Capping a Pulpotomy with Calcium Aluminosilicate Cement: Comparison to Mineral Trioxide Aggregates

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Abstract
Introduction: Calcium aluminosilicate cements have shown little affinity for bacterial growth, low toxicity, and immunogenicity when used as a restoration material, but calcium aluminosilicate cements have not been tested in vivo in pulpotomy procedures. Methods: To address this question, a calcium aluminosilicate cement (Quick-Set) was tested along with 2 mineral trioxide aggregates, ProRoot MTA and MTA Plus. These cements were used as a capping agent after pulpotomy. Control rats had no pulpotomy, or the pulpotomy was not capped. Proinflammatory cytokines interleukin (IL)-1β and IL-1α were measured, and histology was performed at 30 and 60 days after capping. The nociceptive response was determined by measuring the lengthening of the rat’s meal duration. Results: and Conclusions: IL-1β and IL-1α concentrations were reduced in the capped teeth, but no differences were observed among the 3 cements. Dentinal bridging could be detected at both 30 and 60 days with each of the 3 cements, and the pulps were still vital 60 days after capping. Meal duration significantly shortened after placement of the 3 different cements, indicating a nociceptive response, but there were no differences among the materials. Calcium aluminosilicate cement had similar properties to mineral trioxide aggregates and is a viable option for pulpotomy procedures. (End 2014;1–6)

Key Words
Calcium aluminosilicate cement, calcium silicate, endodontic, mineral trioxide aggregate, pain, tri-calcium silicate

Calcium aluminosilicate cements show little immunogenicity, toxicity, or affinity for bacterial growth when tested as a restoration material (1–3). Unfortunately, calcium aluminosilicate cements have a higher failure rate than commonly used restoration materials (4–6). Recently, calcium aluminosilicate cements have been tested in vitro and in vivo as an endodontic material (7–9). In these studies calcium aluminosilicate cement showed little immunogenicity or affinity for bacterial growth, but to date, no study has tested calcium aluminosilicate cements as a pulp-capping material in vivo. In addition to studying immunogenicity and bacterial static properties, pain after capping pulpotomies with mineral trioxide aggregates (MTAs) was measured in patients (10). To our knowledge, no study has compared the nociceptive response after capping pulpotomies with different hydraulic cements (a cement that hardens on hydration), and a pain study has not included calcium aluminosilicate cement. To address this knowledge gap, we hypothesized that calcium aluminosilicate cement is a viable pulp-capping material that shows low immunogenicity and little pain and has a high biocompatibility when in contact with tooth pulp.

In this study, a calcium aluminosilicate cement and MTAs were used as a capping material after pulpotomy, and the inflammatory, biocompatibility, and nociceptive responses were measured after placement of these cements. Inflammation was measured by quantifying proinflammatory cytokines interleukin (IL)-1β and IL-1α in the pulp of the treated teeth. Histology was also performed to assess dentinal bridging, the presence of bacteria, and pulp vitality. The nociceptive response was measured by using a behavioral assay, specifically the rat’s meal duration. Previous studies have shown that a lengthening of the rat’s meal duration correlates to orofacial pain in rats (11–16), and that meal duration has been shown to be significantly longer after pulpotomy (12).

Materials and Methods

Animals
All animal experiments were approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee in accordance with the guidelines of the United States Department of Agriculture, National Institutes of Health Office of Laboratory Animal Welfare, and National Research Council’s “Guide for Care and Use of Laboratory Animals.” Male Sprague-Dawley rats (250–300 g) were purchased from Harlan Industries, Houston, TX. On arrival, the animals were housed individually in a temperature-controlled room (23°C) and kept on a 14:10 light/dark cycle with lights on at 6:00 AM. The rats were given chow (Harlan Industries, Indianapolis, IN) and water ad libitum.

Pulpotomy and Cement Placement
After administering ketamine (90 mg/kg) and xylazine (9 mg/kg), an occlusal pulpotomy on the 6 maxillary molars was completed by a board-certified endodontist by using a ½-round carbide bur. The teeth were immediately capped with 1 of 3 cement materials: ProRoot MTA (Dentsply, Tulsa Dental Specialties, York, PA) mixed 3:1 powder:water, Quick-Set (Avalon Biomed Inc, Bradenton, FL, patent pending) (17), or MTA Plus (Avalon Biomed Inc) mixed at 2:5:1 powder:gel by weight with their respective gels. The mixed cements were placed in the cavity created by the iatrogenic pulpotomy. A self-adhering flowable composite resin (VertiseFlow; Kerr Corporation, Orange, CA) was placed immediately over the cements, and the resin was ultraviolet cured (18). The negative control group had no pulpotomy, and an untreated pulpotomy was used as a...
positive control. Previous studies had groups with exposed untreated pulp for 28 days or longer (19, 20). Treatment groups in this study included the control, the exposed pulps, the exposed pulps capped with ProRoot MTA, the exposed pulps capped with MTA Plus, and the exposed pulps capped with Quick-Set. Twelve animals were in each of these treatment groups. Six animals in each treatment group were killed at 30 days, and 6 animals were killed at 60 days after capping. Death was completed by exposure to CO2. After breathing was observed to stop, the animals were decapitated. The maxillae were isolated and fractured along the midline; half of the maxilla was placed in 4% paraformaldehyde and 1 × phosphate-buffered saline for histology, and the other half was placed in liquid nitrogen for storage and later analysis. The maxilla that was either fixed or frozen was randomly chosen for each animal in each treatment group.

Enzyme-linked Immunosorbent Assay

IL-1α was quantified because this cytokine was elevated after pulpotomy, whereas the common proinflammatory cytokine IL-1β was not found to be elevated in some instances (21).

Three to four out of a total of 6 animals from each treatment group were chosen randomly for measurement of the cytokine levels (pg/mL) by enzyme-linked immunosorbent assay (ELISA). To quantitate the cytokines in the pulp tissue, a maxilla from the rat was removed from the liquid nitrogen, and the molars (3 molars per maxilla) were extracted. The teeth were ground and placed in 300 μL T-Per tissue protein extraction reagent containing Halt Protease Inhibitor (Thermo Scientific, Rockford, IL). The lysates were frozen, thawed, vortexed, and centrifuged for 10 minutes at 4°C, and the supernatant was decanted. Total protein in the supernatant was determined in each sample by using a BCA protein assay (Thermo Scientific, Waltham, MA). Quantitation was completed on duplicate 50-μL samples of supernatant by using ELISA (R&D Systems, Minneapolis, MN) following the manufacturer’s directions. Values were given as pg IL-1α or IL-1β per μg total protein.

Histology

Histology was performed by using a randomly chosen maxilla from 3 rats per treatment group. First, the maxilla was immersed in 4% paraformaldehyde and 1 × phosphate-buffered saline continuously for 1 week, and then the samples were demineralized in 0.5 mol/L EDTA until radiographic examination revealed an absence of radiopaque structures. The demineralized samples were dehydrated and embedded in paraaffin blocks. Serial sections, 6 μm thick, were sliced with a Leitz 1512 rotary microtome (Leica, Buffalo Grove, IL) in a buccolingual longitudinal orientation. Every 20th section was collected and stained with hematoxylin-eosin or a Brown and Hopsps stain. Briefly, paraffin was removed with xylene, and the slides were hydrated in a series of ethanol/water treatments. A portion of the slides were immersed in hematoxylin, rinsed, immersed in eosin, rinsed, and dehydrated, and a non-aqueous mounting medium was added. Alternate slides were immersed in crystal violet, rinsed, immersed in Gram iodine, rinsed, and finally immersed in fuchs in then rinsed and mounted. For the Brown and Hopsps stain, gram-positive bacteria were blue, and the gram-negative bacteria were red. Two examiners blinded to tooth position and treatment scored the teeth for immune cell infiltrate, presence of bacteria, or dentinal bridging. Imaging was completed with a Zeiss Axioplan microscope (Thornwood, NY) and an Insight 2 Spot camera (SPOT Imaging Solutions, Sterling Heights, MI). Images were captured and analyzed with Spot Advanced software (SPOT Imaging Solutions).

Bacteria and Pulp Vitality Measurements

Every 20th section was collected for the maxilla of 3 rats in each treatment group, and the sections were stained with either Brown and Hopsps stain or hematoxylin-eosin stain. After the Brown and Hopsps staining, the bacteria on each slide were counted, and all the bacteria for all the sections of a single maxilla were added together. This total bacteria count for maxilla was reported for each of the 3 rats per treatment group. After the hematoxylin-eosin staining, the percentage of pulp vitality remaining after treatment for each rat was calculated by dividing the area of the vital pulp in the maxillary molars by the average area of vital pulp in the 3 control rats, and then the result was multiplied by 100.

Meal Duration

Meal duration was measured before and after pulpotomy by placing rats in individual, sound-attenuated chambers equipped with computer-activated pellet feeders (Med Assoc Inc, East Fairfield, VT). The feeder units dispensed 45 mg rodent Chow pellets (product no. FO 165; Bioserv, Frenchtown, NJ). When a rat removed a pellet from the feeder trough, a photo beam placed at the bottom of the trough was no longer blocked, signaling the computer-controlled system to drop another pellet. The computer recorded the date and time each pellet was dropped, and the computer kept a running tally of the total daily food consumption. A record of the pellets dropped over time established the meal duration. Meal duration was monitored in this study 2 days before and for 9 days after the pulpotomy. Nine days after...
each pulpotomy there was no significant difference compared with the control group. Meal duration was measured in the 12 animals in each treatment group.

**Statistics**

Meal duration and cytokine data were analyzed by two-way analysis of variance, with treatment and time as independent variables and meal duration and cytokine concentration as dependent variables. Bonferroni post hoc tests were completed on the groups that showed significant main effects. Values were the means ± standard error of the mean.

**Results**

Proinflammatory cytokines were quantified from 3 extracted teeth per rat. Thirty days after capping and composite overlay, IL-1β and IL-1α levels were significantly higher in the rats with exposed pulps versus rats that had the pulps capped or the control group (Fig. 1A: $F_{1,28} = 6.7$, $P < .001$, $n = 4$ and Fig. 1B: $F_{1,24} = 10.95$, $P < .01$, $n = 3$). Sixty days after placing the cement, all the groups had significantly higher amounts of proinflammatory cytokines versus the controls (Fig. 1). The control group was not significantly different when comparing the 30-day and 60-day groups for either IL-1β or IL-1α. Bacteria and immune cells that can stimulate and produce these cytokines were present in the exposed pulps (Fig. 1). Few bacteria were observed in the capped teeth (Fig. 2G); however, clusters of immune cells were present in the margins, and this was noted at the interface between the hydraulic cement and the dentin of less than 20% of the teeth (Fig. 2D–F, open arrows).

After 60 days, 1.6 ± 0.26 of the 6 teeth that were capped with the hydraulic cement ProRoot MTA, on average, lost this material (5 animals were analyzed in each treatment group, each with 6 molars for analysis). On average, 2.6 ± 0.71 of the 6 teeth that were capped with MTA Plus lost this material ($n = 5$), and 2.0 ± 0.44 of the 6 teeth capped with Quick-Set lost the material ($n = 5$). Loss of material was likely because of physical forces applied during mastication. Histology of the teeth indicated that pulp cells were viable when in contact with the hydraulic cements at both 30 and 60 days (Fig. 3, arrows; Table 1). The finer particle size of the MTA Plus and Quick-Set groups compared with the ProRoot MTA cement group can be observed (compare regions labeled “m” in Fig. 3). Exposed teeth exhibited large areas of dead pulp at 30 days, and often these teeth exhibited the absence of viable pulp tissue after 60 days (Fig. 3, second row of panels; Table 1).

![Figure 2. Staining and measurement of bacteria in tooth pulp. Brown and Hopps stain of maxillary sagittal sections 30 days after capping are shown in (A–F). (A–C) Sections are from exposed tooth, and (D–F) sections are from Quick-Set capped tooth. Open arrows point to representative immune cells in the population, and arrows point to representative bacteria present in the section. B, bone; D, dentin; m, hydraulic cement material; P, pulp. A defect is shown by dotted line in (A), and material border is shown by dotted line in (D). Scale bar = 400 μm for (A and D), 25 μm for (B, E, and F), and 10 μm for (C). (G) Bacteria levels in pulp for 3 rats in each treatment group at both 30 and 60 days.](image-url)
Dentinal bridging appeared as early as 30 days after placing the hydraulic cements (Fig. 3, open arrows), and this new dentin was present after capping with each of the 3 cements at 30 and 60 days (Fig. 3).

Meal duration (ie, the nociceptive response) was significantly reduced, $F_{4,66} = 10.8, P < .001$ ($n = 12$), after capping with hydraulic cement versus animals with exposed pulp (Fig. 4). Comparison between the 3 hydraulic cements showed no significant difference in meal duration (Fig. 4); however, the nociceptive response significantly increased for 3 days ($P < .05$) in animals that received a cement pulp cap compared with untreated control animals (Fig. 4).

**Discussion**

Despite the differences in composition, the 3 hydraulic cements had equivalent inflammatory (IL-1$\beta$, IL-1$\alpha$), cellular (dentinal bridging and cellular viability), and nociceptive responses (meal duration). This suggests an equivalent efficacy when the cements were used for capping a pulpotomy procedure in the clinic. In a previous study, pain was reported to be similar for MTA and zinc oxide–eugenol cement when placing a retrofill (22), and similarly in our study, we found that different materials do not affect the pain response. Pain has also been measured in humans when placing MTA cement in a pulpotomy procedure, but to date, no study has measured pain associated with the multiple cements used in endodontic procedures (10).

A very relevant danger for dental pulp tissue after direct pulp capping is a bacterial infection, and bacterial infections of vital pulp tissue can cause inflammation and pulp necrosis (23–25). In rats, the oral bacterial flora is more comparable to that in humans than other commonly used research species (26–29). An improper seal resulting in greater microorganism infiltration at the margins of the material and dentin interface would be indicated by the presence of immune cells. Quick-Set is a calcium aluminosilicate cement with a...
lower pH (10.9 ± 0.5) than MTA (11.6 ± 0.5), and it demonstrates
greater infiltration of the dentinal tubules by the cement (17). Tubule
penetration by MTA blocks bacterial access to the nutrients of the
pulp, and this blockage can inhibit bacterial growth, reducing inflam-
mation and possibly infection (24, 25, 30, 31). Thus, we expected
Quick-Set would have improved bacteriostatic properties compared
with MTAs. Thirty days after capping, Quick-Set did not show improved
infection control when compared with the other cements. It is more
difficult to compare the materials (ie, infection/vitality) after 60 days
because on average, 2 of the 6 capped teeth lost the cement, leading
to bacterial access and infection of the pulp. Bacterial infection caused
by a poor cement/dentin interface and loss of cement likely resulted in
the high inflammatory cytokine levels 60 days after capping. Minimizing
this infection would enhance dentinal bridging and improve pulp-
capping success (23). A future experiment would be to place a perma-
nent restorative material such as amalgam after pulp capping. Perma-
nent restorative materials have been shown to improve the seal after
pulp capping and reduce infection and inflammation (32).

When vital pulp tissue is exposed in the rat, an inflammatory reac-
tion results that is very similar to the reaction in humans; however, the
biological response is faster in rats than in humans (33). Determining
the inflammatory and healing properties after pulp capping a mechan-
ical lesion in a rat model would be expected to provide results repres-
entative of human patients. The hydraulic cements all decreased
inflammation 30 days after pulp capping a mechanical lesion. This
decrease in inflammation was associated with a decrease in orofacial
pain, which is consistent with previous studies (11,13–16). Future
studies could determine whether a reduction in postoperative pain,
an indicator of reduced pulpal damage, can be correlated to a
reduction in the 30% failure rate resulting from pulp capping of
mechanical lesions (32). Note that no differences were measured in
the nociceptive response between the 3 cements in this study; therefore,
we could not correlate the quality of pain to differences in healing.

Reparative dentin found in the tested rats after pulp capping was
shown to be identical to the reparative dentin found in human teeth
(34). In this study we observed signs of dentinal regeneration for all
3 materials at 30 and 60 days. These results are consistent with previous
work that showed that reparative hard tissue formation can be detected
in 83% of rat molar teeth 28 days after pulp capping (35). Consistent
with our results, dentinal bridging has been shown to occur with trical-
cium silicates (36), and bioactivity has been shown for calcium alumi-
nates (37) such as the calcium aluminosilicate Quick-Set (18).

Another factor measured in this study was the nociceptive
response. The response was significantly higher in rats that had cement
placed over the exposed pulps compared with the control teeth. One
explanation for the higher nociceptive response would be compression
and pressure on the pulp that resulted when the cement was placed.
Such compression (eg, vasoconstriction) might lead to inflammation,
although studies suggest this pressure should be reduced by fluid leav-
ing the pulp chamber through the vasculature (38, 39).

In conclusion, hydraulic cements improve the inflammatory and
nociceptive responses after pulp capping, and the newly developed cement,
calcium aluminosilicate or Quick-Set, has equivalent properties to older
MTAs that are used for endodontic pulp-capping procedures.

Figure 4. Twenty-four meal duration of rats treated with 3 different hydraulic
cements. Meal duration was measured 2 days before pulpotomy, and these
pretreatment days were numbered -2 and -1. Days after pulpotomy were
numbered 1–9. Figure 1 shows treatment groups. Significant differences of
P < .05 are indicated on the graph with * and by letters.

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High, >95% of pulp vital; low, <10% pulp vital; medium, >10% but <95% pulp vital.

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The authors report the following potential conflict of interest: Dr Primus owns the intellectual property for the dental materials
MTA Plus and Quick-Set materials and has a financial interest in
Avalon Biomed Inc.

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