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ORIGINAL ARTICLE

Clinical Comparison of Eggshell Derived Calcium Hydroxyapatite with Dycal® as Indirect Pulp Capping Agents in Primary Molars

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Abstract

Objective: To evaluate and compare the efficacy of eggshell derived calcium hydroxyapatite with Dycal® as indirect pulp capping material in primary molars. **Material and Methods:** A total of 32 carious primary molars from 22 children (6-10-years) were screened, of which 26 primary molars meeting inclusion criteria were selected and equally divided into two groups. At the first appointment, the infected dentin was excavated using a spoon excavator after treating the carious part with Carie-Care™ chemomechanical caries removal agent. After this, eggshell derived calcium hydroxyapatite in Group 1 and Dycal® in Group 2 were used as liners followed by restoration of the cavity with type IX glass ionomer cement. Clinical assessment for pain and radiographic assessment for measurement of the amount of reparative dentin thickness formation was performed at baseline, 8 weeks and 3 months. The data were subjected to statistical analysis by one-way ANOVA and Kruskal-Wallis tests. **Results:** Higher mean reparative dentin formation was found in eggshell derived calcium hydroxyapatite group than Dycal® group at the end of 8 weeks and 3 months and the difference was significant statistically ($p<0.001$). **Conclusion:** Eggshell derived calcium hydroxyapatite seems to be a suitable alternative to Dycal® (calcium hydroxide) that can be used as a liner for indirect pulp capping in primary molars.

Keywords: Tooth, Deciduous; Dental Pulp Capping; Dentin, Secondary; Durapatite.

Introduction

Deciduous teeth in the dental arch are essential for the proper growth of facial and skeletal complex. To preserve the deciduous teeth with deep dental caries until their natural exfoliation, pulp therapy has to be performed [1]. Pulp therapy is a treatment objective to maintain the integrity and health of teeth and their supporting tissues and also to maintain the vitality of the pulp of a tooth affected by caries, traumatic injury, or other causes [2].

Treating reversible pulpal injuries and maintaining pulp vitality/function are the primary objectives of vital pulp therapy (VPT) in deciduous teeth. Three VPT therapeutic approaches include indirect pulp capping (IDPC) for teeth with dentinal cavities and reversible pulpitis, direct pulp capping (DPC) and pulpotomy, which are considered in cases of pulp exposure [3]. "*Indirect pulp treatment is a procedure performed in a tooth without signs or symptoms of pulp degeneration but with a deep carious lesion approximating the pulp. The surface of pulp is covered with a biocompatible material and caries surrounding the pulp is left in place to avoid pulp exposure*" [2].

Most commonly used materials for indirect pulp capping are Calcium hydroxide, zinc oxide eugenol cement, corticosteroids, calcium phosphate, and CO2 lasers. Each of these materials have their own drawbacks. Calcium Hydroxide ($Ca(OH)_2$) was introduced by Hermann into dentistry [4] and according to Ca(OH)2 compounds are the gold standard for pulp capping in human teeth [5]. Calcium hydroxide exhibits poor sealing ability and zinc oxide eugenol produces chronic inflammation and also lacks calcific barrier formation [6]. However, due to the reduced biological efficiency and hazardous nature of some of the used dental cements, newer and safer substitutions are needed, particularly those possessing higher antimicrobial activity than their traditional complements [6]. Many animal and human studies postulated mineral trioxide aggregate (MTA) to be superior and good substitute to $Ca(OH)_2$ as a pulp-capping agent. But MTA has certain disadvantages such as instance, it is difficult to handle, has a long setting time, and has high material cost [7,8].

Various bio inductive materials were developed in recent times for use as pulp capping agents in primary teeth. Biodentine, new bioactive cement, with dentin-like mechanical properties, stimulates tertiary dentin formation, making it an alternative to conventional calcium hydroxide-based materials. However, Biodentine is expensive and is not indicated for root caries. Theracal LC is a resin-modified calcium silicate filled liner consisting of tricalcium silicate particles that provide calcium release. Drawback of Theracal LC is its opaqueness and whitish color, so it should be kept thin so as not to show through composite materials that are very translucent affecting final restoration shading. Novel Endodontic Cement is a water-based tooth-colored white powder consisting of hydroxyapatite particles possessing high pH due to this calcium ions which are released react with endogenous phosphate creating a colloidal gel that solidifies and forms hydroxyapatite. But further assessment is required for evaluation of pulp response to this material in the inflamed pulp [1].

Hydroxyapatite (HAp) is the most thermodynamically stable of the synthetic calcium phosphate ceramics having good biocompatibility with neutral pH -7.0 [9]. Eggshell is considered a rich source for calcium as carbonates and oxides that qualify them as excellent sources of hydroxyapatite. Eggshell provides a cost-effective and renewable as well as a sustainable source of biological Hap [10]. The effects of eggshell derived calcium hydroxyapatite on reparative and reactionary dentin formation in primary molars following application as an indirect pulp capping agent in deep carious lesions has not been documented.

In clinical practice, calcium hydroxide has been reported as a liner of choice in patients with deep cavities [11,12]. So, the purpose of this study was to evaluate and compare the efficacy of eggshell derived

calcium hydroxyapatite as indirect pulp capping agent with Dycal® (Calcium hydroxide) in deep carious lesions of primary molars.

Material and Methods

Study Design

Among the 42 primary molars screened in 22 healthy cooperative children (6–10 years) who attended the Outpatient Department of Pediatric and Preventive Dentistry, 26 deep carious primary molars with a history of dull, reversible pain, or mild discomfort on chewing were included in the present study.

The criteria for participation in the research were: teeth with deep carious lesions without involvement of pulp; teeth exhibiting symptoms of reversible pulpitis; on radiographic interpretation, the depth of caries should be greater than two-third of dentin thickness approaching pulp with no radiolucency in the periapical or furcation area of the teeth [13]; and children with carious primary molar teeth belonging to the ICDAS-3 to ICDAS-4 category on occlusal surfaces according to the ADA Caries Classification System [14].

The exclusion criteria were teeth with history indicating irreversible pulpitis, teeth with deep carious lesions involving pulp, teeth with radiolucency in the periapical or furcation area, teeth near exfoliation and patients with systemic medical conditions.

Sampling

The sample size was calculated based on the level of precision at ± 5, the level of confidence at 95% and the desired level of confidence interval was taken as 95%. The selected 26 carious primary molars were randomly assigned and equally divided into two groups: Group 1: Eggshell derived calcium hydroxyapatite and Group 2: Dycal® - calcium hydroxide (Dentsply Caulk, Milford, DE, USA).

Eggshell Derived Calcium Hydroxyapatite Preparation

After thorough washing, hen's eggshells were heated inbox furnace at 900°C for 2 hrs to decompose organic matter. After exposure to the atmosphere, it gets converted into calcium hydroxide. In an agate pestle and mortar, the product was finely ground. Calcium hydroxide was weighed and mixed with distilled water to form 0.3 M suspension. Later the suspension was treated with 0.5 M diammonium hydrogen phosphate solution corresponding to the stoichiometric ratio of Ca/p = 1.67. The mixed reactants were irradiated in a domestic microwave oven and the obtained product was then washed repeatedly with distilled water. This process removed the unwanted ions and was allowed to dry overnight in an oven at 100°C. The end product obtained after this whole process is eggshell derived calcium Hydroxyapatite (EHA) (Figure 1) [5].

Clinical Procedure

The teeth selected after thorough diagnosis were divided into two groups randomly. Indirect pulp capping treatments for both groups in this study were carried out by a single operator. The selected teeth were isolated with cotton rolls followed by application of chemomechanical caries removal agent Carie-Care™ (Eco Dental Works Pvt. Ltd, Bengaluru, India) for 1 minute. Later, as Carie-Care™ gel turns cloudy only softened infected carious dentin was scraped gently without application of vertical pressure with sharp spoon excavator. The process was continued until the gel was no longer cloudy. The cavity was checked for complete removal of caries by absence of any discoloration, a catch, or a tug back position on smooth passage of a blunt probe or curved end of an explorer [15]. After satisfactory caries removal was achieved, the remaining gel was rinsed

away with water and cavity was dried with sterile cotton pellet. In Group 1, eggshell derived calcium hydroxyapatite was mixed with saline and applied as a liner over the cavity floor with the help of cement carrier and in Group 2 Dycal® was placed as a liner to the cavity floor with a ball-ended condenser after mixing 1:1 proportions of both base and catalyst paste on the mixing pad for ten seconds until achievement of uniform color. The final restoration was done with glass ionomer cement (GC Gold Label Type IX, GC Corporation, Tokyo, Japan). An immediate postoperative radiograph was taken.

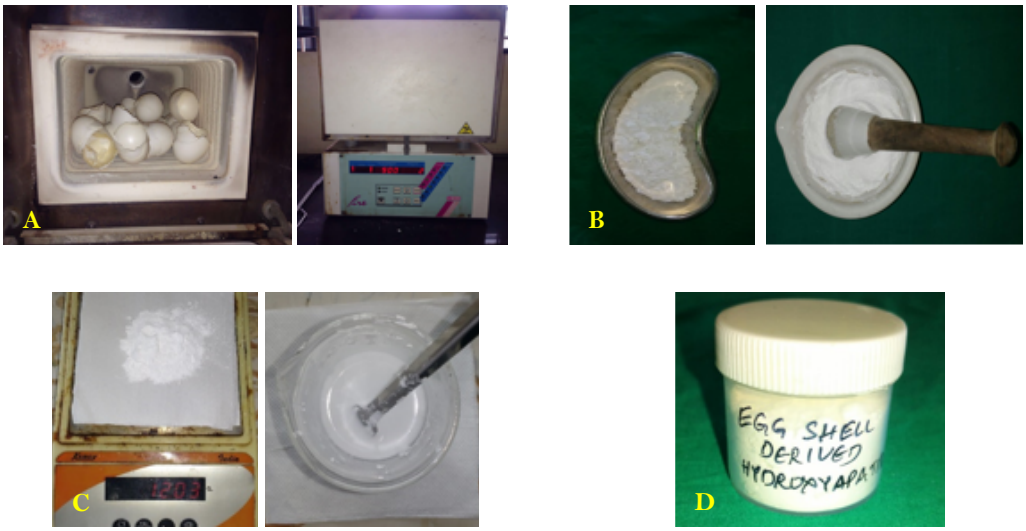

Figure 1. Preparation of eggshell derived calcium hydroxyapatite. A: Eggshells were heated in box furnace. B: Grounded calcium hydroxide. C: Weighed calcium hydroxide is mixed with 0.5 M diammonium hydrogen phosphate solution. D: Eggshell derived calcium hydroxide.

Clinical and Radiographic Assessment

The treated teeth were followed up clinically for evaluation of pain and radiographically for the amount of reparative dentin thickness formed at intervals of 8 weeks and 3 months, respectively. Pain rating was scored as 1 for the presence of pain and 0 in the absence of pain. Each radiograph was captured on Vatech® RVG with AMS® dental X-Ray machine with a tube current of 7mA and 230 V with exposure time adjusted to 440ms. Later the obtained digital radiograph was magnified to 5X and the formed reparative dentin thickness was measured as the difference between the thickness at initial visit subtracted with thickness of dentin at follow-ups. The resultant dentin thickness was then divided by 5 to obtain the original thickness of reparative dentin formed (Figure 2).

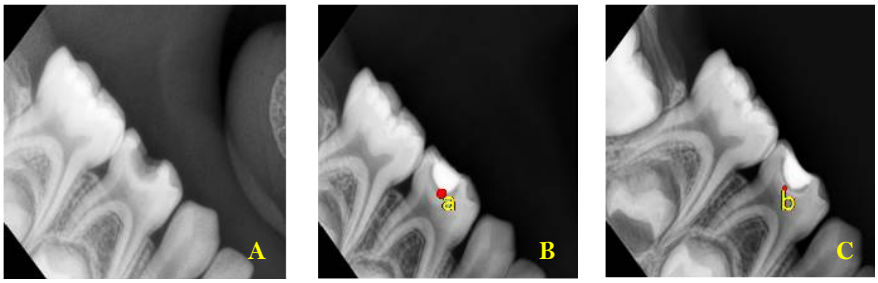

Figure 2. Measurement of the amount of reparative dentin formed. A: Initial radiograph. B: Radiograph immediate. C: Radiograph at follow-up. The radiograph is zoomed by 5X. The distance between restorative material and dentine is calculated post operative (a) and at next follow-up (b). The amount of reparative dentin formed is (b-a)/5.

Data Analysis

The obtained data were subjected to statistical analysis by one-way ANOVA test and Kruskal-Wallis test using SPSS Software (IBM Corp, Armonk, NY, USA), version 16.

Ethical Aspects

Ethical clearance was obtained from the Institutional Ethical Committee (Protocol/ IRB/35/2014-17). Appropriate informed consent was obtained from the parents of the selected children prior to treatment.

Results

The mean calculated for pain rating in Group 1 was 0 at baseline, 0.05 at 8 weeks and 0 after 3 months. The mean calculated for pain rating in Group 2 was 0 at baseline, 0.25 at 8 weeks and 0.20 after 3 months. The p-value was 0.952 and 0.356, respectively, for Groups 1 and 2 (Table 1).

Table 1. Comparison of mean pain rating scores at baseline, 8 weeks and 3 months between groups 1 and 2.

Groups	Baseline	8 Weeks	3 Months	p-value*
G1	0	0.05	0	0.952
G2	0	0.25	0.20	0.356

*Kruskal-Wallis Test.

When the mean thickness of reparative dentin formed in Group 1 was calculated, it showed a mean of 0.062 ± 0.001 in the first 8 weeks and 0.091±0.003 at 3 months follow-up. The mean thickness of reparative dentin formed in Group 2 was calculated, which showed a mean of 0.050 ± 0.001 in the first 8 weeks and 0.061 ± 0.001 at 3 months follow-up. For Groups 1 and 2, the p-value was E-2.12 ($p<0.001$) and E-3.69 ($p<0.001$), respectively, when reparative dentin thickness formed was compared at follow-ups of 8 weeks and 3 months (Table 2).

Table 2. Comparison of reparative dentin thickness deposited within two groups at 8 weeks and 3-month intervals.

Groups	Time Interval	Mean	SD	p-value	F-value
G1	8 Weeks	0.062	0.001	E-2.12	62.49
	3 Months	0.091	0.003		
G2	8 Weeks	0.050	0.001	E-3.69	10.08
	3 Months	0.061	0.001		

*One-way ANOVA; $p<0.001$ – Highly significant; E-Standard notation for powers of 10.

When the inter-group comparison was made at the end of 8 weeks and 3 months, the p-value was E-1.4 ($p<0.001$) and E-6.38 ($p<0.001$), respectively, which was found to be highly significant (Table 3).

Table 3. Intergroup comparison of reparative dentin thickness deposited at 8 weeks and 3-month intervals.

Groups	Time Interval	Mean	SD	p-value	F-value
G1	8 Weeks	0.062	0.001	E-1.7	11.17
G2		0.050	0.001		
G1	3 Months	0.091	0.003	E-6.38	75.99
G2		0.061	0.001		

*One-way ANOVA; $p<0.001$ – Highly significant; E-Standard notation for powers of 10.

Discussion

Dental caries is the localized destruction of susceptible dental hard tissues by acidic by-products from the bacterial fermentation of dietary carbohydrates. Dental caries is a chronic disease that progresses slowly in most people, resulting from an ecological imbalance in the equilibrium between tooth minerals and oral biofilm. The goal of treatment is to preserve tooth structures and prevent further destruction of the tooth. Most importantly, whether the carious lesion is cavitated or non-cavitated dictates the management [10].

Deep caries may induce reversible and or irreversible inflammatory changes in the pulp. Therefore, Indirect Pulp Treatment (IPT) based on a diagnosis of reversible pulpitis, may improve the prognosis for the tooth and maintain its vitality. A previous study evaluated 133 primary molars with deep caries approaching the pulp, which were treated with either Formocresol Pulpotomy (FP) (N=78) or IPT (N=55) and follow up was done for 2-7 years. They found success of IPT significantly higher than FP (P=0.01) in the treatment of deep caries approaching pulp [16]. IPT is less expensive, has fewer potential side effects, and does not exhibit early exfoliation as pulpotomy does [17].

Calcium hydroxide (CaOH) was originally introduced to the field of endodontics by Herman in 1920 as a root canal filling and antimicrobial agent. Since then, CaOH has been used for various endodontic procedures, including pulp capping, pulpotomy, intracanal medication, apexification, root perforation, and is found in some root canal sealants [18]. It has been shown that cements containing calcium hydroxide could provide antibacterial activity and sterilize carious dentin [6].

Calcium hydroxide (Dycal®) has been a gold standard for pulp capping following Zander's initial publication in 1939. The unfavorable effects of Dycal® like internal resorption, degradation over time, tunnel defects through dentinal bridges under it, and poor sealing properties had resulted in failures of the treatment. So, favor for this conservative treatment approach had declined with time [19]. Previous authors stated that when calcium hydroxide was placed in permanent teeth, it resulted in calcific (dentin) bridge formation, but in primary teeth, it is likely to cause internal resorption [18]. Hence, a new material other than calcium hydroxide with lesser limitations as pulp capping agent in primary teeth is needed.

The most important factor in the success of VPT is maintenance of vitality of the pulp and, in particular, the presence of proper vascularization, which is necessary for active formation/function of the odontoblasts [3]. When CaOH is placed in deciduous teeth, the same high alkaline pH could likely trigger existing pre odontoclasts (stromal undifferentiated mesenchymal cells) to transform into odontoclasts [18]. MTA, biodentine and few other materials are being used as alternatives to calcium hydroxide, but they were not cost-effective. So, the need for a new cost-effective indirect pulp capping agent that exhibits similar beneficial properties of all these materials with lesser side effects has araised.

Restored teeth with partial caries removal have equal success compared to restored teeth with complete caries removal [20]. In the present study, chemomechanical caries removal was done with Carie-Care™ for the preservation of the affected dentin. Carie-Care™ softens the carious dentine, which is then hand excavated and claims that it will not affect the underneath healthy affected dentine. Carie-Care™ not only softens infected dentine but also gives additional advantage of anti-inflammatory activity and aroma [21].

The bioavailability of calcium (Ca) ions plays a key role in the various biological events on cells involved in the new formation of mineralized hard tissues. Ca ions stimulate the expression of bone-associated proteins mediated by calcium channels and large quantities of Ca ions could activate adenosine triphosphate (ATP), which plays a significant role in the mineralization process [22]. The essential part of eggshell constituents is represented by mineral with 95.1%, proteins (3.3%) and water (1.6%). With 37.3% of the total

weight, calcium is the main mineral component - mostly in crystalline form as the existing calcium is calcium carbonate ($CaCO_3$) with 93.6% followed by calcium triphosphate (0.8%) and magnesium carbonate) [23].

Calcium plays an active role in remineralization of enamel and eggshell derived calcium hydroxyapatite has a very high percentage of bio-available calcium. The calcination process obtains pure powder free of pathogens. Previous authors evaluated the remineralization potential of enamel surface lesion using chicken eggshell powder (CESP) solution and concluded that the high pH along with the rich bioavailable calcium content of the chicken eggshell solution has the potential to favor remineralization [24].

It has been shown that hydroxyapatite (HA) has antibacterial properties against cariogenic bacteria and that it would be best to harness the antibacterial property of HA, by using it as a base for the treatment of carious cavities, to act against residual cariogenic bacteria [25]. Some authors have suggested that HA can be used as a prospective candidate for dental applications such as pulp capping or as a baseliner material [26].

Some authors evaluated the pH and antimicrobial properties of newly prepared calcium-based cement from eggshell, polycarboxylate cement and biodentine material and concluded that new calcium-based cement from eggshell has better antimicrobial properties than biodentine and polycarboxylate cement. Calcium-based cement from eggshell gave 9.4 pH after five minutes from the reaction and ended in 11.4 pH after 24 hours of reaction [27].

In the present study, the amount of reparative dentin formed after 3 months for Group 2 was 0.061 ± 0.001 which was similar to a study conducted by George V et al where the increment in dentin deposited was 0.068 mm at the first 3 months with Dycal® as a liner [19]. A Njeh studied the reactionary and reparative dentin formation after pulp capping with Dycal®. The thickness of reparative dentin varied between 100 and 200 µm (0.1-0.2 mm) after 4 weeks [28]. Whereas, in the present study the reparative dentin thickness formed by eggshell derived calcium hydroxyapatite (0.091 ± 0.003) was much higher than Dycal® (0.061 ± 0.001) at the end of 3-months review. For remineralization to occur, bioavailable calcium and phosphates are essential [29,30]. Therefore, the rich bioavailability of calcium along with the high concentration of phosphates present in EHA coupled with its increased pH, may have been responsible for increased reparative dentine formation [31].

In the present study, only 1 case reported with pain in Group 1 at 8 weeks follow-up. Because of eggshell derived calcium hydroxyapatite's superior ability of reparative dentin formation, biocompatibility, lack of disease transfer risks, ease of use, easily autoclavable without any changes in biological properties and unlimited availability makes EHA a viable choice as indirect pulp capping material for primary molars [32].

The present study involved a small sample size and the results of this study need to be confirmed in large population-based longitudinal studies. The shelf life and effectiveness of eggshell derived calcium hydroxyapatite as an indirect pulp capping agent in permanent teeth needs to be evaluated further.

Conclusion

Eggshell derived calcium hydroxyapatite yielded better results compared to Dycal® in the parameters of pain and reparative dentin thickness formation. So, EHA can be suggested as a very cost-effective, efficient substitute to Dycal® as indirect pulp capping agent in primary molars.

Authors' Contributions

SPR	0000-0001-7264-7207	Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing – Review and Editing.

MGP	0000-0001-9280-402X	Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing – Review and Editing.
ANR	0000-0002-7006-2587	Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing – Review and Editing.
RVS	0000-0002-1810-505X	Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing – Review and Editing.
DVD	0000-0002-2547-5024	Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing – Review and Editing.
KVKSK	0000-0002-0206-8805	Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing – Review and Editing.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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None.

Conflict of Interest

The authors declare no conflicts of interest.

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