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ORIGINAL ARTICLE

Effect of different adhesive protocols vs calcium hydroxide on primary tooth pulp with different remaining dentin thicknesses:24-month results

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Abstract The aim of this randomized, controlled, single-blind and prospective study was to evaluate the clinical and radiographic success rates of three different bonding protocols vs calcium hydroxide liner for protection of the dentin–pulp complex of primary molars with different remaining dentin thicknesses. Two hundred forty primary molar teeth with moderate to deep occlusal caries were restored in 97 children who met inclusion criteria. After cavity preparation, the teeth were randomly assigned into four groups (n=60/group) with respect to the material used for protection of the dentin–pulp complex: (1) total-etching with 36% phosphoric acid followed by an acetone-based adhesive (Prime&Bond NT), (2) a self-etch adhesive system (Xeno III), (3) an acetone-based adhesive (Prime&Bond NT) without prior acid conditioning, and (4) control: calcium hydroxide cement (Dycal). Teeth in groups 1–3 were restored with a polyacid-modified resin-based composite (Dyract AP) and those in group 4 with amalgam. The remaining dentin thickness was calculated using image analysis software (ImageJ). The teeth were evaluated clinically and radiographically for 24 months. The distribution of restored teeth with minimal remaining dentin thickness (≤0.5 mm) was 3.3, 8.3, 8.3, and 10% for groups 1, 2, 3, and 4, respectively. Despite the absence of pulpal protection in groups 1–3, none of those teeth exhibited any significant clinical or radiographic symptom during the study period. After 2 years, the clinical and radiographic success rate of restorative treatments was 100%. Protection of the dentin–pulp complex with the tested bonding protocols resulted in similar outcomes in mainly shallow and medium deep cavities as compared to calcium hydroxide amalgam in more deep cavities, when indirect pulp treatment was performed in class I compomer restorations.

Keywords Remaining dentin thickness · Indirect pulp treatment · Adhesive resin · Primary teeth · Calcium hydroxide

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Introduction

Complete removal of deep carious lesions might result in exposure of the dental pulp. In an attempt to prevent such exposures, the indirect pulp capping procedure has been advocated as a conservative therapy of the dentin–pulp complex more than 200 years ago [41]. The term indirect pulp capping was recently replaced by the term indirect pulp treatment (IPT) and has been defined as the procedure in which the non-remineralizable carious tissue is removed and a thin layer of caries is left at the deepest sites of the cavity preparation where complete caries removal would result in pulp exposure [4]. Studies have shown the effectiveness of IPT for the treatment of deep caries in primary molars [3, 5, 15, 16, 25, 29, 33, 38, 46]. Calcium hydroxide remains as the most commonly used lining material in IPT, as it is biocompatible, induces pulp–dentin remineralization, and decreases bacterial infection [5, 7, 8, 28, 37, 38]. However, it is not clear whether the long-term success rate of IPT depends solely on the calcium hydroxide liner placed over the remaining tissue [38], as calcium hydroxide is mechanically weak and soluble over time [11, 26]. Results of recent clinical studies suggest that

a good marginal seal, preventing infiltration of bacterial substrate to affected dentin, is technically more important than the type of lining material in achieving clinical success [15, 29, 38]. Thus, IPT should not be considered a material-dependent technique [29]. Indeed, protection of the dentin–pulp complex with an adhesive resin system has been shown to result in similar clinical and radiographic 24-month outcomes as compared to calcium hydroxide liner when IPT was performed in class I composite restorations [15]. Likewise, resin-modified glass ionomer cement and even gutta-percha have demonstrated similar success rates as with calcium hydroxide liners in clinical trials [29, 38].

When adhesive restorative techniques are used in IPT, the acid etchant, and/or the adhesive resins used for bonding procedures may suggest the reduction of bacterial contamination from tooth structure [24, 37, 42] and allow a similar initial bactericidal effect as compared to the effect of calcium hydroxide [26, 38, 40]. On the other hand, the use of acidic conditioners and adhesive resins in the absence of pulpal protection may lead to irreversible pulp reactions in deep cavities [20, 22, 23, 31, 34], especially if the remaining dentin thickness (RDT) is below 0.5 mm [9, 23]. While the skill and expertise of the clinician can help minimize removal of such iatrogenic dentin [32, 47], exact judgement of the cavity depth (and thus, the RDT) is often impossible, as most assessments are made simply by visual examination, and the limitation of visual perception may render such judgments inaccurate and subject to variation [14, 47]. Even in RDT-related in vivo research, cavity dimensions have been estimated during cutting [31, 32], and the variations in the RDT of those teeth as measured from histological slides [9, 31, 32] confirm the difficulty in achieving standardized cavity depths. Finally, clinical situations in which the color of the affected carious lesion left at the deepest site of cavity preparation may disguise a functional pulp exposure [36, 37] and thus complicate estimation of exact cavity depth.

In light of these observations, the purpose of this randomized, controlled, single-blind and prospective study was to evaluate the clinical and radiographic success rates of three different bonding protocols vs calcium hydroxide liner for protection of the dentin–pulp complex of primary molars with different RDT. The null hypothesis tested was that protection of the dentin–pulp complex of primary molars with the tested bonding protocols results in similar clinical and radiographic outcomes as compared to calcium hydroxide amalgam when IPT is performed in class I compomer restorations.

Materials and methods

Operative procedures

Ninty-seven children between the ages of 5 and 10 (mean, 8 years) from both sexes participated in the study. The main criteria for inclusion was the presence of at least one pulpally healthy primary molar with a carious lesion limited to the occlusal surface of the tooth, as diagnosed clinically and radiographically. The degree of physiological root resorption was not a decisive factor [3]. Informed consent was obtained from parents, and both the consent form and the research protocol were performed upon approval by the Institutional Human Subject Review Committee.

After local anesthesia, class I cavity preparations were made using #330 carbide bur at high speed and ISO 012 to 018 carbide burs at low speed. The size of restorations was not recorded. Caries removal at the site of "risk for pulp exposure" [15] was performed with ISO 016 or 018 carbide burs. Teeth were included if infected dentin was removed and affected dentin was left at the deepest cavity area without any visible pulp exposure, which enabled the operator to perform indirect pulp therapy with either the tested adhesive resins or calcium hydroxide. Prepared cavities, with the pink outline of a pulp horn seen through dentin, were regarded as a functional exposure [37] (despite the absence of visible bleeding) and thus were excluded from the study. Prepared teeth were randomly assigned into one of the following restorative treatment protocols using sequentially numbered opaque-sealed envelopes (SNOSEs) [2, 13] prepared with unrestricted (simple) randomization [13]. The envelopes were opened by another operator who was blinded to the final cavity preparation:

Group 1: Enamel and dentin surfaces were etched with 36% phosphoric acid gel (DeTrey Conditioner 36, DeTrey/Dentsply, Konstanz, Germany) for 30 and 15 s, respectively and washed with air-water jet for 15 s. A single-bottle adhesive (Prime&Bond NT, DeTrey/Dentsply) was applied on the entire cavity and margins and light-cured as per the manufacturer's instructions. Then, a polyacid-modified resin-based composite material (Shade A2, Dyract AP, DeTrey/Dentsply) was applied with a maximum of 2-mm-thick increments, each photopolymerized for 40 s. After occlusal adjustments and finishing, the tooth-restoration margins were re-etched with phosphoric acid for 30 s, rinsed with water for 15 s, and dried and sealed with a thin layer of unfilled resin (Heliobond, Vivadent, Schaan, Liechtenstein) to prevent short-term microleakage.

Group 2: A self-etch adhesive system (Xeno III, DeTrey/Dentsply) was applied and light-cured according to the manufacturer's instructions. The remaining procedures for restoring the cavity with Dyract AP and marginal sealing were accomplished in accordance with the protocol followed in group 1.

Group 3: Prime&Bond NT was applied on the entire cavity and margins without prior acid conditioning and light-cured as per the manufacturer's instructions. The

cavities were further restored, finished, and sealed as with groups 1 and 2.

Group 4 (control): A small amount of CH cement (Dycal, DeTrey/Dentsply) was applied on the deepest region of the cavity. A non-gamma II type amalgam (Permite, SDI, Victoria, Australia) was placed into the cavity in small increments with special care not to damage the CH cement during condensation. The tooth-amalgam margins were etched as with group1, dried and sealed with a light-cured fissure sealant (Helioseal, Vivadent, Schaan, Liechtenstein) to prevent short-term microleakage that could affect healing.

All restorative treatments were made by one experienced operator. A new quartz–tungsten light-curing source (Optilux 401, Kerr/Demetron, Orange CA, USA), whose output was controlled with a Model 100 radiometer (Demetron), was used to ensure optimal polymerization of all resin-based materials used.

Image analysis

Postoperative radiographs of teeth were obtained with E-Speed films (Eastman Kodak, Rochester, NY, USA) by an assistant with a paralleling device (Dentsply Rinn, Rinn, Elgin, IL, USA) at 70 kVp and 0.1 s exposure duration using a Gendex GX dental X-ray unit (Gendex, Milwaukee, WI, USA). All films were processed under the same automatic conditions. Radiographs were scanned in an Epson Perfection 4990 scanner (Epson, Tokyo, Japan) The RDT of each tooth was measured using ImageJ V.1.34 software [1]. The reference for exact calibration of the scale of ImageJ was provided by a 2-mm stainless-steel orthodontic wire, attached to the radiographs of all teeth [10]. The RDT was measured (in mm) between the deepest region of the cavity and the dentin–pulp border. Two additional measurements were made 0.5 mm mesial and distal to the initial measurement point, and the mean value of three measurements was recorded as the RDT for each tooth. The teeth were subsequently divided into four groups [9]: (1) RDT<0.5 mm; (2) RDT ranging from 0.5 to 1.0 mm; (3) RDT between 1.0 and 1.5 mm, and (4) RDT>1.5 mm. Statistical comparisons between the treatment groups with respect to RDT were made with one-way analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) tests at $P<0.05$.

Clinical and radiographic evaluations

The following criteria were used for the determination of the clinical and radiographic success of the treatments at 1, 3, 6, 9, 12, 18 and 24 months [15]: (1) absence of clinical symptoms (spontaneous pain and/or sensitivity to pressure/percussion, fistula, and/or edema, abnormal mobility); (2) absence of radiolucencies at the interradicular and/or periapical regions, as determined by radiographs; and (3) absence of internal or external (pathologic) resorption that was not compatible with the expected resorption due to the exfoliation process. When one or more of the aforementioned sings was detected, the treatment was recorded as a failure. The data was analyzed by Fisher's exact test at $P<0.05$ to examine the effect of the treatments in each recall period.

The marginal quality of the restorations were evaluated according to the modified US Public Health Service clinical rating system [12] Comparisons among the treatment groups with respect to marginal integrity criteria and recall periods (baseline and 3, 6, 9, 12, 24 months) were made with Fisher's exact test at $P<0.05$. All data were analyzed with SPSS statistical software (Ver. 11.5, SPSS, Chicago, IL, USA).

Table 1 RDT of teeth with respect to treatment groups

	RDT (mm)		
Group No.	Mean±SD	Minimum	Maximum
1	1.07±0.38	0.44	2.15
2	1.11±0.48	0.25	2.30
3	1.13±0.51	0.34	2.33
4	0.71±0.15	0.42	0.95

Results

The RDT of teeth with respect to treatment groups are presented in Table 1 as mean±SD, minimum and maximum (mm). Distribution of teeth with respect to RDT groups (≤0.5 mm, $0.5>X\leq1$ mm, $1>X\leq1.5$ mm and >1.5 mm) is presented in Table 2. The distribution of restored teeth with minimal RDT (≤0.5 mm) was 3.3, 8.3, 8.3, and 10% for groups 1, 2, 3, and 4, respectively (Table 2). One-way analysis of variance indicated a significant difference between the mean RDT values of all treatment groups ($F=13.99$; $P=0.000$). Accordingly, the mean RDT of the

Table 2 Distribution of teeth with respect to RDT

Group/remaining dentin thickness (mm)	Group 1, *n* (%)	Group 2, *n* (%)	Group 3, *n* (%)	Group 4, *n* (%)
≤0.5	2 (3.3)	5 (8.3)	5 (8.3)	6 (10)
$0.5>X\leq1$	24 (40)	24 (40)	25 (41.7)	54 (90)
$1>X\leq1.5$	27 (45)	18 (30)	17 (28.3)	–
>1.5	7 (11.7)	13 (21.7)	13 (21.7)	–
Total	60 (100)	60 (100)	60 (100)	60 (100)

Values are expressed as number (*n*) and percentage (parentheses)

control group was significantly smaller in group 4 (control) than those of groups 1–3 (Tukey HSD test, $P<0.05$), while the difference between the mean RDT values of groups 1–3 was not significant (Tukey HSD test, $P>0.05$).

A total of 240 teeth were restored in 97 children (60 teeth/group). A maximum of three restorations were placed per patient, and when inclusion criteria were favorable, more than one restoration was placed in one quadrant. A split mouth design could not be utilized due to the insufficient number of patients with bilaterally involved deep occlusal caries. The majority of restored teeth were maxillary and mandibular first molars (55 and 99 teeth, respectively). In group 1, a total of five teeth had exfoliated uneventfully, as recorded at the 12th- and 18th-month recalls. Natural exfoliations were also observed in group 2 at 12 months (one tooth), in group 3 at 12, 18, and 24 months (two, one, and two teeth, respectively), and in group 4 at 18 months (one tooth). In group 1, four teeth presented with infrequent episodes of sensitivity to cold water at 1 month. Because there was no other clinical or radiographic sign that could be indicative of irreversible pulpal involvement, no intervention was made. In three of those teeth, the sensitivity was reported to have subsided at the second month recall and in the remaining one tooth at the fourth month recall. A comparison of postoperative sensitivity (as a clinical symptom) among all treatment groups failed to show any significant difference at 1–4 months (Fisher's exact test, $P=1.0$). The mean RDT of the four teeth presenting with postoperative sensitivity (mean=1.49± 0.4 mm; minimum=1.14 and maximum=2.02) was significantly greater than that of the remaining 56 teeth (mean=1.04±0.36 mm; minimum=0.44 and maximum= 2.15) in the same treatment group (Mann–Whitney U test, $P=0.036$). There was no evidence of failure with respect to the other clinical and radiographic criteria evaluated. Thus, when all groups were pooled and evaluated together, the overall success rate of IPT was 100% (228 teeth, excluding exfoliations) after 24 months. Neither the significantly higher mean RDT values of the experimental groups (1–3), nor the significantly lower RDT of the control group (4) had any influence on the clinical and radiographic outcome.

The rationale behind marginal integrity assessments was to evaluate a possible correlation between indirect evidence of "clinical microleakage" [45] and clinical/radiographic failure. However, the results were not compatible with expectations. Despite a significantly greater tendency toward "Bravo" marginal discoloration scores after 12 months (Fisher's exact test, $P<0.05$) and "Bravo" marginal integrity scores after 9 months (Fisher's exact test, $P<0,05$), no teeth presented with a diagnosis of clinical and/or radiographic failure during the 24-month follow-up period.

Discussion

In the present study, both the parents and the patient were blinded to treatments, while operator blinding to restorative procedures has not been possible to establish, due to the differences in adhesive and/or final restorative materials used. Nevertheless, it may be possible to consider the operator "semi-blinded" in terms of exact cavity depth and thus of the RDT, as the latter could only be measured postoperatively [9]. As the randomization was made exclusively on the basis of restorative treatment (and not the RDT), the significantly lower RDT values of the control group can be explained by the chance variation [27], which confirms that proper randomization does not always provide approximate distribution [27]. These findings also add to the existing knowledge that the color of affected dentin left at the deepest site of the cavity preparation may prevent perception of a pulpally critical cavity depth. The "intentional bias" made herein was the exclusion of teeth with the pink outline of a pulp horn as seen through dentin, as this clinical situation undoubtedly indicates a functional pulp exposure [37]. In such regions, the manufacturer of the tested adhesives stipulate placement of a calcium hydroxide base before etching/bonding procedures. Obviously, inclusion of such teeth would not conform to the primary aim of the present study, which investigated the treatment effect of such adhesive restorations in the absence of pulpal protection.

Despite the common judgment that clinical trials are the ultimate test, pulp-related studies may only provide direct evidence for the ongoing physiological/pathological processes if the tooth is extracted and investigated at the histological level. Due to ethical limitations, however, this may not always be possible. Under such circumstances, the investigator has to rely on clinical and radiographic findings for the interpretation of the studied variables. Undoubtedly, this study presents a typical example to this dilemma, as it is extremely difficult to isolate the exact factor(s) contributing to a 100% clinical and radiographic success rate in presence of adhesively restored teeth, especially in those with a RDT less than 0.5 mm. Murray et al. [32] studied the effect of RDT in 217 human premolars restored with calcium hydroxide (Dycal), zinc-oxide eugenol (ZOE) cement, different total-etch adhesives resin composite, and a resin-modified glass ionomer cement (RMGI) by measuring their influence on pulp injury in terms of odontoblast numbers and dentin repair by measuring reactionary dentin area. The authors showed that the number of odontoblasts beneath cavities prepared with a RDT between 0.5–0.25 mm were 5.6% less than cavities prepared with a RDT between 1–0.5 mm. However, maximal reactionary dentinogenic activity was observed in those cavity preparations (0.5–0.25 mm), demonstrating the

powerful effect of reduced RDT on the induction of reactionary dentin. The rank order of materials from greatest to the least stimulatory effect on production of reactionary dentin was calcium hydroxide, composite resin, RMGI, and ZOE cements [32]. These findings may help explain the possible mechanisms by which the adhesively restored primary molars with a RDT between 0.5–0.25 mm were able to survive without endodontic complications and/or compromised physiologic root resorption. While the buffering effect of RDT is critical for protection of the pulp from the possible cytotoxic effects of adhesive resins, the stimulation of reactionary dentinogenic activity in primary molars with a RDT between 0.5–0.25 mm may have assisted in providing pulp protection in the early phase of healing [32]. Over time, the pulpal response would decrease [9], owing to the spontaneous decrease in dentin permeability [35] through the deposition of sclerotic dentin [44]. These responses are believed to be mediated by the activation endogenous signaling molecules such as TGF-Bs [17], which can be found at the dentinal matrix and are solubilized either by cavity conditioning agents or calcium hydroxide [15, 43].

In the present study, marginal integrity assessments were made in an attempt to evaluate a possible correlation between indirect evidence of "clinical microleakage" [45] and clinical/radiographic failure. The results indicated that the gradual increase in marginal discoloration and loss of marginal integrity did not correlate with the clinical outcome during 24 months. Nevertheless, microleakage and mechanical failure aspects of filling materials can take years to become apparent in patients [32]. Therefore, longer follow-up periods and, if possible, histological investigation of restored primary teeth after exfoliation may be necessary to confirm the effect of reduced marginal seal on the pulpal status.

The clinical and radiographic success rates obtained herein should be looked on with some reservation, with special regard to cavity type. In an attempt to delay the effects of inevitable postoperative microleakage especially during the critical period of healing [32], the study protocol stipulated confinement of the cavities to the occlusal surface (class I), with all margins surrounded by enamel and supersealed with a bonding resin including those of group 4 amalgam restorations (with a fissure sealant material), due to the inferior resistance of amalgam to microleakage in the short term [19]. Guelmann et al [21] have shown that new amalgam restorations sealed with an unfilled sealant demonstrated significantly less microleakage than their unsealed versions after storage in an acid environment, which conformed to our primary aim of preventing microleakage in the short term. In fact, amalgam for small children is rather seldom used in some countries, and this approach was solely made for experimental purposes. The results of bonded class II restorations in primary teeth are still less optimistic due to microleakage at the cervical margin [6, 18]. Moreover, axial dentin is more permeable than pulpal floors of class II cavities [30, 37, 39]. Thus, the present results, especially those obtained with an RDT less than 0.5 mm, cannot be extrapolated to adhesive class II IPT restorations until favorable long-term results have been reported.

Comparisons of the present clinical/radiographic results with those of previous IPT studies have not been possible, as the cavity depth or RDT were not recorded in those investigations [15, 29]. Nevertheless, the high success rate obtained herein corroborates with those of Falster et al. [15], who reported 96% success after 2 years in adhesive class I primary molar IPTs without pulpal protection. The present study also confirms that the application of calcium hydroxide over the affected dentin is not a determinant of the successful outcome of IPT [15] and leads to the conditional acceptance of the null hypothesis that similar reactions have been observed in groups 1–3 in mainly shallow and medium deep cavities compared to calcium-hydroxide/amalgam in more deep cavities.

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