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Response of Human Dental Pulp Capped with MTA and Calcium Hydroxide Powder

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Clinical Relevance

Calcium hydroxide powder and MTA healed the pulp exposures comparably. However, calcium hydroxide powder showed a tendency towards faster dentin bridge formation.

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SUMMARY

Objectives: To compare the response of human dental pulp capped with a mineral trioxide aggregate (MTA) and $Ca(OH)_2$ powder.

Methods and Material: Pulp exposures were performed on the occlusal floor of 40 permanent premolars. The pulp was then capped with either $Ca(OH)_2$ powder (CH) or MTA and restored with resin composite. After 30 days (groups CH30 and MTA30) and 60 days (groups CH60 and MTA60), the teeth were extracted and processed for HE and categorized in a histological score system.

The data were subjected to Kruskal-Wallis and Conover tests (α=0.05).

Results: In regard to dentin bridge formation, CH30 showed a tendency towards superior performance compared to MTA30 (p>0.05), although the products showed comparable results at day 60. In the item "Inflammation" and "General State of the Pulp" (p>0.05), CH showed a tendency towards presenting a higher inflammatory response. In the item "Other Pulpal Findings," MTA and $Ca(OH)_2$ showed equal and excellent performance after 30 and 60 days (p>0.05).

Conclusion: After 30 days, $Ca(OH)_2$ powder covered with calcium hydroxide cement showed faster hard tissue bridge formation compared to MTA. After 60 days, $Ca(OH)_2$ powder or MTA materials showed a similar and excellent histological response with the formation of a hard tissue bridge in almost all cases with low inflammatory infiltrate.

INTRODUCTION

Pulp capping is a treatment where a protective agent is applied to pulp exposed by traumatic injuries, mechanical factors or dental caries, in order to allow pulp healing and the maintenance of pulp vitality and function.[1] Many studies indicate that calcium hydroxide and calcium hydroxide compounds are the gold standard against which new materials should be tested, mainly in human teeth.[2-4]

Despite the growing success and use of calcium hydroxide in vital pulp therapy, considerable confusion and condemnation of its use still persists.[5] Recent attempts to develop different pulp capping materials[6] have resulted in the development of mineral trioxide aggregate (MTA), first proposed for pulp capping in 1996.[7]

Compared to calcium hydroxide cement, MTA has demonstrated a greater ability to maintain the integrity of pulp tissue. Histological evaluations of exposed pulp tissue from animals capped with MTA have shown the formation of a thicker dentinal bridge, with low inflammatory response, hyperemia and pulpal necrosis compared to calcium hydroxide cement.[7-8] MTA also appears to induce the formation of a dentin bridge at a faster rate than calcium hydroxide.[7,9] However, the mechanism by which MTA acts to induce dentin bridge formation is not fully understood, although it has been hypothesized that it is similar to that of calcium hydroxide.[9]

Only a select number of studies have attempted to compare the response of human teeth capped with MTA and calcium hydroxide,[10-12] most of which have concluded that MTA performed better than $Ca(OH)_2$. A closer analysis of these studies shows that MTA was always compared to calcium hydroxide cement, as Dycal (LD Caulk, Milford, DE, USA) or Life (Kerr, Romulus, MI, USA). To the author's knowledge, only one article has used calcium hydroxide powder for comparison purposes.[13]

When calcium hydroxide cements are employed, many other components presented in the cement may interfere with the outcome.[14] Although it is well established that calcium hydroxide is considered a good control material for pulp capping, Holland and others[15-16] showed that calcium hydroxide powder is more effective than calcium hydroxide cement. Therefore, this clinical study compared the histological features of MTA and calcium hydroxide powder covered with calcium hydroxide cement after 30 and 60 days. The null hypothesis tested was that no significant difference would be observed in pulps capped with MTA or calcium hydroxide during the two evaluation periods.

METHODS AND MATERIALS

Forty healthy human premolars scheduled for extraction for orthodontic reasons were selected from volunteers ranging from 15 to 30 years of age. All the teeth were examined clinically and radiographically to ensure the absence of proximal caries and periapical lesions. The volunteers and their parents signed consent forms after receiving a detailed explanation about the experimental rationale, clinical procedures and possible risks. Both the consent form and the research protocol were performed according to the Human Subject Review Committee from the University of Oeste of Santa Catarina, SC, Brazil.

For the thermal testing, ENDO-ICE frozen gas (Coltène/Whaledent Inc, Mahwah, NJ, USA) was applied for five seconds on the buccal surface of the teeth scheduled for pulp therapy, as well as on contra lateral and adjacent teeth. After local anesthesia (Citanest 3%, Merrel Lepetit, São Paulo, Brazil), rubber dam isolation was installed and each tooth was pumiced with a rubber cup at low speed. Occlusal cavities were prepared by means of sterile diamond burs (#1095, KG Sorensen, Barueri, São Paulo, Brazil) at high speed under water/spray coolant. The dimensions of the cavity were: occlusal depth: 3.0 ± 0.2 mm, mesiodistal width: 4.0 ± 0.5 mm and faciolingual width: 3.0 ± 0.2 mm. Pulp exposure was performed in the center of the pulpal floor by means of a round diamond bur under water-cooling (#1014, ϕ 1.2, KG Sorensen). One bur was used for each cavity. The teeth were then divided into four experimental groups, (n=10) as shown in Figure 1.

Hemostasis was established with a sterile cotton pellet soaked in saline solution. In Groups 1 (CH30) and 2 (CH60), calcium hydroxide powder was applied on the occlusal floor with a small amalgam carrier and, subsequently, one coat of calcium hydroxide cement (Life,

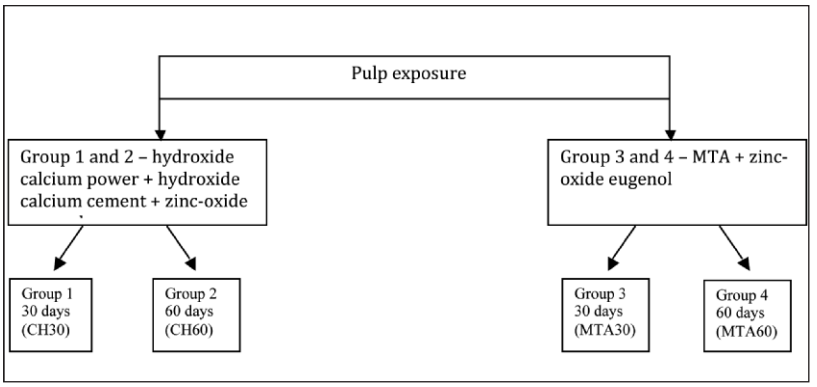

Figure 1: *Experimental design.*

Table 1: *Scores Used During the Histological Exams of Dental Pulp: Hard Tissue Bridge*

Scores	Continuity
1	Complete
2	Little communication of the capping material with dental pulp
3	Only lateral deposition of hard tissue on the walls of the cavity of pulp exposition
4	Absence of a hard tissue bridge and the absence of lateral deposition of hard tissue
Scores	Morphology
1	Dentin or dentin associated with irregular hard tissue
2	Only irregular hard tissue deposition
3	Only a very thin layer of hard tissue deposition
4	No hard tissue deposition
Scores	Thickness*
1	Up to 250 µm
2	From 150 to 249 µm
3	From 1 to 149 µm
4	Partial or absent bridge
Scores	Localization
1	Closure of the exposition area without invading the pulp space
2	Bridge invading pulp space adjacent to the opposite dentin wall
3	Bridge reached the opposite dentin wall
4	No bridge or only hard tissue deposition on the walls of the exposition cavity

**Evaluated with a micrometric ocular in three different points of the bridge.*

Kerr, Romulus, MI, USA) was applied. In Groups 3 (MTA30) and 4 (MTA60), MTA (Angelus, Londrina, PR, Brazil) was applied in the occlusal floor. Then, zinc-oxide eugenol (IRM, Dentsply Caulk, Milford, DE, USA) was placed. When necessary, the excess material was removed using an ultra-fine diamond bur at high speed under water-cooling (KG Sorensen).

Teeth from Groups 1 and 3 were extracted after 30 days, while teeth from Groups 2 and 4 were extracted after 60 days. The patients were asked whether they had postoperative sensitivity during the study period. The extraction was performed under local anesthesia. The roots of all the teeth were sectioned at approximately 5 mm in order to facilitate fixation in 10% buffered formalin solution for 72 hours. The teeth were decalcified in 50% formic acid-sodium citrate for six-to-eight weeks, prepared according to normal histological techniques and embedded in paraffin. Six-micron thick sections were cut with a microtome parallel to the main vertical axis of the tooth. The sections, placed on glass slides, were stained with hematoxylin and eosin (H/E). The Brown and Brenn technique was used to detect bacteria. The sections were blindly evaluated by an experienced pathologist according to the criteria described in Tables 1-3.[17] Each histomorphological event was evaluated using a 1-to-4 scoring system, with 1 being the best result and 4 the worst. Multiple sections were used to achieve an overall assessment for each tooth.

The scores attributed to each group were subjected to non-parametric Kruskal-Wallis analysis. This test was performed separately for each histological exam (hard tissue bridge, inflammatory response and other pulpal findings) (Tables 1-3). The comparisons between averages were performed by Conover test, comparing the ranks with appropriately computed critical values (α=0.05).[18]

RESULTS

The percentages of scores observed for each group are shown in Tables 4, 5 and 6. In regard to dentin bridge formation, CH30 showed more of a tendency towards superior performance than MTA30. However, no significant difference was observed among groups in this item (Table 4) (p>0.05).

In regard to the item "Inflammation" and "General State of Pulp," no significant difference was observed among the groups (Table 5) (p>0.05). However, MTA30 and MTA60 showed a tendency toward superior performance compared to CH30 and CH60. In the item "Other Pulpal Findings," MTA and $Ca(OH)_2$ showed comparable and excellent performance after 30 and 60 days (p>0.05) (Table 6).

Histomorphological Features

Group CH30: All specimens exhibited either total (n=6) or partial (n=4) dentin bridge formation (Figure 2). The hard tissue bridge was usually thin and close to the exposure site. A chronic inflammatory infiltrate

Table 2: *Scores Used During the Histological Exams of Dental Pulp: Inflammatory Response*

Scores	Intensity of Inflammatory Reaction* (acute and chronic processes)
1	Absent or very few cells
2	Mild: average number less than 10 cells
3	Moderate: average number 10-25 cells
4	Severe: average number greater than 25 cells
Scores	Extension of the Inflammatory Reaction (acute and chronic processes)
1	Absent
2	Mild: inflammatory cells only adjacent to the dentin bridge or area of pulp exposition
3	Moderate: inflammatory cells are observed in part of the coronal pulp
4	Severe: all coronal pulp is infiltrated or necrotic
Scores	General State of the Pulp
1	No inflammatory reaction
2	With inflammatory reaction
3	Abscess
4	Necrosis

**Item evaluated in different areas at a magnification of 400x.*

Table 3: *Scores Used During Histological Exams of Dental Pulp: Other Pulpal Findings*

Scores	Giant Cells
1	Absent
2	Mild
3	Moderate
4	Pulp necrosis
Scores	Particles of the Capping Materials
1	Absent
2	Mild
3	Moderate
4	Large number
Scores	Presence of Microorganisms
1	Absent
4	Present

was observed throughout the pulp tissue near the capping material or the hard tissue bridge. In only one specimen, giant cells were observed. Micro-organisms were also found in only one specimen.

Group CH60: Eight specimens exhibited completely hard tissue bridges (Figure 3) and, in two cases, the hard tissue bridge formation was partial. The hard tissue bridge was usually thin and close to the exposure site with an aspect of normality (Figure 4). A chronic inflammatory response was observed in nine specimens. The presence of capping material associated with macrophages was observed in six specimens. Giant cells were present in only one case. No micro-organisms or particles of the capping material were found in this group.

Group MTA30: Four specimens exhibited complete hard tissue bridge, while four specimens showed partial formation

Table 4: *Percentage of Scores (%) Attributed to Each Group in Each Criteria for Hard Tissue Bridge and Multiple Comparisons*

Groups	Continuity				Morphology				Thickness				Localization				(*)
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
CH30	60	20	20	--	60	30	10	--	--	20	60	20	80	10	--	10	1.5 a
CH60	80	10	10	--	70	20	--	10	--	10	70	20	90	10	--	--	1 a
MTA30	40	30	10	20	60	20	--	20	--	10	70	20	80	--	--	20	2 a
MTA60	70	10	20	--	80	10	10	--	--	10	70	20	80	--	--	20	1 a

(*) Overall medians for the criteria. No significant difference was observed (p>0.05).

Table 5: *Percentage of Scores (%) Attributed to Each Group in Each Criteria for Inflammatory Response and Multiple Comparisons*

Groups	Acute Inflammation								Chronic Inflammation								General State of Pulp				(*)
	Intensity				Extension				Intensity				Extension								
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
CH30	80	10	10	--	80	20	0	0	10	60	20	10	10	60	20	10	10	90	--	--	2 a
CH60	80	10	10	--	80	20	--	--	10	60	20	10	10	60	20	10	40	60	--	--	2 a
MTA30	80	10	--	10	80	10	10	--	30	50	--	20	30	50	10	10	30	60	10	--	1.5 a
MTA60	100	--	--	--	100	--	--	--	20	50	10	20	40	10	30	20	60	40	--	--	1 a

(*) Overall medians for the criteria. No significant difference was observed (p>0.05).

Table 6: *Percentage of Scores (%) Attributed to Each Group in Each Criteria for Other Pulpal Findings and Multiple Comparisons*

Groups	Giant Cells				Particles of the Capping Materials				Presence of Microorganisms				(*)
	1	2	3	4	1	2	3	4	1	2	3	4	
CH30	90	10	--	--	100	--	--	--	90	--	--	10	1 a
CH60	90	10	--	--	100	--	--	--	100	--	--	--	1 a
MTA30	90	10	--	--	100	--	--	--	80	--	--	20	1 a
MTA60	100	--	--	--	100	--	--	--	100	--	--	--	1 a

(*) Overall medians for the criteria. No significant difference was observed (p>0.05).

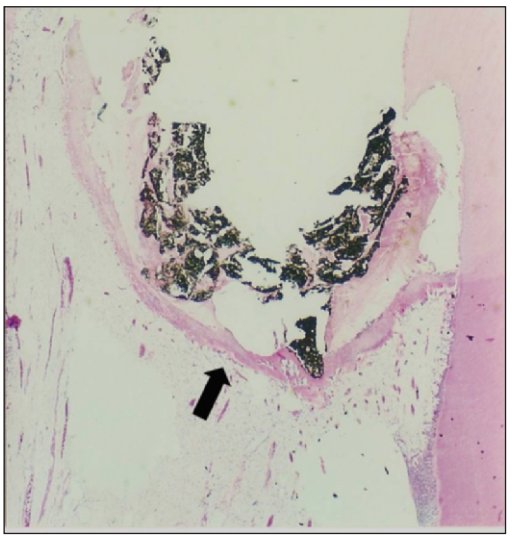

Figure 2: *$Ca(OH)_2$, 30 days. There is a complete, irregular and thin hard tissue bridge (black arrow). Observe no inflammatory infiltrate inside the pulpal tissue (HE, 100x).*

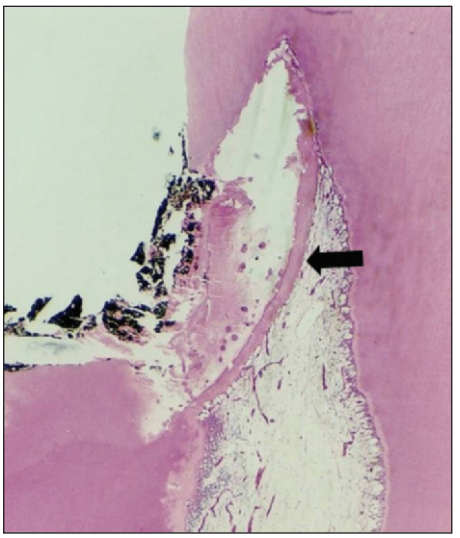

Figure 3: *$Ca(OH)_2$ 60 days. There is a complete, regular and thin hard tissue bridge with no communication of the capping material with the dental pulp (black arrow) (HE, 40x).*

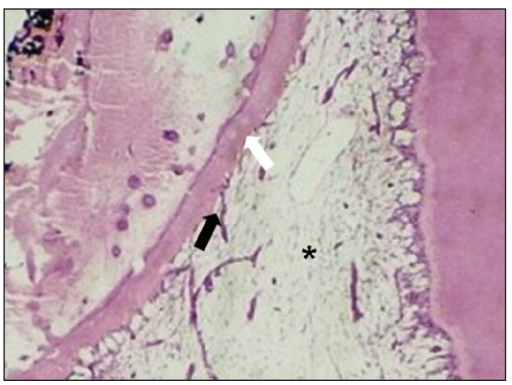

Figure 4: *$Ca(OH)_2$, 60 days. Higher magnification of Figure 3. Observe the presence of hard bridge tissue (white arrow), a new odontoblast-like cell layer in contact with the hard bridge (black arrow) and normal pulpal tissue close to the hard bridge tissue (black*) (HE, 100x).*

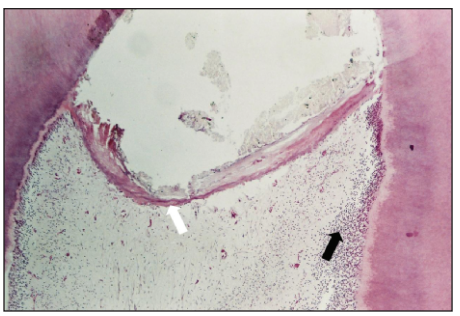

Figure 5: *MTA, 30 days. A complete, irregular and thin hard tissue bridge is shown (white arrow). Only chronic inflammatory infiltrate can be seen close to the dentin walls (black arrow) (HE, 40x).*

(Figures 5 and 6). Only two cases showed no hard tissue bridge. In seven specimens, a chronic inflammatory infiltrate with different intensities and extensions was seen (Figures 5 and 6). Giant cells were found in only one case. Gram-negative microorganisms were observed in two specimens (Figure 7).

Group MTA60: Seven specimens exhibited complete hard tissue bridges (Figure 8) and, in three specimens, the hard tissue bridges were partial and the capping material communicated with the pulpal tissue. The hard tissue bridge was usually thick and close to the exposure site, similar to the other groups. In seven specimens, a chronic inflammatory infiltrate was observed (Figure 8). The presence of capping material, giant cells and microorganisms were not found in this group.

DISCUSSION

Although no significant difference was observed between MTA and calcium hydroxide powder in regard to hard tissue formation, calcium hydroxide powder showed a tendency towards faster healing, as the median score presented by MTA (2.0) was slightly higher than calcium hydroxide (1.5) on day 30. This tendency was not observed in studies conducted in animal[7-8,13] or human teeth.[10-12] One of the reasons for this difference is the type of calcium hydroxide compound used, as previous literature findings have employed cement instead of calcium hydroxide powder in human teeth.[10-12]

Regardless of the way that calcium hydroxide is delivered (cement or powder), its mechanism of action is similar and comparable to MTA. Holland and others[9] theorized that the tricalcium oxide in MTA reacts with tissue fluids to form calcium hydroxide, resulting in hard tissue formation similar to that of calcium hydroxide. This was also suggested by other authors.[8,19-21]

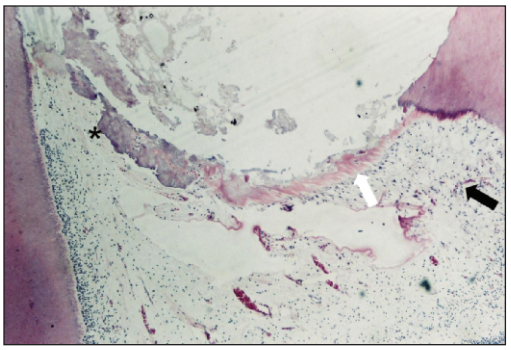

Figure 6: *MTA, 30 days. In this case, a partial and irregular hard tissue bridge is shown (white arrow). In general, a chronic inflammatory infiltrate is presented (black arrow). Observe on the left side that there is contact between the capping material and the pulpal tissue (*) (HE, 40x).*

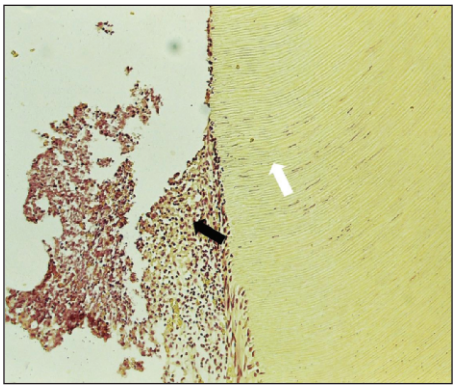

Figure 7: *MTA, 30 days. Microorganisms can be seen in dentin (white arrow) close to the micro abscess in dentin (black arrow) (Brown, Brain HE, 100x) (HE, 40x).*

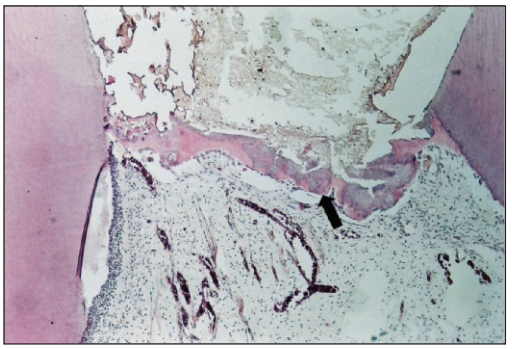

Figure 8: *MTA, 60 days. A complete, irregular and thicker hard tissue bridge is shown (black arrow). There are many dentin fragments inside the pulp tissue (HE, 40x).*

Pulp healing can therefore be attributed to the ability of calcium hydroxide to promote the induction and up-regulation of odontoblast-like cell differentiation for new matrix deposition through its solubilizing effect of growth factors from the dentin matrix.[6] Its caustic effect completely deranged and distorted the pulp tissue in immediate contact with $Ca(OH)_2$,[22-23] producing a mummified zone, which stimulated the subjacent vital pulp tissue to respond with all its healing potential to produce a dentin bridge.

This caustic effect could be the reason why calcium hydroxide powder induced a slightly higher inflammatory response than MTA. The progression of tissue healing is basically the same as what would be expected from wounded connective tissue, starting with vascular changes, inflammatory cell migration and infiltration to control and eliminate irritating agents.[14,22-23]

Despite a similarity in the mechanism of action between calcium hydroxide powder and cement, a more alkaline environment is known to favor the further differentiation of fibroblasts into odontoblasts, inducing calcified dentin bridge formation. Calcium hydroxide powder has a pH ranging from 11 to 13, while MTA and calcium hydroxide cement possess a pH of approximately 10.[14,24] The higher the pH of the material, the thicker the mummified zone, the more complete the dentin bridge formation and the higher the inflammatory infiltrate.[8,12]

This could be one reason why, in the current investigation, the calcium hydroxide powder showed a slight tendency to produce a faster hard tissue formation than MTA at day 30. Another contributing factor is that calcium hydroxide powder has a higher antibacterial property when compared to $Ca(OH)_2$ cement and MTA.[25-28]

Microorganisms were found in two specimens for MTA and one specimen for CH groups at day 30. Unfortunately, in order to detect microorganisms in the pulp tissue, it is necessary to scan as many as 80 serial sections,[14] due to the fact that microorganisms are easily washed out during the process of preparing the tissue for light microscopy.[29-30]

Briso and others[13] evaluated the response of a dog's dental pulp capped with $Ca(OH)_2$ powder or MTA. Contrary to the findings of the current study, Briso and others reported that the MTA was significantly better than $Ca(OH)_2$ after 60 days, due to the higher occurrence of dentin bridge formation and reduced inflammatory infiltrate. Methodological differences, such as the kind of teeth employed, could explain the different results between the current investigation and the previously reported study.[13]

It is likely that the pulpal response in animals is quite different from that of human teeth. For instance, acceptable biocompatibility of adhesive systems as pulp capping agents was observed in animals,[31-32] albeit these findings were not confirmed by studies conducted in human teeth.[2-4,32] Although studies in animals are necessary and their results may provide valuable information, caution must be exercised if results from usage tests in animals are extrapolated to humans.[32]

It must be emphasized that the current investigation was performed in sound teeth. In a clinical situation, pulp exposure frequently occurs from a different process, wherein the level of inflammation is much higher and more difficult to predict than in clinical evaluations of pulp therapies. This fact merits further evaluation. Another limitation that should be mentioned is the short follow-up time period for teeth that were pulp

capped. Long-term clinical evaluations in human pulp capping with MTA should be conducted.

CONCLUSIONS

Although calcium hydroxide powder covered with calcium hydroxide cement showed a tendency toward faster pulpal healing at 30 days, both materials allowed comparable tissue healing after 60 days.

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