PERIAPICAL MICROSURGERY:

AN IN-VIVO EVALUATION OF

ENDODONTIC ROOT-END FILLING MATERIALS

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ABSTRACT

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AN IN-VIVO EVALUATION OF ENDODONTIC ROOT-END FILLING MATERIALS

(Under the Direction of Fabricio Teixeira, Alice Curran, Daniel Caplan & Martin Trope)

The purpose of this study was to assess the efficacy of three apical sealing materials (IRM™, Geristore® and MTA™) following apical microsurgery in an animal model. Periradicular healing was evaluated using radiographic and histologic indicators. Eighty mandibular premolar roots of beagle dogs were instrumented and intentionally infected. Apical microsurgeries were performed after thirty days without prior disinfection of the root canals. The root ends were resected, apical preparations were made and the retrofill materials were placed with the aid of a Surgical Operating Microscope. After a healing period of six months, the periradicular areas in question were assessed using digital radiographic images and prepared histologic sections. Although Geristore showed no radiographic difference when compared to the other groups, it demonstrated the least favorable healing results in the histologic evaluation. IRM achieved the most favorable healing response both radiographically and histologically but these results were not statistically different from MTA.

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I. THESIS INTRODUCTION

Apical surgery is an important part of the modern endodontic practice (1,2). Periradicular surgery should be considered an extension of nonsurgical treatment since the etiology of the disease process and the objectives of treatment for both is ultimately the treatment of apical periodontitis. Periradicular surgery has evolved into a precise, biologically based adjunct to nonsurgical root canal therapy in the treatment of apical periodontitis.

Successful root canal therapy is dependent on the ability to remove intracanal microbes by adequately instrumenting, disinfecting and obturating the root canal system. On occasion, conventional root canal therapy is not able or is not sufficient to eradicate these microbes and surgical root canal treatment is then indicated. This involves surgical exposure of the apical portion of the root, resection of the infected apical segment, and placement of a root-end filling to establish an apical seal.

Apical surgery is ideally performed with the placement of a retrofilling material which seals off toxins in the root canal from the surrounding periapical tissues (3). In the last decade, significant improvements have been made in the development of new instruments, new materials, new techniques, along with a better understanding of the

biology of the root canal system. Many requirements have been specified for an ideal retrofilling material: sealing ability, handling properties, working time, radiopacity, antibacterial activity, biocompatibility, induction of the PDL complex, and many other. Ultimately, one of the most critical properties is the creation of a proper periapical seal (3-7). Molven et al. found that the efficacy of the apical seal was the most critical factor for successful apical surgeries (7).

Given the fact that an effective apical seal is an important factor in successful surgical treatment, much research has been devoted to the search for an ideal surgical material over the years. This search involved many materials, including but not limited to: Cavit (8,9), zinc oxide-eugenol (10), gold foil (11), EBA cement (12), heat sealed gutta-percha (13) and amalgam (10). Amalgam was used as a standard retro-filling material for many years, but has since been shown to have disadvantages including shrinkage (14), corrosion breakdown and leakage (15). More recent clinical human outcome studies have shown that amalgam does not offer good long-term outcomes in periradicular surgery (16,17). Dissatisfaction with amalgam and other current materials has led to research for implementing new restorative materials in periradicular surgery.

Many of the previous in-vivo animal studies that analyzed retro-filling materials did a microbial control phase prior to the surgical portion. Thus, these studies were only evaluating the biocompatibility of the material in question and not its sealing ability. In a patient-based clinical scenario, surgical therapy is then done when a microbial challenge persists inside the canal, preventing the normal healing of the periodontium. In order to

properly test the in-vivo sealing efficiency of the tested material, a microbial challenge has to be present in the canal (42). Therefore, more in-vivo endodontic surgery research qualitatively comparing the seal and the efficacy of different modern retrofilling material seemed to be warranted.

The purpose of this study was to evaluate the sealing ability of IRM, MTA and Geristore as retrofilling materials by analyzing the wound healing responses of periradicular tissues following periradicular microsurgery. The study examined the radiographic changes and focused on the histologic evaluation of the excisional wound healing response associated with these three contemporary retro-filling materials. The responses were compared and contrasted between the three materials.

II. REVIEW OF LITERATURE

i. Wound healing:

Wound healing was eloquently described in 1991 by Harrison and Jurosky (23). The tissues involved in periradicular surgery are oral tissues, periradicular tissues and the radicular tissues. As for the type of surgical wounds that are created during apical surgery, they are the incision, the blunt dissection and the excision. Excisional wound healing is unique in that its progress is dependent on the healing events that precede it (23). It depends on the establishment of a proper epithelial seal that allows the underlying excisional wound healing to progress with no interference from any ingress of irritants from the oral cavity (24).

The creation of the coagulum is an integral part of a complex series of healing events. First comes the clotting and the inflammation, followed by the connective tissue healing and finally the osseous healing (23). The entire healing process starts with acute inflammation that entails changes in vessel permeability and changes in the location and the concentration of white cells (25). The polymorphonuclear leukocyte (PMN) is the predominant cell in this early stage. It rapidly destroys microorganisms and phagocytizes particulate matter and cellular debris (23). Six to twelve hours after surgical injury, the macrophage appears in increasing numbers. This cell is transient and active in both

inflammation and repair (26). It creates an environment in which connective tissue healing can occur. The macrophage becomes the predominant cell between day five and seven after the injury. This cell was shown in previous studies to be a prerequisite for early fibroblast attraction (27) and proliferation (28). This process continues until a transition from granulomatous tissue (tissue with a predominant inflammatory infiltrate) to granulation tissue (highly vascular tissue with fibrous tissue predominance) occurs. When this occurs, it signals a successful progress of connective tissue healing (23).

One must keep in mind that all the healing events occur within two interacting regions: the apical dentoalveolar (area including the apical resected root surface and the re-forming alveolar bone proper) and the osseous access wound (area created when cortical and cancellous bone are removed with a rotary instrument) (23). The dentinal surface of the resected root apex is believed to provide an inductive force necessary for new cementum deposition (29). The formation of a functional PDL after the excisonal wound is dependent on cementum deposition and the degree of reformation of the apical dentoalveolar apparatus. Concerning the osseous access, its repair is primarily the responsibility of osteogenic cells from the endosteum (30). Osseous access repair will start with woven bone that will fill the access moving from the internal toward the external surface (31).

ii. Wound healing and the root-end filling material:

When a root-end filling material is placed, it may have an effect on the dynamics of the excisional wound healing and the overall periradicular health. A root-end filling

exists as an implant that has to seal intraradicular toxins and be biocompatible. Many requirements have been stated for the ideal retrofilling material. Some of the main specifications in a retro-filling that clinicians look for are: Sealing ability, handling properties, working time, radiopacity, antibacterial activity, biocompatibility, induction of PDL complex, and many other. Ultimately, one of the most critical properties is the creation of a proper seal (3-7). Molven et al. found that the efficacy of the apical seal was the most critical factor for successful apical surgeries (7).

iii. Review of the root-end filling material literature:

The sealing ability of various retrofilling materials has been studied in many different in-vitro designs (6,14,32-34). Extensive research has been done with different methodologies as electrochemical techniques, fluorometry, fluid filtration techniques, salivary penetration models, culture techniques to detect bacterial penetration and dye leakage. (35). The variety of methods and their particular assessment of success might be a reason for the poor agreement between the different studies (36). Factors such as the choice of storage solutions, the size of the dye particles and many other variables can come into play in these studies (37). The conclusions of these studies have often been inconsistent and confusing. This is in part due to the fact that the results are often non-reproducible with relatively high standard deviations (36). Each in-vitro model has its supporters and detractors, but there seems to be a general agreement that there is no common understanding of their clinical implications (38). The Editorial Board of the Journal of Endodontics has agreed to restrict publications of leakage studies using these in-vitro techniques starting July 1, 2008 (38).

Concerning in-vivo studies, some prospective and retrospective human clinical studies have been reported comparing the outcome of periradicular surgery involving the placement of various root-end filling materials (16,20,22,39-41). It is often hard to compare the results of these human studies due different evaluation criteria and observation periods used by the different authors. Furthermore, no available study has yet evaluated directly Geristore, IRM and MTA against each other.

Some other in-vivo studies have been done using animal research (23,42-44). Animal use (specifically the use of the canine specie) is well documented in the endodontic literature. The healing pattern of dogs has been shown to be similar to that of humans (23,42-44). Dogs provide a suitable model to evaluate histologically the periapical healing which cannot be obtained in an in-vitro or human study. Furthermore, the occlusion pattern of the dog allows the use of multiple teeth in each animal without interfering with or compromising their ability to chew and function. This allows sufficient numbers of teeth to be treated and provides statistically valid results with relatively few animals (42,45).

iv. The microbial challenge:

Many of the previous in-vivo animal studies that analyzed retro-filling materials did a microbial control phase prior to the surgical portion. Thus, these studies were only evaluating the biocompatibility of the material in question and not its sealing ability. In a patient-based clinical scenario, periapical surgery is done in order to seal microbes that

could not be eliminated by the conventional orthograde root canal therapy. Surgical therapy is then done when a microbial challenge persists inside the canal, preventing the normal healing of the periodontium. In order to properly test the in-vivo sealing efficiency of the tested material, a microbial challenge has to be present in the canal (42). Therefore, more in-vivo endodontic surgery research qualitatively comparing the seal and the efficacy of different modern retrofilling material seemed to be warranted.

v. Surgical magnification:

Magnification (microscopes and loupes) was used appropriately at each step during this experiment to further simulate an actual clinical scenario. Previous studies have never before employed the use of microscopic magnification in an animal model. The importance and advantages of using magnification during endodontic surgery has been described and stressed by several authors (4,46-49).

vi. Review of the root-end filling materials used in this study:

Glass ionomer was created by Wilson and Kent in 1969 (50). It was introduced to the dental community in 1972 (50) and was brought to North America in 1977 (51). The cement components of glass ionomer are an aluminosilicate glass powder and a polycarboxylate copolymer liquid that, when combined, form a cross-linked gel matrix that surrounds the partially reacted powder particles (52). Glass ionomer has several properties that make it a valid potential root-end filling material. It possesses the potential for permanent adhesion to dentin and provides an excellent seal (53). Furthermore, in vitro cell culture studies showed that glass ionomer to be biocompatible and caused

potentially less inflammation than other restorative materials (54,55). There are no long-term studies on the use of glass-ionomer as a retrofilling material. Glass ionomer was compared to other materials in a few studies showing variable results (42,56-58).

Resins have already been shown to be a promising option as a retrofilling material (20,40,59,60). Furthermore, the bond established between dentin and resins was shown to be stable over a nine year observation period in apical surgeries (41). Geristore® (Den-Mat, Santa Maria, CA) (Figure 2), a new resin-ionomer suspension material, has recently been introduced to the market with a new auto-mix syringe delivery tip. Geristore is a dual-curing paste/paste formulation comprised of: hydrophilic Bis-GMA with long-term fluoride release. The attraction to glass ionomer cement as a retrofilling material is mainly due to its dentin-adhesive property, antibacterial activity and mild cytotoxic effect. Several case reports have been published with remarkably favorable results (61-64). Moreover, a recent study by Camp in 2003 showed the possible adhesion of human fibroblasts to this material (65). The published literature on Geristore is limited but this material appears to be very promising.

Mineral Trioxide Aggregate (ProRoot MTA™; Dentsply Tulsa Dental Specialties, Tulsa, OK) (Figure 3) is a powder consisting mainly of: bismuth oxide (Bi_2O_3), calcium silicate ($CaSiO_4$), calcium carbonate ($CaCO_3$), calcium sulfate ($CaSO_4$), and calcium aluminate ($CaAl_2O_4$) (66). MTA has been analyzed extensively. Several leakage studies have shown this material to provide a remarkable seal (67-70). MTA appears to show a favorable biologic response (45,71,72). A series of studies in

different animals (dogs and monkeys) have shown MTA to be a promising new material (45,71). Further in-vivo studies have indicated that MTA has a greater healing capacity and the potential to induce bone, dentin and cementum formation (73,74). Its high pH is a possible explanation for the induction of hard tissue formation (66). Another theory is that the calcium oxide in MTA may react with water and dissociate into calcium ions and hydroxyl ions. The calcium ions react with the carbon dioxide in the tissues and form calcium carbonate granulations, presenting as calcite crystals, which would stimulate the deposition of hard tissue (75).

IRM™ (L.D. Caulk Inc., Dentsply International Inc., Milford, DE) (Figure 1) has become a popular proposed retrofilling material (12,16). IRM powder is made from zinc oxide and polymethylmethacrylate; the liquid is mainly eugenol with less than 1% acetic acid. IRM has the advantage of being cost effective, easy to mix and easy to handle. Furthermore, it performs well in moist conditions. A recent clinical study has shown no statistical difference in healing when IRM was compared to MTA (22). It has been stated that a hard mix of IRM is recommended when being used as a retrofilling material to minimize the toxic effect on surrounding tissues from the eugenol release (76,77).

vii. Study design:

Beagle dogs were used in this study. At three years of age, their premolars were fully erupted and apical root formation was complete. The apical portion of these premolars has an extensive number of lateral canals; this portion was resected during surgery and should not have been a factor. Beagle dogs have eight lower premolars. The

first premolars are single rooted and the remaining six premolars (second, third and fourth premolar) have two well-demarcated roots (one mesial root and one distal root). All mandibular premolar teeth were used. Upper maxillary premolars could not be used due to the proximity of the sinus, which could compromise the healing as well as interfere with histological and radiographical assessment due to lack of bone mass and density.

III FORMATED SECTIONS FOR THE JOURNAL OF ENDODONTICS

i. Introduction

Apical surgery is an important part of the modern endodontic practice (1,2). Periradicular surgery must be considered an extension of nonsurgical treatment since the etiology of the disease process and the objectives of treatment are the same. Periradicular surgery has evolved into a precise, biologically based adjunct to nonsurgical root canal therapy in the treatment of apical periodontitis. Modern surgical endodontic protocols (4) along with contemporary retrofilling materials (IRM, Super-EBA, Retroplast, MTA) have been shown to be very predictable with a radiographic and clinical success rate of 91.2% to 92.5% (18,20-22,78).

Apical surgery is ideally performed with the placement of a retrofilling material which seals off toxins in the root canal from the surrounding periapical tissues (3). In the last decade improvement was made in the development of new instruments, new materials, new techniques, along with a better understanding of the biology. Some of the main specifications that clinicians look for in a retrofilling are: Sealing ability, handling properties, working time, radiopacity, antibacterial activity, biocompatibility and the induction of the PDL complex. One of the most critical properties is ultimately the

creation of a proper seal (3-7). Molven et al. found that the efficacy of the apical seal was the most critical factor in successful apical surgeries (7).

The sealing ability of various retrofilling materials has been studied in many different in-vitro designs (6,14,32-34). Extensive research has been done with different methodologies such as electrochemical techniques, fluorometry, fluid filtration techniques, salivary penetration models and culture techniques to detect bacterial penetration and dye leakage. (35). The conclusions of these studies have often been inconsistent and confusing. This is in part due to the fact that the results are often non-reproducible with relatively high standard deviations (36). Each in-vitro model has its supporters and detractors, but there seems to be a general agreement that there is no common understanding of their clinical implications (38). The Editorial Board of the Journal of Endodontics has agreed to restrict publications of leakage studies using these in-vitro techniques starting July 1, 2008 (38).

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Other in-vivo studies have been done using animal research (23,42-44,79,80). Animal use (specifically the use of the canine specie) is well-documented in the endodontic literature. The advantage of this type of research is the potential for histological assessment at the end of the experiment. Many of the previous in-vivo animal studies that analyzed retrofilling materials had a microbial control phase prior to the surgical portion. Thus, these studies were evaluating the biocompatibility of the material in question and not its sealing ability. In a patient based clinical scenario, periapical surgery is done in order to seal out microbes that could not be eliminated by the conventional orthograde root canal therapy. When a microbial challenge persists inside the canal, preventing the normal healing of the periodontium, surgical therapy is then performed. In order to test the in-vivo sealing efficiency of the tested material, a microbial challenge has to be present in the canal (42). Therefore, more in-vivo endodontic surgery research comparing quantitatively the seal and the efficacy of different modern retrofilling material seemed to be warranted.

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Resins have already been shown to be a suitable promising option as a retrofilling material (20,40,41,59,60,81). Furthermore, the bond established between dentin and resins was shown to be stable over a nine year observation period in apical surgeries (41). Geristore® (Den-Mat, Santa Maria, CA), a new resin-ionomer suspension material, has recently been introduced to the market with a new auto-mix syringe delivery tip. Geristore is a dual-curing paste/paste formulation composed of hydrophilic Bis-GMA with long-term fluoride release. The attraction to glass ionomer cement as a retrofilling material is mainly due to its dentin-adhesive property, antibacterial activity and mild cytotoxic effect. Several case reports have been published with remarkably favorable results (62-64). Moreover, a recent study by Camp in 2003 showed the possible adhesion of human fibroblasts to this material (65). The published literature on Geristore is limited but this material appears to be very promising.

The purpose of the present study was to evaluate the efficacy of three different contemporary retrofilling materials using a modern endodontic surgical protocol (4) in beagle dogs. This was done using radiographic and histologic assessment of the healing of the apical areas in question six months after surgery.

ii. Material 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. Forty-eight premolar teeth in six purpose-bred beagle dogs of approximately three year of age were randomly divided into three treatment and one control group.

Pre-surgical phase:

Randomization of teeth was done in each dog. Eight cards were placed in a box representing two IRM, two Geristore, two MTA and two controls. The cards were pulled out of the box and were assigned to the tooth in question. After randomization was completed the teeth were divided in a total of 36 experimental and 12 control teeth.

Under general anesthesia induction by Pentothal [Abbott Laboratories, North Chicago, IL] (13.5 mg/kg administered intravenously), intubation and maintenance with Isoflurane [Halocarbon Laboratories, River Edge, NJ] supplemented with local anesthesia (Bupivacaine plain 0.5%, Abbott Laboratories). All lower premolars were removed from occlusion (Figure 4), the pulps of all roots were mechanically exposed with a no. 2 round carbide bur (Brassler USA, Savannah, GA) in a high-speed hand piece (Midwest, Mondovi, WI) and the access was made (Figure 5) under non-aseptic conditions. Canals were instrumented to a size 40/04 NiTi with Profile GT files (Dentsply Tulsa Dental, Tulsa, OK) (Figure 6). Supra-gingival plaque scaled from the dog's teeth was mixed with saline (0.9% sodium chloride; Hospira Inc., Lake Forest, IL) and was introduced into the

canals with a lentulo spiral (Dentsply, Maillefer, Johnson City, TN) (Figure 7). Sponges (Dentsply Maillefer, Johnson City, TN) soaked in the plaque solution were placed on the floor of the access. The access was then closed with IRM (L.D. Caulk Inc., Dentsply International Inc., Milford, DE) (Figure 8). The involved teeth were then radiographed (Figure 33) using custom bite registrations (Regisil; Dentsply Caulk, Milford, DE) and radiographic 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 animals were given analgesics (Torbugesic 0.2mg/kg; Butorphanol Tartrate, Fort Dodge Animal Health, Fort Dodge, IA) postoperatively following all procedures and were monitored by the staff of the Department of Laboratory and Animal Medicine in the postoperative period.

The teeth were monitored radiographically (Figure 34, 35) using the original custom bite registrations and paralleling devices until there was radiographic evidence of apical periodontitis (approximately 30 days). All previously infected teeth were re-accessed with the animals under general and local anesthesia. After removal of the IRM and sponge, the canals were rinsed with a saline solution. No canal debridement or microbial control was performed at that stage (42). The canals were dried with paper-points (Figure 9) and a Gutta-Percha cone was then inserted into the canals, approximating the apex as much as possible (Figure 10, 11); this provided a matrix to pack against in order to have a proper dense retrofilling material. The access was then

closed with glass ionomer cement (Fuji IX GP, GC Corporation, Tokyo, Japan) (Figure 12).

Surgical phase:

A surgical aseptic field was implemented (Figure 14, 15) and a surgical operating microscope (OPMI pico, Carl Zeiss Meditec, Inc.) was used appropriately at each step during this surgical phase (Figure 13). The importance and advantages of using magnification during endodontic surgery has been described and stressed by several authors (4,46,48,49).

A sulcular incision (Figure 16) with a vertical release was done at the canine and the first molar region. Buccal full thickness flaps were reflected (Figure 17) and surgical bony windows were created (Figure 18, 19). Access cavities were prepared with a sterile round no. 2, 4 and 6 tungsten carbide burs (Brassler USA, Savannah, GA) at high speed and with water spray cooling (82-84). Periapical granulomatous tissue was curetted out, the root ends were resected 3mm (85,86) (Figure 20) and the resection was made with a multi-purpose carbide bur (Dentsply Maillefer, Johnson City, TN) (89) as perpendicular as possible to the long axis of the tooth (87,88). Retrograde cavities of 3mm depth (Figure 20) (90) were prepared with a Satelec P5 ultrasonic unit (Dentsply Maillefer, Johnson City, TN) at a medium power setting (91,92) with CT surgical ultrasonic tips (SybronEndo Corporation, Orange, CA) (93-96).

To obtain a proper hemostasis during the retrofilling material placement all bone cavities had Racellet epinephrine pellets (Pascal Co., Bellevue, WA) applied with pressure in the area for five minutes (97-99). After hemostasis was obtained, the retro-cavities were rinsed and dried with the Stropko irrigator (SybronEndo Corporation, Orange, CA).

Group I: IRM (Figure 1): IRM was mixed to a hard putty consistency (77). The cement was then packed with a flat plastic instrument. After setting, the excess material was carefully removed and polished with a carbide bur (100) (Figure 21).

Group II: Geristore (Figure 2): Due to the bonding ability of this material, the circumferential cavity design of the root tip was slightly concave as described by Rud to provide an increased dentinal surface (81). After completing the retrograde cavities of 3mm depth with the ultrasonics, an H379 football shape carbide bur (Brassler USA, Savannah, GA) was used to create the concave finish of the retro prep (Figure 22). Geristore was used with the auto-mix syringe delivery tip, following the manufacturer instructions, to fill the retro prep and it extended over the whole circumference of the root as a cap (Figure 22).

Group III: White ProRoot MTA (Figure 3): White ProRoot MTA was mixed following the manufacturer instructions and placed into the retro prep (Figure 23) with the MAP system (Roydent Dental Products, Johnson City, TN) (Figure 24).

Control group: In each dog two teeth with apical periodontitis were resected, polished with a carbide bur (100) and left without retrofill.

The flaps were repositioned and secured with interrupted 5.0 Chromic Gut sutures (Hu-Friedy Manufacturing Company, Inc.) To verify the bony window and avoid a confounding factor in the final healing analysis, radiographs were taken with the custom bite registrations and paralleling devices (Figure: 35-45).

Post-surgical phase:

Following surgery, each animal received an intramuscular injection of 10^6 units of penicillin G. In addition, for possible post-surgical pain each animal was given an initial dose of 0.2mg/kg Meloxicam (Metacam) by mouth then 0.1mg/kg by mouth for three days. Furthermore, for one week, the animals were monitored for possible post-surgical complications and they were placed on a soft diet.

A follow up with a dental cleaning session was done two months post-surgery. The dogs were put under general anesthesia induction by Pentothal (13.5 mg/kg intravenously) and intubation and maintenance with isoflurane. Scaling and prophylaxis was completed (Figure: 25-32).

Six months post surgery the animals were sacrificed under deep general anesthesia with the use of pentobarbital (Butler Company, Columbus, OH) at 30 mg/kg intravenously. The left and right carotid arteries were exposed and canulated. Additional

pentobarbital (Socumb, Butler Company) at a dose of 90 mg/kg intravenously was used to euthanize the animals. The animals were then prefused with 10% buffered formalin (Fisher Scientific, Fair Lawn, NJ). Radiographs were taken with the custom bite registrations and paralleling devices (Figure: 35-43). Jaw blocks containing the treated teeth were resected, fixed in 10% buffered formalin and decalcified in 10% EDTA. Decalcification was monitored with a pressure needle and radiographs (Figure: 46, 47). After four weeks, once decalcification was completed, the samples were embedded in paraffin and prepared for histologic evaluation. On removal from the decalcification solution, the specimens were rinse in a sterile saline solution 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, embedded in paraffin and sectioned on a Leica Jung RM 2045 microtome. Serial longitudinal sections of five to seven microns were made in a mesio-distal orientation to include the apical foramen, the root canal space and the periapical tissue. The cuts that showed the best possible view of the root canal system, the apical filling material as well as the surrounding tissues were chosen to be stained with hematoxylin and eosin. The prepared histologic slides were then examined under light microscopy up to 10X magnification (Figure: 48-61). Two blinded evaluators analyzed the radiographs and the histologic sections following predetermined scales.

Radiographic assessment:

Radiographs of the six month follow-up were compared to post-surgical radiographs. The following ordinal scale was used:

0: Healed, complete healing of the periapical radiolucency with a healthy lamina dura

1: Healing, reduction of the periapical radiolucency size (healing is not complete)

2: Uncertain, no apparent changes in the size of the pariapical radiolucency

3: Failure, increase of the periapical radiolucency size or loss of tooth.

Histological assessment:

Histology sections were evaluated according to the following predetermined scale:

1: Absence of inflammation with presence of PDL regeneration.

2: Presence of inflammation with absence of PDL regeneration.

Data analysis:

All data was entered into Microsoft® Office Excel Program (2003). Data analysis was performed using SAS/STAT® Software. Within each of the six dogs the teeth were considered as the treatment units. Fisher's Exact Test and Mantel-Haenszel Test were used to compare proportions. Analysis were done for possible associations between the tooth location "TOOTH response", the jaw side "RORL" and between dogs "DOG response". P-value of less than 0.05 was considered to be statistically significant.

iii. Results

Radiographic Assessment of Healing:

Table 1 presents the detailed radiographic results for this study. Three teeth were discarded in this study due to their periodontal condition at the time of surgery. A histogram was done summarizing the aggregated results for the radiographic portion (Figure 64). The histogram demonstrates that all teeth with retrofilling (Geristore, MTA and IRM) had an overall better healing response compared to the control group.

After carrying out a stratified analysis by dog "DOG response", jaw side "RORL" and tooth location "TOOTH response", we did not find the response to differ by dog or jaw. The healing was better on posterior premolars compared to anterior premolars. The tooth location "TOOTH response" was significant at the level of 0.05.

Cochran-Mantel-Haenszel tests of association were then used for the "Treatment" and the "Radiographic Response" controlling for "TOOTH response" and stratifying by dog. All experimental groups showed superior healing compared to the control group ($p<0.01$). IRM showed a better healing response than Geristore ($p<0.04$). There was no statistically significant difference between IRM and MTA and between MTA and Geristore.

Histopathologic Assessment of Healing:

Table 5 shows the detailed histology results where four additional teeth were lost during the histology processing. A histogram was done summarizing the aggregated histological results (Figure 64). We could see in that histogram that IRM and MTA had a more favorable response then the control and the Geristore group.

Cochran-Mantel-Haenszel tests of association were then used for the "Treatment" and the "Histological Response" controlling for the "TOOTH response" and stratifying by dog. Geristore demonstrated the worst healing results and had no statistical difference when compared to the control group. Both IRM and MTA demonstrated better healing responses than Geristore ($p<0.04$, $p<0.01$). There was no statistical difference between IRM and MTA.

Additional Findings:

Three teeth were discarded in this study due to their periodontal condition at the time of surgery and were removed from the radiographic and histopathologic assessment (Table 1). Four additional teeth had to be removed from the histopathologic analysis due to technical processing errors during the preparation of the slides (Table 5).

iv. Discussion

MTA has previously been attributed with the unique potential to induce or attach to the newly regenerating periodontal ligament (45,74). Some studies have even shown this to be possible with Geristore (62,65). In this study the proximity between all experimental materials and the newly developed periodontal ligament was frequently observed. However, the presence of the adjacent tissue is not enough to argue of a good surgical outcome. The more appropriate material should improve seal as well as tissue compatibility in order not to see an inflammatory reaction. IRM and MTA fulfilled both these requirements as seen in our findings, while Geristore apparently did not.

Even though Geristore showed a favorable response radiographically, when analyzed histologically, inflammation was seen. This highlights the limitation of radiographic images and the superior level of information provided by a histologic assessment. This finding concurs with previous studies done on human cadavers showing the more accurate assessment provided from histology (101-103).

According to the results of our study, both IRM and MTA resulted in better healing responses than Geristore. The positive outcome from IRM and MTA concurs with some previous in-vivo human studies (18,20-22,78). Furthermore, a previous study done by Trope et al (42) had similar results where Super EBA and IRM resulted in better healing than composite resin and glass ionomer. Comparing their results to other studies where resins were used (40,41), they explained that the difference in results might be due

to the cavity design or possible moisture at the time of surgery. Unlike that study (42), our study employed a concave cavity design (81) and a dry cavity preparation that was confirmed under the Operating Dental Microscope at the time of placement of our material. The disparity between our results and the results by Rud et al. (40,41) could be attributed to the fact that the canals in their study were not as infected as they were in our model. Nevertheless, Retroplast has a different composition and properties than Geristore and the results from this study cannot be directly applied to it. Further histological research with an intra-canal microbial challenge as done in this study is warranted for Retroplast as a potential root-end filling material.

One limitation of our model is that beagle dogs are susceptible to periodontal disease (42) and this was the cause for the loss of three teeth in this study. Furthermore, the fast decalcification method with EDTA used in the study offered less control on the decalcification status of the teeth in question and made the histology slide preparation more challenging, causing the loss of four additional teeth. In addition, this decalcification method was very aggressive toward the inflammatory cells; it resulted in the loss of their distinct purple color and made them hard to see under light microscopy. A binary scale was thus used for the reading in order to facilitate the objective reading of the histology slides.

v. Conclusion

Although Geristore showed no radiographic difference when compared to the other groups, it demonstrated the least favorable healing results in the histologic evaluation. IRM achieved the most favorable healing response both radiographically and histologically but these results were not statistically different from MTA.

TABLES

Table 1: Radiographic Results

Radiographic Results

Dog #	Tooth #	R/L	Treatment	Score
1	1	0	2	N/A
1	2	0	3	0
1	3	0	2	1
1	4	0	3	0
1	1	1	0	2
1	2	1	0	1
1	3	1	1	0
1	4	1	1	1
2	1	0	0	3
2	2	0	3	0
2	3	0	2	1
2	4	0	1	0
2	1	1	0	1
2	2	1	2	1
2	3	1	3	0
2	4	1	1	1
3	1	0	3	0
3	2	0	0	3
3	3	0	1	1
3	4	0	2	0
3	1	1	0	1
3	2	1	2	0
3	3	1	1	0
3	4	1	3	0
4	1	0	1	N/A
4	2	0	0	1
4	3	0	1	1
4	4	0	3	0
4	1	1	2	N/A
4	2	1	0	3
4	3	1	3	0
4	4	1	2	0
5	1	0	0	0
5	2	0	3	1
5	3	0	1	1
5	4	0	3	0

5	1	1	2	0
5	2	1	0	3
5	3	1	1	1
5	4	1	2	0
6	1	0	1	0
6	2	0	0	1
6	3	0	2	0
6	4	0	1	1
6	1	1	3	0
6	2	1	0	3
6	3	1	3	1
6	4	1	2	1

Legend:	
Dog # :	1=ZBQ4 (Suzie) 2=PQK4 (Monica) 3=RVK4 (Cindy) 4=JSW4 (Becky) 5=YOI4 (Donna) 6=IXG4 (Sarah)
Tooth # :	1=PM1 2=PM2 3=PM3 4=PM4
R/L :	0=Right 1=Left
Treatment:	0=Control 1=Geristore 2=MTA 3=IRM
Score:	Original Score per Tooth based on a 0 to 3 ordinal score
N/A:	Teeth excluded from study: Technical error

Table 2: p-value of tests of association for DOG, TOOTH, and RORL by response

Test	Score	
	Fisher	Mantel-Haenszel
DOG response	0.9935	0.9944
TOOTH response	**0.0730**	**0.0360**
RORL response	1.0000	0.5079

Table 3: p-value of tests of association for the TREATMENT and the response.
0=Control, 1= Geristrore, 2=MTA, 3=IRM

Test Treatment	Score	
	Fisher	Mantel-Haenszel
0,1,2,3	**<0.0001**	**<0.0001**
0,1,2	**0.0118**	**0.0012**
0,1,3	**<0.0001**	**0.0002**
0,2,3	**<0.0001**	**0.0002**
1,2,3	0.0916	0.0764
0,1	**0.0326**	**0.0065**
0,2	**0.0165**	**0.0033**
0,3	**<0.0001**	**0.0005**
1,2	0.3949	0.2905
1,3	**0.0361**	**0.0241**
2,3	0.3476	0.2319

Table 4: p-value for Cochran-Mantel-Haenszel tests of association for the TREATMENT and the response controlling for TOOTH
0=Control, 1= Geristrore, 2=MTA, 3=IRM

Test Treatment	Score
0,1,2,3	**0.0088**
0,1,2	0.0625
0,1,3	**0.0088**
0,2,3	**0.0107**
1,2,3	0.1100
0,1	0.2743
0,2	**0.0431**
0,3	**0.0115**
1,2	0.3340
1,3	**0.0381**
2,3	0.2438

Table 5: Histology Results

Histology Results

Dog #	Tooth #	R/L	Treatment	Score
1	1	0	2	N/A
1	2	0	3	2
1	3	0	2	1
1	4	0	3	2
1	1	1	0	N/A
1	2	1	0	1
1	3	1	1	2
1	4	1	1	1
2	1	0	0	2
2	2	0	3	1
2	3	0	2	2
2	4	0	1	N/A
2	1	1	0	2
2	2	1	2	2
2	3	1	3	1
2	4	1	1	2
3	1	0	3	1
3	2	0	0	2
3	3	0	1	2
3	4	0	2	1
3	1	1	0	1
3	2	1	2	2
3	3	1	1	2
3	4	1	3	1
4	1	0	1	N/A
4	2	0	0	1
4	3	0	1	2
4	4	0	3	1
4	1	1	2	N/A
4	2	1	0	2
4	3	1	3	1
4	4	1	2	1
5	1	0	0	N/A
5	2	0	3	1
5	3	0	1	2
5	4	0	3	1
5	1	1	2	1
5	2	1	0	2
5	3	1	1	N/A
5	4	1	2	1
6	1	0	1	1

6	2	0	0	2
6	3	0	2	1
6	4	0	1	2
6	1	1	3	1
6	2	1	0	2
6	3	1	3	2
6	4	1	2	1

Legend:	
Dog # :	1=ZBQ4 (Suzie) 2=PQK4 (Monica) 3=RVK4 (Cindy) 4=JSW4 (Becky) 5=YOI4 (Donna) 6=IXG4 (Sarah)
Tooth # :	1=PM1 2=PM2 3=PM3 4=PM4
R/L :	0=Right 1=Left
Treatment:	0=Control 1=Geristore 2=MTA 3=IRM
Score:	1=PDL healing (with none or mild inflammation) / 2= PDL failing (with moderate or severe inflammation)
N/A:	Teeth excluded from study: Technical error (Surgical or Histological)

Table 6: p-value of tests of association for DOG, TOOTH, and RORL by response

Test	Score	
	Fisher	Mantel-Haenszel
DOG response	0.7873	0.7715
TOOTH response	0.1404	0.1357
RORL response	1	0.8803

Table 7: p-value of tests of association for the TREATMENT and the response.
0=Control, 1= Geristrore, 2=MTA, 3=IRM

Test	SCORE	
Treatment	Fisher	Mantel-Haenszel
0,1,2,3	**0.0354**	**0.0330**
0,1,2	0.1107	0.0784
0,1,3	**0.0335**	**0.0314**
0,2,3	0.1093	0.0783
1,2,3	**0.0434**	**0.0382**
0,1	1	0.7083
0,2	0.1789	0.0812
0,3	0.0836	**0.0392**
1,2	0.0698	**0.0427**
1,3	**0.0300**	**0.0193**
2,3	1	0.7978

Table 8: p-value for Cochran-Mantel-Haenszel tests of association for the TREATMENT and the response controlling for TOOTH.
0=Control, 1= Geristrore, 2=MTA, 3=IRM

Test Treatment	SCORE
0,1,2,3	**0.0470**
0,1,2	0.1027
0,1,3	**0.0368**
0,2,3	0.4262
1,2,3	**0.0206**
0,1	0.3173
0,2	0.9404
0,3	0.0953
1,2	**0.0078**
1,3	**0.0375**
2,3	0.7653

FIGURES

EXPERIMENTAL MATERIALS

Figure 1: IRM™ (L.D. Caulk Inc., Dentsply International Inc., Milford, DE)

Figure 2: Geristore® (Den-Mat, Santa Maria, CA)

Figure 3: ProRoot MTA™ (Dentsply Tulsa Dental Specialties, Tulsa, OK)

PRESURGICAL PHASE

Figure 4: Occlusal reduction of the lower premolar

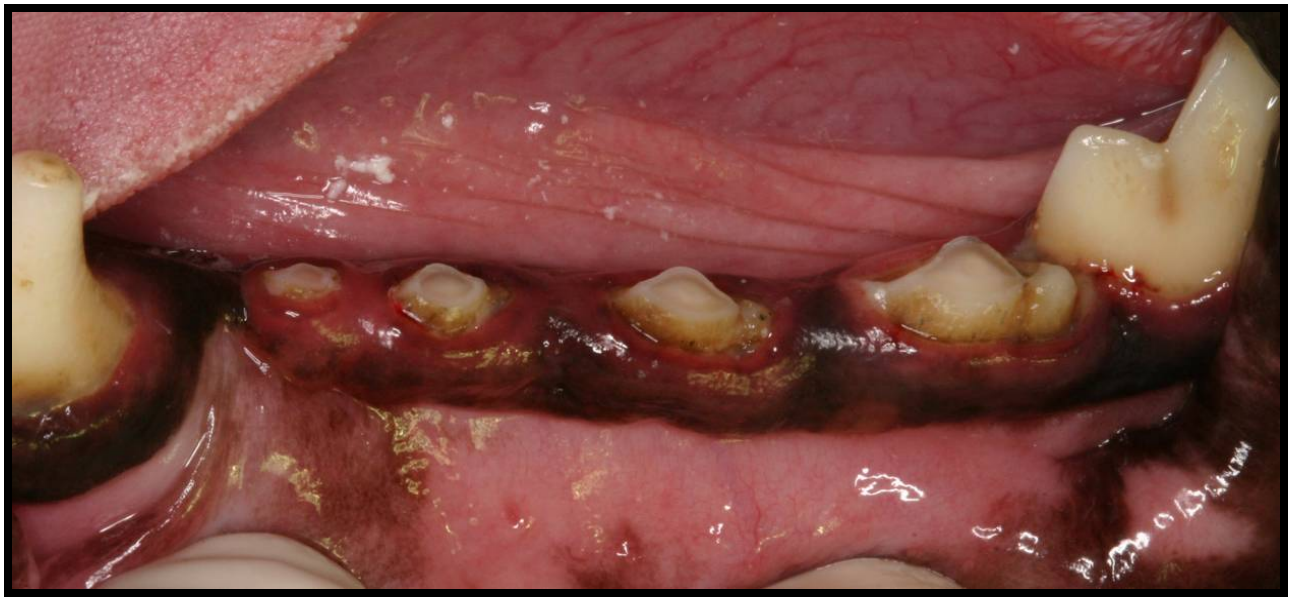


Figure 5: Access

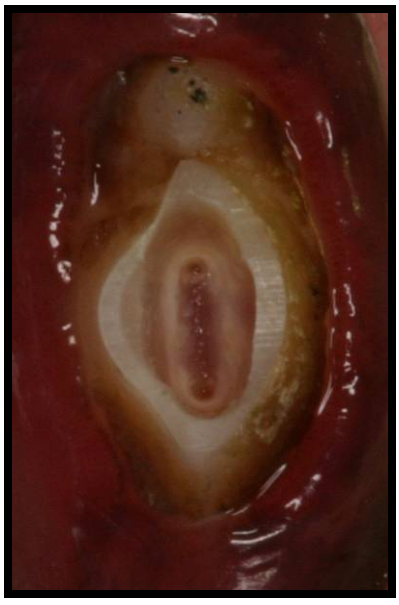


Figure 6: Endodontic NiTi rotary instrumentation

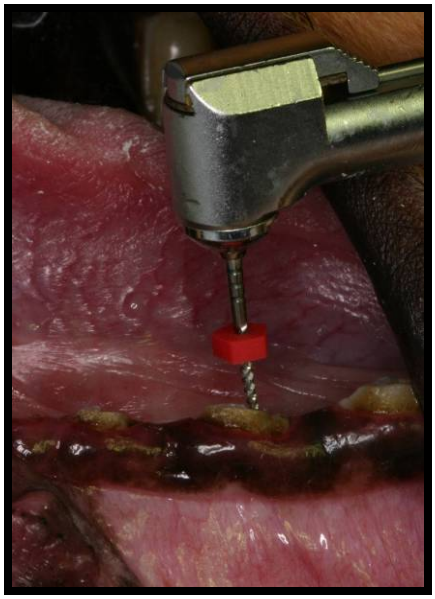


Figure 7: Lentulo spiral introducing the plaque that was mixed with saline

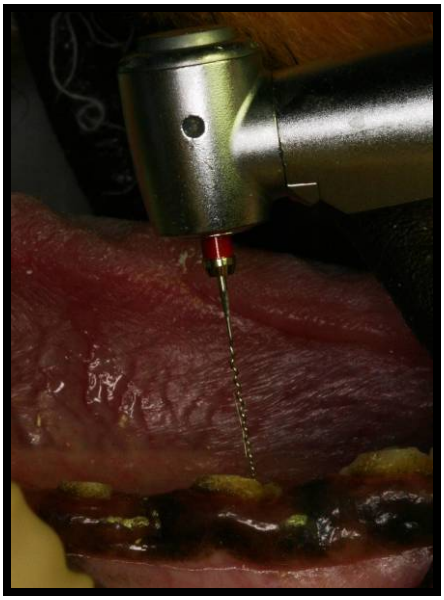


Figure 8: Access closed with IRM

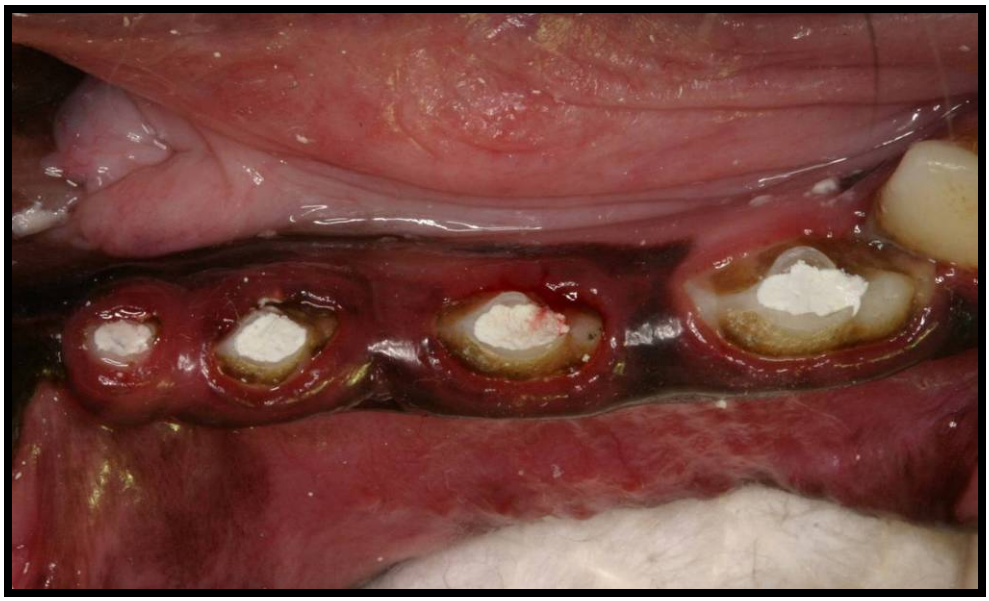


Figure 9: The canals were dried with paper points

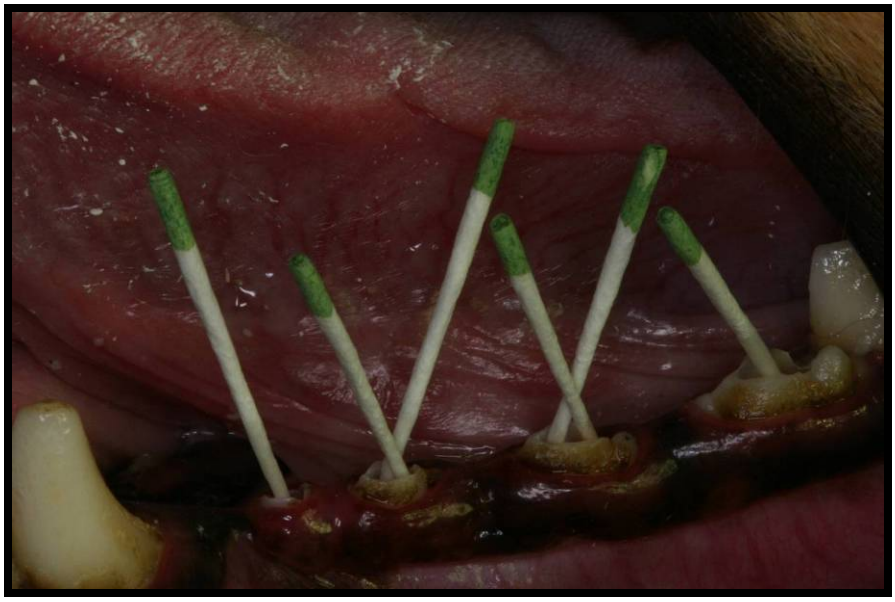


Figure 10: Gutta-Percha point inserted into the canals

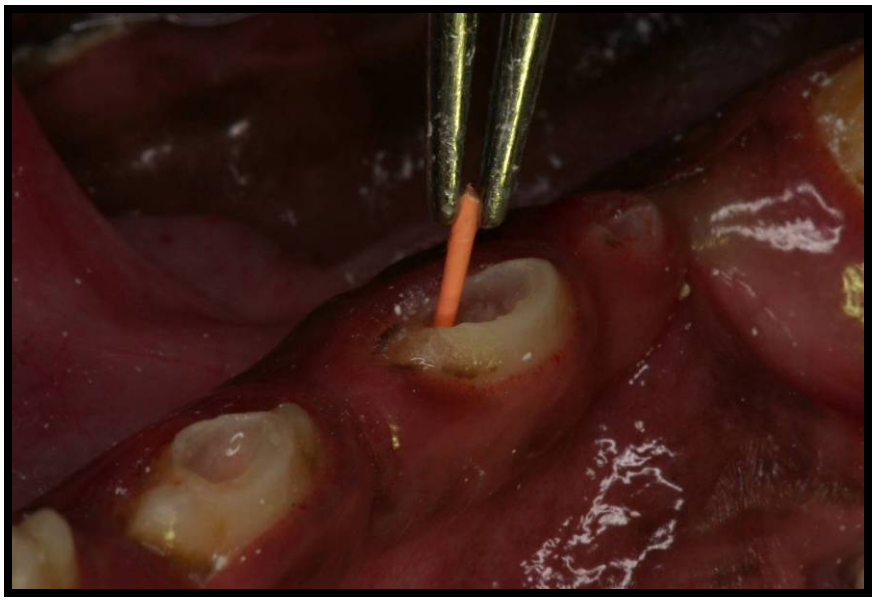


Figure 11: Gutta-Percha points seared into the canals

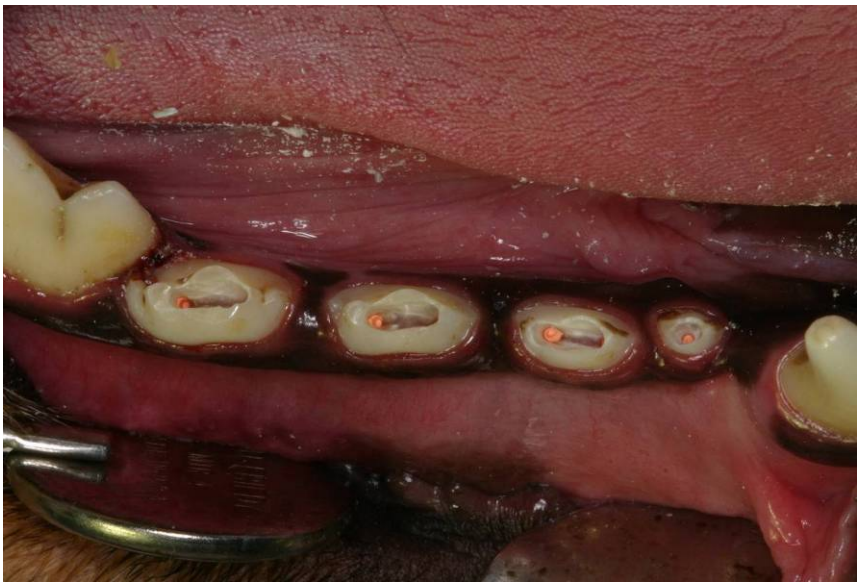
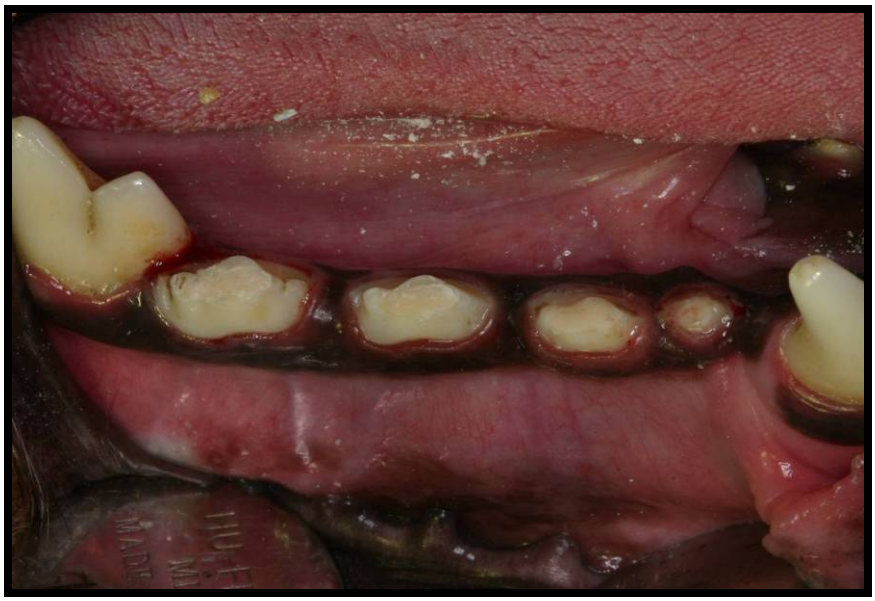


Figure 12: Access closed with glass-ionomer



SURGICAL PHASE

Figure 13: Microscope surgical setting

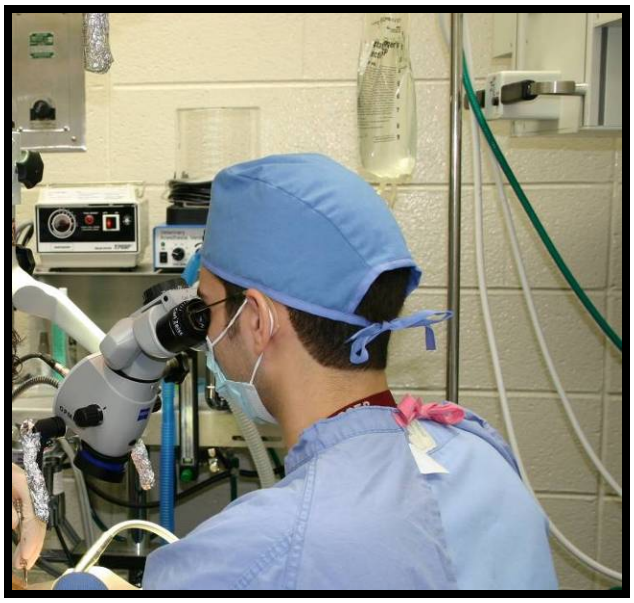


Figure 14: Surgical field (view 1)

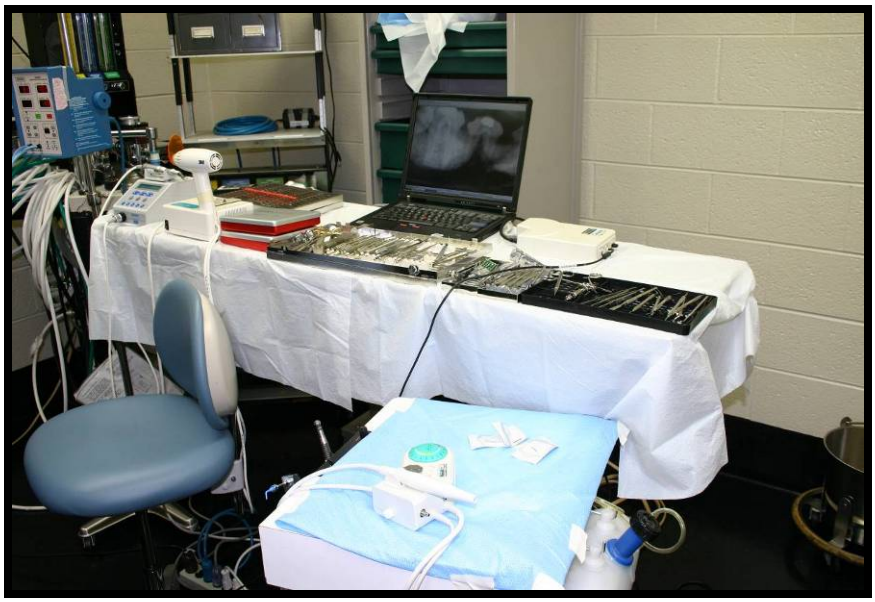


Figure 15: Surgical Field (view 2)

Figure 16: Primary incision

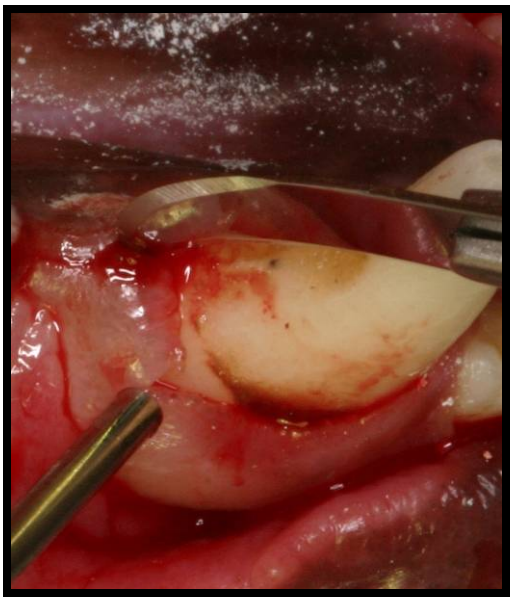


Figure 17: Full thickness flap elevation

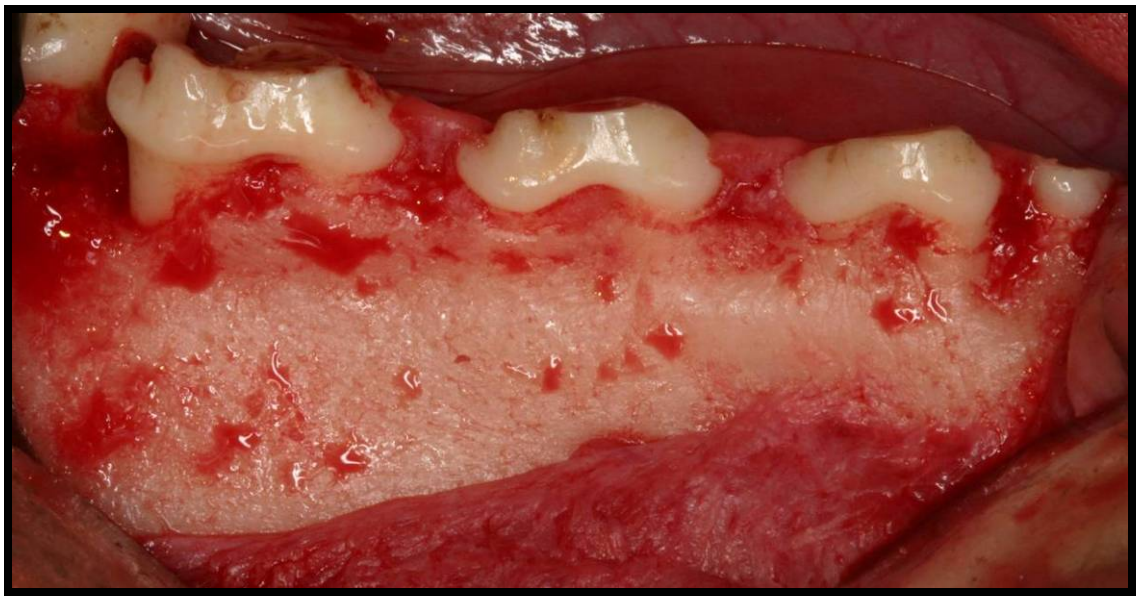


Figure 18: Start of the bony window

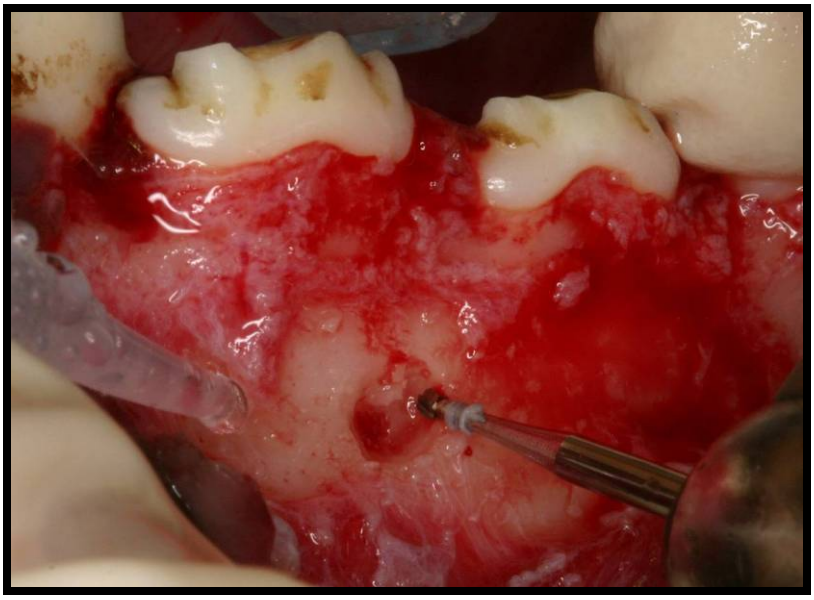


Figure 19: Bony windows

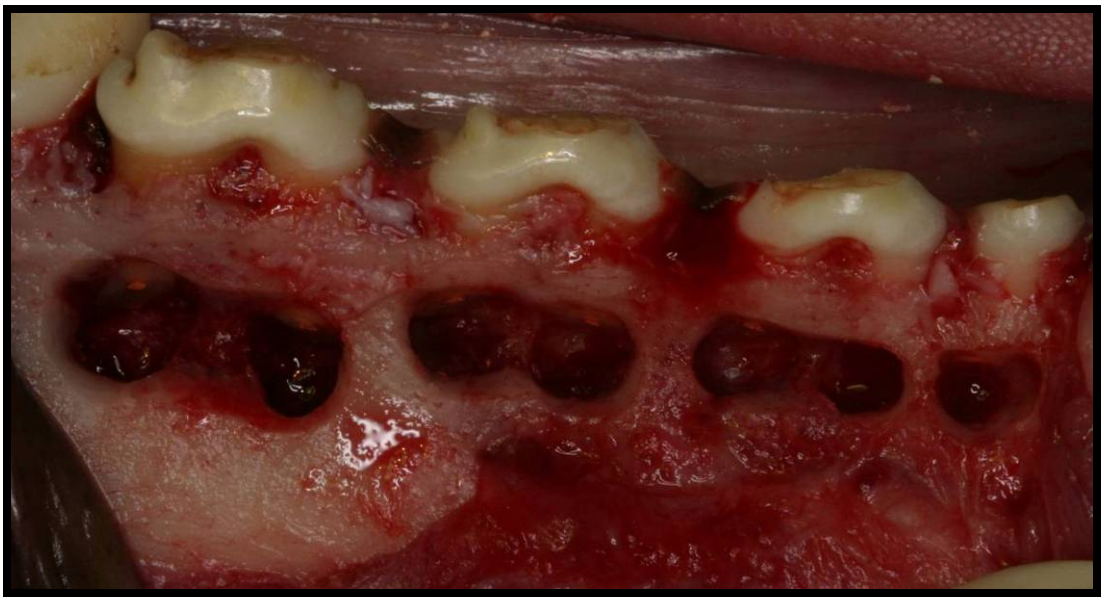


Figure 20: Root end resection and ultrasonic preparation

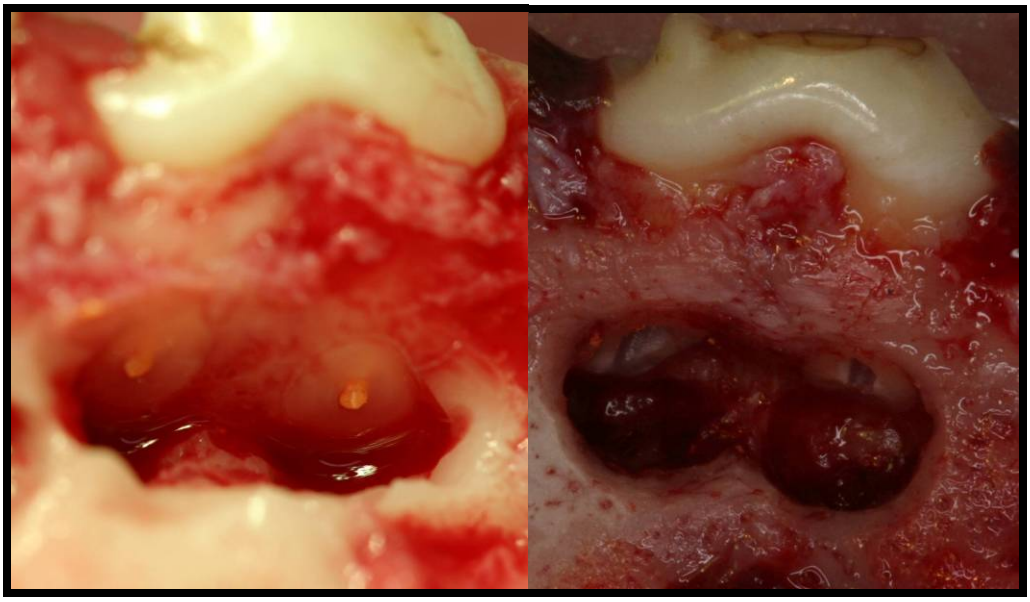


Figure 21: IRM retrofill

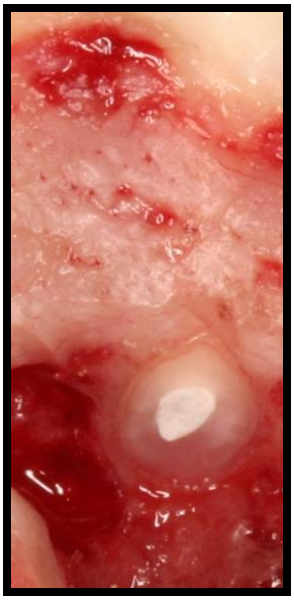


Figure 22: Geristore prep and Geristore retrofilling in place

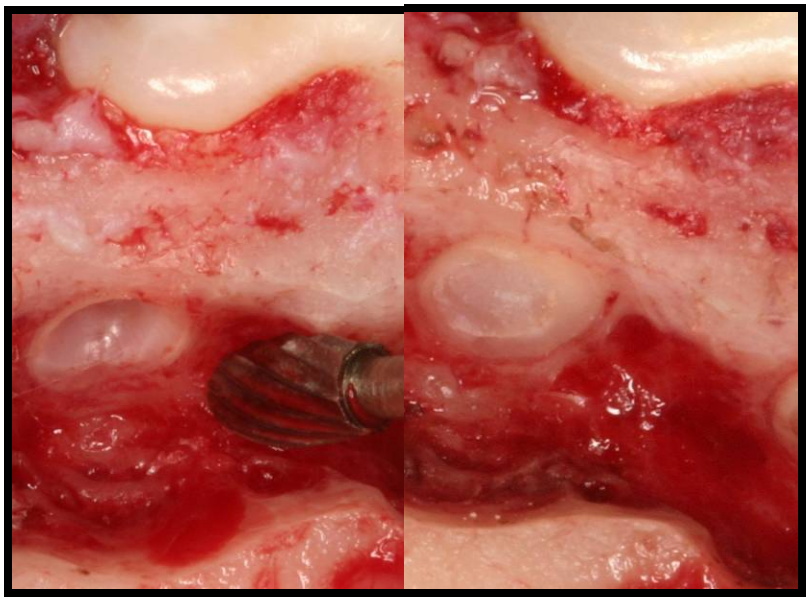


Figure 23: MTA retrofill

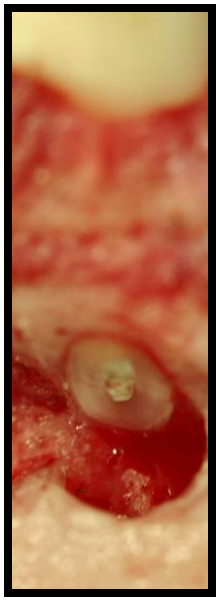
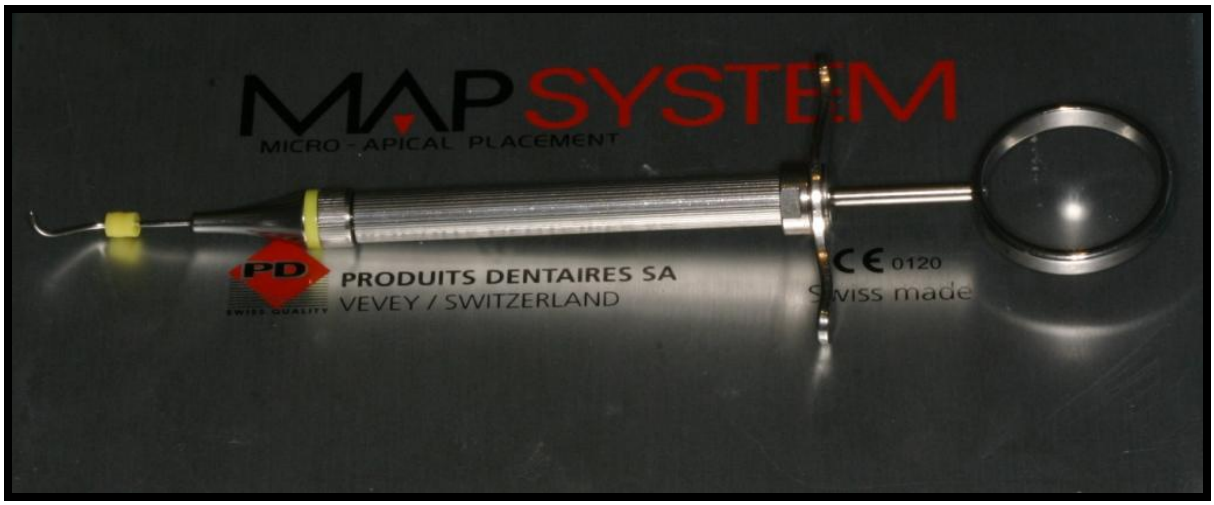


Figure 24: MAP System



2 MONTHS FOLLOW UP AND CLEANING SESSION

Figure 25: Favorable post-surgical healing with healthy gingival tissue 1

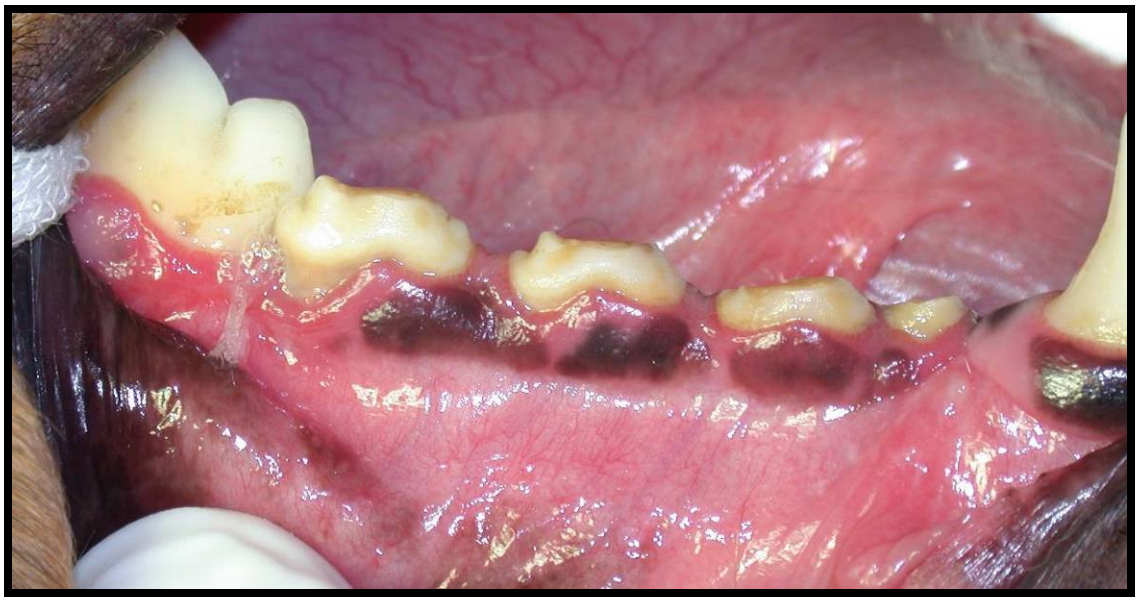


Figure 26: Favorable post-surgical healing with healthy gingival tissue 2

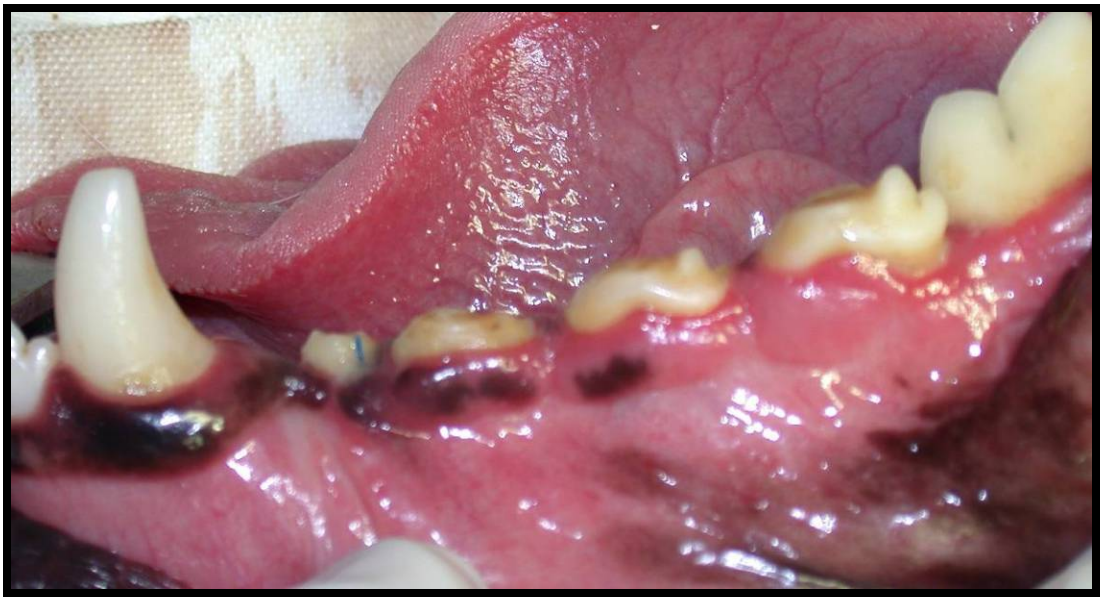


Figure 27: Post-surgical secondary healing 1

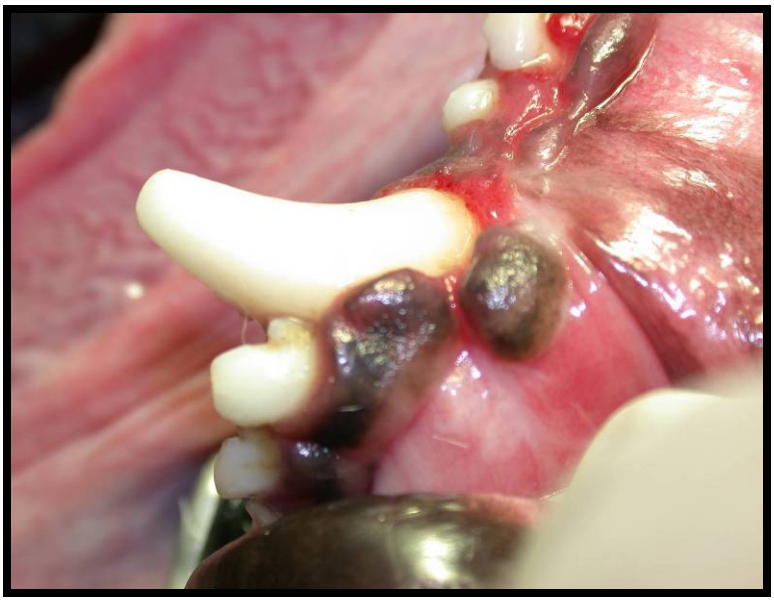


Figure 28: Post-surgical secondary healing 2

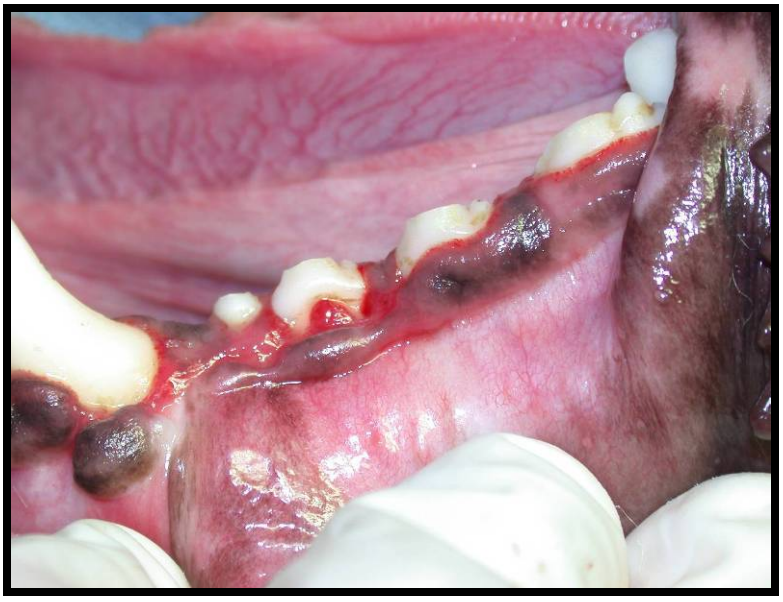


Figure 29: Post-surgical periodontal defects before scaling and prophylaxis 1

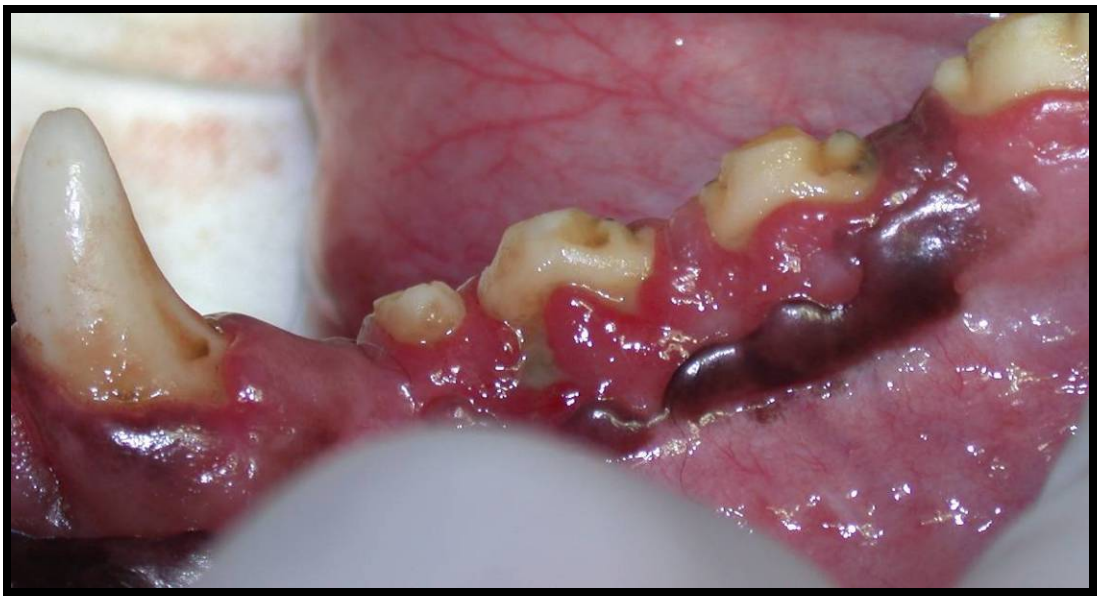


Figure 30: Post-surgical periodontal defects after scaling and prophylaxis 1

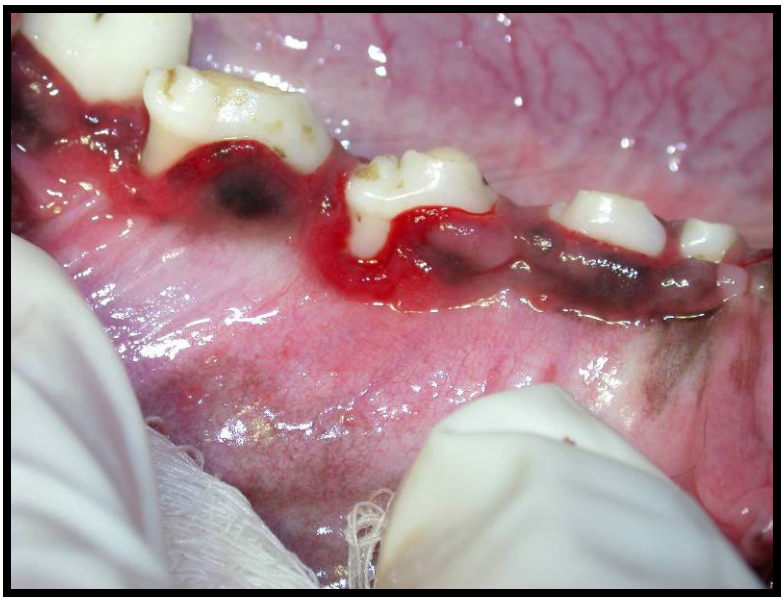


Figure 31: Post-surgical periodontal defects before scaling and prophylaxis 2

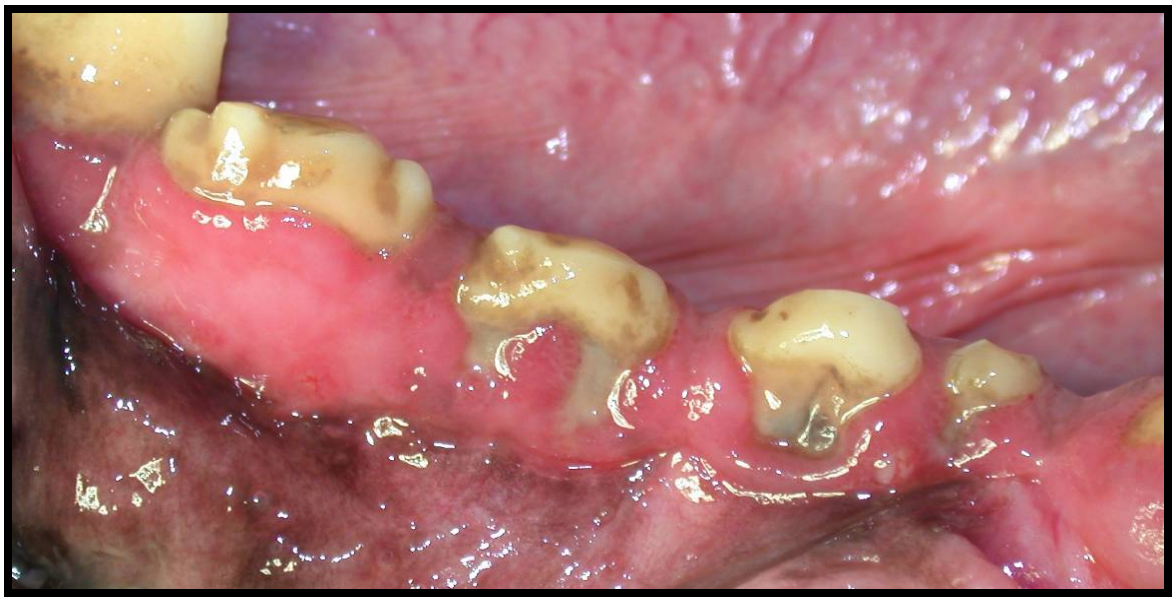
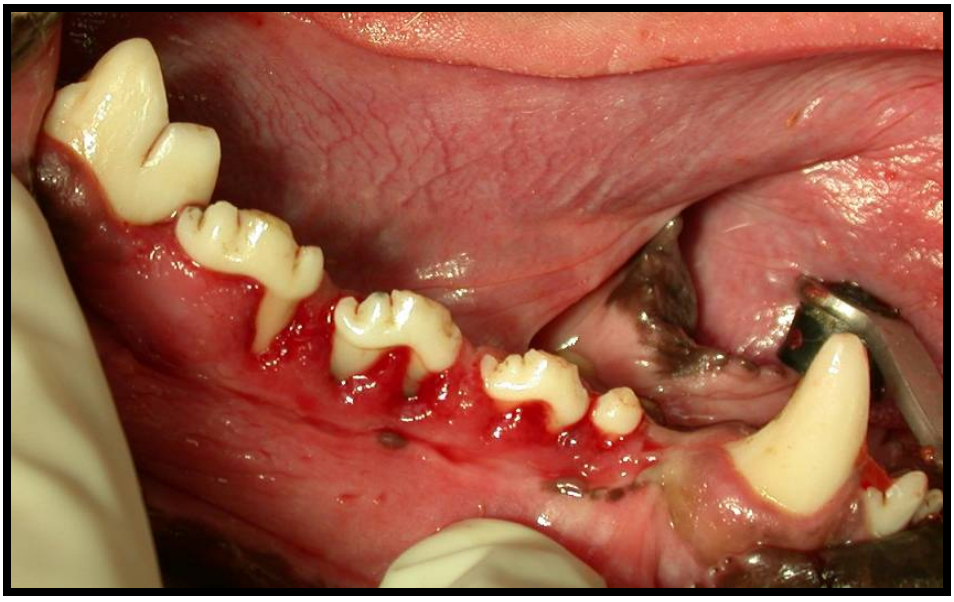


Figure 32: Post-surgical periodontal defects after scaling and prophylaxis 2



RADIOGRAPHIC IMAGES

Figure 33: Pre-op radiographs showing the lower right mandibular pre-molars

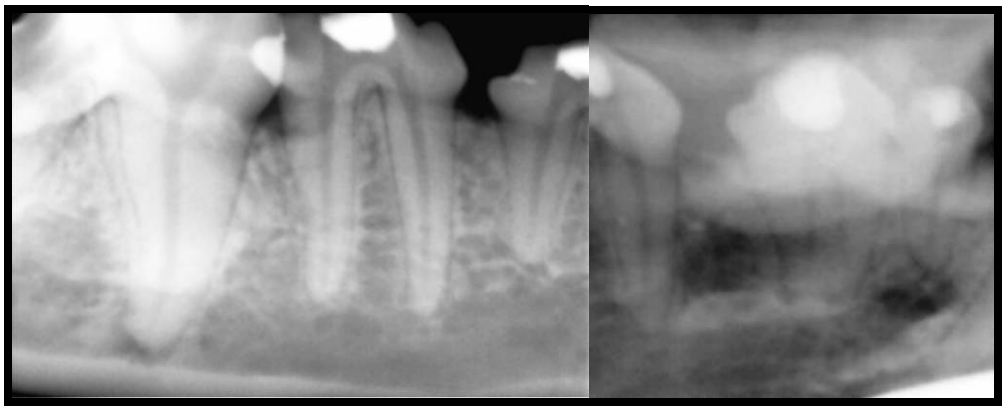


Figure 34: Example of radiographic series 1

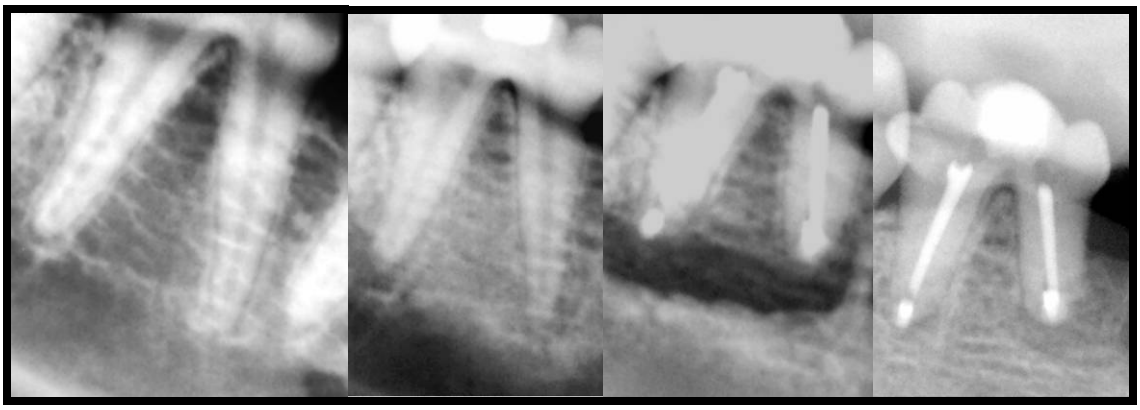


Figure 35: Example of radiographic series 2

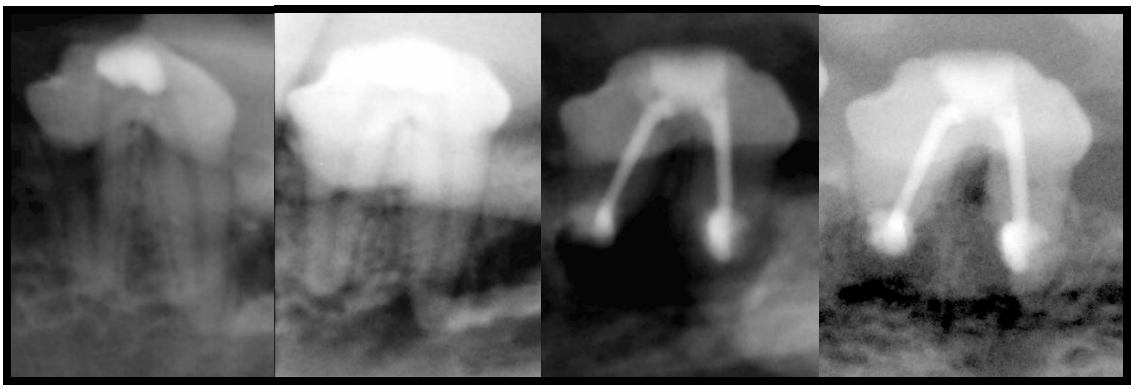


Figure 36: “Control tooth” radiographs post-surgical vs. 6 month follow-up

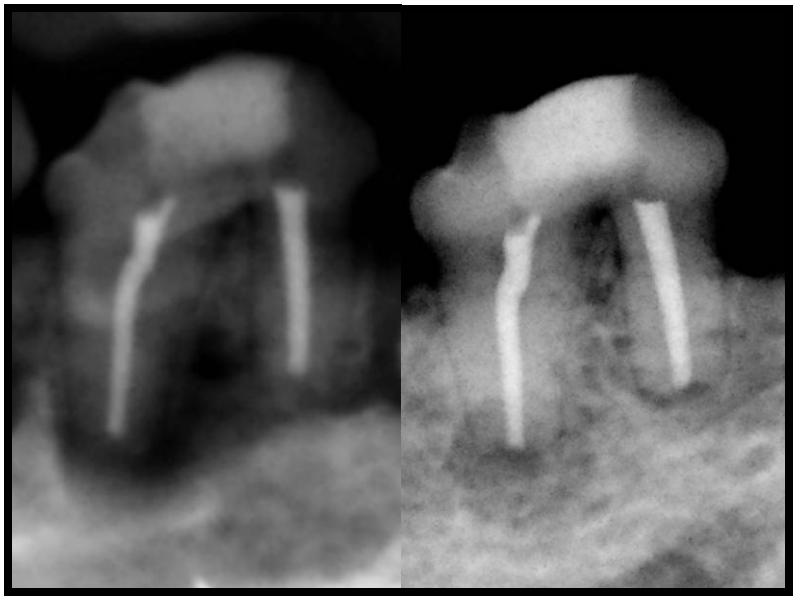


Figure 37: “Geristore tooth” radiographs post-surgical vs. 6 month follow-up 1

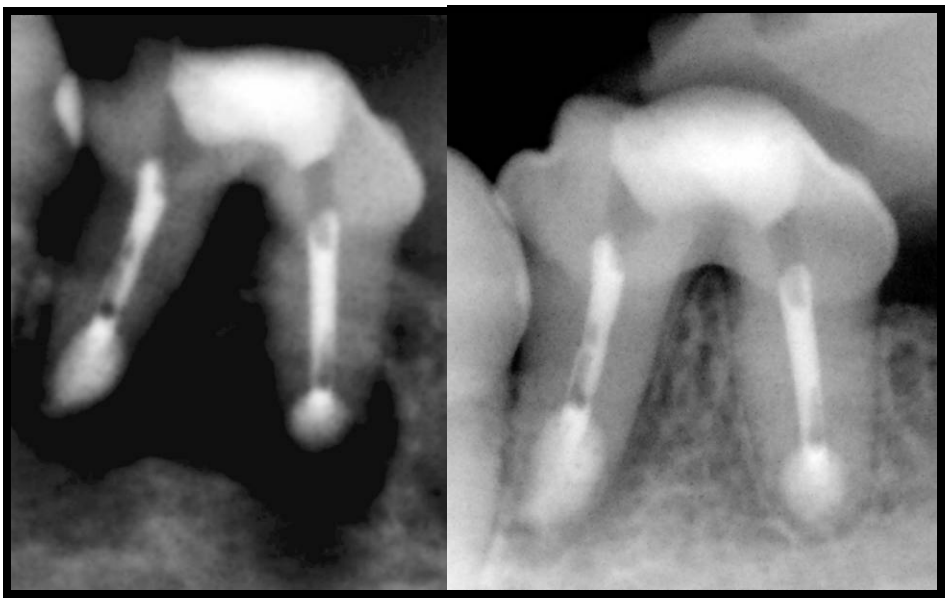


Figure 38: “Geristore tooth” radiographs post-surgical vs. 6 month follow-up 2

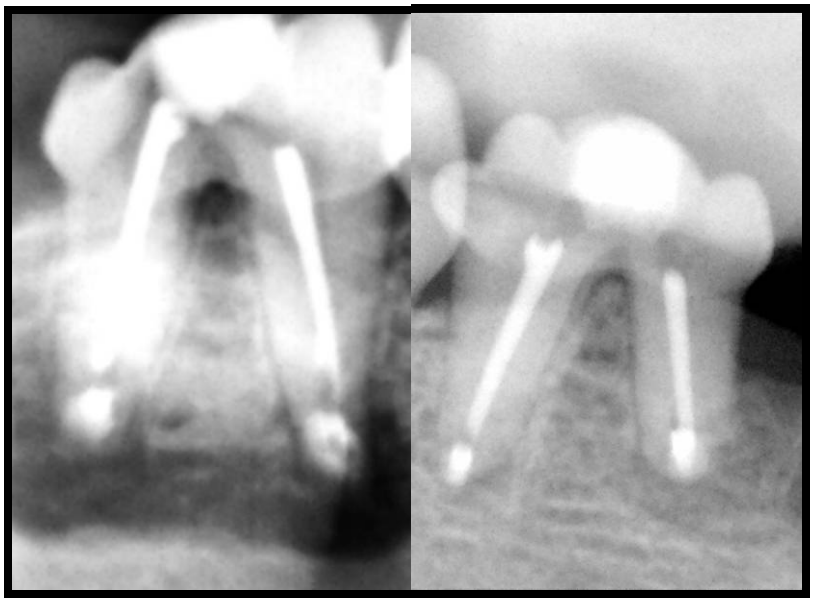


Figure 39: “Geristore tooth” radiographs post-surgical vs. 6 month follow-up 3

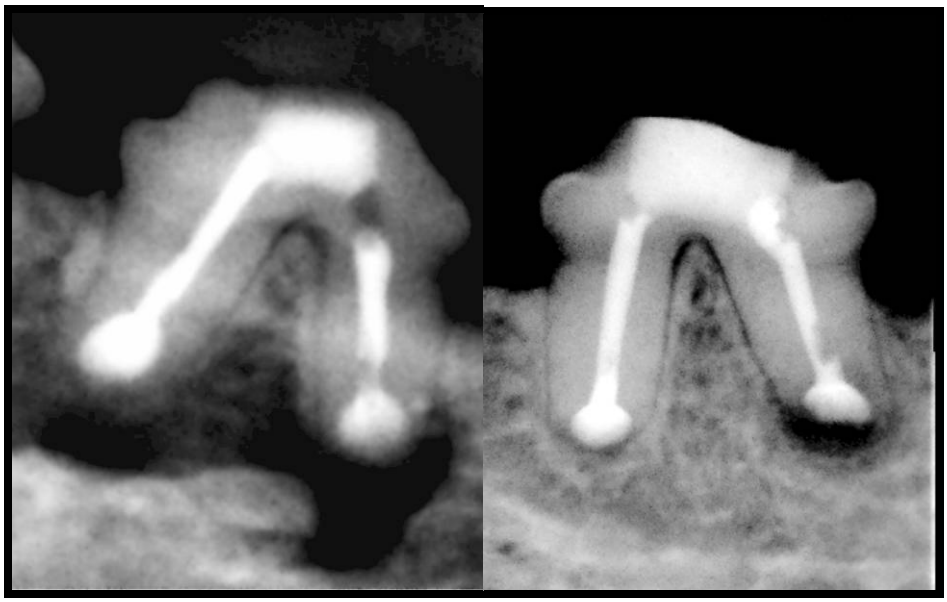


Figure 40: “IRM tooth” radiographs post-surgical vs. 6 month follow-up 1

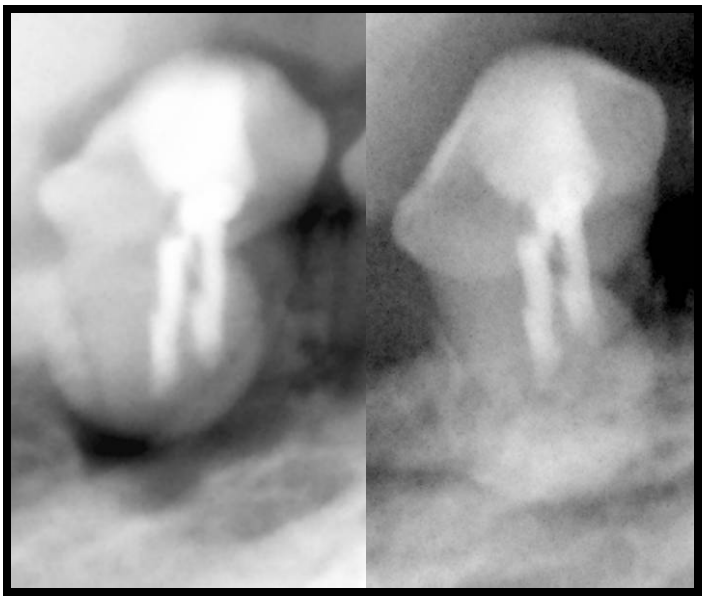


Figure 41: “IRM tooth” radiographs post-surgical vs. 6 month follow-up 2

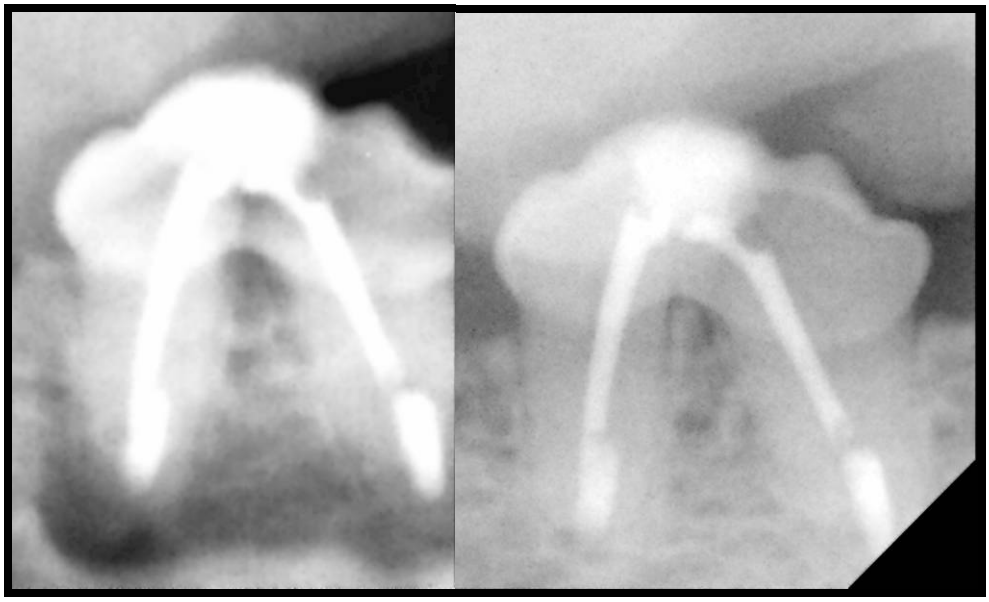


Figure 42: “IRM tooth” radiographs post-surgical vs. 6 month follow-up 3

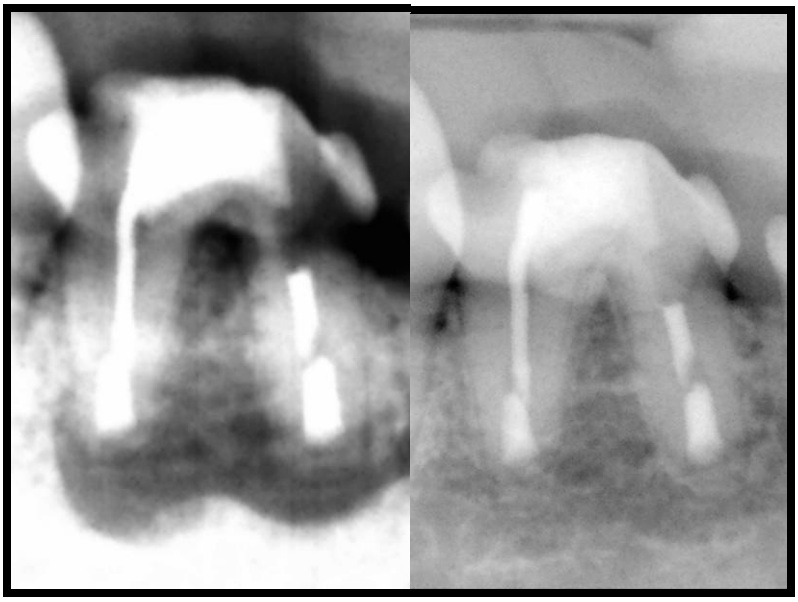


Figure 43: “IRM tooth” radiographs post-surgical vs. 6 month follow-up 4

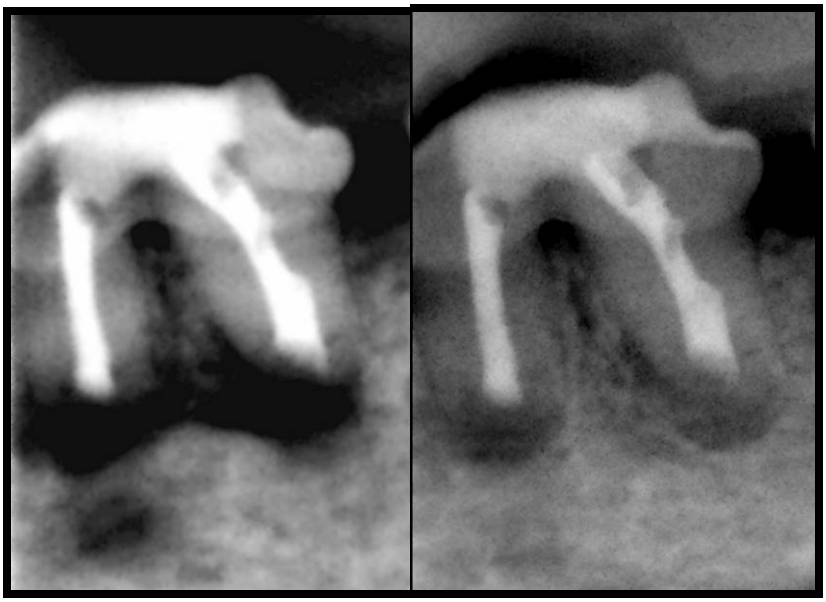


Figure 44: “MTA tooth” radiographs post-surgical vs. 6 month follow-up 1

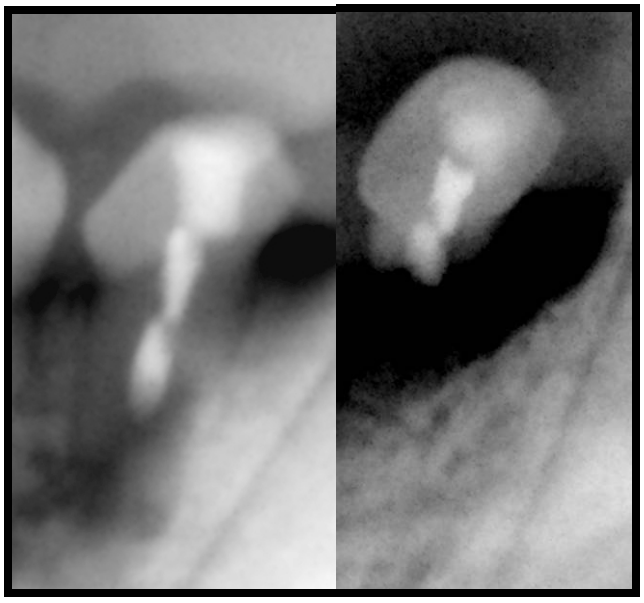


Figure 45: “MTA tooth” radiographs post-surgical vs. 6 month follow-up 2

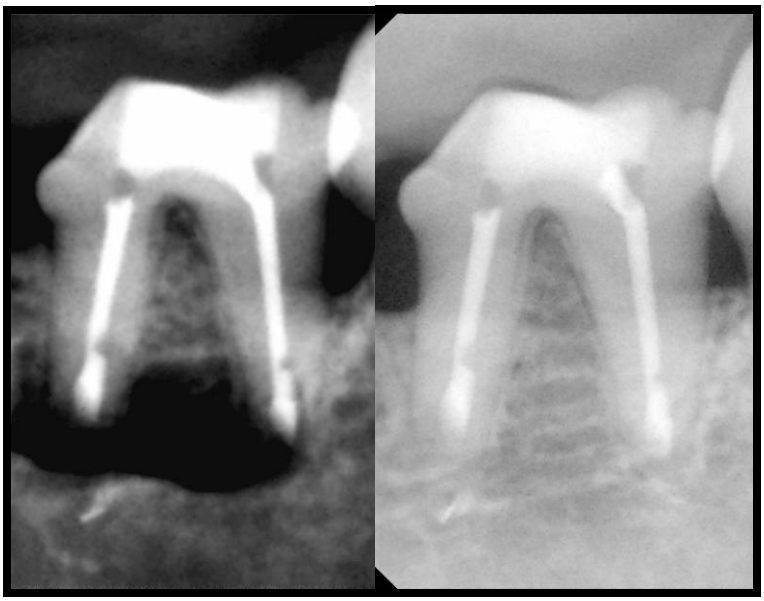
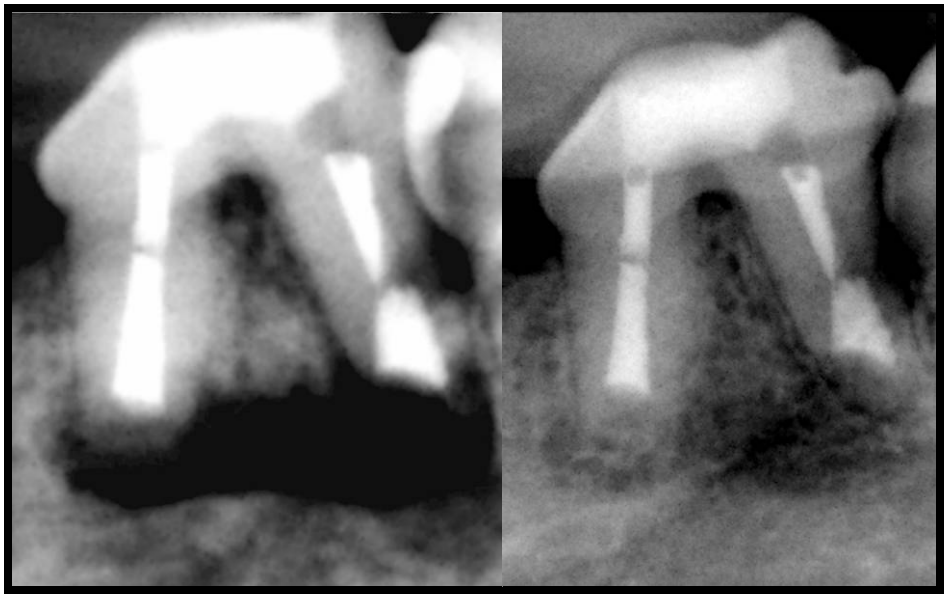


Figure 46: “MTA tooth” radiographs post-surgical vs. 6 month follow-up 3



HISTOLOGY PREPARATION

Acid Decal confirmation

Figure 47: 2 weeks in EDTA

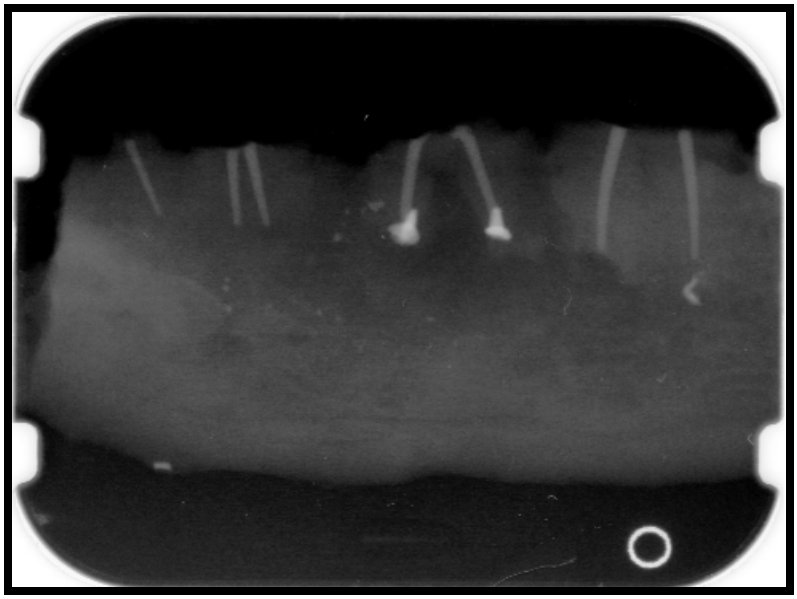
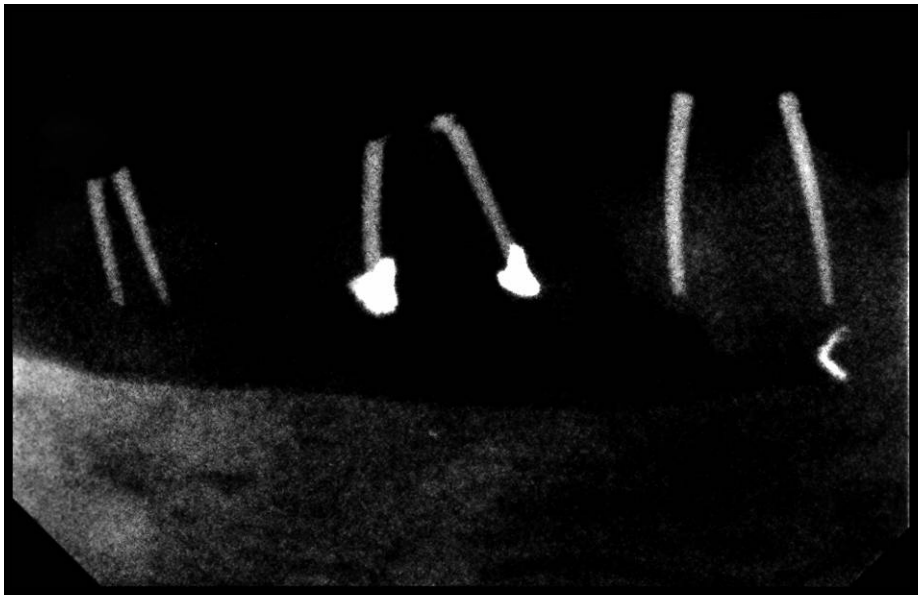


Figure 48: 3 weeks in EDTA



HISTOLOGY SLIDES

Figure 49: 2X Positive Control 1

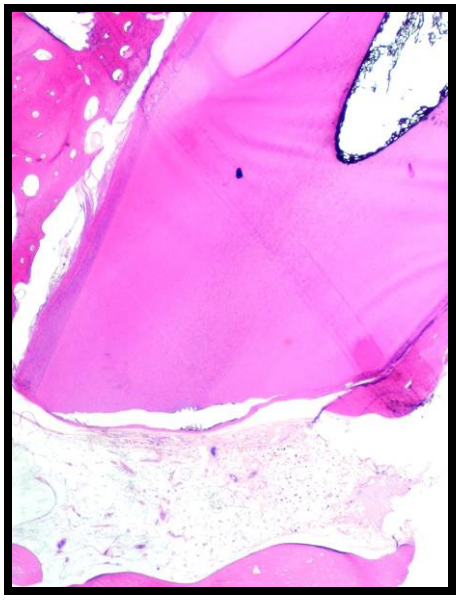


Figure 50: 2X Positive Control 2

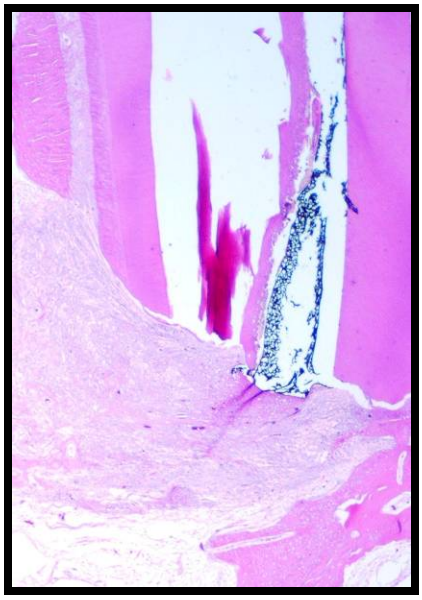


Figure 51 and 52: 10X and 20X showing inflammation cells in positive control group

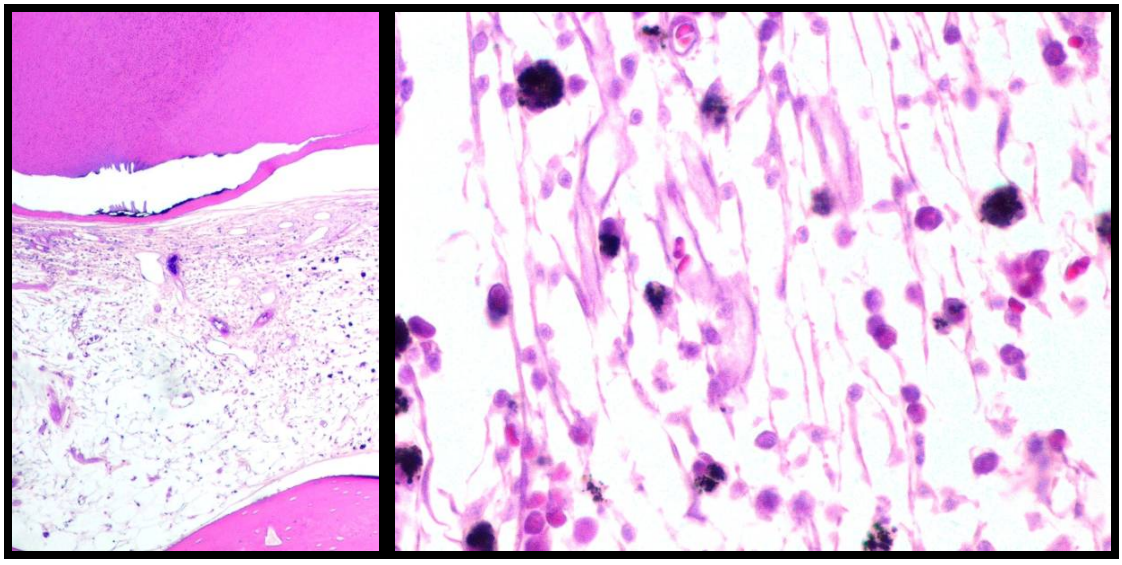


Figure 53: 2X Geristore

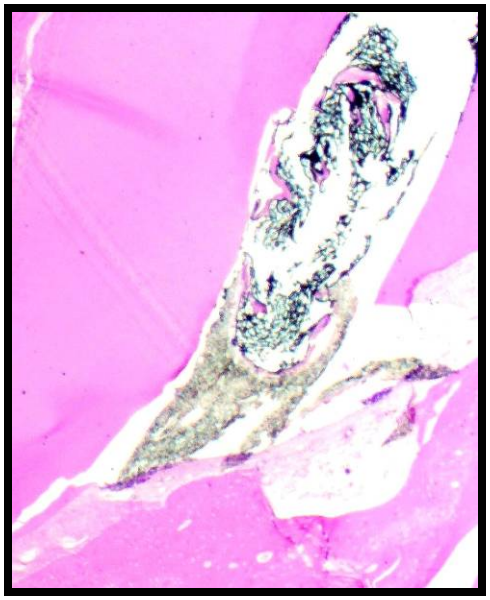


Figure 54: 4X Geristore

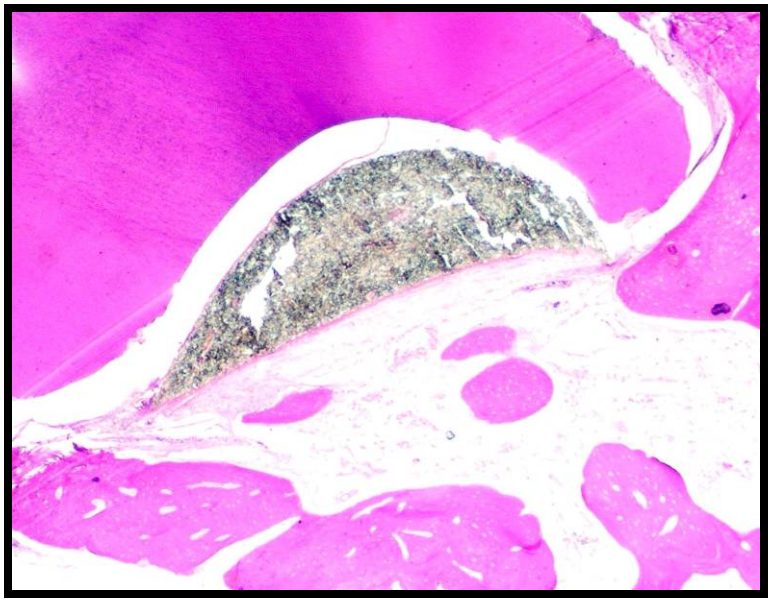


Figure 55: 2X MTA 1

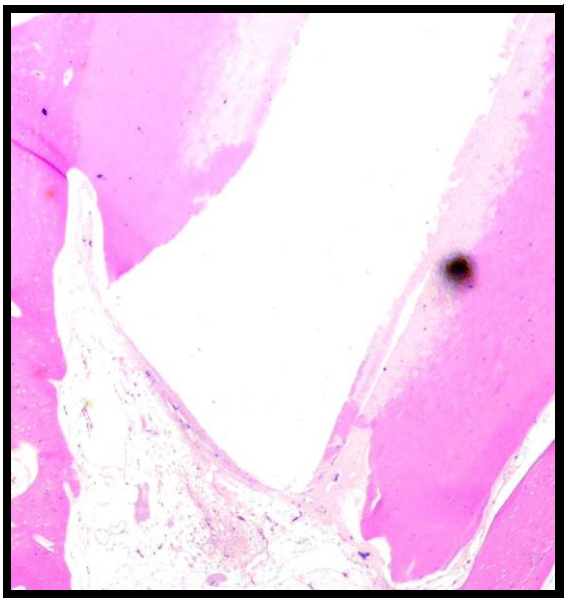


Figure 56: 2X MTA 2

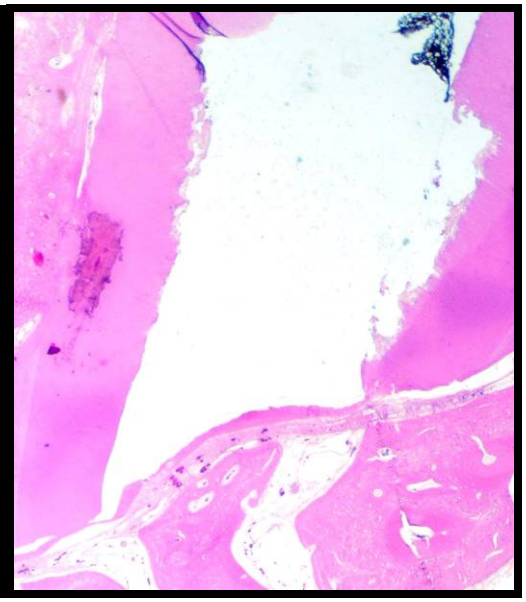


Figure 57: 4X IRM

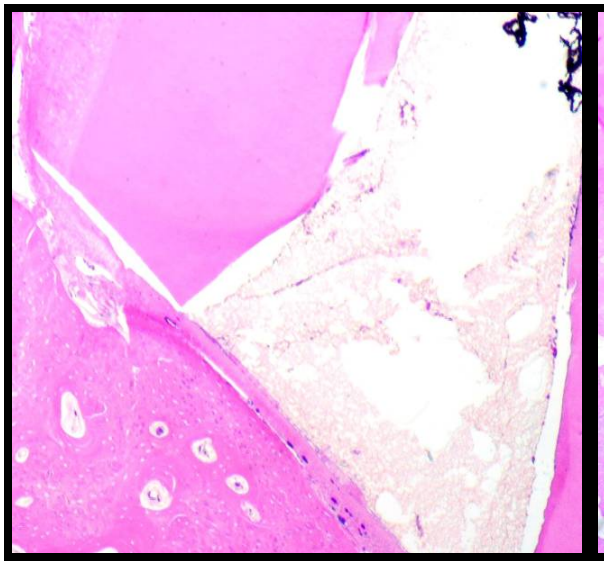


Figure 58: 2X IRM

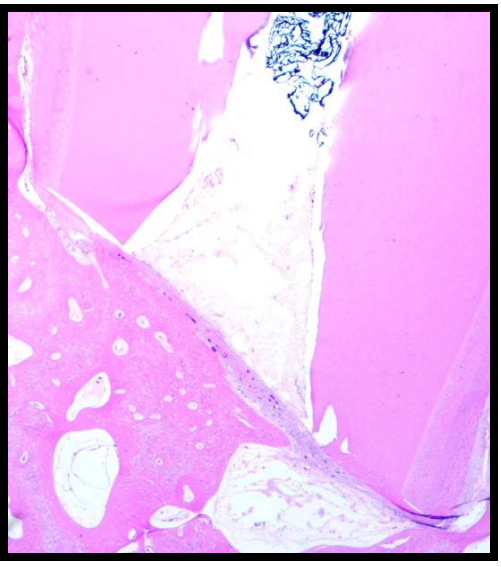


Figure 59: 10X showing tissue separation during histology preparation 1

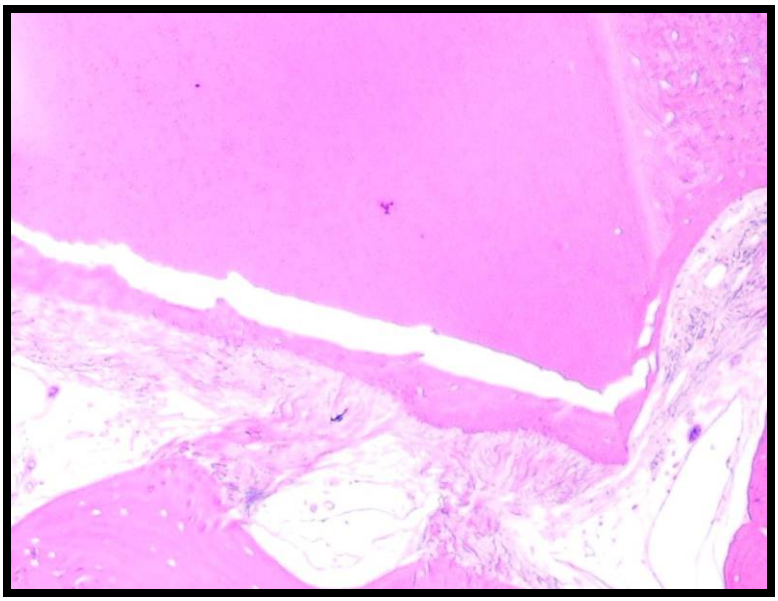


Figure 60: 10X showing tissue separation during histology preparation 2

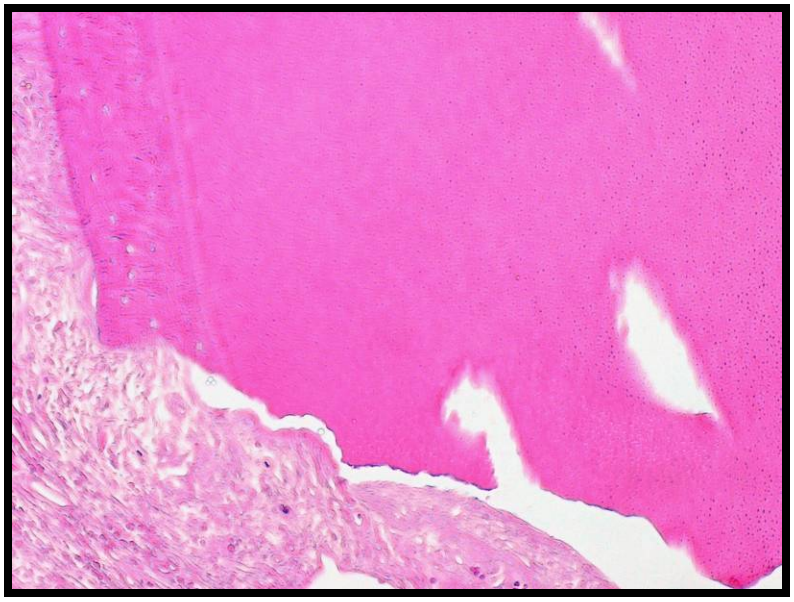


Figure 61 2 X epithelium lining 1

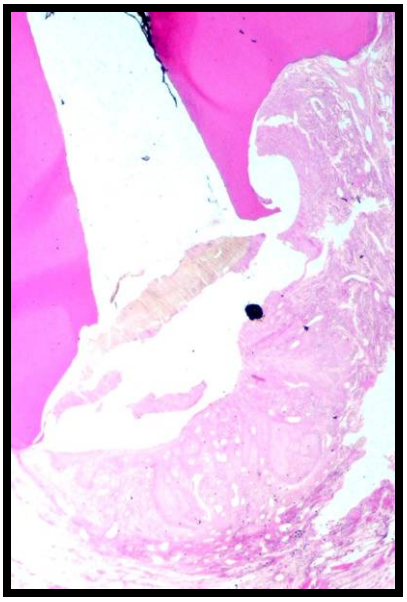
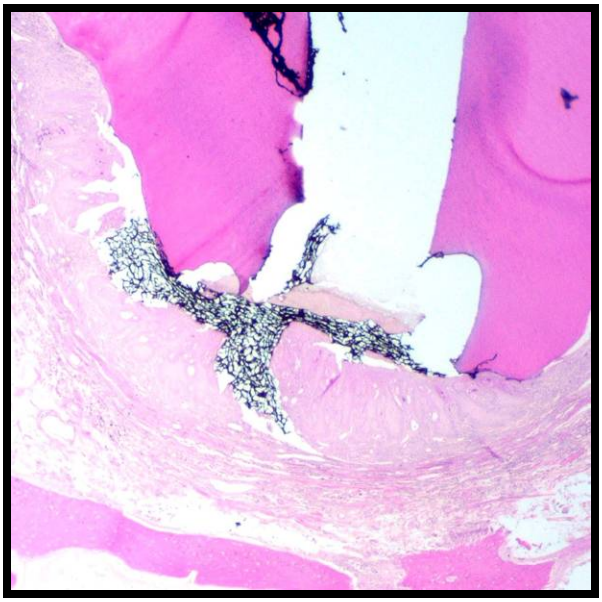


Figure 62: 2X epithelium lining 2



HISTOGRAMS

Figure 63: Number of Teeth after randomization (Geristore, MTA, IRM and Control)

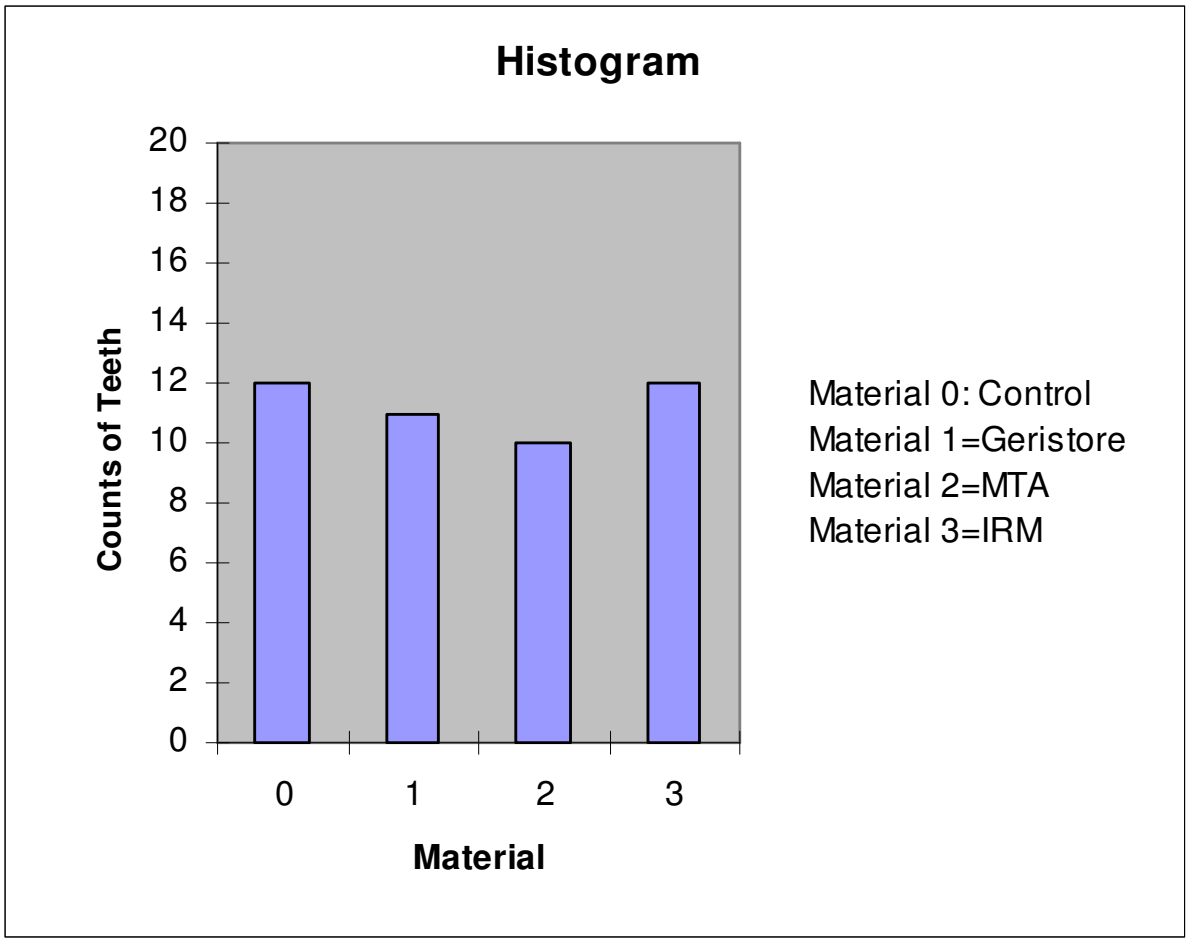


Figure 64:

Radiographic Score Counts of Teeth vs. Treatment (Geristore, MTA, IRM and Control)

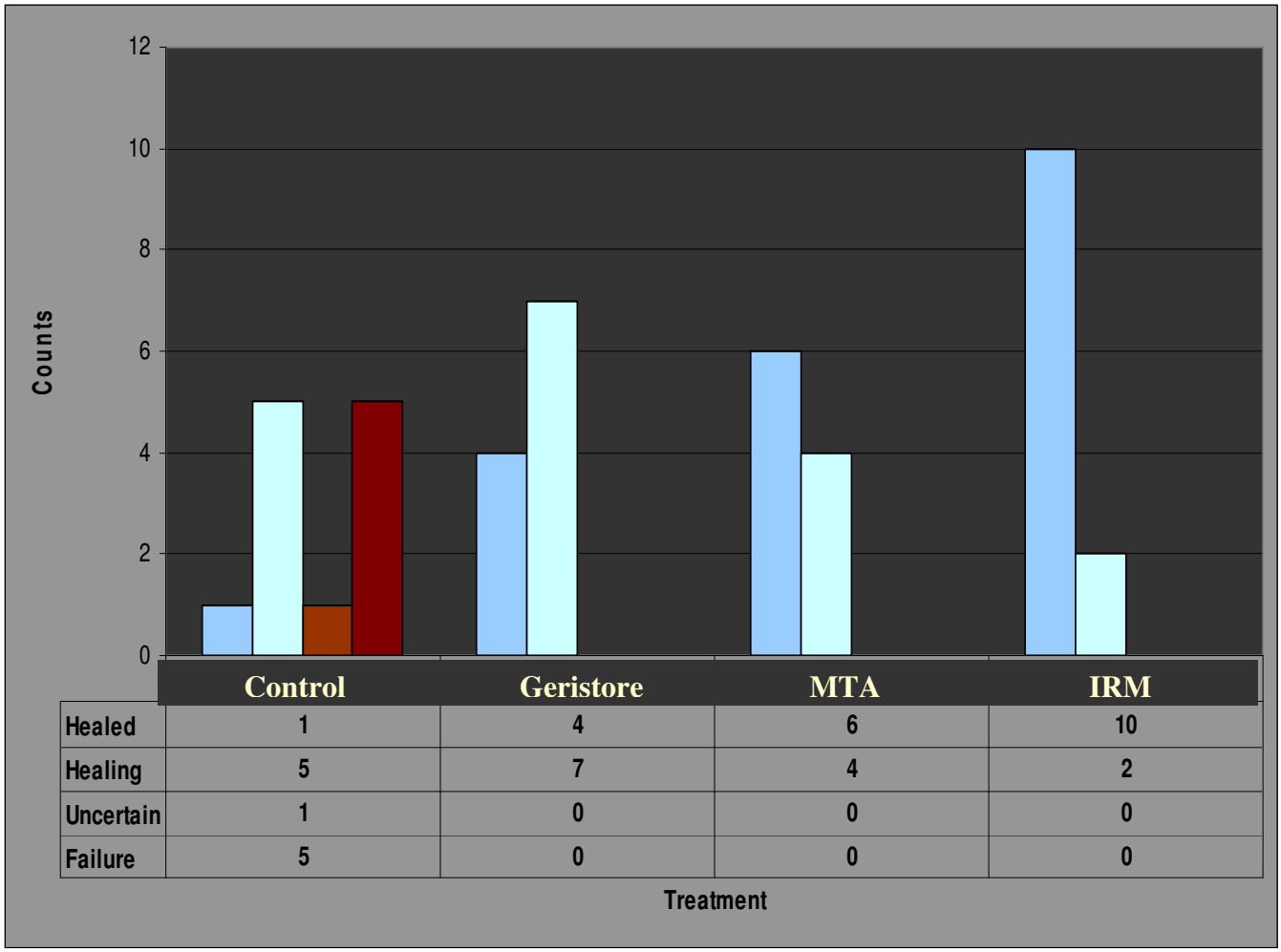
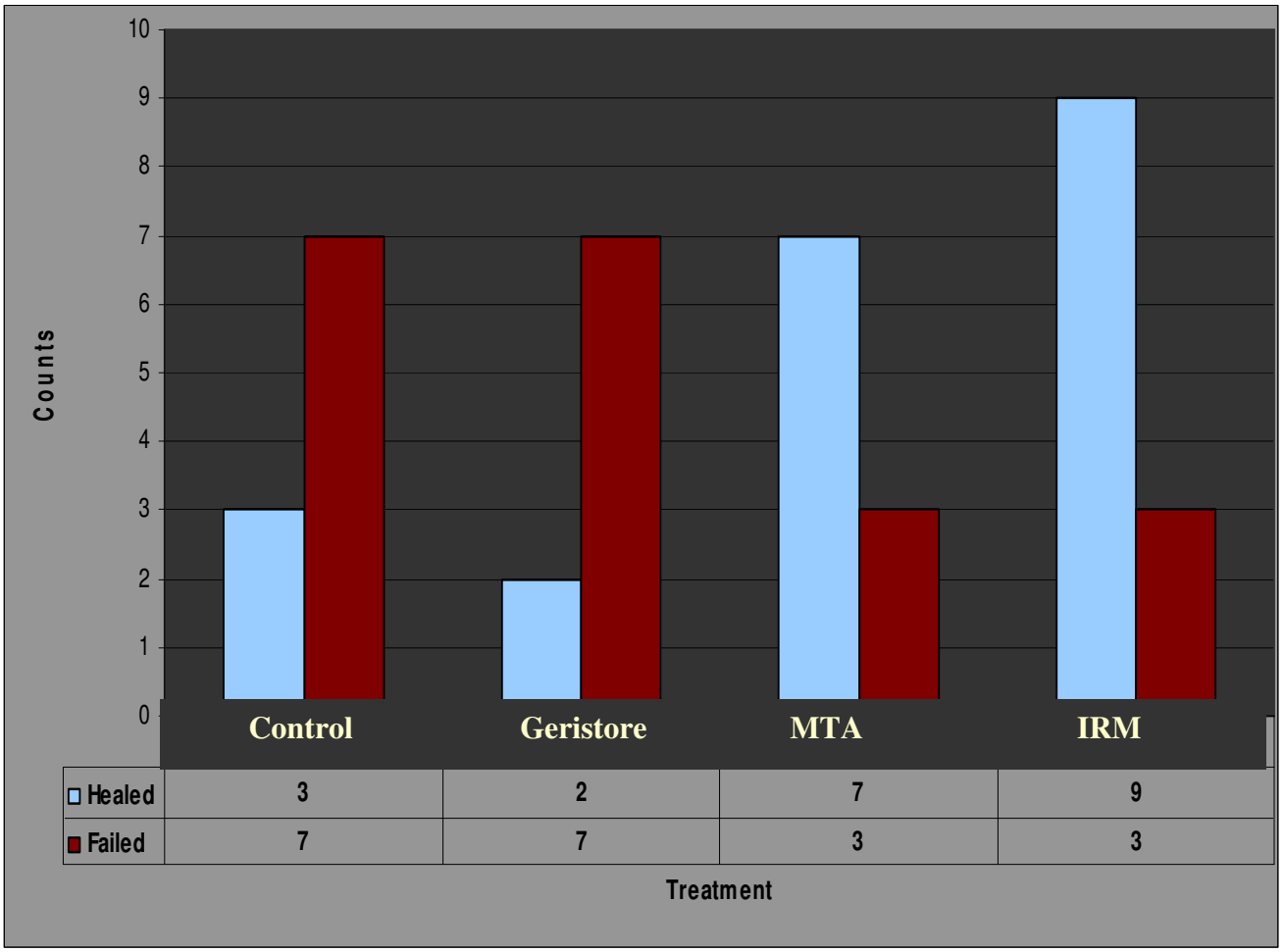


Figure 65:

Histological Score Counts of teeth vs. Treatment (Geristore, MTA, IRM and Control)



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