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Direct Pulp Capping with a Dentin Bonding System in Human Teeth: A Clinical and Histological Evaluation

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Clinical Relevance

Adhesive systems should not be recommended for vital pulp therapy, while $Ca(OH)_2$ remains the capping agent of choice for mechanically exposed human dental pulp.

SUMMARY

This study evaluated the pulpal response in human dental pulp to direct pulp capping with the Single Bond Adhesive System (SBAS) after 10% or 37% phosphoric acid etching and after capping with Calcium Hydroxide (CH). The degree of bleeding and hemostasis conditions was considered during the adhesive technique. The pulps of 78 sound premolars were capped with SBAS after 37% phosphoric acid etching (Group I) or 10% phosphoric acid etching (Group II) and CH (Group III-control). The cavities were restored with a resin composite (Charisma). After 1, 3, 7 and 30 days, the teeth were extracted and processed for light microscopical examination (H/E, AgNOR silver stain and Brown-Brenn). The patients were followed for postoperative symptomatology evaluation. Clinical results showed the possibility of hemostasis with saline solution only. There was no statistical difference between bleeding generated by 10% and 37% acid solutions. In some cases, contact of the pulp tissue with SASB started the bleeding process, thus damaging the adhesive technique. The histological response was similar in Groups I and II, without signs of cellular differentiation and dentin neoformation up to 30 days. Bacteria were not observed in any specimens. In the control group (CH) at day 7, the pulps exhibited cells with high synthetic activity (Ag-NOR-positive) underneath the area of coagulation necrosis. Dentin bridging was observed at the thirtieth day. The postoperative period was asymptomatic for all groups. In conclusion, SBAS should be

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avoided for vital pulp therapy, while CH remains the capping agent of choice for mechanically exposed human dental pulp.

INTRODUCTION

Despite advancements in the field of pulp biology, the technique and philosophy of direct pulp capping (DPC) remains a controversial subject. Calcium hydroxide (CH) is generally the material of choice for the capping of vital pulp that has been accidentally injured (Stanley, 1998). However, in the last few years, the possibility of direct pulp capping with adhesive systems has been advocated. Preliminary clinical and histological experiments have revealed that resin-based composite systems may be as effective as calcium hydroxide (Kashiwada & Takagi, 1991; Heitmann & Unterbrink, 1995; Tsuneda & others, 1995). Both CH and dentinal adhesives have their proponents and adversaries. Proponents of DPC with bonding agents point out that response of the pulp-dentin complex does not depend on the dental materials used as the pulp capping agent, but rather on its capacity to avoid microleakage and the reparative capacity of mechanically exposed dental pulp, regardless of the capping material used when a proper biological seal was provided (Brannstrom & Nyborg, 1972; Cox, 1987). CH is unable to provide a long-term seal against microleakage (Schuurs, Gruythuysen & Wesselink, 2000).

Several investigations carried out in teeth from animals, such as rodents, dogs and cats (Tsuneda & others, 1995; Olmez & others, 1998; Kiba & others, 2000; Costa & others, 2003; Ghavamnasiri & others, 2004) have shown that pulps capped with bonding agents exhibit an initial inflammatory response, followed by pulp repair coupled with dentin bridge formation, even after short-term evaluation. In primates, histological studies have generated controversy from positive (Akimoto & others, 1998; Cox & others, 1998; Kitasako & others, 1998; Tarim & others, 1998; Kitasako, Inokoshi & Tagami, 1999) and negative findings (Pameijer & Stanley, 1998). It has been suggested that data obtained from animal teeth should not be directly extrapolated to human clinical conditions (Costa, Hebling & Hanks, 2000). It is likely that resin components produce more detrimental effects on human pulp than they do in monkeys and other animals (Accorinte & others, 2005a).

Some clinical studies in humans have reported successful results based on tests of pulp vitality, the absence of post-operative symptomatology and favorable radiographic aspects (Heitmann & Unterbrink, 1995; Kanca, 1993, 1996; Gonçalves & Freire, 1997). However, histological studies have demonstrated a chronic inflammatory response, triggered by cytotoxic bonding agent fragments scattered into the pulp space (Gwinnett & Tay, 1998; Porto-Neto & others, 1999; Hebling, Giro & Costa, 1999; Pereira, Segala & Costa, 2000; Costa & others, 2001). Components of the adhesive systems, individually or in combination, seem to harm pulp healing and prevent dentin bridge formation (Accorinte & others, 2005a; Hebling & others, 1999; Subay & Demirci; 2005).

Possible variables during the DPC clinical procedure, such as bleeding level and hemostasis difficulty, bacterial contamination and differences in protocols and materials used may influence and compromise the adhesive technique, contributing to the discrepant results reported in the literature. The clinical and biological success of direct pulp capping with adhesive resin systems depends on the exudation and hemostasis control of the exposed pulp (Tsuneda & others, 1995; Kiba & others, 2000; Cox & others, 1998; Matsuo & others, 1996). In this context, the inherent hemostatic properties of dental etchants and primers would be extremely critical. The placement of acid directly onto the mechanically exposed pulp may aggravate the bleeding process, which, in turn, makes application of the pulp capping material in a dry field very difficult (Pameijer & Stanley, 1998).

This study clinical and histologically evaluated the short-term response of mechanically exposed human dental pulps capped with a dentin adhesive system after 10% or 37% phosphoric acid gel etching when compared with CH.

METHODS AND MATERIALS

Subjects

A total of 81 healthy first human premolars scheduled for extraction for orthodontic reasons were selected from patients ranging in age from 12 to 20 years (mean 15 years). The teeth were randomly distributed into three groups (I, II, III) according to Table 1. All teeth were clinically and radiographically examined to assure the absence of proximal caries and periapical lesions. Three intact teeth were extracted to evaluate normal pulp tissue and for purposes of control of the histological processing. Patients and their parents gave signed consent after they received a thorough explanation related to the study. This research was approved by the Ethic Research Committee of the MG Federal University (COEP/MG), under document nº ETIC 010/98.

Clinical Method

After local anesthesia with 3% prilocaine containing 0.03 UI/ml felipressin, the teeth were isolated with a rubber dam and polished with a rubber cup and a low abrasion prophylactic paste. Finally, the operating field was cleaned with 70% isopropyl alcohol. Class I cavities were prepared as deeply as possible without exposing the underlying pulp tissue using a sterile cylindrical #2068 diamond point (KG Sorensen ISO size 014; Ind.e Com Ltda, SP, Brazil) in an air turbine with distilled

Table 1: *Teeth Distribution According to Groups and Experimental Periods*

		Experimental Periods (days)						
		1	3	7		30		
	Groups	HE	HE	HE	AgNOR	HE	AgNOR	Total
I	37% phosphoric acid[1] + SBAS[2]	n=5	n=5	n=5	n=3	n=5	n=3	**26** 1st UPM = 15 1st LPM = 11
II	10% phosphoric acid[3] + SBAS	n=5	n=5	n=5	n=3	n=5	n=3	**26** 1st UPM = 12 1st LPM = 14
III	Calcium Hydroxide[4] + Dycal[5] + GIC[6]	n=5	n=5	n=5	n=3	n=5	n=3	**26** 1st UPM = 12 1st LPM = 14
	Histological control (n=3)							**3** 1st UPM = 12 1st LPM = 14

Commercial Products:
[1]Email Gel Blue-Vivadent,Vigodent SA, RJ, Brazil
[2]Single Bond Adhesive System - 3M Dental Products, St Paul, MN, USA
[3]All-Etch – BISCO, Schaumburg, IL, USA
[4]Calcium Hydroxide, PA–Biodinâmica Química e Farmacêutica Ltda, Ibiporã, PR, Brazil
[5]Calcium Hydroxide Cement-Dycal-Dentsply Ind e Com Ltda, RJ, Brazil
[6]Glass Ionomer Cement-Vitrebond, 3M Dental Products, St Paul, MN, USA
n= number of teeth/group; UPM = Upper premolar; LPM = Lower premolar

Table 2: *Classification of Bleeding on Pulp Exposure During DPC Procedures*

Scores	Bleeding
Absent	None apparent from the pulp exposure site.
Slight	Slight but apparent from the pulp exposure site—easily controlled with only an irrigation phase with saline and drying with sterile cotton pellets.
Moderate	Stopped in up to 30 seconds, with 3 to 5 irrigation phases with saline and drying with sterile cotton pellets.
Abundant	Persistent for more than 30 seconds. More than 5 irrigation phases were necessary for complete hemorrhage control.

water spray. An exposure approximately 1 mm in diameter was made in the coronal pulp chamber using a sterile round #1010 diamond bur (KG Sorensen, ISO size 010) under air-water spray, applied with a gentle technique to avoid deep penetration into the pulp tissue. Bleeding was arrested by rinsing with physiological saline, along with the application of dry sterile cotton pellets or pellets moistened with saline solution kept in place for 10 to 20 seconds. Pulpal bleeding was examined as clinical findings during the steps of mechanical pulp exposure, acid etching and adhesive application. The degree of bleeding was classified into four categories, adapted from Matsuo and others (1996), according to Table 2.

In Group I (n=26), the enamel was etched for 30 seconds with 37% phosphoric acid gel. Dentin and exposed pulp tissue were etched for 15 seconds. The cavities were rinsed with distilled water spray for 20 seconds and dried with sterile cotton pellets. Re-bleeding was recorded as being absent, slight, moderate or abundant.

After complete hemostasis, the dentin surface was lightly moist, without visible excess. The dentin bonding agent, Single Bond Adhesive System, was used according to the manufacturer's instructions. Briefly, a first layer was gently applied over the perforation site. A second layer was applied in the same way and light-cured for 20 seconds at 500-600 mW/cm^2. Shortly after application of the first adhesive layer, the clinical aspect of the exposed tissue was evaluated according to Table 2. If bleeding was recorded following application of the capping material, the wound was dried using sterile cotton pellets with slight compression. In cases of re-bleeding after application of the dentin bond agent, the wound was dried using sterile cotton pellets with slight compression, and the capping step with the adhesive agent was repeated (without acid conditioning). Increments of Charisma were used to restore the cavities. Each increment, 1 mm in thickness, was light cured for 20 seconds in all buccal, palatal and occlusal surfaces. Light intensity was monitored with a radiometer that was coupled to the light-curing unit (KM 200R—DG Line, K&M Ind Com Equipamentos Ltda, SP, Brazil).

In Group II (n=26), the enamel was submitted to the same procedure as the first group. Dentin and exposed pulp tissue were etched for 15 seconds with 10% phos-

Table 3: *Criteria and Scores for Histological Evaluation*

	Scores	Characterization
Inflammatory Cell Response	0	None or a few scattered inflammatory cells present in the pulp area corresponding to the pulp exposure, characteristic of normal tissue.
	1	Slight inflammatory cell infiltrate with polymorphonuclear leukocytes.
	2	Moderate inflammatory cell infiltrate involving the subjacent area to pulp exposure.
	3	Severe inflammatory cell infiltrate involving the coronal pulp or characterizing abscess.
Tissue Disorganization	0	Normal tissue, with preservation of histological layers.
	1	Slight alterations with superficial odontoblastic layer disorganization and retraction of pulp tissue. The deeper pulp tissue is normal.
	2	Loss of primay odontoblasts below the exposure site and moderate alterations as hialinization and vacuolization of matrix, in the coronal pulp.
	3	Total disorganization of pulp tissue morphology, with areas occupied by monomers in the coronal pulp.
	4	Severe alterarions in histological aspects over the pulp or necrosis in deeper pulp.
Hemorrhage Areas	0	Absence of hemorrhage areas.
	1	Hemorrhage areas present in the subjacent pulp area corresponding to the pulp exposure.
	2	Hemorrhage areas present in deep pulp tissue.
Reparative Dentin Formation (dentin bridge)	0	Absence of dental bridging.
	1	Organization of odontoblast-like layer and initial deposition of reparative dentinal matrix beneath the exposed area.
	2	Presence of hard tissue beneath the exposed area characterizing the deposition of dentin bridge.
Bacteria Presence	0	Absence of bacteria.
	1	Presence of stained bacteria along the cavity walls or within the cut dentin tubules.
	2	Presence of stained bacteria within the dental pulp.

Table 4: *Bleeding Levels After Mechanical Pulp Exposure and Direct Acid Etch*

		Bleeding Level								
	Procedure	**Absent**		**Slight**		**Moderate**		**Abundant**		
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**Total**
Pulp Exposure		-	0	66	84.6	8	10.3	4	5.1	**78**
Group I	37% phosphoric acid etchng	11	42.3	11	42.3	4	15.4	-	0	**26**
Group II	10% phosphoric acid etchng	12	46.1	12	46.1	2	7.7	-	0	**26**

Bleeding caused by mechanical pulp exposure > (I=II)
p<0,001 (The "p" value refers to the Kruskal-Wallis test)

Table 5: *Bleeding Levels Immediately After Capping Phase*

	Bleeding Level								
	Absent		**Slight**		**Moderate**		**Abundant**		
Capping Materials	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**Total**
SBAS after 37% acid etching (Group I)	18	69.2	3	11.5	5	19.3	-	0	**26**
SBAS after 10% acid etching (Group II)	14	53.9	5	19.2	7	26.9	-	0	**26**
Calcium Hydroxide (Group III)	24	92.3	2	7.7	-	0	-	0	**26**

(I=II)>III
p=0.006 (The "p" value refers to the Kruskal-Wallis test)

phoric acid gel. The following steps were performed as described for Group I.

In Group III (n=26), CH powder was applied over the pulp wound using a sterile plastic amalgam carrier with a small point without pressure. CH cement was applied in the occlusal cavity floor. Then, a thin layer of light cured glass ionomer was applied and light-cured for 20 seconds. The cavities were restored as in the other groups.

Prior to extraction, radiographs were taken, and the patients were asked whether they had experienced any pain or postoperative sensitivity. Another radiograph of

each tooth was taken after extraction to assess whether a dentin bridge was present.

Histological Method

After extraction under local anesthesia, the apical third of the teeth was cut off in order to facilitate fixation. Five teeth were extracted after 1, 3, 7 and 30 days (Table 1) and fixed in 2.5% glutaraldehyde (0.1 Mol/L phosphate buffer, pH 7.4) for 48 hours for Hematoxylin-Eosin (HE) and the Brown-Brenn technique for bacterial assessment. Three teeth from each group were extracted after 7 and 30 days (Table 1) and fixed with 10% buffered neutral formalin for the Ag-NOR technique (Linder, 1978) in order to assess the nucleolar organizer region-associated proteins. The teeth were demineralized in 10% EDTA (Ethylene-diamine tetraacetic acid, pH 7.3) for 120 to 180 days and embedded in paraffin. Twenty 6-µm thick sections from each sample were observed under a light microscope (Photomicroscope model Olympus BX50-Japan). Each section was independently examined by three previously calibrated investigators and five histological features (Table 3) were assessed according to criteria adapted from previous studies (Costa & others, 2003; Hebling & others, 1999; Costa & others, 2001; Subay & others, 2000).

Statistical Method

The degree of bleeding on the mechanical pulp exposition, acid etching or adhesive system application was analyzed using a non-parametric statistical Kruskal-Wallis test (Sampaio, 1998). A $p<0.05$ value probability was considered to be statistically significant.

RESULTS

Clinical Findings

The different degrees of bleeding recorded during the procedural steps are shown in Tables 4 and 5. Mechanical pulp exposition resulted in slight bleeding, which was easily controlled in most cases (Table 4). The level of bleeding after acid etching was similar between Groups I and II. In none of the cases was abundant bleeding observed (Table 4). In most cases, the adhesive agent did not restart the bleeding process. When bleeding occurred after capping, it was considered slight or mod-

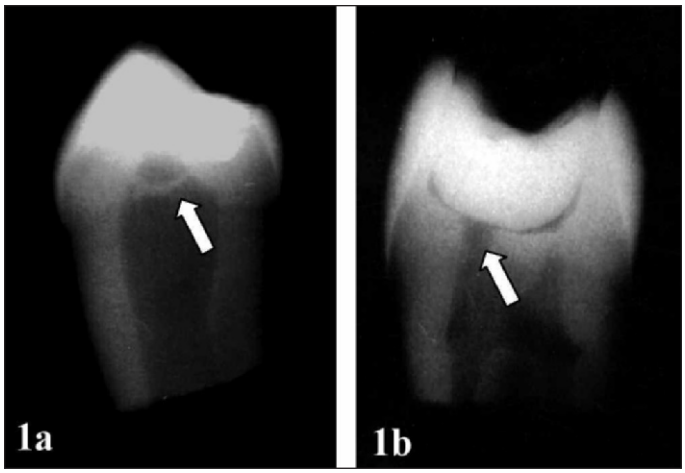

Figure 1: *Radiographs of extracted teeth at day 30. (a) Group III (CH); note the dentin barrier (arrow); (b) Tooth capped with SBAS; no image suggestive of dentin barrier (arrow).*

Table 6: *Grading of Histopathologic Features of Sections*

		Time Intervals											
		Day 1			Day 3			Day 7			Day 30		
Evaluation Criteria		**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
	Score												
Inflammatory	0	1	1	0	0	0	0	0	0	0	0	0	0
Cell Response	1	3	3	0	4	0	3	1	0	3	2	2	4
	2	1	1	5	1	5	2	4	5	2	3	3	1
	3	0	0	0	0	0	0	0	0	0	0	0	0
Tissue Disorganization	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	5	5	0	3	5	0	4	2	0	4	4	0
	3	0	0	5	1	0	5	1	3	5	0	1	5
	4	0	0	0	1	0	0	0	0	0	1	0	0
Hemorrhage	0	1	1	2	1	0	1	1	1	0	4	5	2
	1	3	1	3	2	4	4	2	3	4	1	0	3
	2	1	3	0	2	1	0	2	1	1	0	0	0
Hard Tissue Formation	0	5	5	5	5	5	5	5	5	1	5	5	0
	1	0	0	0	0	0	0	0	0	4	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	5
Bacteria Presence	0	5	5	5	5	5	5	5	4	5	5	5	5
	1	0	0	0	0	0	0	0	1	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0

Groups identified by I, II, III (n=5 for each sample)

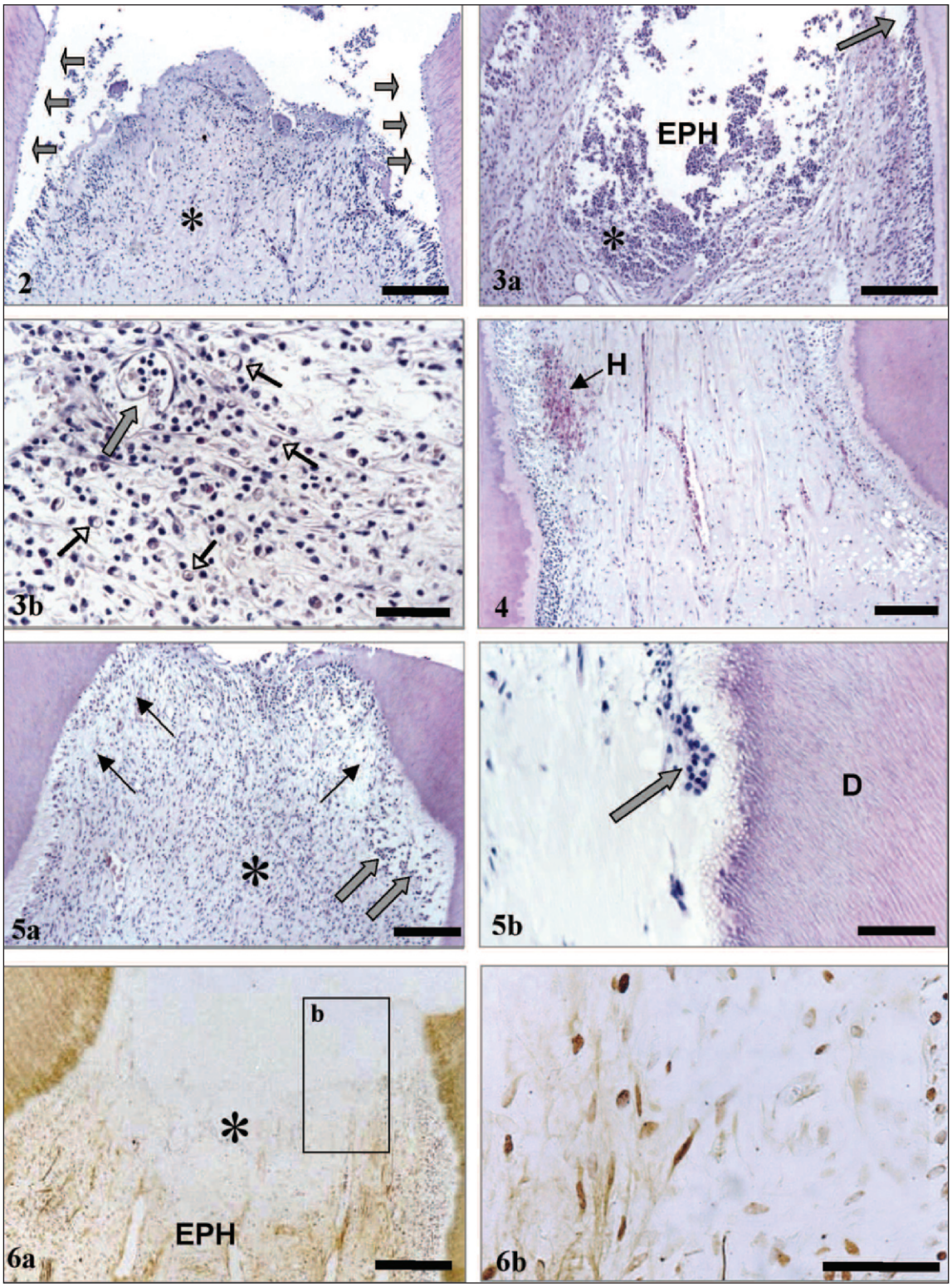

Figure 2. *Histological aspect of pulp tissue capped with SBAS (1st day). Panoramic view of the exposed pulp horn. The odontoblast layer close to the exposure is reduced or eliminated (arrows). Note the discrete inflammatory cell infiltrate (*). HE. Bar=200 µm.*

Figure 3. *Histological aspect of pulp tissue capped with SBAS (3-7 days). Panoramic view of the Exposed Pulp Horn (EPH). (a) Note the dense infiltrate close to the exposure area (*) and loss of odontoblast layer (arrow). HE. Bar=200 µm. (b) Inflammatory response mediated by macrophages (small arrows). Note the arrival of inflammatory cells in the blood vessel (big arrow). HE. Bar=50 µm.*

Figure 4. *Histological aspect of pulp tissue capped with SBAS (day 7). Note the hemorrhagic areas deeper in the pulp (H).*

Figure 5. *Histological aspect of pulp tissue capped with SBAS (day 30). (a) Panoramic view of the exposed pulp horn. Note the persistent inflammatory pulp response (*), absence of new dentin-like tissue (dentin bridge), loss of organization of predentin and cell layers in the lateral walls of the exposed area HE (thin arrows). HE. Bar=200 µm. (b) Higher magnification of multinuclear giant cells close to the odontoblast layer and the pulpal-dentin limit (thick arrows). D=Dentin. HE. Bar=50 µm.*

Figure 6. *AgNOR technique for pulp tissue capped with SBAS (day 30). (a) Panoramic view of the Exposed Pulp Horn (EPH). Absence of odontoblast-like cells with positive labeling for NORs (*). Bar=200 µm. (b) Higher magnification of (a). Transition area between the pulp tissue and the adhesive material. Absence of AgNOR-positive cells. Bar=50 µm.*

erate (Table 5). Clinically, the instantaneous CH hemostatic effect was considered as effective (Table 5).

The postoperative period was asymptomatic for all groups. No correlation could be established between pain and the microscopic findings. No detectable periradicular radiographic changes were observed in any teeth from either the experimental or control group. At day 30, only teeth

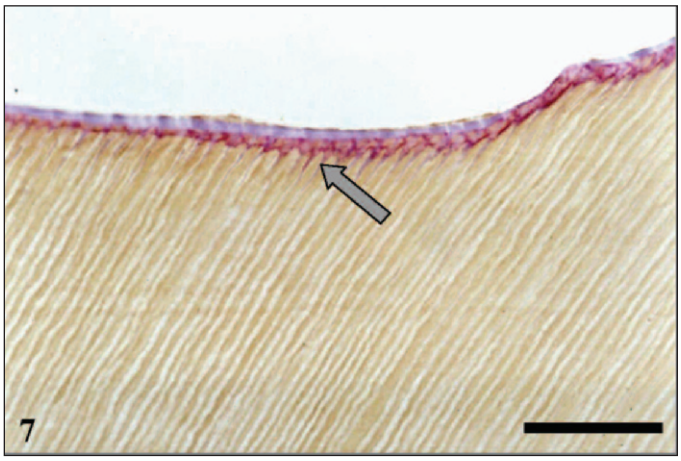

Figure 7: *Evaluation of bacterial microleakage. Representative section of the results obtained in all samples. No bacteria are present on the cavity floor and dentinary tubules. The red-stained layer is the hybrid layer with projections of resin in the opening of the dentinary tubules (arrow). The light pink-stained zone is sign of the Single Bond adhesive system. Brown-Brenn bacterial stain. Bar=50 µm.*

gestive image of a dentin barrier (Figure 1).

Histological Findings

Table 6 summarizes the histopathological findings graded according to criteria defined for both experimental (SBAS) and control (CH) groups at each time interval.

Pulp Reactions to SBAS

The histological aspects of pulp tissue after SBAS capping are shown in Figures 2-6. The pulp response was similar in Groups I and II. In these cases, pulp tissue exhibited discrete disorganization associated with a slight inflammatory process on the first day (Figure 2). The odontoblast layer subjacent to the pulp exposure site was disrupted or sometimes absent. After three to seven days, more dense inflammatory infiltrate and superficial degenerated neutrophils were observed (Figure 3a). The polymorph response was short, and the defense initial line was subsequently replaced with the recruitment of macrophages (Figure 3b). Resin globules were observed within these phagocytes. The presence of hemorrhagic foci in the pulp tissue or extravasated erythrocytes could be observed in all periods, especially at days three to seven (Figure 4).

At day 30, a slight-to-moderate mononuclear inflammatory infiltrate was persistent (Figure 5a). The foreign-body-type giant cells were evident in both the superficial tissue around the exposure site and close to the predentin (Figure 5b). The pulp tissue exhibited a significant disorganization zone, with loss of the odontoblast layer and predentin. There were no signs of cellular differentiation and dentin neoformation in the proximity of the injured area at day 30 (Figure 5a). The Ag-NOR silver staining showed the absence of cells with synthetic activity under the exposed area in both periods evaluated (Figure 6a,b).

The Brown-Brenn technique revealed negative staining for bacteria in all samples (Figure 7).

Pulp Reactions to Calcium Hydroxide

The histological aspects are shown in Figures 8-13. Superficial necrotic tissue with total disorganization was observed on the first day, separated from the subjacent vital-pulp tissue by the evident demarcation line (Figure 8). The remaining pulp tissue exhibited a moderate acute inflammatory infiltrate.

At day 3, the specimens exhibited a conspicuous necrotic area, with cell debris and fragments of coagulation necrosis extending from the exposure site to the deeper zone between the dentin walls (Figure 9). The subjacent pulp tissue showed a slightly more inflammatory response and the presence of hemorrhaged areas, as seen on the first day. On the seventh postoperative day, proliferated fibroblast and elongated pulp cells migrated toward the demarcation line between the normal pulp tissue and the necrotic layer (Figure 10a). The recently differentiated cells deposited a discontinuous layer of dentin matrix beneath the necrotic zone (Figure 10b). At day 30, the subjacent pulp tissue exhibited normal histological characteristics, similar to the control samples (Figure 11a,b). The inflammatory process showed signs of remission with few scattered mononuclear cells (Figure 11a). The dentin bridge deposition was a common finding in all samples (Figure 11 a,b). The Ag-NOR technique showed cells with high synthetic activity over the necrotic area in both evaluated periods (Figures 12 and 13). Bacteria were not detected in Brown and Brenn-stained sections in any specimens capped with CH for either time interval.

DISCUSSION

Currently, many professionals have recommended the application of adhesive systems as pulp capping materials in spite of the manufacturer's instructions to the contrary. Several factors, such as etching acid, toxicity of the leached components, microleakage due to polymerization shrinkage and demineralized dentin incompletely penetrated by the bonding (nanoleakage), sensitization and sudden temperature rise during setting may be harmful to pulp (Schuurs & others, 2000). Moreover, the precarious clinical conditions during DPC procedures, such as bleeding and difficulty with hemostasis maintenance, may contribute to capping failures. In this study, the bleeding generated by both acid solutions (Groups I and II) was similar (Table 4) and, in all cases, only irrigation with saline solution and drying with sterile cotton pellets were sufficient to reach hemostasis. The humidity and bleeding control at this moment in the adhesive technique are essential for the success of DPC. In this study, only saline solution

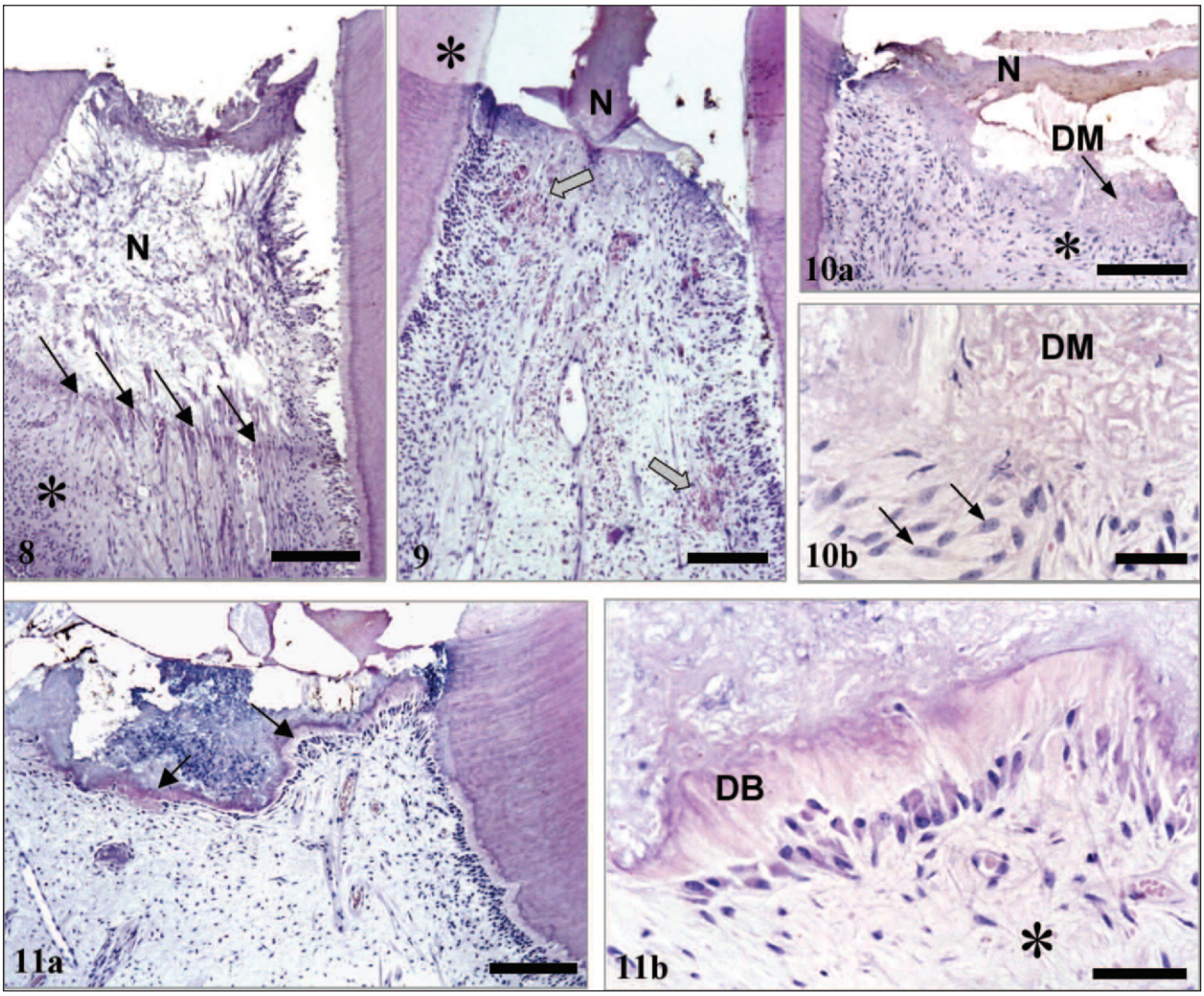

Figure 8: *Histological aspect of pulp tissue capped with CH (day 1). Exposed pulp horn with necrotic area (N), revealing total loss of histological characteristics. Note the demarcation line at the limit with the remaining pulp tissue (arrows) and inflammatory process (*). HE. Bar=200 µm.*

Figure 9. *Histological aspect of pulp tissue capped with CH (day 3). Panoramic bucco-lingual view of the exposed pulp horn. Note the debris of the necrotic tissue (N) and the effects of washing the prolonged odontoblasts on the lateral walls of the dentin (*). On the pulp tissue, the presence of a slight inflammatory cell infiltrate and extravasated erythrocytes (arrows). HE. Bar=200 µm.*

Figure 10. *Histological aspect of pulp tissue capped with CH (day 7). (a) Proliferation of pulpal cells (*), which migrate and elaborate new dentinal matrix (DM) under necrotic zone (N). HE. Bar=200 µm. (b) Elongated cells, that is, odontoblast-like cells (arrows), are adjacent to the newly deposited dentinal matrix (DM), which appears as an irregular acidofilic network. HE. Bar=50 µm.*

Figure 11. *Histological aspect of pulpal tissue capped with CH (day 30). (a) Panoramic bucco-lingual view of the exposed pulp horn. Note the remanescent pulp tissue isolated by the dentin bridge (arrows). HE. Bar=200 µm. (b) Higher magnification of newly-differentiated odontoblast-like cells and its relation with the dentin bridge (DB). Note extracellular matrix with normal aspect (*). HE. Bar=50 µm.*

the success of DPC. In this study, only saline solution was used in hemorrhage control. However, Accorinte and others (2005b) showed that the pulp response after direct pulp capping with adhesive systems is unfavorable, even after the use of different hemostatic agents.

The authors also observed that application of the adhesive system after acid etching, washing and drying did not induce bleeding in most cases. Recent studies have shown the ability of a number of single-bottle dentin adhesives to produce vasoconstriction in the rat carotid artery model (Tasman & others, 2000). Accordingly, Single Bond, which uses ethanol as a solvent, did not produce smooth muscle contraction, while one water-based and acetone-based adhesive systems, it caused dose-dependent contraction (Tasman & others, 2000). The potential capability of some adhesive systems to control hemorrhaging through vasoconstriction, similar to epinephrine, may alter the DPC success favorably (Onur & others, 2004). Despite this, Abebe, Pashley and Rueggeberg (2005) showed that some single-bottle dentin bonding systems may interfere with

vascular function, and this effect may promote bleeding when placed on the pulp exposure. However, this hypothesis was based on a study using rat aortic ring preparations, where the Single Bond caused endothelium-dependent relaxation in a concentration-dependent manner (Abebe & others, 2005).

In this study, patients submitted to direct pulp capping were all asymptomatic, despite the significant inflammation levels observed in the initial periods. This feature confirmed the lack of a relationship between clinical symptoms and histological pulp tissue characteristics (Matsuo & others, 1996). In addition, the authors showed that there was no radiographic evidence of periapical alterations. This data supports the hypothesis that the clinical and radiographic evaluation of teeth submitted to a variable range of pulp treatments can neither predict the biocompatibility of dental material nor indicate the safety of new pulp therapy (Accorinte & others, 2005a; Hebling & others, 1999; Horsted-Bindslev, Vilkinis & Sidlauskas, 2003).

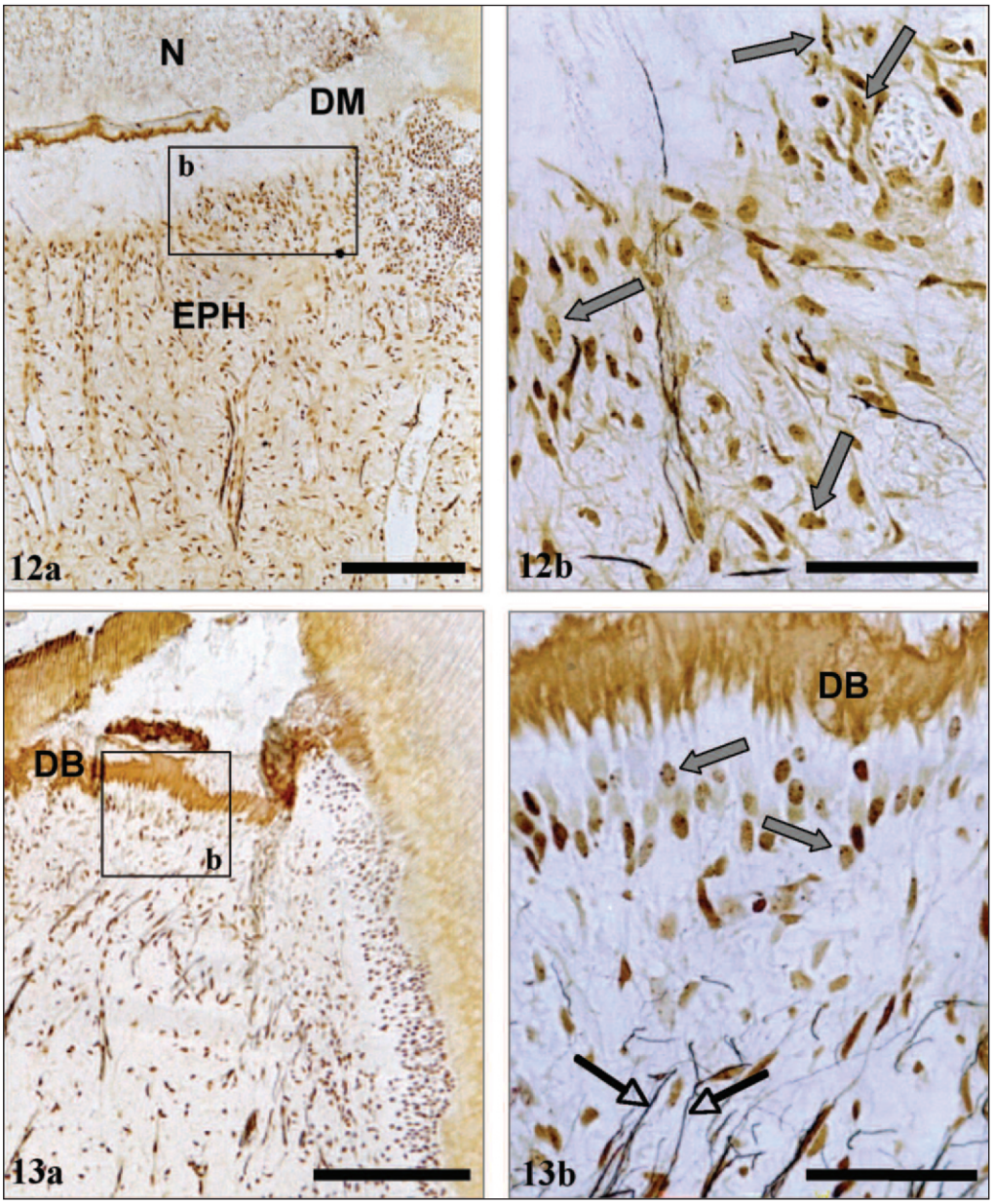

Figure 12: *AgNOR technique for pulpal tissue capped with CH (7th day). (a) Panoramic view of the Exposed Pulp Horn (EPH). N= Necrotic area. DM= newly produced dentinal matrix. Bar=200 µm. (b) Amplification of the previous picture (a). Detail of the odontoblast-like cells positive for AgNOR (arrows). AgNOR. Bar=50 µm.*

Figure 13. *AgNOR technique for pulpal tissue capped with CH (day 30). (a) Panoramic view of the exposed pulp horn, with excellent bridge formation (DB). Bar=200 µm. (b) Higher magnification of the area subjacent to the dentin bridge (DB). Note the newly differentiated odontoblasts with positive reaction in NORs (thick arrows) and the presence of rich innervation in the subodontoblastic zone, shown with irregular fibers stained in black (thin arrows). Bar=50 µm.*

The presence of inflammatory process, macrophages and globules of several sizes, suggesting unpolymerized residual monomers and a disrupted odontoblast layer subjacent to the exposure site observed in samples from Groups I and II, are indicative of low tolerance of the connective tissue in proximity to the adhesive system. In all periods evaluated, no signs of migration and cell differentiation on the exposed area were observed in teeth capped with SBAS. The absence of primary odontoblasts and newly organized odontoblast-like cells result in the lack of dentin bridge formation. Similar pulpal responses have been described after direct pulp capping in human teeth (Gwinnett & Tay, 1998; Porto-Neto & others, 1999; Hebling & others, 1999; Costa & others, 2001) and dog teeth (Koliniotou-Koumpia & Tziafas, 2005) using different adhesive agents.

In vivo studies have shown a strong relationship between bacterial microleakage and inflammatory pulp response (Akimoto & others, 1998; Cox & others, 1987). In this study, the Brown-Brenn technique revealed negative staining for bacteria in three groups. Thus, the authors may speculate that the inflammatory process

materials used. In fact, a recent study showed that all components of adhesive solutions and resin composite may result in degenerative pulp alterations (Accorinte & others, 2005a).

Samples capped with CH presented remarkably different results. During the early time intervals, the specimens exhibited a conspicuous coagulation necrotic zone (Stanley, 1998) as a line delimiting the tissue in repair under the necrotic zone (Figure 8). Despite the caustic property of the calcium hydroxide powder and the consequent loss of pulpal volume as observed at day 3, the material was less irritating than Single Bond and better tolerated by the remnant connective tissue over time. The ability of CH to stimulate the fibrodentinal matrix deposition associated with the histological layer of odontoblast-like cells integrate and AgNOR positive cells observed during the experimental periods are indicative of the repair potential of the remaining pulp tissue.

Deposition of the reparative dentin was an evident aspect observed at day 30 in all samples from Group III. It has been suggested that the dentin bridge formation itself is not a criteria for successful pulp healing once tunnel defects after DPC with CH may lead to leakage unlike the "permanent" seal by bonding agents (Schuurs & others, 2000; Akimoto & others, 1998; Cox & others, 1987). However, in this study, the deposition of reparative dentin was not isolated. At day 30, the remaining pulp tissue exhibited normal histological characteristics, with only discrete mononuclear inflammatory cells (Figure 11). A perfect integration of the new odontoblasts with the bridge and lateral walls of the dentin was observed (Figure 11b). Such features were indicative of recovery and maintenance of the remaining pulp tissue's vitality. The authors agree with the statement by Pereira and others (2000) that the presence of a dentin bridge must be recognized not only as a barrier to future injuries, but also as a sign of biological recovery, represented by the odontoblast activity. The activity of the odontoblast-like cells was confirmed by positive AgNOR labeling.

It is important to point out that the results presented here were obtained from sound teeth and young patients with larger repair potential. The intensity of the pulp reactions demonstrated in sound human teeth must be lower than those observed in carious teeth and/or teeth submitted to irritating stimuli (Costa & others, 2000; Horsted-Bindslev & others, 2003). The selection of intact teeth for this study allowed for homogeneous sampling, without previous clinical intercurrence. Nevertheless, direct pulp capping with the adhesive agent tested (SBAS) did not show aspects indicative of a positive prognostic.

Based on the experimental conditions, the authors believe that the pulp response shall develop in the long-term for aseptic necrosis and that adhesive agents do not represent a biologically acceptable alternative. Dentin adhesives for direct pulp capping should be avoided as long as more favorable human studies have been reported (Ranly & García-Godoy, 2000). This study is a short-term evaluation. Therefore, the transference of data obtained in this study, for clinical conditions, should be viewed with caution.

CONCLUSIONS

Calcium hydroxide remains the agent of choice for the capping of mechanically exposed pulps, and the Single Bond Adhesive System should not be applied directly on pulp connective tissue in human teeth.

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