AN *IN-VITRO* COMPARISON OF THE MICROLEAKAGE OF REALSEAL/ RESILON AND REALSEAL SELF-ETCH/RESILON ROOT CANAL OBTURATION SYSTEM

by

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INTRODUCTION

The goals of root canal obturation are to eliminate bacterial leakage from the oral cavity and to achieve a fluid-tight seal along the dentinal wall.[1] Conventional obturation materials commonly used in endodontics are gutta-percha and root canal sealers. However, gutta-percha has no dentinal adhesion, lacks adequate rigidity, and has a tendency to shrink.[2] A new polymer-based obturation material, Resilon (RealSeal; SybronEndo, Orange, CA,) has been developed as a substitute for gutta-percha. The Resilon cones consist of a vinyl polyester material with methacrylate polymer, glass filler particles, and opacifiers added.[1] By combining methacrylate-based resin sealers with Resilon, the manufacturer claims that the system is based on a 'monoblock-bonding' concept. This concept entails that the obturation material is completely unified with the root canal dentin, which has obvious implications on microleakage and bond strength. Studies have been published demonstrating that Resilon exhibits less microbial leakage compared with gutta percha.[3, 4]

Since the 1980s, successful dentin adhesives have traditionally utilized a three-step system: etch and rinse, primer, and adhesive. This system based on three-step etch and rinse is marketed as a fourth-generation adhesive system.[5] Simplified adhesives were later developed and were classified as a two-step etch and rinse or fifth generation; a two-step self-etch or sixth generation; and a one-step self-etch or seventh generation.[5] These materials all depend on micro-mechanical interlocking from the collagen matrix for

retention. The traditional three-step adhesive system is still considered the gold standard because a combination of steps has generally been shown to result in an inferior bond.[6-8]

Currently in endodontics, one of the resin obturation systems using dentin adhesive technology is RealSeal (SybronEndo, Orange, CA), formerly known as Epiphany (Pentron Clinical Technologies, Wallingford, CT). The two-step self-etching (sixth generation) adhesive system utilizes an acidic primer applied to the dentin surface.[5] The acid and primer have been combined that eliminates one step in the process. After the acidic primer is dried, RealSeal sealer, a dual-cured resin cement, is applied to the root canal system and polymerized.

Recently, a new version of the RealSeal sealer, based on the self-adhesive cement concept (seventh generation) was introduced with the promise of optimizing clinical performance with a simplified one-step application procedure. Thus, the manufacturers have stated that the new RealSeal Self-Etch sealer (RealSeal SE; Pentron Clinical Technologies LLC, Wallingford, CT) could bond simultaneously to both radicular dentin and Resilon points.

Adhesive materials are commonly compared using bond strength and microleakage tests. According to Schwartz,[5] microleakage is considered more important for endodontic applications than bond strength. Studies have been done comparing the push-out bond strength of RealSeal/Resilon and RealSeal Self-Etch/Resilon to root dentin.[9] However, no study has yet been done comparing the microleakage of root canal systems obturated by using RealSeal/Resilon versus RealSeal SE/Resilon. Hence, the present study was designed to compare the microleakage of the root canal system when obturated with either RealSeal/Resilon or RealSeal SE/Resilon.

PURPOSE OF THE PRESENT STUDY

The purpose of this investigation was to evaluate and compare microleakage from the root canal system when obturated by using either RealSeal/Resilon or RealSeal SE/Resilon. The goal is to determine whether a significant difference exists in microleakage between these two groups.

HYPOTHESES

Null Hypothesis: There is no significant difference in microleakage between the RealSeal/Resilon group and RealSeal SE/Resilon group.

Alternative Hypothesis: Our alternative hypothesis is that Real SE/Resilon will show less microleakage in comparison with the RealSeal/Resilon group.

REVIEW OF LITERATURE

HISTORY OF ENDODONTICS

Mankind's fight against tooth pain has been waged since antiquity, and during this long battle, the evolution of endodontics has occurred. Our ancestors have extensively written about their excruciating experience with tooth pain. As Ingle points out, "[O]n Egyptian tablets, in Hebrew books, and from Chinese, Greek, and Roman medical writings are recorded descriptions and causes of this scourge."[10] Different approaches were taken, such as that of the Egyptians in 1500 BC, who wrote the ingredients for the medicament to cure the "gnawing of the blood in the tooth." [10] This cure was based on a combination of Gebu plant, onion, cake, dough, anest plant, and water. Not to be outdone, the Roman civilization came up with novel ideas ranging from a mouthwash made with boiled gallnuts to a concoction of roasted earthworms and spikenard ointment mixed with crushed eggs of spiders.[10]

In the dark, medieval age in Europe, there was a strong emphasis on the "tooth worm" theory as the main etiology behind tooth decay and pain.[11] The tooth worm was thought to be living in the "hollow portion of the tooth . . . gnawing at the structures of the tooth."[11,12] This led to the evolution in treating tooth pain based on "deworming technique,"[13] designed to use the perfume of the wax to draw out the tooth worms subsequently slaughtered by nails.The Arab civilization during the same time period was going through its age of scientific renaissance, and emphasis was placed on devitalizing the pulp through various means to cure toothache. For example, Abulcasis (1105-1122)

used a red-hot needle into the pulp through a tube aimed at cauterizing the pulp to control toothache.[10]

In the 18th and 19th century, definitive progress was made in the effort to treat tooth pain. Initially, some dentists like Shaerjab Spooner (1836) were using arsenic trioxide (protoplasmic poison) for pulp destruction. However, arsenic often leaked from the cavities, which lead to sloughing of the gingiva and eventual tooth extrusion along with severe pain.[14] Edwin Maynard (1838) developed the first root canal broach, which enabled dentists to enter canals and extirpate the pulp.[14] In 1867 Dr. Bowman popularized the use of gutta percha for filling root canals, and the use of arsenic was finally stopped.[10]

Until the 20th century, the process of extirpation of pulp to cure toothache was still an excruciating experience for the patient. This started to change in 1905 when Einhorn developed Novocain, used effectively in dentistry since 1925 by the usage of conduction anesthesia.[14] In the same era, Wilhelm Roentgen discovered x-rays, which enabled dentists not only to diagnose, but to visualize the results after the extirpation of pulp. Dentists were also starting to suspect the role of bacteria in causing pulpal infections, and various medicaments from phenols and formocresol to Dankin's solution (sodium hypochlorite) began to be used during root canal therapies.

Rapid progress in the treatment of tooth infections and subsequent pain management posed a challenge in the early 20th century as well. In the fall of 1910, Dr. William Hunter, an English physician, gave his famous lecture about the theory of focal infection, in which microorganisms from a localized, focal area of infection such as a tooth were labeled the source of serious systemic diseases.[10,15,16] Dr. Hunter unleashed a

contemptuous attack on dentistry and mocked the prosthetic efforts to preserve and maintain teeth. He termed the gold crown, "a mausoleum of gold over the mass of sepsis." Thus, all pulpless teeth were also suspected to be reservoirs of bacteria and in the dental community, "one hundred percenters" ensured that all such teeth were extracted.[15]

It was not until the 1930s that dentistry began to recover from the black eye given to it by a medical professional. Dentists by using radiographic practices, bacteriological culturing, and aseptic techniques started to push back against the theory of focal infection. Research by Dr. Logan (1937) concluded that the presence of bacteria does not necessarily imply infection, because bacteria are also found in normal tissues without a disease state.[17] Cecil and Burket were other pioneers who concluded that removal of localized foci had little effect on systemic diseases such as arthritis.[15] Thus, an era of witch hunt in which teeth were extracted routinely came to an end as a result of scientific research and evidence-based dentistry.

For most of the latter 20th century, dentistry continued to evolve into different specialties. Harry B. Johnston of Atlanta, GA, is credited with formulating the term "endodontics" derived from the Greek, *en* (in) and *odous* (tooth) – thus the meaning of working within the tooth.[10] In 1943, a group of eminent dentists interested in root canal therapy met in Chicago and formed an organization, the American Association of Endodontists (AAE). The seed for the organized specialty of endodontics in the US had been planted, and in 1963 the House of Delegates of the American Dental Association recognized endodontics as a specialty in dentistry.

SUCCESS OF ENDODONTIC THERAPY

Numerous studies have been done in the past evaluating success of endodontic therapy with different criteria. The classic criteria for success of endodontic therapy were listed by Bender[18] as follows:

1. Absence of pain or swelling.
2. Disappearance of any sinus tracts.
3. No loss of function.
4. Radiographic evidence of resolved or reduced areas of radiolucency at one year of recall.

Traditionally, endodontic therapy has enjoyed a high success rate when these studies were initiated by the pioneers in endodontics in the mid-20th century. In the "Washington study," success rate of 91.4 percent was reported for both surgical and non-surgical endodontic therapy for the 1229 cases completed during the mid-1950s.[19] Another classic and extensive study by Seltzer et al.[20] reported a high success rate of 92 percent for endodontic therapy when no radiolucent lesions were present, and a 76-percent rate with teeth with preexisting lesions. Similarly, Grossman et al.[21] reported a high success rate of 89.3 percent for necrotic cases without periapical lesions and 85.7 percent for cases with definitive periapical radiolucencies. These traditional studies were also crucial in delineating the factors that led to endodontic failure: age of the patient, overfilled canal, lack of proper restoration, and pre-existing radiolucency were associated with negatively affecting the outcome of endodontic therapy.[18-21]

Recently, the specialty of endodontics has been under increased scrutiny, especially after the reported high success rates for dental implants. Comprehensive, large-

scale studies have been undertaken to evaluate the clinical success of endodontics. A massive study that reviewed Delta Dental insurance claims involving more than 1,400,000 root canal-treated teeth revealed that 97 percent of root canal-treated teeth were retained within an eight-year recall period.[22] Similarly, another large scale study of 44,000 patients demonstrated that 94 percent of the root canal-treated teeth were retained with an average three-and-a-half-year follow-up period.[23] Interestingly, these recent studies also show that clinical success of endodontic therapy is not completely dependent on the technical quality of endodontics alone. It was found that teeth with no permanent restoration placed after endodontic therapy were about two to four times more likely to be extracted than teeth with permanent restorations.[23] A momentous study conducted by Ray and Trope[24] concluded that the quality of the coronal restoration was significantly more important than the quality of the endodontic treatment for the long-term clinical success of root canal treatment. Thus, the current emphasis of the endodontic specialty is on enhancing not only good endodontic treatment but also the follow-up restorative treatment to maximize the long-term prognosis of root-canal treated natural teeth.

ROOT CANAL ANATOMY

Endodontic success hinges on the proper knowledge of the myriad root canal morphologies and frequent variations in different teeth. As Dr. Kuttler puts it succinctly, it is the proper understanding of anatomy that serves as the "foundation of the art and science of healing."[25] Numerous monumental studies on the root canal anatomies have demonstrated that "a root with a tapering canal and a single foramen is the exception rather than the rule."[26] The complexities within the root canal system include multiple

foramina, intricate apical anatomies with accessory canals, fins, deltas, C-shaped canals etc.

G.V. Black is widely considered to be one of the early pioneers to discuss root canal anatomy. In 1890, in his book titled *Descriptive Anatomy of Human Teeth,* sectioned teeth were used to elucidate different root canal configurations.[27] Various innovative methods were subsequently developed to study root canal anatomies. Preiswork poured molten "Wood's metal" into the root canals of the teeth, after which strong acid was used to dissolve the teeth to show the internal anatomy.[28] Fisher developed a celluloid-acetone method to show apical delta and multiplicities within the root canals.[29] However, it was the monumental study by Hess that illustrated the complexities of the root canal system. Hess injected vulcanized rubber into the root canals of teeth, which were subsequently decalcified to obtain impressions of the canal spaces.[29] This study demonstrated that root canal anatomies consist of isthmuses, intercanal connections, fins, and irregular canal spaces, rather than the smooth-tapered or cylindrical spaces.

Various researchers have attempted to devise a system to classify the root canal system. Weine et al.[30] during his examination of the mesio-buccal root of the maxillary first molar described three canal configurations:

- Type I: A single canal from the pulp chamber to the apex.
- Type II: Two canals which merge before the apex.
- Type III: Two separate canals with two distinct apical foramina.

He subsequently described the fourth type of canal configuration in which one canal separates into two canals with two different apical foramina.

Vertucci et al.[31] published another classical study to categorize root canal anatomies in which cleared teeth were stained with hematoxalin dye. Vertucci's classification is much more intricate than Weine's classification and reflects the complex root canal anatomies that need to be considered prior to endodontic treatment. Eight canal space configurations were identified and elaborated in Vertucci's classifications:

- Type I: A single canal from the pulp chamber to the apex.
- Type II: Two separate canals leaving the pulp chamber that join to form one canal before the apex.
- Type III: One canal that divides into two in the root and then merges as one canal through a single apical foramen.
- Type IV: Two separate canals extending from the pulp chamber to the apex.
- Type V: One canal that divides into two distinct canals with separate apical foramina.
- Type VI: Two separate canals joining in the body of the root, and then dividing to exit as two distinct canals.
- Type VII: One canal divides and then rejoins in the body of the root, and finally redivides and exits as two separate canals.
- Type VIII: Three separate canals leaving the pulp chamber to the apex.

It is important to remember that various canal morphologies appear to occur in different frequencies among diverse ethnic and racial groups. For example, the black population appears to have a greater incidence of mandibular premolars with extra canals than the Caucasian population.[32] Asians tend to have greater frequency of single-rooted

and C-shaped mandibular second molars.[33] Dens evaginatus, a pulpal anomaly associated commonly with mandibular premolars, is found mostly among Native American, Hispanic, and Oriental populations.[34] Sert and Bayrili[35] evaluated 2800 teeth and found that even gender plays a role in the determination of canal morphology and should be considered during pre-operative endodontic evaluation.

Prior to endodontic therapy, even after the thorough understanding of the various canal configurations, a systematic and predictable approach is needed to precisely determine the actual number and location of root canals present in the tooth. Krasner and Rankow,[36] in their monumental study of 500 pulp chambers, concluded that the cemento-enamel junction (CEJ) was the most consistent anatomic landmark for access preparation and serves as the ultimate "Northstar" for the location of the pulp chamber and the root canal orifices. They noted specific anatomic patterns, the presence of which were expressed as laws in determining the number and location of orifices on the chamber floor[26, 36]:

1. Law of symmetry I: The orifices of the canals are equidistant from a line drawn in a mesiodistal direction through the pulp chamber floor, except for maxillary molars.

2. Law of symmetry II: The orifices of the canals lie on a line perpendicular to a line drawn in a mesiodistal direction across the center of the floor of the pulp chamber, except for maxillary molars.

3. Law of color change: The pulp chamber floor is always darker in color than the walls.

4. Law of orifices location 1: The orifices of the root canals are located at the junction of the walls and the floor.

5. Law of orifices location 2: The orifices of the root canals are located at the angles in the floor-wall junction.

6. Law of orifices location 3: The orifices of the root canals are located at the terminus of the roots' developmental fusion lines.

Historically, from early 1900s to 1950s the focus of the endodontic specialty was exclusively on the number of root canals in the tooth and their subsequent divisions. Scant attention was paid to the terminal part of the root canal, which plays a paramount role in the success of non-surgical endodontic treatment. Kuttler[25] was one of the early pioneers who focused his research on the microscopic investigation of the root apex. He found that the minor diameter of the canal also known as the apical constriction (AC) is usually found in the dentin, just before the canal penetrates the cementum at the cemento-dentinal junction (CDJ). From this point, Kuttler noted that the canal progressively widens to the apical foramen (AF) and takes on a funnel shape. He found that the average thickness of the cementum was usually above 0.5 mm, and he concluded that root canals should be filled "only as far 0.5 mm before reaching the foraman [AF]."[25] Thus, this research was fundamental in informing dentists about the reference point for apically terminating their shaping, cleaning, and obturation procedures.

Although there has been considerable debate concerning the exact termination point for endodontic therapy, it is universally accepted that it is the apical constriction (AC), which has the smallest diameter inside the root canal and is generally located 0.5 mm to 1.5 mm inside the apical foramen (AF).[26] Studies have shown that the most

favorable prognosis for success of endodontic therapy was found when the procedures were finished at the apical constriction (AC), while the worst prognosis was found when the treatment was terminated beyond the apical constriction.[37, 38]

ROOT CANAL PREPARATION

One of the most critical steps in endodontic therapy is proper root canal preparation. It involves the removal of vital and necrotic tissues from the pulpal system and systematic enlargement of the individual canals, which leads to the removal of infected dentin as well. Thus, the canal space is created for thorough disinfection by irrigating solutions and medicaments. This mechanical preparation of the canal system and subsequent chemical disinfection are described by the contemporary terms of 'chemo-mechanical' or 'bio-mechanical' preparation of the root canal system.[39] It is only via the combined dual strategy of chemo-mechanical preparation that bacterial load in the canal system is significantly reduced, which promotes the success of the endodontic procedure.

Ingle was one of the initial pioneers who discussed successful canal preparation through his organization of the methods employed in performing endodontic therapy.[40, 41] First, a correct outline form is required to allow complete access for instrumentation of the root canal system. Next, a proper convenience form is needed to allow direct access to the canal orifices. The root canal system should be continuously rinsed throughout the treatment in order to attain successful results. Ingle also described his 'standardized technique' in which endodontic instruments matched the fillings materials in "diameter, taper, and graduated size increments," leading to much more simplicity and uniformity in the preparation of root canal systems.[40]

Schilder was also one of the key visionaries who delineated the key objectives of root canal preparation. He envisioned the ideal preparation to not only reflect the unique anatomy of each root canal but also to facilitate the three-dimensional obturation of the root canal system. In 1974, Schilder[42] described five design objectives for an ideal root canal preparation:

1. Continuously tapering funnel from the apex to the cement-enamel junction.
2. Cross-sectional diameter of the preparation should be narrower at each point apically.
3. The root canal preparation should flow with the original canal shape.
4. The apical foramen should remain in its original location.
5. The apical opening should remain as small as practical.

Four key biological objectives were also elucidated by Schilder as well:

1. Preparation should be confined to the roots themselves.
2. Necrotic debris should not be forced beyond the apical foramen.
3. Removal of all tissue from the root canal system.
4. Sufficient space should be created to allow adequate irrigation by intra-canal medicaments.

These criteria for preparation are now considered the gold standard for root canal preparation and form the basis of contemporary operational techniques.[10] Various assortments of instruments have evolved over time to help achieve the root canal preparation objectives. Hand instruments that include K-type reamers and files, broaches and Hedstrom files have been the traditional and oldest instruments for root canal

preparation. K-type files are made from stainless steel wire, which is ground into various geometrical blanks such as square, triangle, or rhomboid. The blanks are twisted into a file or a reamer, with files having more twists per millimeter of length. In contrast, Hedstrom files are machined from a circular steel blank with sharp, positive rake angles making them much more aggressive than K-files in cutting and shaping dentin.

One of the major limitations of the stainless-steel-based hand instruments is in the preparation of root canal systems with curvatures. Small-diameter files are more flexible and are extensively used in these cases with less likelihood of transportation during preparation. However, the small-preparation diameters as explained by Roane[43] are not the ideal preparation, due to the "reduced amount of mechanical and chemical cleansing of the canal space." In order to circumvent this intrinsic limitation posed by stainless steel hand files, Roane[43] developed a novel technique of instrumentation termed the "balanced force concept." The technique involved using a triangular configured K-file advanced into the canal with a 90-degree clockwise rotation. The file is then rotated 120° to 180° counterclockwise with apical pressure leading to the enlargement of the canal. The sequence is repeated until the desired working length of the canal is achieved. The final motion consists of clockwise rotation without apical advancement, which is intended to facilitate debris removal from the canal. Thus, the balanced force technique allowed the clinicians to use the stainless steel hand files in enlarging curved canals without transportation or ledge formation.

One of the major innovations in root canal preparation occurred in 1988, when Walia[44] used nickel-titanium orthodontic wire alloy to fabricate endodontic files. It was observed that nickel-titanium hand files had "two to three times the elastic flexibility of

the stainless steel files as well as superior resistance to fracture in clockwise torsion and counterclockwise torsion."[44] This breakthrough made it possible to clinically prepare and enlarge curved canals via nickel-titanium instruments without any of the procedural accidents that were inherent with the usage of stainless steel hand files. The nickel titanium files were later modified to be used as engine-driven or rotary instruments utilizing 360-degree rotation at low speed. This advancement truly revolutionized the field of endodontics, making the root canal preparation much more simple, efficient, and predictable.

IRRIGATION

Irrigation regimen plays a critical aspect in the clinical success of endodontics. As pointed out by the landmark study of Kakehashi et al.,[45] the absence of microbial flora was the key factor in the healing of exposed rodent pulps. This study has been the basis of the current emphasis on chemo-mechanical strategies for managing endodontic infections. Mechanical instrumentation cannot solely achieve an adequately clean root canal. Siqueria et al.[46] demonstrated that although nickel titanium instrumentation significantly reduced the bacterial load within the root canal system, bacteria were never eliminated from the root canals regardless of the instrumentation technique and file sizes used. Bacteria located inside dentin tubules, fins, and other ramifications are commonly unaffected by instrumentation alone.[1] In another study by Peters at al.,[47] it was found that after rotary instrumentation, about 35 percent of the root canal surfaces remained uninstrumented. Therefore, adjunct use of antibacterial irrigating solutions and medicaments are imperative to maximize bacterial elimination.

Walton[34] has listed the main properties of an ideal irrigating solution which are listed as follows:

1. Tissue solvent.
2. Non- toxicity.
3. Disinfection agent.
4. Removal of smear layer.

To date, no irrigation solution has been formulated that fulfills all of these conditions.

Sodium Hypochlorite (NaOCl)

Hypochlorite was first produced in 1789 in Javelle, France when choline gas was passed through a solution of sodium carbonate.[1] The resulting solution termed as "Eau de Javelle" was a weak solution of sodium hypochlorite (NaOCl). Its first medical use was reported in 1915, when Dankin recommended a 0.5-percent solution of NaOCl to debride infected injuries during World War I.[48] The introduction of NaOCl to clinical endodontics was pioneered by Dr. Coolidge.[1]

NaOCl is used in concentrations varying from 0.5 percent to 5.25 percent and is commercially buffered to pH values ranging from 11 to 12.5. It is a broad-spectrum antimicrobial agent capable of killing bacteria, spores, fungi, and viruses.[49] It is also a potent tissue agent primarily the organic portion such as collagen, pulp tissue and predentin.[1,50] Some studies have reported that full-strength sodium hypochlorite at 5.25 percent can enhance its antimicrobial effects as well as tissue-dissolving properties.[50, 51] Clinically, it is important to continuously replenish NaOCl as its antimicrobial potency is derived from free chlorine from disassociation of NaOCl.[48] The major clinical

weaknesses of NaOCl are its unpleasant taste, cytotoxicity, and its lack of effect on removing the inorganic aspect of smear layer.[52, 53]

Chlorohexidine (CHX)

Chlorohexidine gluconate, a bisguanide, is a broad-spectrum antimicrobial agent capable of eliminating both Gram-positive and Gram-negative bacteria.[54] It is a cationic compound capable of causing cell lysis by attaching to the negatively charged cell membrane.[55] Some studies have concluded that CHX may be as potent as NaOCl in its antimicrobial effect. Gomes et al.[56] demonstrated through an *in-vitro* study that 5.25 percent of NAOCl and 0.2 percent to 2.0 percent of CHX were equally effective in killing *E. faecalis* within 30 seconds. Chlorohexidine also possesses the property of substantivity (long-term continued effect) in its antimicrobial effect. Oncag and colleagues[57] showed more residual antibacterial effects for 2.0-percent chlorohexidine than for NaOCl. Another important advantage of CHX is its relatively low cytotoxicity in comparison with NaOCl. However, one major drawback of using CHX in clinical endodontics is its inability to dissolve tissue remnants as NaOCl does. Due to this reason, CHX is not recommended as a sole irrigating solution in root canal therapy, but can be a valuable adjunct to the use of NaOCl.

Ethylenediamine Tetra-Acetic Acid (EDTA)

EDTA started to be used in clinical endodontics in 1957.[1] Neutral EDTA (17-percent concentration) is a disodium salt that chelates calcium ions leading to the decalcification of the smear layer along the canal walls and increases the diameter of the dentinal tubules.[58] This property of EDTA facilitates cleaning and removal of infected

tissues by NAOCl. The chelating effect of EDTA is also useful in negotiating narrow, calcified canals with the demineralization effect helping to establish patency. Studies have demonstrated that a 17-percent solution of EDTA is capable of removing smear layer within one minute of contact time.[1] However, it is also important to point out that the action of EDTA is self-limiting[59] and about 50 µm of demineralization into the dentin has been demonstrated according to research studies.[60] EDTA is also slightly antimicrobial as it combines with metal ions in the bacterial cell envelope, which can lead to cell death through the release of bacterial surface proteins.[10]

OBTURATION

Successful endodontic treatment hinges on the complete sealing of the root canal spaces to prevent egress of bacteria and their by-products, which can lead to persistence of apical periodontitis. Historically, obturation of the root canal system was viewed as the factor most behind the success of endodontic treatment as highlighted by the classical 'Washington study,'[10] in which 58.66 percent of endodontic failures were attributed to incomplete obturation. However, contemporary research points to the infection control via chemo-mechanical instrumentation as the most critical factor behind endodontic success.[61] Nevertheless, complete obturation of the root canal system is important to secure the short- and long-term success of endodontic therapy.

Grossman[62] was one of the early pioneers who outlined the ideal properties of an obturation material, which are listed as follows:

- Easily manipulated and provides sufficient working time.
- Dimensionally stable with no shrinkage upon insertion.

- Seals the canal laterally and apically, conforming to its complicated internal anatomy.
- Nonirritating to the periapical tissues.
- Impervious to moisture.
- Easy to remove from the root canal.
- Bactericidal.
- Radiopaque.
- Does not stain tooth structure.
- Sterile or easily sterilized

These criteria have guided the development of obturation materials, which have ranged from paste filling materials, semi-solid core filling materials to solid-core filling materials.[10] The solid-core materials such as silver points and paste materials such as N2 or Sargenti paste have been historically used as root canal filling materials. Lack of adequate seal and toxicity associated with these obturation systems led to the discontinuation of use of these materials.[1,63] In contemporary endodontics, it is the semi-solid core filling material that is considered as the closest in replicating Grossman's original criteria for an obturation material.[62] Gutta percha and Resilon obturation systems are examples of semi-solid filling materials extensively used in current endodontic therapy.

Gutta percha is one of the oldest dental materials in use today with an interesting history behind its development. In 1656, an Englishman named John Trandescant referred to gutta percha as "mazer wood" which conformed to various shapes after the application of heat.[64] It was this property of gutta percha of being pliable under warmth

and stable under cold that led to its use as the first successful insulation material for underwater cable lines in 1848.[64] The application of gutta percha in dentistry is historically credited to Dr. Asa Hill in 1847, when he devised gutta-percha-based obturation material termed "Hill's Stopping."[1] In 1942, Bunn discovered that the crystalline phase of gutta percha existed in two forms: alpha and beta phases.[64] Natural gutta percha derived from the trees exists in the alpha phase. If the alpha form is heated above 65°C and cooled normally will recrystallize into the beta phase.[10] This is significant as it is the beta phase of gutta percha, which is used clinically in endodontics. The disadvantage of the cooling phase to beta formulation is that it leads to significant shrinkage of the gutta percha, which is compensated for by forceful compaction of the gutta percha. The components of modern dental gutta percha are listed as follows[1, 65]:

1. Gutta-percha – 20 percent.
2. Zinc oxide – 65 percent.
3. Radiopacifiers (heavy metal salts) – 10 percent.
4. Plasticizers (waxes and resins) – 5 percent.

The large proportion of zinc oxide in dental gutta percha ensures not only radioopacity, but more importantly, its antimicrobial properties. Thus, gutta percha can be regarded as "self-sterilizing, as it will not support bacterial growth."[34] However, gutta percha does not possess any adhesive qualities to bind to dentin, and thus when solely used, it cannot hermetically seal a canal. Sealer is required to provide a closure of the canal-gutta percha interface.

Resilon is a novel core obturation material which has been introduced in the dental market as leading to the formation of a "monoblock" obturation.[4] This new

paradigm shift in obturation is touted to be superior to the traditional obturation by gutta percha. This rationale is based on the belief that not only does the Resilon core bond to its specific sealer, but the sealer itself bonds to the root canal dentin, which in essence is creating the "monoblock" obturation. This important difference between the two obturation systems has led to assertions of improved seal, higher fracture resistance, and overall greater clinical success in endodontics.

Resilon is a thermoplastic, synthetic polymer-based polycaprolactone core material containing bioactive glass, bismuth oxide, barium sulfate and coloring agents.[66] The filler content within Resilon is considered to be approximately 65 percent by weight.[48] Resilon has similar handling properties to gutta percha and can be used with the same obturation techniques. According to Ingle,[10] if warm vertical obturation technique is used, the temperature required for Resilon is much lower at 150°C as compared with gutta percha at 200°C. Resilon, like gutta percha also appears to be biocompatible and non-toxic.

Resilon is traditionally obturated with RealSeal sealer, formally known as Epiphany, which incorporates the use of self-etching primers. RealSeal is a "dual-cure sealer, composed of urethane dimethacrylate (UDMA), poly dimethacrylate (PEGDMA), ethoxylated bisphenol A dimethacrylate (EBPADMA), and bisphenol A glycidyl, methacrylate (BIS-GMA), barium borosilicate, barium sulfate, bismuth oxychloride, calcium hydroxide, photo initiators, and a thinning resin."[66] The self-etch primer contains "sulfonic acid terminated functional monomer, HEMA, water, and polymerization initiator."[4] The self-etch primer when applied to dentin of the root canal system penetrates through the smear layer and demineralizes the superficial dentin. The self-etch

primer forms a hybrid layer with the dentin, which bonds to the RealSeal sealer, which then bonds to the Resilon core. Thus, the manufacturers have claimed that a 'monoblock' unit, literally meaning a single unit, is formed in the Resilon obturation system. This has potentially positive implications in reducing bacterial microleakage through root canal systems.

Several studies have been done to investigate RealSeal sealer in comparison with conventional non-bonding sealers to evaluate microleakage. A review of these studies using the bacterial leakage model has shown mixed results. Shipper et al.[4] noted that the Resilon group showed minimal bacterial leakage in contrast with the gutta percha group, in which AH Plus sealer was used during the 30-day time period. In contrast, Williamson et al.[67] used a polymicrobial leakage model comparing Resilon/Real Seal (Epiphany) and gutta percha/AH Plus groups for 40 days and concluded that no difference was noted in microleakage between the two groups. Similarly, Baumgartner et al.[68] compared microleakage of root canals filled with Resilon/Real Seal (Epiphany) and gutta percha/AH Plus using *Enterococcus faecalis* for 50 days and found no significant difference as well.

Recently, RealSeal self-etch (SE) has been introduced, leading to the elimination of the separate etching/bonding step.[69] The acidic resin monomers in the self-etch primer are incorporated in the RealSeal SE sealer, thus making the technique an all-in-one step. RealSeal SE uses a polymerizable methacrylate carboxylic acid anhydrite (4-META) as the acidic resin monomer, which etches through the smear layer into the underlying radicular dentin.[70-73] The sealer is claimed to bond to both the Resilon core and radicular dentin via hybrid layers in both substrates leading to a 'monoblock' unit.

Extensive studies have been done recently comparing the etching capabilities of RealSeal and RealSeal SE. Kim et al.[72] have found the self-etch potential of RealSeal SE to be inferior to that of RealSeal. The self-etch sealer was unable to etch beyond smear layers to create micromechanical retention via a hybrid layer in intact dentin. Mai et al.[73] also found a similar result in which the self-adhesive sealer (MetaSEAL) was determined to be incapable of etching beyond the 1-mm to 2-mm thick smear layer. Incomplete smear layer removal from instrumented root canal system that is not reached by calcium chelating agents can thus jeopardize the performance of RealSeal SE. These findings have raised serious clinical concerns about the bonding and sealing capabilities of RealSeal SE with obvious implications on microleakage. To date, no research has been done evaluating the microleakage of RealSeal/Resilon versus RealSeal SE/Resilon which gave the impetus to the present study.

MICROBIOLOGY OF ENDODONTIC INFECTIONS

The success of endodontic therapy is completely dependent on the understanding the microbial etiology of the disease. Since the advent of civilization, it is remarkable to see that only within the last two centuries of scientific and technological revolution did the dental profession start to recognize the infectious basis of endodontic disease, which subsequently led to the successful endodontic management of the disease.

In the 17th century Dutch amateur microscope builder Antony van Leeuwenhoek observed “animalcules” with his microscope from the dental plaque and exposed pulp cavity.[1,10] However, the association between bacteria and pulpal disease was only established in 1890 when Willoughby Dayton Miller, an American dentist, reported through microscopy three basic morphological bacteria known at that time: bacilli, cocci,

and spirochetes.[1,10] It was the elegant study in the 1960s from Kakehashi et al.[45] that conclusively established the pivotal relation between bacteria and pulpal infection. The authors evaluated the response of the dental pulps in normal and germ-free rats in which the pulps were intentionally exposed to oral cavities. Pulp necrosis and apical periodontitis lesions were observed histologically in the pulps of normal rats. In contrast, no pathological changes were noted for the exposed pulps in the germ-free rats and even healing via dentinal bridging was seen in these pulps. Thus, the key determinant for endodontic healing and success was found to be the presence of bacteria. This remarkable finding rightly makes the study of Kakehashi et al.[45] the foundation upon which the entire specialty of endodontics is built.

In the last 50 years, the advances in culturing techniques, electron microscopy, and molecular methods have greatly enhanced the knowledge of the endodontic microbiota in various clinical conditions. Prior to 1970, "few strains of strict anerobes were isolated and identified because of inadequate anerobic culturing methods."[10] However, now it is universally accepted that strict anerobes comprise the majority of the endodontic infections. Pulpal infections usually lead to liquefactive necrosis of the pulp, which is an ample source of nutrition for anerobic bacteria that thrive in the low-oxygen tension environment within the pulp. Sundqvist et al.[74] demonstrated the anaerobic bacteria comprised more than 90 percent of the isolates in teeth showing radiographic evidence of apical periodontitis. Black pigmented bacteria (BPB), which are non-motile, Gram negative rods, have been extensively reported to be associated with clinical symptoms in numerous studies.[75,76]

Currently, the focus of endodontics is on the role of biofilm formation and its role in persistent endodontic disease. Studies have shown the microorganisms in biofilms can be up to 1000 times more resistant than the corresponding free-floating (planktonic) bacterial cells.[77,78] Bacterial biofilms via genetic exchange and quorum sensing have enhanced pathogenicity and virulence, increased resistance to antimicrobial agents, and a broader habitat range of growth.[1] In endodontics, recent studies using SEM (scanning electron microscope) and TEM (transmission electron microscopy) technologies are demonstrating the presence of biofilms not only within the root canal system (intracanal) but also on the external root surfaces (extraradicular).[10] In "refractory periapical endodontic lesions,"[79] the periapical biofilm flora of these teeth was found to be very different from the flora that usually heal with endodontic therapy. Some conspicuous findings of this biofilm were the domination of Gram-positive bacteria (80 percent) and the presence of sulfur granules.[79, 80] Thus, with the technological evolution, the understanding of the microbiology associated with endodontic disease is getting more complex and intriguing and the future success of the specialty will hinge on innovative treatment strategies to overcome the current limitations of endodontic therapy.

Enterococcus faecalis is reported to be the most common species with post-treatment disease (PTD) meaning teeth with failed root canal treatment and persistent endodontic infections.[10, 81] *E. faecalis* are spherical, Gram-positive, catalase-negative, facultative anaerobic cells with inherent resistance to numerous antibiotics like β-lactams, aminoglycosides, and clindamycin.[10, 82] Numerous virulence factors have been identified with *E. faecalis* such as aggregation substance (AS), gelatinase, cytolysin toxin, extracellular superoxide production, and capsular polysaccharides.[81]

E. faecalis is not identified as a dominant bacterial species in primary apical periodontitis.[81,83,84] Siqueira et al.[85] reported an incidence of 7.5 percent of *E. faecalis* in samples collected from 53 infected teeth with primary root canal infections. However, in root canal treated teeth, *E. faecalis* has been reported to be the most frequent species, "with prevalence values reaching up to 90 percent of cases. Root canal treated teeth are about nine times more likely to harbor *E. faecalis* than cases of primary infections."[1]

Various reasons have been attributed for the survival of *E. faecalis* after endodontic therapy. NaOCl is reported to be effective in eliminating *E. faecalis* in both buffered and unbuffered solutions.[86] However, *E. faecalis* is reported to penetrate deeply within the dentin tubules, due to which it is able to resist the current chemo-mechanical model of endodontic therapy.[87, 88] Moreover, *E. faecalis* is reported to be capable of forming biofilms in root canals, which can greatly enhance its resistance to antimicrobial regimens.[89] During endodontic retreatment, inter-appointment placement of calcium hydroxide medicament is a widely used regimen among endodontists. However, *E. faecalis* is capable of resisting high pH values induced by calcium hydroxide, which has been attributed to the species' proton pump mechanism. Thus, all these properties help in explaining why *E. faecalis* is currently crowned as the "root canal survivor and 'star' in post-treatment disease."[81]

ENDODONTIC MICROLEAKAGE STUDIES

Leakage associated with endodontic obturation has been studied extensively and literature is replete with different methodologies to determine leakage such as "assessment of linear and volumetric dye penetration, autoradiographic detection of isotope penetration, radionuclide detection, culture techniques to detect bacterial

penetration, salivary penetration models, fluid filtration techniques, fluorometry, intracanal reservoir techniques, electrochemical techniques, etc."[90] However, these different laboratory studies had poor agreement amongst each other and their clinical implications on endodontic success are still being debated. In 2007 the Editorial Board of the *Journal of Endodontics* made a statement regarding endodontic microleakage studies and decided that "sealability studies comparing endodontic procedures using the penetration of dyes, chemicals, etc are not useful to endodontic science" and subsequently restricted publication of studies using these methods.[90] It is important to discuss the reasons behind this important decision, and which method is currently deemed acceptable to evaluate endodontic microleakage.

Dye and Radioisotope Leakage Studies

Dye and radioisotope leakage studies had been used extensively in the past to evaluate the sealing properties of endodontic materials. In 1993 when Wu published his review article, he found that "in 82 percent of the endodontic leakage studies, dye or radioisotope penetration has been determined."[91] However, there are inherent weaknesses in this methodology that lead to inconsistent and clinically irrelevant results. The molecular size of the dye or radioactive ions is typically much smaller than the bacteria colony itself, a disparity that can give clinically inaccurate results in leakage studies. Other variable factors confounding these leakage studies were the pH of the tracers, chemical reactivity, and immersion time.[92] For example, the methylene blue dye extensively used in the published dye studies is acidic and can increase the amount of leakage through its inherent effect in demineralizing dentin.[91] For the leakage studies involving radioisotopes, Torabinejad critiqued the isotopes as "indicators of ion

exchange, diffusion, or metabolism within the tissues rather than indicators of true leakage"[93] for evaluating endodontic leakage. For the dye/radioisotopes studies, it was later found that the air bubbles in voids within the obturation material can hinder the leakage of these materials and lead to inaccurate results.[94] Thus, for myriad reasons, the implications of dye/radioisotope studies on sealability of obturation materials were questioned, and subsequently, these studies are no longer published in respected endodontic journals.

Fluid Transport/Filtration Studies

In a bid to improve the design of the endodontic leakage model, Pashley[95] devised a different model system based on convective fluid transport under positive pressure, which causes the displacement of an air bubble in a fluid-filled capillary tube between the test specimen and the pressurized reservoir.[96] This new model was touted to be superior to the previous methods; no tracers were necessary, which eliminated the problems related to molecular size, pH, and other chemical properties; samples no longer needed to be destroyed, making it possible to evaluate leakage along extended time periods, and most importantly, the leakage could be measured quantitatively.[97] However, as Wu et al.[96] discussed, although the fluid transport/filtration method has greatly accelerated leakage detection, the application of pressure has no clinical relevance, which is a major shortcoming of this leakage design. Moreover, the fluid transport model is not only extremely sensitive to the length and anatomy of the root canals, but also to the patency and apical diameter after instrumentation, which greatly increases the variables confounding this methodology.[97]

Bacterial Leakage Studies

Leakage studies employing bacterial cultures are considered to be more reliable and clinically relevant than the previously described models.[92] Although this model is criticized as a limited static model that does not simulate conditions in the oral cavity, such as temperature variations, dietary influences, and salivary flow, the bacterial leakage model is still considered one of the best methods for evaluating leakage. This model was pioneered in the field of restorative dentistry in which restorations were evaluated through bacterial leakage in 1965.[98] In the 1980s, this model started to be applied in endodontics.[99] Currently, the most common apparatus for this model is a dual-chambered model, with the tooth sealed in between the upper and lower chamber. Turbidity or a color reaction in the sterile broth in the lower chamber indicates the microleakage of viable microorganisms from the upper chamber.[100]

The proponents of this model argue that clinically, the most common mode of endodontic failure is crown-down leakage of oral bacteria, so that the bacterial leakage model is more reflective of the clinical situation. However, in an important review article, Rechenberg et al.[100] discussed serious potential weaknesses within this model that need to be addressed. The seal between the upper and lower chamber is arguably a potential source of leakage itself, which can confound the results of bacterial leakage studies published so far. Previous researchers have not traced the routes of microleakage histologically, resulting in inconclusive findings on the exact route of microbial leakage through the endodontic obturation material. Another critique of this model is the basis of quantification of leakage through the extent of time for turbidity in the lower chamber. Most of the published bacterial leakage studies are conducted for short periods of time,

which as Wu et al. notes, the clinical relevancy of "early or postponed leakage within a short experimental period is unknown."[101]

STERILIZATION METHODS

The pulp and radicular tissues of extracted human teeth may contain vegetative bacteria and endospores, fungi, viruses, and myriad other blood-borne pathogens. The primary goal of sterilization is to completely eliminate all these microorganisms. There are three methods currently used to sterilize extracted teeth for dental research [102]:

1. Steam autoclave.
2. Gamma or ultraviolet radiation.
3. Ethyelene oxide.

In dental research, it is *Bacillis subtilis* spores that are commonly used as a biological indicator of sterilization.[103] Spores of *Bacillus* are generally induced by lower levels of nutrients in the environment. The spore formation is a defensive mechanism by which the bacteria can survive for extended periods with little or no nutrition.[104] No metabolic activity or any repair mechanism to macromolecules such as DNA or proteins is detected in the dormant spores. If excessive damage is accumulated during the spore dormancy, the repair-system capacity is overwhelmed upon germination, which leads to the death of the spore.[105, 106]

Another important aspect of sterilization of extracted teeth for research purposes is that minimal alteration to the structural properties of enamel and dentin is imperative. This is particularly important for dentin, which is composed of 70-percent inorganic material and 20-percent organic and 10-percent water, which cannot be compared with enamel composed of 96-percent inorganic.[1] Thus, dentin due to its higher organic

composition of delicate collagen fibrils and ground substance of mucopolysacharides is much more sensitive to the harsh effects of sterilization. Therefore, it is critical to understand whether any sterilization methods will affect the structural aspects of dentin, which can subsequently impact the bond strength between the adhesive interphase between resin and dentin surfaces.

In the present study, the choice of the correct sterilization protocol was critical as the sealing property of the bonded obturation system could easily be modified by an aggressive sterilization protocol. Microleakage evaluation would be affected if the chosen sterilization method would alter the interface between Resilon sealers and dentin, which would allow the test organism *E. faecalis* to penetrate through the apex in a short period of time.

Steam Autoclave

Autoclaving is the common method of sterilization in most dental facilities because pressurized steam is used to sterilize instruments. The instruments or extracted teeth are usually wrapped in gauze and autoclaved for 20 minutes and 30 minutes at 121°C at 15 psi.[1] Steam created in an autoclave goes through a series of valves, and this process increases the pressure and superheats the steam. When the steam contacts microbes, it condenses and instantly releases the stored heat energy, which denatures vital cell proteins.[107] The damage to cellular proteins kills all bacteria, spores, and viruses.[108] Clinically, the spores of *B. subtilis* are regularly used as biological monitors to test the reliability of steam sterilization. McGuckin et al.[109] evaluated the bond strength to dentin in teeth that had been autoclaved and found that autoclaving teeth prior to bonding produced significantly lower bond strengths to dentin compared with the control teeth.

Ultraviolet and Gamma Radiation

The antimicrobial efficacy of ultraviolet (UV) irradiation is well-known and has been traditionally applied to disinfect water, air, and surface disinfection. UV radiation, however, possesses lower energy relative to gamma rays, because its penetration is limited and widely absorbed by glass and plastics. There is also a strong reliance on distance from the UV source, which can lead to inconsistent antimicrobial efficacy.[110] The most effective wavelength for UV sterilization is considered to be 254 nm, but it has been found that spores are 10 times to 50 times more resistant to UV irradiation compared with growing cells.[111] This has been attributed to the UV-absorbing pigments found in the spore's outer layers, which shield the UV sensitive macromolecules such as DNA.[112] For *B. subtilis*, research has demonstrated that UV irradiation energies ranging from 10 $nW/cm^2$to 60 nW/cm^2 are lethal doses for the spores.[110]

Gamma radiation is another alternative sterilization method commonly used for the sterilization of medical devices and food treatment.[113] Gamma radiation is at a higher end of the electromagnetic spectrum compared with UV radiation; it has shorter wavelength and higher frequency, which translates into higher energy. White et al.[114] were able to obtain complete sterilization against *B. subtilis* at gamma radiation dosages of 1730 Gy. However, concerns have been raised whether a high dose of ionizing radiation by gamma rays can possibly denature the organic aspect of dentin. In a classic study by White et al.,[114] effects of gamma irradiation on both the structure of dentin and the dental obturation materials within the root canal were evaluated. Gutta percha and Grossman's sealer appeared "unaffected by the gamma irradiation sterilization

process."[114] No major changes in the structure and function of dentin were seen in this study.

It is important to note that if radiation sterilization is used, the total dosage of radiation is the single most critical factor in the achievement of sterilization. Due to the high mineral content of teeth, the time required to sterilize teeth via radiation will be much longer compared with other biological tissues. Thus, typical sterilization time via radiation can be as long as 24 hours, which is significantly longer than steam autoclaving.[114] Radiation, especially high-energy gamma rays, can break the chemical bonds of polymers such as the peptide bonding in collagen.[115] However, Brauer et al.[113] found no significant dose-response relation in the nano-mechanical properties of both enamel and dentin. Thus, radiation may be the ideal method to achieve sterilization for teeth and for heat-sensitive materials such as Resilon.

Ethylene Oxide (EtO)

Ethylene oxide (EtO) sterilization is one of the oldest methods to sterilize extracted teeth; in 1974 it was reported that bovine enamel blocks were getting sterilized by using 1.0-percent ethylene oxide.[103,116] Ethylene oxide (EtO) sterilization process is traditionally performed in three steps[103,107]:

1. Sixteen hour pre-conditioning under 50-percent to 80-percent relative humidity at 38°C.

2. EtO gas cycles for three hours at 625 mg/L concentration under 40°C to 50°C, which is effective for killing all microorganisms including spores.

3. Aeration period of minimal 72 hours at 40°C, which is done because ethylene-oxide-exposed materials can be highly toxic to human tissues.

Studies have shown that EtO sterilization does not affect dentin bond strength or dentin permeability, which makes it an ideal sterilization method for microleakage studies.[102, 117] However, conflicting results appear in dental literature about the sterilization of EtO against the spores of *B. subtilis.* White and Hays in 1995[117] found that both warm (63°C) and cold (30°C) sterilization with EtO did not completely eliminate the spores of *B. subtilis.* The concentration of EtO was able to penetrate the internal tissues of teeth to kill vegetative bacteria, but could not completely kill the endospores. As recently as of 2007,[103] studies are still debating the efficacy of EtO to render extracted teeth free of bacterial spores. It is imperative that future studies address this issue, because a large number of contemporary microleakage studies in endodontics are based on the hypothesis of complete elimination of both bacteria and spores via EtO sterilization, which may not be a correct assumption.

MATERIALS AND METHODS

SELECTION OF TEETH

Sixty-two human, single-rooted maxillary anterior teeth extracted for periodontal considerations were used for this study. The teeth selected were caries-free, and the root length ranged from 20 mm to 25 mm. All teeth were collected from the Oral Health Research Institute under Clarian/IUPUI IRB #NS1005-03. All teeth were stored in sealed containers with 0.1-percent thymol and refrigerated prior to the study. Radiographs were taken in a mesial-distal direction to confirm that a Type I root canal system is present. Teeth with abnormal canal anatomy, abnormal root morphology, extensive caries, or root fractures were discarded.

Once the teeth were selected, calculus and soft tissue debris was removed from the root surface with hand-scaling instruments. Following debridement of the root surface, the teeth were immersed in 6.0-percent sodium hypochlorite (Clorox Co., Oakland, CA) for 30 minutes and mechanically debrided with a soft brush. A No.10 K-type endodontic file (Kerr, Romulus, MI) was inserted into the root canal and advanced out the apical foramen of all teeth (Figure 2). All teeth with canals that could not be negotiated with a No.10 K-type endodontic file were excluded from the study.

CANAL INSTRUMENTATION AND IRRIGATION

Ideal endodontic access was performed for all teeth to gain straight line access. Working length determination was accomplished by passing a No.10 K- type endodontic file into the root canal until the file was just visible at the apical foramen and then

subtracting 1 mm from this file length measurement (Figure 2). The root canals were instrumented using K-type endodontic files (Kerr, Romulus, MI), specifically International Standards Organization (ISO) Sequence rotary files, 0.06 taper, size 40 to size 20 (Dentsply, Tulsa, OK). The #15 and #20 K-type files were instrumented to the working length. Profile orifice shapers sequenced from 30/.06 to 50/.07 to 40/.06 were used to flare the coronal half of the canal. Instrumentation of all teeth was performed using a crown-down technique implementing Sequence 0.06 tapered rotary files with MAF at 35.06 (Figures 2 and 3). Irrigation with 1 ml of 6.0-percent NaOCl was used between each file. A No. 10 K-type file was used to maintain apical patency. After instrumentation, irrigation using 2 ml of a 17-percent EDTA solution was done for 1 minute, after which the canals were flushed with 5 ml of sterile water. The canals were then dried with sterile, medium paper points. To prevent dehydration, all roots were handled using water-moistened gauze during resection and instrumentation.

ASSIGNMENT OF TEETH

Specimens were randomly assigned to two groups of 27 teeth. The two groups were classified as Group I, RealSeal/Resilon and Group II, RealSeal SE/Resilon. In addition, two groups each containing four specimens served as positive and negative controls, Group (+) and Group (-) respectively. The positive and negative control groups ensured that the microleakage model was functioning properly. All grouped teeth were sterilized using ethylene oxide at the Indiana University School of Medicine.

GROUP I

After canal instrumentation and irrigation, Group I RealSeal/Resilon was obturated in an orthograde fashion using a 35/.06 Resilon cone. A paper point conditioned with RealSeal Primer was placed to length. Dry paper points were used to absorb excess primer. A 35/.06 Resilon cone coated with RealSeal sealer was then placed in the canal. Obturation was completed using a System B heat source (Tulsa Dental, Tulsa, OK) and Schilder pluggers. The System-B was used to sear the master cone at 6 mm from the established working length. The canal was filled to a total of 15 mm using an Obtura II (Sybron Endo, Orange, CA) system and condensed with Schilder pluggers with rubber stops. The coronal surface was light-cured for 40 seconds using LED curing light source at the output range of 440 nm to 480 nm. Radiographs were taken to confirm successful length and density of the fill.

GROUP II

After canal instrumentation and irrigation, Group II RealSeal/Resilon was obturated in an orthograde fashion using a 35/.06 Resilon cone. A 35/.06 Resilon cone coated with RealSeal SE sealer was placed in the canal. Obturation was completed using a System B heat source (Tulsa Dental, Tulsa, OK) and Schilder pluggers. The System-B tip was used to sear the master cone at 6 mm from the established working length. The canal was filled to a total of 15 mm using an Obtura II (Sybron Endo, Orange, CA) system and condensed with Schilder pluggers with rubber stops. The coronal surface was light cured for 40 seconds using LED curing light source at the output range of 440 nm to 480 nm. Radiographs were taken to confirm successful length and density of the fill.

POSITIVE CONTROL GROUP

The positive control consisted of four prepared teeth that had a single master cone of Resilon but no sealer. This allowed free communication of the bacteria in the upper chamber with the growth medium in the lower chamber.

NEGATIVE CONTROL GROUP

The negative control consisted of four teeth with two teeth obturated with RealSeal/Resilon and two teeth obturated with RealSeal SE/Resilon. All of these four teeth were coated with cyanoacrylate to seal the apical opening and the dentinal tubules. The negative control was not expected to exhibit leakage.

MICROBIAL LEAKAGE APPARATUS

All experimental teeth were stored for 20 days under room temperature conditions to ensure complete setting of the sealer prior to microleakage testing. The individual components of the microbial leakage apparatus were sterilized using ethylene oxide prior to the assembly of this apparatus. *E. faecalis* [ATCC # 29212] was the species of test bacteria used in this study to determine microleakage. To prevent contamination from other microbes during the study, novel protocols were used to address this issue. Based on the previous studies of Butaye et al.,[118] colonies of *E. faecalis* were successfully cultured in tryptic soy broth (TSB) containing streptomycin at 2000 ug/ml. The choice of streptomycin as the antibiotic of choice was based on the research of Zhu et al.,[119] which determined that *E. faecalis* was sensitive to penicillin, ampicillin, and vancomycin, but resistant to streptomycin. Thus, use of the selective strain of *E. faecalis* with media

containing streptomycin will prevent contamination from other bacterial species during the microleakage experiment.

A microbial leakage apparatus was constructed using a 20-ml scintillation vial. An opening was drilled into the cap of the vial through which the sample tooth protruded into the vial, whereas the space around the tooth was sealed using wax. A small polyethylene-based delivery tube was inserted into the access opening of the tooth and the interface was sealed with flowable resin. The lower chamber of the apparatus, created by the space between the root tip and floor of the scintillation vial, was filled with 15 ml of sterile, modified TSB containing a concentration of streptomycin at 2000 ug/ml. The upper chamber of the apparatus consisted of the space above the obturation material up to the end of delivery tube. The assembled microleakage model is illustrated individually and collectively in Figure 6 and Figure 7, respectively. The microbial leakage apparatus was subsequently sterilized using ultraviolet radiation for 12 hours.

The experimental apparatus was initiated with the upper chamber volume inoculated with a fresh 16-hour culture of Streptomycin-resistant *E. faecalis* in 5- ml-modified TSB containing streptomycin. Freshly modified TSB medium containing *E. faecalis* was added to the upper chamber every three to four days to ensure live bacteria were present during the entire investigation period. Replenishing of the upper chamber was accomplished using sterile technique. All experimental samples were placed in a 5.0-percent CO_2 incubator at 37^oC and 100-percent humidity (Figure 8).

Evidence of leakage was determined by visual turbidity of the growth medium in the lower chamber (Figure 9). The microleakage experiment was conducted for 33 days and the medium in the bottom chamber was examined daily for visual turbidity changes.

SAMPLE SIZE AND STATISTICAL METHODS

With a sample size of 27 teeth in each of the two groups, the study had 80-percent power to detect a difference in the proportion with no microleakage (40 percent vs. 80 percent at 10 days based on a previous study) assuming a two-sized, 5.0-percent significance level using a log-rank test. The presence of microleakage was compared between groups using Fisher's Exact tests. The time to microleakage was compared between groups using log-rank tests.

RESULTS

The experiment was completed in 33 days. Table I demonstrates the data for the microleakage of experimental and control groups over the time period of 33 days. Results of the study at 7 days, 14 days, 21 days, 28 days, and 33 days are discussed in details below.

MICROLEAKAGE AT DAY 7

As shown in Table I, RealSeal SE and RealSeal groups both exhibited microleakage of only one sample out of the total 27 samples for each group. The positive control group showed microleakage in one sample out of the four teeth, whereas the negative control group showed no microleakage in any of the four teeth.

MICROLEAKAGE AT DAY 14

As shown in Table I, RealSeal SE group demonstrated microleakage in three of the 27 sample teeth, whereas RealSeal group showed microleakage in five of the 27 sample teeth. The positive control group showed microleakage in all four teeth, whereas the negative control group showed no microleakage in any of the four teeth.

MICROLEAKAGE AT DAY 21

As shown in Table I, RealSeal SE group demonstrated microleakage in four of the 27 sample teeth, whereas RealSeal group showed microleakage in six of the 27 sample teeth. The positive control group showed microleakage in all four teeth, whereas the negative control group showed no microleakage in any of the four teeth.

MICROLEAKAGE AT DAY 28

As shown in Table I, RealSeal SE group demonstrated dramatically increased microleakage in 24 of the 27 sample teeth, whereas RealSeal group showed microleakage in 10 of the 27 sample teeth. The positive control group showed microleakage in all of the four teeth, whereas the negative control group showed no microleakage in any of the four teeth.

MICROLEAKAGE AT DAY 33

As shown in Table I, RealSeal SE group demonstrated microleakage in 25 of the 27 sample teeth, whereas RealSeal group showed microleakage in 11 of the 27 sample teeth. The positive control group showed microleakage in all four teeth, whereas the negative control group showed no microleakage in any of the four teeth.

Collectively, Figure 11 illustrates the percentage of samples exhibiting microleakage in the study. No microleakage was observed in the negative control and microleakage was observed in all four samples in the positive control. RealSeal Self-Etch Group II had 25 out of 27 samples exhibiting microleakage. Thus, 92.5 percent of the Group II samples showed microleakage by the end of the 33 days. In contrast, Real Seal Group I had 11 out of 27 samples exhibiting microleakage, which was 40.7 percent of the samples showing microleakage by the end of 33 days. Thus, RealSeal Self-Etch Group II had a significantly higher proportion of samples with microleakage than Real Seal Group I ($p < 0.0001$).

In summary, Figure 12 illustrates the microleakage of the experimental groups plotted over the experimental time period of 33 days. The negative controls exhibited no microleakage throughout the study. Interestingly, the positive controls started to exhibit

microleakage starting at the seventh day of the study with all four samples showing microleakage at the 14th day of the study. This delay may be attributed to the presence of the Resilon master cone in 35.06 percent of the positive control teeth. RealSeal SE Group II showed dramatic increase in microleakage at the last 10 days of the study, whereas RealSeal Group I had a relatively steady increase in microleakage over time (Figure 12). Thus, the time to microleakage was also found to be significantly lower in RealSeal Self-Etch Group II than in the Real Seal Group I ($p = 0.0004$)

FIGURES AND TABLES

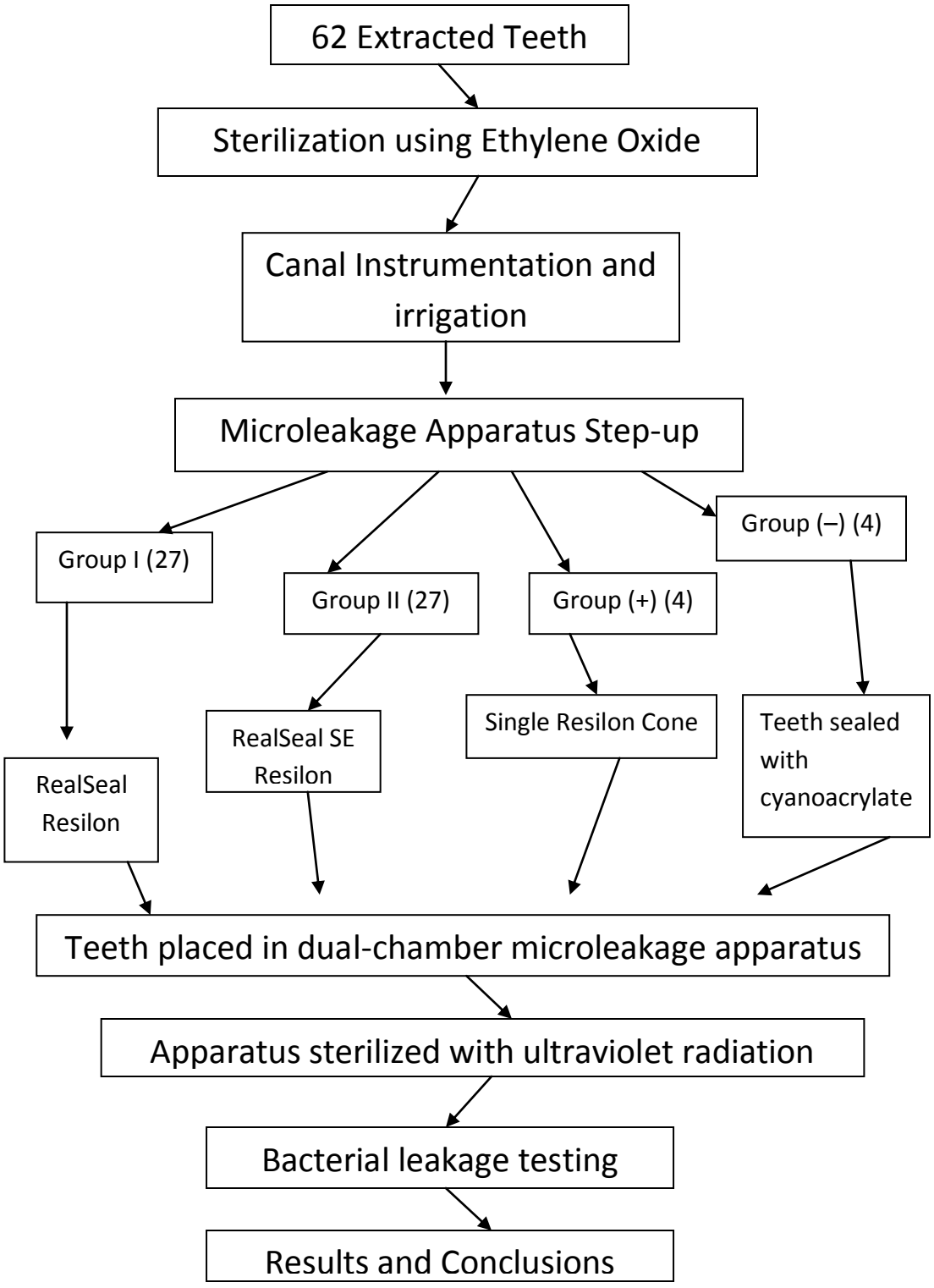

FIGURE 1. Summary of experimental design.

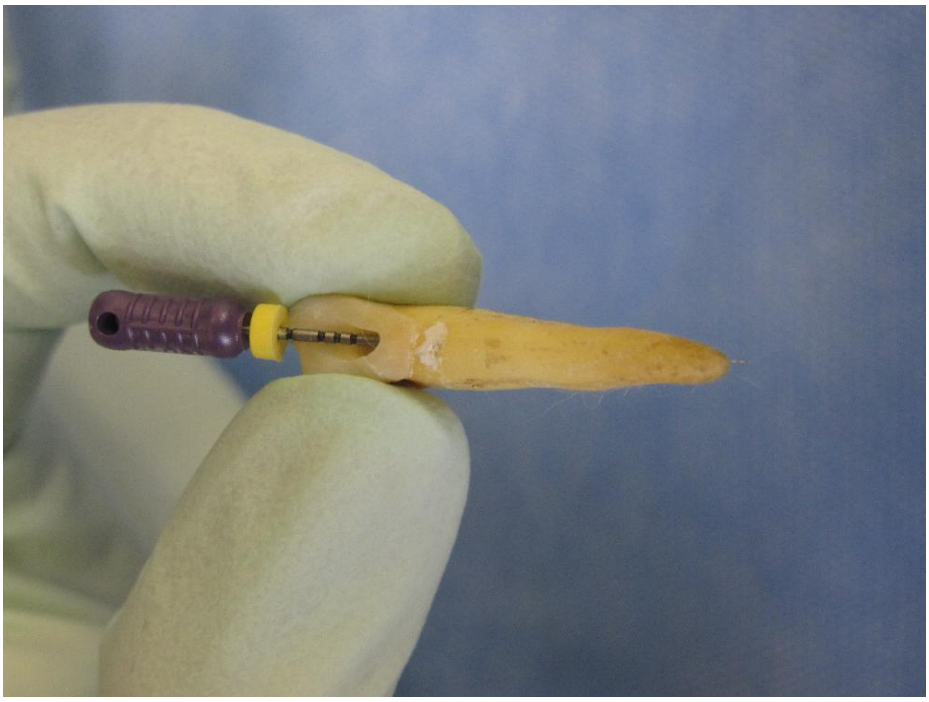

FIGURE 2. Working length determined by passing a #10 file past the apex.

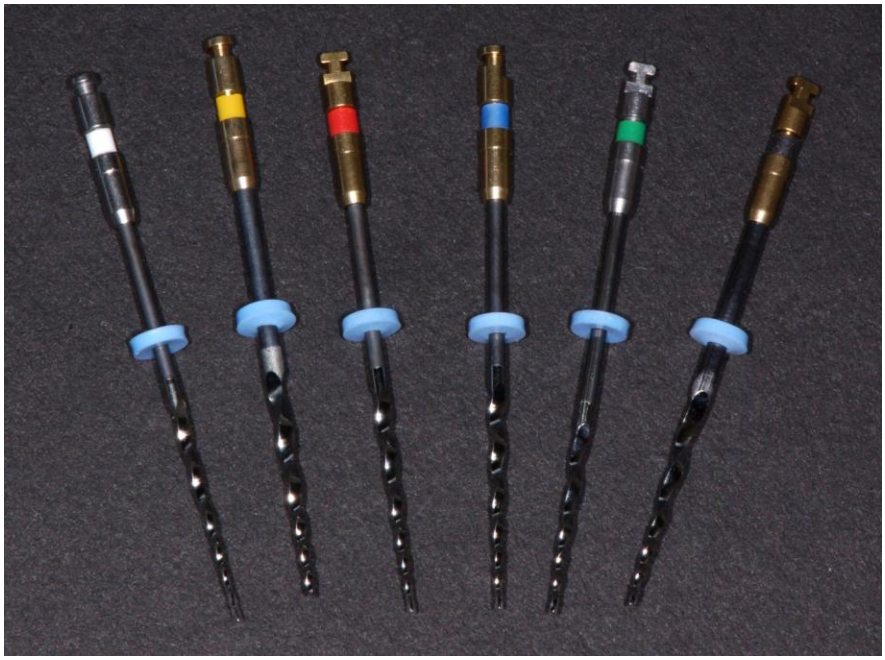

FIGURE 3. Sequence rotary instruments used for instrumentation.

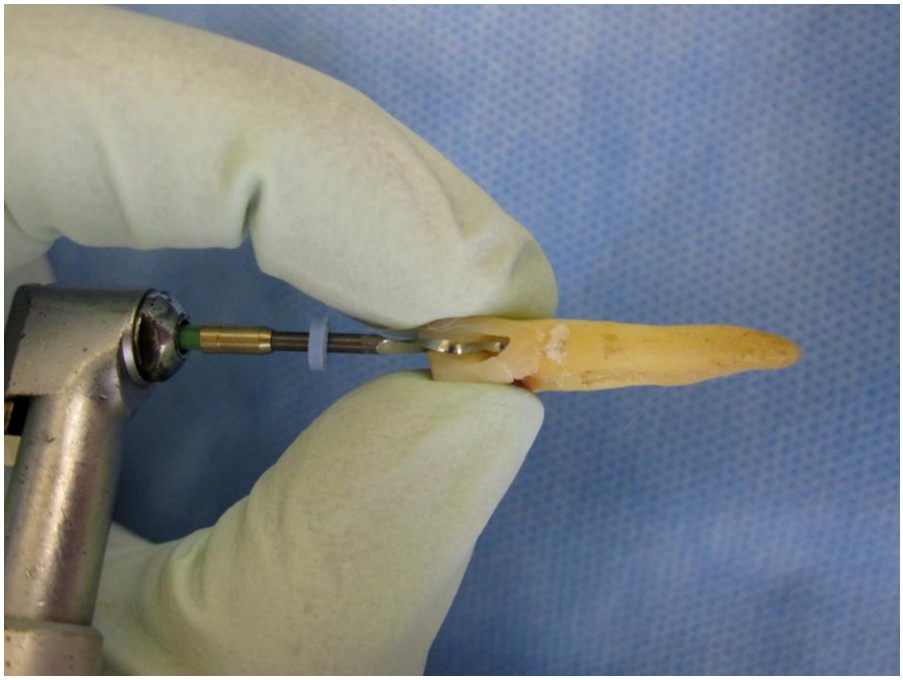

FIGURE 4. Rotary instrumentation with Sequence 35.06.

FIGURE 5. Resilon master cones in different ISO sizes with 0.06 tapers at the periphery. Resilon pellets at the center.

FIGURE 6. At top, RealSeal primer and RealSeal sealer; at bottom, RealSeal SE sealer.

FIGURE 7. Microleakage apparatus prior to loading of upper and lower chambers.

FIGURE 8. Microleakage models assembled prior to the experimental run.

FIGURE 9. Experimental samples in 5.0-percent CO_2 incubator at 37^{o}C and 100-percent humidity.

FIGURE 10. Visual turbidity of the growth medium in the lower chamber observed at right; at left, no visual turbidity of the growth medium.

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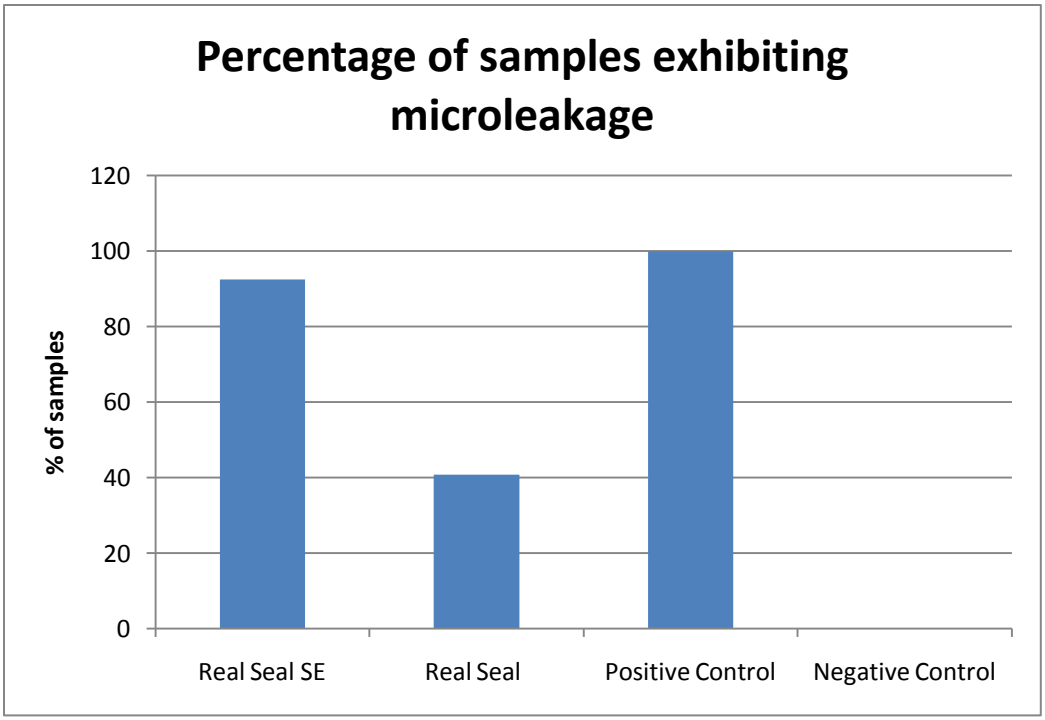

FIGURE 11. Percentage of samples exhibiting microleakage.

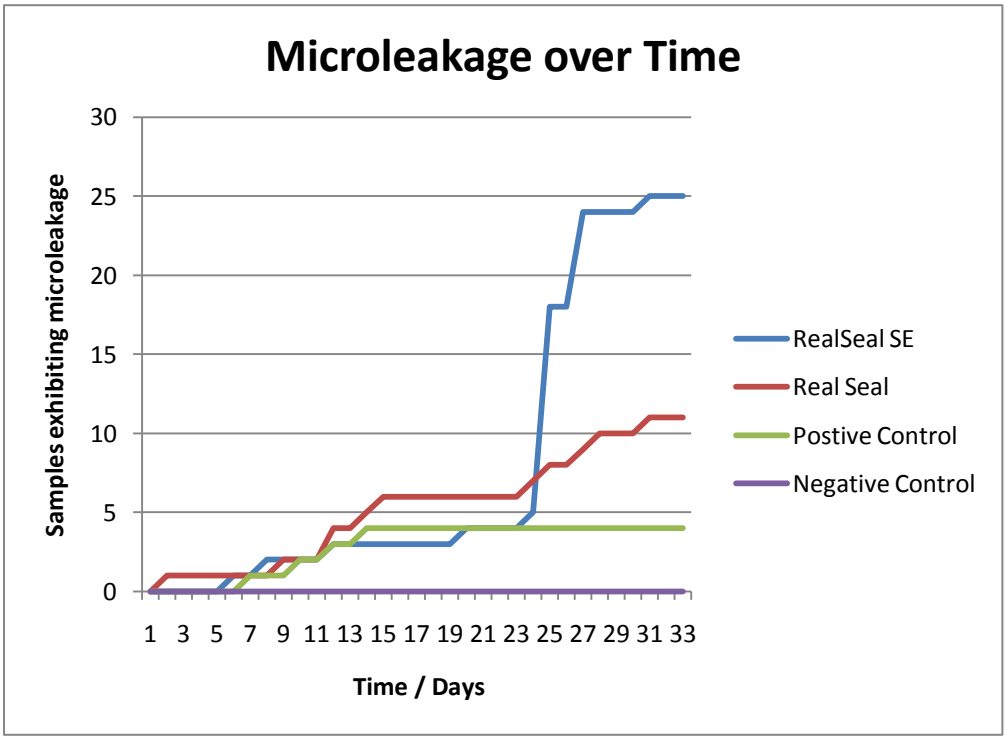

FIGURE 12. Microleakage of experimental groups over time.

TABLE I

Microleakage for the experimental and control groups over time (days)

Days	RealSeal SE	Real Seal	Positive Control	Negative Control
1	0	0	0	0
2	0	1	0	0
3	0	1	0	0
4	0	1	0	0
5	0	1	0	0
6	1	1	0	0
7	1	1	1	0
8	2	1	1	0
9	2	2	1	0
10	2	2	2	0
11	2	2	2	0
12	3	4	3	0
13	3	4	3	0
14	3	5	4	0
15	3	6	4	0
16	3	6	4	0
17	3	6	4	0
18	3	6	4	0
19	3	6	4	0
20	4	6	4	0
21	4	6	4	0
22	4	6	4	0
23	4	6	4	0
24	5	7	4	0
25	18	8	4	0
26	18	8	4	0
27	24	9	4	0
28	24	10	4	0
29	24	10	4	0
30	24	10	4	0
31	25	11	4	0
32	25	11	4	0
33	25	11	4	0

DISCUSSION

The design and execution of the microbial leakage apparatus is critical to the success of any microleakage study.[99] Leakage associated with endodontic obturation has been studied extensively and literature is replete with different methodologies to determine leakage such as "assessment of linear and volumetric dye penetration, autoradiographic detection of isotope penetration, radionuclide detection, culture techniques to detect bacterial penetration, salivary penetration models, fluid filtration techniques, fluorometry, intracanal reservoir techniques, electrochemical techniques, etc."[90] However, these different laboratory studies had poor agreement among each other and their clinical implications for endodontic success are still being debated. For the leakage studies based on dye or isotope penetration, the molecular size of the dye or the radioactive ion was determined to be much smaller than the bacteria, thus making the results clinically insignificant. Other variable factors that confounded these leakage studies were the pH of the tracers, chemical reactivity, and immersion time.[92] For the fluid transport model, it was determined that this model was not only extremely sensitive to the length and anatomy of the root canals, but also to the patency and apical diameter after instrumentation, which greatly increased the confounding variables in this methodology.[97] The weaknesses in these traditional models led the Editorial Board of the *Journal of Endodontics* to make a conclusive statement regarding endodontic microleakage studies. In 2007, it was decided that "sealability studies comparing endodontic procedures using the penetration of dyes, chemicals, etc." were not useful to

endodontic science, and subsequently, the studies using these models are no longer being published in the *Journal of Endodontics*.[90]

Currently, leakage studies employing bacterial cultures are considered to be more reliable and clinically relevant.[92] The proponents of this model argue that clinically, the most common mode of endodontic failure is crown-down leakage of oral bacteria, so that the bacterial leakage model is more reflective of the clinical situation. The most common apparatus for this model is a dual-chambered model, with the tooth sealed in between the upper and lower chamber.[100] Turbidity or a color reaction in the sterile broth in the lower chamber indicates the microleakage of viable microorganisms from the upper chamber. However, in an important review article, Rechenberg et al.[100] discussed serious potential weaknesses within this model that need to be addressed. The seal between the upper and lower chamber is alleged to be a potential source of leakage itself, which can confound the results of bacterial leakage studies published so far. Previous researchers have not traced the routes of microleakage histologically, resulting in inconclusive findings on the exact route of microbial leakage through the endodontic obturation material. Rechenberg et al.[120] in a recent study attempted to assess routes of bacterial leakage in the commonly used two-chamber model designed to evaluate microleakage. In the study, sticky wax was utilized to separate the two chambers, because 23 of the 67 recently reviewed studies on microbial leakage through obturated teeth used sticky wax as the separating medium.[100] Rechenberg et al.[120] found "a blackened discoloration between the sticky wax cuff and the cementum" of the experimental teeth. The discoloration indicated "either the presence of viable *E. faecalis* cells or leakage of blackened enterococci-selective broth from the

lower chamber"[120] which illustrated the major weakness in the traditional dual-chambered apparatus.

In this study, novel ideas were incorporated to bypass the methodological pitfalls inherent in a traditional dual-chambered apparatus. *E. faecalis* was used as the test bacteria in this study, because it is reported to be the most common species associated with failed root canal treatment and persistent endodontic infections, thus improving the clinical relevance of this study.[10, 81] Streptomycin was used as the antibiotic of choice to improve the selectivity of the growth medium. *E. faecalis* was determined to be sensitive to penicillin, ampicillin, and vancomycin, but resistant to streptomycin.[119] Lastly, but most importantly, the interface between the upper and lower chamber was completely eliminated in order to provide only one route for microleakage: through the tested obturated material. This was done through the placement of growth medium broth containing *E. faecalis* directly in the access cavity of the experimental teeth, with the delivery tip providing additional reservoir. The success of the negative controls in showing no leakage and the failure of positive controls in showing any leakage has validated the successful experimental design of the apparatus. The modified dual-chambered apparatus used in this study has overcome the potential weaknesses in the traditional model and thus can now potentially serve as a blueprint for future microleakage studies.

Our results demonstrated that teeth samples obturated with RealSeal SE sealer system had a significantly more microleakage than teeth obturated with RealSeal sealer system. Self-etching adhesive sealers contain an acidic monomer-based primer, which bypasses the need for the traditional etch-and-rinse phase making the technique clinically

less sensitive, as well as more convenient and efficient. Recent studies have shown limited aggressiveness of self-etch resin composites, which results in less hybridization of dentin as compared with the traditional etch-and-rinse adhesives.[71, 121] These higher pH based self-adhesives have been shown to demineralize dentin to only 1 µm, producing much smaller hybrid layers.[122] In coronal dentin bonding, this aspect of self-adhesive composites has been shown to be of minor significance.[123]

The micromechanical strength of the resin/dentin bond is significantly dependent on the complete resin infiltration into the demineralized collagen matrix of dentin, which is also termed the hybrid layer. In the Resilon-based obturation system, the use of EDTA is required to remove the thick smear layer created by hand and rotary instruments within the root canal system. The penetration depth of methacrylate-based adhesives into subsurface dentin varies according to the acidity of the sealers. According to Kim et al.[72] in the absence of EDTA, RealSeal SE with a measured pH of 3.9 could not diffuse through the complete thickness of the smear layer. RealSeal, in contrast, etched through the superficial smear layer to demineralize underlying radicular dentin to a depth of 0.3 µm due to the lower pH of 2.5 of RealSeal self-etching primer.[72] It is this micromechanical retention created at the radicular dentin that is critical for superior bond strength, and thus, lower microleakage at the resin/dentin interface. Thus, the adjunct use of EDTA especially with Real SE is critical to uncover the true self-etching capacity of the sealer to the radicular dentin.

Instrumented root canal systems contain areas that can be inaccessible to canal irrigating solutions such as EDTA. This can result in retention of debris and smear layers especially along the apical third of the canal walls, isthmi, fins, and accessory root

canals.[124, 125] It is in these secluded regions of the root canal system that the etching of radicular dentin through the thick smear layer is particularly critical due to the absence of the demineralizing effects of calcium chelating agent. An absence of seal in these crucial areas will invariably lead to higher microleakage. Our results demonstrate this concept of the poor etching capacity of RealSeal SE, which led to the increased microleakage in contrast to RealSeal.

The geometry of radicular dentin in the root canal system is also considered to be unfavorable to dentin bonding. Configuration factor or C-factor is an important quantitative measure calculated as the ratio of bonded to un-bonded resin surfaces.[126] Any ratio greater than 3:1 is not considered favorable for bonding.[71] Among studies of the root canal system, some reports have estimated the C-factor ratio to be 100:1 and greater, which makes the geometry extremely unfavorable for any type of dentin bonding.[127, 128] According to these studies, the polymerization shrinkage intrinsic in any resin system is greatly magnified in radicular dentin due to the high C-factor ratio and makes the claims of a 'mono-block' concept more of a myth rather than reality. Further research needs to investigate and validate these claims for significant implications for microleakage associated with adhesive obturation systems currently used in root canals.

Concerns have also been raised recently about the potential for alkaline or enzymatic hydrolysis of the polycaprolactone core material. Tay et al.[129] found that the surface resinous component of Resilon was hydrolyzed within 20 minutes of exposure to sodium ethoxide exposing the polymer structure and its fillers. Gutta percha, in contrast, was unaffected by the alkaline hydrolysis of sodium ethoxide. In another study by Tay et al.,[130] susceptibility of Resilon and gutta percha to hydrolytic enzymes was evaluated.

Resilon exhibited extensive surface dissolution of the polymer matrix, which led to exposure of its glass-filler particles. Gutta-percha was again found to be relatively inert against the activities of the hydrolytic enzymes. Thus, as Hiraishi et al.[131] points out, the clinical success of Resilon may also be affected through the mechanism of "enzymatic hydrolysis by endodontic bacteria and fungi via cleavage of the ester bonds within the polymer" core of Resilon. The current study for evaluating microleakage was performed for 33 days and the enzymatic hydrolysis of the Resilon core over such a short-term is considered to be unlikely. However, the gradual biodegradability of the Resilon core can have significant implications on long-term microleakage studies and further studies need to investigate this critical issue.

A potential confounding factor in this study is the possibility that the obturation materials could impede microbial leakage not because of their sealing ability, but rather because of the antimicrobial properties intrinsic within the sealers.[132] However, Siqueira et al.[133] have determined that the currently used sealers displayed "some antimicrobial effect during setting." After setting, the antibacterial effect of the sealers is considered to be short-lived and minimal. In this study, the experimental teeth that were obturated were kept under room temperature settings for 20 days before the initiation of the microleakage study. Thus, the confounding implication of antimicrobial properties on the microleakage results is considered unlikely.[100, 133]

To date, this is the first study that has evaluated and compared the microleakage of RealSeal/Resilon and RealSeal SE/Resilon systems. The higher microleakage associated with RealSeal SE is attributed to the higher pH of the self-etch sealer in comparison to the primer of RealSeal. Further research needs to be done to corroborate

the microleakage results from this study. The microbial leakage apparatus devised in this study utilizing selective growth medium through the use of streptomycin has also been validated by the results of the study. The bacterial leakage apparatus has been considered to be clinically relevant and acceptable by the *Journal of Endodontics*.[90] Thus, the modified dual-chambered microleakage apparatus with selective growth medium used in this research can be easily replicated in future microleakage studies.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to evaluate and compare microleakage of teeth obturated using either RealSeal/Resilon or RealSeal Self-Etch/Resilon systems. The goal was to determine whether a significant difference exists in microleakage between these two groups. To date, no study has been done comparing the microleakage of root canal systems obturated with the use of RealSeal/Resilon versus RealSeal SE/Resilon.

Sixty-two human, single-rooted, anterior teeth were accessed and instrumented for non-surgical root canal therapy. Teeth were randomly assigned to two experimental groups of 27 teeth each. Group I consisted of teeth obturated with the RealSeal/Resilon system, whereas Group II consisted of teeth obturated with the RealSeal SE/Resilon system. In addition, two control groups containing four teeth each served as positive and negative controls, Group (+) and Group (-), respectively. The teeth were then be evaluated for microleakage using a dual-chamber microleakage model. Visual turbidity in the lower chamber denoted microleakage within the experimental groups which were observed for 33 days.

RealSeal SE Group II had a significantly higher proportion of samples with lower time to microleakage than Real Seal Group I. Time to microleakage was also significantly lower in RealSeal SE Group II than Real Seal Group I. No microleakage was observed in the negative control and microleakage was observed in all four samples in the positive control. The higher microleakage associated with RealSeal SE is attributed to the higher pH of the self-etch (SE) sealer in comparison with the self-etch primer of RealSeal. Further research is needed to corroborate the microleakage results from this

study. The microbial leakage apparatus devised in this study utilizing selective growth medium through streptomycin has also been validated by the results of the study and can easily be replicated in future microleakage studies.

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ABSTRACT

AN *IN-VITRO* COMPARISON OF THE MICROLEAKAGE OF REALSEAL/ RESILON AND REALSEAL SELF-ETCH/RESILON ROOT CANAL OBTURATION SYSTEM

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The purpose of this investigation was to evaluate and compare microleakage of teeth obturated using either RealSeal/Resilon or RealSeal Self-Etch/Resilon systems. The goal was to determine whether a significant difference in microleakage exists between these two groups. To date, no study has been done comparing the microleakage of root canal systems obturated with using RealSeal/Resilon versus RealSeal SE/Resilon.

Sixty-two human, single-rooted, anterior teeth were accessed and instrumented for non-surgical root canal therapy. Teeth were randomly assigned to two experimental groups of 27 teeth each. Group I consisted of teeth obturated with the RealSeal/Resilon system, whereas Group II consisted of teeth obturated with the RealSeal SE/Resilon system. In addition, two control groups containing four teeth each served as positive and negative controls, Group (+) and Group (-), respectively. The teeth were then evaluated

for microleakage using a dual-chamber microleakage model. Visual turbidity in the lower chamber denoted microleakage within the experimental groups observed for 33 days.

RealSeal SE Group II had a significantly higher proportion of samples than Real Seal Group I. Time to microleakage was also significantly lower in RealSeal SE Group II than in Real Seal Group I. No microleakage was observed in the negative control and microleakage was observed in all four samples in the positive control.

To date, this is the first study comparing the microleakage of RealSeal/Resilon and RealSeal SE/Resilon systems. The higher microleakage associated with RealSeal SE is attributed to the higher pH of the self-etch (SE) sealer in comparison with the self-etch primer of RealSeal. The self-etching potential of the sealer system is particularly critical in areas inaccessible to calcium chelating agents such as EDTA in root canal systems. Further research needs to be done to corroborate the microleakage results from this study.

The microbial leakage apparatus devised in this study, which used a selective growth medium with streptomycin, has also been validated by the results of the study. The bacterial leakage apparatus has been considered to be clinically relevant and acceptable by the *Journal of Endodontics*. Thus, the modified dual-chambered microleakage apparatus with a selective growth medium used in this research can be replicated easily in future microleakage studies.

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