AN *IN-VITRO* COMPARISON OF BACTERIAL MICROLEAKAGE OF ZINC-OXIDE EUGENOL AND BRASSELER BIOCERAMIC SEALER

by

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INTRODUCTION

One of the keys to successful root canal therapy is adequate obturation of the prepared root canal space.[1, 2] Obturation of the canal system has historically been achieved with gutta-percha and sealer.[3] The goal of root canal obturation is to provide a complete filling of the canal in all dimensions in order to create a fluid-tight seal to prevent ingress of bacteria and their toxins into the periapical tissues.[3-5] Development and maintenance of the seal are essential to optimize the long-term success rate of root canal treatment.[6] This success rate is predicated upon a well-prepared and thoroughly obturated root canal system.[7, 8] Studies suggest that the ideal root canal obturating material should be well adapted to the canal walls and that the entire length of the canal be densely compacted with a homogeneous mass of gutta-percha.[7, 8] Gutta-percha is the most widely used root canal filling material in dentistry. Ingle stated that the most common cause of endodontic failure is incomplete obturation of the root canal system. [9]

Grossman[10] first outlined the ideal root canal filling material in 1940. The characteristics of this material are: 1) ease of introduction, 2) presenting as a liquid or semisolid state that becomes solid, 3) seals the canal laterally and apically, 4) does not shrink, 5) impervious to moisture, 6) bacteriostatic, 7) non-staining, 8) nonirritating to periapical tissues, 9) easily removable, 10) sterile or sterilizable and 11) radiopaque. McElroy[11] then expanded on these key properties of the ideal obturation material by underscoring the importance of biocompatibility, insolubility in tissue fluids, ease of manipulation and adaptation to root canal irregularities. Studies have also found that the concept of warm vertical condensation of gutta-percha improved the sealing properties of

the obturation material.[12, 13] In 1967 Schilder[5] first introduced the concept of warm vertical condensation of gutta-percha in attempt to remedy the lack of 3-D obturation created with lateral condensation. Further research performed by Reader et al.[14] found that warm vertical condensation of gutta-percha filled significantly more lateral canals than cold lateral condensation.

Root canal sealers are an important component in sealing the interface between the dentinal walls and the obturation material. The goal is to obtain a hermetic seal after adequate cleaning and shaping of the canal. This hermetic seal cannot be obtained without the use of a sealer because gutta-percha as mentioned previously does not bond to the dentin walls.[5, 15] Early sealers were modified zinc oxide-eugenol cements based on Grossman and Rickert's formulas. These sealers are still used today.[16] New sealers have been placed on the market to improve the property of this hermetic seal and decrease the gap between the gutta-percha and dentin wall. An ideal endodontic sealer should, in part, adhere firmly both to dentin and gutta-percha. Differences in the adhesive properties of endodontic sealers may be expected because their interaction with either dentin or gutta-percha may vary with their chemical composition. No specific interaction either with dentin or gutta-percha is expected from the setting reaction of calcium hydroxide based sealers and the epoxy-based sealers. In contrast, the zinc oxide-eugenol sealer should firmly bond to dentin and gutta-percha. The setting reaction of the zinc oxide-eugenol mixtures is a chelation reaction occurring with the zinc ion of the zinc oxide.

PURPOSE

The purpose of this investigation is to evaluate the sealing properties of a new bioceramic sealer using gutta-percha with warm vertical condensation and using single

cone technique. The model used is one that was first described by Torabinejad et al.[13] and later refined by Pitout et al.[17] The specimens will be exposed to *E. faecalis*. The goal is to see if there is a significant difference in microleakage between teeth sealed with bioceramic sealer and teeth sealed with Roth Sealer.

HYPOTHESES

Null Hypothesis: There is no significant difference in microleakage between bioceramic sealer and the Roth sealer.

Alternative Hypothesis: There is significantly less microleakage with the bioceramic sealer compared with the Roth sealer.

REVIEW OF LITERATURE

HISTORY OF ENDODONTICS

"A profession that is ignorant of its past experiences has lost a valuable asset because it's missed its best guide to the future," a statement by B.W. Weinberger that we could still apply today.[18]

The American Association of Endodontics defines endodontics as the branch of dentistry revolving around the morphology, physiology, and pathology of the human dental pulp and periapical tissues[19] Dentistry has a long and fascinating history; many of the remarkable techniques in modern dentistry can be traced to the very earliest of times in every culture.

It was the relief of odontogenic pain that played a major role in the specialty branch of dentistry. One of the earliest descriptions of the toothache was credited to Fu-His in 2953 s leading to the advent of various "cures" and "remedies.[20] Hesi-Re was the earliest dentist who practiced in 3000 B.C. and was called "Chief of Toothers." The Egyptian pharaohs were also known to have suffered from periodontal disease. Radiographs of mummies confirm this.[21]

The tooth worm theory began since the Babylonian times and thought to reside in the hollow portion of the tooth. It was thought to cause a toothache by gnawing at the structure of the tooth.[22] In an ancient Babylonian epic, the worm comes weeping to the goddess Ea, asking what is to be its food and, on being told that it is to have fruits for sustenance, cried out:

"Me! What are these ripe figs to me?

And soft pomegranates?

Lift me up, between the teeth, and the

Jaw bone set me,

That I may destroy the blood of the teeth, and ruin their strength,

Grasp the prong and seize the root."[23]

Many methods were used to "drive the worm from his hiding place in the hollow tooth."[23] One method was to place honey on the outside of the tooth. They thought that the tooth worm would emerge to eat the honey and then "plucked" from the tooth. Another, and most common method was to burn henbane seeds, and direct the fumes into the mouth and smoke the tooth worm out. The priest physician Andrew Boorde described the treatment in the following words:

> … and if it (toothache) do come by wormes, make a candell of waxe with Henbane seeds and light it and let it perfume of the candell entre into the tooth and gape over a dyshe of cold water and than you may take the wormes out of the water and kyll them on your nayle.[23]

Evidence of this belief has also been found in ancient India, Egypt, Japan, and China.[20] The legend of the worm is also found in the writings of Homer, and as late as the 14th century the surgeon Guy de Chauliac still promoted the belief that worms cause tooth decay. The Edwin Smith Papyrus, written in the 17th-century B.C. but which may reflect previous manuscripts from as early as 3000 B.C., includes the treatment of several dental ailments.

The tooth worm theory was proven false in 1200 by a physician named Gaubari in his *Book of the Elite Concerning the Unmasking of Mysteries and Tearing of Veils* which dedicated a chapter to dentistry. He was the first to reject the idea of caries being caused

by tooth worms, and he stated that tooth worms in fact do not even exist. The theory of the tooth worm was thus no longer accepted in the Islamic medical community from the 13th century onwards.

The Indus valley civilization has yielded evidence of dentistry being practiced as far back as 7000 B.C.. This earliest form of dentistry involved curing tooth related disorders with bow drills operated, perhaps, by skilled bead craftsmen. The reconstruction of this ancient form of dentistry showed that the methods used were reliable and effective.

In the 18th century B.C., the Code of Hammurabi referenced dental extraction twice as it related to punishment. Examination of the remains of some ancient Egyptians and Greco-Romans reveals early attempts at dental prosthetics and surgery.[24]

Ancient Greek scholars Hippocrates and Aristotle wrote about dentistry, including the eruption pattern of teeth, treating decayed teeth and gum disease, extracting teeth with forceps, and using wires to stabilize loose teeth and fractured jaws. Hippocrates (460 B.C. -377 B.C.) earned the title of "Father of Medicine." Aristotle (384-322 B.C.) the great philosopher, referred to teeth in many of his writings. Diocles of Carystus, physician of Aristotle's time recommended "rubbing the teeth and gums: as oral hygiene instructions. Roman medical writer Cornelius Celsus wrote extensively of oral diseases as well as dental treatments such as narcotic-containing emollients and astringents. By 2000 B.C. the Chinese were practicing dentistry and around the 2nd century A.D the Chinese developed a silver amalgam paste for fillings. This was more than 1000 years before dentists in the west. Leonardo DaVinci studied human anatomy and sketched every part of the human body. He was the first to differentiate between molars and premolars.

It has been said that Abraham Lincoln was afraid of dentists. In 1862 Lincoln developed a severe toothache and consulted Dr. G. S. Wolf, a dentist who had an office near the White House. As Wolf prepared to pull the tooth, Lincoln asked him to wait. Lincoln "took a container of chloroform from his pocket, inhaled it deeply, and sleepily gave the signal for the dentist to proceed." Experiences of the first US president, George Washington battled significant tooth pain, only to eventually succumb to it by wearing dentures. During the Revolutionary War, Washington requested the services of a dentist because of tooth and gum pain that the general wanted "relieved by a man of skill." Even some of Washington's portraits reveal his terrible state of tooth affairs. A painting by Charles Willson Peale of Washington at age 44 shows a scar on his left cheek from an abscessed tooth.[25, 26]

In 1678 Charles Allen, describing the techniques of dental transplants, wrote the first English language book on dentistry. At the time, there was no mention of endodontics as we know it today. It was necessary to experiment with new techniques, materials, and instruments even though the aim of endodontics has been to relieve pain, maintain exposed pulp, and preserve teeth. Often, these attempts were successful. It was not until Pierre Fauchard who was considered the founder of modern dentistry wrote "Le chirugien dentiste." *The Surgeon Dentist* in 1728 described dental pulp and dispelled the idea of "tooth worm" which has been considered the cause of caries and toothaches since the time of Assyrians. He described the technique of opening and draining teeth followed by packing the chamber with lead foil. He also described the removal of pulp tissue in 1746. In 1725 Lazare Rieviere introduced the use of oil of cloves or cinnamon for its sedative properties. He also used opium with the oil to relieve pain.[27]

In 1756 a German dentist, Phillip Pfaff first mentioned pulp-capping procedure. He cut out a piece of gold or lead to approximate the opening over the pulp and placed this over the exposure so that the surface nearest the pulp was concave. It prevented the metal from contacting the nerve. This was an improvement to Fauchard's method of filling the cavity directly over the exposure.[28]

In 1757 Bourdet, the dentist to Louis XV of France, described the procedure for extracting carious teeth, filling the root canals with gold or lead, and replanting them. He also described "intentional" endodontic treatment in which he dislocated a symptomatic tooth in order to sever the nerve and then immediately placed it back into the socket.[29] This was mentioned hundreds of years earlier by the Arabian physician Avicenna.[27]

Avicenna dedicated many chapters of *The Canon of Medicine* to dentistry, particularly dental restoration. Influenced by al-Gazzar, he provided his own treatment for dental caries, stating that carious teeth should be filled with cypress, grass, mastix, myrrh, or styrax among others, with gallnut, yellow sulfur, pepper, camphor, and with drugs for pain relief, like arsenic or wolf's milk. He further stated that arsenic boiled in oil should be dripped into the carious defect.

In 1766 the American physician Robert Woofendale was the first to speak of endodontic therapy by cauterizing the pulp with a hot instrument.[30, 31] He came to New York from England in 1766. The Greeks and the Romans used this method in the past. In addition to hot instruments, they also used boiling oil, herbs, opium, and arsenic to desiccate the dental pulp.[27] By the end of the 18th century, Frederick Hirsch, a German practitioner, wrote of diagnosing occult dental diseases by tapping the suspected teeth. His findings were diseased teeth elicited pain on percussion. The treatment he

recommended was perforation of the tooth at its neck followed by repeated insertion of a red-hot probe. He considered the tooth cured after the cavity was filled with lead.[27]

In 1802 B.T.Longbothom from Charleston, SC, recommended filling roots of teeth when it was deemed inadvisable to extract them.[30] Later, Edward Hudson an Irish man practicing in Philadelphia, has been given credit for the first to place fillings in root canals in 1809. He packed the canals with gold foil, using instruments of his own design.[30, 32, 33] In 1805 J.B. Gariot became one of the first to recognize vitality in connection with pulp treatment.

In 1819 John Callow credited Charles Bew with describing the flow of blood into the pulp through the dentinal wall and periodontal membrane.[22] In 1807 a young German immigrant opened a dental office in Philadelphia. Eleazar Parmly, the founder and co-editor of the first dental journal. He described the first extraction as such "he grasped the tooth with an instrument, shut his eyes, and turning his head from the patient, made a strong effort which dislodged the tooth." This was the beginning for Leonard Koecker, who became a successful dentist and wrote *Principles of Dental Surgery* in 1862. This textbook became the standard work for the next 50 years.[34]

Koecker believed that when the pulp is destroyed by disease or by artificial means, the whole dentinal core is immediately destroyed. This was thought to need an extraction to prevent inflammation and subsequent suppuration and death of vital tissues around. This is when Koecker popularized the pulp capping procedure. It is the same treatment that Pfaff described in 1756.[33, 35, 36] Koecker believed that living tissue could not remain healthy and viable beside dead tissue. This theory dominated pulp treatment procedures for 50 years.[22] Koecker stated he required for the operation a thin iron wire,

fastened to an ivory handle; and a tallow candle with a thick wick. “I put the candle into his (the patient’s) left hand, and direct him to hold it so that the flame of it may be in a position horizontal with his mouth, and about 8 inches from it.” [30]

In 1829 S.S. Fitch believed that teeth were like hollow bones and had an outer periosteum (periodontal membrane) and an inner periosteum or lining membrane that lay between the pulp and dentin. The crown was therefore nourished by the dental pulp membrane on the interior and by the alveolar membrane on the exterior. When the pulp was removed, only the crown lost vitality but the roots continued to be nourished by the periodontal membrane. This lead to the practice of removing the crown of teeth after extirpation of the pulp placing a “pivot crown” on the remaining roots.[33, 35] John Hunter a great British surgeon and anatomist believed that dentin had no circulation, sensibility, capability or repair, and did not posses any of the properties of a living tissue.[35]

In 1836 extirpation of the pulp was extremely painful procedure. Before that time, a symptomatic pulp was cauterized with a hot instrument or corrosive acids. Then, Shearjashub Spooner from New York used arsenic trioxide to devitalize the pulp before removing it. The use of arsenic dates back to the ancient Chinese empire where it was described as a treatment for conditions similar to alveolar abscesses.[27] This therapy became immediate success because it was painless. Leakage of arsenic through the root canal destroyed supporting tissues of the periodontium. Arsenic was still being used until the 1920s.[32, 37] In 1837 Jacob Linderer and his son Joseph published a manual recommending the use of an essential or narcotic oil to render the pulp insensible before attempting to place a permanent filing in a tooth with an exposed pulp.[28]

1838 Edwin Maynard from Washington DC developed the first root canal broach which he fabricated by filling a watch spring. He also developed Hoe-like instruments that could be used for enlarging and for shaping root canals.[30, 38] In 1939 Baker wrote in the *American Journal of Dental Science* that his treatment for an exposed nerve was to remove the nerve, clean the canal, and fill the canal with gold foil. He is credited with writing the first published account of pulpal extirpation, canal cleaning and root canal filling.[27] In the same year S.P.Hullihen classified the causes for toothaches as: exposure of the nerve; fungus of the nerve; confinement of pus inside the tooth; diseases of the periosteum covering the fang; and sympathy. According to his classical description of the "fungus of the nerve" he probably was referring to what is now called a pulp polyp.[27]

Although "mazer wood" had been brought to England as a curiosity for 200 years, it was not until 1847 that Edwin Truman introduced gutta-percha as a filling and denture base material.[34, 39] Throughout the 1850s plugs of wood soaked in creosote were used to fill canals. A solution of Hills stopping and chloroform or eucalyptus oil was used as a liquid cement to seal the wooden plug. This was an early attempt to obturate the canals with a solid root-filling point and cementing medium or sealer.[27] Hill's stopping (restoration) was composed of gutta-percha, quicklime, powdered glass, feldspar, and metal fillings. This was routinely used as a temporary restoration.[37]

In 1821 Codman was the first to claim the ultimate objective of deposition of secondary dentin.[33] In 1851 S.P Hullihen described an procedure he claimed to have performed since 1845 known as the "Hullihen's operation." The procedure was a venting technique used to treat a congested pulp. After a flap was raised over the root of the

symptomatic tooth, a spearlike drill was used to bore through the alveolus and root to gain access to the canal and to induce hemorrhage of the pulp.

Hunter and Fox also had used this technique of drilling into the pulp cavities earlier to allow pus to escape; whereas, Hullihen did so to deplete a congested pulp and to allow its preservation.[33] In 1864 Branum from New York devised a thin sheet of rubber to isolate a tooth during gold foil operations. This became the "rubber dam" that we use today. This quickly became part of endodontic treatment to provide greater aseptic treatment.[27, 30]

In 1863 Clarke from Iowa and others were obturating teeth with a hot mass of base plate gutta-percha. The technique was to heat the material as hot as possible without burning it and then stir it into the canals with a hot instrument. Many authors have credited Bowman as the first to use gutta-percha as the sole material for filling the root canal.[30, 32, 40] In 1867 Joseph Lister used carbolic acid as an antiseptic during surgical procedures. At the same time, Leber and Rottenstein from Berlin proved the existence of a parasite they called *Leptothric buccalis* during the same year. This existed on tooth surfaces, in carious lesions. This finding concluded that tooth decay could cause "gangrene" of the pulp. At that time attempts were made to transfer Listers newfound antiseptic treatment of wounds unto the pulp treatment. In the same year Magitot recommended the use of an electric current for pulp testing.[28, 36]

In 1878 Rogers suggested in an article that pathogenic organisms might be the most common causes of disease of the pulp. He concluded that successful treatment required total destruction of these organisms. This was based on the recognition of the pathogenicity of bacteria, led to the vitalism theory and consequently opened the door to

the new septic theory.[35] Charles S. Tomes in 1879 attempted to incorporate the recent discoveries in pulpal bacterial pathology with the older theory that the etiologic factor of pulp sequelae was a lack of vitality in the tooth. The old "dead tooth" theory was supplanted by the septic theory.[41]

In 1882 Arthur Underwood developed the septic theory. He proposed that suppuration of the pulp with its resultant alveolar abscess depends on the toxic effects of the pathogens. He believed that whether the pulp was vital made no difference as long as the contents of the pulp chamber and canal were sterilized with antiseptics.

In 1883 G.A. Mills described a procedure in which dental pulps were extirpated by driving a hickory of orangewood stick into the root canal. The wooden stick first was tapered to allow it to reach the apex. It was then dipped in Creosote or in Carbolic acid. A quick blow with a mallet drive the tapered wedge to the apex, where it was left for a few seconds before being withdrawn with the pulp firmly attached. In 1895 wooden pints for "knocking out the pulps" were commercially available.[30] That same year Dr. Bowman introduced a solution of choloform and gutta-percha, termed chloropercha. This was used with gutta-percha cones to obdurate root canals.

In 1884 Dr. Cassius taught a method of filling root canals based on the following[41]:

1) Root canal (and apical area when infected) was sterilized as much as possible with phenol and iodine.

2) The foramen was sealed with a sterile, solid material that did not protrude from the apex.

3) The remaining canal and chamber were filled with antiseptic cement.

Cassius was the first to use carbolized orangewood as a root canal filling material.[42] The same year Koller introduced cocaine as a topical anesthetic. It did not gain wide acceptance because of its extreme toxicity.[38] In the mid-1880s nonvital pulps were amputated at the floor of the pulp chamber leaving a nonvital pulp stump in the root canal. They were covered with a drying agent to prevent decompositional changes from taking place. Arsenic usually was used for this. Lepkowski in 1885 introduced formalin as a drying agent, which allowed better fixation of the pulp stump with the caustic side effects of arsenic.[43]

In 1886 Dr. Evans explained a method of disinfecting devitalized teeth with heat. A silver bulb was attached to a broachlike appendage to expel moisture from the root canal before applying disinfectants and filling material. This instrument became known as the Evans root drier.[33] In the same year G.V.Black advocated the total amputation of roots of molars that were severely periodontally involved. Root canal fillings were placed in the remaining healthy roots preserved these structures.[44]

Before the end of the century, crown and bridge restorations had become popular. All these procedures at the time required the use of a dowel in the root canal, which created a need for more endodontic therapy.

In 1888 Otto Walkoff invented his famous “thermometric” using a water bath of variable temperature; he claimed to be able to diagnose the condition of the dental pulp according to the patient’s reaction to the changing thermal stimuli.[28, 36]

In 1890 C.T. Gramm began using copper points for root canal fillings. Later, he improved the points by goldplating them to prevent oxidation and subsequent discoloration.[30] In 1891 Marshal suggested and popularized the electric pulp tester.

Miller, one of the foremost proponents of septic theory, described the human mouth as a focus of infection. After studying the use of antiseptics for sterilizing dentin, he concluded that more time should be spent sterilizing the carious dentin.[45, 46]

In 1891 Otto Walkoff recommended using chlorophenol to sterilize root canals. In 1899 Hermann Prinz introduced the medicament to American dentists.[43]

In 1892 E.C. Kirk suggested the use of sodium dioxide as a root canal cleaning agent for bleaching discolored pulpless teeth.[30] A year later Emil Schreier introduced a mixture of metallic sodium and potassium for cleaning and disinfecting root canals. When the two alkali metals were inserted into the root canal, they contacted the moisture and produced an explosive, exothermic reaction. This had an effervescent effect of expelling the contents and debris from within the canal.[33, 43]

In 1894 J.R.Callahan, suggested the use of 20 percent to 40 percent sulfuric acid to enlarge and to clean root canals. He sealed a small cotton pellet and soaked it in sulfuric acid within the canal for 24 to 48 hours. The acid helped to sterilize the pulp chamber and canal. He was not concerned about the caustic effect through the apical foramen into the supporting structures. He believed that if an abscess existed, some acid should be forced through the foramen and into the periapical lesion.[30, 33, 47] In the same year John Wessler advocated "Pulpol" to the list of pulp capping remedies, which consisted of 80 percent to 90 percent eugenol and zinc oxide.[28]

Breuer was credited to sterilize root canals by using electromedication. Later it became known as ionization. This was an important step to steer the profession away from the caustic and sometimes damaging sterilizing agents.[30, 43, 48] Dr. Herman Prinz

perfected these techniques in 1917 by the use of 1.0-percent sodium chloride solution during electrolization.[48]

In the same year William Roentgen accidentally discovered x-rays. His discovery was a major event in the development of endodontics. For the first time, dentists were able to visualize the results of their root canal procedures. This was not introduced to the American Dental Profession until after 1910. It slowly became accepted as a diagnostic tool that disclosed previously unknown pathologic conditions in the oral cavity.[37, 38, 49]

A physician, W.J. Morton, took the first radiograph in America, shortly after Roentgen's discovery. About the same time Edmund Kells began to use the x-ray in his dental practice. In July 1896 Kells gave the first dental x-ray clinic at Charlotte, NC. Kells was the first to use x-rays to study root canals filled with lead wires.

In 1898 Thomas Alva Edison discovered that calcium tungstate could be used to manufacture florescent dental mirrors. Dentists used these mirrors to observe pathologic conditions and endodontic results. However, the danger of excessive radiation discouraged their use in 1930s.[34]

In 1900 A.W. Harlan recommended the use of papain for digesting dead pulp tissue. This was made from paw leaves. The papain was mixed with glycerol and hydrochloric acid to from a thick paste. The paste had no side effects on vital pulp tissue and was sealed in the chamber and canals for five to eight days. This was advocated in early 1893 when it was used in the treatment of diphtheria, lupus, and tuberculosis.[30, 33] In the same year Zierler and Lehmann provided a detailed account of the action of galvanic current on bacterial infection of root canals.[48] He also suggested the term electrosterilization.

Price worked on x-rays in the 1900 and coined the term ‘blind abscess’ to describe a radiolucent area in the periapical region with no clinical evidence of drainage. He later advocated the use of radiographs to diagnose pulpless teeth.[36]

T.W. Onderdonk in 1901 he supported examining the root canal for bacteria before filling the canal. He listed absence of pain under temporary restoration and absence of bacteriologic culture after disinfection as the two conditions necessary before root canal filling. When proponents of the “focal infection” theory criticized root canal therapy this was reemphasized about 1919.[30, 36, 43]

In 1904 attention was directed to the relationship between oral sepsis and bacterial endocarditis. Vaughn was the first to use infiltration anesthesia before pulpal extirpation.[32] In 1905 Einhorn developed Procaine (Novocaine). For mucosal injections, a tablet had to be dissolved in solution, boiled, cooled and aspirated into a syringe. Nearly a quarter century later, block anesthesia techniques were perfected.[30, 36, 50]

In 1908 Dr. Meyer L. Rhein, a physician and dentist from New York, devised a technique to determine the canal length and degree of obturation. He used a diagnostic wire in conjunction with radiographs to determine whether the apex has been reached.[30, 36, 50] G.V. Black also suggested a measurement control to determine the length of the canal and the size of the apical foramen so that overfilling could be prevented.[43]

In 1909 Rosenow developed the theory of focal infection. He showed that streptococci were present in many diseased organs and that they were capable of spreading through the bloodstream to establish yet another infection at some distant site.[43] In the same year, Mayrhofer linked the nature of pulpal infection with specific microorganisms. He found that streptococci were involved in approximately 96 percent

of cases studied.[48] He later was associated with the Mayo Clinic where he developed the theory of elective affinity of organisms to tissue. He believed that organisms could pass from an apical granuloma to reach organs in the periphery of the body by elective affinity.[46]

In October of 1910 William Hunter, an English physician and pathologist, gave a lecture on focal infection to the faculty of McGill University in Montreal. His lecture was about "The Role of Sepsis and Antisepsis in Medicine" and it was published a year later. He termed the gold crown "a mausoleum of gold over a mass of sepsis." The focal infection theory reigned for 25 years in the American dental profession. Fortunately, there were a few who instead chose to improve their current procedures by using aseptic techniques, bacteriologic and histological methods and diagnostic x-rays. These people included Coolidge, Johnson, Rhein, Callahan, Grove, Prinz and others. Mainly through their efforts, the principle of preserving the pulpless tooth survived. A year after Hunters lecture, Frank Billings replaced the term "oral sepsis" with "focal infection" At the same time Charles Rosenow defined "focus" as a well circumscribed tissue containing pathogen organisms. They both identified two types of foci: 1) primary ones in skin; and 2) mucous membranes and secondary ones which develop by metastasis from the primary foci.[46]

In 1911 Callahan presented his rosin-chloroform technique for filling root canals. He advocated this as a means to penetrate and to seal the dentinal tubules to provide a better hermetic seal. He later advocated the use of rosin for pulp capping.[47] This never became as popular as his rosin-chloroform technique. In 1912 Rhein was one of the first to provide a rebuttal to Hunter. He admitted that defective root canal treatment was

partially to blame for the “oral sepsis” He also related it to a low fee schedule that forced dentists to use easier and quicker means, such as arsenic paste.

The first dental x-ray unit was available in 1913. It was adopted from a medical unit. In 1919 a conventional dental x-ray machine became commercially available. In 1914 Dr. Grove performed experimental studies in root canal therapy using various mild and strong antiseptics. He found that formalin and formocresol produced severe inflammatory changes in the periodontal ligament. He also found that sodium-potassium caused necrosis of the periodontal ligament, whereas mild antiseptics produced no change.[36] Black and Noyes declared that deposition of cementum about the root end was physiologic, whereas Rhein believed it was pathologic.[42]

In 1917 Percy Howe recommended the use of silver nitrate and ammonia in a 25-percent formalin solution to sterilize root canals. However this mixture caused silver to precipitate and this discolored the tooth structure. This was also caustic to the soft tissues.[30] Silver nitrate was used for 30 years even with all these disadvantages.

In 1917 dental researchers questioned the efficiency of medicaments for sterilizing tooth structure. They advised that no medicament sealed in an infected tooth would sterilize the tooth. From here, root canal therapy began to turn from instrumentation and medication to a more careful examination of the biologic principles. Hermann[51] began using calcium hydroxide mixture, called Calxyl, for filling root canals in 1920. He condemned the use of substances such as phenol, tricresol-formol, paraformaldehyde, camphor and other medicaments that were considered foreign to the body. He believed that non-biological medicaments were cytotoxic and would precipitate the formation of various lesions.[52] In 1921 Rosenow and Meisser proved that the apexes

of healthy teeth could be infected through external contaminants. Rosenow maintained that once a tooth became infected, it always remained infected.

Rickert in 1925 proposed the use of a cementing medium, or sealer, in conjunction with gutta-percha. For this method a pre-fitted gutta-percha cone was passed to the dentiocemental junction was coated with the sealer. The cone was then inserted and passed into place, which forced the sealer laterally and apically, so as to obturate and to void an accessory foramen. This technique was later improved by using an instrument designed to facilitate lateral condensation, which, when used, provide space for additional cones to be inserted. The same year, Lentulo introduced his rotary paste inserter. It was made form a length of flexible steel wire in the form of a spiral and was mounted on a mandrel. When rotated counterclockwise direction, the soft paste was carried apically through the root cabal. Later, this instrument was used by some to carry the root canal sealer advocated by Rickert.[53]

In 1928 Walkoff was already using a solution of chlorophenolcamphormenthol, with iodoform, creating a resorbable root canal filling paste. He reported that this iodoformized paste would not irritate the tissues, was resorbable, stopped secretions, and was a long-lasting antiseptic. It proved to be mild and nonirritating filling material. Four cases were reported in which the paste was inadvertently carried beyond the apex and into the sinus without any demonstrable clinical reaction.[53]

Until 1929 human pulp was thought to have little capacity for healing. Then, Balint Orban, using histologic studies of pulpal tissue, he found that the same tissue and blood cells of defense and repair that are in the pulp also are contained in other connective tissue. This did not gain wide acceptance in America. During the same year

Dr. Carl Grove[54] called for standardization of sizes of root canal filling materials and instruments. In 1930 the pendulum began to swing toward a more conservative approach to root canal therapy with improved and more universally used radiographic practices, bacteriological culturing, and a more pronounced emphasis on definitive diagnosis and aseptic techniques.[55] Although a change was occurring, it took approximately a decade before a more conservative approach to the pulpless tooth would be generally accepted in practice and widely taught in dental schools. Rickert and Dixon began a series of experiments in 1931 that were the main support for the hypothesis of the "hollow tube effect" This postulates that if a void is left in a root canal filling, the space may be filled with tissue fluids which undergo enzymatic breakdown in the absence of microorganisms. These products were believed to have the capability of reaching the periapical tissues and to precipitate an inflammatory reaction.[36, 56]

Logan in 1937 believed that the presence of microorganisms did not necessarily imply the presence of infection, but that bacteria are often present in normal tissue without having pathological significance.[56] In the same year, Tunnicliff and Hammond found that microorganisms were present in the pulps of extracted teeth without any evidence of inflammatory tissue changes.[43, 56] In the same year, Cecil at Cornell Medical College reported 200 cases of arthritis in which treatment consisted of removing suspected foci. He found little benefit from surgical intervention. Burket concluded that any clinical improvement after removal of foci suggested a casual relationship between the foci and disease process.

With more scientific evidence based on histological, biological and pathological findings were able to usher the new era in root canal therapy and to stop the wholesale

extraction of nonvital teeth. In 1940 Sommer and Crowley compared the radiographic appearance of periapical lesions with the bacteriological status of the pulp cavity. In finding no correlation between the two, they helped establish the principle that a radiolucent area is not synonymous with infection and a type of microorganism should not be diagnosed from an x-ray.[36] Wais investigated more in 1958 to show that there is no correlation between the type of radiographic lesion and histopathologic findings.

By the end of World War I, chloramine-T solutions was being used for treating wound infections, and were adapted for use as root canal medicaments. In 1941 Fred Adams helped usher the use of antibiotics in root canal therapy by reporting his experience with sulfanilamide in treating periapical infections. He was also credited with being the first to use penicillin in pulp canal therapy in 1944. Grossman also played a major role in the early application of penicillin for root canal treatment. He suggested that if penicillin were used in endodontic procedure, a non-aqueous solution would be more economical. He later used absorbent paper points impregnated with penicillin to sterilize root canals.[32]

Organized endodontics began in 1943 when a group of 25 men met in Chicago and formed the American Association of Endodontics.[57] The term endodontia was unknown before this time. Harry B. Johnston has been given credit for coining the term which is the combination of the greek words: “endo” (within) and “ho dontas (a tooth). He was also one of the first dentists to have limited his practice to endodontics.[58]

The first dental journal devoted entirely to the subject of endodontics, *The Journal of Endodontia*, was published in 1946. Balint Orban was the first editor. Although the publication was discontinued in 1948, arrangements were made at that time

to establish a section in *The Journal of Oral Surgery, Oral Medicine, and Oral Pathology*.

In 1953 many new antibiotics and combinations were found to completely eliminate all bacteria within the root canal. In 1953 Auerbach helped stem the antibiotic tide by reemphasizing the importance of thorough cleaning of the pulp chamber rather than sole dependence on drug therapy.[59] In 1949 the American Association of Endodontists had formed a committee to study the possibility of establishing a specialty board in endodontics. After conferring with the American Dental Association's Council on Dental Education for several years, the American Board of Endodontics was organized in 1956 and incorporated in the state of Illinois.[46]

Sargenti and Richter introduced N_2 (nitrogen gas), a sealer and a medicament to the American Dental Profession in 1959, and, in doing so presented controversy to the practitioners of endodontics that still exists today. Many authors have cited evidence that N_2 is highly irritating to the remaining pulpal and periapical tissue. In addition, the Council on Dental Therapeutics of the American Dental Association has refuted the claims that N_2 has unusual antiseptic properties.[60]

In 1963 more than 200 American dentists were limiting their practice to endodontics.[57] The American Dental Association recognized endodontics as a special area of dentistry that same year. The first examination and the names of Diplomats occurred two years later in 1965.[58]

An Old Italian proverb summarizes the basic theme of this section "chi lascia la via perde e non sa quell che trova" (He who gives up the old ways for the new, knows what he has lost, but not what lies ahead).

ENDODONTIC THEORY

As endodontists we are concerned with the morphology, physiology, and pathology of the human dental pulp and periapical tissues.[6] This specialty encompasses the basic clinical sciences including biology of the normal pulp, etiology, diagnosis, prevention and treatment of diseases and injuries of the pulp, and associated with periapical conditions. The goal of endodontic therapy is to prevent and cure apical pathosis with the goal of restoring and retaining the tooth in a healthy state to allow for proper form and function in the masticatory apparatus.[50]

A classical article by Kakahashi et al.[61] discovered the true correlation between pulpal pathosis and bacteria. This study concluded that bacteria needs to be present for pulpal and periapical pathosis. Therefore, rendering the canal free of bacteria through the use of biomechanical cleaning and shaping, various irrigation solutions, and intracanal medicaments are essential for healing. This study demonstrated exposing the dental pulps in rats in a germ-free environment only led to mild pulpal inflammation and no abscess formation. On the other hand, conventional rats that were not germ-free exhibited abscess formation and the development of purulence in less than 10 days in all cases.

In 1975 Wittgow[62] investigated the pulpal chambers of intact teeth with necrotic pulps as the result of trauma. They were opened and sampled using clinical techniques that minimized the possibility of contamination by oral flora. Samples were placed in an anaerobic environment at chairside and were cultured and identified with the techniques and criteria developed by the Virginia Polytechnic Institute Anaerobe Laboratory. Thirty-two of the 40 teeth sampled had infected pulpal chambers. Three or more species of microorganisms were cultivated from 50 percent of the infected pulpal chambers. The

presence of opportunistic pathogens in such large proportion of intact teeth with pulplal necrosis as a result of trauma should dictate caution when initiating endodontic therapy to prevent instrumentation beyond the apex. Over instrumentation was thought to have the effect of injecting these microorganisms into the alveolar bone. Such manipulation was thought to play a factor in flare-ups that occurs with asymptomatic teeth.

In 1976 Keudell[63] sampled pulp chambers for bacteria. His results were similar to Wittgow, where anaerobes were not detected in vital pulp chambers containing bacteria, 64 percent of the pulps contained obligate anaerobes. Most of the teeth with necrotic pulps as a result of trauma were infected with anaerobes. The genera and species of many anaerobes were similar to many previous studies.[62] The data in this article emphasized the importance of complete instrumentation in endodontic procedures.

Sundqvist et al.[64] in 1977 reinforced the concept of pulpal pathosis and how it can only occur in the presence of bacteria. He found that apical periodontitis could only be demonstrated in teeth with bacteria present in canal systems, whereas periapical pathosis was not present in any necrotic tooth with intact crowns in a sterile environment.

In 1979 Matusow[65] demonstrated an appreciation of the pathogenic potential of oral microorganisms. A wide spectrum of microbes was demonstrated as etiologic factors in a severe form of acute pulpal-alveolar infection; acute cellulitis. Streptococci are the major microbes associated with acute odontogenic infection. Anaerobes, primarily gram negative are also significant factors. This study discussed a culture technique for specific microbial growth that could be used in later studies.

Griffee et al.[66] in 1980 identified that *B. melaninogenicus* was found to be significantly related to pain, sinus tract formation and foul odor. Suggested relationships

were found between the organism and the presence of apical sensitivity and local swelling. A year later, Matusow[67] screened 10 antibiotics for effectiveness against 105 microbes, specifically isolated from 78 teeth involved with acute pulpal-alveolar cellulitis. On the basis of effectiveness and compatibility, erythromycin was regarded as the drug of choice for initial therapy. Streptococci were the predominant class of microbes isolated and enterococci was the most resistant. No resistance was noted with erythromycin but found 52 percent resistant with clindamycin. Tetracycline was the most effective of the common drugs for the aerobic gram-negative rods.

In 1981 Moller[68]confirmed that bacteria are necessary for periapical pathosis. He studied 78 teeth in 9 monkeys. These teeth were aseptically necrotized. Twenty six of the pulp chambers were kept bacteria-free by sealing, while 52 were infected by the indigenous oral flora. The results were recorded clinically, radiographically and microbiologically at the beginning of the experiment and after 6-7 months. The final examination also included histologic recordings. It was shown that non-infected necrotic pulp tissue did not induce inflammatory reactions in the apical tissues. By contrast, teeth with infected pulp tissue showed inflammatory reactions clinically and radiographically. Facultative anaerobic streptococci, coliform rods and obligate anaerobic bacterial strains were most frequently found. In the final samples the number of obligate anaerobic strains increased.

In 1983 Matusow[69] published Part 3 of a study on acute pulpal-alveolar cellulitis. This involved a drug-resistant *Candida albicans* that resolved cellulitis successfully with endodontic treatment in 6 days. This emphasized that effective debridement, irrigation, and intracanal medication were significant factors in obtaining a negative culture at

completion of treatment. This case illustrated that clinical procedures and judgment can be major factors in the resolution of serious infection, where antibiotic therapy is not feasible.

Benjamin Schein[70] in 1986 suggested that excision of necrotic tissue and drainage are necessary and may be the only treatment required for minor infections. Yoshida et al.[71] in 1987 showed a positive correlation between bacterial growth and clinical symptoms. *Peptococcus magnus* and *Bacteroides* species were commonly found in clinically acute cases, while oral streptococci and enteric bacteria were frequently isolated from clinically asymptomatic cases. Faber[72] in 1988 studied the etiology of endodontic infections. At the time, work elucidated specific mechanisms by which bacterial components such as endotoxin and other cell wall components can contribute to the inflammatory processes.

In 1989 Sundqvist[73] evaluated the prevalence of black-pigmented *Bacteroides* in the root canals of 72 teeth with apical periodontitis. Twenty-two of the canals contained one or more species of black-pigmented *Bacteroides. Bacteroides intermedium* (14 strains) and *Bacteroides endodontalis* (5 strains) were most common. Of the species *Bacteroides gingivalis, Bacteroides loeschei,* and *Bacteroides denticola,* 2, 3 and 1 strains, respectively, were isolated. Sixteen of the 22 root canals containing black-pigmented *Bacteroides* species were associated with acute apical abscesses and purulent drainage through the root canal. The other six teeth with black-pigmented *Bacteroides* were asymptomatic. One additional abscess was present among the 72 cases. This root canal contained *Actinomyces israelii* and *Actinomyces naeslundii.* It was then proposed by Shah[74] in 1990 that the genus *Bacteroides* species be reclassified in a new genus,

Prevotella. The type species is *Prevotella melaninogenica*. Baumgartner[75] in 1991 demonstrated in his study the presence of predominantly anaerobic bacteria in the apical 5 mm of infected root canals in teeth with carious pulpal exposures and periapical lesions.

Sundqvist[76] in 1992 studied the ecology of root canal flora and confirmed that, endodontic treatment, apart from directly eliminating bacteria, can completely disrupt the delicate ecology and deprive persisting bacteria of their nutritional source. Later in 1994 he stated that pathogenicity of the polymicrobial root canal flora is dependent on bacterial synergy. The associations are most likely based on nutritional demands and nutritional relationships.[77] Molander[78] in 1998 concluded that the microflora of the obturated canal differs from that found normally in untreated necrotic dental pulp and suggested reconsideration of nonsurgical retreatment strategies. In the same year Sundqvist[79] reinforced how microbial flora in canals after failed endodontic therapy differed. In his sample, infection at the time of root filling and size of the periapical lesion were factors that had a negative influence on the prognosis. Three of four endodontic failures were successfully managed by retreatment.

Love[80] in 2001 postulated that the virulence factor of *E. faecalis* in failed endodontically treated teeth may be related to the ability of *E. faecalis* cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum.[80] *E. faecalis* possesses many virulence factors such as lytic enzymes, aggregation substance, pheromones and lipoteichoic acid. Collagen binding proteins helps *E. faecalis* bind to collagen in dentin. Gelatinase and serine protease is secreted by *E. faecalis* and

contributes to bone resorption and degradation of dentin organic matrix thus playing a role in periapical inflammation.

The question now was no longer whether microorganisms are involved in the pathogenesis of such diseases but which specific microbial species. Siqueria[81] discussed this in 2002 and stated that molecular methods have contributed significantly to the knowledge about microbial species involved. A great deal of additional research was needed to define the specific role played by suspected endodontic pathogens in the etiology of each form of periradicular disease and to determine the best therapeutic measure for the pathogens eradication. He acknowledged how bacterial resistance was emerging against most known antibiotics and how their use in endodontics should be limited and restricted to a few cases. In 2003 he addressed the idea of molecular technology for microbial identification and how it's allowed the detection of microbial taxa that has never been previously found in endodontic infections.[82]

Prior to molecular technology, culturing was studied by Zeldow, Sletzer and Bender in 1963 and 1964. In the 1964 study by Seltzer and Bender[83] the results of root canal therapy in 2335 teeth after 6 months and 706 teeth after 2 years were studied statistically in an attempt to uncover a significant relationship between success of repair in teeth with and without areas of rarefaction, positive or negative cultures, method of filling canals, and other variables. Among root canals of teeth which in the previous visit had yielded a negative culture 16.6 percent yielded positive cultures immediately prior to filling of the canal. Healing was successful in 82 percent of the teeth in all categories. On the basis of radiographic evidence alone, the prognosis for successful repair was less favorable in teeth with areas of rarefaction, regardless of the bacteriologic status of the

root canal. Finally there was no statistically significant difference between successes of repair in teeth yielding positive or negative cultures prior to filling, differences in degree of success in this study were dependent upon radiographic interpretation and not on other clinical factors.

In 2005 Kaufman[84] revealed that that certain bacteria are significantly associated with endodontic treatment failure. *E. faecalis* is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infections ranges from 24 percent to 77 percent. This finding can be explained by various survival and virulence factors possessed by *E. faecalis*, including its ability to compete with other microorganisms, invade dentinal tubules, and resist nutritional deprivation. Use of good aseptic technique, increased apical preparation sizes, and inclusion of 2.0-percent chlorhexidine after the use of sodium hypochlorite and EDTA, are currently the most effective methods to combat *E. faecalis* within the root canal systems of teeth. Stuart in 2006 advocated continued research on *E. faecalis* and its elimination from the dental apparatus may define the future of endodontics.

ANTIBIOTICS

Antibiotics, along with appropriate surgical procedure, provide an effective means for restoration of oral health. In dentistry, Antibiotics are indicated for either treatment of acute infections or for prophylactic coverage of patients at risk for developing bacterial endocarditis or other infections as a result of bacterimia caused by dental procedures. In 1984 Montgomery[85] looked at the principles of anti-infective therapy. He stated that a bactericidal agent is preferred because of greater effectiveness and less reliance on host defense mechanism. For maximal effectiveness of antibiotic therapy of orofacial

infections, several factors must be evaluated in choosing an antibiotic: (1) antibacterial spectrum and specificity of the agent; (2) degree of bacterial resistance reported for the antibiotic; (3) concentrations achieved at various sites; (4) age, type, and extent of infection; and (5) various host factors. The same year Montgomery[86] discussed how penicillin G administered parenterally or penicillin V administered orally are currently the antibiotics of choice for treatment of dental infections of usual etiology. Infections caused by penicillinase-producing staphylococci or those involving gram-negative bacteria should be treated with a penicillinase-resistant penicillin or an ampicillin-like derivative, respectively. Erythromycin was the second-choice bacteriostatic antibiotic, becoming first choice for treating dental infections in patients allergic to penicillin. The cephalosporins, similar in action to ampicillin-like penicillin derivatives, may be used with caution in patients who have exhibited delayed-type allergic reactions to penicillin and when erythromycin cannot be used. Tetracyclines are, at best, third-choice agents for usual dental infections. However, they are useful for cases of acute necrotizing ulcerative gingivitis requiring systemic antibiotic therapy when penicillin is precluded. For candidal infections, nystatin remains a first-choice agent for treatment. Ketoconazole, an orally active systemic antifungal agent, may be used for monilial infections (also known as Candida infections) of the oral cavity refractory to nystatin. Most orally administered antibiotics may cause gastrointestinal disturbances. Superinfections occur with broad-spectrum antibiotics and a severe form of superinfection, antibiotic-associated colitis, has occurred with almost all antibiotics. Allergic reactions of all degrees of severity can occur with most antibiotics. The penicillins, followed by the cephalosporins and tetracyclines, are most frequently implicated in these reactions.

Olson[87] in 1990 provided a rationale for selecting the appropriate antibiotic for odontogenic infections. Increasing reports of antibiotic-resistant bacteria and the introduction of new antibiotics have resulted in a change in prescribing antibiotics in many dental offices. Later, Hersh[88] in 1999 studied the potentially serious adverse drug interactions that can occur between antimicrobial agents used in dental practice and other drugs patients are taking for a variety of medical conditions. It then became important for dentists to stay abreast of potential drug interactions involving antibiotics to avoid serious morbidity among their patients.

As the culturing methods and the susceptibility tests improved throughout the years, Baumgartner[89] in 2003 investigated antibiotic susceptibility tests on a panel of bacteria isolated from endodontic infections. The bacteria was aseptically aspirated with a needle from endodontic abscesses, cultivated, and identified at the species level. Ninety-eight species of bacteria were tested for antibiotic susceptibility. Metronidazole had the greatest amount of bacterial resistance; however, if it is used in combination with penicillin V or amoxicillin, susceptibility of the combination with penicillin V or amoxicillin increased to 93 percent and 99 percent, respectively. Clarithromycin seems to have efficacy, but it is still considered an antibiotic under investigation because the minimum inhibitory concentration has not been established.

SUCCESS AND FAILURE OF ENDODONTIC THERAPY

When attempting to review the outcome studies on nonsurgical endodontic treatment and surgical endodontic treatment, the definitions and classifications have been inconsistent. This created variability of the reported “success” rates. In the majority of

these outcome studies successful outcome is defined by the complete absence of radiolucency and absence of clinical signs and symptoms.

The classic study originating in the University of Washington School[90] of Dentistry comprehensively examined endodontic success and failure as well as its influencing factors. A total of 3678 patients were contacted via mail to participate in a "free x-ray" at follow-up intervals of six months, one year, two years, and five years after non-surgical or surgical endodontic therapy was performed. The two-year study group proved most ideal, because periradicular repair was often not completed for the middle-aged and elderly patient's within one year, and only 302 cases returned at five years. There were a total of 1,229 patients who actually returned at two years, which accounted for 33.41 percent of the original study population. Recall radiographs were evaluated deeming success to those cases that exhibited periradicular improvement and failure to those cases that demonstrated an unimproved or deteriorated periradicular status. The overall success rate of non-surgical root canal therapy at two year follow-up was 91.54 percent. Although examiners found multiple etiologies of root canal failure, incomplete obturation accounted for 58.66 percent. Other causes of failure included: root perforation (9.61 percent), external root resorption (7.7 percent), coexistent periodontal-periradicular lesion (5.78 percent), canal grossly overfilled or overextended (3.85 percent) and other causes. Surgical endodontic treatment was also evaluated at two-year follow-up providing a success rate of 92.88 percent. Sjogren[91] in 1990 looked at the influence of various factors that may affect the outcome of root canal therapy. Three-hundred and fifty-six patients were evaluated 8 to 10 years after treatment. They concluded the results were directly dependent on the preoperative status of the pulp and periapical status. The

rate of success for cases with vital or nonvital pulps that had no periapical radiolucency exceeded 96 percent, whereas only 86 percent of the cases with pulp necrosis and periapical radiolucency exhibited apical healing. Teeth that required retreatment were found to have a 62-percent healing rate.

Lin[92] in 1992 analyzed 236 cases of endodontic treatment failures, none of which had advanced periodontal disease, post perforations, or root or crown fractures. Cases were analyzed clinically, radiographically, and histologically to determine the major factor for treatment failure. It was established that there is a correlation between bacterial infection in the canal system and the presence of periradicular rarefaction in endodontic failures. The apical extent of root canal fillings i.e underfilled, flush-filled, or overfilled, seems to have no correlation to treatment failures.

Smiths[93] retrospective study in 1993 on the outcome of conventional root canal therapy was carried out on patients attending the Eastman Dental Hospital between 1970 and 1982. A minimum follow-up period of 5 years was required for patients to be included in the survey. Factors analyzed were characteristics of the resulting sample group, techniques for canal preparation and obturation and the obturating materials. The type of obturating material used had no demonstrable effect on success rate but sex, age, preoperative vitality and periapical pathology were associated with significantly differing success rates. Another factor that influenced success was technique used. The method of canal preparation and position of the apical seal relative to the radiographic apex were both found to be significant. The overall success rate was 84.3 percent representing 692 of 821 teeth that were included in the survey.

Another influential study by Ray and Trope[94] in 1995 discussed the importance of the quality of the coronal restoration and the root canal obturation on the radiographic periapical status of endodontically treated teeth. Radiographs from randomly selected new patient records at Temple University Dental School were examined. Two examiners evaluated 1010 endodontically treated teeth restored with a permanent restoration independently. The quality of the root filling and coronal restoration was scored as either good or poor. The apical one third of the root and surrounding structures were then evaluated radiographically and the periradicular status categorized as: a) absence of periradicular inflammation (API); and b) presence of periradicular inflammation (PPI). The results concluded that the rate of API for all endodontically treated teeth was 61.1 percent. A good restoration resulted in API cases than good endodontics 80 percent vs. 75 percent. Poor restoration resulted in significantly more PPI cases than poor endodontics, 30.2 percent vs. 48.6 percent. The combination of good restoration and good endodontics had the highest API rate 91.4 percent, significantly higher than poor restoration and poor endodontics with API rate of 18.1 percent.

Sjogren[95] in 1997 investigated the role of infection on the prognosis of endodontic therapy by evaluating teeth that had root canal therapy in a single appointment. Fifty-five single rooted teeth with apical periodontitis were thoroughly instrumented and irrigated with sodium hypochlorite solution and samples were taken for bacteriological sampling. Post-treatment samples were taken and the teeth were then root filled during the same appointment. Healing was evaluated for five years. Complete periapical healing occurred in 94 percent of cases that yielded a negative culture. Whereas samples that yielded a positive culture prior to root filling had a success rate of 86 percent. Further investigation

of three failures revealed the presence of *Actinomyces* species in each case. No other specific bacteria were implicated in failure cases. This emphasized the importance of completely eliminating bacteria from the root canal system before obturating. It was concluded that one-visit root canal therapy could not completely eradicate all infection without the support of inter-appointment antimicrobial dressing.

Trope[96] in 1999 evaluated radiographic healing of teeth with apical periodontitis treated in one or two visits, with or without calcium hydroxide as an intracanal medicament. Patients were followed up for 52 weeks and The Periapical Index (PAI) Scoring Method was used to compare the differences in periapical status. Teeth left empty between visits had inferior healing results. Two visit appointments with calcium hydroxide had an improved PAI score compared to the one step group (74 percent vs. 64 percent).

Lazarski[97] used an insurance company database of 110,766 nonsurgical root canal procedures that were completed by endodontists and their referring general dentists as the basis for an outcome study. Ninety-four percent of nonsurgical root canal treated teeth remained functional over an average follow-up time of 3.5 years. A subset of 44,613 cases, with a minimum required follow-up time of two years, showed incidences of extraction, retreatment and periradicular surgery equal to 5.56 percent, 2.47 percent, and 1.41 percent, respectively. Teeth that were not restored after root canal therapy were significantly more likely to undergo extraction than restored teeth. This strongly supports that the specialist practice provides similar rates of clinical success compared with other providers, even when treating significantly more complex NSRCT cases.

Salehrabi and Rotstein[98] studied the outcomes of initial endodontic treatment done in 1,462,936 teeth of 1,126,288 patients from Delta Dental records and were assessed over a period of 8 years. They found that 97 percent of teeth were retained in the oral cavity 8 years after initial nonsurgical endodontic treatment. Analysis of the extracted teeth revealed that 85 percent had no full coronal coverage. A significant difference was found between full coverage and non-covered teeth for all tooth groups tested. It appears that initial nonsurgical endodontic treatment is a predictable procedure with high incidence of tooth retention.

The Toronto Study[99] assessed 450 teeth over a 4-6 year period. The teeth were obturated using the Schilder technique and step-back with lateral condensation. An independent examiner evaluated all teeth clinically and radiographically. The overall "healed" rate of 81 percent. This was significantly higher for teeth without apical periodontitis (92 percent) than with apical periodontitis (74 percent). This confirmed that apical periodontitis is the main prognostic factor in initial endodontic treatment.

Torabinejad[100] in 2005 evaluated a systemic review and looked to a) search for clinical articles pertaining to success and failure of nonsurgical root canal therapy and b) to assign levels of evidence to these studies. The articles collected were published between January 1966 and September 2004. Articles were reviewed and graded for strength of level of evidence from 1 (being the highest) to 5 (being the lowest). Three hundred and six articles were found, 6 articles were randomized controlled trials (LOE 1). Twelve low quality RCT's (LOE 2), 14-cohort studies (LOE 2), 5 case-control and 8 cross sectional studies (LOE 3). 4 low-cohort studies (LOE 4) and 5 low quality case control studies (LOE 4). The majority (73) of the often-quoted "success and failure"

studies were case series (LOE 4). The rest of the articles were descriptive epidemiological studies (42), case reports (114), expert opinions (18), literature reviews (4), and one meta-analysis. From these results, it can be concluded that few high-level studies have been published in the past four decades related to the success and failure of nonsurgical root canal therapy. This data can be used in future studies relevant to the outcome of nonsurgical root canal treatment.

During the past 40 years, dental implants have evolved to where they are now considered to be a reliable treatment for missing teeth. Dental implant therapy is rapidly changing in the field of dentistry. One of the major issues confronting dentists is the choice of treatment for severely compromised teeth. Even the criteria for defining a tooth as compromised are controversial and subject to differences in interpretation. However, a careful and extensive consideration of indications, contraindications, risks, and benefits of both single-tooth implants and the naturally restored tooth is of critical importance if an accurate evaluation of treatment options is to be presented to the patient for their informed consent. Kim and Iqbal[101] in 2008 reviewed the literature regarding single-tooth implants and restored natural teeth. The major studies published to date indicate that there is no difference in long-term prognosis between single-tooth implants and restored root canal treated teeth. They emphasized that the decision to treat a tooth endodontically or to place a single-tooth implant should be based on the following criteria: prosthetic restorability of the tooth, quality of bone, esthetic demands, cost-benefit ratio, systemic factors, potential for adverse effects, and patient preferences.

ROOT CANAL ANATOMY

Successful endodontic therapy depends to a large extent on the thorough

mechanical cleansing of each root canal. One of the main reasons for failure in root canal therapy is a lack of knowledge of the anatomy of the pulp cavity.[102] Complex canal configuration provides the majority of challenges during endodontic therapy and over the years there have been many methods to study the anatomy of root canals. The methods used can be classified into nine groups: 1) direct observation; 2) direct observation with the aid of a microscope;[103] 3) macroscopic sections;[104] 4) microscopic sections;[102] 5) transverse sections and micrometric measurements;[105] 6) examination of intraoral radiographs;[106, 107] 7) filling and decalcification;[108, 109] 8) filling and clearing; and 9) grinding and radiographs. These studies presented a range of variations throughout the canal system and have increased our success in endodontic therapy. It is not possible to debride, prepare, and fill root canals correctly without first knowing in detail the anatomy of the root canals, because the operator encounters root canals varying in number, size, shape, and having different divisions, fusions, directions, and stages of development. Therefore, no one technique exists for correctly treating all of them in the same manner.

According to Ingle,[57] the most common cause of endodontic failure is apical percolation with the largest percentage of eases failing due to incomplete canal obliteration. Other reasons for failure are frequency of occurrence of the number of canals, shape of the canal system, and the number of roots and the incidence of root fusion. Another reason includes leaving a canal completely unfilled which can be attributed to operator has failing to recognize its presence. Rankine-Wilson and Henry[109] described the common finding of a bifurcated canal in mandibular incisors, cuspids, and premolars. Green[110] gave an exhaustive description of the presence of auxiliary and secondary canals among all the teeth in the dental arch. Therefore, it is our obligation to

be thoroughly familiar with root canal anatomy in both normal and abnormal situations in order to decrease root canal failures.

Green[110] emphasized variations in the morphology of the pulp cavity due to age and functional changes. He studied variations in tooth lengths and how they can vary in proportion to the height of man and often to bear a relationship to the size and shape of the head. He stated that teeth are usually shorter in the female than in the male. It is noteworthy that the average ratio of root length and crown length is about 1.5 to 1 with slight variations.

The apical constriction (cementodentinal junction or CEJ) has long been advocated as the terminal end of instrumentation and obturation.[111, 112] It is in theory the narrowest part of the canal and the location where the pulp ends and the periodontium begins. Ricucci[113] advocated instrumenting to the apical constriction because impingement outside this junction may delay wound healing or result in adverse effects on the outcome of endodontic therapy. Materials or medications extruded beyond the constriction may promote inflammation and a foreign body reaction. Ricucci and Langeland[114] demonstrated that instrumentation and obturation to the apical constriction gave the best prognosis. A poorer prognosis was observed when obturating material extended beyond the apical constriction. A literature review by Wu et al.[115] agreed with the major findings of Ricucci and Langeland. However, it is worth noting that the apical constriction may not always be present or easily identifiable.[112, 116]

In 1955 Kuttler[102] studied 402 apexes of healthy teeth from corpses between the ages of 18 and 25, or more than 55 years of age, using an ocular micrometer, observed that the center of the foramen comes away from the apical vertex with an increase in age.

He also observed that the symmetry of the cone does not follow the same tooth orientation, and also does not end in the apex vertex, deviating to one of its sides at a distance of 0.5 mm, thus justifying a root canal in the referred to limit.

In the same year Green[117] studied the diameter of apical foramina. It was found to range anywhere from 0.03 mm to as much as 2 mm. The average value was 0.57 mm. The major canal foramina was found to be measuring up to 3 mm short of the apex and multiple foramina generally were found to be smaller than accessory foramina with the accessory foramina being smaller than major foramina. Later in 1960, Green[118] studied 877 teeth to determine the incidence of foramen deviation from the anatomic apex. 92.4 percent of the major foramina opened short of the anatomic apex with an average distance of 0.59 mm. This is important to the clinician during instrumentation and filling of root canals in order to avoid encroachment of the periodontal ligament space.

The final apical preparation size remains controversial despite considerable clinical and *in vitro* research. The astute clinician must be aware of this research before choosing any instrumentation system because the informed clinician's decision must be guided by the best available evidenced-based information. Baugh in 2005 provided a review article generated from a Medline-based search strategy to disclose these studies and provide a summary of the results.

In 1969 Weine[104] studied the mesiobuccal roots of 208 teeth that were sectioned buccolingually from the mesial aspect and the canal configurations were categorized. One hundred one had a single canal; seventy-eight showed two canals that merged toward a single apical foramen and twenty-nine displayed two distinct canals with separate apical foramina. The frequency of occurrence of the bifurcated or double canal must be taken in

consideration when surgical and nonsurgical treatment is planned. He later added another canal configuration in which a single canal further divides into two canals then exits with distinct apical foramina.

Schneider[119] in 1971 looked at 29 single root teeth and were classified based on the degree of root curvature, straight, moderate and severe. The canals were instrumented and cross sections were made from 1 mm to 5 mm from the apical foramen. They were then evaluated under a dissecting microscope to determine whether round or triangular preparations had been made. He found that straight canals were more readily prepared round than were curved canals, round preparations were obtained 50 percent of the time at the 1-mm level as compared to 17 percent at the 5-mm level.

In 1972 Pineda and Kuttler[120] took radiographs from both mesiodistal and buccolingual directions to study root canal morphology. A total of 7275 canals of 6219 roots from 4183 teeth were used. All root canals were cone shaped and this conical shape is mostly marked in the middle and cervical thirds of the buccolingual plane. The buccolingual diameter of the apical third of the dentinal canal was often larger than the mesiodistal diameter. Thirty percent had ramifications in the apical third of the main root canal system. Ramifications in the bifurcation or trifurcation of the multirooted teeth were not seen. Only 3 percent of canals were straight in a mesiodistal and buccolingual directions and most of these were maxillary central incisors. In 83 percent of cases, foramen of the main root canal was located to one side of the apical vertex, sometimes to a distance 2 mm or 3 mm. In the remaining 16.9 percent, foramina of the main root canal were located in the top of the apex. The influence of age on root canals was obvious in

the reduction of their diameters. It was concluded that radiographs are a poor representation of the canal system because they are in two dimensions only.

Later in 1974 Vertucci[121, 122] studied 200 maxillary second premolars that were decalcified, injected with dye, and cleared. He classified the canal configuration into eight types:

Type I – Single canal from pulp chamber to the apex.

Type II – Two separate canals leaving the pulp chamber but joining short of the apex to form one canal.

Type III – One canal leaving the pulp chamber, dividing into two within the body of the root, and merging again to exit as one canal.

Type IV – Two separate and distinct canals from the pulp chamber to the apex

Type V – One canal leaving the pulp chamber and dividing short of the apex into two separate and distinct canals with separate apical foramen.

Type VI – Two separate canals leaving the pulp chamber, merging in the body of the root and re-dividing into two distinct canals short of the apex.

Type VII – One canal leaving the pulp chamber, dividing and then rejoining within the body of the root, and finally re-dividing into two distinct canals short of the apex.

Type VIII – Three separate and distinct canals from the pulp chamber to the apex.

Locating the number and position of orifices on pulp-chamber floors can be challenging, this is especially true when the tooth that is being treated is heavily restored, malposed, or calcified. Krasner and Rankow[123] evaluated 500 pulp chambers of extracted teeth and formulated the following laws of symmetry and laws of orifice location:

1) Law of Symmetry #1: The orifices of the canals are equidistant from a line in a mesiodistal direction through the floor, except for maxillary molars.

2) Law of Symmetry #2: The orifices of the canals are located 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 color of the pulp chamber floor is always darker than the walls.

4) Law of Orifices Location #1: The orifices at the canals are always located at the junction of the walls and the floor.

5) Law of Orifices Location #2: The orifices of the canals are located at the angles in the floor-wall junction.

6) Law of Orifices Location #3: The orifices of the canals are located at the terminus of the root developmental fusion lines.

Stropko[124] in 1999 examined 1732 conventionally treated maxillary molars to determine the percentage of mesiolingual (MB2) canals that could be located routinely. The teeth examined were 1,096 first molars, 611 of second molars, and 25 third molars. The MB2 canal was found 73.2 percent of first molars, 50.7 percent in second molars and 20 percent in third molars. It occurred as a separate canal in 54.9 percent of first molars, 45.6 percent of second molars, and joined in third molars. However, as the operator became more experienced and used the dental operating microscope with appropriate instruments, MB2 were located 93 percent of the time in first molars and 60.4 percent in second molars. Prior to this study Kulild and Peters[125] also studied the anatomy of the MB root of 51 maxillary first and 32 maxillary second molars. He found that the location

of the MB2 canals was 1.82 mm lingual to the main MB canal orifice. Baldassari-Cruz et al.[126] indicated that the dental operating microscope provides increased opportunity for the dentist to detect canal orifices.

An accurate knowledge of the morphology of the pulp cavity is essential before one can rationally approach any endodontic procedure. The frequency with which root canals unite should be taken into consideration during the enlargement and filling procedures. One should also be aware of the common occurrence of bifurcated and double canals whenever surgical procedures are planned and as possible causes for unexplained failure. Knowledge of these variations will assist the dentist in reaching conclusions in his diagnosis and treatment of endodontic cases.

CHEMO-MECHANICAL PREPARATION OF THE CANAL SYSTEM

Stewart[127] demonstrated the importance of chemo-mechanical preparation in 1955. He divided root canal therapy into 3 phases: chemo-mechanical preparation, microbial control, and complete obturation of the root canal system. Each phase plays a key role in the healing of the periodontium. As the root canal system is enlarged, the more debris is removed and there is a reduction in the number of viable bacteria by the delivery of our irrigation solutions and medicaments He determined root canal success by obtaining a negative culture before obturation. The goal of treatment is to render the canal free of bacteria.

Endodontic instruments and techniques have evolved over time. Instruments such as K files, reamers, H-type instruments, and barbed broaches were popular and still are. Over time, low-speed instruments, such as Gates-Glidden drills and Peeso reamers were being used. The last group is engine-driven instruments in an electric hand piece, such as

nickel-titanium rotary instruments. Several instrumentation techniques are used including step-back technique, circumferential filing, step-down technique, and balanced force technique.

In 1970 Molven[128] concluded that the ability to shape dentin is not only dependent upon the technique of operation but also the design of the instrument. Roane et al.[129] proposed the use of a K type file with a triangular cross section and a modified tip. They concluded that a file with a triangular cross-section has increased flexibility because of decreased cross sectional area and that a modified tip produces less transportation and ledging of the canal. Ciucchi et al.[130] reported that the use of modified instruments eliminated ledging and transportation effects seen with conventional rotating instruments used in curved canals. The tip of each cutting edge, namely the transition angle, had been removed. The use of modified tip instruments prevented apical deviations and preserved the flow of the preparations in contrast to the undesirable ledging and gouging that are frequently observed when using conventional tipped instruments. Goerig et al.[131] in 1982 described a step-down technique of radicular access. He concluded that there were various advantages to this method. First, the vast majority of microorganisms and pulpal tissues are removed early during cleaning and shaping, thereby reducing the potential for extruding material into the periapical area. Second, instruments pass unhindered into the apical area once the coronal two-thirds are enlarged. Apical shaping is completed quickly and efficiently. Last, the methodology allows for better penetration of the irrigation solution.[131] All instruments must have the property to trace curvature and not form undesirable outcomes like transportation, ledges, and blockages. Roane[132] in 1998 proposed combining the crown-down technique with a file possessing tip guidance as one

of its features. He reported that aberrations in canal shape after instrumentation were significantly reduced and instrument breakage was minimized by this combination. He deduced that shaping is accomplished primarily with the tip of each instrument, and lighter loads are produced on the files.

In 1974 Schilder[5] recommended five components that a canal should be created at the time of the instrumentation phase.

1) The root canal should have continuous taper.

2) The cross-sectional diameter of the canal should be smaller at every point when progressing apically.

3) The root canal preparation should follow the shape of the original canal.

4) The apical foramen should remain in its original position.

5) The apical foramen should be kept as small as practical.

From his study he stated that instrumentation should allow for complete removal of debris and tissue without extrusion past the apex. He also stated that the shape of the canal should be created in relation to the obturation technique.

In 1975 Weine[133] believed that proper root canal preparation was the most important phase, he used clear casting resin blocks to allow visualization of simulated canal preparation procedures on curved canals. Despite the instrument or technique used, the canals prepared showed common undesirable characteristics. This showed hourglass rather than funnel shapes and increased removal of material on the outer portion of the curve near the apex. If instruments were overextended then a teardrop appearance developed at the site of the canal exit. To modify the typical preparations, alteration of

the enlarging flutes, use of rasping rather than rotation of the instruments, and a flaring technique were recommended.

In the same year Coffae and Brilliant[134] evaluated serial (step-back) and non-serial root canal preparations. The teeth were then sectioned and examined microscopically at 1 mm, 3 mm and the 5 mm for remaining pulp tissue. They concluded that serial preparations were significantly more effective than non-serial preparations in removal of tissue at all three levels studied. Where isthmuses were observed, the teeth prepared serially were not significantly cleaned than those prepared serially.

Walton[135] in 1976 evaluated different methods of enlarging the pulp canal space histologically. The methods included filing, reaming and step-back filling (flaring). The teeth were studied and the adequacy of debridement was assessed. He concluded that step-back filing resulted in significantly the highest percentage of pulp walls planed during canal enlargement. This was more significant when comparing methods used in the moderately to severely curved teeth. He also stated that filing tended to be the least effective method of dentin removal. More walls were prepared in the straight canal to slightly curved group than in the moderately to severely curved group regardless of the method used. However, no method of enlargement used in this study did better than to plane 85 percent of the dentin walls. He also stated that reaming and filing both tended to produce a more uniform shape in cross section than did step-back filing. However, the greater the uniformity was related to fewer walls planed; step-back filed canals were more irregular but the walls were cleaned better. Finally he also reported that canals were prepared until all walls felt smooth. When white dentin shavings were obtained, it

showed a wide variety in the percentage of the walls that were actually planed with files. This was an inaccurate determinant of total debridement.

Allison, Weber, and Walton[136] in 1979 assessed the amount of microleakage by autoradiography and concluded that the quality of the apical seal was related directly to the method of canal preparation. The method that permitted deeper penetration of the spreader resulted in a seal closer to the prepared length. The distance that the Ca^{2+} leaked in to the canal from the prepared length was closely related to the depth of the spreader penetration. Microleakage generally extended close to the point where the tip of the spreader penetrated. The coronal seal, obtained by vertical condensation of warmed gutta-percha was complete. They concluded that the method of canal preparation evidently had no effect. In the clinical situation, a method of canal preparation that results in deep penetration, and therefore better apical access, should ultimately result in better obturation.

Moodnik[137] in 1976 looked at biomechanical instrumentation using K-type and Hedstrom files under scanning electron microscopy. It was concluded that the walls of the root canals contain many irregularities that trap debris and harbor pulp tissue that current endodontic instruments are unable to remove. One side of a root canal is not necessarily better instrumented than the other side and there was no level of the root canal, whether coronal, middle or apical, that was cleaner than any other level. There was no difference in the efficacy of the K and Hedstrom files under conditions, which they were used but a layer of sludge covered all instrument canal surfaces.

In 1983 Leeb[138] studied the effect of orifice enlargement prior to biomechanical canal preparation of maxillary and mandibular molars. After orifice enlargement files

could be passed more easily to the apex, indicating the elimination of interferences cervical to the apical constriction. Peezo reamers and Gates-Glidden drills were used for this study. Philippas[139] first made the observation that the orifice may have restrictive effect on canal exploration and instrumentation. He stated that subsequent files are easier to insert after orifice enlargement. The mesial canals in molars generally curve towards the distal producing a root of dentin, which restricts the range of motion of the file. Efforts to open the orifice mesially by placing mesial pressure on the instrument result in dentin acting as a fulcrum. This will lead to uneven instrumentation and lead to zipping of the apex as described by Weine.[133]

Leseberg and Montgomery[140] in 1991 compared the Canal Master Instrument and the instrumentation technique to Flex-R Files used with a "balanced forced" technique and K-Flex files used with a step-back filing technique. Teeth were sectioned, photographed and evaluated before and after instrumentation. Evaluation included canal shape, direction and extent of transportation, amount of dentin removed and centering ability. Instruments with the "balanced forced" technique performed significantly better than files with a modified step-back filling method. In the mid-root sections instrumented to a size #45, canal Master instrumentation transported significantly less, removed less dentin, and remained centered better than the other two techniques.

Torabinejad[141] described the step by step method of the passive step-back technique in 1991. This used a combination of hand and rotary instruments to clean and shape root canals. Clem in 1969 first described step-back technique. In this technique the canal is prepared to a small size at the apical region, and subsequently larger files are used to decreasing lengths. This results in a canal preparation with a small apical segment

and a progressively larger apicocoronal taper. Crown-down, step-down and balanced force techniques are essentially modifications of step-back technique. The major advantage of a flared preparation is to allow effective removal of canal contents and cleaner canals. Previous studies mentioned that most hand instruments transport the original shape of the apical portion of the root canals.[133, 142, 143] This could be because o the apical enlargement prior to adequate coronal flaring or taking small curved canals to file sizes larger than 25 to 30.

Recently Siqueira et al.[144] evaluated whether or not hand-rotary instrumentation was more efficient in reducing bacterial loads compared to just plain hand instrumentation. Root canals inoculated with *E. faecalis* suspension were instrumented using hand nitiflex files, greater (GT) taper files, and ProFile 0.06 taper Series 29 rotary instruments. Root canals were sampled before and after instrumentation and showed that all techniques and instruments were able to reduce significantly the number of bacterial cells in the root canals by 90 percent. They concluded that while larger preparations could incorporate more anatomical irregularities and allow for eradication of bacteria within the root canal system.

Davis[145] in 2002 investigated pre and post instrumentation working length measurements in curved root canals. A combination of: a) stainless steel hand files and Gates Glidden drills vs. NiTi rotary; and b) early coronal flaring vs. late coronal flaring was taken into consideration. Results indicated that working length decreased for all canals as a result of canal preparation. Less change in working length occurred in both groups when initial working length was determined after coronal flaring.

IRRIGATION SOLUTIONS

Sodium Hypochlorite (NaOCl)

Instrumentation removes a great number of microbes from the accessible parts of the main root canal by direct mechanical cleaning action. Moreover, our instrumentation allows for greater chemical eradication of the infection by irrigation. The use of irrigating solutions facilitates removal of necrotic tissue, microorganisms and dentin chips from the root canal by flushing action. They can also help prevent packing infected hard and soft tissue apically in the root canal and into the periapical area. Some irrigating solutions dissolve either organic or inorganic tissue. Several irrigating solutions exhibit antimicrobial activity by actively killing bacteria and yeasts when in direct contact with microorganisms. Disadvantages of irrigating solutions have shown cytotoxic activity and may cause severe pain reaction if they gain access into the periapical tissues.[146] This has been based on previous cytotoxicity tests,[147, 148] conjunctival inflammatory tests,[149-151] and implantation or injection into animal subcutaneous tissue.[151-155]

Several irrigation solutions have been advocated such as saline, but the most popular in our endodontic literature are sodium hypochlorite (NaOCl), chlorohexidine gluconate (CHX), ethylene-diamine tetraacetic acid (EDTA), and BioPure MTAD.

An ideal irrigation solution should dissolve organic and inorganic tissue, differentiate between necrotic and vital tissue, lubricate the canal, prevent and remove smear layer, exhibit low surface tension, provide broad-spectrum antimicrobial action, retain its effectiveness with dental hard tissue and when mixed with other irrigation solutions, and inactive endotoxin while remaining local and systemically nontoxic to normal host tissues with little potential to cause an allergic reaction.[40]

Sodium hypochlorite was first recommended as an antiseptic solution by Henry Dakin[156] during World War I to irrigate wounds. Taylor and Austin[157, 158] demonstrated the solvent action of sodium hypochlorite on nonvital tissue while noting that the solution was only mildly inflammatory to normal tissue. Harrison[159] in 1984 indicated that sodium hypochlorite exhibits powerful antimicrobial activity, excellent necrotic tissue solvent, and is the most efficient irrigation solution in removing organic debris from the root canal system.

Various concentrations of sodium hypochlorite have been reported in the endodontic literature, 0.5 percent, 1.0 percent, 2.5 percent and 5.25 percent. Lower concentrations seem favorable, as they are less tissue toxic than full strength. However, the antimicrobial properties and tissue dissolution ability is diminished at lower concentrations. Full-strength concentration is commonly used. It has been reported that it takes 7 minutes of tissue contact time with sodium hypochlorite to dissolve 75 percent of tissue plug *in-vitro*.[160] Sodium hypochlorite has a pH of 11 in an unbuffered solution. It is proteolytic and is very effective in dissolving organic debris and it is a very potent antimicrobial. This action is predicated on the availability of free chlorine (Cl^-) ions in solution. NaOCl is ionized into sodium (Na+) and hypochlorite ion (OCl-) in water. The hypochlorite ion, (OCl-), establishes an equilibrium with hypochlorous acid (HOCl), that is responsible for bacterial inactivation. Hypochlorous acid (HOCL) has a profound effect on oxidative phosphorylation and other membrane-associated activities as well as DNA synthesis.[161] Due to this property NaOCl is considered self-limiting so it must be frequently replenished during the procedure. The effect of NaOCl is enhanced with increased contact time and volume administered.

The classic study by Bystrom and Sundqvist[162] in 1981 from Umea, Sweden greatly influenced our understanding of instrumentation and irrigation of the canal system. The presences of bacteria in 17 single-rooted teeth, with periapical lesions were studied. The authors demonstrated that thorough instrumentation together with irrigation with physiological saline was not adequate to eliminate all bacteria. The most commonly isolated species were *Peptostreptococcus micros, Peptostreptococcus anaerobius, Fusobacterium nucleatum, Bacteroides oralism, Bacteroides melaninogenicus subsp intermedius and Eubacterium alactolyticum.* Orstavik[163] and Cvek[164] also reported similar results in teeth with closed and immature apices. The antibacterial effect of mechanical cleansing with sterile saline was reported to be very low and limited to teeth with fully developed roots. NaOCl increased the antibacterial effect as compared with saline irrigation. No statistical difference was found in the antibacterial effect between 0.5-percent and 5.0-percent NaOCl solutions.[164]

Senia[165] in 1971 reported that the solvent action of full strength sodium hypochlorite on pulp tissue of extracted teeth was more effective than normal saline on the human pulp tissue. Time was not a limiting factor in this study since increasing time from 15 min and 30 min did not significantly change results when Clorox was in contact with tissue. Without direct contact, the deeper protected tissues were dissolved more slowly and less completely which showed Clorox cleaned more effectively at 5 mm. Where an isthmus was found between two canals, more pulp tissue was dissolved by Clorox than by normal saline solution at all three levels. Also Clorox was found to be more effective in the larger diameters of the root canals then in the smaller apical constrictions. The value of sodium hypochlorite in the apical 3 mm of narrow root canals

was questionable. Three factors to consider when irrigating with sodium hypochlorite are surface contact, volume and exchange of solution.

In 1978 a clinical investigation was conducted to determine the effect of various endodontic irrigation solutions on inter-appointment pain.[166] The results showed no relationship between inter-appointment pain and the type of irrigation solution used. In the same year, Hand[160] analyzed the effect of dilution on the property of sodium hypochlorite to dissolve necrotic tissue and compared the necrotic tissue dissolution properties with that of normal saline solution, distilled water, and 3.0-percent hydrogen peroxide. He concluded that dilution of 5.25-percent NaOCl resulted in a significant decrease in the ability to dissolve necrotic tissue and emphasized that the surface contact of NaOCl with tissue is important.

Several authors concluded that higher concentrations of NaOCl are more effective to reduce microflora and loose debris.[167-168] Svec and Harrison[169] found that 5.25-percent NaOCl in combination with 3.0-percent H_2O_2 was significantly more effective than normal saline solution in cleansing the canal system.

Another study by Gambarini et al.[170] investigated the effect of heating sodium hypochlorite to 50^{o}C on the stability of the solution. Several previous studies[171-173] investigated the advantage on tissue dissolving and antimicrobial properties of sodium hypochlorite. However, it is known that the chemical stability of sodium hypochlorite is adversely affected by exposure to high temperature. An idometric titration test was used to evaluate the decomposition rates of heated and non-heated solutions over 30 days. After 30 days both heated and non-heated solutions maintained high available chlorine content and pH values consistent with excellent tissue-dissolving and antibacterial

properties. Waltimo et al.[174] compared the effects of 5.0-percent and 0.5-percent-NaOCl against resistant forms of *Candida albicans.* The results showed that both concentrations killed the yeast in 30 seconds. When 0.05-percent and 0.005-percent NaOCl were used *Candida albicans* was not affected after 24 hour period. Vianna et al.[175] found similar results when 5.25-percent NaOCl was used. Their research indicated that the concentration eliminated all yeast cells in 15 seconds.

Another clinical use for sodium hypochlorite is for disinfection of gutta-percha cones. A one min exposure to 1.0 percent or five minute exposure to 0.5 percent has been shown to be an effective disinfectant.[176]

Currently studies such as Radcliffe[177] studied the antimicrobial activity of various concentrations of NaOCl on endodontic organisms such as *Actinomyces israelii, A. naeslundii, Candida albicans and E. faecalis.* His results showed that *E. faecalis* was eradicated with 0.5-percent NaOCl after 30 min, 1.0 percent for 10 min, 2.5 percent for 5 min, 5.25 percent for 2 min. Naenni[178] in 2004 evaluated the effects of CHX, H2O2, citric acid and NaOCl on pulp tissue and, only NaOCl had any significant tissue dissolution activity. Recently, Christensen[179] reported that lowering the pH can improve the antimicrobial properties of NaOCl. However, once the pH is lowered past 9 its ability to dissolve organic debris is lessened. Basrani[180] stated that when NaOCl is mixed with CHX it produces a precipitate called parachloraalanine (PCA). PCA has been shown to be toxic and carcinogenic in animals and mixture of these two solutions is to be avoided. In addition, Bui et al.[181] showed that PCA can occlude dentinal tubules making removal difficult.

Weaknesses of NaOCl include unpleasant taste, inability to remove smear layer, resistance of microorganisms to its antimicrobial effects. These disadvantages lead researchers to add other irrigation solutions to enhance the antimicrobial properties.

Chlorohexidine Gluconate (CHX)

This solution was developed over 50 years ago at Imperial Chemical Industries in England. It was first marketed in the UK as an antiseptic cream in 1953. Since 1957 it has been used for general disinfection purposes and the treatment of skin, eye and throat infections in humans and animals.[182, 183]

CHX is a strongly basic molecule and is stable as a salt. It belongs to the polybiguanide antibacterial family. It is a wide-spectrum antimicrobial agent, active against gram-positive and gram-negative bacteria as well as all yeasts. CHX has a cationic nature and capable of electrostatically binding to negatively charged surfaces of bacteria[184] damaging the outer layers of the call wall and rendering it permeable.[185-187] CHX can have both bacteriostatic and bacteriocidal effects depending on its concentration. At high concentrations CHX acts as a detergent. By damaging the cell membrane, it causes precipitation of the cytoplasm and thereby exhibiting a bactericidal effect. At low concentrations, CHX is bacteriostatic causing low molecular weight substances such as potassium and phosphorous to leak out without the cell being irreversibly damaged. It can also affect bacterial metabolism such as abolishing the sugar phosphotransferase system transport activity and inhibiting acid production in some bacteria.[188]

Due to the cationic nature of the CHX molecule, it can be absorbed by anionic substrates such as oral mucosa.[189, 190] CHX has the property to bind to proteins such as

albumin in serum or saliva, pellicle found on the tooth surface, salivary glycoproteins, and mucous membranes.[191, 192] This reaction is reversible. CHX can also be adsorbed onto hydroxyapatite and teeth. The substantivity of CHX is due to the reversible reaction of uptake and release of CHX. Lin et al.[193] attributed the substantivity of CHX to its ability to adsorb onto the dentin during the first hour. They stated that its saturation point is reached after the first hour and that the antimicrobial capability of CHX increases with time. Furthermore, Komorowski et al.[194] revealed that a 5-minute application of CHX did not induce substantivity, so dentin should be treated with CHX for 7 days.

CHX has been used as an endodontic irrigation solution and intracanal medicament. Two percent CHX is has been reported to be effective in eliminating a biofilm of *E. faecalis,*[195] it inhibits inflammatory external root resorption when applied for 4 weeks.[196] In infected root canals, it reduces bacteria as effectively as $Ca(OH)_2$ when applied for 1 week.[197] Unlike $Ca(OH)_2$, CHX has substantive antimicrobial activity that has the potential to prevent bacterial colonization of root canal walls for prolonged periods of time.[194, 198]

CHX comes in liquid and gel form and many investigations have conducted studies on the antibacterial effectiveness of CHX in different concentrations. It has been shown that 2.0-percent CHX as an irrigation solution has a better antibacterial efficacy than 0.12-percent CHX *in vitro,* so the antimicrobial efficacy depends on its concentration.[199]

Ethylenediamine Tetra-Acetic Acid

This has been recommended in our irrigation regiment because of its property to chelate and remove the mineralized portion of the smear layer. It is a

polyaminocarboxylic acid. It is colorless, water-soluble and odorless. Its prominence as a chelating agent arises from its property to sequester di- and tricationic metal ions such as Ca^{2+} and Fe^{3+}. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity.

This was first described and prepared by Ferdinand Munz in 1935. On direct exposure for extended period of time, EDTA extracts bacterial surface proteins by combining with metal ions from the cell envelope that lead to bacterial death. EDTA alone cannot completely remove the smear layer.[200] However, a proteolytic component such as NaOCl must be incorporated into our irrigation regiment to remove the organic portion of the smear layer. Two irrigation solutions, MTAD and Smear Clear have also been studied. Smear Clear is colorless, odorless and water soluble, has 17-percent EDTA salts, a cationic surfactant, and anionic surfactants.

EDTA is normally used in 17-percent concentration and it takes 1 min to remove smear layer when in direct contact. It is self-limiting and is capable of decalcifying up to 50 μm, it can open up an occluded, fine canal. EDTA's interaction with NaOCl has also been studied.[201] It was concluded that EDTA retained its calcium-complexing ability when mixed with NaOCl, but EDTA caused NaOCl to lose its tissue-dissolving capacity and virtually no free chlorine was detected in combinations. Clinically this suggests that EDTA and NaOCl should be used separately. In an alternating irrigating regimen, copious amounts of NaOCl should be administered to wash out remnants of the EDTA.

OBTURATION MATERIALS

After the root canal system has been appropriately prepared, it must be obturated with a material capable of completely preventing communication between the oral cavity

and the periapical tissue wound.[202] Historically, before 1800, materials used were tin foil, gold foil, lead foil, cotton pallets with various medicaments, wood, spunk, plaster of Paris, oxychloride of zinc, oxyphosphate of zinc, zinc oxide, paraffin, and copper points.[203]

In 1931 Rickert and Dixon[204] formulated the "hollow tube theory," to which an empty space within a living organism tends to fill with tissue fluids in a short period of time. This theory was based on the observation of an inflammatory reaction around the ends of hollow steel and platinum anesthetic needle fragments implanted in experimental animals. This reaction did not occur if the implant was made of solid, non-porous material. Two years later Coolidge[43] showed bacteria reaching empty spaces within the root canal system by means of the phenomenon "anachoresis." This is where bacteria transported by the blood circulation colonized these areas. They remained sheltered from phagocytosis by the host's defenses. In the tissue fluids that had collected, the bacteria found a nutritional source that could sustain them. The irritating substances derived from the breakdown of the organic material contained in the tissue fluid and from the products of the bacterial metabolism were supposedly the cause of the surrounding inflammatory reaction.

More recent studies questioned this theory[205] and demonstrated that it is possible in experimental animals to implant sterile empty glass or polyethylene tubes or even empty root canals, causing only mild inflammation or none at all around the open ends of the tube. Other authors demonstrated that empty spaces made inside plastic teeth implanted in fresh sockets did not produce any inflammation around the open ends; while

in other cases these spaces were subsequently filled up with fibrous tissue or bone. The later occurred more frequently with large size apical openings.[206]

Hatton[207] believed that there was no technical procedure in dentistry in which more depends on a strict adherence to high ideals than that of obturating a root canal system. Grossman modified Brownlee's[208]criteria for the idea root canal filling material. He listed the following criteria for an idea root canal filling:

1) It should be easily introduced into the root canal.
2) It should seal the canal laterally and apically.
3) It should not shrink after being inserted.
4) It should be impervious to moisture.
5) It should be bacteriostatic or at least not encourage bacterial growth.
6) It should be radio-opaque.
7) It should not stain tooth structure.
8) It should not irritate periradicular tissues.
9) It should be sterile, or easily and quickly sterilized, immediately before insertion.
10) It should be easily removed from the root canal, if necessary.

Gutmann and Witherspoon[209] explained that there are two key reasons to achieve proper obturation of the root canal system: 1) To abolish all avenues of leakage from the oral cavity or the periradicular tissues into the root canal, and, 2) To seal within the system any irritants that cannot be entirely removed during the cleaning and shaping phase of therapy.

In turn, they advocated that gutta-percha with sealer or cement was the material of choice.

Root canals filling materials have been classified as solid-core filling materials, semisolid core, and paste filling materials. Silver points are an example of solid-core materials. Gutta-percha is the most widely used semisolid-core filling material. Paste systems used in the past are zinc-oxide containing pastes.

Silver Points

Introduced by Jasper in 1933[210] silver points were widely used in the 1930s to the 1960s, especially in smaller canals. Silver points had the same diameter and taper as hand files and reamers. They were fabricated to the same size as the last file used to prepare the canal. The advantages of silver points thought of were easy to insert and length control was easier. The main drawbacks of silver points were that they did not seal well laterally or apically because of their lack of plasticity. Silver points cannot be compacted into spaces or voids within the root canal system leaving too much space to be filled by sealer or cement. The leakage associated with silver points allows for corrosion of the silver points and formation of silver salts. These were found to be cytotoxic. Seltzer et al.[211] found products such as silver sulfides or silver sulfates from silver points being removed from canals. Tissue culture indicated that the corrosion products were highly toxic. The mechanisms for the formation of the corrosion products have been postulated as being due to plastic deformation and metal transfer to the silver cones, plus contact of the silver with tissue fluids. Brady and del Rio[212] found corrosion products of sulfer and chlorides by microanalysis of failed silver points. Gutierrez et al.[213] reported that canal irrigation solutions could corrode silver points.

With the advent of different instrumentation techniques that allowed for successful obturation of smaller canals with gutta-percha, the use of silver points has declined because of these disadvantages. Its use currently is extremely rare and there is no indication or justification for its use today.

Gutta-Percha

This material is the most popular and commonly used root canal filling material. Although it does not meet all the requirements, it does fulfill most of them. The major disadvantage of gutta-percha is its lack of rigidity. The smaller sizes of gutta-percha will bend easily under lateral pressure.

Gutta-percha known as "mazer wood" was introduced to England from Asia in the 1600s. It was not until 1848 that Ernst Werner von Siemans used gutta-percha as insulation for underwater cable. With its desirable properties, it was being used in many different ways and in different products such as, corks, cements, threads, surgical instruments, garments, pipes, musical instruments, candelabras, gaiters, garters, suspenders, window shades, carpets, gloves, mattresses, pillows, tents, umbrellas, and sheathing for ships.[39] The best known use of gutta-percha was in golf balls, introduced later in the 19th century, they were called "gutties."[39]

Natural gutta-percha has been described as the product of various species of rubber trees from Malaysia, Borneo, Indonesia and South America, mainly Brazil. It is the purified coagulated exudate from *Palaquium gutta* tree. Gutta-percha is a linear crystalline polymer that melts at a set temperature, with a random but distinct change in structure. It occurs normally as 1,4-polyisoprene and is harder, more brittle, and less elastic than natural rubber. The crystalline phase has two forms, alpha and beta. The

alpha form is material that comes from the natural tree product. The beta form is the processed form used in obturations. When heated, gutta-percha undergoes phase transitions. The transition from beta phase to alpha phase occurs around 115^{o}F. An amorphous phase develops around 130^{o}F to 140^{o}F. When cooled very slowly (i.e; 1^{o} per hour), gutta-percha crystallized to the alpha phase. Normal cooling returns gutta-percha to its beta phase. These cones can be easily dissolved by chloroform and halothane.[39, 214]

Friedman et al.[215] investigated the ingredients of five commercial brands of gutta-percha. They were found to contain approximately 20-percent gutta-percha (matrix), 66-percent zinc oxide (filler), 11-percent heavy metal sulfates (radiopacifier), and 3.0-percent waxes and/or resins (plasticizer). The mechanical properties were indicative of a partially crystalline viscoelastic polymeric material. They were found to obey Hooke's law and displayed a prominent upper and lower yield point when stressed beyond the proportional limit. The essential differences in mechanical properties of individual brands were found to be a function of the gutta-percha and zinc oxide concentration. Although gutta-percha is not the main ingredient, it serves as a matrix, zinc oxide acts as filler, whereas the waxes and resins serve as plasticizers. Metal sulfates such as barium sulfate, provide some radioopacity to be able to identify the material radiographically.

This obturation material is considered acceptable because of its biocompatibility and a low degree of toxicity.[216] After subcutaneous implantation, gutta- perch is normally surrounded by a defined capsule rich in cells, although some macrophages are present.[216-218] Nevertheless, gutta-percha in the form of very small particles induces an intensive foreign body reaction, with massive accumulation of mononucleotide and multinucleated macrophages.[217]

Tavares et al.[219] studied the reaction caused in the connective tissue of rats by implantation of two brands of gutta-percha cones, and thermo plasticized gutta-percha cylinders. Thirty-six rats were used, in groups of 12, for periods of 15 days, 30 days, and 60 days. They concluded that gutta-percha was well tolerated by connective tissue. Another similar study by Leonardo et al.[220] observed the subcutaneous connective tissue response of 24 white rats to three different formulations of gutta-percha was undertaken. The prepared specimens were examined under the light microscope after intervals of 7 days, 21 days, 60 days, and 120 days. The results indicated identical tissue responses after the initial period of 7 days. However, after 120 days the gutta-percha supplied with the Ultrafil system presented mature granulation tissue with neither edema nor vascular congestion, in contrast to the responses observed with the McSpadden and Obtura formulations.

Other investigators have postulated that the extension of gutta-percha into the periapical tissue may slow periapical healing as well as induce a moderate, localized tissue response. Sjogren et al.[91] investigated various factors that may affect the outcome of root canal therapy was evaluated in 356 patients 8 years to 10 years after the treatment. The results of treatment were directly dependent on the preoperative status of the pulp and periapical tissues. The rate of success for cases with vital or nonvital pulps but having no periapical radiolucency exceeded 96 percent, whereas only 86 percent of the cases with pulp necrosis and periapical radiolucency showed apical healing. The possibility of instrumenting the root canal to its full length and the level of root filling significantly affected the outcome of treatment. Of all of the periapical lesions present on previously root-filled teeth, only 62 percent healed after retreatment. It was concluded

that extension of the obturating material beyond the radiographic apex of the tooth resulted in significantly lower rate treatment success. Seltzer et al.[221] found similar results in 2921 endodontically treated teeth recalled in 6 months. It was concluded that overfilled root canals were significantly less successful than obturated flush with the apex or slightly underfilled.

One of the major controversies in root canal therapy concerns the apical limit of instrumentation and obturation. Ricucci[113] looked at the results of longitudinal prognostic studies, basic anatomical knowledge of the apical third of the root canal, and the histological pulp reaction to caries progression demonstrated the presence of a vital apical pulp remnant even in the presence of a periapical lesion. Finally necrosis and bacteria establish themselves in the periapical lesion. In the same year Ricucci[114] also studied an *in-vivo* histological study involving apical and periapical tissues following root canal therapy. Favorable histological conditions were seen when the instrumentation and obturation remained at or short of the apical constriction. This was the case in the presence of vital or necrotic pulps and when bacteria had penetrated the foramen and were present in the periapical tissues. When the sealer and the gutta-percha were extruded into the periapical tissue, the lateral canals and the apical ramifications, there was always a severe inflammatory reaction, including a foreign body reaction despite a clinical absence of pain.

RESIN BASED OBTURATION MATERIAL

Resilon

Resilon is a polycaprolactone core material with difunctional methacrylate resin, bioactive glass, bismuth and barium salts as fillers, and pigments, which is used with a resin sealer (Epiphany or RealSeal/SE). The rationale behind this is to create a "monoblock" consisting of resin sealer with resin tags that enter into and bond to dentinal tubule, and to the dentin on the canal wall, as well as adhesively bonding to the core material. Resilon can also be light cured and sealed coronally. The obturation technique consists of a primer, sealer and synthetic polymer points or pellets. The temperatures used in the thermoplastisized techniques are lower than what is used with gutta-percha. (150°C compared with 200°C). The handling characteristics are similar to gutta-percha and allows for lateral compaction or warm vertical compaction.

The susceptibility of a polycaprolactone-based material to degradation has been an ongoing investigation. Tay et al.[222] examined what effect alkaline hydrolysis had on disks of Resilon or gutta-percha using SEM and energy dispersive x-ray analysis. They found that for Resilon, the surface resinous component was hydrolyzed after 20 min of sodium ethoxide immersion, exposing the spherulitic polymer structure and subsurface glass and bismuth oxychloride fillers. More severe erosion occurred after 60 min of sodium ethoxide treatment. Gutta-percha was unaffected after immersion in sodium ethoxide. As Resilon is susceptible to alkaline hydrolysis, it is possible that enzymatic hydrolysis may occur. Biodegradation of Resilon by bacterial/salivary enzymes and endodontically relevant bacteria warrants further investigation.

In the same year Tay et al.[223] evaluated the susceptibility of Resilon, gutta-percha, and polycaprolactone disks to hydrolytic enzymes present in saliva. The materials exhibited slight weight gains when incubated in phosphate-buffered saline that can be attributed to water sorption. Gutta-percha exhibited similar weight gains in the two enzymes but Resilon and polycaprolactone exhibited extensive surface thinning and weight losses after incubation in lipase and cholesterol esterase. Glass filler particles in Resilon were exposed following surface dissolution of the polymer matrix, creating rough surface topography. They concluded that biodegradation of Resilon by bacterial and salivary enzymes warrants further investigation of their activities using cultures of endodontically relevant microbes and human saliva extracts.

Many studies have looked at the interfacial strengths Resilon with Epiphany sealer being compared to AH Plus and gutta-percha. Gesi et al.[224] The gutta-percha group exhibited significantly higher interfacial strength than the Resilon group. The gutta-percha root slices failed exclusively along the gutta-percha/sealer interface. The Resilon root slices failed predominantly along the sealer/dentin interface with recognizable, fractured resin tags.

Stratton et al.[225] looked at the sealing property of Resilon and Epiphany versus gutta-percha and AH Plus by fluid filtration testing and found that there was less leakage using Resilon and Epiphany as compared to the gutta-percha and AH Plus. Long term results and clinical trials will still need to be investigated to improve this material.

Coated Gutta-Percha

ActiV Gutta-percha Precision Obturation System (Brasseler USA, Savannah, GA) is a new glass ionomer (GI) based obturation system. The manufacturer claims the

product to be superior to previous GI-based systems in terms of handling characteristics, working time, and radiopacity.[226] Inadequate bonding between GI and Activ GP is a drawback with GI-based sealers.[226] To enhance the GP-GI bonding, ActiV GP has a 2-μm coating of GI particles on its surface; these particles are also incorporated into the body of the cone. As with Resilon/Epiphany, the bond to both the dentin and core via the sealer is referred to as a "monoblock." ActiV GP cones are designed to match Sequence NiTi rotary files (Brasseler USA); in turn, a single-cone obturation technique is recommended for ActiV GP.

The manufacturer claims that this will inhibit leakage between the solid core and the sealer creating a "monoblock." This material with a resin sealer was compared with gutta-percha for microleakage. The results indicated a bonding between core and sealer resulting in far less microleakage than gutta-percha.[227] A bacterial microleakage study comparing ActivGP/glass ionomer sealer, Resilon/epiphany, and Gutta-Percha/AH plus showed that there was no significant difference in leakage in 65 days.

OBTURATION TECHNIQUES

The ultimate objective of root canal therapy is a three-dimensional obturation of the endodontic space after it has been cleaned, shaped and disinfected. There is little evidence in the endodontic literature that supports one method of obturation as being superior to another and the influence of treatment technique on success/failure has yet to be determined.[228] No matter what technique is used for obturation, the primary goal is a good aseptic technique. Gutta-percha is the preferred by most endodontists as a filling material because of its permanency and its capacity to be condensed against the

irregularly shaped walls of the canals.[40, 50] There are many different techniques of filling root canals with gutta-percha.

Cold Lateral Condensation

Lateral compaction is a common method for obturation.[229] This technique is considered to be the standard against which other methods of obturation are measured.[230] The method is generic, encompassing a range of approaches in terms of master cone design and adaptation, spreader and accessory cone selection, choice of sealer and spreader application. There is little clear evidence on how it is best done.[231] Glickman and Guttman discussed five important conditions that must be met:

1) The shape of the prepared root canal must be continuous to allow for proper placement of the master cone, spreader, and accessory cones.

2) Forces in lateral condensation are both lateral and vertical.

3) The placement of the spreader must reach the appropriate depth without touching the canal walls to ensure continuous taper prior to condensation.

4) The master cone should fit within 0.5 mm to 1.0 mm of the radiographic apex and should have tug back.

5) The placement of the spreader should be within 1 mm of the working length adjacent to the master cone.

Cold lateral condensation commences by liberally coating canal walls with sealer cement.[231] *In-vitro* evidence suggests that application with a spiral paste filler, fine injection needle, or an ultrasonically energized file ensures the most complete wall coverage,[232] although application on the master cone, a paper point or a small file are popular alternatives. The master cone is placed to working length and the measured

spreader applied under vertical loading for 10 sec to 60 sec to deform material apically and laterally.[233] A sealer coated accessory cone is then placed in the prepared space followed by re-insertion of the spreader. The sequence of accessory cone placement and spreader insertion is continued until the spreader advances no further than 2 mm to 3 mm into the canal. Heat is then applied to remove excess obturation material to the canal orifices level, followed by apical compaction with a cold plugger.[209]

Seltzer and Bender[221] investigated the outcome of 2,921 endodontically treated teeth to determine if single cone obturation was as successful as cold lateral condensation. This study demonstrated that cold lateral condensation had a success rate of 84.4 percent as compared with an 83.7 percent success rate found in single-cone technique. In 1997 Sjogren et al.[95] investigated the role of infection on the prognosis of endodontic therapy by following-up teeth that had their canals cleaned and obturated during a single cone appointment. The root canals of 55 single-rooted teeth with apical periodontitis were thoroughly instrumented and irrigated with sodium hypochlorite solution. Microbial samples were taken from the teeth prior to obturation and bacteria were detected in 22 of 55 root canals. After a five-year follow up period, periapical healing was observed in 94 percent of cases that yielded a negative culture.

De Moor and Hommez[234] found that the hybrid gutta-percha condensation technique was superior to the other four obturation techniques in respect to apical leakage. Coronal leakage was significantly greater during the first four months for the Thermafil system as compared to the three condensation techniques; coronal leakage was significantly greater at all time periods for the Soft-core system. There were no significant differences between the Thermafil and the Soft-core system.

There are several disadvantages to the cold lateral condensation technique that have been reported. In this technique, the final obturation is composed of a large number of gutta-percha cones coupled by a frictional grip and cementing material, rather than a homogenous mass of gutta-percha.[235] Poor root canal preparation, inadequate lateral pressure during condensation, curved canals, or mismatches between the gutta-percha cones and the prepared root canal can cause voids between the gutta-percha and the canal wall. In such cases, the resulting obturation would lack homogeneity and rely heavily on the sealer to fill the voids, which would implicate a poorer prognosis.[236, 237]

Warm Vertical Condensation

Herbert Schilder was the first clinician to teach and advocate warm vertical condensation of gutta-percha. He believed the technique produced consistently dense, dimensionally stable, three-dimensional root canal fillings in which lateral canals were filled with extraordinary frequency.[5] Prior to the introduction of warm vertical condensation of gutta-pecha, cold lateral condensation was used almost exclusively for obturating root canals with gutta-percha. Ruddle[208] lists the criteria for satisfactory canal shaping as:

1) Continuously tapered preparation.
2) Original anatomy maintained.
3) Position of the apical foramen maintained
4) Foramen diameter as small as practicable.

The tapered canal preparation allows softened materials to enter anatomical complexities as heat and pressure are applied in a canal of rapidly diminishing diameter.

Resistance to over-filling is achieved by creation of an apical control zone as opposed to a traditional ISO stop.

His technique consisted of first selecting a master gutta-percha cone that closely approximates the length and taper of the canal. Sealer is then applied to the canal and the apical portion of the master cone. The cone is then seated into the canal. Heated pluggers are then used to remove the coronal aspect and transfer heat to the remaining apical portion of the cone. The remaining heated gutta-percha is condensed vertically and laterally with cold pluggers. This process is continued until the gutta-percha has been condensed in the apical extent of the canal. The remaining portion of the canal is then obturated by condensing heated gutta-percha segments from the apical to coronal portion of the canal.[5]

In this technique, opponents have historically argued that the cost in terms of the dentin removal to accommodate appropriate pluggers was excessive compared with the required for lateral condensation. This seems to be less of an issue in current practice with controlled canal flaring by contemporary instruments, and a welcome range of sufficiently narrow and efficient heat carries, pluggers (including nickel titanium pluggers) and backfill needles. Additional concerns have been raised with regard to thermal damage to the periodontal ligament during thermal compaction techniques, particularly if temperature guidelines are exceeded.[238-241] Evidence on this appears to be equivocal[242, 243] with most of the modeling taking place in the laboratory setting where the heat buffering effects of a richly perfused periodontium cannot properly be taken into account. There is little clinical evidence of adverse periodontal responses following thermoplastic obturation in clinical practice.

Buchanan[244] improved upon this technique by incorporating the System B heat source that delivers a continuous heat source for extended periods of time. Known as the continuous-wave-of-condensation technique, various pluggers are used to match the taper of the non-standardized gutta-percha cones. After placement of the cone, the System B is activated and heat is delivered to the corresponding plugger at 200^0 C. The heated plugger is advanced through the gutta-percha until it is 5 mm to 7 mm from the apical extent of the canal. Once at this level, the plugger is removed along with the coronal aspect of the gutta-percha cone. The remaining portion of the canal is obturated using the System B and small increments of gutta-percha, or with an injectable system of gutta-percha such as the Obtura or the Hot Shot.

Yee et al.[245] was the first to introduce the technique of using heat plasticized gutta-percha for obturating the root canal system. His study investigated the feasibility of root canal obturation by using thermoplasticized, injectable gutta-percha. An experimental method was devised for introducing thermoplasticized gutta-percha into prepared root canals with the endodontic pressure syringe. The suitability of injectable, warm gutta-percha as an endodontic filling material was then evaluated *in vitro* by two methods: direct visual examination of recovered root canal fillings achieved with the adopted technique, and assessment of the adaptation between the filling material and the root canal walls by means of a dye. The findings showed that the injection-molding technique produced a seal comparable to that of conventional approaches. The Obura II is currently the most well known system used to deliver heat plasticized gutta-percha to the root canal system. With this device gutta-percha is delivered at a temperature between

80°C and 135°C. Studies have also found that the levels of heat generated by the plasticized gutta-percha do not appear to clinically have any negative effects.[246-248]

In 2006 Aqrabawi[246] assess the treatment results following endodontic therapy of teeth filled with lateral condensation versus teeth filled with step-back technique for canal preparation, which were filled with either lateral condensation of vertical compaction in one single session. Five years later, the treatment results were assessed clinically and radio graphically. Of the 340 teeth that were examined, 160 teeth were filled with lateral condensation, and 180 teeth were filled with vertical condensation. The results showed a significantly higher success rate for the vertical compaction vs. the lateral condensation technique of teeth presented with preoperative periapical lesions. Regardless of the preoperative periapical status of the teeth, no statistically significant difference was found between the two techniques. The overall success rate of both filling techniques was 80.3 percent. When the density of the standard cold lateral gutta-percha compaction and warm vertical condensation were quantitatively compared, Lea et al.[249] demonstrated that warm vertical condensation resulted in a greater gutta-percha fill by weight.

Ventura and Breschi[250] evaluated the quality of endodontic sealing in the apical 4 mm of narrow and curved canals using different filling techniques. Human teeth were selected and assigned to four different techniques: Group a, Schuler's warm vertical condensation; Group B, Schuler's technique modified by using an electric heater; Group C, Schuler's technique modified by compaction of the apical tract at body temperature; and Group D, a modified vertical compaction with apical backfilling. A dye penetration test was performed, and specimens of Group D showed increased apical sealing and reduced extension of voids. The use of the vertical compaction with apical back-filling

technique allowed the creation of an effective apical plug and an excellent adaptation of back-filling to apical gutta-percha and to root canal walls.

Carrier Systems

Carrier systems represent another convenient means of delivering thermally softened gutta-percha to canal systems with some degree of control. This approach is typified by Thermopile, which has developed from involving the coverage of a conventional file with regular gutta-percha.[251, 252]

Thermopile originally manufactured with a metal core and a coating of gutta-percha, the carrier was heated over an open flame. This technique was popular because the core material provided a rigid mechanism to facilitate the placement of the gutta-percha.[202] Advantages included ease of placement and the pliable properties of gutta-percha. Disadvantages were that the metal core made placement of a post challenging and retreatment procedures were difficult. In addition, the gutta-percha was often stripped from the carrier, leaving the carrier as the objurgating material in the apical area of the canal.

Joplin et al.[253] examined in curved canals, the intracranial relationships of 1) a metal carrier; 2) the surrounding gutta-percha; and 3) the sealer. Twenty resin blocks that had canals prepared were obdurate and cross-sections were made in the cervical, middle and apical third. These were evaluated under stereomicroscope for adaptation at the apical extent of the preparation. In most preparations, the carrier abutted the canal wall in the cervical and middle sections but was usually surrounded by gutta-percha in the apical one third. The adaptation of components showed that most variability at the most apical extent of the preparation. Complete encasement of the carrier did not occur in any

specimen. Sealer distribution was variable throughout, usually being absent in the apical canal.

ENDODONTIC SEALERS

In endodontic therapy, sealers are responsible for filling irregularities in the prepared canals, entombment of remaining bacteria, and the sealing of the root canal system.[254] The sealer occupies the space left between the root canal wall and the core material in most obscuration techniques. Due to the physical limitations of the core obscuration material, voids are inevitably left in the root canal system and must be filled to ensure successful therapy. An ideal sealer should also have both adhesive properties, between dentin and the core material, and cohesive properties to bond the objurgating material together. Properties of an ideal sealer are[255]:

1) Exhibits tackiness when mixed to provide a good adhesion between it and the canal wall when set.
2) Establishes a hermetic seal.
3) Radio-opaque, so that it can be seen on a radiograph.
4) Very fine powder, so that it can mix easily with liquid.
5) No shrinkage on setting.
6) No staining of tooth structure.
7) Bacteriostatic, or at least does not encourage bacterial growth.
8) Exhibits a slow set.
9) Insoluble in tissue fluids.
10) Tissue tolerant; that is, nonirritating to periradicular tissue.

11) Soluble in a common solvent if it is necessary to remove the root canal filling.

Orstavik[254] categorized sealers into five different types based on their chemical formulation: zinc-oxide-eugenol based sealers; glass-ionomer based sealers; resin-based sealers; silicone-based sealers; calcium hydroxide-based sealers.

Zinc-oxide-eugenol-based materials have long been considered the sealer of choice in enodontic therapy. They will absorb if extruded into the periradicular tissues.[256] They exhibit a slow setting time, shrinkage on setting,[257] solubility,[236, 258] and they can stain tooth structure.[259] An advantage to this sealer group is its antimicrobial activity.[260] Proto types are Rickert's sealer, commercial in the form of Kerr Pulp Canal Sealer and Grossman's sealer, which has several commercial variants, among them Roth sealer and ProcoSol. An early zinc oxide-eugenol sealer was introduced by Rickert and Dixon. This powder/liquid sealer contained silver particles for radiopacity. Although it was possible to demonstrate the presence of lateral and accessory canals, the sealer had the distinct advantage of staining tooth structure if not completely removed. Grossman, on the other hand used bismuth and barium salts.[254] Similar to gutta-percha, zinc oxide contained within sealers can confer antimicrobial properties. Crane et al.[261] studied the biological and physical properties of a root canal sealer without eugenol compared to sealers with eugenol. The setting times were similar at 37°C after 96 hours, the non-eugenol sealer was more tissue compatible. After six months, no significant difference between sealers was found in relation to biocompatibility. Zinc oxide-eugenol sealers were found to be both anti-inflammatory and toxic depending if the eugenol contacted tissue.[262] Al-Khatib et al.[260] compared the antimicrobial activity of Grossman's sealer, Tubliseal, calciobiotic,

Sealapex, Hypocal, Eucapercha, Nogenol and AH26. These sealers were placed into the prepared wells of agar plates inoculated with the test microorganisms. After varying periods of incubation, the zones of inhibition of bacterial growth were observed and measured. Grossman's sealer had the greatest overall antibacterial activity. However, AH26 had the greatest activity against *B. endodontalis*. The zinc oxide-eugenol based sealers had more antimicrobial activity than either the calcium hydroxide based sealers of eucopercha. Leonardo et al.[263] found similar results when the antimicrobial activity of four zinc oxide-eugenol sealers and pastes were evaluated.

Glass-ionomer sealers have been advocated because of their biocompatibility and adhesive properties towards dentin.[254] Ray and Seltzer[264] investigated various physical characteristics of a new glass ionomer root canal sealer. These included setting time, ease of delivery to the root canal, adaptability and adhesion to the dentinal wall of the root canal and radioopacity. Scanning electron micrographs and electron microprobe analyses were made. The characteristics were compared with those of Grossman's sealer. The results indicated that, with respect to the properties tested, the glass ionomer sealer was superior to Grossman's sealer. Friedman et al.[265] assessed the treatment results following endodontic therapy using a glass ionomer cement sealer and related the results to various clinical factors. A total of 186 teeth were treated by three operators, using the "standardized technique" for canal preparation and either single cone or laterally condensed gutta-percha in multiple treatment sessions. Six to eighteen months postoperatively, the treatment results were assessed clinically and radiographically, and related to preoperative, intraoperative, and postoperative factors. Of 378 followed-up teeth, there was a 78.3-percent success, 15.6-percent incomplete healing and 6.1-percent

failure. It was concluded that these treatment results were compatible with those reported in previous studies, and supported the clinical use of Ketac-Endo as an acceptable endodontic sealer. On the other hand Schafer et al.[266] compared the weight loss of eight different root canal sealers in water and in artificial saliva with different pH values. They concluded that AH Plus showed the least weight loss of all sealers tested, independent of the solubility medium used and Ketac-Endo had marked weight loss in all liquids when immersed in double distilled water or artificial saliva. These results revealed that glass ionomer based sealers could be predisposed to leakage as well as some degree of disintegration.

The more recent available sealers are resin polymers, which include AH26, AH Plus, Diaket and Epiphany. The AH series has been considered the most successful of the traditional resin-based sealers.[254] AH-26 is a slow-setting epoxy resin that was found to release formaldehyde when setting.[267] AH Plus is a modified formulation of AH-26 in which formaldehyde is not released. AH-26 and AH Plus have been shown to have lower solubility than zinc oxide-eugenol and calcium hydroxide sealers along with an adequate working time and flow rate.[268] Using electrical resistance to measure microleakage with and without smear layer removal for AH-26 and Roth's 811 sealers, AH-26 showed a decrease in microleakage with smear layer removal compared to sealing with an intact smear layer. Roth's 811 sealer showed no differences with or without smear layer removal.[269]

Resin-based sealers also provide a significantly better apical seal when compared with other non-resin based sealers. Using a dye leakage model, Stewart[270] found that Diaket showed significantly less shrinkage than two zinc oxide-eugenol sealers. Diaket is

a polyvinyl resin, consists of a powder composed of bismuth phosphate and zinc oxide and a liquid consisting of dichlorophen, triethanolamine, propionylacetophenone, and copolymers of vinyl acetate, vinyl chloride, and vinylisobutyl ether. Resin-based root canal sealers were found more effective in sealing root canals than the zinc oxide-eugenol- based sealer.[271]

Other resin-based sealers, Epiphany and RealSeal/SE have been introduced for use with a new core material, Resilon. It has been proposed that they bond to the canal wall and to the core material to create a "monoblock." One study indicated that the bond strength to dentin can be influenced by the irrigation solution used. Water and chlorohexidine decreased the bond strength compared with NaOCl, NaOCl/EDTA, and NaOCL/MTAD. The use of EDTA and MTAD did not improve the bond strength compared with NaOCl alone.[272]

Calcium hydroxide sealers were developed for therapeutic activity. It was thought that these would exhibit antimicrobial activity and have osteogenic-cementogenic potential. These have not been demonstrated yet. Solubility is required for release of calcium hydroxide and sustained activity; this is inconsistent with the purpose of a sealer. Examples of these are Calciobiotic root canal sealer, Apexit and Sealapex. Four noneugenol sealers were developed from a periodontal dressing, Nongenol. This is a sealer without any irritating effects of the eugenol.[273]

Bioceramic (BC) sealer is the most recent sealer composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, and various filling and thickening agents. It is available in a premixed syringe with calibrated intracanal tips. This sealer utilized moisture within the canal to complete the setting reaction ad it does

not shrink on setting. It is biocompatible and exhibits antimicrobial properties during the setting reaction. The manufacturer advocates expressing the sealer into the coronal one third to one half of the canal and then seating the master gutta-percha cone. Bioceramic materials having nano-sized particles achieved excellent adhesion to the canals dentinal walls and, more importantly, from a chemical bond with dentin. Structure of these materials during their mixing with water allows a very good consistency to be achieved or optimal consistency is already guaranteed within the premixed syringes.[274] Koch and Brave[275] discussed in an introductory article many benefits of bioceramics in both surgical and nonsurgical endodontics. The benefits are significant that the BC sealer is now an integral component of the EndoSequence instrumentation and obturation system and, along with the new root repair material, has evolved into the realm of surgical endodontics. This is a natural progression given the fact that these particular bioceramics have exceptional dimensional stability and do not shrink upon setting and, consequently, remains non-resorbable inside the root canal and retro-preparation. Furthermore, the formation of calcium hydroxide as a by-product of the setting reaction produces a very high pH (12.8) rendering the material anti-bacterial during its setting time. This is an important physical property for cement, particularly if it is being used as an endodontic sealer.[276] Recently Zuang et al.[277]demonstrated that BC sealer killed all bacteria within two minutes of contact. The authors proceed to explain that its potent anti-bacterial effect may be a combination of its high pH, hydrophilic nature and its active calcium hydroxide diffusion. The BC sealer sets in three to four hours and this provides ample time for clinical use in surgical and non-surgical applications. Another study in China[278] evaluated the sealing property of BC sealer compared to AH Plus using the fluid filtration

technique. They found that there was no significant difference in fluid leakage among the groups, as well as no time effect on leakage. SEM revealed both gap-free regions and gap-containing regions in canal filled with both materials.

CORONAL LEAKAGE

A commonly suggested cause for failure of root canal therapy is percolation of microleakage due to inadequate apical seal.[279] This allows for periapical fluids, proteins, and bacteria access to the root canal. This will result in radiographic and clinical signs of failure of root canal therapy.[280] Many investigators have compared materials and techniques in an attempt to identify a means of attaining an impermeable seal at the root canal terminus.[281, 282] Techniques such as radioisotopes, bacteria, and dyes to evaluate the integrity of the apical seal have been studied. Messing[283] evaluated coronal and apical seal using a fluorescent dye and reported no leakage with the experimental materials used.[283] The results were contrary to the studies obtained by Swanson and Madison[284] which compared coronal microleakage at various time periods when the endodontic access preparation and root canal obturation materials were exposed to synthetic saliva. Their results showed considerable dye penetration from 79 percent to 85 percent of the root length in all specimens exposed to the artificial saliva. No leakage was observed in the control group with any exposure to this fluid.

In-Vitro Isotope Studies

Isotopes have been used as tracers for coronal leakage. In 1955, Dow and Ingle[279] immersed obturated teeth without temporary restorations in radioiodone (^{131}I) for 120 hours. Teeth were obturated by lateral condensation to obtain a good seal and others were

obturated leaving voids in the canal. The teeth were split longitudinally to assess isotope penetration by autoradiographs. Their results showed that radioisotope penetration was evident in the poorly obturated specimens.

Marshall and Massler[285] used several isotopes to examine the marginal seal of different types of obturating fillings. Two-hundred-sixty-one teeth were obturated with various materials and placed in ^{131}I, ^{86}Rb, ^{22}Na, ^{35}S, ^{45}Ca, or ^{32}P solutions. Autoradiographs were used to determine leakage. ^{35}S penetrated the most, and the best objurgating material was gutta-percha and sealer, preventing isotope penetration in 100 percent of the specimens. Yee, Legacy, and Peterson[286] also studied the *in-vitro* permeability of 12 root canal fillings to radioactive isotopes. They claimed that the root canal could always be sealed against the ingress of radioactive ions. The validity of Marshall and Masker's results may be questioned because evaluations were made immediately after the root canals were filled. Dimensional changes in cements after setting could have altered their findings. In the study by Lee, Lugassy, and Peterson, the teeth were stored for 24 hours at 100-percent relative humidity.

In radioactive isotope studies, the penetration of the tracer may be partly a function of polarity or it may be related to particle size.[287] Kapsimalis and Evans[288] modified the autoradiographic technique using ^{35}S and nonpolar tritiated glucose because of the difference in polarity and particle size. They found leakage in all but two of eight sealers.

In 1976 Younis and Hembree[289] used ^{45}Ca to compare leakage between teeth obturated with laterally condensed gutta-percha and paste filled root canals. One hundred five single-rooted teeth were prepared and filled with 10 commercially available root

canal paste sealers. Five teeth were filled with only gutta-percha points, 50 teeth were filled with a particular type of cement, and the rest of the teeth were filled with a particular type of cement in conjunction with gutta-percha points. It was found that the gutta-percha alone is not sufficient for sealing the periapical foramen; nor are N2 (Sargenti), Riebler's paste, and the iodoform paste. Apical portions of the roots were exposed to ^{45}Ca for two hours. In general it was found that the combination of gutta-percha and cement is more effective in sealing the periapical foramen.

Three similar studies compared different obturating techniques to evaluate sealing efficiency of endodontic treatment. In 1981 Rhome et al.[290] used ^{14}C and compared coronal leakage in root canals obturated with either vertical or lateral condensation or filled with Hydrom. Hydrom leaked the most. Czonstkowsky et al.[291] used ^{14}C starch solution and compared the sealing properties of injectable gutta-percha with and without sealer to lateral condensation with Grossman's sealer. Results indicated that the injection technique demonstrated significantly more leakage if sealer were not used. The third study by Fuss et al.[292] used ^{125}I- labeled human serum albumin and compared laterally condensed gutta-percha and sealer to gutta-percha compacted with either the McSpadden compactor or an engine driven plugger. They showed that the apical one-third of the roots of all specimens demonstrated the same effective seal.

Many variables in a study by Blaney et al.[293] in 1981 questioned the true representation of radioisotopes and dyes to evaluate coronal leakage. Variables discussed such as molecular size, pH, polarity, and capillary action influenced microleakage by radioactive labeled elements or dyes but did not indicate how microorganisms would penetrate. Penetration by microorganisms, rather than by dyes or radioactive elements,

seems to be a more biologically significant approach. Going, Massler, and Dute[287] showed that the charge on the ion and its chemical affinity greatly influenced its absorption on the surface of the filling material and on the tooth surface. It also influenced its permeability through the margins of the restorations. Change in functional group, accompanied by a change in pH had some influence on results.[288] Finally, the studies mentioned above did not take into account the effects of moisture or warmth of vital tissues. Although isotopes may be a good tool for comparing relative leakage, they cannot give a true picture of the leakage, which occurs clinically. This is because the ions used are much smaller than dye molecules and they diffuse more rapidly than other small molecules.[13]

In Vivo and *In Vitro* Dye Studies

A number of methods, employing dyes and radioactive isotopes have been used to measure the property of various root canal filling techniques and materials to seal the canal. In the study by Matloff et al.,[294] a comparison of methods used were studied in root canal sealability. This study was conducted in vitro; 63 extracted, single-rooted teeth were instrumented and filled in a standardized manner. Teeth were then divided into groups of twenty and exposed to solutions containing methylene blue dye, ^{45}Ca, ^{14}C labeled urea, and ^{125}I- labeled albumin for 48 hours to compare the degree of leakage indicated by each technique. Methylene blue dye was found to penetrate farther up the canal than any of the isotope tracers. Carbon-fourteen-labeled urea penetrated farther than the ^{45}Ca or ^{125}I-labeled albumin. Linear dye penetration became popular because of its sensitivity, ease of use, and convenience. Investigators believed the depth of dye penetration represents the extent of the gap between the root filling and the canal wall.

From here it has been concluded that greater dye penetration implies greater coronal leakage.[294, 295] Schnell[296] and Ford[297] pointed out that dye penetration into small voids would be by capillary force. Such action is created by collapse pressure at the liquid-air interface. In endodontic situations the capillary force is modified by the fact that the capillary lumen is closed on one end. On the other hand, Spradling and Senia[298] found that dye solutions did not penetrate fully even in canal specimens open in both ends. They proposed that air trapped in the canal might have prevented dye from progressing the complete length of the lumen. Unless entrapped air is totally evacuated from the void, dye penetration by capillary force will not occur more at the point where balance is reached between the collapse pressure and the surface tension of the fluid. Therefore, they concluded that passive soaking in dye solution does not reveal the entire voids present, and therefore is an unreliable method.

In 1988, Madison and Wilcox[12] performed root canal therapy on posterior teeth in monkeys using gutta-percha and various sealers for obturation. The access openings were restored with zinc oxide-eugenol. Seventy-two hours later, the temporary restorations were removed and the coronal openings exposed to the oral environment for 1 week. Following removal, the teeth were placed in dye and cleared to allow visualization of dye penetration. Linear measurements of dye penetration were made and compared by groups. The results showed the presence of dye in all groups with no significant differences among the groups. Investigators explained the results to be due to monkey chow filled in the access openings. They suggested that the animals food due to its consistency and its packing during mastication acted as a barrier preventing dye penetration into the canals. They also suggested that proteins and mucins contained in

animal saliva were not present in the artificial saliva used in the *in-vitro* study. They concluded that perhaps the whole saliva occluded the canal orifices adequately to retard or prevent microleakage. The results of this study did not confirm the *in-vitro* findings reported earlier comparing coronal microleakage in canals obturated with gutta-percha and various sealers.[280] In that study, the AH 26 group demonstrated significantly more dye penetration than Sealpex or Roth sealer when teeth were exposed to artificial sealer. Although the tendencies were the same, for example the dye penetration observed in AH26 group was higher than that seen with the other two sealers. The difference was not significant.

Massler and Ostrovsky[299] tested the sealing property of various filling materials against a glass interface with gentian violet and methylene blue dyes. They found that baseplate gutta-percha, temporary stopping, zinc phosphate cement, and cold-curing acrylic resin were not effective sealers against dye penetration. In a similar study, Curson and Kirk[300] observed the depth of penetration of methylene blue between glass tube walls and various root canal cements. They found that zinc oxide-eugenol, Rickert's cement, Diaket, Kerr Tubliseal, and AH-26 were all satisfactory sealers. However, in both these experiments, the interface between a filling material (or cement) and glass, not dentin, was investigated.

Antoniazzi, Mjor, and Nygaard-Ostby[301] studied methylene blue penetration along the interface between root canal sealer and dentin. The root canals of extracted teeth were filled with gutta-percha and sealer, and some with sealer only. Kloroperka, AH-26, and Kerr sealer were tested both immediately after setting and after a 48-hour setting time. They found that none of the tested materials were impermeable to dye penetration.

However, they admitted that due to difficulties in standardizing the method of evaluation, it might be concluded that only limited information can be obtained from *in-vitro* studies.

Variables such as variance in pH, pulpal architecture, time of immersion of the material in the dye solution, and difficulty in rendering the external cementum impermeable, were difficult to control. Furthermore, the validity of the use of dyes in diffusion studies is questionable because of the molecular size of the dye particles.[237]

Other studies used methylene blue to evaluate coronal leakage using different temporary restorations. In 1988 Teplistsky and Meimaris[302] used 10-percent methylene blue to compare coronal leakage between TERM and Cavit. Half the teeth were subjected one-hour thermocycles of 4°C to 60°C for 56 cycles. The teeth were then removed from the dye after seven days, sectioned, and evaluated under stereomicroscopy. They found that thermocycling did not affect Cavit but lead to an increased incidence of leakage with TERM and that Cavit maintained a better seal then TERM.

Fogel[303] in 1977 tested the adaptation of various filling materials to the walls of 210 instrumented root canals. The materials tested were Adaptic, AH-26, Cavit, Durelon, and ZOE. These materials were placed in the canals via an endodontic pressure syringe, then subjected to methylene blue dye for intervals of 1,7 and 30 days. Canals filled with a silver cone and laterally condensed gutta-percha and sealer with a master gutta-percha cone and laterally condensed gutta-percha and sealer were used as controls. Teeth were then sectioned longitudinally at each time interval, and the marginal penetration of the dye was measured. Of all the materials tested, the silver point control exhibited the least amount of marginal leakage at each time period. AH-26 exhibited the least amount of marginal leakage after 30 days as compared to the other experimental materials and the

gutta-percha control and was the easiest material to handle of all those tested. Goldman[304] investigated the usefulness of dye-penetration studies. There are two parts to his study; in Part 1, teeth were prepared through the foramen and then left open, either apically, coronals, or both ends. In all cases, the dye did not penetrate the full length of the canal. The only exception was when both ends were left open and the teeth were placed into the dye in an upright position. In Part 2, a measurable defect filling the length of the canal was created and left open apically. When the tooth was immersed in dye, the penetration was incomplete. When the air was removed by vacuum pump prior to placement in the dye, the penetration was total. The validity of dye studies that do not consider entrapped air is open to question.

A study by Zakariasen, Douglas and Stadem[305] developed a dye recovery method that allowed penetration to the apical foramen and leakage into the root canal system. The teeth were then decalcified, and the dye present within the root canal system is returned to solution. Spectrophotometric readings are taken, and from these readings the actual volume of dye can be calculated. It was thought that this method provides for a direct quantitative measurement of the material leaking into the radicular space or spaces. In a similar method by Johnson and Zakariasen[306] looked at mesial roots from 40 human mandibular molars. Teeth were assigned into two groups and obturated with laterally condensed gutta-percha or silver coned. Each group was subdivided into two subgroups. The first subgroup consisted of specimens with two canals and separate foramina. All specimens were obturated and exposed to 2.0-percent methylene blue dye at 37°C for a period of 30 days. A dye-recovery, spectrophotometric method was used to evaluate the

apical microleakage. There was no significant difference in leakage between the groups and subgroups.

In 1989 Kersten and Moorer wanted to find out if and to what extent, different types of root fillings prevent leakage of bacteria-sized particles and large protein molecules; and second, if the amount of leakage of methylene blue, a commonly used indicator, was comparable with the leakage of bacterial product of low molecular weight that had an established pathogenicity. Forty-six human extracted incisor roots had root canals prepared in a standardized method, obturated in four ways using gutta-percha. The roots were then sectioned 9-mm long. The sections were mounted in the middle of tubes that were closed at both ends with rubber membrane stoppers. Those permitted sampling from the apical reservoir. The coronal reservoir was filled with a solution of 1 mg/ml latex beads, 4 μg/ml endotoxin, 0.5-percent butyric acid, 0.1-percent valeric acid and 0.1-percent methylene blue water. Samples were taken from the apical reservoir after 1 week and 2 weeks. Solutions were tested for the presence of latex beads phase-contrast microscopy, for endotoxin with a limulus lysate test, for butyric acid with gas-chromatography and for methylene blue with spectrophotometric analysis. Leakage of bacteria-sized particles and large-sized protein molecules could be prevented only when both sealer and pressure were used in obturating root canals with gutta-percha leakage of butyric acid proved to be comparable with leakage of methylene blue. Microleakage of these small molecules could not be prevented in this study regardless of the method of filling.

As mentioned previously, a commercial product that incorporates a central carrier coated with alpha-phase-gutta-percha in a pre-packaged form, known as Thermafil uses a

flexible stainless steel file. When heated the gutta-percha surrounding the carrier becomes thermoplastic and adheres to the carrier. When inserted into the root canal, the carrier transports the gutta-percha in the root canal and retains in the canal as part of the obturation. Beatty et al.[307] showed Thermalfil to be more effective in restricting apical dye penetration than laterally condensed gutta-percha or the single cone obturation techniques. On the other hand Lares[308] and El Deeb and Haddix et al.[309] showed significantly less leakage with lateral condensation than with Thermafil. Bahambhani and Sprechman[310] studied the effect of two different sealers on the quality of the apical seal obturated with Thermalfil obturators and vertical condensation was tested. Fifty extracted human maxillary anterior teeth were treated. The root canals were obturated using Thermaseal or Kerr sealer, and Thermafil or vertical condensation. All roots were placed in 1.0-percent methylene blue dye for 2 weeks. The roots were cleaned, and the degree of linear dye penetration was measured using a Unitron light microscope with a digital read out. The study showed that under *in-vitro* conditions, the Thermafil technique was equivalent in linear leakage to vertical condensation. It showed that there was no statistical difference in the mean apical dye penetration between the two sealers and the two obturation techniques.

Fluorometric Studies

Fluorescent spectroscopy is an analytical technique of quantitative application to chemical solutions. On exposure to ultraviolet or visible light, certain compounds will emit light of a longer wavelength. This emission is fluorescent.

Ainley[311] in 1970 designed an *in-vitro* investigation technique that would render a quantitative result and to utilize this technique in an evaluation of the sealing property

of gutta-percha and of both the chilled and unchilled, sealed and unsealed split silver cone. This study tested single rooted anterior teeth for microleakage from within root canals of rhodamine B, a fluorescent dye. The choice of Rhoda mine B was chosen for its high degree of fluorescence and water solubility. The teeth were instrumented and a reservoir was created for the dye in the coronal two thirds of the canal internally. Externally the root surface was coated with colloid ion and sealed with sticky wax. Silver cones were adjusted to be 0.5 mm form the apex. The cones were notched 5 mm form the tapered end and seated, wither chilled or unshelled and with or without sealer, as appropriate. The cores were then twisted off and the canals were injected with Rhodamine B, stock solution from a 1-ml tuberculin syringe. The access opening was sealed with Cavit and covered with sticky wax. From this study it was concluded that the apical leakage of sealed gutta-percha and sealed and unsealed split silver cones was minute, the values falling within a similar range. No determination could be made of a superior obturating technique because of the overlapping range of leakage values between the test groups and broad inconsistent range of values in a given test group.

In vitro dye and fluorescent studies may have many limitations and flaws. Therefore, no filling material or method of filling has been proven to be superior to others.

In Vivo and *In Vitro* Bacterial Studies

Mortensen et al.[312] stated that microorganism penetration might be more appropriate than dye or isotope penetration for studying leakage *in vivo*. Goldman et al.[304] have pointed out that bacteria performed better than dye penetration in testing for leakage of hydrophilic materials and that dyes could give a false positive reading if their

molecules were small enough. Because air bubbles can prevent dye leakage, the results of dye studies have been questioned. It appears that bacterial studies can provide more accurate information in clinical situations. In 1987 Madison et al.[280] found that microleakage in endodontically treated teeth by using artificial saliva would cause coronal microleakage in 7 days depending on the type of sealer. Khayat et al.[313] determined coronal leakage with natural human saliva would thoroughly penetrate the entire length of the root canal in less than 30 days.

In 1980 Lamers et al.[314] tested the sealing properties of Cavit-W temporary filling material *in vivo* in the control group of a usage experiment in which root canal disinfectants were studied. Histological examination of the root canals showed a statistical significance in the occurrence if microorganisms with increase in time. Microleakage past the filling material was considered to be responsible.

Torabinejad et al.[13] cleaned and shaped 45 canals, obturated using Roth sealer and gutta-percha by lateral condensation. The coronal portions of the root filling materials were placed in contact with *Staphylococcus epidermidis* and *Proteus vulgaris*. The number of days required for these bacteria to penetrate the entire root canal system was determined. Results showed that over 50 percent of the root canals were completely contaminated after 19 day exposure to *S. epidermidis*. Fifty percent of the root canals were also totally contaminated when the coronal surfaces of their fillings were exposed to *P. vulgaris* for 42 days.

In 1992 Deveaux et al.[315] a model system was developed and tested to evaluate the sealing property of temporary restorative materials used in endodontic access preparations. The materials Cavit, IRM, and TERM were tested on 40 premolars against a

known bacterial species, *Streptococcus sanguis*. Leakage of bacteria was checked every 4 days to 8 days after initial immersion in the culture. Thermo cycling was introduced on the fourth day. Cement thicknesses were measured after 8 days and after the teeth had been longitudinally sectioned. IRM was less leak-proof than Cavity and TERM. Thermo cycling aggravated percolation in the case of IRM and decreased the tightness of Cavity, whereas TERM remained leak-proof. The thickness for Cavit was 3.73 mm; IRM, 3.45 mm; and TERM, 5.49 mm. There was no statistically significant relationship between thickness and tightness.

Magura et al.[316] evaluated coronal leakage in obturated root canals using fresh human saliva. They found that salivary leakage was slower then dye penetration. They also reported that salivary penetration at 90 days was significantly greater than that seen after 2, 7, 14 and 28 days. Later in 1994, Gish[317] stated that coronal leakage of bacteria from saliva into root canal filling materials is a potential cause of failure. This problem may be more pronounced when only a small volume of obturating material remains in the canal, such as after post-preparation. In this study, coronal leakage of bacteria through unsealed apically obturated canals was investigated using a new *in-vitro* model system.

In 1996 Chailertvanitkul et al.[318] investigated the coronal leakage of obligate anaerobes into root canals obturated with lateral condensation of cold gutta-percha with two root canal sealers. Sixty single rooted canals were prepared using the modified double-flared technique with balanced force. Twenty teeth were obturated with lateral condensation of cold gutta-percha and AH26 sealer and 20 teeth were obturated with the same technique using TubliSeal EWT sealer. Negative control teeth were completely sealed and the positive control was not obturated. The root surface of each tooth was

sealed with nail varnish except the apical 2 mm. The coronal part of each root canal was sealed with the cut end of a polypropylene tube and placed in a glass bottle containing sterile Fastidious Anaerobe Broth (FAB). An inoculum of *Fusobacterium nucleatum* in the FAB was placed in each coronal chamber at 7-day intervals and daily observed to determine bacterial growth for 12 weeks. All positive control teeth showed bacterial leakage within a week, while the negative control teeth remained uncontaminated. All experimental teeth exhibited leakage of bacterial metabolites within 12 weeks, ranging from 1 to 12 weeks. The time period for complete leakage in AH26 and TubliSeal EWT groups was 8.4 and 8.2 weeks. There was no statistical difference between the groups. They reported later that 30 percent and 75 percent of Apexit and TubliSeal EWT groups, respectively, showed bacterial leakage at 90 days.[319]

In 2001 Barthel et al.[320] examined whether obturated roots canals combined with several adhesive and temporary filling materials, can be bypassed by bacteria. The teeth were coronally sealed with Clearfill, CoreRestore, IRM, Ketac Fil, or a combination of IRM/Wax or Ketac Fil/Wax. The roots were then placed between a top and bottom chamber. The top chamber contained soy broth with *Staphylococcus epidermis,* and the bottom chamber contained sterile soy broth. For one year the lower chamber was checked on a regular basis for turbidity, which indicated bacterial growth. Only three samples from the CoreRestore group and two sampled from the Clearfil group resisted leakage. There was no significant difference in number of leaking samples among the groups. At the beginning of the experiment IRM performed the worst. Between 5 months and 10 months, Clearfil exhibited the least leaking sampled.

Ricucci and Bergenholtz[321] in 2003 described histological and microbiological findings in teeth where root fillings have been exposed to caries and the oral environment for prolonged period. Teeth with a follow up period of three years or more and those that has been without proper restoration for at least a period of three months were considered. Some root fillings have been without restoration for several years. In all 39 roots representing 32 teeth were examined by histology. It was concluded that well-prepared teeth and filled root canals resist bacterial penetration even upon frank and long-standing oral exposure by caries, fracture or loss of restoration.

STERILIZATION OF HUMAN TEETH

The Centers for Disease Control and Prevention (CDC) recommends teeth that will be extracted and used in dental educational exercises to be cleaned and disinfected before use.[322] Extracted teeth used for education contain blood. All persons who collect, transport, or manipulate extracted teeth should handle them with the same precautions as a specimen for biopsy. Several sterilization methods have been used to remove debris of extracted teeth. The CDC recommends scrubbing with detergent and water or by using an ultrasonic cleaner to remove any debris of extracted teeth. Teeth should be immersed in sodium hypochlorite diluted to 1:10 with tap water or any liquid germicide.[323]

The method of autoclaving was studied by Pantera and Schuster [324] and Pashley,[325] who determined that autoclaving is an effective method for sterilizing teeth for routine pre-clinical courses. A recommendation of 40 minutes at 121°C and 15 pounds psi either in saline or autoclave bags. Pashley evaluated extracted human teeth and divided them into three groups. Group one were the controls, teeth were untreated. Group 2 were sterilized in a steam autoclave and group 3 used ethylene oxide gas.

Crowns of teeth were resected at the CEJ. The permeability of the dentin was determined to be similar in all three groups. It was determined that ethylene oxide or autoclaving resulted in no change in the intrinsic permeability of the dentin.

Toro et al.[326] wanted to determine if autoclaved or gas-sterilized enamel responds to fluoride in the same way as alcohol-disinfected enamel lesions do. She suggested that gas sterilization is the preferred method for sterilizing enamel specimens that will be used in intra-oral studied.

Another method of sterilization used is gamma irradiation. Brauer[327] recently examined the effect of gamma radiation on the nana-mechanical properties of dentin and enamel of extracted teeth. Whole teeth were sterilized with gamma radiation doses of 7 kGy and 35 kGy respectively. The control group teeth were not treated. Crowns were then sectioned and polished. The elastic modulus and hardness were tested using atomic force microscopy with nano-indentations under wet conditions. The results showed that gamma radiation is an efficient way to sterilize extracted teeth while alteration of dentin and enamel mechanical properties are minimized.

Ethylene oxide is a well-known sterilizing agent and it is only recently that its use significantly emerged. It has been showed that it still remains a promising field to explore and develop.[328] Thomas et al.[329] looked at the effect of ethylene oxide sterilization on enamel and dentin demineralization *in vitro*. The aim of their study was to assess the effect of sterilizing sound human enamel and dentin with ethylene oxide on the depth of lesion and loss before and after *in-vitro* demineralization. The results showed that there was a significant reduction of lesion depth due to sterilization in demineralized enamel.

MATERIALS AND METHODS

SELECTION OF TEETH

Eighty-five single rooted maxillary anterior teeth in which extraction was indicated for orthodontic purposes were used for this study. All teeth were collected from the Oral Health Department under the Clarian/IUPUI IRB (IRB # NS1003-03). All teeth were stored in a sealed container with sterile saline and refrigerated prior to the study. Radiographs were taken in the mesial-distal direction to confirm a type 1 root canal system was present. Teeth with abnormal canal anatomy, abnormal root morphology including obvious lateral canals, extensive decay, and root fracture were discarded.

Once the teeth were selected, calculus and soft tissue debris were removed from the root surface with hand scaling instruments. Following debridement of the root surface, teeth were immersed in 6.0-percent sodium hypochlorite (Clorox Co., Oakland, CA) for thirty minutes and mechanically derided with a soft brush. Teeth were accessed (Figure 1) and#10 K- type endodontic file (Kerr, Romulus, MI) was inserted into the root canal and advanced out of the apical foramen of all teeth (Figures 2, 3 and 4). All teeth with canals that could not be negotiated with a #10 K-type file were excluded from the study.

CANAL INSTRUMENTATION

Working length was determined by passing a #10 K-type endodontic file into the root canal until the file is just visible at the apical foramen, then subtracting 1 mm to establish the file length measurement. The root canals were cleaned and shaped using K-type endodontic files, Gates Glidden drills (Brasseler, Savannah, GA), EndoSequence

Rotary Instrument System (Brasseler, Savannah, GA), 0.06 taper, size 20-35 (Figure 5 and 6), while irrigating with 6.0-percent sodium hypochlorite. K-type #15 and #20 files were used to instrument to the working length, then Gates Glidden drills numbered 2 and 3 were used to flare the coronal half of the canal. Instrumentation of all teeth was performed with a crown-up technique using the EndoSequence 0.06 tapered rotary files, number 20-35, until the #35 file was instrumented to the working length. RC prep, a lubricant to facilitate movement of rotary instruments in the canal was used between all files. Root canal irrigation was performed using 1 ml of 6.0-percent sodium hypochlorite between each file. A #10 K-type file was used to maintain apical patency. Upon completion of instrumentation, the smear layer was removed from the canals by rinsing with 2 ml of a 17-percent EDTA solution for 5 minutes with the use of the Endo activator. The teeth were irrigated with a final rinse of 2.0-percent chlorohexidine (Figure 7 and 8). Following final irrigation, the canals were dried with sterile, coarse paper points (Figure 9). To prevent dehydration, all roots were handled using water-moistened gauze during resection and instrumentation. All teeth were then sterilized by ethylene oxide after canal preparation

ASSIGNMENT OF TEETH

Specimens were randomly assigned to a total of 5 groups. Three groups, designated 27 teeth in group A (Bioceramic sealer using warm vertical technique), 27 teeth in group B (Roth Sealer using warm vertical technique), and 27 teeth in group C (Bioceramic sealer using single cone technique) served as the experimental groups. Two other groups Group + and Group – served as positive and negative controls, respectively

with two specimens in each group. The positive and negative controls insured the apparatus model was working properly.

CANAL OBTURATION

Single cone obturation and warm vertical condensation of gutta-percha (Dentsply, Tulsa, OK) were the techniques used for obturation. Materials and reagent tube tips and all instruments were either alcohol wiped or autoclaved as appropriate. Obturation of the root canal was done with aseptic techniques.

Group A was obturated in an orthograde fashion using a #35/.06 bioceramic impregnated and coated gutta-percha cones (Brasseler, Savannah, GA) and the bioceramic sealer (Brasseler, Savannah, GA) (Figure 10, 11 and 12) with warm vertical condensation. A master cone (Figure 14 and 15) was coated with the bioceramic Sealer and fitted to length. The System B heat source (Tulsa Dental, Tulsa, OK) plugger was placed at the canal orifice and activated; this heated the tip to 200^{o}C (Figure 16 and 17). While holding the heat activator, the plugger was advanced apically. At 6 mm from the working length, the heat activator was released and apical pressure was held for ten seconds to allow the apical plug of gutta-percha to cool without shrinking. Heat was activated for one second, which provided a separation burst of heat, and the plugger was then removed from the canal with the excess gutta-percha adhered to the plugger. A #8 and #9 Schilder pluggers (Dentsply/Caulk, Milford, Del) were used to condense gutta-percha. The canal was then obturated to the orifice with the HotShot gun (Discus Dental) using conventional gutta-percha pellets (Figure 18 and 19). Radiographs were taken to ensure length and density of the fill. Group B was obturated in an orthograde fashion in

the same technique previously described with the exception of using Roth sealer and non-coated gutta-percha.

Group C was obturated in an orthograde fashion using a #35/.06 bioceramic impregnated and coated gutta-percha cones (Brasseler, Savannah, GA) and the bioceramic sealer (Brasseler, Savannah, GA) with a single cone technique, following manufacturer's instructions. A master cone was coated with the bioceramic sealer and fitted to length. The gutta-percha is machined to precisely match the preparation, thereby reducing leakage and achieving a hermetic seal. To achieve proper setting of the sealer, all samples were allowed to set for eight weeks. [257]

The positive control included two teeth that had a single cone with no sealer. This allowed free communication of the bacteria in the upper chamber with the growth medium in the lower chamber.

The negative control also included two teeth that were obturated with sealer. The samples in this group were coated with bonding agent to seal the apical opening and the dentinal tubules to prevent any leakage.

Again, radiographs were taken to ensure length and density of the fill. All specimens in all groups were placed in a humidor with 100-percent humidity at 37ºC for 24 hours to allow for adequate setting prior to bacterial leakage testing.

All groups (Group A, B, C, positive and negative control groups) were placed in sterile bags with wet gauze to maintain hydration and allow setting of the bioceramic sealer for 8 weeks.

MICROBIAL LEAKAGE TEST

A microbial leakage apparatus (Figure 20 and 21) was constructed using a similar two-chamber method described by Torabinejad et al.[13] *E. faecalis* ATCC 29212 was the test bacterium used in this study to determine microleakage. To prevent contamination from other microbes during the study, an antibiotic and an antifungal was added to the autoclaved tryptic soy broth described by Butaye et al.[20] and Odds et al.[21] The choice of antibiotic and antifungal was streptomycin and ketoconazole respectively. Upon further investigation it was found that ketoconazole is not soluable in water like streptomycin. To reduce variables that could affect microleakage, ketoconazole was eliminated. Success was obtained in growing colonies of *E. faecalis* in the trypticase soy broth using concentrations of streptomycin at 2000 ug/ml was tested. The strain of *E. faecalis* was tested to determine if it could grow at this concentration of streptomycin. Thus, this mechanism should prevent contamination from both stray bacterial and fungal species during our microleakage experiment.

Similar to Torabinejad et al.,[13] 20-ml scintillation vials were used to suspend the prepared teeth. A high-speed handpiece with a #6 round bur was used to make a hole through the center of every cap. Each tooth was placed into the fabricated hole in the cap, up to its cementoenamel junction, and secured to the cap using sticky wax. When the tooth was placed in the vials, 3 mm of apical root structure was submerged into the selective broth without contacting the floor of the vial. Dentin bonding agent was painted along the root surface excluding the apical 3 mm. The upper chamber consisted of a delivery tip secured with composite in the access of the tooth outside the vial. Securing

each tooth to the vial cap in this manner allowed the root of the tooth to be within the vial and its crown outside of the vial.

The lower chamber of the apparatus, created by the space between the root tip and floor of the scintillation vial, was filled with 7 ml of filter-sterilized modified trypticase soy broth containing streptomycin at 2000 ug/ml. The streptomycin was dissolved in 10ml of distilled water. Prior to placing the streptomycin, the trypticase soy broth was autoclaved and the streptomycin was added via filter sterilization. After all samples were prepared they were placed under ultraviolet light overnight for final sterilization.

The upper chamber of the apparatus, the space above the canal orifice, was filled with 0.1 ml of the modified Trypticase Soy Broth and inoculated with resistant *E. faecalis*. Fresh medium and *E. faecalis* was added to the upper chamber every 4 days to ensure viable bacteria are present. Replenishing of the upper chamber was accomplished using sterile technique using sterile pipette tips every time. All of the experimental samples were placed in a 5.0-percent CO_2 incubator at 37^{o}C and 100-percent humidity.

A positive incidence of leakage visualized by the naked eye was determined by turbidity of the growth medium in the lower chamber (Figure 22, 23 and 24). The microleakage experiment was conducted for 33 days and the medium in the bottom chamber was examined daily for turbidity changes. Based on previous studies, leakage can occur in 3 days when gutta-percha is directly exposed to any contaminants. Thirty-three days was sufficient enough to show significant microleakage.

STATISTICAL METHODS

The outcome of interest (turbidity), and time-to-leakage (in days), was determined for each of the samples. Survival analysis was used to compare the three groups, with a Kaplan-Meier plot to visualize the results and a nonparametric log-rank test for the group comparison.

SAMPLE SIZE

With a sample size of 27 teeth in each of the three groups, the study has 80-percent power to detect a difference in the proportion without microleakage (40 percent vs. 80 percent at 10 days based on Williamson et al.[330]), assuming a two-sized 5.0-percent significance level using a log-rank test.

The survival analysis for the study examined the time to microleakage. Samples were followed until microleakage for 33 days. For sample size calculations for a survival analysis, the sample size calculations do not need to be performed using the proportion surviving at the end of the study. Based on the graph in Williamson's paper the sample size calculations are based on her data through 10 days because those estimates seem fairly robust. The power calculations were applicable even though the current study followed the samples for more than 10 days.

RESULTS

The presence of microleakage was compared between groups using the Fishers Exact test. The time to microleakage was compared using a log-rank test. The information described in Table 1 shows the microleakage observed in the three groups. No microleakage was observed in the negative control or the group that was obturated using Roth sealer with warm vertical condensation (Group B). Microleakage was observed in the two groups using the bioceramic sealer with the single cone technique (Group C) and the warm vertical technique (Group A). Groups A and C had a significantly higher proportion of samples with microleakage than group B ($p < 0.0001$), but groups A and C were not significantly different from each other ($p = 0.50$)

The information described in Table 2 lists the time to leakage between all three groups. The time to leakage was significantly lower in the bioceramic group using the warm vertical condensation (Group A) and the bioceramic group using the single cone technique (Group C) than the Roth's sealer group using warm vertical condensation (Group 1) ($p < 0.0001$). The two groups using the bioceramic sealer with different obturation techniques (Group A and C) were not significantly different from each other ($p = 0.37$).

FIGURES AND TABLES

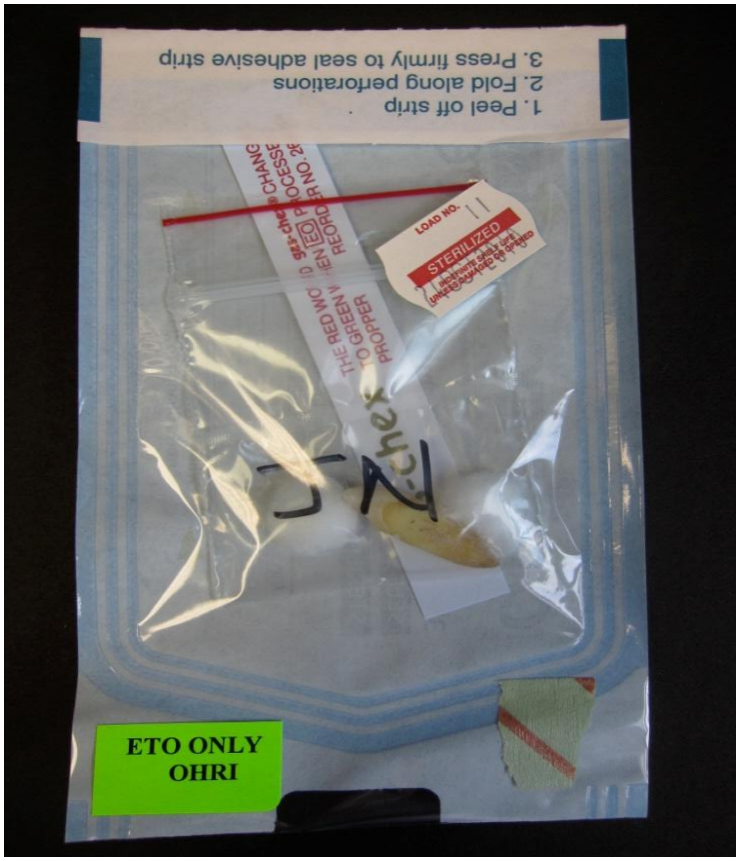

FIGURE 1. Specimens sterilized with ethylene oxide.

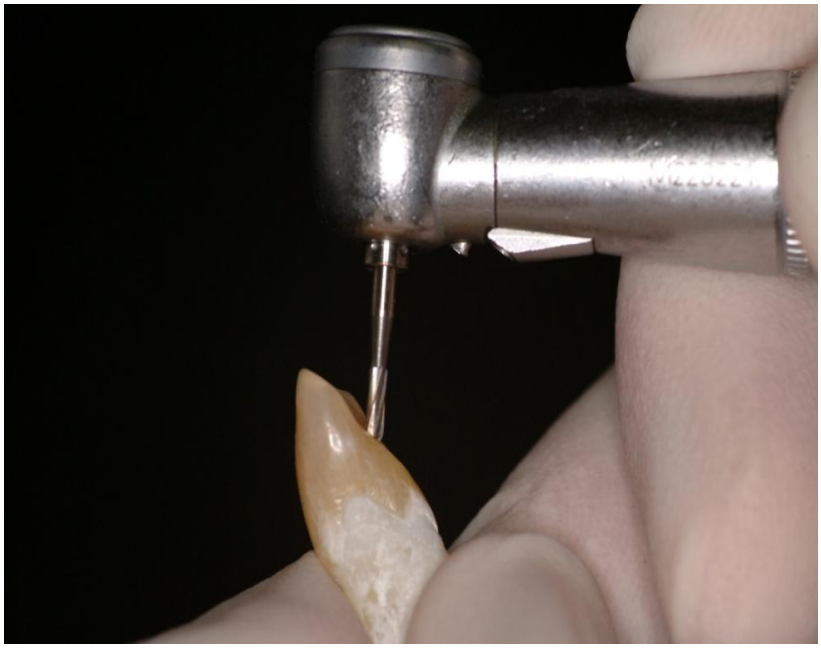

FIGURE 2. Tooth accessed.

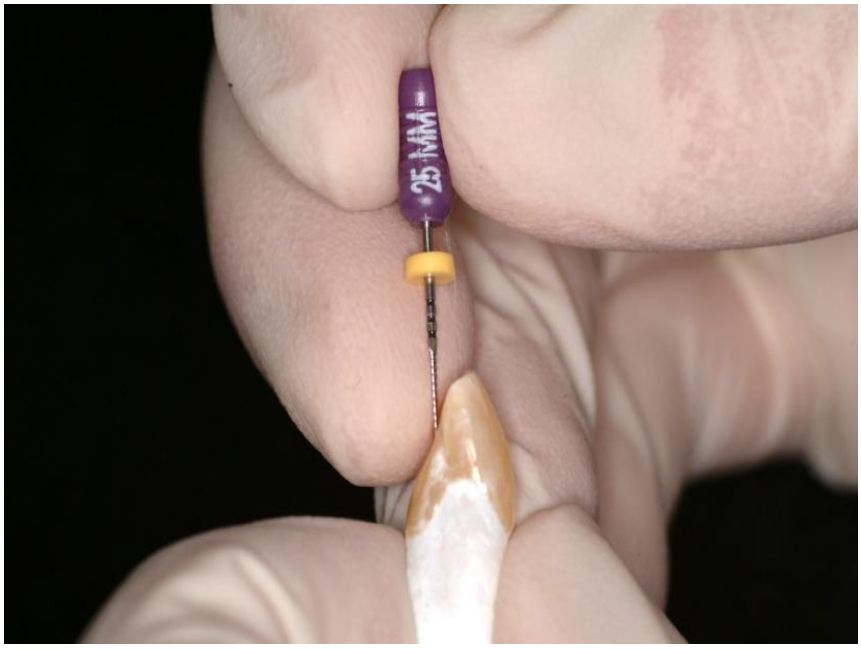

FIGURE 3. Canal located and #10 K-file placed in canal.

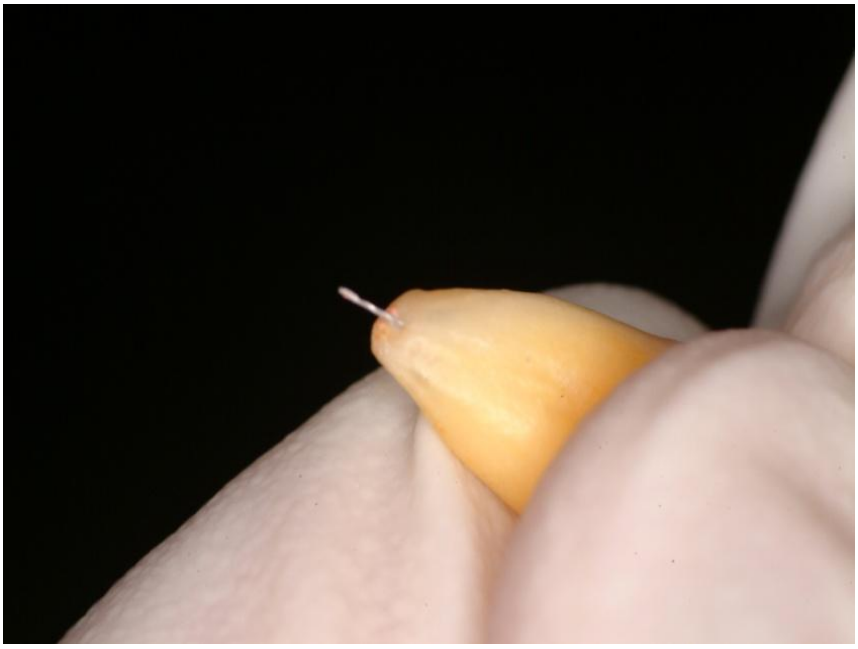

FIGURE 4. Working length established by passing a #10 file past the apex to gain patency and then by pulling back 0.5 mm from anatomic apex.

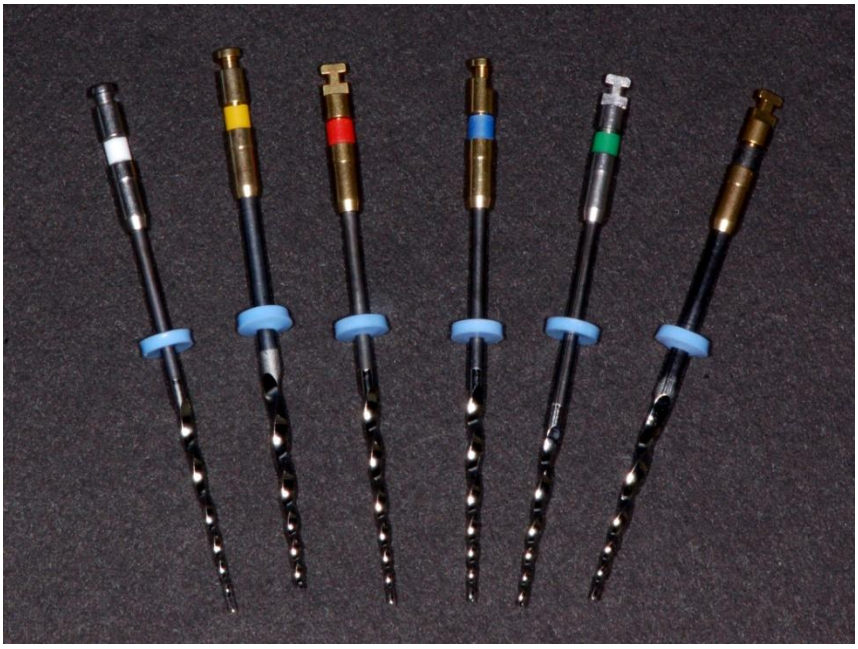

FIGURE 5. Brasseler EndoSequence rotary used to instrument canals to a 35/.06.

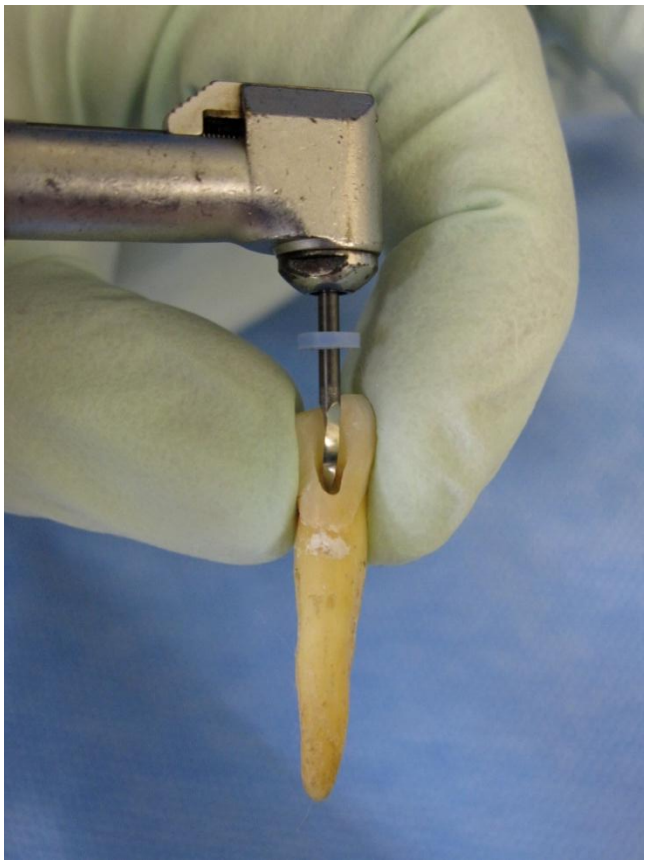

FIGURE 6. Instrumentation using the EndoSequence Rotary files.

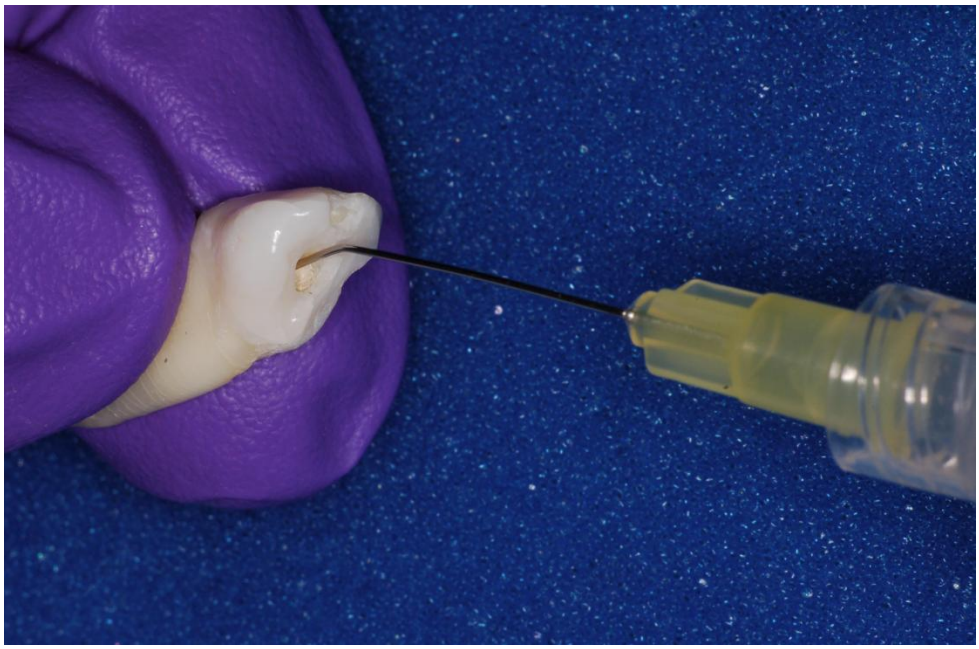

FIGURE 7. Irrigation using 6% NaOCl, 17% EDTA and 2% CHX.

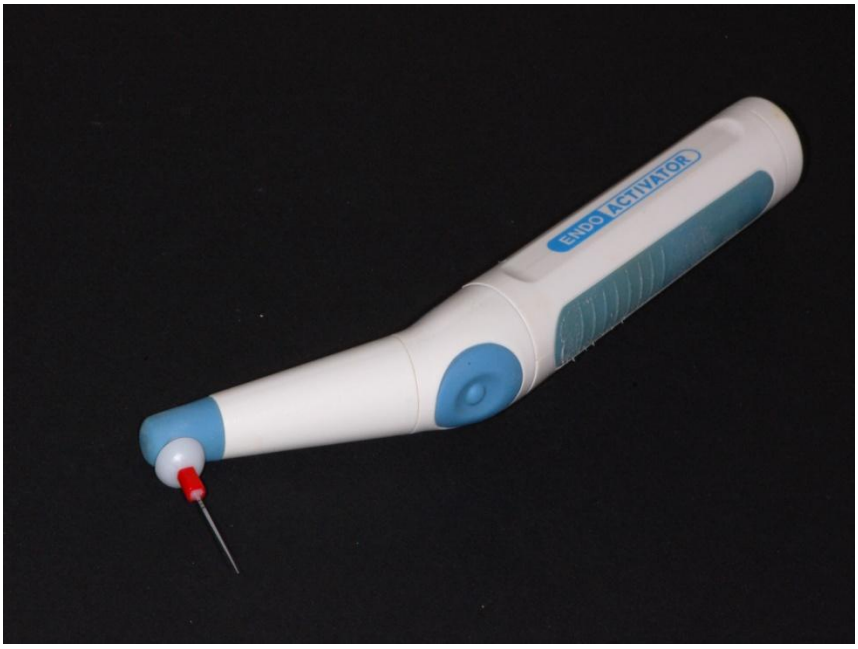

FIGURE 8. Endoactivator used with irrigation solutions.

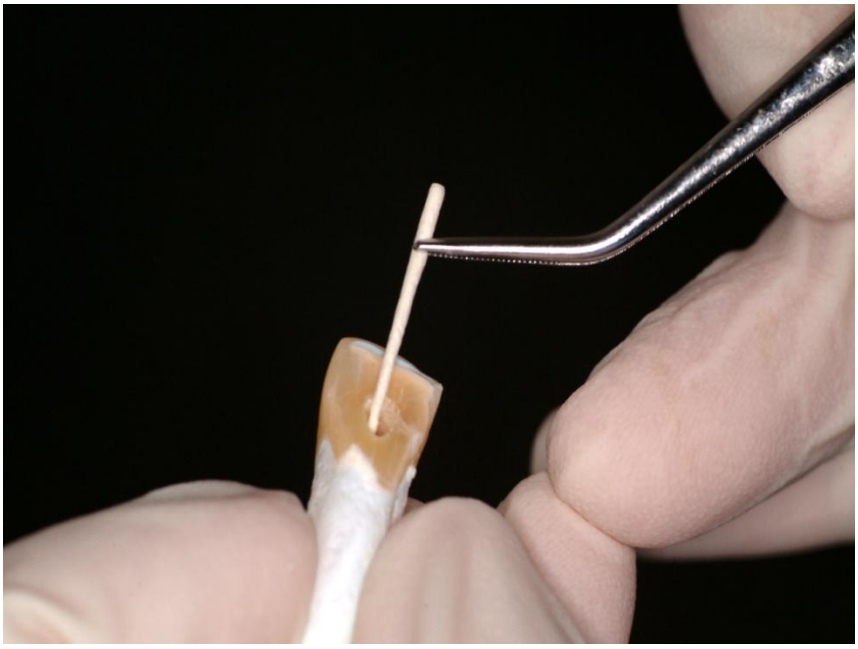

FIGURE 9. Drying canals with paper points.

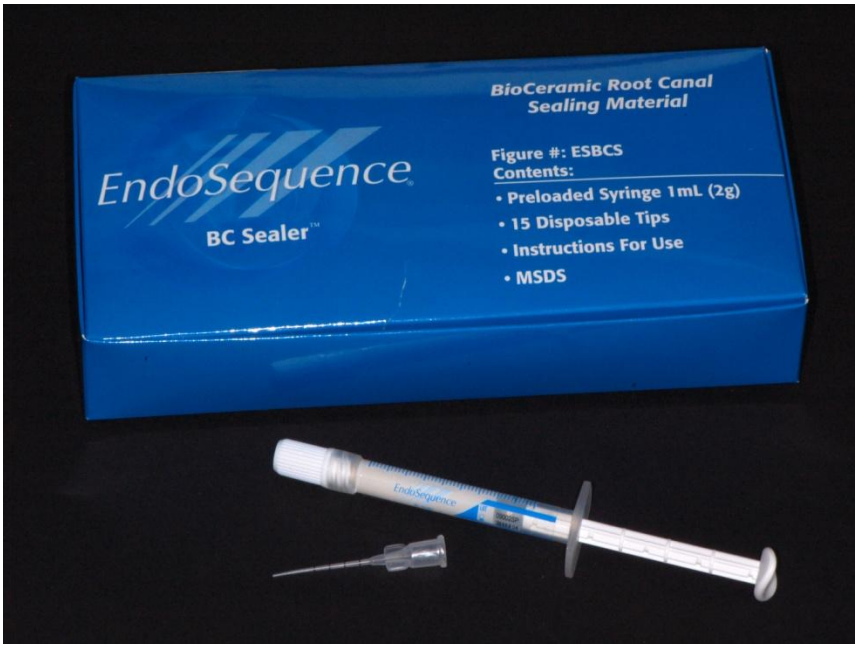

FIGURE 10. Bioceramic sealer.

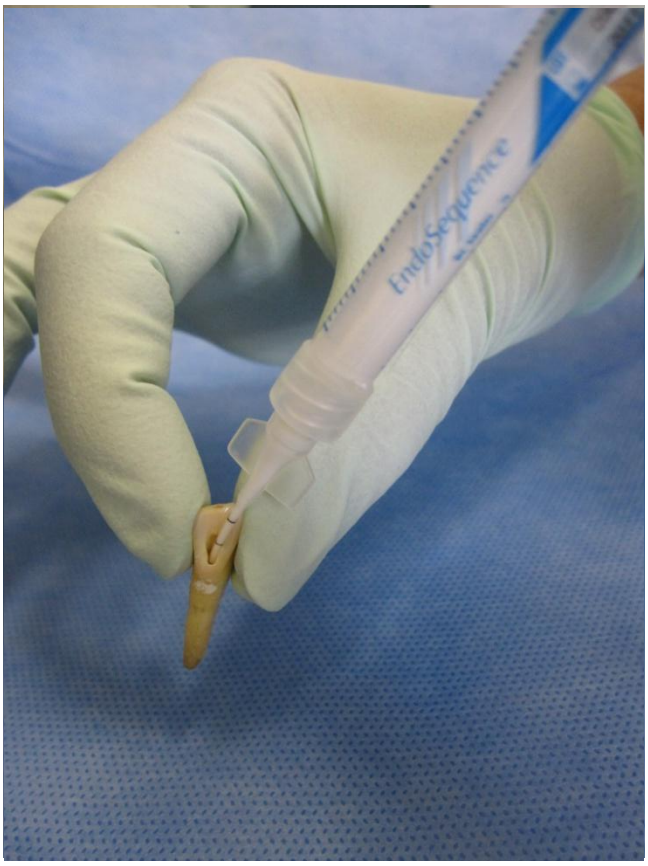

FIGURE 11. Placement of bioceramic sealer.

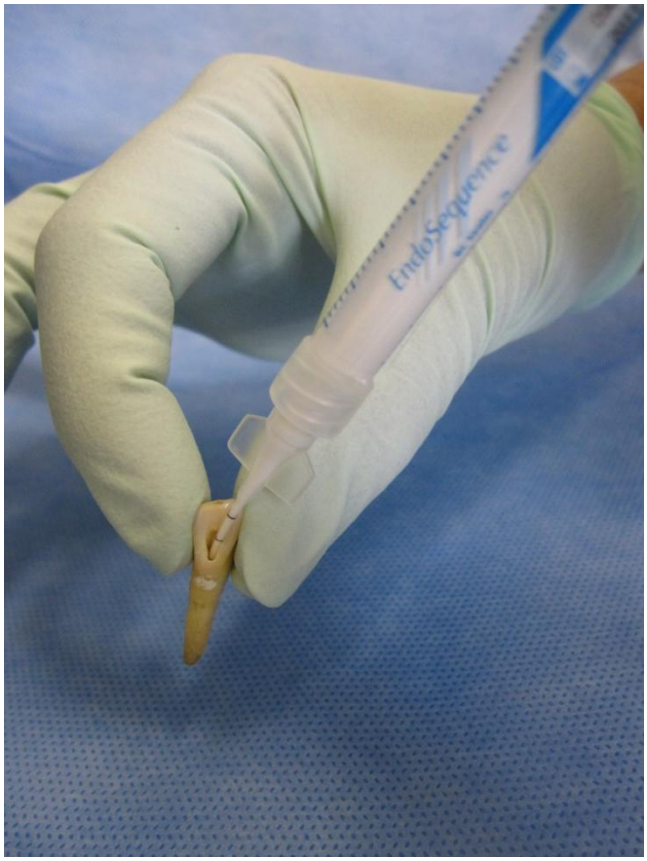

FIGURE 12. Placement of bioceramic sealer.

FIGURE 13. Roth sealer.

FIGURE 14. Bioceramic-impregnated and coated gutta-percha points.

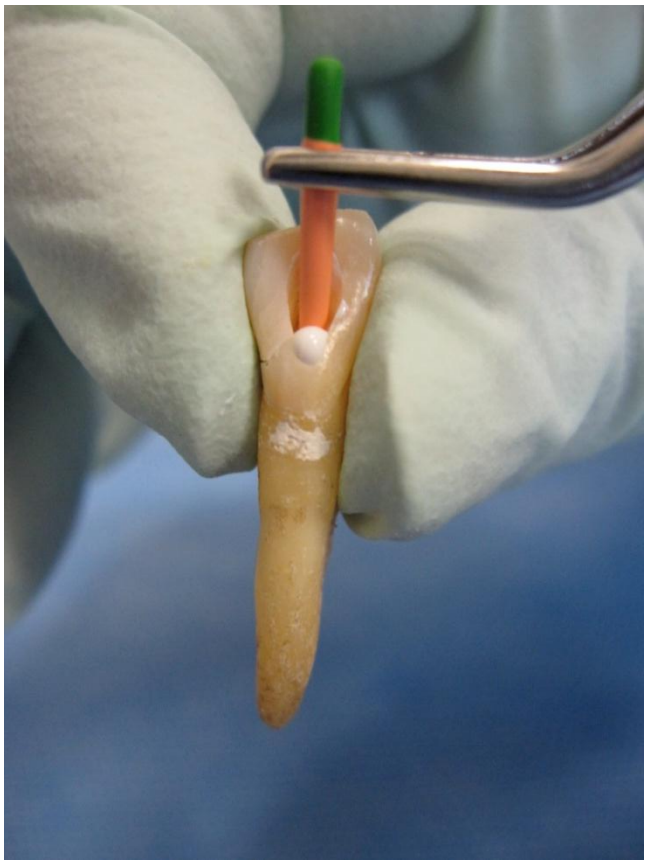

FIGURE 15. Placement of bioceramic impregnated and coated gutta-percha points.

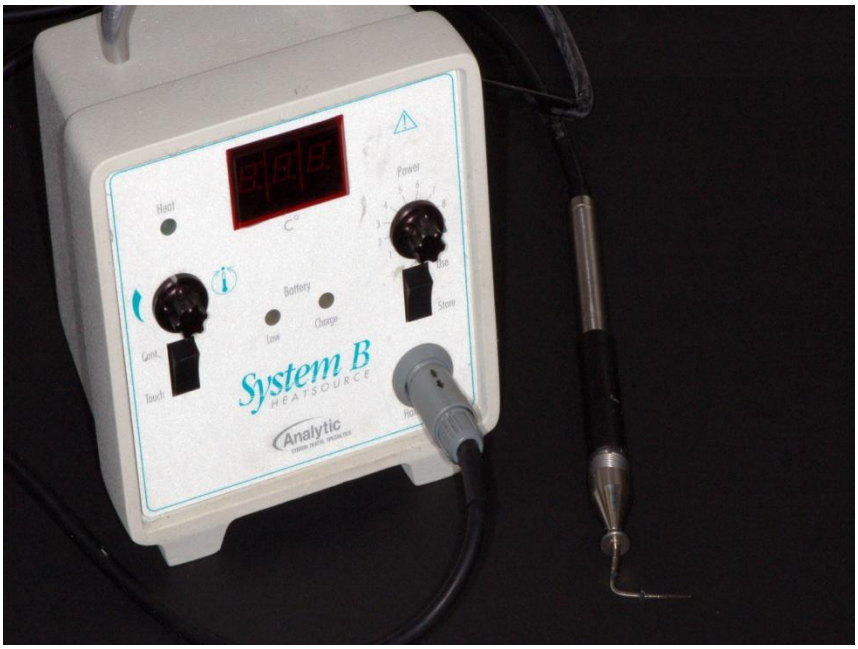

FIGURE 16. System B to downpack.

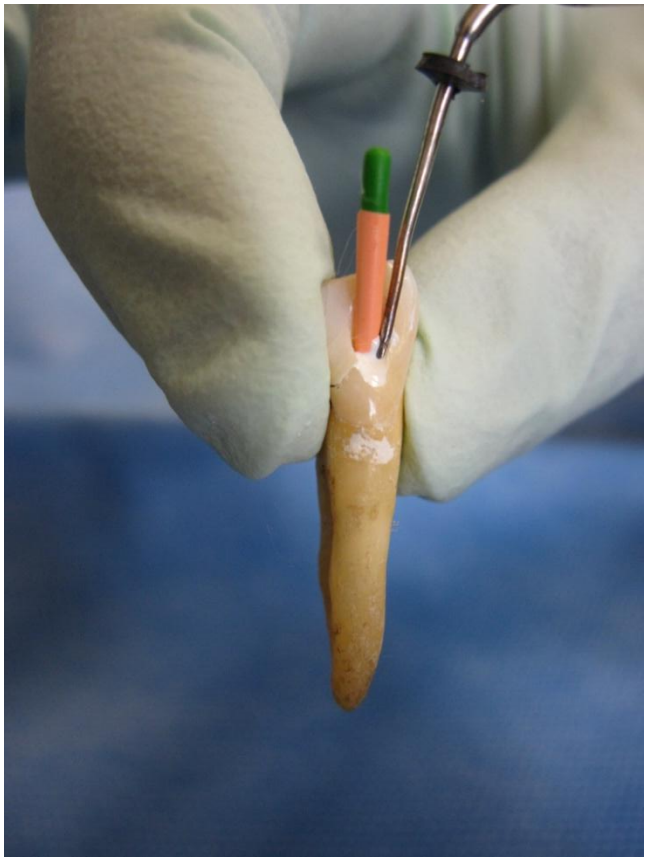

FIGURE 17. System B to downpack.

FIGURE 18. Hot shot used to backfill root canal.

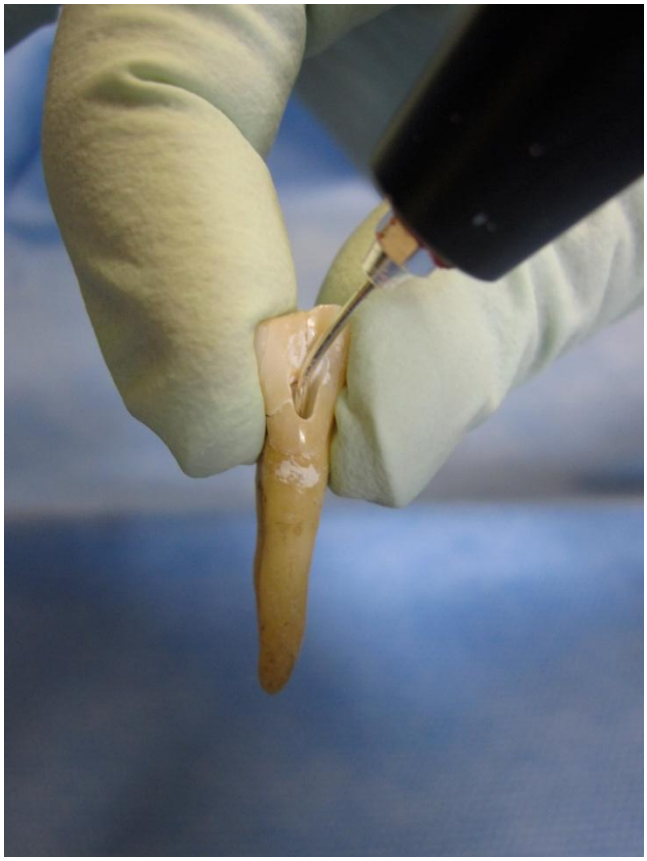

FIGURE 19. Hot Shot used to backfill root canal to provide 5 mm of root canal filling.

FIGURE 20. Apparatus for bacterial microleakage.

FIGURE 21. Apparatus grouped together.

FIGURE 22. Apparatus on the left with turbid broth in the lower chamber indicative of bacterial microleakage. Apparatus on the right with clear broth in the lower chamber indicative of no leakage.

FIGURE 23. Inoculated broth with *E. faecalis* to place in upper chamber.

FIGURE 24. Incubator with all groups covered with sterile foil.

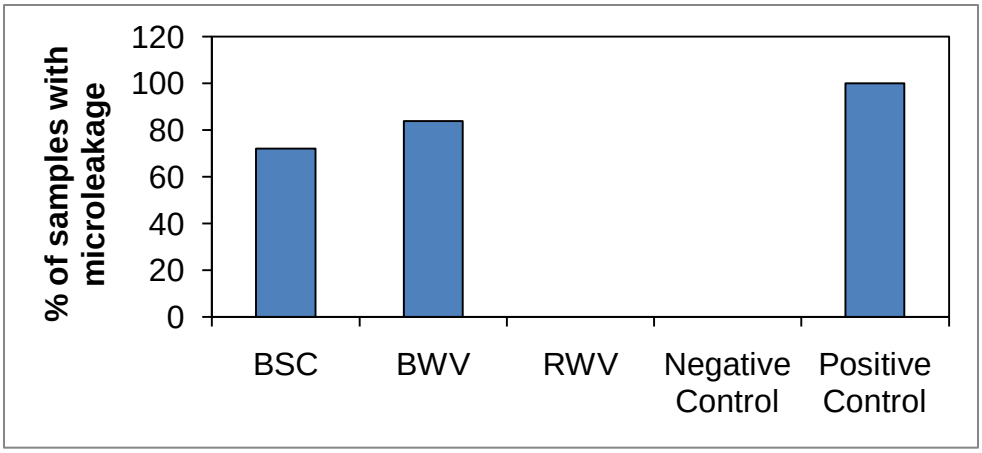

FIGURE 25. Samples with microleakage. Bioceramic sealer with a single cone (BSC), Bioceramic sealer using warm vertical condensation (BWV) and Roth warm vertical (RWV).

TABLE I

Percentage of microleakage in each group

Group	Microleakage, N (% Indicating leakage)
BSC (Bioceramic sealer using single cone)	18 (67%)
BWV (Bioceramic sealer using warm vertical)	21 (78%)
RWV (Roths sealer using warm vertical)	0 (0%)
Negative Control	0 (0%)
Positive Control	2 (100%)

TABLE II

Time in days to microleakage in each group

Group	Time (days) to Microleakage Median (95% CI)
BSC (Bioceramic sealer using single cone)	14 (7, 29)
BWV (Bioceramic sealer using warm vertical)	12 (7, 22)
RWV (Roth using warm vertical)	No microleakage within 33 days

DISCUSSION

Sealers have been extensively studied in the past and play an important role in root canal obturation; however, an ideal sealer that meets all properties has not been found. Filling the space between the core material and dentin is critical coronal leakage. Grossmans[60] description of the ideal properties of a sealer were discussed previously. He states that a good sealer adheres strongly to the dentin and the core material; it must also have strength to hold the obturation together. Sealers should be antimicrobial, biocompatible and one that can be dissolved in solvent if retreatment is warranted. Sealers should also have minimal toxicity that may be irritating to the tissues. The sealer must have some degree of radio-opacity to be visible on x-rays.

EndoSequence BC Sealer is a new sealer that is popular and claims to have many advantages: 1) Outstanding sealing properties due to small particle size, which allows the sealer to flow into the dentinal tubules, lateral canals and webs as well as conforming to the surface of gutta-percha; 2) Gap-free interface between the gutta-percha and the sealer; 3) Hydrophilicity and antimicrobial properties. This bioceramic Sealer is an aluminum-free sealer based on calcium silicate composition. Its inorganic component includes tricalcium silicate, dicalcium silicate, calcium phosphates, colloidal silica, and calcium hydroxide. The radioopacifier is zirconium oxide and contains water-free thickening vehicles to enable the sealer to be delivered in the form of a premixed paste. This study evaluated the sealing properties of EndoSequence BC sealer, a bioceramic sealer using gutta-percha with warm vertical condensation and using the single cone technique in

comparison with Roth sealer using warm vertical condensation.

Prior to finalizing the methods and materials section, four pilot studies were completed for the duration of 4 weeks. The purpose of the pilot studies was to minimize uncontrollable variables in the apparatus and ensure reliable results.

The first pilot study, originally described by Williamson et al.[330] was reproduced with gamma irradiation for sterilization of the teeth, with the apparatus assembled as described by Torabinejad et al.[13] The results demonstrated that all negative controls had both bacterial and fungal contamination. The second pilot study was constructed using a strain of *E. faecalis* that was antibiotic and antifungal resistant. The fresh medium contained streptomycin and ketaconazole with the resistant strain of *E. faecalis*. This was used to inoculate the upper chamber every three to four days. This broth was chosen to reduce the risk of contamination by selecting for this resistant strain. The *E. faecalis* grew in this medium. After further investigation, ketoconazole was found to be only soluble in methanol, acids or ethanol. To limit variables in our study it was decided to eliminate ketoconazole and keep streptomycin in the broth. A third pilot study was completed with different materials between the upper chamber and the tooth. Materials used were polyvinyl siloxane, sticky wax, glass ionomer, packable composite and packable composite with flowable. All groups failed except for the sticky wax, which lasted 40 days. The final pilot study was completed using a modified version of the apparatus eliminating the interface between the upper chamber and the lower chamber. The upper chamber became the access of the tooth with a delivery tip. The fourth and final pilot study yielded desirable results and an apparatus that was used in this research.

Development and maintenance of the seal are essential to optimize the long-term success rate of root canal treatment.[8] There have been many studies that address the subject of coronal leakage.[331] [332] The importance of the quality of the coronal restoration was significantly more critical than the quality of endodontic treatment.[331] This study evaluated the sealing properties of EndoSequence BC sealer, a bioceramic sealer using bioceramic impregnated and coated gutta-percha points with warm vertical condensation and using the single cone technique in comparison with Roth's sealer using warm vertical condensation.

The results in this study have shown that Roth sealer with gutta-percha using the warm vertical condensation technique has superior properties to those of the bioceramic sealer using in the single cone technique and the warm vertical condensation technique. In the past Roth has been known for its antibacterial properties and that may have contributed to the results in this study. Also, important variations in the obturation technique using the bioceramic sealer could have affected the seal. Heat using the warm vertical condensation technique could have affected the sealability of the bioceramic sealer; however, there are no studies to show the effect of the heat on the bioceramic sealer or the Activ GP. This needs further research.

Studies have compared the bioceramic sealer with other sealers. The most recent study done in China by Zhang et al.[333] investigated the apical sealing of a bioceramic sealer in comparison to AH Plus using the fluid filtration method. They showed that there was no significant difference in fluid leakage among their groups, as well as no time effect on leakage. They also used SEM to reveal gap free and gap containing regions in canals filled with both materials. They concluded that the bioceramic sealer was

equivalent to AH Plus sealer in the apical sealing. Although in this study no SEM was used, the results conflicted with the results obtained in this study where there was a statistical difference between the Roth group and the bioceramic Sealer.

In a recent study, Loushine et al.[334] evaluated the setting properties and cytotoxicity of the premixed bioceramic root canal sealer. It was concluded that changes in environmental water content adversely affect the setting time and microhardness of the bioceramic sealer. Further studies should be conducted to evaluate the byproduct components produced upon setting in order to accurately assess the cytotoxicity of this sealer. Another study by Zhang at al.[278] also conflicted with our results. They compared the bioceramic sealer to AH plus and found no significant difference in fluid leakage among the groups as well as no time effect on leakage.

In this study a period of 8 weeks was allotted for the sealer to set. The obturated specimens were placed in a sterilized bag with moist 2 x 2 gauze to allow for a moist environment. The inventor of the bioceramic sealer states that 4 hours are needed for the bioceramic sealer to set but in the study mentioned by Loushine et al.[334] the setting time of the sealer was anywhere between 72 hours to achieve initial set and 240 hours to achieve final set.[334, 335] It is also important to consider the setting time and microhardness of the bioceramic sealer in the presence of different moisture contents. According to the manufacturers' recommendations, the bioceramic sealer uses the moisture that remains within the dentinal tubules after canal irrigation to initiate a complete setting time. The amount of moisture may vary upon drying with paper points. A smear layer or tubular sclerosis can affect the amount of moisture also. The manufacturers of bioceramic sealer suggest 4 hours for the setting time of the sealer but this can be skewed with the amount

of moisture within the canal. When the amount of water was included the initial setting time increased and the final setting time decreased. The microhardness was also affected in this study when water was included. They concluded that further studies are required to evaluate the correlation between the length of setting time of bioceramic sealer and its degree of cytotoxicity. In *in-vitro* studies it is difficult to simulate the environment of an *in-vivo* study due to lack of moisture available to aid in setting of the sealer. Hence, for such studies it may be more clinically relevant for the bioceramic sealer to perform an *in-vivo* study.

Gutta-percha has been the conventional obturation material used in endodontics used in combination with a sealer. Recently, obturating materials have been developed that provide a better seal of a root canal by the "monoblock" theory that adheres and bonds to the dentinal wall. With the parameters of this study, it did not appear that there was a complete seal with the bioceramic sealer. These results may be due to unfavorable geometry of the canals for bonding as well as the potential gapping along the sealer and dentin interface. In 2007 Monticelli[336] studied two contemporary single-cone filling techniques using Activ GP (bioceramic impregnated and coated gutta-percha points) and Gutta-Flow and monitored bacterial leakage for 100 days. His results proved that warm vertical compaction technique using thermoplasticized gutta-percha and AH Plus sealer appears to be more effective in minimizing bacterial leakage. Another study by Monticelli[337] in the same year compared sealing of root canals filled with two single-cone obturation systems using Active GP and Gutta flow and a warm vertical compaction with AH Plus. He hypothesized that the poor coronal seal of these single-cone techniques may be improved with the placement of accessory cones to reduce sealer thickness or an

immediate coronal adhesive restoration. These conflicting results with the Active GP/GI sealer using the single-cone technique need further improvements. It has also been reported that the coating of GI filler on the surface of Active GP/GI sealer cones is nonhomogenous, which may affect bonding.[337] In addition, there has not been any data to confirm if there is shrinkage upon setting of the ActivGP/GI sealer.[336]

Different irrigation regimes may cause alteration in the chemical and structural composition of human dentin. This changes its permeability and solubility characteristics affecting adhesion of materials to dentin. Hashem et al.[338] studied the bond strength on Activ GP after different irrigation protocols. It was concluded that the bond strength of Activ GP was improved using 2.0-percent CHX in the final irrigation after 17-percent EDTA, where as CHX did not enhance the effect of MTAD on the bond strength of the material. In this *in vitro* study the final rinse used was 2.0-percent CHX.

Techniques for evaluation of endodontic leakage have yielded conflicting results. Wu et al.[339] demonstrated the lack of correlation between bacterial penetration and fluid transport in root canal fillings. Similarly, Barthel et al.[340] found no difference between bacterial and dye leakage. It has been shown that a bacterial leakage model is more clinically relevant compared to other microleakage techniques. The correlation of the amount of microleakage to the clinical outcomes of endodontic treatment is still in question and warrants further research. Therefore, the efficacy of contemporary single-cone filling endodontic techniques with Active GP/GI sealer should be further evaluated in randomized clinical trials.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to compare microleakage of teeth obturated with bioceramic impregnated and coated gutta-percha points using Brasseler's bioceramic sealer, with microleakage of teeth obturated with conventional gutta-percha and Roth sealer. To date there are few studies that have evaluated the sealability of this bioceramic sealer.

Eighty-five single-rooted maxillary anterior teeth were used for this study. Endodontic cleaning and shaping of each root canal system was accomplished using K-type hand files and NiTi Rotary files. Group A consisted of 27 anterior teeth, which were obturated using a bioceramic sealer and gutta-percha with System B and the HotShot. Another 27 anterior teeth, Group B, was obturated using Roth's sealer and gutta-percha with System B and the HotShot. A final group, Group C with 27 teeth was obturated using the single cone technique by means of a single gutta-percha point with the bioceramic sealer. Two teeth were used as a positive control group, and two other teeth as a negative control group. A microbial leakage apparatus was constructed using a similar two-chamber method described by Torabinejad et al.[13] *E. faecalis* ATCC 29212 was the test bacterium, which was used in this study to determine microleakage.

This study did demonstrate a significant difference in microleakage between teeth obturated with the bioceramic sealer compared with teeth obturated with Roth sealer. The results suggested higher sealing capability with the Roth sealer. This is clinically significant when considering materials to be used during obturation. Further investigation

is needed to improve dentin bonding in the root canal system, setting time of the bioceramic sealer and heat application to the bioceramic-impregnated and coated gutta-percha points used with the bioceramic sealer.

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ABSTRACT

AN *IN-VITRO* COMPARISON OF BACTERIAL MICROLEAKAGE OF ZINC-OXIDE-EUGENOL SEALER AND BRASSELER BIOCERAMIC SEALER

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The purpose of this study was to evaluate the sealing properties of EndoSequence BC sealer, a bioceramic sealer, and Roth's sealer using gutta-percha with warm vertical condensation and using the single cone technique with the EndoSequence BC sealer only.

Eighty-five single-rooted maxillary anterior teeth were used for this study. Endodontic cleaning and shaping of each root canal system was accomplished using K-type hand files and NiTi Rotary files. Group A consisted of 27 anterior teeth, which

weres obturated using EndoSequence BC sealer and gutta-percha with System B and Obtura. Another 27 anterior teeth forming Group B were obturated using Roth's sealer and gutta-percha with System B and Obtura. In the final group, Group C, 27 teeth were obturated using the single-cone technique by means of a single gutta-percha point with EndoSequence BC sealer. Two teeth were used as a positive control group, and two other teeth as a negative control group. A microbial leakage apparatus was constructed using a similar two-chamber method. The test bacterium used to determine microleakage was *E. faecalis* ATCC 29212.

The outcome of interest (bacterial turbidity) and time-to-leakage (in days), was 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 results from this study indicated no microleakage was observed in the negative control or Group B. Microleakage was observed in all positive controls. Group A and C had a significantly higher proportion of samples with microleakage than group B ($p < 0.0001$), but Group A and Group C were not significantly different from each other ($p = 0.50$). Time to microleakage was also significantly lower in Group A and Group C than Group B ($p < 0.0001$), but group A and C were not significantly different from each other ($p = 0.37$).

In conclusion, using *E. faecalis* as our test bacteria, the microleakage of canals obturated with gutta-percha and Roth sealer was significantly less than in canals obturated with gutta-percha and EndoSequence BC sealer.

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