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Apexification: a review

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Abstract – This paper reviews the rationale and techniques for treatment of the non-vital immature tooth. The importance of careful case assessment and accurate pulpal diagnosis in the treatment of immature teeth with pulpal injury cannot be overemphasized. The treatment of choice for necrotic teeth is apexification, which is induction of apical closure to produce more favorable conditions for conventional root canal filling. The most commonly advocated medicament is calcium hydroxide, although recently considerable interest has been expressed in the use of mineral trioxide aggregate. Introduction of techniques for one-visit apexification provide an alternative treatment option in these cases. Success rates for calcium hydroxide apexification are high although risks such as reinfection and tooth fracture exist. Prospective clinical trials comparing this and one-visit apexification techniques are required.

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The completion of root development and closure of the apex occurs up to 3 years after eruption of the tooth (1). The treatment of pulpal injury during this period provides a significant challenge for the clinician. Depending upon the vitality of the affected pulp, two approaches are possible – apexogenesis or apexification. Apexogenesis is 'a vital pulp therapy procedure performed to encourage continued physiological development and formation of the root end' (2). 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' (2). As always, success is related to accurate diagnosis and a full understanding of the biological processes to be facilitated by the treatment.

Root development

Root development begins when enamel and dentin formation has reached the future cementoenamel junction. At this stage the inner and outer enamel epithelium are no longer separated by the stratum intermedium and stellate reticulum, but develop as a two layered epithelial wall to form Hertwig's epithelial root sheath. When the differentiation of radicular cells into odontoblasts has been induced and the first layer of dentin has been laid down, Hertwig's epithelial root sheath begins to disintegrate and lose its continuity and close relationship to the root surface. Its remnants persist as an epithelial network of strands or tubules near the external surface of the root (1).

Hertwig's epithelial root sheath is responsible for determining the shape of the root or roots. The epithelial diaphragm surrounds the apical opening to the pulp and eventually becomes the apical foramen. An open apex is found in the developing roots of immature teeth until apical closure occurs approximately 3 years after eruption (1).

Pulpal injury in teeth with developing roots

Unfortunately traumatic injuries to young permanent teeth are not uncommon and are said to affect 30% of children (3). The majority of these incidents occur before root formation is complete (4) and may result in pulpal inflammation or necrosis. The root sheath of Hertwig is usually sensitive to trauma but because of the degree of vascularity and cellularity in the apical region, root formation can continue even in the presence of pulpal inflammation and necrosis (5, 6). Because of the important role of Hertwig's epithelial root sheath in continued root development after pulpal injury, every effort should be made to maintain its viability. It is thought to

provide a source of undifferentiated cells that could give rise to further hard tissue formation. It may also protect against the ingrowth of periodontal ligament cells into the root canal, which would result in intracanal bone formation and arrest of root development (7).

Complete destruction of Hertwig's epithelial root sheath results in cessation of normal root development. This does not however mean that there is an end to deposition of hard tissue in the region of the root apex. Once the sheath has been destroyed there can be no further differentiation of odontoblasts. However, hard tissue can be formed by cementoblasts that are normally present in the apical region and by fibroblasts of the dental follicle and periodontal ligament that undergo differentiation after the injury to become hard tissue producing cells (8).

Diagnosis and case assessment

The importance of careful case assessment and accurate pulpal diagnosis in the treatment of immature teeth with pulpal injury cannot be overemphasized. Clinical assessment of pulpal status requires a thorough history of subjective symptoms, careful clinical and radiographic examination and performance of diagnostic tests. An accurate pain history must be obtained. The duration and character of the pain and aggravating and relieving factors should be considered. Duration of pain may vary but pain that lasts for more than a brief period (a few seconds) in a tooth with a vital pulp has been thought to be indicative of irreversible pulpitis. When pain is spontaneous and severe, as well as long lasting, this diagnosis is almost certain. If the pain is throbbing in character and the tooth is tender to touch, pulpal necrosis with apical periodontitis or acute abscess is likely. Confirmation from objective tests is necessary. These include visual examination, percussion testing and thermal and electric pulp testing. The presence of a swelling or sinus tract indicates pulpal necrosis and acute or chronic abscess respectively. Tenderness to percussion signifies inflammation in the periapical tissues. Vitality testing in the immature tooth is inherently unreliable as these teeth provide unpredictable responses to pulp testing. Prior to completion of root formation, the sensory plexus of nerves in the subodontoblastic region is not well developed and as the injury itself can lead to erratic responses (9) over reliance on the results of clinical tests of pulp vitality, particularly by the use of electric pulp testing devices, is not recommended (10). Radiographic interpretation can be difficult. A radiolucent area normally surrounds the developing open apex of an immature tooth with a healthy pulp. It may be difficult to differentiate between this finding and a pathologic radiolucency resulting from a necrotic pulp. Comparison with the periapex of the contralateral tooth may be helpful.

Unfortunately, it has not been possible to establish a close correlation between the results of these individual tests and the histological diagnosis (11–13) but it is hoped that by combining the results of the history, examination and diagnostic tests, an accurate clinical diagnosis of pulpal vitality can be made in most cases. When the pulp is deemed vital, apexogenesis techniques can be attempted. A necrotic pulp condemns the tooth to apexification.

Apexogenesis

Apexogenesis involves removal of the inflamed pulp and the placement of calcium hydroxide on the remaining healthy pulp tissue. Traditionally this has implied removal of the coronal portion of the pulp. However, the depth to which the tissue is removed should be determined by clinical judgment. Only the inflamed tissue should be removed, but the difficulty in assessing the level of inflammation is widely acknowledged. However a number of investigators have demonstrated that, following mechanical exposures of the pulp that were left untreated for up to 168 h, inflammation was limited to the coronal 2–3 mm of the pulp (14). This has led to the development of the so-called Cvek or shallow pulpotomy in which only the most superficial pulp is removed. The goals of apexogenesis, as stated by Webber (15) are as follows:

1. Sustaining a viable Hertwig's sheath, thus allowing continued development of root length for a more favorable crown-to-root ratio.
2. Maintaining pulpal vitality, thus allowing the remaining odontoblasts to lay down dentine, producing a thicker root and decreasing the chance of root fracture.
3. Promoting root end closure, thus creating a natural apical constriction for root canal filling.
4. Generating a dentinal bridge at the site of the pulpotomy. While the bridging is not essential for the success of the procedure, it does suggest that the pulp has maintained its vitality.

The total time for achievement of the goals of the apexogenesis ranges between 1 and 2 years depending on the degree of tooth development at the time of the procedure. The patient should be recalled at 3-monthly intervals in order to determine the vitality of the pulp and the extent of apical maturation. If it is determined that the pulp has become irreversibly inflamed or necrotic, or if internal resorption is evident, the pulp should be extirpated and apexification therapy initiated.

Apexification

In the past, techniques for management of the open apex in non-vital teeth were confined to custom fitting the filling material (16, 17), paste fills (18) and apical surgery (19). A number of authors (16, 17) have described the use of custom fitted gutta-percha cones, but this is not advisable as the apical portion of the root is frequently wider than the coronal portion, making proper condensation of the gutta-percha impossible. Sufficient widening of the coronal segment to make its diameter greater than that of the apical portion would significantly weaken the root and increase the risk of fracture. The disadvantages of surgical intervention include the difficulty of obtaining the necessary apical seal in the young pulpless tooth with its thin, fragile, irregular walls at the root apex. These walls may shatter during preparation of the retrocavity or condensation of the filling material. The wide foramen results in a large volume of filling material and a compromised seal. Apicoectomy further reduces the root length resulting in a very unfavorable crown root ratio. The limited success enjoyed by these procedures resulted in significant interest in the phenomenon of continued apical development or establishment of an apical barrier, first proposed in the 1960s (20, 21). Many techniques have been suggested for induction of apical closure in pulpless teeth to produce more favorable conditions for conventional root canal filling.

Most of these techniques involve removal of the necrotic tissue followed by debridement of the canal and placement of a medicament. However, it has not been conclusively demonstrated that a medicament is necessary for induction of apical barrier formation. Nygaard-Ostby hypothesized that laceration of the periapical tissues until bleeding occurred might produce new vital vascularized tissue in the canal. He suggested that this treatment 'may result in further development of the apex' (22). Moller et al. (23) have shown that infected necrotic pulp tissue induces strong inflammatory reactions in the periapical tissues. Therefore removal of the infected pulp tissue should create an environment conducive to apical closure without use of a medication. McCormick et al. (24) have hypothesized that debridement of the root canal and removal of the necrotic pulp tissue and microorganisms along with a decrease in pulp space are the critical factors in apexification. A number of authors (25–28) have described apical closure without the use of a medicament. Some believe that instrumentation may in fact hamper root development and that preparation of these canals should be done cautiously, if at all (29). Cooke and Robotham (30) hypothesize that the remnants of Hertwig's epithelial root sheath, under favorable conditions, may organize the apical mesodermal tissue into root components. They advise avoidance of trauma to the tissue around the apex. This theory is supported by Vojinovic (31) and Dylewski (32).

Much of the early work in the area of induced apical closure focused on the use of antiseptic and antibiotic pastes. A number of investigators (33, 34) demonstrated apical closure using an antiseptic paste as a temporary filling material following root canal debridement and Ball (35) successfully reproduced these results using an antibiotic paste.

Calcium hydroxide

Although a variety of materials have been proposed for induction of apical barrier formation, calcium hydroxide has gained the widest acceptance. The use of calcium hydroxide was first introduced by Kaiser (20) in 1964 who proposed that this material mixed with camphorated parachlorophenol (CMCP) would induce the formation of a calcified barrier across the apex. This procedure was popularized by Frank (21) who emphasized the importance of reducing contamination within the root canal by instrumentation and medication and decreasing the canal space temporarily with a resorbable paste seal. A number of studies (32, 36, 37) have reported a high level of clinical success with the use of calcium hydroxide in combination with CMCP. Klein and Levy (38) and others (39, 40) described successful induction of an apical barrier using calcium hydroxide and Cresatin (Premier Dental Products). Cresatin had been shown to have minimal inflammatory potential as a root canal medicament (41) and to be significantly less toxic than CMCP (42). To further reduce the potential for cytotoxicity, the use of calcium hydroxide mixed with saline (43), sterile water (44, 45) or distilled water (46) has been investigated with similar clinical success. Heithersay (47, 48) and others (49, 50) have used calcium hydroxide in combination with methylcellulose (Pulpdent Corporation, Watertown, MA, USA). Pulpdent has the advantage of decreased solubility in tissue fluids and a firm physical consistency (51).

As the calcium ions from the calcium hydroxide dressing do not come from the calcium hydroxide but from the bloodstream (52, 53) the mechanism of action of calcium hydroxide in induction of an apical barrier remains controversial. Mitchell and Shankwalker (54) studied the osteogenic potential of calcium hydroxide when implanted into the connective tissue of rats. They concluded that calcium hydroxide had a unique potential to induce formation of heterotopic bone in this situation. Of 11 other materials used in comparative studies, only

plaster of Paris (calcium sulfate hemihydrate) and magnesium hydroxide demonstrated any osteogenic potential.

Holland et al. (55) have demonstrated that the reaction of the periapical tissues to calcium hydroxide is similar to that of pulp tissue. Calcium hydroxide produces a multilayered necrosis with subjacent mineralization. Schroder and Granath (56) have postulated that the layer of firm necrosis generates a low-grade irritation of the underlying tissue sufficient to produce a matrix that mineralizes. Calcium is attracted to the area and mineralization of newly formed collagenous matrix is initiated from the calcified foci.

It appears that the high pH of calcium hydroxide is an important factor in its ability to induce hard tissue formation. Javelet et al (57) compared the ability of calcium hydroxide (pH 11.8) and calcium chloride (pH 4.4) to induce formation of a hard tissue barrier in pulpless immature monkey teeth. Periapical repair and apical barrier formation occurred more readily in the presence of calcium hydroxide.

It has been demonstrated that apical barrier formation is more successful in the absence of microorganisms (58) and the antibacterial efficacy of calcium hydroxide has been established (59–64). The antimicrobial activity is related to the release of hydroxyl ions, which are highly oxidant and show extreme reactivity. These ions cause damage to the bacterial cytoplasmic membrane, protein denaturation and damage to bacterial DNA.

Heithersay (47, 48, 51) has postulated that calcium hydroxide may act by increasing the calcium concentration at the precapillary sphincter, reducing the plasma flow. In addition, the calcium ion can affect the enzyme pyrophosphatase, which is involved in collagen synthesis. Stimulation of this enzyme can facilitate repair mechanisms.

The hard tissue barrier has been described by Ghose et al. (65) as a cap, bridge or ingrown wedge and may be composed of cementum, dentin, bone or 'osteodentin' (32). This osteodentin appears to be formed by connective tissue at the apices, in that Hertwig's epithelial sheath is not seen. Torneck et al. (66) reported that a bonelike material was deposited on the inner walls of the canal while Steiner and Van Hassel (67) demonstrated apical closure by formation of a calcific bridge that satisfied the usual histological criteria for identification as cementum. Study of the serial sections gave the impression that cementum formation proceeds from the periphery of the original apex towards the center in decreasing concentric circles. In spite of radiographic and clinical evidence of complete apical bridge formation, histological examination reveals that the barrier is porous (67–69). Scanning electron microscopy and histological analysis of the apical barrier (70) demonstrated that the outer surface of the bridge extended in a 'cap like' fashion over the root apex, displaying irregular topography with indentations and convexities throughout. The histological sections showed distinct layers. The outer layer appeared to be composed of a dense acellular cementum-like tissue. This surrounded a more central mix of irregular dense fibrocollagenous connective tissue containing foreign material with irregular fragments of highly mineralized calcifications.

Controversy exists as to whether or how often the calcium hydroxide dressing should be changed. Chawla (71) suggests that that it suffices to place the paste only once and wait for radiographic evidence of barrier formation while Chosack et al. (72) found that after the initial root filling with calcium hydroxide there was nothing to be gained by repeated root filling either monthly or after 3 months. Proponents of a single application claim that the calcium hydroxide is only required to initiate the healing reaction and therefore repeated applications are not warranted. A number of authors (73, 74) propose that the calcium hydroxide should be replaced only when symptoms develop or the material appears to have washed out of the canal when viewed radiographically. Abbot (75) points out that radiographs cannot be relied upon to determine the amount of calcium hydroxide remaining in the canal or to demonstrate whether or not the barrier is complete. He concludes that regular replacement of the dressing has a number of advantages. It allows clinical assessment of barrier formation and may increase the speed of bridge formation (76–78). Abbot (75) suggests that the ideal time to replace a dressing depends on the stage of treatment and the size of the foramen opening. This must be assessed for each individual tooth at each stage of development.

Studies vary in assessment of the time required for apical barrier formation in apexification using calcium hydroxide. In a review of ten studies, Sheehy and Roberts (79), reported an average length of time for apical barrier formation ranging from 5 to 20 months. Finucane and Kinirons (78) reviewed 44 non-vital immature incisors undergoing calcium hydroxide apexification and found that the mean time to barrier formation was 34.2 weeks (range 13–67 weeks). The strongest predictor of rapid barrier formation was the rate of change of calcium hydroxide and a barrier also formed more rapidly in cases with narrower initial apical width. Age may be inversely related to the time required for apical barrier formation. In one study patients who were 11 years or older had significantly shorter treatment times (76). Others, however, refute this

finding (80, 81). Cvek (73) has reported that infection and/or the presence of a periapical radiolucency at the start of treatment increases the time required for barrier formation but other studies indicate no relationship between pretreatment infection and periapical radiolucency and barrier formation time (65, 76, 80, 81). Kleier and Barr (80) found that in the presence of symptoms the time required for apical closure was extended by approximately 5 months to an average of 15.9 months.

In a review of 10 studies, Sheehy and Roberts (79) reported that the use of calcium hydroxide for apical barrier formation was successful in 74–100% of cases irrespective of the proprietary brand used. They do point out that follow-up is necessary and information regarding long-term outcomes is limited. Problems such as reinfection and cervical root fracture may occur.

Mineral trioxide aggregate

Although calcium hydroxide has been the material of choice for apexification, a number of authors have worked with other materials. In the 1970s interest was expressed in the use of tricalcium phosphate for induction of apical barrier formation with some success (82, 83). Nevins et al. (84) reported favorable outcomes using collagen-calcium phosphate gel. In recent times interest has centered on the use of mineral trioxide aggregate (MTA) for apexification. This material was first introduced in 1993 and received Food and Drug Administration (FDA) approval in 1998. MTA is a powder consisting of fine hydrophilic particles of tricalcium silicate, tricalcium oxide and silicate oxide. It has low solubility and a radiopacity that is slightly greater than that of dentin (85). This material has demonstrated good sealability and biocompatibility (86, 87). MTA has a pH of 12.5 after setting which is similar to the pH of calcium hydroxide and it has been suggested that this may impart some antimicrobial properties (88). It has been used in both surgical and non-surgical applications including root end fillings (86, 87, 89), direct pulp caps (90), perforation repairs in roots (91) or furcations (92, 93) and apexification (94, 95). Shababhang et al. (94) compared the efficacy of osteogenic protein-1 and MTA with that of calcium hydroxide in the formation of hard tissue in immature roots of dogs. They concluded that MTA produced apical hard tissue formation with significantly greater consistency. The difference in the amount of hard tissue formed among the three test materials was not statistically significant.

One visit apexification

Induction of apical healing, regardless of the material used, takes at least 3–4 months and requires multiple appointments. Patient compliance with this regimen may be poor and many fail to return for scheduled visits. The temporary seal may fail resulting in reinfection and prolongation or failure of treatment. The importance of the coronal seal in preventing endodontic failure is well established (96–98). For these reasons one-visit apexification has been suggested. Morse et al. (99) define one-visit apexification as the non-surgical condensation of a biocompatible material into the apical end of the root canal. The rationale is to establish an apical stop that would enable the root canal to be filled immediately. There is no attempt at root end closure. Rather an artificial apical stop is created. A number of materials have been proposed for this purpose including tricalcium phosphate (100, 101), calcium hydroxide (100, 102), freeze dried bone (103) and freeze-dried dentin (104). Favorable results have been reported. Recently there have been a number of reports describing the use of MTA in one-visit apexification. Witherspoon and Ham (105) describe a technique using MTA. They assert that MTA provides scaffolding for the formation of hard tissue and the potential of a better biological seal. They conclude that this technique is a viable option for treating immature teeth with necrotic pulps and should be considered as an effective alternative to calcium hydroxide apexification. Steinig, Regan and Gutmann (106) consider that the importance of this technique lies in the expedient cleaning and shaping of the root canal system, followed by its apical seal with a material that favors regeneration. Furthermore the potential for fractures of immature teeth with thin roots is reduced, as a bonded core can be placed immediately within the root canal. A number of authors (95, 107, 108) have reported clinical success using MTA for one visit apexification.

While the objective of apexification is to stimulate apical barrier formation, in the belief that continued root formation cannot occur, there are a number of reports of continued apical development in spite of a necrotic pulp (109, 110). Yang et al. (111) reported a case in which apical barrier formation was accompanied by a separate disto-apically growing root. Histological evaluation revealed immature hard tissue mixed with calcium hydroxide, connective tissue and bone apically in the original root canal. In the separate newly formed part of the root, pulp tissue, odontoblasts, predentin, cementum and an apical foramen could be identified. Selden (112) also described a case in

which the outcome morphologically closely resembled normal root formation. It has been suggested that for continued root development to occur the area of calcific scarring must not extend to Hertwig's root sheath or to the odontoblasts in the apical area (113).

Tooth restoration following apexification

Because of the thin dentinal walls there is a high incidence of root fractures in teeth after apexification. Restorative efforts should be directed towards strengthening the immature root. A number of studies have demonstrated that the use of the newer dentin bonding techniques can significantly increase the resistance to fracture of these teeth to levels close to that of intact teeth (114). Goldberg et al. (115) have recently demonstrated the reinforcing effect of a resin glass ionomer in the restoration of immature roots. The risk of root fracture during apexification is a concern, but during this time it is essential that access to the apical portion of the canal is preserved. Katebzadeh et al. (116) have described a technique in which the access is restored with a composite restoration. A clear curing post is inserted into the soft composite and cured. The post is then removed leaving a patent channel for calcium hydroxide replacement and subsequent obturation of the canal.

Conclusions

Calcium hydroxide apexification remains the most widely used technique for treatment of necrotic teeth with immature apices. Success rates are high. However techniques for one-visit apexification provide an alternative treatment option in these cases. Prospective clinical trials comparing these alternative techniques are required.

References

1. Bhasker SN. Orban's oral histology and embryology, 11th edn. St. Louis: Mosby-Year Book; 1991.
2. American Association of Endodontists. Glossary of endodontic terms, 7th edn. Chicago: American Association of Endodontists; 2003.
3. Andreasen JO, Andreasen FM. Textbook and color atlas of traumatic injuries to the teeth, 3rd edn. Copenhagen: Munksgaard; 1994.
4. Andreasen JO, Ravn JJ. Epidemiology of traumatic dental injuries to primary and permanent teeth in a Danish population sample. Int J Oral Surg 1972;1:235–9.
5. Andreasen JO, Hjorting-Hansen R. Intra-alveolar root fractures: radiographic and histologic study of 50 cases. J Oral Surg 1967;25:414–26.
6. Pindborg JJ. Clinical, radiographic and histologic aspects of intra-alveolar fractures of upper central incisors. Acta Odontol Scand 1956;13:41–7.
7. Andreasen JO, Borum MK, Andreasen FM. Replantation of 400 avulsed permanent incisors. Endod Dent Traumatol 1992;8:45–55.
8. Torneck CD. Effects of trauma to the developing permanent dentition. Dent Clin N Am 1982;26:481–504.
9. Klein H. Pulp response to an electric pulp stimulator in the developing permanent anterior dentition. J Dent Child 1978;45:23–5.
10. Jacobsen I, Kerekes K. Long-term prognosis of traumatized permanent anterior teeth showing calcifying processes in the pulp cavity. Scand J Dent Res 1975;83:355–64.
11. Seltzer S, Bender IB, Ziontz M. The dynamics of pulp inflammation: correlation between diagnostic data and actual histologic findings in the pulp. Part I. Oral Surg 1963;16:846–71.
12. Seltzer S, Bender IB, Ziontz M. The dynamics of pulp inflammation: correlation between diagnostic data and actual histologic findings in the pulp. Part II. Oral Surg 1963;16:969–77.
13. Hyman JJ, Cohen ME. The predictive value of endodontic diagnostic tests. Oral Surg 1984;58:343–6.
14. Cvek M, Cleaton-Jones PE, et al. Pulp reactions to exposure after experimental crown fracture or grinding in the adult monkey. J Endod 1982;8:391–7.
15. Webber RT. Apexogenesis versus apexification. Dent Clin N Am 1984;28:669–97.
16. Stewart DJ. Root canal therapy in incisor teeth with open apices. Br Dent J 1963;114:249–54.
17. Friend LA. The root treatment of teeth with open apices. Proc R Soc Med 1966;19:1035–6.
18. Friend LA. Treatment of immature teeth and non-vital pulps. J Br Endod Soc 1967;1:28–33.
19. Ingle JI. Endodontics. Philadelphia: Lea and Febiger; 1965:504.
20. Kaiser HJ. Management of wide open apex canals with calcium hydroxide. Presented at the 21st Annual Meeting of the American Association of Endodontists, Washington DC April 17 1964.
21. Frank AL. Therapy for the divergent pulpless tooth by continued apical formation. J Am Dent Assoc 1966;72:87–93.
22. Nygaard-Ostby B. The role of the blood clot in endodontic therapy. Acta Odontol Scand 1961;19:323–46.
23. Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89:475–84.
24. McCormick JE, Weine FS, Maggio JD. Tissue pH of developing periapical lesions in dogs. J Endod 1983;9:47–51.
25. Chawla HS, Tewari A, Ramakrishnan E. A study of apexification without a catalyst paste. J Dent Child 1980;47:431–4.
26. England MC, Best E. Non-induced apical closure in immature roots of dogs' teeth. J Endod 1977;3:411–7.
27. Whittle M. Apexification of an infected untreated immature tooth. J Endod 2000;26:245–7.
28. Das S. Apexification in a non-vital tooth by control of infection. J Am Dent Assoc 1980;100:880–1.
29. Das S, Das AK, Murphy RA. Experimental apexogenesis in baboons. Endod Dent Traumatol 1997;13:31–5.
30. Cooke C, Rowbotham TC. The closure of open apices in non-vital immature incisor teeth. Br Dent J 1988;165:420–1.
31. Vojinovic O. Induction of apical formation in immature teeth by different endodontic methods of treatment. Experimental pathohistolological study. J Oral Rehab 1974;1:85–97.

32. Dylewski JJ. Apical closure of non-vital teeth. Oral Surg 1971;32:82–9.
33. Cooke C, Rowbotham TC. Root canal therapy in non-vital teeth with open apices. Brit Dent J 1960;108:147–50.
34. Holland R, de Souza V, Tugliavini RL, Milanezi LA. Healing process of teeth with open apices: histologic study. Bull Tokyo Dent Coll 1971;12:333–8.
35. Ball JS. Apical root formation in non-vital immature permanent incisor. Report of a case. Brit Dent J 1964;116:166–7.
36. Steiner JC, Dow PR, Cathey GM. Inducing root end closure of non-vital permanent teeth. J Dent Child 1968;35:47–54.
37. Van Hassel HJ, Natkin E. Induction of root end closure. J Dent Child 1970;37:57–9.
38. Klein SH, Levy BA. Histologic evaluation of induced apical closure of a human pulpless tooth. Oral Surg 1974;38:954–9.
39. Stewart GG. Calcium hydroxide induced root healing. J Am Dent Assoc 1975;90:793–800.
40. West NM, Lieb RJ. Biologic root-end closure on a traumatized and surgically resected maxillary central incisor: an alternative method of treatment. Endod Dent Traumatol 1985;1:146–9.
41. Schilder H, Amsterdam M. Inflammatory potential of root canal medicaments: Preliminary report including non-specific drugs. Oral Surg 1959;12:211–21.
42. Vander Wall GL, Dowson J, Shipman C. Antibacterial efficacy and cytotoxicity of three endodontic drugs. Oral Surg 1972;33:230–41.
43. Citrome GP, Kaminski EJ, Heuer MA. A comparative study of tooth apexification in the dog. J Endod 1979;5:290–7.
44. Michanowicz J, Michanowicz A. A conservative approach and procedure to fill an incompletely formed root using calcium hydroxide as an adjunct. J Dent Child 1967;32:42–7.
45. Wechsler SM, Fishelberg G, Opderbeck WR, et al. Apexification: a valuable and effective clinical procedure. Gen Dent 1978;26:40–3.
46. Binnie WH, Rowe AHR. A histologic study of the periapical tissues of incompletely formed pulpless teeth filled with calcium hydroxide. J Dent Res 1973;52:1110–6.
47. Heithersay GS. Periapical repair following conservative endodontic therapy. Aus Dent J 1970;15:511–8.
48. Heithersay GS. Stimulation of root formation in incompletely developed pulpless teeth. Oral Surg 1970;29:620–30.
49. Anthony DR, Senia ES. The use of calcium hydroxide as a paste fill. Tex Dent J 1981;99:6–10.
50. Feiglin B. Differences in apex formation during apexification with calcium hydroxide paste. Endod Dent Traumatol 1985;1:195–9.
51. Heithersay GS. Calcium hydroxide in the treatment of pulpless teeth with associated pathology. J Br Endod Soc 1975;8:74–93.
52. Sciaky I, Pisanti S. Localization of calcium placed over amputated pulps in dog's teeth. J Dent Res 1960;39:1128–32.
53. Pisanti S, Sciaky I. Origin of calcium in the repair wall after pulp exposure in the dog. J Dent Res 1964;43:641–44.
54. Mitchell DF, Shankwalker GB. Osteogenic potential of calcium hydroxide and other materials in soft tissue and bone wounds. J Dent Res 1958;37:1157–63.
55. Holland R, de Mello W, Nery MJ, et al. Reaction of human periapical tissue to pulp extirpation and immediate root canal filling with calcium hydroxide. J Endod 1977;3:63–7.
56. Schroder U, Granath L. Early reaction of intact human teeth to calcium hydroxide following experimental pulpotomy and its significance to the development of hard tissue barrier. Odontol Revy 1971;22:379–95.
57. Javelet J, Torabinejad M, Bakland L. Comparison of two pH levels for the induction of apical barriers in immature teeth of monkeys. J Endod 1985;11:375–8.
58. Ham JW, Patterson SS, Mitchell DF. Induced apical closure of immature pulpless teeth in monkeys. Oral Surg 1972;33:438–49.
59. Barthel CR, Levin LG, Reisner HM, Trope M. TNF-alpha release in monocytes after exposure to calcium hydroxide treated *E. coli* LPS. Int Endod J 1997;30:155–9.
60. Bystrom RH, Claesson R, Sundqvist G. The antibacterial effect of calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;1:170–5.
61. Estrela C, Pimento FC, Ito IY, Bammann LL. *In vitro* determination of direct antimicrobial effect of calcium hydroxide. J Endod 1998;24:15–7.
62. Jiang J, Zuo J, Chen SH, Holliday LS. Calcium hydroxide reduces lipopolysaccharide-stimulated osteoclast formation. Oral Surg 2003;95:348–54.
63. Kontakiotis E, Nakou M, Georgopoulou M. *In vitro* study of the indirect action of calcium hydroxide on the anaerobic flora of the root canal. Int Endod J 1995;28:285–9.
64. Safavi KE, Nicholls FC. Alteration of biological properties of bacterial lipopolysaccharide by calcium hydroxide. J Endod 1994;20:127–9.
65. Ghose LJ, Bagdady VS, Hikmat BYM. Apexification of immature apices of pulpless permanent anterior teeth with calcium hydroxide. J Endod 1987;13:285–90.
66. Torneck CD, Smith JS, Grindall P. Biological effects of endodontic procedures on developing incisor teeth. IV. Effect of debridement procedures and calcium hydroxide CPCP paste in the treatment of experimentally induced pulp and periapical disease. Oral Surg 1973;35:541–54.
67. Steiner JC, Van Hassel HJ. Experimental root apexification in primates. Oral Surg 1971;31:409–15.
68. Walia T, Chawla HS, Gauba K. Management of wide open apices in non-vital permanent teeth with calcium hydroxide paste. J Clin Pediatr Dent 2000;25:51–6.
69. Lieberman J, Trowbridge H. Apical closure of non vital permanent incisor teeth where no treatment was performed: a case report. J Endod 1987;9:257–60.
70. Baldassari-Cruz LA, Walton RE, Johnson WT. Scanning electron microscopy and histologic analysis of an apexification 'cap'. Oral Surg 1998;86:465–8.
71. Chawla HS. Apical closure in a non-vital permanent tooth using one calcium hydroxide dressing. J Dent Child 1986;53:44–7.
72. Chosack A, Sela J, Cleaton-Jones P. A histological and quantitative histomorphometric study of apexification of nonvital permanent incisors of vervet monkeys after repeated root filling with a calcium hydroxide paste. Endod Dent Traumatol 1997;13:211–7.
73. Cvek M. Treatment of non-vital permanent incisors with calcium hydroxide. I. Follow-up of periapical repair and apical closure of immature roots. Odonotol Revy 1972;23:27–44.
74. Feiglin B. Differences in apex formation during apexification with calcium hydroxide paste. Endod Dent Traumatol 1985;1:195–9.
75. Abbot P. Apexification with calcium hydroxide – when should the dressing be changed? The case for regular dressing changes. Aust Endod J 1998;24:27–32.
76. Mackie IC, Bentley EM, Worthington HV. The closure of open apices in non-vital immature teeth. Brit Dent J 1988;165:169–73.

77. Kinirons MJ, Srinivasan V, Welbury RR, Finucane D. A study in two centers of variations in the time of apical barrier detection and barrier position in nonvital immature permanent incisors. Int J Pediatr Dent 2001;11: 447–51.
78. Finucane D, Kinirons MJ. Non-vital immature permanent incisors: factors that may influence treatment outcome. Endod Dent Traumatol 1999;15:273–7.
79. Sheehy EC, Roberts GJ. Use of calcium hydroxide for apical barrier formation and healing in non-vital immature permanent teeth: a review. Br Dent J 1997;183:241–6.
80. Kleier DJ, Barr ES. A study of endodontically apexified teeth. Endod Dent Traumatol 1991;7:112–7.
81. Yates JA. Barrier formation time in non-vital teeth with open apices. Int Endod J 1988;21:313–9.
82. Koenigs JF, Heller AL, Brilliant JD, Melfi PC, Driskell TD. Induced apical closure of permanent teeth in adult primates using resorbable form of tricalcium phosphate ceramic. J Endod 1975;1:102–6.
83. Roberts SC, Brilliant JD. Tricalcium phosphate as an adjunct to apical closure in adult permanent teeth. J Endod 1975;1:263–9.
84. Nevins A, Finkelstein F, Laporta R, Borden BG. Induction of hard tissue into pulpless open-apex teeth using collagen-calcium phosphate gel. J Endod 1978;4:76–81.
85. Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root end filling material. J Endod 1995;21:349–53.
86. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of mineral trioxide aggregate when used as a root end filling material. J Endod 1993;19:591–5.
87. Torabinejad M, Hong CU, Lee SJ. Investigation of mineral trioxide aggregate for root end filling in dogs. J Endod 1995;21:603–8.
88. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root-end filling materials. J Endod 1995;21:403–6.
89. Torabinejad M, Pitt Ford TR, McKendry DJ, Abedi HR, Miller DA, Kariyawasam SP. Histologic assessment of mineral trioxide aggregate as a root end filling material in monkeys. J Endod 1997;23:225–8.
90. Pitt Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp capping material. J Am Dent Assoc 1996;127:1491–4.
91. Lee SJ, Monsef M, Torabinejad M. Sealing ability of mineral trioxide aggregate for repair of lateral root perforations. J Endod 1993;19:541–4.
92. Arens DE, Torabinejad M. Repair of furcal perforations with mineral trioxide aggregate: two case reports. Oral Surg 1996;82:84–8.
93. Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. Oral Surg 1995;79:756–63.
94. Shabahang S, Torabinejad M, Boyne PP, Abedi HR, McMillan P. A comparative study of root-end induction using osteogenic protein-1, calcium hydroxide and mineral trioxide aggregate in dogs. J Endod 199;25:1–5.
95. Shabahang S, Torabinejad M. Treatment of teeth with open apices using mineral trioxide aggregate. Pract Periodontics Aesthet Dent 2000;12:315–20.
96. Saunders WP, Saunders EM. Coronal leakage as a cause of failure in root canal therapy: a review. Endod Dent Traumatol 1994;10:105–8.
97. Ray H, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. Int Endod J 1995;28:12–8.
98. Magura ME, Kafrawy AH, Brown CE Jr, Newton C. Human saliva coronal microleakage in obturated root canals: an *in vitro* study. J Endod 1991;17:324–31.
99. Morse DR, O'Larnic J, Yesilsoy C. Apexification: review of the literature. Quintessence Int 1990;21:589–98.
100. Coveillo J, Brilliant JD. A preliminary clinical study on the use of calcium phosphate as an apical barrier. J Endod 1979;5:6–13.
101. Harbert H. One step apexification without calcium hydroxide. J Endod 1996;22:690–2.
102. Schumacher JW, Rutledge RE. An alternative to apexification. J Endod 1993;19:529–31.
103. Rossmeisl R, Reader A, Melfi R, et al. A study of freeze-dried (lyophilized) cortical bone used as an apical barrier in adult monkey teeth. J Endod 1982;8:219–26.
104. Rossmeisl R, Reader A, Melfi R, et al. A study of freeze-dried dentin used as an apical barrier in adult monkey teeth. Oral Surg 1982;53:303–10.
105. Witherspoon DE, Ham K. One-visit apexification: technique for inducing root end barrier formation in apical closures. Pract Proced Aesthet Dent 2001;13:455–60.
106. Steinig TH, Regan JD, Gutmann JL. The use and predictable placement of mineral trioxide aggregate in one-visit apexification cases. Aust Endod J 2003;29: 34–42.
107. Giuliani V, Baccetti T, Pace R, Pagavino G. The use of MTA in teeth with necrotic pulps and open apices. Dent Traumatol 2002;18:217–21.
108. Maroto M, Barberia E, Planells P, Vera V. Treatment of a non-vital immature incisor with mineral trioxide aggregate (MTA). Dent Traumatol 2003;19:165–9.
109. Herbert WE. Three cases of disturbance of calcification of a tooth and infection of the dental pulp following trauma. Dent Pract 1959;9:176–80.
110. Rule DC, Winter GB. Root growth and apical repair subsequent to pulpal necrosis in children. Br Dent J 1966;120:586–90.
111. Yang S-F, Yang Z-P, Chang K-W. Continuing root formation following apexification treatment. Endod Dent Traumatol 1990;6:232–5.
112. Selden HA. Apexification: an interesting case. J Endod 2002;28:44–5.
113. Gupta S, Sharma A, Dang N. Apical bridging in association with regular root formation following single visit apexification. Quintessence Int 1999;30:560–2.
114. Hernandez R, Bader S, Boston D, Trope M. Resistance to fracture of endodontically treated premolars restored with new generation dentin bonding systems. Int Endod J 1994;27:281–4.
115. Goldberg F, Kaplan A, Roitman M, Monfre S, Picca M. Reinforcing effect of a resin glass ionomer in the restoration of immature roots in vitro. Dent Traumatol 2002;18:70–2.
116. Katebzadeh N, Dalton C, Trope M. Strengthening immature teeth during and after apexification. J Endod 1998;24:256–9.