Chapter 8

Experimental Studies of Root Canal Therapy for Infected Nonvital Permanent Teeth with Incompletely Formed Apices

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《Abstract》

A total of 160 root canals in 90 teeth with incompletely formed roots of puppies were prepared for infected root canal therapy. The experimental group was treated with Vitapex and the control group was treated with calcium hydroxide-CMCP paste root canal filling material. Histopathological sections from decalcified specimens were prepared after observation periods of 30, 60, 120 and 180 days. In addition, 24 root canals in 16 teeth were treated with Vitapex on the opposite side with sound teeth and subjected to tetracycline administration. Observations were carried out by contact microradiography and tetracycline labeling and resulted in the following conclusions.

1. Almost no root growth was observed using both root canal filling materials, and the addition of newly-formed hard tissue was observed close to the root apical foramen involved.
2. The addition of hard tissue after root canal filling was thought to be primarily by cementum.
3. A greater amount of hard tissue formation resulting in closure of the apex and a more favorable outcome was observed in the control group than in the experimental group.
4. The control group had a higher percentage of hard tissue formation outside the root apex close to the root tip than did the experimental group.
5. The experimental group showed good results in each observation period. The control group showed better results with a shorter observation period than did the experimental group, but in longer observation periods, tended to decrease in cases with poor results.
6. Undecalcified specimens were examined using contact microradiography and tetracycline labeling. In the experimental group, the addition of newly-formed hard tissue was observed close to

the apical foramen near the root tip formed during the course of treatment. Normal root formation in the dental arch was observed in all samples in the experimental and control groups.

Introduction

A permanent tooth with an incompletely formed root has a short and thin root, open apex, and funnel-shaped opening with large periodontal tissue. Root canal enlargement during conventional root canal therapy with apical infection is difficult and may increase the risk of irritating the periodontal tissues. Furthermore, excess root canal medication at the root tip can stimulate apical periodontal tissues. Moreover, because of the root canal morphology of permanent teeth with incompletely formed roots, application of solid root canal filling material like gutta percha point is not practical. Currently, calcium hydroxide preparation is used during root canal treatment of infected root in permanent teeth with incompletely formed roots, since it is believed to induce closure of the apical foramen with hard tissue formation at the root apex [1-5].

The efficacy of calcium hydroxide formulation used as root canal filling has been known. Calcium hydroxide promotes hard tissue formation at the root apex leading to closure or narrowing of the apical foramen. However, reports from the early 1970s on the occasional application of calcium hydroxide as root canal filling material in permanent teeth with incompletely formed roots were mostly case reports using conventional treatment [6-13], clinical studies [14-29] and experimental studies [30-42]. According to those studies, calcium hydroxide preparation is an effective root canal filling agent in permanent teeth with incompletely formed roots.

In this study, therefore, limitations of calcium hydroxide preparation as root canal filling material have been achieved resulting from the use of a calcium hydroxide and iodoform preparation known as Vitapex. This has already been proven effective in deciduous teeth [43-47] and permanent teeth [48, 49] used as root canal filling material for infected root canals with incomplete root formation when compared to calcium hydroxide-CMCP paste.

This study induced infected root canals in permanent teeth with incompletely formed roots using dogs, and then performed infected root canal treatment and root canal filling using calcium hydroxide-CMCP paste and Vitapex, respectively, after each observation period of 30, 60, 120, 180 days. Root formation and closure of the apex in different stages of periodontal healing as well as differences in the amount of hard tissue formation were compared histologically. Contact microradiography and tetracycline labeling at the end of the application of Vitapex were carried out to determine the addition of new hard tissue causing root formation compared to sound teeth.

Examination methods

1 Experimental animals

Twenty-two 6 month-old mongrel puppies were used in this experiment with 122 maxillary incisors and mandibular molars with 208 root canals. From 22 heads, 18 heads with 90 teeth and 160 root canals were decalcified for histopathological examination. In the remaining 4 dogs with 16 teeth and 24 root canals, undecalcified specimens were examined using contact microradiogram and tetracycline (TC) fluorescent labeling.

The root canal filling material Vitapex and calcium hydroxide-CMCP paste formulation are as follows:

Vitapex (100 g)	
Calcium hydroxide (Japan Pharmacopoeia)	30.3g
Iodoform	40.4g
Silicone oil	22.4g
Others	6.9g
Calcium hydroxide-CMCP paste	
Powder	
Calcium hydroxide	
Liquid	
CMCP (USP 56)	100g
Paramonocholorophenol	35g
Camphor	65g

2 Experimental procedures

First, the dogs were subjected to general anesthesia with 5% sodium pentobarbital (Nembutal, Dainippon Pharmaceutical Co.). The dog teeth and surrounding periodontal tissue were washed and cleaned. Access preparation was done with a diamond point and the pulp chamber was opened and enlarged with a round bur. Next, the pulp was removed by using a barbed broach and the canal was left opened for 14 days to induce infection. After 14 days, the dogs were again subjected to general anesthesia. Tincture of iodine and 70% ethanol were used to sterilize the experimental teeth; 10% sodium hypochlorite and 2% hydrogen peroxide aqueous solution, broach, reamer and file were used simultaneously for root canal cleaning and then dried with sterile cotton plugs. Then the experimental teeth were filled with Vitapex and the control teeth were treated with calcium hydroxide-CMCP paste. Finally, the canal was lined with zinc oxide eugenol followed by zinc phosphate cement and restored with amalgam to complete the treatment.

In addition, TC labeling was performed in 4 subjects prepared for undecalcified sections after the experimental teeth were treated with Vitapex and on the other side (control), no treatment was done.

In this experiment, radiographs were taken 14 days after opening the root canal, after root canal therapy and at the end of each observation period.

3 Observation method

After root canal therapy, subjects were observed at 30, 60, 120 and 180 days and then sacrificed with an overdose of 5% sodium pentobarbital. Experimental teeth and alveolar bone were cut and immediately fixed in 10% neutral formalin solution.

Decalcification of specimens for pathological observation was made by the following methods. First, samples were fixed in 5% formalin solution and formic acid decalcification after dehydration using alcohol, embedded in ceroidin, and serially sectioned. Sections were stained with hematoxylin and eosin, and with thionine-picric acid. Subsequent microscopic examination of these specimens was then carried out.

For the histopathological observation, detailed observation of the changes in the periodontal tissues and newly-formed tissues at the root apex were recorded. For hard tissue formation at the root apex, the type and location were also recorded using a new type of instrument, an all-purpose projector for tissue thickness V16-A (Nikon Co.) at a magnification of

50. For measurement sites, the maximum thickness and diameter of hard tissue formation was measured from the axial to the middle third of the tooth in all samples. For radiographic findings, the conditions of the root canal filling material after treatment and resorption of the root canal filling material after the closure of the root apex were likewise investigated.

Contact microradiogram and TC fluorescent labeling for undecalcified specimens were prepared by the following methods. First, the samples were fixed, dehydrated in alcohol and embedded in polyester resin (Rigolac2004 and Rigolac70F). Specimens were then sectioned thinly, about 200 mm, using a Buehler low speed precision cutting machine (ISOMET11-1180) and polished using a wheel. The final thickness of undecalcified specimens for observation was about 60 μm.

Contact microradiogram was performed under the following conditions. Images were taken using Softex CMR type focus film at a distance of 8 cm, 7kV tube voltage, 3mA current for 60 min. The film used was Spectroscopic Safety Film Type 649-0 (Kodak). After specifying the development process, the specimen was sealed in Canada balsam and microscopically examined. Undecalcified specimens were further sealed in non-fluorescent glycerol for further observation using fluorescent microscope VDF-T (Nikon Co.) with BV filter. The 515W filter was attached to the eyepiece and TC labeling was observed in fluorescent image.

Labeling was carried out using TC administration. The administrating TC-dose and administration method were as follows. The Oxytetracycline (Terramycin, Pfizer Co. Tokyo) 20mg/kg was subcutaneous injected at intervals of 7 days (5 times). The administration was carried out after immediately after the treatment for 30 day group; after day 30 for day 60 group; after day 90 for day 120 group; and after day 150 for day 180 group.

4 Histopathological criteria

The extent of lesion was graded according to the following four categories: ± (minor), + (mild), ++ (moderate), +++ (strong). However, the degree of root apex closure was established using separate criteria to determine whether there was addition of hard tissue formation and incomplete or complete closure. So we could observe in detail the tissue changes, the pathologic results for each patient were judged according to the criteria below. The histopathological scores are as follows:

Good – no or very minor residual inflammation of periodontal tissues, with remarkable reparative changes at the root apex and apical closure or a tendency for closure of root apex

Fair – with some residual inflammation of periodontal tissues but with reparative changes at the root apex and a tendency for closure of root apex

Poor – marked inflammation of periodontal tissues at the root apex, no sign of repair with remarkable destructive lesion

Results

1 Pathological findings

Typical results of the experimental group were compared to those of the control group in each observation period.

1) 30-day observation period

Figure 1-A shows the histological view of case 18 of the experimental group (distal root of left mandibular 4th premolar). Vacuolation can be seen immediately below the root apex but inflammatory changes were localized. In addition, a portion of the periodontal tissue entered the root canal wall separating the newly added and calcified tissue closing the root apex. Accordingly, this case was given a pathological score of good.

Figure 1-B shows the histological view of case 18 of control group (distal root of right mandibular 4th premolar). No inflammatory changes in the periodontal tissue can be observed from the case. Addition of newly formed cementum-like tissue, which calcified and almost closed the root apex with repair of alveolar bone, can be observed. This case was given a score of good.

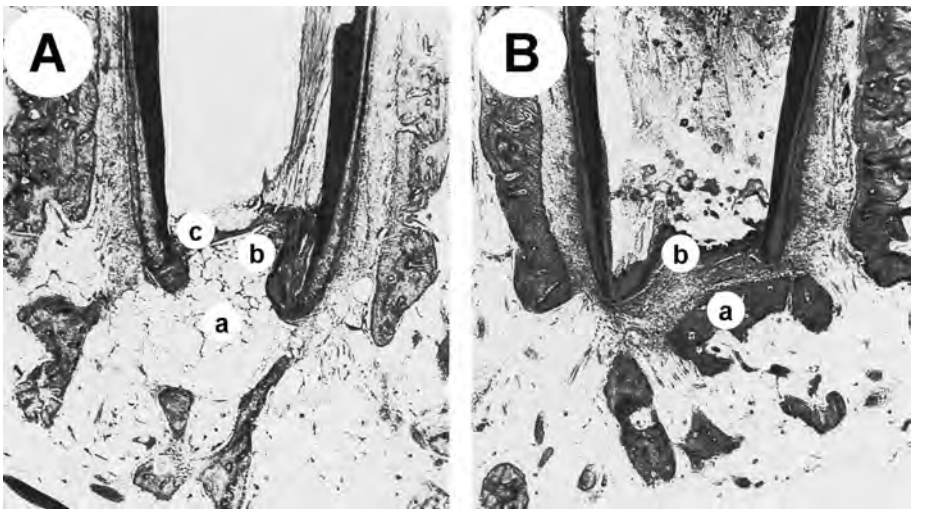

Figure 1 Case 18. Observation period: 30 days.
A: Experimental, left mandibular PM4, distal root, histopathological result: good; a: vacuoles, b: root canal polyp, c: closing hard tissue
B: Control, right mandibular PM4, distal root, histopathological result: good; a: alveolar bone deposition; b: closing hard tissue

2) 60-day observation period

Figure 2-A shows the histological view of case 31 of experimental group (mesial root of left mandibular 3rd premolar). Mild inflammatory cell infiltration of periodontal tissues at the root apex can be seen but the root is surrounded by newly formed tissue in addition to alveolar bone repair. The newly formed tissue is concentrated at the root apex, which is cementum-like tissue closing the apex of the root. This case was given a score of good.

Figure 2-B shows the histological view of case 31 of control group (mesial root of right mandibular 3rd premolar). Few changes in the root were observed with no damage in alveolar bone and periodontal tissues. Newly added active tissue regularly formed encircling the root. The apical foramen has been almost completely closed by the addition and calcification of newly formed cementum-like tissue at the root apex. This case was given a score of good.

3) 120-day observation period

Figure 3-A shows the histological view of case 50 of experimental group (distal root of left mandibular 3rd premolar). No damage is seen in periodontal tissues. Granulation tissue formed at the distal root canal and root apex remained open with the existence of excess root canal filler. Inside the root canal wall, a relatively thick cementum-like tissue borders the canal wall. Moreover, granulation tissue and thin deposits of cementum-like tissues line the root canal surface. The shape becomes irregular as it gets closer to the root apex. This case was given a score of good.

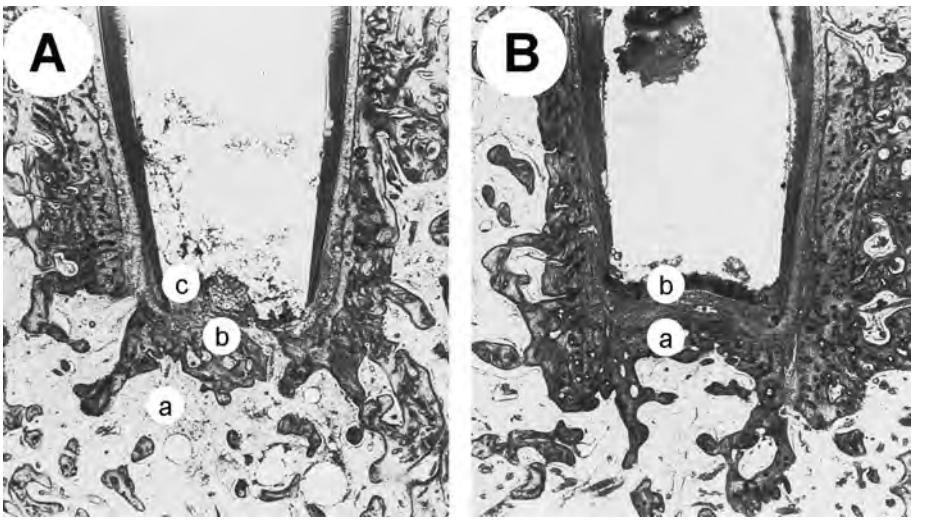

Figure 2 Case 31. Observation period: 60 days.
A: Experimental, left mandibular PM3, mesial root, histopathological result: good; a: Inflammatory cell infiltration, b: alveolar bone deposition, c: closing hard tissue
B: Control, right mandibular PM3, mesial root; histopathological result: good; a: alveolar bone deposition, b: closing hard tissue

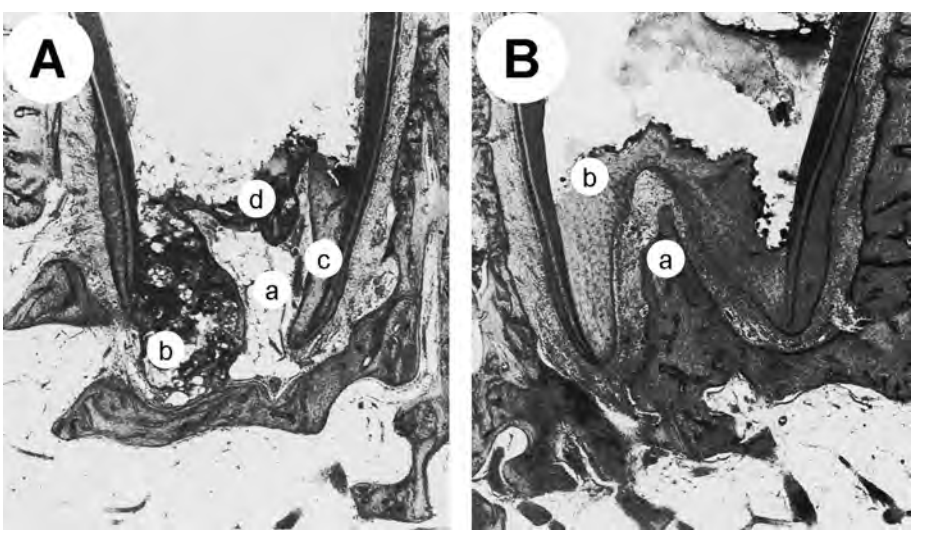

Figure 3 Case 50. Observation period: 120 days.
A: Experimental, left mandibular PM3, distal root, histopathological resultgood; a: root canal polyp, b: penetrated root canal filling material, c: cementum-like hard tissue, d: calcium deposition
B: Control, right mandibular PM4, distal root, histopathological result: good; a: alveolar bone deposition, b: closing hard tissue

Figure 3-B shows the histological view of case 50 of control group (distal root of right mandibular 3rd premolar). Active regenerative changes in the periodontal tissue at the root apex characterized by alveolar bone repair can be clearly seen, some of which are beyond the root apex and have penetrated the root canal. The root apex shows the addition of active, newly-formed cementum-like tissue, which has completely closed the root apex. This case was given a score of good.

4) 180-day observation period

Figure 4-A shows the histological view of case 68 of experimental group (distal root of left mandibular 4th premolars). No inflammatory changes of the periodontal tissues at the root apex can be observed at all. Newly formed irregular and calcified cementum-like tissues in large amount are present at the root canal walls. More hard tissue formation was observed continuously and beside the root apex, which completely closed the root apex. This case was given a score of good.

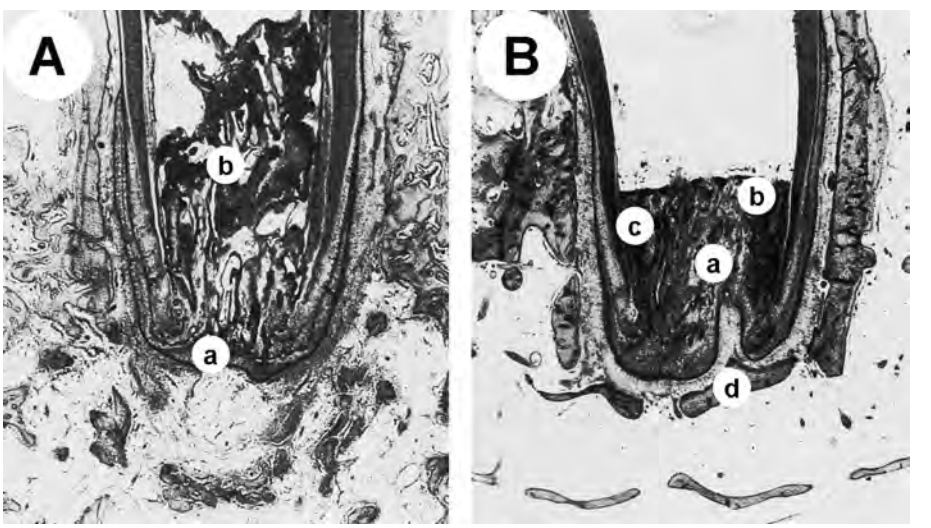

Figure 4 Case 68. Observation period: 180 days.
A: Experimental, left mandibular PM4, distal root, histopathological result: good; a: cementum-like hard tissue, b: calcium deposition
B: Control, right mandibular PM4, distal root, histopathological result: good; a: cementum-like hard tissue, b: calcium deposition, c: dentin, d: alveolar bone deposition

Figure 4-B shows the histological view of case 68 of control group (distal root of right mandibular 4th premolar). Root damage near the periodontal tissue is not observed at all. In some areas of the root canal wall, formation of cementum-like hard tissue and calcification, including branching structure of dentin formation were observed and the root apex was completely closed. This case was given a score of good.

Figure 5 shows the histological view of case 62 of the control group (distal root of right mandibular 3rd premolar). Granulation tissue infiltration into the root canal was observed, accompanied by strong inflammatory changes. Periodontal tissue inflammation beneath the root apex is strong. Suppuration around the nest of inflammatory cells is severe and diffuse. Hard tissue and bone formation around the root apex are present adjacent to bone resorption. This case was given a score of poor.

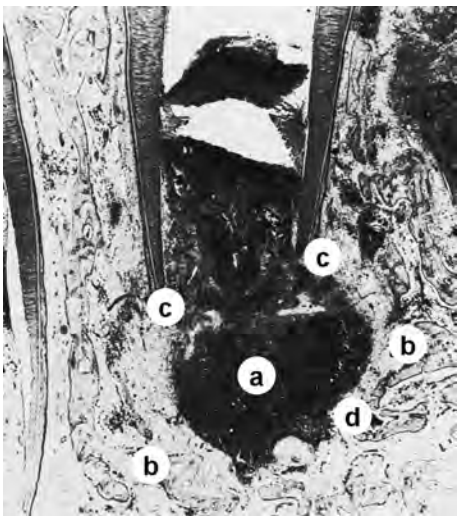

Figure 5 Case 62. Observation period: 180 days. Control, right mandibular PM3, distal root, histopathological result: poor; a: purulent, b: inflammatory cell infiltration, c: root apex hard tissue resorption, d: alveolar bone resorption

2 Contact microradiogram and TC fluorescent labeling

The use of contact microradiogram and TC labeling makes it is possible to observe the changes over time due to the presence of newly-added site or hard tissue morphology. This study also employed the use of control teeth by observing the formation of normal and healthy tooth and the changes in the alveolar bone, attempting to accurately capture the changes thought to be caused by the experiment. The following are typical examples.

1) 30-day observation period

Figure 6-A and Figure 6-B are the contact microradiogram and TC labeling, respectively, of a control case (mesial root of the left mandibular 3rd premolar). Alveolar bone formed regularly along the outer surface of the root. With the contact microradiogram, alveolar bone around the mesial of the root apex was observed with low levels of opacity and roughness coefficient compared to the other bone. With TC labeling, labeling of the bands showed the addition of new bone. According to image labeling, 5 almost equally-spaced layers or zones in the root canal wall were clearly observed. Weak labeling of a surface layer in the root canal wall and four layers of strong labeling clearly reveal the extent of root formation in each period when TC labeling was administered. The edge of the root is slightly obscured compared to the edges in the dentin, but the 4 zones labeled are cementum-like tissues involved in root apex closure.

Figure 6-C and Figure 6-D are contact microradiogram and TC labeling, respectively, of an experimental case (mesial root of right mandibular 3rd premolar). In the contact microradiogram, root growth was not observed at all. Radiopaque finding at root apex seems to be root canal filler, but the newly formed tissue responsible for closing the apex is difficult to distinguish from the filler. The alveolar bone at the root apex is denser compared to the control side, but in the lower part of the root apex, the alveolar bone formed exhibits a very extensive and irregular form. For

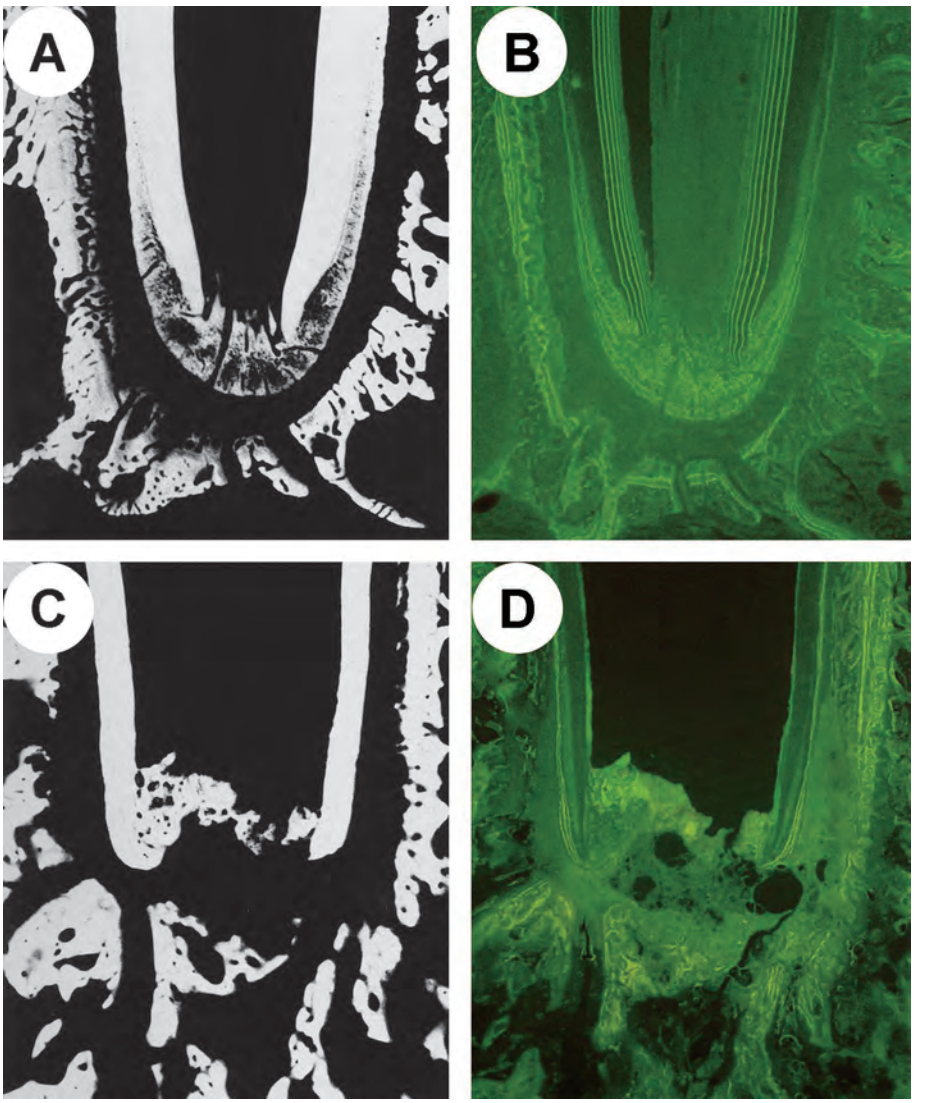

Figure 6 A: Control (left mandibular PM4, distal root) contact microradiogram; B: Control (left mandibular PM4, distal root) TC labeling image; C: Experimental (right mandibular PM4, distal root) contact microradiogram; ; D: Control (right mandibular PM4, distal root) TC labeling image

TC labeling, layered bands in the root canal wall, which did not occur in the control group, were observed, indicating that there was formation of root canal dentin. The distal part of the inside of root canal is randomly labeled with new hard tissues. In addition, 2 to 3 bands were labeled in the outer root formation characterized by cementum-like layers. In addition, 1 to 5 layers of labeled band mostly bone surrounding the root was observed indicating the addition of actively produced new bone. The newly-formed bone was, however, very irregular compared to the control side.
4) 180-day observation period

Figure 7-A and Figure 7-B show the contact microradiogram and TC labeling, respectively, of a control case (mesial root of left mandibular 3rd premolar). In the contact microradiogram, no

continuity of specific alveolar bone at the vicinity of the root apex was observed. In TC labeling, only 1 to 2 layers could be observed in the root canal. Furthermore, only labeled sites were observed near the root tip and they were confined to the trabecular bone.

Figure 7-C and Figure 7-D show the contact microradiogram and TC labeling, respectively, of an experimental case (mesial root of right maxillary 3rd premolar). In the contact microradiogram, addition of new hard tissue was found at the root apex. Part of the root apex below the root canal filler that overextended was found surrounded by bone formation. In TC labeling, root canal filler can be seen in the root canal with borders clearly labeled but hard tissue closure cannot be observed. The site-specific labeled bone in alveolar bone around the root apex

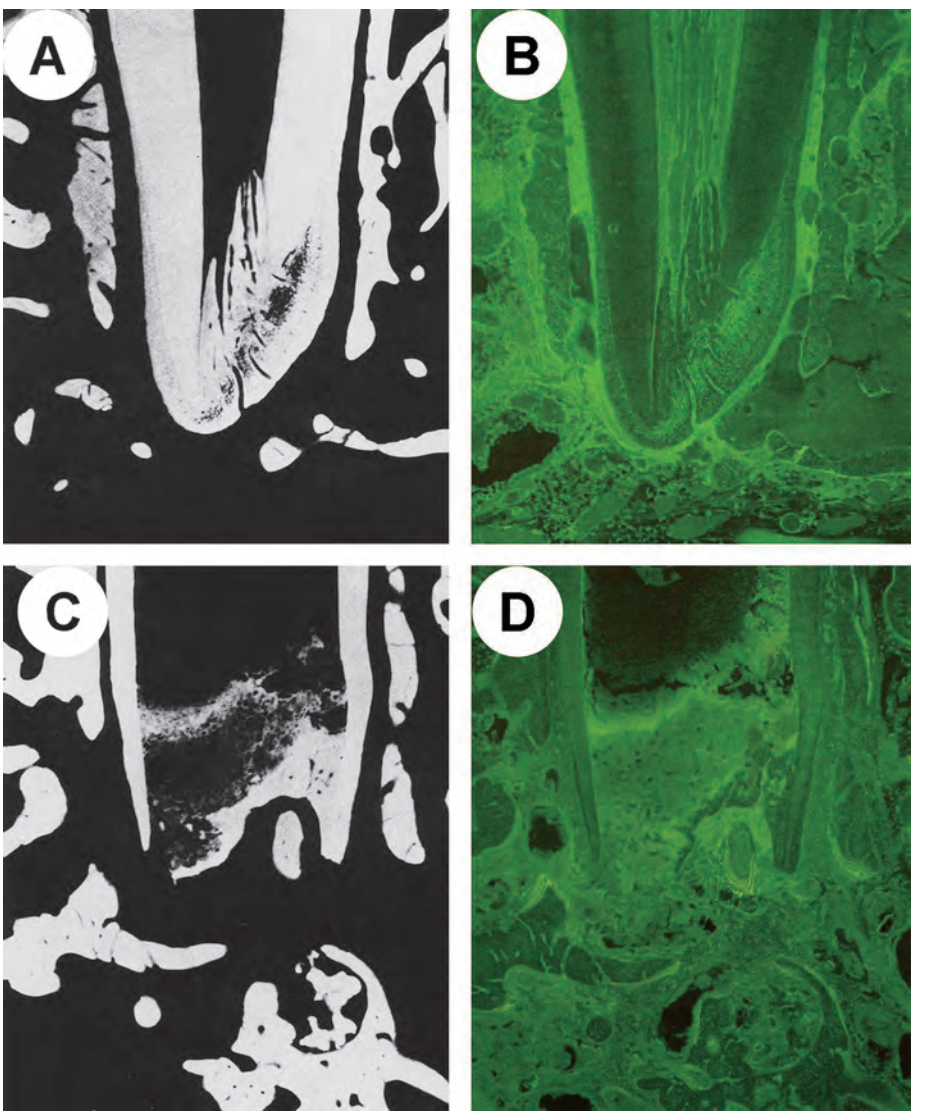

Figure 7 A: Control (left mandibular PM4, mesial root) contact microradiogram; B: Control (left mandibular PM4, mesial root) TC labeling image; C: Experimental (right mandibular PM4, mesial root) contact microradiogram; ; D: Control (right mandibular PM4, mesial root) TC labeling image

and trabecular bone of lateral roots are very narrow.

Discussion

1 Histopathological findings

1) Pathological findings of the periodontal tissues at the root apex

Observed periodontal diseases at the root apex are shown in Tables 1 and 2. The following is the summary of each disease and discussion.

(1) Congestion and hemorrhage

Although congestion was frequently observed in both groups, it was mostly mild. However, when comparing the 2 groups, the incidence and severity in the experimental group is lower than the control group. In addition, at each observation period, the incidence and extent in both groups showed no consistent trend.

During root formation, there is quite a number of natural blood vessels in the pulp and development of bone in periodontal tissues. In this experiment, the pulp was removed early, which caused inflammatory changes in the periapical region. Therefore, the congestion observed in this experiment was due to the inflammatory changes, alteration, repair and resistance, which were more likely expected to exist physiologically.

(2) Inflammatory cell infiltration and suppuration

We also created root canal infections in this study where the canals were left opened intentionally for 2 weeks after pulp extirpation. Thus, at that time, inflammatory changes in periodontal tissues at the root apecies in nearly all cases of infected root canal treatment were considered. Therefore, a high incidence of inflammatory changes in both experimental and control groups wase found to remain, the extent of which, however, has been significantly

Table 1 Pathological findings of apical periodontal tissues (experimental group)

Pathological findings		Congestion	Hemorrhage	Inflammatory cell infiltration	Suppuration	Granulation tissue	Alveolar bone resorption	Alveolar bone deposition	Hard tissue formation at the root apex
Degree	±	32	11	34	7	10	8	32	6
	+	3	2	19	4	17	7	33	11
	++	1	0	5	2	6	1	13	2
	+++	0	0	2	1	0	0	1	0
No of cases		36 (45.0%)	13 (16.3%)	60 (75.0%)	14 (17.5%)	33 (41.3%)	16 (20.0%)	79 (98.8%)	19 (23.8%)

Table 2 Pathologocal findings of apical periodontal tissues (control group)

Pathological findings		Congestion	Hemorrhage	Inflammatory cell infiltration	Suppuration	Granulation tissue	Alveolar bone resorption	Alveolar bone deposition	Hard tissue formation at the root apex
Degree	±	24	17	17	7	9	6	29	7
	+	13	5	10	7	10	7	31	10
	++	8	3	7	5	10	5	18	6
	+++	0	0	9	4	0	3	1	4
No of cases		45 (56.3%)	25 (31.3%)	43 (53.8%)	23 (28.8%)	29 (36.3%)	21 (26.3%)	79 (98.8%)	27 (33.8%)

improved. Thus, it can easily be inferred that the treatment of infected root canal using the filler is effective in controlling inflammatory changes. If experimental and control groups are compared, the incidence of inflammatory cell infiltration is higher in the experimental group but the extent is slightly less whereas most of the intensity in the control group ranges from minor to strong. Therefore, considering the difference in the degree of inflammatory changes between the 2 groups, the experimental group has less residual inflammation considering intensity. This may be due to the persistence of anti-inflammatory action of the root canal filler used in each group.

(3) Granulation tissue in the root canal wall

The presence of granulation tissue in the root canal wall was relatively low in both groups, ranging from minor to moderate. There is a high capacity to repair compared with mature adult teeth, yet because of the incomplete root formation with large open apical foramen, vigorous growth of granulation tissue probably occurred. In the present study, the low incidence of 33-40% is from necrosis due to tissue liquefaction, root canal filler and residual inflammation that took place during the formation of hard tissue involved in closure of the root apex.

(4) Alveolar bone resorption and deposition

Originally, bone tissues respond to local conditions and systemic functional requirement; thus, structural remodeling and resorption in opposite directions are constantly emerging. Accordingly, any observation about these changes must be expressed. In this study, we attempted to capture only the changes that seemed to be affected by this experiment. As a result, it was determined that the incidence of pathological alveolar bone loss is very weak; many of these bone tissues were present at the periphery of residual inflammation. On the other hand, alveolar bone deposition was seen at a very high rate; alveolar bone regeneration, in addition to the healing of periodontal tissue inflammation at the root apex, was observed and it was even active in adding new bone due to changes in morphology and function after treatment.

(5) Resorption of hard tissues at the root apex

Formation of soft tissue is relatively low at the root apex and is slightly more in the control group than in the experimental group. This change is often based on inflammatory changes seen at the periapical region, and thus a higher than moderate degree of hard tissue resorption at the root apex until the final observation day with residual inflammation was observed only in some patients.

2) Pathological findings regarding the closure of the root apex

(1) Closure of the root apex

Tables 3 and 4 show the closure of the root apex in different observation periods.

Complete closure of the apical foramen of root is expressed frequently in both control and experimental groups. However, if the sums of the partial and total closure are considered, closure is more frequently expressed in the experimental group. Also, no hard tissue formation was observed in only 2 cases in the experimental group compared to 13 cases in the control group, which is a relatively large difference. The experimental group showed an increased number of cases with complete closure due to the prolonged observation period. In contrast, in the control group, even with the extension of observation period, no increase in closure of the root apex due to hard tissue formation was observed at all. This occurred despite the temporary addition of newly-formed hard tissues after the initial treatment. With the extension of

Table 3 Closure of the root apex (experimental group)

Type of closure \ Observation periods (days)	30	60	120	180	Total
Hard tissue formation (−)	0	1 (5.0%)	0	1 (5.0%)	2 (2.5%)
Addition of some hard tissue	6 (30.0%)	9 (45.0%)	1 (5.0%)	1 (5.0%)	17 (21.3%)
Incomplete closure	13 (65.0%)	8 (40.4%)	14 (70.0%)	12 (60.0%)	47 (58.7%)
Complete closure	1 (5.0%)	2 (10.0%)	5 (25.0%)	6 (30.0%)	14 (17.5%)
No of cases	20	20	20	20	80

Table 4 Closure of the root apex (control group)

Type of closure \ Observation periods (days)	30	60	120	180	Total
Hard tissue formation (−)	0	3 (15.0%)	4 (20.0%)	6 (30.0%)	13 (16.3%)
Addition of some hard tissue	6 (30.0%)	4 (20.0%)	5 (25.0%)	3 (15.0%)	18 (22.5%)
Incomplete closure	13 (65.0%)	11 (55.0%)	4 (20.0%)	6 (30.0%)	34 (42.5%)
Complete closure	1 (5.0%)	2 (10.0%)	7 (35.0%)	5 (25.0%)	15 (18.7%)
No of cases	20	20	20	20	80

observation period, more patients had resorption of hard tissue in the presence of inflammatory change, which seems to be due to the deterioration of residual inflammatory tissues.

In permanent teeth with incompletely formed roots, calcium hydroxide preparation, when applied as a root canal filler after pulp extirpation and root canal therapy, can cause closure of the root apex, which is clear from previous research reports. However, the mechanism of hard tissue closure of the root apex has not been elucidated.

In this study, the continuous formation of root dentin in patients in non-vital teeth with incompletely formed roots was not observed; only the addition of hard tissue was observed in the root apex involved in its closure. Thus, in non-vital teeth, the involvement of Hertwig's epithelial root sheath can be considered. However, this experiment only focused on the effect of root canal filler in the treatment of infected canals after root canal therapy. However, after pulp extirpation, in the course of root apex closure after treatment, the involvement of Hertwig's epithelial root sheath cannot be denied.

(2) Closure of the apex by hard tissue formation

Most the hard tissues involved in closure of the root apex observed in this experiment are structurally similar to cellular cementum. During closure of the root apex with hard tissue and bone, if periodontal ligament was present, continuous closure formed by hard tissue and alveolar bone did not occur in any case. Moreover, such cases were observed by continuous closure of the hard tissue and by cementum-like tissues at the sides of the roots. The hard tissue closure by fibroblasts derived from periodontal ligament was supposed to form chalky cementum. In the present study, it further showed that formation of root canal dentin was observed in some cases. However, a very small mass of dentin was formed; most of the dentin is probably due to the remaining vital pulp at the time of pulp extirpation because root formation was incomplete

during treatment. Necrosis of the residual pulp or granuloma seemed to be formed when the root canal was opened. Thus, the dentin caused by the root canal filler and root canal therapy as well as the subsequent infection are thought to be extremely low. However, calcification of newly formed hard tissue was observed in the closure of the apex in many cases, and the tendency is very high, especially in the experimental group.

(3) Closure of the apex and the amount of root formation

Concerning the closure of the root apex, this study attempted to capture the difference in the amount of new hard tissue and its morphology in the control and experimental groups. The location of hard tissue formation relative to the position of the apical closure was classified according to the location of the hard tissue in the root canal (Figure 1-A), near the root apex (Fiure 1-B) or outside the root apex from the apical foramen (Figure 8).

Following the above criteria as shown in Table 5, the classification of hard tissue formation in relation to the root apex was examined in this experiment.

In most cases in the experimental group, hard tissues formed around the root apex, causing closure of the apical foramen in more than half of the cases. In contrast in the control group, hard

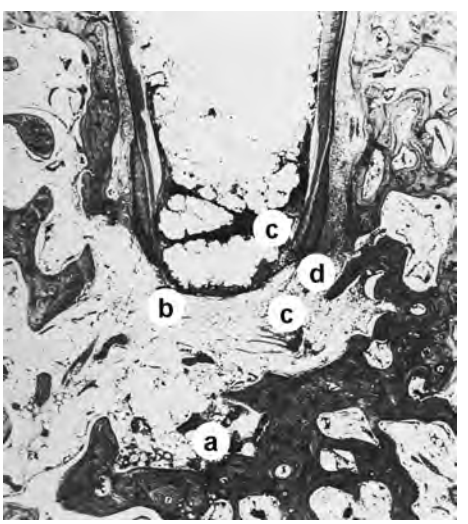

Figure 8 Case 78. Observation period: 180 days. Experimental, left mandibular PM3 dostal root, histopathological result: fair; a: penetrated root canal filling material, b: closing hard tissue, c: inflammatory cell infiltration, d: root apex hard tissue resorption

Table 5 Location of hard tissue formation in relation to root apex

Location / Study group	Within the root canal	At the root apex	Outside the root apex	No of cases with hard tissue closure
Experimental group	12 (15.4%)	48 (61.5%)	18 (23.1%)	78
Control group	9 (13.4%)	30 (44.8%)	28 (41.8%)	67

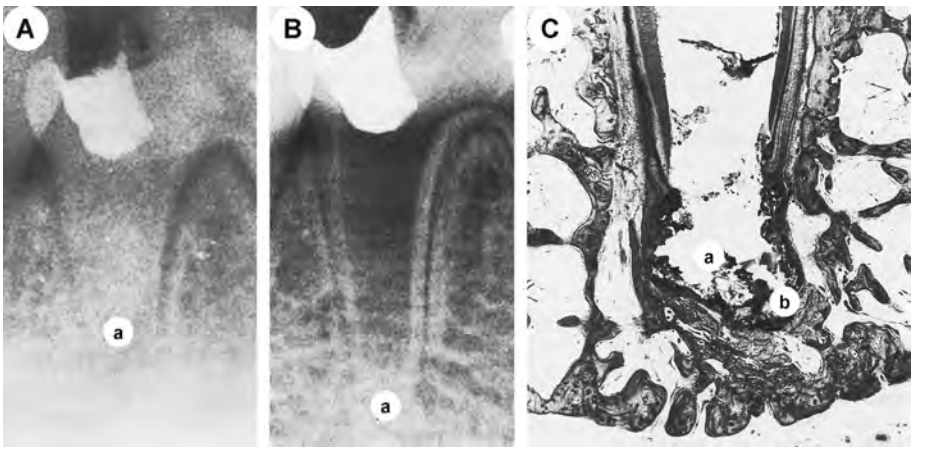

Figure 9 Case 56. Control, right mandibular PM4, distal root, observation period: 120 days, histopathological result: good.

A: Radiograph immediately after filling, a: no appearance of the filling material;

B: Radiograph at the day 120, a: radiopaque image due to hard tissue formation.

C: Histopathological photograph, a: Vacuoles due to disappearance of the penetrated root canal filling material, b: Calcium deposition and newly-formed hard tissue around the root canal filling material.

tissues formed at the root apex and outside the root apex. In the control group, hard tissue that formed outside the apex is more than in the experimental group. This is because the consistency of the filler used in the control group is difficult to manipulate and prone to overfilling because it is not radiopaque. Moreover, calcium hydroxide is less likely to be resorbed initially, as shown in Figures 9 and 10, and is believed to form a hard tissue closure which seems to encapsulate the root canal filler.

The form of hard tissue closure in each case in the experimental group is characterized by irregular tissue formation in large amounts, including the cementum-like hard tissue not in alignment with the layer of hard tissue. In addition, many of those were interspersed by soft tissues. In contrast, the control group is relatively regular in structure; the layer of hard tissue can be seen, and in many instances it took the form of a gentle curve or linear form.

As a means of determining the amount of hard tissue closure, we measured the diameter of the thickest part of the hard tissue formed. In each case of serial sections, which account for the central section of root apex, the thickest part was almost always measured at the middle third of the soft tissue closure. These measurements were calculated as the average value in each observation period. The results are shown in Table 6. In the experimental group, the amount of hard tissue in 60 days was less than in 30, 120, and 180 days. Significantly greater values were obtained in short-term cases. In the control group, the average value in each observation period showed higher values than did the experimental groups, and it showed an increased in amount with prolonged observation periods. Thus the control group was better and superior than the experimental group if only hard tissue formation were considered.

3) Summary of radiographic findings and root canal filler

The root canal filler used in this study, called Vitapex, has a very distinctradiographic contrast; thus, it is possible to determine the condition of the filler after root canal therapy. Calcium hydroxide-CMCP paste used in the control group, as shown in Figure 9, is not

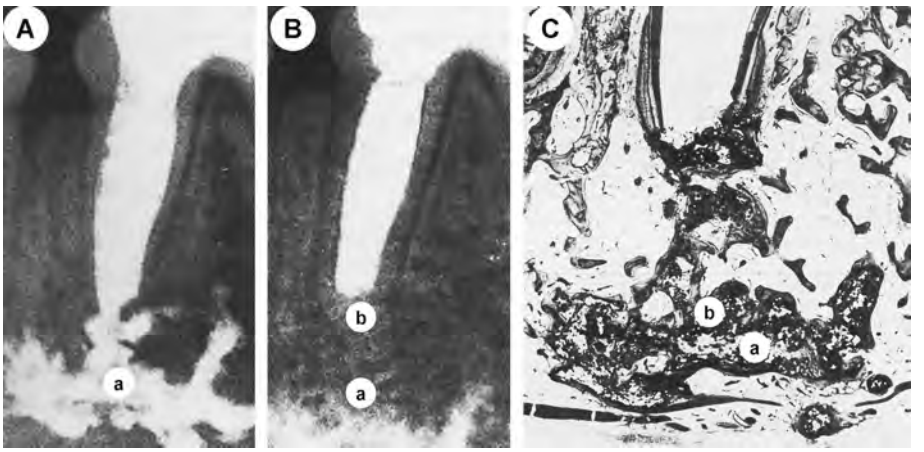

Figure 10 Case 47. Experimental, left mandibular PM4, mesial root, observation period: 120 days, histopathological result: fair.

A: Radiograph immediately after the filling; a: large amount of penetrated root canal filling material.

B: Radiograph at day 120; a: disappearance of radiopacity of the root canal material, b: radiopaque image due to hard tissue formation.

C: Histopathological photograph, a: penetrated root canal filling material, b: calcium deposition and alveolar bone around the root canal filling material.

Table 6 Average thickness of the hard tissue closure

Observation periods (days) / Study group	30	60	120	180	Average
Experimental group	0.34mm (0.339)	0.23mm	0.46mm	0.55mm	0.39mm
Control group	0.34mm (0.344)	0.73mm	0.60mm	0.68mm	0.48mm

radiopaque and confirmation of the condition of the root canal filler after treatment is almost impossible.

Therefore, in this section, the relationship between the radiographic findings and the condition after root canal therapy in experimental group was confirmed. Normalization was performed in relation to the morphology of the mandible in 2 cases in 30 days and 4 cases in 60 days because the area around the root apex could not fit completely in the radiograph and thus we could not obtain the findings. Radiographic findings were obtained in 76 cases, except in 4 or more cases.

Results on the condition of the root canal filler after treatment are shown in Table 7. The high incidence of overfilling may be due to the incompletely formed roots and the presence of lesions formed on the root end.

In cases of overfilling, radiographs revealed 40 cases of overfilling, except in 1 case, which showed loss of contrast. Figure 11-A, B shows excess amount of root canal filler. Even if there is loss of contrast inradiograph, the remaining root canal filler can still be seen histologically where in it is encapsulated by calcified tissue or bone, as shown in Figure 12. Therefore, intentional

Table 7 Condition of root canal filling in radiograph after treatment

Condition of root canal filler	No. of cases
Overfilled	40 (52.6%)
Flush	24 (31.6%)
Underfilled	12 (15.8%)
Total no. of cases	76

overfilling should be avoided.

Underfilling during root canal treatment can be observed in radiograph with the loss of root canal filler in some cases. However, underfilling is about 1 mm short from the root apex before and after the loss of contrast. When observed histologically, parts of the loss of root canal filler is occupied by the growth of soft tissue in the root canal wall in many cases. In this study, granulation tissue was not observed and root canal filler was observed to remain intact even though there was loss of contrast. Shibuya [48] reported that the loss of contrast is supposed to be due to the loss of iodoform.

In cases where underfilling was determined, the hollow portion of the root canal without contrast is hard to envision. In other words, granulation tissue in incompletely formed root that is considered to be relatively high during treatment of the infected root canal. Therefore, if these cases will be subjected to root canal treatment, radiograph would presumably reveal underfilling. We frequently observed such images in our histopathological examination.

2 Pathological results

1) Pathological scores

Pathological scores obtained in this experiment are shown in Table 8. Pathological score in the experimental group, in deciduous teeth of puppies that were anesthetized and immediate root canal filling with Vitapex suggest superior results compared to the results of Fuchino's study 6). Root canal treatment performed using this drug in the treatment of infected root canal in dog teeth showed almost similar results with Shibuya's study [48]. Overall, the experimental group is superior compared to the control group.

2) Relationship between pathological scores and observation period

The relationship between pathological scores and observation period obtained in this experiment is shown in Tables 9 and 10. In the experimental group, the total number of cases with good and fair pathological scores was almost the same in each observation period, with very little differences in each period. In the control group, the total number of cases with good and fair pathological scores was seen more in 30 days and is better than experimental group. However, in 60 days, the experimental group became better than the control group. In long-term cases in the control group, the pathological score became worse and gradually decreased with a prolonged observation period. Thus, the application of Vitapex in the experimental group is considered suitable root canal filler in infected root canals with incompletely formed roots in permanent teeth. In contrast, calcium hydroxide-CMCP paste applied to the control group is initially superior but becomes inferior with prolonged healing and in long-term application.

Table 8 Pathological scores

Type	No. of cases	Pathological scores		
		Good	Fair	Poor
Experimental group	80	51 (63.7%)	23 (28.8%)	6 (7.5%)
		74 (92.5%)		
Control group	80	50 (62.4%)	15 (18.8%)	15 (18.8%)
		65 (81.2%)		
Fuchino's study (1980) [46] Deciduous teeth	81	41 (50.6%)	20 (24.7%)	20 (24.7%)
		61 (75.3%)		
Shibuya's study (1980) [48] Permanent teeth	40	27 (67.5%)	11 (27.5%)	2 (5.0%)
		38 (95.0%)		

Table 9 Relationship between pathological scores and observation period (experimental group)

Observation periods (days) / Pathological score	30		60		120		180		Total	
Good	10 (50.0%)	19 (95.0%)	12 (60.0%)	19 (95.0%)	16 (80.0%)	19 (95.0%)	13 (65.0%)	17 (85.0%)	50 (62.5%)	74 (92.5%)
Fair	9 (45.0%)		7 (35.0%)		3 (15.0%)		4 (20.0%)		24 (30.0)	
Poor	1 (5.0%)		1 (5.0%)		1 (5.0%)		3 (15.0%)		6 (7.5%)	
No of cases	20		20		20		20		80	

Table 10 Relationship between pathological scors and observation period (control group)

Observation periods (days) / Pathological score	30		60		120		180		Total	
Good	16 (80.0%)	20 (100%)	15 (75.0%)	16 (80.0%)	16 (80.0%)	15 (75.0%)	7 (35.0%)	14 (70.0%)	51 (63.7%)	65 (81.2%)
Fair	4 (20.0%)		7 (5.0%)		3 (15.0%)		7 (35.0%)		14 (17.5)	
Poor	0		4 (20.0%)		5 (25.0%)		6 (30.0%)		15 (18.8%)	
No of cases	20		20		20		20		80	

3) Relationship between pathological score and condition of the root canal filling material

Table 11 shows the relationship between pathological score and condition of the root canal filling material. Cases with flush obturation have the highest tendency for a good pathological score while overfilling and underfilling resulted to lower pathological scores, both of which have

Table 11 Relationship between the condition of root canal filling and pathological scores(experimental group)

Condition of root canal filling material	No of cases	Pathological scores		
		Good	Fair	Poor
Overfilled	40	26	10	4 (10.0%)
		36 (90.0%)		
Flush	24	13	10	1 (4.2%)
		23 (95.8%)		
Underfilled	12	9	2	1 (8.3%)
		11 (91.7%)		

the same tendency.

4) Relationship between pathological score and closure of the root apex

Tables 12 and 13 show the relationship between the pathological score and closure of the root apex. Complete closure of the apex and good pathological score were the same for both groups with no evidence of soft tissue closure. All cases with no hard tissue formation were classified as poor in both groups. In other words, the results indicate a trend with good pathological score with complete closure of the apex.

3 Results of contact microradiogram and TC fluorescent labeling

Root formation in the control was observed as follows. Root canal dentin turned inward and formed downward and cementum also formed on the lateral part of the root during elongation of the root. The original root length was reached just before cementum formation increased; the length of root dentin formation was completed and cementum was added to cover the root apex. At root formation completion, dentin was further formed inward and cementum was added outside to prepare the outline of the root.

Table 12 Relationship between closure of the root apex and pathological scores (experimental group)

Closure of the root apex	No of cases	Pathological scores		
		Good	Fair	Poor
Hard tissue formation (−)	2	0	0	2 (100%)
		0		
Addition of some hard tissue	17	8	6	3 (17.6%)
		14 (82.4%)		
Incomplete closure	47	30	16	1 (2.1%)
		46 (97.9%)		
Complete closure	14	13	1	0
		14 (100%)		

Table 13 Relationship between closure of the root apex and pathological scores (control group)

Closure of the root apex	No of cases	Pathological scores		
		Good	Fair	Poor
Hard tissue formation (−)	13	0	0	13 (100%)
		0		
Addition of some hard tissue	18	10	7	1 (5.6%)
		17 (94.4%)		
Incomplete closure	34	26	7	1 (2.9%)
		33 (97.1%)		
Complete closure	15	14	1	0
		15 (100%)		

In the alveolar bone, growth of the jaw, active bone resorption and deposition occurred, especially if accompanied by physiologic tooth movement. Newly-formed bone was formed on the tension side. Tooth formation and bone growth and development were completed, and bone remodeling became weak; alveolar bone deposition was only being observed in a circumscribed region.

In contrast in the experimental group, the formation of dentin after treatment could not quite be seen; hard tissue formation was initiated near the root end closure formed at the root apex during healing. The increase in the thickness of hard tissues was prolonged. Lateral root cementum formation, although small, continued when compared to control group.

In alveolar bone, during growth and development of the jaw and associated changes, damage and repair might have occurred as a sense of modification in which there was no control and the addition of new alveolar bone in the lower portion was markedly produced. Furthermore, the healing of the tooth root due to the formation of new bone became slow and after a long period each alveolar bone might have increased in the density thereby increased in contrast.

Conclusion

The authors conclude the following based on the results. In both cases, the application of the root canal filler showed little addition of newly-formed hard tissue and did not bring about root apex closure. The addition of hard tissue after root canal filling was primarily due to cementum. In addition, through closure of the apex by hard tissue formation, a favorable outcome in patients is more regular and greater in the control group than in the experimental group. However, the control group had a higher percentage of hard tissue formed outside the apex close to the root tip than did the experimental group. In addition, the experimental group showed good results in each observation period; the control group showed better results in short-term cases than did the experimental group, but with an increased observation period, the poor pathological grade also increased.

Undecalcified specimens observed in contact microradiogram, as well as tetracycline labeling in the experimental group during the healing process, showed newly-formed hard tissue at the apex of the root. However, the normal root formation in the control group was not observed in the experimental group.

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