

AN *IN-VITRO* COMPARISON OF MICROLEAKAGE WITH *E. FAECALIS* IN TEETH  
WITH ROOT-END FILLINGS OF PROROOT MTA AND BRASSELER'S  
ENDOSEQUENCE ROOT REPAIR PUTTY

by

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## INTRODUCTION

When non-surgical root canal therapy fails to allow for healing of the periapical tissues, or restorative materials prevent orthograde endodontic retreatment, periradicular surgery may be employed to save a tooth that might otherwise have required extraction.

The goal of periradicular surgery is to “remove the causes of disease and to provide a favorable environment for healing of the surgical wound.”<sup>1</sup> Advances in root-end filling materials along with improved surgical technique and armamentarium have allowed this goal to be accomplished more completely with enhanced outcomes for periradicular surgery. Patients are then saved the time, the expense, the trauma, and the psychological burden that might have been incurred had a surgical approach not been used.

One critical component of modern endodontic surgery is to seal the canal with a root-end filling material. The root-end filling material provides a physical seal after root-end resection that can prevent the passage of microorganisms to the periodontium and allows for the re-establishing of the attachment apparatus. The qualities of the ideal root-end filling material have been described by Gartner and Dorn,<sup>7</sup> Kim et al.<sup>8</sup> and Chong<sup>9</sup> as the following: 1) Adheres or bonds to tooth tissue and seals the root end three-dimensionally; 2) Inhibits the growth of pathogenic microorganisms; 3) Is dimensionally stable and unaffected by moisture in either the set or unset state; 4) Is well-tolerated by periradicular tissues with no inflammatory reactions; 5) Stimulates the regeneration of normal periodontium; 6) Is nontoxic both locally and systemically; 7) Is not corrosive

or electrochemically active; 8) Does not stain the tooth or the periradicular tissues; 9) Is easily distinguishable on radiographs; 10) Has a long shelf life and is easy to handle.

Mineral trioxide aggregate (MTA) has been thoroughly investigated in a variety of clinical endodontic applications. No dental material previously available to endodontists has demonstrated such a desirable combination of biocompatibility, hydrophilicity, sealability, strength, and antibacterial action. MTA clinical applications include direct pulp capping, apexogenesis, apexification, regenerative endodontics, root perforation repair, and surgical root-end filling.<sup>4,5</sup> The clinical success of MTA in these applications is well-studied, but many authors describe the poor handling characteristics of MTA and the resulting technique sensitivity of its application as the major disadvantage of this outstanding material.

EndoSequence Root Repair Material (ERRM) (Brasseler; Savannah GA) is stated by the manufacturer to bond to adjacent dentin, to have no shrinkage, and to be highly biocompatible, hydrophilic, radiopaque, and antibacterial due to a high pH during setting. Brasseler's ERRM comes premixed from the manufacturer in a jar as putty and in preloaded syringes as a flowable paste and sets within 30 minutes. The major advantages of this material are improved handling characteristics over traditional MTA and the delivery of a consistent product with each application. The current research on ERRM is limited and warrants further investigation. ERRM is composed of calcium silicates, monobasic calcium phosphate, zirconium oxide, tantalum oxide, proprietary fillers, and thickening agents. Investigations on the sealing properties of this material have not yet been conducted.

## PURPOSE OF THE PRESENT STUDY

The present study seeks to compare the materials ProRoot MTA and Brasseler's ERRM by testing their ability to seal the root end three-dimensionally from bacterial leakage.

The study compares the microbial leakage of *Enterococcus faecalis* in teeth with root-end fillings by using ProRoot MTA and Brasseler's ERRM in a dual-chamber bacterial leakage model as described by Torabinejad and colleagues.

## HYPOTHESES

Null Hypothesis: There is no significant difference in microleakage between the ProRoot MTA group and the Brasseler ERRM group.

Alternative Hypothesis: There is a significant difference in microleakage between the ProRoot MTA group and the Brasseler ERRM group.



REVIEW OF LITERATURE

## HISTORY OF ENDODONTICS

As early as 2953 BC, Fu His is credited with one of the earliest descriptions of a toothache, complete with pain caused by cold and pain with mastication.<sup>2</sup> The Egyptian Eber's papyrus from 1550 BC contains several remedies "to strengthen the teeth," including a "mixture powder of the fruit of the dum-palm, green lead, and honey, to be mixed and the teeth rubbed with it," but oral surgical procedures were not mentioned.<sup>3</sup> However, by fifth-century BC, Herodotus of Halicarnassus recorded that the Egyptians had a well-developed dental community, indicating that some doctors were specializing in teeth.<sup>3</sup> Moreover, the theory of a "tooth worm" residing in the hollow portion of a tooth and gnawing at the structure of the tooth pervaded dental theory from the time of the Babylonians to the modern era.<sup>4</sup> Anton von Leeuwenhoek, the "father of modern microscopy," helped to discredit the Worm Theory of tooth decay, when he identified worm-infested cheese as the source of contamination in 1700.<sup>5</sup>

In 1687 Charles Allen wrote the first textbook in English devoted entirely to dentistry.<sup>4</sup> Although he described no endodontic procedures, he did share a crude method of dental allotransplantation involving "taking out the rotten teeth or stumps and putting in their place some sound ones drawn immediately out of some poor body's head."<sup>6</sup>

In 1728 Pierre Fauchard, the "founder" of modern dentistry, wrote and published his landmark book, *The Surgeon Dentist*. He described the pulp chambers and canal anatomy of several teeth accurately. He described a method of pulp extirpation using a small pin and advised the application of oil of cloves or oil of cinnamon to the area for

several weeks. Fauchard outlined a method for the relief of pain associated with a dental abscess. He opened the tooth and left it open for two to three months to relieve pressure and evacuate pus. Then, he would fill the pulp chamber with lead foil.<sup>4</sup> In cases of vital pulp exposure, Fauchard advocated applying filling material directly to the exposed nerve. Phillip Pfaff, a German dentist who treated Kaiser Frederick the Great, first mentioned a pulp-capping procedure in 1756 in which he would fashion a concave piece of gold or lead foil to approximate the size of the exposure and place the restoration on top of this cap.<sup>7</sup>

In 1783 a New York dentist from England, Robert Woofendale, published *Practical Observations on the Human Teeth*. He is credited with the first recorded description of an endodontic procedure in the US. Woofendale described a method of using a hot instrument to cauterize the pulp. He also stated that “a small bit of lint, dipped in the oil of cinnamon, cloves, turpentine, or any chemical oil, frequently gives relief, and if repeated for sometime, often destroys the nerve.”<sup>4</sup>

Frederick Hirsch of Germany first described the percussion test in 1800 to diagnose dental disease. He advocated tapping teeth to elicit pain in the diseased tooth. Once diagnosed, his treatment of choice was to perforate the offending tooth at the neck, insert a red-hot instrument into the access repeatedly, and then fill the cavity with lead.<sup>4</sup>

Edward Hudson of Philadelphia is credited with having been the first dentist to place fillings in root canals when he used gold foil placed with instruments of his own design in 1809.<sup>8</sup>

## SURGICAL ENDODONTICS

### History of Endodontic Surgery

Intentional replantation, a procedure practiced today within the scope of the specialty of endodontics, was first recorded in the 11<sup>th</sup> century by Abulcasis.<sup>9</sup> Fauchard in 1712 recorded a detailed account of intentional replantation with indications, precautions, and descriptions in his book, “Le Chirurgien dentiste ou traité des dents.”<sup>10</sup> Hunter in 1778 concluded that a vital periodontal ligament was required for the procedure to be successful, and if it was damaged, resorption of the replanted tooth would inevitably ensue.<sup>11</sup>

Incision and drainage of a dental abscess was described by Harris in 1839.<sup>12</sup> He recommended the use of a “lancet or sharp, bistrory-pointed knife” to drain a “tumor of the gums” when fluctuant. Hullihen described the “Hullihen operation” in 1845,<sup>13</sup> a method of draining intraobony pressure that is similar to modern surgical trephination, in which the operator would puncture the gingiva, the buccal cortical plate, and the tooth root to expose the canal space in an effort to relieve pressure in infected teeth. Farrar in 1880<sup>14</sup> advocated a procedure for surgical trephination of the buccal cortical plate after the elevation of a mucosal flap with vertical-releasing incisions.

Endodontic surgery in the form of root resection was probably first practiced in France in 1843 by Desirabode<sup>15</sup> and in the US by Farrar<sup>16</sup> as early as 1884. Farrar recommended the amputation of any portion of a root that was in lesion and not surrounded by bone, and he had detailed drawings for his procedure. In 1890, Rhein<sup>17</sup> published his paper, “The Amputation of Roots as [a] Radical Cure of Chronic Alveolar

Abscess,” and he is credited with popularizing the procedure by advocating its widespread acceptance.

The advancement of endodontics was nearly stopped in 1910 when Sir William Hunter popularized the focal infection theory. In his lecture “The Role of Sepsis and Antisepsis in Medicine,” he accused dentists of creating “a veritable mausoleum of gold over a mass of sepsis to which there is no parallel in the whole realm of medicine or surgery.”<sup>18</sup> His ideas grew in popularity at the time, and for more than 20 years, physicians and dentists agreed that extraction of pulpally compromised teeth should be the treatment of choice in an effort to avoid complications in systemic disease. By the 1930s, sufficient evidence had been amassed to prove that retaining teeth after endodontic therapy was a viable treatment option.<sup>19</sup>

In February 1943, a group of 20 dentists met in the Palmer House Hotel in Chicago to form the American Association of Endodontists. By 1963, endodontics had been officially recognized as a specialty by the American Dental Association.<sup>20</sup>

#### Modern Endodontic Surgery: Rationale and Explanation

Nonsurgical retreatment is generally believed to be the preferred first line of retreatment for teeth with persistent apical periodontitis after initial non-surgical root canal therapy.<sup>21-23</sup> However, clinical judgment may dictate retreatment by surgery when non-surgical retreatment is impractical or undesirable. Specifically, surgery may be important for teeth with long posts; in cases of irretrievable separated instruments, non-negotiable ledges, canal blockages, transportation, and hard cement filling materials; after failure of previous non-surgical retreatment, and in cases of suspected vertical root

fracture, when a biopsy is indicated, if the risks and the costs of retreatment are considered excessive.<sup>24</sup>

### Indications for Surgical Endodontics

Leubke in 1964<sup>25</sup> published an extensive list of indications for endodontic surgery as follows:

1. Necessity for drainage.
  - a. Elimination of toxic material.
  - b. Alleviation of pain.
2. Postoperative failure of conventional therapy.
  - a. Obviously inadequate filling.
  - b. Apparently adequate filling.
  - c. Persistent postoperative discomfort.
3. Predictable failure with conventional therapy.
  - a. Flaring apex.
  - b. Severely curved root end.
  - c. Internal, external, or apical resorption.
  - d. Fractures in the apical third.
  - e. Persistent infection.
  - f. Persistent suppuration or exudation.
  - g. Forecast of acute abscess.
  - h. Apical cyst.
4. Impracticality of conventional therapy.
  - a. Porcelain jacket crown.

- b. Fixed partial denture attachment.
  - c. Dowel-retention crown.
  - d. Excessive calcification.
  - e. Associated periodontal lesion.
5. Procedural accidents.
- a. Instrument fragmentation.
  - b. Perforation.
  - c. Overinstrumentation.
  - d. Gross overfilling.

The aim, therefore, of endodontic surgery is “to correct problems and successfully eliminate inflammatory processes that [can]not otherwise be successfully treated with nonsurgical root canal treatment.”<sup>26</sup> Treatment is then necessarily focused on eliminating the etiology, which could be persistent or secondary intraradicular infection or extraradicular infection.<sup>22</sup> Even with the most strenuous non-surgical efforts, bacteria can persist in the root canal system, finding refuge in the dentinal tubules, irregularities, isthmuses, or apical deltas and cause persistent disease.<sup>27</sup> Sundqvist et al.<sup>23</sup> in 1998 conducted a clinical study to determine the microbial flora present in teeth with failed root canal therapy. In his study, he selected 54 teeth with previous root canal therapy and persisting periapical radiolucencies and took samples from these teeth during retreatment. He found that persistent infections were mainly single-species infections and usually gram-positive organisms with *E. faecalis* being the most commonly isolated bacteria in persistent endodontic disease. In a more recent study using ribosomal RNA analysis, Sakamoto et al.<sup>28</sup> in 2008 identified mixed infections in persistent root canal infections

with some species being yet unidentified. Viruses as well as fungi have also been implicated in persistent periapical pathosis.<sup>29, 30</sup>

Extraradicular colonies of microorganisms not reached by non-surgical endodontic therapy and host defenses incapable of eliminating bacteria are other potential reasons for failure. Sequiera<sup>31</sup> examined the apical root surfaces of untreated teeth with chronic periradicular lesions and found extraradicular bacteria organized into mature corn cob colonies in 4 percent of cases. In 2010 Ricucci<sup>32</sup> evaluated the prevalence of bacterial biofilms on both treated and untreated teeth with apical periodontitis and found an incidence of 6 percent. Ferreira<sup>33</sup> published a case study of a maxillary premolar that had been treated for one year, receiving replacement of intracanal medicament several times over the course of the year with no resolution of periapical pathosis. Periapical surgery with root resection was completed, and scanning electron microscopy (SEM) showed colonization of the root tip with cocci and fungi. Twelve months after the surgery, the periapical lesion was healing.

Nair<sup>34</sup> found that overextension of filling materials in the absence of microorganisms can cause persistent disease through chronic inflammatory reactions of multinucleated giant cells, especially if those fillings contain irritating substances. In 1999 Nair<sup>35</sup> also found that cholesterol crystals from large periapical lesions may accumulate in the periapical tissues and make resolution of the lesion impossible, even after adequate non-surgical treatment. He advised surgery for the treatment of these lesions.



## THE SURGICAL PROCEDURE

### Pre-Operative Preparation

Veksler et al.<sup>36</sup> found that dual rinses of 0.12-percent chlorhexidine gluconate prior to surgery reduced the salivary bacterial load 97 percent, and this reduction lasted for 60 minutes. Jackson and Hargreaves<sup>37</sup> found that pre-operative 800-mg ibuprofen immediately before the surgery and QID after the surgery for 48 hours after the procedure reduced post-operative discomfort compared with controls without increasing bleeding during the surgery.

### Local Anesthetic

Claffey et al.<sup>38</sup> compared the efficacy of 2.0-percent lidocaine with 1:100,000 epinephrine and 4-percent articaine with 1:100,000 epinephrine administered as an inferior alveolar nerve block in patients with irreversible pulpitis and found no significant difference in efficacy between the two anesthetics. In a separate study, Haase et al.<sup>39</sup> compared the anesthetic efficacy of 4.0-percent articaine with 1:100,000 epinephrine against the efficacy of 2.0-percent lidocaine with 1:100,000 epinephrine as supplemental buccal infiltrations after inferior alveolar nerve blocks with 2.0-percent lidocaine with 1:100,000 epinephrine for mandibular first molars. They found that articaine resulted in anesthesia 88 percent of the time compared with 71 percent for lidocaine. In a similar study, Evans et al.<sup>40</sup> compared the anesthetic efficacy of 4.0-percent articaine with 1:100,000 epinephrine and 2.0-percent lidocaine with 1:100,000 epinephrine in infiltrations of maxillary lateral incisors and maxillary first molars. They found that articaine was statistically significantly more effective than lidocaine for the lateral

incisors, but no difference was observed for the first molar. Paschley et al.<sup>41</sup> observed the systemic effects of the periodontal ligament (PDL) supplemental injection and found that the PDL injection is in fact an intraosseous injection and that solutions administered via the PDL injection rapidly enter the systemic circulation. Kim<sup>42</sup> proposed that the PDL injection induces significant reduction of pulpal blood due to vasoconstriction from the anesthetic solution and should therefore only be used in endodontics and extractions, but not for restoration of vital teeth. Reisman et al.<sup>43</sup> studied the anesthetic efficacy in mandibular molars with irreversible pulpitis of 3.0-percent mepivacaine administered as an intraosseous injection after inferior alveolar nerve block (IANB) with 2.0-percent lidocaine and 1:100,000 epinephrine. They found that IANB was 25-percent successful; the first intraosseous injection was 80-percent successful, and the second was 98-percent successful. Replogle et al.<sup>44</sup> also found that the use of the plain anesthetic did not result in cardiovascular changes after administration of intraosseous anesthetic. In a similar study, Replogle et al.<sup>45</sup> compared anesthetic efficacy of 2.0-percent lidocaine with 1:100,000 epinephrine and 3.0-percent mepivacaine and in human mandibular first molars administered as primary intraosseous injections. They found that lidocaine was successful 74 percent of the time compared with 45 percent for mepivacaine.

### Magnification

Modern endodontic surgery combines the magnification and illumination of the surgical operating microscope with new microinstruments, microheaded ultrasonic tips, and advanced materials.<sup>46</sup> The use of the microscope improves endodontic surgery by allowing high magnification inspection of the surgical field, precise and complete removal of diseased tissues, distinction between the bone and root tip, smaller osteotomy,

reduced occupational and physical stress on the operator, reduced radiographs due to the ability of the operator to directly inspect the apex, enhanced documentation, and enhanced communication with the referring dentist.<sup>47</sup> Kim<sup>48</sup> even went so far to say that “performing apical surgery without magnification is no longer adequate or defensible” in light of the improvements it offers to the surgical endodontist. However, it is interesting to note that the only time Kim recommends high magnification outside the range of loupes during endodontic surgery is to inspect the resected root surface and root-end filling.<sup>48</sup>

#### Soft Tissue Management

Soft-tissue management and flap design in surgical endodontics enhance the access to and the healing of periapical tissues and the surgical field.<sup>49</sup> Lubow et al.<sup>50</sup> recommended using the sulcular full-thickness flap and vertical-releasing incisions. This design allows excellent access, but has a disadvantage of possible gingival recession and shrinkage of the papilla due to compromised blood flow.<sup>51</sup> Velvart<sup>52</sup> proposed the use of the papilla-based incision to prevent this shrinkage. Vreeland and Tidwell<sup>53</sup> recommend a flap length-width ratio of 2:1 with a base wider than the free margin of the flap to avoid a convergence of the vertical-releasing incisions.

The submarginal flap was designed by Ochsenbein and Luebke<sup>54</sup> whereby the horizontal incision scallops the architecture of the free gingival margin in the attached gingival, but the marginal gingival is left untouched. This design is only to be used when the attached gingival is a minimum of 2 mm and the surgical bony access does not extend to the flap margins.<sup>55</sup> The purported advantage of this flap design is that it does not move the free gingival margin to expose restoration margins and therefore results in less

recession. The recession is minimized because the flap design does not require crestal bone to be denuded; however, postoperative necrosis of the unreflected marginal gingival is a risk, because the blood supply is only coming from the periodontal ligament.<sup>56, 57</sup>

The semilunar flap design using a curved horizontal incision in the unattached gingival is no longer recommended because of limited access to the surgical area, the inability to close the wound over sound bone, the presence of secondary healing with scar formation, and the severance of a maximum number of blood vessels.<sup>58</sup>

### Osteotomy

Boyne et al.<sup>59</sup> observed healing of 21 periapical defects in the anterior region with at least one cortical plate intact and found that defects of 9 mm to 12 mm healed with a fibrous scar, while smaller lesions healed with complete bone regeneration. Hjørting-Hansen and Andreason<sup>60</sup> conducted a study in dogs comparing healing in osseous defects of varying sizes and with two, one, or no cortical plates intact. They found complete healing in lesions of 5 mm with one cortical plate intact, but if lesions were larger or had both cortical plates missing, healing with fibrous tissue resulted. Osteotomy size can also affect the rate of healing. Rubenstein and Kim<sup>61</sup> found that average lesions smaller than 5 mm require 6.4 months to heal; lesions of 6 mm to 10 mm take 7.25 months, and those of 10 mm require 11 months. Kim<sup>62</sup> recommends an optimal osteotomy site with 4-mm diameter to allow free movement of a 3-mm ultrasonic tip within the surgical crypt while minimizing the time to healing and fibrous-healing defect creation.

## Hemostasis

Ferric sulfate aids in hemostasis through a still-debated mechanism involving the chemical reaction of ferric and sulfate ions and the acidic pH of the solution with blood proteins resulting in agglutination of the blood proteins that plug small vessels.<sup>63</sup> Ferric sulfate is easy to use, effective, unlikely to be absorbed systemically, but is known to be cytotoxic, cause tissue necrosis, and have adverse effects on osseous healing if left in the surgical site.<sup>64</sup>

The use of epinephrine for surgical hemostasis is possible because the predominant receptors in the oral tissues are alpha receptors, which bind epinephrine and result in vasoconstriction.<sup>48</sup> Lindorf<sup>65</sup> stated this vasoconstriction is relatively short-lived and results in rebound vasodilation and increased bleeding at 15 min to 30 min. Pellets containing racemic epinephrine hydrochloride do not cause systemic effects, because the topical vasoconstriction is almost immediate and results in very little uptake into the systemic circulation.<sup>66</sup>

## Root Resection and Retropreparation

Root resection with a steep root bevel angle of 45° to 60° was necessary with traditional rotary burs for access and visibility, but the introduction of ultrasonic surgical tips has allowed for a reduction of this bevel.<sup>67, 68</sup> Tidmarsh and Arrowsmith<sup>69</sup> recommended minimal bevel of root resection due to opening of dentinal tubules. Gilheany et al.<sup>70</sup> evaluated bevel angles of root resection and the required lengths of retroprep and found that increasing the amount of bevel required an increase in the depth of retrograde filling to decrease apical leakage. He concluded that retroprep depth should be a minimum of 3.5 mm. Vertucci<sup>71</sup> studied human permanent teeth and found that

resection of the apical 3 mm of root would result in removing 93 percent of lateral canals and 98 percent of apical ramifications.

Carr<sup>72</sup> defines the ideal root-end preparation as a class I cavity at least 3 mm into the root dentin, parallel to the long axis of the root and coincident with the anatomic root canal space. Ultrasonic instruments were first introduced to endodontics in 1957 by Richman.<sup>73</sup> Carr later introduced retrotips designed specifically for root-end cavity preparation during endodontic surgery.<sup>74</sup> These tips offered superior operator control, decreased risk of perforation, and increased ability to stay centered in the canal as compared with microheaded handpieces.<sup>75</sup> Wuchenich et al.<sup>76</sup> conducted a SEM comparison study in human cadavers of retropreparations with ultrasonic and microheaded handpieces. They found that ultrasonic tips made cleaner and deeper root-end cavity preparations, aided in the retention of root-end filling materials, and improved disinfection by removing infected dentin. Saunders et al.<sup>77</sup> reported crack formation on extracted teeth after using ultrasonic instruments for apical retropreparation. Layton<sup>78</sup> later substantiated these findings with a similar study and found that a higher prevalence of microfractures was observed when the power setting of the ultrasonic handpiece was increased. Because of this phenomenon, it has been recommended to complete the apical retropreparation at low to moderate power settings for two minutes in an effort to reduce the risk for crack formation.<sup>79</sup> Ultrasonic tips were originally constructed of smooth stainless steel, but many manufacturers have diamond-coated and zirconium oxide-coated tips in an effort to increase cutting efficiency and reduce the risk of crack formation.<sup>80</sup> Studies by Peters et al.<sup>81</sup> and Baumgartner et al.<sup>82</sup> compared the microfracture formation of stainless steel tips with coated tips and found that neither tip produced a significant

number of microcracks. They did, however, observe that coated tips took less time to complete the preparation, and that the surface of the preparation was roughened and could have helped to retain the retrofilling material.

## OUTCOMES

Randomized-controlled studies comparing of success rates between surgical and non-surgical retreatment have been conducted. Danin et al.<sup>83</sup> completed a randomized-controlled clinical trial comparing clinical and radiographic signs of healing for non-surgical retreatments and apicoectomies over the course of one year and found that surgical retreatments resulted in complete healing 58 percent of the time versus only 28 percent for non-surgical retreatments. Kvist<sup>84</sup> designed a randomized clinical trial to compare surgical and nonsurgical retreatment followed for four years. He found a statistically significant higher healing rate at 12 months for surgically retreated teeth than in non-surgically retreated teeth, but these differences were not present at 48 months. He postulated the differences in findings at 12 months could be explained by higher healing dynamics of surgical retreatment. Although Kvist did not show any significant difference in outcome for surgical and nonsurgical retreatment, he discovered the length of follow-up may influence the conclusions made by outcomes studies. The type of surgical procedure also makes a difference in surgical outcome. In 1999, Rubenstein and Kim<sup>61</sup> used a modern microsurgical technique and followed 94 cases for 1 year. They found that 96.8 percent of cases had healed to the point of a completely restored lamina dura in an average time of 7.2 months. In 2006 Tsesis<sup>85</sup> published a study comparing a traditional periapical surgery with a 45-degree bevel, carbide round bur retropreparation, and no magnification against a modern technique utilizing minimal or no bevel, retrograde

preparation with ultrasonic retro-tips, and a dental operating microscope. Complete healing was found in 91.1 percent of the modern cases and in only 44 percent with traditional technique. Likewise, Maddalone<sup>86</sup> conducted a prospective study of periapical surgery using modern magnification and ultrasonic instrumentation and found an overall success rate of 92.5 percent at three years. In 2011 Song<sup>87</sup> published a prospective study with eight-year follow-up of surgical retreatments using modern treatment techniques and found a success rate of 92.9 percent.

#### Clinical Consequences of Apical Leakage

Siqueira<sup>88</sup> stated that “for any bacterial species to cause disease, they have to reach a populational density (load) that is conducive to tissue damage either caused by the bacteria themselves or by the host defense mechanisms in response to infection.” The aim of the apical retropreparation is to deny the pathogens that may reside in the canal space from coming in contact with the periapical tissues.<sup>24</sup> In a separate article, Siqueira<sup>88</sup> reviewed the microbiology and implications of bacterial persistence after treatment procedures and showed that bacteria have specialized means and are able to evade nearly every effort to disinfect the canal system and are able to survive in a viable but noncultivable state in the hostile environment of the obturated canal for years, and then able to resume division when favorable conditions are restored. Hoen<sup>89</sup> screened 1100 failing endodontically treated teeth and analyzed them for causes of failure and found that while the cause of failure was often multifactorial, leakage of irritants into the periapical tissues caused persistent periapical pathosis.



## ROOT END FILLING MATERIALS

### The Ideal Root-End Filling Material

Torabinejad<sup>90</sup> states the qualities of an ideal filling material will:

- 1) Adhere to dentin.
- 2) Maintain a sufficient seal.
- 3) Be insoluble in tissue fluids
- 4) Be dimensionally stable
- 5) Be nonresorbable over time
- 6) Be radiopaque
- 7) Be easily manipulated
- 8) Be adequately compressible
- 9) Have an adequate working time
- 10) Have a quick setting time
- 11) Be biocompatible with human tissue.

Gutmann and Harrison<sup>91</sup> state that the purpose of a root-end filling is “to establish, as well as possible, a hermetic seal of all apical avenues in the tooth from the oral environment to the periradicular tissues.”

### Burnished Gutta-Percha

Gutta-percha obturation cones contain 21.8 percent to 18.9 percent gutta-percha, 56.1 percent to 75.3 percent zinc oxide, 1.5 percent to 17.3 percent heavy metal sulfates, and 1.0 percent to 4.1 percent waxes and resins.<sup>92</sup> Gutta-percha is generally considered to be non-resorbable and does not dissolve in tissue fluids.<sup>93</sup> The biocompatibility of

gutta-percha has been studied by several studies. Spangberg<sup>94</sup> implanted samples of gutta-percha into bone and found acceptable compatibility with fibrous connective tissue healing adjacent to the sample. Marcotte et al.<sup>95</sup> used Rhesus monkeys with gutta-percha root-end fillings and histologically observed healing over a period of 15 weeks and found “only minimal inflammatory response.”

Burnishing gutta-percha for a root-end filling has been practiced since at least 1880, when Brophy<sup>96</sup> published his recommendation of smoothing gutta-percha at the resected root end during surgical endodontics. Blum<sup>97</sup> indicated that the act of resecting the root end with a surgical bur would self-burnish the gutta-percha at the root end and advocated the use of radiographs to assess the quality of the root-end filling.

Cunningham<sup>98</sup> later disproved this idea of a surgical bur self-burnishing gutta-percha during root resection, showing that the bur tears and drags the gutta-percha as it cuts and results in a gapped and poorly adapted root-end filling. Harrison and Tood,<sup>99</sup> however, were able to demonstrate acceptable sealing properties in teeth obturated with well-condensed sealer and gutta-percha combinations and resected with high-speed rotary instruments. Gutmann<sup>91</sup> hypothesized that the cause for the differences in the findings of these studies has to do with the type of gutta-percha used, nature of the sealer used, condensation technique, the type of bur used, and the operator skill. Another technique for placing gutta-percha in the root-end preparation involved pulling the gutta-percha through the root end in an effort to create a tight seal.<sup>100</sup> Peters and Cunningham<sup>101</sup> conducted an SEM study comparing the adaptation of gutta-percha when placed by coronal condensation or apical tension and found that tension-placed gutta-percha resulted in significant gaps, retraction from dentin walls, and voids. Barry et al.<sup>102</sup>

advocated the use of a heated burnisher to seal the gutta-percha exposed by root resection and conducted a study between amalgam and hot burnished gutta-percha which found that the seals of the two materials were not significantly different. Tanzilli et al.<sup>103</sup> set out to determine if the marginal adaptation of cold-burnished and hot-burnished gutta-percha was superior using an SEM. His study found that cold burnished gutta-percha had an average void size of 1.8µm compared with 22 µm for hot-burnished gutta-percha. He also noted that the heat sealed gutta-percha produced defects or ‘blisters’ in the gutta-percha that resulted in surface defects of 62 µm and the gutta-percha pulling away from the dentinal wall some 104 µm.<sup>102</sup> Kaplan et al.<sup>104</sup> conducted a methylene blue dye leakage study to compare cold-burnished gutta-percha and heat-sealed gutta-percha and found that cold-burnished gutta-percha yielded a better seal. Another study by Szeremeta-Browar et al.<sup>105</sup> used radioactive calcium in an autoradiographic leakage study and found contradictory results. The heat-sealed gutta-percha had a superior apical seal to cold-burnished gutta-percha. Bramwell and Hicks<sup>106</sup> conducted a methylene blue leakage study *in vivo* with rhesus monkeys and found an inconsistent variety of dye penetration among the samples. Their findings suggested that the quality of the seal was dependent on not only the technique employed, but also on the skill of the operator.

## Amalgam

Prior to the introduction of more advance dental materials, silver amalgam was the material of choice for surgical retrofills, and had even been called “the standard to which new materials are usually compared.”<sup>107</sup> It was desirable because it did not demonstrate excessive expansion as a result of moisture contamination, was widely available, and inexpensive to obtain.<sup>108</sup> Amalgam was also familiar to the dentist, and the

material shows highly radiopaque on the radiograph.<sup>109</sup> The composition of the amalgam can affect the sealing ability of the material.<sup>110</sup> Amalgam can be broadly classified base upon the percentage of copper by weight. Low-copper amalgams have less than 6 percent copper by weight, and high-copper amalgams have increased amounts of copper, from 9 percent to 20 percent by weight, to achieve better mechanical properties, lower corrodibility, and less cytotoxicity.<sup>111</sup> When an alloy of silver tin ( $\text{Ag}_3\text{Sn}$ ) is triturated with elemental mercury (Hg), a three phased amalgamation of silver tin ( $\text{Ag}_3\text{Sn}$ ), silver mercury ( $\text{Ag}_2\text{Hg}_3$ ) and tin mercury ( $\text{Sn}_8\text{Hg}$ ) is produced.<sup>112</sup> The silver mercury phase, known as gamma 2 is responsible for setting shrinkage<sup>112</sup> and passive corrosion of the material in an aqueous environment.<sup>113</sup> The introduction of an element to the to the alloy or admix silver and tin that has a higher affinity for tin prior to amalgamation eliminates the weak and corrosion prone gamma 2 phase.<sup>114</sup> When high-copper alloys are amalgamated, the weak gamma 2 phase is replaced with ( $\text{Cu}_6\text{Sn}_5$ ), called the eta phase.<sup>115</sup> Zinc may be added to the amalgam to scavenge oxygen and reduce the formation of oxides.<sup>116</sup>

Omnell<sup>117</sup> in 1959 published a case report where a zinc containing amalgam had been used for an endodontic retrofilling material and a radiopaque halo of zinc carbonate had precipitated to the periapical tissues. He hypothesized that the reaction was the result of electrolytic flow between the zinc and other metals in the amalgam. This single case report resulted in a virtual ban of the use of zinc-containing amalgam in endodontic surgery until 1980 when Liggett<sup>118</sup> found no histological reaction difference between zinc and zinc free amalgam. Liggett pointed out that Omnell's alternate hypothesis that the zinc carbonate was from the cement of the root canal post was likely a more plausible

explanation for the unusual zinc carbonate halo.<sup>118</sup> Kimura<sup>119</sup> also conducted a comparative analysis of zinc-containing and non-zinc alloys used in retrograde endodontic surgery, and found that both zinc and non-zinc alloys elicited a similar inflammatory response.

Skinner and Phillips<sup>120</sup> stated that water contamination of zinc-containing amalgams while setting results in significantly increased expansion. However, after the first day, dimensional change was the same for both zinc containing and non-zinc alloys.

The clinical performance of amalgam compared to contemporary materials has been relatively poor, with higher leakage, lower biocompatibility, higher corrosion, and staining.<sup>121</sup> Pitt Ford et al.<sup>122</sup> compared amalgam as a root-end filling material with both Super EBA and Cavit, and found that the amalgam showed the most severe immune response. Dorn and Gartner<sup>123</sup> compared clinical success rates of teeth with root-end fillings of SuperEBA, IRM, and zinc-free high-copper spherical amalgam and found success rates of 75 percent for amalgam, 91 percent for IRM, and 95 percent for SuperEBA. Tronstad and Wennberg<sup>124</sup> tested the cytotoxicity of conventional amalgam and high copper amalgam (Dispersalloy) on mouse fibroblasts and found that both types of amalgam were initially toxic, but that the toxicity decreased after 24 hours. They noted that high copper alloys were more toxic than low-copper alloys. Frank et al.<sup>125</sup> had a 10-year follow-up on surgical endodontic cases with amalgam as a retrofill and found that all cases showed clinical success early; only 57.7 percent were successful at 10 years.

#### Cavit

Cavit is commonly used as a provisional restorative material and contains zinc oxide, calcium sulfate, zinc sulfate, glycol acetate, polyvinyl acetate, polyvinyl chloride-

acetate, triethanolamine, and red dye.<sup>126</sup> Cavit comes premixed from the manufacturer as a soft putty, and undergoes a hygroscopic setting reaction when in contact with water that results in an 18-percent linear setting expansion.<sup>127</sup> Studies demonstrating the sealability of Cavit have mixed results. Parris et al.<sup>128</sup> tested the seal of Cavit after thermocycling the material 10 times from 60°C and 4°C and found that the material demonstrated excellent sealing ability when tested with aniline blue dye. Delivanis and Tabibi<sup>129</sup> studied the sealing properties of Cavit when used as a root-end filling material in dogs' teeth over a period of six months and found deterioration of Cavit seal at six months. They determined that Cavit leaked more than amalgam, and that the deterioration of Cavit was significant.

Studies on the biocompatibility of Cavit are also ambivalent. Wennberg and Hasselgren<sup>130</sup> evaluated the cytotoxicity of various temporary restorative materials and found Cavit to be toxic. Al-Nazhan, Spaounas, and Spangberg used mouse fibroblasts to evaluate the cytotoxicity of fresh Cavit, 1-day set Cavit, and 7-day set Cavit and found that it had not toxic effect in any of the samples studied. Finne et al.<sup>131</sup> reviewed 218 teeth on a three-year recall with root-end fillings of either amalgam or Cavit, and found that the amalgam group demonstrated significantly better results than the Cavit group. Their hypothesis was that the seal of the Cavit was not durable and could deteriorate over time, while the amalgam would obliterate the canal space and lead to an improved seal over time. Nord conducted a clinical study of 354 teeth treated with Cavit root-end fills and found complete healing with 61 percent of teeth, incomplete healing in 17 percent, and no healing in 22 percent of cases.<sup>132</sup>

## Polycarboxylate Cements

Zinc polycarboxylate cements were first introduced by Smith in 1968.<sup>133</sup> They are produced as a powder that contains zinc oxide, magnesium oxide, and stannous fluoride that sets to form a cement of zinc oxide set in a crosslinked matrix of zinc polycarboxylate when mixed with an aqueous solution of polyacrylic acid.<sup>116</sup> The setting reaction between polycarboxylate cements results in available free carboxyl groups that can chelate calcium which results in the material's ability to adhere to tooth structure.<sup>134</sup> The solubility of the material in an aqueous environment depends on the powder to liquid ratio used when mixing the cement. Moore et al.<sup>135</sup> demonstrated that decreasing the powder by one-third resulted in a three-fold increase in cement solubility. Freshly mixed polycarboxylate cement has an extremely acidic pH of 1.7 which quickly rises during the setting reaction to a neutral pH.<sup>136</sup> Zartner et al.<sup>137</sup> studied bony tissue responses to set polycarboxylate cement implanted in rabbit tibias and found that set polycarboxylate cement is very well tolerated by viable bone and observed no destruction of osteocytes. They did note that tissue in direct contact with the polycarboxylate cement demonstrated decalcification of the bone, which they hypothesized to be due to the chelating property of the material. Seltzer et al.<sup>138</sup> filled dogs' teeth with polycarboxylate cement and extruded excess cement into the periapical tissues and conducted a histological examination of the tissue response. They found severe and persistent inflammation adjacent to the cement even after 225 days and concluded that there would be 'no advantages' to adopting polycarboxylate cement as a root canal filling material. Leakage studies of polycarboxylate cement have demonstrated that the material has a poor apical seal when compared to either amalgam or gutta-percha.<sup>139, 140</sup> Gutmann

hypothesizes that decalcification of the dentin at the dentin-cement interface in a similar fashion to the bony decalcification observed by Zartner may be responsible for the increased leakage seen with this material.<sup>91</sup>

### Glass Ionomer Cements

Glass ionomer cement is composed of a calcium fluoroaluminosilicate glass powder that sets via an acid-base reaction with an aqueous solution of an acrylic acid homo- or copolymer.<sup>141</sup> McLean<sup>142</sup> commented that a more accurate name for the material could be glass polyalkenoate cement, as the set material is not truly an ionomer. Commercially available glass-ionomer cements can be subdivided into two broad groups: Conventional glass ionomer cements and resin modified glass ionomer cements.<sup>143</sup> The addition of acrylic acid-itaconic acid copolymers and acrylic acid-maleic acid copolymers in the resin-modified glass ionomer cements results in better mechanical properties than conventional glass ionomer cements.<sup>144</sup> The setting reaction of glass-ionomer cements begins when polyacrylic acid reactions with calcium and aluminum ions to form a firm gel that provides initial adhesion to tooth structure and metal.<sup>145</sup> The reaction continues from 30 minutes to 24 hours as aluminum polycarboxylate is formed and the material improves its physical properties.<sup>146</sup> The reaction generates no heat<sup>147</sup>, and demonstrates no shrinkage while setting.<sup>148</sup> The material is susceptible to moisture contamination and dehydration during the first 60 minutes of the setting reaction, both of which can result in decreased physical properties, surface hardness, and color stability.<sup>149</sup> In an effort to overcome this adverse outcome, manufacturers have advocated coating the material in a surface varnish that protects the material from moisture contamination and dehydration



while setting.<sup>149</sup> Fully set glass ionomer cement contains a matrix of calcium aluminum polysalts with silica gel coated fluoroaluminosilicate glass embedded within.<sup>150</sup>

Fukazawa et al.<sup>151</sup> demonstrated that glass ionomer cement will leach aluminum, fluoride, silicon, and calcium ions if allowed to set in an acidic environment. Smith<sup>152</sup> conducted a SEM evaluation of the surface of glass ionomer cement allowed to set in an acidic environment and observed pores in the surface of the material, which could have contributed to marginal leakage if the material was placed and set in an acid environment. Due to glass ionomer cement's sensitivity to pH and moisture, Friedman<sup>153</sup> questioned the suitability of the material as a root-end filling material in which moisture contamination is highly likely, and when inflammation in the periapical tissues can lead to an acidic environment. Furthermore, Beltes et al.<sup>154</sup> said the material was sticky and difficult to adapt to root-end preparations during endodontic surgery.

Pitt Ford first suggested the use of glass ionomer cement in endodontics in 1979 as a sealer for a single-cone obturation technique, because the working time was too short to be used with lateral condensation.<sup>155</sup>

Zetterqvist et al.<sup>156</sup> studied tissue reaction to glass ionomer cement when used as a root-end filling material in monkeys and found complete healing of the periradicular tissues by three months with no inflammatory reaction, and mature alveolar bone surrounding the root apices by six months.

### Composite Resins

Composite resins have been used, with limited acceptance, in endodontics for retrograde fillings.<sup>157</sup> One possible reason for this limited acceptance is due to polymerization shrinkage that can result in a marginal gap leading to apical leakage.<sup>158</sup>

Rud et al.<sup>159</sup> proposed the use of a dentin bonding agent composed of a water-based solution of 5.0-percent glutaraldehyde and 35-percent 2-hydroxyethyl methacrylate (HEMA), which reacts with dentin collagen and bonds methacrylate groups to the NH-groups in collagen. This dentin-bonded hybrid layer can then bond to the dimethacrylates in restorative composite resin by copolymerization and can reduce the risk of a marginal gap allowing for leakage.<sup>160</sup> Rud's study found that a tight seal had been formed between the dentin and composite, and in some cases even cementum and Sharpey's fibers formed in contact with the filling.<sup>159</sup> In a second publication on composite resin, Rud et al.<sup>161</sup> demonstrated healing in 78 percent of 388 cases at one year following apical surgery with composite as a retrograde filling. In his analysis of the failures, half were caused by "loose retrograde composite fillings" due to handling the composite beyond its working time, or because of moisture contamination of the dentin bonding agent during the surgery.<sup>161</sup>

## IRM

Intermediate restorative material (IRM) consists of a powder with more than 75-percent zinc oxide and 20-percent polymethacrylate mixed with a liquid that contains 99-percent eugenol and less than 1.0-percent acetic acid.<sup>24</sup> Crooks et al.<sup>162</sup> in 1994 conducted a study to evaluate the seal of IRM root-end fillings prepared with various powder- to-liquid ratios and found that varying the powder-to-liquid ration did not have an effect on the microleakage of the material. Safavi et al.<sup>163</sup> evaluated the adherence of enamel matrix derivatives to IRM and found that the proteins do not adhere to IRM.

## EBA

SuperEBA was first recommended as a retrofilling material by Hendra in 1970.<sup>164</sup> Oynick and Oynick in 1978 advocated EBA a material that would offer the superior sealing ability of zinc oxide and eugenol without being resorbable.<sup>165</sup> Super-EBA cement is composed of a powder of 60-percent zinc oxide, 34-percent silicone dioxide, 6.0-percent natural resin and a liquid composed of 62.5-percent ethoxybenzoic acid, and 37.5-percent eugenol.<sup>166</sup> It has desirable handling characteristics, high compressive strength, high tensional strength, neutral pH, is radiopaque, and low solubility.<sup>165</sup> In their histological analysis, Oynick and Oynick<sup>165</sup> were even able to demonstrate Sharpey's fibers inserting on the Super-EBA.

Testori et al.<sup>167</sup> compared healing at five-year follow-up of ultrasonic retropreparation filled with SuperEBA to microhandpiece preparation filled with amalgam and found the Super-EBA group demonstrated 85-percent healing while amalgam only had 68 percent. Dorn et al.<sup>123</sup> found 95-percent success with Superb, 91-percent with IRM, and 75-percent with amalgam. Pitt Ford, Andresen, Dorn and Karlyawasam<sup>168</sup> used monkeys to observe the affect of Super-EBA on tissue healing and concluded that the tissue response to Super-EBA as a root-end filling is acceptable and superior to amalgam.

## Mineral Trioxide Aggregate

According to the US patent published by Torabinejad et al.<sup>169</sup> in 1998, mineral trioxide aggregate is a Type I Portland cement available commercially as the Colton Fast-Set brand of the California Portland Cement Co. This cement has the following dry composition by weight:

Component	Percentage by weight
SiO <sub>2</sub>	21%
Al <sub>2</sub> O <sub>3</sub>	4%
Fe <sub>2</sub> O <sub>3</sub>	5%
CaO	65%
MgO	2%
SO <sub>3</sub>	2.5%
Alkalies (Na <sub>2</sub> O, K <sub>2</sub> O)	0.5%

As Portland cement is not radiopaque by itself, bismuth oxide (Bi<sub>2</sub>O<sub>3</sub>) was added to the mix at a ratio of one part bismuth oxide to four parts Portland cement and the whole mixture was sterilized by autoclave.<sup>169</sup> Camilleri<sup>170</sup> studied the constitution of MTA and found that the primary difference between MTA and Portland cement was the lack of potassium and the presence of bismuth oxide. After the introduction of water to the mix, a colloidal gel is formed with particles of less than 1 µg, and calcium hydroxide with calcium silicate hydrate transforms into a poorly crystallized solid gel.<sup>171</sup> Then, a calcium precipitate is formed, reducing the ratio of calcium silicate and increasing the proportion of calcium hydroxide, which increases the pH of the compound.<sup>172</sup> The exact source of the calcium hydroxide produced during the hydration of MTA has been thought to be either tricalcium silicate<sup>172</sup> or tricalcium aluminate hydrogenation.<sup>173</sup>

Clinical dental applications for MTA are numerous. Arens et al.<sup>174</sup> published two case reports of repair of successful furcal perforations with MTA. When planning to be

used as a retrofilling material, the recommended powder-to-water ratio is between 10 percent and 40 percent, with the ideal being 3:1 or 25 percent by weight.<sup>169</sup>

MTA is available in both white mineral trioxide aggregate (WMTA) and gray mineral trioxide aggregate (GMTA) types. Asgary et al.<sup>175</sup> investigated chemical composition of WMTA and GMTA with the use of electron probe microanalysis and found that GMTA had higher concentrations of FeO (+1000%), Al<sub>2</sub>O<sub>3</sub> (+122%), and MgO (+130%). The markedly higher levels of FeO in GMTA are responsible for discoloration and staining of teeth, so the WMTA may be a more suitable material for treatments in the esthetic zone.<sup>176</sup> In another study, Asgary<sup>177</sup> used qualitative x-ray analysis of WMTA and GMTA and found that the crystal size of WMTA is eight times smaller than that of GMTA.

Some concerns over MTA's biocompatibility have been raised. Camilleri<sup>171</sup> showed that the presence of bismuth in MTA has posed some concerns over the material's biocompatibility when placed in an acid environment, such as inflamed periapical tissues. Bismuth oxide dissolves in an acidic environment, and the release of bismuth has been shown to negatively affect cell culture proliferations.<sup>178</sup> Also, Dammaschke<sup>173</sup> showed that surface sulfur in hydrated MTA is three times higher than in the dry powder, and that this surface layer may inhibit the uptake of more water and lengthen the setting reaction.

In a comprehensive review of the literature for MTA, the material's inventor, Mahmoud Torabinejad,<sup>179</sup> states that the differences among published studies regarding the chemical composition of MTA are related to the various liquids used to mix the MTA and the various equipment used to test its composition.

The physical properties of MTA can be influenced by the amount of liquid used and the pH of the liquid used when hydrating the powder.<sup>180, 181</sup> Walker<sup>182</sup> studied the flexural strength of MTA placed with one moistened surface versus two-sided hydration at time intervals up to 72 hours and found that 24 hour two-sided hydration was significantly stronger than all one-sided hydration samples and two-sided hydration samples of different durations. Both GMTA and WMTA expand while setting but research is conflicting about which material expands more. Chng et al.<sup>183</sup> found that WMTA expanding slightly more than GMTA. Storm et al.<sup>184</sup> investigated setting expansions for WMTA and GMTA in samples covered with sterile saline or Hank's balanced salt solution (HBSS) and found that GMTA expanded significantly more than WMTA in either water or HBSS. The pH value of MTA while mixing is 10.2 and then rises to a peak level of 12.5 at 3 hours.<sup>185</sup> Islam et al.<sup>186</sup> compared the pH of WMTA and GMTA and found WMTA to have a slightly higher pH of 13.0 compared to 12.8 for GMTA at 60 minutes. Firdland<sup>187</sup> conducted a 78-day solubility study of MTA and found that the pH of the solute was 11.88 at 24 hours and maintained a high pH ranging between 11.65 and 11.72 for the entire length of the study. The radiopacity of MTA has been reported at 7.17 mm aluminum equivalent.<sup>185</sup>

### Bioceramics

Bioceramics is a term applied to special ceramic materials that have been developed for applications in medicine and dentistry.<sup>188</sup> Depending upon the composition of ceramic utilized, these compounds may be bioinert (alumina, zirconium), restorable (tricalcium phosphate), bioactive (hydroxyapatite, bioactive glasses, or glass-ceramics) and porous for ingrowths of tissue (hydroxyapatite-coated metals, alumina).<sup>189</sup>

'Bioactive' bioceramics serve as a permanent scaffold that can become vascularized for new bone formation.<sup>188</sup> Brasseler USA (Savannah, GA) developed and introduced a new bioceramic putty called EndoSequence Root Repair Material (ERRM) that can be used as a retrofilling material for surgical endodontics. The material is composed of calcium silicates, monobasic calcium phosphate, zirconium oxide, tantalum oxide, proprietary fillers and thickening agents.<sup>190</sup> The material has nanosphere particles with a maximum diameter of  $1 \times 10^{-3} \mu\text{m}$  that allow for the material to enter dentinal tubules, be moistened by dentin liquid, and create a mechanical bond upon setting.<sup>26</sup> The manufacturer claims material does not shrink upon setting, has 30+ minutes of working time, is available as a putty and as a syringible paste, is bright white for easy identification clinically, and is highly radiopaque for easy identification on radiographs.<sup>191</sup> The tissue compatibility of Brasseler's bioceramic material has been investigated independently. AlAnezi et al.<sup>190</sup> used cultured mouse fibroblast cells to determine the cytotoxicity of EndoSequence Root Repair Material as compared with gray and white MTA and found that both set and fresh samples showed no significant cell viability differences. Damas et al.<sup>26</sup> investigated the cytotoxicity of EndoSequence Root Repair Material and EndoSequence Root Repair Putty compared to white MTA and MTA-Angelus using human dermal fibroblasts and found that both of the EndoSequence Root Repair Materials had similar cytotoxicity levels to those of ProRoot MTA and MTA-Angelus.

In 2011 Hansen et al.<sup>192</sup> compared the diffusion of hydroxyl ions for ERRM and WMTA through root dentin and found that although both materials showed diffusion of ions through dentin, the effect was less pronounced and of shorter duration for ERRM than WMTA.

## MICROLEAKAGE STUDIES

Even with continuing advancements in canal debridement and disinfection, microorganisms may persist in root canal systems after treatment is complete.<sup>193</sup> These organisms can possess virulence factors that, when allowed to contact the periapical tissues, may lead to continued or new endodontic infections.<sup>194</sup> It is therefore desirable to create a hermetic seal to entomb the microorganisms that may be left behind after thorough chemomechanical debridement.<sup>195, 196</sup> Many methods for obturation have been suggested, but given the complexities of the root canal system, vast flora of bacteria involved in the development of apical periodontitis, variations in operator skill, and divergent protocols for assessment, direct comparison of various obturation methods are often difficult to perform.<sup>196</sup>

*In-vitro* methodologies in microleakage assessment allow for a more direct comparison of materials, standardizing canal length and shape, operator technique, type of leakage challenge, with their main goal being that the results are often reproducible.<sup>197</sup> Often, however, the results are not reproducible. Tamse et al.<sup>198</sup> conducted a study comparing the apical leakage in teeth obturated in the exact same fashion as shown by four different dyes with two different evaluation methods and found that the method of assessment caused a significant difference in the leakage observed. His data indicated that dye leakage study designs influence the outcomes of the study and are not standardized. Criticisms like these in study design must have garnered some attention,



because in 2007 the Editorial Board of the *Journal of Endodontics* published an editorial stating that it would decline publication of sealability studies.<sup>199</sup>

### Radioisotope Studies

The use of radioactive isotopes and autoradiography to demonstrate permeability of tooth structure was first conducted in 1950 by Wainwright and Lemoine.<sup>200</sup>

Fremlin and Mathieson<sup>201</sup> demonstrated that contamination of samples is possible as the radioactive solutions were shown to cross various materials commonly used as barriers. Dow et al.<sup>202</sup> used radioactive iodine I<sup>131</sup> in a water-soluble solution that was allowed to permeate obturated root canals. The radioactive isotopes release of radiation was able to expose dental radiography films, a process called ‘autoradiography,’ and then the intensity of these developed images were used as an indicator of dye leakage. Other authors have used various radioactive isotopes including <sup>45</sup>calcium, <sup>14</sup>carbon, and <sup>125</sup>Iodine.<sup>203</sup> The development and use of radioactive isotopes as a tracer was based on the theory that the isotopes could more easily penetrate the test materials than traditional dye tests, but the results of a study by Matloff et al.<sup>203</sup> would demonstrate this not to be the case. Their study found little difference between the isotope tracers and traditional dye leakage results. Going et al.<sup>204</sup> were able to demonstrate that the penetration of dyes into the margins of dental fillings was controlled in part by the molecular size and ionic charge of the particle.

### Dye Studies

Dye leakage studies have been performed on the gamut of retrofilling materials utilizing several types of dye including methylene blue,<sup>205-209</sup> fuchsin, rhodamine B,<sup>209,210</sup>

silver nitrate,<sup>211</sup> India ink,<sup>212</sup> and Pelican ink.<sup>213</sup> Critics of the dye leakage study say that the dye's molecular size, pH, and chemical reactivity will all affect the degree to which the dye will penetrate in leakage assessments.<sup>214</sup> For example, India ink molecules are much smaller and lighter than many bacterial virulence factors, which could lead to an overestimation of a material's leakage,<sup>215</sup> and methylene blue dye can be dissolved during the demineralization and clearing process leading to an underestimation of a material's leakage.<sup>216</sup> However, in a study by Barthel et al.<sup>217</sup> the molecular size of the penetrating agent was not a significant factor in determining the root canal fillings sealability. Wu, De Gee, and Wesselink<sup>218</sup> criticized dye penetration studies by submitting that penetration of dye along root canal fillings may be hindered by air entrapped in voids within the root canal system. They recommended that dye penetration should be performed under reduced pressure.

Some authors have questioned the clinical relevance of dye leakage studies. Pichardo<sup>212</sup> in 2006 determined that storing teeth in formalin for four weeks prior to a dye leakage study significantly decreases the amount of dye leakage in comparison to freshly extracted teeth. In 2001 Oliver and Abbott<sup>219</sup> conducted a study to determine if a correlation exists between apical dye penetration and the clinical performance of root fillings. In their study, they performed apical dye tests on 116 recently extracted teeth classified as having either successful or unsuccessful endodontic treatment based on clinical signs and symptoms and found that 99.5 percent of all teeth studied showed dye penetration. They concluded that clinically placed fillings do not provide an apical seal that prevents fluid penetration, and therefore the outcomes of treatment cannot be predicted from the results of apical dye leakage studies. In 2006, Susini<sup>220</sup> compared 84

endodontically filled-teeth to see if a correlation existed between the presence of an *in-vivo* periapical radiolucency and *ex-vivo* apical dye penetration on the same human teeth and found no correlation between apical dye penetration and the presence of a periapical radiolucency.

#### Electrochemical Studies

Jacobson<sup>221</sup> developed an electrochemical technique where flaws around fillings would allow for the flow of electricity when an electrolytic solution penetrated the flaw. The apparatus consisted of obturated teeth which had their external root surfaces coated with lacomite resin sparing the apex suspended in a 1.0-percent solution of potassium chloride. A steel rod anode was then placed in the access of the tooth and a steel cathode was placed in the solution. Leads connected the anode and cathode to a zero-resistance ohmmeter. As leakage occurred and an electrolytic current was generated, the ohmmeter would detect the flow, and a quantitative value for leakage could be recorded.<sup>221</sup> This basic method was used by von Fraunhofer to evaluate the electrochemical leakage of endodontic sealers/cements,<sup>222</sup> retrograde amalgams,<sup>223</sup> and to determine the effect of post space preparation on the endodontic seal.<sup>224</sup>

#### Fluid Filtration Studies

Fluid filtration was developed in 1986 by Pashley<sup>225</sup> and modified for use in root canals by Wu<sup>226</sup> in 1993 as a method whereby the sealing capacity of a material is measured by means of an air bubble moving inside a capillary tube. The apparatus consists of an obturated tooth with its apex sealed to a glass capillary tube filled with water at atmospheric pressure and its coronal access sealed to a tube filled with water that

is pressurized to force fluid through the tooth. If leakage occurs, the volume of water expressed through the capillary tube is measured as a function of time, giving values in  $\mu\text{l}/\text{min}$  and allowing for comparisons of different materials.<sup>227</sup> Advantages of this method are that the samples are not destroyed; results are recorded automatically; results are precise, and system sensitivity can be adjusted by adjusting the pressure through the system.<sup>228</sup>

Pommel and Camps<sup>227</sup> are critics of the method stating that some authors use pressures 100 times greater than the physiologic environment, leading to unreasonable conclusions. Miletic et al.<sup>229</sup> was critical of the lack of setting time that materials are allowed prior to testing, and so stored their samples in saline at 37°C for 1 year prior to testing and found results that were significantly different than similar studies with shorter setting times.

### Bacterial Studies

Timpawat et al.<sup>230</sup> considered the use of bacteria penetration in leakage studies to be of superior clinical significance and more biologically relevant than dye leakage studies. The basic study design was devised by Goldman et al.<sup>231</sup> in 1980 consists of two chambers, one inoculated with bacteria and one sterile, separated by the test specimen whereby passage of bacteria from the inoculum to the sterile chamber occur by leakage along the test specimen and as indicated by turbidity of the previously sterile chamber.<sup>232</sup> The study design produces qualitative rather than quantitative results, as even a single bacteria will multiply to create turbidity in the lower chamber.<sup>233</sup> Many different microorganisms have been used, including *Staphylococcus epidermidis*,<sup>217</sup> *Enterococcus faecalis*,<sup>230</sup> *Proteus mirabilis*,<sup>234</sup> *Staphylococcus epidermidis*,<sup>234</sup> *Candida albicans*,<sup>229</sup>

*Streptococcus mutans*,<sup>229</sup> *Streptococcus mitis*,<sup>229</sup> *Prevotella melaninogenica*,<sup>229</sup> *Lactobacillus acidophilus*,<sup>229</sup> *Actinomyces odontolyticus*,<sup>235</sup> *Pseudomonas fluorescens*,<sup>235</sup> *Fusobacterium nucleatum*,<sup>233</sup> and *Streptococcus salivarius*.<sup>232</sup> *Enterococcus faecalis* is often used, because it has been frequently identified in canals with persistent endodontic infections and is part of the normal oral flora.<sup>236</sup>

Weaknesses in this study design exist. In a bacterial leakage study comparing Epiphany and Resilon with Roth's sealer and gutta-percha, Pitout et al.<sup>237</sup> found no difference between the groups, but commented that his results were unreliable owing to the known antibacterial effect of Roth's root canal sealer. Slutzky-Goldberg et al.<sup>238</sup> evaluated the antibacterial properties of four endodontic sealers due after hypothesizing that the antimicrobial property of the materials may have as much or more effect on the perceived seal as the material's property to adhere to dentin. Even though no viable bacteria may have leaked through to be detected by turbidity, their toxins may have still made it through, which can result clinically in periradicular pathosis.<sup>239</sup> After a failed attempt at keeping negative controls from becoming turbid in their own *in-vitro* evaluation, Rechenberg and Zehnder<sup>240</sup> conducted a systematic review of microbial leakage studies and found that most microbial leakage studies do not have sufficient controls to account for the possibility of accidental leakage via a route other than through the root canal space. They concluded that microbial leakage study designs published prior to 2011 are not suitable to compare differences in permanent root canal fillings, and that further investigation should be performed to address the problem of alternate routes of microbial leakage in a two-chamber model.

## METHODS AND MATERIALS

## SELECTION OF TEETH

Sixty-two human, single-rooted, mandibular premolars were used for this study (Figure 3). All teeth were collected without identifiers from the Oral Health Research Institute's teeth collection program (IRB approval #NS0911-07). Specific criteria were met for tooth selection. Radiographs were taken in the mesial-distal direction to confirm that a Type I root canal system was present (Figure 4). Teeth with abnormal canal anatomy and abnormal root morphology, including obvious lateral canals, extensive caries, or root fracture were excluded. Teeth were inspected for root surface cracks using the surgical operating microscope at X5 and X20 magnification (Figures 6 and 7).

Once the teeth were selected, calculus and soft tissue debris were removed from the root surface with hand-scaling instruments (Figure 5). Following debridement of the root surface, the teeth were immersed in 6.0-percent sodium hypochlorite (Clorox Co., Oakland, CA) for 30 minutes and then mechanically debrided with a soft brush. A size No.-10 K-type endodontic file (Kerr, Romulus, MI) was inserted into the root canal and advanced out the apical foramen of all teeth. All teeth with canals that could not be negotiated with a No.-10 K-type endodontic file were excluded from the study.

## CANAL INSTRUMENTATION

Working length determination was accomplished by inserting a No.-10 K-file into the canal and then allowing it to exit the apical foramen until just visible, and then after this distance was obtained, 1 mm was subtracted. The root canals were cleaned and

shaped using K-type endodontic files (Kerr, Romulus, MI), Gates Glidden drills (Brasseler, Savannah, GA), EndoSequence rotary files, 0.06 taper, size 20 to size 35 (Brasseler, Savannah, GA), while irrigating with 6.0-percent sodium hypochlorite (Figure 8). Number 15 and No.-20 K-type files were instrumented to the working length. Instrumentation of all teeth was performed using a crown-down technique with EndoSequence 0.06 tapered rotary files, size 20 to size 40, until a No.-35 file was instrumented to working length. Root canal irrigation was performed using 1 ml of 6.0-percent sodium hypochlorite between each file. A No.-10 K-type file was used to maintain apical patency. Upon completion of instrumentation, the smear layer was removed by rinsing with 2 ml of a 17-percent EDTA solution for 3 minutes with sonic activation from the EndoActivator (Figure 9). The teeth were then irrigated with a final rinse of 5 ml of 2.0-percent chlorhexidine gluconate. Following final irrigation, the canals were dried with sterile, coarse paper points. To prevent dehydration, all roots were handled using water-moistened gauze during resection and instrumentation.

#### ROOT-END RESECTION

Master gutta-percha points without sealer were placed in the prepared canal at working length prior to root-end resection. The remaining coronal gutta-percha cone served as a matrix for retrofill placement. Resection of the apical 3 mm of root was completed using carbide burs in high-speed handpieces (Figure 10). Apical retropreparation was then completed by using zirconium-coated ultrasonic instrumentation to a depth of 3 mm (Figure 11). Adequate preparation of the root end was verified by the passive seating of root-end pluggers for retrofill. The external root surfaces of the prepared teeth were then coated in dentin bonding agent (Figure 12).



## ROOT-END FILLING

Root-end filling was accomplished with either ProRoot MTA or Brasseler's ERRM according to the manufacturer's instructions. ProRoot MTA was mixed in a 3:1 powder-to-liquid ratio with sterile water. The MTA was carried to the preparation with the Micro Apical Placement (MAP) system carrier and condensed with a tight-fitting microplugger. Brasseler's ERRM was rolled into thin aliquots on a glass slab and carried to the preparation on the tip of a CK2 surgical knife. The ERRM was then condensed in the preparation with a tight-fitting microplugger and sections of sterile paper points held in cotton forceps. Radiographs of root-end fillings were taken to verify length and density of restoration (Figure 13). Root-end fillings with visible voids, with less than 3 mm of material, or with more than 3 mm of material were excluded. Restored roots were then allowed to set for 24 hours at 37°C and 100-percent humidity prior to the investigation of microleakage. Cotton pledgets soaked in sterile water were applied to root-end fillings after placement to provide setting moisture for materials during the entire setting period.

## ASSIGNMENT OF TEETH

Specimens will be randomly assigned to two groups of 27 teeth. The two groups, designated Group A (ProRoot MTA) and Group B (Brasseler EndoSequence Root Repair Material or ERRM) served as the experimental groups. Two groups each containing two specimens served as positive and negative controls, Group (+) and Group (-), respectively. The positive and negative control groups ensured the bacterial microleakage apparatus was working properly. The positive control consisted of two teeth prepared, but not obturated or retrofilled to allow free communication of the bacteria in the canal with the growth medium in the lower chamber. The negative control

consisted of two unprepared teeth coated with dentin bonding agent to seal the apical opening and the dentinal tubules. The negative control was to have no leakage. These controls were to verify the proper set-up of the fluid filtration apparatus.

#### MICROBIAL LEAKAGE APPARATUS

A microbial leakage apparatus was constructed using a 20-ml glass scintillation vial in which a tooth is seated in the vial opening and sealed using sticky wax (Figures 14 and 15). The end of the tooth was suspended in the lid of the plastic vial so that when a tooth was placed in the lid of the vial, the root protruded into the vial without contacting the floor of the vial. An impression material application tip was cemented in the orifice of the tooth and secured with resin. The lower chamber of the apparatus, created by the space between the root tip and floor of the plastic vial, was filled with sterile tryptic soy broth (TSB) containing streptomycin (2000 µg/ml) (Figure 17). The upper chamber of the apparatus, that space above the canal orifice of the tooth, was filled with TSB-inoculated with *Enterococcus faecalis* (Figure 18). The strain of *E. faecalis* used in this experiment was resistant to the concentration of streptomycin mentioned above. Fresh medium and *E. faecalis* were added to the upper chamber every three days to ensure live bacteria were present during the entire investigation period.

A positive incidence of leakage was determined by turbidity of the growth medium in the lower chamber (Figure 16). Samples of the lower chamber medium were collected and plated on the day that turbidity was observed to verify the presence of *E. faecalis*.

## STATISTICAL METHODS

The presence of microleakage was compared between groups using a Fisher's Exact test. The time to microleakage was compared between groups using a log-rank test.

## RESULTS

The determination of bacterial microleakage was made by the observation of turbidity in the lower chamber. Observations for turbidity were made and recorded daily for 40 days. At Day 0, no turbidity was observed in any of the experimental samples or in the positive and negative controls. At Day 1, both positive controls showed visible turbidity in the lower chamber, and none of the experimental groups or negative controls had become turbid. At Day 2, one sample from the EndoSequence Root Repair Material (ERRM) had become turbid and all other samples and negative controls were still uncontaminated. At Day 3, no leakage was observed. At Day 4, turbidity was observed in one sample in the ProRoot MTA group with all other samples and negative controls showing no signs of leakage. At Day 5, a second sample in the ProRoot MTA group became turbid, and all other samples and negative controls remained uncontaminated. For Day 6 through Day 40, none of the other samples and none of the controls leaked. In this study, all the positive controls became turbid within 24 hours of inoculation, and all negative controls stayed uncontaminated for the entire observational period.

Four percent of the ERRM group leaked and 7 percent of the ProRoot MTA group leaked during the observational period. One of the ERRM group leaked and 2 of the ProRoot MTA group leaked during the observational period. This difference of proportion of samples with microleakage ( $p = 1.00$ ) and time to microleakage ( $p = 0.57$ ) were not significantly different between ERRM and MTA.

FIGURES AND TABLES



FIGURE 1. Manufacturer's packaging for ProRoot Mineral Trioxide Aggregate.



FIGURE 2. Manufacturer's packaging for Brasseler's EndoSequence Root Repair Putty.





FIGURE 3. Sixty-two single-rooted human teeth selected for this study.



FIGURE 4. Digital radiograph of tooth in proximal view to ensure type I system.

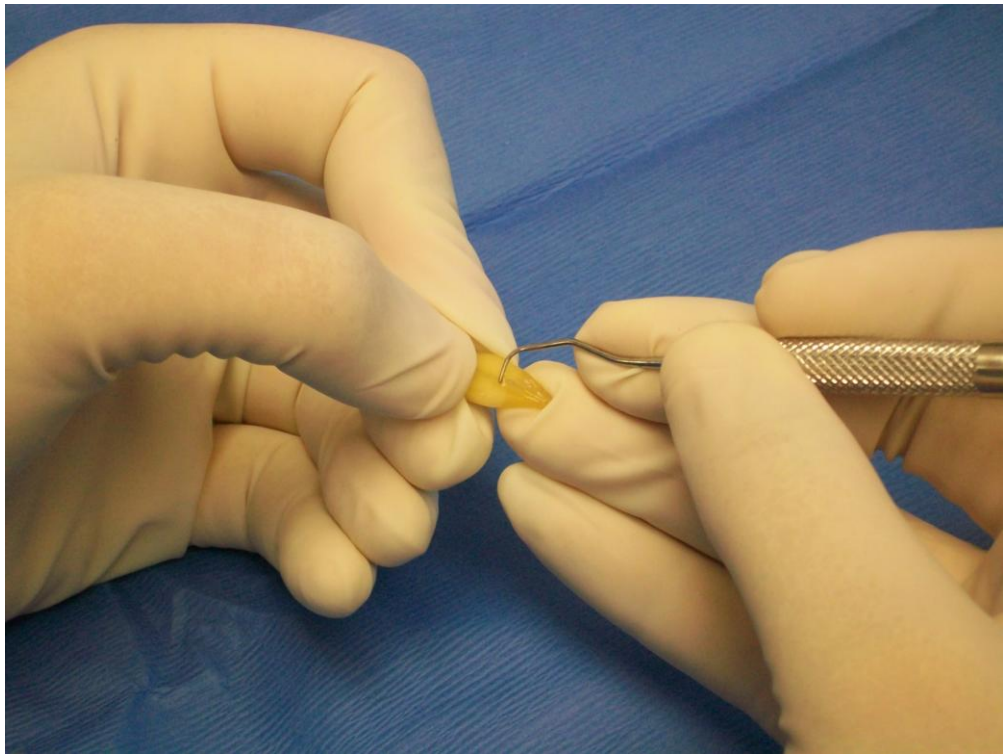


FIGURE 5. Scaling of debris from external root surface.



FIGURE 6. Inspection of external root surface to screen for cracks at X5 magnification.



FIGURE 7. Inspection of external root surface at X20 magnification to screen for cracks.



FIGURE 8. Brasseler EndoSequence NiTi rotary files, 0.06 taper.





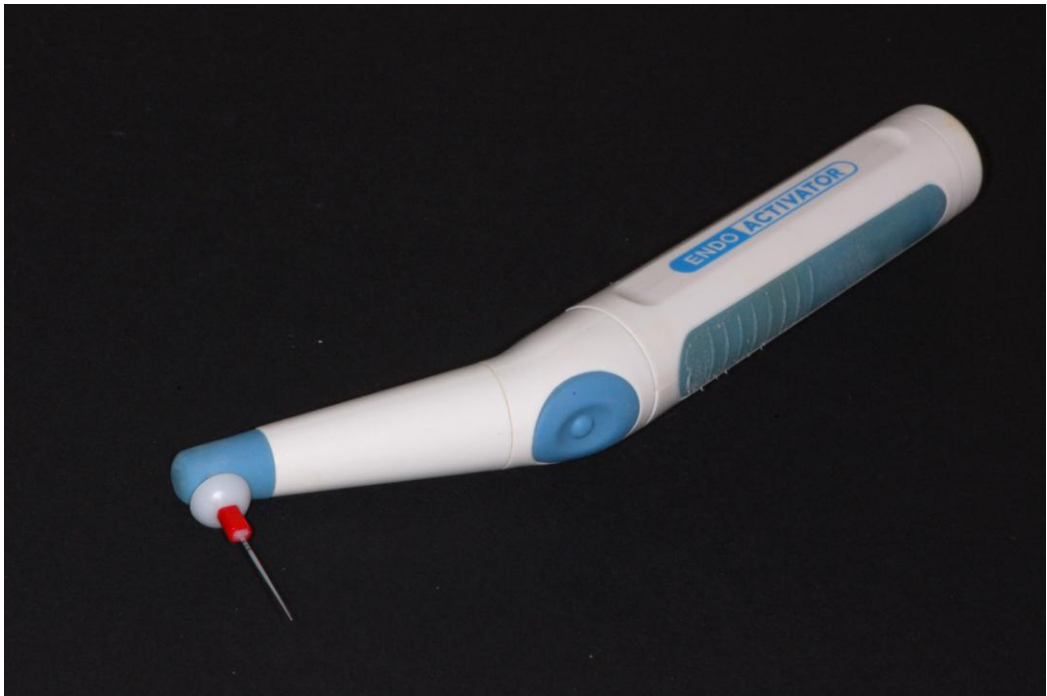


FIGURE 9. EndoActivator.



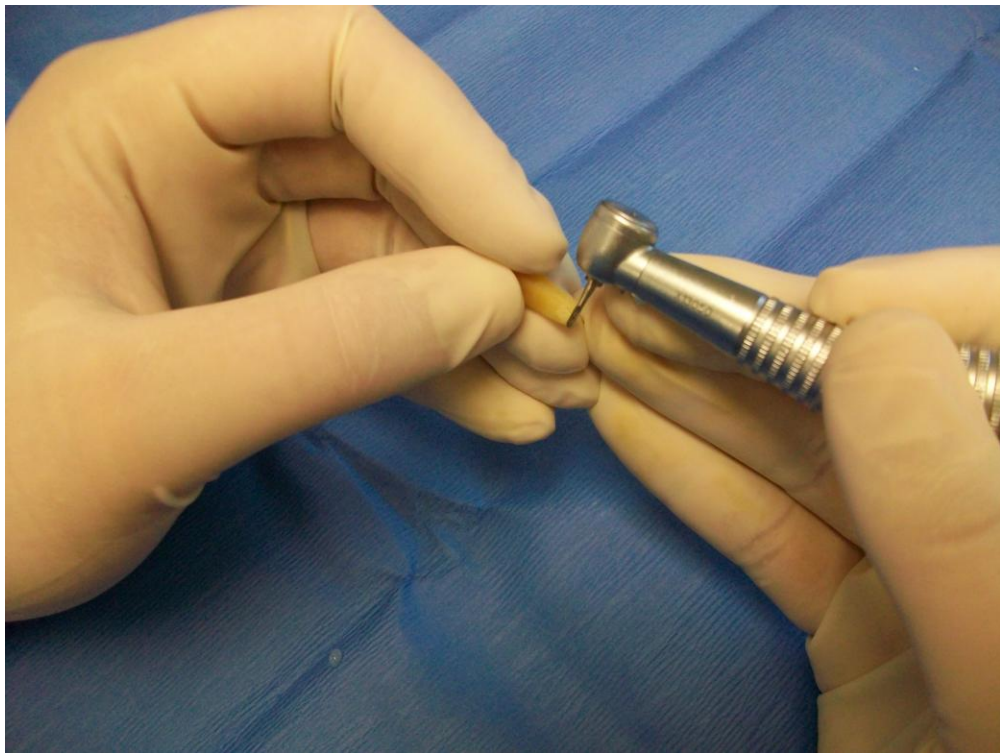


FIGURE 10. Root resection of apical 3 mm by using high-speed carbide burs.

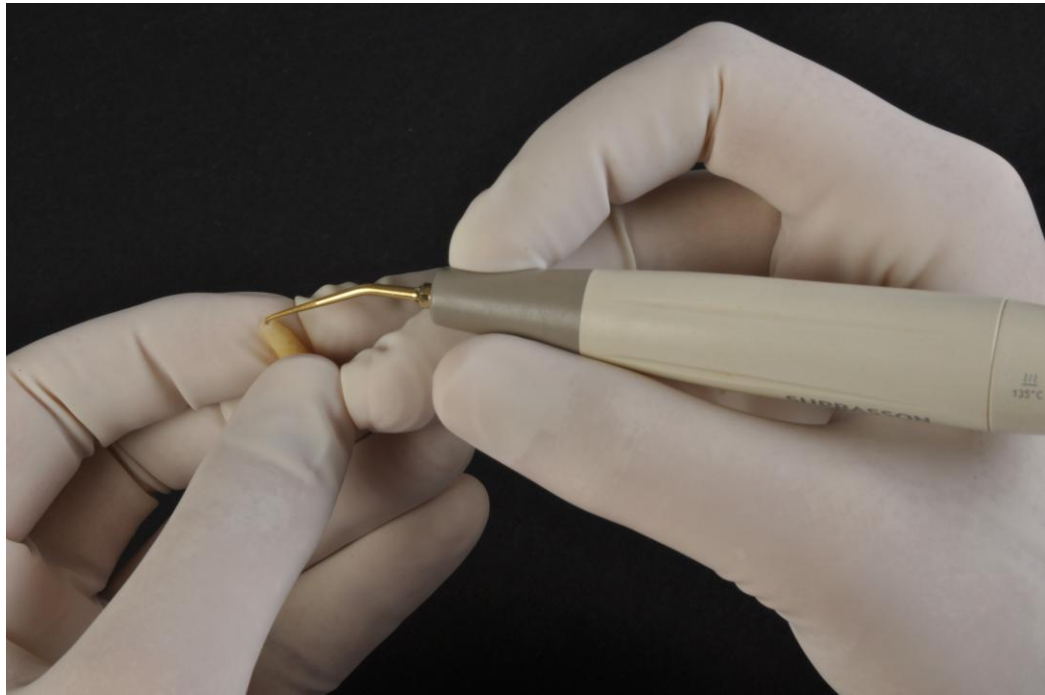


FIGURE 11. Ultrasonic root end preparation.

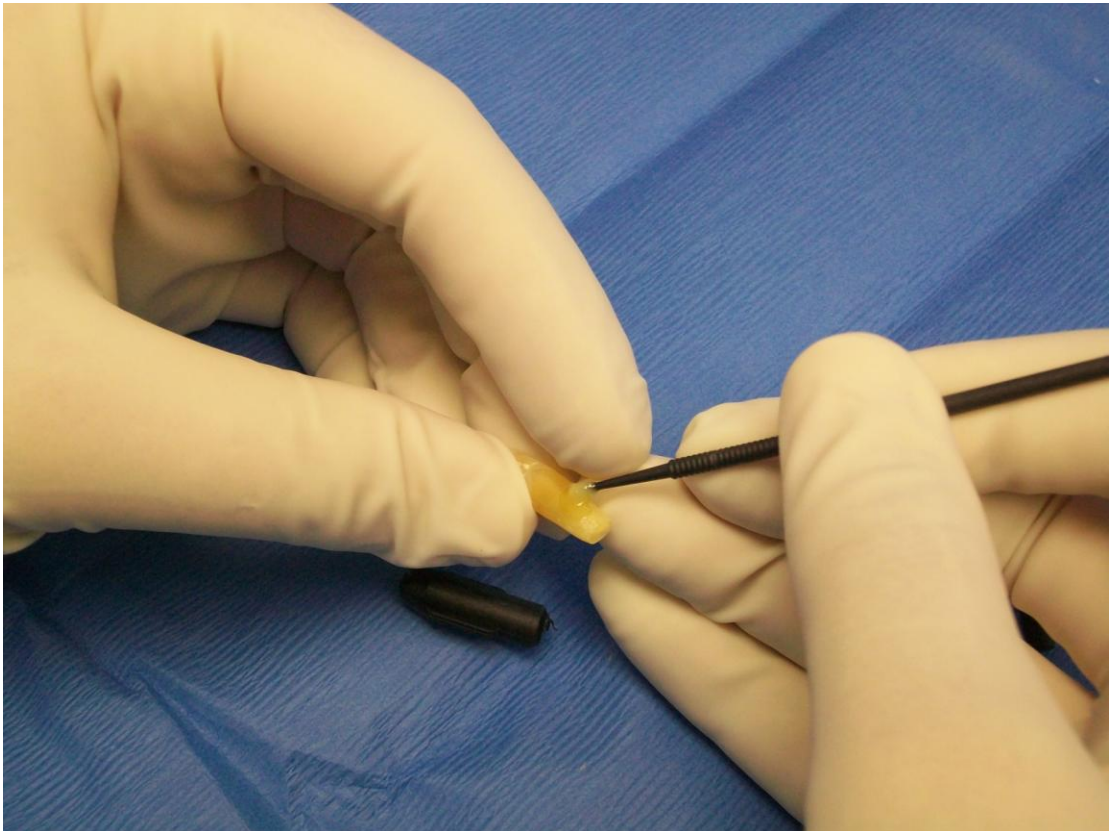


FIGURE 12. Application of dentin bonding agent to external root surface.

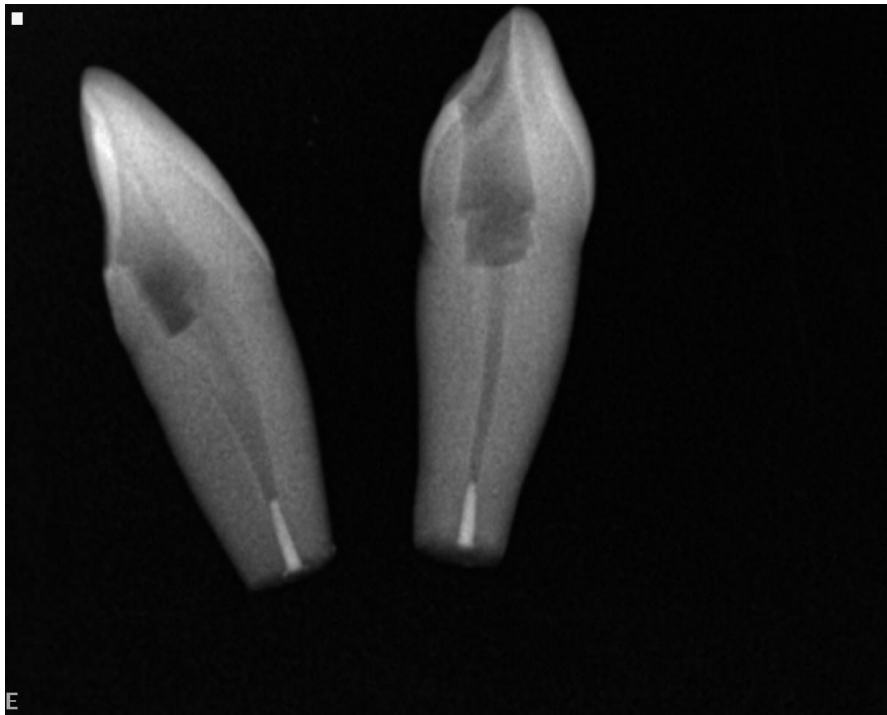


FIGURE 13. Radiographic inspection of root end filling materials.

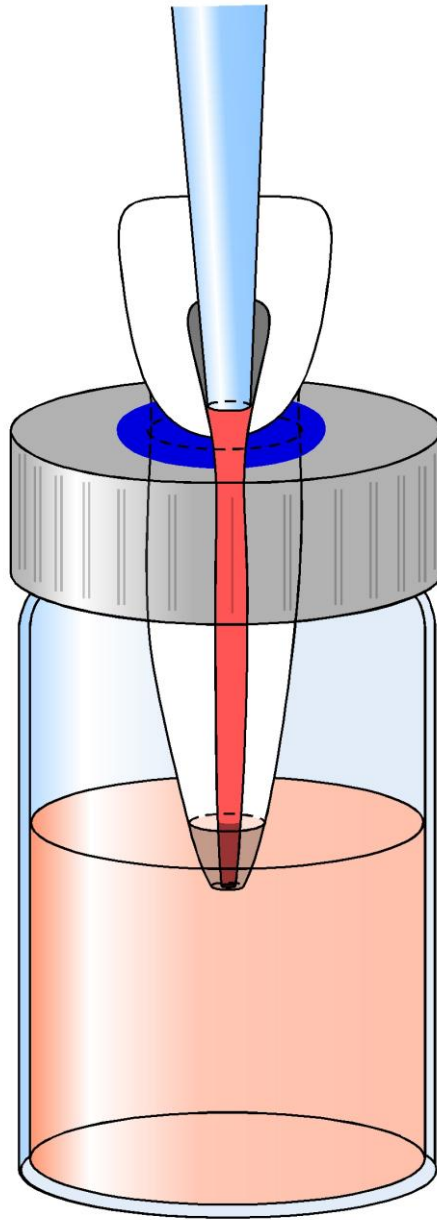


FIGURE 14. Schematic of assembled microbial leakage apparatus.



FIGURE 15. Photograph of assembled microbial leakage apparatus.



FIGURE 16. Photograph of turbid apparatus (left) and clear apparatus (right).



FIGURE 17. Photograph of assembled apparatuses ready for inoculation.





FIGURE 18. Scanning electron microscope image of *Enterococcus faecalis*.

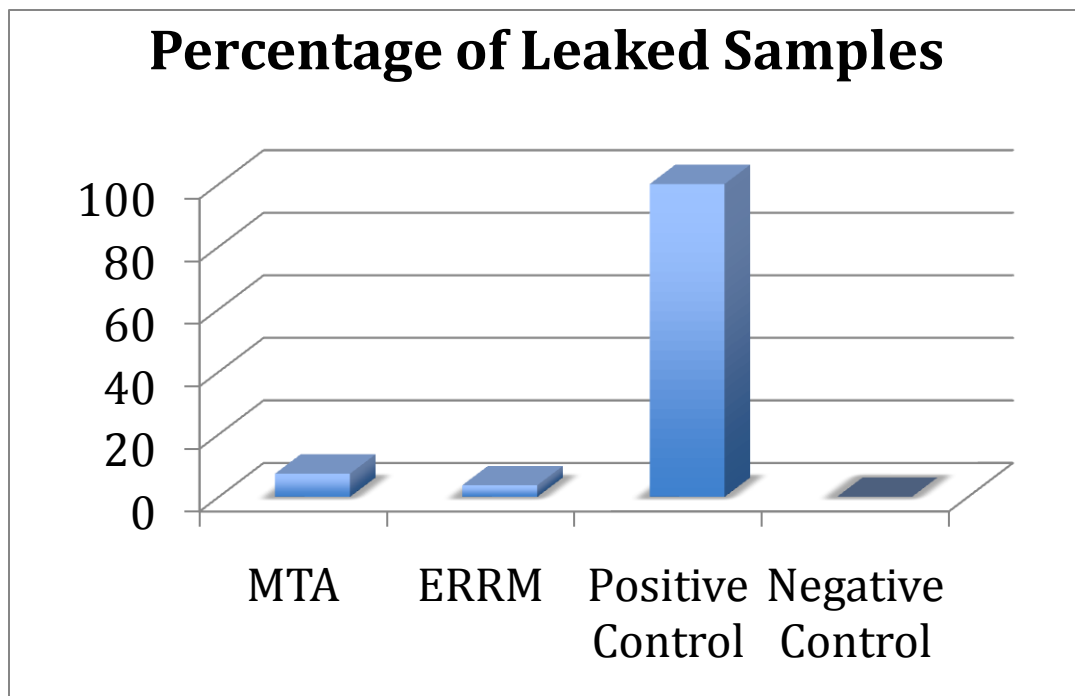


FIGURE 19. Bar graph showing percentage of leaked samples for ProRoot Mineral Trioxide Aggregate (MTA), EndoSequence Root Repair Material (ERRM), positive control, and negative control.

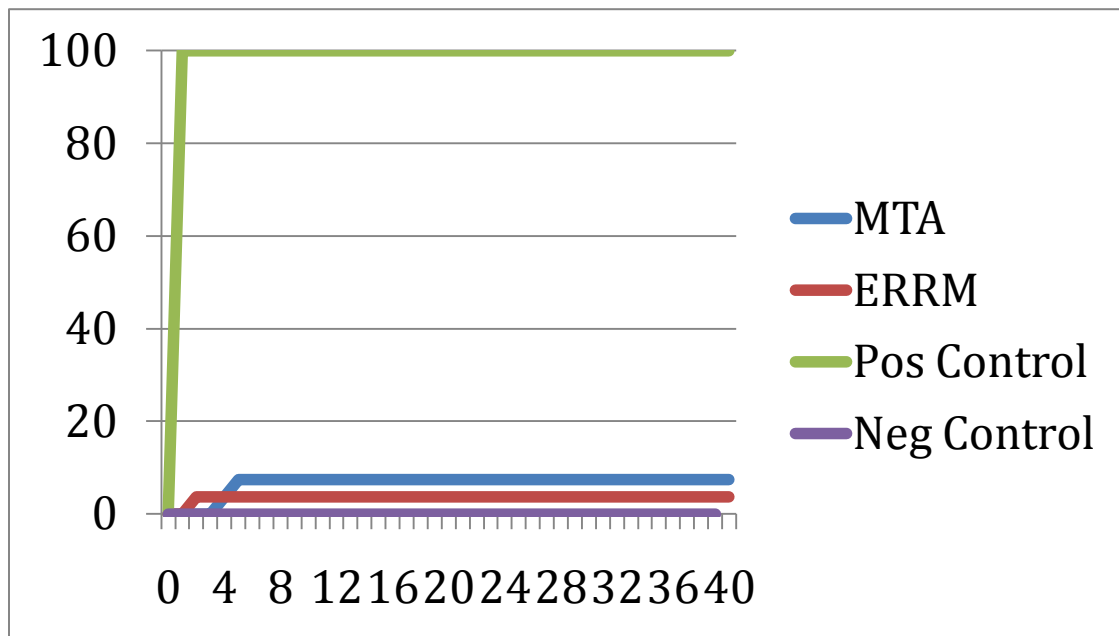


FIGURE 20. Line graph showing days to leakage for samples of ProRoot Mineral Trioxide Aggregate (MTA), EndoSequence Root Repair Material (ERRM), positive control, and negative control.

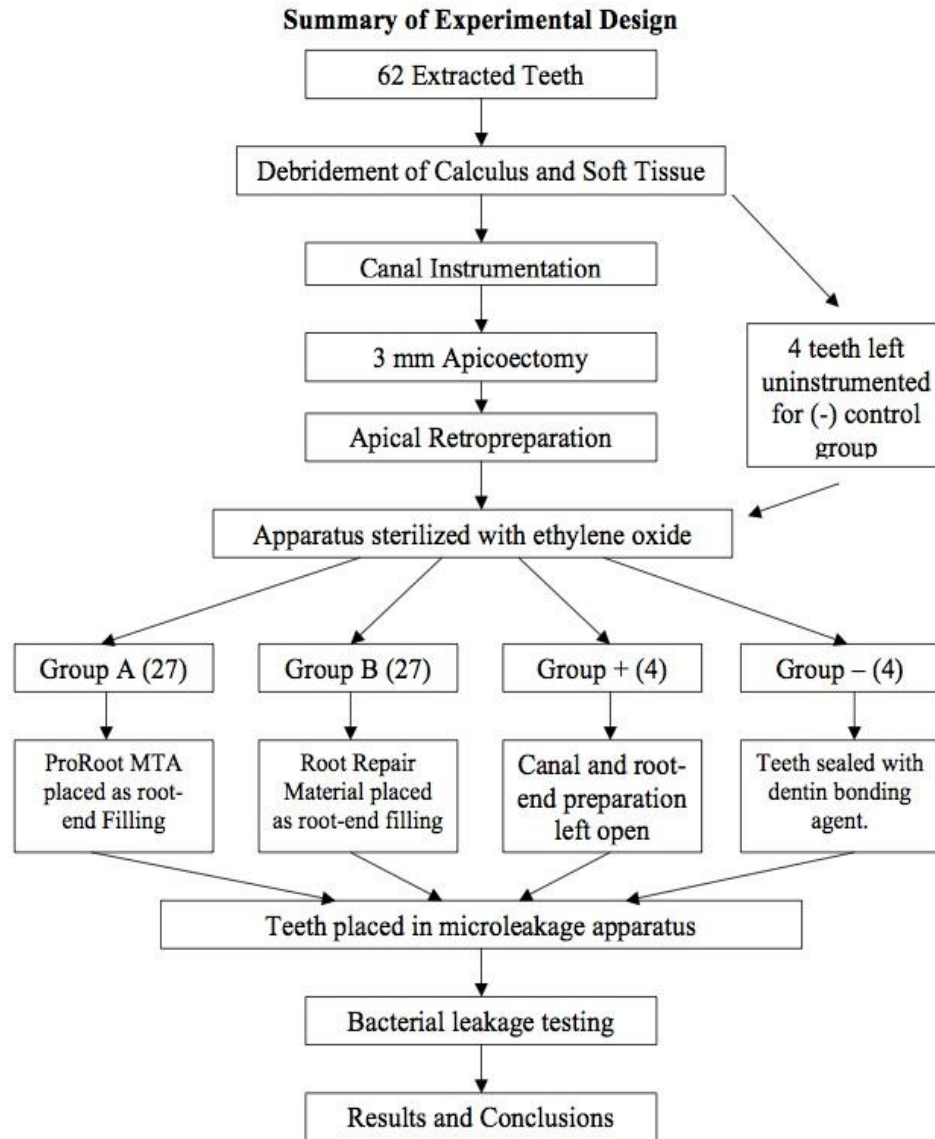


FIGURE 21. Summary of experimental design.

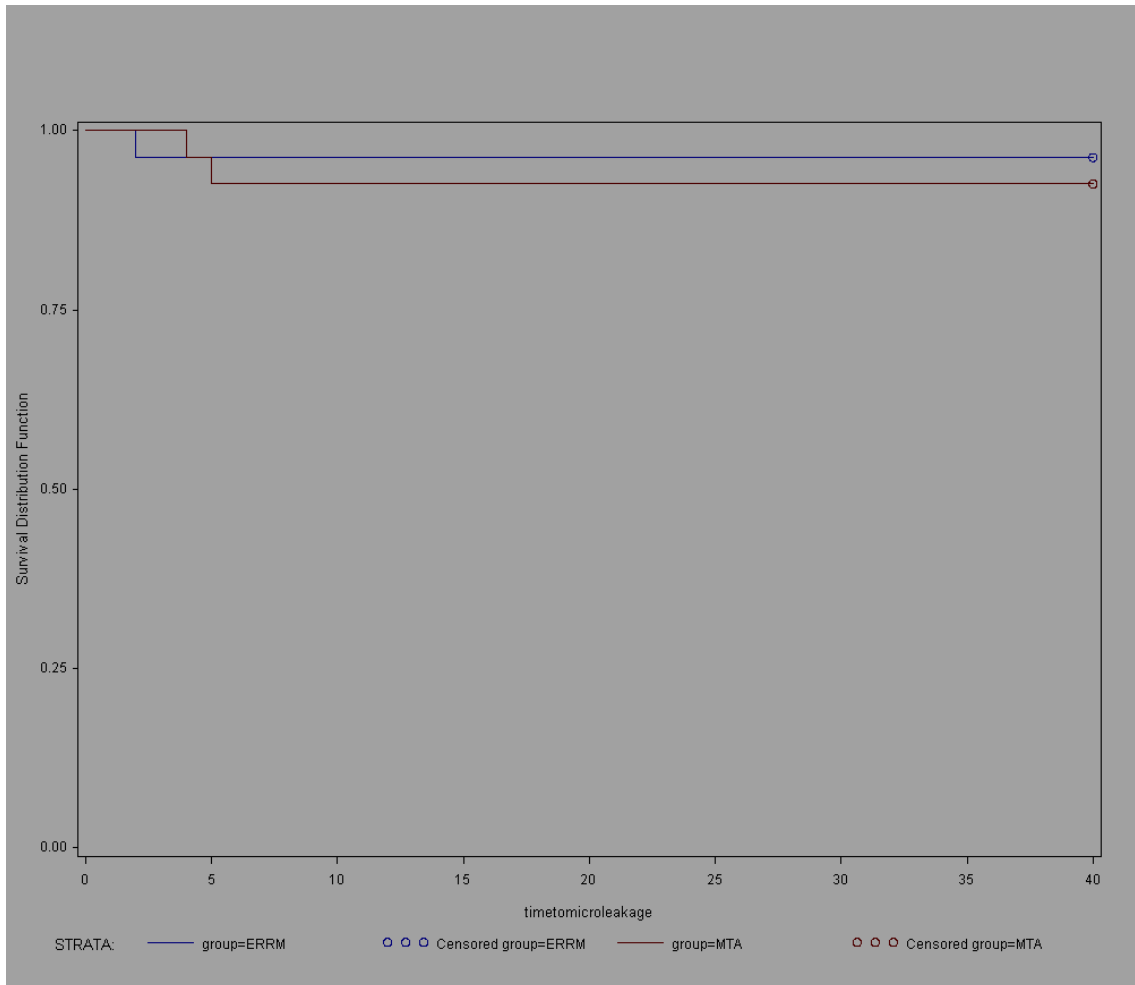


FIGURE 22. Graph showing survival distribution factor as a function of time to leakage.

TABLE I

Percentage of ERRM and MTA samples with micoleakage

Group	Microleakage
ERRM	4%
MTA	7%

TABLE II

Number of ERRM and MTA samples with micoleakage

Group	Microleakage
ERRM	1
MTA	2

DISCUSSION



Dental material development is fundamental to the improvement of clinical outcomes in dentistry. Along with advancements in equipment technology, procedural improvements, and therapeutic knowledge, the integration of these new materials into clinical practice can open treatment horizons that would have been otherwise impossible to envision.

Mineral trioxide aggregate (MTA) has been a mainstay of clinical endodontics since its introduction in the mid-1990s. Clinical application of the material opened new doors for dramatically improved outcomes for surgical endodontics, perforation repair, immature apex management, vital pulp therapy, resorption repair, and regenerative endodontics.<sup>179</sup> In fact, the development of this material created such a quantum leap forward in success rates for surgical endodontics that studies describing outcomes for the apicoectomy with root-end filling have to be categorized according to the material being used.<sup>48</sup> The so-called traditional endodontic surgery with no MTA, no ultrasonic handpieces, and no surgical operating microscope had a success rate of 57 percent,<sup>241</sup> while the modern endodontic surgery with MTA, ultrasonic handpieces, and surgical operating microscope boasts success rates of 92 percent or higher.<sup>242</sup> The improvement in these success rates is due in large part to the application of MTA.

MTA, however, is not perfect. In preparing teeth for this investigation, the handling differences between the MTA and ERRM became very apparent. Variations in water-to-powder ratio for MTA could produce a material that was either too fluid to load

in a carrier, or too dry and brittle to place. The ERRM, on the other hand, came from the manufacturer as pre-mixed putty with an ideal consistency for root-end filling placement. The MTA was often difficult to condense in the root-end preparation without void production, while the ERRM putty was easily condensed with root-end pluggers and trimmed paper points to create a dense, void-free filling. Rinsing or burnishing the MTA samples prior to setting would often result in a washout of material from the preparation, which then required replacement of the missing material. ERRM appeared more radiopaque than MTA in radiographs of the root-end fillings of the samples. The ERRM, however, could be rinsed and burnished with no material loss. These differences in handling characteristics became very noticeable during preparation of the samples for the study. In this research, the ease and speed with which the ERRM samples were completed are preferred as compared with the relatively technique sensitive and slower preparation of the MTA samples. The superior handling of the ERRM is a significant advantage at chairside that should not be understated. Even though MTA investigations have demonstrated excellent chemical, physical, and biological properties, all these benefits can be nullified if the material is not properly placed and well-adapted to the root-end preparation. The development of the ERRM putty increases the ease with which root-end fillings will be adequate to seal the root canal system.

Development of a suitable experimental apparatus for this investigation was a challenge. Several experimental designs from different authors were considered, reproduced, and tested. Initially, an apparatus design by Williamson et al.<sup>243</sup> was considered in which obturated teeth were situated in an upper chamber comprised of an Eppendorf tube with the tip removed so that a tooth might be sealed in the tube and

suspended in a larger scintillation vial that served as the lower chamber. This particular study's design was appealing because it modeled the work of earlier authors,<sup>244, 245</sup> but addressed the problem that sterilization of the teeth may have on the experimental dental materials, the root dentin, and the viability of the inoculated bacteria through gamma irradiation of the assembled experimental apparatus. A pilot study attempted to recreate Williamson's apparatus with her gamma irradiation protocol, but the results showed exuberant growth of contamination bacteria and fungi. The pilot study was therefore a categorical failure.

As a second pilot study, we pursued the development of a selective broth and resistant bacteria to serve as a biological indicator of leakage in the present investigation. Tryptic soy broth containing streptomycin was chosen as the selective broth, and *E. faecalis* was used as the test bacteria due to its presence in persistent endodontic infection and its antimicrobial resistance. A strain of *E. faecalis* was grown on plates of agar with increasing concentrations of the streptomycin in order to develop a streptomycin-resistant strain of *E. faecalis*. This strain was then tested for growth in tryptic soy broth with streptomycin added to a concentration of 2000 µg/ml, and it survived. As an additional effort to avoid contamination with fungus as observed in the first pilot study, 30 mg/ml of ketoconazole was also added to the broth and the *E. faecalis* still grew in the selective broth milieu. During the preparation and filter sterilization of the selective broth, it was noticed that the ketoconazole was not completely dissolving in the selective broth. The chemical properties of ketoconazole were then researched, and it was found that ketoconazole is insoluble in water. The results of the second pilot study led to the development of a selective tryptic soy broth with streptomycin at a concentration of 2000

µg/ml and a recognized strain of *E. faecalis* resistant to streptomycin at this concentration.

A third pilot study was conducted to determine the most suitable material for creating a bacteria-tight seal between the prepared tooth and the Eppendorf tube in the upper chamber. Samples were prepared using sticky wax, vinylpolysiloxane impression material, nail polish, Super glue, flowable resin with dentin bonding agent, packable resin with bonding agent, and petroleum jelly. The samples were then sterilized using ethylene oxide sterilization, loaded with sterile broth in the lower chamber, and inoculated in the upper chamber with *E. faecalis* containing broth. The samples were inoculated with fresh broth every three days for 30 days, and of the samples, seal failure was noted in every sample except for the samples with sticky wax. The results of this pilot study were as surprising as they were encouraging, because the sticky wax was obviously affected by the ethylene oxide sterilization process with the development of voids within the seal during the sterilization.

An abstract prepared by Zehnder and Rechenberg<sup>246</sup> for the 2010 American Association of Endodontists Annual Session titled “Bacterial Leakage Studies: Where is the Leak?” described the use of an apparatus identical to the apparatus used in the third pilot study. They showed that leakage in these designs occurred at eight weeks and resulted from a failure of the seal between the tooth and the upper chamber. Prompted by the findings of Zehnder and Rechenberg, the investigators in the present study attempted to find an alternative study design. A 1995 study by Torabinejad et al.<sup>247</sup> used the internal canal space of the tooth itself as the upper chamber, and the tooth could then be suspended over a second chamber with no interface between the upper and lower

chambers except through the root canal system. Their study design was adapted by the investigators in the present study and modified to include the selective broth and resistant bacteria from the previous pilot studies to create an acceptable apparatus.

A fourth pilot study was conducted to test the negative controls in an apparatus adapted from Torabinejad using external root sealing with dentin bonding agent, ethylene oxide sterilization, UV sterilization, selective broth, and resistant *E. faecalis*. The results of this pilot study demonstrated no contamination of the system and produced stable negative controls that remained sterile over the time period for the present investigation.

Much thought, research, and effort were spent in the development of the experimental apparatus for the present study. The apparatus used in this investigation should be considered a reliable method to test *in-vitro* bacterial microleakage with root-end filling materials and orthograde obturation materials in future studies.

The results of this investigation indicated no statistically significant difference ( $P > 0.05$ ) in bacterial leakage between the MTA and ERRM groups. Both groups were extremely resistant to leakage. The few specimens that leaked (#1 and #2 out of 27) did so in the beginning of the investigation, but no other specimens leaked after the first five days of the observation period. It is hypothesized that the early failure of these specimens could have been due to undetected cracks in the experimental teeth, undetected voids in the obturation material, or exposure of accessory canals of the apical delta. The samples that did leak were collected and examined again with radiographs and magnification, but no errors in obturation or cracks were observed.

The potential of a material to create a bacteria-tight seal at the root apex is highly desirable in the alleviation of apical periodontitis. The results of this investigation

showed that ERRM was at least as good as MTA in the prevention of bacterial leakage. This excellent seal coupled with the improved handling characteristics of the ERRM make it a highly desirable material for root-end fillings.

SUMMARY AND CONCLUSIONS

In this study, 62 human, single-rooted teeth were prepared for root-end fillings and placed in a dual-chamber bacterial microleakage apparatus for 40 days to determine time to leakage. Survival analysis was used to compare the two groups with a Kaplan-Meier plot to visualize the results and a nonparametric log-rank test for the group comparison.

This study was the first to study bacterial microleakage of Brasseler's EndoSequence Root Repair Material. In our present study, we compared the bacterial microleakage of Brasseler's EndoSequence Root Repair Material (ERRM) with ProRoot Mineral Trioxide Aggregate (MTA) using an *in-vitro* simulation of root-end fillings. This *in-vitro* study used a novel adaptation of previous bacterial leakage apparatus to overcome the limitations of past designs and deliver meaningful data on leakage.

ERRM was as good as MTA in resisting bacterial leakage, with no statistically significant differences ( $p > 0.05$ ) in time to leakage observed between the two materials. ERRM also exhibited superior handling characteristics to MTA and was noticeably easier to place in the root-end preparation. Based on the findings of this study, ERRM is a superior alternative to MTA for use as a root-end filling material.



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ABSTRACT

AN *IN-VITRO* COMPARISON OF MICROLEAKAGE WITH *E. FAECALIS* IN TEETH  
WITH ROOT-END FILLINGS OF PROROOT MTA AND BRASSELER'S  
ENDOSEQUENCE ROOT REPAIR PUTTY

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Brasseler USA (Savannah, GA) developed and introduced a bioceramic putty called EndoSequence Root Repair Material (ERRM) that can be used as a retrofilling material for surgical endodontics. The material is said to have many of the same chemical, physical, and biological properties as mineral trioxide aggregate (MTA), but with superior handling characteristics. The material is composed of calcium silicates, monobasic calcium phosphate, zirconium oxide, tantalum oxide, proprietary fillers, and thickening agents. ERRM is said by the manufacturer to bond to adjacent dentin, have no shrinkage, be highly biocompatible, hydrophilic, radiopaque, and antibacterial due to a

high pH during setting. Investigations on the sealing properties of this material have not yet been conducted.

The purpose of this study was to compare the microbial leakage of *Enterococcus faecalis* in teeth with root-end fillings using ProRoot MTA and Brasseler's ERRM in a dual-chamber bacterial leakage model as described by Torabinejad and colleagues. The aim of this investigation was to compare the bacterial microleakage of these two root-end filling materials exists.

Sixty-two human, single-rooted, mandibular premolars in which extraction was indicated were accessed and instrumented in an orthograde fashion with hand and rotary files. Root resection of the apical 3 mm was then completed and root-end retropreparations were created for placement of root-end filling material. Twenty-seven of these premolars had root-end fillings using ProRoot MTA and 27 had root-end fillings using ERRM. Two teeth were used as a positive control group with no root-end filling, and two other teeth were used as a negative control group and were sealed and coated with dentin bonding agent. The teeth were then evaluated for microleakage using a dual-chamber bacterial microleakage model for 40 days as described by Torabinejad and colleagues. Microleakage was determined by the presence of turbidity in the lower chamber of the apparatus and was assessed each day. Fresh samples of *E. faecalis* were used every three days to inoculate the apparatus and serve as a bacterial challenge for the materials. Results were recorded every day for 30 days. The outcome of interest (bacterial turbidity) and time-to-leakage (in days) were determined for each of the samples. Survival analysis was used to compare the two groups with a Kaplan-Meier plot to visualize the results and a nonparametric log-rank test for the group comparison.

The microleakage of ERRM was not statistically different ( $p > 0.05$ ) than leakage of ProRoot MTA when subjected to *E. faecalis* over the 40 day observation period. Both groups had a small number of early failures (within 4 days) and no leakage was observed for the remaining 40 days of the study. Therefore, the null hypothesis was rejected.

The results of this research support the use of either of these two materials when compared with the controls. The microleakage of Brasseler's EndoSequence Root Repair Material was at least as good as ProRoot Mineral Trioxide Aggregate when tested with *E. faecalis*.

## CURRICULUM VITAE



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