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Comparative evaluation of calcium ion release of two commercially available pulp capping agents at different time periods: An in vitro study

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Abstract

INTRODUCTION. Vital pulp therapy is a significant approach in restorative dentistry, enabling the preservation of pulp vitality and the stimulation of hard tissue repair. Modern pulp capping materials, particularly silicate-based cements, facilitate dentin bridge formation and promote tissue remineralization. TheraCal LC and ApaCal ART are two commercially available materials with bioactive properties and the ability to release calcium ions, contributing to pulp healing. However, there is limited literature on the bioactivity of ApaCal ART. This in vitro study aims to comparatively evaluate the calcium ion release from TheraCal LC and ApaCal ART using the EDTA titration method.

AIM. The study aimed to evaluate and compare the calcium ion release of two commercially available pulp capping agents TheraCal LC and ApaCal ART at different time periods.

MATERIALS AND METHOD. This in vitro study was conducted following good laboratory practice guidelines and approved by the institutional review board (Approval No. [DYPDCH/DPU/EC/582/142/2023]). Twenty cylindrical specimens (6 mm × 3 mm) were prepared using silicon molds and divided into two groups: TheraCal LC (n = 10) and ApaCal ART (n = 10). A dental floss was incorporated into each mold before filling with the respective material. TheraCal LC was light-cured for 20 seconds, and ApaCal ART for 40 seconds. The specimens were weighed for standardization and incubated in deionized water at 37°C and 100% humidity for 24 hours. Specimens were immersed in 5 ml of distilled water and assessed at 24 hours, 7 days, and 21 days. The solution was refreshed at each time point, and calcium ion concentration was measured using the EDTA titration method.

RESULTS. The mean (± SD) calcium ion release for TheraCal LC group was 17.07 ± 0.48 at 24 hours, 18.36 ± 0.51 at 7 days and 20.95 ± 0.38 at 21 days which was significantly higher compared to ApaCal ART at all time intervals ($p \leqslant 0.001$).

CONCLUSIONS. The study demonstrated that TheraCal LC and ApaCal ART exhibited a progressive increase in calcium ion release over time, reaching a peak on day 21. TheraCal LC released significantly more calcium ions at all time points and may be preferable for indirect pulp capping due to its enhanced stimulation of hard tissue formation.

Keywords: calcium hydroxide, TheraCal LC, ApaCal ART, vital pulp therapy, indirect pulp capping

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Сравнительная оценка высвобождения ионов кальция двумя коммерчески доступными материалами для покрытия пульпы в разные временные периоды: in vitro исследование

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Резюме

ВВЕДЕНИЕ. Витальная терапия пульпы является важным направлением в восстановительной стоматологии, позволяя сохранить жизнеспособность пульпы и стимулировать репарацию твердых тканей зуба. Современные материалы для покрытия пульпы, в частности силикатные цементы, способствуют

образованию дентинного мостика и реминерализации тканей. TheraCal LC и ApaCal ART – два коммерчески доступных материала, обладающие биоактивными свойствами и способностью высвобождать ионы кальция, что способствует заживлению пульпы. Однако в литературе имеется ограниченное количество данных о биоактивности ApaCal ART.
Настоящее in vitro исследование направлено на сравнительную оценку высвобождения ионов кальция из TheraCal LC и ApaCal ART с использованием метода титрования ЭДТА.
ЦЕЛЬ. Оценить высвобождение ионов кальция из TheraCal LC и ApaCal ART в in vitro условиях с использованием метода титрования ЭДТА, учитывая ограниченное количество данных в литературе о биоактивных свойствах ApaCal.
МАТЕРИАЛЫ И МЕТОДЫ. Настоящее in vitro исследование проводилось в соответствии с принципами надлежащей лабораторной практики и было одобрено институциональным этическим комитетом (номер одобрения: [DYPDCH/DPU/EC/582/142/2023]). Были подготовлены 20 цилиндрических образцов (6 мм × 3 мм) с использованием силиконовых форм и разделены на две группы: TheraCal LC (n = 10) и ApaCal ART (n = 10)**.** В каждую форму перед заполнением исследуемым материалом помещалась зубная нить. Световая полимеризация проводилась в соответствии с рекомендациями производителя: TheraCal LC – 20 секунд, ApaCal ART – 40 секунд. Образцы взвешивались для стандартизации, после чего инкубировались в деионизированной воде при температуре 37°C и влажности 100% в течение 24 часов. Образцы погружали в 5 мл дистиллированной воды и проводили анализ через 24 часа, 7 и 21 день. На каждом этапе раствор заменяли свежим, а концентрацию ионов кальция определяли методом титрования ЭДТА.
РЕЗУЛЬТАТЫ. Среднее значение (± SD) высвобождения ионов кальция для группы TheraCal LC составило 17,07 ± 0,48 через 24 часа, 18,36 ± 0,51 через 7 дней и 20,95 ± 0,38 через 21 день, что было значительно выше по сравнению с ApaCal ART во всех временных интервалах ($p \leqslant 0{,}001$).
ВЫВОДЫ. Исследование показало, что TheraCal LC и ApaCal ART увеличивали высвобождение ионов кальция со временем, достигая пика на 21-й день. TheraCal LC высвобождал значительно больше ионов кальция на всех этапах и может быть предпочтительным для непрямого покрытия пульпы благодаря стимулирующему воздействию на твердые ткани.

Ключевые слова: гидроксид кальция, TheraCal LC, ApaCal ART, витальная терапия пульпы, непрямое покрытие пульпы.

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INTRODUCTION

In modern restorative dentistry, it is imperative to employ materials that not only restore the structure of teeth in an aesthetically pleasing manner but also possess the ability to repair hard dental tissue that has been affected by caries [1].

The preservation and maintenance of healthy pulp tissue is an essential goal that can be attained through the implementation of vital pulp therapy. This therapeutic approach has been specifically designed to address compromised tissue that may arise as a result of caries, trauma, or restorative procedures. For teeth with an inflamed but vital pulp, vital pulp therapy is a feasible alternative to root canal treatment [2]. The efficacy of vital pulp therapy largely depends on the quality of the dentin bridge and the pulpal response to the capping material which stimulates the production of reparative dentin, thereby ensuring the preservation of the tooth as a functional unit [3].

Vital pulp therapy has entered a new era with the advent of bioactive agents, which allow for the remineralization of caries-affected hard tissue [4]. In an effort to find the best material for vital pulp therapy, researchers have studied a wide range of substances including calcium hydroxide, zinc oxide, resin-modified glass ionomers, calcium phosphate, tricalcium silicate, calcium-tetracycline, hydroxyapatite and more recently, bioactive agents that enhance pulpal defences [4].

The application of novel calcium silicate cements has gained momentum in vital pulp therapy which are known to considerably enhance the clinical efficacy of both direct and indirect pulp capping procedures [5]. Clinical results show that permanent teeth with symptomatic or asymptomatic irreversible pulpitis consistently exhibit success rates between 85% and 100% at 1–2 years [6].

TheraCal LC (TLC), (Bisco, Schaumburg, USA) is a resin modified, calcium silicate liner utilized in direct and indirect pulp capping techniques. The active calcium ion release is known to promote healing and apatite formation[1]. ApaCal ART [Prevest DenPRO Limited, India] is resin modified, tricalcium phosphate pulp protectant fortified with nano-hydroxyapatite with its antibacterial effect and calcium release properties comparable

[1] Seal and Protect with TheraCal LC Pulp Capping Material and Liner. Available at: https://www.bisco.com/assets/1/22/TheraCal_LC_Brochure3.pdf (accessed: 27.12.2024).

to those of TheraCal LC[2]. The inclusion of light curable monomers, in TheraCal LC and ApaCal ART offers the ability to command cure the material and enhances the bonding of composite to the liner [7].

Due to the paucity of documented literature on the bioactivity of ApaCal, this in vitro study aims to evaluate the calcium ion release from TheraCal LC and relatively newer material, ApaCal ART using the EDTA titration method.

A null hypothesis proposed was that there is no difference in the calcium ion release between TheraCal LC and ApaCal ART.

AIM

The study aimed to evaluate and compare the calcium ion release of two commercially available pulp capping agents TheraCal LC and ApaCal ART at different time periods.

[2] ApaCal ART Cement and Liners. Available at: https://www.prevestdenpro.com/product/apacal-art/ (accessed: 27.12.2024).

MATERIALS AND METHODS

This in-vitro study was conducted according to the guidelines of good laboratory practice and executed with an ethical approval from the institutional review board committee, under the approval No. [DYPDCH/DPU/EC/582/142/2023].

Specimen preparation

Total of 20 cylindrical molds with a height of 6 mm and a diameter of 3 mm were made with silicon tubes [Fig. 1A]. The specimens were allocated into two groups:

– Group A: TheraCal LC [*n* =10];

– Group B: ApaCal ART [*n* = 10].

A dental floss was placed in the silicon tubes and these were filled with respective material to be tested in each group (Fig. 1B). The tip of the syringe was placed inside the silicon tubes to avoid incorporation of air bubbles. As recommended by the manufacturer, the specimens in Group A were light cured for 20 seconds, and Group B for 40 seconds (Fig. 1C, 1D). Specimens were weighed to ensure standardization within each group using a digital balance (Wensar, India).

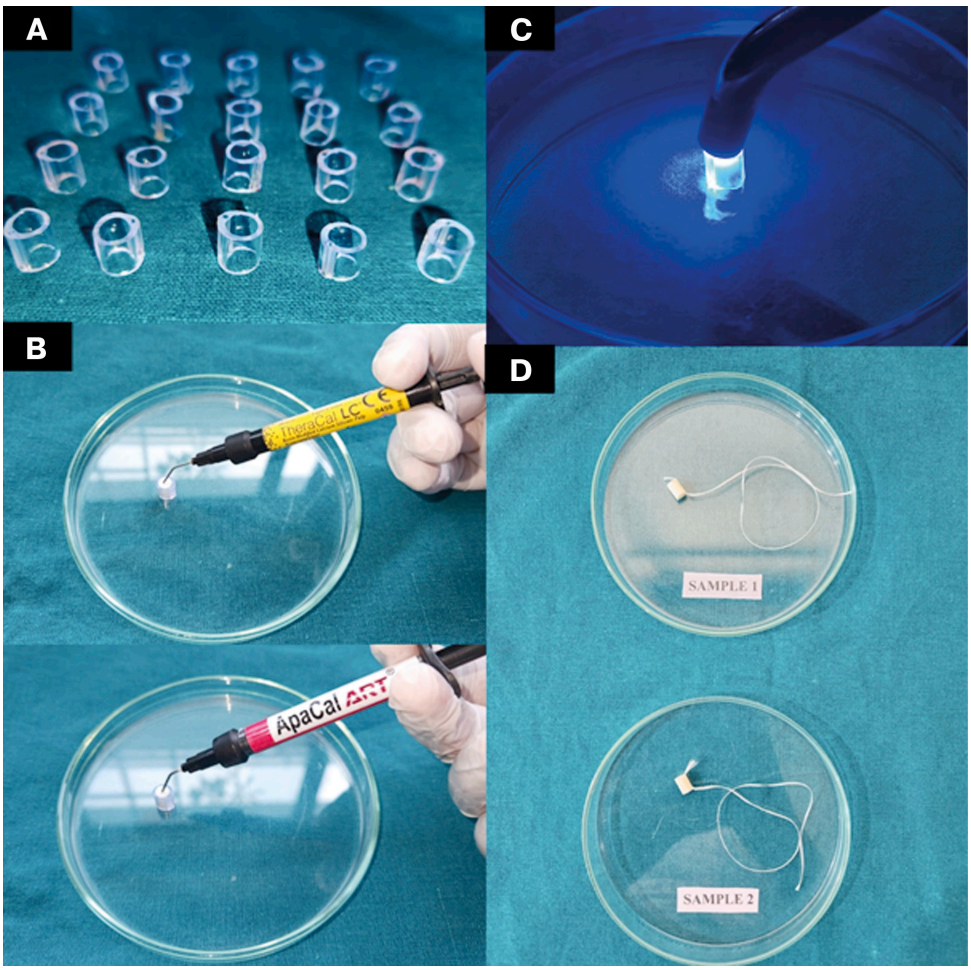

Fig. 1. Depicting procedure steps in specimen preparation: *A* – cylindrical molds used for sample preparation; *B* – cylindrical molds filled with TheraCal LC and ApaCal ART; *C* – light curing of the samples; *D* – light cured samples incorporated with dental floss

Рис. 1. Иллюстрация этапов подготовки образцов: *A* – цилиндрические формы, используемые для подготовки образцов; *B* – формы, заполненные TheraCal LC и ApaCal ART; *C* – световая полимеризация образцов; *D* – полимеризованные образцы с встроенной зубной нитью

Sample incubation and storage

The specimens were suspended in deionized water and were subjected to storage conditions of 37°C and 100% relative humidity using an incubator (Bio Technics®BTI25, D. Haridas and Company, India) for duration of 24 hours to enable the initial setting of the materials [8].

Calcium ion measurement

The individual specimens were subsequently immersed in 5 ml of distilled water and assessed at specific intervals; 24 hours, 7 days, and 21 days. It was ensured that all the tubes were transferred to fresh solutions at the commencement of each respective period. At the end of each evaluation period, the medium was collected, and its calcium ion concentration was measured using an ethylenediaminetetraacetic acid [EDTA] titration method [8; 9].

EDTA titration method

EDTA solution (Loba Chemie® Pvt. Ltd. India) was taken in a burette and 10 ml of this mixture was pipetted out into a conical flask. To this mixture, 5 ml ammonium chloride (Rankem chemicals Pvt. Ltd., India) and sodium hydroxide (Rankem chemicals Pvt. Ltd., India) buffer solutions were added. Subsequently, 3 to 4 drops of Erio chromic black T indicator (Labogen's Fine Chem Industry, India) were added and the solution was heated to 600°C. The solution was immediately titrated with 0.01M EDTA until the red wine colour of the solution completely disappeared and a sky-blue colour appeared [10–12].

Statistical analysis

The data was subjected to statistical analysis using IBM Corp. 2012, IBM SPSS® Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

The mean and standard deviation (SD) was obtained for the Calcium ion release in both TheraCal LC and ApaCal ART group at different time intervals. For intragroup comparison at different time interval Repeated measure ANOVA and Tukey post hoc was applied. Intergroup comparison was done using Unpaired T Test. All the statistical tests were carried out with confidence interval at 95% and $p < 0.05$ was considered statistically significant.

RESULTS

Total of 10 specimens were tested in each group for mean release of calcium ions. The mean (± SD) calcium ion release for TheraCal LC group was 17.07 ± 0.48 at 24 hours, 18.36 ± 0.51 at 7 days and 20.95 ± 0.38 at 21 days which was significantly higher compared to ApaCal ART at all time intervals ($p \leqslant 0.001$), (Fig. 2). The mean difference between TheraCal LC and ApaCal ART group at 24 hours, 7 days and 21 days was found to be 11.88, 10.86, 9.61 respectively. The unpaired t-test showed a significant difference among the tested groups ($p \leqslant 0.001$). Repeated Measure ANOVA analysis revealed a statistically significant difference at various time intervals followed by Tukey's post hoc analysis ($p < 0.001$). When a pairwise comparison of the calcium release was done for TheraCal LC and ApaCal ART group at different time intervals, an increase in the release of calcium ions was observed over the course of days as compared to a 24-hour period, which was statistically significant ($p < 0.05$) (Table 1).

All values are expressed as mean ± standard deviation (SD) (in parentheses). The statistical test used: Repeated Measure ANOVA; level of significance: $p \leqslant 0.001$ is considered statistically significant.

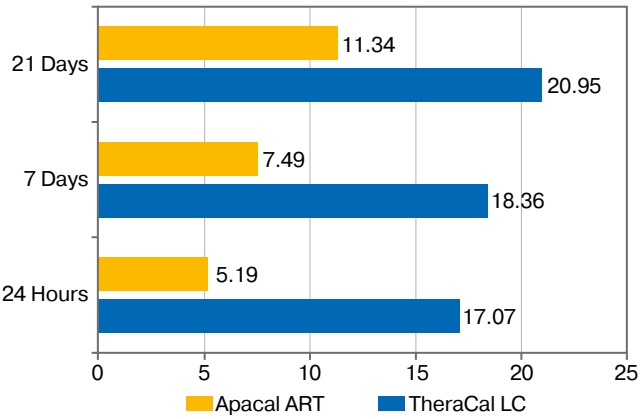

Fig. 2. The mean Calcium ion release for TheraCal LC and ApaCal ART at different time periods

Рис. 2. Среднее высвобождение ионов кальция для TheraCal LC и ApaCal ART в разные временные периоды

Table 1. Comparison of the Calcium ion release between and TheraCal LC and ApaCal ART at different time intervals

Таблица 1. Сравнение высвобождения ионов кальция между TheraCal LC и ApaCal ART в разные временные интервалы

Time Interval	Groups	Mean	Std. Deviation	Std. Error Mean	Mean Difference	*t*	*p*-value
24 Hours	TheraCal LC	17.07	0.48	0.15	11.88	70.51	⩽0.001
	ApaCal ART	5.19	0.24	0.07			
7 Days	TheraCal LC	18.36	0.51	0.16	10.86	63.17	⩽0.001
	ApaCal ART	7.50	0.19	0.06			
21 Days	TheraCal LC	20.95	0.38	0.12	9.61	66.56	⩽0.001
	ApaCal ART	11.34	0.26	0.08			

DISCUSSION

Vital pulp therapy is a biologic and conservative treatment approach which aims to preserve the health and function of the pulp-dentin complex [13]. A vital pulp can promote formation of reparative dentin and reduce inflammation. In this procedure, a protective biomaterial known as a pulp capping agent may be applied to the thin layer of remaining dentin over an exposed coronal pulp (direct capping), a nearly exposed pulp (indirect capping), or partially exposed coronal pulp tissue (pulpotomy) [14].

TheraCal LC is a resin-modified, light-cured calcium silicate material containing 45% wt. mineral material [type III Portland cement.], 10% wt. radiopaque agent, 5% wt. hydrophilic thickener [fumed silica] and estimated 45% resin [15]. It is classified as IV generation calcium silicate material according to ISO 9917-2017 – part 2 clause 4.1 [16]. TheraCal LC has an opaque shade and should thus be placed in a thin layer under the composite restoration. The manufacturers suggest applying it in a layer of 1mm and curing it for 20 seconds with light. However, Gandolfi et al. stated that the material can be placed in a thickness of 1.7 mm after an exposure with visible light for 20 seconds.

TheraCal LC has been used in the present study owing to its immediate setting, ease of usage and low solubility. TLC is a hydraulic silicate material "in which the setting reaction of the polymerizable component is light-activated" [16]. The setting commences when the material comes into contact with water. Since water is not included in TheraCal LC for material hydration, it is dependent on the amount of water that is absorbed from the surroundings and how it diffuses through the material [16]. Thus, the material is applied to moist dentin as recommended by the manufacturer. The resin modification in TheraCal LC is known to accelerate the hydration reaction of the material, thus resulting in low solubility and substantial calcium release within the first several hours. The calcium ions release is pivotal for effective pulp capping procedures due to the effects of these ions in the differentiation of pulp cells and mineralization of the hard tissue [16; 17]. In addition to stimulating dental pulp cell proliferation in a dose-dependent manner, the calcium ions eluted, also boost pyrophosphatase activity which helps in forming a dentin bridge [17; 18].

Camilleri et al. found that the limited moisture diffused from within the pulp-dentine complex into the obtained set mixture results in incomplete hydration of TheraCal LC [19]. When used in pulp capping procedures, the polymerization in TheraCal LC is linked to lower heat generation, which minimizes deleterious pulpal effects [20].

ApaCal ART a novel pulp capping agent which is light-cured and is primarily indicated for various pulp capping techniques. It is comprised of calcium hydroxide as a primary component, a resin matrix of triethylene glycol dimethacrylate [TEGDMA] and urethane dimethacrylate [UDMA] fortified with tricalcium phosphate and hydroxypatite fillers, barium zirconate oxide and silanated barium glass powder which serve as radiopacifiers, photoinitiator and amine accelerator.

Tricalcium phosphate has the potential to function as a phosphate reservoir and enhance cement reactivity through the nucleation of calcium phosphate nanoapatite, which stimulates pulpal cells to aid in the dentin bridge formation [21; 22]. Additionally, calcium phosphate granules have also been detected in ApaCal ART. Calcium phosphate accelerates the formation of hydroxyapatite as it provides additional phosphate from the biological fluid for this process [23].

It has been proposed that the alkaline pH functions as a regional buffer to neutralize the inflammatory process's acidic responses in addition to activating the alkaline phosphatase [ALP] that play an important role in hard-tissue formation [24].

Research has indicated that a high concentration of hydroxyl ions from calcium hydroxide is necessary for the initial changes that lead to the differentiation of pulp cells into odontoblasts [25]. A minimum of six to eight weeks is needed for adequate remineralization of the cavity floor after the pulp capping procedure. The ability of the provisional and final restorations to maintain a hermetic seal against microleakage is crucial for a satisfactory outcome [26; 27].

The use of newer light cured pulp capping agents permit the clinician to etch and bond the lining material to aid in the placement of final restorations, thus increasing the efficiency of the clinician. These advancements have translated to excellent clinical outcomes for pulp capping procedures. In this study, it has been observed that TheraCal LC had an increased calcium ion release when compared to ApaCal ART. An increased calcium ion release aids in rapid tertiary dentin formation and the dentin bridge formed, acts as a protective barrier to the pulp space in deep restorations. A novel method for digitally evaluating dentin bridge formation with CorelDRAW X7 software has been developed [28]. The results of the study provide the opportunity to compare the materials by further clinical and experimental studies for various clinical applications.

Inevitably, the limitations of this in vitro study include difficulty to precisely simulate the biological aspects and the multitude of intraoral conditions which are not accounted. Future studies should investigate the long-term performance of these materials in vivo, focusing on clinical outcomes expanding to include other bioactive materials.

CONCLUSION

In line with the study findings, it was observed that both TheraCal LC and ApaCal ART showed a sustained increase in calcium ion release over time with the highest calcium ion release noted at 21 days.

TheraCal LC demonstrated a significantly higher calcium ion release when compared to ApaCal ART at 24 hours, 7 days and 21 days and may be preferable for indirect pulp capping because of their greater ion-releasing ability and stimulation of hard tissue formation.

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