FULL PAPER *Surgery*

Efficacy of Enamel Matrix Proteins on Apical Periodontal Regeneration after Experimental Apicoectomy in Dogs

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ABSTRACT. Adult dogs have a complex apical delta structure in all root apexes of teeth. This complex structure may affect the formation of apical lesions in the teeth such as apical abscesses. The purpose of this study was to evaluate the efficacy of enamel matrix protein (EMP) which was used for periodontal regeneration therapy after an experimental apicoectomy for an assumed apical lesions of the teeth in dogs. The maxillar canine roots and maxillar fourth premolar buccal mesial roots in five beagles were experimentally apicoectomized under general inhalation anesthesia. After the root apex was exposed and excised, EMP was applied on the surface of the exposed dentin. After 12 weeks, dogs were euthanized, and the experimental teeth together with the surrounding soft and hard periodontal tissues were collected for histological evaluation under a light microscope. In the EMP group, the size of the defect where the root apex was removed was smaller than that of the control group. New cementum was dominantly achieved in the EMP group compared to the control group. Furthermore, new collagen fibers that bridged area between the new cementum and new alveolar bone were detected only in the EMP group. The present results demonstrated marked apical periodontal regeneration after apicoectomy in the EMP group. These results, therefore, suggest that the application of EMP can effectively induce the regeneration of periodontal structures in apicoectomized dogs.
KEY WORDS: apical lesion, apicoectomy, canine, enamel matrix protein.

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In dogs, apical lesions (apical abscesses) frequently result in toxic tissues and products of bacterial breakdown that can drain to the ventral sites of the orbit and the root apex part of the canine eminence [5, 10, 18]. The apical delta is a complex structure of the root apex in adult dogs, and this may be a factor in causing these apical lesions of the tooth [26]. The presence of many blood vessels and nerve passages through the apical delta makes it difficult to eliminate apical lesions completely by routine root canal treatment [1, 7, 14, 19, 23–26]. We confirmed that mature apical delta was formed in all roots of the teeth in beagle dogs over 8 months of age [27]. Apicoectomy is indicated as a surgical endodontic therapy for the elimination of apical lesions [5, 10, 18]. This treatment repairs the periodontal tissues of the root apex, but does not induce regeneration of the original structure [2, 3, 6]. The goal in human dental therapy is now not only repairing defective tissue but also inducing regeneration of it. Periodontal tissue regeneration of the root apex in dogs is not expected by routine surgical treatment. It has been suggested that the tissue healed by regeneration is more resistant to recurrence than that of the tissue healed by repair [28, 29].

It has been reported that enamel matrix protein (EMP) applied onto the surface of the tooth root induces a series of phenomena leading to the regeneration of the adhesion system of the periodontal tissues and provides an environment for periodontal regeneration [8, 9, 15–17, 20, 21, 28]. EMP is known to have a common molecule structure in most mammals [4, 22, 30]. Recently, EMP extracted and refined from the tooth germ of the piglet has been applied for periodontal regeneration therapy in human dentistry.

This study focused on the efficacy of EMP used for periodontal regeneration therapy and histologically investigated apical periodontal regeneration after experimental apicoectomy as a surgical endodontic therapy in dogs.

MATERIALS AND METHODS

Animals: Five clinically healthy beagles, 4 males and 1 female weighing 9–13 kg and 14–31 months of age were used for the experiments.

All experiments were done according to the approved guidelines in the handling of experimental animals at the Graduate School of Veterinary Medicine, Hokkaido University.

Pretreatment of the tooth root: The left and right maxillary canine and maxillary fourth premolar mesiobuccal tooth roots were used for the experiments. Seven days before the experimental apicoectomy, the pulps of these roots were extirpated, and their root canals were obturated with a gutta percha point under general inhalation anesthesia.

EMP preparation: EMDOGAIN® (BIORA AB, Malmö, Sweden) was used as the EMP. Thirty mg of the freeze-dried enamel matrix derivative preparation was reconstituted with 1.0 m*l* of propylene glycol alginate solution according to the operating instructions at 15 min before the application.

Surgical procedures: The surgical procedure is shown in Fig. 1. Experimental apicoectomy was done under general

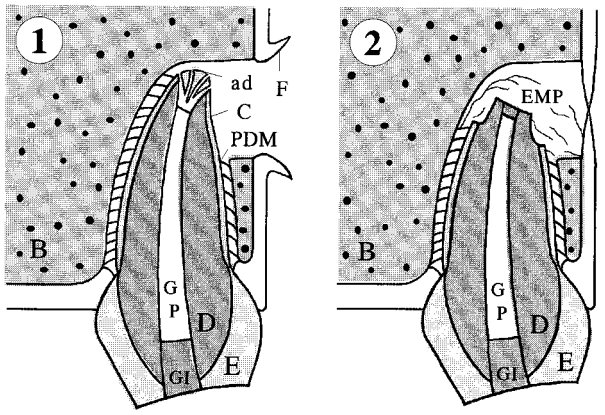

Fig. 1. Surgical procedure. 1: A flap was formed and the gum along the root apex was cut to expose the alveolar bone covering the root apex of these tooth. A dental bur was used and 13 mm and 10 mm holes were opened to reach the root apex of the canine and premolar teeth, respectively. After being exposed, the root apex was cut 4 mm or 5 mm from the tip, the exposed root canal was closed with glass ionomer cement, and the exposed side of the remaining root apex corresponding to the tooth cement was removed for 3 mm and 4 mm for the premolar and canine tooth, respectively. 2: The flap was returned to the original position, and sutured using a 5–0 nylon suture. EMP was then injected into the defect space through the gap of the suture in the EMP group. In the control group, application of EMP was done alternately such that when the tooth of one side was used as a control while its counterpart on the other side received EMP. ad: apical delta, B: alveolar bone, C: cementum, D: dentin, E: enamelum, EMP: enamel matrix protein, F: flap, GI: glass ionomer cement, GP: gutta-percha point, PDM: periodontal membrane.

inhalation anesthesia at 7 days after the preparative surgery. The animals were intramuscularly injected with atropine sulfate (0.03 mg/kg) and intravenously (iv) injected with flunitrazepam (0.03 mg/kg). After 10 min, the animals were injected intravenously with thiopental-Na (10 mg/kg) and intubated. Anesthesia was maintained with oxygen and isoflurane. Lidocaine containing epinephrine as a local anaesthetic agent was injected into the surgical area of the gum.

The right and left maxillary canine and maxillary fourth premolar buccomesial-laterostral roots of the dogs were apicoectomized. EMP was then injected into the space of the defect through the gap of the suture in the EMP group. Their counterparts on the other side were used for the control with no EMP.

Postoperative management: The animals were administered ampicillin (20 mg/kg, iv) preoperatively and for 3 days postoperatively to prevent infection, and flunixin meglumine (1 mg/kg, subcutaneously) for 3 days postoperatively as an analgesic. Disinfection of the oral cavity was done by glucuronic acid chlorhexidine for 3 days postoperatively after meals. The dogs were fed a dry diet.

Clinical evaluation: Radiographs were taken at the perioperative oral inspection, before and immediately after surgery, and then every 1 week until the 12th week. On the oral inspection and treatments, the animals were sedated with iv injection of medetomidine hydrochloride (0.03 mg/kg) and midazolam (0.15 mg/kg).

Histologic examination: At 12 weeks postoperatively, the dogs were euthanized by intravenous injection of an excessive dose of an anesthetic agent. The jaws containing the experimental teeth with adjacent teeth and alveolar bone were examined. The tissues were fixed in 10% buffered formalin, decalcified in 10% formic acid and embedded in paraffin. The serial sections of the roots were cut at 5 μm in thickness parallel to their long axes in the buccopalatine plane and were stained with hematoxylin and eosin solutions. The apical periodontal region was then examined under a light microscope at $\times 40$ magnification. The widest samples with root canals in the serial sections were used for the histologic evaluation according to the observation items shown below.

Observation items:

1) The size of the defect where the root apex was removed

The distance between the middle point of the excision line of the root apex and the newly formed tissue was measured as shown in Fig. 2.

2) Evaluation of the newly formed cementum and collagen fibers (Fig. 2)

The new cementum and collagen fibers bridging the area between the new cementum and alveolar bone were evaluated.

Statistical analysis: Results for the EMP and control groups are expressed as mean ± standard deviation (SD), and their data were analyzed using the Mann-Whitney *u*-test. Differences were considered significant at $P<0.05$.

RESULTS

The findings of oral inspection: Sutures in the mucosal flap could be removed at 1 or 2 weeks after surgery in both the EMP and control groups, and no infection of surgical area was observed on oral inspection in any dog. On palpation of the alveolar bone holes of the gums, the margin of the bone defect was evident at the 1st week, slightly palpable at the 2nd week, and unpalpable at the 3rd week after operation in both groups.

Radiographic findings of the root apex: The bone defect space became gradually smaller until 12 weeks, but there was no obvious difference between the two groups.

Histologic findings:

1) The defect size of the roots

Histologically, defect size of the root apex was smaller in the EMP group than in the control group (Fig. 3).

In the canine root, the distances between the root apex excision lines and the newly formed tissues for the EMP and control groups were 0.35 ± 0.16 mm and 1.15 ± 0.53 mm, respectively (Table 1). The size in the EMP group was significantly smaller than that in the control group ($P<0.01$). In the maxillary fourth premolar root, the distances in the EMP and control groups were 0.25 ± 0.10 mm and 0.54 ± 0.39

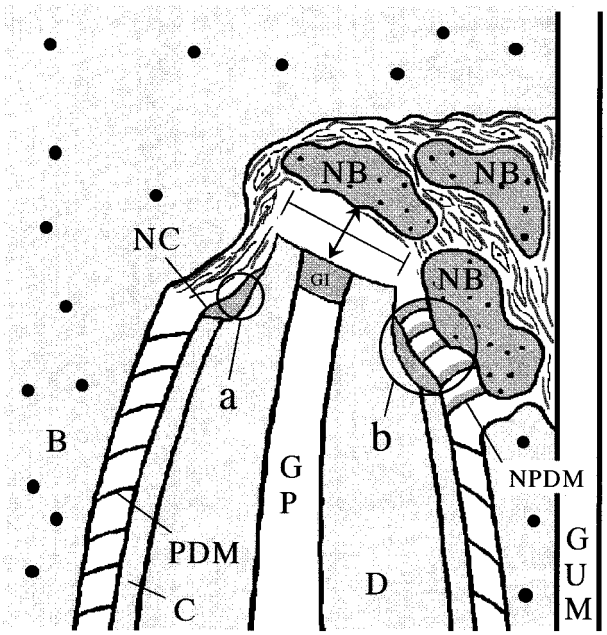

Fig. 2. The methods for evaluation of the size of the defect after the root apex was removed and for the newly formed tissues. ⊢⊣: Diameter of the dentin exposed after the root apex and the apical cementum were removed. ←→: Distance between the central point of the excision line of the root apex and newly formed tissue in the right angle (measurement position). B: alveolar bone, NB: new alveolar bone, C: cementum, NC: new cementum, D: dentin, GI: glass ionomer cement, GP: gutta-percha point, GUM: gum, PDM: periodontal membrane, NPDM: new periodontal membrane (the formation of the new collagen fibers bridged the area between the new cementum and the newly formed alveolar bone) a: the formation of new cementum b: the formation of new cementum and new collagen fibers bridging the area between the new cementum and the newly formed alveolar bone.

mm, respectively. The defect size of all roots in the control group was bigger than that of the contralateral EMP-treated roots.

2) Histlogic findings of the newly formed cementum and collagen fibers

In the EMP group, the newly formed alveolar bone covered the surface of the dentin exposed by the apicoectomy, and the new cementum bridged the area between the exposed dentin and the new alveolar bone (Fig. 4). Additionaly, newly formed collagen fibers extending from the inner layer of the new cementum to the new alveolar bone were observed in several cases of the EMP group, but were not in the control group, while irregular collagen fibers encapsulating the root were observed. New alveolar bone approaching along the surface of the exposed dentin was not observered either (Fig. 5).

Formation of new cementum was found in 8 of the 10 roots in the EMP group, and in 4 of the 10 roots in the control group. The newly formed collagen fibers bridged the area between the new cementum and the new alveolar bone in 7 of the 10 roots of the EMP group, but in none of those in the control group (Table 2).

DISCUSSION

Apical lesions of the maxillary fourth premolar (buccomesial-laterostral root), the maxillary canine, and the mandibular canine teeth are common in dogs [10]. In this study, these 2 kinds of teeth were therefore used for the experimental endodontic therapy.

It was described that 98% of the apical ramifications and 93% of the collateral branches in tooth roots can be removed when the root is cut 3 mm distant from the root apex in humans [12, 13], so removal of the affected root apex is recommended to be at least this distance from the root apex.

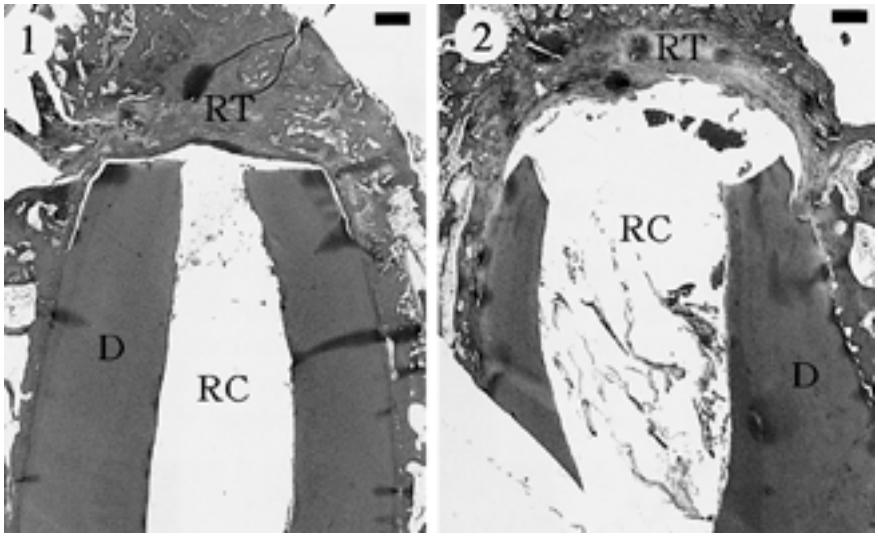

Fig. 3. Histologic findings of the defect where the root apex was removed. In the EMP group, the size of the defect where the root apex is removed was smaller than that in the control group. D: dentin, RT: repaired tissues, RC: root canal, bar=1 mm 1: EMP group, dog No. 2, canine tooth 2: control group, dog No. 4, canine tooth.

Table 1. The size of the defect where the root apex was removed

		Dog No.					
		1	2	3	4	5	M ± SD
Maxillar canine	EMP group	0.15	0.35	0.30	0.35	0.60	0.35 ± 0.16*
	Control group	0.40	1.00	1.05	1.80	1.50	1.15 ± 0.53
Maxillar fourth premolar	EMP group	0.40	0.20	0.20	0.15	0.30	0.25 ± 0.10
	Control group	0.50	0.25	1.20	0.25	0.50	0.54 ± 0.39

The data are shown as the distance (mm) between the middle point of the excision line of the root apex and the newly formed tissue was measured as shown in Fig. 2.
M ± SD: mean ± standard deviation. * $P<0.05$.

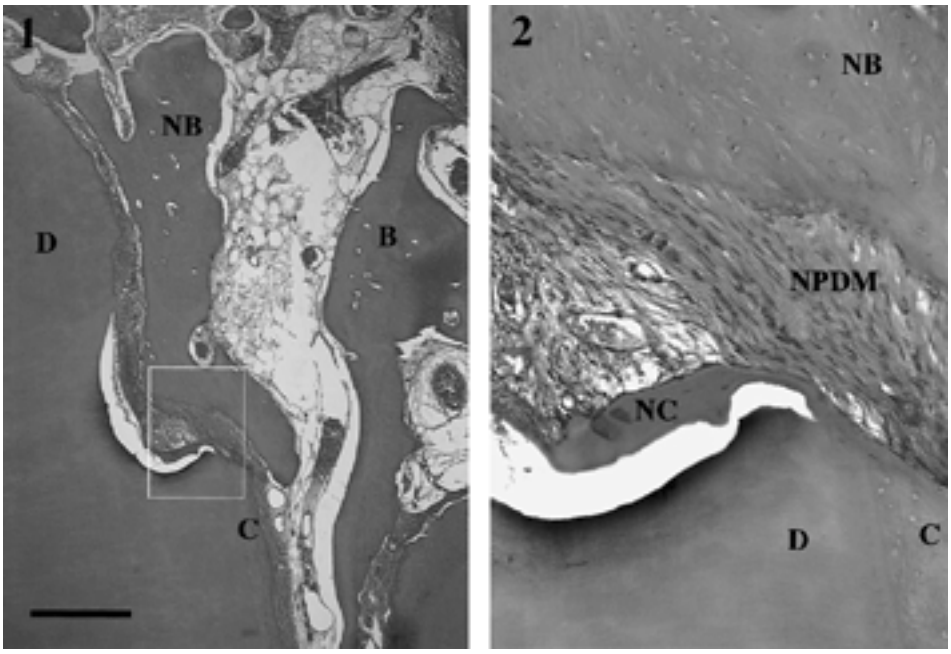

Fig. 4. Histologic findings of the exposed dentin where the cementum was taken in the EMP group. In the EMP group, the new alveolar bone approaches along the surface of exposed dentin where the cementum was burred, and the new cementum is seen between them. Furthermore, new collagen fibers which extended from the inner layer of the new cementum to the new alveolar bone can be observed. There is no infiltration of immune-related cells. B: alveolar bone, NB: new alveolar bone, C: cementum, NC: new cementum, D: dentin, NPDM: new periodontal membrane (the formation of the new collagen fibers bridging the area between the new cementum and the newly formed alveolar bone), bar=0.5 mm. 1: EMP group, dog No. 1, canine tooth, 2: EMP group, enlargment of the area inside the white rectangle of "1".

Gamm *et al*. reported that the lengths of most apical deltas from the root apex in canine teeth were less than 3 mm in dogs [7]. In our previous report [27], apical deltas in canine teeth and in the premolar teeth were found to be less than 5 mm and 4 mm distant from root apex, respectively. The root apex was therefore removed 5 mm from the root apex in the canine teeth and 4 mm from the apex in the premolars in this study.

Histologically, the size of the defect in apicoectomized region was smaller in the EMP group than in the control group. Distance from the root apex excision line to the newly formed tissue was significantly shorter in maxillar canine root of the EMP group. In the EMP group, newly formed cementum was observed in 80%, and collagen fibers extending from the inner layer of it to the new alveolar bone were found in 70% of the cases. On the other hand, newly formed cementum was found in 40% of the cases, but collagen fibers extending from the inner layer of the newly formed cementum to the new alveolar bone were not seen in any cases of the control group. These findings suggest that the defective region after apicoectomy was rapidly replaced with newly formed cementum, alveolar bone and collagen fibers that connected with each other as a result of the application of EMP.

The EMP used in this study is known to play an important role in the regeneration of the original structure of the peri-

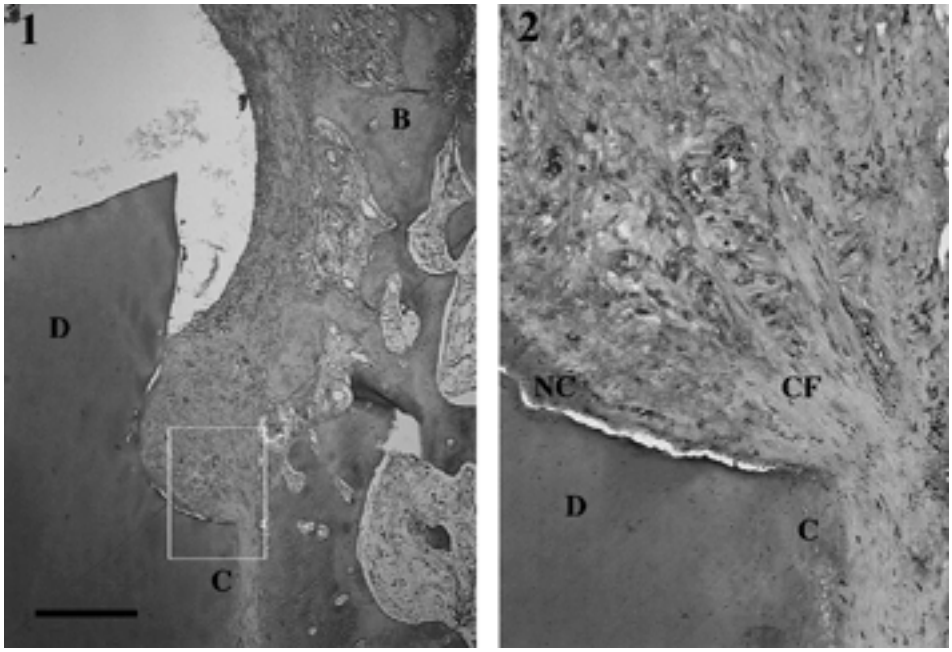

Fig. 5. Histologic findings of the exposed dentin where the cementum was taken in the control group. New cementum is observed, but collagen fibers extending from the inner layer of the new cementum to the new alveolar bone are not seen, and irregular collagen fibers which capsuled the root are found. B: alveolar bone, C: cementum, NC: new cementum, D: dentin, CF: capsuled fibers, bar=0.5 mm. 1: control group, dog No. 4, canine tooth, 2: control group, enlargment of the area inside the white rectangle of "1".

Table 2. The evaluation of the newly formed cementum and collagen fibers

		Dog No.					
		1	2	3	4	5	M ± SD
Maxillar canine	EMP group	2	2	2	2	0	1.60 ± 0.89
	Control group	1	0	0	1	1	0.60 ± 0.55
Maxillar fourth premolar	EMP group	2	2	2	1	0	1.40 ± 0.89*
	Control group	0	1	0	0	0	0.20 ± 0.45

Score
2: Formation of new cementum and new collagen fibers between the new cementum and the new alveolar bone were seen (Fig. 2-b).
1: Only formation of the new cementum was observed (Fig. 2-a).
0: None (formation of the new cementum and new collagen fibers between the new cementum and the newly formed alveolar bone were not seen).
M ± SD: mean ± standard deviation. * $P<0.05$.

odontal tissues [8, 9]. Functional periodontal regeneration was induced in a buccal dehiscence monkey model by application of EMP [9]. Additionally, the same results were obtained in human clinical research [11]. Regeneration is defined as a re-formation or re-construction of the injured or defective tissue [28]. For periodontal treatment, it means that new cementum consisting of the periodontal membrane and alveolar bone are re-formed [8, 28]. Most periodontal diseases take a healing form of repair [8, 28]. Repair is defined as wound healing in which the structure and function of the organization are not recovered to the same degree as those in the original [28]. Repair does not restore the original structures of the new cementum, periodontal membrane and alveolar bone as well as the adhesion mechanism of new periodontal tissues [28, 29]. Regeneration of the organs is thought to be very important, because it is known that the organization formed by regeneration shows resistance against recurrence [28].

In some cases of canine periodontal disease, only the root apex is affected, but periodontal tissues are almost normal [5, 10, 18]. In these cases, EMP is therefore expected to induce regeneration of apical periodontal tissues after the

root apex is surgically removed in dogs.

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