Chapter 6

Histopathological Studies of Pulpectomy in the Deciduous Teeth of Dogs

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《Abstract》

A study was performed to examine the impact of resorbable root canal filling material, Vitapex, containing calcium hydroxide and iodoform, on the physiological resorption of its roots, succedaneous permanent tooth buds and periodontal tissues. A total of 14 puppies aged 2-5 months were used; teeth were anesthetized, pulps were extirpated and immediate root canal filling was performed. These procedures were carried out in 53 deciduous teeth with 81 root canals, and contralateral intact teeth were used as controls. The animals were bred for 5 to 60 days and were tracked with radiographical and histological examinations.

Results obtained were the following:

1. The form of healing observed after the experimental procedures was divided into 2 groups: those healed either by remaining vital pulp tissue or by the periodontal ligament, with 17 and 29 cases, respectively.
2. The resorption of root canal filling material and the physiological root resorption were almost the same in the majority of cases.
3. The rate of physiological root resorption in the experimental group was slightly delayed compared to that of the control group.
4. The remaining pulp tissue lesions, either in short or long term cases, comprised 5 cases of congestion, 3 cases of hemorrhage, 5 cases of mild inflammation, 11 cases of purulent inflammation, and 1 case of atrophy.
5. Periapical lesions, either in short or long term cases, comprised 6 cases with congestion, 12 cases with mild inflammation, 17 cases with purulent inflammation and 7 cases with pathological alveolar bone resorption.
6. In most cases, the lesions did not significantly affect the permanent tooth buds, except for 6 of 81 cases.
7. Pathological grades obtained in this experiment, in a total of 81

cases, were good in 41 cases (50.6%), fair in 20 cases (24.7%) and a failure in 20 cases (24.7%).

8. The best pathological grades after the experimental procedure were obtained in cases in which the root canal filling material was limited within the canal itself (flush) and in those cases which were underfilled. Overextension of root canal filling should be avoided as much as possible.
9. The relationship between root status and pathological grades showed a slightly lower incidence and favorable results with physiological root resorption compared to those without physiological root resorption.
10. In this study, the use of Vitapex for immediate root canal filling after pulp extirpation in canine deciduous teeth produced good results. Vitality of the remaining pulp and periodontal tissue was restored with subsequent physiological root resorption and without adverse effect on succedaneous permanent tooth buds.

Introduction

The study of pulpectomy in deciduous teeth is still inadequate. This is because it is difficult for physiological root resorption of deciduous teeth and resorption of root canal filling material to occur at the same time. Furthermore, during pulpectomy root canals are complicated and hard tissue of roots forms thin walls. In addition, deciduous teeth have large canal openings when the tooth has incompletely formed roots or begins physiological root resorption. Moreover, the rate of root resorption varies in every deciduous tooth root. To confirm the position of the root canal filling and to determine the extent of the apical foramen and root resorption in radiographs is not easy because of the possibility of overlapping of deciduous teeth and permanent tooth buds.

During the treatment of infected pulp in deciduous tooth, it is best to consider physiological root resorption and vitality of the remaining pulp. Pulpotomy is recommended to save the remaining pulp as much as possible and if it is indicated [1-8]. Accordingly, there are many reports about this treatment done in deciduous teeth.

However, most cases encountered in clinical practice require pulpectomy for the treatment of infected pulp. Clinical studies on immediate root canal treatment in deciduous teeth after anesthesia and pulp extirpation [9-23], as well as experimental pathological studies [24-28], have not yet been reported.

The authors considered conducting a study using resorbable root canal filling material, Vitapex, based on calcium hydroxide and iodoform, for root canal filling on deciduous teeth through clinical observation and the use of clinical radiographs. We first reported the efficacy of Vitapex as root canal filling material in deciduous teeth [29]. Several studies subsequently reported using this material as root canal filling [30-39], and it is now being introduced for root canal treatment in pediatric dentistry [2, 7]. The effect of this material has been studied and reported in mature permanent teeth as root canal filling material, used both pathologically [40] and clinically with radiographic examination [41], and in immature permanent teeth with incompletely formed roots pathologically [42] and clinically with radiographic examination [43]. Tissue reactions have also been studied [44-47].

The authors confirmed histologically the validity of Vitapex as root canal filling material in

deciduous teeth of dogs where immediate root canal treatment was done after anesthesia and pulp extirpation. The impact of Vitapex on periapical tissues and succedaneous permanent tooth buds has been studied in detail histologically.

Examination methods

Fourteen mongrel puppies, about 2 to 5 months of age, were used in this experiment. A total of 53 deciduous teeth and 81 experimental root canals were obtained. The deciduous teeth were all from the mandibular arch as i1, i2, i3 and m2, m3. The experimental group consisted of 25 incisors with 25 root canals and 28 molars with 56 root canals. For the control, untreated teeth on the contralateral side were used. The control group had 49 teeth with 76 root canals consisting of 22 incisors with 22 canals and 27 molars with 54 root canals. A total of 102 teeth with 157 root canals were used in this study.

Animals were subjected to general anesthesia using 5% sodium pentobarbital intravenously. The oral cavity was washed with 2% hydrogen peroxide solution, and the surrounding teeth were sterilized with iodine solution. Local anesthesia was done with 2% Xylocaine. Then the experimental teeth were disinfected with 70% ethanol. Access preparation was done according to the prescribed method, the coronal pulp was removed, and the remaining pulp in the root canal was extirpated with a barbed broach. After pulp extirpation, the root canals were irrigated with 10% sodium hypochlorite and 2% hydrogen peroxide and dried with paper point. The canals were filled with Vitapex following the manufacturer's instructions. This was followed by zinc phosphate lining cement and amalgam filling to complete the treatment.

After treatment, root canal obturation was checked with a radiograph. The observation periods ranged from 5 to 60 days. After the experimental periods, the animals were sacrificed with large doses of 5% sodium pentobarbital. Mandibles were removed and immediately fixed in 10% neutral formalin. Radiographs were again taken in order to compare the root canal filling. After fixation and decalcification in formic acid formalin solution, the mandible was divided, embedded in paraffin wax, serial sections were cut and stained with hematoxylin and eosin.

Results of the experiment are presented below. For convenience in understanding postoperative changes, samples were divided into 3 groups: 1) 5 to 10 days, 2) 14 to 20 days and 3) 34 to 60 days.

Results

Histopathological examination focused on the remaining pulp, periodontal ligament, tooth root, alveolar bone and the permanent tooth buds. Pathological grades were determined according to tissue changes and the condition. Following are the grades and criteria.

Good – no or very minor inflammation, with remarkable healing and tissue repair, without presumed adverse effect on permanent tooth bud or root resorption of deciduous teeth

Fair – mild inflammation, tissues have a healing tendency over time, no resorption on permanent toot bud or root of deciduous teeth

Poor – marked inflammation, necrosis and tissue destruction, no chance or capacity for tissue repair, growth inhibition of the deciduous tooth root and permanent tooth bud, with pathological root resorption

For the controls, histological examination of dental hard tissues, dental pulp, periodontal ligament, alveolar bone revealed no abnormal tissue changes and the permanent teeth and tooth buds were omitted.

1) Cases 5 to 10 days postoperatively

Out of 23 cases, 14 were good (60.9%), 7 were fair (30.4%) and 2 were poor (8.7%).

Figure 1 shows the histopathological view of a case with a good result (case 10, mesial root of mandibular right m3, 7 days postoperatively). The present case has an incomplete root. Pulp extirpation was done in the root canal and radiograph shows underfilling of the root canal. No inflammatory change was observed in the periodontal ligament and alveolar bone around the root apex. Granulation tissue formation penetrating the surrounding periodontal ligament and tissue scarring were observed.

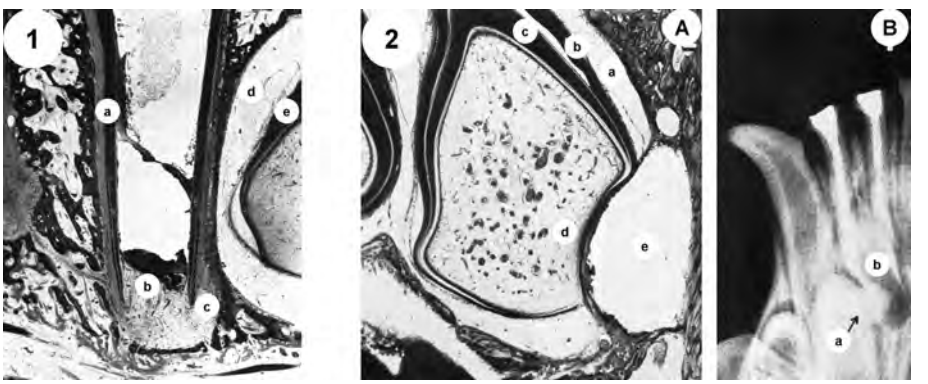

Figure 1 a: Deciduous tooth root; b: Root canal polyp showing fibrous change; c: Periodontal membrane; d: Dental follicle; e: Hard tissue formation of permanent tooth.

Figure 2 A: a: Dental follicle; b: Permanent tooth enamel; c: Permanent tooth dentin; d: Deformed permanent tooth due to the penetrating root canal filling material; e: Penetrating area of root canal filling material. B: Radiograph of day 5 after operation. a: Penetrating root canal filing material from root apex; b: Injured permanent tooth.

Figure 2 shows the histopathological view of a case with a poor result (case 2, mandibular right i3, 5 days postoperatively). After treatment, the apical foramen was filled with excess root canal filling material, which remained at the root tip, as seen in radiograph. Excess filling material caused pressure on the tooth bud of the succeeding permanent tooth bud sac, some of which disappeared and induced more deformation on the permanent tooth bud.

2) Cases 14 to 20 days postoperatively

Out of 27 cases, 11 were good (40.8%), 8 were fair (29.6%) and 8 were poor (29.6%). The following are the typical examples of this group.

Figure 3 shows the histopathological view of a case with a good result (case 36, mandibular right i1, 19 days postoperatively). This case has a complete root with underfilling of the root canal, as seen in the radiograph. However, histologically, no dead space was found at the root apex and so no underfilling was created. The pulp at the root tip was vital with incomplete dentin formation on the surface. There was no notable periodontal ligament change, alveolar bone resorption surrounding the root, resorption of deciduous root or subsequent damage to the growth and development of permanent tooth buds.

Figure 4 shows the histopathological view of a case with poor result (case 34, mesial root of mandibular right m3, 15 days postoperatively). The root was incomplete, and radiograph immediately taken after treatment showed underfilling of the root canal. The dentin wall was

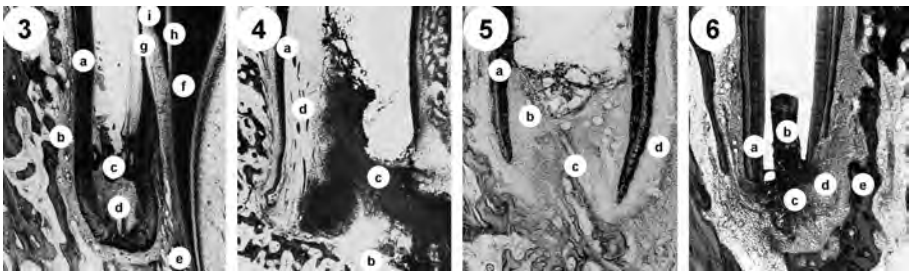

Figure 3 a: Deciduous tooth root; b: Periodontal membrane; c: Uncompleted dentin bridge; d: Apical dental pulp; e: Dental follicle; f: Permanent tooth dentin; g: Physiological tooth root resorption; h: Crack due to artifact; i: Permanent tooth enamel.

Figure 4 a: Thin dentin of the deciduous tooth root; b: Alveolar bone resorption; c: Suppuration; d: Remaining vital pulp.

Figure 5 a: Deciduous tooth root; b: Root canal polyp showing fibrous change; c: Formation of alveolar bone into root canal; d: periodontal membrane.

Figure 6 a: Resorbed deciduous tooth root; b: Young root canal polyp; c: Suppuration; d: Inflammatory cell infiltration; e: Alveolar bone.

very thin; the other side of the pulp canal near root tip was very large and therefore was not removed along the root canal wall. Purulent nodules were detected in the remaining pulp and in periodontal ligament. Purulent materials surrounding the bone and alveolar bone resorption were also observed.

3) Cases 34 to 60 days postoperatively

Out of 31 cases, 16 were good (51.6%), 5 were fair (16.1%) and 10 were poor (32.3%). The following are typical examples of this group.

Figure 5 shows the histopathological view of a case with a good result (case 65, distal root of mandibular right m2, 42 days postoperatively). This case has an incomplete root; the radiograph shows underfilling of root canal. Proliferation of granulation tissue surrounding the apical foramen with scar formation can also be seen. Active proliferation and hyperplasia of the alveolar bone in the granulation tissue has reached the edge of the root canal and entered into it.

Figure 6 shows the histopathological view of a case with poor result (case 52, mandibular right i3, 34 days postoperatively). This example shows underfilling of the root canal in the radiograph taken immediately after treatment. However, histopathological examination 34 days after treatment showed root resorption, inflammatory cell infiltration and suppuration in the root canal as well in the periodontal ligament of the root apex. Granulation tissue from the immature periodontal ligament entered the root canal.

Discussion

❶ Form of healing

The healing that was observed after pulpal extirpation and immediate root canal filling was divided into 2 types: that with vitality of the remaining pulp tissues, and that with healing of the periodontal ligament.

During healing of the remaining pulp tissue, vitality of the pulp was maintained; scarring and formation of dentin tissue on the surface exposed to the vital pulp occurred during the process. Figure 7 shows the morphology of a case with healing of the remaining vital pulp. In some cases with vital pulp remaining at the root end, the surface was formed by incomplete

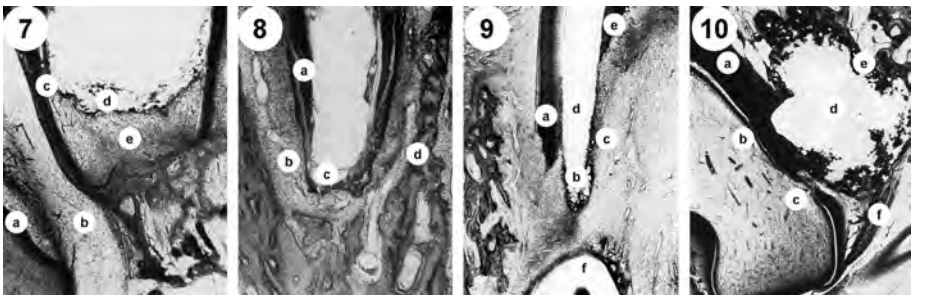

Figure 7 a: Hard tissue of permanent tooth; b: Dental follicle; c: Deciduous tooth root; d: Uncompleted dentin bridge; e: Remaining vital pulp.

Figure 8 a: Deciduous tooth root; b: Periodontal membrane; c: Uncompleted cementum bridge; d: Alveolar bone.

Figure 9 a: Resorbed deciduous tooth root; b: Remaning root canal filling material after tooth resorption; c: Tissues attched the root canal filling material; d: Empty area due to the disappearance of root canal filling material at the histopathological preparation; e: Resorbed deciduous tooth root; f: Permanent tooth enamel area.

Figure 10 a: Hard tissues of permanent tooth; b: Odontoblast layer of permanent tooth; c: Deformed permanent tooth due to the penetrating root canal filling material; d: Penetrating area of root canal filling material; e: Calcified deposition around the penetrating area; f: Alveolar bone.

Table 1 Form of Healing

Healing type	Remaining vital pulp type	Periodontal ligament type	Others	Total
Number of cases	17 (21.0%)	29 (35.8%)	35 (43.2%)	81

dentin tissue.

During healing of the periodontal ligament, granulation tissue entered the root canal with scarring of the periodontal ligament. At that time, the apical foramen was closed at the end of hard tissue formation or during the process. Figure 8 shows that pulp extirpation was done almost entirely of the root tip. The apical foramen was observed with incomplete root by cementum formation, but periodontal ligament took the form of healing and brought about scarring of the periodontal ligament.

In 81 root canals studies, 17 cases showed vitality of the remaining pulp as a form of healing, and 29 cases showed healing of the periodontal ligament (Table 1). However, in the remaining 35 cases, a form of root resorption took place at that moment and was classified as unknown, or else no healing took place.

2 Relationship between physiological root resorption and resorption of root canal filling material

In this study, cases with physiological root resorption were observed in 30 out of 81 cases radiographically and histopathologically. However, after observations were made, only 16 cases had not resorbed the filling material but had made physiological root resorption.

The rate at which root canal filling material resorption reached the physiological root resorption was observed in 14 cases radiographically and histologically. As shown in Table 2, in

Table 2 Relation between the physiological root resorption and root canal filling material resorption

Resorption speed	Same as root resorption	Ahead of root resorption	Delay from root resorption	Total
Number of cases	10 (71.4%)	2 (14.3%)	2 (14.3%)	14

10 cases the rate of resorption of the root canal filling material was the same as the physiological root resorption. A slightly faster physiological root resorption occurred in 2 cases. Furthermore, as shown in figure 9, resorption of the filling material somewhat delayed physiological root resorption in 2 cases.

Although physiological root resorption was not recognized after treatment and at the end of the experiment in radiographs, the filling material was resorbed in 9 cases. Radiographs showed underfilling of the root canal, and it seems that the disappearance of the iodoform was the cause of the lack of necessary radiographic contrast.

3 Rate of physiological root resorption of experimental and control teeth

Table 3 shows the physiological root resorption rate of the experimental and control teeth observed radiographically and histologically. Root resorption in the control teeth took place in 16 out of 30 cases. A more rapid rate of resorption was observed in experimental teeth in 2 cases. The remaining 12 cases showed a similar resorption rate.

In conclusion, the experimental non-vital teeth have a greater tendency toward slightly delayed physiological root resorption than control vital teeth.

Table 3 Rate of physiological root resorption of experimental and control teeth

Rate of resorption	Nearly equal	Ahead of experimental	Ahead of control	Total
Number of cases	12 (40.0%)	2 (6.7%)	16 (53.3%)	30

4 Pathological condition

1) Lesions in the remaining vital pulp

Major lesions observed in the remaining vital pulp tissue are shown in Table 4. Five cases had congestion, 3 cases had hemorrhage, 5 cases had mild inflammation, 11 cases had suppurative inflammation, and 1 case had atrophy.

The difference between lesions in the remaining vital pulp in short and long-term experimental periods was not specified. This is because the relatively large number of cases of root resorption appeared to be expected in long-term cases.

2) Incidence of lesions in the periapical tissues

Table 5 shows the major lesions found at the periapical tissues. These are 6 cases of congestion, 12 cases of mild inflammation, 17 cases of suppurative inflammation and 7 cases of pathological alveolar bone resorption. No case of hemorrhage was found.

Table 4 Histopathological changes appearing in the remaining vital pulp

Histopathological changes	Experimental periods	5-10 days	14-20 days	34-60 days
	Number of cases	23	27	31
Congestion	5	1	0	4
Hemorrhage	3	2	0	1
Mild inflammation	5	1	3	1
Suppurative inflammation	11	3	4	4
Atrophy	1	0	0	1

Table 5 Histopathological changes appearing in the periapical tissues

Histopathological changes	Experimental periods	5-10 days	14-20 days	34-60 days
	Number of cases	23	27	31
Congestion	6	6	0	0
Hemorrhage	0	0	0	0
Mild inflammation	12	3	6	3
Suppurative inflammation	17	0	7	10
Alveolar bone resorption	7	2	4	1

Periapical lesions were observed in both short and long term cases. Congestion was observed in short-term cases only. Suppurative inflammation was observed in many long-term cases; in 4 cases it even reached the bone.

Ankylosis was not observed at the root apex, but was observed at the center of the cervical root in 6 out of 81 cases.

5 Impact on the permanent tooth bud

An effect on permanent tooth buds was observed in 6 out of 18 cases. Table 6 shows the results. Among those, 4 cases had changes in enamel epithelium, 5 had changes in dental follicle and 2 had hard tissue disorders. However, most did not reveal a significant impact on the permanent tooth buds. However, as shown in Figure 10, extravagated root canal filling material caused the failure of the permanent tooth bud to procedure hard tissue formation, thus caution must be observed.

Table 6 Effect on the permanent tooth buds

Experimental periods	Number of cases	Enamel epithelium	Dental follicle	Hard tissue disorder
5-10 days	23	0	1	1
14-20 days	27	2	2	0
34-60 days	31	2	2	1
Total	81	4	5	2

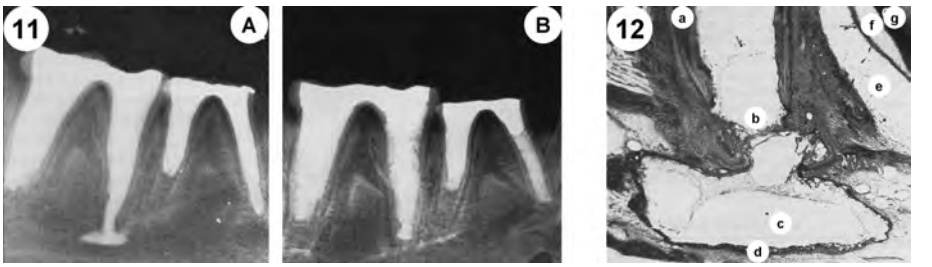

Figure 11 A: Radiograph of just after the operation. Over-filling of the root of right mandibular third molar; B: Radiograph of day 10. Disappearance of the penetrating root canal filling material.

Figure 12 a: Deciduous tooth root; b: Root apex. c: Area of penetrating root canal filling material; d: Calcified zone around the area; e: Dental follicle; f: Enamel; g: Hard tissue of permanent tooth.

6 Summary of the pathological results

Table 7 shows all pathological results obtained in this experiment. Of 81 cases, 41 obtained good results (50.6%), 20 obtained fair results (24.7%) and 20 obtained poor results (24.7%). Therefore, satisfactory results were obtained in 61 cases (75.3%).

Table 7 Pathological results

Results	Good	Fair	Poor
Number of cases	41	20	20 (24.7%)
	61 (75.3%)		
Total	81		

7 Relationship between conditions of the root canal filling immediately after treatment and pathological results

After treatment, radiographs were immediately taken to compare the relationship between pathological results and conditions of the root canal filling. After root canal treatment, the conditions were divided into 3 types and we examined the relationship between the grade and pathological conditions.

Table 8 shows the results. Specifically, in 12 cases with overfilled root canals, 2 were good (16.7%), 4 were fair (33.3%) and 6 were poor (50.0%). In addition, in 14 cases where root canal filling was flush, 9 were good (64.3%), 4 were fair (28.6%) and 1 was poor (7.1%). Of 55 underfilled cases, 30 were good (54.6%), 12 were fair (21.8%) and 13 were poor (23.6%).

In short, the best pathological results were obtained in root canals filled just enough, followed by a slight underfilled root canal. In case of overfilling, half of the cases (6/12 cases [50. 0%]) were associated with unfavorable results. Thus, excessive root canal filling should be avoided as much as possible.

Although in 12 cases extravasations of the root canal filling material were observed in the radiograph, at the end of the experiment, excess root canal filling material was not fully resorbed in 4 cases, as shown in Figure 2. However, a radiograph of the case, Figure 11A, shows excess root canal filling material at the edge of the foramen. However, at the end of day 10 of the experimental period, excess root canal filling material had been resorbed, as shown in Figure

Table 8 Pathological results of groups by filling appearance examined by X-ray photography immediately after treatment

Filling appearance	Number of cases	Pathological results		
		Good	Fair	Poor
Over	12	2	4	6 (50.0%)
		6 (50.0%)		
Flush	14	9	4	1 (7.1%)
		13 (92.9%)		
Under	55	30	12	13 (23.6)
		44 (76.4%)		

11B. In Figure 12, after root canal treatment, the presence of histopathological cavity adjacent to the foramen led to almost the same morphology, with the root apex showing overextended filling, while in proliferation of calcified bone around the apex, it was not observed at all. Based on the above findings, possible overfilling should be avoided. However, it is quite difficult to perform root canal filling exactly the length of the root. Overfilling was completely replaced by granulation tissues, and wound healing was observed in long-term cases.

8 Relationship between conditions of the root and pathological results

The cases in this experiment were divided into 2 types: cases with physiological root resorption, and cases without during experimental periods. We investigated the relationship between conditions of the root and pathological results observed in this study. To determine the status of the root, radiographs were taken immediately after treatment and at the end of the experimental period, and correlated with histological findings.

Table 9 shows the results. Specifically, in 51 cases without physiological root resorption, 28 were good (54.9%), 11 were fair (21.6%) and 12 were poor (23.9%). In 30 cases observed with physiological root resorption, 13 were good (43.3%), 11 were fair (21.6%) and 12 were poor (23. 9%).

Therefore, those with physiological root resorption had a slightly lower incidence and favorable pathological grade, and a slightly higher incidence of fair and poor pathological grades. For this reason, cases that exhibit root resorption seemed to be influenced by the contact with root canal filling material being constantly absorbed by the resorbing tissue. However, root

Table 9 Relation between conditions of the root and pathological results

Condition of the root	Results / Number of cases	Good	Fair	Poor
Non physiological root resorption	51	28 (54.9%)	11 (21.6%)	12 (23.5%)
Physiological root resorption	30	13 (43.3%)	9 (30.3%)	8 (26.7%)
Total	81	41 (50.6%)	20 (24.7%)	20 (24.7%)

resorption of deciduous teeth in young dogs progresses in the short term more rapidly than in humans. In case of root resorption of human deciduous teeth, the impact of root canal filling material on resorption is probably lesser than in young dogs.

Conclusions

In this study, the use of Vitapex adopted in root canal filling was done immediately after use of anesthesia and pulp extirpation in deciduous teeth of puppies and produced good results. The vitality of the remaining pulp was restored with subsequent physiological root resorption and without adverse effects on permanent tooth buds.

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