Journal of Biomedical Engineering MANUSCRIPT
ISSN: 1001-5515 Vol. 40 No. 3 (2023)

Evaluation and comparison of release of calcium, phosphate ion and pH of three commercially available pulp capping materials - An Invitro study

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Abstract:
Background: The ultimate goal of direct pulp capping is to safeguard the underlying pulp and sustain pulp vitality by rebuilding reparative dentin at the Materio Pulpal Complex, which acts as a "biological seal" to protect the underlying pulp tissues. Calcium hydroxide has been the material of choice for the purpose. However, due to varioius disadvantages Tricalcium silica-based cements are being developed.
Aims: To evaluate and compare pH, calcium, phosphate ion release of premixed Neoputty MTA, dual cured calcium silicate cement (TheraCal PT) and light cured calcium silicate cement (TheraCal LC).
Materials and Methods
Neoputty MTA, dual cured calcium silicate cement (TheraCal PT) and light cured calcium silicate cement (TheraCal LC) were prepared and immersed in distilled water. Calcium, phosphate and pH were measured at 6h, 12h, 24h, 7 days, 14 days, 28 days. Following each evaluation, the water was discarded, and the samples were immersed in fresh deionized water. One-way analysis of variance with Tukey's Honest significant was performed.
Result:
pH of the system increased in the initial seven days. The peak was hit on the seventh day followed by gradual decrease for next 28 days. Calcium release was highest in the initial 6 hours and gradual decrease was seen till 28 days. Phosphate release was highest in the initial 6 hours and gradual decrease was seen till 28 days.
Conclusion
Primarily, TheraCal LC shows highest Calcium and phosphate release, which is beneficial for regeneration of pulp. However, it also shows high pH, more than 8, and hence need to be a concern during clinical use.

Keywords: Calcium; Phosphate; pH, Theracal, Neoputty.

1. Introduction

Dental caries is one of the most common infectious oral diseases, affecting more than 90% of adults. (1) Removal of dental caries is obviously the most common routine dental practise due to its high prevalence.

DOI: 10.105515/JBE.40.3.18

During such caries removal processes, particularly on deep caries, either indirect or direct pulp-capping techniques are frequently required. The goal is to stimulate the formation of tertiary (reactionary or reparative) dentin. (2) This essentially preserves the pulp in order to avoid root canal therapy. Pulp preservation is a critical goal in the therapy of young permanent dentition. The goal of pulp therapy is to keep the teeth and their supporting tissues healthy and intact.

In 1756, Phillip pfaff performed the first pulp capping procedure, installing a tiny gold fragment over an exposed vital pulp in order to induce healing. Many materials have been developed and introduced since then. The procedure's success, however, is highly dependent on the conditions under which it is performed, and the outcome relies on the age, type, location, and size of pulp exposure. Furthermore, the pulp capping material must be able to foster reparative dentin formation, maintain pulpal vitality, emit fluoride for preventing secondary caries, possess a bactericidal or bacteriostatic impact, adhere to dentin and the restorative material, oppose forces both during and following restoration placement, be radiopaque, and offer a bacterial seal. (3)

Several pulp capping materials are used for this purpose, including calcium hydroxide and hydraulic calcium silicate cements like mineral trioxide aggregate. These materials are placed on the "unexposed" pulp for indirect pulp capping to encourage reactionary dentin formation from the existing odontoblasts in the dentino-pulpal complex. Direct pulp capping, on the contrary, refers to the placement of pulp-capping materials on "exposed" pulp - where odontoblast layers have been breached - to promote reparative dentin development mediated by odontoblast-like cells differentiated from dental pulp stem cells at the materio-pulpal complex.

Unlike indirect pulp capping, which typically has foreseeable clinical results, direct pulp capping often has variable outcomes based on the operator technique, material properties, and host pulpal responses. The ultimate goal of direct pulp capping is to safeguard the underlying pulp and sustain pulp vitality by rebuilding reparative dentin at the Materio Pulpal Complex, which acts as a "biological seal" to protect the underlying pulp tissues, increase tooth life expectancy, and improve overall oral health. An effective pulp capping procedure can help to avoid more invasive and costly dental procedures like root canal therapy. Hence, to maximise regeneration of reparative dentin, it is critical to optimise direct pulp-capping techniques, improve material biocompatibility, and improve biological responses of pulp tissues.

Indirect Pulp Capping, which induces remineralization, calcium hydroxide has been the material of choice. Calcium hydroxide's disadvantages include its poor physical properties, high solubility, and slow dissolution. Tricalcium silica-based cements are being developed to compensate for the shortcomings of calcium hydroxide-based materials. Calcium silicate-based liners that can be used as indirect pulp capping materials include MTA, Biodentine, and Theracal LC. TheraCal LC (Bisco, USA) is a light-cured resin-modified calcium silicate-filled material that is made up of Type III Portland cement, fumed silica, strontium glass, barium sulphate (BaSO4), barium zirconate (BaZrO3), and polyethylene-glycol dimethacrylate monomers. TheraCal LC has good sealing properties, a high alkalinity for pulpal healing and reparative dentine formation, but it quickly returns to a neutral pH. Theracal PT (ThPT, Bisco Inc) is a dual-cured resin modified calcium silicate-based material. It is most commonly used for pulpotomies, but it can also be used for indirect and direct pulp capping. TheraCal LC is toxic to pulpal fibroblasts and has lower bioactivity, a high inflammatory tendency, and partial or complete disorganisation of underlying pulp tissue. When used in pulp capping, TheraCal LC has lower antibacterial activity against Streptococcus mutans. To address these shortcomings, Neoputty MTA was recently introduced. Neoputty MTA is a premixed syringe material with disposable plunger tips that was introduced in 2020. It is a bioactive tricalcium silicate-based bioceramic material with excellent handling properties. It encourages the formation of hydroxyapatite by releasing calcium and hydroxide ions. Because it is available as a premixed syringe, it can be placed directly into the canal, reducing material waste and chair side time. The aim of this In-vitro study is to evaluate and compare pH, calcium, phosphate ion release of premixed Neoputty MTA, dual cured calcium silicate cement (TheraCal PT) and light cured calcium silicate cement (TheraCal LC).

2. Materials and Methods

Materials:

Neoputty MTA, dual cured calcium silicate cement (TheraCal PT) and light cured calcium silicate cement (TheraCal LC) were purchased and used as received according to manufacturer's instructions. All other chemicals used were of analytical grade.

Formation of samples:

DOI: 10.105515/JBE.40.3.18

Polyethylene tubes were cut into 30 tubes of equal sizes, with each tube measuring 5.0 mm length × 4.0 mm diameter using bard parker blade and digital Vernier caliper. The tubes were pre-weighed by a digital weighing balance machine to select similar weight tubes. The tubes were prewashed with 5% nitric acid to prevent interference with phosphate ions and alkaline metals. The polyethylene mounted tubes were divided as:

- Group 1 (n = 10) – tubes filled with TheraCal LC
- Group 2 (n = 10) - tubes filled with TheraCal PT
- Group 3 (n = 10) - tubes filled with Neoputty MTA

Freshly mixed materials were prepared according to manufacturer's instructions. After complete filling of the tubes, the materials were condensed with the hand pluggers to avoid any voids in the inserted material. The filled polyethylene tubes were weighed to ensure equal quantity of material in each sample. Samples were placed in polypropylene flasks, containing 10 ml of deionized water. The deionized water is verified for the total absence of calcium ions and the presence of neutral pH (6.8). The flask was closed with the lid, and the samples were subsequently stored in an incubator at 37°C . At 6h, 12h, 24h, 7 days, 14 days, 28 days the deionized water was measured for pH by a Digital pH meter and released calcium and phosphate ions were measured by atomic absorption spectrophotometer. Following each evaluation, the water was discarded, and the samples were immersed in fresh deionized water.

3. Statistical analysis:

Data regarding values of calcium, phosphate ion release and pH of 3 groups were entered into Microsoft Excel and analysed using IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, N.Y., USA). The calcium, phosphate ion release and pH of 3 groups were analysed using One-way analysis of variance (ANOVA) followed by multiple comparisons with Tukey's Honest significant difference test (α=0.05). The level of statistical significance was determined at $p \leq 0.05$.

4. Results:

Table 1 : pH at different time interval were analysed using One-way analysis of variance (ANOVA).

Groups	Mean					
	6 hrs	12 hrs	24 hrs	7 days	14 days	28 days
Group 1	8.568	8.974	9.238	10.574	9.594	9.250
Group 2	7.140	7.446	7.848	8.152	7.646	7.436
Group 3	7.750	8.072	8.748	8.974	7.450	7.142
P value	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
1 vs 2	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
1 vs 3	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
2 vs 3	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

*Statistically Significant ($p \leq 0.05$)

Table 2 : Calcium ion release of three groups

Groups	Mean					
	6 hrs	12 hrs	24 hrs	7 days	14 days	28 days
Group 1	110.200	99.536	83.602	51.250	33.542	15.250
Group 2	70.950	65.262	55.544	35.360	19.548	7.266
Group 3	94.558	91.256	82.432	49.462	31.618	12.068
P value	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
1 vs 2	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
1 vs 3	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
2 vs 3	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

*Statistically Significant ($p \leq 0.05$)

Table 3 : Phosphate ion release of three groups

	Mean

DOI: 10.105515/JBE.40.3.18

Groups	6 hrs	12 hrs	24 hrs	7 days	14 days	28 days
Group 1	97.282	91.500	85.514	57.242	25.466	9.270
Group 2	62.528	53.156	33.392	17.836	8.240	4.690
Group 3	83.556	73.568	62.802	33.240	17.958	8.232
P value	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
1 vs 2	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
1 vs 3	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
2 vs 3	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

***Statistically Significant ($p \leq 0.05$)**

5. Discussion

The pulp capping procedure aims to produce high-quality reparative dentin that covers the exposed pulp. Pulpal wound healing, including reparative dentin formation, is a multi-factorial, complex process coordinated by discrete but overlapping steps of pulp cell migration, proliferation, and mineralization. (4) Unlike reactionary dentin, which is formed by existing odontoblasts, reparative dentin is formed when the pulp is exposed and the existing odontoblastic layers are breached by odontoblast-like cells presumably differentiated from DPSCs. These DPSCs are anticipated to migrate to and expand on the materio-pulpal complex, where they will eventually undergo odontogenic differentiation to form reparative dentin.

Although pulp capping agents are thought to be odontoconductive in the sense that they act as scaffolds for the correct performance of these processes by dental pulp stem cells, clinical and molecular studies show that they are also odontoinductive in the sense that they may encourage dental pulp stem cells to go through odontogenic differentiation and mineralization.

Although an alkaline environment is required to create an osteoinductive environment, mineralization is extremely sensitive to pH change; alkaline phosphatase activity spikes at pH 7.37 and decreases significantly above this physiologic level. (5) Furthermore, pH levels above 8.0 have been shown to inhibit mineralization both in vitro and in vivo. (6)

In the current study, pH of the system increased in the initial seven days. The peak was hit on the seventh day followed by gradual decrease for next 28 days. Although the trend was similar, there was a significant difference in pH between the groups with group 1 having the highest pH (TheraCal LC). Group 2 and 3 followed the group 1.

According to Lin et al., (2022)(7), after 90 days of initiation, Biodentine® and MTA Angelus® showed appreciably higher pH, while Bio-Cap® and Lime-Lite™ had lower effects in raising the pH. Beegum et al., (2021)(8) showed that all 3 materials TheraCal LC, ProRoot MTA and Calcimol LC showed slightly alkaline pH after 28 days.

Extracellular Ca2+ stimulates the differentiation of dental mesenchymal cells into odontogenic cells. Ca2+ therapy alone caused osteogenic gene expression in dental pulp cells, such as osteopontin and BMP2. (9) Mizuno et al. also demonstrated that Ca2+ released by direct pulp capping agents triggered fibronectin gene expression in dental pulp cells, a process that could induce differentiation of these cells into mineralized tissue forming cells. (10) Elevated Ca2+ levels have also been shown to promote differentiation and the mineralization in different dental mesenchymal cells, such as cementoblasts, by raising fgf-2 expression.(11)

In the current study calcium release was highest in the initial 6 hours and gradual decrease was seen till 28 days. Compared to 24 hours, at seven days, the release was almost half of the first day level. Group 1 (TheraCal LC) had the high amount of release in all time intervals followed by group 3and group 2.

Biodentine® and MTA Angelus® released significantly more cumulative calcium ions, followed by Ceramir® Protect LC and Dycal®; Lime-LiteTM had the least amount of calcium ions, according to Lin et al., (2022).(7) According to Beegum et al., (2021), TheraCal LC had the highest calcium release among TheraCal LC, ProRoot MTA, and Calcimol LC.(8)

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Phosphate release from pulp capping agents have certain roles to play in pulpal regeneration. It may serve as a source of phosphate ion for reparative dentin formation. As a component of natural bone it can be a cue for differentiation of pulpal cells to form the initial dentinal bridge. Various ratios of phosphorus with calcium show various properties towards the target cells. It should also be noted that materials without phosphorus are also successfully used for the purpose like calcium silicate. However, calcium phosphate cements continue to remain as gold standard for the purpose.(12)

In the current study phosphate release was highest in the initial 6 hours and gradual decrease was seen till 28 days. Compared to 24 hours, at seven days, the release was almost two thirds of the first day level. Group 1 (TheraCal LC) had the high amount of release in all time intervals followed by group 3and group 2. This study is one of the pioneering works in evaluating phosphate release as most of the studies have only seen calcium and pH. Therefore, it has thrown new light into the literature on how phosphate release changes with time.

6. Conclusion

Within the limitations of the study, following conclusions can be made. Primarily, TheraCal LC shows highest Calcium and phosphate release, which is beneficial for regeneration of pulp. However, it also shows high pH, more than 8, and hence need to be a concern during clinical use.

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DOI: 10.105515/JBE.40.3.18