Factors influencing pulpal response to cavity restorations

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Abstract

Objectives: The purposes of this retrospective work were: (1) to determine the relative importance of bacteria on cavity walls, remaining dentin thickness and post-operative time on pulpal inflammation after cavity restoration; (2) to compare the respective influences of bacterial microleakage and the restorative material itself on pulp reaction severity.

Methods: 317 class V cavities, in human bicuspid scheduled for extraction for orthodontic reasons were used for this study. Nine different materials were included. The severity of the pulpal reaction was ranked on hematoxylin/eosin stained sections according to FDI standards. The further parameters recorded were: (1) the presence or absence of bacteria on the cavity walls was noted on Brown and Brenn stained sections; (2) the remaining dentin thickness was measured and the teeth classified into three groups (<500 , $500-1000$, >1000 μm); and (3) the post-operative delay before extraction was recorded and classified as short time (<5 weeks) or long time (>5 weeks). Three two-way analyses of variance (ANOVA) followed by Kruskal and Wallis tests evaluated the influence of the three parameters on pulpal reaction severity. The third ANOVA also compared pulpal reactions under the different materials when the teeth were pooled, on bacteria free teeth and on bacteria contaminated teeth.

Results: The first ANOVA ranked by decreasing order of importance: the presence of bacteria ($p < 0.0001$), the remaining dentin thickness ($p = 0.02$) and the post-operative delay ($p = 0.04$). The second ANOVA showed no difference among the restorative materials when bacteria were present on the cavity walls

Significance: The presence of bacteria on the cavity walls is the main factor influencing pulpal reaction under restorative materials, but does not account for 100% of the cases. © 2000 Academy of Dental Materials.

Keywords: Pulpal reaction; Bacterial microleakage; Dentin thickness

1. Introduction

According to the bacterial microleakage concept, pulpal reactions observed after cavity filling are mainly due to the passage of bacteria between the dentinal walls and the filling material towards the pulp. Many studies showed strong evidence that this theory is valid. Kakehashi et al. [1], Watts [2], Paterson [3] and Watts and Paterson [4] found spontaneous and better pulpal healing after pulpal exposure or cavity filling on germ free animals than on conventional animals. A correlation has also been shown between the pulpal inflammation and the presence of bacteria entrapped between the restorative material and the cavity floor [5,6]. Recently Cox et al. have shown [7,8] that pulpal inflammation subsequent to cavity filling was only transient if a zinc-oxide eugenol cement was placed on the outer surface of the cavity, thus preventing any bacterial contamination. However, if pulpal inflammation was due solely to bacteria

leakage, no pulpal healing should occur, since a continuously renewed flow of bacteria should permanently maintain pulp inflammation. Some studies have shown pulpal inflammation in the absence of bacteria [9,10]. Other factors are responsible for pulp injury after restorative treatment, even if it is to a lesser extent than bacterial microleakage.

The remaining dentin thickness has been shown to modify the pulp reaction: the thinner the dentin the more severe the pulp inflammation [11]. The mechanical procedures of deep cavity preparation or filling may be traumatic and induce pulpal irritation [12,13]. Pulpal inflammation may also be provoked by inflammation mediators such as calcitonin gene-related peptide and substance P released by sensory nerve fibers [14,15] aggravated by cavity preparation. A thin dentin means a high dentin permeability allowing the uncured monomers from composite to reach the pulpal space [16] and exert their cytotoxicity [17].

Many histological studies evaluating pulp response under various restorative materials also demonstrated the influence of post-operative time. Since pulpal response often decreases over time, FDI standards [18] recommend the

Table 1
Restorative materials used in this study

Restorative material	Number of teeth	Material number
Scotchbond + Silux ^a	43	1
37% H ₃ PO ₄ for 60 s + Scotchbond + Silux ^a	27	2
15% EDTA for 60 s + Scotchbond + Silux ^a	41	3
Scotchbond 2 + Silux ^a	40	4
Gluma Bond + Lumifor ^b	44	5
Syntac + Heliomolar ^c	33	6
Vitrabond ^a	27	7
Vitremer ^a	27	8
XR-Bond ^d	35	9
IRM ^e	21	10

^a 3M Dental Products, St Paul, MN, USA.

^b Bayer Dental, Leverkusen, Germany.

^c Vivadent, Schaan, Liechtenstein.

^d Kerr, Romulus, MI, USA.

^e De Trey Dentsply, Zurich, Switzerland.

inclusion of a short and a long time group to take into account the likely pulp healing over time. In line with this idea, an in vivo study demonstrated a strict reduction of thermal sensitivity 6 months postoperatively [19] which may be related to the spontaneous decrease in dentin permeability observed in vivo after cavity preparation [20].

Bacterial leakage, remaining dentin thickness and postoperative time, all affecting pulpal response, have been extensively studied but their relative importance has never been compared. In addition, their effects have never been compared to that of the material itself. Only a large scale study may allow us to compare these factors. Many of the histological studies are realized on primates, thus with a reduced number of available teeth for ethical reasons. Several histological studies on human bicuspid were performed in our university during the eighties before the introduction of ethical laws forbidding this kind of experiment. More than 500 teeth were prepared for histological evaluation of the pulpal response under various restorative materials but only two studies were reported [21,22].

The purpose of this retrospective study was to use this very large number of human teeth to: (1) evaluate the relative influence on pulpal response of the presence of bacteria, remaining dentin thickness and postoperative delay; and (2) compare the effects of material cytotoxicity and those of bacterial microleakage on pulpal reaction.

2. Materials and methods

All the teeth were sound human bicuspid, scheduled for orthodontic extraction in patients aged from 12 to 16 years and were prepared according to the same protocol; briefly, after cleaning the tooth surface with pumice-water paste, buccal class V cavities were prepared with a carbide bur under water spray according to FDI standards [18]. The

teeth were filled with various restorative materials according to the recommendations of the manufacturer (the list of the materials is given in Table 1). Extractions were performed without forceps at various time intervals. The teeth were immediately sectioned with a high-speed bur at the apical third and immersed in a neutral buffered 10% formalin solution for 7 days before demineralization, dehydration and embedding. Serial sections (6 µm thick) were mounted on slides. Every ten sections, three successive sections were selected, one was stained with hematoxylin/eosin, one with Masson's trichrom to stain in bright green the collagen of dentin for better discrimination of the results and one with the Brown and Brenn technique for recognition of the bacteria [23]. Unused sections are still available for further analysis.

Pulpal inflammation was recorded by two different operators, using the criteria proposed by Mjör [24,25] and Stanley [11] which classifies the reactions as absent, slight, moderate and severe. In cases of disagreement between the two examiners, the tooth was re-examined and the worst reaction chosen.

Three factors were recorded:

Factor 1: The presence/absence of microorganisms at the interface between the tooth and the restorative material was recorded on the Brown and Brenn stained sections. The presence was noted as 1 and absence as 0.

Factor 2: The remaining dentin thickness was measured using a calibrated eyepiece. Teeth were subsequently divided into three groups: group 1 with a remaining dentin thickness smaller than 500 µm; group 2 with a remaining dentin thickness ranging from 500 to 1000 µm; and group 3 with a remaining dentin thickness greater than 1000 µm.

Factor 3: In the the postoperative delay, teeth fell into two groups: the short-term group was extracted within 5 weeks of filling; and the long-term group was extracted 6–19 weeks after filling.

Many teeth were discarded because of histological artifacts, pulpal perforation or missing information, thus leaving 317 teeth, meeting the requirements of this study. 21 teeth were also prepared under the same conditions, to be used as control for the operative techniques, but were filled with IRM (De Trey Dentsply, Zurich, Switzerland) known for being well tolerated by the dentin pulp organ [26].

Statistical analysis was two-fold to follow the two purposes of the study.

1. Three two-way analyses of variance (ANOVA), at the 95% confidence level, were performed to evaluate the effects of the three factors (presence of bacteria, remaining dentin thickness and postoperative time) on the pulpal response. The first way was one of the three factors and the second way was the material itself. The purpose of this two-way ANOVA was not to compare the

Fig. 1. The effects of the presence of bacteria between the restorative materials and the cavity walls on the inflammatory response. Pulpal inflammation was more severe when bacteria were present between the cavity walls and the restorative materials.

- materials but to ensure that there was no interaction between the material and the tested parameter allowing us to pool together the 338 teeth. Thus, for each of these three ANOVA, only the results of the first way and those of the interaction were given. Within each of the three factors, secondary Kruskal–Wallis tests were performed to evaluate the effects of the two other factors.
- To know to what extent bacterial microleakage and the cytotoxicity of the restorative material influenced the pulpal response, three analyses of variance were performed to compare pulpal response severity under the various restorative materials: one including the 338 teeth, one including the 237 teeth without bacteria and one including the 101 teeth with bacteria. The three analyses of variance were followed by a secondary Duncan test for the comparison of the restorative materials.

3. Results

Thirty eight percent of the pulpal reactions were slight, 56% were moderate and 6% were severe. For the control group 81% of slight reactions were observed associated with

Fig. 2. The effects of residual dentin thickness on the inflammatory response. The remaining dentin thickness did not affect the pulpal response in the presence of bacteria while, in the absence of bacteria, the inflammatory response decreased with increasing residual dentin thickness.

19% of moderate reactions, while no severe reaction occurred.

3.1. Influence of the three factors

The results of ANOVA showed no significant interaction between the restorative materials and the three factors: the presence of bacteria, remaining dentin thickness and post-operative time influenced pulpal response in the same manner, whatever the material. Thus, the 338 teeth could be pooled together for the three following analyses.

3.1.1. Factor 1: the presence of microorganisms at the interface between the tooth walls and the restorative material

No bacteria were observed on the walls of the cavity in the control group. Thirty two percent of the 317 other teeth contained microorganisms at the interface between the cavity walls and the restorative material. Only 7 out of the 10 groups presented bacteria on the cavity walls: 1, 2, 3, 4, 5, 6 and 9 which respectively displayed 57, 41, 67, 29, 29, 42 and 20% of contaminated teeth. The distribution was as follows: 9% of the slight reactions, 42% of the moderate reactions and 96% of the severe reactions presented bacteria. The presence of bacteria significantly increased pulpal inflammation ($p < 0.0001$) (Fig. 1). In the presence of bacteria the remaining dentin thickness did not influence the pulpal response (ns) (Fig. 2), but in the absence of bacteria, pulpal response decreased with increasing remaining dentin thickness ($p = 0.01$). However, either with or without bacteria, the pulpal reactions decreased with time: there was a statistically significant difference between the short-term and the long-term group in both cases ($p = 0.02$) (Fig. 3).

3.1.2. Factor 2: the remaining dentin thickness

A more severe reaction was statistically associated with a thinner remaining dentin thickness in the control group ($p = 0.047$). Twenty two percent of the cavities belonged to group 1 ($<500 \mu\text{m}$), 53% to group 2 ($500\text{--}1000 \mu\text{m}$) and 25% percent to group 3 ($>1000 \mu\text{m}$). Sixty percent of the severe reactions belonged to group 1, 35% to group 2 and 5% to group 3. On the contrary 40% of the slight reactions belonged to group 3, 35% to group 2 and 25% to group 1. The remaining dentin thickness significantly modified the pulpal response ($p = 0.02$): the thicker the remaining dentin the lower the pulpal reaction (Fig. 4). In group 1 ($p = 0.001$) and group 2 ($p = 0.02$) pulpal response severity decreased with time, while in group 3 postoperative delay had no effect on pulpal healing (ns) (Fig. 5). Whatever the residual dentin thickness (group 1: $p = 0.03$, group 2: $p = 0.02$ and group 3: $p = 0.01$) the presence of bacteria significantly increased the pulpal response (Fig. 6).

3.1.3. Factor 3: the post-operative delay

Post-operative time did not influence pulpal healing (ns)

Fig. 3. The effects of post-operative delay on pulp response. Pulpal response severity decreased either in the presence or absence of bacteria.

in the control group. Thirty eight percent of the teeth were in the short-term group and 62% in the long-term group. Only 11% of the severe reactions belonged to the long-term group while 89% belonged to the short-term group. On the contrary, 39% of the slight reactions belonged to the short-term group and 61% to the long-term group. Pulpal healing was significantly better in the long-term group than in the short-term group ($p = 0.04$) (Fig. 7). In the short-term group the remaining dentin thickness significantly modified the pulpal response severity ($p = 0.03$), which increased as residual dentin thickness decreased (Fig. 8). On the contrary, in the long-term group the remaining dentin thickness had no effect on pulpal healing (ns). In the short-term as well as in the long-term group, the presence of bacteria significantly increased the severity of the pulpal response independent of the post-operative delay ($p = 0.01$) (Fig. 9).

3.2. Cytotoxicity of the restorative material and bacterial microleakage

The pulpal reactions under the different restorative materials showed no statistically significant difference when the 101 teeth presenting bacteria (ns) were compared according to restorative material. On the contrary, a statistically significant difference as a function of the restorative material was found among the pulpal reactions either when the 338 teeth were pooled ($p = 0.03$) or when comparing the 237 teeth without microorganisms on the cavity walls ($p =$

Fig. 4. Effects of residual dentin thickness on pulp reaction. Pulpal reaction severity decreased as residual dentin thickness increased.

Fig. 5. Effects of post-operative delay on pulp reaction severity as a function of residual dentin thickness. Pulpal reaction severity decreased after longer post-operative delay, except with high ($>1000 \mu\text{m}$) residual dentin thickness.

0.039). Sub groupings by the secondary Duncan test are given in Table 2.

4. Discussion

This histological study on 338 human teeth restored with 10 different materials, presents the largest scale ever published. Nowadays ethical considerations no longer allow us to perform this kind of experiment on human subjects. In addition to the ethical problem, economical reasons always reduce the number of subjects to 4 or 5 when working on primates. Against this background, this study permitted us to confirm on human beings, older knowledge, which had already been demonstrated on primates and most of all to evaluate the relative importance of some factors affecting pulpal response to various restorative materials.

All of the three factors: bacterial microleakage ($p < 0.001$), remaining dentin thickness ($p = 0.02$) and post-operative delay ($p = 0.04$), significantly influenced the pulpal response. These results support those of Fujitani et al. [10] and Bergenholtz et al. [6] who have already shown a

Fig. 6. Effects of the presence of bacteria on pulp reaction. The presence of bacteria increased pulp reaction severity independent of residual dentin thickness.

Fig. 7. Effects of post-operative delay on pulp reaction. Pulp reaction decreased when post-operative delay increased.

statistical correlation between the presence of entrapped bacteria and the severity of pulpal inflammation, respectively on 105 and 79 teeth from 4 monkeys. When looking at the secondary Kruskal and Wallis tests, bacterial microleakage always significantly increased the pulpal response whatever the postoperative time or remaining dentin thickness. On the contrary, this was not the case for the two other factors: remaining dentin thickness did not influence pulpal response in the long-term group and conversely pulpal response did not decrease over time when remaining dentin thickness was larger than 1000 μm . This leads us to think that bacterial microleakage is probably the key factor and corroborates the studies by Cox who showed that pulp always recovers when bacterial microleakage is prevented [7,8]. Pulpal reactions of the teeth presenting no bacteria must be compared in rank to the two other factors, i.e. remaining dentin thickness and post-operative time. In the absence of bacteria, whatever the postoperative delay, no severe pulpal reaction was observed when the remaining dentin thickness was larger than 1 mm. On the contrary, severe reactions occurred in the long-term group when remaining dentin thickness was inferior to 1 mm. Thus, between the two other factors, the remaining dentin thickness seems to be more important than postoperative delay. The factors affecting pulpal response, evaluated in our work, can therefore be ranged by decreasing order:

Fig. 8. Effects of post-operative delay on pulp reaction severity as a function of post-operative delay. A decrease in residual dentin thickness increased the severity of the pulp reaction after a short post-operative delay while it had no effect after a long post-operative delay.

bacterial microleakage > remaining dentin thickness > postoperative time.

However, the bacterial microleakage theory does not account for 100% of the cases. For instance 9% of the teeth with a slight reaction presented bacteria (Fig. 10) and on the contrary 4% of the teeth with a severe reaction did not present bacteria (Fig. 11). Fujitani had already noticed some rare cases of the presence of bacteria associated with slight pulpal reactions [10]. The 9% of slight reactions with bacteria can be considered false positive responses, since the Brown and Brenn staining does not allow us to know whether or not the bacteria were still alive. The bacteria present in these teeth might have been dead bacteria, which were not removed due to lack of rinsing, but thus triggering no pulpal inflammation. On the contrary, the 4% of teeth with a severe reaction cannot be considered false negative since the Brown and Brenn staining stains both viable and dead bacteria. They corroborate the studies, which already showed pulpal inflammation in the absence of bacteria [10]. The chemical toxicity of the composite itself, on permeable teeth [17] may be responsible for these severe reactions. However it must be kept in mind that only 3% of the teeth (9% of the slight reactions + 4% of the severe reactions) are not in line with microbacterial theory, thereby reinforcing it.

In our study, 34% of the teeth presented bacteria. This is much higher than in the study of Blosser et al. [27] who only evaluated composite resins, but corresponds to the 44% of contaminated teeth found by Bergenholtz et al. [6] who worked on various materials, as in our protocol. This percentage also corresponds to the occurrence of bacterial leakage reported by Qvist et al. [9] who also showed great variations, caused by variations in the etching technique. The relatively high percentage of teeth presenting bacteria on the cavity floor is likely to be related to the lack of hermeticity of these older materials. The comparison of the materials given by the Duncan test is interesting (Table 2):

Two groups seem to present a better pulp reaction when the 338 teeth are pooled. Both groups presented no bacteria at the dentin-material interface: the teeth filled with IRM (group 10), probably because IRM releases enough eugenol, which is cytotoxic at concentrations above 10^{-5} M, to prevent any contamination [28] and the teeth filled with Vitrabond (group 7), possibly as a result of fluoride release. On the contrary, the groups containing bacteria (1, 2, 3, 4, 5, 6 and 9) showed pulpal reactions, which were not statistically different. The only exception was the Vitremer (group 8) which showed no bacteria and almost no pulpal inflammation, but was poorly noted because a lack of reactionary dentin associated with a disturbance of the odontoblast layer was observed in 50% of the cases, raising doubt on the functional aspect of odontoblasts. This result is in contradiction with those of Cox et al. who concluded there was a

Fig. 9. Effects of the presence of bacteria on pulp response as a function of post-operative delay. The presence of bacteria increased the severity of the pulpal response whatever the post-operative delay.

lack of effect of the material on the secretion of reparative dentin [29]. However, this shows once more the prime importance of bacterial etiology of pulpal responses under restorative materials.

Surprisingly, the teeth filled with Scotchbond + Silux without removing the smear layer (group 1) presented no difference in biocompatibility with the same material when dentin was etched with 37% H_3PO_4 (group 2) or was treated with EDTA (group 3). The bonding resin was a mixture of BisGMA- $POCl_2$ (chlorophosphate ester of bisphenol glycidyl dimethacrylate) with tetraethylene glycol dimethacrylate and benzoyl peroxide, which was supposed to bond to the $-OH$ or $-NH_2$ terminal groups of dentin collagen. Ethanol was not able to carry the modified hydrophobic BisGMA through the smear layer [30], leading to a lack of interaction with the underlying dentin walls. This was the likely pathway for the bacteria towards the pulp (56%). When the smear layer was removed either with H_3PO_4 or with EDTA it was recommended to wash dentin to stop dentin etching and dentin chelation, which were thought to be harmful for the pulp [31]. Dentin was then very thoroughly dried to obtain the same aspect on dentin as on etched enamel. This drying was probably excessive, allowing the collagen scaffold to collapse [32], permitting a high percentage of bacterial leakage, respectively 43 and 66%.

Table 2

Results of the secondary test, following the analyses of variance showing that pulpal reactions under the restorative materials were statistically different when the teeth were pooled together and when the teeth did not present any bacteria

	Grouping according to the Duncan test
Teeth pooled together ($n = 338$)	10, 7 7, 1 1, 2, 3, 4, 5, 6, 8, 9
Teeth without bacteria ($n = 237$)	1, 2, 7, 8, 10 1, 2, 3, 5, 6, 7, 8 1, 2, 3, 4, 6, 8, 9 3, 4, 5, 6, 8, 9

Smear layer was removed with EDTA in two groups: Scotchbond + EDTA (group 3) and Gluma (group 5). No statistical difference was found when pooling the teeth despite a two times lower bacterial leakage with Gluma (30%) than with Scotchbond. Gluma is a mixture of 5% glutaraldehyde and 35% hydroxyethylmethacrylate (HEMA) in water. The lower bacterial leakage is probably due to bacterial growth inhibition under the material due to glutaraldehyde [33]. In addition, two dentin bonding agents may exert a different antimicrobial activity according to the composite subsequently placed [34], and one group was filled with Silux and the other with Lumifor. Despite this lower bacterial leakage, no difference was found between the materials. The cytotoxicity of the restorative material, placed onto the bonding agent, may be involved since it has been shown that the monomers of the composite resin may diffuse through the bonding agent and reach the pulp [35].

Syntac (group 6) and Scotchbond 2 (group 4) are two third generation dentin bonding agents. Both of the primers contain maleic acid and a hydrophilic monomer. Scotchbond 2 bonding agent contains a mixture of BisGMA and HEMA while Syntac adhesive contains an aqueous solution of polyethylene glycol dimethacrylate and glutaraldehyde and its application is followed with that of a fluid resin over the adhesive. The increased shear bond strength observed between the second and the third generation does not allow for a better biocompatibility. Both present the same antimicrobial activity [35].

Among the glass ionomer cements Vitrabond (group 7) behaved correctly and showed no bacteria. XR-Bond (group 9), despite known antimicrobial activity [36], showed 20% of bacteria leading to a more severe pulpal reaction and Vitremer (group 8) showed no bacteria but triggered a modification of the activity of the odontoblast layer.

The severity of pulpal reactions decreased with an increase in remaining dentin thickness and corresponds to previous results. When bacteria were absent no severe pulpal reaction was observed in the short-term and long-term groups when remaining dentin thickness was larger than 1 mm. It has already been shown that a higher dentin thickness reduces the diffusion of uncured monomers [16] and therefore the cytotoxicity of the restorative materials [37,38]. Dentin permeability which is related to dentin thickness [39] has also been shown to influence the cytotoxicity of resinous monomers [17,40] but not that of eugenol based restorative materials [28] probably because the diffusion of molecules through dentin does not only depend on molecular size [41] and because dentin does not behave like a simple filter but can also interact with the diffusing molecules.

The severity of pulpal reactions decreased with time. This result corresponds to those of previous studies, which showed a decrease of inflammation over time. For example,

Fig. 10. Histological sections of a tooth presenting a slight pulpal reaction, despite the presence of bacteria on the cavity walls. The bacteria (arrow) present at the interface between the dentin and restorative material are seen on the hematoxylin/eosin (d) as well as on the Brown and Brenn stained sections (c). No pulpal inflammation is observed either on the Brown and Brenn (a) or the hematoxylin/eosin stained sections (b): the odontoblastic layer is intact and the pulpal core shows good pulpal healing. Abbreviations: p, predentin; od, odontoblast cells layer. Original magnification $\times 20$.

Felton et al. [42] found no difference at 90 days, between the pulpal reactions of teeth immediately restored with a composite versus teeth, which had been exposed to the oral environment for 48 h prior to restoration. In the short-term group 16% of severe reactions were recorded and the percentage fell to 2.6% in the long-term group. The FDI recommended in 1980 to plan two observation periods and more recently the ISO standards renewed this requirement [43]. Decrease in pulpal inflammation over time shows the outstanding ability of dental pulp to organize its defenses and manage bacterial infection as well as chemical injury [44].

This study compared to what extent bacterial microleakage and the cytotoxicity of restorative materials could influence pulpal response. This comparison was possible only owing to the large number of teeth, allowing us to build large groups, leading to a satisfying statistical analysis.

Differences in pulpal reactions under the various materials were not observed on teeth presenting bacteria. On the contrary, bacteria free teeth presented pulpal reactions varying according to the material ($p = 0.039$). This led us to conclude that differences in cytotoxicity leading to differences in pulpal responses were masked by the presence of bacteria. Thus, bacterial microleakage seems to be a more important factor than the cytotoxicity of the material itself. However, it must be kept in mind that the recent materials permit us to obtain a tight sealing of the cavity. Moreover, with the progress of tissue engineering, future materials will be designed to interact with dental pulp in order to obtain a positive pulpal response [45]. Under these conditions in vitro studies are still of prime interest to better understand the interactions between dentin, dental pulp and the diffusing molecules.

The evaluation of pulpal reactions was based on

Fig. 11. Histological sections of a short-term tooth presenting a severe pulpal reaction despite the absence of bacteria at the interface between restorative material and cavity walls. No bacteria could be detected on the Brown and Brenn stained section (c) or on the hematoxylin/eosin stained section (d). The higher magnifications on the Brown and Brenn (a) and the hematoxylin/eosin stained sections (b) show the absence of predentin, a complete disappearance of the odontoblastic layer (arrow) and a loosened, fibrous pulpal core.

histological criteria determined by experienced researchers. However this evaluation suffers from the limitations of classical histological staining usually used in the assessment of the pulpal reaction to restorative materials, e.g. cell misinterpretation or miscounting. A quantitative assessment of pulpal response involving measurement of the thickness of the buccal and lingual odontoblastic layers has been proposed [46]. A quantitative immunohistochemical technique has also been proposed [47] but the cost and the time necessary for these techniques are incompatible with the aim of this study, which was to record data from the largest number of teeth. However, these techniques could be performed on teeth showing a pulpal reaction, which was not in line with the microbacterial theory, to evaluate the subpopulations of the inflammatory cells.

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