Primate Pulpal Healing after Exposure and TheraCal Application

Cannon M*/ Gerodias N**/ Vieira A***/ Percinoto C****/ Jurado R*****

Aim: *The purpose of this in vivo study was to compare the effectiveness of a new light cured resin based dicalcium/tricalcium silicate pulp capping material (TheraCal LC, Bisco), pure Portland cement, resin based calcium hydroxide or glass ionomer in the healing of bacterially contaminated primate pulps.* ***Study design:*** *The experiment required four primates each having 12 teeth prepared with buccal penetrations into the pulpal tissues with an exposure of approximately 1.0 mm. The exposed pulps of the primate teeth were covered with cotton pellets soaked in a bacterial mixture consisting of microorganisms normally found in human pulpal abscesses. After removal of the pellet, hemostasis was obtained and the pulp capping agents applied. The light cured resin based pulp capping material (TheraCal LC) was applied to the pulpal tissue of twelve teeth with a needle tip syringe and light cured for 15 seconds. Pure Portland cement mixed with a 2% Chlorhexidine solution was placed on the exposed pulpal tissues of another twelve teeth. Twelve additional teeth had a base of GIC applied (Triage, Fuji VII GC America) and another twelve had a pulp cap with VLC DYCAL (Dentsply), a light cured calcium hydroxide resin based material. The pulp capping bases were then covered with a RMGI (Fuji II LC GC America). The tissue samples were collected at 4 weeks. The samples were demineriliz ed, sectioned, stained and histologically graded.* ***Results****: There were no statistically significant differences between the groups in regard to pulpal inflammation (H= 0.679, P=1.00). However, both the Portland cement and light cured TheraCal LC groups had significantly more frequent hard tissue bridge formation at 28 days than the GIC and VLC Dycal groups (H= 11.989, P=0.009). The measured thickness of the hard tissue bridges with the pure Portland and light cured TheraCal LC groups were statistically greater than that of the other two groups (H= 15.849, P=0.002). In addition, the occurrence of pulpal necrosis was greater with the GIC group than the others. Four premolars, one each treated according to the protocols were analyzed with a microCT machine. The premolar treated with the light cured TheraCal LC demonstrated a complete hard tissue bridge. The premolar treated with the GIC did not show a complete hard tissue bridge while the premolar treated with VLC Dycal had an incomplete bridge. The pure Portland with Chlorhexidine mixture created extensive hard tissue bridging.*

Conclusion: *TheraCal LC applied to primate pulps created dentin bridges and mild inflammation acceptable for pulp capping.*

Key words: pulp exposures, pulp response, bacteria, primate

INTRODUCTION

The ideal material for pulp treatment would be bactericidal, creating no injury to the pulp or adjacent oral structure, and promote regeneration of the pulp tissue. New substances, considered to be more biologically kind, such as dehydrated bone, collagen, morpho-genetic proteins and allogenic dentin matrix have been proposed, as well as non-pharmacological techniques for the treatment of the exposed pulp, including laser and electro-surgery. Calcium hydroxide, considered biocompatible, has a highly alkaline pH and demonstrates capability of inducing a mineralized barrier while also conferring a bactericidal effect, is considered to be a pulp tissue regenerating material [1].

A new material called mineral trioxide aggregate (MTA®) was proposed by the University of Loma Linda, California, to seal communications between the root canal system and the external surfaces of teeth at different levels[2]. MTA is made up of fine hydrophilic particles (powder) that harden in the presence of water. Its main components are tricalcium aluminate, tricalcium silicate,

*Mark L Cannon, DDS MS, Professor, Northwestern University, Feinberg School of Medicine, Chicago, IL USA. E-mail: mark-cannon@att.net
**Nate Gerodias, formerly resident, Ann and Robert Lurie Children's Hospital, Chicago, IL USA.
***Ray Jurado DDS, Director of Pediatric Dentistry, Ann and Robert Lurie Children's Hospital, Chicago, IL USA.
****Ana Elisa de Mello Vieira, DDS PhD, University of Sao Paulo, Aracatuba, UNESP, Departments of Pediatric Dentistry and Endodontics, Aracatuba, Brasil.
*****Celio Percinoto, DDS PhD, University of Sao Paulo, Aracatuba, UNESP, Departments of Pediatric Dentistry and Endodontics, Aracatuba, Brasil.

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tricalcium oxide and silicate oxide, all found in Portland cement, with the addition of bismuth oxide to confer radio-opaque characteristics. Electronic micro-analysis revealed that the main molecules present in MTA are calcium and phosphate ions, and as these are also components of the dental tissues, they may confer biocompatibility to this material. After mixing with water, the pH progresses from 10.2 to 12.5 in three subsequent hours, providing evidence, among other properties, of an antimicrobial effect on some facultative anaerobic bacteria, due to its highly alkaline pH [3].

This material is characterized by demonstrating excellent sealing capacity [4, 5] biocompatibility [6, 7, 8] not having mutagenic potential [9], low cytotoxicity [10] and for stimulating the activation of cellular response [11], the deposition of cementum [12], which may facilitate the regeneration of the periodontal tissue and the formation of mineralized tissue [13, 14].

Holland *et al* [14] observed that MTA has a similar mechanism to that of calcium hydroxide, with calcium oxide as one of its main components which, after mixing the powder with water, would be converted into calcium hydroxide. After contacting the tissue fluids the calcium hydroxide dissociates itself into Ca and OH ions. The calcium ions react with carbon dioxide in the tissues giving rise to the calcite granulations. Together with these granulations there would be an accumulation of fibronectine, which would allow cellular adhesion and differentiation. In time a hard tissue bridge is formed [15].

Pitt Ford *et al* [16] evaluated the reaction of pulp tissue after capping with MTA and Dycal® and found the presence of a dentin bridge in all the pulps treated with MTA after six months, whereas in the group in which Dycal was used, all showed severe chronic inflammation and in only 2 the formation of a dentin bridge was detected. Abedi *et al* [18] found less inflammatory reaction and greater formation of calcified tissue after the use of MTA in comparison with Dycal in direct capping of tooth pulp in dogs and monkeys. Earlier studies have shown dentine bridge formation after a direct pulp cap with VLC Dycal®, a resin based light cured calcium hydroxide formulation. Pitt Ford and Roberts [17] studied the pulpal response to mechanical exposure and capping either immediately or after 24 hours in 64 teeth of four cynomolgus monkeys with the use of Dycal, VLC Dycal, or Prisma-Bond®. Dentine bridges were present in almost all teeth filled with Dycal or VLC Dycal, and pulpal inflammation was observed in only one tooth that showed evidence of infection. The success rate of pulp capping delayed for 24 hours was as high as that for immediate capping.

Holland [18] reported that both MTA and PC allow for dentin bridge formation after pulpotomies on dogs. Estrela [19] reported that MTA and PC have comparable antibacterial activity. Whereas Saidon [20], and Menezes [21] also demonstrated that MTA and PC have similar histological results when used as pulp capping agents. This would not be unexpected as Funteas [22] found no significant differences when comparing 14 different elements between Portland cement and MTA. De Deus [23] compared the cytotoxicity of Pro-Root MTA and MTA Angelus® to Portland cement and found no statistically significant differences between any of the experimental materials.

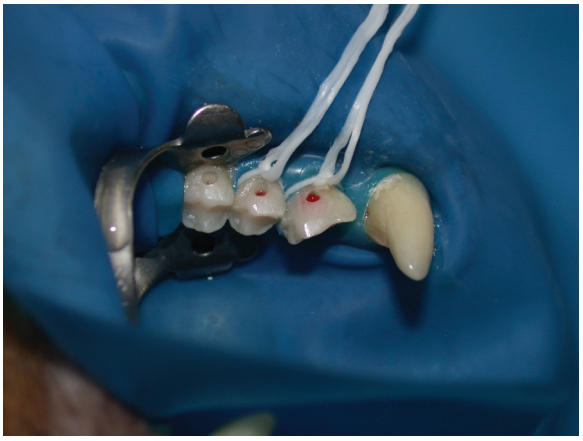
The purpose of the present *in vivo* study was to analyze a new light cured resin based dicalcium/tricalcium silicate pulp capping material (TheraCal LC, Bisco) as a pulp capping agent.

MATERIALS AND METHOD

The experiment required four primates, young adult alpha male Capucin Cebus Opella, who were randomly selected from the primate population of the Aracatuba, Sao Paulo, Monkey Research facility. The research project was presented to and approved by the Animal Research Committee of Sao Paulista University, Aracatuba, Sao Paulo, UNESP, Brasil. Throughout the research project the animals were cared for according to International Standards for Animal Care. All procedures were performed in the Primate Operating Rooms at the Monkey Research Facility. Following standardization of the two principal investigators, each primate had 12 teeth prepared with buccal penetrations into the pulpal tissues. The preparations were performed under general anesthesia administered by an experienced animal anesthetist utilizing Thiopental 30mg/kg IV along with Diazepam .17cc IM and with as sterile conditions as practical. The teeth were isolated with rubber dam, pumice prophylaxis performed and the operative area disinfected with providone-iodine. Small pediatric size high speed handpieces (Dabi Allante) were utilized to create the preparations. The handpieces were autoclaved prior to each use. A new sterile small round bur was used to make the preparation with sterile saline as the coolant. Cavity preparations bordered by enamel but extending into the mesial/distal surfaces of the teeth were created and a pulpal exposure of approximately 1.0 mm diameter was accomplished in the center of the cavity. After the preparations were performed, the exposed pulpal tissue was rinsed with sterile saline to remove operative debris.

The exposed pulps of the primate teeth were blotted with cotton pellets soaked in a bacterial mixture consisting of microorganisms normally found in human pulpal abscesses. The microorganism solution consisted of anaerobic and aerobic bacteria, obtained from the Endodontic Clinic of UNESP. The bacterial mixture also contained *Porphyromonas gingivalis* and *Fusobacterium nucleatum* as both are known pathogens responsible for acute dental pulpitis and alveolar abscesses. Following bacterial inoculation (30 minute exposure) the pulpal tissue was immediately rinsed with sterile saline and treated with Cipro HC Otic solution. A cotton pellet soaked with Cipro HC solution was applied to the inoculated pulp for five minutes. After removal of the pellet, hemostasis was obtained and the pulp capping agents applied.

Figure 1: Isolation, disinfection and preparation of primate premolars demonstrated above. Pulp exposure of approximately 1 mm. placed in center of cavity preparation.



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The light cured resin based pulp capping resin based dicalcium/tricalcium silicate (TheraCal LC) was applied to the pulpal tissue of twelve teeth with a needle tip syringe and light cured for 15 seconds. Pure Portland cement mixed with a 2% Chlorhexidine solution was placed on the exposed pulpal tissues of another twelve teeth. Twelve more teeth had a base of GIC applied (Triage, Fuji VII GC America) and another twelve had a pulp cap with VLC DYCAL (Dentsply), a light cured calcium hydroxide resin based material. The pulp capping bases were then covered with a RMGI (Fuji II LC GC America).

Table 1. Four Treatment Groups- each consisting of 12 teeth, primate premolars

- Fuji VII
- Fuji VII + VLC Dycal (LC CaOH)
- Fuji VII + LC resin based di-calcium/tri-calcium silicate (TheraCal)
- Fuji VII + PC/2% CHX

The monkeys were cared for according to International Standards for Animal Care. The primates were observed for any changes in eating habits or signs of inflammation or suppuration in the oral tissues. The primates were to be medicated with analgesics if determined necessary by the care givers. The primates' behavior was closely monitored and recorded. There were no behavioral changes noted in any of the animals.

Table 2. Distribution of Treatment Groups

Monkey 1	
LC TheraCal	PC + CHX
GIC	VLC Dycal
Monkey 2	
PC + CHX	VLC Dycal
LC TheraCal	GIC
Monkey 3	
VLC Dycal	GIC
PC + CHX	LC TheraCal
Monkey 4	
GIC	LC TheraCal
VLC Dycal	PC + CHX

The tissue samples were collected at 4 weeks after animal sacrifice. The tissues were cut into serial sections of 6 µm with a Leica BM 2025 microtome. The sections were then stained using the following methods: Hematoxylin and Eosin, Brown and Breen, Masson-Trichrome. The samples were transported to Northwestern University for independent histological evaluation utilizing a Leitz Dialux 20 microscope. The evaluators were unaware of the materials and technique utilized as all the samples were assigned identification by location only. The histological analysis consisted of the following parameters: necrosis, hyperemia, quantity and quality of hard tissue bridging, presence of odontoblasts, other calcifications, presence of giant cells, particles of capping agent, and a ranking of the inflammation; no inflammation-0, mild inflammation-1, moderate inflammation-2, severe inflammation-3, abscess formation-4. The thickness of the hard tissue bridge was measured using the phase contrast microscope using three randomly chosen points on two separate sections of each sample. The data was statistically analyzed by a statistician unaware of the samples constituents.

Four premolars, one each treated according to the protocols were not sectioned but removed in toto and analyzed with a microCT machine, a Scanco MicroCT-40 at Northwestern University, Chicago, Illinois. Computerized micro-radiographic sections were created of the four teeth and analyzed for hard tissue development, "dentin bridging". The sections were "sliced" at regular intervals and reconstructed with a 6 um voxel and a 3-D image created with the system's software.

RESULTS

Table 3: Histologic Results

Inflammatory Scale	Light Cure TheraCal	Portland Chlorhexidine	Glass Ionomer Cement	Light Cured Dycal
0	7	4	3	2
1	1	4	1	2
2	1	1	3	4
3	1	1	1	3
4	1	1	3	0

Figure 2: Example of histologic sample treated with the light cure pulp capping material demonstrating the measurements of the dentin like bridges. Also note the odontoblast like cells lining the bridge and the lack of inflammation.

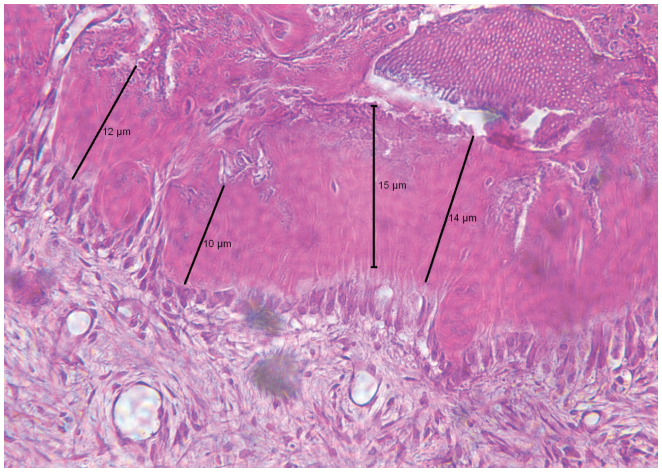

Table 4: Hard tissue bridge formation at 28 days

Hard Tissue Bridge Presence	Light Cure TheraCal	Portland Chlorhexidine	Glass Ionomer Cement	Light Cured Dycal
Yes	11	12	4	4
No	1	0	8	8

The average depth of the hard tissue bridge was obtained by measuring the apparent thickness of the hard tissue over the exposure in three different areas, with an attempt at achieving representative

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results. The light cure Theracal had an average dentin-like bridge of approximately 60.0 µ as did the Pure Portland (averaging 52.7 versus 67.2), Glass Ionomer Cement averaged 17.2 µ and Light Cure Dycal averaged 19.0 µ. Large dentin chips stimulated dentin development in several instances increasing the standard deviation. Sections of the samples not influenced by the extraneous chips of dentin chips were not found for evaluation preventing a more true measurement of the hard tissue bridge. One tooth pulp that was capped with VLC Dycal demonstrated remarkable dentin-like hard tissue bridging along with odontoblastic cell lining. This also increased the standard deviation found in the data result.

The microCT analysis demonstrated hard tissue formation with the pure Portland and light cured TheraCal formulations. The pure Portland cement mixed with 2% chlorhexidine produced extensive hard tissue deposition as seen in Figure 4. The "scout" view of a premolar is demonstrated in Figure 5 for better orientation of a sample by the observer. Unfortunately, neither the samples treated with GIC nor VLC Dycal appeared to have generated a hard tissue bridge as analyzed by the micro CT. An example of the lack of bridging may be seen in Figure 6, with the entire preparation filled by GIC, and with no hard tissue bridge formed, the shape of the canal and density would seen to indicate that the pulp became necrotic.

Figure 3: MicroCT of sample with pure Portland cement mixed with 2% chlorhexidene and sealed with Fuji II LC, a resin modified glass ionomer.

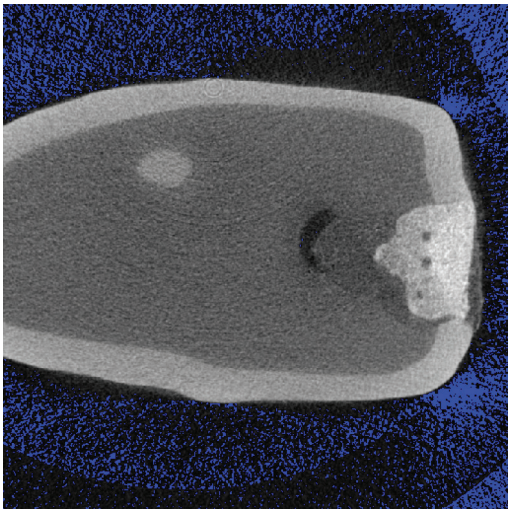


Figure 4: Scout view of a sample to demonstrate orientation of "slices".

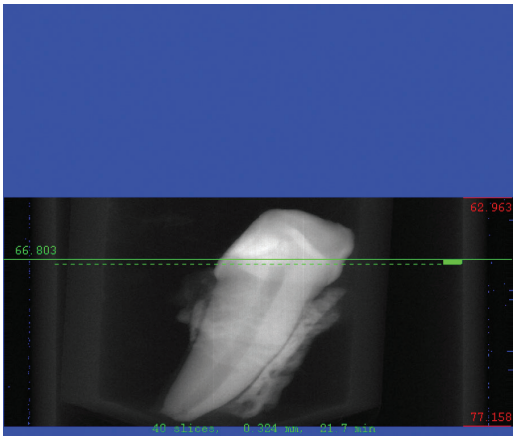
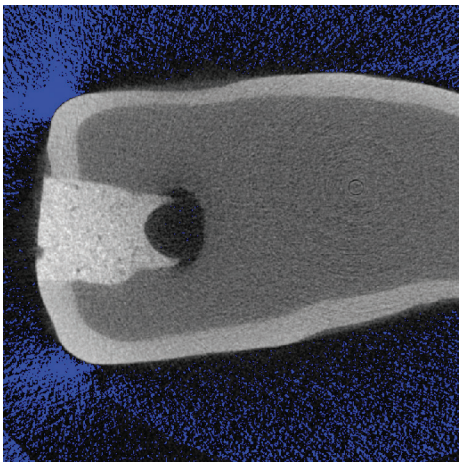


Figure 5: MicroCT section demonstrating the apparent lack of a hard tissue bridge. A GIC was used for the pulp capping.



Statistical analysis of the results was performed with the Kruskal-Wallis test. There were no statistically significant differences between the groups in regard to pulpal inflammation (H= 0.679 with 3 degrees of freedom, P=1.00). However, both the Portland cement and light cure TheraCal LC groups had significantly more frequent hard tissue bridge formation at 28 days than the GIC and VLC Dycal groups (H= 11.989 with 3 degrees of freedom, P=0.009). The measured thickness of the hard tissue bridges with the pure Portland and light cured TheraCal LC groups were statistically greater than that of the other two groups (H= 15.849 with 3 degrees of freedom, P=0.002). In addition, the occurrence of pulpal necrosis was greater with the GIC group than the others.

DISCUSSION

Treatment that preserves the dental pulp vitality is quite desirable and Portland cement (MTA) does fulfill that requirement. Although both the glass ionomer cements and visible light cured resin based calcium hydroxide materials have been recommended for deep cavity lining, they may not be the best in direct contact with pulpal tissues. However, MTA and pure Portland cement are difficult to place and require a setting time that is currently much too long. The light cure resin based TheraCal is easily placed and has an initial set within 20 seconds after light exposure begins. The TheraCal powder continues to set due to water penetrating the hydrophilic resin matrix, increasing the mechanical properties of the materials. In addition, the alkaline pH and calcium oxide availability maintains its biocompatibility and anti-bacterial qualities.

Only one of the light cured TheraCal pulp caps appeared not to have a hard tissue dentin-like bridge formation (due to pulpal necrosis, possibly secondary to restoration leakage) suggesting a superior dentin stimulating quality to this new material. All groups had a mixed result in regard to the inflammatory reaction. However, the light cure TheraCal pulp capping agent had more positive responses to the pulp exposure and bacterial contamination than the other treatment materials. The use of bacterial contamination is essential in properly gauging the pulp response to capping agents. With good isolation and the same bacterial broth used to contaminate all the exposed pulps and the same treatment with Cipro HC placement, the capping agents were the only variable. Cipro HC was applied to the exposures due to the results of a previous

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study, submitted for publication, demonstrating the superior pulpal response to Cipro HC over other pulp medicaments, such as, formocresol, which de-vitalize the pulpal tissue and would have prevented healing. Agents that inhibit full contact with the biocompatible pulp capping materials, such as ferric sulfate with the resultant coagulum, may have also provided skewed data.

Micro CT analysis proved to be very useful and provided a 3-D view of the pulp capped teeth. The use of microCT in pulpal studies will become more commonplace as researchers become accustomed to this newer technology. Future studies will involve the use of synchotronic radiation for an increasingly more detailed observation of the pulp and its interactions with newer medicaments.

The clinical application of the light cured resin based dicalcium/tricalcium material, TheraCal LC, was exceedingly straight forward as the material adheres well to a moist substrate. A small increment may be precisely placed upon a pulpal exposure.

CONCLUSION

Both the Portland cement and light cure TheraCal groups had significantly more frequent hard tissue bridge formation than the GIC and VLC Dycal groups and the measured thickness of the hard tissue bridges with the pure Portland and light cured TheraCal groups were statistically greater than that of the other two groups. Also, the occurrence of pulpal necrosis was greater with the GIC group than the others. The TheraCal LC histologically appeared to be more pulpally kind than the glass ionomer cement, Triage, or the light cure resin based calcium hydroxide product, VLC Dycal. Although there was a wide variance in the response of the pulpal tissues to the pulp capping agents, the lack of a statistical difference between the pure Portland cement and the resin based light cured TheraCal proves that the new light activated material is as effective as the pure material, chemically the same as MTA. The added clinical advantage of precise placement and command set means that the new light cured resin based TheraCal will be an important addition to dentistry's treatment of the compromised pulp.

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REFERENCES

1 Ranly, DM. Pulpotomy therapy in primary teeth: new modalities for old relationales. Pediatr. Dent. v.16, p.403-9, 1994.
2 Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J. Endod. v.12, p.591-95, 1993.
3 Torabinejad, M. et al. Physical and chemical properties of a new root-end filling material. J. Endod. v.21, n.7, p. 349-353, 1995.
4 Lee S, Monsef M., Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J. Endod. v.19, n.11, nov. 1993.
5 Aqrabawi J. Sealing ability of amalgam, Super EBA cement, and MTA when used as retrograde filling materials. Br. Dent. J, v.188, n.5, p.266-268, 2000.
6 Torabinejad M, Ford TR, Abedi HR, Kariyawasam SP, Tang HM.. Tissue reaction to implanted Super EBA and mineral trioxide aggregate in the mandible of guinea pigs: a preliminary report. J. Endod. v.21, n.11, p.569-71, 1995.
7 Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F. Mineral trioxide aggregate stimulates a biological response in human osteoblasts. J. Biomed. Mater Res.v.37, n.3, p.432-9, 1997.
8 Moretton TR, Brown CE JR, Legan JJ, Kafrawy AH. Tissue reactions after subcutaneous and intraosseous implantation of mineral trioxide aggregate and ethoxybenzoic acid cement. J. Biomed. Mater. Res. v.52, n.3, p.528-533, 2000.
9 Kettering JD, Torabinejad M. Investigation of mutagenicity of mineral trioxide aggregate and other commonly used root-end filling materials. J. Endod, v.21, n.11, 1995.
10 Keiser K, Johnson C, Tipton DA. Citotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. J. Endod, v.26, n.5, p. 288-291, 2000.
11 Koh ET, McDonald F, Pitt Ford TR, Torabinejad M. Cellular response to mineral trioxide aggregate. J. Endod. v.24, n.8, p.543-7, 1998.
12 Schwartz RS, Mauger M, Clement DJ, Walker WA 3rd.. Mineral trioxide aggregate: anew material for endodontics. J. Am. Dent. Assoc, v.30, p.969-75, 1999.
13 Abedi HR et al. The use of mineral trioxide aggregate cement as a direct pulp capping agent. J. Endod. v.22, n.4, p.199, 1996.
14 Holland R, Souza V, Nery MJ, Faraco Junior IM, Bernabe PF, Otoboni Filho JA, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tube filled with mineral trioxide aggregate, portland cement or calcium hydroxide. Braz. Dent. J. v.112, p.3-8, 2001.
15 Holland R, Souza V, Nery MJ, Faraco Junior IM, Bernabe PF, Otoboni Filho JA, Dezan Junior E.. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J. Endod. v.25, n.3, p.161-6, 1999.
16 Pitt Ford TR, Torabinjad M, Abedi HR, Bakland LK, Kaylyawawam SP. Using mineral trioxide aggregate as a pulp capping material. J. Am. Dent. Assoc. v.127, n.10, p.1491-4, 1996.
17 Pitt Ford TR, Roberts GJ. Immediate and delayed direct pulp capping with the use of a new visible light-cured calcium hydroxide preparation. Oral Surg Oral Med Oral Pathol. 1991: Mar; 71(3):338-42.
18 Holland R, de Souza V, Murata SS, Nery MJ, Bernabe PF, Otoboni Filho JA, Dezan Junior E. Healing process of dog dental pulps after pulpotomy and pulp covering with mineral trioxide aggregate or Portland cement. Braz Dent J. 2001, 12(2):109-13.
19 Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J. 2000;11(1):3-9.
20 Saidon J, He J, Zhu Q, Safavi K, Sapangberg LS. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. Oral Surg Oral Med Oral Path Oral Radio Endod. 2003 Apr,95(4):483-9.
21 Menezes R, Bramante CM, Letra A, Carvalho VG, Garcai RB. Histologic evaluation of pulpotomies in dog using two types of mineral trioxide aggregate and regular and white Portland cements as wound dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004 Sep;98(3):376-9.
22 Funteas UR,Wallace JA, Fochtman EW. A comparative analysis of Mineral Trioxide Aggregate and Portland cement. Aust Endod J. 2003 Apr; 29(1):43-4.
23 De Deus, G. et al. Cytotoxicity of MTA and Portland cement on human ECV 304 endothelial cells. *Int Endod J.* v. 38 Issue 9 p. 604, 2005.

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