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ORIGINAL ARTICLE

Response of human pulps capped with different self-etch adhesive systems

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Abstract The aim of this study was to evaluate the response of human pulps capped with a calcium hydroxide hard-setting cement or with two-step self-etch adhesive systems. Pulp exposures were performed on the occlusal floor, and the bleeding control was performed with saline solution. The exposed pulp tissue was capped with Clearfil LB 2V (2V) or Clearfil SE Bond (SE) and restored with a composite resin. In control group, the pulpal wound was capped with $Ca(OH)_2$ cement and restored with Clearfil LB 2V or Clearfil SE Bond + composite resin. After 30 and 90 days, the teeth were extracted, processed for hematoxylin and eosin, and categorized in a histological score system. The pulpal response was worse for groups capped with the self-etch adhesive systems (2V and SE) in both periods of evaluation, when compared to their respective control groups at 90 days ($p<0.05$). For both self-etch systems evaluated, the pulp tissue exhibited moderate to severe inflammatory cell infiltrate involving the coronal pulp with chronic abscesses. Dentin bridging was observed in a few specimens. For the calcium hydroxide groups, almost all specimens showed dentin bridge formation, with few scattered inflammatory cells and normal tissue below the pulp exposure site. Calcium hydroxide should be used as the material of choice for pulp capping, and the use of two-step self-etch adhesives for human pulp capping is contraindicated.

Keywords Pulp exposure · Self-etch adhesive systems · Pulpal response

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Introduction

Pulp exposure usually occurs in daily clinical practice due to cavity preparations, caries removal, or trauma. In such situations, there is a need to protect the pulp with a material that stimulates the organ to repair. Calcium hydroxide has been considered the outstanding material for pulp capping [7, 15]. The antibacterial activity, biocompatibility, stimulation of dentin bridge formation, reparative dentin induction, stimulation of the cellular activity, and release of bioactive molecules have been associated with the success of calcium hydroxide [15].

However, several concerns have been listed when calcium hydroxide is used for pulp capping. Presence of tunnel defects in the dentin barrier, extensive dentin matrix deposition obliterating the pulp chamber, short-time effect, solubility in oral fluids, lack of adhesion to the dental tissues, and degradation under acid etching are some of the limitations related to this material [10, 12]. The apposition of a blood clot between the pulp tissue and the pulp-capping material has been partially blamed for such limitations, as it inhibits the intimate contact of the calcium hydroxide with the pulp tissue and does not prevent the pulp from having a persistent chronic inflammatory reaction which may impair the pulp healing [37].

The reported disadvantages listed for calcium hydroxide have led researchers to investigate the potential of adhesive systems as pulp-capping agents [12, 13]. These studies supported the concept that microleakage of bacteria and their products around composite restorations, rather than the capping agent itself, play the most important role in pulpal inflammation [5, 11].

Opposite findings were observed when etch-and-rinse adhesives were applied in human pulps [1–3, 8, 17, 19, 28]. In these studies, etch-and-rinse bonding agents applied on pulp exposures following acid etching elicited a moderate inflammatory response at short-term evaluation, which sometimes leads to necrosis. Over time, a persistent mild inflammatory pulp response mediated by macrophages and giant cells was seen adjacent to the pulp exposure site. This chronic inflammatory response seems to play a role in the lack of complete dentin bridging formation. In long-term evaluations, the lack of dentin bridge formation is a common histological finding [8, 28].

Recently, Demarco et al. [14] reported that contrary to etch-and-rinse adhesives, direct pulp capping with a self-etch adhesive evoked less inflammatory response and induced dentin bridge formation in some cases. The authors concluded that the self-etch adhesives can provide a more favorable response of pulps than that achieved by etch-and-rinse systems. This was also observed in cytotoxicity studies [2, 40]. Unfortunately, most of the authors have evaluated the effects of pulp capping with self-etch adhesives in teeth of animals [4, 10, 18], and only few studies in human teeth have been conducted to evaluate the response of human pulps capped with self-etch adhesives [8, 14]. Consequently, the aim of the present in vivo study was to assess the response of human pulps capped with two contemporary two-step self-etching systems.

Materials and methods

Thirty-four healthy human premolars scheduled to be extracted for orthodontic reasons were selected from patients ranging from 15 to 30 years old. All teeth were examined clinically and radiographically to assure absence of proximal caries and periapical lesions. The patients and their parents signed consent forms after receiving a throughout explanation about the experimental rationale, clinical procedures, and possible risks. The parents and adult volunteers were asked to read and sign a consent form allowing the clinical procedure. Both the consent form and the research protocol were performed according to the Human Subject Review Committee from the University of Oeste de Santa Catarina, Santa Catarina, Brazil.

For the thermal testing, Endo-Ice frozen gas (Coltène/Whaledent, Mahwah, NJ, USA) was applied for 5 s on the buccal surface of the teeth scheduled for the pulp therapy and adjacent teeth. After local anesthesia (Citanest 3%; Merrel Lepetit, São Paulo, Brazil), the rubber dam isolation was installed, and each tooth was pumiced with a rubber cup at low speed. Occlusal cavities were prepared by means of sterile diamond burs (# 1095, KG Sorensen, Barueri, São Paulo, Brazil) at high speed under water/spray coolant. The dimensions of the cavity were: occlusal depth, 3.0± 0.2 mm; mesiodistal width, 4.0±0.5 mm; and faciolingual width, 3.0±0.2 mm. Pulp exposure was performed in the center of the pulpal floor by means of a round diamond bur under water-cooling (#1014, ϕ 1.2, KG Sorensen). One bur was used for each cavity. The teeth were then divided into six experimental groups as shown in Fig. 1.

The bleeding was only controlled by abundant irrigation with sterile saline solution (Fórmula e Ação, São Paulo, São Paulo, Brazil) followed by the application of damp cotton pellet embedded in saline solution. In groups 1 to 4, the pulp was capped with the self-etch adhesives Clearfil Liner Bond 2V (2V) or Clearfil SE Bond (SE). The capping materials and resin composite used in the present in vivo study and their chemical compositions are described in Table 1.

In groups 1 (2V30, n=6) and 2 (2V90, n=6), Clearfil Liner Bond 2V (Kuraray Medical, Osaka, Japan) was used. Equal amounts of primers A and B were mixed and immediately applied on the pulpal wound and cavity walls for 30 s. This layer of primer was gently air-dried for 20 s. The bonding resin was subsequently applied and light-cured for 10 s. In groups 3 (SE30, n=6) and 4 (SE90, n=6), Clearfil SE Bond (Kuraray Medical) was used. The primer was applied on the pulpal wound and cavity walls for 20 s, followed by gentle air-drying for 20 s. The bonding resin was subsequently applied and light-cured for 10 s.

In groups 5 [calcium hydroxide (CH) + 2V90, n=5] and 6 (CH + SE90, n=5), a calcium hydroxide hard-setting cement (Dycal Dentsply, Petrópolis, Rio de Janeiro, Brazil) was applied on the pulpal wound, and Clearfil Liner Bond 2V or Clearfil SE Bond was applied on the cavity walls, respectively, as previously described. Then, increments of Z-100 (3M ESPE, St. Paul, MN, USA) were used to restore the cavities. Each increment (±2 mm) was light-cured for 40 s. All light-cured procedures were made with at light-curing device with 450 mW/cm^2 (Ultralux Electronic, Dabi Atlante, Ribeirão Preto, São Paulo, Brazil). A radiometer (Model 100P-Demetron Research, Kerr, Danbury, CT, USA) was used to check the light intensity immediately before each clinical appointment. When necessary, the material excesses were removed using an ultra-fine diamond bur at high speed under water cooling (KG Sorensen).

Teeth from groups 1 (2V30) and 3 (SE30) were extracted after 30 days, while teeth from groups 2, 4, 5, and 6 (2V90, SE90, CH + 2V90, and CH + SE90) were extracted after 90 days. The patients were asked about the presence of

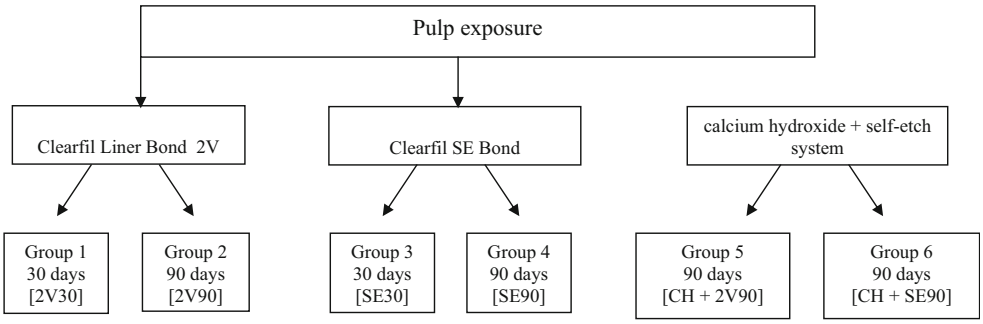

Fig. 1 Experimental design

postoperative sensitivity throughout the study period. The extraction was performed under local anesthesia. The roots of the teeth were sectioned midway between cement–enamel junction and the apex in order to allow adequate fixation of the specimens which were immersed for 96 h in 10% buffered formalin solution. The teeth were demineralized with Morse's solution (50% formic acid + 20% sodium citrate) for 60 days. The teeth were then vacuum-infiltrated with paraffin wax and finally embedded in paraffin, sectioned at 5–6 μm thickness, stained with hematoxylin and eosin and Masson's trichrome stains, and treated with Brown and Breen technique to assess bacteria. Single-blind evaluation of the microscopic sections was performed. For all sections, four histological features were assessed, which were recorded as shown in Tables 2, 3, 4, and 5, using a light microscope (Carl Zeiss, Oberkochen, Germany). Each histomorphological event was evaluated in a 0 to 3 score system, with 0 being the best result and 3 the worst result. The multiple sections were used to achieve an overall assessment for each tooth.

The mean of all the scores attributed to the sub-items of each one of the four criteria (inflammatory cell response, tissue disorganization, hard-tissue bridge, and stained bacteria) were subjected to nonparametric Kruskal–Wallis analysis (Tables 2, 3, 4, and 5). The comparisons between ranks were performed by Mann–Whitney *U* test (α=0.05).

Results

The percentages of scores for the experimental groups are shown in Table 6. Overall, the histomorphological features from groups capped only with both adhesive systems (2V and SE) in both periods of evaluation were inferior to the response from the groups capped with calcium hydroxide hard-setting cement ($p<0.05$). The statistical analysis demonstrated no significant difference between Clearfil Liner Bond 2V and Clearfil SE Bond in both periods (30 and 90 days) of evaluation ($p>0.05$).

Group 1—2V30 It was observed that half of the specimens (50%) presented moderate inflammatory pulpal response mediated by mononuclear cells along with tissue disorganization (Fig. 2a,b). In these specimens, only one specimen presented a complete hard-barrier formation. In only one specimen (17%), bacteria were seen along the cavity walls, and in this case, a severe inflammatory pulp response occurred (Fig. 2c).

Group 2—2V90 In this experimental group, three specimens exhibited slight inflammatory response and tissue disorganization. Proliferation of the connective tissue out of the pulp chamber was frequently observed in most of specimens (Fig. 2d). In 17% of specimens that presented no

Table 1 Products, commercial name, and composition

Product/commercial name	Composition
Clearfil SE Bond (Kuraray Medical)	Primer: water, MDP, HEMA, camphoroquinone, hydrophilic dimethacrylate, *N*,*N*-diethanol *p*-toluidine
	Adhesive: MDP, HEMA, Bis-GMA, camphoroquinone, hydrophobic dimethacrylate, *N*,*N*-diethanol *p*-toluidine, silanated colloidal silica
Clearfil Liner Bond 2V (Kuraray Medical)	Primer A: water, MDP, HEMA, camphoroquinone, hydrophilic dimethacrylate, *N*,*N*-diethanol *p*-toluidine
	Primer B: HEMA and water
	Adhesive: MDP, HEMA, Bis-GMA, camphoroquinone hydrophobic dimethacrylate, *N*,*N*-diethanol *p*-toluidine; silanated colloidal silica
Z-100 (3M ESPE)	Bis-GMA, TEGDMA, and silica/zirconium filler
Dycal Dentsply	Base past: ester glycol salicylate, calcium phosphate, Ca tungstate, and ZnO
	Catalyst past: ethylene toluene sulfonamide, Ca $(OH)_2$, ZnO, Ti_2O, and Zn stearate

Table 2 Scores used during the histological exams: inflammatory cell response

Scores	Characterization
0	None or a few scattered inflammatory cells presented in the pulp exposure site or below the hard-barrier formation, characterizing a normal tissue
1	Slight inflammatory cell infiltrate with polymorphonuclear or mononuclear leukocytes below the pulp exposure site
2	Moderate inflammatory cell infiltrate below the pulp exposure site
3	Severe inflammatory cell infiltrate involving the whole radicular pulp, characterizing pulpal necrosis or abscess

inflammatory response or tissue disorganization, a complete hard-barrier formation was observed (Fig. 2e,f). In another 17% of the specimens in which bacteria were found on the exposed pulp tissue, a severe inflammatory pulp response was presented.

Group 3—SE30 At 30 days, 50% of the specimens exhibited slight inflammatory pulpal response and tissue disorganization. In 33% of the specimens, moderate inflammatory response was observed. Only 17% of the specimens presented severe inflammatory reaction associated with noticeable disorganization of the coronary pulp tissue. For all specimens of this experimental group, neither bacteria were seen at the cavity walls nor hard-barrier formation was observed (Fig. 3a,b).

Group 4—SE90 In this experimental group, 50% of the specimens presented mild inflammatory response adjacent to the pulp exposure site, which exhibited slight tissue disorganization. It was determined that 83% of the specimens exhibited discrete, moderate, or intense deposition of dentin matrix at the pulp exposure site characterizing the partial or complete hard-barrier formation (Fig. 3c–f). No bacteria were observed at the cavity walls or over the pulp tissue in 66% of the specimens.

Group 5—CH + 2V90 In 20% of the specimens, a moderate to intense inflammatory response mediated by mononuclear cells was observed below the pulp capping site. No cell differentiation or deposition of dentin matrix occurred. The subjacent pulp tissue exhibited scattered inflammatory cells associated with only a few dilated blood vessels. Bacteria were evidenced at the lateral cavity walls and close to the pulpal wound. In 80% of the specimens, a complete hard-barrier formation was observed (Fig. 4a). However, the displacement of the capping agent (Dycal) gave rise to a hard barrier with irregular shape which was partially underlined by elongated odontoblast-like cells (Fig. 4b). No microorganisms were found at the lateral walls or over the exposed pulp tissue.

Table 3 Scores used during the histological exams: tissue disorganization

Scores	Characterization
0	Normal tissue below the pulp exposure site
1	Slight disorganization immediately below the pulp exposure site or adjacent to the hard-tissue formation
2	Moderate disorganization evolving 2/3 of the pulp tissue below the pulp exposure site or adjacent to the hard-tissue formation
3	Total disorganization associated to pulp breakdown

Table 4 Scores used during the histological exams: hard-barrier formation

Scores	Characterization
0	Intense hard-tissue deposition beneath the pulp exposure site characterizing complete dentin bridge
1	Moderate hard-tissue deposition beneath the pulp exposure site
2	Discrete hard-tissue deposition beneath the pulp exposure site
3	Absence

Group 6—CH + SE90 Similar histological findings described above for the control group 5 were also observed in group 6. However, 20% of the specimens were lost during histological processing. The remaining 80% of the specimens showed none or few scattered inflammatory cells in the pulp tissue. In these specimens, noticeable deposition of dentin matrix characterized the irregular hard barrier formed adjacent to the capping agent. No microorganisms were found in this control group.

Discussion

The control of bleeding has been considered one of the most important clinical conditions to allow pulpal healing [4, 12, 24]. The lack of adequate bleeding control after acid etching has been blamed for the high failure rates detected in pulp-capping studies using etch-and-rinse adhesive systems [13, 18]. Both self-etch adhesives used in the

Table 5 Scores used during the histological exams: stained bacteria

Scores	Characterization
0	Absence
1	Presence of stained bacteria along the cavity lateral walls
2	Presence of stained bacteria along all the cavity walls
3	Presence of stained bacteria along the cavity walls and over the exposed pulp tissue

Table 6 Number of specimens attributed for each group in each period of evaluation and multiple comparisons

Groups	Inflammatory cell response					Tissue disorganization					Hard-tissue barrier					Bacterial presence					Mean[b]
	0	1	2	3	Mean[a]	0	1	2	3	Mean[a]	0	1	2	3	Mean[a]	0	1	2	3	Mean[a]	
C2V (30)	0	2	3	1	1.8	0	2	4	0	1.3	1	0	2	3	2.2	5	0	1	0	0.3	1.4 b,c
CSE (30)	0	3	2	1	1.7	0	3	2	1	1.7	0	0	0	5	3.0	6	0	0	0	0.0	1.6c
C2V (90)	1	3	1	1	1.3	1	3	1	1	1.3	1	3	1	1	1.3	5	0	0	1	1.5	1.3 b
CSE (90)	1	3	2	0	1.2	0	5	0	1	1.3	1	1	3	1	1.7	4	1	1	0	1.5	1.4 b,c
CH C2V (90)	4	0	1	0	0.4	4	1	0	0	0.4	4	0	1	0	0.4	4	0	0	1	0.6	0.4 a
CH + CSE (90)	4	0	0	0	0.0	4	0	0	0	0.0	4	0	0	0	0.0	4	0	0	0	0.0	0.0 a

[a] Means for each group in each sub-item of the criteria
[b] Overall means for the criteria. Different letters mean significant differences ($p<0.05$)

present investigation (Clearfil Liner Bond 2V and Cleafil SE Bond) do not require a separate acid etching step which reduces the technique sensibility and prevents the occurrence of new bleeding after etching. Also, self-etch agents are less acidic than the phosphoric acid, reducing their deleterious potential over the pulp tissue. It is worth mentioning that self-etch systems are also easier to be buffered than the phosphoric acid [23].

Current studies have shown the ability of adhesive systems to produce vasoconstriction in the rat carotid artery model [38]. The potential capacity of adhesive systems to control hemorrhaging through vasoconstriction, similar to epinephrine, may alter the direct pulp capping favorably [26]. This seems to be the reason for the higher success rates of self-etch systems, when used as pulp-capping agents, than the etch-and-rinse adhesives [24, 39].

In the present investigation, dentin bridging was observed in few specimens in which the pulps were capped with the self-etch systems. This finding agrees with other published studies. For instance, Akimoto et al. [4] showed that Clearfil Liner Bond 2, when placed in direct contact with pulp tissue in primate animals, allowed differentiation of new pulpal cells that stratified, polarized (reoriented), and laid down a dentinal bridge. Demarco et al. [14] reported that direct pulp capping of human pulps with Clearfil Liner Bond 2 showed a more favorable pulp response than that usually seen for etch-and-rinse systems. The authors highlighted the inherent pulp capacity to heal and the release of growth factors by dentin demineralization, due to primer conditioning, as possible reasons for the dentin barrier formation with the adhesive material.

However, despite the aforementioned finding, a greater inflammatory response and more tissue disorganization was observed with the two self-etch systems when compared to the hard-setting calcium hydroxide cement. This is possibly associated with the cytotoxic effects of uncured residual monomers presented in the adhesive systems. As seen in Table 1, the main constituents of primers and adhesive agents are hydroxyethylmethacrylate (HEMA), hydrophilic monomers, 10-methacryloxydecyldihydrogen phosphate (MDP), and bisphenol A diglycidylether methacrylate (Bis-GMA). In a ranking of toxicity, HEMA, which is the main chemical component of the bonding agents, is the least toxic substance in comparison with other monomers such as Bis-GMA, urethane dymethacrylate (UDMA) and triethyleneglycol-dimethacrylate (TEGDMA) after 24 and 72 h exposures [31]. When monomers are photoactivated, the amount of unreacted products leached out is reduced and thus, their cytotoxic effects over cells are dramatically diminished [6, 9, 14]. Although the primer is not light-cured, this solution contains camphoroquinone, and therefore, the monomers from this layer can copolymerize with the bonding resin by the time the latter is light-cured. However, when primers are applied to pulp wounds, the cytotoxic effects of resinous monomers may promote pulp damage because the residual monomers in self-etch adhesives are not rinsed out clinically. Although these components were light-cured before restoration of the cavity, it is well known that the conversion of monomer to polymer is never complete [30]. All the organic components of the adhesive systems, photo-initiators, and chemical constituents generated during the setting process are released shortly after setting [16, 21, 31, 42].

It is known that oxygen acts as an inhibitor of resin polymerization [32]. It has been previously reported that an unfilled resin cured in room atmosphere had a significantly greater thickness of polymerization-inhibited material than did resin cured in argon atmosphere [32]. Other studies have demonstrated that the wet environment (as occurs to monomers when in contact with the pulp tissue) may preclude the adequate polymerization of resin-based materials [16, 35]. The addition of approximately 20 μl of water per milliliter of adhesive solution reduces the conversion degree of a water-free bonding resin from 53 to about 25% [22]. The residual water, characterized as the local edema at the pulp exposures, may dilute the priming components and preclude the production of highly cross-linked polymer chains, compromising in turn the integrity of the dentin interface over time [27].

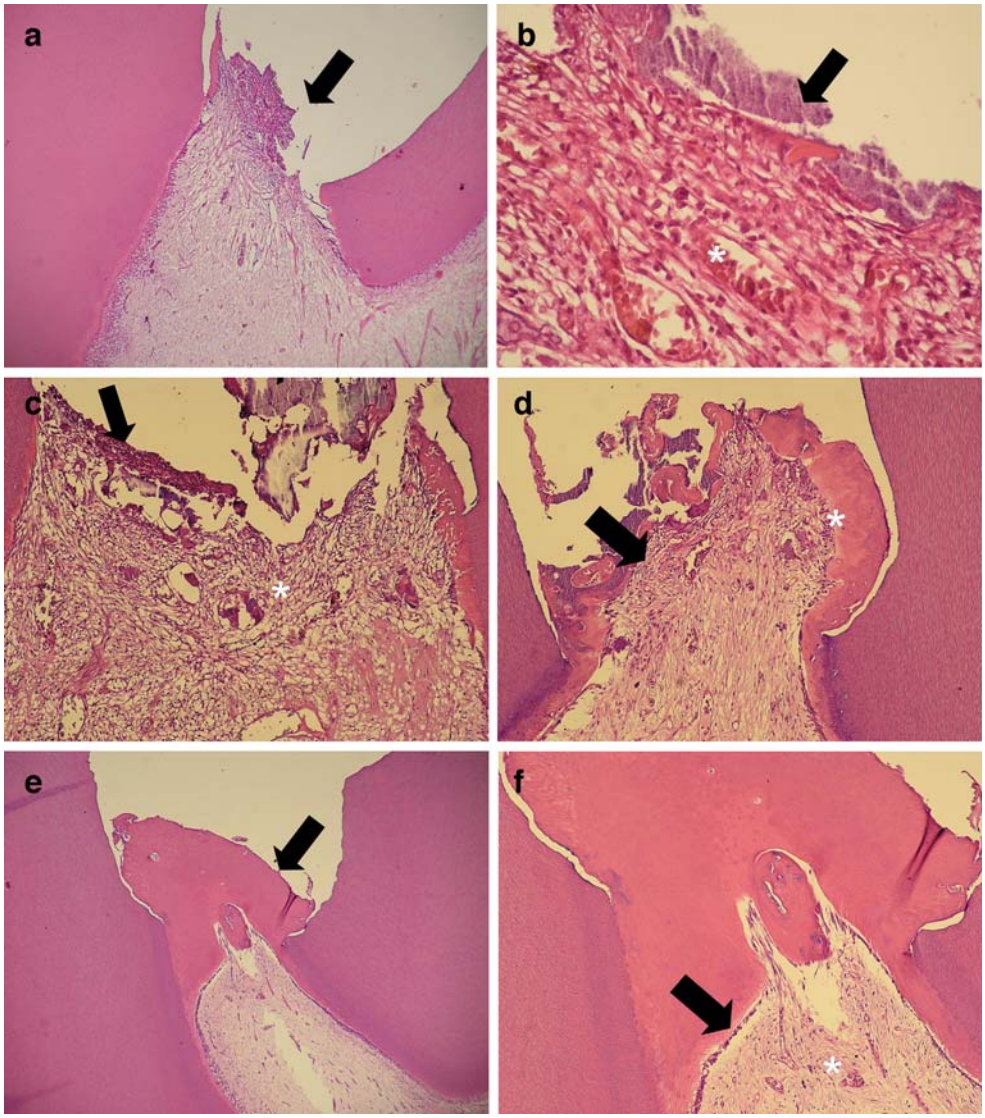

Fig. 2 **a**–**c** Group 1 (C2V, 30 days). **a** General view of the pulp horn exposure on which the self-etching adhesive system was applied. A small part of the connective tissue grew out of the coronary pulp chamber, and no hard-tissue bridge can be seen (*black arrow*). H/E, ±32×. **b** High magnification of **a**. Note the residual components of the bonding agent on the pulp tissue (*black arrow*) which exhibits moderate inflammatory response mediated by mononuclear cells. A number of dilated and congested blood vessels can also be observed associated with local edema (*white asterisk*). H/E, ±125×. **c** Intense inflammatory response and tissue disorganization is observed adjacent to the capping agent (*white asterisk*). In this specimen in which bacteria were evidenced, no hard-barrier formation occurred (*black arrow*). H/E, ±125×. **d**–**f** Group 2 (C2V, 90 days). **d** Notable amount of pulp tissue is observed out of the coronary pulp chamber (*black arrow*). Note that heterogeneous deposition of dentin matrix by elongated pulp cells occurred (*white asterisk*). The subjacent pulp tissue exhibits local inflammatory reaction. H/E, ±64×. **e** General view of the complete hard barrier deposited in the pulp tissue that grew out of the coronary pulp chamber (*black arrow*). H/E, ±32×. **f** High magnification of **e**. Note that a layer of odontoblast cells are underlying the hard barrier (*black arrow*). Normal tissue below the pulp exposure site with no inflammatory pulpal response is observed (*white asterisk*). H/E, ±64×

However, after 30 days, the pulp response of Clearfil SE Bond was worse than Clearfil Liner Bond 2V. Although these systems have similar compositions, the primer of the former is presented in two bottles that should be mixed prior use. The composition of primer A (which is similar to the Clearfil SE Bond primer) is diluted by primer B, since the latter is mainly composed of water. Therefore, it may be speculated that the better response of Clearfil Liner Bond 2V could be due to the lower concentration of monomers. After 90 days, a better pulp response was observed for both systems. Hard-barrier formation also occurred in some specimens, as previously demonstrated by Demarco et al. [14].

However, the literature is controversial in this matter. Costa et al. [8] did not find pulpal healing after application of Clearfil Liner Bond 2V on mechanically exposed pulp tissue. On the contrary, the pulps capped with this self-etch

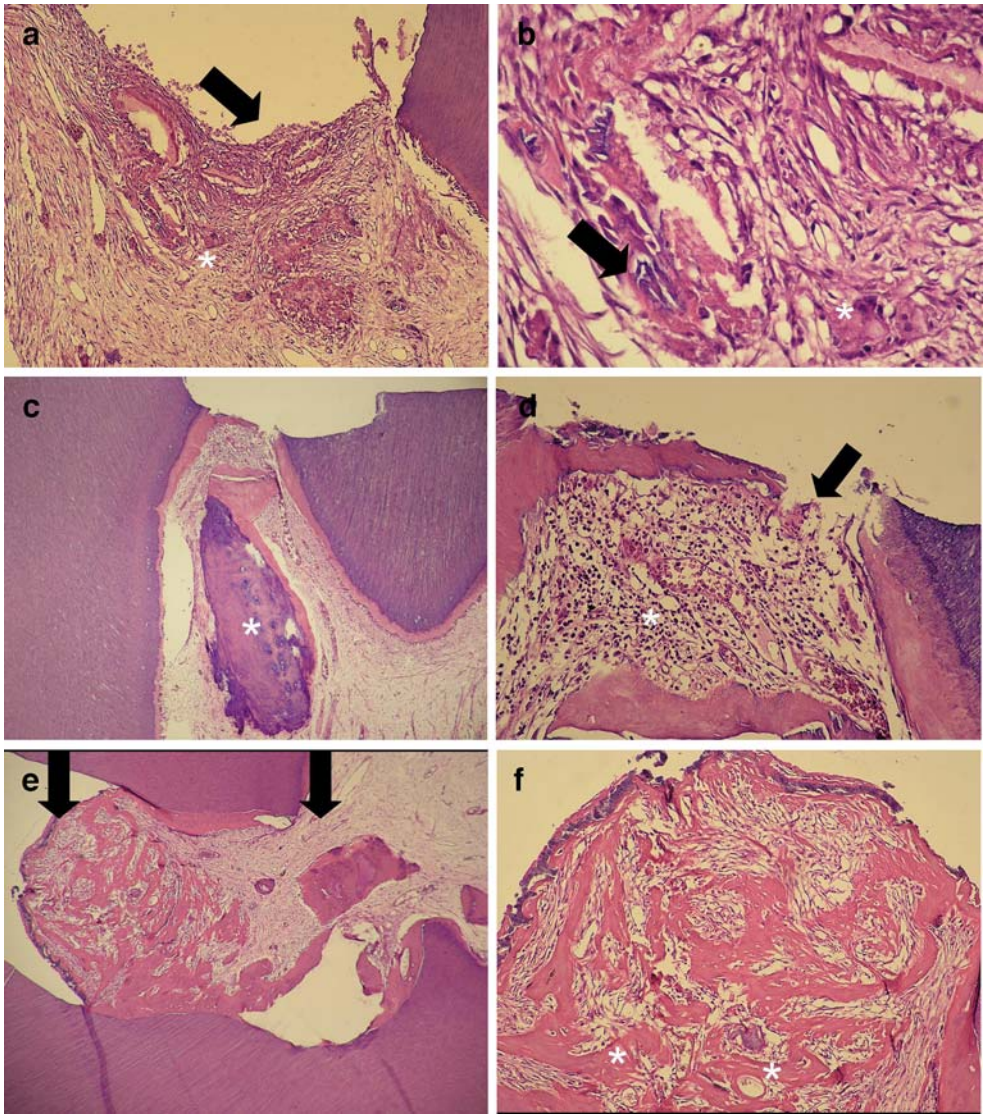

Fig. 3 **a**, **b** Group 3 (CSE, 30 days). **a** Moderate inflammatory response is observed at the pulpal wound, as well as moderate disorganization near to the pulp exposure site (*white asterisk*). No hard-barrier deposition occurred (*black arrow*). H/E, ±125×. **b** High magnification of **a**. Note that components of bonding agent displaced within the pulp tissue (*black arrow*) triggered an inflammatory reaction mediated by mononuclear and giant cells (*white asterisk*). H/E, ±310×. **c**–**f** Group 4 (CSE, 90 days). **c** General view of the pulpal wound capped with the bonding agent. Note the presence of a calcified pulpal nodule in the central zone of the pulp horn (*white asterisk*). H/E, ±32×. **d** High magnification of **c**. Moderate deposition of dentin matrix at the pulp capping site gave rise to the incomplete hard barrier. Observe that the pulp tissue between the pulpal nodule and the partial hard barrier present (*black arrow*) local disorganization associated with moderate inflammatory response (*white asterisk*). H/E, ±125×. **e** Note that the pulp tissue migrated out of the coronary pulp chamber (*between two black arrows*). H/E, ±32×. **f** Detail of the connective tissue that migrated from the pulp horn. Ectopic deposition of dentin matrix is observed inside of the pulp tissue (*white asterisk*). H/E, ±125×

adhesive system were associated with chronic inflammatory response, triggered by residual components of bonding agent displaced into the pulp space. The presence of resin particles at the exposure site has been linked to persistent inflammatory reaction. Such reaction could avoid the odontoblast-like cell differentiation resulting in lack of complete hard-barrier formation [17].

Several authors have reported that the staining methodology used to detect bacteria is not sensitive [1–3]. This fact makes the identification of bacteria difficult, mainly when a small number of such microorganisms are presented. Moreover, bacteria are easily removed from pulp tissue during laboratorial processing of the teeth [43]. Another disadvantage is that the bacteria-staining technique does not differentiate microorganism types, their pathogenic effect and viability [17].

Despite these considerations, this in vivo study detected bacteria in three out of four groups capped with the self-etch systems. This may be due to contamination during or after the restorative procedure. As the number of bacteria

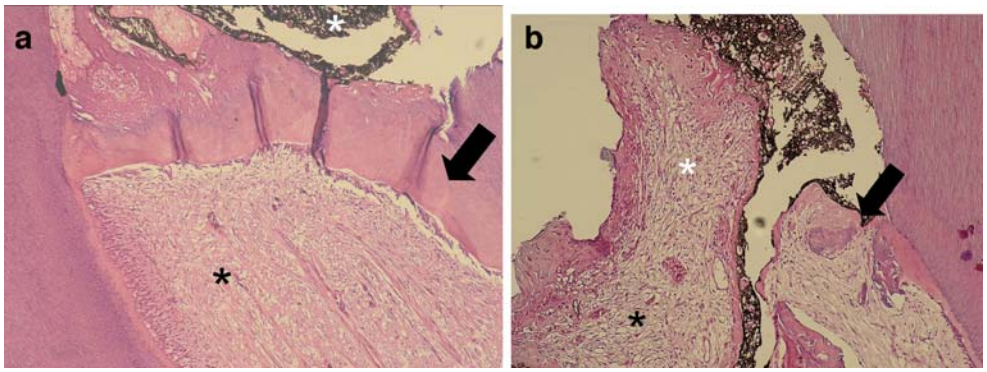

Fig. 4 **a**, **b** Control group 5 (C2V, 90 days). **a** Thin necrotic tissue adjacent to the pulp-capping agent (Dycal) is observed (*white asterisk*). Note the complete hard barrier between the capping agent and the pulp tissue (*black arrow*), which does not exhibit inflammation and normal tissue below the pulp exposure site (*black asterisk*). H/E, 125×. **b** Note the deposition of irregular dentin matrix (*black arrow*) adjacent to the capping agent (Dycal) which displaced from the capping site (*white asterisk*). The hard barrier with irregular shape is partially underlined by elongated pulp cells. The subjacent pulp tissue exhibits a number of mononuclear inflammatory pulp cells (*black asterisk*). H/E, 125×

was lower in the specimens examined at 30-day period, one may hypothesize that the microleakage took place at the resin-based material/cavity wall interface after the restorative procedure. This indicates that the sealing provided by the self-etch adhesives evaluated in the present investigation is not as effective as desired, particularly when the margins are located in enamel [29, 41]. In the present in vivo study, it was not demonstrated to have statistical difference between the two experimental self-etch adhesive systems regarding the presence of bacteria. This information did not corroborate with the data provided by some previous clinical trials, in which better marginal sealing was observed with Clearfil SE Bond when compared to Clearfil Liner Bond 2V. Probably, 90-day period is a short time of evaluation when compared to the long-term assessment of the clinical behavior of specific adhesive restorations [29, 41].

In recent years, several researchers have recommended the use of adhesive systems for pulp capping [10, 25, 34]. They supported the concept that microleakage of bacteria and its products around composite restorations, rather than the cytotoxic effects of materials, is responsible for pulpal inflammation [5, 11]. This statement cannot be corroborated by the current in vivo study as many samples which presented pulpal breakdown after pulp-capping with the adhesive systems did not exhibit microleakage. On the contrary, bacteria were observed in one of the two control groups, and this fact did not prevent the pulpal healing and dentin bridge formation.

The results observed after pulp capping with calcium hydroxide were not surprising. The ability of different calcium hydroxide formulations to allow pulp repair and hard-barrier deposition, as shown in the present investigation, has already been observed in several previous histological studies [1–3, 8, 17, 19, 20, 28]. Calcium hydroxide and its compounds are the materials against which new candidates for pulp capping are tested because of their long record of success in clinical and histologic studies. Calcium hydroxide is considered an outstanding material for direct pulp capping because it preserves pulp viability. The caustic effect of calcium hydroxide completely deranges and distorts the pulp tissue in immediate contact with the material [33]. The sequence of tissue healing after application of calcium hydroxide as pulp-capping agent is basically that which is expected of wounded connective tissue, starting with vascular changes and inflammatory cell migration and infiltration to control and eliminate irritating agents [33, 37]. The more basic, alkaline environment appears to favor the further differentiation of pulp cells into odontoblast-like cells which make the synthesis and deposition of dentin matrix, giving rise to the calcified dentin bridge. In the present investigation, all specimens capped with calcium hydroxide showed dentin bridge formation [36].

Conclusions

According to the experimental conditions and the methodology employed in the present investigation, it may be concluded that calcium hydroxide should be used as the material of choice for pulp capping. On the other hand, the use of two-step self-etch adhesive systems in vital pulp capping is not indicated.

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