

Pulpal evaluation of two adhesive systems in rat teeth

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Formocresol is the agent most frequently used with pulpotomies in primary teeth, but its use is currently under discussion in regard to its possible toxic effects. Some current works suggest the use of dentinal adhesives in cavities with minimal dentinal thickness and even in small pulpal exposures. Our objective was to evaluate pulpal response to the application of two dentinal adhesives Syntac® and Prime & Bond NT® in pulpal exposures in rat teeth. Sprague Dawley rats were used, male, 150 to 200gm in weight. The maxillary first molar, left and right, was employed in each case, a total of twelve teeth. Duration of the study was 45 days. Sections were evaluated using a light microscope in order to determine pulpal response to the two adhesives. In both groups the persistence of chronic inflammation was associated with the absence of dentinal bridges in the affected specimens. Areas of necrosis and absence of regularity of the odontoblastic layer could also be seen.

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INTRODUCTION

Pulpotomy is one of most frequent pulpal treatments employed in pediatric dentistry. It consists of the amputation of the affected coronal pulp and the application to the radicular stump of an agent that permits the vitality and function of this tissue to be maintained. The agent must be harmless, bactericidal, promote the healing of the radicular pulp, and not interfere in the resorption process.¹

Formocresol, either concentrated or a 20% dilution thereof, is the agent most frequently used, mainly because of the proven clinical and radiological effectiveness.^{2,4} However, use of formocresol is currently under discussion regarding to the toxic effects: pulpal response with resulting inflammation and necrosis,^{5,6} cytotoxicity,^{7,8} systemic absorption and changes in organs such as kidney and liver,^{9,10} mutagenic and carcinogenic potential,^{11,12} and immunological response.¹³

Alternative therapies are available and, with the study by Ranly¹⁴ in mind, these may be classified according to the objectives achieved.

If the objective is the devitalization of the remaining radicular tissue then the alternatives are: total pulpectomy¹⁵, pulpotomy with electrocoagulation,^{16,17} and laser;¹⁸ however, the studies mentioned tend to show that these techniques are not reliable alternatives to formocresol.

If the objective is to minimize damage to the radicular tissue, the most frequently used agents are glutaraldehyde^{4,19,20,21} and ferric sulfate.^{22,23} In the case of glutaraldehyde there are reservations as to the undesirable side-effects, which can be similar to those induced by formocresol.^{7,24,25}

Finally, if the objective is to maintain the vitality of the radicular pulp without any inflammation, biocompatible materials such as collagen,^{26,27} freeze dried bone,²⁸ and, more recently, bone morphogenetic proteins,^{29,30,31} have been used.

On the other hand, some current works suggest the use of dentinal adhesives in cavities with minimal dentinal thickness and even in small pulpal exposures, based on the formation of a hybrid layer to prevent the passage of bacteria and the consequent pulpal damage. These authors consider microleakage to be more important in the etiology of pulpal pathology than the potential toxicity of the material *per se*.³²⁻³⁵

Heitmann and Unterbrink³⁶ did a study on human bicuspid and permanent molars in which they performed direct pulp capping using a glutaraldehyde-based adhesive (Syntac®) after acid etching of enamel and dentin, with results satisfactory after six months.

However, Gwinnett and Tay³⁷, after preparing human bicuspid with and without pulpal exposure, applied pulp capping with an adhesive system (All-Bond®) after hemorrhage control and acid-etching with 10% orthophos-

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phoric acid and in most cases results showed generalized chronic inflammation and foreign body inflammatory reaction localized close to resin particulates.

More recently, Cox *et al.*³⁸ did a study using 9 adhesive systems on teeth prepared with and without pulpal exposure. They found that the materials were compatible with pulpal tissue.

In the light of these previous studies our objective was to evaluate pulpal response to the application of two dentinal adhesives (Syntac[®] and Prime & Bond NT[®]) in pulpal exposures in rat teeth, and to evaluate the possible utilization in pulpotomies of human primary teeth.

MATERIALS AND METHODS

Six male Sprague Dawley rats were used that weighed 150 to 200gm. The maxillary first molar, left and right, was employed in each case, a total of twelve teeth. Duration of the study was 45 days.

Materials employed were: Syntac[®] (Vivadent, Liechtenstein), and Prime & Bond NT[®] adhesive systems (Dentsply/DeTrey, Konstanz, Germany), and Tetric Flow[®] (Vivadent, Liechtenstein) as a restorative material. Right-side maxillary first molars were treated with Prime & Bond NT and left-side maxillary first molars with Syntac. Thus, each rat became its own control.

Animals were anesthetized by intramuscular injection of ketamine (Ketolar[®], Parke-Davis, Morris Plains) at 75 mg/kg and were placed on an operating table. Tongue and cheeks were reflected from the operative field. Access to the pulpal chamber was opened from the occlusal surface and small pulpal exposures were effected. Care was taken to avoid perforation of the chamber floor. Hemorrhage was controlled with endodontic paper points.

In each group of teeth the corresponding adhesive was applied, strictly according to instructions of the manufacturer. Acid-etching of surfaces was not performed. Restoration was done using a fluid material (Tetric Flow) for ease of application and for avoidance of excessive pressure on the exposure during application.

Animals were sacrificed after 45 days. Dissection of the hemimaxillae was performed and specimens were fixed in 10% formalin and were subsequently decalcified in a solution of formic and chlorhydric acid. Specimens were washed, dried, embedded in paraffin, and sectioned using a microtome. Sections were stained with hematoxylin and eosin.

Sections were evaluated using a light microscope in order to determine pulpal response to the two adhesives. For evaluation, the criteria described by Fuks *et al.*¹⁹ were used. These criteria are clear indicators of any alteration to the normality of the pulp and are as follows: degree of inflammation, presence and regularity of the odontoblastic layer, presence or absence of dentinal bridge and presence or absence reactive dentin and fibrotic tissue.

RESULTS

In the clinical exploration two teeth in the Syntac group were seen with a partial loss of the restoration. Neither of the two groups presented localized abscesses in the treated teeth.

In the Prime & Bond NT[®] group the most common pattern was a wide necrotic zone at the point of exposure (Figure 1). Below this necrotic layer there was evidence of tissue with little cellular structure and, in some sections, areas of severe inflammation penetrating deep into the tissue (Figure 2). Dentinal bridge formation was not seen. However, some specimens in this group showed and inflammatory foreign-body reaction and the presence of some giant cells, possibly associated with the presence of small resin particulates.

The Syntac group presented a similar histological reaction with wide areas of necrosis (Figure 3) and absence of regularity of the odontoblastic layer (Figure 4). Areas with formation of fibrotic tissue surrounded by an inflammatory infiltrate could also be seen. Dys-trophic calcification lesions were observed.

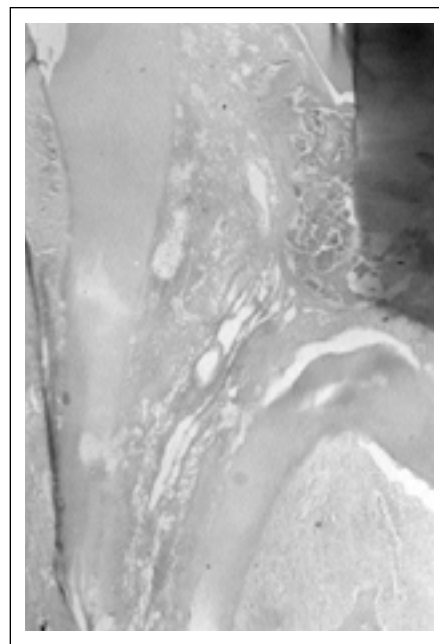


Figure 1. Photomicrograph 45 days following treatment with Prime & Bond NT. Necrosis at exposure site. (H & E, X100)

In both groups, the persistence of chronic inflammation was associated with the absence of dentinal bridges in the affected specimens, but there were no findings related to internal resorption.

DISCUSSION

At present, many agents are being used in the performing of pulpotomies in primary dentition. A few, such as

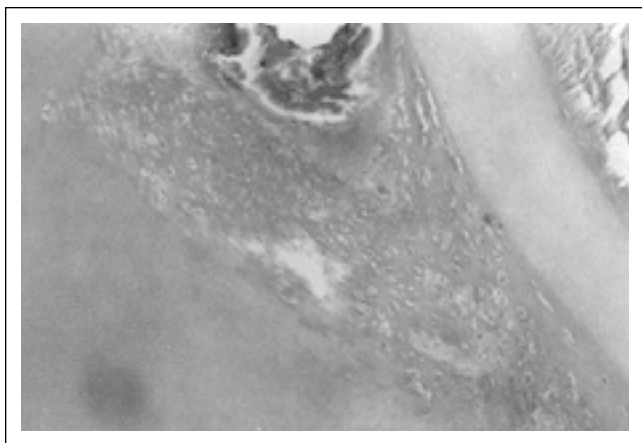


Figure 2. Photomicrograph 45 days following treatment with Prime & Bond NT. Inflammatory lesion adjacent to exposure penetrated deep into pulp tissue. (H&E, X200)

formocresol, have been widely studied and consequently the limitations are better known.

Several studies have suggested that dental adhesives may be effective for direct pulp capping.^{32,33,36} The formation of a hybrid layer can avoid bacterial microleakage and at the same time increase the adhesion of the material and diminish postoperative sensitivity. The possibility of hybridizing the exposed pulpal tissue exists as an analog to hybridizing dentin.^{34,35,38,39}

After the application of various adhesive systems Cox *et al.*³⁸ observed the formation of dentinal bridges, and a pulpal tissue with scarcely any inflammation.

The results of the present study are similar to those of Gwinnett and Palmeijer^{37,40} where the histological study revealed wide necrotic zones and areas of chronic inflammation and no repair of the pulpal tissue.

There are several factors to consider. The use of rats in the present study resulted in a certain amount of difficulty in obtaining good isolation, free from saliva contamination, given the small oral cavity available for experimentation.

Total acid-etching of pulp and dentinal surface is a topic addressed in several previous studies. Recently, Parmeijer⁴⁰ observed highly negative histological effects in total acid-etching of the pulpal tissue of primates and subsequent capping with adhesive systems. He suggests that acid-etching of the pulp may induce hemorrhage that is difficult to contain and that this may interfere with pulpal healing.

Cox *et al.*³⁸ stated that other factors are more important: the presence of bacteria, the use of irritant bactericides, and even the manner of placement of the restorative material can influence the histological results.

In the present study the total acid-etching technique was not employed. This factor must be considered for its influence both on the formation of a hybrid layer and on the failure of the restoration in two of the treated teeth.

A further point to be considered is the disinfection of the surfaces, as the previous presence of bacteria is a



Figure 3. Photomicrograph 45 days following treatment with Syntac. The coronal pulp has undergone complete necrosis. (H&E, X100)

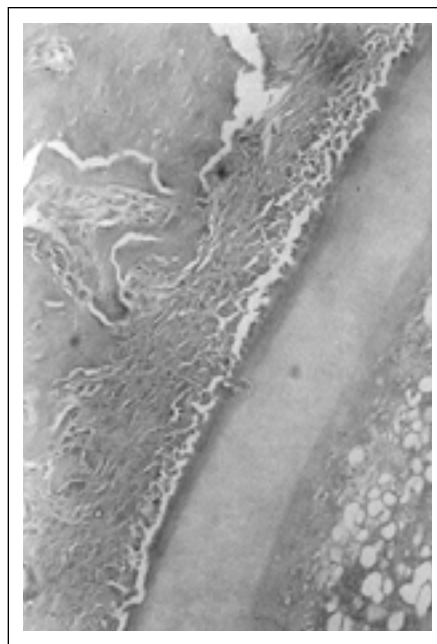


Figure 4. Photomicrograph 45 days following treatment with Syntac. In the middle third of the radicular pulp, presence of irregular odontoblastic layer. (H&E, X200)

determining indicator of capping failure. Parmeijer⁴⁰ used a 2% chlorhexidine solution as disinfectant, with highly negative results. In contrast, Cox *et al.*³⁸ used 6% NaOCl, which in addition to being a disinfectant agent also enabled hemorrhage control. These results were highly positive. There are also dental adhesives containing glutaraldehyde (e.g. Syntac), which has a bactericidal effect and also produces a layer of fixation that

may favor the repair of the pulpal tissue.^{36,41} However, in one of the two groups in the present study, Syntac® was applied and the results were not significantly different from those obtained in the group where Prime & Bond® was used.

Appropriate control of hemorrhage at the point of exposure is important, as clot formation can interfere with the normal process of tissue repair.^{37,38,40} Thus, the use of 2.5% NaOCl is suggested, or, alternatively, the application of slight pressure to the exposure zone.

The effect of the heat generated by the light and the exothermic reaction of the material during light-curing might also damage pulpal vitality.³⁶

As in the present study, after application of the adhesive to the pulp other authors have observed slight inflammatory foreign-body reaction (apparently triggered by resin particulates). Further studies will be needed to evaluate the biocompatibility of adhesives and the effect on the process of pulpal repair, and to determine the possible causes of these reactions. It will be essential to take other factors into account, and not just, the presence or absence of bacteria.^{37,42}

More long-term studies will be needed, taking these factors into account, and clinical, radiological and histological evaluations will need to be performed. The possibility of the successful utilization of adhesives as agents for pulpotomies is a distant prospect. Much more research will be needed in this field, not only in pulpal exposures, but also in pulpotomies of primary teeth.

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