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Comparative Evaluation of Dental Pulp Tissue Response to Different Direct Pulp Capping Materials: An In Vivo Histological Study

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ABSTRACT

Aim: The aim of this study was to evaluate and compare the inflammatory changes of the dental pulp and formation of Dentin Bridge to calcium hydroxide cement, dentin bonding agent, bioactive glass and dentinal chips as a direct pulp capping agent.

Materials and Methods: Forty human premolar teeth scheduled for orthodontic extraction were mechanically exposed. Teeth were divided into four groups (ten each) and were capped with Dentin bonding agent, calcium hydroxide, Bioactive glass and Dentin chips. The cavities were filled with Glass ionomer cement. After 40 days, the teeth were extracted and processed for histologic evaluation and the results were compared statistically by Kruskal Wallis Test and Mann-Whitney Test.

Results: Calcium hydroxide showed the minimum inflammation, maximum number of teeth with Dentin bridge formation, minimum number of cases with bacteria compared to other groups.

Conclusion: Calcium Hydroxide continues to be the gold standard material for pulp capping. Future clinical studies with larger sample sizes and longer evaluation period will confirm the effect of Dentin Bonding agent and Bio active Glass for direct pulp capping.

Keywords: Calcium hydroxide, Bioactive glass, Dentin chips, Dentin bonding agent, Direct pulp capping, Dentin bridge.

INTRODUCTION

The dental pulp has myriad, yet understated functions throughout its life. It produces secondary dentin, peritubular dentin (sclerosis) and reparative dentin under biological and pathological stimuli. It also keeps the dentin supple (less brittle), moist, and resilient; through its circulation extending into the tubular dentin, which in turn protects tooth from masticatory forces. Without a pulp, all these functions cease. [1]

This vitality of the pulp is under attack from various sources, the most common being caries. An attempt to save the tooth itself may lead to an onslaught on the 'life-force' of the tooth i.e. a pulpal exposure. Through vital pulp capping, the emphasis has shifted from the 'doomed organ' concept of an exposed pulp to one of 'hope and

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recovery'.[2] Direct pulp capping involves 'the application of a medicament, dressing or dental material to the exposed pulp in an attempt to preserve the pulp vitality'.[3]

Various materials have been tried as direct pulp capping agents. Calcium hydroxide, which has an anti bacterial effect because of its high pH, is considered to be the 'Gold standard' material for pulp capping of normal vital pulp tissue[4]. Nevertheless, calcium hydroxide has some limitations such as presence of tunnels in dentin barrier, extensive dentin formation obliterating the pulp chamber, high solubility in oral fluids, lack of adhesion and degradation after acid etching.[5]

In an incidental finding in 1921, it was distinguished that the formation of hard tissue in dental pulp can be promoted by dentin chips. These annotations lead some investigators to use dentin chips purposely as a dressing material for pulpotomy and pulp capping.[6]. On the basis of this philosophy, dentine bonding agents have been proposed for the treatment of pulp exposure and it has been demonstrated that they provide promising results[7]. Recently, Bioactive glasses (BAGs) were introduced in the field of dentistry as it can repair bone lesions through osteoblastic potential, thus it is sound that BAGs can refurbish exposed pulp through odontoblastic activity.[4]

In this study the old and new have been pitted against the established and proven. the 'gold standard' calcium hydroxide , 'newer' dentin bonding agent, 'older' dentin chips and the revolutionary Bio active glass have been compared, in vivo and histologically to assess the degree of reparative dentin formation after placement for 40 days.

MATERIALS AND METHODS

Prior to the commencement of the study, ethical clearance was obtained from the Institutional Ethical Committee. The patients for the present study were selected from the outpatient section of Department of Orthodontics,. Ten patients with forty healthy premolars scheduled to be extracted for orthodontic reasons were selected.

Inclusion criteria for the study was patient age ranging from 15-30 years, Clinically intact and non carious tooth, No radiographic evidence of caries, No superficial attrition, No signs of trauma, No history of pain, No systemic disease/medical condition precluding dental treatment

Exclusion criteria for the study was Restored or carious tooth, Tooth with open apex, Tooth showing periapical changes in radiograph, Tooth with root resorption, Allergy to local anesthesia, Unwilling or unable to consent for the study, symptoms suggestive of irreversible pulpitis, reversible pulpitis, pulpal necrosis or periradicular pathology

For all selected teeth, good quality preoperative intra oral periapical radiographs were taken using extension cone paralleling technique to ensure absence of proximal caries and periapical lesions.

Clinical procedure:

Local anesthetic agent containing 2% lignocaine hydrochloride with adrenaline 1:80000 was used. A 2ml disposable single use syringe with a needle size of 0.55 × 25 mm was used to administer anesthesia. For maxillary premolar 0.4-0.6 ml of solution was deposited by infiltration near the mucobuccal fold of maxillary premolar. For mandibular premolar conventional inferior alveolar nerve block was given by administering 1.2 ml of solution at the target nerve block area.

After confirming the onset of local anesthesia, rubber dam isolation of the tooth to be treated was done. Standard occlusal cavities were prepared by means of sterile straight fissure diamond burs (ISO # 107, 1.2mm diameter) at high speed under water coolant. Depth of the cavity was determined by a study of the pre-operative radiograph. The cavities were extending as deep as 3 to 3.5 mm without exposing the pulp tissue. Pulp exposure was performed in the pulpal floor by means of a round diamond bur (ISO # 1, 0.8 mm diameter) under water cooling. One bur was used for each cavity. Homeostasis was established with a sterile cotton pellet soaked in saline solution. The teeth were divided into 4 experimental groups.

Group 1 – pulp capping with Dentin bonding agent
Group 2 – pulp capping with Calcium hydroxide
Group 3 – pulp capping with Bioactive glass

Group 4 – pulp capping with Dentin chips

Pulp capping with Dentin bonding agent:

Adper single bond 2 adhesive system, a two step total etch adhesive system, was used as a pulp capping material in maxillary right first premolar of all ten patients. The Adper single bond was used according to the manufacturer's instructions i.e. the cavity and pulp were etched with 37% Phosphoric acid for 15 seconds and rinsed for 10 seconds. The excess water was blotted using a cotton pellet. Immediately after blotting, 2 consecutive coats of adhesive were applied to etched surface for 15 seconds with gentle agitation using a fully saturated applicator. The cavity was air thinned gently for five seconds to evaporate solvents. Then the adhesive was cured using a curing light (emitting 350 mW/cm^2) for 10 seconds. The cavity was then restored with Glass ionomer cement.

Pulp capping with Calcium hydroxide:

Dycal was used as a pulp capping material in maxillary left first premolar of all ten patients. The Dycal was used according to the manufacturer's instructions i.e. equal volumes of base and catalyst paste were mixed and were applied to the exposed pulp. The cavity was restored with Glass ionomer cement.

Pulp capping with Bioactive glass:

Perioglass was used as a pulp capping material in mandibular left first premolar of all ten patients. Perioglass was used according to the manufacturer's instructions i.e. 8-12 drops of saline were mixed with 1cc of bioactive glass and applied to the exposed pulp. The cavity was then restored with Glass ionomer cement.

Pulp capping with Dentin chips:

Dentin chips were used as a pulp capping material in mandibular right first premolar of all ten patients. Round carbide bur (ISO # 2, 1mm diameter) was used with micro motor hand piece at slow speed to get dentin chips from the cavity walls which was then applied to the exposed pulp. The cavity was restored with Glass ionomer cement.

After 40 days, the treated teeth were extracted in the Department of Oral Surgery. Radiographs were taken before extraction, to detect any periradicular tissue changes subsequent to the restorations. The subjects were also asked to report pain histories for the treated teeth, which may indicate pulpal inflammation, before the extractions were performed.

Histological procedure

The apical third (5 mm) of all roots was sectioned to facilitate fixation in 10% buffered formalin solution for 72 hours. The teeth were decalcified in 10% Nitric acid for 1-2 weeks, then dehydrated in ascending grades of N-butyl alcohol and embedded in paraffin. Six-micrometer-thick sections were cut with a microtome parallel to the main vertical axis of the tooth. The sections, mounted on glass slides, were stained with hematoxylin-eosin. The sections were then evaluated by an oral pathologist according to the criteria used by Cox et al.: [8]

The following statistical methods were applied in the present study:

- Kruskal Wallis Test
- Mann-Whitney Test

RESULTS

The following statistical methods were applied in the present study

- Kruskal Wallis Test
- Mann-Whitney Test

Comparing the Pulpal response among different groups

Inflammatory cell response among four groups:

Inflammatory responses among the four groups were not statistically significant (P value 0.423). (Table I, Graph 1)

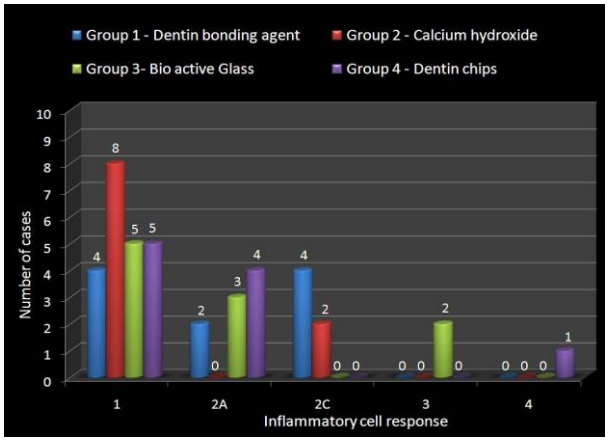
Table I: Inflammatory cell response among four groups.

Inflammatory cell response	Group 1 - Dentin bonding agent	Group 2 - Calcium hydroxide	Group 3- Bio active Glass	Group 4 - Dentin chips
1	4	8	5	5

2A	2	0	3	4
2C	4	2	0	0
3	0	0	2	0
4	0	0	0	1
Total	10	10	10	10
Mean Rank	23.80	16.10	21.55	20.55
P value#	0.423NS			

Kruskal Wallis Test; NS: p > 0.05; Not Significant

Graph 1: Inflammatory cell response among four groups.



The inter group comparison of Inflammatory Cell response among all the groups showed no statistically significant value (Table II).

Table II: Inter-Group Comparison of Inflammatory Cell response using Mann-Whitney Test.

Comparison	Z value	P Value	Significance
Group 1 vs 2	1.563	0.118	Not Significant
Group 1 vs 3	0.401	0.688	Not Significant
Group 1 vs 4	0.808	0.419	Not Significant
Group 2 vs 3	1.246	0.213	Not Significant
Group 2 vs 4	1.070	0.284	Not Significant
Group 3 vs 4	0.124	0.901	Not Significant

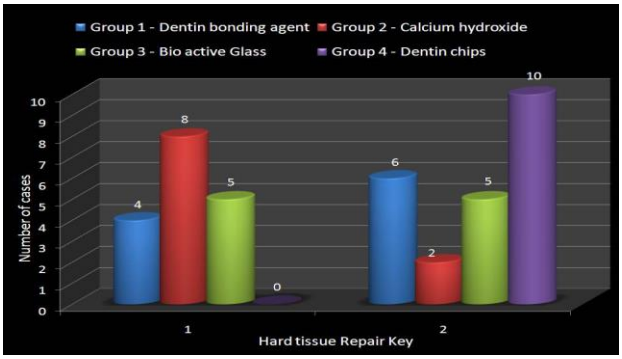
Hard tissue Repair key response among four groups

Table III: Hard tissue Repair key response among four groups.

Hard tissue Repair key	Group 1 - Dentin bonding agent	Group 2 - Calcium hydroxide	Group 3 - Bio active Glass	Group 4 - Dentin chips
1	4	8	5	0
2	6	2	5	10
Total	10	10	10	10
Mean Rank	21.00	13.00	19.00	29.00
P value#	0.004*			

Kruskal Wallis Test; *p < 0.01; Significant at 1%

Graph 2: Hard tissue Repair key response among four groups.



The difference in results between the Calcium hydroxide and Dentin chips was statistically highly significant (P Value <0.001). Dentin bonding agent and Calcium hydroxide displayed results that were statistically significant (P Value 0.029). The result difference between Bio active glass and Dentin chips also was statistically significant (P Value 0.012) (Table IV).

Table IV: Inter-Group Comparison of Hard Tissue Repair key response using Mann-Whitney Test.

Comparison	Z value	P Value	Significance
Group 1 vs 2	1.780	0.075	Not Significant
Group 1 vs 3	0.438	0.661	Not Significant
Group 1 vs 4	2.179	0.029	**Significant**
Group 2 vs 3	1.371	0.170	Not Significant
Group 2 vs 4	3.559	<0.001	**Highly Significant**
Group 3 vs 4	2.517	0.012	**Significant**

Stained bacteria key response among four groups

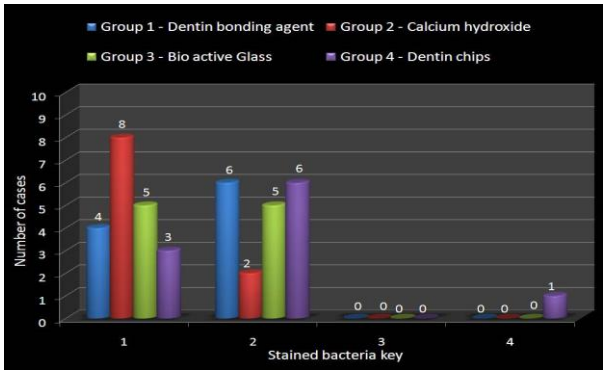
Stained bacteria key response among four groups were not statistically significant (P Value 0.117) (Table V, Graph 3)

Table V: Stained bacteria key response among four groups.

Stained bacteria key	Group 1 - Dentin bonding agent	Group 2 - Calcium hydroxide	Group 3 - Bio active Glass	Group 4 - Dentin chips
1	4	8	5	3
2	6	2	5	6
3	0	0	0	0
4	0	0	0	1
Total	10	10	10	10
Mean Rank	22.20	14.40	20.25	25.15
P value#	0.117NS			

Kruskal Wallis Test; NS: p > 0.05; Not Significant

Graph 3: Stained bacteria key response among four group.



Calcium Hydroxide and Dentin chips were found to have results that were statistically significant (P Value 0.025) on comparison (Table VI).

Table VI: Inter-Group Comparison of Stained Bacteria Key response using Mann-Whitney Test.

Comparison	Z value	P Value	Significance
Group 1 vs 2	1.780	0.075	Not Significant
Group 1 vs 3	0.438	0.661	Not Significant
Group 1 vs 4	0.702	0.483	Not Significant
Group 2 vs 3	1.371	0.170	Not Significant
Group 2 vs 4	2.238	0.025	**Significant**
Group 3 vs 4	1.076	0.282	Not Significant

DISCUSSION

The success of direct capping depends to a large degree on the histological state of the pulp. The healthy, youthful and therefore cell-rich pulp affords the best prerequisites for the formation of hard tissue such as osteo, or canalicular dentine. In this study therefore healthy, young pulp were mechanically exposed. A study evaluated the influence of rubber dam isolation on the response of human pulps capped with calcium hydroxide and an adhesive system. They concluded that Calcium hydroxide should be used as the material of choice for pulp capping with or without rubber dam isolation[9]. Therefore in this study Rubber dam is placed from the start of the operative procedure.

The reduction in clinical success if a direct pulp capping is not followed immediately with permanent restoration has been shown in other clinical studies.[10,11,12] So in this study Glass Ionomer cement has been used as permanent restoration immediately after pulp capping to prevent microleakage. Calcium hydroxide (CH) is certainly one of the most studied dental materials and it is classically used as the gold standard in biocompatibility tests due to its direct or indirect effect on exposed pulp repair. It is the material of choice for all pulp conservative treatment because of its biological and therapeutic potential. Due to its high pH, CH induces a coagulation necrosis layer when in direct contact with pulp tissue[13]. In this study 8 teeth showed none or a few scattered inflammatory cells present in the pulp at the exposure site or beneath new hard tissue formation and showed presence of new hard tissue directly adjacent or near to some portion of the material interface.

However teeth showed an inflammatory cell infiltrate predominated with mononuclear leukocytes (MNLs) as a chronic lesion and showed no evidence of hard tissue formation in any section. Also 2 teeth showed presence of stained bacteria along the cavity including the axial wall and axial floor or within the dental tubules and the dental materials. This can be due to calcium hydroxide is

soluble in water and its physical properties are deficient.

A study reported that during pulp capping procedures with adhesive systems, interaction of resin components with plasma proteins, tissue proteins, and connective tissue cells may provide appropriate substrate for precursor- pulp cells to complete their phenotic expression to fully differentiated odontoblasts[14]. In this study with dentin bonding agent as pulp capping agent 4 teeth showed none or a few scattered inflammatory cells present in the pulp at the exposure site or beneath new hard tissue formation and showed presence of new hard tissue directly adjacent or near to some portion of the material interface. Also 4 teeth showed absence of stained bacteria. This result of the present study is consistent with most other studies in which DBAs have been used on exposed human pulps. A study reported that direct pulp capping with Clear Liner Bond 2 evoked no inflammation in most of the teeth and induced dentin bridge formation in some teeth[15].

A study reported that the self-etching adhesive system Clearfil Liner Bond 2V (Kuraray Co., Tokyo, Japan) and the resin-modified glass-ionomer cement Vitrebond (3M/ESPE) allowed pulpal healing characterized by cell-rich fibrodentin and tertiary dentin deposition as well as calcified barrier formation[16]. The results of the present study are consistent with most other studies in which DBAs have been used on exposed human pulps. [17,18,19,20]. Bioactive glasses (BAGs), as opposed to most technical glasses, are characterized by the materials' reactivity in water and in aqueous liquids. The bioactivity of BAGs is derived from their reactions with tissue fluids, resulting in the formation of a hydroxycarbonate apatite (HCA) layer on the glass. BAGs is biocompatible and can bind to the bone. BAGs can be the material of choice for pulp capping and peri apical bone healing because it is biocompatible and has antibacterial property.[4]

In this study 5 teeth showed none or a few scattered inflammatory cells present in the pulp at the exposure site or beneath new hard tissue formation and showed presence of new hard tissue directly adjacent or near to some portion of the material interface. This result of the present study is consistent with other studies in which bio active glass have been used as direct pulp capping agent. study compared Calcium Hydroxide and Bioactive Glass after Direct Pulp Capping in Primary Teeth. Out of 10 teeth Inflammation was seen in three Bioactive glass samples and dentinal bridge in seven teeth[4].

A study has shown that dentin chips which get into the pulp by chance were completely surrounded with reparative dentin. The tissue was free of infammation. Hence, the use of dentin chips for direct pulp capping was proposed by several authors[6]. In this study 5 teeth showed none or a few scattered inflammatory cells present in the pulp at the exposure site or beneath new hard tissue formation and 4 teeth showed an inflammatory cell infiltrate predominated with poly morphonucleur leucocytes (PMNs) as an acute lesion. 1 tooth showed a necrotic pulp. 10 teeth showed no evidence of hard tissue formation in any section. 3 teeth showed Absence of stained bacteria and 6 teeth showed Presence of stained bacteria along the cavity including the axial wall and axial floor or within the dental tubules and the dental materials.

This could be due to dentin chips could contain microorganisms. In a study, it was shown that lyophilized dentin chips strongly stimulate dentin production by the pulp and when used together with prednisolone and neomycin, such chips exerted a stimulating effect. So, sterilization of dentin chips is utmost important when used as pulp capping agent[21].

The difference in results between the Calcium hydroxide and Dentin chips was statistically highly significant (P Value <0.001) which suggested that Calcium Hydroxide continues to be the gold standard material for pulp capping The difference in result between Calcium hydroxide and Bio active glass was statistically not significant (P Value 0.170) which suggested that future clinical studies with larger sample sizes and longer evaluation period will confirm the effect of Dentin Bonding agent and Bio active Glass for direct pulp capping. Based on the histomorphologic results of the present study, it can be concluded that:

- Calcium Hydroxide continues to be the gold standard material for pulp capping.

- Future clinical studies with larger sample sizes and longer evaluation period will confirm the effect of Dentin Bonding agent and Bio active Glass for direct pulp capping.

The histological findings had no direct correlation with clinical observations. Clinical and radiographic evidence alone cannot support the introduction of any new direct pulp capping agent, further clinical correlation must be done.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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