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Effects of Dentin Chips, Hydroxyapatite, and Demineralized Dentin as Apical Plugs

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Abstract

EFFECTS OF DENTIN CHIPS, HYDROXYAPATITE,
AND DEMINERALIZED DENTIN AS APICAL PLUGS

by

D. Wylie Brandell

Two mm plugs of demineralized dentin, hydroxyapatite, or dentin chips were condensed into the apical perforations of 48 anterior teeth of eight adult cynamolgus monkeys. The remainder of the canals were obturated with gutta percha and Grossmans sealer using lateral condensation technique. The degree of hard tissue formation and inflammation was evaluated at three and six months. No significant changes were noted between various materials at three months. However, after six months, the samples with apical plugs of hydroxyapatite had more hard tissue formation and less inflammation than others.

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EFFECTS OF DENTIN CHIPS, HYDROXYAPATITE, AND DEMINERALIZED DENTIN AS APICAL PLUGS

by

D. Wylie Brandell

A Manuscript Submitted in Partial Fulfillment
of the Requirements for the Degree Master of Science
in Endodontics

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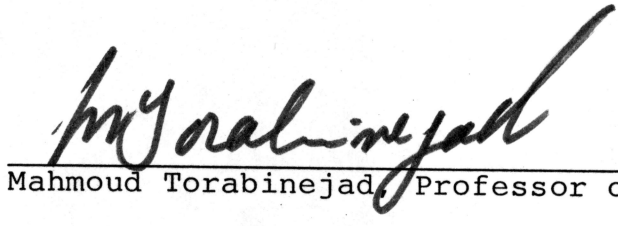
Each person whose signature appears below certifies that this manuscript in his opinion is adequate, in scope and quality, in lieu of a thesis for the degree Master of Science.



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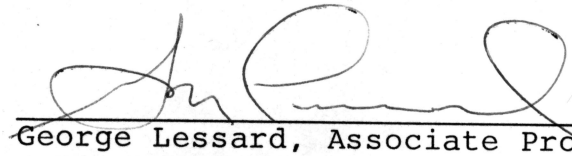

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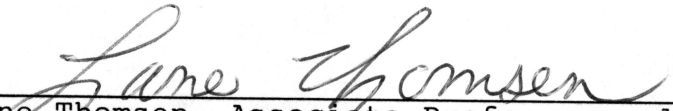

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INTRODUCTION

Obturation of the root canal system is an important phast of endodontic therapy. Presence of an apical stop aids in achieving such a goal. Inadventant apical perforations or pulp necrosis of young teeth can result in the lack of an apical stop for obturation. Presence of an open apex does not allow thorough condensation and it permits extention of filling materials into the periapical tissues.

Attempts at solving this problem have centered around materials and techniques that somehow stimulate the formation of a hard tissue barrier across the apical opening. These attempts include the use of calcium hydroxide,[1-4] tricalcium phosphate,[5-7] bovine skin collagen,[8,9] collagen calcium phosphate gels,[10] and decalcified allogenic bone matrix.[11,12]

Another approach has been to pack dentin chips into the apical portions of canals to form a physical barrier against which filling materials can be condensed immediately.[13-19]

Histologic evaluation of teeth treated with this procedure has revealed deposition of a cementoid hard tissue subjacent to, and in contact with, the apical end of the dentin plugs.[17-19] Although it has been shown that dentin chips seem to stimulate formation of such hard tissue, it is not known which component of the dentin chip plug (organic or inorganic) is mainly responsible for this action.

The purpose of this investigation was to study the ef-

fects of dentin chips, hydroxyapatite and demineralized dentin as apical plugs.

METHODS AND MATERIALS

Forty-eight mandibular and maxillary incisor teeth with closed apices in eight female, cynomolgus monkeys (2-4 kg) were used for this study. The monkeys were housed individually and fed Purina Monkey Chow* and water ad libitum.

Each monkey was anesthetized with an intramuscular injection of ketamine hydrochloride, (40 mg/kg) and Xylazine, (2 mg/kg) and preoperative periapical radiographs were taken of the maxillary and mandibular anterior teeth.

The maxillary central and lateral incisors together with the mandibular central incisor teeth from each monkey were randomly assigned to one of four groups and distributed evenly among all eight monkeys. Each group consisted of 12 canals with the following distribution of test materials condensed into the apical 2 mm of each canal:

Group 1. Demineralized dentin material

Group 2. Hydroxyapatite

Group 3. Dentin Chips

Group 4. The apical 2 mm left empty

The materials used to produce apical plugs in this study were prepared in the following manner.

*Ralston purina Company, St. Louis, Missouri

Eight weeks before root canal treatment, four to six posterior teeth from each monkey were extracted to obtain autologous demineralized dentin. The groups of extractions from each animal were sequestered from the others in order that the prepared material could be utilized in monkeys from which it originally was derived. The anatomic crowns were removed from the teeth and the remaining root structures were immediately fixed in a 10% buffered formalin solution for a period of ten days. The teeth were then decalcified in RDO solution* for 24 hours, removed and rinsed with sterile water and dried in sterile glass jars for a period of 48 hours. The samples were individually ground with an acrylic bur in a low speed handpiece to a powdered consistancy and placed in labeled glass vials for use as organic apical plugs.

Hydroxyapatite [$Ca_{10}(PO_4)_6OH_2$] has been demonstrated to be closely related to the inorganic component of dentin.[20] Accordingly, a commerical preparation of this material was used as the inorganic apical plugs. The autologous dentin chips were obtained from the canal orifices by rotating a #5 Gates-Glidden bur against the canal walls after root canal preparation. This powder was then immediately compacted into the apical 2 mm in those canals designated for this test material.

*Dupage Kinetic Laboratories, Plainsville, Illinois

After anesthetizing the monkeys in the manner previously described, access to the coronal pulp champbers were made with a #4 high speed round bur and the pulps extirpated with barbed broaches. The canals were irrigated with sterile water. After determining working length, each tooth was perforated apically with a #15 or #20 K-file, the length verified radiographically and the canal was instrumented 1 mm beyond the radiographic apex to a size #70 K-file. The length of the largest working file was verified radiogrpahically, and the canal rinsed with sterile water and dried with sterile paper points.

After cleaning and shaping, the test materials were condensed into the apical 2 mm of the prepared root canals with the aid of endodontic pluggers. The integrity of the apical plug was considered acceptable when a #25 K-file could not penetrate through the material. At that time the rest of each canal was obturated with gutta percha and Grossman's sealer using lateral condensation technique. Control teeth were instrumented as described above but were obturated 2 mm short of the radiographic apices with no apical plugs. The access openings were sealed with silver amalgam.

After three months, four of the monkeys were anesthetized with ketamine and xylazine and then sacrificed with intracardiac injections of euthanasia solution. The re-

mainder were sacrificed after six months with the same procedure. Block sections of the maxillary and mandibular teeth and surrounding tissues were removed from each monkey and radiographed. The coronal amalgams were removed and the samples immediately immersed in a 10% buffered formalin solution for ten days. The samples were then rinsed with sterile water and decalcified in RDO for 72 hours. After complete decalcification, the blocks were embedded in paraffin. Serial sections were prepared at six microns and every tenth section was slide mounted and stained with hematoxylin and eosin for microscopic examinations.

The apical regions of the teeth in each section were evaluated for establishment of hard tissue across the apical foramen and for the degree of inflammation. The amount of apical closure was graded as being none or complete. When all the examined histologic sections showed presence of a bridge, it was termed complete. Lack of a bridge in any section was graded as no closure.

The degree of apical inflammation was designated as none, mild, moderate, or severe responses. The evaluation of the sections was performed in a double blind manner and in most instances there was agreement among the three examiners. In cases of disagreement, a majority decision was used.

RESULTS

Three Months

The tissue samples of the three month evaluation consisted of 24 teeth from four monkeys. The results are summarized in Table 1. Of the 24 teeth, only one tooth demonstrated complete closure of the apex with cementoid hard tissue. This tooth was filled with decalcified dentin. The remainder of the samples studied in the three month group failed to demonstrate complete closure across the apices.

One of the six canals obturated with demineralized dentin plugs was associated with an apical mild inflammatory infiltrate of plasma cells, lymphocytes and occasional polymorphonuclear leukocytes. Three of the samples showed a moderate inflammatory response and two demonstrated severe inflammatory reactions along with obvious abscess formation. Of the six canals with hydroxyapatite plugs, three were associated with mild inflammatory responses apically, one with a moderate response and two with severe responses.

The degree of inflammation was more pronounced in the samples which had apical dentin chips. Four of six samples with dentin chip plugs had moderate inflammation. The remaining two were divided evenly between mild and severe inflammatory responses.

In the periapical tissues of the teeth with no plugs, three of the six samples exhibited moderate signs of in-

flammation, two had mild reactions and one had a severe inflammatory response.

Although none of the samples from the three month group was totally free of inflammatory infiltrate, those with hydroxyapatite appeared to have more fibroblasts and fibrous connective tissue in the apical regions compared to the other experimental groups. (Figure 1)

Six Months

The degree of closure and inflammation in the six months samples is shown in Table 2.

The canals which had demineralized dentin plugs failed to exhibit hard tissue formation. The periapical tissues of four teeth showed a moderate chronic and acute inflammatory infiltrate, while one had a mild inflammatory reaction and one had severe inflammation. The cellular infiltrate consisted of plasma cells, lymphocytes, polymorphonuclear leukocytes with proliferating strands of epithelium. (Figure 2)

Of the canals that had apical plugs of hydroxyapatite, four exhibited complete closure of the apical region with hard tissue formation (Figure 3) and two had no evidence of bridge formation. Five of the six samples did not demonstrate any significant periapical inflammation. The periodontal spaces appeared intact with a normal arrangement of fibrous tissue. The remaining sample demonstrated a mild inflammatory response.

Half of the canals obturated with dentin chip plugs showed complete closure and had no evidence of periapical inflammation. Of the remainder, one exhibited a mild inflammatory response and two demonstrated severe inflammation.

One of the six control teeth had complete apical closure with no periapical inflammation. The remainder had no apical closure. One had mild apical inflammation. Three showed moderate responses and one was characterized by severe inflammatory signs.

DISCUSSION

The purpose of this study was to compare the effects of dentin chips, hydroxyapatite and demineralized dentin on the induction of apical hard tissue and the degree of apical inflammation. As control, the three test materials were compared to the results of no plug, or empty apical canals.

By using a total of 48 teeth, random assignment of procedures was assured and enabled the division of the samples into four groups. This allowed evaluation of the responses over two time periods, three and six months.

The use of demineralized dentin chips as an apical plug elicited a more intense inflammatory response in the radicular tissues than that demonstrated in the control teeth or when dentin chips or hydroxyapatite were used.

Most of the organic samples examined after three months demonstrated severe cellular inflammatory infiltrates with occasional abscess formation. There was usually resorption of the apical tooth structure and adjacent osseous tissues with replacement by chords of fibrous connective tissue and occasional strands of epithelial cells. This shows that the organic component of dentin is not effective in inducing apical hard tissue formation and appears to prolong the inflammatory response produced by apical overinstrumentation.

After six months, the demineralized dentin plugs were not effective in inducing hard tissue formation and most of the cases developed moderate inflammatory responses to the placement of the test material. In canals condensed with hydroxyapatite and with dentin chips, the degree of apical closure was more dramatic and overall the amount of apical inflammation and inflammatory cell infiltration was less.

By using RDO as a decalcifying agent in this study, the composition of the demineralized dentin may have been physically or chemically altered in such a manner that the tissues responded with more intense inflammation. The actual effects of denaturing dentin in this manner for use as apical plugs has not yet been examined thoroughly.

Urist[21] described the preparation of demineralized dentin for his studies to induce the formation of bone by decalcifying the dentin in 0.6N HCL, then washing, ster-

ilizing and storing it in 70% alcohol before implantation. The demineralization process used in our study employed a commercial decalcifying agent to remove calcium slats from the dentin. Although some controversy exists as to the amounts of inorganic material that can be removed by either method, our technique of preparation appears not to have the severe denaturant effect on the protein structure of the organic portion that decalcification in HCL acid would have.[22]

Our study indicates that the amount of hard tissue growth that occurs across the apex against condensed dentin chips is negligable after a period of three months. Even after six months the formation of hard tissue is not consistant - in our study only half of the samples showed bridging. Previous investigations by Tronstad[17] and by Rossmeisl et al.[19] indicate that histologic responses to the condensation of dentin chips into apical preparations and the effects that they have on the induction of hard tissue growth in endodontically treated teeth over a similar period of time is more predictable and effective. The degree of inflammation that we noted after three months in the samples with dentin chips resembled the inflammatory cellular and tissue responses that the above authors had noted. However, after six months, the degree of inflammation was more extensive in our study. A reason for these differences may

lie in variations in experimental designs. Tronstad[17] did not perforate the apices of the roots and therefore the inflammatory response would not be expected to be as severe. Oswald and Friedman[18] achieved very rapid development of hard tissue but that may be due to the species difference between their study and ours.

In our study, autologous dentin chips were condensed into the apical ends of the root canals. The resultant hard tissue formation and the degree of apical inflammation was observed to be less than that observed in the study by Rossmeisl et al.[20] In that investigation the apical portions of canals were condensed with freeze dried dentin. The freeze drying of the material may have affected the physiologic nature of the implanted material and may have lessened the extent to which inflammatory reactions were observed.

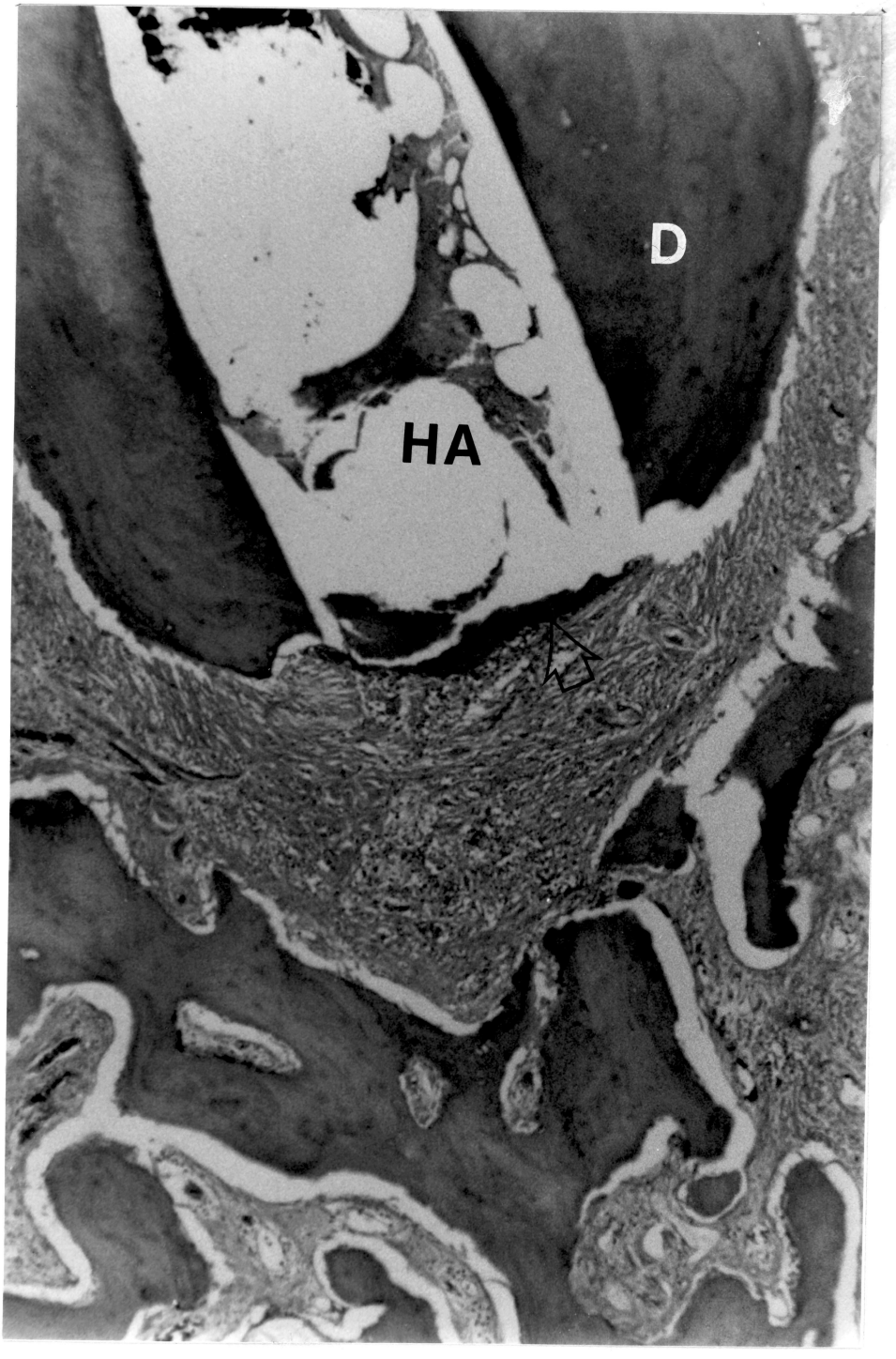
The hydroxyapatite plugs in this study, although not developing any hard tissue at the apex of the sample teeth after three months, did show only mild inflammatory responses. The control teeth and those compacted with dentin chips had moderate levels of inflammation after the same period of time. Compared to the inflammatory responses seen with the demineralized dentin, the post-operative response to hydroxyapatite was more favorable. Increased levels of fibrous connective tissue with humerous fibroblasts and coarse bundles of collagen were seen in the periapical re-

gions of these teeth and the numbers of acute and chronic inflammatory cells were significantly less.

After six months, the hydroxyapatite plugs proved effective in inducing hard tissue in almost all of the sections. Closure was demonstrated in half of the dentin chip samples and in one control which was less than that obtained with hydroxyapatite. Inflammation was less frequent in hydroxyapatite samples than the rest. Therefore, it appears that the periapical inflammatory response to condensation of hydroxyapatite plugs is less severe than that of the demineralized dentin samples, dentin chips, or the controls.

Our results indicate that hydroxyapatite may be a useful material for apical plugs in cases of apical perforations and in cases where the apex has failed to close as a result of pulp necrosis. Hydroxyapatite may also have potential use in apexification procedures in divergent canals with open apices.

Figure 1. Three month sample with hydroxyapatite; HA, hydroxyapatite (material removed during tissue processing), D, dentin. Fibrous connective tissue next to area of hydroxyapatite plug. Note beginning of hard tissue formation (arrow). H and E stain, original x 10 magnification.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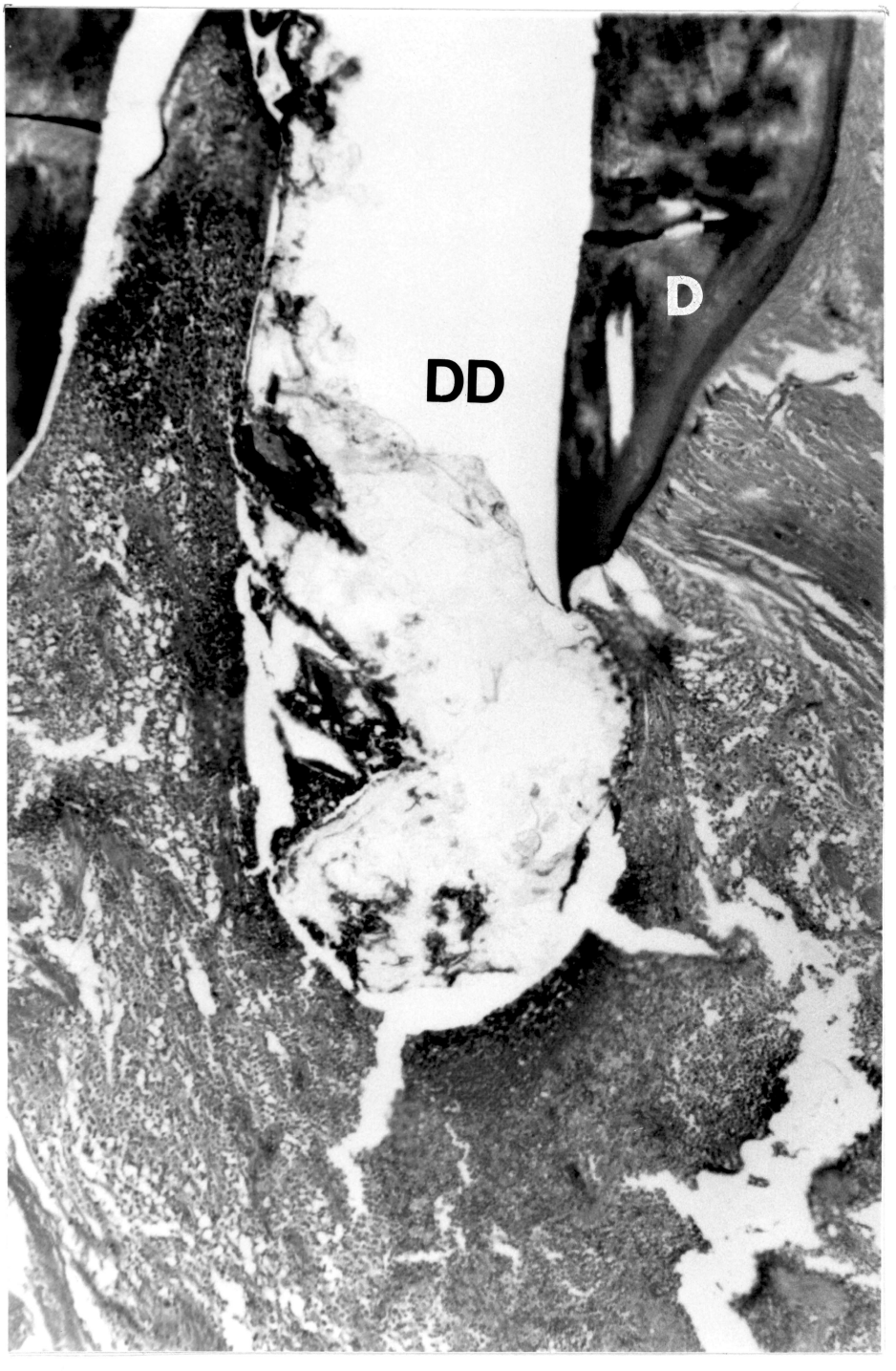
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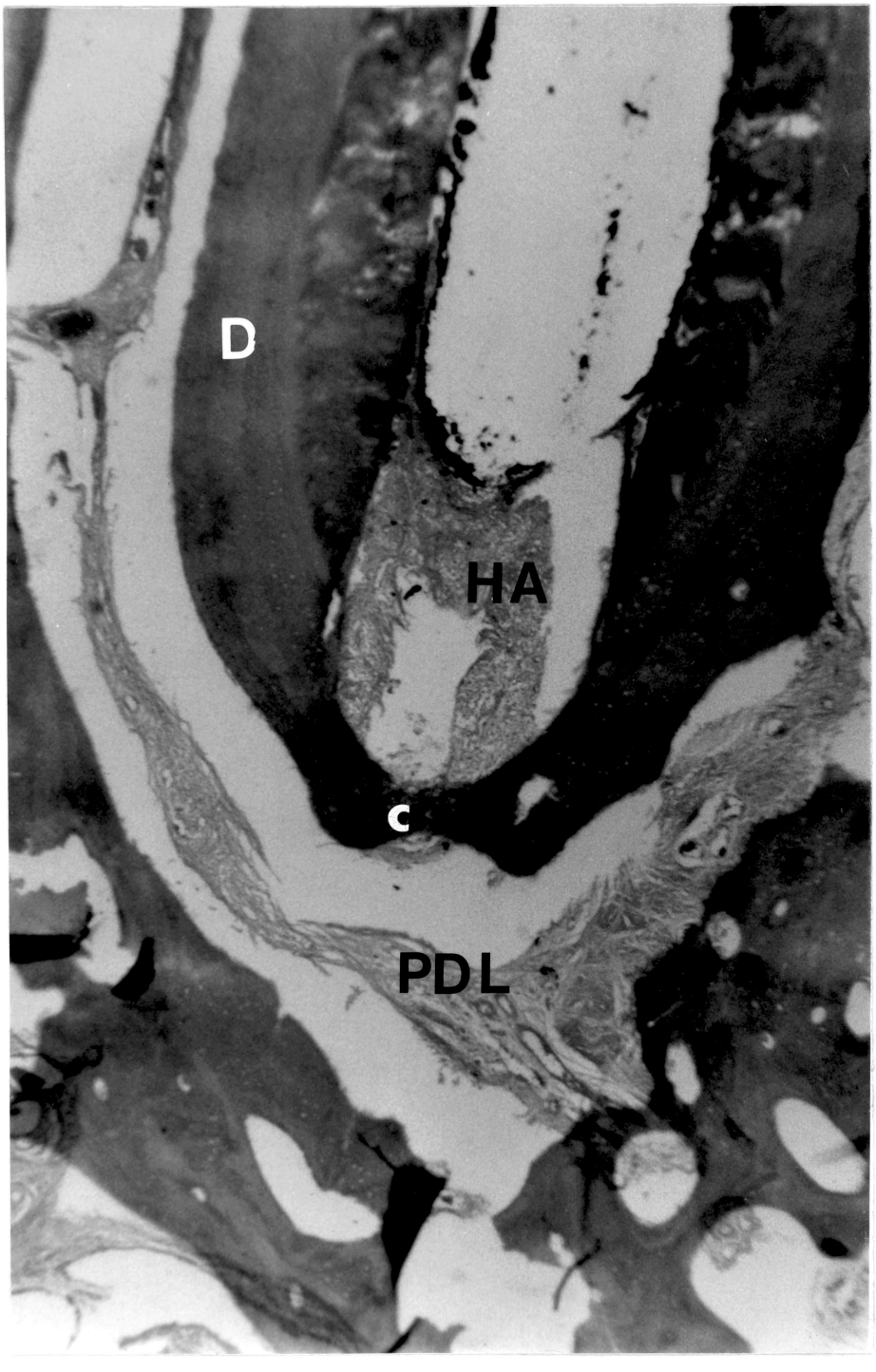
D
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TABLE 1

Three Month Samples

Substance	N	Closure		Inflammation			
		None	Complete	None	Mild	Moderate	Severe
Demineralized Dentin	6	5	1	0	1	3	2
Hydroxyapatite	6	6	0	0	3	1	2
Dentin Chips	6	6	0	0	1	4	1
Controls	6	6	0	0	2	3	1

TABLE 2

Six Month Samples

Substance	N	Closure		Inflammation			
		None	Complete	None	Mild	Moderate	Severe
Demineralized Dentin	6	6	0	0	1	4	1
Hydroxyapatite	6	2	4	5	1	0	0
Dentin Chips	6	3	3	3	1	2	0
Controls	6	5	1	1	1	3	1

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