Comparison of Diaket and MTA when used as root-end filling materials to support regeneration of the periradicular tissues

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Abstract

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Aim The objective of this study was to evaluate on a comparative basis the potential for mineral trioxide aggregate (MTA) and Diaket to promote periradicular tissue regeneration when used as surgical root-end filling materials.

Methodology Seven dogs weighing between 15 and 25 kg were anesthetized prior to having the root canals of their mandibular premolars accessed, cleaned, shaped and obturated. Coronal access cavities were restored with IRM. Surgical access to the root ends was established and the root ends were resected and prepared with ultrasonic tips. Root-end fillings of either MTA or thickly mixed Diaket were randomly assigned to the preparations. Reflected tissues were repositioned and sutured with 4–0 vicryl sutures. Sixty days postsurgery, the animals were killed, perfused with 10% neutral buffered formalin and the third and fourth premolars removed in block sections. The specimens were demineralized and sectioned at 6-μm intervals for histological assessment. Sections were stained with either haematoxylin and eosin or Gomori's one step trichrome stain and examined under the light microscope. All evaluations were made by two calibrated examiners and gradings were scored based on established criteria. The raw data was evaluated statistically using ANOVA after adjusting for the animal block effect.

Results Statistical evaluation indicated that there were no statistical differences between the observed regenerative responses of the tissues to the two root-end filling materials.

Conclusions Both Diaket and MTA can support almost complete regeneration of the periradicular periodontium when used as root-end filling materials in periradicular surgery on noninfected teeth.

Keywords: diaket, mineral trioxide aggregate, periradicular surgery, regeneration, root-end fillings.

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Introduction

A significant number of research and clinical studies have attempted to identify the ideal root-end filling material (Gutmann & Harrison 1994). These studies focused primarily on the physical properties and materials usage in the surgical site and the radiographic assessment of their periradicular tissue responses.

Andreasen *et al.* (1993) addressed the biological parameters necessary for the ideal root-end filling materials and concluded that regeneration should be added to the list of desirable properties. Regeneration has been defined as the replacement of tissue components in their appropriate location, amounts and relationship to each other (Aukhil 1991). This implies the reformation of the bone in the surgical site, adjacent to a fully reconstituted periodontal ligament (cells and fibres), which is attached to newly formed cementum over the resected root end and the root-ending filling material. The occurrence of this type of tissue regeneration has not been demonstrated when amalgam, gutta-percha, glass ionomers, intermediate restorative material (IRM) or ethoxy benzoic acid cement (EBA) have been used as root-end filling

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materials, as none of these materials have exhibited a cemental regrowth over their surfaces. In an animal histological study, Andreasen *et al.* (1989) identified the regeneration of cementum over a bonded composite root-end filling material (Retroplast). This was subsequently confirmed in a number of human histology cases (Andreasen *et al.* 1993) and in multiple radiographic studies (Rud *et al.* 1996a,b; 1997, Rud & Rud 1998).

Inherent in the tissue response to the root-end filling is the potential for the material or its constituents to influence the extracellular matrix (ECM) (Craig *et al.* 1997). This influence will ultimately determine specific cellular induction and matrix formation characteristics of the phenotypic expression of osteoblasts, fibroblasts and cementoblasts. Recent histological studies have demonstrated the strong possibility of cemental regeneration over the root-end filling when either mineral trioxide aggregate (MTA) (Dentsply/Tulsa Dental, Tulsa, OK, USA) (Torabinejad *et al.* 1995a,b,c) or Diaket (ESPE, Seefeld, Germany) (Snyder Williams & Gutmann 1996a) were used. Whilst neither study specifically characterized immunologically the nature of this covering material, total tissue regeneration in the surgical site appeared most promising, when either material was used.

The purpose of this study was to evaluate further, on a comparative basis, the potential for MTA and Diaket to promote periradicular tissue regeneration when used as surgical root-end filling materials.

Materials and methods

Ethical approval for this study was sought and received from the Institutional Animal Care and Use Committee, Baylor College of Dentistry, Texas A&M University Health Science Centre. Seven dogs weighing between 15 and 25 kg were quarantined for 10 days before commencement of the experiment procedures. The animals were anesthetized by intramuscular injection of 1 mg kg^{-1} of rompun (Mowbay Corporation, Shawnee, KS, USA) and 20 mg kg^{-1} of ketamine (Mowbay Corporation). Intraoral anaesthesia was achieved with a mandibular nerve block injection of 1.8 mL of 2% lidocaine containing 1 : 100 000 epinephrine (Astra Pharmaceutical Products, Westborough, MA, USA).

Following preoperative radiographic assessment of the third and fourth mandibular premolar region, the teeth were isolated with surgical gauze. The pulp chambers were accessed, pulpectomies were performed and the root-canal systems were cleaned and shaped chemomechanically using Gates Glidden drills (Brasseler, Savannah, GA, USA) and Flexofiles (Dentsply/Maillefer, Ballaigues, Switzerland). Copious amounts of sodium hypochlorite (5.25% NaOCl) (The Excelex Corporation, Dallas, TX, USA) were used for irrigation. The canals were dried with paper points and obturated with a thick mixture of Diaket (Tetsch 1986, Snyder Williams & Gutmann 1996a). The coronal access cavities were restored with IRM (Caulk Dentsply, Milford, DE, USA).

Following completion of the nonsurgical phase and immediately prior to the surgical phase of treatment, a local infiltration of 1.8 mL of 2% lidocaine containing 1 : 50 000 epinephrine (Astra Pharmaceutical Products) was used to enhance haemostasis in the surgical sites (Buckley *et al.* 1984, Gutmann 1993). A full thickness mucoperiosteal flap was reflected and an osteotomy was performed exposing the apical third of the experimental tooth roots. The root ends were resected using a Lindeman bone-cutting bur (Brasseler, Savannah, GA, USA). Root-end cavity preparations, approximately 1.0–1.5 mm in diameter and 1.5–2.0 mm deep, were prepared using ultrasonically energized tips held in a Spartan ultrasonic device (Excellence in Endodontics, Orange, CA, USA). Collaplug (Colla-Tec Inc., Plainsboro, NJ, USA) was placed in the bone crypt to enhance the local haemostasis and increase visibility during the root-end preparation and filling. A solution of 1% methylene blue (Excellence in Endodontics) was applied to the resected root face to outline the periodontal ligament (Cambruzzi & Marshall 1983). The resected root faces were burnished with a sponge applicator soaked in 10% solution of citric acid (pH 1) for 2 min (Register 1975, Polson & Proye 1982, Craig & Harrison 1993) and washed with sterile saline.

The root-end filling materials Diaket (ESPE) or MTA (Dentsply/Tulsa Dental) were prepared and allocated randomly for placement in the root-end preparation. The reflected tissues were repositioned and compressed with moist gauze for 5 min and sutured with 4–0 vicryl suture. The animals were placed on a soft diet and approximately 7.5 mg kg^{-1} ibuprofen twice daily for 2 days.

Sixty days postsurgery, the animals were killed after sedation and anaesthesia with intramuscular injections of 1 mg kg^{-1} of rompun and 20 mg kg^{-1} of ketamine. The head and neck were perfused with 1.5 L of 10% phosphate-buffered formalin maintained at a pressure of between 110 and 140 mm Hg. The mandible was dissected free and block sections of each of the third and fourth premolar roots were formed and placed in 10% formalin for further fixation.

The resected blocks were gently demineralized in 0.5 M ethylene diamine tetraacetic acid (EDTA), embedded in paraffin and 6-μm serial sections were made in a bucco-lingual longitudinal orientation using a Leitz 1512 rotary microtome (Ernst Leitz Wetlzar, Rockleigh, NJ, USA). The sections were stained alternatively with either haematoxylin and eosin stain (Man & Marshallbeck 1995) or Gomori's one step trichome (Masson's trichome method) stain.

The tissue sections were evaluated by two calibrated examiners using a light microscope (CHS Biological Microscope, Olympus America Inc., Melville, NY, USA). Specific parameters of evaluation and grading are indicated in Table 1 and described in a previous study (Regan *et al*. 1999). When a discrepancy between grading was noted, a second evaluation was performed jointly until agreement was reached. The raw data was accumulated, tabulated and entered into a spreadsheet for statistical analysis using the SAS programme (SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) was applied to each group of data after adjusting for the animal block effect. A statistical model was created and the sum of the squares partitioned to take into account the difference between the animals whilst adjusting for correlation within animal. Analysis of the data indicated that the use of ANOVA could be applied to

Table 1 Quantitative and qualitative scoring criteria

Histological parameter	Score	Description
Acute inflammation	1	<25%, PMN infiltrate adjacent to the resect root-end and root-end filling
	2	≥25% < 50%, PMN infiltrate adjacent to the resect root-end and root-end filling
	3	≥50% < 75%, PMN infiltrate adjacent to the resect root-end and root-end filling
	4	≥75%, PMN infiltrate adjacent to the resect root-end and root-end filling
Chronic inflammation	1	<25%, PMN infiltrate adjacent to the resect root-end and root-end filling
	2	≥25% < 50%, PMN infiltrate adjacent to the resect root-end and root-end filling
	3	≥50% < 75%, PMN infiltrate adjacent to the resect root-end and root-end filling
	4	≥75%, PMN infiltrate adjacent to the resect root-end and root-end filling
Abscess formation	1	None
	2	Micro-abscess
	3	Extensive
Bone formation in the body of the wound	1	<25%, new bone formation in the body of the wound
	2	≥25% < 50%, new bone formation in the body of the wound
	3	≥50% < 75%, new bone formation in the body of the wound
	4	≥75%, new bone formation in the body of the wound
New bone formation adjacent to root-end	1	<25%, new bone formation adjacent to the resected root end
	2	≥25% < 50%, new bone formation adjacent to the resected root end
	3	≥50% < 75%, new bone formation adjacent to the resected root end
	4	≥75%, new bone formation adjacent to the resected root end
New bone formation adjacent to root-end fill	1	<25%, new bone formation adjacent to root end fill
	2	≥25% < 50%, new bone formation adjacent to root end fill
	3	≥50% < 75%, new bone formation adjacent to the resected root end
	4	≥75%, new bone formation adjacent to the resected root end
Periodontal ligament (PDL) formation	1	PDL adjacent to <25% of the resected root end and root-end filling
	2	PDL adjacent to ≥25% < 50% of the resected root end and root-end filling
	3	PDL adjacent to ≥50% < 75% of the resected root end and root-end filling
	4	PDL adjacent to ≥75% of the resected root end and root-end filling
Cementum deposition	1	Cementum formation on < 25% of the resected root-end dentine
	2	Cementum formation on ≥ 25% < 50% of the resected root-end dentine
	3	Cementum formation on ≥ 50% < 75% of the resected root-end dentine
	4	Cementum formation on ≥ 75% of the resected root-end dentine
	5	Cementum formation on 100% of the resected root-end dentine & ≤50% of the root-end fill
	6	Cementum formation on 100% of the resected root-end dentine & >50% of the root-end fill

the data even though the data were ordinal. This was because the sample size was relatively large and that two independent examiners evaluated each of the sections.

Results

Statistical evaluation of the tissue response to the root-end filling materials indicated that no significant differences existed at the $P < 0.05$ level (Table 2) between the Diaket or MTA root-end filling materials in terms of the designated histological parameters (Table 1). In particular, the following were observed.

- No significant difference was found between MTA and Diaket for the presence of inflammation or abscess formation (Table 2).
- The greater amount of bone formation in the body of the wound was associated with the Diaket root-end filling cases. However, there was no statistically significant difference between the materials (Table 2). The degree of new bone formation in the body of the excisional wounds was relatively complete with both materials (Figs 1 and 2).
- New periodontal ligament formation (Fig. 2) was greater, adjacent to the Diaket root-end filling materials; however, there was no statistically significant difference (Table 2).
- MTA demonstrated more new bone formation adjacent to the root-end filling than did Diaket. Once again however, there was no statistically significant difference (Table 2).
- The presence of a complete cemental covering of the root apex and filling material was variable and unpredictable with both materials. Some excellent examples of new cementum-like formation over the root-end filling material were observed for both materials (Figs 2–5).

Table 2 Results of statistical analysis

Dependent variable	*P*-values
Acute inflammation	0.3739
Chronic inflammation	0.3739
Abscess formation	0.3739
Bone formation – body of wound	0.3046
Bone formation – adjacent to root end	0.8149
Bone formation – adjacent to root-end fill	0.1087
Cementum formation	0.2302
Periodontal ligament formation	0.4766

Significance ($P < 0.005$).

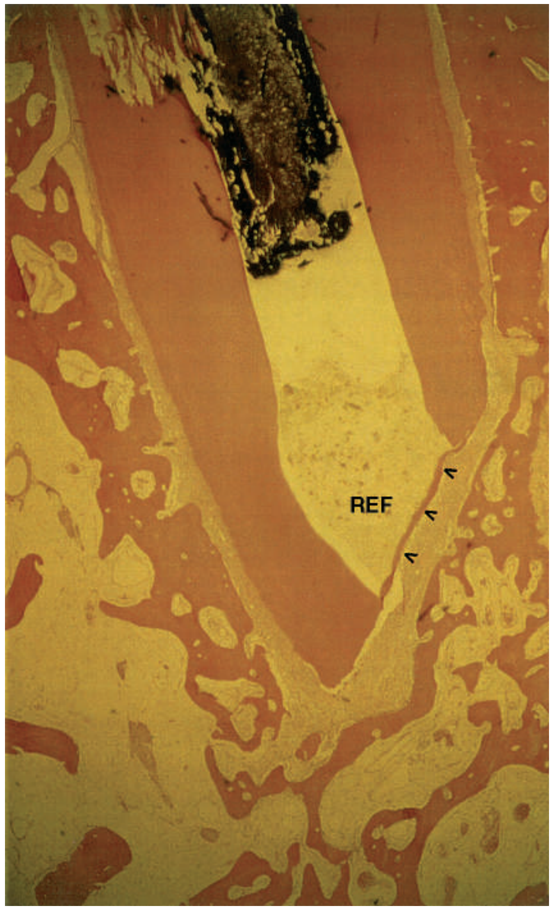

Figure 1 A 60-day MTA section showing significant periodontal architecture regeneration in the surgical site adjacent to the root-end fill (REF), cementum-like material (arrows). Haematoxylin and eosin (×20).

A wide range of inflammatory responses was observed with some sections showing little inflammatory cell infiltrate, whilst others presented with a marked inflammatory cell presence.

Discussion

The two materials chosen for this evaluation have been shown to support varying degrees of regeneration under different experimental conditions (Torabinejad *et al.* 1995a,b,c, Snyder Williams & Gutmann 1996a, Moretton *et al.* 2000). Diaket is a polyvinyl resin that was initially formulated as a root-canal sealer, whilst used as a root-canal sealer and empirically as a root-end filling material for over two decades, Tetsch (1986) first documented the use of Diaket as a root-end filling material, suggesting that it be mixed to a very thick consistency.

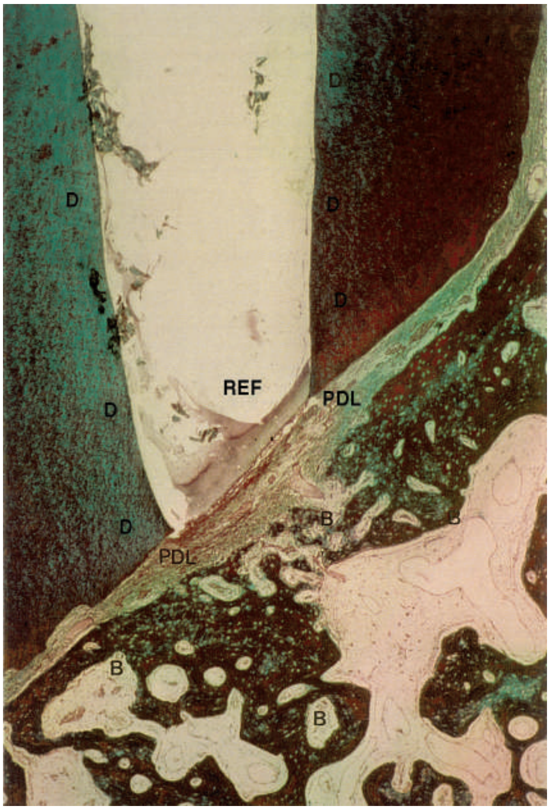

Figure 2 A 60-day Diaket specimen showing extensive regeneration of all component of the periodontal architecture adjacent to the resected root end and root-end filling. Masson's Trichrome (×40). B, bone; PDL, periodontal ligament; REF, root-end filling; D, dentin.

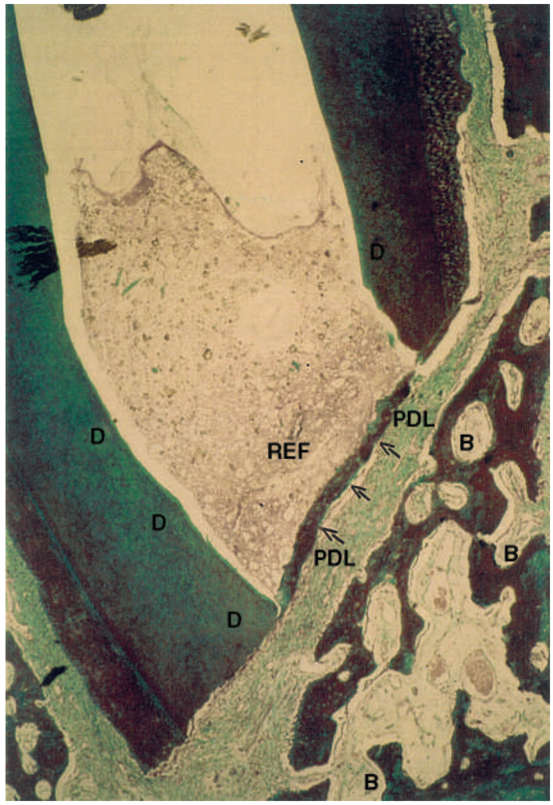

Figure 3 A 60-day MTA specimen showing extensive regeneration of all components of the periodontal architecture adjacent to the resected root end and root-end filling. Masson's Trichrome (×40). B, bone; PDL, periodontal ligament; REF, root-end filling; D, dentine. Cementum-like material (arrows) in intimate contact with the root-end filling material.

As a root-canal sealer, the material was mixed in a ratio of 1 : 1, powder to liquid. Walia *et al.* (1995) showed that Diaket used in a ratio of two to three parts powder to one part liquid produced a material that provided a better apical seal than either IRM or Super–EBA in both 1 and 3-mm deep root-end preparations. The tissue response to Diaket mixed in this manner was evaluated by Nencka *et al.* (1995). They found the material to be biocompatible with bone. This same favourable response was noted by Snyder Williams & Gutmann (1996a,b), Douthitt (1996) and Witherspoon & Gutmann (2000) when Diaket was used as a root-end filling material in a dog model. An 'osteoid' type material was consistently formed adjacent to the Diaket root-end filling (Fig. 4). This material has not yet been fully characterized using immuno-histochemical techniques or specific matrix antigens and these evaluative tests remain to be performed.

Based on previous animal studies using the dog model, it would appear that 60 days is an appropriate time to wait between completion of experimental procedure and sacrifice. Previous dog studies have evaluated healing at intervals shorter than 60 days (Craig & Harrison 1993, Torabinejad *et al.* 1995a,b,c, Douthitt *et al.* 2001), equal to 60 days (Regan *et al.* 1999, Snyder Williams & Gutmann 1996b) and more than 60 days (Torabinejad *et al.* 1995a,b,c, Trope *et al.* 1996, Ne 2001).

MTA was developed 'to seal communications between the tooth and the external surface' (Lee *et al.* 1993). The main constituents of this material are tricalcium silicate ($CaSiO_4$), bismuth oxide (Bi_2O_3), dicalcium silicate ($2CaOSiO_2$), calcium sulphate ($CaSO_4$), tricalcium aluminate ($3CaOAl_2O_3$), tetra calcium aluminoferrite ($4CaOAl_2O_3FeO_3$) and an amorphous structure consisting of 33% calcium, 49% phosphate, 2% carbon,

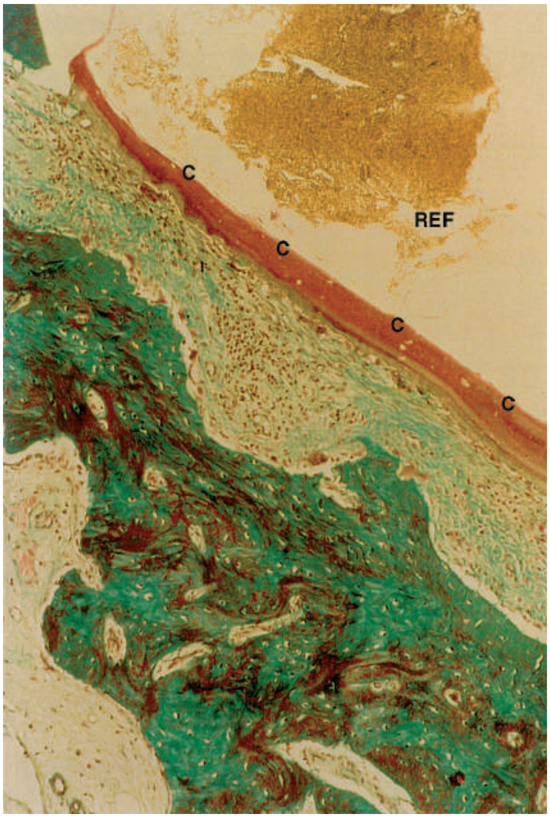

Figure 4 Masson's Trichrome specimen demonstrating cementum-like material (C) adjacent to the Diaket root-end filling and newly formed periodontal ligament (×100).

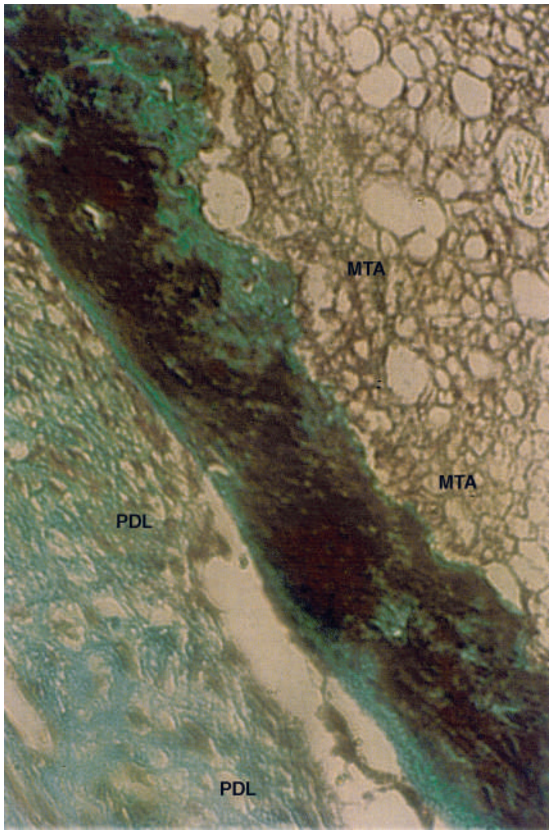

Figure 5 A ×400 view of the cementum-like material interposed between the MTA root-end filling material and the newly formed periodontal ligament. Masson's Trichrome stain.

3% chloride and 6% silica (Torabinejad *et al.* 1995a,b,c). Torabinejad *et al.* (1993; 1994a,b) further recommended the use of MTA as a root-end filling material based on its sealing properties when compared to amalgam, IRM and Super-EBA. In a series of *in vitro* and *in vivo* experiments, investigating the physical properties and biocompability of MTA, the same group reported that MTA compared favourably with both Super-EBA and IRM (Torabinejad *et al.* 1994a,b; 1995a,b,c). Further studies have confirmed the biocompability of the MTA in cultures of osteoblasts (Koh *et al.* 1998, Mitchell *et al.* 1999, Zhu *et al.* 2000). In response to the material, cell growth was favourable and the expression of IL-6 and IL-8 suggests that it may promote healing through the stimulation of bone metabolism. The presence of IL-8 has been shown to stimulate angiogenesis, which is necessary for connective tissue healing (Hu *et al.* 1993).

Takata *et al.* (1998) have shown that transforming growth factor-β1 (TGF-β1) influences the development, remodelling and regeneration of cells. MacNeil *et al.* (1995) noted that the phosphorylated glycoprotein osteopontin is expressed and localized to the root surface during cementogenesis. Ne (2001) demonstrated marked immunoreactivity for both TGF-β1 and osteopontin in response to the presence of MTA when used as a root-end filling in a dog model. Whilst these studies may suggest that MTA is osteoinductive, Moretton *et al.* (2000) found that subcutaneous and intraosseous implants of EBA and MTA were not osteoinductive but rather osteoconductive.

Clinically, both materials are markedly different. Whilst the MTA material is easily mixed, its handling characteristics are not ideal. It can be difficult to place and compact in many root-end preparations. Diaket, on the other hand is very easy to place and compact as a root-end filling in virtually all prepared root ends. It sets hard in a short time, after which it can be polished with a fine diamond rotary instrument to produce a smooth finish. It is also more radiopaque than MTA, providing a clear radiographic image of the final restoration.

Ideally, the healing responses both in the presence or absence of superimposed infection and inflammation should be evaluated. However, in practical terms, this is not always possible owing to financial and temporal constraints. In addition, surgery is occasionally performed in the absence of an active infection (e.g. multirooted teeth, in teeth with root fractures, perforations, fractured instruments).

The evidence collected during this histological study would suggest that formation of a complete cemental coverage over both the root end and the root-end filling material was possible, though not predictable. However, this indicates that it is feasible to promote 'a double seal' following root-end surgery, incorporating both a physical and biological covering or 'seal' of the resected root end.

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