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The use of mineral trioxide aggregate in one-visit apexification treatment: a prospective study

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Abstract

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Aim To assess the outcome of apexification using mineral trioxide aggregate (MTA).

Methodology Fifty-seven teeth with open apices on 50 patients referred for root canal treatment received an apexification procedure in one appointment with MTA by the same operator. Patients were recalled at 6 months, 12 months and every year thereafter. Blind to the treatment record, two examiners assessed the pre-treatment, post-treatment and control radiographs of the study patients in a dark room using a magnifier. Each apex visible on the radiographs was scored with the periapical index (PAI), and the size of the apical lesion was measured. The presence of an apical bridge was also noted. Kappa-Cohen test was used for examiners calibration. The paired *t*-test was used for statistical analysis of apical healing.

Results Forty-three cases were included with at least 12 months follow-up. When considering the PAI score and the decrease in size of the apical lesion, healing occurred in 81% of cases.

Conclusion Apexification in one step using an apical plug of MTA can be considered a predictable treatment, and may be an alternative to the use of calcium hydroxide.

Keywords: apexification, immature tooth, mineral trioxide aggregate, prospective clinical study.

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Introduction

The completion of root development and closure of the apex occurs up to 3 years following eruption of the tooth (Nolla 1960). After crown formation, the inner and outer enamel epithelium develop as a two-layered epithelial wall to form Hertwig's epithelial root sheath (HERS), which plays a key role in the differentiation of odontoblasts. When the first layer of dentine has been laid down, HERS begins to disintegrate and only the cell rests of Malassez persist in the periodontal ligament. At the same time, HERS progresses in an apical direction until complete formation of the root.

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When teeth with incomplete root formation suffer pulp necrosis, the root development ceases and apical closure cannot be achieved. Root canal treatment at this time is a significant challenge, because of the size of the canal, the thin and fragile dentine walls and the large open apex. Apexification is defined as 'a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incomplete root in teeth with necrotic pulp' (American Association of Endodontists 2003). The goal of this treatment was to obtain an apical barrier to prevent the passage of toxins and bacteria into the periapical tissues from the root canal. Technically, this barrier is also necessary to allow the compaction of the root filling material.

Calcium hydroxide pastes have been considered as the material of choice to induce the formation of a hard tissue apical barrier. Its efficiency has been demonstrated by many authors, even in the presence of an apical lesion (Chosack *et al.* 1997, Felippe *et al.* 2006). This chemical has several disadvantages, such as variability of treatment time (average 12.9 months) (Dominguez Reyes *et al.* 2005), difficulty of the patient's recall management, delay in the treatment and increase in the risk of tooth fracture after dressing with calcium hydroxide for extended periods (Andreasen *et al.* 2002, Felippe *et al.* 2005, Andreasen *et al.* 2006).

Alternatives to calcium hydroxide have been proposed; the most promising being mineral trioxide aggregate (MTA®) (Shabahang *et al.* 1999, Shabahang & Torabinejad 2000, Witherspoon & Ham 2001, Steinig *et al.* 2003). The advantages of this material are multiple: (i) reduction in treatment time, (ii) possibility to restore the tooth with a minimal delay, and thus to prevent the fracture of the root and (iii) it also avoids changes in the mechanical properties of dentine because of the prolonged use of calcium hydroxide.

In addition, because of its noncytotoxicity (Osorio *et al.* 1998), MTA has good biological properties (Torabinejad *et al.* 1995, Torabinejad *et al.* 1998) and stimulates repair (Economides *et al.* 2003). When used in dogs' teeth with incomplete root formation and contaminated canals, MTA induced the formation of an apical barrier with hard tissue (Shabahang *et al.* 1999).

In a prospective study on 26 immature teeth apexification treatment with calcium hydroxide showed a high prevalence of apical healing and apical closure (Felippe *et al.* 2006) with the formation of a hard tissue barrier. To date, no clinical study has been published to evaluate the outcome of a one-visit apexification treatment with MTA.

The aim of this prospective study was to verify the effectiveness of apexification using MTA in one appointment, by reviewing 50 patients who had received this treatment.

Material and methods

The study period was from June 2001 to June 2005. All the patients referred for root canal treatment on a tooth with an open apex were included in the study, after obtaining informed consent. Alternative treatment options were discussed with the patients and their referring dentists. Main exclusion criteria were general medical contraindications (one case of heart disease and one case of severe immunodeficiency) or a contraindication for endodontic treatment (fracture of the tooth, tooth unrestorable).

Fifty-seven teeth were treated on 50 patients (20 female and 30 male). Forty-three patients (26 male, 17 female) received one treatment and seven patients (three female and four male) had two teeth treated. Distribution of the treated teeth is shown in Table 1.

Root canal treatment

All treatments were performed using the same protocol by the same operator (Figs 1–5):

1. A preoperative radiograph was taken with a film-holder using a paralleling technique.
2. Infiltration for local anaesthesia with articaine and 1/100 000 adrenaline (Ubistesin – 3MESPE, Cergy Pontoise, France).
3. Restoration of the tooth if needed and isolation of the tooth with rubber dam.
4. Access cavity with round diamond bur (diameter 014), Endo Z® bur (Dentsply-France Ballaigues, Switzerland) and Sonic inserts (Sonic Flex, Kavo, Roissy, France).
5. Canal debridement with Hedström file.
6. Copious irrigation with sodium hypochlorite (5%).
7. Determination of the working length on radiograph with the carrier (Messing Gun® – Produits Dentaires, Vevey, Switzerland) placed in the canal.
8. Drying of the canal with a sterile blunt paper point (Size X Coarse, Henry Schein, Paris, France).
9. Placement of the MTA (White Pro-Root MTA®, Dentsply Maillefer, Ballaigues, Switzerland) apical plug. The cases number 1–11 were treated with Grey MTA and the cases number 11–57 with White MTA, the marketing of the grey one having been suspended.

Table 1 Distribution and follow-up of the treated teeth

Tooth	Total	Preoperative radiograph	Preoperative + 1 follow-up	Preoperative + 2 follow-up	Preoperative + 3 follow-up
11, 21	43	38	31	11	3
12, 22	1	1	1	1	0
14, 15, 24, 25	1	1	1	1	0
16, 17, 26, 27	9	8	8	1	0
31, 41	1	1	1	1	0
36, 46	2	1	1	0	0
Total	57	50	43	15	3

Figure 1 Tooth treated by renewing calcium hydroxide in the canal. After 1 year of treatment no signs of healing were evident, and the patient complained of recurrent swelling.

10. Radiological examination of the apical plug, and adjustment if needed with an endodontic plugger.
11. Completion of the filling of the apical third with MTA, to 5 mm from the radiographic apex.
12. Temporary filling with a moist cotton pellet and Cavit® (ESPE, Cergy Pontoise, France).
13. Postoperative instructions and nonsteroidal analgesics were prescribed if necessary.
14. Filling of the whole canal was completed by Schilder's warm vertical compaction of gutta-percha and Pulp canal sealer (Kerr Manufacturing Co., Romulus, MI, USA), and corono-radicular restoration was completed with a bonded resin composite in the following 7 days.

Evaluation

Patients were recalled for clinical and radiographic examinations every 6 months for 2 years, and every year thereafter. Periapical radiographs were taken using a paralleling technique with a film holder. At the end of the study, the last review occurred at 6 months for 14 teeth (25%), at 12 months for 21 teeth (37%), at 18 months for six teeth (10.5%), 24 months for 10 teeth (17%) and 36 months for six teeth (10.5%). Blind to the treatment record, two examiners assessed the pre-treatment, post-treatment and review radiographs of the study patients in a

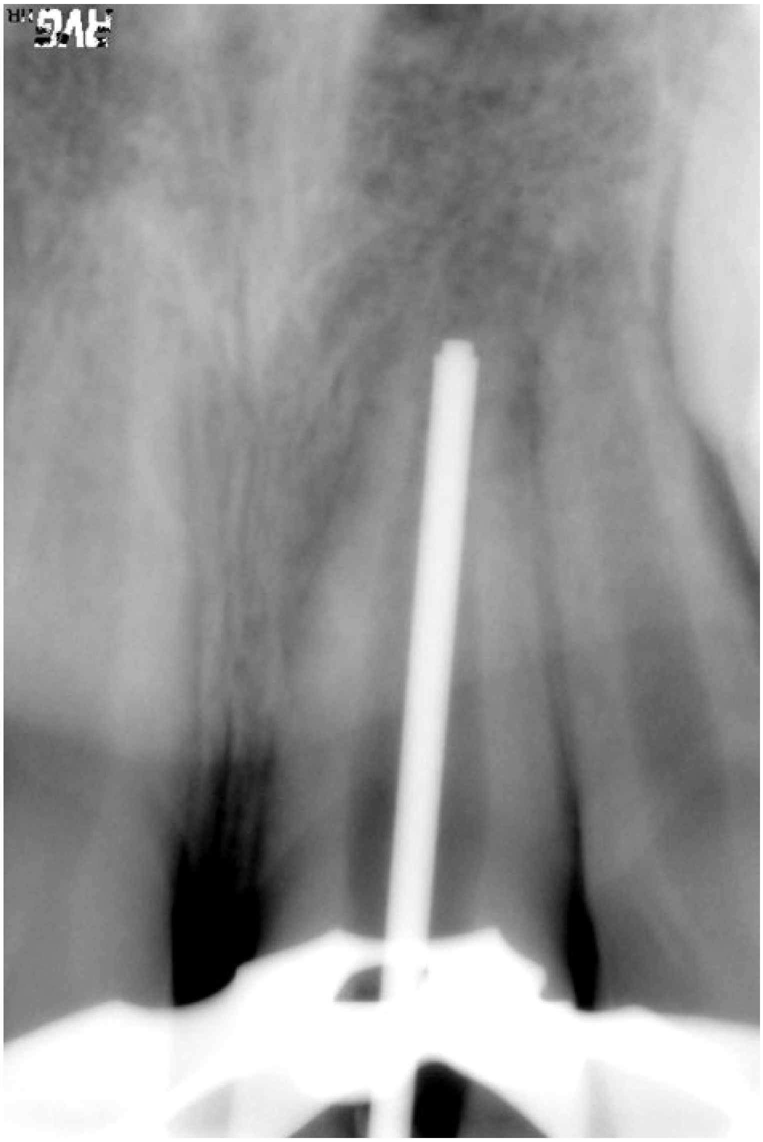

Figure 2 After canal disinfection, the MTA carrier is placed in the canal to determine the working length on the radiograph.

darkened room using a magnifier (2.5×). Periapical status of each root was categorized with the periapical index (PAI) (Ørstavik *et al.* 1986). When an apical lesion was present, its largest dimension was recorded. The presence or absence of a visible apical closure was also recorded.

Randomization

The operator (SS) randomized the entire sample using odd numbers. Two examiners (P.M., F.R.) assessed the PAI scoring, lesion diameter and the presence of apical closure for all teeth in the sample, blinded to unaware of the stage of the treatment.

Statistical analysis

Data were recorded on Excel 2002® (Microsoft Inc., Redmond, WA, USA). The Kappa-Cohen test was used for examiner calibration, following the recommendations described by Landis & Koch (1977). The paired *t*-test was used for analysis of quantitative data, i.e. PAI scoring and apical lesion diameter, between preoperative and postoperative radiographic examinations. The

Figure 3 An apical plug of MTA deposited in the apical 2 mm of the root canal and condensed with sterile paper cones (X Coarse Size).

chi-squared test of Pearson was used for distribution comparison. The Kappa test was performed with Microsoft Excel draft product of Department of Psychology, Université de Québec à Montréal (http://www.er.uqam.ca/nobel/r30574/). Statistical analysis was completed with Sigma Stat. 2.03, (SPSS Inc., Chicago, IL, USA).

Results

The mean age of the patients was 18 years (SD = 12), 16 years (SD = 10) for male patients and 20 years (SD = 14) for female patients (Table 2). There was no statistically significant difference in distribution by gender, or age between the genders.

Fifty-seven teeth were examined preoperatively and 43 teeth for 12 months or more postoperatively (exhaustivity = 86%).

Examiner calibration

The result of the first calibration, on 10 radiographs, was $\kappa = 0.87$ (Po = 0.95, Fc = 0.65). Then 108 randomized radiographs were analysed by the two examiners, and the apical status of 394 roots was recorded by each examiner. When the periapical status

Figure 4 The length of the apical plug of MTA must be at least 5 mm.

was not recordable because of radiographic imprecision, the score not interpretable (NI) was attributed.

After the first analysis, the Kappa-Cohen test value was $\kappa = 0.69$ ($n = 394$, Po = 0.79, Fc = 0.32); without the score NI, $\kappa = 0.74$ ($n = 316$, Po = 0.85, Fc = 0.41). κ being inferior to 0.81, a second observation of cases with divergent results ($n = 34$) was undertaken by each examiner. Examiner 1 changed his score for 13 cases, and Examiner 2 changed his score for 21 cases. After this rereading, there was no significant difference between the two examiners (χ^2, 1ddl, =0.95, $P < 0.40$), suggesting that there was no influence of one examiner to the other. With this analysis, the Kappa test value was $\kappa = 0.83$ (Po = 0.88, Fc = 0.31) with the NI score and $\kappa = 0.94$ (Po = 0.96, Fc = 0.39) without the NI score. The new Kappa-Cohen score was superior to 0.81 and the results could be used for the statistical analysis. It was decided to eliminate the NI score.

PAI score

The comparison of the PAI scores between the preoperative radiograph and the last review radiograph, analysed with the paired *t*-test, showed a statistically significant reduction in the pathology on the whole of

Figure 5 Root canal filling completed during a second visit by warm compaction of gutta-percha.

the sample ($t = 4.83$, 42ddl, $P < 0.001$). On the first control, the PAI increased for six teeth (14%) and remained stable for 10 teeth (26%). No improvement in the PAI was noted in seven cases (16%), and the score decreased for 27 teeth (63%). On the first review, the rate of failure to heal was thus 30% (13 teeth: seven with increasing score of PAI and 6 teeth which did not present any sign of healing).

In this study, 'absolute rate' was defined as the increase (absolute failure rate) or decrease (absolute success rate) in the PAI score; 'relative rate' corresponded to the absolute rate added to the cases with stability of the PAI but different of 1 (relative failure rate) or cases with stability of PAI = 1 (relative success rate).

On final review, the PAI increased in six cases (14%), was stable (but >1) in 6 cases (14%), decreased in 29 cases (67%) and remained equal to 1 in two cases. So, the absolute failure rate was 14%, the relative failure rate was 28%; the absolute success rate was 67% and the relative success rate was 72%.

Size of the apical lesion

A comparison of the size of apical lesion (=0 by defect) at baseline and following review showed a statistically

Table 2 Summary of the clinical cases and results*

				JO		6 months		12 months		18 months		24 months		36 months					Apical barrier	
Case number	Male/ female	Age (years)	Tooth number	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	Last recall (months)	Final PAI -initial PAI	Size of lesion (Final diameter-initial diameter) (mm)	Formation	Time (months)
1	M	16	11	3	1					2				2		40	−1	−1	×	18
2	M	14	11	2				2						1		36	−1	0		
3	M	18	21	2								2				21	0	0		
4	M	17	11	5	13			4	7							13	−1	−5.5		
5	F	26	21	5	4			2								12	−3	−4		
6[a]			11	5	4.5			4	5							13	−1	0.5		
7	M	9	11	4	5			1								12	−3	−5	×	12
8	F	53	27	3	2			3								15	0	−2		
9	M	10	11	3	3					2				1		36	−2	−3	×	18
10	F	51	11	4	4.5			2								12	−2	−4.5		
11	M	34	15	4	6.5	3				2						19	−2	−6.5		
12	F	42	11	2								1				22	−1	0		
13	M	10	11	4	4.5					3	2	2				24	−2	−2.5		
14	M	34	31	5	9			3				2				24	−3	−9		
15	F	9	11	1				1								14	0	0	×	12
16	F	8	11	2				1		1		1				22	−1	0	×	12
17	M	7	11	4	4			3				1	0			22	−3	−4		
18	M	10	21	4	4			3	2.5	2						18	−2	0	×	18
19	M	12	11	3	2.5					4	4	1		1		35	−2	−2.5	×	24
20	M	8	11	4	5			1	1							14	−3	−4		
21	F	30	17	4	5.5			4	5			4	3			24	0	−2.5		
22			27	4	7					4	5					18	0	−2		
23	M	42	16	5	3.5			3								12	−2	−3.5		
24[b]	F	8	36	4	5			5	15							13	1	10		
25	F	12	11	5	11							3	3.5			24	−2	−7.5		
26	M	9	11	5	4					1						18	−4	−4	×	18
27	M	37	16	4	2.5			4	2							12	0	−0.5		
28	M	10	16	1				1								12	0	0		
29	M	22	11	4	6			4	4							12	0	−2		
30	F	17	11	1						2						17	1	0		
31	F	26	11	4	5			3	2.5							12	−1	−2.5		
32			21	5	8			3	2.5							12	−2	−5.5		
33	M	35	16	1						3						15	2	0		
34			26	1				3								12	2	0		

Table 2 Continued

Case number	Male/ female	Age (years)	Tooth number	JO		6 months		12 months		18 months		24 months		36 months		Last recall (months)	Final PAI -initial PAI	Size of lesion (Final diameter-initial diameter) (mm)	Apical barrier	
				PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)	PAI	Size of lesion (mm)				Formation	Time (months)
35	M	8	11	1				1				3		3	2.5	36	2	0		
36	M	7	21	3				1				1		1		36	−2	0	×	12
37	M	14	11	4	5			2								12	−2	−5		
38	M	10	12	1				2				2				24	1	0	×	24
39	M	7	11	4	4			2				2				22	−2	−4		
40	F	19	11	2				1								12	−1	0		
41	F	15	21	5	4.5			4	4							13	−1	−0.5		
42	M	12	21	3				1								12	−2	0	×	12
43	M	8	11	5	8.5			2								12		−8.5		

*The cases with less than 12 months follow-up (14 teeth) do not appear in this table because they have not been retained for the study.
[a]An apical surgery was underteken at 12 months (recurrent swelling).
[b]The tooth was extracted at 12 months.

significant reduction on the whole of the sample ($t = 4.34$, 42ddl, $P < 0.001$).

In 28 cases (65%), the observers measured an increase in the width of the ligament ≥ 1 mm on the preoperative radiographs; the diameter decreased in 26 samples and increased in two samples between the initial radiograph and the last review. There was no case of development of a lesion after treatment.

With reference to the size of the lesion, the absolute success rate (completely healed lesions) was 65%, the relative success rate (healed lesions and lesions in process of healing) was 95%; and the absolute failure rate (lesions enlarging) was 5%.

Size of the lesion and PAI

By considering that the addition of absolute success of the two criteria were required to consider the treatment as successful, the apexification treatment with MTA was successful in 77% of the cases (33 teeth); the relative success rate (including the healing in process was 81%. Absolute failure rate was 16% (seven cases) and relative failure rate was 19% (eight cases) (Table 3).

Apical foramen closure

An apical barrier was distinguishable in 11 cases (26%). In nine cases, this barrier appeared in a context of absolute success. In one case, the bridge was visible in a context of relative success, the PAI being stable and no lesion being present. In the last case (case 38), an apical barrier was present, despite the PAI score increasing from 1 to 2. This case was considered as an absolute failure. Considering that the appearance of a dentinal bridge is a sign of success, the success rate should be 88% (38 cases out of 43).

In case 6, the PAI score decreased (5 to 4) but the size of the lesion increased (4.5–5 mm). The last review was at 12 months postoperativly and because of the recurrent clinical symptoms and the complaint of the patient, apical surgery was undertaken.

Discussion

Apexification has been investigated in many studies (Goldman 1974, Morse *et al.* 1990, Rafter 2005), and especially the clinical use of calcium hydroxide (Farhad & Mohammadi 2005). A recent prospective clinical study showed a success rate of 100% for this technique, the mean time necessary for the formation of an apical

Table 3 Correlation between Periapical index (PAI) score and size of the lesion

	PAI decreasing (absolute success)	PAI stable = 1 (relative success)	PAI stable > 1 (relative success)	PAI increasing (absolute failure)
Size of lesion decreasing (absolute success)	21	0	5	0
Size of lesion = 0 (relative success)	7	2	1	5
Size of lesion stable > 0 (relative failure)	0	0	0	0
Size of lesion increasing (absolute failure)	1	0	0	1

barrier being 12.19 months (Dominguez Reyes *et al.* 2005). In such a treatment, the formation of the apical barrier is necessary to allow the filling of the root canal system without risk of over filling. Failure of this technique may be because of several factors: (i) a repeated overfilling with the material with a high pH (12.7) can induce a necrotic zone in the periapical bone; (ii) the lack of coronoradicular restoration and thus of an appropriate coronal seal whilst the canal system is not filled; (iii) a prolonged contact with calcium hydroxide induces a significant decrease in intrinsic properties of the exposed dentine. These last two factors are directly responsible for many root fractures occurring before the end of the treatment (Rafter 2005).

To avoid this risk of fracture, several authors (Witherspoon & Ham 2001, Linsuwanont 2003, Andreasen *et al.* 2006) proposed a technique of apexification in one visit, by placing an apical plug of MTA in the last 5 mm of the canal. Obturation of the root canal system and placement of a coronal restoration in the tooth immediately are thus possible, and are regarded as key elements for the long-term conservation of the treated tooth (Goldberg *et al.* 2002, Steinig *et al.* 2003).

Several case reports have been published. Biological apical closure appears later, i.e. after the filling of the root canal, contrary to the technique with calcium hydroxide, where obtaining the apical barrier is necessary to complete the root canal treatment. Recently, in an experiment on dogs, Felippe *et al.* (2006) concluded that MTA induced in all the treated teeth the formation of an apical barrier. It would appear that no clinical study on outcome of apexification with MTA has been published. Nevertheless, treatment outcome is an important part of evidence based practice. It is the basis of treatment planning and prognostic considerations (Peak *et al.* 2001). The aim of this work was to determine whether the treatment of apexification with MTA in one stage has constant and predictable results after a sufficient period of follow-up.

The results of the present study indicate a success rate of 81%; thus, it can be considered as predictable. Nevertheless, further studies including more patients and a longer period of survey should be undertaken to confirm these results. In this work, all the treatments were carried out by the same operator, a specialist in endodontics. The validation of this procedure for general practitioners is necessary.

The PAI score was used to evaluate the periapical health and the healing process because it was considered as the most appropriate of all the evaluation techniques validated in endodontics. However, PAI scoring is not ideal for the evaluation of immature teeth; histologically the periapical area during the root formation is different from the mature tooth due to cell differentiation, the presence of HERS and bone reorganization. The sensitivity of interpretation of radiographs on immature teeth seems to be insufficient to allow precise scoring of 1 or 2 for example. Nevertheless, the results presented here regarded a score 1 as health apically. Limits of the PAI score in such a study should affect the results, by decreasing the success rate.

Contrary to the study of Dominguez Reyes *et al.* (2005), a healing response occurred in 81% of cases. Dominguez Reyes *et al.* (2005) considered only incisors in young patients whereas the present study included all teeth with a large canal and open apex, in young patients and adults. This difference in patient and case selection could explain the difference in the results.

The study ended at a defined date, and took into consideration those cases that were followed up for at least 12 months, as recommended by Ørstavik (1996). That is why 57 teeth were initially included in the study, but 43 were retained for the prospective analysis. In one case, the PAI was stable but greater than 1, and the size of apical lesion decreased at 1 year. This case was considered as a relative failure; a review in few months will be necessary to confirm continuing healing. Thus, the success rate was affected by the limit of the observation time.

Biological process of apical closure

Histologically, induction of apical hard barrier tissue formation has been described and validated by Felippe (Felippe *et al.* 2006) in 100% of the animals treated. In the present study, apical closure was noted only in 26% of cases. This difference could be because of the interpretation of the radiograph and the limited thickness of the dentine bridge that was too thin to be clearly distinguishable.

Biological process

Investigations have been done on the HERS (Zeichner-David *et al.* 2003) and cell differentiation during root and apical odontogenesis, but none about the real biological process of apical barrier formation. To date, no explanation about the process of apical formation after pulp necrosis nor about the process of activity of MTA is available. The material is proposed for several applications (pulp capping, apical surgery and perforation treatment). In all these situations, cell differentiation seems to appear. Felippe *et al.* (2006) showed that the bridge seems to be formed by bone and not dentine.

In the pulp capping procedure with MTA, the dentine bridge obtained is thicker, and its direct contact with the dentine walls ensures a better sealing than that obtained with calcium hydroxide. For apexification, no histological investigations have been done to compare the quality of the apical barrier obtained with MTA and calcium hydroxide.

A better comprehension of the biological process and especially about the role of MTA is necessary to understand the healing process and eventually to develop new clinical procedures.

Conclusion

These results show that apexification in one visit by placing an apical plug of MTA is a predictable and reproducible clinical procedure. More investigations are necessary about the biological process of apexification, and the activity of MTA in contact with cells, especially its potential role in cell differentiation and wound healing.

References

AAE (2003) *Glossary of endodontic terms*. Chicago: American Association of Endodontists.

Andreasen JO, Farik B, Munksgaard EC (2002) Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dental Traumatology* **18**, 134–7.

Andreasen JO, Munksgaard EC, Bakland LK (2006) Comparison of fracture resistance in root canals of immature sheep teeth after filling with calcium hydroxide or MTA. *Dental Traumatology* **22**, 154–6.

Chosack A, Sela J, Cleaton-Jones P (1997) A histological and quantitative histomorphometric study of apexification of nonvital permanent incisors of vervet monkeys after repeated root filling with a calcium hydroxide paste. *Endodontics and Dental Traumatology* **13**, 211–7.

Dominguez Reyes A, Munoz Munoz L, Aznar Martin T (2005) Study of calcium hydroxide apexification in 26 young permanent incisors. *Dental Traumatology* **21**, 141–5.

Economides N, Pantelidou O, Kokkas A, Tziafas D (2003) Short-term periradicular tissue response to mineral trioxide aggregate (MTA) as root-end filling material. *International Endodontic Journal* **36**, 44–8.

Farhad A, Mohammadi Z (2005) Calcium hydroxide: a review. *International Endodontic Journal* **55**, 293–301.

Felippe MC, Felippe WT, Marques MM, Antoniazzi JH (2005) The effect of the renewal of calcium hydroxide paste on the apexification and periapical healing of teeth with incomplete root formation. *International Endodontic Journal* **38**, 436–42.

Felippe WT, Felippe MC, Rocha MJ (2006) The effect of mineral trioxide aggregate on the apexification and periapical healing of teeth with incomplete root formation. *International Endodontic Journal* **39**, 2–9.

Goldberg F, Kaplan A, Roitman M, Manfre S, Picca M (2002) Reinforcing effect of a resin glass ionomer in the restoration of immature roots in vitro. *Dental Traumatology* **18**, 70–2.

Goldman M (1974) Root-end closure techniques including apexification. *Dental Clinics of North America* **18**, 297–308.

Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics* **33**, 159–74.

Linsuwanont P (2003) MTA apexification combined with conventional root canal retreatment. *Australian Endodontic Journal* **29**, 45–9.

Morse DR, O'Larnic J, Yesilsoy C (1990) Apexification: review of the literature. *Quintessence International* **21**, 589–98.

Nolla C (1960) The development of the permanent teeth. *Journal of Dentistry for Children* **27**, 245–66.

Ørstavik D (1996) Time-course and risk analyses of the development and healing of chronic apical periodontitis in man. *International Endodontic Journal* **29**, 150–5.

Ørstavik D, Kerekes K, Eriksen HM (1986) The periapical index: a scoring system for radiographic assessment of apical periodontitis. *Endodontics and Dental Traumatology* **2**, 20–34.

Osorio RM, Hefti A, Vertucci FJ, Shawley AL (1998) Cytotoxicity of endodontic materials. *Journal of Endodontics* **24**, 91–6.

Peak JD, Hayes SJ, Bryant ST, Dummer PM (2001) The outcome of root canal treatment. A retrospective study within the armed forces (Royal Air Force). *British Dental Journal* **190**, 140–4.

Rafter M (2005) Apexification: a review. *Dental Traumatology* **21**, 1–8.

Shabahang S, Torabinejad M (2000) Treatment of teeth with open apices using mineral trioxide aggregate. *Practical Periodontics and Aesthetic Dentistry* **12**, 315–20

Shabahang S, Torabinejad M, Boyne PP, Abedi H, McMillan P (1999) A comparative study of root-end induction using osteogenic protein-1, calcium hydroxide, and mineral trioxide aggregate in dogs. *Journal of Endodontics* **25**, 1–5.

Steinig TH, Regan JD, Gutmann JL (2003) The use and predictable placement of Mineral Trioxide Aggregate in one-visit apexification cases. *Australian Endodontic Journal* **29**, 34–42.

Torabinejad M, Hong CU, Pitt Ford TR, Kaiyawasam SP (1995) Tissue reaction to implanted super-EBA and mineral trioxide aggregate in the mandible of guinea pigs: a preliminary report. *Journal of Endodontcs* **21**, 569–71.

Torabinejad M, Ford TR, Abedi HR, Kariyawasam SP, Tang HM (1998) Tissue reaction to implanted root-end filling materials in the tibia and mandible of guinea pigs. *Journal of Endodontics* **24**, 468–71.

Witherspoon DE, Ham K (2001) One-visit apexification: technique for inducing root-end barrier formation in apical closures. *Practical Periodontics and Aesthetic Dentistry* **13**, 455–60.

Zeichner-David M, Oishi K, Su Z, Zakartchenko V, Chen LS, Arzate H, Bringas P Jr (2003) Role of Hertwig's epithelial root sheath cells in tooth root development. *Developmental Dynamics* **228**, 651–63.