THE EFFECT OF INTRACANAL CORTICOSTEROIDS ON HEALING OF REPLANTED DOG TEETH AFTER EXTENDED DRY TIMES

Anna Kirakozova, DDS

"A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the School of Dentistry (Endodontics)."

Chapel Hill
2008

Approved by:

Dr. Martin Trope

Dr. Fabricio Teixeira

Dr. Alice Curran

ABSTRACT

ANNA KIRAKOZOVA, DDS.: The effect of intracanal corticosteroids on healing of replanted dog teeth after extended dry times
(Under the direction of Dr. Martin Trope)

Corticosteroids may inhibit unfavorable periodontal healing of replanted avulsed teeth. **Aims:** 1) investigate the effect of potent intracanal corticosteroids on periodontal healing; 2) evaluate systemic absorption of intracanal corticosteroids. **Methods:** 67 dog premolar roots were extracted. Groups 1-3 were filled with gutta-percha and replanted immediately, after 40- and 60 min, respectively. Groups 4 and 5 were filled with 0.05% clobetasol. Groups 6 and 7 were filled with 0.05 % fluocinonide. Groups 4 and 6 were replanted after 40 min; groups 5 and 7 after 60 minutes. After 4 months, roots were evaluated histologically for type of periodontal healing. **Results:** Groups treated with clobetasol and fluocinonide healed more favorably than groups filled with gutta-percha and were different from each other at 60 min. No change in the systemic corticosteroid blood concentration was observed with any of the intracanal corticosteroids. **Conclusion:** Corticosteroids were efficacious as intracanal medicaments for promoting favorable periodontal healing.

AKNOWLEDGMENTS

Thesis Mentor: Dr. Martin Trope

Thesis Advisor: Dr. Fabricio B. Teixeira

Thesis Advisor: Dr. Alice E. Curran

Professional assistance: Dr. Peter Tawil and Dr. Derek Duggan

Animal care and surgery: UNC DLAM - Anissa Anderson and staff

Histology slide preparation: Janice Weaver

Microscopic evaluation: Dr. Alice E. Curran

Statistical analysis: Dr. Fang Gu

Expert counseling: Dr. Hong Chen

Text editing: Dr. Shannon Vann and Ryan Vann

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

REVIEW OF THE LITERATURE

Tooth avulsion, or complete dislodging of a tooth from the socket, accounts for up 16% of all traumatic dental injuries to the permanent dentition in humans (1). Rupture of the periodontal ligament, damage to the cementum, and severance of the apical neurovascular bundle leading to inevitable pulpal necrosis occur as a result of tooth avulsion. Previous studies report that periodontal ligament healing and long-term prognosis of replanted teeth depend on several factors that include length of extraoral dry time (2-4), extra-alveolar storage media (2, 3), stage of root development (2), socket management (5), type and duration of splinting (6, 7), and timely control of root canal infection (8). Extraoral dry time is defined as the time during which avulsed teeth are allowed to air dry before being replanted into a socket or placed in a proper storage medium. Length of extraoral dry time appears to be the most critical factor since it directly affects the viability of periodontal ligament (PDL) cells remaining on the root surface. It has been shown that the chance of PDL healing is inversely proportional to the amount of time spent out of the tooth socket. Periodontal ligament healing is expected if the dry time is less than 15-20 minutes (9, 10), while PDL cells are unlikely to survive after 60 minutes of dry time (2, 11). Neither complete periodontal healing nor complete failure is expected if the extraoral dry time period is more than 20 but less than 60 min. Therefore, immediate replantation of an avulsed tooth provides the best treatment.

If immediate tooth replantation is not feasible however, proper storage medium of a tooth may maximize the viability of PDL cells and minimize the total extraoral dry time.

Milk has been shown to be an excellent storage medium as its osmolarity is compatible with PDL cell survival (12, 13). Water is the worst storage medium due to its hypotonicity that leads to the lysis of PDL cell (13). Several studies have demonstrated that specialized storage media such as Hank's Balance Salt Solution (a cell culture medium) and Viaspan (a liver transport medium) prolongs the survival of PDL cells, but these media are not easily available at sites where most dental injuries occur (14-16).

Inflammation is the first response after replantation of a tooth with a damaged root surface (17). Initial inflammatory response is provoked by dead or dying PDL cells, debris, and bacterial contamination of the root surface and alveolus. Previous studies have shown that the extent of the initial inflammatory response can determine the type of periodontal healing after replantation (7, 18). If the area of damaged cementum is small and extraoral dry time is minimized, inflammation is localized, and favorable healing with new cementum is expected. On the other hand, if excessive cemental damage or PDL drying of a large area of the root surface occurs, a severe inflammatory response will develop, leading to unfavorable healing with osseous replacement (ankylosis) or external root resorption.

During the actual tooth avulsion and replantation, only small areas of mechanical damage to the cementum are present. This leads to localized inflammation and consequently localized areas of root resorption (termed "surface resorption") (19, 20). Under ideal circumstances that include viable PDL, bacteria-free root surface and pulp space, these areas of root resorption will heal with new reparative cementum within fourteen days (12, 21, 22). However, if an avulsed tooth has been left dry for an extended period of time (>60 min) prior to its replantation, necrosis of periodontal ligament and mechanical damage to a large area of cementum will occur. After the initial inflammatory response, the type of cells that

repopulate the denuded root surface will determine the clinical outcome and long-term prognosis of a replanted tooth. It has been shown that the bone-producing cells often repopulate the root surface at a faster rate than PDL or cementum-producing cells. This leads to fusion between the bone and the root surface, a process termed dentoalveolar ankylosis (23). It has been reported that damage to more than 20 percent of the total root surface is required for ankylosis to occur (23). As a result of initial ankylosis and physiologic bone remodeling, the root is resorbed by osteoclasts and new bone is deposited instead of dentin. This process is termed osseous replacement (7). Eventually, complete replacement of root dentin occurs, which ultimately leads to fracture of unsupported coronal tooth structure. This process usually takes about 5-7 years in a growing individual (24, 25). Therefore, the goal of the treatment, especially in young patients, is to slow down the osseous replacement of the root, to retain the tooth long enough to gain time for optimal restorative treatment plan, and to allow for the alveolar bone growth (25).

Treatment procedures for delayed replanted teeth include the complete removal of PDL and debris from the root surface either by curettage or acid (7) followed by topical application of various pharmacological agents prior to its replantation into the socket. In animal models, soaking the avulsed tooth in fluoride has been shown to be beneficial in retarding the osseous replacement (26-29). Also, topical effect of bisphosphonates on root surfaces of replanted teeth experiencing extended dry times has been investigated (30, 31). Alendronate, a drug that inhibits action of osteoclasts, has shown promising results in slowing down osseous replacement (30). Conflicting results have been reported with the use of Emdogain®, an enamel matrix derivative gel, widely used in periodontal surgeries for regeneration of lost periodontal attachment (32, 33). Several studies investigated the effect of

Emdogain® on teeth that lack periodontal ligament (>60 minutes of extraoral dry times) and found that it did not significantly reduce incidences of osseous replacement (34-37). It appears that Emdogain® may improve the outcome of replanted teeth experiencing shorter extra oral dry times (38, 39).

Following the replantation of an avulsed tooth, the condition of the pulp is a factor in success. Inevitable loss of blood supply renders pulp necrotic. Pulp revascularization is possible in teeth with open apex that are replanted within 60 minutes of injury, but it will not occur in mature teeth with a closed apex (40, 41). While necrotic uninfected pulp is not of consequence (42), its susceptibility to the bacterial contamination is well-established (43, 44). If left untreated, necrotic pulp will become infected within 3 weeks following the trauma (43). Necrotic infected pulp in conjunction with damaged root surface serves as an additional stimulus for periodontal ligament inflammation and promotes osteoclast-mediated external root resorption.

External root resorption is the loss of root structure from the outer surface of the root that results from odontoclast cells formed by fusion of macrophages (45). Due to their histochemical and ultrastructural similarities, odontoclasts are believed to belong to the same cell population as osteoclasts (46). However, odontoclasts differ slightly from osteoclasts in that they appear morphologically smaller and have fewer nuclei (47, 48).

Root resorption rarely occurs in permanent teeth (49). Even in the presence of periradicular inflammation that results in activation of osteoclasts/odontoclasts, resorption of the alveolar bone will occur while root surface itself will often remain intact (50). The exact mechanism for the resistance of the root surface to resorption is not fully understood. Many studies support the theory that odontoclasts/osteoclasts cannot bind to the unmineralized

matrix of pre-cementum (24, 49, 51). Pre-cementum, the most external aspect of cementum, consists of a layer of cementoblasts covering the organic unmineralized cementoid. To function, osteoclasts need to adhere to specific extracellular proteins that contain the arginin-glycin-aspartic (RGD) sequence of amino acids. RGD sequences are bound to calcium salt crystals of mineralized matrix and serve as osteoclast binding sites (48). Therefore, lack of RGD peptides in pre-cementum prevents osteoclast binding and subsequently renders the root surface resistant to the resorption. If, however, damage to the pre-cementum occurs either directly due to trauma or indirectly due to the secondary inflammatory response, then the root surface becomes vulnerable to odontoclasts/osteoclast-induced resorption.

Another theory for the resistance of the root to the resorption takes into account the presence of intermediate cementum (24), the innermost layer of cementum, which in health serves as a barrier between the dentinal tubules and periodontal ligament (52). If intermediate cementum is damaged, then bacteria and bacterial byproducts can travel from the pulp space through the dentinal tubules and stimulate the inflammatory response in corresponding periodontal ligament, leading to resorption of root and bone (45, 48). In summary, combination of both the damage to the protective layer of the root surface (pre-cementum and intermediate cementum) and the presence of the inflammatory stimulus (bacteria in the pulp space) are required for the initiation of external root resorption. If the inflammatory stimulus is maintained for a prolonged period of time, external resorption eventually will lead to the loss of the tooth (53). Therefore, emergency treatment following the tooth avulsion is aimed at minimizing the initial inflammatory response, limiting the size of damaged root and hence promoting favorable rather than unfavorable healing.

Calcium hydroxide remains the intracanal medicament of choice for a replanted permanent tooth with a closed apex (8). Current recommendations include the initiation of root canal therapy within seven to ten days, and placement of calcium hydroxide for up to one month followed by the root canal obturation (54, 55). If, however, external inflammatory root resorption is suspected prior to the placement of root canal filling, or if initiation of the root canal treatment has been delayed beyond the ideal 7-10 day period, then long-term calcium hydroxide treatment is recommended for a period of 6 to 24 months or until the healing of resorption can be confirmed radiographically and clinically (7, 54, 56). Long-term calcium hydroxide treatment has been shown to be more effective than short-term treatment in cases of established inflammatory root resorption. (56). Although calcium hydroxide possesses excellent antibacterial properties (57-59), it inhibits the root resorption initiated by intracanal bacteria only (60), and has no effect on the inflammation produced by damaged periodontal ligament cells, as observed in avulsion injury. In addition, it was reported that calcium hydroxide therapy of teeth with severely traumatized periodontium may interfere with the healing process of periodontal ligament and lead to PDL and reparative cells' necrosis and osseous replacement (61-64). Some animal studies have shown that there were not statistically significant differences in resorption indices between the replanted teeth obturated with gutta percha or with calcium hydroxide (65). Furthermore, replanted teeth filled with calcium hydroxide showed higher percentages of ankylosed root surface area than those treated with gutta-percha and sealer (62, 64).

Corticosteroids, as anti-inflammatory drugs, have been used in endodontics for several decades. In 1965, Schroeder described the ability of corticosteroids to treat endodontic disease of inflammatory origin (66). The anti-inflammatory action of

corticosteroids can be attributed to their ability to block all pathways of eicosanoid synthesis (67). Eicosanoids are metabolic products of arachidonic acid. Arachidonic acid is released from the cell membrane phospholipids of macrophages and polymorphonuclear leukocytes (PMNs) by the action of phospholipase A_2 in response to injury by noxious stimuli. There are two major groups of eicosanoids. The first is prostaglandins (PGs) that are formed from arachidonic acid by cyclooxygenase pathway and the second is leukotrienes (LTs) formed from lipoxygenase pathway. Among other functions, eicosanoids mediate the inflammatory response. On a cellular level, corticosteroids are believed to induce the production of anti-inflammatory proteins called lipocortins that antagonize the action of phospholipase A_2 (68, 69). This action decreases the subsequent production and release of lipid inflammatory mediators by microphages. Corticosteroids also increase the production of interleukin-10 (IL-10), the anti-inflammatory cytokine, and interleukin-1 (IL-1) receptor antagonist, the cytokine that counteracts the effect of pro-inflammatory cytokine, interleukin-1 (IL-1) (70). PGE_2 and PGI_2, produced mainly by PMNs during acute inflammation, are also potent activators of osteoclasts that lead to bone resorption (45).

It has been reported that local administration of corticosteroids reduces the osteoclastic bone resorption by affecting recruitment and promoting the receptor-mediated apoptosis of osteoclasts (71, 72). Another study confirmed this finding by investigating the direct effect of the synthetic glucocorticosteroid dexamethasone on cultures of human mature osteoclasts *in vitro* and found that it reduces the bone-resorbing activity of mature osteoclasts, probably through the specific cytotoxic action of the drug, which increases osteoclast apoptosis (73). Given the anti-inflammatory and anti-resorptive properties, corticosteroids could potentially be useful in manipulating the initial inflammatory response

in teeth with damaged periodontal attachment, thereby promoting the favorable healing with cementum rather than osseous replacement and/or osteoclast-mediated inflammatory root resorption (17).

Topical and systemic effects of dexamethasone were compared in healing of replanted dog's teeth (74). It was found that teeth soaked in dexamethasone exhibited significantly more favorable healing and less root resorption than systemic dexamethasone. The same positive effect of topical dexamethasone was also confirmed in rats' teeth following the delayed replantation (75).

Tetracycline has been widely used in periodontics due to its sustained broad-spectrum antimicrobial effect. Tetracycline also promotes fibroblast adhesion and connective tissue attachment, thus facilitating periodontal ligament regeneration (76). In addition, it was shown that tetracycline possesses anti-resorptive properties due to its direct effect on osteoclasts and matrix metalloproteinases, enzymes responsible for collagen degradation (77, 78). In endodontics, when used in an osseous remodeling study in dogs, systemic tetracycline was found to result in significantly more cemental healing than systemic penicillin (79). While it appears that tetracycline affects the osteoclasts present at the site of resorption and corticosteroids regulate osteoclast recruitment to the site of injury, synergistic effect of antibiotic and corticosteroid might enhance the inhibition of root resorption (17).

Ledermix ™, a corticosteroid/tetracycline water-soluble paste, was first introduced for endodontic use in 1962 as a pulp capping agent and intracanal interappointment medication by Schroeder (66). Since then, several studies have demonstrated that intracanal Ledermix ™ is an effective anti-bacterial and anti-inflammatory medicament that promotes favorable periodontal healing and inhibits inflammatory root resorption following the

experimental avulsion and replantation in an animal dental trauma model (80-83). It has been shown that Ledermix™ is more effective in inhibiting the inflammatory root resorption than calcium hydroxide in traumatized teeth (82, 84). The release of active components of Ledermix ™ through the dentinal tubules, lateral canals, and the apical foramen to the external root surface can take as long as 14 weeks (85). After a rapid initial release, the antibiotic component of Ledermix ™, 3.21% demethylclortetracycline, remains effective against microorganisms in the dentinal tubules for the first week with 66% release after 14 wks. The corticosteroid component, 1% triamcinolone, is slowly released into periodontal tissues with 98% release after 14wks (85). In the absence of cementum on the root surface, the diffusion rate of Ledermix™ is significantly higher (85). While steroids generally suppress inflammation by inhibiting all pathways of eicosanoid synthesis, the steroid component of Ledermix™ paste has demonstrated a direct inhibitory effect on clastic cells without damaging the cells of periodontal ligament (86). The antibiotic component is effective in attenuating root resorption, in part due to the elimination of a wide spectrum of bacteria and their toxins from dentinal tubules (87).

However, tetracycline in Ledermix™ paste has been shown to cause the discoloration of mature and immature teeth, especially when exposed to sunlight (88, 89). Discoloration is the major side effect of Ledermix ™ that should be taken into consideration since majority of dental trauma occurs to the anterior dentition of children and adolescents (90, 91).

Because of the clinically unacceptable tooth discoloration of Ledermix™, use of alternative therapies has been investigated. Chen *et al*. studied the individual influences of tetracycline and corticosteroid components of Ledermix™ to determine whether either would be as effective as a combination of both in inhibiting the root resorption in delayed replanted

dogs' teeth (83). It was shown that the corticosteroid at the same dosage as the one found in the Ledermix™ as an intracanal medicament was as effective as Ledermix™ itself in inhibiting external root resorption and promoting favorable healing, thus eliminating the side effect of tooth discoloration. Although concern exists that the use of corticosteroids within the root canal system may have deleterious systemic effects including the suppression of the hypothalamus-pituitary-adrenal axis, it has been reported that the highest possible amounts used intrapulpally are unlikely to result in any systemic side-effects (92).

INTRODUCTION

Periodontal ligament healing and long term prognosis of replanted avulsed teeth depend on several factors, most importantly on the length of extraoral dry time (2-4). Periodontal ligament healing is expected if the dry time is less than 15-20 min (9, 10). Periodontal ligament (PDL) cells are unlikely to survive after 60 min of dry time (2, 11). Severe inflammatory response following the delayed replantation of a tooth with the damaged root surface is predicted to result in unfavorable healing with osseous replacement or external inflammatory root resorption (7, 18). Therefore, the emergency treatment following the tooth avulsion is aimed at minimizing the initial inflammatory response, limiting the size of damaged root and hence promoting the favorable rather than unfavorable healing.

The pharmacological manipulation of the inflammatory response following tooth replantation has been widely investigated. Calcium hydroxide remains the intracanal medicament of choice for a replanted permanent tooth with a closed apex (8). Although it possesses excellent antibacterial properties (57, 58), calcium hydroxide inhibits the root resorption initiated by intracanal bacteria only and has no effect on the inflammation produced by damaged periodontal ligament cells as observed in avulsion injury. Several studies have demonstrated that intracanal Ledermix ™, a corticosteroid/tetracycline water-soluble paste, is an effective anti-bacterial and anti-inflammatory medicament that promotes favorable periodontal healing and inhibits inflammatory root resorption following the experimental avulsion and replantation in an animal dental trauma model (80-83). However,

tetracycline in Ledermix™ paste has been shown to cause the unwanted discoloration of mature and immature teeth (88, 89).

Few studies have addressed the individual effect of corticosteroids on the periodontal healing pattern of replanted teeth. While steroids in general suppress inflammation by inhibiting all pathways of eicosanoid synthesis, the steroid component of Ledermix paste, 1% triamcinolone, has been demonstrated to have a direct inhibitory effect on clastic cells (86). Chen *et al.* showed that the corticosteroid at the same dosage as the one found in the Ledermix™ as an intracanal medicament was as effective as Ledermix™ itself in inhibiting external root resorption and promoting favorable periodontal healing, thus eliminating the side effect of tooth discoloration (83). It still remains unclear if the anti-resorptive effect of corticosteroids can be further enhanced by increasing the corticosteroid potency and its anti-inflammatory properties. Although concern exists that the use of corticosteroids within the root canal system may have deleterious systemic effects including the suppression of the hypothalamus-pituitary-adrenal axis, it has been reported that the highest possible amounts used intrapulpally are unlikely to result in any systemic side-effects (92). However, to date, no *in vivo* studies have addressed the systemic absorption and adverse effects caused by intrapulpal application of corticosteroids or medications containing corticosteroids (e.g. Ledermix ™).

The purpose of this study was: 1) to investigate the effect of potent intracanal corticosteroids 0.05% clobetasol (Temovate®) cream and 0.05 % fluocinonide (Lidex®) cream on the periodontal healing pattern of dog teeth following simulated avulsion and replantation after various dry times; 2) to assess changes in the corticosteroid blood

concentration following the intracanal use of these corticosteroids to determine if there are systemic effects.

MATERIAL AND METHODS

Prior to initiation of the study, the use of the dog model had been approved by the Institutional Animal Care and Use Committee (IACUC) of the University of North Carolina at Chapel Hill. The surgical phase of the study was conducted in the facilities of the Division of Laboratory Animal Medicine (DLAM), University of North Carolina at Chapel Hill. Creams of 0.05% clobetasol and 0.05 % fluocinonide were prepared by the Central Pharmacy in Durham, North Carolina. Blood samples were analyzed by the Antech Diagnostics Laboratory in Lake Success, New York. The experimental protocol of this study was based on the previous studies conducted at the Department of Endodontics, University of North Carolina Chapel Hill (82, 83).

1. Surgical Procedure

One hundred five mature premolar roots in six beagle dogs were used in this study. All surgical procedures were performed under the general anesthesia, which was achieved by intravenous injection of 6% sodium pentobarbital (30mg/ kg body weight). Two-rooted premolars were hemisected. Each hemisected root was treated as a separate treatment unit to maximize the number of teeth used in each dog (Figure 1). All study roots were extracted as atraumatically as possible. Following extraction, each root canal was accessed and instrumented in aseptic fashion to minimize the pulpal infection as a stimulus for external inflammatory root resorption. Canal instrumentation was performed with nickel- titanium

rotary files and 1% sodium hypochlorite for irrigation and the canals were dried with paper points. Then each root was randomly assigned to one of the seven groups (Table 1). Randomized scheme was stratified in each dog such as all seven treatment groups were equally represented in each dog:

- **Group 1** (negative control-15 roots): The roots were filled with Gutta- Percha and Roth's sealer using lateral condensation technique and replanted in their sockets within a total extraoral dry time of approximately 5 minutes.
- **Group 2** (positive control-15 roots): The roots were filled with Gutta-percha and Roth's sealer and replanted after 40 minutes of extraoral dry time.
- **Group 3** (positive control-15 roots): The roots were filled with Gutta-percha and Roth's sealer and replanted after 60 minutes of extraoral dry time.
- **Group 4** (experimental group- 15 roots): The roots were filled with 0.05% clobetasol propionate (Temovate®) cream using a lentulo spiral and replanted after 40 minutes of extraoral dry time.
- **Group 5** (experimental group-15 roots): The roots were filled with 0.05% clobetasol propionate (Temovate ®) cream using a lentulo spiral and replanted after 60 minutes of extraoral dry time.
- **Group 6** (experimental group-15 roots): The roots were filled with 0.05 % fluocinonide (Lidex®) cream using a lentulo spiral and replanted after 40 minutes extraoral dry time.
- **Group 7** (experimental group-15 roots): The roots were filled with 0.05 % fluocinonide (Lidex ®) cream using a lentulo spiral and replanted after 60 minutes of extraoral dry time.

Table 1. Allocation of teeth to treatment groups

Treatment Group	Extraoral dry time (minutes)	Root canal filling	Group Abbreviation *
1- negative control	0-5	Gutta-percha + Roth's sealer	NC
2- positive control	40	Gutta-percha + Roth's sealer	PC40
3-positive control	60	Gutta-percha + Roth's sealer	PC60
4- experimental	40	Temovate® cream	T40
5- experimental	60	Temovate® cream	T60
6- experimental	40	Lidex® cream	L40
7- experimental	60	Lidex® cream	L60

* group abbreviations will be used further in the text to refer to the treatment groups of the study

The access cavities were filled with Fuji IX glass ionomer prior to the replantation of roots in their sockets. Splinting was not used due to the stability of the replanted teeth.

Surgery was performed in two sessions per dog to minimize postoperative discomfort. During the first session, roots in two quadrants (either upper right and lower left or upper left and lower right) were extracted and replanted. Two weeks later, the same procedure was performed on the other quadrants. Dogs were maintained on a regular diet following the procedure.

Systemic absorption of intracanal corticosteroids was assessed by evaluating changes in plasma concentration of endogenous cortisol (93, 94). Blood samples (5ml) were taken immediately prior to the surgical procedure (day 0) to determine the baseline plasma levels of cortisol in each dog. In addition, blood was drawn 24 hours (day 1) (92) and 30 days postoperatively in order to determine possible changes in the systemic cortisol blood levels. Plasma concentration of cortisol was determined by use of radioimmunoassay (95).

Dogs were sacrificed after 4 months by overdose of 6% sodium pentobarbital solution administered intravenously. Jaw blocks containing the roots and surrounding alveolar bone were prepared and fixed in 10% formalin. After removal of soft tissues, the hard tissue blocks were first decalcified in Formical™ (Decal Chemical Corporation, Congers, NY) solution for 1 week. Then the specimens were slowly decalcified in Immunocal™ (Decal Chemical Corporation, Tallman, NY) solution for 2 months, undergoing solution change every 2 weeks over that time. Subsequently, specimens were dehydrated in alcohol and embedded in paraffin. Sections were made perpendicular to the long axis of the roots at a thickness of 5 µm at 90µm and stained with hematoxylin and eosin. Two mid-root cross-sections per root were examined with light microscopy. The middle cross sections of the root were chosen for histological evaluation to avoid the apical root and its delta-like anatomy. Coronal sections of the root were also excluded from the analysis due to potential similarities in histological appearances of the resorption caused by sulcular infection and external inflammatory root resorption.

Figure 1. Tooth hemisection prior to extraction

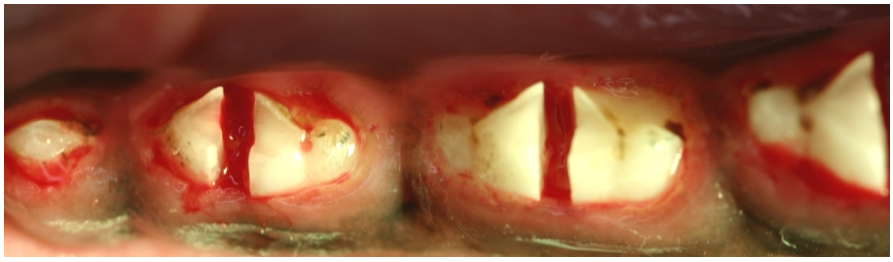


Figure 2. Replantation of teeth

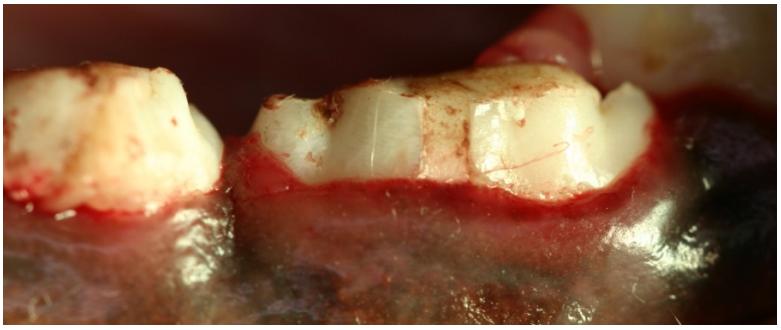


Figure 3. Chart used for monitoring extraoral dry times

2. Histological evaluation

Histological evaluation was conducted by two blinded examiners. Each cross section of the root was analyzed for the type of periodonal healing based on the status of the interface between the root and the surrounding tissue. Each root was scored as favorable healing vs. unfavorable healing.

To assess the type of periodontal healing in each section, an eight-point grid was superimposed on each root cross-section. The root/ surrounding tissue interface at each of the eight circumferential points was identified (96). Presence of cementum over dentin was judged as **favorable healing**, whereas areas of direct fusion of bone to the root (osseous replacement) or active inflammatory root resorption was identified as **unfavorable healing.**

Figure 4. Histological evaluation: eight-point grid

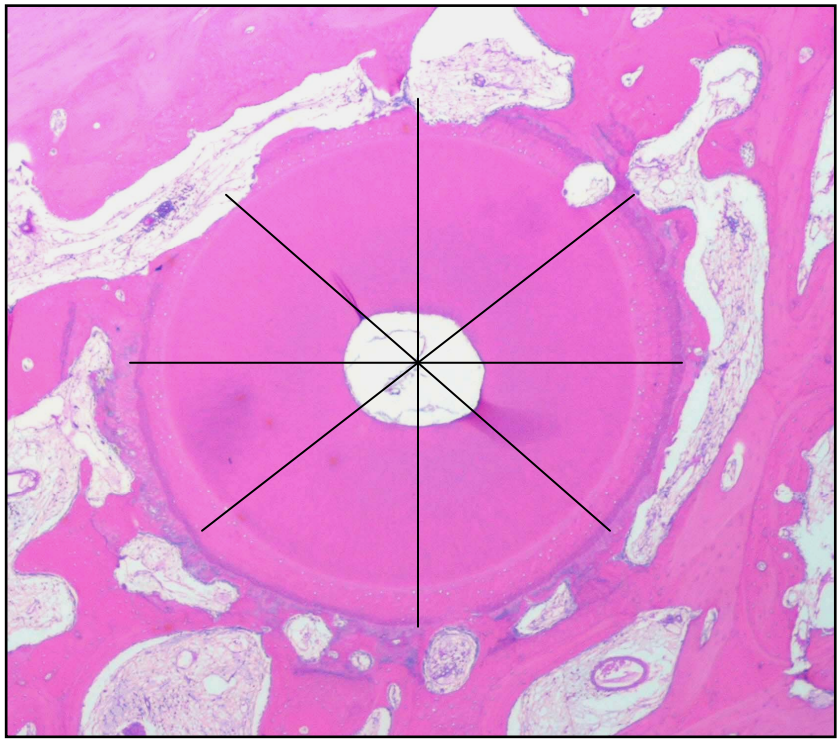


Figure 5. Favorable healing: cementum

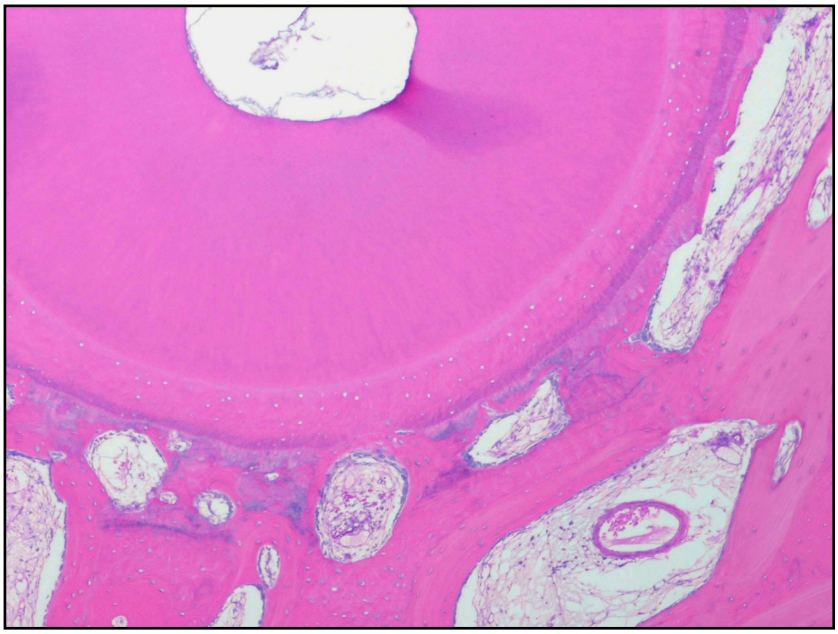


Figure 6. Unfavorable healing: osseous replacement

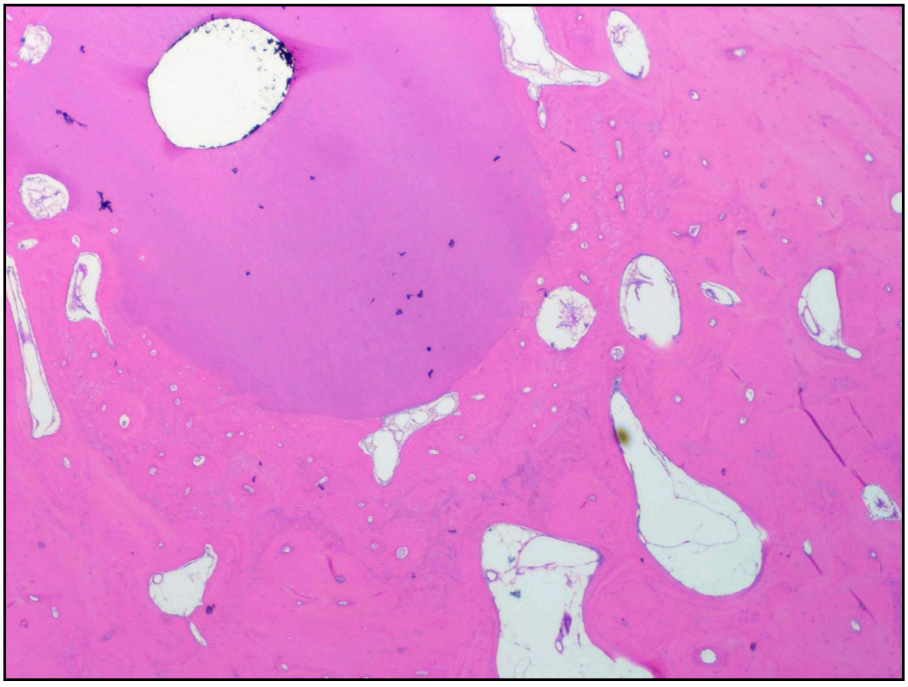


Figure 7. Unfavorable healing: external inflammatory root resorption

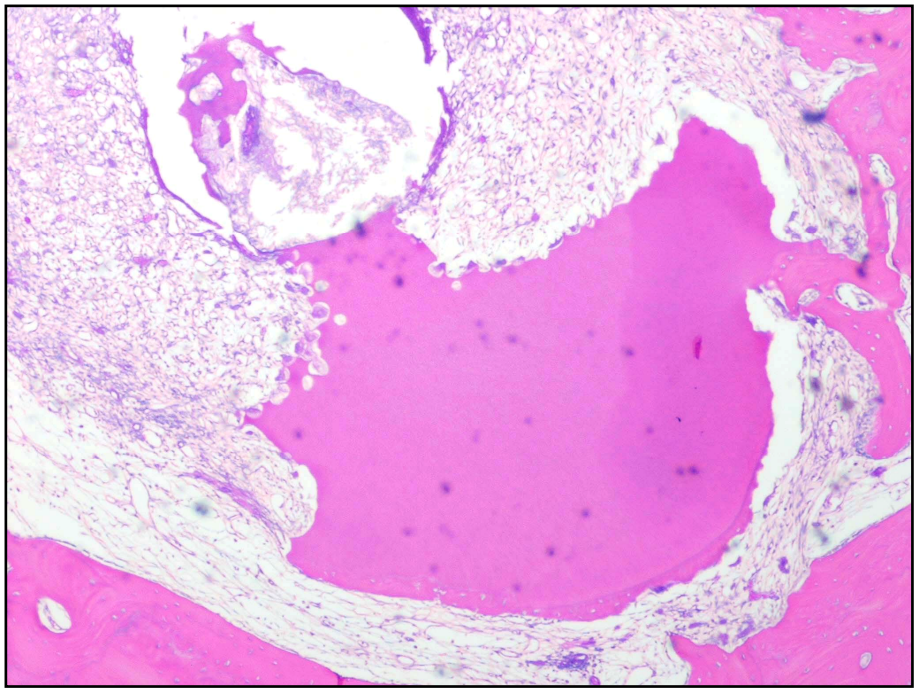
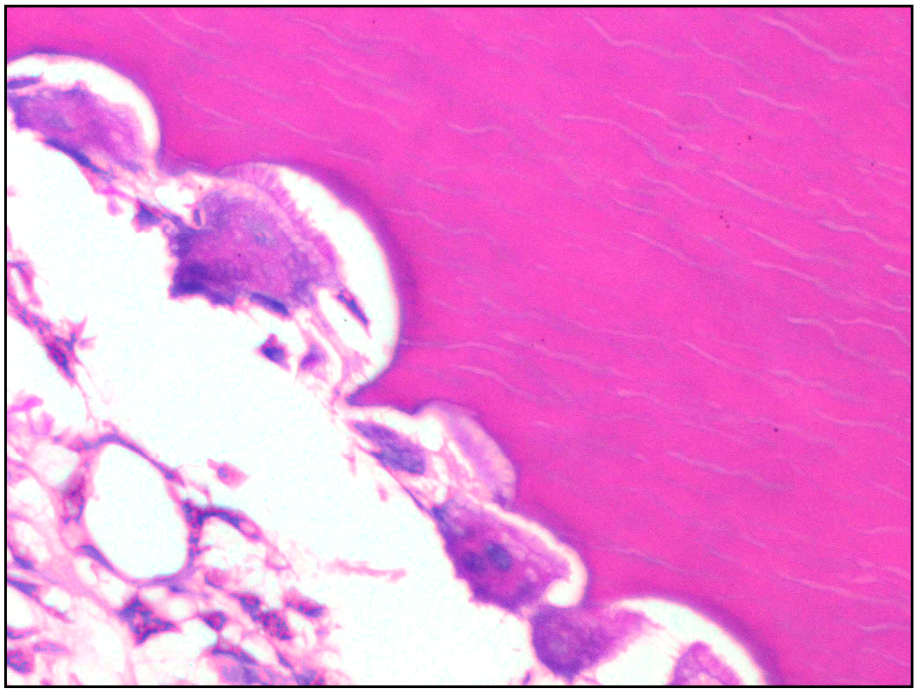


Figure 8. Unfavorable healing: osteoclasts



3. Data analysis

Periodontal healing pattern:

The measurements for favorable (healing with cementum) and unfavorable healing (inflammatory root resorption and osseous replacement) were collected as percentages of the total number of evaluation points on the root cross-section for each experimental group. The model based approach, Generalized Estimating Equations (GEE) analysis, was used to investigate whether the periodontal healing pattern is different among treatment groups. A likelihood ratio test (score test for GEE analysis) was used to compare the mean responses for three different types of healing (cementum vs resorption vs osseous replacement) among experimental groups (97). Regression analysis with GEE methodology was used because the outcome of interest is discrete (3 types of periodontal healing) and it takes into account possible within- dog correlation of healing responses.

Blood cortisol concentration:

Repeated measurement procedure (Proc mixed model) was used to analyze the differences in plasma cortisol levels between different time points (day 0, day 1 and day 30). Repeated measures analysis deals with response outcomes measured on the same experimental unit at different times and under different conditions. Measurements on the same experimental unit are likely to be correlated and repeated measurement analysis can account for this correlation.

SAS® version 9.1 (Cary, NC, USA) was used for the analysis. The level of statistical significance was set at $P< 0.05$.

RESULTS

The dogs tolerated the treatment well and did not show any signs of distress following the surgical procedure. Twelve roots were fractured during the extraction and were not included in the experiment. Twenty six roots were lost postoperatively during the 4 months between the time of surgery and sacrifice. A total of thirty-eight roots were not included in the experiment. Therefore, of initial target of 15 roots per each treatment group, the following number of roots per group remained for final statistical analysis of periodontal pattern of healing (Table 2):

Table 2. Number of roots (n) per treatment group used for statistical analysis

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
	Negative Control	Positive Control (40 min)	Positive Control (60min)	Temovate (40min)	Temovate (60min)	Lidex (40min)	Lidex (60min)
Number of roots (n)	13	13	9	10	9	5	8

Periodontal healing pattern:

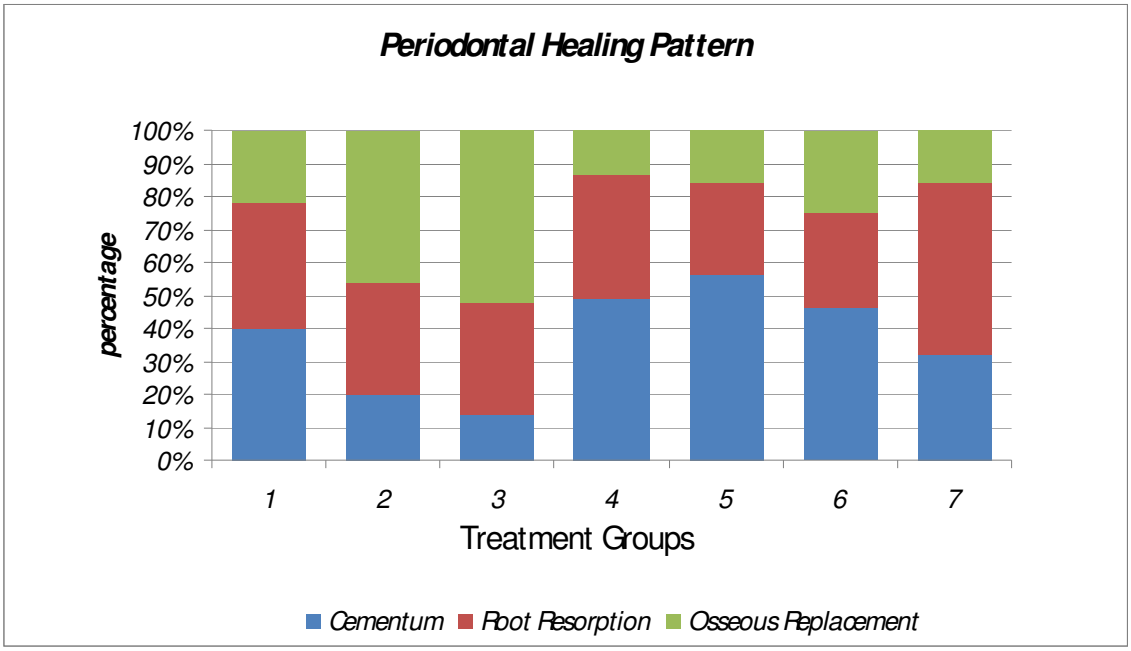
1. Overall healing: cemental healing vs root resorption vs osseous replacement

Table 3 and Figure 9 show the percentages of overall healing by treatment.

Table 3. Percentage of evaluation points with overall healing by treatment

	Treatment groups						
	Group1	Group2	Group3	Group4	Group5	Group6	Group7
	Negative Control	Positive Control (40 min)	Positive Control (60min)	Temovate® (40min)	Temovate® (60min)	Lidex® (40min)	Lidex® (60min)
Cementum	39.90	20.19	13.89	49.38	56.25	46.25	32.03
Root Resorption	37.98	33.65	34.03	37.50	27.78	28.75	52.34
Osseous replacement	22.12	46.15	52.08	13.13	15.97	25.00	15.63

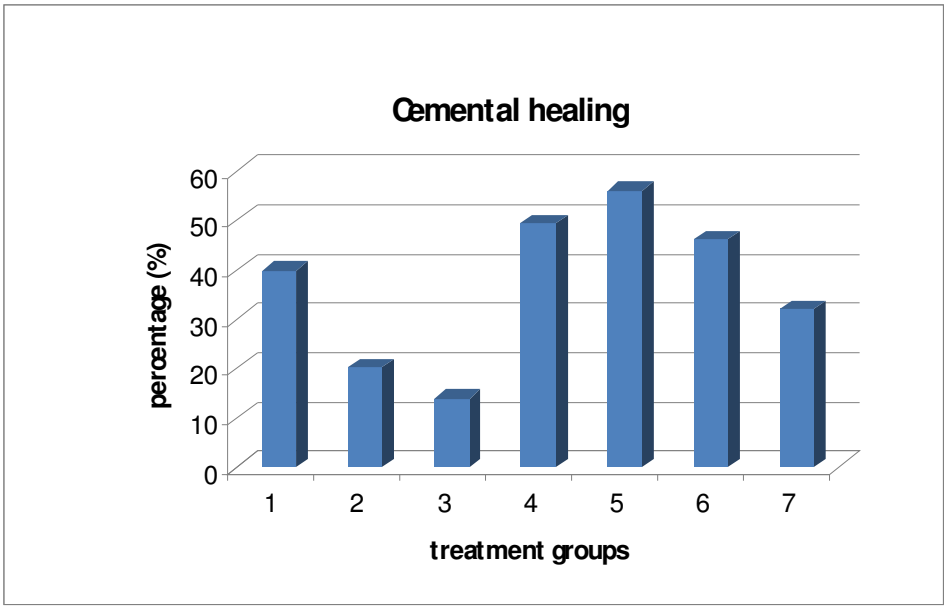
Figure 9. Percentage of evaluation points with overall healing by treatment



2. Periodontal Healing Pattern : Favorable Healing (Cementum) (Figure 10)

There were significant differences between treatment groups in term of favorable healing (P<0.001). As expected, group 1 (NC) showed significantly more cemental healing that groups 2 (PC40) and 3 (PC60) (P<0.001). Pairwise comparison showed that groups 4 (T40), 5 (T60), 6 (L40) and 7 (L60) had significantly more favorable healing than groups 2 (PC 40) and 3 (PC60) (P<0.05). Groups 4 (T40) and 5 (T60) had significantly more favorable healing than group 7 (L60) (P<0.05); group 5 (T60) had significantly more healing than group 1 (NC) (P=0.028).

Figure 10. Percentage of evaluation points with favorable healing (cementum) by treatment

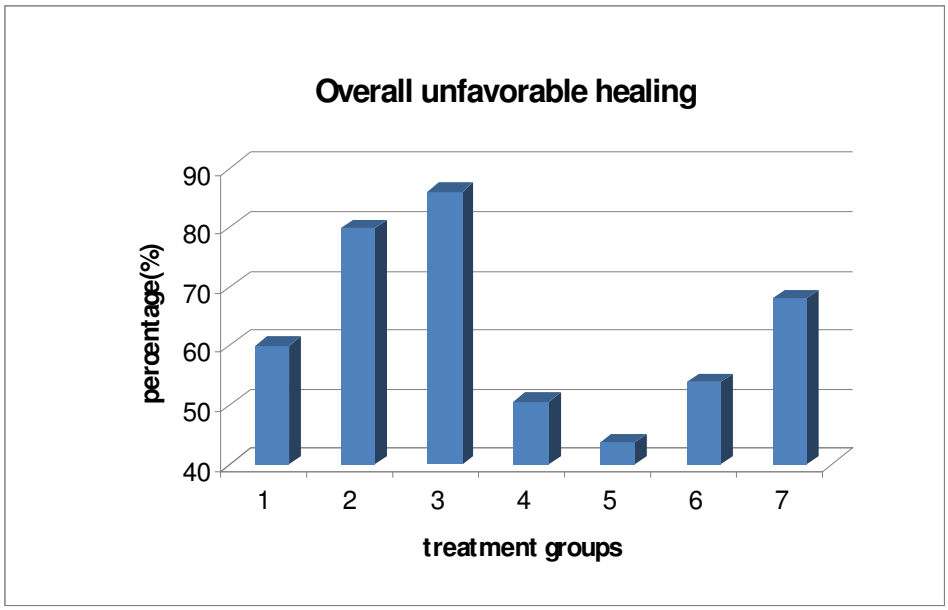


3. Periodontal Healing Pattern : Unfavorable Healing

A. Overall Unfavorable healing: Combined inflammatory root resorption and osseous replacement (Figure 11)

There were statistically significant differences between treatment groups in terms of unfavorable healing (combined inflammatory root resorption and osseous replacement) (P<0.001). Pairwise comparison showed that groups 2 (PC40) and 3 (PC60) had significantly more unfavorable healing than groups 1(NC), 4 (T40), 5 (T60) and 6 (L40) (P<0.05). Group 5 (T60) had significantly less unfavorable healing than groups 1 (NC) and 7(L60) (P<0.05).

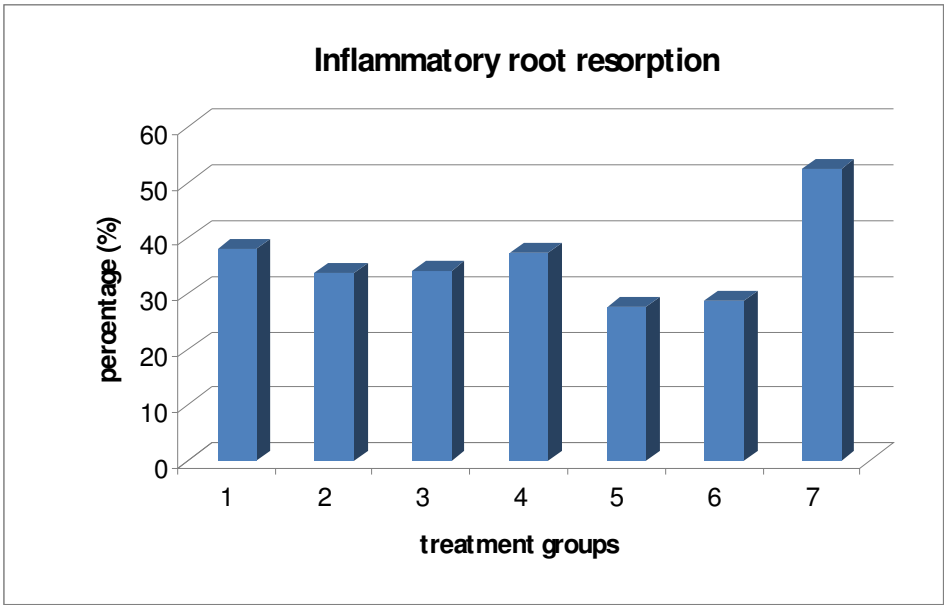
Figure 11. Percentage of evaluation points with unfavorable healing by treatment



B. <u>Unfavorable Healing</u>: External inflammatory root resorption (Figure 12)

There were statistically significant differences between treatment groups in terms of inflammatory root resorption (P<0.001). Pairwise comparison showed that groups 2(PC40), 3(PC60), 5(T60) and 6(L40) had significantly less resorption than group 7(L60) (P<0.05).

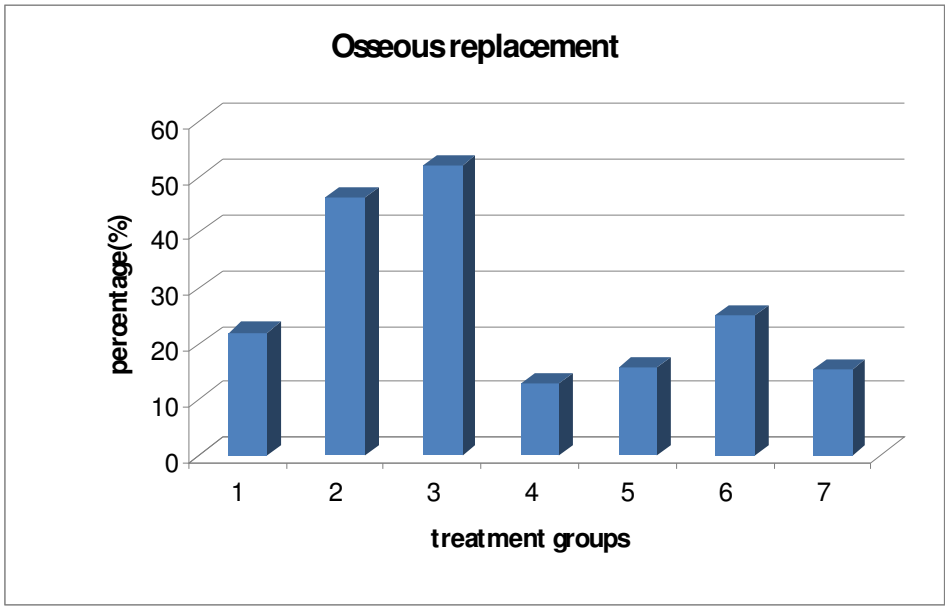
Figure 12. Percentage of evaluation points with inflammatory root resorption by treatment



C. <u>Unfavorable healing</u> : Osseous replacement (Figure 13)

There were statistically significant differences between treatment groups in terms of osseous replacement (P<0.001). Pairwise comparison showed groups 2(PC40) and 3(PC60) had significantly more osseous replacement than groups 1(NC), 4(T40), 5(T60), 6(L40) and 7 (L60) (P<0.05). Group 4(T40) showed less osseous replacement than group 6(L40) (P=0.039).

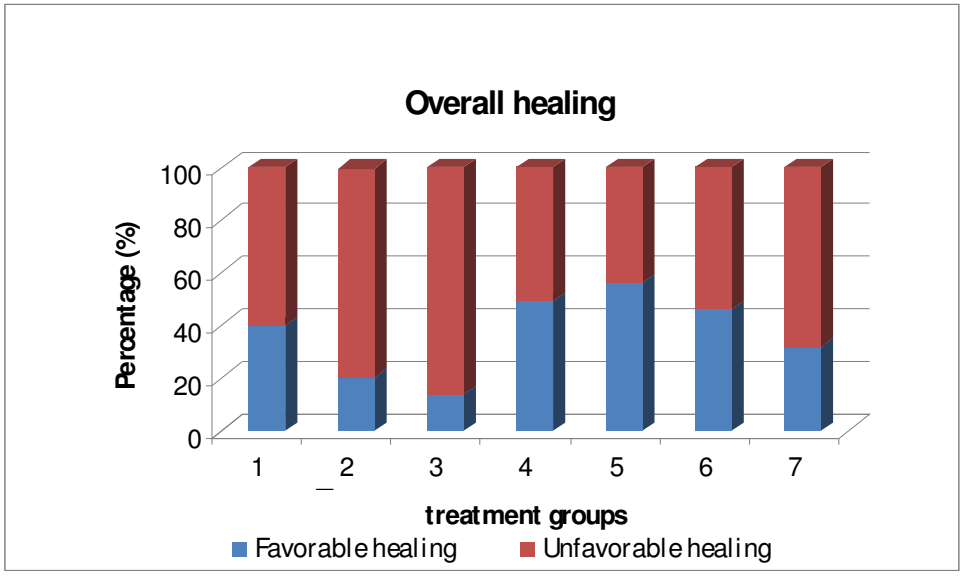
Figure 13. Percentage of evaluation points with osseous replacement by treatment



4. Periodontal Healing Pattern : Favorable vs. Unfavorable Healing

Among all treatment groups, experimental groups 4 (T40), 5 (T60), 6 (L40) showed significantly more favorable and less unfavorable healing than positive control groups 2 (PC40) and 3 (PC60) (P<0.05) (Figure 14).

Figure 14. Percentage of evaluation points with overall healing by treatment



5. Evaluation of favorable healing by extraoral dry time

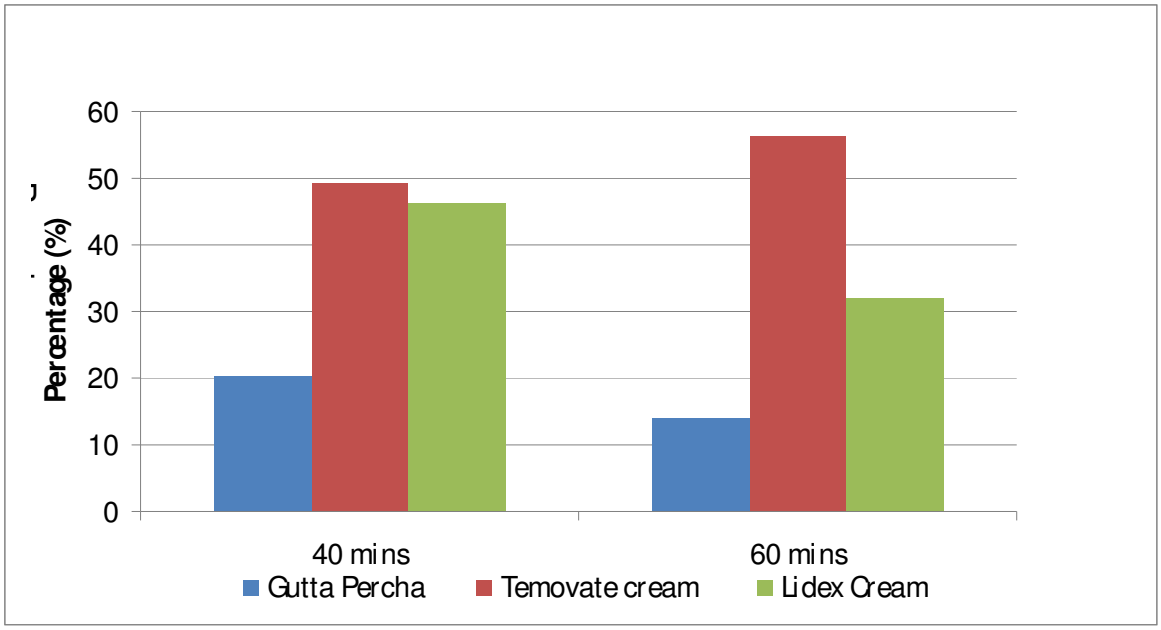
Table 4 and Figure 15 show the percentage of evaluation points with favorable healing by dry time.

No significant differences in favorable healing was found between the 40- and 60-min dry time groups in either treamtent group (Gutta- percha, Temovate®, Lidex®).

Table 4. Percentage of evaluation points with favorable healing by dry time

	40 min dry (% healed)	60 min dry (% healed)
Gutta-percha+sealer	20.19	13.89
Temovate® cream	49.38	56.25
Lidex® cream	46.25	32.03

Figure 15. Percentage of evaluation points with favorable healing by dry time

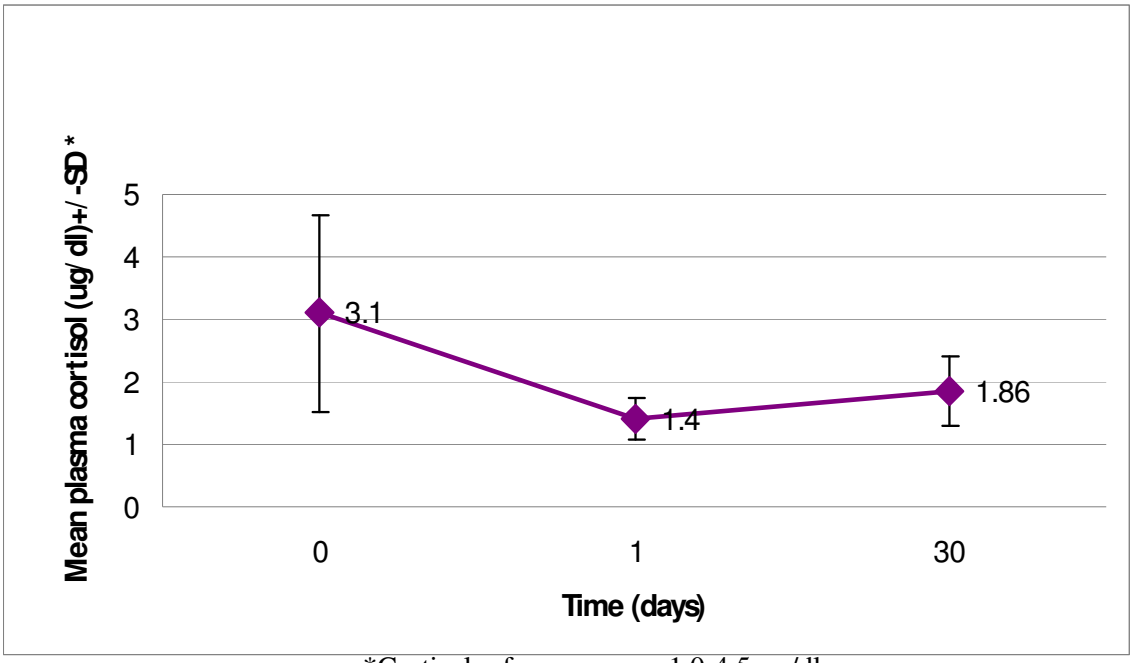


Plasma cortisol concentration

Data from one dog was not included in the statistical analysis and that dog was considered to be an outlier since its baseline endogenous cortisol was abnormally high (7.1 µg/ml). This increase in cortisol level can most likely be attributed to the paw injury that the dog sustained several days prior to the experiment. However, the dog's cortisol plasma concentration returned to the normal range following the surgery: 2.2 µg/ml and 4.1 µg/ml on day 1 and day 30, respectively.

The statistical analysis was performed on the data collected from the remaining five dogs. Figure 16 shows the plasma concentration of cortisol in dogs before (day 0) and after (days 1 and 30) the surgery. There was no statistically significant difference in plasma cortisol levels between different time points (day 0,1,30) (P=0.1005).

Figure 16. Plasma Concentration of cortisol in dogs before (day 0) and after (days 1 and 30) the surgery (µg/dl±SD)



*Cortisol reference range 1.0-4.5 µg/dl

DISCUSSION

The canine trauma model provides a reproducible and affordable system to investigate the pathophysiology of dental trauma (27, 29). This study is one in a series of experiments that uses the canine trauma model to examine the effect of various intracanal chemotherapeutic agents on periodontal healing pattern following simulated avulsion and replantation of teeth after various dry times (82, 83). In the present study, the effect of high potency intracanal corticosteroids on periodontal healing pattern in dogs has been investigated. Some concerns have been raised that due to differences in the apical root anatomy between dog and human teeth, the release and availability of the intracanal medicaments in the periapical tissues would be greater in dogs than humans. Although the presence of many accessories canals in the apical root of dogs' teeth (delta-like anatomy) may influence the rate of diffusion of the intracanal medicaments, the object of this study is not the treatment of apical periodontitis but rather the healing response of the attachment apparatus of the lateral root to the prolonged desiccation and secondary injury caused by inflammation (29).

In this study, the choice of experimental corticosteroids was based on their relative anti-inflammatory potency. Our objective was to determine if the most potent anti-inflammatory agents were also the most anti-resoptive. Based on the topical corticosteroid potency classification, 0.05% clobetasol (Temovate®) belongs to the 'very high' potency corticosteroid group. 0.05% fluocinonide (Lidex®) belongs to the 'high' potency group (98). In comparison, 1% triamcinolone as used in previous study belongs to the 'intermediate'

potency group (83). Changing the concentration of the particular corticosteroids does not qualify them to be assigned to the higher potency group; hence, no appreciable additional anti-inflammatory effect would be expected. For that reason the higher concentration of triamcinolone was not tested in this study, as its anti-inflammatory effect likely would not be very different from what it was found previously (83).

Results of the present study demonstrate that the use of intracanal corticosteroids promoted significantly more favorable and less unfavorable healing of replanted dogs' teeth at 40- and 60-minute dry time periods as compared to positive control groups. Roots treated with higher potency corticosteroid (Temovate®) showed more favorable and less unfavorable healing than roots treated with weaker corticosteroid (Lidex®); this difference was statistically significant at 60 min of dry time (56% vs 32% for favorable and 44% vs 68% for unfavorable healing). Surprisingly, the 60 min dry group treated with Temovate® showed significantly more favorable than the negative control group (56% vs 40%). The possible explanation for this could be that although the intended extraoral time for the negative control group was estimated to be less than 5 min, the completion of root canal treatment in that time period was not always possible. It would be prudent in future similar studies to perform the root canal treatment intraorally either before or after the extraction, thus minimizing the extraoral dry time and the damage to the cementum and PDL cells that results from manipulation of the root during canal instrumentation, irrigation and obturation. Moreover, treatment of the root canal following the replantation of the tooth will represent the clinical situation better since the root canal treatment on teeth that are avulsed and replanted in less than 60 minutes would be usually performed within 7-10 days following the replantation.

The choice for extraoral dry times of 40 and 60 minutes in this study was based on the amount of viable periodontal ligament cells expected to remain on the root surface prior to the replantation. It has been shown that if the root is left dry for more than 60 minutes, no PDL cells are expected to survive and unfavorable healing with osseous replacement is predicted (3). The healing pattern remains unpredictable for teeth experiencing the extraoral dry times between 20 and 60 minutes. The dead or dying PDL cells on the roots of replanted teeth provoke the destructive inflammatory response that results in root resorption. We hypothesized that the degree of inflammatory response and hence the amount of root resorption induced by non-viable cells after 60 minutes of dry time would be greater as compared to 40 minutes. As a result, we expected to observe more favorable healing in 40 min group as compared to 60 min, especially after the manipulation of the inflammatory response with high potency corticosteroids. The results of this study showed that, despite the lack of statistical differences between the 40- and 60 min dry time groups (groups 2, 4, 6 vs groups 3, 5, 7), those groups treated with Gutta- Percha and Lidex® showed more favorable healing at 40 min dry time as compared to 60 min. Interestingly, the roots treated with Temovate® showed a reverse relationship with more favorable healing observed in 60 min dry group as compared to 40 min group. This can be attributed to the groups' sample size.

Findings of this study confirmed previous study results of the favorable effect of corticosteroids on periodontal healing (83). In study by Chen *et al*., roots treated with 1% triamcinolone showed 69% of favorable healing. On average, the amount of favorable healing achieved in Chen's study among different treatment groups appears to be higher than what was found in this study. Although the same experimental protocol was followed throughout the study, the number of teeth lost during 4 month postoperative period was

larger in present study (26 lost roots out of 93). This can be attributed to the increased susceptibility of dogs to the periodontal disease (20, 53, 99). The other possibility could be that teeth became infected due to coronal microleakage of the access opening restorations and consequently exfoliated as a result of severe periradicular periodontitis or external inflammatory root resorption. Therefore, the comparison between the effects of the weaker corticosteroid, 1% triamcinolone, used in Chen's study and stronger ones used in present study is not possible. However, based on the results of the present study, the stronger corticosteroid Temovate® produced more favorably healed surfaces than the weaker one, Lidex®.

The result of this study also showed that certain amount of inflammatory root resorption was observed in each treatment group. That was a surprising finding since the root canal treatment was performed in vital teeth in aseptic fashion in order to minimize the pulpal infection as a stimulus for external inflammatory root resorption. The possible explanation for the presence of root resorption is the bacterial contamination of the root canal space likely through coronal microleakage or sulcular area during 4 month postoperative period.

Results of this study demonstrate that corticosteroids are not absorbed systemically when they are applied intrapulpally. Systemic absorption of intracanal corticosteroids was assessed by evaluating changes in plasma concentration of endogenous cortisol, since the potency of the systemic effects of exogenous corticosteroids is often expressed by their ability to suppress cortisol production by the adrenal glands (93, 94). The mean cortisol levels among dogs were 3.1µg/dl, preoperatively, 1.4µg/dl after 24 hours postoperatively, and 1.86µg/dl at 30 days postoperatively. All measurements were within the normal range of

the levels of endogenous cortisol for dogs (1.0-4.5µg/dl). In this study, on average, 10-12 roots per dog have been treated with corticosteroids, which makes the amount of corticosteroid applied intrapulpally much greater than what it would be typically used clinically when treating patients with avulsion injuries. To date, no *in vivo* studies have been published that assess the systemic absorption and adverse effects caused by intrapulpal application of corticosteroids or medications containing corticosteroids (e.g. Ledermix ™).

The results of this study support the findings of an *in vitro* study by Abbott who investigated the systemic release of 1% triamcinolone from Ledermix™ paste following its intrapulpal use (92). Calculation of the highest possible amount of corticosteroid that could be used intrapulpally appears to constitute to only a fraction of endogenous cortisol produced daily by a healthy individual. The author concluded that the highest possible amounts of corticosteroids such as in Ledermix ™ used inside the canal are unlikely to result in any systemic side effects. Once the corticosteroid reaches the systemic circulation in periradicular tissues, its concentration is further reduced making the amount measured in *in vitro* much higher that what it would be otherwise observed *in vivo* (92).

In the present study the choice of corticosteroids was based on their high anti-inflammatory potency when compared to that of endogenous hormone cortisol. The potency of 0.05% clobetasol (Temovate®) is about 25 times that of endogenous cortisol, while the potency of 1% triamcinolone as used in Ledermix™ is 5 times that of cortisol (98). The potency of 0.05% fluocinonide (Lidex®) appears to fall in between that of 1% triamcinolone and 0.05% clobetasol (Temovate®). This study further confirms the finding that, even when high potencies corticosteroids are used in root canals of multiple teeth *in vivo,* no changes in the systemic concentration of cortisol are observed. Therefore, our results indicate that

neither pituitary- adrenocortical suppression nor associated adverse effects are likely to occur with intrapulpal application of high potency corticosteroids.

In conclusion, the present study found that:

- Teeth treated with 0.05% clobetasol (Temovate®) and 0.05% fluocinonide (Lidex®) exhibited significantly more favorable healing than teeth filled with Gutta percha at 40 min (49%, 46% vs 20%) and 60 min (56%, 32% vs 14%).
- The higher potency corticosteroid (Temovate®) showed significantly more favorable healing than the lower potency (Lidex®) at 60 min dry time (56% vs 32%)
- No change in the systemic corticosteroid blood concentration following intracanal use of high potency corticosteroids was observed.
- Corticosteroids were efficacious in the beagle model as intracanal medicaments for promoting favorable healing. Additional controlled trials are needed to determine clinical effectiveness, before immediate placement of intracanal corticosteroid can become a standard emergency treatment for dental injuries.

This research project was supported by an Endodontic Research Grant from the American Association of Endodontists Foundation

REFERENCES

1. Andreasen JO. Etiology and pathogenesis of traumatic dental injuries. A clinical study of 1,298 cases. Scand J Dent Res. 1970;78(4):329-42.

2. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 4. factors related to periodontal ligament healing. Endod Dent Traumatol. 1995 Apr;11(2):76-89.

3. 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 Feb;10(1):43-53.

4. Boyd DH, Kinirons MJ, Gregg TA. A prospective study of factors affecting survival of replanted permanent incisors in children. Int J Paediatr Dent. 2000 Sep;10(3):200-5.

5. Trope M, Hupp JG, Mesaros SV. The role of the socket in the periodontal healing of replanted dogs' teeth stored in ViaSpan for extended periods. Endod Dent Traumatol. 1997 Aug;13(4):171-5.

6. Kinirons MJ, Boyd DH, Gregg TA. Inflammatory and replacement resorption in reimplanted permanent incisor teeth: A study of the characteristics of 84 teeth. Endod Dent Traumatol. 1999 Dec;15(6):269-72.

7. Trope M. Clinical management of the avulsed tooth: Present strategies and future directions. Dent Traumatol. 2002 Feb;18(1):1-11.

8. Trope M, Yesilsoy C, Koren L, Moshonov J, Friedman S. Effect of different endodontic treatment protocols on periodontal repair and root resorption of replanted dog teeth. J Endod. 1992 Oct;18(10):492-6.

9. Andersson L, Bodin I. Avulsed human teeth replanted within 15 minutes--a long-term clinical follow-up study. Endod Dent Traumatol. 1990 Feb;6(1):37-42.

10. Donaldson M, Kinirons MJ. Factors affecting the time of onset of resorption in avulsed and replanted incisor teeth in children. Dent Traumatol. 2001 Oct;17(5):205-9.

11. Soder PO, Otteskog P, Andreasen JO, Modeer T. Effect of drying on viability of periodontal membrane. Scand J Dent Res. 1977 Mar;85(3):164-8.

12. Lindskog S, Blomlof L. Influence of osmolality and composition of some storage media on human periodontal ligament cells. Acta Odontol Scand. 1982;40(6):435-41.

13. Blomlof L, Lindskog S, Hammarstrom L. Periodontal healing of exarticulated monkey teeth stored in milk or saliva. Scand J Dent Res. 1981 Jun;89(3):251-9.

14. Trope M, Friedman S. Periodontal healing of replanted dog teeth stored in viaspan, milk and hank's balanced salt solution. Endod Dent Traumatol. 1992 Oct;8(5):183-8.

15. Pettiette M, Hupp J, Mesaros S, Trope M. Periodontal healing of extracted dogs' teeth air-dried for extended periods and soaked in various media. Endod Dent Traumatol. 1997 Jun;13(3):113-8.

16. Hupp JG, Mesaros SV, Aukhil I, Trope M. Periodontal ligament vitality and histologic healing of teeth stored for extended periods before transplantation. Endod Dent Traumatol. 1998 Apr;14(2):79-83.

17. Trope M. Root resorption due to dental trauma. Endod Topics. 2002(1):79-100.

18. Andreasen JO, Kristerson L. The effect of limited drying or removal of the periodontal ligament. periodontal healing after replantation of mature permanent incisors in monkeys. Acta Odontol Scand. 1981;39(1):1-13.

19. Andreasen JO. Analysis of topography of surface- and inflammatory root resorption after replantation of mature permanent incisors in monkeys. Swed Dent J. 1980;4(4):135-44.

20. Trope M. Root resorption of dental and traumatic origin: Classification based on etiology. Pract Periodontics Aesthet Dent. 1998 May;10(4):515-22.

21. Hammarstrom L, Pierce A, Blomlof L, Feiglin B, Lindskog S. Tooth avulsion and replantation--a review. Endod Dent Traumatol. 1986 Feb;2(1):1-8.

22. Lindskog S, Blomlof L, Hammarstrom L. Cellular colonization of denuded root surfaces in vivo: Cell morphology in dentin resorption and cementum repair. J Clin Periodontol. 1987 Aug;14(7):390-5.

23. Lindskog S, Pierce AM, Blomlof L, Hammarstrom L. The role of the necrotic periodontal membrane in cementum resorption and ankylosis. Endod Dent Traumatol. 1985 Jun;1(3):96-101.

24. Andersson L, Bodin I, Sorensen S. Progression of root resorption following replantation of human teeth after extended extraoral storage. Endod Dent Traumatol. 1989 Feb;5(1):38-47.

25. Ebeleseder KA, Friehs S, Ruda C, Pertl C, Glockner K, Hulla H. A study of replanted permanent teeth in different age groups. Endod Dent Traumatol. 1998 Dec;14(6):274-8.

26. Barbakow FH, Cleaton-Jones PE, Austin JC, Vieira E. Healing of replanted teeth following typical treatment with fluoride solutions and systemic admission of thyrocalcitonin: A histometric analysis. J Endod. 1981 Jul;7(7):302-8.

27. Bjorvatn K, Selvig KA, Klinge B. Effect of tetracycline and SnF2 on root resorption in replanted incisors in dogs. Scand J Dent Res. 1989 Dec;97(6):477-82.

28. Shulman LB, Kalis P, Goldhaber P. Fluoride inhibition of tooth-replant root resorption in cebus monkeys. J Oral Ther Pharmacol. 1968 May;4(5):331-7.

29. Selvig KA, Bjorvatn K, Claffey N. Effect of stannous fluoride and tetracycline on repair after delayed replantation of root-planed teeth in dogs. Acta Odontol Scand. 1990 Apr;48(2):107-12.

30. Levin L, Bryson EC, Caplan D, Trope M. Effect of topical alendronate on root resorption of dried replanted dog teeth. Dent Traumatol. 2001 Jun;17(3):120-6.

31. Lustosa-Pereira A, Garcia RB, de Moraes IG, Bernardineli N, Bramante CM, Bortoluzzi EA. Evaluation of the topical effect of alendronate on the root surface of extracted and replanted teeth. microscopic analysis on rats' teeth. Dent Traumatol. 2006 Feb;22(1):30-5.

32. Pontoriero R, Wennstrom J, Lindhe J. The use of barrier membranes and enamel matrix proteins in the treatment of angular bone defects. A prospective controlled clinical study. J Clin Periodontol. 1999 Dec;26(12):833-40.

33. Heijl L, Heden G, Svardstrom G, Ostgren A. Enamel matrix derivative (EMDOGAIN) in the treatment of intrabony periodontal defects. J Clin Periodontol. 1997 Sep;24(9 Pt 2):705-14.

34. Araujo M, Hayacibara R, Sonohara M, Cardaropoli G, Lindhe J. Effect of enamel matrix proteins (emdogain') on healing after re-implantation of "periodontally compromised" roots. an experimental study in the dog. J Clin Periodontol. 2003 Oct;30(10):855-61.

35. Lam K, Sae-Lim V. The effect of emdogain gel on periodontal healing in replanted monkeys' teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004 Jan;97(1):100-7.

36. Schjott M, Andreasen JO. Emdogain does not prevent progressive root resorption after replantation of avulsed teeth: A clinical study. Dent Traumatol. 2005 Feb;21(1):46-50.

37. Poi WR, Carvalho RM, Panzarini SR, Sonoda CK, Manfrin TM, Rodrigues Tda S. Influence of enamel matrix derivative (emdogain) and sodium fluoride on the healing process in delayed tooth replantation: Histologic and histometric analysis in rats. Dent Traumatol. 2007 Feb;23(1):35-41.

38. Iqbal MK, Bamaas N. Effect of enamel matrix derivative (EMDOGAIN) upon periodontal healing after replantation of permanent incisors in beagle dogs. Dent Traumatol. 2001 Feb;17(1):36-45.

39. Filippi A, Pohl Y, von Arx T. Treatment of replacement resorption with emdogain--a prospective clinical study. Dent Traumatol. 2002 Jun;18(3):138-43.

40. Kling M, Cvek M, Mejare I. Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. Endod Dent Traumatol. 1986 Jun;2(3):83-9.

41. 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 Aug;6(4):157-69.

42. Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res. 1981 Dec;89(6):475-84.

43. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odontol Revy. 1974;25(4):347-58.

44. Sundqvist G. Bacteriological studies of necrotic dental pulps. . 1976.

45. Nair PN. Pathobiology of primary apical periodontitis. In: Cohen S, Hargreaves KM. Pathways of the pulp. 9th ed. St. Louis, Mo.: Mosby Elsevier; 2006.

46. Ryan GB, Majno G. Acute inflammation. A review. Am J Pathol. 1977 Jan;86(1):183-276.

47. Lindskog S, Blomlof L, Hammarstrom L. Repair of periodontal tissues in vivo and in vitro. J Clin Periodontol. 1983 Mar;10(2):188-205.

48. Levin L, Trope M. Root Resorption. In: Hargreaves KM, Goodis HE, Seltzer S, Gender IB. Seltzer and bender's dental pulp. Chicago: Quintessence Pub. Co.; 2002.

49. Hammarstrom L, Lindskog S. General morphological aspects of resorption of teeth and alveolar bone. Int Endod J. 1985 Apr;18(2):93-108.

50. Seltzer S, Krasner P. Endodontology :Biologic considerations in endodontic procedures. 2nd ed. Philadelphia: Lea & Febiger; 1988.

51. Nakamura I, Takahashi N, Sasaki T, Jimi E, Kurokawa T, Suda T. Chemical and physical properties of the extracellular matrix are required for the actin ring formation in osteoclasts. J Bone Miner Res. 1996 Dec;11(12):1873-9.

52. Saygin NE, Giannobile WV, Somerman MJ. Molecular and cell biology of cementum. Periodontol 2000. 2000 Oct;24:73-98.

53. Tronstad L. Root resorption--etiology, terminology and clinical manifestations. Endod Dent Traumatol. 1988 Dec;4(6):241-52.

54. American Association of Endodontists. Recommended guidelines of the american associaton of endodontists for the treatment of traumatic dental injuries. 2004.

55. Flores MT, Andersson L, Andreasen JO, Bakland LK, Malmgren B, Barnett F, et al. Guidelines for the management of traumatic dental injuries. II. avulsion of permanent teeth. Dent Traumatol. 2007 Jun;23(3):130-6.

56. Trope M, Moshonov J, Nissan R, Buxt P, Yesilsoy C. Short vs. long-term calcium hydroxide treatment of established inflammatory root resorption in replanted dog teeth. Endod Dent Traumatol. 1995 Jun;11(3):124-8.

57. Sjogren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J. 1991 May;24(3):119-25.

58. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol. 1985 Oct;1(5):170-5.

59. Safavi KE, Nichols FC. Effect of calcium hydroxide on bacterial lipopolysaccharide. J Endod. 1993 Feb;19(2):76-8.

60. Tronstad L, Andreasen JO, Hasselgren G, Kristerson L, Riis I. pH changes in dental tissues after root canal filling with calcium hydroxide. J Endod. 1981 Jan;7(1):17-21.

61. Lengheden A, Blomlof L, Lindskog S. Effect of delayed calcium hydroxide treatment on periodontal healing in contaminated replanted teeth. Scand J Dent Res. 1991 Apr;99(2):147-53.

62. Lengheden A, Blomlof L, Lindskog S. Effect of immediate calcium hydroxide treatment and permanent root-filling on periodontal healing in contaminated replanted teeth. Scand J Dent Res. 1991 Apr;99(2):139-46.

63. Massarstrom LE, Blomlof LB, Feiglin B, Lindskog SF. Effect of calcium hydroxide treatment on periodontal repair and root resorption. Endod Dent Traumatol. 1986 Oct;2(5):184-9.

64. Andreasen JO, Kristerson L. The effect of extra-alveolar root filling with calcium hydroxide on periodontal healing after replantation of permanent incisors in monkeys. J Endod. 1981 Aug;7(8):349-54.

65. Dumsha T, Hovland EJ. Evaluation of long-term calcium hydroxide treatment in avulsed teeth--an in vivo study. Int Endod J. 1995 Jan;28(1):7-11.

66. Schroeder A. Corticosteroids in endodontics. J Oral Ther Pharmacol. 1965 Nov;2(3):171-9.

67. Hong SL, Levine L. Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. Proc Natl Acad Sci U S A. 1976 May;73(5):1730-4.

68. Flower RJ, Rothwell NJ. Lipocortin-1: Cellular mechanisms and clinical relevance. Trends Pharmacol Sci. 1994 Mar;15(3):71-6.

69. De Caterina R, Sicari R, Giannessi D, Paggiaro PL, Paoletti P, Lazzerini G, et al. Macrophage-specific eicosanoid synthesis inhibition and lipocortin-1 induction by glucocorticoids. J Appl Physiol. 1993 Dec;75(6):2368-75.

70. Barnes PJ. Anti-inflammatory actions of glucocorticoids: Molecular mechanisms. Clin Sci (Lond). 1998 Jun;94(6):557-72.

71. Tobias J, Chambers TJ. Glucocorticoids impair bone resorptive activity and viability of osteoclasts disaggregated from neonatal rat long bones. Endocrinology. 1989 Sep;125(3):1290-5.

72. Dempster DW, Moonga BS, Stein LS, Horbert WR, Antakly T. Glucocorticoids inhibit bone resorption by isolated rat osteoclasts by enhancing apoptosis. J Endocrinol. 1997 Sep;154(3):397-406.

73. Hirayama T, Sabokbar A, Athanasou NA. Effect of corticosteroids on human osteoclast formation and activity. J Endocrinol. 2002 Oct;175(1):155-63.

74. Sae-Lim V, Metzger Z, Trope M. Local dexamethasone improves periodontal healing of replanted dogs' teeth. Endod Dent Traumatol. 1998 Oct;14(5):232-6.

75. Keum KY, Kwon OT, Spangberg LS, Kim CK, Kim J, Cho MI, et al. Effect of dexamethasone on root resorption after delayed replantation of rat tooth. J Endod. 2003 Dec;29(12):810-3.

76. 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 Periodontal Res. 1986 Jul;21(4):330-7.

77. 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(3):297-321.

78. Keller DC, Carano A. Tetracycline effect on osteoclastic and osteoblastic activity. Gen Dent. 1995 Jan-Feb;43(1):60-3.

79. Sae-Lim V, Wang CY, Choi GW, Trope M. The effect of systemic tetracycline on resorption of dried replanted dogs' teeth. Endod Dent Traumatol. 1998 Jun;14(3):127-32.

80. Pierce A, Lindskog S. The effect of an antibiotic/corticosteroid paste on inflammatory root resorption in vivo. Oral Surg Oral Med Oral Pathol. 1987 Aug;64(2):216-20.

81. Wong KS, Sae-Lim V. The effect of intracanal ledermix on root resorption of delayed-replanted monkey teeth. Dent Traumatol. 2002 Dec;18(6):309-15.

82. Bryson EC, Levin L, Banchs F, Abbott PV, Trope M. Effect of immediate intracanal placement of ledermix paste(R) on healing of replanted dog teeth after extended dry times. Dent Traumatol. 2002 Dec;18(6):316-21.

83. Chen H, Teixeira FB, Ritter AL, Levin L, Trope M. The effect of intracanal anti-inflammatory medicaments on external root resorption of replanted dog teeth after extended extra-oral dry time. Dent Traumatol. 2008 Feb;24(1):74-8.

84. Thong YL, Messer HH, Siar CH, Saw LH. Periodontal response to two intracanal medicaments in replanted monkey incisors. Dent Traumatol. 2001 Dec;17(6):254-9.

85. Abbott PV, Heithersay GS, Hume WR. Release and diffusion through human tooth roots in vitro of corticosteroid and tetracycline trace molecules from ledermix paste. Endod Dent Traumatol. 1988 Apr;4(2):55-62.

86. Pierce A, Heithersay G, Lindskog S. Evidence for direct inhibition of dentinoclasts by a corticosteroid/antibiotic endodontic paste. Endod Dent Traumatol. 1988 Feb;4(1):44-5.

87. Hammarstrom L, Blomlof L, Feiglin B, Andersson L, Lindskog S. Replantation of teeth and antibiotic treatment. Endod Dent Traumatol. 1986 Apr;2(2):51-7.

88. Kim ST, Abbott PV, McGinley P. The effects of ledermix paste on discolouration of mature teeth. Int Endod J. 2000 May;33(3):227-32.

89. Kim ST, Abbott PV, McGinley P. The effects of ledermix paste on discolouration of immature teeth. Int Endod J. 2000 May;33(3):233-7.

90. Andreasen JO, Hjorting-Hansen E. Replantation of teeth. I. radiographic and clinical study of 110 human teeth replanted after accidental loss. Acta Odontol Scand. 1966 Nov;24(3):263-86.

91. Andreasen JO, Ravn JJ. Epidemiology of traumatic dental injuries to primary and permanent teeth in a danish population sample. Int J Oral Surg. 1972;1(5):235-9.

92. Abbott PV. Systemic release of corticosteroids following intra-dental use. Int Endod J. 1992 Jul;25(4):189-91.

93. Downie WW, Dixon JS, Lowe JR, Rhind VM, Leatham PA, Pickup ME. Adrenocortical suppression by synthetic corticosteroid drugs: A comparative study of prednisolone and betamethasone. Br J Clin Pharmacol. 1978 Nov;6(5):397-9.

94. Zenoble RD, Kemppainen RJ. Adrenocortical suppression by topically applied corticosteroids in healthy dogs. J Am Vet Med Assoc. 1987 Sep 15;191(6):685-8.

95. Johnston SD, Mather EC. Canine plasma cortisol (hydrocortisone) measured by radioimmunoassay: Clinical absence of diurnal variation and results of ACTH stimulation and dexamethasone suppression tests. Am J Vet Res. 1978 Nov;39(11):1766-70.

96. Andreasen JO. Experimental dental traumatology: Development of a model for external root resorption. Endod Dent Traumatol. 1987 Dec;3(6):269-87.

97. Twisk JWR. Applied longitudinal data analysis for epidemiology :A practical guide. Cambridge, UK; New York: Cambridge University Press; 2003.

98. Goodman LS, Gilman A, Brunton LL, Lazo JS, Parker KL, Teton Data Systems. Goodman & gilman's the pharmacological basis of therapeutics. 11th ed. New York: McGraw-Hill; 2006.

99. Gold SI, Hasselgren G. Peripheral inflammatory root resorption. A review of the literature with case reports. J Clin Periodontol. 1992 Sep;19(8):523-34.