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The Adverse Effects of Nano bond Adhesive Systems Used as Direct Pulp Capping Materials

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ABSTRACT

The purpose of this study was to evaluate the histopathological changes in mechanically exposed dog's pulps capped with Nano bond[®], Clearfil SE Bond Self-etching adhesive systems in comparison to Dycal. Six dogs were used in this study. Class V cavities were prepared on the buccal surface of 15 intact teeth in each dog. Pulps were then mechanically exposed, then capped according to each group: Group (1): Control, pulp capped with CH Dycal (lower right side), Group (2): Pulp capped with Nano bond[®], Group (3): Pulp capped with Clearfil SE Bond (SE). The cavities were restored with Nano composite Filtek Supreme XT. Dogs were sacrificed at 7, 14, 30 days postoperatively. Teeth were fixed, decalcified, processed and stained with hematoxylin and eosin. The histopathological features were examined and graded using light microscopy. The data were statistically analyzed using Kruskal-Wallis test. For both adhesive systems, the pulp tissue exhibited moderate to severe inflammatory infiltrate involving the coronal pulp. Dycal induced a less severe inflammatory response and more consistent formation of reparative dentine. Statistically significant differences in inflammatory response, tissue disorganization and hard tissue formation were observed among teeth treated with adhesive systems and Dycal. A less favorable pulpal response was noted in the experimental groups in comparison to the control. Aiming to avoid the limited physical properties of Ca(OH)₂-based materials, self etching restorative materials developed by nanotechnology were evaluated as potential pulp capping material. Unfortunately, the tested materials failed to produce a favorable pulp response.

Key words: Nano bond adhesive system, nanocomposite, pulp capping, histological changes, pulp reaction

INTRODUCTION

Exposure of the dental pulp may be occurring accidentally during cavity preparation and removal of carious dentin. Direct pulp capping may be indicated in selected cases for maintaining pulp health and function (Lu *et al.*, 2008). Ca(OH)₂-containing materials were traditionally used in pulp capping. These materials stimulate reparative dentine formation with high rates of pulpal survival (more than 80% of the treated teeth) (Horsted *et al.*, 1985). However, Ca(OH)₂-based materials are hindered by physical limitations, such as non-adherence to dentine, dissolution in tissue fluids or other dental materials and degradation upon tooth flexure. Therefore, new treatment modalities have been attempted (Leinfelder, 1994; Cox and Suzuki, 1994).

To avoid the rinsing and drying steps, scientists developed self-etching systems which made a substantial improvement in adhesive restorative dentistry (Watanabe *et al.*, 1994; Perdigao and Lopes, 1999). Primers of self-etching systems decalcify the inorganic component and infiltrate the collagen fibers *in situ*, thus providing a good seal between the restoration and the tooth substance. Moreover, the smear plugs are left intact, thus preventing collapse of air-dried, demineralized collagen (Nakabayashi and Saimi, 1996; Ferrari *et al.*, 1997). Over the past decade, new self-adhesive materials were developed. Several researchers questioned the biological effects of these materials on exposed or unexposed pulps. Studies pointed to the importance of eliminating bacterial microleakage in order to achieve pulp recovery in resin capped teeth (Kitasako *et al.*, 1999; Olmez *et al.*, 2006). Since, pulp has the ability to heal itself in the absence of bacteria, adhesive systems appear promising in indirect and direct pulp capping, being able to reduce microleakage, (Kitasako *et al.*, 1999; Median *et al.*, 2002).

The term “Nanotechnology” is currently used to refer to the research and development of an applied science at the atomic, molecular, or macromolecular levels. In the field of adhesive restorative dentistry, Nano bond nanoparticulate reinforced adhesive system have been introduced as the 6th generation self-etch bonding system that can be used for direct or indirect bonding applications (Terry, 2002).

Chemically, the Nano bond system is composed of a self-etch primer and a nanoparticulate reinforced adhesive. Both components work together to achieve a tight bond to the tooth. For proper etching of the tooth surface, the self-etch primer component of the Nano bond’s HEMA produce a pH of 1 at the start of its application. However, this acidic pH quickly neutralizes, leaving the tooth surface properly etched and occluded with an aqueous layer containing HEMA. This optimally moist surface provides a superior environment for effective resin penetration of the tubules (Pentron, 2009).

The purpose of this study was to evaluate the histopathological changes in mechanically exposed dogs' pulps capped with the newly developed Nano bond Adhesive system (Pentron), the self-etching adhesive systems Clearfil SE-Bond (Kuraray) in comparison with Dycal (Dentsply) at 7,14,30 days postoperatively.

MATERIALS AND METHODS

Experimental procedure: Six young healthy non pedigree dogs, aged between one and two years old with an average weight of 10 kg were used in this study. Radiographs demonstrated that the tooth apices had formed completely. The experimental protocol was conducted according to the ethical guidelines for animal care in the Kasr Alainy animal and experimental laboratory (Faculty of Medicine, Cairo University). Adequate measures were taken to minimize the pain or discomfort to the animals. Animals of all groups were supplied a diet composed of fresh vegetables, powdered milk and water *ad libitum*.

The animals were sedated by an intravenous injection of ketamine (Amoun Pharmaceutical Co., El-Obour City, Egypt) at a dose of 1 mg kg⁻¹ body weight. Ten minutes later, general anesthesia was induced and maintained by using Thiopental sodium (Egyptian Interpharmaceutical Industries Co., 10th of Ramadan City, Egypt) at a dose of 5 mg kg⁻¹ of 2.5% solution intravenously.

Fifteen teeth in each dog were selected (upper and lower corner incisor, canine, second and third premolar and first molar) in three quadrants.

Table 1: The commercial name, the manufacturer and the composition of the materials used

Materials	Manufacturer	Composition
Dycal (DY)	Dentsply 023302 023303, Caulk, Milford, DE, USA	Base: substitute phenol, titanium dioxide calcium sulfate, pigment, Catalyst: calcium hydroxide, stearate, plasticizer, zinc oxide
Nano bond®	Pentron Clinica Wallingford, CT 06492 USA	Self-Etch Primer: Mixture of Functional Sulfonic acid resin, HEMA and other multi-functional methacrylate resins with water and initiator Nano bond Adhesive: PMGDM, HEMA, BisGMA, Surface functional nanosized particulate material, acetone and polymerization initiators
Clearfil SE Bond (SE)	Kuraray Medical, Osaka, Japan 00220A	Self-etch Primer: MDP, HEMA, Hydrophilic dimethacrylate, DL-camphorquinone, N, N-diethanol-P-toluidine, water, Bond: MDP, Bis-GMA HEMA, Hydrophilic dimethacrylate, DL-camphorquinone, N, N-diethanol-P-toluidine, silanated colloidal silica
Filtek Supreme XT	3M ESPE, Dental St. Paul,	Bis-GMA, bis-EMA, UDMA and TEGDMA, MN, USA, 59.5% in volume (clusters of 0.6–1.4 µm, individual particle size of 5–20 µm), zirconia and silica

Group 1: Control, pulp capped with CH Dycal (lower right side)

Group 2: Pulp capped with Nano bond (Upper right side)

Group 3: Pulp capped with Clearfil SE-Bond (Upper left side)

Selected teeth on each dog were scaled and polished with a rubber cup. After rubber dam placement, Class V cavities were prepared on the buccal surface of intact teeth with a fissure bur (ISO 700L, Dentsply) at high speed under copious sterile water spray. A new bur was used on every fourth tooth to reduce heat. When the unexposed pulp was seen shining through the dentin as a pink spot, cavity cutting was stopped. After rinsing with 3% hydrogen peroxide and physiologic saline alternatively, the cavities were disinfected with 3% sodium hypochlorite. The pulps were then mechanically exposed cautiously by small round bur (ISO1/2SS, Dentsply) at high speed under water spray coolant. Hemorrhage was controlled with sterile cotton wool. The exposed pulps on upper right and left side were capped with Nano bond and Clearfil SE-Bond. In the control group, the exposed pulps were directly capped with Dycal. The cavities were restored with nanocomposite Filtek Supreme XT. All the materials were used according to the manufacturers' instructions (Table 1). The experiment duration was 30 days after application of the tested materials.

Histological procedures: At 7, 14 and 30 days postoperatively, two dogs were sacrificed by using an overdose (0.5 grams) of 10% solution of Thiopental sodium intravenously. The jaws were immediately dissected free and the teeth (5 teeth for each group in 2 dogs, providing a total of 10 teeth in each group at each observation period) were separated from the jaws as tissue blocks by the use of saw. The teeth were immediately disinfected using 2% glutaraldehyde and then stored in normal saline 0.9% to keep them moist.

The apical root third of each tooth was amputated, the teeth were immediately placed in 10% neutral-buffered formalin solution for 48-72 h to allow proper fixation of pulp tissue, then teeth were decalcified with 10% EDTA at room temperature for two months till complete softening of enamel is assured by a special blunt stylus. The teeth were dehydrated in ascending grades of ethanol and embedded in paraffin. Serial section of 6-8 µm thicknesses were cut longitudinally, buccolingually and stained with hematoxylin and eosin. The histopathological features were evaluated using light microscopy. The examiner was blinded to the identity of the specimens. Each section was graded according to criteria listed as follows (Murray *et al.*, 2000; Cui *et al.*, 2009):

Inflammatory cell infiltrate:

- None (0):** No or few inflammatory cells scattered in the pulp
Mild (1): A small number of inflammatory cells gathered at the exposure area, including neutrophils (acute period) or mononuclear leukocytes (chronic period)
Moderate (2): A number of inflammatory cells infiltrated in the coronal pulp
Severe (3): Necrosis or abscess formation

Pulp tissue disorganization:

- None (0):** Normal pulp tissue morphology
Mild (1): Discrete pulp disorganization close to the pulp exposure site but normal central pulp
Moderate (2): More widespread disorganization of the pulp tissue morphology
Severe (3): Total pulp tissue disorganization or pulp necrosis.

Hard tissue formation:

- None (0):** No hard tissue formation
Initial (1): Limited hard tissue deposition below or around the exposure, extending to no more than half of the exposure site
Partial (2): Hard tissue deposition below or around the exposure, extending to more than half but not completely closing the exposure site
Complete (3): Hard tissue formed across the exposure site completely

Statistical analysis: Scores were assigned for the obtained qualitative data (as explained above). Scores were submitted to statistical analysis using the non-parametric one way analysis of variance Kruskal-Wallis test. A p value below 0.05 was considered significant.

RESULTS

First observation period: Seven days after pulp capping using Dycal, the pulp tissue immediately subjacent to the exposure site revealed mild hyperaemia. The blood vessels were intact with no evidence of haemorrhage. A mild inflammatory response was observed in 60% of the cases while a moderate to severe inflammatory response was noted in the remaining specimens. This consisted of monocytic cells (mainly lymphocytes), with only a few neutrophilic leucocytes and was located in the vicinity of the exposure site. Tissue necrosis was mainly noted in the coronal pulp, just beneath the exposure site. Limited hard tissue deposition attempting to occlude the exposure was noted in 40% of the specimens (Fig. 1a, Table 2).

The tissue adjacent to the exposed area of the Nano bond group was in general characterized by a moderate to severe inflammatory reaction mainly consisting of lymphocytes with few neutrophils. The odontoblastic layer close to the exposure was often interrupted and vacuolization was noted within the surviving cells. Various numbers of dilated blood vessels and extravasated erythrocytes were seen. Areas of necrosis tissue and marked tissue disorganization were noted. There was no evidence of hard tissue formation (Fig. 1b, Table 2).

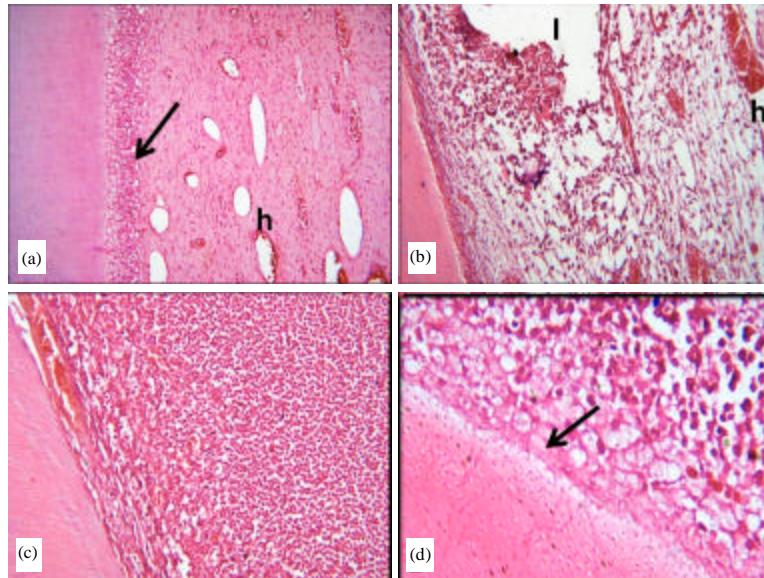


Fig. 1(a-d): Pulp reaction seven days post-operatively. (a) Dycal group revealing intact odontoblastic layer (arrow), pulp hyperaemia (h) and mild inflammatory response (H and E x100), (b) Nano bond® group revealing moderate inflammatory reaction (I) dilated blood vessels (h) and interrupted odontoblastic layer (H and E x200) and (c-d) Clearfil SE bond (SE) group revealing severe inflammatory response chiefly consisting of lymphocytes with few neutrophils, the odontoblasts are absent or vacuolated (arrow) (H and E x200, 400, respectively)

Table 2: Grading of the histological features of the examined sections

Materials	Days	Inflammatory response				Tissue disorganization				Hard tissue formation			
		0	1	2	3	0	1	2	3	0	1	2	3
Nano bond®	7	0	0	4	6	0	2	2	6	10	0	0	0
	14	0	2	2	6	0	2	3	5	10	0	0	0
	30	3	0	3	4	0	4	2	4	9	1	0	0
Clearfil SE Bond	7	0	2	4	4	0	3	2	5	10	0	0	0
	14	0	3	3	4	0	3	2	5	9	1	0	0
	30	1	3	4	2	5	2	2	1	8	2	0	0
Dycal	7	0	6	2	2	0	8	1	1	6	2	2	0
	14	4	6	0	0	4	4	2	0	5	0	3	2
	30	4	6	0	0	4	4	2	0	3	0	4	3

In the Clearfil SE-Bond (SE) group, a moderate to severe inflammatory response was noted in the coronal pulp in 80% of the specimens. The inflammatory cells were mainly of the chronic type (lymphocytes) with few neutrophils. At the periphery of the exposure site, the odontoblasts were absent or vacuolated. Tissue disorganization of varying extent altered the pulp morphology. No hard tissue formation could be detected (Fig. 1c-d, Table 2).

Kruskal-Wallis test showed significant difference between experimental and control groups regarding the inflammatory response ($p = 0.0285$), tissue disorganization ($p = 0.0182$) and hard tissue formation ($p = 0.0118$).

Second observation period: At 14 days, a mild inflammatory response was noted in 60% of Dycal-capped pulps. The inflammatory response, consisting of chronic inflammatory cells (mainly lymphocytes), was confined to a limited area beneath the exposure site. In 40% of the specimens only few scattered inflammatory cells could be detected in the coronal pulp. The odontoblastic layer adjacent to the exposure area displayed normal morphology. Mild hyperaemia was still noted in the blood vessels. Normal pulp morphology was noted in 40% of the specimens while tissue disorganization of variable extent was noted in the remaining cases. Two specimens (20%) exhibited complete dentine bridge formation across the exposure site while partial hard tissue deposition was noted in three additional specimens (Fig. 2a, Table 2).

In the Nano bond group, a moderate to severe inflammatory reaction was noted in most of the specimens. The odontoblastic layer adjacent to the exposure site was interrupted. Dilated and congested blood vessels were noted. Widespread disorganization of the pulp tissue and areas of necrosis were evident. No hard tissue formation could be detected (Fig. 2b, Table 2).

In the Clearfil SE-Bond (SE) group, a moderate to severe chronic inflammatory response was still observed in the coronal pulp in 70% of the specimens. At the periphery of the exposure site, the odontoblasts were absent or vacuolated. Tissue disorganization was evident in the coronal pulp. A thin layer of partially calcified dentine was noted at the exposure site of a single case (Fig. 2c-d, Table 2).

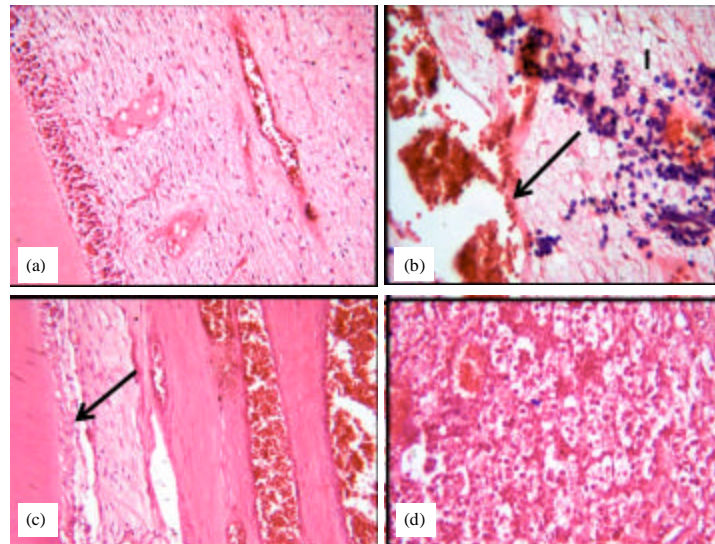


Fig. 2(a-d): Pulp reaction 14 days post-operatively, (a) Dycal group revealing almost normal pulp morphology with mild hyperaemia (H and E x200), (b) Nano bond® group revealing dilated and congested blood vessels (arrow) and chronic inflammatory cell infiltration (I), (H and E x400), (c) Clearfil SE bond (SE) group revealing tissue disorganization with dilated and congested blood vessels, absence of odontoblasts (arrow), (H and E x200) and (d) Clearfil SE bond (SE) group revealing severe chronic inflammatory response (H and E x200)

Kruskal-Wallis test showed significant difference between experimental and control groups regarding the inflammatory response ($p = 0.0004$), tissue disorganization ($p = 0.0027$) and hard tissue formation ($p = 0.0102$).

Third observation period: At 30 days, Dycal-capped pulps demonstrated a nearly normal appearance. A mild chronic inflammatory response confined to a limited area beneath the exposure site was still observed in 60% of the cases. Blood vessels had a normal size with no evidence of hemorrhage. Discrete tissue disorganization close to the pulp exposure site was noted in almost half of the cases. Partial or complete hard tissue formation across the exposure site was detected in 70% of the specimens. The newly formed dentin appeared homogenous with no tubular structure. Variation in thickness of the hard tissue was also noted (Fig. 3a, Table 2).

In the Nano bond group, an inflammatory reaction of variable intensity and extension was still noted in only 70% of the specimens. The dental pulp was free from inflammation in the remaining cases. The odontoblastic layer adjacent demonstrated an altered morphology with total absence in some areas. Hyperemia and edema was still observed. Tissue disorganization of varying extent was noted within the pulp. Limited hard tissue formation was detected in a single specimen (Fig. 3b, Table 2).

In the Clearfil SE-Bond (SE) group, a moderate to severe inflammatory response was noted in the coronal pulp in 60% of the specimens. A normal pulp morphology was noted in 50% of the cases while tissue disorganization of varying extent affected the rest of the specimens. A limited amount of hard tissue was laid down on the dentin walls close to the exposure in only 2 cases (Fig. 3c-d, Table 2).

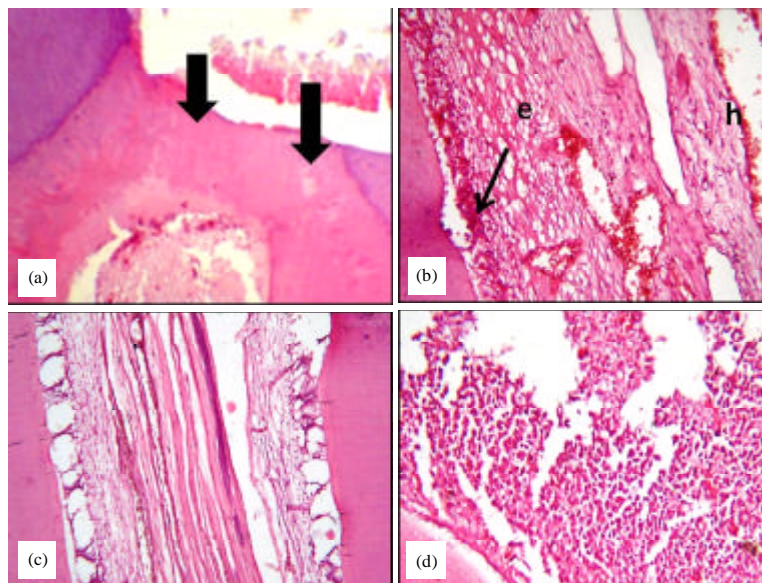


Fig. 3(a-d): Pulp reaction 30 days post-operatively, (a) Dycal-group revealing complete hard tissue formation across the exposure site (arrows), (H and E x200), (b) Nano bond® group revealing altered morphology of the odontoblastic layer (arrow), hyperaemia (h) and edema (e), (H and E x200), (c) Clearfil SE bond (SE) group revealing disorganization of pulp tissue (H and E x100) and (d) Clearfil SE bond (SE) group revealing a severe chronic inflammatory response (H and E x200)

Kruskal-Wallis test showed significant difference between experimental and control groups regarding the inflammatory response ($p = 0.0270$), tissue disorganization ($p = 0.0229$) and hard tissue formation ($p = 0.0025$).

DISCUSSION

Carious exposure of the pulp is by far the most common reason for pulp capping although it is also used to treat mechanical and traumatic exposures. Various materials have been used for pulp capping, the aim being to induce reparative dentinogenesis across the exposed pulp. Calcium hydroxide is generally accepted as the material of choice, since it has the most consistent ability to form a hard tissue barrier (Olsson *et al.*, 2006). Moreover, the antibacterial activity, biocompatibility, stimulation of the cellular activity and release of bioactive molecules have been associated with the success of calcium hydroxide (Foreman and Barnes, 1990) justifying its choice as a control material in the current study.

A dog model was chosen for this experiment since it has been shown that the pulpal, apical and periapical healing process in dogs is similar to that in human (Perirokh *et al.*, 2005; Queiroz *et al.*, 2005).

In this study, the pulp was exposed mechanically through class V preparations according to recommendations of ISO regarding usage tests on animals (Murray *et al.*, 2000). This technique helped to avoid the high occlusal forces on the restorations during biting throughout the follow-up period.

The success for vital pulp therapy after pulp exposure depends on several factors, such as the pulp status, extent and degree of the injury, capping agents and bacterial infection (Murray *et al.*, 2002). In the present study, measures were taken to standardize the pulp status and the extent of the injury. This was achieved through selection of healthy animals of nearly the same age and weight and through strict standardization of the operative procedure. To control contamination, a series of measures were taken during the procedure, such as disinfecting the oral cavity before operation, using rubber dam and rinsing with solution during the operation. Since it has been suggested that provision of a seal against bacterial ingress is probably the most critical factor in the success of vital pulp therapy (Cox, 1992) the nanocomposite (Filtek Supreme XT) was used in the current study to provide a tight seal at the cavity margins after pulp capping. On the other hand, proper haemorrhage control was ensured to prevent blood clot formation at the exposure site, since a blood clot may act as substrate for microorganisms leading to pulp infection (Kopel, 1992).

Pulpal reaction can originate with many factors, such as operative procedure, the toxicity of the material and bacterial contamination at the material cavity interface (Kitasako *et al.*, 1999). In the current study, an inflammatory response of varying intensity was noted in all groups in the first observation period (7 days post operatively). A less intense inflammatory reaction that tended to subside by time was noted in pulps capped with Dycal compared to the dentine adhesive systems. It has been demonstrated that pulp inflammation shortly after treatment is usually caused by mechanical trauma and operative procedures, whereas, inflammation over longer periods is mainly due to the presence of bacteria and bacterial products introduced by microleakage around the restoration or is due to material toxicity (De Souza *et al.*, 2001).

The greater inflammatory response noted in the dentine adhesive system groups can be attributed to the chemical composition and cytotoxicity of these materials. Some researches pointed out that the major components of adhesive system, such as bisglycidyl methacrylate (bis-GMA), urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (HEMA), have cytotoxicity

when applied to fibroblasts *in vitro* (Hanks *et al.*, 1991; Costa *et al.*, 1999). Costa *et al.* (1999) suggested that the adhesive systems have the possibility of injury to pulp tissue, because of the cytotoxicity of the resinous materials and their components to pulp cells. Furthermore, when resins are placed directly on exposed pulps, the high lipid solubility of the resins in the lipid-phase of biologic membranes may permit the resin monomers to reach cytotoxic concentrations within cell membranes (Pashley *et al.*, 1996). In addition, resin monomers may affect the immune system adversely and induce immunosuppression which is often correlated with decreased host resistance to infection (Luster, 1989).

Since previous studies have demonstrated that these systems do not appear to result in perfect sealing between resin and dentin, resulting in the bacterial invasion (Cui *et al.*, 2009; Osorio *et al.*, 2003) the more intense inflammatory reaction noted in the adhesive systems can be attributed to microleakage of bacteria after pulp capping. On the other hand, the persistent moderate to severe inflammatory response noted in pulps capped with the dentine adhesive systems contradicts the previous *in vitro* evidence that suggested that self-etching primers may exert a bactericidal effect within a short time of contact and even provide long term bacteriostatic action (Imazato *et al.*, 1998).

Whenever the pulp is affected by caries or mechanical trauma, the immune system will trigger an inflammatory response to limit tissue damage by eliminating and ingesting invading microorganisms and cell debris. These inflammatory reactions can injure the pulpal cell populations and in the most severe cases obliterate the whole tooth pulp by a process of necrosis (Bergenholtz, 1990). This deleterious effect was observed in some specimens capped by adhesive systems in the current study. In addition, it might be reasonable to account for the possibility that any factor inducing a persistent inflammatory pulp response following direct pulp capping can lead to the attraction of blood-borne microorganisms through an anachoretic effect (Tay and Pashley, 2001) this will consequently result in an unfavorable outcome.

A clear association among the pulpal inflammation, tissue disorganization and hard tissue formation was observed in the present study. This association has been also highlighted by Cui *et al.* (2009). It has been suggested that the pulpal tissue injury and inflammation were inhibitory to the pulpal regenerative processes (Cui *et al.*, 2009; Rutherford and Gu, 2000).

In the current study, odontoblasts were observed in relation to the dentine bridge formed at the exposure site in the Ca(OH)₂ group. Previous studies have demonstrated that following pulp exposure, the irreversibly injured odontoblasts are replaced by odontoblast-like cells derived from within other pulp cells by a process of differentiation (Fitzgerald *et al.*, 1990; Goldberg and Smith, 2004). The high alkaline pH of Ca(OH)₂ has been implicated in reducing the acidic pH of inflamed tissue (Schroder, 1985) and this reduction may be beneficial for odontoblast-like cell proliferation, differentiation and migration. On the other hand, Mathieu *et al.* (2005) reported that endothelial cell injury is involved in the recruitment of odontoblast-like cells at the injury site. These new odontoblast-like cells will secrete a tertiary dentin matrix termed reparative dentin (Teles *et al.*, 2005) and the number of these cells was found to be the most important factor influencing the area of dentine bridge formation (Murray *et al.*, 2002).

In the current study, dentin bridging was observed in few specimens capped by the dentine adhesive systems. In addition to the cytotoxicity of these materials, the presence of resin particles at the exposure site has been linked to a persistent inflammatory reaction that hinders the odontoblast-like cell differentiation resulting in lack of complete hard-barrier formation (Gwinnett and Tay, 1998). Considering the fact that resin-dentine bonds undergo degradation

in vivo over time, risk of pulp infection by invaded microorganisms may be greater in cases without a hard tissue barrier than in cases with a dentinal bridge (Lu *et al.*, 2008).

From a comparison of the results between the groups of teeth treated with dentine adhesive systems and those treated with Ca(OH)₂, it may be stated that a low success rate (more intense inflammatory reaction and tissue disorganization, together with a limited potential for reparative dentin formation) might be expected from pulp capping with the investigated dentine adhesive systems. These findings are in consistence with previous studies (Cui *et al.*, 2009; Koliniotou-Koumpia and Tziapas, 2005; Accorinte *et al.*, 2008). Despite reporting a comparable or even less severe inflammatory reaction in beagles and human teeth capped with Clearfil SE-Bond in comparison to Ca(OH)₂ (Lu *et al.*, 2008; Lu *et al.*, 2006) also observed a limited ability for hard tissue formation in relation to the dentine adhesive systems. These findings justify the use of calcium hydroxide as the material of choice for pulp capping.

CONCLUSION

Based on the results of the present investigation, it can be concluded that the Nano bond adhesive systems applied in direct contact with the mechanically-exposed pulp of healthy dogs' teeth were not able to minimize pulpal inflammatory reactions nor to induce dentine bridge secretion and consequently failed to provide proper pulpal healing and protection.

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