A Histopathological Study of Direct Pulp Capping with Adhesive Resins

M. Ghavamnasiri,[1] F. Maleknejad,[1] J. Salhenejad,[2] H. Moosavi[3]

[1] Associate Professor, Department of Operative Dentistry, Faculty of Dentistry , Mashhad University of Medical Sciences, Mashhad, Iran
[2] Assistant Professor, Department of Oral & Maxillofacial Pathology, Faculty of Dentistry , Mashhad University of Medical Sciences, Mashhad, Iran
[3] Assistant Professor, Department of Operative Dentistry, Faculty of Dentistry , Mashhad University of Medical Sciences, Mashhad, Iran

Statement of Problem: Recently, it has been proposed that different adhesive materials can be used for direct pulp capping. Previous studies have demonstrated that multi steps dentin adhesives could form reparative dentin similar to calcium hydroxide (CH).

Purpose: The aim of this study was to evaluate the histological pulp response of ninety mechanically exposed cat pulps to two adhesive resins (Scotch Bond MP and Single Bond 3M) were compared with a calcium hydroxide cement (Dycal, Dentsply).

Materials and Methods: Class V facial cavities with similar pulpal exposures were prepared in canines. In the experimental groups phosphoric acid was used to etch the enamel and dentin and pulp exposure, and after it dentin adhesives was applied. The exposure point of the control group was capped with Dycal then the remainder of the cavities was etched and a dentin adhesive (single bond) was applied. All of the cavities were restored with a composite resin (Z 100) in usual manner. The animals were scarified after 7, 30 and 60 days (n=30), and the pulp evaluated histologically, statistical analysis was carried out with Kruskal- Wallis test (a=0.05).

Results: The data showed that most of the cases had mild inflammation of pulp tissue. There was no significant difference in inflammatory reaction of pulp by Dycal and two adhesive systems, severe inflammatory reaction of pulp was observed only in most of the 30- day Single Bond group. Soft tissue organization of dentin bridge was less than Scotch Bond and Dycal groups, the differentiation of dentin bridge was less than Scotch Bond group after 7 days.

Conclusion: Slight inflammatory cell infiltration was the main reaction of exposed pulp when two commercially available adhesive resins were placed directly on the exposed pulp. There was no significant difference in inflammatory reaction of pulp between Dycal and two adhesive systems after 7 days and 60 days. After 7 days most of the specimens showed an amount of predentin deposition.

Key Words: Direct pulp capping; Adhesive resin; Histopathological study

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When preparing deep cavities, the dental pulp may be mechanically exposed. Calcium hydroxide is commonly used for direct pulp capping of these exposures. However the ultimate failure of Calcium hydroxide is its inability to provide a log-term seal against microleakage. This incomplete seal allows bacterial infection with eventual pulp necrosis.[1,2]

A number of factors were reported to be

responsible for pulpal inflammation, including acid etching, material toxicity, cavity preparation, trauma and bacterial infection. [3,4] Data from most studies have documented that pulpal inflammation and eventual necrosis are due to the injurious effect of bacterial microleakage.[5-9] It is now understood that bacterial microleakage occurs through an unsealed restoration interface and the underlying unsealed dentinal tubules and lead to pulpal involvement. [2]

Newly developed adhesive resin systems can seal dentinal tubules by creating hybridized zones with the use of superficial dentin. [10,11] The attractiveness of these systems is that a polymeric film can be layered over an exposure site without displacing pulp tissue and onto surrounding dentin where it permeates those tubules. [12] These adhesives are hydrophilic, meaning that dentin and pulp need not he dehydrated prior to their application. The adhesive film is cured by light, and then acts as a barrier while a composite resin is gently spread over the pulp on to the surrounding dentin. After the composite is cured, the exposure is sealed against microleakage.[12]

The results of some short-term experiments suggest that direct capping of vital pulps with modern resin-based adhesive systems are as effective as capping with calcium hydroxide.[2,12-18]

The aim of the present investigation was to assess the short-term histological response of the mechanically exposed pulp with two commercially available adhesive resin systems used as direct pulp capping agents.

Materials and Methods

Twenty-five healthy cats (mean aged 2 years and mean weight 3.5 kg) were tranquilized with an intramuscular injection of 10 ml of ketamin hydrochloride (Ketalar, Warner-Lambert) and 0.5 ml of xylozine (Rhompun, Miles Laboratory, Inc, Shawnee, Kans).

All teeth were scaled and polished with a rubber cap and pumice two weeks before and again on the operative procedures. Four canines per cat, for a total of ninety teeth were used. Then teeth were randomly selected for each capping material at the indicated time intervals.

The cat teeth presented difficulties regarding application of the rubber dam, but in as much as salivation was found to be minimal when the animal was anesthetized; a dry field was readily achieved by means of cotton rolls and gauze swabs.

On the facial surfaces, class V cavities were prepared approximately 1mm coronal to the gingival margin with a # 330 carbide bur at high speed under copious sterile water spray. Preparations were at such a level that the unexposed pulp was seen shining through the dentin as a pink spot. A new bur was used on every five teeth to ensure cutting efficiency. All cavity margins were beveled. After rinsing with physiologic saline and drying with cotton pellets and air spray, an exposure 0.5 to 0.7 mm in diameter at the center of the preparation in Mesiodistally dimension was made into the coronal pulp chamber with a sterile sharp probe. Bleeding was controlled with a sterile cotton pellet soaked in 2.5% sodium hypochlorite. Sodium hypochlorite was used for 20 seconds.[20]

The exposure area was rinsed with normal saline, the materials was then placed gently over the exposure site.

In experimental group, the enamel, dentin and pulp exposure of the cavities were initially etched with 37% phosphoric acid gel for 15 seconds. After washing for 20 seconds and semidrying the cavities, with cotton pellet dentin- bonding agents: Scotch Bond Mp (3M Dental Products St. Paul, MN, USA), and Single Bond (3M Dental Products St. Paul, MN , USA) were used according to manufacturers' instruction. Globular structures in pulp were reported to be polymerized primer globules which were often surrounded by macrophages and the dentin bridge formation around these

structures was inhibited. [21] In control groups after pulp capping by a thick layer of Dycal (Dentsply, Caulk, Milford, DE, USA), etching was preformed. The acid was washed off with water for 30 seconds. After semidrying, the cavities were sealed with Single Bond. A hybrid composite resin Z100 (3M Dental Products St. Paul, MN, USA) in two oblique increments were inserted into the cavity. Each increment was light cured for 60 seconds.

At 7, 30 and 60 days after the procedure, the cats were killed with 10% Nembutal. The mandible and maxilla of each animal were dissected free. The teeth were immediately removed from the jaws with a diamond disk. To fix the pulp, the apical thirds of the roots were removed. The teeth were placed in 10% formaldehyde at room temperature for 5 days. After washing and dehydration in an ascending series of ethanol, the teeth were cleaved in xylene and embedded in paraffin. Facio-lingual sections (5 microns in thickness) were prepared form mesial to distal. Serial sections that showed the deepest part of the cavity and the underlying pulp were selected. Among every 10 sections those numbered 1 through 5 were stained and those numbered 6 through 10 were set aside. Sections 1,2,3,4 and 5 were stained with Hematoxylin and Eosin. Selected sections were evaluated with light microscopy, with at least 15 to 20 sections from each tooth being assessed. Each specimen was examined by a blind independent investigator according to criteria, which were described by Kitasako et al and Demarco et al (Table I) [14,19]

Comparison among the capping materials in each time interval and also among three time intervals for each capping material were made using Kruskal-Wallis statistical test (a=0.05)

Table I- Evaluation criteria of study for pulp capping success

Inflammatory Cell Response
1. No, or a few scattered inflammatory cells are present in the tissue beneath the exposed area.
2a. Acute inflammatory cell predominated by polymorph nuclear leukocytes.
2b. Chronic inflammatory cell predominated by mononuclear lymphocytes.
3. Severe inflammatory cell appears as an abscess or dense infiltrate of polymorpho nulcear leukocytes involving one third or more of the pulp system.
4. A compartmentalized zone of necrotic pulp is present.
Soft Tissue Organization
1. Tissue morphology is normal or almost normal below the exposed area or throughout the pulp
2. There is a lack of normal tissue morphology below the exposed area and deeper pulpal tissue is normal.
3. There is loss of general pulp morphology and cellular organization below the exposed area.
4. Necrosis is present in the coronal third of the exposed area
Dentin Bridge Formation
1. None
2. Initial dentin bridging (I.D.B)
3. Partial dentin bridging (P.DB)
4. Almost complete dentin bridging (A.C.DB)
5. Complete dentin bridging (C.DB)
Differentiation of Dentin Bridge
1. Osteodentin (OD)
2. Osteodentin + Partial tubular dentin (OD+P.TD)
3. Tubular dentin+ partial osteodentin (TD+P.OD)

Results

Kruskal Willis test showed that in most of the cases slight inflammatory cell infiltration was the main inflammatory reaction and there was no statistically significant difference among different materials (P>0.05).

Only in Single Bond group there was a statistically significant difference among the time interval in inflammatory cell reaction (P<0.05) and also soft tissue organization (P<0.05). Thirty days time interval had the highest mean rank. This means that in Single Bond group, inflammatory cell reaction was the highest and soft tissue organization was the lowest (Fig. 1C, 2C, 3C).

The normal soft tissue organization was just under exposure site in most cases, but deeper pulpal tissue was normal in all groups (Fig 2C).

There was no significant difference among materials and time intervals in mean rank of soft tissue organization and dentin bridge formation (P>0.05). In single Bond and Dycal groups, the highest mean rank of differentiation of dentin bridge was observed at 7 days group (P<0.05). This means that osteodentin did not form after seven days of pulp capping by these two capping materials (Fig 1B).

Discussion

The aim of this study was comparing the two different adhesive resins with calcium hydroxide in direct pulp capping of cat teeth.

In this study cat teeth were selected because it has been recognized that their pulp are structurally similar to human's one [22] and such as the other studies of direct pulp capping, class V cavity preparations were prepared [14,15,17,23] and pulpal exposure was done by the tip of a probe. [15]

It was thought that the most critical issue in treatment of pulpal exposure was the control of bleeding at the exposure site and it had not been observed histological pulpa1 response such as necrosis after using sodium hypochlorite for control of hemorrhage [26-28].

In this study enamel, dentin and pulp were etched before direct pulpal capping with adhesive.

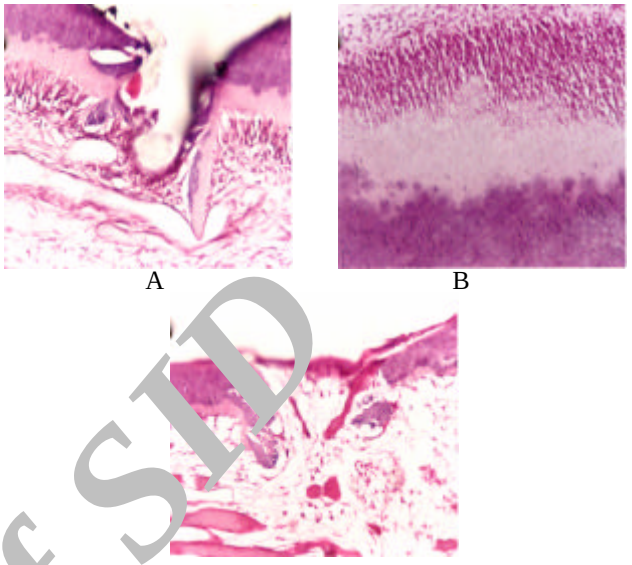


Fig 1- Seven days after operation

(A) Dycal Group: dentin chips, without inflammatory cell infiltration, Vasodilatation (×400). **(B)** Scotch Bond MP Group: Odontoblasts and high thickness of predentin (×400). **(C)** Single Bond Group: dentin chips; hyperemia; without inflammatory cells; Globular structure and macrophages (×400).

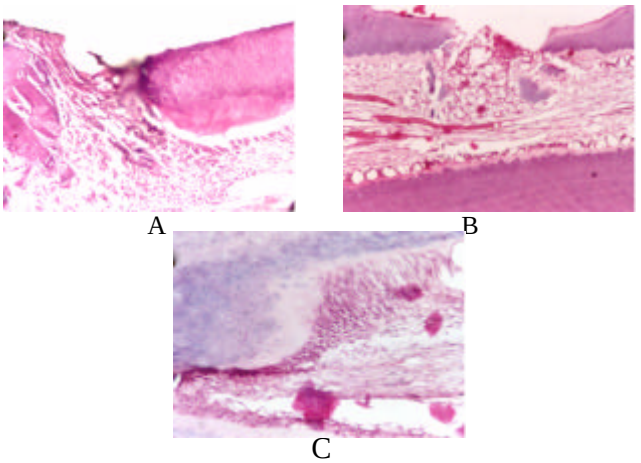
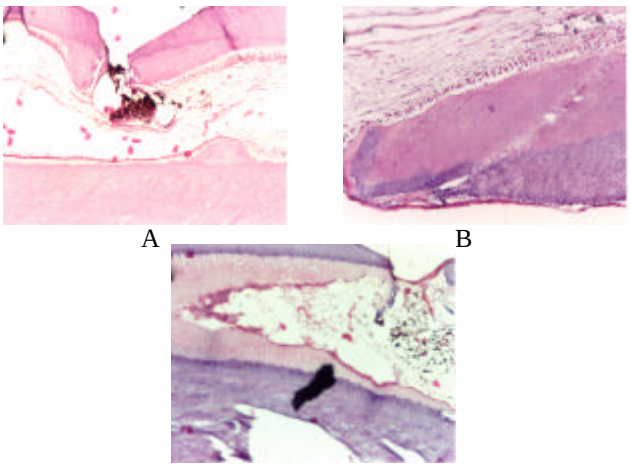


Fig 2- Thirty days after operation

(A) Dycal Group: mild protrusion of Pulp tissue; overgrowth of Odontoblasts and increasing of dentin bridging in border of wound area; chronic inflammatory cells (×400). **(B)** Scotch Bond MP Group: granulation tissue in the exposure site; odontogenesis around exposure site. (×400). **(C)** Single Bond Group: without inflammatory cell; soft tissue organization; differentiation of dentin bridge (TD+P.OD) (×400).



A B C

Fig 3- Sixty days after operation

(A) Dycal Group: globular structure; dentin bridging in the opposite site of wound area; differentiation of dentin bridge (TD + P.OD) (×100). **(B)** Scotch Bond MP Group: differentiation of dentin bridge (osteodentin); dentin chips (×400). **(C)** Single Bond Group: a thick layer of predentine (×100).

Studies have shown that etching with phosphoric acid followed by application of dentin-bonding agent and composite resin could create a suitable seal and prevent microleakage.[2,12,27,29,30].

The results of earlier studies showed that Dycal caused a necrotic Zone in superficial tissue.[31,32] It appeared that this layer had been required for formation of reparative dentin.[33,13]

In present study the necrotic zone was not observed, Probably the cavity preparation or properties of capping material could be act as stimulating factors for formation of reparative dentin. [34,35] Pulpal necrosis was observed only in a suitable seal of margin and microleakage occurrence and also degradation of calcium hydroxide after etching or because of tubular liquid flow. [8,12,16,27]

In the 7-day Single Bond and Scotch Bond MP groups, a thin layer of predentin and a mild inflammatory reaction of pulp were observed. After 30 days, there were 4 necrotic pulps in Single Bond group and 3 cases of necrosis in Scotch Bond MP Group, but in 60-day groups only one necrosis was seen in each group similar to Olmez and others. [15] This seemed to be due to technical error during application of adhesive or composite resin or was because of microleakage.[14] Pulpal response to testing materials in animal experiments have been classified as acceptable when no or only slight reaction was observed and as unacceptable when moderate or severe reactions prevailed. [36] All inflammatory reaction of the present study were classified as acceptable after 60 days according to this criteria.

In Single Bond group after 30 days, the inflammatory cell reaction was higher than the 7 and 60 day of the group, which was probably due to the presence, stability and maximum effect of HEMA and also release of monomers into the pulp that it might be cytotoxic. [37,38]

In all 60-day groups the amounts of osteodentin were similar to a study by Kitasak and others. [2] Soft tissue organization was dependent on the rate of pulpal inflammation, this means that the less inflammatory reaction, the faster the cell organization. [17] Dentin bridge formation of all groups had not statistically significant difference for any kind of materials, but the dentin bridging in adhesive groups was lower than that of Dycal group at 30 days however it became comparable to the Dycal group after 60 days. The formation of reparative dentin after pulp capping by adhesives compared with Dycal occurred later and it has also been proved by other studies. [14,17]

This study reported the presence of small particles of calcium hydroxide cement in pulp tissue similar to Cox and others. [39]

In future, some animal and clinical researches will be needed to compare the effect of long-term dentin adhesives with calcium hydroxide for DPC in respect to evaluation of microleakage and pulpal response.

Conclusion

Direct pulp capping by different adhesive resins was studied histological with the following results:

- Slight inflammatory cell infiltration was the main reaction of exposed pulp when two commercially available adhesive resins were placed directly on the exposed cat pulp.
- There was no significant difference in inflammatory reaction of pulp by Dycal and two adhesive systems.
- In 30 days Single Bond group, inflammatory cell reaction was higher and soft tissue organization was lower, than 7 and 60 day Single Bond groups
- After 30 days, Dycal Group showed deposition of osteodentin while other experimental groups showed a thick layer of predentin.
- The lowest dentin bridge differentiation was related to the 7-day Dycal, and Single Bond groups.

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