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DENTAL TRAUMATOLOGY

Pulp revascularization of replanted immature dog teeth after treatment with minocycline and doxycycline assessed by laser Doppler flowmetry, radiography, and histology

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Abstract – This study investigated the effect of topical antibiotic treatment on pulp revascularization in replanted teeth. Thirty-four immature teeth were selected from three young dogs. Baseline radiographs and laser Doppler flowmetry (LDF) readings were obtained. Specimens were randomly divided into four groups: Thirty-eight teeth were extracted, kept dry for 5 min, and either (Group 1) covered with minocycline mixture (G1, $n = 11$), (Group 2) soaked in doxycycline (G2, $n = 11$), or (Group 3) soaked in saline (G3-negative control, $n = 6$), and replanted. Teeth in Group 4 were not extracted (positive control, $n = 6$). Postoperative radiographs and LDF readings were obtained for 2 months after replantation. After sacrifice, the jaws were collected and processed for light microscopy. Pre- and postreplantation LDF readings and radiographs, and histologic findings were analyzed to assess revascularization. Pulp revascularization occurred in 91% (G1), 73% (G2), and 33% (G3) of the specimens. In conclusion, minocycline facilitates pulp revascularization in replanted immature teeth after replantation.

Alessandra Luisa de Souza Ritter[1], André Vicente Ritter[2], Valerie Murrah[3], Asgeir Sigurdsson[1], Martin Trope[1]

Departments of [1]Endodontics, [2]Operative Dentistry and [3]Diagnostic Sciences, School of Dentistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Key words: dental trauma; replantation; revascularization; minocycline; doxycycline; laser Doppler flowmetry

Dr Alessandra L. S. Ritter, Department of Endodontics, School of Dentistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7450, USA
Tel./fax: +1 919 966 6344
e-mail: Alessandra.Ritter@dentistry.unc.edu

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Avulsion is a traumatic injury characterized by the total displacement of the tooth from its socket. Upon avulsion of a vital tooth, the pulp's neurovascular supply is severed, and the periodontal ligament (PDL) is ruptured. The most ideal outcome after replantation of the tooth into its alveolar socket would be pulp regeneration and reattachment of healthy PDL. Severe attachment damage, pulp necrosis, and pulp infection are common consequences of tooth avulsion that frequently result in ankylosis with osseous replacement or progressive inflammatory root resorption (1, 2).

Pulp necrosis is particularly harmful in immature teeth. Without a viable pulp, tooth development stops resulting in open apex and thin dentin walls. Endodontic treatment of teeth with open apices is less successful than that of teeth with closed apices (3). Although apexification might be successful in some cases, it takes 6–18 months for apical development to complete (4). Also, even if the treatment is successful, it has been reported that up to 30% of apexification-treated teeth will fracture during or after treatment because of fragile root walls and challenging restorability (5, 6).

Revascularization of the necrotic pulp is highly desirable after tooth avulsion. When pulp revascularization occurs, the vital tissue within the pulp space prevents infection and the continued root development ensures a thick dentinal wall that is resistant to fracture and a closed apex that makes subsequent

endodontic therapy, if required at a later time, more predictable than that in open apex. It has been conclusively shown that revascularization of a necrotic pulp would be possible if the avulsed teeth were vital before avulsion and the replantation performed under certain conditions (1).

Previous experimental studies have reported successful revascularization in 18–41% of the cases (7–10). Pulp revascularization is favored when the apical foramen is not completely formed (immature stage of root development) (7, 8, 11–13). The duration of extraoral time and storage medium also appear to affect the potential for pulp revascularization (14, 15). If the tooth is replanted within 45 min of the injury, the prognosis for pulp revascularization is favorable (8).

Cvek et al. (9, 13) showed that pulp revascularization is highly dependent on the presence or absence of bacteria in the pulpal lumen. Various pathways of bacterial penetration to the pulp have been proposed. One of the suggested pathways is through enamel and dentin cracks in the crown of the traumatized avulsed tooth. This has been observed by Love (16) in an *in vitro* study but not confirmed by Yanpiset & Trope (17). The movement of bacteria along the blood clot that develops between the root and the socket when the tooth is replanted has been proposed by Cvek's group (9, 13). These authors raised the hypothesis that microorganisms from the oral cavity (or from contaminated root surfaces during extraoral time) reach the apical area of the recently replanted tooth through the blood clot present on the alveolar socket before replantation or formed during the initial phase of the healing process. 'Contaminated teeth were replanted into the alveoli, most of which filled with coagulated blood, known as an excellent substrate for the growth of microorganisms' (13).

Tetracyclines have been used as an adjunct to periodontal surgical procedures. Topical tetracycline application to root surfaces has been shown to assist in decontamination and periodontal regeneration (18). Studies have linked the presence of microorganisms to the inhibition of revascularization of avulsed replanted teeth and to inflammatory root resorption (13, 19). Tetracyclines, which exert their antimicrobial activity by inhibiting protein synthesis, are broad-spectrum antibiotics providing action against anaerobes and facultative organisms. Tetracyclines are bacteriostatic at levels found in the crevicular fluid after systemic administration (20) but provide bactericidal action at the high concentration occurring with local delivery of the drug (21).

Based on the hypothesis that microorganisms reach the apical area of the recently replanted tooth from the oral cavity (or from contaminated root surfaces during extraoral time), and on the action of tetracyclines to potentially inhibit this route of bacterial contamination, Cvek developed a new protocol for topical treatment of the exposed root with doxycycline before replantation, aiming to locally eliminate microorganisms from the root surface of an avulsed tooth in order to decrease the frequency of inflammatory response (13). These authors showed that topical doxycycline significantly increased the chances of successful pulp revascularization. The beneficial effect of soaking a tooth in doxycycline was recently confirmed by Yanpiset & Trope (17).

Although the results obtained from the topical use of doxycycline were significantly better than those of other treatment protocols, they are not yet the ideal (17). Approximately 40% of the replanted immature teeth in the animal model undergo necrosis, even under optimum conditions of avulsion/replantation (short extraoral dry time, atraumatical extraction, absence of gross contamination during extraoral time, and careful manipulation of teeth) (13, 17). Bacterial penetration into the pulp canal space seems to be the cause of the revascularization failure in the majority of the cases.

The time needed for the initial healing of the periodontal ligament to occur under normal conditions is approximately 10 days after replantation (22). Skoglund & Tronstad, observing pulpal changes in replanted and auto-transplanted immature teeth, demonstrated that repair occurs by ingrowth of a cell-rich, highly vascularized connective tissue (22). Ten days after replantation, pulp tissue is present at the apical half of the pulp space, and after 30 days, revascularization of the whole pulp can occur (22). During this time, bacteria may travel from the oral cavity or the external root surface, contaminating the pulpal lumen and impairing revascularization.

Minocycline is a broad-spectrum, semisynthetic tetracycline. A number of studies show that the local administration of a topical minocycline is effective in the treatment of periodontitis (23–26). Minocycline is available in the form of Arestin™ Microspheres (OraPharma, Inc., Warminster, PA, USA), which is a sustained-release product containing minocycline hydrochloride into a bioabsorbable polymer (polyglycolide-co-DL lactide). The resulting microspheres combine the minocycline and bioabsorbable polymer in a powder form. Immediately upon contact with moisture, the polymer hydrolyzes, releasing minocycline. Concentrations of 340 μg minocycline ml^{-1} have been measured in human crevicular fluid after 14 days, exceeding the minimum inhibitory concentrations for many pathogens (27).

Minocycline has been shown to slow down periodontal bone loss when administered topically (27). Its utility in the treatment of traumatized teeth, however, has not been tested. Particularly, the usefulness of minocycline in pulp revascularization after

replantation of avulsed teeth has not been investigated. Its slow-release action may keep bacteria from entering the pulp space long enough to allow revascularization to occur at a higher rate than with doxycycline-treated replanted teeth, as the later has a shorter period of action.

The purpose of this study was to evaluate the efficacy of topical minocycline application in pulp revascularization and PDL healing of avulsed and replanted immature teeth in a dog dental trauma model. In addition, we analyzed histologically the different healing patterns that occur after successful revascularization.

Materials and methods

This research was conducted with the support of The University of North Carolina (UNC) Animal Facility. The use of live vertebrate animals for the purposes of this study was reviewed and approved by the UNC Institutional Animal Care and Use Committee (IACUC protocol #00-097.0-A).

Three mongrel dogs approximately 5 months old and weighing approximately 11 kg each were obtained. At this age, the animals' teeth are expected to have incomplete root development and open apices. Twenty-two incisors and 12 premolars were selected, and open apices were confirmed radiographically. These radiographs provided radiographical baseline information.

The animals were anesthetized during all experimental procedures by intravenous administration of Pentothal IV and Isofluorine maintenance. The selected teeth were randomly divided into three experimental groups and one control group. To reduce the influence of an individual animal on the outcome of a specific treatment, specific teeth from each animal were assigned to each of the four research groups. Teeth were treated as follows:

- Group 1 ($n = 11$): The teeth were extracted, kept dry for 5 min, covered with minocycline hydrochloride microspheres (Arestin™, OraPharma Inc.) for 5 min, and replanted (Fig. 1);
- Group 2 ($n = 11$): The teeth were extracted, kept dry for 5 min, soaked in a doxycycline solution (Doxy 100, American Pharmaceutical Partners, Inc., Los Angeles, CA, USA) for 5 min, and replanted (Fig. 2A,B);
- Group 3 (negative control, $n = 6$): The teeth were extracted, kept dry for 5 min, soaked in saline for 5 min, and replanted; and

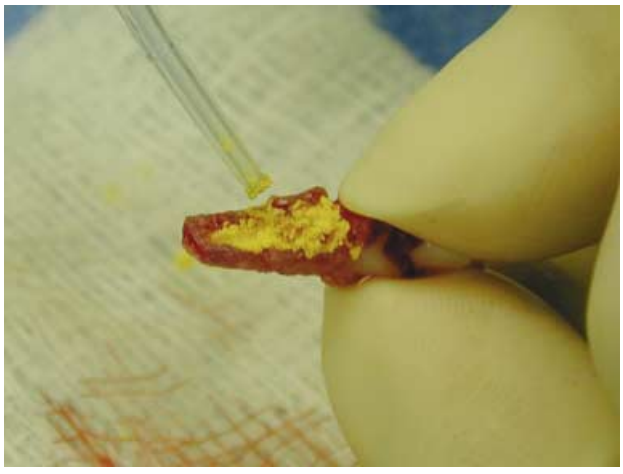

Fig. 1. Minocycline HCl microspheres (Arestin™) being applied to extracted tooth immediately before replantation (Group 1).

(A)

(B)

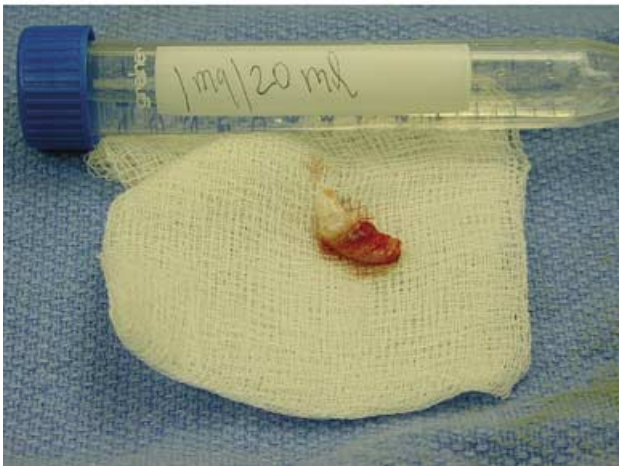

Fig. 2. (A) Doxycycline hyclate (Doxy 100) in the powder form to be diluted. (B) Doxycycline hyclate (Doxy 100) diluted 1 mg per 20 ml and used in Group 2 to treat the roots of the specimens before replantation.

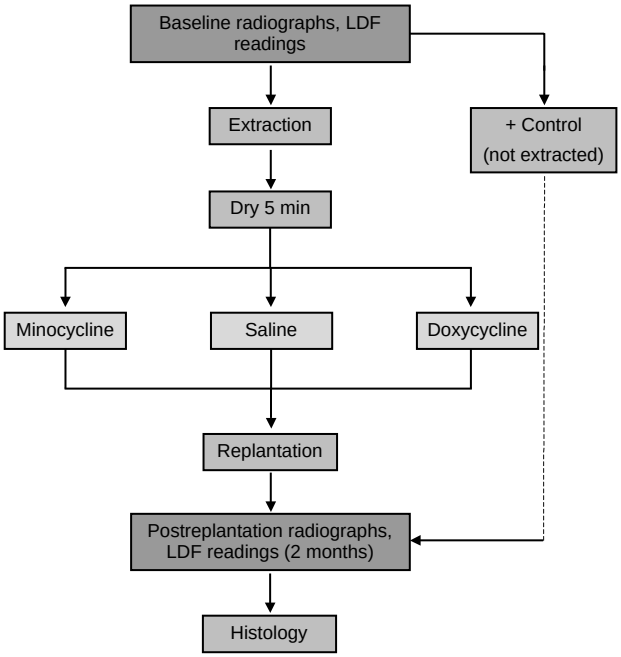

Fig. 3. Flowchart – experimental groups/procedures.

- Group 4 (positive control, $n = 6$): The teeth were not extracted.

Extractions were accomplished using elevators and forceps as needed. Care was exercised to extract the teeth with little trauma to the PDL. The teeth used had incomplete root formation, which favors an atraumatic extraction technique.

Figure 3 shows a flow chart depicting the experimental procedures. Table 1 shows the distribution of teeth per experimental group.

The replanted teeth were not splinted. A #4.0 gut suture was placed at both proximal sites of single rooted teeth in order to prevent postoperative loss while allowing mobility of the tooth during function. The dogs were pain-medicated with buprenorphine (0.01–0.03 mg kg^{-1} i.m.) for 2 days after the surgical procedures and received a soft diet for 3 days after the replantation.

The animals were clinically evaluated at 7, 15, 25, 35, 45, and 60 days after replantation and checked for pulp vitality with the laser Doppler. Sixty days after replantation, follow-up periapical radiographs were taken to evaluate presence/absence of periapical radiolucency and integrity of lamina dura. All radiographs were evaluated in a light box with magnifying loupes.

Table 1. Number of teeth (*n*) per experimental group

Group	Procedure/treatment	*n*
G1	Minocycline HCl	11
G2	Doxycycline	11
G3	Saline (−control)	6
G4	Not extracted (+control)	6

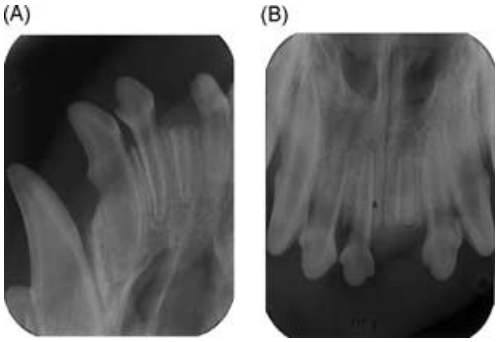

Fig. 4. Representative radiographs showing presence of intact lamina dura (A, arrow) and presence of periapical radiolucency (B, arrow) in replanted teeth.

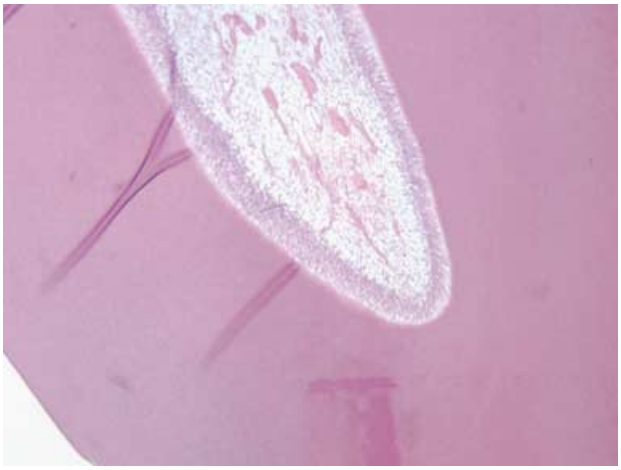

Fig. 5. Histological appearance of vital pulp with normal odontoblastic layer (4× magnification, H&E).

The following were used as radiology criteria:

1 intact lamina dura (Fig. 4A) and

2 presence of periapical radiolucency (Fig. 4B).

The animals were then sacrificed by perfusion of the left and right common carotid arteries with 4%

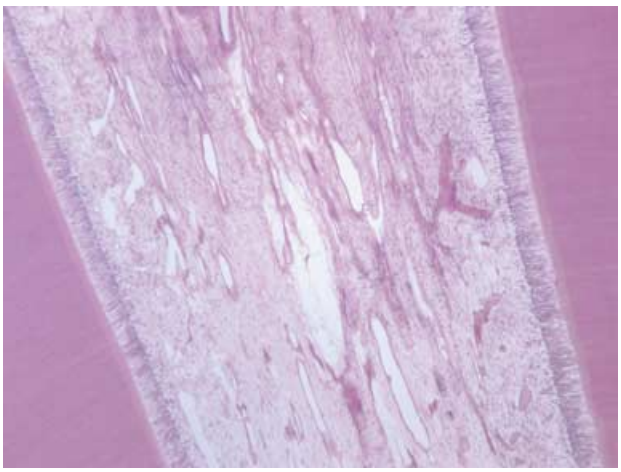

Fig. 6. Histological appearance of vital pulp with normal odontoblastic layer (10× magnification, H&E).

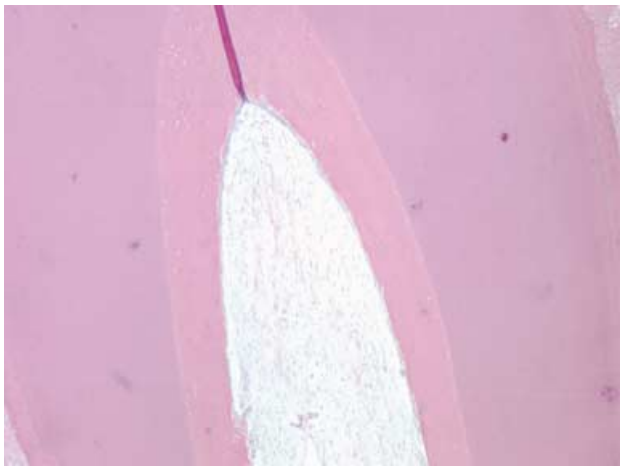

Fig. 7. Histological appearance of reactive dentin and flattening of 'odontoblasts' (4× magnification, H&E).

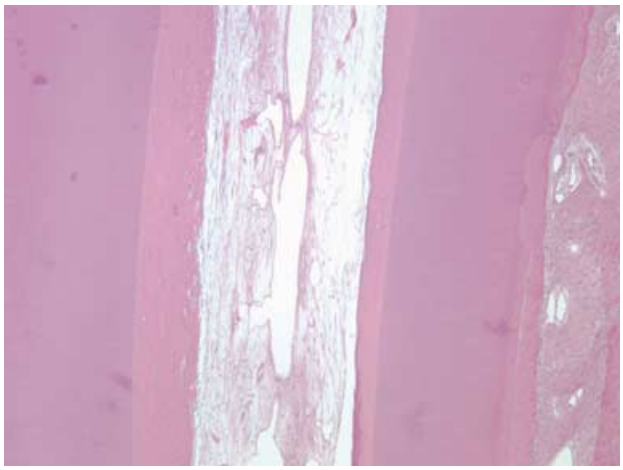

Fig. 9. Histological appearance of reactive dentin and flattening of 'odontoblasts' (4× magnification, H&E).

neutral buffered formaldehyde (pH = 7.4), and the jaws were resected, fixed in 10% formalin, decalcified in EDTA, and embedded in paraffin.

Histological analysis was performed as described by Yanpiset & Trope (17). Briefly, longitudinal 0.5-μm thick sections were obtained from the embedded specimens, two slices being collected every 50 μm within the pulp. The slides were stained for H&E and evaluated with a light microscope for the presence or absence of healthy, vital tissue and revascularization pattern.

Two evaluators performed histologic evaluations. Only teeth with vital tissue occupying the entire pulp space were considered vital, regardless of the nature of the tissue, i.e. pulp tissue with normal odontoblastic layer, connective tissue with reactive dentin layer, or mineralized connective tissue (osteodentin).

The following were used as histology criteria:

1.1 vital pulp tissue characterized by normal odontoblast layer (Figs 5 and 6);

1.2 vital connective tissue with reactive dentin layer (Figs 7, 8, and 9);

1.3 vital mineralized connective tissue (Figs 10, 11, and 12); and

2 necrotic pulp (Figs 13, 14, and 15).

Fischer's exact test was used to compare the presence of vital tissue between groups with and without antibiotic soak, using the criteria described above. The level of significance of the overall difference and pairwise comparisons of treatment was set at $P = 0.05$.

Results

Results are presented in Tables 2 and 3, and illustrated in Fig. 16. A 94% positive correlation was observed between histology and radiographs concerning tooth vitality. 100% correlation was observed between vital tissue within the pulp space and normal PDL at apical area, and a 75% correlation was observed between

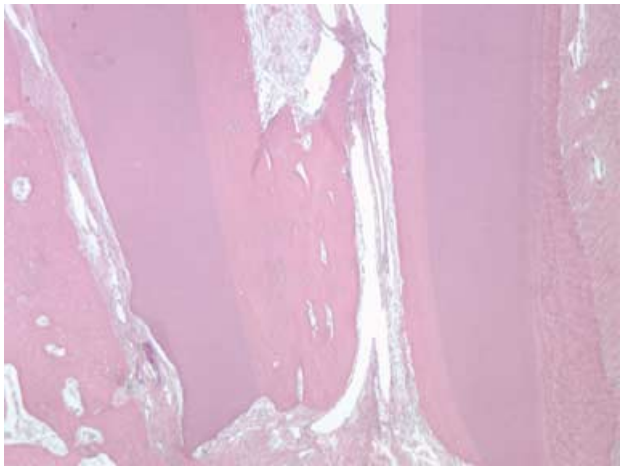

Fig. 8. Histological appearance of reactive dentin and 'osteoid tissue' (4× magnification, H&E).

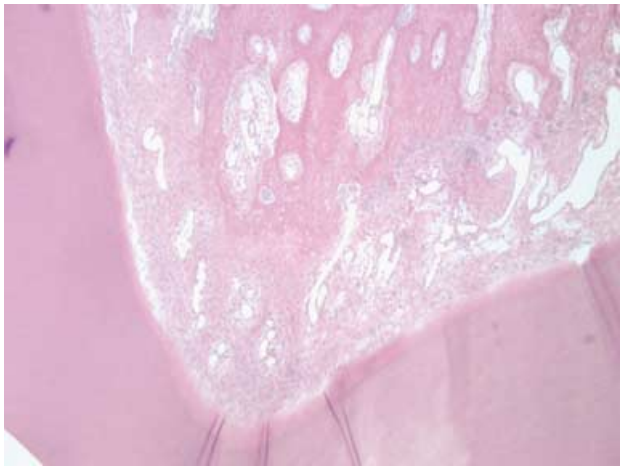

Fig. 10. Histological appearance of central metaplastic bone formation (osteodentin) (2× magnification, H&E).

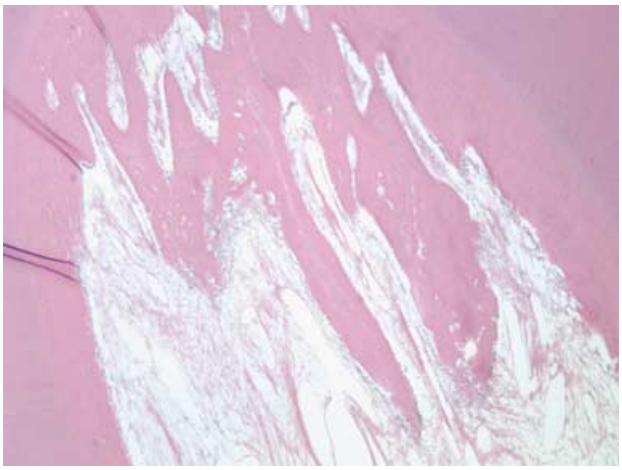

Fig. 11. Histological appearance of reactive dentin formation with loss of typical odontoblasts (4× magnification, H&E).

Fig. 13. Histological appearance of coronal pulpal abscess with fibrosis and bone formation in more apical portion (4× magnification, H&E).

necrotic tissue within the pulp and the presence of apical radiolucency.

Statistical analysis of the histology data (Table 2) revealed that minocycline-treated specimens presented a significantly higher number of vital teeth when compared to saline-treated specimens ($P = 0.027$). Doxycycline-treated specimens presented a number of vital teeth statistically comparable to that of the saline-treated specimens ($P = 0.144$) and to the minocycline-treated specimens ($P = 0.29$). Histologically, the control (non-avulsed) specimens resulted in a significantly higher number of vital teeth when compared to the saline-treated specimens ($P = 0.03$). Neither minocycline- nor doxycycline-treated specimens resulted in number of vital teeth significantly different from that in the case of control specimens ($P > 0.05$).

Statistical analysis of the radiology data revealed that minocycline-treated specimens presented a significantly higher number of vital teeth when compared to saline-treated specimens ($P = 0.03$). Doxycycline-treated specimens presented number of vital teeth statistically comparable to the saline-treated specimens ($P = 0.34$) and to the minocycline-treated specimens ($P = 0.10$). Representative examples of radiology findings are presented in Fig. 4 (A,B).

Histology (number of vital and non-vital teeth) and radiology (presence or absence of periapical radiolucency) outcomes revealed no statistically significant disagreement between both observations ($P = 0.81$).

The following results are presented by group:

Group 1 (minocycline treatment)

From the 11 specimens replanted, 10 (91%) showed vital tissue occupying the pulp canal space. From these vital specimens, seven presented with reactive dentin, dense fibrous connective tissue and osteoden-

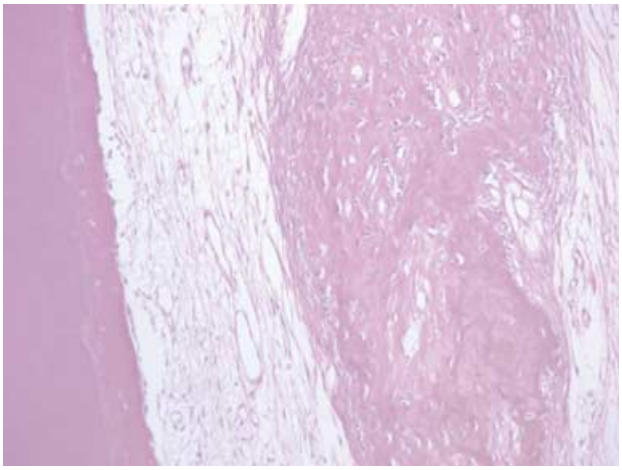

Fig. 12. Histological appearance of central osteodentin and loss of odontoblasts (10× magnification, H&E).

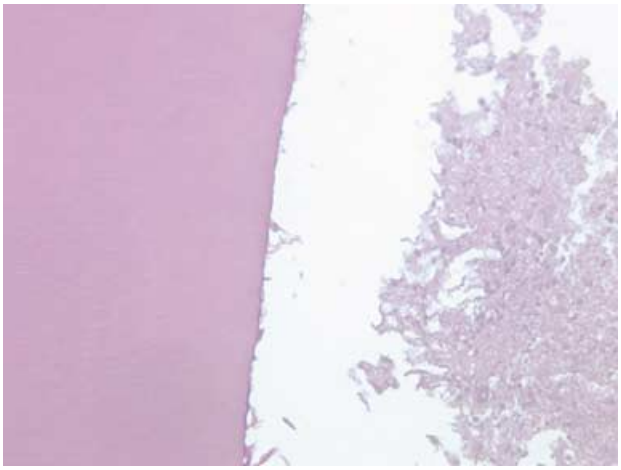

Fig. 14. Histological appearance of pulpal necrosis (20× magnification, H&E).

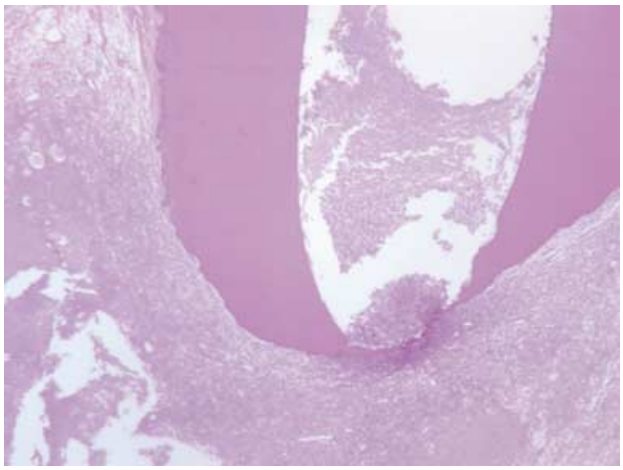

Fig. 15. Histological appearance of pulpal necrosis and periapical abscess (4× magnification, H&E).

Table 2. Final total percentage of vital pulp findings after histological evaluation

Group	Procedure/treatment	*N*	Vital pulp (*n*)	% vital pulp
G1	Minocycline HCl	11	10	91
G2	Doxycycline	11	8	73
G3	Saline (−control)	6	2	33
G4	Not extracted (+control)	6	6	100
Total		34	26	

tin tissue, two showed a reactive dentin layer and loose connective tissue, and one showed normal pulp tissue with normal odontoblastic layer. Only one (9%) specimen in this group failed to revascularize.

Group 2 (doxycycline treatment)

From the 11 specimens replanted, eight (72.7%) showed vital tissue occupying the pulp canal space. From these vital specimens, five presented internal cement/predentin layer, connective tissue and bone/osteoid tissue, and three specimens showed cement layer and loose connective tissue. Three (27.3%) specimens in this group showed necrotic pulp.

Group 3 (saline)

From the six (33.3%) specimens replanted, two showed vital tissue occupying the pulp canal space. From these vital specimens, one specimen showed internal cementum/predentin layer and loose connective tissue, and one specimen showed normal pulp tissue with odontoblastic layer. Four (66.7%) specimens in this group presented necrotic pulp.

Group 4 (positive control – no treatment)

Radiographically, all specimens in this group continued root development with pulp space narrowing. Histologically, normal pulp tissue with intact odontoblastic layer was present in all the specimens.

Discussion

The appropriateness of the dog model for use in endodontic research is often questioned because of the difficulty in extracting dog's teeth as well as the web-like anatomy of the root apex. In the present study, there was no 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 characteristic irrelevant. Therefore, we felt that the dog model was a suitable model for this replantation revascularization study as compared to the primate model used in other replantation revascularization studies (11, 28). Only two teeth were lost during extraction procedures, but were substituted by other two teeth on the same dog that were not originally planned to be included in the study. This substitution permitted to continue the study without changes in number of teeth per dog per group.

One of the difficulties encountered was in using radiographic observation of continued root develop-

Table 3. Histological evaluation of the different healing patterns that occurred after successful revascularization

Group	Procedure/treatment	*N*	Vital tissue (*n*)	Different healing patterns
G1	Minocycline HCl	11	10	7 – Reactive dentin, dense fibrous connective tissue and osteodentin tissue, 2 – Reactive dentin layer and loose connective tissue, 1 – Normal pulp tissue with normal odontoblastic layer
G2	Doxycycline	11	8	5 – Reactive dentin, dense fibrous connective tissue and osteodentin tissue, 3 – Reactive dentin layer and loose connective tissue
G3	Saline (−control)	6	2	1 – Reactive dentin layer and loose connective tissue , 1 – Normal pulp tissue with normal odontoblastic layer
G4	Not extracted (+control)	6	6	6 – Normal pulp tissue with normal odontoblastic layer
Total		34	26	

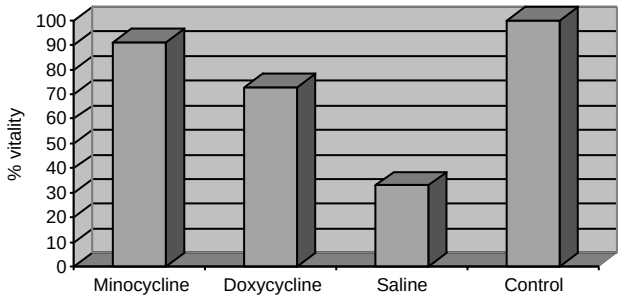

Fig. 16. Percentage of vital teeth among groups.

ment as our criteria for revascularization. We worked with incisors and first premolars only, and because of the technical difficulties in obtaining the same film position and the relatively short evaluation time, it was not possible to distinguish between arrested root formation and complete development. Therefore, our criteria for assessment of revascularization radiographically were the presence or absence of periapical radiolucency and presence of continuous and intact lamina dura.

We observed 100% correlation between presence of periapical radiolucency on the radiographic analysis and histological diagnosis of necrotic pulp. We could not find the same correlation between presence of intact lamina dura and presence of vital pulpal tissue, mainly because some of the specimens presented vital tissue at the apical and middle portion of the pulp canal space and were diagnosed as necrotic.

Our criteria for considering revascularization histologically was the presence of vital tissue (loosely collagenized pulp tissue with presence of odontoblastic layer, or more densely collagenized connective tissue and/or mineralized tissue with presence of a reactive dentin layer) without evidence of significant inflammatory reaction and occupying the entire pulp canal space. Any inflammatory tissue, localized abscess formation, or lack of complete revascularization was diagnosed as failure to revascularize.

The patterns of revascularization found were: (i) presence of pulp tissue with odontoblastic layer and continuous root development; (ii) presence of a mineralized tissue layer deposited along the pulp space walls, consistent with reactive dentin deposition and a fibrous connective tissue with blood vessels and fibroblasts in the central portion of the pulp space; and (iii) presence of bone formation in the central portion of the pulp space surrounded by fibrous connective tissue and reactive dentin layer. Skoglund & Tronstad (11) found the same patterns of revascularization. They reported that less than 12% of replanted teeth in dogs exhibited normal pulp upon revascularization. The majority of the teeth observed after 180 days showed the pulp tissue markedly reduced in cells and blood vessels. Cell-containing atubular hard tissue occupying most of the original pulp space and large hard tissue deposition on the root canal walls were seen. Also, ingrowth of bone and formation of an internal periodontal ligament were found in teeth related to arrested root formation. In previous studies, it has been found that the pulp of replanted and auto-transplanted teeth becomes necrotic immediately after the extraction and revascularization with periodontal-like tissue can then occur in teeth with immature roots (2, 11). Retrospective studies have shown that the pulp often obliterates after these injuries (7, 10, 29, 30). We propose that teeth presenting additional variations of vital tissue and arrested root development can be considered as revascularized, consistently with other studies.

Topical treatment with doxycycline before replantation doubled the frequency of pulp revascularization when compared to saline, confirming the results from Yanpiset & Trope (17) and Cvek in a previous monkey study (13). The reason attributed to the increase of revascularization was the antimicrobial affect of doxycycline decreasing the number of microorganisms in the pulpal lumen. Studies have linked the presence of microorganisms with revascularization of avulsed replanted teeth and a stimulus for inflammatory root resorption (12, 19).

In our results, we obtained 90% (10 out of 11 teeth) revascularization of replanted immature teeth after coating the extracted teeth with minocycline. Doxycycline and minocycline are broad-spectrum antibiotics synthetically derived from oxytetracycline. They exert their antimicrobial activity by inhibiting protein synthesis and provide action against anaerobes and facultatives, and against a wide range of Gram-positive and Gram-negative organisms.

They are bacteriostatic at levels found in the crevicular fluid after systemic administration (20) but provide bactericidal action at the high concentration occurring with local delivery of the drug (21). Tetracyclines have wide therapeutic usage as antimicrobial agents, and remain useful as adjuncts in periodontal therapy.

However, tetracyclines also have non-antimicrobial properties, which appear to modulate host response. In that regard, tetracyclines and their chemically modified analogs have been shown to inhibit the activity of the matrix metalloproteinase (MMP), which is a collagenase. The activity of this enzyme appears crucial in the destruction of the major structural protein of connective tissues, collagen. They have the ability to bind to the tooth surface and then be slowly released in active form (31). Tetracyclines also promote fibroblast and connective tissue attachment, enhancing regeneration of periodontal attachment lost to pathologic processes (32). Systemic tetracyclines provide benefit to cutaneous diseases of a non-bacterial origin by their anti-inflammatory effects,

which may include suppressing neutrophil function, inhibiting phospholipase A2 activity, and scavenging reactive oxygen species produced by PMNs, which are present in inflammation and tissue destruction (33, 34).

Tetracyclines inhibit collagenase activity and osteoclast function (26, 35), which is beneficial to a replanted tooth. Their effects on the osteoclasts include diminished acid production, decreased ruffled border area, and decreased adhesive properties, all of which inhibit bone resorption (36).

A number of studies show that the local administration of a 2% minocycline gel is effective in the treatment of periodontitis (23–25). Minocycline, in the form of Arestin™ Microspheres (OraPharma, Inc.) is a sustained-release product containing minocycline hydrochloride into a bioabsorbable polymer (polyglycolide-co-DL lactide) that has been shown to decrease periodontal bone loss when administered topically.

These properties of tetracyclines added to the fact that Arestin™ stays in place for a longer time and, at a higher concentration, may be responsible for the revascularization results obtained with immature teeth. Its slow release action may keep bacteria from entering the pulp space for long enough to allow revascularization to occur at a higher rate than the doxycycline solution as it has a shorter period of action.

Conclusions

Based on the results of this study, it can be concluded that:

- Topical treatment of avulsed immature dog teeth with minocycline improves the chances of revascularization after replantation when compared to doxycycline and saline.
- The healing patterns observed in the replanted dog teeth consisted of (i) internal reactive dentin layer with connective tissue and bone/osteoid tissue, (ii), reactive dentin layer and loose connective tissue, and (iii) normal pulp tissue with odontoblastic layer.

References

1. Trope M. Clinical management of the avulsed tooth: present strategies and future directions. Dent Traumatol 2002;18(1):1–11.
2. Andreasen JO, Andreasen FM. Textbook and color atlas of traumatic injuries to the teeth, 3rd edn. Copenhagen: Munksgaard; 1994. p. 383–425.
3. Kerekes K, Heide S, Jacobsen I. Follow-up examination of endodontic treatment in traumatized juvenile incisors. J Endod 1980;6:744–8.
4. Frank AL. Therapy for the divergent pulpless tooth by continued apical formation. J Am Dent Assoc 1966;72:87–93.
5. Stormer K, Jacobsen I. Hvor funksjonsdyktige blir rotfylte unge permanente incisiver? Nordisk forening for pedodonti. Bergen, Norway: Arsmote; 1988.
6. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Endod Dent Traumatol 1992;8:45–55.
7. Öhman A. Healing and sensitivity to pain in young replanted human teeth. An experimental, clinical and histological study. Odontol Tidskr 1965;73:168–227.
8. Kling M, Cvek M, Mejare I. Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. Endod Dent Traumatol 1986;2:83–9.
9. Cvek M, Cleaton-Jones P, Austin J, Lownie J, Kling M, Fatti P. Pulp revascularization in reimplanted immature monkey incisors-predictability and the effect of antibiotic systemic prophylaxis. Endod Dent Traumatol 1990;6:157–69.
10. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. Part I. Diagnosis of healing complications. Endod Dent Traumatol 1995;11:501–8.
11. Skoglund A, Tronstad L. Pulpal changes in replanted and autotransplanted immature teeth of dogs. J Endod 1981;7:309–16.
12. Kristerson L, Andreasen JO. Influence of root development on periodontal and pulpal healing after replantation of incisors in monkeys. Int J Oral Surg 1984;13:313–23.
13. Cvek M, Cleaton-Jones P, Austin J, Kling M, Lownie J, Fatti P. Effect of topical application of doxycycline on pulp revascularization and periodontal healing in reimplanted monkey incisors. Endod Dent Traumatol 1990;6:170–6.
14. Sheppard PR, Burich RL. Effects of extra-alveolar exposure and multiple avulsions on revascularization of reimplanted teeth in dogs. J Dent Res 1980;59:140.
15. Andreasen JO. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. Int J Oral Surg 1981;10:43–53.
16. Love RM. Bacterial penetration of the root canal of intact incisor teeth after a simulated traumatic injury. Endod Dent Traumatol 1996;12:289–93.
17. Yanpiset K, Trope M. Pulp revascularization of replanted immature dog teeth after different treatment methods. Endod Dent Traumatol 2000;16:211–7.
18. Trombelli L, Scabbia A, Zangari F, Griselli A, Wikesjo UM, Calura G. Effect of tetracycline HCl on periodontally-affected human root surfaces. J Periodontol 1995;66:685–91.
19. Andreasen JO. Relationship between surface and inflammatory root resorption and pathologic changes in the pulp after replantation of mature incisors in monkeys. J Endod 1981;7:194–201.
20. Sutter VL, Jones MJ, Ghoneim AT. Antimicrobial susceptibilities of bacteria associated with periodontal diseases. Antimicrob Agents Chemother 1983;23:483–6.
21. Greenstein G, Polson A. The role of local drug delivery in the management of periodontal diseases: a comprehensive review. J Periodontol 1998;69:507–20.
22. Skoglund A, Tronstad L. A microangiographic study of vascular changes in replanted and autotransplanted teeth of young dogs. Oral Surg Oral Med Oral Pathol 1978;45:17–28.
23. Hirasawa M, Hayashi K, Takada K. Measurement of peptidase activity and evaluation of effectiveness of administration of Minocycline for treatment of dogs with periodontitis. Am J Vet Res 2000;61:1349–52.
24. Thomas BS, Varma BR, Bhat KM. Efficacy of Minocycline as a root conditioner in comparison to citric acid and tetracycline. Indian J Dent Res 1999;10:69–75.
25. van Steenberghe D, Rosling B, Soder PO, Landry RG, van der Velden U, Timmerman MF, et al. A 15-month evaluation of the effects of repeated subgingival Minocycline in chronic adult periodontitis. J Periodontol 1999;70:657–67.

26. Golub LM, Ramamurthy NS, McNamara TF, Greenwald RA, Rifkin BR. Tetracyclines inhibit connective tissue breakdown: new therapeutic implications for an old family of drugs. Crit Rev Oral Biol Med 1991;2:297–322.
27. Williams RC, Paquette DW, Offenbacher S, Adams DF, Armitage GC, Bray K, et al. Treatment of periodontitis by local administration of Minocycline microspheres: a controlled clinical trial. J Periodontol 2001;72:1535–44.
28. Johnson DS, Burich RL. Revascularization of reimplanted teeth in dogs. J Dent Res 1979;58:671.
29. Kristensen L. Autotransplantation of human premolars. A clinical and radiographic study of 100 teeth. Int J Oral Surg 1985;14:200–13.
30. Johnson WT, Goodrich JL, James GA. Replantation of avulsed teeth with immature root development. Oral Surg Oral Med Oral Pathol 1985;60:420–7.
31. Baker PJ, Evans RT, Coburn RA, Genco RJ. Tetracycline and its derivatives strongly bind to and are released from the tooth surface in an active form. J Periodontol 1983;54: 580–5.
32. Terranova VP, Franzetti LC, Hic S, DiFlorio RM, Lyall RM, Wikesjo UM, et al. A biochemical approach to periodontal regeneration: tetracycline treatment of dentin promotes fibroblast adhesion and growth. J Periodont Res 1986;21:330–7.
33. Gabler WL, Creamer HR. Suppression of human neutrophil functions by tetracyclines. J Periodont Res 1991;26: 52–8.
34. Pruzanski W, Greenwald RA, Street IP, Laliberte F, Stefanski E, Vadas P. Inhibition of enzymatic activity of phospholipases A2 by Minocycline and doxycycline. Biochem Pharmacol 1992;44:1165–70.
35. Rifkin BR, Golub LM, Sanaui F, Vernillo AT, Kleckner AP, McNamara TF, et al. Effects of tetracyclines on rat osteoblast collagenase activity and bone resorption *in vitro*. In: Davidovitch, Z, editor. The biological mechanisms of tooth movement and craniofacial adaptation. Birmingham: EBSCO Media; 1991. p. 85–90.
36. Rifkin BR, Vernillo AT, Golub LM. Blocking periodontal disease progression by inhibiting tissue-destructive enzymes: a potential therapeutic role for tetracyclines and their chemically-modified analogs. J Periodontol 1993;64: 819–27.