

Histological Analysis of Human Pulp by Comparing Propolis, Mineral Trioxide Aggregate and Calcium Enriched Mixture as a Direct Pulp Capping Agent in Primary Molars

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ABSTRACT

Aim: To evaluate the histological pulp response to three direct pulp capping agents (Propolis, Mineral Trioxide Aggregate and Calcium Enriched Mixture) in primary molars.

Study design: Comparative study

Place and duration of study: Pediatric Dentistry department and Oral Pathology department at de'Montmorency College of Dentistry, Lahore from 1st May 2016 to 31st December 2016.

Methodology: Fifty-seven human vital primary first molars were selected from patients aged 8 years. They were divided into three groups of nineteen each. Propolis, mineral trioxide aggregate (MTA) and calcium enriched mixture (CEM) were used as direct pulp capping (DPC) agents in primary molars. After pinpoint exposure, the teeth were directly pulp capped with above mentioned materials. Teeth were extracted after fifteen days for histological examination of inflammatory pulpal cell response.

Results: In Propolis group (15 days duration), 15 teeth (78.9%) were in grade 1 inflammation, 1 tooth (5%) was in grade 2 inflammation and 3 teeth (15.7%) were in grade 3 inflammation. In Mineral Trioxide Aggregate group 14 teeth (73.7%) were in grade 1 inflammation, 3 teeth (15.7%) were in grade 2 inflammation and 2 teeth (10.5%) were in grade 3 inflammation. In Calcium Enriched Mixture group 15 teeth (78.9%) were in grade 1 inflammation, 3 teeth (15.7%) were in grade 2 inflammation and 1 tooth (5.3%) were in grade 3 inflammation.

Conclusion: The effects of Propolis, Mineral Trioxide Aggregate (MTA) and Calcium Enriched Mixture (CEM) on pulpal tissue are comparable to each other. Propolis is a cheaper material as compared to other direct pulp capping agents and easily available. Therefore, it may be used as an alternative pulp capping agent however, further investigation is required to analyze human pulpal response.

Keywords: Direct pulp capping, Propolis, Mineral trioxide aggregate, Calcium enriched mixture, Primary molars

INTRODUCTION

Despite advancements in dentistry, teeth are still lost at a premature age. Primary teeth maintenance is absolutely necessary until their natural exfoliation. If primary teeth are lost prematurely, it can cause malocclusion leading to esthetic concerns. Therefore, preservice of primary teeth vitality until natural exfoliation duration is important for keeping the arch integrity. Vital pulp therapy (VPT) is a way of saving primary teeth. Vital pulp therapy includes direct pulp capping (DPC), pulpotomy and pulpectomy. Direct pulp capping or pulpotomy are used for retention of primary dentition by most of the dentists¹.

Direct pulp capping is more conservative than pulpotomy or pulpectomy². It involves putting a biocompatible material on the pulpal exposure site. The advantages of this therapy are to protect the pulp against invasion of microorganisms resulting in dentine bridge formation which leads to maintenance of healthy pulpal tissue³. Elements affecting pulp healing are bacteria penetrating filling-tooth junction, harmful effects of materials, vulnerability of dental operations and condition of the pulp⁴. It is an established fact that the microorganisms are responsible for peri-radicular disease and they usually access through carious lesions. An adequate coronal seal and regenerative capacity of peri-radicular tissues are mandatory

for healing of dental pulp and formation of reparative dentine.⁵ Numerous materials have been nominated for protection of exposed pulps. Ca(OH)₂ cement is regarded as a benchmark for DPC as suggested by different studies. In 1930, Herman introduced Ca(OH)₂ as a DPC agent². Numerous publications showed astounding outcomes using Ca(OH)₂ for DPC in milk teeth. However, some disadvantages with Ca(OH)₂ cement are the formation of channels in the tertiary dentine, sclerosed dentine obliterating the chamber, enhanced disintegration in oral fluids, absence of adhesion and degeneration following acidetch⁶.

Due to restricted properties, different materials have been developed for DPC; one of them is MTA. MTA is a better material for pulp capping. MTA is cement made of tri-calcium oxide, tri-calcium aluminates, tri-calcium silicate, silicate oxide and bismuth. It activates regeneration of pulp, periodontal-ligament (PDL) and alveolar bone. However, it is high-priced with extensive setting time and possible discoloration^{7,8}.

A newly introduced endodontic calcium-enriched mixture (CEM) cement has same clinical application as that of MTA, but it has different chemical composition compared to MTA. CEM consists of calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide and calcium chloride⁹. This novel cement has an antibacterial like that of calcium hydroxide and superior to MTA and sealing ability like MTA and can set within 1 hour of duration, more flow and less film thickness than MTA⁹.

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Lately, an organic product named Propolis (Russian penicillin) has demonstrated strong antimicrobial and anti-inflammatory properties. Propolis is physically, a resinous wax-like substance used to fill up cracks, which differs from yellowish to brown in color, and is collected by bees from plants and buds. Usually, raw Propolis constitutes 50% resin/balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, 5% other substances and fragments of wood. Flavonoids and phenolic acids are biologically active molecules in Propolis. Flavonoids have properties against bacteria, fungus, viruses and anti-inflammatory, properties. Propolis has the property to stop the manufacturing of prostaglandins. It contributes to the immune system by enhancing phagocytosis and cell immunity. Moreover, it contains zinc and iron which help in production of collagen^{3,10}.

MATERIALS AND METHODS

This study was conducted in the Pediatric Dentistry Department and Oral Pathology Department at de'Montmorency College of Dentistry, Lahore after approval from Ethical Committee. Fifty-seven human vital primary first molars were selected from patients aged 8 years. They were divided into three groups of nineteen each. After pinpoint exposure, the teeth were directly pulp capped with above mentioned materials. Teeth were extracted after fifteen days for histological examination of inflammatory pulpal cell response. All patients age of 8 years, vital sound primary teeth, space-supervision for mesial-step class-1 cases, permanent canine having 6+ or 7Nolla's stage, possible to restore the tooth and tenderness to percussion is nil were included. The patients complaining of abrupt pain, absence of succedaneous tooth were excluded.

The procedure started with the application of local anesthesia (Lidocaine HCL 2%) (Medicaine). A 0.2% chlorhexidine rinse was done. Teeth were isolated with rubber dam. Class I cavities were made using sterile diamond straight fissure bur (0.8mm X 3mm) (Shofu, Japan). Pulp was exposed with a sterile round ¼ diamond bur (Shofu, Japan). Hemostasis was gained by sterile saline moistened cotton pellets. After the bleeding stopped, the exposed area was capped with materials directly contacting the pulp tissue. In Group I: 100% Propolis extract powder was manipulated with 96% ethyl-alcohol. Then used for direct pulp capping. In Group II: Pro Root Mineral Trioxide Aggregate (MTA) (Angelus) was mixed and used for direct pulp capping. In Group III: Calcium Enriched Mixture (CEM) mixed according to manufacturer's instructions. The mixture was then applied to the site in metallic carrier.

Teeth were then lined with Resin Modified Glass Ionomer Cement (GC Universal Restorative) and then restored with Nano Hybrid Composite Resin (Meta Biomed Nexcomp). After 15 days, teeth were extracted under local anesthesia for orthodontic reason.¹¹ Once extraction was done, the apical thirds of the teeth were removed and fixed in 10% formalin. 20% formic acid was used to demineralize the teeth for 6-8 weeks following which the teeth were washed with distilled water and dehydration was done in ascending grades of N-butyl alcohol and embedded paraffin.

A microtome was used to cut serial sections of 6 µm in width, which were then stained with haematoxylin and eosin on gelatin-coated slides. An experienced pathologist examined and evaluated the slides according to the criteria: Inflammatory cell response grading³: Grade 1: No or few inflammatory cells, Grade 2: Less than ten inflammatory cells, Grade 3: Abscess

in one-third or more of the coronal pulp and Grade 4: Complete necrosis of pulp. The data was analyzed through SPSS-23.

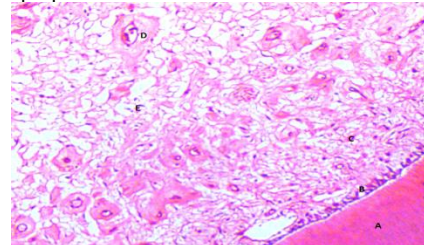
RESULTS

Propolis group (15 day duration), 15 teeth (78.9%) were in grade 1 inflammation, 1 tooth (5%) was in grade 2 inflammation and 3 teeth (15.7%) were in grade 3 inflammation. In Mineral Trioxide Aggregate group 14 teeth (73.7%) were in grade 1 inflammation, 3 teeth (15.7%) were in grade 2 inflammation and 2 teeth (10.5%) were in grade 3 inflammation. In Calcium Enriched Mixture group 15 teeth (78.9%) were in grade 1 inflammation, 3 teeth (15.7%) were in grade 2 inflammation and 1 tooth (5.3%) were in grade 3 inflammation (Table 1). Results are also presented as graphical form in Figures 1-3. There was no statistically significant difference between the type of pulp capping agent and degree of inflammation (p=0.7). Grade 4 inflammation was not included in the results as we found no grade 4 response in any histological slides.

Table 1: Inflammatory cell response for 3 materials after 15 days

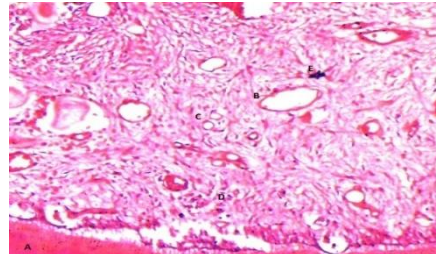
Materials	Grade 1	Grade 2	Grade 3
Propolis	15 (78.9%)	1 (5.2%)	3 (15.7%)
MTA	14 (73.7%)	3 (15.7%)	2 (10.5%)
CEM	15 (78.9%)	3 (15.7%)	1 (5.2%)

Fig. 1: Histological slide of pulpal inflammation in primary 1st molar capped with propolis



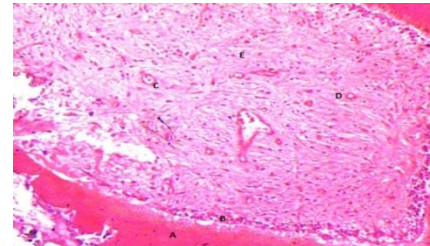
(A=Dentine, B=Odontoblastic layer, C=Fibroblasts, D=Venule, E=Mild inflammation)

Fig. 2: Histological slide of Pulpal inflammation in primary 1st molar capped with MTA



(A=Dentine, B=Venule, C=Arterioles, D=Fibroblasts, E=Few Inflammatory Cells)

Fig. 3: Histological Slide of Pulpal inflammation in primary 1st molar capped with CEM



(A=Dentine, B=Odontoblasts, C=Venule, D=Arteriole, E=Minimal Inflammation)

DISCUSSION

Dental pulp has the ability of repair and regenerate. Clinicians often disagreed the importance of dental pulp in long term prognosis of tooth. In pulp less/root filled teeth, bacteria gain access to the root canal system quickly¹².

Currently all sensibility tests have limitations when it comes to reliability, accuracy, and reproducibility¹³. Vitality tests are also not reliable in children due to variable patient's response¹⁴. Assessing inflammation and presence of necrosis on histopathological sections of pulpal specimen remains the most reliable method in determining pulpal health¹³.

In this study Propolis, MTA and CEM were used as DPC materials. MTA and CEM is a dental material used extensively for vital pulp therapies⁸ and Propolis is a natural substance accumulated by honeybees from several plants¹⁵. In the present study less, inflammation was noted in teeth capped with Propolis and CEM in comparison to MTA however, the difference in dento-pulpal response between three DPC materials was not significant ($p=0.7$).

American Academy of Pediatric Dentistry do not advise, DPC of curiously exposed pulp of a primary tooth¹⁶. Direct pulp capping of deciduous dentition has been reported to be significantly less successful in comparison to pulpotomy, despite high healing capacity of vital primary pulp. High failure rates of direct pulp capping in deciduous teeth may be the result of differentiation of mesenchymal cells to odontoclasts which may lead to internal resorption. It has been previously concluded that direct pulp capping of deciduous teeth should be further investigated as a viable treatment option¹⁶.

In the past different agents have been successfully used for DPC of curiously and iatrogenically exposed deciduous teeth. Caicedo et al¹⁴ reported MTA as a suitable agent in deciduous teeth for DPC and pulpotomies.

Bodem and his colleagues¹⁷ reported a case of lower first deciduous molar in which MTA was used as direct pulp capping material. They found no pathological findings radiographically on 1 year follow up and clinically after 18 months. The tooth remained vital after the procedure. Similarly, Tuna and Olmez¹⁸ compared mineral trioxide aggregate and calcium hydroxide in deciduous dentition. The authors reported no clinical or radiographic failure on 1 year follow up.

Similarly, Ghajari et al¹⁹ successfully used calcium enriched mixture in primary teeth as a direct pulp capping material.

The present study compared Propolis, MTA and CEM for direct pulp capping in deciduous dentition for the very first time. In the past, Parolia and colleagues³ used Propolis, MTA and Dycal as direct pulp capping agents in permanent teeth. So this research was conducted to popularize DPC procedure in children and use of a natural material such as Propolis.

This study lacked in obtaining results regarding dentine bridge formation as done by Parolia et al³. The present study was conducted on iatrogenically exposed teeth, whereas, in case of carious exposure, results would have been different. Also, this study was not done on

extracted teeth under ideal conditions as done by Parolia et al³. This study was done in vivo making it more realistic.

CONCLUSION

The effects of Propolis, MTA and CEM upon pulpal tissue are comparable to each other. Propolis is a cheaper material as compared to other direct pulp capping materials and easily available. It is recommended as a natural direct pulp capping material and suggested for further investigations in treatment of dental diseases.

Conflict of interest: Nil

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