Light-cured calcium hydroxide cements release of calcium ions using argon based induction coupled mass spectroscopy - an in vitro study

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Abstract
Calcium ion-releasing ability of different calcium hydroxide-based pulp capping materials was comparatively evaluated in this study. Different brands of cements were taken from different manufacturers and categorized into three groups. Three different brands of Ca(OH)2 cements (Dycal, Theracal, and Cal LC) were taken prepared by mixing and curing the cements as per the manufacturer’s instructions. Consequently, ion release was measured after 7, 14, and 21 days by argon-based induction coupled plasma mass spectroscopy test. Within the limitations of this study, light-cured Ca(OH)2 cements released a higher amount of calcium ions compared with self-cured Ca(OH)2 cements. Theracal was found to be the highest light-cured calcium ion releasing materials throughout the period of 21 days. In conclusion, further clinical studies are warranted to substantiate the findings of this study.

Key words: calcium ion; indirect pulp capping; Theracal; Ca(OH)2; Dycal; Cal LC; vital pulp therapy; pulp

INTRODUCTION
Vital pulp therapy has been the mainstay of restorative dentistry since time immemorial and has reiterated its significance with dually fulfilling esthetic, functional and psychological needs of the patients. Different strategies have been used for vital pulp therapy, namely indirect or direct pulp capping and pulpotomy with the former being the minimally invasive and most viable option. With the advent of new bioactive materials and biomimetic approach towards dentistry pulp capping procedure has reclaimed its significance like never before and has shown tremendous potential with respect to preservation and protection of pulp vitality respectively. The protective biomaterials should have specific properties such as biocompatibility, biointeractivity (biologically relevant ions releasing), and bioactivity (apatite forming ability) to activate the pulp cells and the formation of reparative dentin.1 Calcium hydroxide has been the longest in service pulp capping agent to compare the calcium release of two light-cured Ca(OH)2 cements on the other hand impart friendly benefits for practitioners. Newer light-cured agents containing calcium trisilicate compounds have been shown to release calcium ions.1 There is an increasing need for current pulp capping material to be evaluated on its calcium releasing ability for positive and consistent clinical outcomes.

So the above-mentioned factors and respective reasoning formed the basis of our study. Hence, the aim of the study was to compare the calcium release of two light-cured Ca(OH)2 cements (Cal LC and Theracal) and self-cured cements Ca(OH)2 (Dycal).

MATERIALS AND METHODS

Mold preparation
Cylindrical molds measuring 3 mm in diameter and 1.5 mm in height dimension of polyvinylchloride were prepared for the study. The specimens were made by mixing and curing three batches of Ca(OH)2 cement. They were then filled in standard mold (polyvinylchloride) sizes of 3 × 1.5 mm2. The specimens were prepared by mixing and curing three Ca(OH)2 cements (Self-cured Ca(OH)2—Dycal (Dentsply Caulk, Milford, DE, USA) Theracal (BISCO, Chicago, IL, USA) light-cured
Ca(OH)\textsubscript{2}, and light-cured Ca(OH)\textsubscript{2}-Cal LC (Prevest Denpro Ltd., New Delhi, India) according to manufacturer’s instructions and were filled in molds of standardized dimensions and each group contained 10 specimens.

**Specimen preparation**

For Group I specimen in this group was by mixing in equal proportion of base i.e. titanium dioxide and barium sulfate in glycol disalicylate and catalyst (i.e. 1:1), i.e. calcium hydroxide, zinc oxide and zinc stearate in ethyl toluene sulphonamide to a condensable consistency with plastic spatula on oil impervious paper-pad. For Group II (calcium trisilicate) and Group III (urethane dimethacrylate, triethylene glycol dimethacrylate, silanated barium glass, amorphous fumed silica, barium sulfate, calcium hydroxide), they were prepared by dispensing the cement from the syringe and bulk light cured with the light-emitting diode probe vertically placed as close as possible to the specimen for 20 seconds as recommended by the manufacturer, the different cement pastes were placed into the plastic molds (3 mm in diameter and 1.5 mm in height). Each mold was placed on the bottom part of a standard test tube, which was filled with de-ionized water. The water was collected for analysis according to the predetermined periods. Each filled mold was placed on the bottom of the standard test tube which was filled with 15 mL of de-ionized water at 37°C. The stored water was collected for Ca analysis and replaced after 7, 14, and 21 days respectively.

**Analysis**

After each time interval 5 mL of calcium sample from each group was carried for analysis and quantification by argon-based induction coupled plasma mass spectroscopy (A600, Shimadzu, Osaka, Japan) test and the values calibrated in the designated parts per million (ppm) units.

In the present study, a simulated intrapulpal pressure of 0.29 kPa was produced by the addition of 0.1 mL HNO\textsubscript{3} within a test tube of 15 mL deionized water (3 cm H\textsubscript{2}O) to achieve calcium quantization up to two decimal places before the analysis respectively.

**Statistical analysis**

Calcium ion release at various time durations from all the groups was measured and mean along with standard deviation was calculated. These values were compared using two-way repeated analysis of variance and Tukey’s post hoc test under SPSS20 (IBM, Armonk, NY, USA).

**RESULTS**

The results of our study showed that all the materials we evaluated are calcium ion releasing. Light cured Ca(OH)\textsubscript{2} cements were proved to release significantly more calcium ions during all the test periods when compared with Dycal group (Table 1).

**DISCUSSION**

To improve the handling properties of conventional calcium hydroxide cements, resin-based cements containing calcium hydroxide were developed. These materials are light-cured, highly resistant to etchants, present superior physical properties, and handling characteristics. So, two such materials namely Cal LC (Prevest Denpro) and Theracal (Bisco) were the experimental groups in this study respectively where Cal LC is a light-cured radiopaque calcium hydroxide paste which is composed of urethane dimethacrylate, triethylene glycol dimethacrylate, silanated barium glass, amorphous fumed silica, barium sulfate and calcium hydroxide, and Theracal LC is a unique light-cured, radiopaque, dentin adhering liner and base material containing calcium hydroxide and calcium hydroxyapatite in a urethane dimethacrylate base. It is a highly filled resin with minimal shrinkage and water absorption unlike conventional Ca(OH)\textsubscript{2} cements making it more compatible as a part of routine dental practices.

Theracal being the current material has its considerable bioactivity connected with the presence of silanol groups and resin groups that are able to promote the formation of calcium phosphate deposits. In this study, light cured Ca(OH)\textsubscript{2} materials showed high biointeractivity (ion release) and bioactivity with high open pore volume (i.e., porosity). The internal network of high open pore volume provides a large surface area for the leaching process. The high amount of calcium ions released from TheraCal can be related to the presence of a calcium silicate component in a hydrophilic monomer, making it uniquely stable and durable. The faster hydration reaction of TheraCal LC\textsubscript{s} resulted in low solubility and high calcium release during the early few hours.

Mineralized tissue formation due to contact of Ca(OH)\textsubscript{2} and connective tissue has been observed from the 7th to the 10th day after application. The complete antibacterial activity takes place in 7 days by Ca(OH)\textsubscript{2}, and the slight inflammation induced by Ca(OH)\textsubscript{2} is resolved in 14 days. Even though the recommended application period for the Ca(OH)\textsubscript{2}, is 4–5 weeks, it is reported that 4–5 weeks of Ca(OH)\textsubscript{2} application causes necrosis of the normal cells. Thus, the time period of 7, 14, and 21 days for calcium ion release measurement in this study allows to gauge the action of Ca(OH)\textsubscript{2} emulating its usage in clinical setting.

Specimens of 3 × 1.5 mm were used in the present study to simplify the process and avoid the washing-out of the test material during immersion in deionized water and for exposing the entire surface to light-emitting diode curing tip. Also the normal tip of light-emitting diode curing unit is 15 mm and cures to the depth of 2 mm effectively! Thus justifying the chosen dimensions for the study.

Deionized water at neutral pH was chosen for specimen

### Table 1: Calcium ion release (part per million) of three groups

| Days | Group I | Group II | Group III | P-value |
|------|---------|----------|-----------|---------|
| 7    | 122.40±6.32 | 1238.43±64.60 | 245.94±36.12 | <0.001 |
| 14   | 159.15±4.58 | 1320.65±51.39 | 275.33±18.74 | <0.001 |
| 21   | 169.59±5.67 | 1393.92±22.12 | 299.05±15.05 | <0.001 |
immersion to obtain accurate measurements of ion release without ion contamination from the immersion liquid.\textsuperscript{10} Since the study evaluates the calcium ion release a neutral liquid medium was deemed necessary and replaced distilled water which was the choice of medium in previous studies.

Different methods are available for evaluating calcium ion release they are ethylendiaminetetraacetic acid titration method, induction coupled plasma-mass spectrophotometry, atomic absorption spectrophotometry, and potentiometer. Ethylendiaminetetraacetic acid titration method is both technique and operator sensitive with risk of over and under-estimation of calcium ion with high interferences.\textsuperscript{11} Induction coupled plasma-mass spectrophotometry has matrix effects and high cost of instrumentation as its disadvantage\textsuperscript{12} and potentiometer does not show the same capacity of calcium ion detection as spectrophotometer. Argon based Induction coupled plasma mass spectroscopy testis a holistic method used to measure the release of calcium ions, i.e., argon gas used to detect the ionized elements released in an atomized liquid medium providing linear correlation with actual calcium concentration and real-time values. This method allows reproducibility along with continuous monitoring.\textsuperscript{14-16} Hence, was elected as the testing method for this study.

The findings of calcium ion release in our study were not comparable to recent studies because the experimental protocols were different and justified by the respective methodology chosen by the authors of their studies,\textsuperscript{9,11,17-20} which signified that cements containing resin components need to be cured and this causes a reduction in the release of calcium ions and also attributed calcium ion release to the additional hydroxyapatite crystals in the tooth itself rather than the pulp capping agent through their respective potentiometric and randomized control histological analysis which was in contrast to the results of our study.

The results of this study showed that the resin portion in the light cured Ca(OH)\textsubscript{2} cement (containing hydrophobic and hydrophilic monomers) can promote calcium and OH ion release within the wet area on the tooth pulp and/or dentin and favored the interaction with the hydrophilic tooth dentin.\textsuperscript{9,10,19,21-24}

In the present study, a simulated intrapulpal pressure of 0.29 kPa produced by the water in the cylindrical containers (3 cmH\textsubscript{2}O) was used. Normal pulp has a pressure of 1.5 Pa (15 cmH\textsubscript{2}O) and inflamed pulp of 3.5 Pa (36 cmH\textsubscript{2}O).\textsuperscript{25-27} A bare minimum pressure was chosen to prevent dissolution of the released ions and ease out their measurement by the ion-selective probes. A minimum intrapulpal pressure allows the movement of ions towards the pulp via dentinal tubules, whereas the ionic elution from the materials is eventually reduced respectively.

Both the control and experimental groups tested for the study were found to be calcium ion releasing. Light-cured Ca(OH)\textsubscript{2} cements released more calcium ions than the Dycal whereas the Theracal group showed significant higher values throughout the 21 days study period and in all time periods respectively.

Furthermore studies are needed to study the mechanism of the relationship between eluted media and odontoblast differentiation\textsuperscript{28-30} and toxicity of these materials at histological and cellular levels which ultimately determine the quality of dentin bridge formation in the presence of biological fluids glorifying the true status of these ions in pulp capping agents which may unravel its yet to explore potential and utilization of those findings to achieve the best of clinical outcomes and tissue healing feasible respectively.

Within the limitations of the present study, it can be concluded that:

1. All light cured Ca(OH)\textsubscript{2} cements released calcium ions in comparison to conventional Ca(OH)\textsubscript{2} cements despite addition of resin monomers and inherent shrinkage associated with it.
2. Also the calcium ion release of light-cured Ca(OH)\textsubscript{2} cements was found to be higher in all time periods of study than self-cured Ca(OH)\textsubscript{2}.
3. Among Theracal and Cal LC, Theracal significantly released higher calcium ions with respect to entire time period considered for the study which is credited to scaffold action (calcium trisilicate component) and faster hydration reaction rates.

However, further clinical trials are warranted to confirm these findings and the superiority of light-cured over conventional self-curing Ca(OH)\textsubscript{2} based pulp capping materials.

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Author contributions
All the authors has substantially contributed to the design and conduct of the study, manuscript preparation, analysis, and reporting of this study and approved the final manuscript for publication.

Conflicts of interest
None.

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