

Pulp Revascularization of Immature Dog Teeth With Apical Periodontitis

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Abstract

This study examined the ability of a collagen solution to aid revascularization of necrotic-infected root canals in immature dog teeth. Sixty immature teeth from 6 dogs were infected, disinfected, and randomized into experimental groups: 1: no further treatment; 2: blood in canal; 3: collagen solution in canal; 4: collagen solution + blood; and 5: negative controls (left for natural development). Uncorrected chi-square analysis of radiographic results showed no statistical differences ($p \geq 0.05$) between experimental groups regarding healing of radiolucencies but a borderline statistical difference ($p = 0.058$) for group 1 versus group 4 for radicular thickening. Group 2 showed significantly more apical closure than group 1 ($p = 0.03$) and a borderline statistical difference ($p = 0.051$) for group 3 versus group 1. Uncorrected chi-square analysis revealed that there were no statistical differences between experimental groups for histological results. However, some roots in each of groups 1 to 4 (previously infected) showed positive histologic outcomes (thickened walls in 43.9%, apical closure in 54.9%, and new luminal tissue in 29.3%). Revascularization of disinfected immature dog root canal systems is possible. (*J Endod* 2007;33:680–689)

Key Words

Histologic outcomes, pulp revascularization, radiographic

Pulp necrosis of an immature permanent tooth from caries or trauma arrests further development and leaves the tooth with thin, weak walls that are prone to fracture (1–3). Endodontic treatment of such a tooth is difficult because the thin walls do not forgive much mechanical instrumentation (4), and the open apex is difficult or impossible to seal with conventional methods of lateral condensation or thermoplasticized techniques (5). The traditional treatment for these teeth is long-term calcium hydroxide application to induce apexification (an apical hard tissue barrier) (6, 7). More recent treatments have used an artificial barrier of mineral trioxide aggregate (MTA) (8, 9). Both of these techniques are followed by a traditional root filling, but they do not increase the fracture resistance of the walls (2, 3). In fact, one in vitro study has raised the question that long-term calcium hydroxide therapy for apexification may leave the thin walls even more prone to fracture (10). Root-wall-strengthening methods with composite resin have been advocated (11), but they may limit the possibility of root canal retreatment if the need arises in the future (11).

Human avulsion case series (12) and controlled animal studies (13, 14) have shown radiographic and histological evidence of successful revascularization of immature permanent teeth after replantation. In this situation, the necrotic uninfected pulp acts as a scaffold for the in-growth of new tissue from the periapical area. The absence of bacteria is important for successful revascularization because the new tissue will stop at the level it meets bacteria in the canal space (13, 15). Studies to test the ability of topical antibiotics to improve revascularization outcomes in experimental avulsions (13, 14, 16) have shown that topical doxycycline and minocycline can improve radiographic and histological evidence of revascularization in immature avulsed permanent teeth.

Extrapolating from this information, it is hypothesized that disinfection of a necrotic-infected immature permanent tooth with apical periodontitis may render it to the same starting point as a necrotic uninfected avulsed immature permanent tooth (necrotic due to severance of the vascular supply but uninfected by oral bacteria). If disinfection is achieved, then revascularization should be possible for the disinfected canals, just as it is for the uninfected canals in the avulsion scenario (12, 14).

Research with topical antibiotics has shown that a combination of metronidazole, minocycline, and ciprofloxacin is effective in vitro (17) at killing common endodontic pathogens from necrotic-infected root canals. This antibiotic combination is also an effective disinfectant in vivo (18).

An empty canal space will not support in-growth of new tissue from the periapical area on its own (19, 20). Early studies on attempted revascularization used blood or blood substitutes to act as a scaffold to aid the in-growth of new tissue into the empty canal space. Most of these studies used vital teeth with complete pulpectomies followed by partial root-filling procedures (21–23) but did not show a significant benefit of inclusion of a blood clot to improve revascularization. Other studies using necrotic-infected teeth failed at attempted revascularization, primarily because of inadequate disinfection before inducing bleeding into the canal space (15).

Revascularization research has also studied collagen solutions as artificial scaffolds in the canal space. A series of studies (24–28) using bovine collagen with crystals of calcium and phosphate, as nucleation centers for hydroxyapatite formation, achieved some revascularization success; however, most of this work used teeth with vital pulps. Results with necrotic pulps disinfected with calcium hydroxide showed lower success, possibly because of calcium hydroxide-induced limited necrosis of the progenitor cells

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in the periapical area that would have been instrumental in repopulating the empty canal space (29–31).

Radiographic evidence of successful revascularization of immature permanent human teeth with apical periodontitis has been described in the literature (32–34). In these cases, some form of topical antibiotics was used to disinfect the canal space before filling it with a scaffold.

The purpose of this study was to examine the ability of a collagen solution to aid revascularization of necrotic-infected immature dog root canals with apical periodontitis after disinfection with the triple antibiotic combination of metronidazole, minocycline, and ciprofloxacin.

Materials and Methods

Approval for this study was obtained from the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. Sixty double-rooted premolar teeth in six purpose-bred mixed breed canine model dogs aged approximately 6 months were randomly divided into 5 treatment and control groups of 12 teeth each. Before any interventions, the involved teeth were radiographed using custom bite registrations (Regisil; Dentsply Caulk, Milford, DE) and radiograph paralleling devices (Dentsply Rinn, Elgin, IL). These radiographic aids were used for all subsequent radiographs to improve the alignment and position of the films and x-ray beam for direct comparison of the radiographs with minimal distortion or magnification.

The teeth were randomly divided into 48 experimental and 12 control teeth. Under general anesthesia (induction by Pentothal [Abbott Laboratories, North Chicago, IL] 13.5 mg/kg intravenously and intubation and maintenance with isoflurane [Halocarbon Laboratories, River Edge, NJ]) supplemented with local anesthesia (bupivacaine plain 0.5%, Abbott Laboratories); the pulps of all experimental teeth were mechanically exposed with a #2 round carbide bur (Brassler USA, Savannah, GA) in a high-speed hand piece (Midwest, Mondovi, WI) under nonaseptic conditions. A sterile #30 stainless steel endodontic hand file (Dentsply Maillefer, Johnson City, TN) was used to disrupt the pulp tissue in the canal spaces, without removing it from the canals. Supragingival plaque scaled from the dogs' teeth was mixed with sterile saline (0.9% sodium chloride; Hospira Inc., Lake Forest, IL). Sterile sponges (Dentsply Maillefer, Johnson City, TN) soaked in the plaque suspension were sealed temporarily in the pulp chambers of the teeth with IRM (Intermediate Restorative Material, Dentsply Caulk). The animals were given analgesics (Torbugesic 0.2 mg/kg; Butorphanol Tartrate, Fort Dodge Animal Health, Fort Dodge, IA) postoperatively following this and all operative procedures and were monitored by staff of the Department of Laboratory and Animal Medicine in the postoperative period.

The teeth were monitored radiographically by using the original custom bite registrations and paralleling devices until there was radiographic evidence of apical periodontitis (approximately 3 weeks).

All previously infected teeth were re-entered under aseptic conditions of rubber dam isolation with retractors and surface disinfection with 0.12% chlorhexidine (Alpharma USPD, Baltimore, MD) and tincture of iodine (Humco, Texarcana, TX) with the animals under general and local anesthesia. After removal of the IRM and sponge, the canals were irrigated with 10 mL of 1.25% NaOCl (sodium hypochlorite; Clorox, Oakland, CA) per tooth. No mechanical instrumentation was performed in the canals. Canals were dried with sterile paper points (Dentsply Maillefer, Tulsa, OK) and disinfected with a mixture of equal parts of metronidazole, ciprofloxacin, and minocycline (Professional Compounding Centers of America, Houston, TX) mixed with sterile saline (0.9% sodium chloride, Hospira Inc.). The paste was applied to the canal spaces with a sterile lentulo spiral (Dentsply Maillefer, Johnson City, TN) in a slow speed handpiece (Midwest). The 12 teeth (24 roots)

that were randomly assigned to group 1 were closed permanently at that appointment with a double coronal seal consisting of white MTA (Dentsply Tulsa Dental, Johnson City, TN) and silver amalgam (Sybraloy; Kerr Corporation, Orange, CA). The other 36 experimental teeth were closed temporarily with a sterile sponge (Dentsply Maillefer) and IRM (Dentsply Caulk) for 4 weeks to allow disinfection of the canal spaces.

After 4 weeks, under the same conditions of asepsis and general and local anesthesia, the temporary restorations and sponges were removed under rubber dam isolation from the remaining 36 experimental teeth. The antibiotic mixture was irrigated from the canals with 10 mL of 1.25% NaOCl (Clorox) and 10 mL of sterile saline (0.9% sodium chloride, Hospira Inc) per tooth. In the 24 roots randomly assigned to group 2, a sterile #30 stainless steel endodontic hand file (Dentsply Maillefer) was inserted past the canal terminus into the periapical tissues to induce bleeding to fill the canal spaces as much as possible. A type I collagen solution (rat tail type I collagen; BD Biosciences, Bedford, MA, 2.33 mg/mL in 2× phosphate-buffered saline) was placed in the 24 roots randomly assigned to group 3. The 24 roots randomly assigned to group 4 had the collagen solution placed in the canals before induction of bleeding from the periapical tissues into the canal space. All of these teeth were then closed with a double coronal seal of white MTA (Dentsply Tulsa Dental, Johnson City, TN) and silver amalgam (Sybraloy). The 12 teeth randomly assigned to group 5 were negative controls. These teeth were never operated but were left untouched to develop naturally for comparison with the experimental teeth. All of the teeth were monitored radiographically on a monthly basis for 3 months before the animals were sacrificed, and tissues were harvested for histological examination.

The animals were sacrificed under general anesthesia provided by Socumb (pentobarbital; Butler Company, Columbus, OH) at 30 mg/kg intravenously. The carotid arteries were exposed and cannulated. The animals were euthanized with additional pentobarbital (Socumb, Butler Company) at a dose of 90 mg/kg intravenously. The animals were perfused with 10% buffered formalin (Fisher Scientific, Fair Lawn, NJ). The jaws with the involved teeth were resected and placed in formaldehyde (Fisher Scientific).

After removal of all soft tissue and excess hard tissue from the specimens, they were next placed in Formical (Decal Chemical Corporation, Congers, NY) for decalcification for 6 days, including one change of the solution. The specimens were subsequently decalcified in Immunoal (Decal Chemical Corporation, Tallman, NY) for 2 months, undergoing four changes of the solution over that time. On removal from the decalcification solution, the specimens were placed under a running tap water wash for 20 minutes followed by immersion in 70% ethyl alcohol. The specimens were then dehydrated through ascending gradations of ethanol and processed on a Leica TP 1020 dip n' dunk processor (Leica, Wetzlar, Germany) at 45 minutes per station in the following manner: one cycle of 70% ethanol, two cycles of 80% ethanol, two cycles of 95% ethanol, two cycles of 100% ethanol, two cycles of xylene, and two cycles of Paraplast paraffin (Kendall, Mansfield, MA) at 58°C. The tissues were then removed from the storage cassettes and embedded in paraffin and were sectioned on a Leica Jung RM 2045 microtome. Sections were made longitudinally every 5 μ m through the apical foramen of the roots and placed on probe on plus slides. Tissues were stained with hematoxylin and eosin and evaluated under light microscopy at up to 10× magnification for the presence or absence of healthy, vital tissue, and revascularization pattern, if any.

Summary of Treatment and Control Groups

The groups were as follows:

Group 1: 12 teeth: infected → disinfected (Figs. 1 and 2)

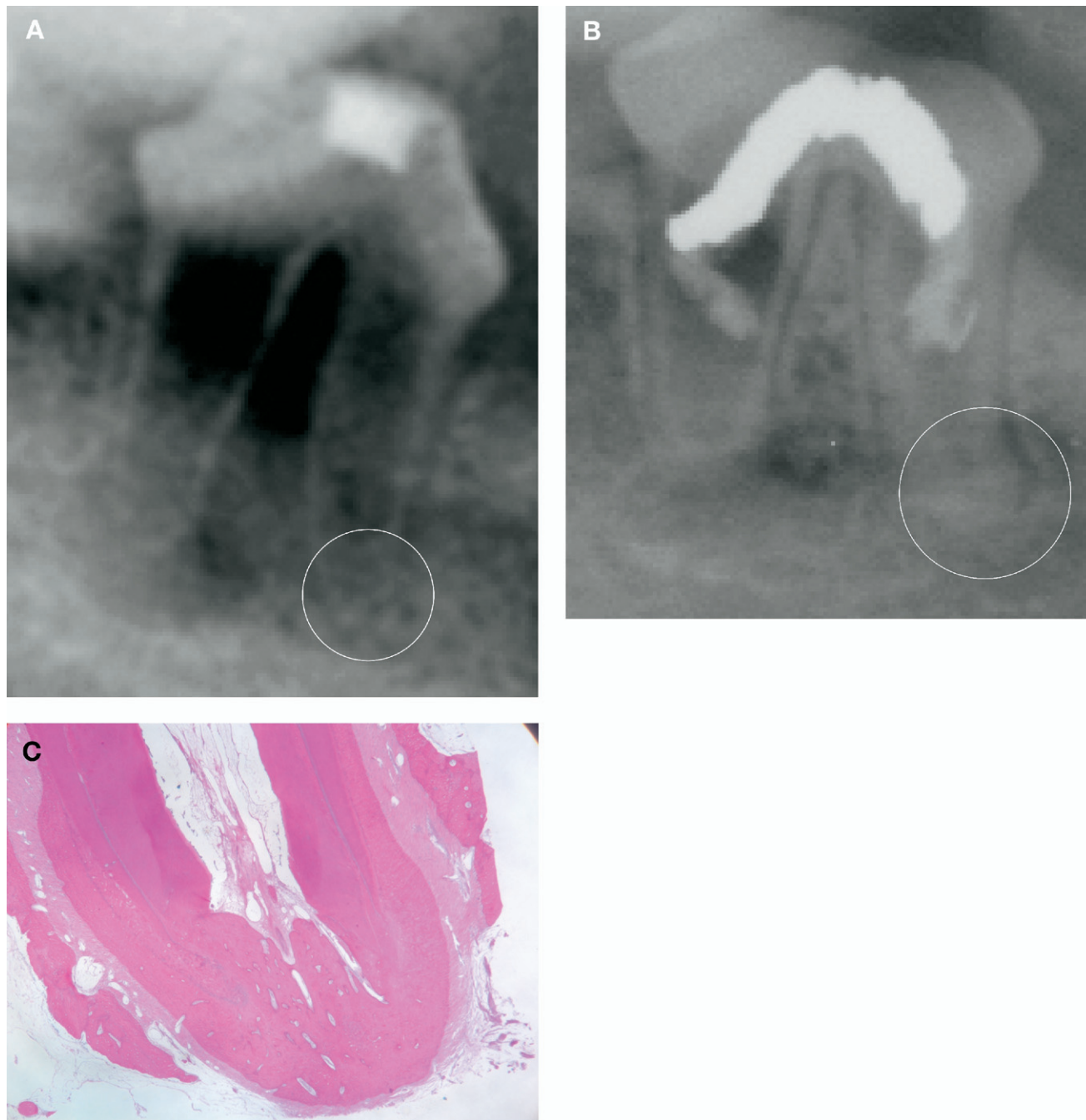


Figure 1. Tooth from group 1 (disinfected only) showing thickened root walls, apical development, and healing of apical periodontitis (original magnification $2\times$).

Group 2: 12 teeth: infected \rightarrow disinfected \rightarrow blood clot (Fig. 3)
 Group 3: 12 teeth: infected \rightarrow disinfected \rightarrow collagen
 Group 4: 12 teeth: infected \rightarrow disinfected \rightarrow collagen + blood clot
 Group 5: 12 teeth: negative control. Untouched teeth left to develop naturally for comparison (Fig. 4)

Each individual root was taken as the unit of measure. Two examiners subjectively evaluated the radiographs independently of one another. Digital radiographs were saved to a computer in jpg format. After a training session explaining the gold standard of the three evaluation parameters, each examiner separately viewed the image exposed preoperatively on the day that the roots were disinfected with an image exposed postmortem. Each examiner graded

each root for the following three parameters: (1) diminished size or absence of a periapical radiolucency, (2) presence or absence of continued thickening of radicular walls, and (3) presence or absence of apical closure. The kappa statistic values comparing the two evaluators were as follows: 0.64 for evaluation of periapical radiolucencies, 0.60 for evaluation of thickness of radicular walls, and 0.60 for evaluation of apical closure, all indicating good agreement between the two evaluators. When there was not agreement between both evaluators, a discussion was undertaken until a consensus was reached. The data were analyzed with chi-square tests, with the level of significance set at $p < 0.05$, to determine if there were any significant differences between the experimental groups.

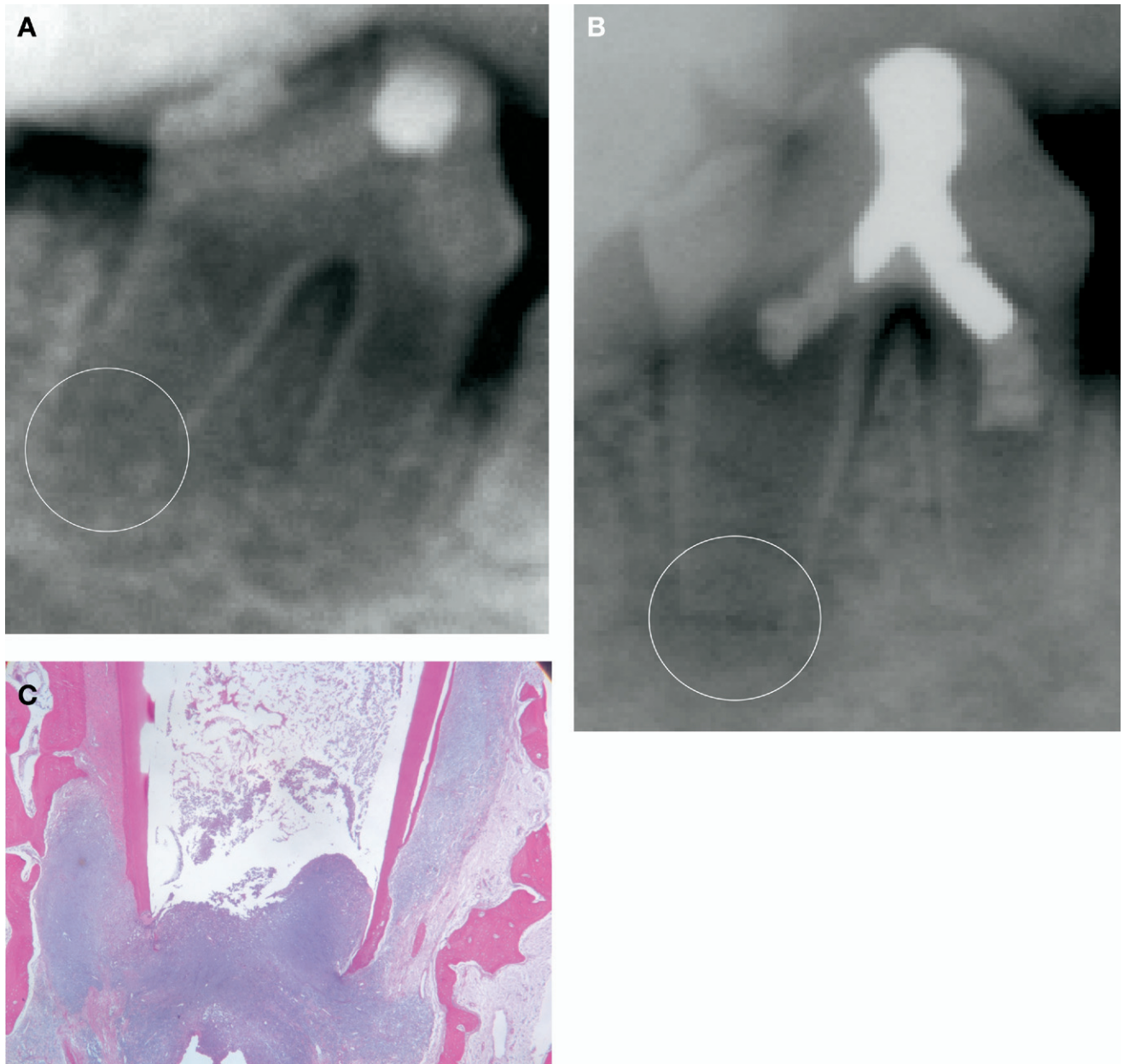


Figure 2. Tooth from group 1 (disinfected only) showing persistence of apical periodontitis, thin root walls, and lack of development of apex (original magnification $2\times$).

Each individual root was taken as a unit of measurement/assessment for the histological evaluations. After a training session explaining the gold standard of the different evaluation parameters, three evaluators examined the histological slides of the roots and graded them for the following three parameters: (1) presence or absence of new hard tissue on the internal root dentinal walls, (2) presence or absence of continued apical closure, and (3) presence or absence of vital tissue within the canal space. All three evaluators examined the same slide independently at the same time under identical lighting and magnification conditions by using a multiheaded microscope. When the results of evaluation of a parameter were not unanimous among all three evaluators, a consensus was reached through discussion. The data were analyzed with chi-square tests, with the level of significance set at $p < 0.05$, to determine if there were any significant

differences between the experimental groups with respect to the histological criteria.

Results

No animal showed any established signs of undue distress (35, 36) from the treatment procedures. However, one animal lost six experimental teeth during the course of the study, whereas another animal lost one experimental tooth. The loss of these teeth did not cause the animals any change in behavior or eating habits, which are recognized animal husbandry parameters for assessment of animal health and well-being (35, 36). The loss of these teeth was not because of any specific experimental treatment but was because of the initial infection procedure as gross mobility occurred after the infection procedure in those teeth that were lost.

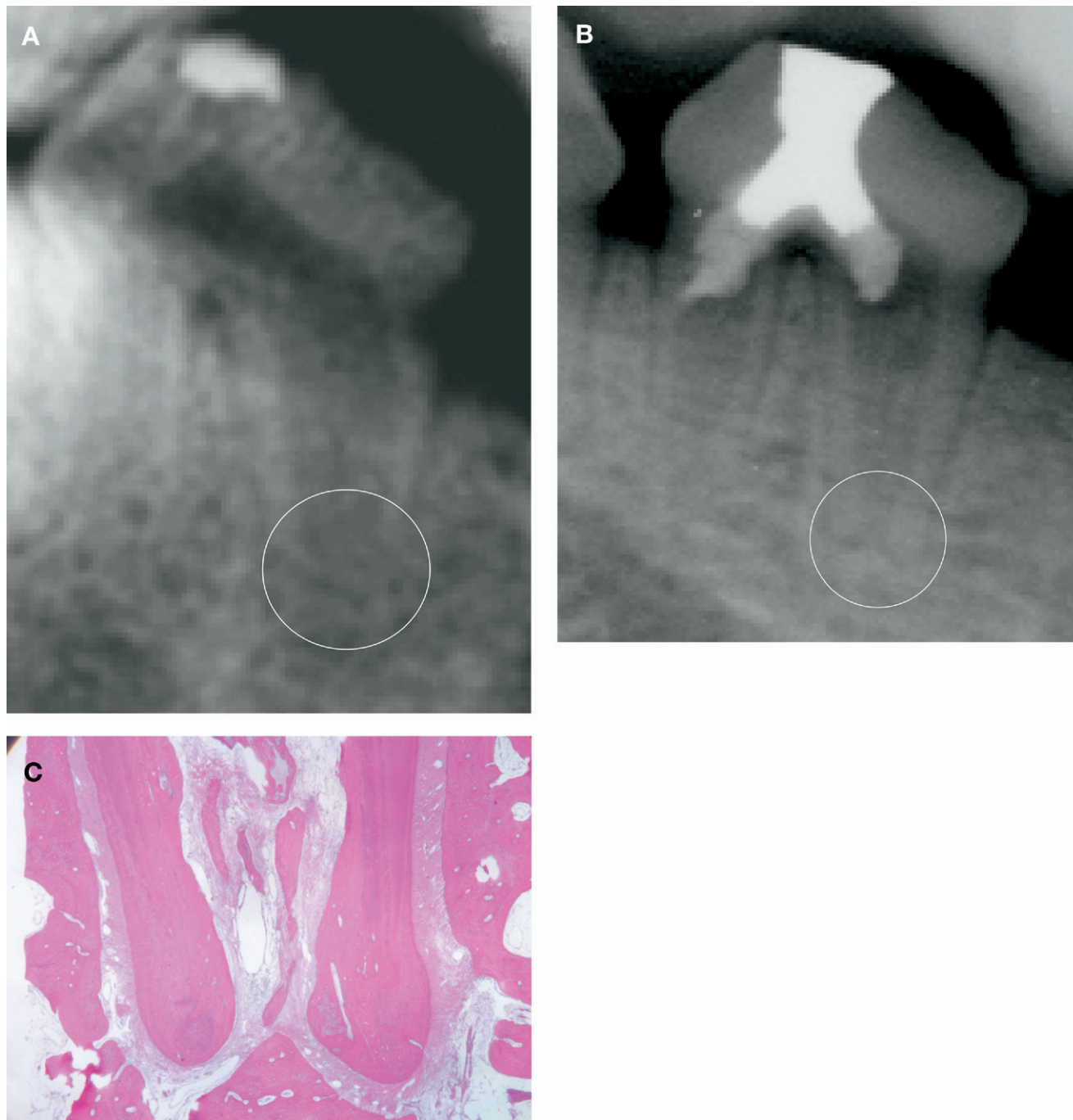


Figure 3. Tooth from group 2 (blood clot) showing thickened root walls, apical development, and healing of apical periodontitis (original magnification $2\times$).

Radiographic Results

Radiographic evidence of continued apical closure, thickened root canal walls, and healed or healing periapical radiolucencies was seen in roots from all four experimental groups.

Radiographic Thickening of Root Canal Walls

Chi-square evaluation of all five groups for continued radiographic thickening of radicular walls showed a significant difference between the groups ($p < 0.01$) (Fig. 5). When the individual experimental groups (groups 1–4) were compared with each other using chi-square tests in 2×2 tables with 1 degree of freedom for thickening of radicular

dentin walls, there was a borderline difference ($p = 0.058$) between group 4 (infected \rightarrow disinfected \rightarrow collagen + blood clot) and group 1 (infected \rightarrow disinfected); group 4 (infected \rightarrow disinfected \rightarrow collagen + blood clot) showed more thickening of radicular walls radiographically than group 1 (infected \rightarrow disinfected). There were no other significant differences between the experimental groups with respect to thickening of radicular dentin walls ($p = 0.26$).

There were 48.8% of the roots from the four experimental groups with radiographic evidence of continued thickening of radicular dentin walls. In groups 2 (infected \rightarrow disinfected \rightarrow blood clot) and 4 (infected \rightarrow disinfected \rightarrow collagen + blood clot), those including use of

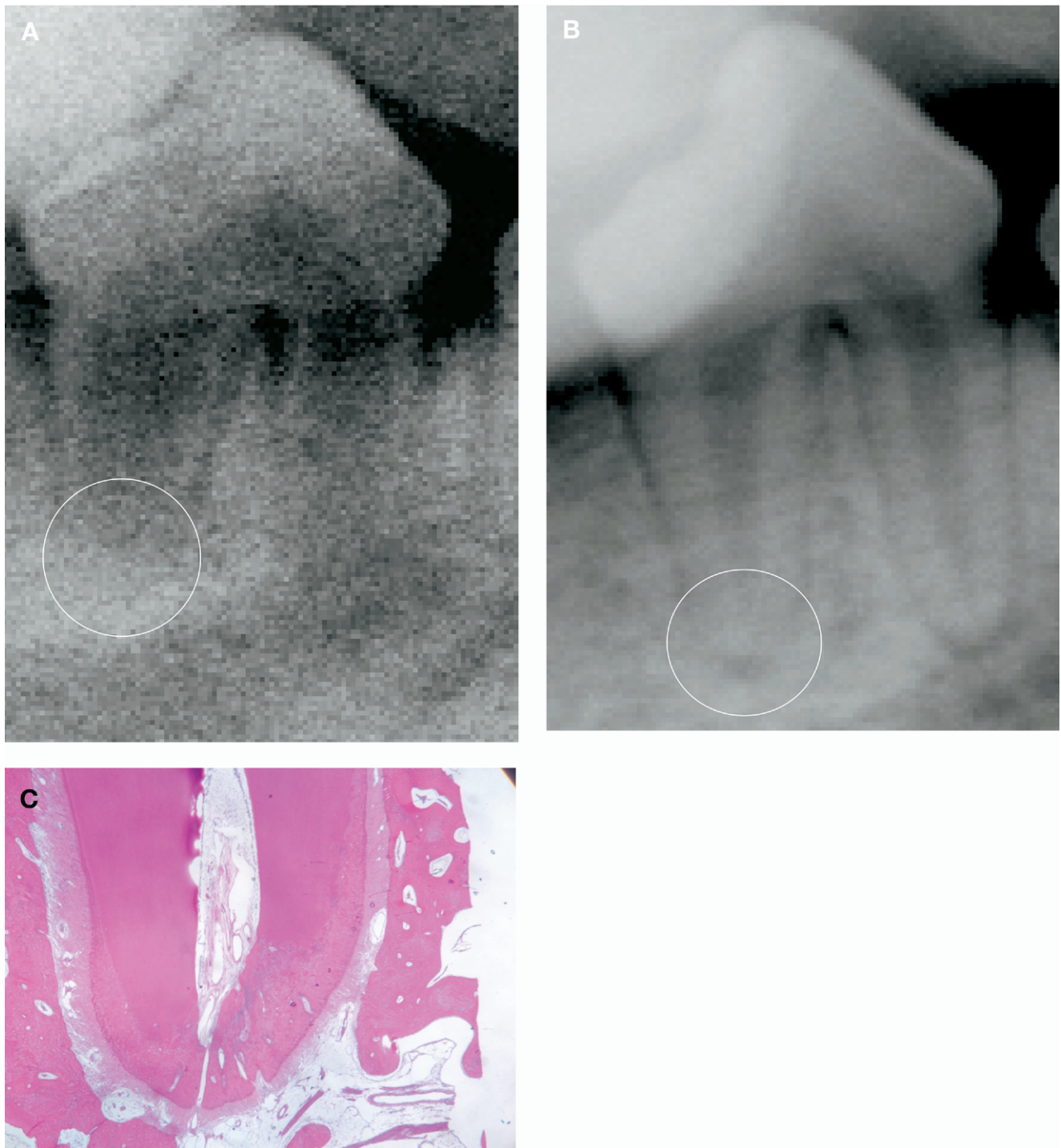


Figure 4. Tooth from group 5 (negative control) showing continued apical and radicular development with normal pulp tissue (original magnification $2\times$).

a blood clot, over 50% of the roots showed radiographic evidence of continued radicular wall development. Group 1 (infected \rightarrow disinfected) had 30%, and group 3 (infected \rightarrow disinfected \rightarrow collagen) had 50% of the roots with continued thickening of radicular dentin walls judged radiographically.

Radiographic Apical Closure

Chi-square evaluation of all five groups for radiographic apical closure showed a significant difference between the groups ($p < 0.01$)

(Fig. 6). When the individual experimental groups (groups 1-4) were compared with each other using chi-square tests in 2×2 tables with 1 degree of freedom for radiographic apical closure, a significant difference ($p = 0.03$) was noted between group 1 (infected \rightarrow disinfected) and group 2 (infected \rightarrow disinfected \rightarrow blood clot), with group 2 (infected \rightarrow disinfected \rightarrow blood clot) showing significantly more apical closure than group 1 (infected \rightarrow disinfected). There was also a borderline difference ($p = 0.051$) between group 3 (infected \rightarrow disinfected \rightarrow collagen) and group 1 (infected \rightarrow disinfected), with

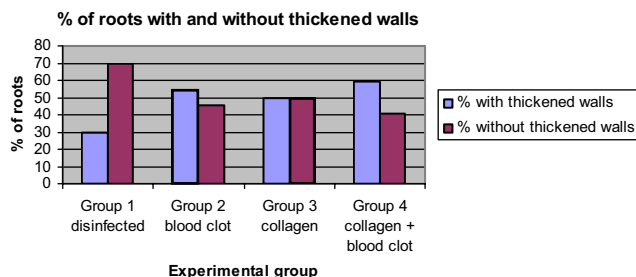


Figure 5. Percentage of roots in experimental groups with and without presence of thickened radicular walls assessed radiographically.

group 3 (infected → disinfected → collagen) showing more apical closure than group 1 (infected → disinfected). There were no other significant differences between the experimental groups with respect to apical closure ($p = 0.11$).

Overall, 54.9% of the roots of the four experimental groups showed radiographic evidence of apical closure, including more than 68% of the roots in group 2 (infected → disinfected → blood clot) and more than 66% of the roots in group 3 (infected → disinfected → collagen).

Radiographic Healing of Periapical Radiolucencies

Chi-square evaluation of all five groups for the presence or absence/improvement of periapical radiolucencies showed a significant difference between the groups ($p = 0.02$) (Fig. 7). However, a chi-square test of the four experimental groups (groups 1–4), not including the negative control group (group 5), did not show any significant differences with respect to presence or absence/improvement of periapical radiolucencies ($p = 0.91$).

After disinfection, 64.6% of the roots from the four experimental groups had no radiographic evidence of periapical radiolucencies or showed improvement/healing of previous periapical radiolucencies.

Histologic Results

Histologic evidence of hard-tissue deposition on internal root canal walls, apical closure, and new vital tissue within the canal spaces was seen in roots from all four experimental groups.

Histological Hard-Tissue Deposition on Radicular Dentin Walls

Chi-square evaluation of all five groups for histological evidence of hard-tissue deposition on internal root dentin walls showed a significant difference between the groups ($p < 0.01$) (Fig. 8). However, a chi-square test comparing the four experimental groups (groups 1–4), not including the negative control group (group 5), did not show any significant differences ($p = 0.22$).

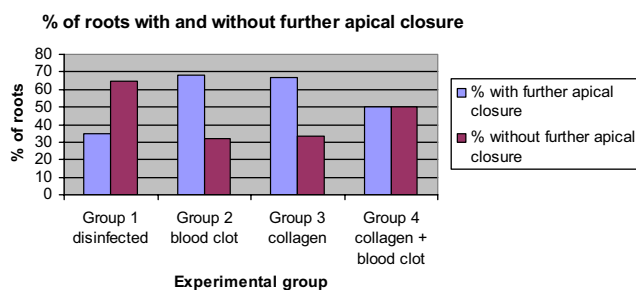


Figure 6. Percentage of roots in experimental groups with and without further apical closure assessed radiographically.

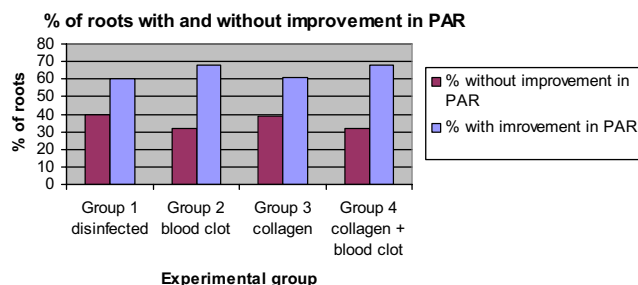


Figure 7. Percentage of roots in experimental groups with and without improvement of periapical radiolucencies assessed radiographically.

In the four experimental groups, 43.9% of the roots showed histological evidence of hard-tissue deposition on internal root dentin walls. In groups 2 (infected → disinfected → blood clot) and 4 (infected → disinfected → collagen + blood clot), those including the use of a blood clot, more than 54% of the roots showed histological evidence of hard tissue deposition on internal dentin walls. Groups 1 (infected → disinfected) and 3 (infected → disinfected → collagen) had approximately 30% of the roots with histological evidence of hard tissue deposited on the internal radicular dentin walls.

Histological Apical Closure

Chi-square evaluation of all five groups for histological evidence of apical closure showed a significant difference between the groups ($p < 0.01$) (Fig. 9). A chi-square test for evaluation of the four experimental groups (groups 1–4), not including the negative control group (group 5), did not show any significant differences between the four experimental groups for histological apical closure ($p = 0.88$).

In the four experimental groups, 54.9% of the roots showed histological evidence of apical closure. In groups 2 (infected → disinfected → blood clot) and 4 (infected → disinfected → collagen + blood clot), those including the use of a blood clot, more than 59% of the roots showed apical closure histologically. Half of the roots (50%) in groups 1 (infected → disinfected) and 3 (infected → disinfected → collagen) showed histological apical closure.

Histological Vital Tissue Within Canal Spaces

Chi-square evaluation of all five groups for histological evidence of vital tissue within the canal space showed a significant difference between the groups ($p < 0.01$). However, a chi-square test for evaluation of the four experimental groups (groups 1–4), not including the negative control group (group 5), did not show any significant differences between the groups ($p = 0.51$) (Fig. 10). In the four experimental groups, 29.3% of the roots showed histological evidence of vital tissue within the canal space. In groups 2 (infected → disinfected → blood

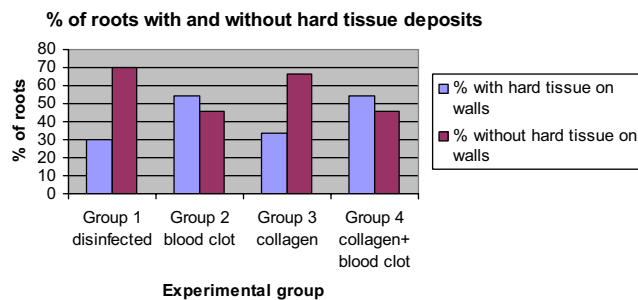


Figure 8. Percentage of roots in experimental groups with and without hard-tissue deposition on radicular dentin walls assessed histologically.

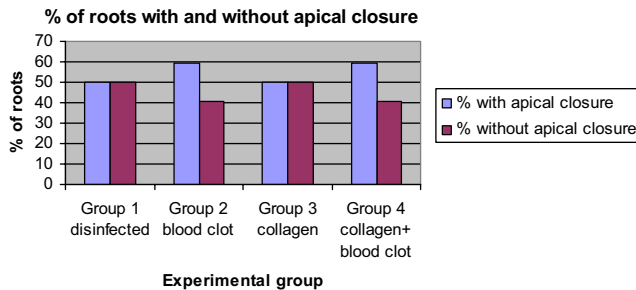


Figure 9. Percentage of roots in experimental groups with and without apical closure assessed histologically.

clot) and 4 (infected → disinfected → collagen + blood clot), those including the use of a blood clot, more than 36% of the roots showed new vital tissue within the canal spaces histologically. Approximately 20% of the roots in groups 1 (infected → disinfected) and 3 (infected → disinfected → collagen) had new vital tissue in the canal spaces.

Discussion

Radiographic Thickening of Root Canal Walls

Although there were no statistical differences between the four experimental groups for thickening of radicular dentin walls, there was a borderline difference between group 1 (infected → disinfected) and group 4 (infected → disinfected → collagen + blood clot), ($p = 0.058$) with group 4 (infected → disinfected → collagen + blood clot) showing more radiographic thickening of root canal walls than group 1 (infected → disinfected). In addition, there was radiographic evidence of continued root-wall thickening among all of the experimental groups that were disinfected with the triple antibiotic paste. Roots that are infected and not disinfected or treated further would not have continued thickening of radicular dentin walls (37, 38). In this study, 48.8% of the roots in groups 1 to 4 (previously infected canals) showed some continued thickening of root walls judged radiographically after disinfection with the triple antibiotic paste. This was because of in-growth of progenitor cells from the periapical area (39) because the pulps in these teeth were all necrotic and infected before disinfection. This was taken as a surrogate outcome measure representing successful revascularization because the new hard tissue was produced by cells that grew in to repopulate the canal space.

Radiographic Apical Closure

There was a statistical difference for radiographic apical closure between groups 1 and 2 ($p = 0.03$), with group 2 (infected → disinfected → blood clot) showing statistically more apical closure than group 1 (infected → disinfected). There was also a tendency whereby group 3 (infected → disinfected → collagen) showed more apical closure ($p = 0.051$) than group 1 (infected → disinfected). There was also radiographic evidence of apical closure among roots of all of the experimentally infected groups (groups 1–4) that were disinfected with the triple antibiotic paste. Roots that were infected and not disinfected or treated further would not have had continued apical closure (37, 38).

Radiographic Healing of Periapical Radiolucencies

There were no statistical differences between the four experimental groups for healing/improvement of apical periodontitis judged radiographically, but there was radiographic evidence of healing apical periodontitis among all of the experimental groups that were disinfected with the triple antibiotic paste. This supports the findings of Windley et al. (18) that the triple antibiotic paste significantly reduces the bacteria

in experimentally infected canals of immature dog teeth. Roots that were infected and not disinfected or treated further would have remained infected with persistent lack of improvement of periapical radiolucencies (37, 38). The time frame of this study may not have been long enough for complete radiographic healing of periapical radiolucencies. It may take up to 4 years for complete healing of apical periodontitis lesions, before which failure should not be assessed (40). In this study, 64.6% of the infected roots in groups 1 to 4 (previously infected canals) showed healing/improvement of existing periapical radiolucencies after treatment with the triple antibiotic paste, with near equal outcomes in each group. This medicament can be relied on to consistently render infected canals effectively free of bacteria. From these results, no radiographic evidence supporting the promotion of revascularization by a collagen solution scaffold was found.

Histological Hard-Tissue Deposition on Radicular Dentin Walls

Although there were no statistical differences between the four experimental groups for new hard tissue deposition on internal root walls, there was histological evidence of hard tissue deposition on internal root dentin walls among all of the experimental groups that were disinfected with the triple antibiotic paste. Roots that are infected and not disinfected or treated further would not have hard tissue deposition on radicular dentin walls (37, 38). In this study, 43.9% of the roots in groups 1 to 4 (previously infected canals) had new hard tissue deposited on the internal root walls. Although some canals only showed thickened root walls and a lack of new vital tissue in the canal space, some measure of successful revascularization was achieved in these cases. Hard-tissue deposition in the absence of new vital tissue in the canal lumen may be because of the limited space available in some canals when the coronal restoration was placed too far apically in the canal space. The physical presence of the coronal restoration too far apically may have precluded the formation of extensive deposits of new hard tissue on the internal canal walls and any significant amounts of new tissue in the canal lumen proper.

New hard tissue deposited on the internal root canal walls is the histologic measure that corresponds to the radiographic measure of thickening of root walls. Comparing the percentage of roots in the four experimental groups (groups 1–4) that exhibited radiographic evidence of continued root wall thickening (48.8%) with the corresponding percentage of roots seen histologically to have hard tissue deposited on the internal canal walls (43.9%) by using the χ^2 test showed there was no significant difference ($p = 0.48$) between the two methods of measurement of continued root wall thickening. This indicates that the radiographic measure of thickened canal walls (normally the only measurable indication of successful revascularization available to the clinician) is representative of the actual histological outcome.

There was a trend showing that the roots treated with a blood clot (groups 2 and 4) after disinfection had better outcomes than those that

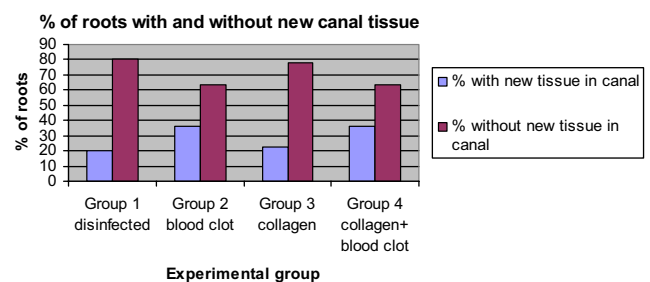


Figure 10. Percentage of roots in experimental groups with and without new vital tissue within the canal space assessed histologically.

did not include a blood clot in the canal space (groups 1 and 3). Although the histological outcomes did not show statistical differences between the groups for continued root wall thickening, they may represent clinical significance because any measure of revascularization represents an improvement over the clinical situation of persistent thin, weak root walls. Inclusion of the blood clot with its constituent growth and differentiation factors (39, 41, 42) may be important for successful revascularization after disinfection.

Histological Apical Closure

Despite the lack of statistical differences between the four experimental groups for histological apical closure, apical closure was seen in all of the experimental groups (groups 1–4) that were disinfected with the triple antibiotic paste. Roots that were infected but not disinfected or treated further would not have had apical closure (37, 38). In the experimental groups (groups 1–4), 54.9% of the roots showed histological apical closure.

Although group 2 (infected → disinfected → blood clot) and group 4 (infected → disinfected → collagen + blood clot) showed marginally better outcomes for histological apical closure than group 1 (infected → disinfected) and group 3 (infected → disinfected → collagen), the four experimental groups (groups 1–4) had similar outcomes. This is because disinfection with the triple antibiotic paste was applied uniformly to all of the roots in the four experimental groups. Apical closure was not because of subsequent treatment after disinfection (blood clot and/or collagen solution application to the canal spaces) but was because of the antibiotic disinfection itself.

Histological Vital Tissue Within Canal Spaces

No statistical differences were seen between the four experimental groups (groups 1–4) for new vital tissue within the canal spaces; however, there was histological evidence of new vital tissue within the canal spaces among all groups that were disinfected with the triple antibiotic paste. Roots that were infected but not disinfected or treated further would not have new vital tissue within their canal spaces (37, 38). After disinfection with the triple antibiotics, 29.3% of the previously necrotic-infected roots (groups 1–4) had new healthy tissue within the canal spaces. Success was higher after disinfection than without disinfection, regardless of the presence or absence of a blood clot or collagen scaffold in the lumen. This is a very significant outcome. It highlights that this protocol rendered previously infected canals effectively bacteria free (18) and allowed in-growth of new vital tissue into the canal spaces. Traditional treatment techniques for an immature tooth with a necrotic-infected pulp space do not involve any attempts at further physiologic development of the tooth. With this protocol, induction of pulp space revascularization with new vital tissue is possible. Although the tissue may not be pulp tissue proper (23, 43), it is vital tissue that can remain in the canal space indefinitely and can provide thickening and consequent strengthening of root canal walls. In addition, this new tissue allows physiologic development of the apices so that if future endodontic treatment does ever become necessary, a more successful outcome can be expected (7).

There was a trend toward more vital tissue within the canal lumen for roots from group 2 (infected → disinfected → blood clot) and group 4 (infected → disinfected → collagen + blood clot) compared with group 1 (infected → disinfected) and group 3 (infected → disinfected → collagen). This suggests the importance of inclusion of a blood clot to improve the chances of revascularization in previously infected canals that are disinfected with the triple antibiotic paste; however, this was not a statistically significant association and highlights the need for further research in this area.

From these results, no statistically significant evidence supporting the promotion of revascularization by a collagen solution scaffold was found. However, inclusion of a blood clot in the canal space tended to improve the revascularization outcome in necrotic-infected immature dog root canal systems that had been disinfected with the triple antibiotic paste of metronidazole, minocycline, and ciprofloxacin. Most importantly, this research showed that revascularization of previously necrotic-infected canals is possible provided they can be effectively disinfected.

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