Analysis of the healing response to gutta-percha and Diaket when used as root-end filling materials in periradicular surgery

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Abstract

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Aim To analyse the healing response to gutta-percha and Diaket when used as root-end filling materials in periradicular surgery.

Methodology Periradicular surgery was completed using the mandibular second, third and fourth premolar teeth from nine male mongrel dogs. The six roots on one side of the mouth were randomly allocated to one of the following groups: group A: a resected root end and a burnished gutta-percha root filling; group B: cavities were prepared to a depth of 4.0 mm, using ultrasonic root-end preparation and filled with Diaket. The response was evaluated histologically at 55 (nine specimens) and 150 (three specimens) days post operatively.

Results The data for the 55-day period was analysed statistically using Wilcoxon's Signed Ranks test. No statistical analysis was carried out on the 150-day group due to the small number of specimens. The level of significance was set at $P < 0.05$. No statistical significance was observed in the healing response between Diaket and gutta-percha in the following categories at 55 days: inflammatory response, angiogenesis, root-end resorption, and cementum deposition. Statistically significant differences were observed in the healing categories: bone apposition ($P < 0.05$) and periodontal ligament formation ($P < 0.05$).

Conclusions At both time intervals, Diaket had a better healing response that was characterized by hard tissue formation adjacent to the root-end filling material bordered by occasional multinucleated giant cells. The nature of both the hard tissue formation and the adjacent cells, however, remains undetermined. Diaket displayed the best healing of either material used in this study.

Keywords: Diaket, endodontic surgery, root-end filling.

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Introduction

Endodontic periradicular surgery is the treatment of choice to manage tooth roots and associated pathosis when nonsurgical root canal treatment has been unsuccessful or is not feasible. Ultimately, the aims of periradicular surgery are to remove the causative agent of pathosis and establish an environment conducive to the regeneration of the wounded tissues. The ability to achieve this later goal is governed by the predictable regenerative properties of specific materials, cells, and growth factors. Management of the resected root end during periradicular surgery is critical to establish conditions favourable to the regeneration of the periodontium. Often a key feature in managing the root end is the placement of a root-end filling. The

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ideal properties of a root-end filling material include being biocompatible, nonresorbable, adaptable to the root-end preparation, easily placed and radiopaque.

The purpose of a root-end restoration placed during periradicular surgery is to establish a barrier between the root canal system and the surrounding supportive tissues (Gutmann & Harrison 1991). This barrier should be permanent and able to prevent the egress of microorganisms from within the root canal system to the periradicular tissues. Ideally, this material should be biocompatible, should not evoke a foreign body response, periradicular inflammation or root resorption and should demonstrate a bioinductive capacity in regeneration of the periodontium (Andreasen *et al.* 1993). Failure to create an environment conducive to this process would result in tissue repair as opposed to regeneration and the potential for less than ideal healing. A cementum layer would also enhance the integrity of the apical barrier, making it more resistant to the penetration of microorganisms and, in effect, establishing a biological barrier (Andreasen 1981, Andreasen *et al.* 1993).

The major function of cementum is for the attachment of periodontal ligament fibres to the root surface. The integrity of these fibres is essential for tooth function (Narayanan *et al.* 1995). The formation of new cementum is thus an integral part in attaining a new functional attachment apparatus (Ratcliff 1966). Studies indicate that without cementum, the periodontium cannot be restored to normal health (Lindskog & Blomlöf 1983, Baad *et al.* 1985). During regeneration of the periradicular tissues, cementum has been shown to form over the surface of surgically resected root ends (Andreasen 1973, Craig & Harrison 1993). Formation of cementum begins at the root periphery and proceeds centrally toward the root canal (Andreasen & Rud 1972). Newly formed periodontal ligament fibres show a functional realignment that involves a reorientation of fibres perpendicular to the plane of the resected root end, extending from the newly formed cementum to the woven bone trabeculae (Gutmann & Harrison 1991, Lowenguth *et al.* 1993).

A plethora of materials have been used in an effort to establish an apical barrier including amalgam, Cavit, composite resin, Diaket, Super EBA, IRM, glass ionomer, gold foil, gutta-percha, polycarboxylates, zinc oxide-eugenol and zinc phosphate (Gutmann & Harrison 1991). To date, predictable regeneration of the periodontium utilizing these materials has not been achieved.

Diaket, a polyvinyl resin initially intended for use as root canal sealer, has been advocated for using as a root-end filling material (Snyder Williams & Gutmann 1996). Leakage studies comparing Diaket to other commonly used root-end filling materials have shown it to have a superior sealing ability (Kadohiro 1984, Walia *et al.* 1995). When used as root canal sealer, biocompatibility studies have shown Diaket to be cytotoxic in cell culture (Kettering & Torabinejad 1984) and generate long-term chronic inflammation in osseous (Spangberg 1969) and subcutaneous tissues (Olsson & Wennberg 1985). However, when mixed at the thicker consistency advocated for use as a root-end filling, Diaket has displayed good biocompatability with osseous tissues (Nencka *et al.* 1995, Snyder Williams & Gutmann 1996). Histologically, a unique tissue barrier has formed across the Diaket at the resected root end, the nature of which is unknown (Snyder Williams & Gutmann 1996). However, this tissue resembles an osteoid or cementoid type of matrix with a close approximation of periodontal tissue fibres, suggesting a regenerative response to the root-end filling material. Therefore, the aim of this study was to assess the nature of the periradicular tissue response to Diaket when used as root-end filling material.

Materials and methods

Nine male mongrel dogs, approximately 50.0 kg, and older than 18 months of age, were obtained and quarantined for seven days to ensure optimal health prior to initiation of research. The animals were cared for and housed by the Baylor College of Dentistry Animal Research Unit. In three animals, a split mouth design was used. The right or left mandibular was allocated randomly to a postsurgical sacrifice time period, either 55 days or 150 days. This was achieved by operating 150 and 55 days prior to the sacrifice. The specimens were then removed simultaneously at the time of sacrifice (Olson *et al.* 1982). The remainder of the animals were observed for 55 days.

The mandibular second, third and fourth premolar teeth from each dog were used in this study. The six roots on one side of the mouth from these teeth were randomly allocated to either group A or B: (see below):

For all phases of treatment, general anaesthesia of the animals was achieved by (i) initial sedation with intramuscular injection of 1.0 mg kg^{-1} to 2.2 mg kg^{-1} of Rompun (Mobay Corporation, Shawnee, KS, USA) and (ii) deep sedation with intramuscular injection of 20.0 mg kg^{-1} of Ketamine (Aveco Company Incorporated, Fort Douglas, IA, USA). Prior to nonsurgical and surgical endodontic procedures, intraoral anaesthesia was achieved with a mandibular block injection of 1.8 mL of 2% lidocaine containing 1 : 100 000 adrenaline (Astra Pharmaceutical Products, Westborough, MA, USA).

The nonsurgical phase of the procedure consisted of full mouth prophylaxis, preoperative radiographs and nonsurgical root canal treatment of the second, third and fourth mandibular premolars. The mesial and distal root canals of the teeth were cleaned and shaped chemomechanically, dried and obturated with Sealapex (Kerr Manufacturing Company, Romulus, Michigan) root canal sealer and thermoplasticized gutta-percha (Obtura Corp., Fenton, MO, USA). Access openings were sealed with fast-set Tytin amalgam (Kerr Manufacturing Company, Romulus, MI, USA) and Copalite varnish (Cooley and Cooley Limited, Houston, TX, USA).

During the surgical phase of treatment, a local infiltration of 1.8 mL of 2% lidocaine containing 1 : 50 000 adrenaline (Astra Pharmaceutical Products, Westborough, MA, USA) was used for haemostasis (Buckley *et al.* 1984). The animals were placed on a soft diet and 200 mg 27.3 kg^{-1} ibuprofen (Whitehall Laboratories, New York, NY, USA) twice daily was administered for a minimum of 2 days post surgery. In cases of severe pain, butorphenol 0.025 mg kg^{-1} (Fort Dodge Laboratories Incorporated, Fort Dodge, IA, USA) was administered intramuscularly every 8 h.

The surgical phase of the procedure consisted of a vertical incision that extended coronally from the alveolar mucosa to the distal line angle of the first premolar and an intrasulcular incision extending posteriorly from the distal of the first premolar to the mid-buccal surface of the first molar. A full thickness mucoperiosteal triangular flap was reflected. The second, third and fourth premolar root ends were accessed using a high speed round surgical bur (Brasseler, Savannah, GA, USA) with copious water spray. The root ends were resected using a high speed flat fissure surgical bur (Brasseler, Savannah, GA, USA) with copious water spray. Following placement of the root-end filling material, the reflected tissues were repositioned and compressed with moist gauze for 5 min and sutured with 4–0 Vycril suture (Johnson & Johnson, Somerville, KY, USA). A further 5 min of postsurgical compression with moist gauze was applied after suture placement.

Group A: Roots were left with a resected root end and a burnished gutta-percha root filling. The smear layer was removed with a 17% EDTA solution (Sigma, St. Louis, MO, USA) (Rud *et al.* 1991, Gutmann & Pitt Ford 1993, Blomlöf & Lindskog 1995).

Group B: Root-end cavities were prepared to a depth of 4.0 mm, using ultrasonic tips (Osada Electric Company Incorporated, Los Angeles, CA, USA). The smear layer was removed with a 17% EDTA solution and the root-end preparation was filled with Diaket (ESPE, Seefeld, Germany).

To demonstrate active deposition of mineralizing tissues all animals were injected intraperitoneally with Procion Brilliant Red Fluorescent dye, M-8BS (Reactive Red 4) (Sigma, St. Louis, MO, USA), 90.0 mg kg^{-1} bodyweight 50 days post operatively.

Six animals were sacrificed 55 days post surgically and three animals at 150 days post surgically using anaesthesia with intravenous administration of sodium pentobarbital (Sigma, St. Louis, MO, USA) 33.0 mg kg^{-1}. The right and left common carotid arteries and external jugular veins were surgically exposed and the common carotid arteries were cannulated with the tubing of a positive pressure perfusion system. Approximately 20.0 mL of 20% potassium chloride solution (Sigma, St. Louis, MO, USA) was injected into the external jugular vein to fibrillate the heart. Following overdose and lack of palpable pulse, the carotid artery was perfused, with 1.0–1.5 L of 10% phosphate buffered formalin (Mallinckrodt, Paris, KY, USA) at a pressure of 120.0–140.0 mm of Hg for the purpose of internal fixation. Block sections of the mandible were obtained using a Stryker autopsy saw (Abbott Laboratories, Chicago, IL, USA).

All sections were placed into 10% formalin solution for further tissue fixation. Specimens were then placed in 0.5 mol L^{-1} EDTA at 4°C for demineralization (Bruce 1992). Demineralization was complete when the section demonstrated an absence of radiopaque structures upon radiographic evaluation. The block specimens were dehydrated in alcohol and infused with Paraplast Plus Paraffin (Monojet, St. Louis, MO, USA) using the Technicon (Technicon Instruments Corporation, Tarrytown, NY, USA). The block sections were embedded in paraffin wax and serially sectioned at 5.0–7.0 mm using a microtome (Leitz, Rockleigh, NJ, USA). Sectioning began at the centre of the root canal and progressed until evaluation material was no longer available from the section. A total of seven randomly selected representative sections were used from the central portion of each root. Six sections stained with haematoxylin and eosin (H & E), and one section per group stained with a modified Attwood's stain (Hess & Villanueva 1983).

Histological evaluation criteria

Two independent examiners graded each H & E section (slide) according to the criteria outlined in Table 1.

Statistical evaluation

Haematoxylin and eosin 55-day histological data was analyzed using Wilcoxon's Signed Ranks test. The level of significance was set at $P < 0.05$. Data was compiled in raw data collection tables and tabulated to give cumulative results. Any significant difference between the healing response as assessed by the outlined criteria resulted in the rejection of the null hypothesis for the given criteria. The sample size for the 150-day specimens was insufficient for statistical analysis, and therefore only descriptive evaluation was performed.

Results

All animals tolerated the nonsurgical and surgical phases of the experiment well. H & E light microscopic assessments of periradicular excisional wound healing for the 55-day specimens are listed in Table 2. The modified Attwood's stain, that differentially stains cementum, dentine, bone and periodontal ligament, was used to enhance the H & E assessment

Day 55 specimens (Diaket)

In the majority of specimens inflammation was rated as being none to minimal. Cementum deposition across the resected root end was greater than 75% in all specimens, however, cementum was not identified adjacent to the root-end filling material. Bone apposition into the surgical wound site was extensive, both in close proximity to the resected root end and root-end filling material. Periodontal ligament

Table 1 Criteria for histological evaluation

Chronic inflammatory response
0 = No inflammation in the apical excisional wound site
1 = Mild inflammation in the apical excisional wound site
2 = Moderate inflammation in the apical excisional wound site
3 = Severe inflammation in the apical excisional wound site
Root-end resorption
0 = No root-end resorption in the apical excisional wound site
1 = Mild root-end resorption in the apical excisional wound site
2 = Moderate root-end resorption in the apical excisional wound site
3 = Severe root-end resorption in the apical excisional wound site
Ankylosis of the root end
0 = Ankylosis of the root end not present in the apical excisional wound site
1 = Ankylosis of the root end present in the apical excisional wound site
Cementum formation
0 = Cementum observed on <25% of the resected root end
1 = Cementum observed on >25 < 50% of the resected root end
2 = Cementum observed on >50 < 75% of the resected root end
3 = Cementum observed on >75% of the resected root end
Apical periodontal ligament (PDL) formation
0 = Functionally oriented collagen fibres inserting into <25% of the new cementum and bone
1 = Functionally oriented collagen fibres inserting into >25 < 50% of the new cementum and bone
2 = Functionally oriented collagen fibres inserting into >50 < 75% of the new cementum and bone
3 = Functionally oriented collagen fibres inserting into >75% of the new cementum and bone.
Bone apposition
0 = New bone apposition observed in <25% of the of the osseous excisional wound
1 = New bone apposition observed in >25 < 50% of the of the osseous excisional wound
2 = New bone apposition observed in >50 < 75% of the of the osseous excisional wound
3 = New bone apposition observed in >75% of the of the osseous excisional wound
Angiogenesis
0 = New blood vessels present in <25% of the wound site
1 = New blood vessels present in >25 < 50% of the wound site
2 = New blood vessels present in >50 < 75% of the wound site
3 = New blood vessels present in >75% of the wound site

Table 2 Results of the histological evaluation (55-day specimens)

Animal	Inflamation		Root resorption		Cementum deposition		Periodontal ligament formation		Bone apposition		Angiogenesis	
	Diaket	GP	Diaket	GP	Diaket	GP	Diaket	GP	Diaket	GP	Diaket	GP
1	1	0	0	0	3	3	2	2	2	2	1	0
2	0	0	0	0	3	3	3	3	3	2	2	0
3	0	1	1	1	2	2	2	2	3	2	2	1
4	0	1	1	0	3	3	2	2	3	3	0	0
5	0	0	0	0	3	3	2	2	2	2	2	2
6	0	1	0	0	3	3	3	1	3	1	1	0
7	0	0	0	2	3	3	3	2	3	3	0	1
8	0	0	0	0	3	3	3	1	3	3	0	0
9	1	0	0	0	3	2	3	2	3	2	0	2

formation was also extensive in the majority of cases with functionally oriented collagen fibres inserting into approximately 75% of the new cementum and bone. Whilst there was no ankylosis or epithelial proliferation, a very mild degree of root resorption was present in some specimens, which is consistent with the normal healing process in the dog. The material within the root canal system at the resected apex appeared to be of mineral form and occasionally had a distinct basophilic line within the substance. A number of different cell types were in close proximity to this substance, including multinucleated giant cells, possibly foreign-body-type cells or clastic-type cells, connective tissue cells, and secretory-type cells.

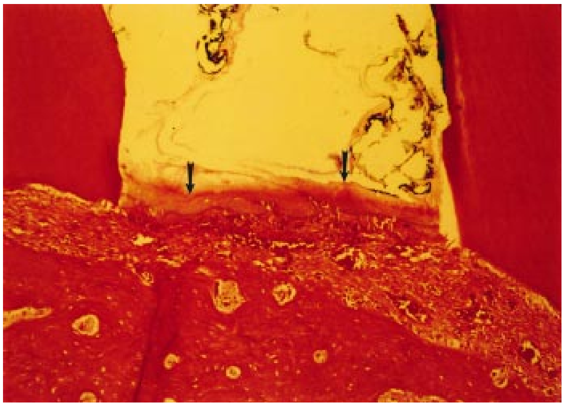

Figure 1 Day 150 Diaket X100. There is reformation of the periodontal ligament with close approximation of the alveolar bone. A homogenous hard tissue barrier (arrows) has formed over the root-end filling material. Minimal to no inflammation is seen.

Day 55 specimens (gutta-percha)

Inflammation was rated as being none to minimal in the majority of specimens, however, a zone of chronic inflammation consistently existed approximating the root-end filling material. Cementum deposition was greater than 75% in all specimens, however, no cementum was identified adjacent to the root-end filling material. Bone apposition into the surgical wound site was approximately 75%, and in the majority of cases, was in close proximity to both the resected root end and root-end filling material. Periodontal ligament formation was also extensive in the majority of cases with functionally oriented collagen fibres inserting into approximately 50–75% of the new cementum and bone. There was no ankylosis or epithelial proliferation, and a very mild degree of root resorption was evident.

Day 150 specimens (Diaket)

Healing was essentially the same as the 55-day period (Fig. 1) except for one specimen. This specimen had atypical healing compared to all other Diaket specimens. The inflammatory infiltrate was severe and extensive, as was the root resorption. Bone apposition, cementum deposition, and periodontal ligament formation was minimal. The roots mesial and distal to this specimen had very extensive cyst and abscess formation. This dog also had extensive periodontal pocketing on teeth not used in the experiment.

Day 150 specimens (gutta-percha)

Healing was essentially the same as at the 55-day period (Fig. 2) except for one specimen. This specimen had atypical healing to all other gutta-percha specimens, exhibiting a severe extensive inflammatory infiltrate and root resorption. Bone apposition, cementum deposition, and periodontal ligament

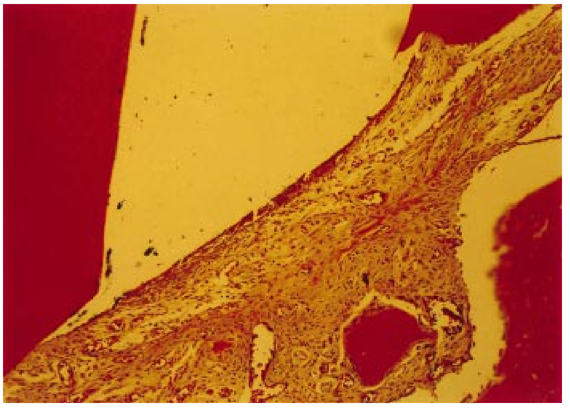

Figure 2 Day 150 Gutta-percha X100. Reformation of the periodontal ligament infiltrated with chronic inflammatory cells. No hard tissue formation is seen over the root filling.

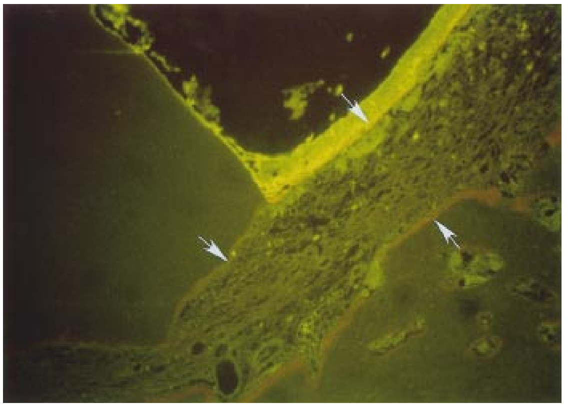

Figure 3 Diaket 100x Procion Brilliant Red: hard tissue deposition evidenced by a red fluorescent line both in cementum and bone (arrows). The line extends from the cementum onto the material at the root end.

formation was minimal. This dog also had extensive periodontal pocketing on teeth not used in the experiment.

Procion Brilliant Red evaluation

Hard tissue deposition was evidenced by a red fluorescing line that occurred both in cementum and bone. In the Diaket specimens, a line was seen extending from the cementum surface into the material that would appear to indicate active hard tissue deposition. The nature of this hard tissue remains undefined. No such line existed with gutta-percha samples (Figs 3, 4).

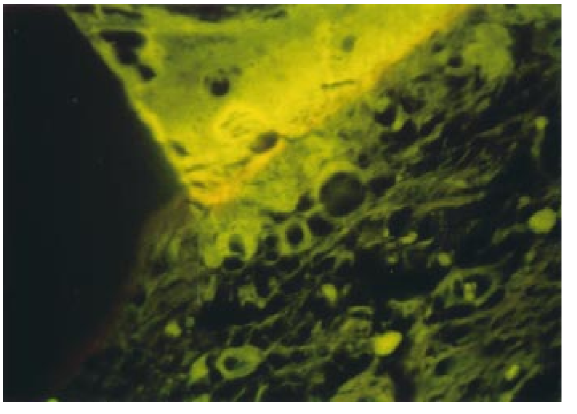

Figure 4 Diaket 400x Procion Brilliant Red. Higher magnification of tissue formation in Fig. 3. Hard tissue deposition evidenced by a red fluorescent line. The line extends from the cementum onto the material at the root end.

Statistical analysis

No statistical significance was observed in the healing response between Diaket and gutta-percha in the following categories: inflammatory response, angiogenesis, root-end resorption, and cementum deposition. Statistically significant differences were observed in bone apposition ($P < 0.05$) and periodontal ligament formation ($P < 0.05$). In both cases Diaket had a better healing response.

Discussion

The key to re-establishing the functional microanatomical architecture of the resected root end is regeneration of the periodontium. The periodontium contains a combination of complex tissues that include alveolar bone, periodontal ligament, and cementum. There is general agreement that tissues of the periodontium harbour cells with the capacity to regenerate the periodontium. The specific cells responsible for stimulating new cementum formation in the postdevelopment stages, however, have yet to be clarified (Aukhil *et al.* 1990). The evidence suggests that progenitor cells with regenerative potential are in close proximity to the vascular spaces (Roberts *et al.* 1987, McCulloch *et al.* 1987). Analysis of cell kinetic patterns during wound healing in experimental periodontal regenerative procedures suggests that the cells are either from the periodontal ligament (Aukhil & Iglhaut 1988) or the alveolar bone proper (Iglhaut *et al.* 1980). Cells with the ability to migrate and attach to the denuded root surface are induced to differentiate into cementoblasts.

Many dentino-osseous wound healing models exist for the investigation of repair and regeneration of tissue. The dog mandible is a good model for testing surgical dental procedures and it has been used extensively to investigate all facets of healing response to oral surgical procedures (Manni *et al.* 1986, Hollinger & Kleinschmidt 1990, Johnson 1991, Bruce 1992, Craig & Harrison 1993).

There are some shortcomings in using this model for the study of periradicular wound healing. The healing rate compared to humans is more rapid and may not be reflective of that which occurs in humans. The healing response amongst the animals is also variable and, to some degree, species dependent. Two animals used in this experiment had a healing response (150 days post operatively) that differed greatly from the other animals. These two dogs had extensive marginal periodontitis throughout the oral cavity. Periodontitis that increases in prevalence and severity with age can occur spontaneously in dogs kept in large colonies and in domestic dogs kept on a commercial dog diet (Page & Schroeder 1982). The beagle breed is known for extensive periodontitis (Anderson 1970). One animal was a beagle and the other a crossbred Doberman of unknown origin. Furthermore, the proximity of the premolar teeth to the inferior dental canal and the multiple exits of the mental foramina in dogs makes surgical injury to the neurovascular bundle more probable. The extensive muscle attachment distal to the first premolar makes reflection and reapproximation of the reflected tissue difficult. As a consequence, there may be a greater probability of periodontal breakdown.

The body of the mandible in the region from the second premolar tooth to the canine tooth rotates from sagittal plane to horizontal plane. The second premolar tooth roots are also angled toward the median plane to a greater extent. The combination of these anatomical features makes osseous access to the periradicular region of this tooth difficult. Often bone has to be removed to a depth of approximately 8.0 mm. A further complication is the small dimension of the resected root end to that of an ultrasonic root-end preparation tip. The aggressive nature of some of these tips makes apical perforation highly possible.

The Procion Red Brilliant dye effectively stained the forming hard tissue (Figs 3, 4). The dye readily forms stable irreversible covalent bonds and cross-links with various hydrogen-active functional groups in organic components of tissue matrix in developing bone and tooth. The dye is incorporated into the collagenous structure of mineralizing tissue and remains in the specimen postdemineralization. Following injection, the dye is deposited within 3 to 6 h at the sites of hard tissue growth. Uptake of dye is then continuous for the next 2 to 3 days.

Surgical wound healing response to gutta-percha is well documented and the response evident in this experiment is consistent with that previously reported (Gutmann & Harrison 1991). The typical periradicular response to gutta-percha at the root apex demonstrates an acceptable tissue tolerance with fibrous connective tissue, fibrous encapsulation or mild chronic inflammation adjacent to the material.

Diaket, when mixed in two parts powder to one part liquid, has ideal handling properties. This material offered a firm working consistency and acceptable working time of 30 min or more, which was consistent with previous studies (Snyder Williams & Gutmann 1996). Histologically, specimens with Diaket as a root-end filling displayed an osteoid-type matrix barrier across the material. The continuation of a procion-forming hard tissue line from the dentinal surface to the material at the resected root suggests that a mineralizing hard tissue is being formed adjacent to the root-end filling material (Figs 3, 4), as is the occasional basophilic line visible within this substance. The nature of the multinucleated cells adjacent to the Diaket root-end filling material is unknown at this stage. However, the appearance at both the 55- and 150-day interval was nearly identical with little evidence of resorption of this material by these cells. Both the 55- and 150-day interval gave favourable results for Diaket used as a root-end filling material.

In summary, the healing response to the gutta-percha was typified by good bone apposition, reformation of periodontal ligament, and deposition of new cementum on the resected root end. However, there consistently existed a fibrous capsule with mild chronic inflammation adjacent to the root-end filling material. The healing response to the Diaket was typified by good bone apposition, reformation of periodontal ligament, and deposition of new cementum on the resected root end. There appeared to be some type of hard tissue formed adjacent to the root-end filling material bordered by an occasional multinucleated giant cells. The nature of both the material and the adjacent cells remains undetermined. Diaket displayed the better healing of either material used in this experiment.

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