International Journal of Scientific Reports
Gopika GJ et al. Int J Sci Rep. 2017 May;3(5):120-127
http://www.sci-rep.com
pISSN 2454-2156 | eISSN 2454-2164

DOI: http://dx.doi.org/10.18203/issn.2454-2156.IntJSciRep20171926

Original Research Article

Histological evaluation of human pulp capped with light-cured calcium based cements: a randomized controlled clinical trial

G. Jeya Gopika[1]*, Sathyanarayanan Ramarao[1], Carounanidy Usha[1], Bindu Meera John[1], N. Vezhavendhan[2]

[1]Department of Conservative Dentistry and Endodontics, [2]Department of Oral pathology and Microbiology, Indira Gandhi Institute of Dental Sciences, Puducherry, India

Received: 18 February 2017
Accepted: 20 April 2017

***Correspondence:**
Dr. G. Jeya Gopika
E-mail: drgopikagopu@gmail.com

ABSTRACT

Background: Calcium hydroxide has traditionally been used as the pulp capping material for pulpal exposures in permanent teeth. The tunnel defects in the barrier and the tendency for dissolution, however, fails to provide permanent protection to the pulp. Light curable resin based cements have been introduced to enable a better marginal seal and lesser dissolution. The purpose of this study was to compare and evaluate the response of human pulp following direct pulp capping with the new resin based Calcium silicate (TheraCal LC) and Calcium hydroxide with hydroxyapatite (Septocal LC) cements compared with calcium hydroxide (Dycal).
Methods: 72 intact human premolars scheduled for orthodontic extractions were exposed to direct pulp capping procedures using three different pulp capping agents. Teeth were randomly divided into 3 groups, Group A: Dycal, Group B: TheraCal LC, Group C: Septocal LC. The teeth were extracted at the end of 15 and 40 days' and were evaluated histologically. They were scored for reparative dentin formation and inflammatory response. Inferential statistics was done using Chi square test**.**
Results: Majority of the specimens in all three groups at the end of 15 days' showed partial to lateral deposition of hard tissue. There was continuous deposition of hard tissue and severe inflammatory response at the end of 40 days' in Dycal. There was partial deposition of hard tissue and reduced inflammatory response at the end of 40 days' in TheraCal LC and Septocal LC. However, the results were not statistically significant between the three groups at two different time periods.
Conclusions: Light cured, Calcium silicate (TheraCal LC) and Calcium hydroxide with hydroxyapatite (Septocal LC) cements were as effective as calcium hydroxide (Dycal) in inducing the formation of reparative dentin and evoking inflammatory response.

Keywords: Pulp capping, Calcium hydroxide, TheraCal LC, Septocal, Pulp therapy

INTRODUCTION

Direct pulp capping (DPC) is defined as the treatment of a mechanical or traumatic vital pulp exposure by sealing the pulpal wound with a biomaterial placed directly on exposed pulp to facilitate formation of reparative dentin and maintenance of the vitality of pulp.[1] Calcium hydroxide has been the gold standard for this purpose. The alkalinity of calcium hydroxide causes superficial necrosis of exposed pulp. This necrosis acts as mild irritation and thus stimulates the pulp to form a reparative dentin bridge. The success rate of calcium hydroxide direct capping was 80.1% after 1 year, 68.0% after 5 years, and 58.7% after 9 years.[2] The reason for failure over time is attributed to tunnel defects that form in the bridge. This fails to provide a permanent barrier and a

long-term seal against bacterial infection. Furthermore, dissolution, formation of dead space and subsequent microleakage are other disadvantages of calcium hydroxide pulp capping.[3-5] Dycal (Dentsply) is a self-setting radiopaque calcium hydroxide material containing sulphonamide (plasticizer) butylene glycol disalicylate (setting activator). Though this material has adequate calcium release, it is hydrolytically unstable and is moisture sensitive.[6]

Alternate materials such as MTA, white Portland cements are self-setting hydrophilic calcium silicate cements. They exhibit lesser pulpal necrosis, lesser inflammation, more and faster deposition of hard tissue barrier that is more homogenous and complete.[7] TheraCal LC (Bisco Inc, Schamburg, IL, USA) is a new light-cured resin-modified calcium silicate-filled base/ liner material. This contains 45% wt. mineral material (type III Portland cement), 10% wt. radiopaque component, 5% wt. hydrophilic thickening agent (fumed silica) and approximately 45% resin. The resin consists of a hydrophobic component (comprising hydrophobic monomers) such as urethane dimethacrylate (UDMA), bisphenol A-glycidyl methacrylate (BisGMA), triethylene glycol dimethacrylate (TriEDMA or TEGDMA) and a hydrophilic component (containing hydrophilic monomers) such as hydroxyethyl methacrylate (HEMA) and polyethylene glycol dimethacrylate (PEGDMA).[8]

Septocal LC (Septodont) is another radio-opaque, resin based, visible light cured calcium hydroxide liner, which contains hydroxyapatite and fluorides. Being resin based this cement is also insoluble. Hydroxy apatite has good biocompatibility with neutral pH -7.0. It can be used as scaffolding for the newly formed mineralised tissue.[9]

Although clinical and histological studies have evaluated the effect of calcium hydroxide for pulp capping in permanent human teeth, either alone or in comparison with other materials such as MTA and propolis, no such reports are available on the pulpal response of visible light cured calcium based pulp capping agents, in terms of dentinal bridge formation and inflammatory response of the pulp.[10-15] Being light cured materials, these materials might be less soluble than calcium hydroxide, and provide better seal. One school of thought in pulp capping is that pulp-dentin response does not depend on the pulp capping agent per se, but rather on its capacity to avoid microleakage providing a biological seal. Use of adhesive materials for direct pulp capping has been reported to evoke severe inflammatory response and absence of dentinal bridge formation.[16] However certain resins such as 4-Meta have been reported to be less cytotoxic and also forms soft tissue hybrid layer on top of the exposed pulp.[17]

A research hypothesis was generated to assess if the light-cured calcium silicate based and calcium hydroxyapatite based pulp capping agents performed better than self-cure calcium hydroxide. The null hypothesis generated was that there was no difference in the hard tissue barrier formation and pulpal response with all the three tested materials.

The objective of the study was to histologically, compare the pulpal response of chemical cured calcium hydroxide (Dycal (Denstply)) with light cured calcium silicate cement (TheraCal LC (Bisco)) and calcium hydroxide liner with hydroxyapatite and fluoride (Septocal LC (Septodont)), on iatrogenically exposed human pulp in permanent premolar teeth, at two different time intervals such as 15 days' and 40 days'.

METHODS

This study is a randomised controlled, double blinded, parallel group, ex-vivo clinical trial, conducted in Indira Gandhi Institute of Dental Sciences Puducherry, India. Study approval and ethical clearance was obtained from the Institutional Review Board (IGIDSIRB2014 NDP01PGJGCDE) and Institutional Ethical committee of the institute. (IGIDSIEC2015 NDP01PGJGCDE). Patients with intact maxillary and mandibular premolars scheduled for orthodontic extraction in the age group of 15-25 years, were included in the study. The sample size was chosen based on the previous study done by Eskandarizadeh et al.[14] The estimated sample size was 12 per group. Twenty-four patients as per inclusion/ exclusion criteria were selected. They were randomly divided into 15 days' follow-up group (Group 1) and 45 days' follow-up group (Group 2). Three teeth from each patient in group 1 or 2 were randomly allocated to 3 subgroups– Group A: Dycal; Group B: TheraCal LC, Group C: Septocal LC, with n=12 per subgroup, using Latin square method. The sample distribution is discussed in the CONSORT flow chart as in Figure 1.

Informed consent was obtained from the patients. The teeth were examined clinically and radiographically to ensure absence of caries, periapical pathosis and immature roots. Vitality was assessed using cold test. Only vital teeth were included.[13] Intentional pulp exposure was made approximately 1 mm using round diamond bur FG 001 (0.08 mm) (HORICO) under water coolant/spray coolant, under local anaesthesia. Haemostasis was achieved with a sterile cotton pellet soaked in saline.[15]

In Groups A, Dycal placed over the exposed pulp as per the manufacturer's instructions. Group B and C were treated with 1 mm layer of TheraCal LC and Septocal LC, respectively and light cured for 20 seconds. Permanent restoration was done using resin modified Glass Ionomer cement (GC, Asia).

Patients were blinded to the material used for capping. Analgesics were prescribed and the patients were instructed to have it only when symptom arises. Patients were also asked about postoperative sensitivity or pain

throughout the study period.[15] Prior to the extraction, after the stipulated follow-up period, the teeth were clinically examined for any signs or symptoms and response to cold test.[16] No treated tooth responded negatively at the end of the follow-up period. Extraction was carried out under LA as atraumatically as possible and subjected to histological examination.

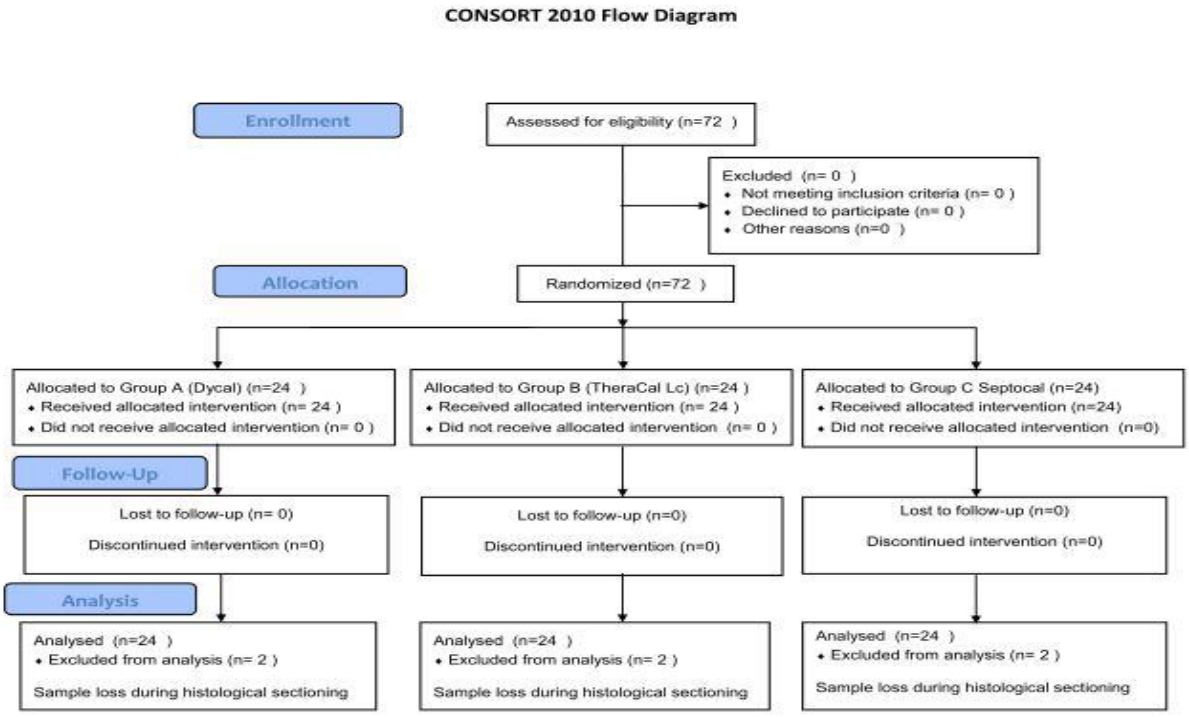

Figure 1: CONSORT flow chart of the study.

The apical portion of the tooth around 5 mm was sectioned using a diamond disc to facilitate fixation in 10% buffered formalin for 48 hours. The specimens were decalcified in 10% nitric acid for 1 week, processed according to the histologic techniques. Serial bucco-lingual sections of 5-6 micron thickness were cut longitudinally through the center of the exposure site, using YSI 060 semiautomatic soft tissue Microtome. The sections were mounted on glass slides, and were stained with haematoxylin-eosin.[16,19]

The section was blindly evaluated by an experienced and calibrated pathologist, according to scoring criteria as described in Table 1. Each histologic feature was graded from 1-4, with 1 being the best result and 4 being the worst. After the scoring, the prime investigator decoded the sample sections and the data was subjected to Chi-square test.

Table 1: Scoring criteria for histological evaluation.

Grade	Inflammation – intensity	Inflammation - extension	Blood vessel dilatation	Hard tissue bridge
1	Absent/very few inflammatory cells	Absent	Absent	Complete hard tissue bridge-closure to the exposure area
2	Mild<10 inflammatory cells	Mild- next to dentin bridge	Mild - Very Few	Partial hard tissue bridge-little communication of the capping material with dental pulp.
3	Moderate 10-25 inflammatory cells	Moderate – coronal pulp	Moderate - Present in adjacent/ coronal pulp	Lateral deposition of hard tissue on the walls of the cavity of pulp exposition.
4	>25 inflammatory cells	Severe - Coronal and radicular pulp	Severe - Present in coronal/ radicular pulp	Absence of hard tissue bridge and absence of lateral deposition of hard tissue

RESULTS

All the patients reported at 15 and 40 days', none of the teeth were non-vital, and all the teeth were extracted atraumatically. All 72 teeth were sectioned for histological evaluation. Six samples were lost while sectioning. Staining was done for 66 samples.

The histological scoring on hard tissue barrier and inflammatory response (intensity, extension and blood vessel dilatation) observed in the samples and test of significance is presented in Table 2 and 3, between the materials and between the two time-periods, respectively.

Reparative dentin formation

Complete hard tissue barrier was evident in Group A (Dycal), at 15 days' follow-up, in 18.2 % of samples and in 54.5% at 40 days' follow-up. In Group B (TheraCal LC), the similar score for hard tissue formation was seen only in 18.2% and 27.3% of samples in the 15 days' and 40 days' follow-up groups respectively. Group C (Septocal LC) showed complete hard tissue barrier only in 9.1% of the samples in the 15 days' and 40 days' follow-up groups. Figure 2a, 2b, 2c shows the hard tissue deposition with Dycal, TheraCal LC and Septocal LC respectively (D-Dentin, P-Pulp, DB-Dentin bridge, C-Cavity).

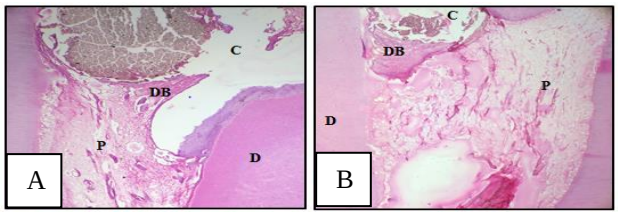

Figure 2a: (A) Dycal -15 days, (B) Dycal -40 days.

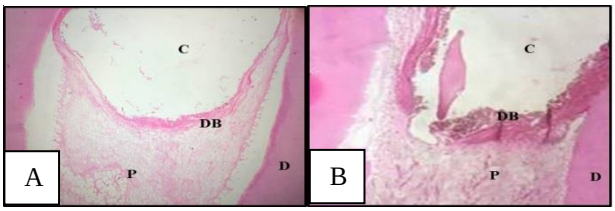

Figure 2b: (A) TheraCal Lc -15 days, (B) TheraCal Lc -40 days.

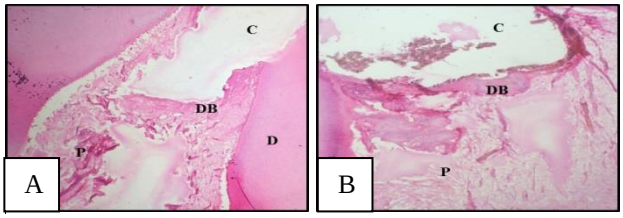

Figure 2c: (A) Septocal Lc -15 days, (B) Septocal Lc -40 days.

Inflammatory response

Severe extent of inflammation was evident at the 15 days' follow-up more frequently in Group C (Septocal LC), in 45.5% of samples, followed by 36.4% of samples in Group B (TheraCal LC). The percentage reduced to 18.2% after 40 days' follow-up of the samples in both groups. High extent of inflammation was evident in 36.4 % of samples in Group A (Dycal) only at 40 days' follow up-than in 15 days' follow-up. Intensity of inflammation was high in all the three groups at the end of 15 days' follow up, more frequently found in Group B (TheraCal LC) in 45.5% of the sample. However only 18.2 % samples in this group showed such severity in the 40 days' follow up period. In the same period, in Group C (Septocal), 63.6% of samples showed no inflammatory cells. Severe blood vessel dilatation was evident in all the three groups in around 50% of the samples at the 15 days' follow up group. Figure 3a, 3b and 3c shows the inflammatory response of the pulp under Dycal, TheraCal LC and Septocal LC.

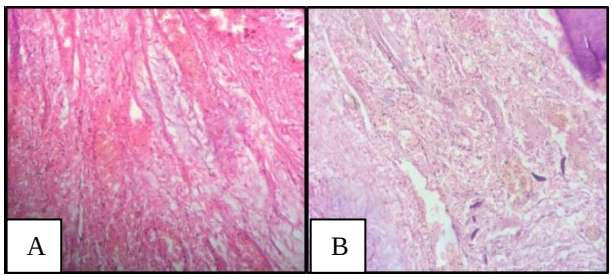

Figure 3a: (A) Dycal -15 days, (B) Dycal -40 days.

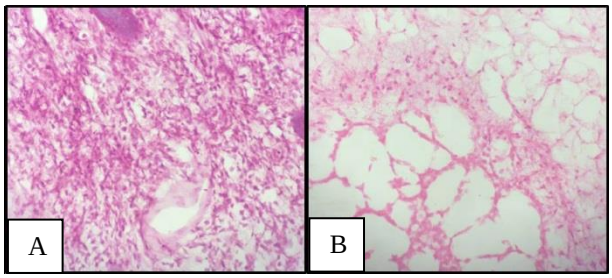

Figure 3b: (A) TheraCal Lc -15 days, (B) TheraCal Lc -40 days.

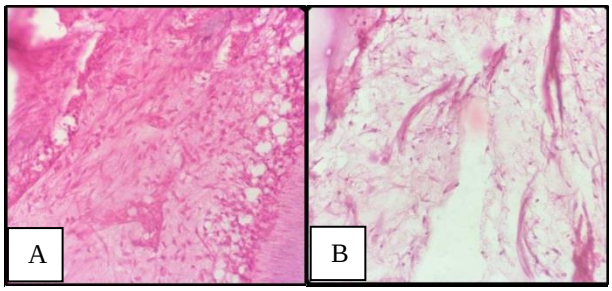

Figure 3c: (A) Septocal Lc -15 days, (B) Septocal Lc -40 days.

Table 2: Intergroup comparison between the experimental groups at 15 and 40 days' follow up.

Parameters	15 Days'				40 Days'			
	Median			Chi-square	Median			Chi-square
	Group A (Dycal)	Group B (TheraCal LC)	Group C (Septocal LC)	(p-value)	Group A (Dycal)	Group B (TheraCal LC)	Group C (Septocal LC)	(p-value)
Reparative dentin	3	2	3	2.663 (0.264^{ns})	1	2	3	3.097 (0.213^{ns})
Inflammation extension	3	2	3	0.547 (0.761^{ns})	2	2	2	1.233 (0.540^{ns})
Inflammation intensity	3	3	3	0.196 (0.907^{ns})	2	2	1	2.013 (0.365^{ns})
Blood vessel dilatation	4	4	2	1.935 (0.380^{ns})	2	2	3	7.043 (0.030^{ns})

ns-not significant.

Table 3: Median scoring and Chi-square values at 15 and 40 days' follow-up.

Parameters	Group A (Dycal)			Group B (TheraCal LC)			Group C (Septocal LC)		
	Median			Median			Median		
	15 days	40 days	Z – Value p - Value	15 days	40 days	Z – Value p - Value	15 days	40 days	Z – Value p - Value
Reparative dentin	3	1	1.237 (0.243^{ns})	2	2	0.319 (0.750^{ns})	3	2	0.349 (0.727^{ns})
Inflammation extension	3	2	0.102 (0.949^{ns})	2	2	0.823 (0.410^{ns})	3	2	1.674 (0.094^{ns})
Inflammation intensity	3	2	0.239 (0.847^{ns})	3	2	1.065 (0.287^{ns})	3	1	1.638 (0.101^{ns})
Blood vessel dilatation	4	2	2.610 (0.010*)	4	3	0.861 (0.390^{ns})	2	3	0.629 (0.529^{ns})

There was no statistically significant difference in the pulpal response and hard tissue barrier formation, in between the three materials as well as in between different time intervals for each material.

DISCUSSION

The null hypothesis generated at the beginning of the study is accepted that there is no significant difference in the performance between Dycal, TheraCal LC and Septocal LC as pulp capping agents.

However, on observation of the median scores, all three experimental materials used in this study have been found to evoke similar severe inflammatory response at the end of 15 days. But in Dycal and TheraCal LC groups the median score for inflammatory intensity reduced after 40 days'. Septocal LC showed no inflammatory cells at all, at the end of 40 days. Blood vessel dilatation or hyperaemia also reduced significantly in the Dycal group in this period. Thus, it can be inferred that the immediate and delayed inflammatory response of the pulp to light cured pulp capping agents, TheraCal LC and Septocal LC, was like that of the response under Dycal. All the experimental materials did not show evidence of complete hard tissue barrier in the 15 days' follow-up. It was evident only in the 40 days' follow-up samples capped with Dycal. Lateral deposition was seen in Septocal LC in both time periods and consistent partial deposition was observed in TheraCal LC in both time periods. These results might indicate that hard tissue induction was less and slower with light cured materials compared to Dycal, though the difference was not statistically significant.

It is well known that calcium hydroxide in powder form or in cement form evokes a severe inflammatory reaction on the exposed pulp due to its alkalinity. Thus, it has been used as a control material in several studies that assessed the pulpal response of pulp capping agents over different time periods. Aeinehchi et al in 2003 studied the effect on pulp that was capped with MTA or calcium hydroxide cement and they were extracted after periods of 1 week, 2 months, 3 months, 4 months and 6 months. Histological evaluation demonstrated inflammatory response under calcium hydroxide that lasted for 6 months, starting with acute inflammation and ending in mild chronic inflammation. The hyperaemia (blood vessel dilatation) also reduced but persisted for 6 months.

Dentinal bridge was formed only after 3 months and that too thinner.[19] Yet another study by Nair et al in 2008, reported chronic inflammation of the pulp until 3 months after capping with Dycal. A heterogenous dentinal bridge with tunnel defects was observed only at the end of 3 months.[15] Similar reports have emerged from studies by Parolia et al in 2010 and Eskandarizadeh et al in 2011.[13,14] The results of our study are in accordance with these reports, that though less, chronic inflammation persisted in the pulpal samples capped with Dycal even after 40 days' follow up and the hard tissue deposition increased with follow-up time.

Both the light cured cements used in this study contain resin components that enable command set as well as better seal at the tooth-agent interface. The severe inflammatory response of the pulp can be attributed to the toxicity of the resin monomers. Accorinte et al in 2005 reported severe inflammatory reaction of the pulp to all the components from total etch and self-etch adhesives and discouraged the use of resin for pulp capping.[20] Monomers present in resin composites and adhesive systems (e.g.: BISGMA, UDMA, TEGDMA, HEMA) have been shown to have cytotoxic effects as reported by de Silva Modena et al in their literature review in 2009.[21] TheraCal LC contains these resin monomers, whereas no information is available on the resin content of Septocal LC. However one recent study in 2014, done on primates by Cannon et al, found out that TheraCal LC showed better pulpal response after 28 days, when compared with Dycal and glass ionomer cement.[22]

Though hydrophobic and hydrophilic resins can be irritant to pulp, a study by Gondolfi et al in 2011, comparing Thercal LC with Dycal and Portland cement, stated that presence of these monomers in TheraCal LC enhanced the calcium release due to hydration. Here the calcium release from TheraCal LC was significantly more than Dycal.[6] Yet another similar study by Chaudari et al in 2016, Septocal LC was tested as another experimental group along with TheraCal LC. The study reported better calcium releasing property of both the cements.[23]

The bioavailability of calcium ions plays a key role in the new formation of mineralized hard tissues. It induces both proliferation and differentiation into odontoblast-like cells.[24] The ability of TheraCal LC to release calcium and alkalinize the surrounding fluids is correlated to the formation of calcium hydroxide that separates into calcium and hydroxyl ions, resulting in increased pH. These attributes have resulted in thick dentinal bridge formation in primates' pulp with TheraCal LC when compared to Dycal and Portland cement, as reported in a study by Cannon et al in 2014.[22] A review on Calcium silicate based cements by Dawood et al in 2015, has elaborated that TheraCal LC has less solubility, less water sorption and better sealability.[25] However, the response of TheraCal LC in our study was not superior to Dycal both in inflammatory response and hard tissue barrier formation.

No studies are available assessing the pulp regenerating capacity of Septocal LC. But studies have been done evaluating their antibacterial effect and their calcium releasing capacity. Septocal LC has been found to have comparable antibacterial effect against *S. aureus, S. mutans*, *P. species* and *B. melaninogenicus*, when compared to calcium hydroxide.[26] Calcium release was also found to be more than calcium hydroxide.[9] The results of our study seem to indicate the use of this material as a pulp capping agent apart from a cavity liner. Presence of hydroxyapatite and fluoride along with calcium hydroxide can be reason for the lateral deposition of hard tissue that was evident in this study. Hydroxyapatite (OH-AP) is a main constitution of dental hard tissues. The possibility of being used as a pulp capping agent was explored in previous studies. One study by Subay et al in 1993 reported no hard tissue formation on exposed pulp with HA crystals, but another study in 1999 by Hayashi et al supported the use of HA as a direct pup capping agent.[27,28] The study by Chaudari et al reported better calcium release from Septocal LC than Dycal, but did not explain the reasons.[23] It is possible that presence of additional calcium ions in the HA along with calcium hydroxide released more calcium ions. However further studies are required on this material's use as potential pulp capping agent.

The authors found difficulty in histological sectioning of the specimens due to the hard set light cured pulp capping agents, which resulted in loss of samples. Advanced imaging technology like micro CT scan could have provided better information on the hard tissue barrier. The properties such as the seal in the tooth-material interface and the bacterial penetration that could influence the outcome should be evaluated in the future studies. Long term clinical follow-up are essential to confirm the performance of newly introduced materials as pulp capping agents.

CONCLUSION

Within the limitations of this study it can be concluded that

1. Calcium hydroxide based chemical cure cement (Dycal) evoked severe and persistent inflammatory response in the pulp. However over a longer period it succeeded in forming the complete hard tissue barrier over the exposed pulp.
2. Light cured, calcium silicate cement (TheraCal LC) evoked lesser inflammation and partial hard tissue barrier formation.
3. Light cured, calcium hydroxide and hydroxyapatite based material (Septocal LC), has the potential to generate hard tissue barrier over the pulp and has shown least inflammatory response.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Gopika GJ, Ramarao S, Usha C, John BM, Vezhavendhan N. Histological evaluation of human pulp capped with light-cured calcium based cements: a randomized controlled clinical trial. Int J Sci Rep 2017;3(5):120-7.