Endod Dent Traumatol 2000; 16: 211–217
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Endodontics & Dental Traumatology
ISSN 0109-2502

Pulp revascularization of replanted immature dog teeth after different treatment methods

Yanpiset K, Trope M. Pulp revascularization of replanted immature dog teeth after different treatment methods. Endod Dent Traumatol 2000; 16: 211–217. © Munksgaard, 2000.

Abstract – The purpose of the present study was to determine the effect of topical treatment with doxycycline and/or the application of unfilled resin to the anatomical crown on the occurrence of revascularization in reimplanted dog teeth. Ninety-six teeth in 4 young mongrel dogs were used. Eighty one teeth were atraumatically extracted and divided into four groups. Group 1, 17 teeth were kept dry for 5 min and then replanted. Group 2, 21 teeth were soaked with a freshly prepared solution of doxycycline (1 mg/20 mL saline) for 5 min before replantation. Group 3, 23 teeth were soaked with the doxycycline solution for 5 min, and then replanted. The crowns were coated with 2 layers of light cured unfilled resin. Group 4, 20 teeth were kept dry for 5 min, and then replanted. The crowns were treated as with the teeth in Group 3. Three months after surgery, radiographic evaluation revealed that 27 teeth had continued root development and 32 teeth showed arrested root development with periradicular pathosis. The remaining 17 teeth, which had arrested root development but no signs of periradicular pathosis, were all histologically evaluated for final assessment. The occurrence of revascularization according to treatment group was 29.4%, 60%, 60%, 36.8% in Group 1, 2, 3, and 4, respectively. A multiple logistic regression analysis in SAS indicated there was no significant association between vitality and dog (P=0.7564). Soaking for 5 min in doxycycline significantly increased the revascularization rate (P=0.024) while the addition of resin to the crown did not result in an increased incidence of pulp revascularization (P=0.823).

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Key words: avulsion; doxycycline; immature teeth; replantation; revascularization

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Accepted February 29, 2000

Avulsion is a traumatic injury with potentially severe consequences. Attachment damage and pulpal infection are two serious sequelae that can result in root resorption and tooth loss after replantation (1). While damage to the periodontium at the time of the accident is uncontrollable, additional periodontal damage and infection of the root canal can be controlled.

In immature teeth necrosis is particularly harmful because tooth development stops. This results in a tooth with an open apex and thin dentinal walls. Endodontic treatment of teeth with open apices is potentially less successful than in teeth with closed apices (2) or it takes 6–18 months for apical development in apexification (3). Also, even if the apexification is successful it has been reported that upto 30% of these teeth will fracture during or after treatment (4, 5). Revascularization, therefore, is beneficial and extremely desirable, not only to maintain a pulp space free of infection, but to allow the tooth to continue to develop and strengthen. Previous experimental and retrospective studies have shown that the frequency of pulp revascularization in immature teeth is 18–41% (6–9).

The occurrence of pulp revascularization is enhanced if the width of apical foramen is greater than 0.5 mm in the monkey (10) or 1.1 mm in humans (8)

Fig. 1. A preoperative radiograph of teeth confirmed open apices.

and if the tooth is reimplanted within 45 min after avulsion regardless of storage conditions (8).

Bacterial contamination appears to be a principle factor for failure of a tooth to revascularize (10). Cvek et al. (9) report that the occurrence of revascularization is highly dependent on the presence or absence of bacteria in the pulpal lumen. Their experimental study has demonstrated that topical treatment with doxycycline before replantation significantly increased the frequency of complete pulp revascularization, presumable due to a decrease in the number of microorganisms that contaminated the root surface during the extra-alveolar period.

Various pathways of bacterial penetration to a pulp have been proposed. One of the suggested pathways is through enamel and dentin cracks in the crown of an otherwise seemingly intact tooth. Therefore, theoretically any barrier to this pathway of bacteria to the pulp space should enhance revascularization. A recent *in vitro* study by Love (11) suggests that trauma induced enamel/dentin infractions are pathways for bacterial invasion of the root canal system and claims that the application of unfilled resin to the anatomical crown blocks bacterial penetration.

Therefore a new protocol, which combines sealing the crown with unfilled resin prior to replantation and soaking the avulsed tooth in doxycycline as proposed by Cvek et al. (9), may enhance pulp revascularization in young permanent teeth. Hopefully, bacterial penetration to the pulp could be prevented for enough time in order to allow the pulp to revascularize. The frequency of revascularization, therefore, should increase.

The purpose of present study was to determine radiographically and histologically the effect of topical treatment with doxycycline and/or the application of unfilled resin to the crown of the tooth on the occurrence of revascularization in reimplanted dog teeth.

Material and methods

The material consisted of 48 incisors and 48 premolars in 4 healthy mongrel dogs approximately 4 months old at the beginning of the experiment. Open apices of the experimental teeth were confirmed radiographically (Fig. 1).

The dogs were anesthetized by intravenous administration of 20 mg/kg body weight of Thiopental, followed by 1–2% Halothane for maintenance of anesthesia. The teeth were randomly divided into 4 experimental groups and 1 negative control group. The distribution of the material is presented in Table 1.

In the experimental groups the teeth were extracted as atraumatically as possible with an elevator and forceps.

Group 1: The teeth were kept dry for 5 min and then replanted.

Group 2: The teeth were soaked in a freshly prepared solution of doxycycline (Doxy 100, Fujisawa, Deerfield, IL, USA) (1 mg/20 mL saline) for 5 min before they were replanted.

Group 3: The teeth were soaked in the doxycycline solution for 5 min, and then replanted. The crowns were acid etched with 37% phosphoric acid for 15 s, washed and air-dried with a triple syringe. Two layers of unfilled resin (Scotchbond, 3M, St. Paul, MN, USA) were painted over all the enamel to the cemento-dentinal junction and light cured.

Group 4: The teeth were kept dry for 5 min, and replanted. The crowns were treated as with the teeth in Group 3.

The negative control group: No treatment was performed on these teeth.

Fixation of the teeth was not used. A 4.0 gut suture was placed at both proximal sites of single rooted teeth in order to prevent postoperative loss while

Table 1. Distribution of 96 teeth in 1 control and 4 experimental groups

	Experimental groups				Control
	Group 1	Group 2	Group 3	Group 4	No treatment
	Dry 5 min	Doxycycline 5 min	Doxycycline and 2-layer dunfilled resin	Dry 5 min and resin	
# of teeth	17	21	23	20	15

allowing a mobility of the tooth during function. The dogs were fed a soft diet for three days.

Three months after the surgery periapical radiographs were taken. The dogs were sacrificed by being deeply anesthetized with 100-mg/kg IV administration of pentobarbital. The left and the right common carotid arteries were exposed/perfused with 4% neutral buffered formaldehyde (pH=7.4) and jaws were resected, fixed in formaldehyde solution and decalcified in 5% formic acid. The crowns were crossectionally cut about 1 mm below the cementoenamel junction and paraffin embedded. Longitudinal serial sections were taken from the crowns at 10 μm in buccolingual direction. Every other section was stained with hematoxylin and eosin using standard methods. The sections were evaluated with a light microscope at ×10 magnification for the presence/absence of vital tissue.

Radiographic evaluation: Vital tooth was indicated if no periradicular pathosis was present and continued root development was seen.

Non-vital tooth was indicated if arrested root development with periradicular pathosis was present.

Histological evaluation was made on selected teeth to verify the radiographic findings.

The evaluations were to assess the presence/absence of vital pulp tissue above the cervical margin of the tooth.

Statistical Analysis

A multiple logistic regression was performed to test if the 4 treatment groups were different from one another and to test the influence of dog on the outcome. A Fisher's exact test was used to test if there was a difference between groups with and without doxycycline soak and with and without resin coating on the crown. The level of significance for the overall differences and pair-wise comparisons of treatment was set at $P<0.05$. All statistical analyses were performed by SAS (Carry, NC, USA).

Results

Control teeth-No treatment

Radiographically, all teeth continued and completed root development with pulp space narrowing (Fig. 2). Histologically, normal pulp tissue with intact odontoblastic layer was present in the entire pulp chamber (Fig. 3).

Experimental teeth

Five of 81 teeth were excluded due to loss of crown/tooth: 4 had crowns broken during extraction and one tooth exfoliated at week 2 after replantation. The remaining 76 teeth were evaluated.

Fig. 2. A tooth (arrow), which has continued to develop.

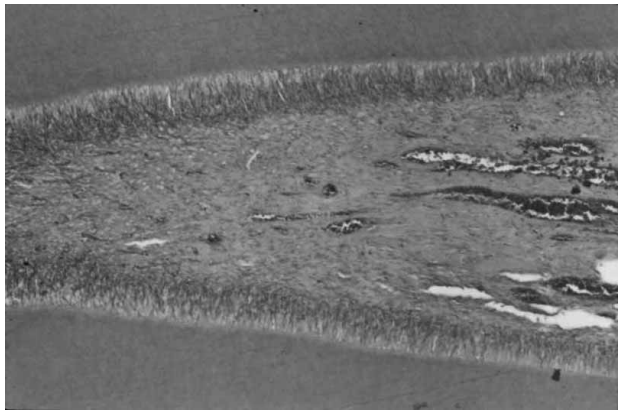

Fig. 3. Histologic evaluation of a control tooth showing continued development. A normal pulp is seen with an intact odontoblastic layer present (H&E, ×40).

Fig. 4. A tooth (arrow) assessed as a non-vital. The roots have not continued to develop and radiographic sign of apical pathosis is present.

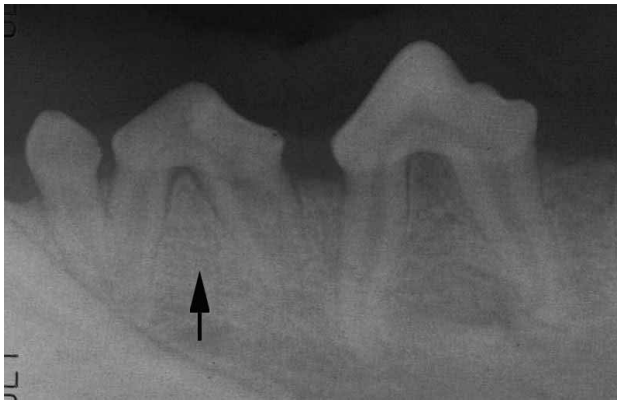

Fig. 5. A tooth (arrow) showing arrested tooth root development but no periradicular pathosis is visible radiographically.

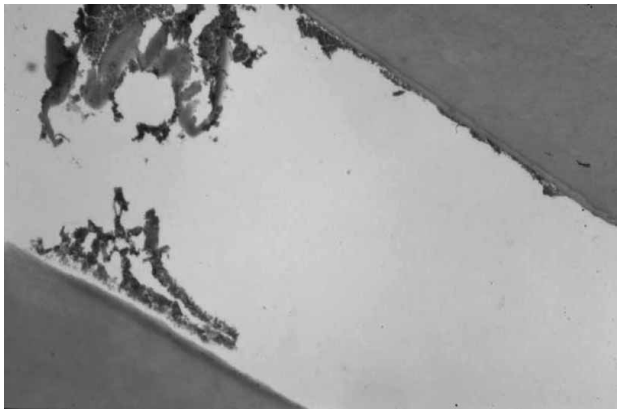

Fig. 6. Histologic evaluation of a tooth with arrested root development. A necrotic pulp is confirmed (H&E, ×100).

Radiographic evaluation

Twenty seven of the 76 teeth (35.5%) were assessed as definitely vital and 32 of the 76 teeth (42.1%) as definitely non-vital (Fig. 4). The remaining 17 teeth had arrested root development but no signs of periradicular pathosis (Fig. 5). These were tentatively assessed as non-vital but were all histologically evaluated before final status was determined.

Histology

Histologic evaluation of the teeth radiographically assessed as definitely vital was not done. Five randomly chosen teeth assessed radiographically as definitely non-vital were confirmed histologically as non-vital (Fig. 6).

Histologic evaluation was performed for all 17 teeth with arrested root development but with no periradicular pathosis. Nine of these teeth contained uninflamed vital tissue. However this tissue was not normal pulp but pink homogeneous tissue indicating newly formed osteoid tissue that resembled bone deposit along pulpal walls with osteogenic-type cells contained in the inner aspect. Blood vessels, fibroblasts and fibrous connective tissue occupied the central portion of the pulp chamber without a significant inflammatory reaction. (Fig. 7A and 7B). Therefore the evaluation of these teeth was changed to vital. The other 8 teeth histologically showed ischemic necrosis and were left in the non-vital category. The final status of the teeth is shown in Table 2. Nine teeth were added to the vital category. Therefore the final number of successful revascularization was 47.3% (36 of 76 teeth). The occurrence of revascularization according to treatment group is shown in Table 3.

Replantation of the extracted teeth without any extra treatment (Group 1) resulted in 29.4% revascularization. The addition of resin to the crown after replantation (Group 4) did not result in a significant increased incidence of pulp revascularization (29.4% vs 36.8%). Soaking for 5 min in doxycycline doubled the revascularization rate to 60% (Group 2) while the addition of resin to the doxycycline treatment (Group 3) failed to increase the rate above 60%. Therefore soaking the teeth in doxycycline, independent of the

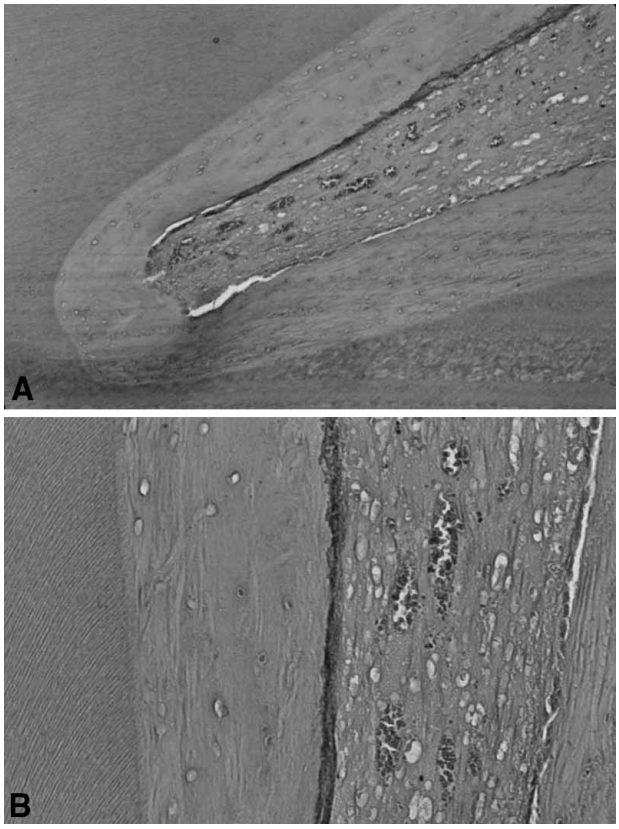

Fig. 7. (A) Histologic evaluation of a tooth with arrested root development but no periradicular pathosis. Osteoid tissue deposit is seen on the pulp chamber walls and osteogenic type cells are seen on the inner aspect of the pulp (H&E, ×40). (B) High magnification (H&E, ×100), osteoid tissue is seen on the periphery and blood vessels, fibrous connective tissue occupy the central pulp space.

Table 2. Vitality assessment of replanted teeth

Radiographic	Vital	Non-vital	Unsure	
# of teeth	Continued root development	Arrested root development with periradicular lesion	Arrested development without periradicular lesion	
76	27	32	17	
			Histologic	
			Vital	Non-vital
			9	8

Table 3. % Revascularization according to treatment group

			Vital		Non-vital	
Group	Treatment	# of teeth	#	%	#	%
1	Dry 5 min	17	5	29.4	12	70.6
2	Doxycycline 5 min	20	12	60	8	40
3	Doxycycline 5 min+resin	20	12	60	8	40
4	Dry 5 min+resin	19	7	36.8	12	63.2
Total		76	36	47.3	40	52.7

addition of resin to the crown, increased the frequency of revascularization from 33.3% (12 out of 36 teeth) to 60% (24 out of 40 teeth). The resin groups did not increase the occurrence of revascularization over the non-resin groups (48.5% [19 out of 39 teeth] vs. 45.9% [17 out of 37 teeth]).

The statistical analysis indicated that there was no significant association between dog and vitality (P= 0.756). This suggested that vitality of the teeth did not vary among dogs. There was no significant overall association between treatment and vitality but there was a suggestive difference (P=0.1432) in favor of the doxycycline groups. Because there was no difference in vitality of teeth that were and were not covered with resin (Group 1 and 4), but there appeared to be beneficial effect of the doxycyline (Group 2 and 3), it was decided to test if there was a significant difference between the doxycyline groups vs. the non-dyxycycline groups. It was found that there was a significant difference between the groups with and without doxycycline soak (P=0.024). For the resin groups there was no significant difference between the groups with and without resin coating on the crowns (P=0.823).

Discussion

The appropriateness of the dog model for use in endodontic research is often questioned because of the difficulty in extracting dog teeth as well as the web-like anatomy of the root apex. In the present study, there was little difficulty extracting the teeth presumably because the dogs were young and the teeth immature. Regarding the web-like anatomy at the root apices, our study was performed in open apex teeth, making this anatomical difference irrelevant. Therefore, we felt that the dog model was as suitable as the primate model for this replantation study and has in fact been successfully utilized in other replantation revascularization studies (7, 12, 13). Five teeth were discarded at the time of extraction. These teeth were spread in 3 of the 4 experimental groups. Therefore, the groups were still "balanced" enough with fairly even spread between the tooth and dog type for statistical evaluation.

When using continued root development, as viewed radiographically as our criteria for revascularization, we had a success rate of 35%. However on histologic examination we added 9 teeth to the vital category. These teeth had arrested root development but showed no periradicular pathosis. These 9 teeth histologically showed osteoid tissue resembling bone deposit along the pulp chamber walls with osteogenic-type cells in the inner aspect of the pulp space. Blood vessels, fibroblasts and fibrous connective tissue occupied the central portion of the pulp space without evidence of significant inflammatory reaction. Even though an odontoblastic layer was not seen, we considered these teeth as vital. The healing of pulp by replacement of periodontal and bone tissue has previously been observed. Skoglund & Tronstad (7) found that less than 12% of replanted teeth in dogs that revascularized exhibited normal pulp. They found that the majority of the teeth observed after 180 days showed the soft tissue of the pulp markedly reduced in cells and blood vessels. Cell-containing atubular hard tissue occupied most of the original pulp origin and large hard tissue deposit on the root canal walls was seen (7). Also ingrowth of bone and

formation of an internal periodontal ligament was found in teeth related to arrested root formation. In previous studies, it has been found that the pulp of replanted and autoreplanted teeth became necrotic immediately after the extraction and that revascularization with periodontal like tissue can then occur in teeth with immature roots (13, 14). Retrospective studies have shown that the pulp often obliterates after these injuries. (6, 15–17). Therefore we hypothesize that these 9 teeth with arrested development but vital tissue in the pulp space contained tissue consistent with other studies and would have obliterated if the follow up time before sacrifice had been extended.

Topical treatment of doxycycline before replantation doubled the frequency of revascularization in our study. According to Cvek et al. (9) who found similar results in monkeys, the reason for the increase in revascularization frequency is that the antibiotic decreases the number of microorganisms in the pulpal lumen. In our study the doxycycline presumably had a similar effect and although our overall revascularization rates (controls and antibiotic treated) were higher than those found by Cvek et al. (9) this might be due to species difference (dog vs. monkey). Therefore we can assume that doxycycline would have a similarly beneficial effect in humans although these studies cannot indicate the true incidences of pulp revascularization in humans either with or without prior soaking in the antibiotic.

We were surprised that coating the crowns of the teeth with unfilled resin did not enhance revascularization in this investigation. A number of hypotheses could be put forward to explain this result: 1. The tooth crown may not be an avenue for bacterial penetration. 2. The doxycycline soak may have removed any possibility for bacteria to move through the crown. 3. Resin may not be an effective barrier, unlike the *in vitro* study by Love (11) that showed the bacterial contamination of the pulp on trauma simulated teeth is prevented when the anatomical crown is sealed with 2 layers of unfilled resin. 4. The resin had possibly come off quickly after placement due to chewing and general function of the dogs allowing for subsequent bacterial penetration into the pulp. 5. Exposed cervical dentin that was not covered by the resin might be the principle pathway for bacterial penetration of dentinal tubules. Cvek et al. (10) have found that mechanical damage of cementum and dentin in the cervical area of the tooth, caused by forceps at the time of extraction, is another pathway of pulp contamination. They demonstrated that the crushed tissue was covered by plaque from which microorganisms, probably attracted by the disintegration products from the necrotizing pulp, penetrated into the dentinal tubules. The resin was applied to the whole clinical crown but did not cover the cervical area where bacteria most likely could penetrate into the pulp. 6. Finally, the crown damage of an extracted tooth might not be the same as that after an avulsion injury. Therefore the possible beneficial effect of the resin might not be demonstrated in this model.

This study confirmed the clinical benefit of doxycycline in enhancing revascularization of replanted dog teeth. However, more research should be done in order to evaluate the pathways of bacterial penetration after traumatic injuries and also to find the treatment method to eliminate this penetration. Presumably the longer the pulp space can be kept free of bacteria, the higher will be the incidence of revascularization of these teeth.

Acknowledgement – The authors thank Dr. Jeff Burkes (Professor of Pathology, Department of Diagnostic Science, University of North Carolina at Chapel Hill) for his assistance in the histological evaluation of our data.

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