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Influence of apicoectomy on the pulps of replanted monkey teeth

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Abstract

A new modified apicoectomy technique developed to improve the revascularization and survival potential of replanted mature teeth was evaluated in this in vivo study comparing the pulpal responses of monkey incisors replanted following the modified apicoectomy with those of monkey incisors replanted without an apicoectomy or following a "wire-cutter" apicoectomy. Histologic analysis of the pulps 1, 4, and 8 weeks after replantation showed that pulpal necrosis with severe periapical inflammation and abscess formation were the predominant findings for all 3 study groups.

The results indicated that the modified apicoectomy technique did not enhance pulpal survival. Possible reasons for failure included damage to the pulp during the apicoectomy procedure, relatively large distances from the pulps of the apicoectomized teeth to their blood supplies at the bases of the alveolar sockets, diminished reparative capacity of mature pulp, bacterial infection, and use of multiple replants in each experimental animal.

The acid-etch technique for bonding resins to dental enamel has had a profound effect on restorative and preventive dental practices. Considerable efforts also have gone into the development of a system for bonding composite resins to dentin. The development of a clinically effective dentinal bonding agent might lead to additional techniques that could preserve tooth structure and provide longer lasting restorations.[1] Materials that adhere to dentin also might reduce significantly or eliminate marginal microleakage.[2]

Two new dentinal bonding techniques have been introduced recently. A glycerol phosphate dimethacrylate-containing dentinal bonding agent[a] has been shown to bond favorably to etched enamel and unetched dentin of permanent human teeth. Bowen et al.[3] have shown that the application of ferric oxalate, NTG-GMA (N-[p-tolyl] glycine-glycidyl methacrylate) and PMDM (pyromelletic acid dianhydride and 2-hydroxyethyl methacrylate) also provide excellent bonding to both enamel and dentin of permanent human teeth. The use of these 2 techniques on dentin surfaces of primary teeth has not been investigated.

The purpose of the present study was to compare the dentin-resin bond strengths obtained with the Scotchbond® system and the Bowen method in primary and permanent teeth in vitro. In addition, microscopic examination was used to evaluate several of the specimens before and after preparation of the dentin surfaces, and following the testing of the retentive bond strengths.

Survival of the dental pulp rarely occurs following the replantation of an avulsed mature tooth with a fully formed root and closed apex, and an ischemic necrosis of the pulp is the usual result. This necrotic response is followed by either a reparative response or by further degeneration of the pulp. In the former, an ingrowth of granulation tissue, cellular connective tissue, and, in many cases, a dentin-like, hard tissue formation gradually replaces the necrotic pulp.[1,2] In the latter, bacterial contamination is implicated in causing a massive inflammatory response resulting in liquefaction necrosis and abscess formation.[3]

Some success in maintaining pulpal vitality has been noted following the replantation of immature teeth with open apices.[4,5] The pulpal survival of immature teeth presumably is due to the larger surface area of pulpal tissues available for revascularization at the wider apical foramina. Therefore, attempts have been made to improve the survival potential of the pulps of mature teeth by increasing the exposed surface area of the pulpal tissues of these teeth before replantation. Thus far, the experimental apicoectomy

[a] Ketaset — Bristol Laboratories: Syracuse, NY.

and apical modification techniques used to enlarge the surface area of exposed pulp all have been unsuccessful. Damage to the apical pulp during the procedures[6,7] and large distances between the apicoectomized pulpal tissues and the blood supplies at the bases of the alveolar sockets[4] are the reasons offered for the failures.

The purpose of this investigation was to study the pulpal responses of teeth replanted following a new modified apicoectomy technique. With this technique the surface area of the pulpal tissues available for revascularization was enlarged, and it was anticipated that the integrity of the apical pulpal tissues would be maintained and a close proximity between the pulpal blood vessels and the blood vessels at the bases of the alveolar sockets would be established.

Methods and Materials

The maxillary and mandibular incisors in 6 cynomolgus monkeys (*Macaca fascicularis*) were selected as experimental and control teeth. Radiographs confirmed that all incisors had fully developed roots with completed apex formation.

Each animal was anesthetized using an injection of ketamine hydrochloride[a] (10mg/kg IM) for immobilization and methoxyflurane as the inhalation agent. When each animal reached a surgical plane of anesthesia, the incisor teeth were scaled thoroughly. The teeth selected for the control group received no further treatment, but the experimental teeth were luxated with a straight elevator,[b] extracted with forceps[c], and then replanted.

The following 3 variations of the replantation procedure (Fig 1) were performed:

1. Replantation without apicoectomy — In this procedure the extracted teeth were replanted intact.
2. Replantation with apicoectomy — In this group the extracted teeth were replanted after removal of the apical 2-3 mm of the root using sterile wire cutting pliers[d] according to the technique described by Skoglund.[4]
3. Replantation with modified apicoectomy — In this variation the extracted teeth were replanted with the apical pulp intact but with the apical hard tissue removed. The initial step in this procedure was to notch the entire circumference of the root to a depth of 0.25-0.75 mm at a point about 2-3 mm from the apex using a separating disc in a slow-speed, belt-driven laboratory handpiece. Next, straight pliers[e] were used to apply force to the apical portion of the root until the remaining dentin surrounding the root canal fractured. The apical portion of the root then was separated from the remainder of the tooth and pulp using an explorer and a gentle tugging motion (Fig 2a). This left the apical pulp attached to the rest of the pulp tissue and protruding from the root (Fig 2b).

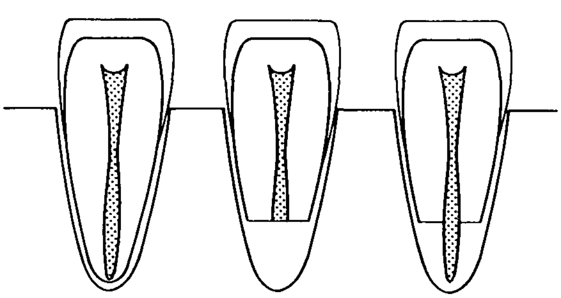


Fig 1. Diagrammatic representation of experimental replantation procedures: (1) replantation without apicoectomy; (2) replantation with apicoectomy; (3) replantation with modified apicoectomy.

The extraoral time was limited to 2 min for each experimental tooth. During this time the teeth were held with the extraction forceps and the root surfaces were irrigated with sterile normal saline.

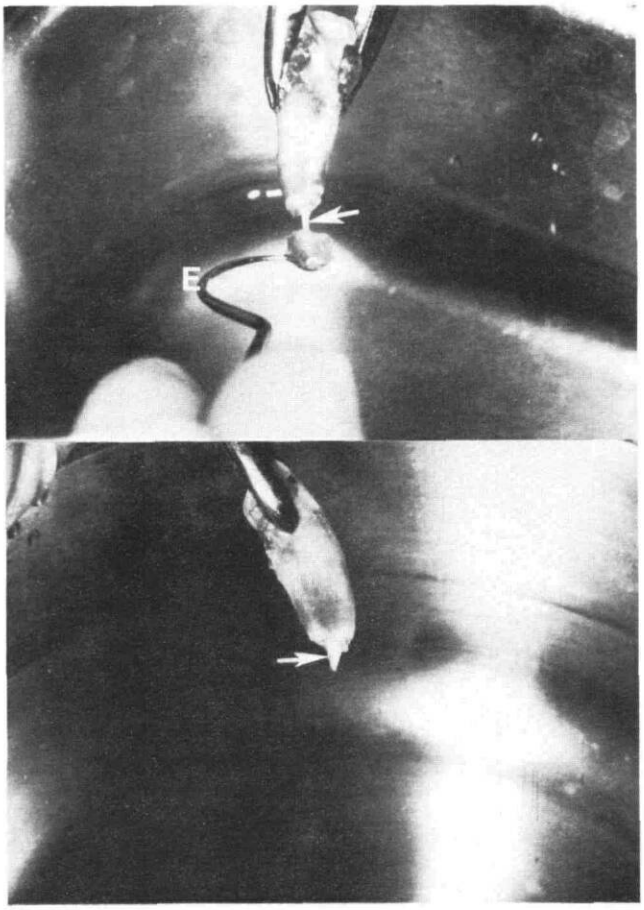
After the designated apical modification, each tooth was returned to its socket using the extraction forceps for the initial placement and finger pressure for the final seating. No form of stabilization was used. For the 2 weeks immediately following surgery, the animals were fed a soft diet.

To prevent infection, each animal received 20,000 units/kg/day of procaine penicillin G IM 1 day prior to the surgical procedures and daily for 6 days after the operations.

The experimental procedures were performed so that there would be follow-up periods of 1, 4, and 8 weeks after which the animals again were anesthetized and then euthanized by IV perfusion of saline and 10% neutral buffered formalin. Twenty minutes after perfusion, block sections of the maxilla and mandible were removed and placed in 10% neutral buffered formalin for 72 hr of further fixation. The fixed specimens then were washed in running tap water for 24 hr and placed in 10% EDTA at 45°C for demineralization.

Following demineralization, the teeth were embedded in paraffin and sectioned in a facial-lingual direction at 5-μ thickness. Step-serial sections representing intervals of 100 μ were stained with Harris' hematoxylin and eosin Y and then evaluated under the light microscope.

[b] Cook-Osseus #1 — Silverman: Plymouth Meeting, PA.
[c] Ash Pedo E — Silverman: Plymouth Meeting, PA.
[d] #45071 — Sears, Roebuck and Co: Chicago, IL.
[e] Howe #110 — Henry Schein Inc: Port Washington, NY.



Figs 2a&b. Experimental procedure 3 — Replantation with modified apicoectomy. a *(top)* — Separation of apical hard tissue from remainder of tooth and pulp (arrow) using an explorer (E); b *(bottom)* — Completed modified apicoectomy with exposed pulp tissue (arrow).

The pulpal responses of each replanted tooth were placed in 1 of the following 3 histopathologic categories:

1. **Normal pulp** (pulpal survival) — loose connective tissue within the pulp chamber arranged into an odontoblastic layer, cell-free zone of Weil (in coronal and middle thirds), cell-rich layer, and pulp core.
2. **Necrotic pulp and replacement connective tissue** (pulpal repair) — ischemic necrosis within the pulp chamber characterized by tissue in which pyknotic nuclei were seen within a structureless or slightly fibrillar stroma lacking in cellular outlines or endothelial linings; ingrowth of vital connective tissue, cellular or fibrillar in nature, from the periapical region.
3. **Necrotic pulp and inflammation/abscess formation** (pulpal degeneration) — ischemic necrosis within the pulp chamber; infiltration of acute and/or chronic inflammatory cells from the periapical region with localized areas of liquefaction and pus formation.

Results

All animals survived the surgical procedures. Of the 48 incisor teeth available from the 6 monkeys, 9 were eliminated from the study — 1 tooth was exfoliated less than 1 day after replantation due to a fracture of the facial alveolar bone which occurred during the extraction procedure; 5 teeth were fractured during the extraction procedure; and 3 teeth were not processed adequately for histologic analysis. Therefore, a total of 33 replanted teeth and 6 control teeth were used in the final analysis. Table 1shows the distribution of the replantation procedures and observation periods for the 33 experimental teeth and the distribution of pulpal responses to the replantation procedures.

Control Group

The 6 control teeth, which were not extracted and replanted, exhibited normal pulpal tissues throughout the pulp chambers, and the characteristic zones of dental pulp were identified easily.

Experimental groups

Normal pulp (pulpal survival). Of the 33 experimental teeth used in this study, only 1 incisor (which was replanted without apicoectomy, procedure 1, and followed for 8 weeks) showed evidence of pulpal survival (Table 1). Normal pulp was seen, but was stained more lightly than that found in the control sections.

Necrotic pulp with replacement connective tissue (pulpal repair). Three of the 33 experimental teeth showed evidence of pulpal repair with an ingrowing connective tissue bordering directly on the necrotic tissue within the pulp chamber (Table 1). This type of response was seen at the 4-week observation period in 1 tooth (replanted without apicoectomy, procedure 1) and at the 1- and 8-week periods in 2 teeth (re-

Table 1. Distribution of Replantation Procedures, Observation Periods, and Pulpal Responses to Replantation

	Observation Period			
Replantation Procedure	*1 week*	*4 weeks*	*8 weeks*	*Total*
1—No apicoectomy	3	6+	4*	13
2—Apicoectomy	3	3	2	8
3—Modified apicoectomy	2+	6	4+	12
Total	8	15	10	33

* One replant in this group exhibited normal pulp (survival).

+ One replant in each of these groups exhibited necrotic pulp and replacement connective tissue (repair).

All other replants exhibited necrotic pulp and inflammation with abscess formation (degeneration).

planted following the modified apicoectomy, procedure 3).

In the necrotic portions of the pulp chambers of all 3 teeth, the cells and fibers appeared to have condensed into a relatively homogeneous eosinophilic mass. Cell outlines were absent and pyknotic nuclei were scattered throughout the stroma (Fig 3).

The appearance of the replacement connective tissue varied with the length of the follow-up period. At the 1-week stage, an immature tissue consisting of cells, randomly oriented fibers, and numerous blood vessels, was seen (Fig 4). At the 4- and 8-week periods, the fibers were considerably more numerous and organized and were arranged parallel to the long axis of the tooth. In the 4-week specimen (Fig 5) the fibers extended into the apical third of the pulp chamber, and in the 8-week sample the connective tissue reached to the middle third of the chamber.

Necrotic pulp with periapical inflammation and abscess formation (pulpal degeneration). Twenty-nine of the 33 experimental teeth exhibited degenerative responses characterized by pulpal necrosis with dense periapical inflammation and abscess formation (Table 1). In most of these teeth, a large abscess outlined with inflammatory cells was seen in the apical third of the pulp chamber. Coronal to the abscess was necrotic pulp, and apical to the abscess was a granulomatous formation consisting of a dense accumulation of round inflammatory cells within a fibrous connective tissue capsule (Fig 6).

Discussion

The results of this experiment showed that the modified apicoectomy technique did not enhance the pulpal survival of replanted mature teeth in monkeys, nor did it seem to overcome the difficulties of previous experimental replantation techniques using surgical modification of the apical portion of the tooth root. As with other studies,[4,6-8] damage to the apical pulp could have occurred at any point in the modified apicoectomy procedure. Also, an approximation of the exposed pulp with the blood vessels at the base of the alveolar socket was not established. Instead, the exposed pulp tended to fold over the apicoectomized surface of the tooth and thus was deflected away from the apical blood supply. Although the possibility of reattachment to the vessels of the periodontal ligament did exist, such revascularization of the pulp evidently did not occur.

The majority of replants in this study exhibited a severe inflammatory response in the periapical regions and in the apical portions of the pulp chambers. Inflammation can be caused by any of several factors including tissue injury, chemical or thermal irritants, or bacterial infection. Andreasen[3] found that the only replanted teeth which demonstrated a heavy inflammatory infiltrate were those with bacterial contamination. To prevent such infection, all animals in the study were given antibiotics pre- and postoperatively. Nevertheless, it was possible that some strains of microorganisms were penicillin-resistant and persisted to cause infection. Further, studies[9-11] have shown that even healthy individuals develop transient bacteremias. Thus, when the antibiotic regimen was stopped postoperatively, the monkeys may have developed bacteremias. The blood-borne microorganism then could have found their way via anachoresis to the necrotic pulps of the replanted teeth where the impaired circulation could not cope effectively with or eliminate them.[9,10,12,13]

Other investigations[4-6,8,14,15] have demonstrated that pulpal survival and repair occurs most often in immature teeth with wide open apices. Success in these cases is attributed to the larger surface area of pulpal tissue available for revascularization. However, it is possible that surface area is not the only factor to contribute to the more favorable prognosis for replanted immature teeth. The mesenchymal nature of the pulpal tissues in the apical region of these teeth also may play a significant role. Such tissue is more vascular than mature pulp and there are more vessels

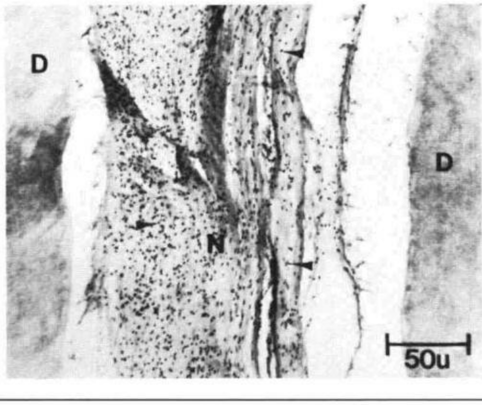
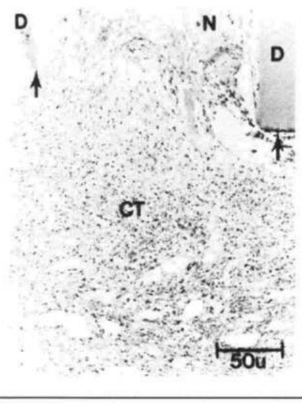


FIG 3 (*left*). Necrotic pulp (N) seen in 33 of 34 replanted incisors. Note pyknotic nuclei (arrows) within homogeneous stroma of necrotic pulp (N). Root dentin (D) surrounds pulp chamber (100×).

FIG 4 (*right*). Necrotic pulp (N) with replacement connective tissue (CT) from incisor replanted following a modified apicoectomy (procedure 3). Note cellular nature of connective tissue and apicoectomized surfaces (arrows) of root dentin (D) in this specimen obtained 1 week after replantation (100×).



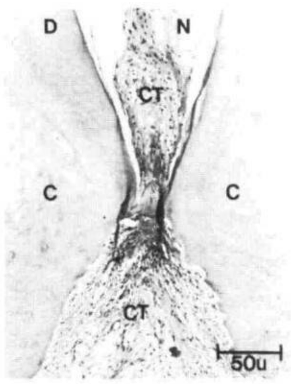
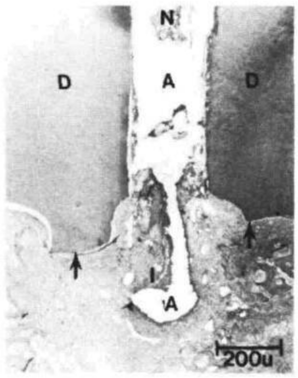


FIG 5 (*left*). Necrotic pulp (N) with replacement connective tissue (CT) from incisor replanted without apicoectomy (procedure 1). Connective tissue in this specimen obtained 4 weeks after replantation is less cellular and more fibrous than that seen in the 1-week sample (Fig 4). Dentin (D) and cementum (C) surround the connective tissue (100×).

FIG 6 (*right*). Necrotic pulp (N) with periapical inflammation (I) and abscess formation (A) typical of pulpal response seen in 29 of 33 replanted teeth. Note apicoectomized surfaces (arrows) of root dentin (D) from experimental incisor replanted following a modified apicoectomy (procedure 3, 100×).



available for reattachment to the apical blood supply. In addition, the pulps of immature teeth are more cellular than those of mature teeth and therefore have greater reparative and regenerative potentials.[17-19] Lastly, the presence of tissue spaces in mesenchymal tissue allows the diffusion of essential nutrients to maintain cell vitality even before revascularization occurs.[16,17] The diffusion of nutrients is much less likely in the more fibrous pulps of mature teeth.

Conclusion

From the results of this and previous studies, it is evident that the survival of the pulpal tissues in replanted mature teeth seldom occurs and that the experimental techniques developed to enhance pulpal survival have not been successful. Therefore, in a clinical setting, pulpal extirpation with a calcium hydroxide fill 7-14 days after replantation, is recommended in order to eliminate the possibility of pulpal necrosis and to prevent other adverse sequelae.[20]

Further research into mechanisms for maintaining pulpal survival in replanted mature teeth is warranted. However, because of the consistent failures with the surgical techniques, other approaches such as Andreasen's use of tissue culture,[21] should be developed.

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