



ORIGINAL ARTICLE

# Comparative evaluation of diffused calcium and hydroxyl ion release from three different Indirect pulp capping agents in permanent teeth – An in vitro study



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## KEYWORDS

Calcium ion;  
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**Abstract** *Background:* Indirect pulp capping therapy has gained increased popularity in paediatric dentistry since it is less invasive, and is of low cost. The aim of the present study was to evaluate and to compare the diffusion of calcium (Ca<sup>2+</sup>) and hydroxyl (OH<sup>-</sup>) ions through coronal dentin into pulp after indirect pulp capping in vitro using TheraCal LC, ProRoot MTA and Calcimol LC.

*Materials and methods:* Total of 60 human caries-free maxillary first premolars were selected for the study. Samples were divided into 4 groups with 15 in each group: Group 1 TheraCal LC; Group 2 ProRoot MTA; Group 3 Calcimol LC; Group 4 Control Group. Indirect pulp capping on the coronal RDT (remaining dentine thickness) system was performed using pulp-capping materials, such as TheraCal LC, ProRoot MTA and Calcimol LC, on the respective samples. The control group was completely filled with composite. Ca<sup>2+</sup> ions (ppm) and OH<sup>-</sup> ions (pH) were analysed in deionized water using a multimeter connected to a calcium probe (calcium ion electrode) and pH metre connected to a temperature-compensated pH probe after 3 h, 24 h, 7 days, 14 days, 28 days and 60 days.

*Results:* Calcium release was significantly higher ( $P < 0.05$ ) in the TheraCal LC group than in the other groups. Slightly alkaline pH values were observed in all the groups except for the control.

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**Conclusion:** TheraCal is a new light-curable pulp capping material that initially releases high  $\text{Ca}^{2+}$  ions and creates an environmental pH close to physiological pH after 60 days.

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## 1. Introduction

Pulp preservation is an essential objective for the treatment of young permanent dentition. The goal of pulp therapy is to maintain the integrity and health of the teeth and their supporting tissues (Modena et al. 2009). Pulp capping has been suggested to be an alternative for root canal treatment. Pulp capping is of two types: direct and indirect pulp capping (IPC). Indirect pulp capping is a treatment in which carious dentin nearest to the pulp is protected to avoid pulpal exposure and covered with a biocompatible material (George et al. 2015).

Generally, calcium hydroxide has been the decisive material in IPC, which in cites pulpo dentin remineralisation by releasing calcium ( $\text{Ca}^{2+}$ ) and hydroxyl ( $\text{OH}^-$ ) ions.  $\text{Ca}^{2+}$  ions are fundamental for the differentiation and mineralisation of pulpal cells, and they regulate osteopontin and bone morphogenetic proteins and invigorate the expansion of human dental pulp cells (Takita et al. 2006).  $\text{OH}^-$  ions (alkaline pH) have bacteriostatic and bactericidal activity, neutralise lactic acid and advance the development of apatite and reparative dentin (Siqueira and Lopes, 1999).

Mineral trioxide aggregate (MTA) is a pulp-capping material that is bioactive and biocompatible and is self-setting hydrophilic calcium silicate cement.

Calcimol LC is a light-cured, resin-modified  $\text{Ca}^{2+}$  ion releasing and pulp-capping material, helps in healing, and serves as an intermediary layer in the cavity walls, which is similar to that of flowable composites (Claudio et al., 2014).

TheraCal LC (Bisco, USA) is a recently introduced light-cured resin-modified calcium silicate-filled base/liner material structured with pulp capping containing approximately 45% wt mineral material (type III Portland cement.), 10% wt radiopaque part, 5% wt hydrophilic thickening agent (fumed silica) and approximately 45% resin (Suh et al. 2008). TheraCal has good sealing capabilities and is well tolerated by immortalized odontoblast cells.

TheraCal LC gives the early high alkalinity, pH 10 to 11, required for pulpal healing but returns to a neutral pH after a few days. The physiological pH of TheraCal LC make pulp cell feasibility and the development of new reparative dentine feasible. High calcium discharge has been demonstrated to be basic for the stimulation of apatite development and secondary dentin bridge formation while giving a mechanical seal of the pulp without adhesive (Griffin 2012).

The aim of the present study was to evaluate and compare the diffusion of calcium and hydroxyl ions through coronal dentin into pulp after indirect pulp capping in vitro using TheraCal LC, ProRoot MTA and Calcimol LC.

## 2. Materials and methods

The study was conducted in the Department of Pedodontics and Preventive Dentistry, PMS College of Dental Science &

Research, India. Ethical clearance was obtained from the Institutional Ethical Committee before proceeding with the study.

Sixty human caries-free extracted maxillary first premolars with perfect occlusal surfaces were selected for the study. Teeth with caries, fractured teeth or developmental defects were excluded.

The tooth was sectioned at the cemento-enamel junction using a diamond disc. Class 1 occlusal cavity (buccolingual width 2 mm and mesiodistal width 3 mm) was prepared on the coronal side using a high-speed, straight fissure (SF-31) diamond bur. Pulp tissue was excised, and the outermost pulp-side dentin was removed to obtain a standardised pulp-side chamber (buccolingual width: 3 mm and mesiodistal width: 5 mm). The RDTs were standardised to  $1 \pm 0.2$  mm and assessed using Boley's gauge. A sample defined as the "coronal RDT system" was obtained. Each sample was immersed in 4 mL of 15% EDTA for 3 min at room temperature to remove the smear layer. Samples were then thoroughly rinsed with deionized water. The external surface and the walls of pulpal cavities were covered by nail varnish (Fig. 1) to hamper the release of  $\text{Ca}^{2+}$  ions from the dental tissues, except the occlusal and floor of pulpal cavities, which were covered by wet cotton pellets to avoid dehydration of the dentin.

Samples were divided into 4 groups with 15 in each group: Group 1- TheraCal LC (Bisco Dental); Group 2- ProRoot MTA (Dentsply); Group 3- Calcimol LC (Prevest Denpro); Group 4 -Composite (Filtek), the control Group (Fig. 2).

In groups 1, 2 and 3, indirect pulp treatment of the coronal RDT system was performed using the respective pulp-capping



**Fig. 1** Class 1 cavity prepared, and external surface, and the walls of pulpal cavities were covered by nail varnish.



Fig. 2 Pulp-capping materials.

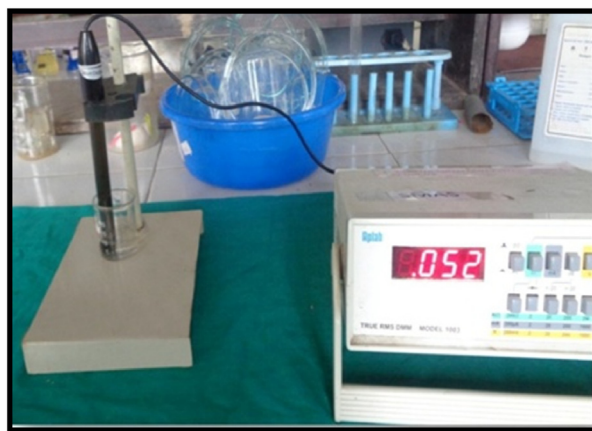


Fig. 4 Multimeter connected to a calcium probe.

materials, TheraCal LC, ProRoot MTA and Calcimol LC. Coronal restoration was completed with composite, and finally nail varnish was applied. The control group was completely filled with composite in the occlusal cavity, and the whole surface of the system was covered by nail varnish.

The samples were stored individually at a constant 37 °C temperature in numbered, sealed, polypropylene containers, which were filled with 10 mL of deionized water to create a simulated intrapulpal pressure (Fig. 3).

Ca<sup>2+</sup> ions in ppm and OH<sup>-</sup> ions (pH) were analysed in deionized water using a multimeter connected to a calcium probe (Fig. 4) (calcium ion electrode ISE-06 Thomas Scientific Brand) and a pH metre connected to a temperature-compensated pH probe after 3 h, 24 h, 7 days, 14 days, 28 days and 60 days, respectively.

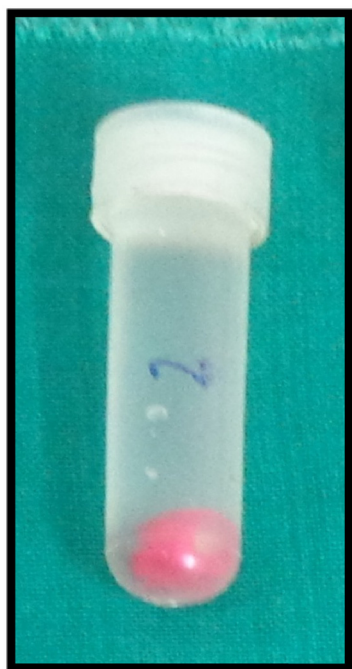


Fig. 3 Samples in polypropylene-sealed containers filled with 10 mL of deionized water.

### 3. Results

The present study evaluated and compared the Ca<sup>2+</sup> and OH<sup>-</sup> ion release of 3 different indirect pulp-capping agents on permanent teeth using a multimeter connected to an ion selective electrode method and a pH metre connected to a total compensated pH probe, respectively. Calcium levels were obtained in potential unit. These values were converted to ppm using a calibration curve. The obtained values were analysed statistically. The statistical calculations were performed using SPSS version 2.1 software using one-way Analysis of Variance (ANOVA) and two-way repeated measures ANOVA with Tukey's test.

The results showed that Ca<sup>2+</sup> ion release was significantly higher in TheraCal (P < 0.05) than in ProRoot MTA, Calcimol LC and Composite, and decreased throughout the tested period (Table 1).

Initially, the pH was slightly alkaline, decreased significantly throughout the time period until the 3rd day, increased from the 7th day until the end of the 60 days and reached an alkaline pH. Compared to ProRoot MTA, Calcimol LC and Composite, there was a significantly (P < 0.05) higher release of OH<sup>-</sup> ions from TheraCal LC initially (Table 2).

### 4. Discussion

One of the goals in the treatment of deep carious lesions is to protect the vitality of the pulp (Miyashita et al. 2007). Indirect pulp treatment is one of the proposed treatments in which contaminated dentin is excavated, leaving a slim layer of more profoundly affected dentin, where complete caries excavation would bring about pulp exposure.

Materials that have bacteriostatic/bactericidal impact have been demonstrated for application over the affected dentin. Hence, the present study compared the bioactivity (release of Ca<sup>2+</sup> and OH<sup>-</sup> ions) of TheraCal LC with ProRoot MTA, Calcimol LC and composite, which is the control group.

In this study, dentin on the pulpal side as well as coronal dentine was evaluated to obtain a normalised RDT with the most extreme permeability (Pashley et al 1981, Pashley et al 1991). The ion selective electrode method was used to analyse calcium ion release.

**Table 1** Comparison of calcium release of the 4 groups.

Groups	N	3 hr (Mean ± SD)	1 day (Mean ± SD)	3 days (Mean ± SD)	7 days (Mean ± SD)	14 days (Mean ± SD)	28 days (Mean ± SD)	60 days (Mean ± SD)	P-Value
TheraCal LC	15	94.07 ± 4.72	43.67 ± 3.17	34.73 ± 3.92	26.8 ± 2.27	22.53 ± 2.69	15.0 ± 1.89	8.93 ± 1.43	0.001*
ProRoot MTA	15	57.6 ± 7.35	38.67 ± 5.62	35.47 ± 4.96	27.2 ± 3.82	18.93 ± 2.4	11.73 ± 1.98	6.4 ± 2.99	
Calcimol LC	15	59.87 ± 10.35	29.8 ± 5.32	22.87 ± 3.56	18.27 ± 1.33	15.67 ± 3.08	11.67 ± 2.55	4.33 ± 1.95	
Control	15	8.53 ± 4.40	8.13 ± 3.42	9.60 ± 4.57	6.53 ± 3.09	4.87 ± 2.77	2.80 ± 2.51	1.00 ± 0.65	

\* Significant at the 0.05 level using 2-way repeated measures ANOVA.

**Table 2** Comparison of thepH of the 4 groups.

Groups	N	3 hr (Mean ± SD)	1 day (Mean ± SD)	3 days (Mean ± SD)	7 days (Mean ± SD)	14 days (Mean ± SD)	28 days (Mean ± SD)	60 days (Mean ± SD)	P-Value
TheraCal LC	15	7.09 ± 0.53	6.43 ± 0.22	6.13 ± 0.36	6.72 ± 0.22	6.60 ± 0.24	7.05 ± 0.38	7.45 ± 0.26	0.001*
ProRoot MTA	15	6.44 ± 0.07	6.18 ± 0.46	5.99 ± 0.30	6.70 ± 0.29	7.06 ± 0.31	7.31 ± 0.32	7.68 ± 0.32	
Calcimol LC	15	6.50 ± 0.15	6.52 ± 0.26	6.51 ± 0.24	6.66 ± 0.21	6.90 ± 0.20	7.16 ± 0.19	7.39 ± 0.24	
Control	15	5.76 ± 0.27	5.34 ± 0.25	5.23 ± 0.19	5.42 ± 0.47	5.95 ± 0.48	5.91 ± 0.63	5.97 ± 0.71	

\* Significant at the 0.05 level using 2-wayrepeated measures ANOVA.

In the present study, TheraCal LC released Ca<sup>2+</sup> and OH<sup>-</sup> ions for at least 60 days, and it discharged essentially more calcium than either ProRoot MTA or Calcimol LC throughout the trial. This result was in accordance with Gandolfi et al. in 2012, who showed that TheraCal releases Ca<sup>2+</sup> and OH<sup>-</sup> were discharged more calcium than either ProRoot MTA or Dycal throughout the study period. The high amount of Ca<sup>2+</sup> ions released from TheraCal can be related to the presence of a calcium silicate component in a hydrophilic monomer, making it a uniquely stable and durable material. The faster hydration reaction of TheraCal LCs resulted in low solubility and high calcium release during the early few hours (Gandolfi et al. 2013).

Compared with Calcimol LC, a significantly increased release of Ca<sup>2+</sup> ions was obtained in ProRoot MTA, which was in accordance with Takita et al. (2006) and Gandolfi et al. (2011), but it was significantly lower than TheraCal LC.

Composite, the control group in the study was not a calcium-releasing material. The low calcium ions demonstrated that these ions had originated from the tooth material only, and not from the control group.

The pH values obtained in this study were slightly alkaline for TheraCal LC, ProRoot MTA and Calcimol LC, except for the control group. The pH values were lower because the ions were diffused through the remaining dentine thickness. This finding is in contrast with the study by Gandolfi et al. (2012). Approachment of physiological pH within 60 days in the present study may provide a positive environment for pulpal cell viability and metabolic movement with the development of reparative dentine.

## 5. Conclusion

TheraCal LC has been demonstrated to discharge high Ca<sup>2+</sup> ions in the beginning and to make a natural pH near the physiological pH after 60 days. Further elaborate clinical trials are definitely required to determine the release of biologically active ions from TheraCal LC, which contributes to its clinical success in vital pulp therapies.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

- Claudio, P., Carla, R.A., Riccardo, B., Annachiara, M., Alberto, D., Marco, L., Livia, V., 2014. Cytocompatibility and antibacterial properties of capping materials. *Sci. World J.* 2014, 1–10. <https://doi.org/10.1155/2014/181945>.
- Gandolfi, M.G., Taddei, P., Siboni, F., Modena, E., Ginebra, M.P., Prati, C., 2011. Fluoride containing nanoporous calcium-silicate MTA cements for endodontics and oral surgery: early fluorapatite

- formation in a phosphate containing solution. *Int Endod J.* 44 (10), 938–949.
- Gandolfi, M., 2012. A new method for evaluating the diffusion of Ca<sup>2+</sup> and OH<sup>-</sup> ions through coronal dentin into the pulp. *Iran. Endodontic J.* 7 (4), 189–197.
- Gandolfi, M., Siboni, F., Polimeni, A., Bossù, M., Riccitiello, F., Rengo, S., Prati, C., 2013. In vitro screening of the apatite-forming ability, biointeractivity and physical properties of a tricalcium silicate material for endodontics and restorative dentistry. *Dent. J.* 1, 41–60.
- Gandolfi, M., Siboni, F., Prati, C., 2012. Chemical–physical properties of TheraCal, a novel light-curable MTA-like material for pulp capping. *Int. Endod. J.* 45, 571–579.
- George, V., Janardhanan, S.K., Varma, B., Kumaran, P., Xavier, A. M., 2015. Clinical and radiographic evaluation of indirect pulp treatment with MTA and calcium hydroxide in primary teeth (in-vivo study). *J. Indian Soc. Pedod. Prev. Dent.* 33, 104–110.
- Griffin, J.D., 2012. Utilizing Bioactive Liners. *Dentistrytoday.com*. October 2012.
- Miyashita, H., Worthington, H.V., Qualtrough, A., Plasschaert, A., 2007. Pulp management for caries in adults: maintaining pulp vitality. *Cochrane Database Syst. Rev.* 18 (2), CD004484.
- Modena, K.C., Casas, A.L.C., Atta, M.T., Costa, C.A., Hebling, J., Sipert, C.R., 2009. Cytotoxicity and biocompatibility of direct and indirect pulp capping materials. *J. Appl. Oral. Sci.* 17, 544–554.
- Pashley, D.H., Michelich, V., Kehl, T., 1981. Dentin permeability: effects of smear layer removal. *J. Prosthet. Dent.* 46 (5), 531–537.
- Pashley, E., Talman, R., Horner, J., Pashley, D., 1991. Permeability of normal versus carious dentin. *Dent Traumatol.* 7 (5), 207–211.
- Siqueira Jr, J.F., Lopes, H.P., 1999. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int. Endod. J.* 32 (5), 361–369.
- Suh, B., Cannon, M., Yin, R., Martin, D., 2008. Polymerizable dental pulp healing, capping, and lining material and method for use. *International Patent A61K33/42*, Application number WO2008US54387 20080220; Publication number WO2008103712 (A2).
- Takita, T., Hayashi, M., Takeichi, O., Ogiso, B., Suzuki, N., Otsuka, K., 2006. Effect of mineral trioxide aggregate on proliferation of cultured human dental pulp cells. *Int. Endod. J.* 39, 415–422.