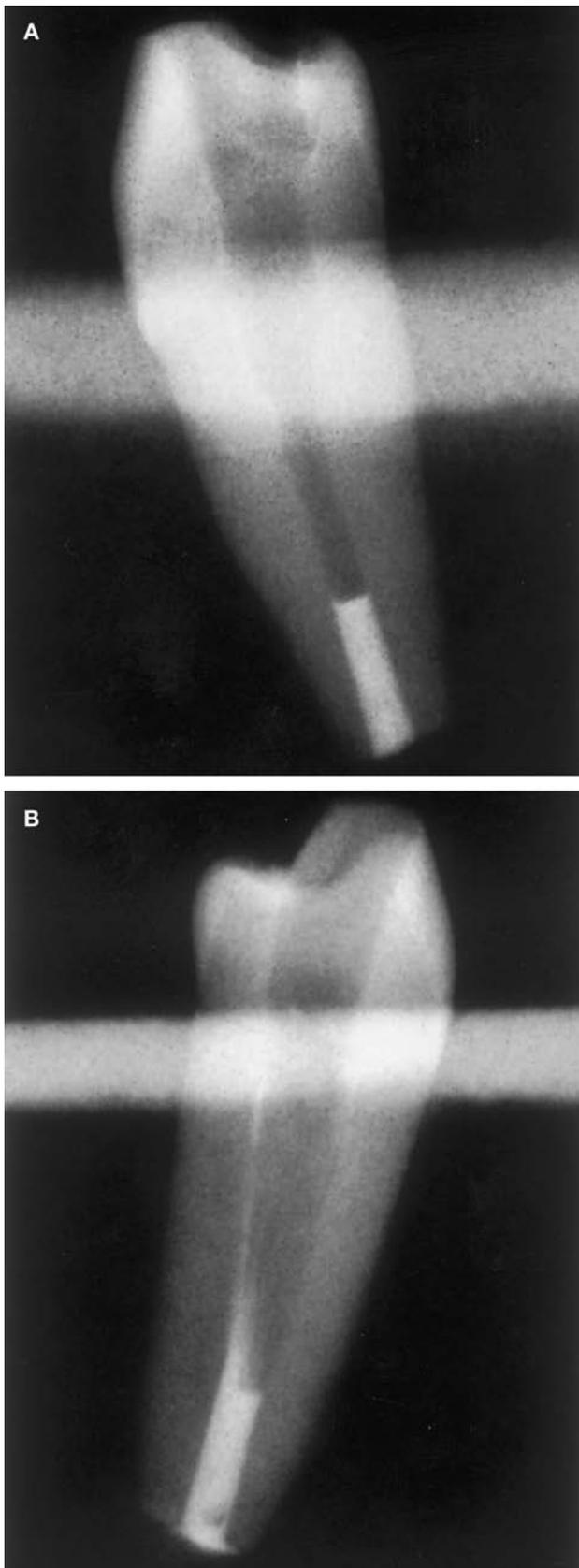
FIG 1. Mineral trioxide aggregate (MTA) apical barriers in open apex model. (a) Ultrasonics aids in the flow and placement of the MTA apically. (b) Without ultrasonics, slumping of the MTA against the canal walls along with voids were frequently seen.

*Enterobacter aerogenes* (ATCC 13048), *Enterococcus faecalis* (ATCC 19433) and *Staphylococcus epidermidis* (ATCC 49741) were used in the leakage study in order to be consistent with previous studies (7). The bacteria were grown overnight in BHI broth. The optical densities of the bacterial suspensions were determined using a spectrophotometer, and the individual bacteria suspensions were diluted with sterile BHI broth to yield concentrations of approximately $2.0 \times 10^6$ bacteria per ml. The three species were mixed in equal numbers and frozen at $-80°C$ with an equal volume of 100% glycerol to provide the bacterial suspensions for use in the experiments. A sample of the frozen cell suspensions was thawed for use in the leakage study and was plated occasionally to test cell viability. There was no loss in viability of the frozen samples over the course of the experiment.

The experimental data were statistically analyzed using Kruskal-Wallis non-parametric ANOVA and Dunn multiple comparison test.

## Fracture Resistance Study

Thirty-six prepared maxillary incisors had the cervical buccal-lingual dimension just below their CEJ measured with a Boley gauge. The teeth were then divided into three experimental groups using a randomized-stratified design. All teeth received a 4-mm apical barrier of ultrasonically placed MTA (as described earlier). A surveyor pin was placed in each canal to the depth of the MTA and secured with a small bead of sticky wax (Kerr). The suspended tooth was then inserted into a polyvinyl ring and mounted in orthodontic acrylic (GAC Dentsply, Tulsa, OK) from the apex to the facial CEJ. The surveyor pin and the long axis of the canal were perpendicular to the top of the acrylic block in the surveyor.

Group 1 (n = 12) received no further treatment and served as the unrestored positive controls. In group 2 (n = 12), each canal was etched with 38% phosphoric acid (Etch-Rite; Pulpdent Corp.), rinsed, dried, and lightly coated with a self-cured dentin bonding agent (All-Bond 2; Bisco) using a microbrush (Bisco). The canal was then backfilled with a flowable self-cured composite (BisFill 2B; Bisco), delivered with a C/R EZ Centrix syringe (Centrix) through a needle tube (Centrix) from the MTA barrier to within 2 mm of the cavosurface margin. Per the manufacturer's recommendation, the remaining 2 mm was filled with a more durable self-cured composite (BisFill II; Bisco) to the oral environment and smoothed with a plastic instrument.

In group 3 (n = 12), the canals were lightly coated with AH Plus sealer (Dentsply Tulsa) with a paper point, and then they received a gutta-percha backfill to the canal orifice using an Obtura II (Spartan, Fenton, MO). The canals were etched with 38% phosphoric acid (Etch-Rite; Pulpdent Corp.), rinsed, dried, lightly coated with a self-cured dentin bonding agent (All-Bond 2; Bisco), and then filled with BisFill II composite (Bisco) from the canal orifice to the cavosurface margin.

Each specimen was marked 3 mm above the lingual CEJ, wrapped in moist gauze, and stored in 100% humidity at 37°C for 24 hr to allow full set of the resin and sealer. An aluminum jig was fabricated to fit each tooth/acrylic block individually. An Instron Universal Testing Machine was used to apply a specified load to each specimen at a crosshead speed of 5.0 mm/min. Each specimen block was fixed in the aluminum jig ensuring a loading angle of 130 degrees to the long axis of the tooth (10). The load was delivered in a lingual-labial direction at a point 3 mm above the CEJ. The force was applied and measured continuously by the

TABLE 1. Bacterial leakage over 90 days

| Day | Group 1: 4 mm MTA US (n = 18) | Group 2: 4 mm MTA No US (n = 18) | Group 3: 4 mm MTA US + Cmpst (n = 18) |
|---|---|---|---|
| 1 | 1 | 3 | 1 |
| 2–10 | 0 | 1 | 0 |
| 11–20 | 0 | 0 | 0 |
| 21–30 | 0 | 1 | 0 |
| 31–40 | 0 | 1 | 0 |
| 41–50 | 1 | 0 | 1 |
| 51–60 | 0 | 0 | 0 |
| 61–70 | 2 | 0 | 0 |
| 71–80 | 0 | 0 | 0 |
| 81–90 | 0 | 0 | 0 |
| Total | 4 | 6 | 2 |

US, ultrasonics; MTA, mineral trioxide aggregate; Cmpst, composite.

Instron machine until catastrophic failure, which was complete fracture of the cervical portion of the root. Fracture loads applied were recorded and statistically analyzed using a one-way analysis of variance (ANOVA), and Newman-Keuls multiple comparison test.

## RESULTS

### Leakage Study

The bacteria placed in the root canals of the positive control group, canals filled with gutta-percha, leaked within 24 h, as shown by turbidity in the BHI broth. Culture and plating of the turbid broth identified growth of cocci morphology similar to the three species of bacteria placed into the sterile canals. No specimens in the negative control group, in which the entire root was sealed with sticky wax, showed turbidity by experiment's end at 90 days. The broth did not become turbid in those samples in which sterile saline was placed into the canals instead of bacteria.

After 45 days, bacterial leakage occurred in 33% of the non-ultrasonic MTA group, 11% of the ultrasonic MTA group, and 6% of the ultrasonic MTA-composite group (Table 1). The addition of ultrasonics provided a significantly better (p < 0.05) MTA seal than without ultrasonics when compared with the positive control (Fig. 2).

After 90 days, only the ultrasonic MTA-composite group provided a significantly better (p < 0.05) MTA seal than without ultrasonics when compared with the positive control (Fig. 3). No significant differences were found among the three groups. Of the 12 turbid samples from the three experimental groups, PCR analysis identified a mixture of *E. faecalis* and *S. epidermidis* in one sample from group 3 and only *E. faecalis* in the remaining 11 samples.

### Fracture Resistance Study

One specimen from each group fractured during the preparation phase and was not used. Of the 33 specimens tested, all teeth showed either horizontal or oblique fractures that went through the cervical area of the root (Fig. 4). In groups 2 and 3, the fracture extended through the gutta-percha or composite materials. The mean peak load was 1494.3 N to fracture the MTA unrestored
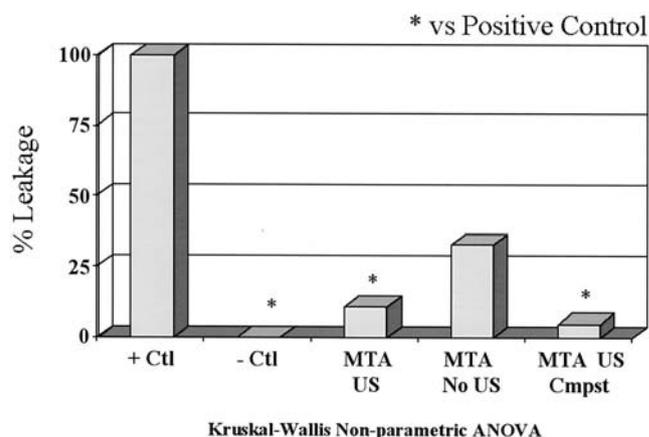


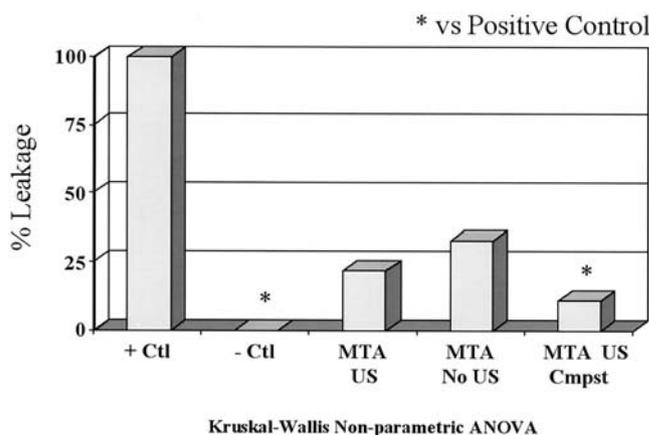FIG 2. Percent leakage at 45 days. *Statistically significant data. US, ultrasonics; Comp, composite.



FIG 3. Percent leakage at 90 days. *Statistically significant data.

positive controls, 2052.4 N to fracture the MTA-composite group, and 1626.7 N to fracture the MTA–gutta-percha group (Fig. 5).

The fracture resistance of the MTA-composite group was significantly stronger than the MTA unrestored positive control (p < 0.001) and the MTA–gutta-percha group (p < 0.01). The MTA–gutta-percha group was not significantly different than the MTA unrestored positive control.

## DISCUSSION

In the present study, we investigated the ability of ultrasonics to improve the placement and seal of MTA as an apical barrier in the pulpless immature root. The inherent irregularities and divergent nature of some open apices may predispose the material to marginal gaps at the dentin interface. The ultrasonic vibration applied to an endodontic condenser was aimed at improving the flow, settling, and compaction of MTA apically. Under our experimental conditions, 33% of the MTA barriers without ultrasonics demonstrated bacterial leakage by day 32. In contrast, only 11% of the MTA/Ultrasonics group leaked by day 32, and only 22% of this group leaked by day 64. Although these differences were not statistically significant, ultrasonics may still be a useful adjunct. During placement of the MTA, the ultrasonic energy seemed to flow the MTA apically more efficiently than with only hand
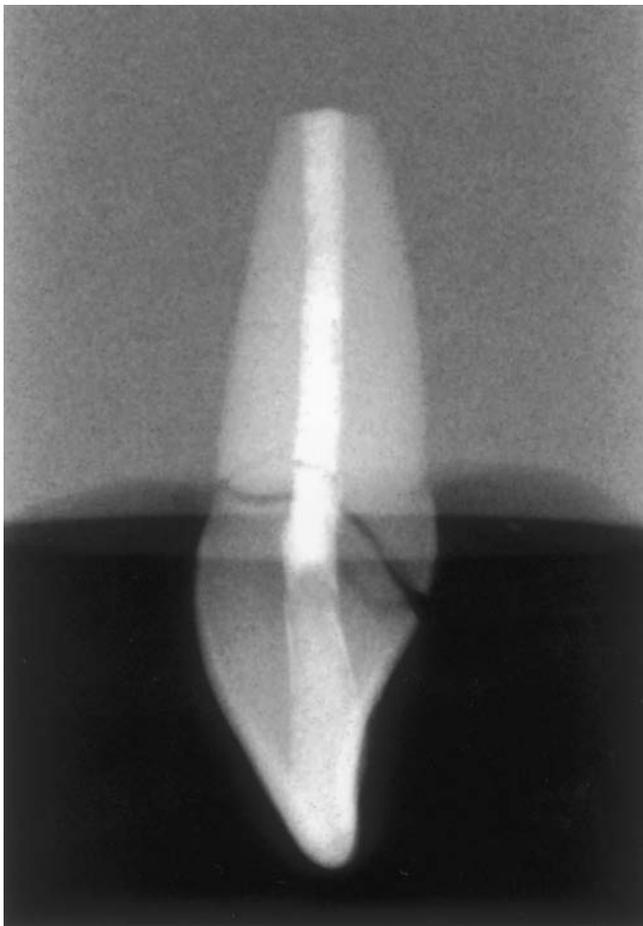
FIG 4. Radiograph of typical tooth fracture seen in the resistance study. All teeth showed either horizontal or oblique fractures that went through the cervical area of the root.
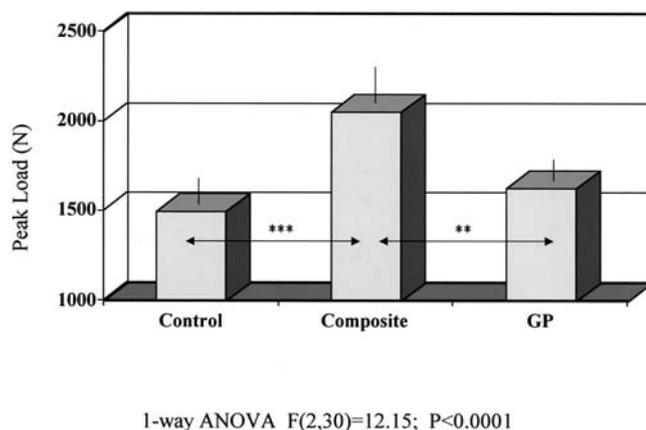


1-way ANOVA F(2,30)=12.15; P<0.0001

FIG 5. The mean N-force required for cervical fracture. ***p < 0.001, **p < 0.01 indicates statistically significant data.

condensation. Also, the ultrasonically condensed MTA appeared denser radiographically with fewer voids (Fig. 1).

Interestingly, only one of the bacterial species, *E. faecalis*, was found in all the experimental samples that leaked. *S. epidermidis* was found in only one sample that leaked, and *E. aerogenes* was found in none. This was in direct contrast to the results of Hachmeister et al. (7), who used the same three bacteria but only

found *E. aerogenes* in their turbid samples. This difference could be explained by the identification method used. Hachmeister et al. (7) used colony morphology to determine which bacteria were present in the turbid samples. However, we could not readily differentiate between the three bacteria used in this experiment by colony morphology alone. Therefore, we used PCR analysis, which clearly identified only *E. faecalis* in all the experimental specimens that had leaked. The *E. faecalis* is a facultative Gram-positive coccus often present in persistent endodontic infections (15). Thus, good chemomechanical preparation of the canal system, along with intracanal medicaments such as calcium hydroxide, are important modalities to combat this resistant organism and increase the chance of success (15).

Prior to placement of MTA for apexification, the manufacturer recommends that the canal be medicated with calcium hydroxide for 1 week, with subsequent removal using sodium hypochlorite and instruments as needed. This may enhance the difficult task of debriding the canal system with an open apex. Porkaew et al. (16) investigated the effects of calcium hydroxide remnants along the canal walls on the sealing ability of gutta-percha with sealer and found a significant decrease in dye leakage in canals medicated with calcium hydroxide. Further, Hachmeister et al. (7) demonstrated that canals medicated with calcium hydroxide for 1 week had no significant effect on MTA leakage times or displacement resistance. We therefore chose to medicate all the prepared canals with calcium hydroxide for 1 week to optimize canal cleanliness and simulate clinical conditions.

The MTA apexification technique can potentially eliminate the lengthy conventional apexification procedure and allow internal bonding to be performed much earlier in the treatment process. The manufacturer of MTA recommends that a 3-5–mm thickness of MTA be placed at the apex for the apexification procedure. Hachmeister et al. (7) found that MTA placed as an apical barrier at a 4-mm thickness significantly resisted displacement forces when compared with only a 1-mm barrier. Therefore, a 4-mm thickness of MTA was chosen for these studies. Our results showed that a 4-mm thickness of ultrasonically placed MTA, followed with an intracanal bonded composite, provided an adequate apical seal, with 89% of the barriers resistant to bacterial leakage over a 90-day observation period. In addition, 4 mm of MTA allowed an adequate thickness of composite to be bonded against, to provide a significant resistance to fracture.

In several in vitro studies, the sealing ability of MTA placed as a root-end filling has been shown to resist bacterial leakage (17), electrical and dye leakage (18), and even endotoxin (19). However, the ability of MTA to seal in an in vitro open apex model has been questioned. Hachmeister et al. (7) showed 92% of the 4-mm MTA apical barriers had bacterial leakage by day 10. Our results showed that 33% of the 4-mm MTA apical barriers had bacterial leakage by day, 90 regardless of ultrasonics. It appears that MTA placed in the pulpless immature root is very technique-sensitive and requires careful delivery to maximize its ability to seal. Although ultrasonics did not significantly improve the prevention of bacterial leakage, it did show some improvement; it was definitely helpful in placing the MTA apically.

The fracture resistance model provided a reproducible system, as all fractures consistently occurred in the cervical third in all specimens (Fig. 4). Cobankara et al. (20) found that canals obturated with gutta-percha and resin-based sealers were significantly more resistant to root fracture than roots in which canals were instrumented but not obturated. Our results showed no significant difference in fracture resistance between roots backfilled with

gutta-percha and AH plus sealer and roots left unrestored. Further, our results demonstrated that a flowable composite resin-bonded intracanal against an MTA apical barrier significantly increased the fracture resistance. A Centrix syringe (Centrix) with a needle tube dispenser allowed consistent intracanal delivery of a flowable dual cure composite (BisFil 2B; Bisco).

In conclusion, treating the immature apex with an MTA apexification procedure followed with an internal bonded composite appears to offer a favorable prognosis. After 90 days, the ultrasonically placed MTA followed with composite provided a significantly better MTA seal than without ultrasonics compared with the positive control. In addition, the ability of a flowable composite resin to be bonded intracanal against a 4-mm apical barrier of MTA significantly increased the fracture resistance, compared with an MTA barrier followed with gutta-percha and sealer.

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