International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Calcium Ions Released from Light - Cured Calcium Hydroxide Cement using Argon - Based Induction Coupled Mass Spectroscopy

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Abstract: ***Introduction: Calcium ions released from calcium hydroxide cements plays an important role in Protecting dental pulp complex and maintain its vitality through the stimulation of the formation of secondary/reparative dentin layer when directly contacted with the exposed pulpal tissues. Ca (OH) 2 cements release hydroxyl (OH) and calcium ions (Ca) upon its dissolution helping and its alkalinity reaches (PH 9 - 11) in favor of the cement to act as a barrier in protection of the pulp (1, 2, 3). Materials and Methods: 3 groups of calcium hydroxide cements were tested in this study. Each group composed of 10 specimens. Group I; self - cured Ca (OH) 2– Dycal (Dentsply Caulk, Milford, DE, USA). Group I; TheraCal (BISCO, Chicago, IL, USA) light - cured Ca (OH) 2and Group III; light - cured Ca (OH) 2 - Cal LC (PrevestDenpro Ltd., New Delhi, India). All specimens (n = 30) were prepared by mixing and curing the cements as per manufacturer's instructions. Each sample was placed on the bottom of a 4 cm high test tube in 10 ml deionized water at 37°C. This stored water was collected for Ca analysis and replaced after 7, 14, and 21 days. In this manner, ion release was measured after 7, 14, and 21 days by inductively coupled plasma-optical emission spectroscopy test. Results: All calcium hydroxide cements release Ca ions yet, it was found that light - cured cements release higher Calcium ions than self - cured cements. TheraCal cement showed the highest amount of Calcium ions released in 21 days period and in all periods. Conclusion: within the limitation of this study, light - cured calcium hydroxide showed to have high amount of Ca ions released and TheraCalshown to be the highest cement in releasing Ca ions.***

Keywords: Calcium hydroxide, light - cured calcium hydroxide cements, vital pulp therapy, direct/indirect pulp capping, inductively coupled plasma-optical emission spectroscopy test

1. Introduction

The main purpose of vital pulp therapy is to promote pulp tissue healing and allow the formation of reparative dentin in order to preserve the pulp vitality and health (4). Vital pulp therapy (VPT) is a restorative dental procedure that aims to remove local irritants and placement of a protective material directly or indirectly over the pulp (5).

Calcium hydroxide has been known as the "gold standard" for long decades by which all the other materials were judged (6), it has been widely used in dental procedures for direct and indirect pulp capping due to its advantages such as; excellent antibacterial properties (7), long - term track record of clinical success as a direct pulp - capping agent in periods of up to 10 years (8), However, considerable limitations of calcium hydroxide such as presence of tunnels in dentin bridge, pulp chamber obliteration, high solubility in oral fluids, poor sealing ability and degradation over time were reported (6, 9).

Pulp capping materials are placed as a protective layer on the vital pulp directly or to the exposed dentin on the floor of deep cavities after removing caries or after exposure to trauma (10). These protective biomaterials should have specific properties such as biocompatibility, bio interactivity

(biologically relevant ions releasing), and bioactivity (apatite-forming ability) to activate the pulp cells and the formation of reparative dentin (10).

Many studies have proposed that released components from pulp capping materials, especially calcium ions, play the mean role in reparative dentin formation via osteoblast differentiation, modulation of Osteo - pontin and bone morphogenetic protein 2 levels (11) and the documented antibacterial efficacy (12), which makes this ion become an indispensable entity for pulp capping procedures. Also, elution of calcium ions and the apatite formation identify the role of pulp capping agents as a scaffold to induce new dentin bridge formation and clinical healing (13). Traditional self - cured Ca (OH) 2cement (Dycal, Dentsply Caulk, Milford, DE, USA) has many drawbacks. Mainly, its solubility, high alkalinity, and formation of necrotic layer at the interface between the material and pulp tissue. It also has significant high chances of microleakage, don't forget to mention the clinical setting of this material in the presence of blood and oral fluids raising valid questions regarding its efficient usage (14). Light - cured Ca (OH) 2cements on the other hand impart pleasant benefits for dentists. Recent introduced light - cured agents containing calcium trisilicate compounds shown to release calciumions (14). There is a demanding require for current pulp capping material to be evaluated on its calcium releasing ability for positive and consistent clinical outcomes (15).

The aim of this in - vitro study was to compare calcium ions released from different calcium hydroxide cements.

2. Materials and Methods

3 groups of calcium hydroxide were used and prepared following manufactures instructions. Each group contained 10 specimens (n=10) and each specimen was filled in cylindrical molds of polyvinylchloride with standardized dimension measuring 3 mm in diameter and 1.5 mm in height. Group I; self - cured Ca (OH) 2– Dycal (Dentsply Caulk, Milford, DE, USA) prepared by mixing equal proportions of base and catalyst (1: 1) to a condensable consistency with plastic spatula on oilimpervious paper - pad. Group II; TheraCal (BISCO, Chicago, IL, USA) light - cured Ca (OH) 2and Group III; light - cured Ca (OH) 2 - Cal LC (PrevestDenpro Ltd., New Delhi, India). Both Group II and Group III were prepared by dispensing the cement from the syringe and bulk light cured with the light - emitting diode probe in a vertical direction placed as close as possible to the specimen for 20 seconds as recommended by the manufacturer. Each cement specimen was placed in 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 15 ml of deionized water at 37°C. The stored water was collected for Ca analysis and replaced after 7, 14, and 21 days respectively.

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). The values calibrated in the designated parts per million (ppm) units. In the present study, a simulated intra - pulpal pressure of 0.29 kPa was produced by the addition of 0.1 mL HNO3 within a test tube of 15 mL deionized water (3 cm H 2O) to achieve calcium quantization up to two decimal places before the analysis respectively.

3. Results

Ca ion release from all groups at various time durations was measured and mean was calculated with the standard deviation [Table 1]. These values were compared using two-way ANOVA and Tukey's *post hoc* test which showed highly significant result with $P < 0.001$.

The result of the current study showed that all three groups have the ability of Ca ions releasing. Light cured Ca (OH) 2cements (group I and group II) showed significantly higher amount of Ca ions releasing during all periods when compared to Dycal (group I). TheraCal (group II) has the highest Ca ions release among all groups in all time periods.

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

Days	Group I	Group II	Group III	P - value
7	100.20±6.32	128.48±64.60	119.94±36.12	<0.001
14	123.05±4.58	168.65±51.39	132.33±18.74	<0.001
21	130.50±5.67	299.92±22.12	177.05±15.05	<0.001

Data are expressed as mean ± SD (n = 10)

4. Discussion

As a part of daily routine dental practice aiming to improve handling properties of conventional calcium hydroxide cements the urge of developing resin - based calcium hydroxide cements occurred. These materials are light cure, have better handling characteristics, Superior physical properties and high etchant resistance (16, 15). It is a highly filled resin with minimal shrinkage and water absorption unlike conventional Ca (OH) 2 cements making it more compatible (16).

In this study a considerable high bio - activity and bio - intra - activity were found in light cure Ca (OH) 2materials. TheraCalis a unique light - cured, radiopaque, dentin adhering liner and base material containing calcium hydroxide and calcium hydroxyapatite in a urethane dimethacrylate base (14). It has considerable bioactivity related to the presence of silanol groups and resin groups that are able to promote the formation of calcium phosphate deposits. While Cal LC; is a light - cured radiopaque calcium hydroxide paste composed of urethane dimethacrylate, triethylene glycol dimethacrylate, silanated barium glass, amorphous fumed silica, barium sulfate and calcium hydroxide (14).

Contact of Ca (OH) 2and connective tissue has been observed to cause the formation of mineralized tissue from the 7 to the 10 day after application. The complete antibacterial activity takes place in 7 days by Ca (OH) 2, and the slight inflammation induced by Ca (OH) 2is resolved in 14 days (13). The recommended application period for the Ca (OH) 2is 4–5 weeks, yet, it is reported that its application for that period of time 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 gauging the action of Ca (OH) 2emulating its usage in clinical setting (15).

As a justification of specimen's dimensions in this study (3 × 1.5 mm) is to prevent the washing out of the test material during immersion in deionized water and to expose the entire surface to light emitting diode curing tip which is 15 ml with a curing depth of 2 mm (14, 17).

Deionized water at neutral pH was chosen as an emission liquid to avoid ion contamination during measuring ion release (17). Argon based Induction coupled plasma mass spectroscopy test was selected to be used in this study to measure the release of calcium ions, as 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 (18, 19, 20).

In the present study, a simulated intra - pulpal pressure of 0.29 KPa produced by the water in the cylindrical containers was used. Normal intra - pulpal pressure is 1.5 KPa (15 cm H2O) and of inflamed pulp is 3.5 KPa (36 cm H2O). A low intra – pulpal pressure favors the movement of ions through the dentinal tubules to the pulp, while the ionic dissociation from the materials is certainly reduced (14).

The findings of Ca release of the current study cannot be comparable to other studies because the experimental protocols are different (13, 17, 21, 22, 23, 24).

All three experimental groups tested in this study were found to be calcium ion releasing. Light - cured Ca (OH) 2cements 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. This can be explained to the presence of calcium silicate components in hydrophilic monomer making it uniquely stable and durable (13).

More studies are needed to study the mechanism of the relationship between eluted materials and the level of its toxicity in both histological and cellular levels which determine the quality of dentin bridge formation and utilizing those findings to achieve the best of clinical outcomes and tissue healing feasible respectively.

5. Conclusion

Within the limitation of the present study, light- cured Ca (OH) 2cements released high amount of Ca ions compared to self- cured Ca (OH) 2cements. TheraCal found to be the highest Caion- releasing materials among them.

Financial support and sponsorship
Nil

Conflicts of interest
There are no conflicts of interest.

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